Cytogenetic and phytochemical aspects of members of Acanthaceae used as the drug 'Sahachara' in Ayurveda

Thesis
submitted to the University of Calicut
for the award of the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

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MARCH 2014

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CERTIFICATE

This is to certify that the thesis entitled "**Cytogenetic and phytochemical aspects of members of Acanthaceae used as the drug 'Sahachara' in Ayurveda**" submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY, is an authentic record of original research work done by RENJANA P. K., during the period of her study (2009-2013) at Cell and Molecular Biology Division, Department of Botany, University of Calicut under my supervision and guidance and that it has not formed the basis for the award of any degree/diploma to any candidate of any university.

Dr JOHN E. THOPPIL
Supervising Teacher

DECLARATION

I, Renjana P. K., hereby declare that the thesis entitled "**Cytogenetic and phytochemical aspects of members of Acanthaceae used as the drug 'Sahachara' in Ayurveda**" submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY is a record of original research work done by me under the supervision and guidance of Dr John E. Thoppil, Associate Professor of Botany, University of Calicut and that it has not formed the basis for the award of any degree/diploma to any candidate of any university.

Date: **Renjana P. K.**

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ACKNOWLEDGEMENT

It is a pleasure to remember and specially mention all those who have helped in various ways to carry out my research work and compilation of the thesis.

First of all, I would like to express my deepest admiration and heartfelt gratitude to my guide Dr John E. Thoppil, Associate Professor of Botany, University of Calicut, for his constant support, timely supervision, valuable suggestions and guidance. His continuous encouragement and inspiration motivated me to put all my efforts and concentration focussed in to the work.

I am greatly obliged to Dr M. Sabu, Head, Dr Nabeesa Salim, Dr K. V. Mohanan and Dr P. V. Madhusoodanan, former Heads, Department of Botany, University of Calicut for providing the administrative assistance and necessary research facilities.

I place on record my sincere gratitude to Dr. S. Nandakumar, Former Head, Department of Botany, University of Calicut for the helpful suggestions and clarifications of my doubts and queries.

The help rendered by Dr A. K. Pradeep, Assistant Professor, Department of Botany, University of Calicut in identifying and authenticating the plant materials used in the study is thankfully acknowledged.

I am greatly indebted to Dr Ramadasan Kuttan, Director and Dr Jose Padikkala, Senior Scientist, Amala Cancer Research Centre, Thrissur for providing facilities to do a part of my work.

I wish to express my very great appreciation to Mr. Prakashan P. M., Librarian, Department of Botany as well as the staff of INFLIBNET, University of Calicut for the help rendered during the literature survey.

I would like to acknowledge the staff of Biochemistry Division, CEPC Laboratory and Technical Division, Cashew Bhavan, Kollam, Kerala for the facilities provided for GC-MS analysis.

I am extremely thankful to Dr Remasree A. B., Deputy Project Director, Centre for Medicinal Plants Research, Kottakkal, Malappuram, Kerala for the help rendered in HPTLC analysis.

I am immensely thankful to the entire teaching faculty as well as the non-teaching staff and the students, Department of Botany, University of Calicut for all the help and generous support I received from them.

The financial support of the University Grants Commission is greatly acknowledged.

I am happy to record my sincere thanks to the Director, Department of Collegiate Education, Thiruvananthapuram and Prof. P. M. Raghavan, Principal and Prof. P. Sushama and Prof. K. Vijayan, Former Principals, Govt. Arts and Science College, Kozhikode who have permitted me to do my Ph. D. work.

I express my deep sense of gratitude to my colleagues Prof K. K. Abdul Majeed, Former Head, Dr Sheela S., Associate Professor and Head, Dr Vasantha V. A., Associate Professor and Sinitha K., Assistant professor, Department of Botany, Govt. Arts and science College for their constant support and encouragement.

I record my immense obligation to Dr P. N. Ravindran, Former Director, Indian Institute of Spices Research (IISR), Calicut; National Coordinator for Spices Research under ICAR and Mrs. M. Shylaja Ravindran, Former Head, Providence Women's College, Calicut for their suggestions and motivation.

I am greatly indebted to all my teachers of St. Joseph's College, Devagiri and Providence Women's College, Kozhikode for their inspiration and encouragement throughout the study.

I owe my sincere gratitude to my labmates at Amala Cancer Research Centre, Thrissur Dr Fijesh P. Vijayan, Adil P. Nafar, Vipin Sivaram, Seema Menon,

Lincy Lawrence, Johannah Natinga and Jayaseelan R. S. for their kind co-operation and scholarly assistance rendered during the antioxidant and anti-inflammatory analysis on mice.

The immeasurable help, co-operation and constructive suggestions extended by my labmates Dr Leeja L., Dr (Sr) Betty K. P., Dr Umesh Balakrishanan B. T., Dr Jija Mathew, Dr Asha Ramachandran, Mrs. Julie John, Mrs. Seema Devi, Ms Sandhya Vincent Neelamkavil, Mrs Anjana S., Mrs Sumitha K. V., Mrs Lasi Das and Ms Prajitha V. are thankfully acknowledged.

A special word of thanks to Mr Alfred Joe, Research Scholar, Department of Botany, University of Calicut for the immense help rendered to take photographs of the plant materials used in the study and also in the making of herbarium.

I would like to express my sincere gratitude to all my friends and well wishers for their whole hearted support and motivation without which it would not have been possible for me to complete this endeavour.

Last, but not the least I wish to express my gratitude towards my family members for their patience, endurance and untiring support. I am dedicating this thesis to my father whom I fondly remember in every walk of my life.

Renjana P. K.

ABBREVIATIONS

ABTS	:	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACL	:	Average Chromosome Length
b. wt	:	body weight
c.	:	circa
CHIAS	:	Chromosome Image Analysing System
cm	:	centimetre
°C	:	Degree Celsius
DI	:	Disparity Index
DMSO	:	Dimethyl Sulphoxide
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DW	:	Dry Weight
EDTA	:	Ethylene Diamine Tetra Acetic acid
Fig.	:	Figure
FRS	:	Free Radical Scavengers
GC	:	Gas Chromatography
GC-MS	:	Gas Chromatography-Mass Spectrometry
h	:	hour
H_2O_2	:	Hydrogen peroxide
HC	:	Higher Concentration
HPTLC	:	High Performance Thin Layer Chromatography
IAEC	:	Institutional Animal Ethics Committee
IC_{50}	:	Inhibitory Concentration 50%
LC	:	Lower Concentration
LC-MS	:	Liquid Chromatography-Mass Spectrometry

LD_{50}	:	Lethal Dose 50%
M	:	Molar
m	:	metre
µg	:	microgram
µg/ml	:	microgram/millilitre
µl	:	microlitre
µm	:	micrometer
µM	:	micro Molar
mg	:	milligram
mg/kg	:	milligram/kilogram
min	:	minute
ml	:	millilitre
mM	:	milli Molar
mm	:	millimetre
mmol/L	:	millimoles per Litre
N	:	Normal
NBT	:	Nitro Blue Tetrazolium
NSAIDs	:	Non Steroidal Anti-Inflammatory Drugs
OD	:	Optical Density
PAI	:	Paired Affinity Index
PBS	:	Phosphate Buffered Saline
PC	:	Primary Constriction
PDB	:	Para-dichlorobenzene
p.o.	:	per oral
RCL	:	Range of Chromosome Length

R_f	:	Retention factor
ROS	:	Reactive Oxygen Species
SE	:	Standard Error
SOD	:	Super Oxide Dismutase
TAE	:	Tris Acetic Acid EDTA
TBA	:	Thiobarbituric Acid
TBARS	:	Thio Barbituric Acid Reacting Substances
TCL	:	Total Chromosome Length
TE	:	Tris EDTA
TF%	:	Total Forma Percentage
U	:	Unit
V	:	Volume
v/v	:	volume per volume
var.	:	variety
VC	:	Variation Coefficient
vs	:	versus
w/v	:	weight per volume
WHO	:	World Health Organization

Appendix 1

Phosphate Buffered saline (PBS)

NaCl	:	8 g
KCl	:	0.2 g
Na_2HPO_4	:	1.44 g
KH_2PO_4	:	0.2 g

Dissolve in 1 litre double distilled water and pH adjusted to 7.4

Appendix 2

Phosphate buffer

A) KH_2PO_4	:	916 mg
B) Na_2HPO_4	:	950 mg

Dissolve both in 100 ml double distilled water separately. Take 8.5 ml of solution A and add

91.5 ml of solution B and pH adjusted to 7.8

Appendix 3

KH_2PO_4 - KOH buffer

A) KH_2PO4	:	0.272 g
B) KOH	:	0.112 g

Dissolve both in 100 ml double distilled water separately. Mix both the solutions and pH adjusted to 7.4

Appendix 4

Tris-HCl buffer

Tris- HCl	:	1.575 g

Dissolve in 100 ml double distilled water and pH adjusted to 7

INTRODUCTION

Man has been dependent on plants in nearly every aspect of life from time immemorial. The World Health Organization (WHO) estimates that about 65-80% of the world's population in developing countries, due to poverty and lack of access to modern medicine, relies exclusively on plants for their primary health care needs (Calixto, 2005). Worldwide, tens of thousands of higher plants and several hundred lower plants are currently been used by humans for a wide variety of purposes. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Rios and Recio, 2005).

Ayurveda, accepted to be the oldest treatise on medical system, based on lifestyle, diet and herbs, came into existence in about 900 B. C. The demand for plant based therapeutic agents has increased manifold in both developing and developed countries due to the growing recognition that they are non-narcotic, having no side-effects and are easily available at affordable prices. The healing techniques and preventive methods of Ayurveda include many herbal formulations that possess long term health promoting and medical qualities, which can help to access our body's innate healing intelligence by strengthening the immune system. The complexity of the newly deriving diseases in the current depleting environment causes rapid expansion in the number of medical prescriptions and also accelerates the acceptance of various forms of alternative medicine, most of them employing herbs of some form.

Nature has bestowed on us a very rich botanical wealth, which includes a large number of medicinal and aromatic plants. Throughout the centuries, herbalists have used specific herbs or combinations of herbs to treat various

diseases. Natural products, their derivatives and analogs represent over 50% of all drugs in clinical use, with higher plant derived natural products representing 25% of the total (Balandrin *et al*., 1993). According to the WHO, around 85% of the traditional medicine involves the use of plant extracts (Farnsworth, 1988). Although synthetic drugs and antibiotics brought about a revolution in controlling different diseases, plants occupy a significant place in modern medicine as judicious use of medicinal herbs could even cure deadly diseases that have long defied synthetic drugs.

Herbal remedies are natural products derived from plants and plant extracts that have been traditionally used to treat various diseases or to promote general health (Tapsel *et al*., 2006). Herbal medicines are the result of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine over hundreds of years and they are known to be the oldest health care products that have been in use worldwide as folklore, traditional or ethnic medicines. The popularity and acceptability of herbal medicines are mainly due to the toxicity and undesirable side effects of the synthetic allopathic medicines. Natural products from plants have provided the pharmaceutical industry with one of its most important sources of lead compounds. Investigation into the natural products and traditional medicines to explore the possibility of developing potent drugs from local resources should be given priority (Umadevi, 2000). Up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version (Hsieh *et al*., 2008). The plant-based formulations have been found to be effective against mild or chronic ailments and have proved biologically more compatible with human system.

Medicinal plants constitute a very important natural resource of India, making it one of the richest plant based ethno-medical traditions in the world (Rajasekharan and Ganesan, 2004). The market of traditional drugs has

enormously increased in India during the last two decades (Mukhopadhyay, 2002); on recognition of the fact that many drugs are manufactured from synthetic raw materials and are considered to be unsafe (Rajamani, 2001). The global market for medicinal plants and herbal medicine is estimated to worth US $800 billion in the North-Eastern States of India (Syiem *et al*., 1999). But, unfortunately, crude drug scenario in India is much unorganized and unsystematic (Agarwal, 1998). Despite the fact that our country has a well recorded and well practiced knowledge of indigenous medicare system, we have not yet been able to capitalize on this rich heritage, mainly due to the non-availability of standardized herbal drugs and their formulations.

Majority of the crude drugs available in the market are supplied by crude drug suppliers who procure the material from collectors and the collectors in turn collect them from the wild (Athneria *et al*., 1987). The collectors as well as the crude drug sellers are generally uneducated and ignorant about the botanical identity of the medicinal plants. Thus, the materials supplied in many cases can be either intentionally or unintentionally adulterated. Botanical names are very rarely used outside the scientific community (Narayana *et al*., 1997), and hence there can be several names in vernacular languages used to denote a single crude drug. The physicians are not directly linked to the plants or the manufacturers who only know the common names which changes frequently. Thus there is every chance for misinterpretation of the plants to be used as raw material sources of the drugs. Proper identification of the plant source and isolation of the genuine raw material from the adulterants or impurities are essential prerequisites for the preparation of genuine drugs. According to Rao and Savithramma (2001), problems in the identification of the medicinal plants have led to the increased use of adulterants. Physical verification is also very difficult mainly because the plant part used in many cases like rhizomes, bark, roots *etc*. show close similarity with that of the adulterants or substitutes. Thus the only way to

discriminate the adulterants would be by chemical, anatomical and pharmacognostical examinations (Raghunathan and Mitra, 1982).

Manufacture of Ayurvedic drugs has become an important industry. The recent global resurgence of interest in herbal medicines has led to an increase in demand for them. But unfortunately, commercialization of the manufacture of these medicines to meet this increasing demand has resulted in a decline of their quality. This necessitates an urgent need to evolve a systematic approach to develop well-designed methodologies for the standardization of herbal raw materials and herbal formulations to ensure their reproducible and safe therapeutic effects (Rajani and Kanaki, 2008).

Quality control standards of herbal drugs have become more relevant now because of the increased commercialization of formulations based on medicinal plants (Yadav *et al*., 2009). This necessitates an urgent need to control and guarantee the quality of such preparations through systematic scientific studies including chemical standardization, biological assays and validated clinical trials (Firenzuoli and Gori, 2000). The United States Food and Drug Administration (USFDA) and European Agency for the Evaluation of Medicinal Products (EMEA) have drafted guidelines on various aspects of quality control of medicinal plants and among these, the chemical standardization of a herbal formulation with respect to its major phytoconstituent (preferably active) has emerged as the most sought after parameter (Bansal and Bansal, 2011). Numerous separation methods coupled with mass spectrometric detection (MS) are employed for herbal drug analyses and standardization (Chen *et al*., 2009). Application of chromatographic techniques ensures consistency in the quality of drug material (Uniyal and Uniyal, 2002). The pattern of spots obtained after TLC is suitable for monitoring the identity and purity of drugs and for detection of adulterants and substitution. Though most of the widely used drug plants are

amply described in the various pharmacopoeias, their standards in terms of chemical markers and Thin Layer Chromatography (TLC) fingerprints have yet to be established (Yadav *et al*., 2009). International Union of Pure and Applied Chemistry (IUPAC) also have published a technical report on safety, efficacy, standardization and documentation of herbal medicines (Mosihuzzaman and Choudhary, 2008).

Acanthaceae is a large pantropical family comprising about 357 genera and 4350 species (Mabberley, 1987). The members are chiefly distributed in the tropics and subtropics and are also found in the Mediterranean regions. The members of Acanthaceae are chiefly perennial, armed or unarmed herbs or shrubs or rarely trees. The family is characterized by the possession of simple opposite decussate leaves, presence of swollen nodes, cystoliths in vegetative parts, prominent bracts and bracteoles and mostly bilabiate corolla with bilocular ovary. In India, the family is represented by 427 species falling in 81 genera (Santapau and Henry, 1973). The important genera coming under Acanthaceae are *Acanthus*, *Andrographis*, *Barleria*, *Thunbergia*, *Ruellia*, *Justicia*, *Ecbolium*, *Strobilanthes etc*. The Acanthaceae family is an important source of several indigenous therapeutic drugs. The ethnopharmacological knowledge of this family requires urgent documentation as quite a lot of its species are at the verge of extinction (Correa and Alcantara, 2012). Though several members of Acanthaceae are reported to be used in traditional Indian herbal and folk medicines, relatively few appear to have been analysed in detail. Authentic phytochemical standards are not available for many of the members of this medicinally important family.

'Sahachara' is a well known Ayurvedic drug widely used against rheumatism and neurological disorders such as paraplegia and sciatica. This drug is also used for curing ulcers, glandular swellings, poisonous affections, itching, leprosy, other skin diseases, cough, edema, tooth ache, gum diseases

and to strengthen nerves (Sivarajan and Balachandran, 1994). The official part of the drug is the root which is used as the major ingredient of several important Ayurvedic preparations.

The correct identity of the species to be used as 'Sahachara' is controversial as several confusing Latin names have been assigned to 'Sahachara' in various texts and Ayurvedic treatises (Warrier, 1996). The crude drug 'Sahachara' is marketed as dried roots and hence it is impossible to ascertain the proper identity of the plant source. It is found that a variety of herbs of the family Acanthaceae are being used as the plant source of 'Sahachara' in different regions. At least around ten different species are presently in use under the name of 'Sahachara' by different people. Many physicians are of the opinion that the drug should be equated with *Strobilanthes*, but the synonyms and qualities ascribed to 'Sahachara' are not mentioned in the case of *Strobilanthes*, while they are cited for *Barleria* by others. On the basis of Ashtangahrudayakosam, many physicians in Kerala equate *Ecbolium viride* with 'Sahachara'. In Ayurvedaviswakosam, *B. cristata* is equated with 'Sahachara'. But in Kerala, *B. cristata* is not used medicinally; it is treated only as a garden plant. In Kerala, *Strobilanthes ciliatus* is used mostly as the plant source of 'Sahachara'. But the Malayalam name 'karinkurinji' is not quite appropriate for *S. ciliatus* because its flowers are white (Nair, 2004). According to Kirtikar and Basu (1987), only the bark and the flowers of *S. ciliatus* are of some medicinal value.

None of the above plants exactly matches with all the indications given in the synonyms. All of them have been in use as 'Sahachara' for years in different places. Though plant taxonomy can very well support the evolution of unambiguous standards for plant sources of drugs, the genuineness of the correct source plant have to be decided only after pharmacological and adequate clinical trials. So, comparative pharmacological investigations of

these herbs are critical to find out which one among them possesses curative properties and are therapeutically more efficacious (Sivarajan and Balachandran, 1994).

According to the texts, 'Sahachara' is a gregarious shrub, having sharp piercing structures like spines and occurring in fully exposed habitats like deserts, naked hill tops or the tidal areas along the sea coast (Sivarajan and Balachandran, 1994). The distinction of different varieties of 'Sahachara' is mainly based on flower colour and these varieties have been equated mostly with species of *Barleria*. Kerala physicians, on the other hand recognize two varieties, the dark variety and the white variety. According to Van Rheed's *Hortus malabaricus*, *E. viride* had been the accepted source of the drug in those days. Nevertheless, currently in Kerala, *S. ciliatus* is increasingly being used as the source of 'Sahachara'. An examination of the market samples brought to light that roots of *Strobilanthes heyneanus* has also been used widely as 'Sahachara' in different formulations (Sivarajan and Balachandran, 1994). According to Nair *et al*. (1985b), the synonyms and descriptions of the source plants of the drug are applicable to these species also. Several other authors are of the opinion that yet another plant of Acanthaceae, *viz*., *Justicia betonica* also can form the source of the drug (Sivarajan and Balachandran, 1994).

Plant derived compounds play a vital role in drug discovery programmes as many novel compounds have been discovered and successfully utilized by the pharmaceutical industry in the manufacture of drugs. The large scale use of medicinal plants and herbs in the preparation of drugs are increasing due to the growing concern about the side effects of chemicals and synthetic substances. The extracts of plants that are found to have specific disease curative properties are increasingly being used to manufacture effective drugs. More than 50% of all successful modern drugs

in clinical use are of natural product origin (Taraphdar *et al*., 2001). The study of crude drugs of vegetable origin, which forms the source of majority of Ayurvedic medicines, is an unavoidable part in the academic learning of indigenous drugs. Immediate effort should be made to tackle the issues pertaining to the controversy of identification of Ayurvedic drugs, their illegal substitutions and adulteration prevalent in the markets which is a huge problem faced by the Ayurvedic physicians and pharmaceutical firms (Ansary, 2005).

To develop new drugs that satisfy the requirements of the modern treatment, the pharmacological properties of the plant need to be evaluated. The assurance that the medicinal herbs are safe, effective and are constantly active is equally important as the identification, isolation and characterization of their active principles. Therefore, before commencing any chemical investigations, it is necessary to check the presumed activity of the plants. Experimental evidence suggests that many plants of the family Acanthaceae possess a wide range of biological and pharmacological activities that may provide protection and cure against several human ailments.

Herbal medicines are frequently used to treat chronic diseases compared to modern medicines (Calixto, 2000) and all chronic diseases have an element of increased inflammatory response in common. The generation of free radicals in excess can cause oxidative damage to functional macromolecules such as DNA, proteins and lipids leading to many diseases such as age-related disorders, cancer, atherosclerosis, neurodegenerative diseases and inflammation (Cai *et al*., 2004; Dragland *et al*., 2003). Antioxidant compounds from plants can minimize the generation of free radicals and alleviate diseases caused by oxidative stress (Akinmoladun *et al*., 2010; Ozen *et al*., 2010).

The inflammatory process may be defined as a sequence of events that occurs in response to noxious stimuli, infection or trauma (Calixto *et al*., 2004). Sometimes, inflammation seems to produce events that are quite serious and become chronic like occurrence of rheumatoid arthritis and hay fever which may be life threatening (Denko, 1992; Singh *et al*., 2008a). Infections and inflammations have long been recognized as related to cancer also. There is extensive data demonstrating the role of inflammation in the establishment, progression and complications of various malignancies (Rayburn *et al*., 2009).

Various synthetic anti-inflammatory drugs have been in use; both steroidal and non-steroidal. The non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs prescribed worldwide for pain, inflammation and fever. Though these have potent activity, long-term administration required for treatment of chronic diseases can cause several adverse effects that include hirsutism, Cushing's habitus, hypersensitivity reactions, peptic ulceration, hyperglycemia, osteoporosis, various immunodeficiency related problems as well as toxic effects on the gastrointestinal tract, kidney and cardiovascular system (Beg *et al*., 2011; Arya and Arya, 2011). The greatest disadvantage of the modern synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their anti-inflammatory activity is the need of the hour and there have been many efforts for finding anti-inflammatory drugs from indigenous medicinal plants (Srinivasan *et al*., 2001).

The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species (ROS) from activated neutrophils and macrophages. In addition, ROS propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor, and

interferon which stimulate recruitment of additional neutrophils and macrophages. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation (Geronikaki and Gavalas, 2006).

The market for use of herbal drugs in the treatment of inflammatory diseases constitutes 83% worldwide and is expected to reach a value of around more than 95% in the forthcoming years due to their increased acceptability (Bent and Ko, 2004; Boullata and Nace, 2000). The inflammatory mediators induce the formation of the enzyme cyclooxygenase-2 (COX-2) in different inflammatory states. Recently many natural products are being evaluated for their ability to selectively inhibit COX-2 and hence could be used as drugs for cancer and Alzheimer's disease.

Natural products with anti-inflammatory activity have long been used as a folk remedy for inflammatory conditions such as fevers, pain, migraine and arthritis. Plant-derived bioactive compounds with anti-inflammatory effects can act as potential source for the development of pharmaceuticals for treating rheumatoid arthritis, asthma, Alzheimer's diseases, multiple sclerosis and ulcerative colitis (Polya *et al*., 2002). The inflammatory response of a tissue is regulated by a variety of regulatory proteins which are the actual potential targets of the anti-inflammatory drugs. The anti-inflammatory treatment given to such auto-immune diseases helps to overcome the accompanying tissue damage and pain to a great extent. The anti-inflammatory bioactives of plant origin are principally phenolics and flavonoids and their effects can be attributed partly to their inhibition of COX and Lipoxygenase (LOX), thereby hindering prostaglandin and leukonetrine synthesis.

Cigarette smoking and chronic inflammation - two major causes of cancer has strong free radical components in their mechanism of action. Recently, the studies on oxidative stress and its adverse effects on human health has become a subject of considerable interest. Reactive intermediates in oxidation processes, particularly free radicals are receiving increased attention in biology, medicine and food chemistry, as well as in environmental areas (Jayaprakasha *et al*., 2006). ROS are produced by cellular metabolism and by exogenous agents in the cells. These ROS can induce oxidative damage to biomolecules and tissues which in turn leads to cardiovascular and neurodegenerative diseases, inflammation, arthritis, diabetes, asthma and others. At least two major problems in human ageing and cancer are mediated by ROS induced DNA damage (Kumar and Chattopadhyay, 2007). As antioxidants can decrease tissue damage by scavenging/neutralizing ROS, they can be of great value in preventing the onset and the propagation of several oxidative diseases (Hafidh *et al*., 2009). Therapeutic effects of several medicinal plants which are commonly used in folk medicine against many diseases are usually attributed to their phenolic contents and antioxidant properties (Coruh *et al*., 2007).

Chemical profiling is a versatile technique and can be used in standardization by generating fingerprints through different analytical procedures (Mukherjee, 2002). The pattern recognition through chromatography can be used to discriminate different kinds of samples of traditional herbal medicines investigated (Giri *et al*., 2010). Following its widespread application in the quality control assessment of crude drugs and foods, chemical fingerprinting has recently gained increased attention (Tao *et al*., 2011; Zheng *et al*., 2009; Locatelli *et al*., 2009). A chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract of some pharmacologically active or inactive chemical components. By using reliable chromatographic fingerprints, the

authentication and identification of herbal medicines can be accurately conducted (Patil and Shettigar, 2010; Liang *et al*., 2004). Fingerprint analysis by HPTLC has become an effective and powerful tool for linking the chemical constituent profile of the plants with botanical identity and for the estimation of chemical and biochemical markers (Narmadha and Devaki, 2012b). HPTLC fingerprint is suitable for rapid and simple authentication and comparison of the subtle difference among samples with identical plant resource but different geographic locations and hence is a very important tool in herbal drug industry (Soni and Naved, 2010). It also offers better resolution and estimation of active constituents with reasonable accuracy in a shorter time.

Chemical features of medicinal plants serve as a fundamental determinant of their pharmacological properties (Lovkova *et al*., 2001). The rapid vanishing of the biodiversity rich tropical forests and other vegetation calls for an urgent isolation and identification of bioactive natural products. The characterization of metabolites in complex mixtures requires sophisticated hyphenated techniques with good sensitivity and selectivity. The plant kingdom represents an extraordinary reservoir of novel molecules that can be a possible source of new and promising lead compounds to be introduced into therapeutics. Therefore, naturally originated agents with very little side effects are the need of the hour to substitute chemical therapeutics (Conforti *et al*., 2008).

Of the plant species so far been estimated worldwide, only a very meager number have been explored phytochemically and the fraction subjected to bioassay or pharmacological screening is even much lower. Techniques like GC/MS and LC/MS provide a great deal of information about the presence and nature of constitution of crude plant extracts. Mass Spectrometry (MS) is a highly sensitive and specific tool for identifying

organic compounds when coupled with separation techniques like GC or HPLC. GC and GC-MS are the most common, unanimously accepted methods for the analysis of volatile constituents of herbal medicines. For the detection of volatile non-polar compounds, Gas Chromatography/Mass Spectrometry (GC/MS) has been applied successfully and routinely. MS detection of non-volatile polar molecules is not simple and demands the coupling of High pressure Liquid Chromatography (HPLC) with Mass spectrometer. The hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents (Teo *et al.*, 2008; Nikam *et al.*, 2012).

During the last decade, Liquid Chromatography/ Mass Spectrometry (LC/MS) techniques were developed employing soft ionization methods like electrospray or photoionization and mass spectrometers have become more sophisticated and robust for daily use. LC/MS can be an extremely powerful technique for screening crude plant extracts with the right ionization conditions carefully optimized (Hostettman and Wolfender, 2001). The combination of the high separation efficiency of HPLC with MS has made possible the acquisition of information about LC peak of interest within a complex mixture as plant extracts. LC/MS has been proven to be highly useful in the field of analytical phytochemistry since mid ‘80s. This progress has been achieved because of its unequalled sensitivity, efficient detection, high speed and wide applicability over a large number of biochemical purposes including natural products monitoring and drug discovery (de Hoffmann and Stroobant, 2007).

Plants produce a great variety of organic compounds that are not directly involved in primary metabolic processes of growth and development. Secondary metabolites are low molecular weight compounds occurring within the plant kingdom derived from the primary metabolites. More than 1,00,000

structures have already been described (Buckingham, 1994; Croteau *et al*., 2000). These plant metabolites are grouped as alkaloids, glycosides, phenols, flavonoids, tannins, mucilage, essential oil *etc*. Due to their large biological activities, plant secondary metabolites have been used for centuries in traditional medicine.

Among the several hundreds of secondary metabolites found in a medicinal plant, the main effective compounds are called active constituents. Quantification of the active ingredient is the first step of standardization of herbal drugs and among the vast diversity of phytoconstituents reported so far, there has been a growing interest in phenolics, including flavonoids, because of their health promoting functions (Qian *et al*., 2004; Chen *et al*., 2006a) and significant biological activities (Robbins and Bean, 2004). Phenolics and flavonoids are known to be responsible for conferring the medicinal herbs much of their curative properties. These two classes of compounds are well reputed for their antioxidant and anti-inflammatory properties and therefore can lower the risks of cardiovascular diseases, arthritis, chronic inflammation and cancers (Chen *et al*., 2005; Zhang *et al*., 2005). Their inhibition of the enzymes COX and LOX result in a decrease of platelet activation and aggregation. Thus it is responsible for their anti-inflammatory activity leading to protection against cardiovascular diseases and cancer chemoprevention. Phenolics and flavonoids are known to exhibit many other biological activities like antiviral, antimicrobial, antihepatotoxic, antiosteoporotic, antiulcer, immunomodulatory, antiproliferative and apoptotic activity (Kim *et al*., 2004; Tapiero *et al*., 2002; Yao *et al*., 2004; Sadik *et al*., 2003).

Though the members of Acanthaceae are widely represented in our flora and are of considerable medicinal and ethnic importance, yet very little attention has been paid to their cytological analysis. Very few detailed studies

have so far been conducted on the chromosome analysis of the different species used as the source plants of 'Sahachara'. Even where the chromosome numbers have been determined, the knowledge concerning the details of karyotype pertaining to chromosome measurements is still fairly insufficient. Cytology is used as a dependable tool for solving taxonomic problems and for elucidating systematic relationships, phylogeny and evolution of related plants. Information regarding chromosome number, ploidy, structure and morphology has been of considerable importance in understanding their relationships and delimitation of taxa. The chromosome numbers tend to exhibit a greater consistency than any other character since they are an integral part of the genetic system (Yoshikane and Naohiro, 1991; Den Hartog *et al*., 1979). Therefore, it can be used in the same way as the morphological characters for identification and standardization. Chromosome research requires a study of the morphology of the individual chromosomes. Karyological studies can reveal the variations in chromosome number and size and suggest the direction of chromosomal evolution in specific taxa (Jones, 1979). Karyotype analysis with computer based Chromosome Image Analysis System (CHIAS) proves to be a powerful tool to study chromosome morphology with great precision and speed. The imaging methods are indispensable in routine chromosome analysis such as karyotyping, detection of chromosome aberrations and also in the basic chromosome research (Fukui and Nakayama, 1998). Hence a comprehensive study of the chromosome constitution of the source plants of 'Sahachara' has also been carried out to understand the cytogenetic constitution with the help of chromosome imaging.

To facilitate the continued development of high-quality, unadulterated drugs that will boost up the credibility of the system, further extensive research deep in the field of medicinal and aromatic plants are needed. The standardization of the herbal drugs can be done by taking up critical

pharmacognostic studies using botanical, pharmacological and chemical parameters.

Since authentic phytochemical standards are yet to be available for the various species that are used as 'Sahachara', presently there is no definite way to ascertain the quality equivalence of the different species used as 'Sahachara', *ie*., whether they are with more or less the same or entirely different properties and chemical quality spectrum. The present study looks forward to a detailed karyomorphological, phytochemical and pharmacological investigations of the source plants of 'Sahachara', that are common in Kerala. This could be helpful in gaining more information about the plants that are being used under the name of this popular drug used in large quantities by the Ayurvedic industry of India.

The present investigation aims to fulfill the following objectives:-

- Chromosome imaging and karyomorphological analysis of few plants of Acanthaceae that are used as the source plants of 'Sahachara'.
- Pharmacological investigations of the plants by screening their comparative antioxidant and anti-inflammatory activities.
- Chemical fingerprinting of the plants by HPTLC.
- GC-MS and LC-MS analyses of the root methanolic extracts for further chemical characterization.
- Measurement of total phenolic and flavonoid content of the extracts.

REVIEW OF LITERATURE

The production, consumption and international trade in medicinal plants and phytomedicines are growing and expected to grow in future quite significantly. Medicinal plant based drugs have the advantage of being simple and effective with broad spectrum activity with an emphasis on the preventive action. The search for herbal remedies and natural substances and understanding their mechanism of action in the body are on the rise, but unfortunately, the area of our indigenous medical system dealing with Ayurvedic drugs has not received adequate attention it deserves. The availability of good quality raw materials free from adulterants in sufficient quantity has been one of the chronic problems of the phyto-pharmaceutical industry. Presently no quality standards have been fixed for the raw materials and so one finds wide variations in the quality specifications (Narayana, 1998). A great deal of adulteration and substitution is met with commercially available raw drugs owing to the confusion caused by the different local names these plants are known by and also due to their diverse geographical locations. This confusion demands an urgent need for standardization of the real drug and identification of its substitutes and adulterants.

There is a growing interest in the investigation of secondary metabolites from food stuffs and other plants as potential therapeutic agents against several human ailments. Chemical profiling is a versatile technique and can be used in standardization by generating fingerprints through different analytical procedures (Mukherjee, 2002). While the plants under study are found to have varied therapeutical properties, phytochemical studies are very much essential for standardization and application on a scientific basis for identifying the active chemical components (Subramanian and Sasidharan, 1997).

In the context of the present work which deals with the cytogenetic, phytochemical and pharmacological aspects of few plants of Acanthaceae used as the source plants of an important Ayurvedic drug 'Sahachara', the quantum of research output already available on their cytological studies, antioxidant as well as anti-inflammatory potential and phytochemical analyses are reviewed in chronological order.

A. CYTOGENETICAL STUDIES

Despite its considerable size, cytological knowledge of Acanthaceae is relatively scanty (Daniel *et al*., 1984). Eventhough many of the Acanthaceae are increasingly being used in traditional, tribal and ethnic medicines, yet very few attempts have been done on their detailed and critical karyological studies. The family includes many plants with alleged folkloric use as antioxidant and anti-inflammatory agents with great potential to be evolved as novel therapeutic agents. Many members of the family have been explored for their activities against the damaging effects of free radicals and inflammation which could be helpful in the development of novel and powerful drugs with low toxicity.

a. CYTOLOGICAL STUDIES

Chromosome number provides indispensable information on genetic discontinuities within and among species (Semple *et al*., 1989). Surveying available genome size and chromosome data of these medicinal plants can provide valuable and consistent information that can be used for solving taxonomic problems, and for elucidating systematic relationships, phylogeny and evolution of related plants.

Although many workers have attempted cytological studies of Acanthaceae, there have not been any proper cytogenetic investigations as far as the South Indian taxa are concerned (Govindarajan and Subramanian,

1983). Most of the chromosome studies of the family were carried out from the North and Central India with only few scattered reports from South India.

Chromosome analyses previously conducted in Acanthaceae suggest that the family is polybasic and reveals the presence of a continuous series of basic numbers between x = 7 and x = 34 (Govindarajan and Subramanian, 1983).

A more or less comprehensive study of chromosomes in the Acanthaceae was made by Grant (1955). He reported chromosome counts of 105 species and varieties representing 33 genera and suggested that poyploidy, and more commonly aneuploidy were responsible for the occurrence of diverse chromosome numbers in the family.

Chromosome numbers and meiotic abnormalities were observed in a few members of Acanthaceae by Joseph (1964). Cytological analysis of some species representing the subtribes Barlerieae, Andrographideae, Eujusticieae and Diclipteraeae of the Justicieae of Bentham & Hooker was carried out by Dutta and Maiti (1970). Their study suggests that Barlerieae is the most primitive subtribe of Justicieae.

Chromosome counts of 30 species belonging to 16 genera of Acanthaceae from South India have been made by Govindarajan and Subramanian (1983). Chromosome numbers determined in the study range from 2n = 16 to 2n = 60. The commonest basic number and the ancestral basic number in the family were suggested to be x = 16 and x = 8 respectively.

Saggoo and Bir (1986) reported meiotic studies in certain members of Acanthaceae including *Barleria* species. They found some relationship among the different taxa and made comparison between plant habit and incidence of polyploidy in perennial and shrubby species to annual and herbaceous ones.

The observations on the chromosome number of *Barleria* species revealed their diploid nature in spite of being shrubby.

Another worth mentioning study on the chromosome analysis of Acanthaceae was carried out by Daniel *et al*. (1990). They made a total of 72 meiotic chromosome counts for 58 taxa representing 23 genera of Acanthaceae from the United States. Among these, 41 counts were reported for the first time. He noted constancy of chromosome numbers on some genera while variation in chromosome number due to polyploidy and aneuploidy occurs in others.

Karyotypes of six species of *Barleria* L. occurring in South India *viz*., *B. acuminata*, *B. buxifolia*, *B. gibsoni*, *B. involucrata* var. *elata*, *B. noctiflora* and *B. strigosa* were studied by Ranganath and Krishnappa (1990). Though all species revealed the same chromosome number of 2n = 40, each species is distinct in respect of relative positions of centromere, presence/absence and position of satellites and total haploid chromatin length.

Cytology of eight species of *Barleria* from South India has been reported by Devi and Mathew (1991). All the species investigated had n = 20 or 2n = 40. Meiosis was normal in all, except one species (*B. courtallica*) in which univalents, bivalents, trivalents and tetravalents were consistently noticed. Somatic chromosomes were large sized, and the karyotypes were fairly symmetrical and unspecialised in all except *B. acwninata*.

Karyomorphological studies were conducted in *Strobilanthes glandulifera* and *S. tashiroi* by Kanemoto (2001). Chromosome numbers of both the species were noted as 2n = 30. Both the plants showed similar karyomorphological features like the interphase nuclei of the simple chromocenter type, mitotic prophase chromosomes of the proximal type and a

gradual size variation in mitotic metaphase chromosomes with a pair of satellite chromosomes.

Meiotic chromosome numbers were reported for 12 species in eight genera of Acanthaceae from Madagascar (Daniel, 2006). Chromosome numbers of 11 species are reported for the first time. A new chromosome number (n = 30) is reported in *Justicia*. Systematic implications of the chromosome counts are addressed and basic chromosome numbers for these eight genera of Malagasy Acanthaceae are discussed.

Sharma *et al*. (2010) recently investigated the chromosome number, chromosome associations, chiasma frequency, tetrads of microspores and pollen grains of *B. cristata* and *B. prionitis*. The chromosome number in both the species was 2n = 32. They also noted minute differences in the frequency of univalents and ring or rod bivalents among the two species. No changes could be observed in the frequency of occurrence of chiasma per chromosome in the two species.

Detailed karyomorphological analysis of *B. grandiflora* has been carried out by Gosavi *et al*. (2011). The species showed 2n = 44, which is the highest chromosome count for the genus so far reported. The study also revealed that the karyotype of *B. grandiflora* is moderately symmetrical.

Cytological investigations on some North Indian Bicarpellatae were carried out by Bala and Gupta (2011). Among a total of 11 species of Acanthaceae investigated, 7 were found to be diploids and 4 polyploids. The meiotic chromosome number (n) of *B. cristata* was determined as 19. They also reported that the meiotic chromosome number of both *Justicia diffusa* and *Strobilanthes alatus* is n = 16.

Cytomorphological studies on 30 populations of 26 species of the gamopetalae from Western Himalayas were done by Gupta *et al*. (2012).

The chromosome number of *B. cristata* was reported to be 2n = 40. It has also been reported that out of the two populations of *S. alatus* studied, one was revealed to be diploid (2n = 2x = 16) and the second one was found to be tetraploid (2n = 2x = 32).

Cytological studies on *Barleria* spp. was conducted by many scientists. Chromosome complements of n = 12, 15, 16, 17, 18, 19, 20 and 21 have been reported by several investigators (Grant, 1955; Goldblatt, 1985, 1988; Govindarajan and Subramanian, 1983; Moore, 1973; Daniel, 2000). However, most counts have been either n = 20 or 2n = 40.

Cytological works on *Ecbolium* are very few and diploid chromosome numbers of 2n = 36, 40 and 44 have been reported. Chromosome number reports on *Strobilanthes* are also relatively little. The haploid chromosome numbers so far reported in the genus are n = 9, 11, 13, 14, 15, 16, 20 and 21, the most common being 16. The highest chromosome number reported for the genus is 2n = 60 by Iwatsubo *et al*. (1993).

Although a wide range of chromosome numbers, from n = 9 to n = 31, has been reported for species of *Justicia*, n = 14 appears to be the most common number (Daniel *et al*., 1984). The species, *J. betonica* has been cytologically studied by many workers in the past and has been shown to have the diploid chromosome number of 2n = 34. But, according to Subramanian and Govindarajan (1980), the sporophytic count of *J. betonica* is 2n = 28.

Chromosome number reports of different members of the genera *Barleria*, *Ecbolium*, *Justicia* and *Strobilanthes* published so far are recorded in Table 1.

Table 1. List of chromosome number reports of different species of the genera *Barleria, Ecbolium, Justicia* and *Strobilanthes*

Botanical Name	2n	n	Author	Year
Genus: *Barleria* L.				
B. acanthoides Vahl		20	Khatoon & Ali	1993
B. acuminata Wight	40		Krishnappa & Basavaraj	1982
		20	Saggoo	1983
	40		Ranganath & Krishnappa	1990
	40		Devi & Mathew	1991
B. buxifolia L.	40		Krishnappa & Basavaraj	1982
	40		Subramanian & Govindarajan	1980
		20	Saggoo	1983
	40		Govindarajan & Subramanian	1983
		20	Saggoo & Bir	1986
	40		Ranganath & Krishnappa	1990
		20	Devi & Mathew	1991
	40	20	Ranganath	1981
B. courtallica Nees		20	Saggoo & Bir	1982
		20	Saggoo & Bir	1986
	40		Devi & Mathew	1991
B. cristata L.		20	Saggoo	1983
		20	Saggoo & Bir	1986
		20	Daniel & Chuang	1989
	40		Devi & Mathew	1991
		20	Khatoon & Ali	1993
		20	Sharma	1970
		20	Vasudevan	1976

B. cuspidata Heyne ex Nees	40		Subramanian & Govindarajan	1980
		20	Saggoo	1983
	40		Govindarajan & Subramanian	1983
		20	Saggoo & Bir	1986
	40	20	Devi & Mathew	1991
B. gibsonii Dalzell	40		Krishnappa & Basavaraj	1982
	40		Ranganath & Krishnappa	1990
		20	Vasudevan	1976
	40		Ranganath	1981
B. involucrata Nees	40		Govindarajan & Subramanian	1985
B. involucrata var. *elata* C. B. Clarke	40		Krishnappa & Basavaraj	1982
	40		Ranganath & Krishnappa	1990
	40		Ranganath	1981
B. longiflora L. f.	40		Govindarajan & Subramanian	1985
B. micans Nees		20	Daniel *et al*.	1990
		20	Daniel & Chuang	1993
B. noctiflora L.	40		Krishnappa & Basavaraj	1982
	24		Govindarajan & Subramanian	1983
	40		Ranganath & Krishnappa	1990
		20	Devi & Mathew	1991
	40		Ranganath	1981
B. prionitis L.		20	Saggoo	1983
	40		Devi & Mathew	1982
		20	Saggoo & Bir	1986
	40		Devi & Mathew	1991
	40	20	Sarkar *et al*.	1980

		20	Vasudevan	1976
B. repens Nees		20	Daniel & Chuang	1998
B. senensis Klotzsch		16	Daniel *et al*.	2000
B. strigosa Willd.	40		de la Bathie	1950
	40		Krishnappa & Basavaraj	1982
	40		Ranganath & Krishnappa	1990
B. tomentosa Roth.	40	20	Devi & Mathew	1991
Genus: *Ecbolium* Kurz				
E. linneanum (L.) Kurz	40		Subramanian & Govindarajan	1980
	36		Devi & Mathew	1982
	44		Govindarajan & Subramanian	1983
		18	Sharma	1970
E. syringifolium (Vahl) Vollesen		18	Daniel	2006
Genus: *Justicia* L.				
J. adenothyrsa (Lindau) T. F. Daniel		28	Daniel *et al*.	1990
J. adhatoda L.		17	Daniel & Chuang	1998
J. alopecuroidea T. F. Daniel		28	Daniel *et al*.	1990
J. anagalloides (Nees) T. Anderson	36	18	Ensermu	1990
J. angustibracteata Leonard		14	Daniel	2000
J. aurea Schltdl.		c.11	Daniel	2000
J. betonica L.	34		Krishnappa & Basavaraj	1982
	28		Subramanian & Govindarajan	1980
		17	Saggoo	1983

	34		Devi & Mathew	1982
	34		Govindarajan & Subramanian	1983
		17	Bir & Saggoo	1981
		17	Saggoo & Bir	1986
		17	Daniel	2000
		17	Daniel *et al*.	2000
		17	Daniel & Chuang	1998
	34	17	Ranganath	1981
	34	17	Bir & Saggoo	1979
J. brandegeeana Wassh. & L. B. Sm.		14	Daniel & Chuang	1989
J. brasiliana Roth.		14	Hunziker *et al*.	1985
	28	14	Piovano & Bernardello	1991
J. brevipila Hedren	c.26		Hedren	1989
J. californica (Benth.) D. N. Gibson		14	Daniel *et al*.	1984
J. calyculata M. Deflers	18		Ensermu	1990
J. candelariae (Oerst.) Leonard		14	Daniel	2000
J. candicans (Nees) L. D. Benson		14	Daniel *et al*.	1984
		14	Daniel & Chuang	1993
J. carnea Lindl.		17	Daniel & Chuang	1989
J. caudata A. Gray		14	Daniel *et al*.	1990
		14	Daniel & Chuang	1993
		14	Daniel & Chuang	1998
J. comata (L.) Lam.	28		Daniel	2000
J. costaricana Leonard		14	Daniel	2000
J. cufodontii (Fiori) Ensermu	22		Ensermu	1990

J. dejecta var. *albipila* Benoist		13	Daniel	2006
J. diclipteroides subsp. a*ethiopica* Hedren	c.26		Hedren	1989
J. diclipteroides subsp. *nierensis* Hedren	c.26		Hedren	1989
J. diffusa Willd.	18		Krishnappa & Basavaraj	1982
		9	Saggoo	1983
		9	Devi & Mathew	1982
		9	Bir & Saggoo	1981
		9	Khatoon & Ali	1993
	18	9	Ranganath	1981
	18	9	Sidhu	1979
	18	9	Bir & Sidhu	1980
		9	Vasudevan	1976
J. diffusa var. *prostrata* Roxb. ex C. B. Clarke		9	Bir & Saggoo	1981
		9	Bir & Saggoo	1979
J. exigua S. Moore		14	Renard *et al*.	1983
		14	Ensermu	1990
J. extensa T. Anderson	34		Daniel & Chuang	1998
J. flava (Forssk.) Vahl	26		Podlech	1986
J. fortunensis T. F. Daniel & Wassh.		14	Daniel & Wasshausen	1990
		14	Daniel & Chuang	1993
J. fulvicoma Schltdl. & Cham.		14	Daniel *et al*.	1990
		14	Daniel & Chuang	1998
J. galapagana Lindau		24	Daniel	2000
J. gendarussa Burm. f.	30		Krishnappa & Basavaraj	1982
	30		Ranganath	1981
	30		Sarkar *et al*.	1980
		14	Vasudevan	1976

J. gilliesii (Nees) Benth.	28		Piovano & Bernardello	1991
J. glabra K. D. Koenig ex Roxb.	54		Krishnappa & Basavaraj	1982
	54		Ranganath	1981
J. glamea Rottler		13	Saggoo	1983
J. glauca Rottler	36		Krishnappa & Basavaraj	1982
	26		Govindarajan & Subramanian	1985
		13	Saggoo & Bir	1986
	26		Ranganath	1981
J. herpetacanthoides Leonard		14	Daniel	2000
J. heterocarpa subsp. *petterssonii* Hedrén	c.26		Hedren	1989
J. hyssopifolia L.	c.70		Suda *et al*.	2005
J. insolita Brandegee		12	Daniel *et al*.	1984
J. insolita subsp. *tastensis* (Brandegee) T. F. Daniel		12	Daniel	1997
J. isthmensis T. F. Daniel		14	Daniel	2000
J. japonica Thunb.		18	Khatoon & Ali	1993
J. ladanoides Lam.	c.28 c.40		Hedren	1989
J. latispica Gamble	18		Krishnappa & Basavaraj	1982
	18		Ranganath	1981
J. leonardii Wassh.		14	Daniel & Chuang	1998
J. linearis B. L. Rob & Greenm.		14	Daniel *et al*.	1990
J. masiaca T. F. Daniel		14	Daniel	2000
J. matammensis (Schweinf.) Oliv.		14	Ensermu	1990
J. micrantha Wall.	18		Krishnappa & Basavaraj	1982
		9	Devi & Mathew	1982

		9	Ranganath	1981
J. montana Wall.	30		Krishnappa & Basavaraj	1982
	30		Ranganath	1981
J. nuttii C. B. Clarke	22		Ensermu	1990
J. odora (Forssk.) Lam.	c.26		Hedren	1989
		14	Daniel *et al.*	2000
J. oerstedii Leonard		22	Daniel *et al.*	2000
		11	Daniel	2000
J. oranensis De Marco & T. Ruiz		14	Piovano and Bernardello	1991
J. ornatopila Ensermu	22		Ensermu	1990
J. palmeri Rose		14	Daniel	1997
J. pectoralis Jacq.		11	Daniel *et al.*	1990
		11	Daniel & Chuang	1993
J. peploides (Nees) T. Anderson	18	9	Khatoon & Ali	1993
J. petiolaris (Nees) T. Anderson		26	Daniel *et al.*	2000
J. procumbens L.	36		Krishnappa & Basavaraj	1982
		18	Bir *et al.*	1982
	18		Govindarajan & Subramanian	1985
	36	18	Ranganath	1981
J. procumbens var. *latispica* C. B. Clarke		9	Devi & Mathew	1982
J. prostrata Gamble	18		Krishnappa & Basavaraj	1982
		9	Saggoo & Bir	1983
		9	Saggoo & Bir	1986
	18	9	Ranganath	1981
	18		Sarkar *et al.*	1980
		9	Sarkar *et al.*	1978

J. pubigera Wall.		14	Saggoo & Bir	1983
		14	Bir & Saggoo	1981
		14	Vasudevan	1976
J. purpusii (Brandegee) D. N. Gibson		14	Daniel *et al.*	1984
J. quinqueangularis Koenig		9	Saggoo & Bir	1983
	18		Govindarajan & Subramanian	1985
	18		Sareen & Sanjota	1976
		9	Vasudevan	1976
J. reflexiflora Vahl		14	Daniel *et al.*	1984
J. rhodoptera Baker		30	Daniel	2006
J. salviiflora Kunth		12	Daniel *et al.*	1984
J. schoensis Lindau	c.32		Ensermu	1990
J. secunda Vahl		14	Daniel *et al.*	1990
		14	Daniel	2000
J. simplex D. Don	18		Krishnappa & Basavaraj	1982
	18	9	Saggoo	1983
		18	Bir and Saggoo	1981
		9	Saggoo & Bir	1986
	18	9,18	Ranganath	1981
	18		Sarkar *et al.*	1980
		18	Vasudevan	1976
J. sonorae Wassh.		11	Daniel *et al.*	1984
		11	Daniel *et al.*	1990
J. squarrosa Griseb.	28	14	Piovano & Bernardello	1991
J. striata subsp. *austromontana* Hedrén	c.26		Hedren	1989
J. striolata Mildbr.	c.50		Hedren	1989
J. tenella (Nees) T.	26		Gadella	1982

Anderson	26		Gadella	1977
J. tranquebariensis L. f.	26		Ranganath	1981
J. trinervia Vahl	34		Krishnappa & Basavaraj	1982
	34		Ranganath	1981
J. tweediana (Nees) Griseb.	56		Piovano & Bernardello	1991
J. unyorensis var. *keniensis* (Rendle) Hedrén	c.26		Hedren	1989
J. *vahlii* Roth		9	Devi & Mathew	1982
J. vahlii var. *scindica* Malik & Ghafoor		9	Khatoon & Ali	1993
J. ventricosa Wall. ex Hook. f.		15	Saggoo	1983
J. whytei S. Moore	c.26		Hedren	1989
J. wynaadensis B. Heyne	28		Krishnappa & Basavaraj	1982
		14	Saggoo	1983
		14	Saggoo & Bir	1982
		14	Saggoo & Bir	1986
	28	14	Ranganath	1981
Genus: *Strobilanthes* Blume				
S. alatus Nees		16	Bir & Saggoo	1981
		16	Bhat *et al*.	1975
		16	Vasudevan	1976
S. asperrimus Nees		16	Devi & Mathew	1997
S. atropurpureus Nees		16	Saggoo	1983
		16	Daniel & Chuang	1998
		16	Vasudevan	1976
S. barbatus Nees	32		Govindarajan & Subramanian	1985
S. callosus Nees		14	Saggoo & Bir	1982

S. consanguineus Clarke		16	Saggoo & Bir	1982
S. cusia (Nees) Kuntze	32		Ge *et al*.	1987
	32		Iwatsubo *et al*.	1993
S. dalhousieanus (Nees) C. B. Clarke		9	Bir & Saggoo	1981
		9	Vasudevan	1976
S. discolor T. Anderson	22	11	Pandey & Pal	1980
S. dyerianus Mast.		15	Daniel & Chuang	1998
S. glandulifera Hatus.	30		Kanemoto	2001
S. heteromallus T. Anderson		16	Devi & Mathew	1997
S. heyneanus Nees		16	Devi & Mathew	1997
S. homotropus Nees	42		Govindarajan & Subramanian	1983
S. isophyllus (Nees) T. Anderson		20	Govindarajan & Subramanian	1985
S. japonica (Thunb.) Miq.	30		Iwatsubo *et al*.	1993
S. kunthianus T. Anderson	32		Govindarajan & Subramanian	1983
		16	Devi & Mathew	1997
S. lawsoni Gamble	30		Govindarajan & Subramanian	1985
		20	Devi & Mathew	1997
S. lupulinus Nees		15	Devi & Mathew	1997
S. luridus Wight	32		Govindarajan & Subramanian	1983
S. oliganthus Miq.	60		Iwatsubo *et al*.	1993
S. pentastemonoides (Nees) T. Anderson		13	Saggoo & Bir	1982
S. pulneyensis C. B. Clarke	40		Govindarajan & Subramanian	1983
S. scaber Nees		16	Saggoo & Bir	1981

S. tashiroi Hayata	30		Kanemoto	2001
S. wakasana Wakasugi & Naruh.	30		Iwatsubo *et al*.	1993
	30		Wakasugi & Naruhashi	1993
S. wallichii Nees		16	Saggoo & Bir	1982
		20	Vasudevan	1976
S. wightianus Nees	30		Govindarajan & Subramanian	1983
		16	Devi & Mathew	1997
S. zenkerianus T. Anderson	32		Govindarajan & Subramanian	1985

b. ANTIOXIDANT AND ANTI-INFLAMMATORY STUDIES

Plants and plant derived drugs play a dominant role in current therapeutics (Kwon *et al*., 2007). However, the lack of scientific and chemical data in support of better understandings of the efficacy and safety of the herbal drugs has become the major hindrance to the use of traditional herbal preparations. Hence, it is necessary to conduct more chemical and pharmacological studies both at organism and molecular levels to investigate the untapped potential of the herbal drugs.

All cells of the human body are exposed to oxidative stress, and thus oxidation and free radicals may be an important cause in the onset and progression of several human diseases (Ames *et al*., 1993). Likewise, inflammatory processes are involved in the pathogenesis of the most common chronic and non-communicable diseases. The antioxidants are known to alleviate oxidative stress by scavenging the free radicals (Sardas, 2003). Antioxidant and anti-inflammatory agents are fundamental in prevention of carcinogenicity, cardiovascular and neurodegenerative changes associated with ageing.

Nair *et al.* (1985a) conducted studies on some South Indian market samples of Ayurvedic drugs, including those of 'Sahachara'.

Shantha *et al*. (1988) carried out detailed pharmacognostical studies of the roots of *Strobilanthes heyneanus*. They identified some of the diagnostic features of the drug like the presence of pigmented cystoliths in the cortex and pith region, yellow coloured cell contents in the cortex region, thick walled and oval-elongated stone cells in the cortex region *etc*.

The hydroalcoholic extract of *Justicia pectoralis* and its main constituents, coumarin and umbelliferone were studied for possible anti-edema activity, employing carrageenan and dextran paw edema in rats (Lino *et al*., 1997). All the tested extracts presented a significant anti-edema effect in the carrageenan model but only coumarin could decrease the rat paw volume in the dextran model, and this effect was not observed with the other two treatments.

The total antioxidant activity of leaves of *S. crispus* was investigated by Ismail *et al.* (2000). Proximate analyses and total antioxidant activity using ferric thiocyanate and thiobarbituric acid methods were employed in the study. The study revealed that the catechins of *S. crispus* leaves showed good antioxidant activity and consumption of the leaf extract daily (5 g/day) as a herbal tea could contribute to the additional nutrients and antioxidants needed in the body to enhance the defense system, especially towards the incidence of degenerative diseases.

The anti-inflammatory activity of the 95% ethanol extract, benzene fraction and isolated triterpenoids of *S. callosus* were investigated by Singh *et al*. (2002). In the carrageenan-induced paw edema inflammation model, taraxerol isolated from *S. callosus* showed a high reduction of edema. The anti-inflammatory activity of taraxerol was apparent as early as 1 h after

carrageenan injection and was maintained until the experiment was terminated at 10 h. The anti-inflammatory effect of taraxerol (48.61%) is similar to that of the positive control, indomethacin (48.7%). These results confirm the use of this plant in folk medicine as an anti-inflammatory herbal drug.

The 'TAF' fraction from the hydromethanolic extract of *Barleria prionitis* whole plant has been reported to have prominent anti-inflammatory activity against different acute and chronic inflammation induced by carrageenan, histamine and dextran (Singh *et al*., 2003). The anti-inflammatory activity in adrenalectomised rats was maintained showing that the effect of fraction, 'TAF' is not activated by the pituitary-adrenal axis. 'TAF' also showed inhibition of vascular permeability and leucocytes migration *in vivo* into the site of inflammatory insult.

Agarwal and Rangari (2003b) evaluated the anti-inflammatory activity of lupeol and 19 α-H-lupeol isolated from the roots of *Strobilanthes callosus* and *S. ixiocephala* respectively using carrageenan induced rat paw edema model for acute inflammation and cotton pellet granuloma model for chronic inflammation. The results showed that lupeol at the doses of 200, 400 and 800 mg/kg produced a dose dependent inhibition *i.e.*, 24%, 40% and 72% whereas 19 α-H- lupeol showed 21%, 47% and 62% inhibition after 24 h in acute model of inflammation. In chronic model of granuloma pouch in rats, lupeol exhibited 33% and 19 α-H lupeol, 38% reduction in granuloma weight. In the arthritis model, lupeol and 19 α-H- lupeol exhibited 29% and 33% inhibition respectively after 21 days of treatment.

Sanmugapriya *et al*. (2005) screened the aqueous and alcoholic extracts of the whole plant of *Justicia prostrata* for their acute and sub-acute anti-inflammatory activities using carrageenan-induced acute inflammation

and cotton-pellet-induced granuloma (sub-acute inflammation), respectively, in rats. At the dose of 500 mg/kg p.o., both extracts showed maximum inhibition (51.39% and 62.5%, respectively) in rat paw edema volume at the first hour of carrageenan-induced acute inflammation. In the cotton pellet granuloma assay, the extracts at the dose of 500 mg/kg p.o. suppressed the transudative, exudative and proliferative phases of chronic inflammation. The study proved that the extracts were able to reduce the lipid peroxide content of exudates and liver. They were also able to normalize the increased activity of acid and alkaline phosphatases in serum and liver of cotton pellet granulomatous rats. Preliminary phytochemical screening of the extracts revealed the presence of lignans, triterpenes and phenolic compounds in the aqueous extract, whereas phenolic compounds and glycosides in the alcoholic one. The anti-inflammatory effects produced by the extracts at the dose of 500 mg/kg, p.o. was comparable with the reference drug diclofenac sodium (5 mg/kg p.o.).

Ho *et al*. (2003) reported the anti-inflammatory potential of the methanolic leaf extract of *Strobilanthes cusia* to decrease the paw edema induced by carrageenan in rats. The anti-inflammatory effect of the methanolic extract of *S. formosanus* was investigated by Kao *et al*. (2003).

Antioxidant activity and cytotoxic effect of essential oil from *S. crispus* has been studied by Rahmat *et al*. (2006). The study revealed that *S. crispus* oil has the potential to be used as a nutraceutical supplement due to its antioxidant activity as well as for the prevention of degenerative diseases.

The antiproliferative properties and antioxidant activity of various types of *S. crispus* tea has been investigated by Mohd Fadzelly *et al*. (2006). A nutraceutical herbal tea from the young and old leaves of *S. crispus* was developed, and the potential antiproliferative properties and antioxidant activity *in vitro* has been studied. The results showed that *S. crispus* tea

inhibited the proliferation of human hormone dependant breast cancer cell lines only but not the non-hormone dependant breast cancer cell line. The antioxidant activity was determined using ferric reducing antioxidant power and DPPH free radical scavenging assay. The results showed that the hot water extract of *S. crispus* tea has high antioxidant activity especially the unfermented tea from old leaves of *S. crispus*.

A study was conducted to assess the anti-inflammatory potential of methanol extract of aerial parts of *Barleria lupulina* in acute and sub-acute inflammation models of albino rats by Suba *et al*. (2005). The extract in all the tested doses caused pronounced inhibition of carrageenan and serotonin induced paw edema volumes when compared with the control. The extract could also significantly reduce granuloma weight in the cotton pellet induced granuloma model when compared to the standard drug indomethacin. The extract demonstrated protection against CCl_4 induced lipid peroxidation as well.

Antioxidant potency of 70% aqueous ethanolic extract of leaves of *Justicia gendarussa* was investigated by Mruthunjaya and Hukkeri (2007) employing DPPH radical scavenging, nitric oxide (NO) scavenging, β-carotene linoleic acid module system (β- CLAMS), hydroxyl (OH) radical scavenging and anti-lipid peroxidation. IC_{50} values were determined in each experiment. Also, ferric ion reduction capacity of extracts in presence and absence of chelating agent (EDTA) also was determined.

Wanikiat *et al*. (2008) studied the anti-inflammatory activity of *B. lupulina* extract employing two neutrophyll dependent acute inflammatory models, carrageenan induced paw edema and ethyl propionate induced ear edema in rats. The underlying cellular mechanisms also were brought out by investigating the effects of the extract on human neutrophyll responsiveness by analyzing the myloperoxidase (MPO) activity. The extract could induce

powerful dose dependent inhibitory effects in both edema models in rats. Moreover a significant reduction in MPO activity in the inflamed tissue was noted which indicates that the anti-inflammatory effect of the extract is mediated by reduced neutrophyll migration.

The anti-inflammatory activities of extracts from different parts of three *Barleria* species of South African origin were investigated by Amoo *et al*. (2009). All the extracts showed broad-spectrum antibacterial activity with minimum inhibitory concentrations ranging from 0.059 to 6.25 mg/ml. Good activity (>70%) was shown by 12 out of 21 crude extracts in the cyclooxygenase-1(COX-1) assay while 10 extracts showed good activity in the COX-2 assay. All the petroleum ether extracts (except *B. prionitis* stem) exhibited good inhibition of prostaglandin synthesis in COX-1. The results demonstrated the therapeutic potential of these plants as an anti-inflammatory agent and also that the effect is mediated by the inhibition of the COX enzymes.

Antioxidant activity of aqueous and methanolic extracts obtained from leaf, stem and flower of *Justicia spicigera* and their contents of phenolic compounds and flavonoids were evaluated in a study by Jimenez *et al*. (2009). The amount of total phenolic compounds was determined using the Folin-Ciocalteu reagent. Total flavonoid content was evaluated with aluminium chloride under basic conditions. The results revealed that extracts prepared with methanol possess a higher antiradical activity than the aqueous one. The leaf and flower extracts were found to have more antioxidant activity than the stem extract prepared with the same solvent. The results prove the ability of *J. spicigera* to be used against various free radical-related and inflammatory disorders.

The anti-inflammatory activity of aqueous extract of *B. cristata* leaves in different experimental screening methods has been reported (Gambhire *et*

al., 2009b). The aqueous extract of *B. cristata* leaves at doses of 125, 250, and 500 mg/kg body weight was evaluated in acute inflammatory models against carrageenan induced paw edema in rats, prostaglandins inhibitory activity and acetic acid induced capillary permeability in mice. The outcome of the study showed significant inhibition of edema induced by carrageenan, prostaglandin activity and vascular permeability in mice.

Gambhire *et al*. (2009a) evaluated the anti-inflammatory activity of methanolic extract of *B. cristata* leaves using *in vivo* and *in vitro* methods. In the *in vivo* inflammation tests, *B. cristata* leaf methanolic extract could inhibit edema produced by histamine and serotonin in rats and reduce acetic acid-induced vascular permeability in mice, dose dependently. In the *in vitro* tests, the probable supporting mode by which the extract mediates its effects on inflammatory conditions was studied on red blood cells (RBCs) exposed to hypotonic solution and thermally induced protein denaturation.

Gambhire *et al*. (2009c) carried out extensive assays on anti-inflammatory and antioxidant potential of different extracts and fractions of *B. cristata* using acute and chronic models of inflammation. Anti-inflammatory activity of petroleum ether, chloroform and methanol fractions of *B. cristata* extract were studied by carrageenan induced rat paw edema and cotton pellet induced granuloma method at the dose levels of 50, 100 and 200 mg/kg body weight. Results of the study showed that chloroform fraction has moderate anti-inflammatory activity where as methanol fraction showed significant and dose dependent anti-inflammatory activity, in both the models studied. Methanol fraction at the dose of 200 mg/kg and indomethacin (10 mg/kg) significantly inhibited (65.21% and 69.07% respectively) rat paw edema at the end of 4 h after carrageenan injection. In the cotton pellet induced granuloma method, all the three fractions and indomethacin showed significant activity when compared with control group. Methanol fraction

(200 mg/kg) showed maximum inhibition of 62.37% (wet cotton) and 53.84% (dry cotton) where as indomethacin (10 mg/kg) showed 68.04% (wet cotton) and 59.61% (dry cotton) inhibition of cotton pellet induced granuloma in rats.

Gambhire *et al*. (2009b) has also analyzed the free radical scavenging activity of *B. cristata* aqueous extract using DPPH (1,1-diphenyl-2-picryl-hydrazyl) and NO (Nitric oxide) radicals. The IC_{50} values for the percentage of inhibition of the formation of the two radicals were 206.61 µg/ml and 289.01 µg/ml respectively.

Sawarkar *et al.* (2009) investigated the antioxidant activity of the aqueous and hydro alcoholic extracts of the leaves of *B. grandiflora* by the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. FTC method was used to measure the amount of peroxide formed at the primary stage of linoleic acid peroxidation. The leaf extracts were found to possess significant antioxidant activity when compared to the standard, Vitamin C and the activity was found to be more for the hydro-alcoholic extract, when compared to the aqueous extract.

Crude extracts (dichloromethane and methanol) of 20 plants of Acanthaceae were screened for their antiplasmodial, cytotoxic, antioxidant and radical scavenging activities by Charoenchai *et al*. (2010). The methanolic extracts of *B. cristata*, *Justicia procumbens* and *Strobilanthes auriculatus* were shown to exhibit excellent antioxidant property in the ORAC antioxidant assay.

Jaiswal *et al*. (2010a) carried out a comparative study on the total phenolic content, reducing power and free radical scavenging activity of aerial parts of *B. prionitis*. They evaluated the antioxidant activity of 50% ethanolic extract of leaf, flower and stem of *B. prionitis* by using β-carotene bleaching assay, reducing power and free radical scavenging activity (DPPH and

hydroxyl radical scavenging activity). The total phenolic content also was analyzed by the Folin–Ciocalteu colorimetric method. The leaves exhibited free radical scavenging activity as evidenced by the low IC_{50} values in both DPPH (336.15 µg/ml) and hydroxyl radical (568.65 µg/ml) methods. The total phenolic content of *B. prionitis* leaves was found to be 67.48 mg/g gallic acid equivalent.

Jaiswal *et al*. (2010b) reported the anti-inflammatory activity of 50% methanolic extract of the flower of *B. prionitis*. The extract in doses of 50, 100 and 200 mg/kg caused a dose-dependent inhibition of swelling caused by carrageenan equivalent to 17.8-48.6% protection and showed 46.2–36.4% protection from inflammation in cotton pellet granuloma.

Lalitha and Sethuraman (2010) reported anti-inflammatory activity of the roots of *E. viride.* The ethyl acetate fraction of the root extract was administered orally to rats. The anti-inflammatory activity was determined by carrageenan-induced paw edema and cotton pellet granuloma models.

The anti-inflammatory activitiy of ethanol extract of aerial parts of *Justicia gendarussa* in animal models was demonstrated by Jothimanivannan *et al*. (2010) using carrageenan-induced rat paw edema and cotton pellet granuloma method. The study was carried out in two different dose levels of 250 and 500 mg /kg orally. The extract at the dose of 500 mg/kg showed maximum inhibition of 52% in carrageenan-induced paw edema and 45% inhibition in dry weight cotton pellet granuloma formation.

In a study by Muslim *et al*. (2010), validation of GC-TOF mass spectrophotometric methods for quantitative determination of phytoconstituents in methanolic and aqueous extracts of *S. crispus* was done. The antioxidant properties of standardized methanolic and aqueous extracts of *S. crispus* were assessed using DPPH free radical, xanthine oxidase activity

and 3-carotene-linoleate model system. The extracts displayed very strong inhibitory activity towards xanthine oxidase enzyme, however, they demonstrated moderate antioxidant properties, which is evidenced by the quenching of DPPH free radical and preventing the bleaching of 3-carotene by linoleic acid.

The anti-inflammatory activity of *Justicia pectoralis* has been reported by Locklear *et al*. (2010) using cyclooxygenase-2 (COX-2) enzyme inhibition assay for evaluating its anti-inflammatory property. The extract inhibited COX-2 catalytic activity with IC_{50} value of 4.8 µg/ml revealing its significant anti-inflammatory effect.

There have been many previous reports on the antioxidant and anti-inflammatory activities of *J. gendarrussa*. The anti-inflammatory activity of the leaves of *J. gendarussa* has been measured by carrageenan induced rat paw edema, formalin-induced paw edema and cotton pellet granuloma after extracting with ethanol (Shikha *et al*., 2010). Krishna *et al*. (2010) reported the antioxidant potential of stem methanolic extract of *J. gendarussa*. The crude methanolic extract was fractionated in petroleum ether, chloroform and methanol fractions. The marc remained was further extracted with double distilled water. All fractions and the aqueous extract were tested for antioxidant activity. Among the different fractions, methanolic fraction showed the highest antioxidant activity.

The anti-inflammatory activity of *J. gendarussa* leaf methanolic extact was evaluated in Freud's complete adjuvant induced arthritic rats. The results of the study suggested that the anti-inflammatory mechanism of *J. gendarussa* leaf extract may be through its free radical scavenging activity, its stabilizing action on lipid peroxide and increased antioxidant levels (Chinna *et al*., 2011). Antioxidant activity of leaf extract of *J. gendarussa* was assayed by Krishna *et al*. (2009) by DPPH free radical scavenging, hydrogen peroxide scavenging

and reduction of ferric ion in presence and absence of EDTA, and the results indicated that the methanolic extract has good antioxidant activity.

The antioxidant potential of methanolic extract of *Ecbolium viride* roots was investigated by employing three different *in vitro* methods such as DPPH radical scavenging activity, nitric oxide radical scavenging activity and reducing power assay (Babu *et al*., 2011). Total flavonoid content was also determined by colorimetric method. The extract was found to be rich in flavonoid content (78 ± 4.8 mg quercetin equivalent/g dry weight of extract). Results obtained in the present study reveal that methanolic extract of *E. viride* possess significant antioxidant potential.

The whole plant extracts of *B. prionitis* was reported to show potent antioxidant activity (Chetan *et al*., 2011). The radical scavenging potential of the aqueous and alcoholic extracts of the whole plant was analyzed using the DPPH radical, ABTS radical, hydroxyl radical, reducing power and nitrous oxide scavenging assays. The ethanol extract was shown to be more potent in scavenging the free radicals than the aqueous extract. The total phenolic content also was determined during the study which showed sharp correlation with the antioxidant activity.

The antioxidant properties of petroleum ether, dichloromethane, ethanol and methanol extracts from different parts of *B. prionitis*, *B. greenii* and *B. albostellata* were studied (Amoo *et al*., 2011). In the DPPH radical scavenging assay, different parts of *Barleria* species showed IC_{50} values ranging from 6.65 to 12.56 µg/ ml. The extract could bring about a reduction of the ferric ion/ferricyanide complex to the ferrous form and reduce the carotenoid bleaching rate suggesting the presence of antioxidant compounds. The phytochemical studies confirmed the presence of flavonoids, iridoids, tannins *etc*. which might be responsible for the observed biological activities of the extract.

Khobragade and Bhande (2012) reported the presence of reactive oxygen scavenging agents in the extract of *B. prionitis* leaf methanolic extract which can disrupt the membranes of bacteria. They investigated the antioxidant and anti-inflammatory potentials of *B. prionitis* leaf extract using DPPH and anti-denaturation assays respectively. The methanolic extract of *B. prionitis* exhibited radical scavenging activity in a concentration dependent manner with an IC_{50} value of 0.30 ± 0.02 mg/ml compared to L-ascorbic acid (IC_{50} = 0.5 ± 0.01 mg/ml) and anti-inflammatory activity with percentage inhibition of 85.77% which was comparable to standard Ibuprofen which showed 90.0% of inhibition at the same dose.

The effect of hydro-alcoholic extract of *B. prionitis* whole plant on inflammatory mediators and cell membrane in response to toxic chemicals was evaluated (Maji *et al*., 2011). The membrane stabilization and mast cell protection activity of the extract was studied. The extract at the dose of 10 µg/ml could significantly reduce the rat mesenteric mast cell degranulation up to 64.91% and prevented hypotonic solution induced hemolysis of rat erythrocytes by 27.10%. The results of the study are a clear evidence for the anti-inflammatory activity of *B. prionitis* whole plant.

Amid *et al*. (2011) observed antioxidant activity of leaves, callus and suspension culture of *J. gendarusa* via DPPH radical scavenging assay. The assays were conducted on aqueous and methanolic extracts of leaves, callus culture and cell suspension culture. Callus induced on NAA showed higher phenolic content and antioxidant activity as compared to the one induced on 2,4-D.

Banu *et al*. (2011) studied the total phenolic content of the methanolic extract of the leaves of *Barleria montana* which was assessed by using Folin-Ciocalteau method employing gallic acid and tannic acid as the calibration standards. The antioxidant potential of the methanolic extract also was also

measured using hydrogen peroxide scavenging and DPPH method. The methanolic extract of *B. montana* leaves showed prominent IC_{50} value of 51 µg/ml by hydrogen peroxide method and 68 µg/ml by DPPH method, and the standard ascorbic acid showed an IC_{50} value of 9 µg/ml by hydrogen peroxide method and IC_{50} value of 7.8 µg/ml by DPPH method.

Chemical constituents and biological activities of species of *Justicia* were reviewd by Correa and Alcantara (2012). The anti-inflammatory and antioxidant activities of various phytochemical constituents reported from the species of *Justicia* like coumarin, flavonoids, alkaloids, triterpenes and lignans have been described in detail. Banerjee *et al*. (2012) has reviewed the traditional uses, phytochemistry, pharmacology and toxicity of *B. prionitis* and has highlighted the different phytochemical constituents and the wide range of pharmacological properties of the plant.

Various extracts of *B. prionitis* roots were screened for anti-inflammatory activity using carragennan induced rat paw edema at the dose of 200 and 400 mg/kg orally (Khadse and Kakde, 2011). The aqueous extract was found most active; it was then fractionated into four major fractions (FR I to IV) and were screened by the same tests. Results showed that at a dose of 400 mg/kg FR-III & IV showed significant activity with 50.64% and 55.76% inhibition of edema respectively at the end of 4 h as compared with reference drug indomethacin with 60.25% inhibition of edema.

The antioxidant property of aqueous and ethanolic extracts of *S. crispus* leaves has been reported by Qader *et al*. (2011). Antioxidant activity was evaluated by DPPH and ferric reducing antioxidant power (FRAP) assays. The results showed that ethanol extract displayed high antioxidant activities, when compared to aqueous extract.

The inhibitory effect of ethanolic extract of *J. gendarrussa* leaves in nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) and matrix metalloproteinase-9 (MMP-9) gene expressions were studied in lipopolysaccharide (LPS) stimulated macrophage cell line RAW 264.7. The extract (200-50 µg/ml) attenuated NO production from macrophages simulated with LPS. Moreover, it significantly suppressed iNOS mRNA expression and also downregulated the MMP-9 gene expression in the macrophages. The results revealed the modulatory function of the extract in inhibiting NO, iNOS and MMP-9, and demonstrate the anti-inflammatory properties of *J. gendarussa* (Varma *et al*., 2011).

In a study by Uddin *et al*. (2011), the crude methanol extract as well as the petroleum ether, carbon tetrachloride and chloroform soluble fractions of the whole plant of *J. gendarussa* were studied for antioxidant activity. Among the different fractions tested for antioxidant activity, maximum activity was observed with the chloroform fraction with IC_{50} value of 18.80 µg/ml followed by the two other fractions carbon tetrachloride (26.00 µg/ml) and petroleum ether (37.64 µg/ml) soluble fractions exhibiting significant antioxidant activity as well.

The antioxidant potential of ethanolic extract of *Justicia tranquebariensis* by eight different *in vitro* models such as DPPH radical, ABTS radical, Ferric reducing power, chelating ability of ferrous iron, reducing power, hydroxyl radical, hydrogen peroxide and nitric oxide radical scavenging activity was analysed, and the results were compared with the standard antioxidants like butylated hydroxyanisole (BHA) and ascorbic acid. The alcoholic leaf extract was found to be more effective in the ABTS radical scavenging activity and its percentage of inhibition was 75.7±0.3 for 300 µg/ml (Suriyavathana *et al*., 2011).

The antioxidant screening of *Justicia wynaadensis* leaf extract was carried out by Medapa *et al*. (2011) and the observed high antioxidant property has been suggested to be due to the presence of phenolics, flavonoids in the extract as well as its catalase and peroxidase enzyme activities.

The ethanolic extract of *E. linneanum* was tested for antioxidant activity in Swiss albino mice and found to possess high antioxidant potential when compared to the control ascorbic acid (Khan *et al*., 2013). Dipankar and Murugan (2012) reported the *in vitro* antioxidant property of leaves and stem extracts of *E. linneanum*.

Narmadha and Devaki (2012a) evaluated the antioxidant activity of ethanolic and aqueous extracts of *B. cristata* leaves and reported good scavenging activity of the ethanolic extract in DPPH and ABTS radical scavenging and FRAP assays.

Yadav *et al*. (2012) reported the antioxidant activity of defatted methanol extract of *Barleria noctiflora* leaf and root extracts using DPPH radical scavenging activity, Fe^{2+} chelating activity assay, nitric oxide radical scavenging activity, $ABTS^{+}$ radical cation decolourisation assay, superoxide anion and hydrogen peroxide radical scavenging activities. The extracts showed good antioxidant capacity in DPPH radical scavenging assay, when compared to other *in vitro* models and the IC_{50} values were found to be 150 µg/ml in leaf extract and 140 µg/ml in root extract. The total phenolic content using Folin-Ciocalteu reagent indicated that 1 mg of leaf and root extracts contain 368 µg and 481 µg gallic acid equivalents and also the total flavonoid content was found to be 240 µg and 410 µg respectively with quercetin equivalence. The results also indicated that the antioxidant potential of the root extract is high, when compared to the leaf.

Marathakam *et al*. (2012**)** investigated the phytochemical constituents and *in vitro* antioxidant potential of different extracts of aerial parts of *Justicia beddomei*. All the extracts were evaluated for their potential antioxidant activities using DPPH, hydroxyl radical, superoxide anion radical scavenging abilities, β-carotene-linoleic acid model, reducing power ability and total phenolic and flavonoid contents. The phenolic and flavonoid content was found to be highest in methanolic extract and lowest in petroleum ether extract.

The anti-inflammatory function and mechanism(s) of action of an ethyl acetate fraction isolated from methanolic extract of *J. gendarussa* roots (EJG) was studied (Kumar *et al*., 2012). Anti-inflammatory studies were conducted on rats using partitioned fractions. In carrageenan-induced rat paw edema, ethyl acetate fraction brought about 80% and 93% edema inhibition at 3rd and 5th h at a dose of 50 mg/kg, when compared to other extracts and the standard. They also investigated whether the extract inhibits the release of cyclooxygenase (COX), 5-lipoxygenase (5-LOX), interleukin-6 (IL-6) and nuclear factor kappa B (NF-κB) in LPS stimulated human peripheral blood mononuclear cells (hPBMCs). Results showed that the extract dose dependently inhibited LPS-activated COX, 5-LOX, IL-6, and NF-κB in hPBMCs. EJG also reduced LPS induced levels of iNOS and COX-2 mRNA expression in hPBMCs.

Desu *et al*. (2011a) has conducted *in-vitro* anti-inflammatory and anti-osteoarthritic studies of ethanolic extracts of *Strobilanthes kunthianus* and *S. cuspidatus* using ‘Human RBC membrane stabilization method’ and ‘Rabbit cartilage explants culture method’ respectively. The results revealed that both the extracts have anti-inflammatory and anti-osteoarthritic activity in another study by Desu *et al*. (2011b), where the anti-inflammatory effects of *S. kunthianus* and *S. cuspidatus* dried leaves alcoholic extracts has been

studied by ‘Carrageenan induced rat paw edema method’ and ‘Cotton pellet induced granuloma formation’ in rats.

The *in vitro* grown stem and leaf derived calli of *J. gendarussa* obtained on solid MS medium supplemented with NAA+BAP were extracted in ethanol, methanol and ether. A significantly highest concentration of phenolics was observed in the solid grown stem callus extracted in methanol and solid grown leaf callus extracted in ethanol. Highest DPPH scavenging activity was observed with methanol extracts of stem derived callus cultured on solid medium at the concentrations of 145.00 ± 5.00 µg/ml (Bhagya and Chandrasekhar, 2013).

The methanol extract of the whole plant of *B. prionitis* was subjected to screening for anti-inflammatory activity using carrageenan-induced rat paw edema model (Singh *et al*., 2013). The methanol extract showed 51.39% inhibition with the administered dose (500 mg/kg, p.o.) as compared to 88.54% of edema inhibition by 20 mg/kg of the standard indomethacin.

The protective efficacy of *Strobilanthes callosus* against the acute and chronic inflammation was assessed on rat model (Kumar *et al*., 2013). Inflammation was induced by carrageenan and Freund’s complete adjuvant model in plantar surface of the rats. The ethanol, chloroform and petroleum ether extracts in three different doses (100, 200 and 400 mg/kg) were administered orally. Only the petroleum ether extract (100 and 400 mg/kg) showed statistically significant effect at every interval of 1h in carrageenan induced model. In Freund’s adjuvant model, petroleum ether and ethanol extract (200 and 400 mg/kg) have shown statistically significant effect. The chloroform extract was shown to be effective only at the dose of 400 mg/kg. The findings of the study clearly show the promising efficacy of *S. callosus* against acute and chronic inflammation.

Malarvizhi *et al*. (2013) evaluated the protective effect of ethanolic and aqueous extract of leaves of *E. viride* on acetaminophen induced toxic hepatitis. Oral administration of acetaminophen at the dose of 2g/kg decreased the activities of antioxidant status and increased lipid peroxidation in hepatic tissue of rats. Treatment with *E. viride* extracts at the dose of (200 & 400 mg/kg p.o.) significantly ameliorated the toxic manifestations to normalcy. The aqueous and ethanolic extracts of *E. viride* could attenuate the induced hepatic oxidative damage possibly by modulating the activities of the antioxidant status and membrane bound phosphatases and lipid peroxidation, by acting as a potent scavenger of free radicals.

The phytochemical screening and evaluation of antioxidant activity of *Barleria mysorensis* leaves were carried out by Jinsu *et al*. (2012). Antioxidant study was performed with DPPH assay and metal chelating activity. Analysis of total phenolic cotent of the extract was also done, which revealed significant amount of phenolics in the extract.

Preliminary phytochemical screening, quantitative determination of secondary metabolites and vitamins as well as evaluation of *in vitro* antioxidant activity of the ethanolic extract of *B. cristata* leaves have been carried out by Amutha and Doss (2009). Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, phenols and tannins. The 50% ethanolic extract of leaves showed significant antioxidant activity which was presumed to be due to the occurrence of secondary metabolites in the extract.

B. PHYTOCHEMICAL STUDIES

Phytochemical attributes of various medicinal plants are based on the nature and constitution of secondary metabolites present in them. Recently, there is a growing interest in correlating phytochemical constituents of plant with its pharmacological activity (Vaidya and Antarkar, 1994). Owing to the new attraction for natural products, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment. Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry, without showing the secondary effects (Carson and Riley, 2003).

The earliest work on the isolation of chemical constituents from the genus *Strobilanthes* dates back to 1979 wherein tryptanthrin, a quinazoline alkaloid has been isolated from the leaves of *Strobilanthes cusia* (Honda and Tabata, 1979).

β-sitosterol and three triterpenoids namely lupeol, betulin and lupenone were isolated from the roots of *S. cusia* by Rong *et al.* (1987). Two quinazolinone alkaloids 4(3H)-quinazolinone, 2, 4 (1H, 3H)- quinazolinedion; two indole alkaloids indigo, idirubin and three triterpenes *viz*., lupeol, betulin and lupenone were isolated from the whole plant of *S. cusia* by Li *et al.* (1993).

The analysis of extracts from the South American plant *J. pectoralis* by de Vries *et al*. (1988) resulted in the identification of coumarin, dihydrocoumarin, umbelliferone and 3-(2-hydroxyphenyl)propionic acid along with other compounds by gas chromatography/mass spectrometry (GC/MS); the acids and phenolic compounds were derivatized with diazomethane. GC/MS of simple coumarins, phenylpropionic acids and their

hydroxylated isomers was performed after derivatization through methylation and trimethylsilylation.

The essential oil from *S. callosus* from Central India was analyzed by a combination of GC, GC/MS and NMR. The oil obtained from pre-flowering plants was found to possess compositional differences to that from post-flowering plants. The oil from post-flowering plants contained trans-sabinene hydrate (3%), cis-sabinene hydrate (9%), terpinen-4-ol (19%), alpha-terpineol (5%) and methyl chavicol (24%). The oil from pre-flowering plants contained trans-sabinene hydrate (5%), cis-sabinene hydrate (14%), terpinen-4-ol (23%) and α-terpineol (5%). Methyl chavicol was not detected in pre-flowering plants (Weyerstahl *et al*., 1992).

Four iridoid glucosides isolated from the leaves of *B. lupulina* have been identified as 6-*O*-*p*-methoxy-cis-cinnamoyl-8-*O*-acetylshanzhiside methyl ester, 6-*O*-*p*-methoxy-trans-cinnamoyl-6-*O*-acetylshanzhiside methyl ester, 6-*O*-*p*-cis-coumaroyl-8-*O*-acetylshanzhiside methyl ester and 6-*O*-*p*-trans-coumaroyl-8-*O*-acetylshanzhiside methyl ester and their structures have been elucidated (Tuntiwachwuttikul *et al*., 1998).

Two new iridoid glycosides, together with the known compounds barlerin and verbascoside, were isolated from *B. prionitis* by Chen *et al*. (1998). The new iridoid glycosides were determined to be 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester and its cis isomer by using spectroscopic, especially 2D NMR, data. A 3:1 mixture of the two was shown to have potent *in vitro* activity against respiratory syncytial virus with EC_{50} 2.46 µg/mL and IC_{50} 42.2 µg/mL.

Venkata Rao *et al*. (1999) identified campesterol, stigmasterol and β-sitosterol as minor components of a sterol mixture which they isolated from the stem and root extract of *B. longiflora* by GC-MS. The extracts were

subjected to column chromatography and that led to the isolation of four anthraquinones. A pentacyclic triterpene namely arnidiol was also isolated with its isomer faradiol.

Two new lignan glycosides, 4-O-[alpha-L-arabinopyranosyl-(1' "→2")-beta-D-xylopyranosyl-(1' " '→5' ')-beta-D-apiofuranosyl]diphyllin (ciliatoside A) and 4-O-¿ [beta-D-apiofuranosyl-(1' " "→3' ")-alpha-L-arabinopyranosyl-(1' "→2' ')][beta-D-xylopyranosyl-(1' " '→5' ')]-beta-D-apiofuranosyl¿ diphyllin (ciliatoside B) with potent anti-inflammatory effect were isolated from the whole plant of *J. ciliata* (Day *et al*., 2000). The structures of the compounds were determined by extensive spectral and chemical methods. Both the compounds strongly inhibited the accumulation of NO(2)(-) in lipopolysaccharide-stimulated RAW 264.7 cells in a concentration-dependent manner with IC_{50} values of 27.1 ± 1.6 and 29.4 ± 1.4 µM, respectively.

Chemical composition of *S. crispus* leaf extract was determined by Ismail *et al*. (2000). Mineral content was determined using the atomic absorption spectrophotometer, whereas the water-soluble vitamins were determined by means of the UV-VIS spectrophotometer (vitamin C) and fluorimeter (vitamins B_1 and B_2). Catechin, tannin, caffeine and alkaloid contents were also studied. The dried leaves contained a high amount of total ash (21.6%) as a result of a high amount of minerals including potassium (51%), calcium (24%), sodium (13%), iron (1%) and phosphorus (1%). High content of water-soluble vitamins (C, B1 and B2) contributed to the high antioxidant activity of the leaves. The leaves also contained a moderate amount of other compounds such as catechins, alkaloids, caffeine and tannin, contributing further to the total antioxidant activity. Catechins of *S. crispus* leaves showed highest antioxidant activity when compared to yerbamate and vitamin E.

From the aerial part of *B. lupulina*, 18 compounds were isolated by Kanchanapoom *et al*. (2001) among which 13 are known compounds. The newly identified compounds are 8-O-acetyl-6-O-trans-p-coumaroylshanzhiside, saletpangponosides A–C and 8-O-acetylmussaenoside. The known compounds included iridoid glucosides, phenyl propanoid glycosides, lignan glucoside, aliphatic glycoside and benzyl alcohol glycoside. The structural elucidations were based on analyses of physical and spectroscopic data. The methanolic extract of aerial part of *B. lupulina* was suspended in water and defatted with diethylether. The aqueous layer was subjected to column chromatography using a highly porous copolymer of styrene and divinylbenzene, and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH was repeatedly subjected to column chromatography using silica gel and octadecylsilyl silica gel, then by preparative HPLC-ODS to obtain 18 compounds.

The phytochemical investigation of roots of *S. callosus* revealed lupeol as a major ingredient to the extent of 50% of the total petroleum ether extract. Stigmasterol β-D-glucopyranoside, crassifolioside and few phenyl propanoid glycosides were also isolated from the chloroform and ethyl acetate extracts of *S. callosus* by Agarwal and Rangari (2001).

Agarwal and Rangari (2003a) has carried out the column chromatographic studies of the essential oil from the flowering tops of *S. ixiocephala* and isolated β-caryophyllene, fenchyl acetate, cadinol and a new sesquiterpene ixiocephol. The structure of ixiocephol was structurally elucidated from its UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

Two new anthraquinones have been isolated from *B. prionitis* and characterized as 1,8, dihydroxy -2,7-dimethyl 3,6-dimethoxy anthraquinone and 1,3,6,8-tetra methoxy-2,7-dimethyl anthraquinone by Ganga Raju *et al*. (2002).

Kanchanapoom *et al*. (2004a) isolated four triterpenoidal glycosides (justiciosides A–D) from the aerial portion of *J. betonica*. Their structures were established through chemical and NMR spectroscopic analyses as olean-12-ene-1β,3β,11α,28-tetraol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, olean-12-ene-1β,3β,11α,28-tetraol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside and 11α-methoxy-olean-12-ene-1β,3β,28-triol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside respectively.

A phenylethanoid (4-hydroxyphenylethyl 4-O-beta-D-glucopyranosyl-(1→3)-O-alpha-L-rhamnopyranoside) and an iridoid (10-O-trans-coumaroyl-eranthemoside) were isolated from an entire *B. strigosa* plant together with verbascoside, isoverbascoside, decaffeoylverbascoside, (+)-lyoniresinol 3α-O-β-D-glucoside, apigenin 7-O-α-L-rhamnosyl-(1-->6)-O-β-D-glucoside, 7-O-acetyl-8-epi-loganic acid and (3R)-1-octen-3-ol-3-O-β-D-xylosyl-(1-->6)-β-D-glucoside. The structural elucidations were based on analyses of physical and spectroscopic data (Kanchanapoom *et al*., 2004b).

A new indolo[3,2-*b*]quinoline alkaloid glycoside, jusbetonin (1), and three known alkaloids, namely, 10*H*-quindoline (2), 6*H*-quinindoline (3), and 5*H*,6*H*-quinindolin-11-one (4) have been isolated from the leaves of *J. betonica*. The structure of (1) was established on the basis of 1D and 2D NMR (^{1}H−^{1}H COSY, HMQC and HMBC) and HRFABMS data. Compound (1) is the first example of a glycosylated indolo[3,2-*b*]quinoline alkaloid, while compound (4) was isolated for the first time from a natural source (Subbaraju *et al*., 2004).

Three phenylethanoid glycosides *viz*., β-[(3′,4′-dihydroxyphenyl)-ethyl]-(4″-*O*-caffeoyl)- β-D-glucoside (desrhamnosylacteoside), β-[(3′,4′-

dihydroxyphenyl)-ethyl]-(3″-*O*-L-rhamnosyl)-(4″-*O*-caffeoyl)-β-D-glucoside (acteoside) and β-[(3′,4′-dihydroxyphenyl)-ethyl]-(3″,6″-*O*-L-dirhamnosyl)-(4″-O-caffeoyl)-β-D-glucoside (poliumoside) were isolated and identified from the callus cultures of *B. cristata* (Abd El-Mawla *et al*., 2005). The structures of the isolated compounds were established by spectroscopic evidence (UV, 1D and 2D-NMR and ESIMS) and further confirmation has been done by comparison with authentic samples. The amount of the above compounds in the callus culture was determined using HPLC.

Three new triterpenoidal glucosides, justiciosides E, F and G were isolated from the aerial portion of *J. betonica* (Kanchanapoom *et al*., 2005). Their structures were established through chemical and spectroscopic analyses and showed an unusual A-nor-B-homo oleanan-12-ene skeleton type for the aglycone moiety as A-nor-B-homo-oleanan-10,12-diene-3β,11α,28-triol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, A-nor-B-homo-oleanan-10,12-diene-3β,11α,28-triol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside and 11α-methoxy-A-nor-B-homo-oleanan-10,12-diene-3β,11α,28-triol 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside respectively.

Khare (2007) has reported that the roots of *B. cristata* contain anthraquinones and flowers contain apigenin, naringenin, quercetin and malvadin. Leaves and stems of *B. prionitis* showed the presence of iridoid glucosides, barlerin and acetyl barlerin. Flowers contain the flavonoid glycoside, scutellarin-7-neohesperidoside. The presence of β-sitosterol also has been reported.

Ata *et al*. (2007) carried out detailed phytochemical analysis of the crude ethanolic extract of *B. prionitis* that led to the isolation of six natural products *viz*., balarenone (1), pipataline (2), lupeol (3), prioniside A (4), prioniside B (5), and prioniside C (6). Compounds 1, 4, 5, and 6 were new

natural products, while 2 and 3 were already known. Compounds 1-6 were screened for glutathione *S*-transferase (GST) acetylcholinesterase (AChE) inhibitory activities and found to be active in these bioassays. Pipataline (2) was isolated on a large scale and it exhibited moderate bioactivity in both the enzyme inhibition assays.

Ata *et al*. (2009) carried out phytochemical studies on the ethanolic extract of the aerial parts of *B. prionitis* that resulted in the isolation of a phenyl ethanoid glycoside, namely, barlerinoside along with six iridoid glycosides, namely shanzhiside methyl ester, 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside. Among them barlerinoside was significantly active in GST inhibition assay with an IC_{50} value of 12.4 µM but weakly active in AChE inhibition assay. The other compounds also exhibited different levels of GST, AChE inhibitory activities. All of these natural products were also evaluated for free radical scavenging activity and only compound 1 was found to be significantly active in this assay with an IC_{50} value of 0.41 µg/ml. Rest of the compounds were weakly active in this assay with IC_{50} values in the range of 5-50 µg/ml.

Jimenez *et al*. (2009) estimated the total phenolic and flavonoid contents of extracts of *J. spicigera*. The total phenolic content ranged from 1.33-5.01 g gallic acid equivalents/100 g dry weight. Leaf and flower extracts obtained with methanol or water had higher amounts of phenolic compounds than stem extract. Total flavonoid content was between 0.18 and 1.30 g catechin equivalents/100 g dry weight and the order for methanol extracts is leaf > flower > stem, whereas for the aqueous extracts this sequence is stem > flower > leaf. This is the first study describing the antioxidant activity from *J. spicigera*. Phenolic compounds and flavonoids contribute to this activity.

Kpoviessi *et al*. (2009) analyzed *Justicia anselliana* essential oils from fresh aerial parts and roots by GC and GC/MS. The analysis revealed the presence of β-phellandrene (51.2%), δ-2-carene (43.4%), dihydrotagetone (0.4%) and propyl butyrate (0.3%) in the aerial parts, whereas, phenylacetaldehyde (39.2%), δ-3-carene (31.7%), 6-methyl-5-hepten-2-one (16.4%) and α-phellandrene (12.6%) were detected in the roots.

A new iridoid glycoside and three known iridoid glycosides were isolated from the aerial parts of *B. trispinosa* by Harraz *et al*. (2009). The structure of the new compound was determined as 6-α-L-rhamnopyranosyl-8-O-acetylshanzihiside methyl ester. The known compounds were identified as 6,8-O,O-diacetylshanhiside methyl ester (acetyl barlerin), 8-O-acetylshanzhiside methyl ester (barlerin) and shanzhiside methyl ester, which were isolated from the plant for the first time. The structures of the isolated compounds were elucidated by spectroscopic evidence, mainly one- and two-dimensional proton and carbon-13 NMR spectroscopy.

Balakrishnan *et al.* (2010) conducted preliminary phytochemical and pharmacognostic studies on *E. viride* to set scientific parameters to ensure its quality by identifying the true plant material. Preliminary phytochemical profile and the necessary pharmacognostic standards for evaluating the plant material have been established. Various parameters like morphology, microscopy, powder analysis, fluorescence characteristics and physico-chemical constants of the roots were studied and the diagnostic features are documented. Many of these diagnostic elements and preliminary phytochemical profile were found to be useful evidences for further scientific investigations of this medicinal plant.

The bioactive flavonoid compounds of *S. crispus* leaves obtained by supercritical carbon dioxide (SC-CO_2) extraction were investigated and the obtained crude extract yields were compared in order to select the best

operation parameters (Liza *et al*., 2010). Since carbon dioxide is a non-polar solvent, ethanol was used as co-solvent to increase the polarity of the fluid. The studied parameters were pressure (100, 150 and 200 bar), temperature (40, 50 and 60°C) and dynamic extraction time (40, 60 and 80 min). The optimum extraction condition occurred at 200 bar, 50°C and 60 min. Based on the mean value, pressure had dominant effect on the extraction yield. HPLC was used to determine the major bioactive flavonoid compounds from *S. crispus*. Under the optimum conditions, eight flavonoid compounds were identified; they were (+)-catechin, (−)-epicatechin, rutin, myricetin, luteolin,
apigenin, naringenin and kaempferol.

Reneela and Sripathi (2010) conducted phytochemical investigation on the stems of *S. ciliatus*. The separation of the chemical components was carried out by chromatography and structures of the compounds were elucidated by spectroscopic methods including nuclear magnetic resonance as well as mass spectrometry. The compounds were identified as lupeol, stigmasterol, betulin and stigmasterol glycoside.

A total of ten biological compounds were isolated from the leaves of *B. montana* by using GC-MS analysis (Natarajan *et al*., 2011). Among them, benzaldehyde,2-hydroxy-6-methyl- and urs-12-en-24-oicacid,3-oxo-,methyl ester,(+)- were identified as major compounds.

Maji *et al*. (2011) carried out preliminary phytochemical analysis of hydro-methanolic extract of *B. prionitis* whole plant which revealed the presence of glycosides, flavonoids, steroids and tannins.

Pino (2011) studied the volatile constituents from leaves of *J. pectoralis* var. *tipo* grown in Cuba. Thirty-two compounds were identified from the volatile oil, of which the most prominent were nonanal (45.9 %), 1-octen-3-ol (8.4 %) and coumarin (7.4 %).

Wang *et al.* (2011) developed HPLC fingerprints for the quality evaluation of *Justicia procumbens* and its compound preparation, Jian-er syrup, together with the simultaneous quantification of eight arylnaphthalide lignans (6'-hydroxy justicidin B, 6'-hydroxy justicidin A, 6'-hydroxy justicidin C, justicidin B, chinensinaphthol methyl ether, justicidin C, taiwanin C and neojusticin A). For fingerprint analysis, 17 peaks were selected as the characteristic peaks for the evaluation of similarities among different *J. procumbens* samples collected from different places. The new method was successfully applied for the chromatographic fingerprint analysis and simultaneous determination of eight lignans in its compound preparation, Jian-er syrup. All the results indicated that HPLC fingerprint assay in combination with multi-marker determination afforded a useful method for the quality control of *J. procumbens* and its compound preparation, Jian-er syrup.

Olean-12-en-3β-24 diol, auranamide, aurantiamide acetate, 2α,3β-dihydroxy-olean-12-en-28-oic acid and quindoline were isolated from the dichloromethane extract of the stems of *Justicia secunda* (Calderon *et al.*, 2013). Liquid chromatography with ultraviolet and mass spectrometric detection was used to acquire more knowledge of the chemical composition of this extract and to monitor variations in profiles of both the isolated and the other non-identified compounds in *J. refractifolia* and *J. graciliflora*. The compound classes, phenolic and olefinic amides, feruloyltyramine amides, 2,5-diaryl-3,4- dimethyltetrahydrofuranoid lignans, peptide alkaloids, phenylalanine derivatives, conjugated ynones, indolquinoline alkaloids, triterpenes and pigments, were tentatively identified based on the LC–DAD–APCI–MS analysis. The most frequently encountered compound among the species was auranamide while the distribution of quindoline was limited to *J. secunda*.

A detailed phytochemical screening (qualitative and quantitative) and HPTLC analysis of various phytochemicals of the crude extracts of *B. cristata* leaves have been carried out by Narmadha and Devaki (2012b). The different analyses revealed the presence of amino acids, carbohydrates, flavonoids, proteins, phenolic groups, saponins, steroids, tannins and terpenoids. Among the different crude extracts tested, most of the phytochemicals were found to be present in the ethanolic and aqueous extracts. The quantitative analysis of secondary metabolites was done by using these two extracts. In the quantitative assays, the ethanolic extract showed the highest content of alkaloids (1.43 ± 0.02), phenols (0.725 ± 0.01), flavonoids (0.47 ± 0.05) and proteins (42.5 ± 5.13). HPTLC profile of the ethanolic extract support the presence of alkaloids, flavonoids and phenolic compounds like quercetin in the leaf extract of this plant.

Two phenolic compounds (*P*-coumaric acid and *α*–tocopherol), two flavonoidal compounds (luetoline and 7-methoxy luetoline) and two iridoidal glycosides (barlerin and schanshiside methyl ester) have been isolated from leaves of *B. cristata* and their structure were established by spectral analysis (Hemalatha *et al*., 2012). Chromatographic resolution of petroleum ether extracts furnished two phenolic compounds, which were characterized as *p*-coumaric acid and *α*- tocopherol, whereas ethyl acetate extract resulted in two flavonoidal compounds, which were further identified and characterized as luteoline and 7-methoxy luteoline and finally, from ethanolic extract yielded another two new iridoidal glycosides like barlerin and shanshiside methyl ester respectively.

Lupeol was found to be the major constituent of *S. ciliatus* and was isolated from the petroleum ether extract of aerial parts of *S. ciliatus* by column chromatography and identified by IR, NMR, and MS spectral data

(Venkatachalapathi and Ravi, 2012). It was quantified in the petroleum ether extract by HPTLC method and found to be 0.16 ± 0.02% w/w.

Sarpate and Tupkari (2012) established a sensitive high performance thin layer chromatographic method for quantification of lupeol which was isolated from the methanolic extract of dried stem powder of *S. callosus*. The developed HPTLC technique is precise, specific, accurate and shows remarkable stability indicating that it can be used for the routine quality control analysis and quantitative determination of lupeol from dried stem powder of *S. callosus* for further scientific work.

According to Correa and Alcantara (2012), a great diversity of chemical classes have been reported to be present in the species of *Justicia* by several authors, mainly alkaloids, lignans, flavonoids and terpenoids (iridoids, diterpenoids and triterpenoids). Other chemical classes have been isolated from species of *Justicia* such as essential oils, vitamins, fatty acids (docosanoic acid) and salicylic acid. The steroids like campesterol, stigmasterol, sitosterol and sitosterol-D-glucoside were also been isolated from the leaves and roots of different species. The other compounds reported to be present in species of *Justicia* are coumarin, flavonoids, alkaloids and triterpenoidal glycosides.

Ghule *et al*. (2012) has developed a method for the quality control of *B. prionitis* which can be employed profitably in place of HPLC. A simple as well as sensitive and reproducible high-performance thin-layer chromatography (HPTLC) method for the simultaneous quantification of shanzhiside methyl ester (SME) and barlerin, the active principles of *B. prionitis* was validated. The marker compounds were isolated, purified and authenticated by spectral analysis. Precoated silica gel 60 F_{254} TLC plates were used as a stationary phase and chloroform-methanol (80:20) as the mobile phase. The method was validated in terms of linearity, precision,

robustness, accuracy and limits of detection, and quantification. Quantification was performed in the absorbance mode at a wavelength of 240 nm using Deuterium lamp. The percentage recoveries for SME and barlerin were in the range of 99.20 to 99.54% and 98.93 to 99.19% respectively. The content (% *w/w*) of SME and barlerin was found to be 4.91% and 4.69% respectively, in methanol extract of *B. prionitis*.

The chemical composition by GC-MS analysis and antibacterial property of *B. lupulina* essential oil were reported by Sarmad *et al*. (2012). The main components of the essential oil included cyclobutane,1,1-dimethyl-2-octyl, 2-Hexyl-1-octanol, 1, 2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and 1-hentetracontanol.

The active compound ecbolin A (a furofuran type of lignan) was isolated from ethyl acetate extract of *E. viride* roots using chromatographic techniques (Cecilia *et al*., 2012). The dried powder of *E. viride* roots has been subjected to sequential extraction with different solvents in the increasing order of polarity. The ethyl acetate extract exhibited significant antimicrobial activity and hence it was subjected to column chromatography, and a single crystal obtained from fraction 7 was identified as ecbolin A, a furofuran type of lignin. The electron impact mass spectrum of ecbolin A indicated the molecular weight of 444.05 corresponding to the molecular formula $C_{23}H_{24}O_9$. The structure of ecbolin A reported in the study was compared and confirmed with previous reports. The compound ecbolin A inhibited the growth of gram positive bacteria, gram-negative bacteria and several fungi.

Tanaka *et al*. (2004) found a new lignin, (+) lyoniresinol 3 α-O-ß-D apiofuranosyl-(1-2)-ß-D-glucopyranoside and two new phenylethanoid glycosides, [2,(3,4-dihydroxyphenylethyl)]-3-O-a-D-apiofuranosyl-(1-4)-4-O-caffeoyl)-ß-D- glucopyranoside (cusianoside A) and [2,(3,4-dihydroxyphenylethyl)]-3-O-ß-D-xylopyranosyl-(1-3)-4-O-caffeoyl)- ß-D-

glucopyranoside (cusianoside B) together with five known compounds lupeol, (+)-5,5'-dimethoxy-9-O- ß-D-glucopyranosyl lariciresinol, (+)-9-O- β -D-glucopyranosyl lyoniresinol, (+)-5,5'-dimethoxy-9-O- β -D-glucopyranosyl secoisolariciresinol and acetoside from *Strobilanthes cusia*. Seco-pregnane steroid glaucogenin C and its monosugar-glycoside cynatratoside A have been isolated from the whole plant of *S. cusia.* These compounds were found to be specifically active against the group of positive strand RNA viruses.

Rahmat *et al*. (2006) investigated the chemical constituents, antioxidant activity and cytotoxic activity of essential oil from *S. crispus* by GC-MS analysis. The analysis revealed the presence of 28 components. The main constituents of the oil were phytol (46.01%), 2-undecanone (5.84%), α-cadinol (3.47%), megastigmatrienone (1.21%), 2,3-dihydrobenzofuran (1.68%) and eugenol (1.08%).

Ponnamma and Manjunath (2012) analyzed the methanolic extract of *Justicia wynaadensis* by GC-MS and twenty four compounds were identified. The major constituents present were dihydrocoumarin, phytol and palmitic acid. Significant quantities of linoleic acid, stearic acid, squalene and phytosterols such as campesterol and stigmasterol were also present.

A simple HPTLC method was developed by Amutha and Doss (2012) to determine the saponin profile of *B. cristata* crude leaf methanolic extract. The HPTLC chromatographic plates were derivatized with anisaldehyde – sulphuric acid reagent, dried and scanned at 500 nm for saponins. The green, violet, yellow, brown coloured zones at Rf value 0.28, 0.32, 0.4, 0.46 and 0.64 present in the chromatogram confirm the presence of saponins in the sample.

Preliminary phytochemical screening and GC-MS analysis were carried out to identify the phytoconstituents of *B. montana* (Sriram and

Sasikumar, 2012). The analysis revealed the presence of coumarin, sterol, quinone, flavonoids, alkaloids, terpenoids and tannin in the plant. The GC-MS analysis of the extract retrieved 26 compounds.

Koay *et al*. (2013) carried out detailed phytochemical investigation of *S. crispus* leaves. The air-dried leaves were extracted sequentially with hexane, dichloromethane and methanol. Each of the extracts, purified by chromatographic techniques has led to the isolation of 1-heptacosanol, tetracosanoic acid, stigmasterol from the hexane extract, a mixture of fatty acid esters of *β*-amyrin, taraxerol, taraxerone, another mixture of fatty acid esters of taraxerol from the dichloromethane extract and 4-acetyl-2,7-dihydroxy-1,4,8-triphenyloctane-3,5-dione and stigmasterol *β*-D-glucopyranoside from the methanol extract. The structures of the compounds were elucidated with the aid of spectroscopic techniques (IR, GC-MS, MS, ^{1}H and ^{13}C NMR) and also by comparison with the previously published data.

MATERIALS AND METHODS

Karyomorphological analysis, antioxidant as well as anti-inflammatory assays and phytochemical characterization of six taxa belonging to four genera of Acanthaceae that are used as the source plants of the Ayurvedic drug 'Sahachara' were undertaken during the current study. Details regarding the assays conducted and their experimental procedures are described in this session.

Experiments of the present study were carried out at Cell and Molecular Biology Division, Department of Botany, University of Calicut, Kerala, India and also at Amala Cancer Research Centre, Thrissur, Kerala, India. The experiments performed in the investigation include the following:-

1. Karyomorphological analysis of the six taxa belonging to four genera of Acanthaceae.
2. Antioxidant and anti-inflammatory assays of root methanolic extracts of these taxa.
3. Characterization of the methanolic extracts of the plants by Gas Chromatography – Mass Spectrometry (GC-MS), High Performance Thin Layer Chromatography (HPTLC) and Liquid Chromatography – Mass Spectrometry (LC-MS).
4. Estimation of the total phenolic and flavonoid content of the methanolic extracts.

1. PLANT MATERIALS

Barleria cristata L., *B. prionitis* L., *Ecbolium viride* (Forssk.) Alston, *Justicia betonica* L., *Strobilanthes ciliatus* Nees and *S. heyneanus* Nees were used for current study (Plate 1). The plants were collected from wild

populations, from different parts of Kozhikode and Wayanad districts of Kerala, India and were kept in the net house attached to Department of Botany, University of Calicut. The plants were authenticated by Dr A. K. Pradeep, Assistant Professor, Department of Botany, University of Calicut, where voucher specimens were deposited. Voucher numbers allotted to each plant is given in brackets.

Barleria cristata L. (CALI- 123738)

Common names: Philippine violet/Bluebell Barleria/Sahachara. Distribution: Common in deciduous forests in all dry plains and districts up to about 3,000 ft. Also grown as an ornamental.

An erect or diffuse herb up to 1 m tall; stems adpressedly hairy, densely hairy at the nodes, bracts and bracteoles spiny. Leaves elliptic-oblong to lanceolate, 6.3-13 cm long and 2.5-4.5 cm wide, acute or acuminate, hairy on both sides. Flowers bluish-purple, pink or white, pubescent outside, borne in 1-4 flowered axillary and terminal spikes; bracts lanceolate, serrate-dentate; calyx lobes 4, spinous-ciliate; corolla funnel-shaped, limb subequally 5-lobed. Fruits (capsules) 1.6 cm long, ellipsoid or long, acute at both ends, 4-seeded; seeds 4 mm in diameter, orbicular, compressed, silky-hairy. Flowering from September to November and fruiting from October to January in central Indian deciduous forests (Parrotta, 2001).

Barleria prionitis L. (CALI- 123737)

Common names: Porcupine flower/Sahachara. Distribution: The plant is found in all plains and districts, especially in the Deccan and Carnatic, chiefly on waste lands and road sides. It is also distributed in Africa, tropical Asia, Sri Lanka and Malacca.

A much branched, usually prickly shrub up to 3 m tall, with whitish stems and rounded branches. Leaves opposite, elliptic, acuminate, lineolate, bristle-tipped, entire, 9-18 cm long and 2.5-5.7 cm wide, glabrous above, young leaves often pubescent beneath. Flowers orange-yellow or cream coloured, sessile, borne in axillary foliaceous bristle-tipped bracts. Fruits (capsules) ovoid, 1.8-2.5 cm long with a tapering beak, 2-seeded; seeds compressed, ovate, clothed with silky appressed hairs. Flowering from September to December and fruiting from January to April in central Indian deciduous forest areas; in this region the plant is leafless from January to April or May (Parrotta, 2001).

Ecbolium viride (Forssk.) Alston (CALI- 123739)

Common names: Green Shrimp Plant/Green Ice Crossandra/ Odiyamadantha/Neelakarinkurunji. Distribution: Usually seen in waste places and exposed habitats. Distributed in Sri Lanka, India, Africa and Malaya.

Woody undershrub, leaves simple, opposite, elliptic-ovate, obtuse or acute, base narrowed down to a short petiole, 11 x 4.5 cms, glabrous green above, pale beneath; flowers greenish blue in terminal spikes; bracts large, green, orbicular or lanceolate; calyx lobes 5, subequal, lanceolate, glandular, pubescent; corolla tube slender, long, limb distinctly bilabiate, upper lip linear, lower lip spreading, 3-lobed; stamens 2, attached at the base of upper lip, exerted; capsule clavate, compressed; seeds 2, orbicular, tuberculate on curved retinacula (Sivarajan and Balachandran, 1994).

Justicia betonica L. (CALI- 123740)

Common name: White shrimp plant/Squirrel’s tail/Sahachara/Vellakurunji. Distribution: A pantropical species, found in all districts in waste lands and forest clearings.

Gregarious tall shrub; leaves simple, opposite, long-petioled, ovate-lanceolate, 22 x 7 cm, entire to crenate dentate, glabrous; flowers white speckled with pink in long terminal bracteate spikes; bracts and bracteoles similar, leafy, elliptic to ovate acute, white with green nerves; calyx lobes 5; corolla tubes short, limb distinctly bilabiate; stamens 2; clavate; seeds spinulose when wetted (Sivarajan and Balachandran, 1994).

Strobilanthes ciliatus Nees (CALI-123736)

Common name: Karvi, Karimkurinji/Sahachara. Distribution: The plant is common in Western Ghats, S. Canara to Travancore, in evergreen forests up to 4,000 ft.

Gregarious shrub, often growing in large colonies; stem and leaves dark green; leaves simple, opposite, elliptic-acuminate, serrate, 17 x 5 cm; flowers pale rose in short, glandular, bracteate, deflexed spikes; calyx lobes five, lanceolate, acuminate; corolla base cylindric, upper part companulate, spreading; stamens didynamous; ovary 2-celled with two ovules in each; capsule clavate, slightly exerted from the glandular fruiting calyx (Sivarajan and Balachandran, 1994).

Strobilanthes heyneanus Nees (CALI- 123735)

Common name: Karvi or Karimkurinji. Distribution: Mostly found in South-West India.

An undershrub, about a meter high, with grooved stem, often covered with hairs. Oppositely arranged, unequal, ovate leaves are hairy and have serrated margin. Blue urn-shaped flowers 1-1.2 cm long, occur in axillary spikes. Five sepals are united at the base. Flower tube is swollen in the middle, and has 5 rounded petals. The swollen part is whitish in color.

Stamens 4, filaments hairy at base. Capsule 6-8 mm long, oblong, 4-seeded. Flowering: September-October (http[1]).

2. METHODOLOGY

In the present investigation, the methods adopted to reveal the cytogenetical and phytochemical aspects of members of Acanthaceae used as the source plants of the drug 'Sahachara' are as per standard references quoted there-upon.

A. CYTOGENETICAL ANALYSIS

In the present study, cytogenetical analyses comprise karyomorphological studies, antioxidant and anti-inflammatory assays.

a. KARYOMORPHOLOGICAL ANALYSIS

Karyomorphological studies are of paramount importance, as they often provide authentic information pertaining to chromosome structure, number and their general gross morphology (Darlington and La Cour, 1970). Besides being helpful in elucidating phylogenetic and evolutionary relationships between species of plants, karyotype analysis can contribute cytological data for the important drug plants of Ayurveda whose correct identity is in dispute. In order to accurately ascertain chromosome homologies and differences, several indices are used by measuring chromosomes as a whole and their arms (Duncan and Smith, 1978).

i. MITOTIC SQUASH PREPARATION

Vegetative cuttings were taken from the experimental plants at their nodes and planted in sand, in wide shallow pots. Young, healthy root tips were collected from the plants under study at the period showing peak mitotic activity (9-10 am). They were thoroughly washed in distilled water and were subjected to pre-treatment in cytostatic chemicals. Saturated aqueous solution

of para-dichlorobenzene (PDB) alone and PDB along with a trace of aesculine were used for pre-treatment of different species. Pre-treatment results in better separation of individual chromosomes, helps in the identification of chromosome arms and ensures a high frequency of metaphase stages (Sharma, 1991).

The pre-treatment solution was initially chilled to 0-5˚C for 5 min and the root tips were dipped in it. This was kept at 12-15˚C for 1-2 h to obtain best results. After pre-treatment, root tips were thoroughly washed with distilled water and were fixed in modified Carnoy's fluid (1 acetic acid: 2 ethyl alcohol) for 2 h. Fixed root tips were transferred to 70% alcohol and stored under refrigeration.

Mitotic squash experiments were conducted with the help of improved techniques (Sharma and Sharma, 1990). The fixed root tips were washed with distilled water and hydrolyzed in 1N HCl for 25-30 min at room temperature. Traces of acid were removed by thorough washing with distilled water. After hydrolysis, the root tips were stained in 2% aceto-orcein for 2-3 h, destained and squashed in 45% acetic acid. All the slides were scanned in Olympus microscope CX 21 and the photographs were taken with Olympus Camedia C-4000 Zoom digital compact camera attached to the microscope.

ii. KARYOMORPHOMETRICAL ANALYSIS

Karyograms were generated from the photomicrographs with the aid of computer based programmes such as Adobe Photoshop, AutoCAD and a data based analyzing system (Microsoft Excel). Photographs taken were scanned and stored as digital images. These digital images were converted to gray scale images using Photoshop program. Identification numbers were allotted to each chromosome and then loaded to AutoCAD for karyomorphometrical analysis. Centromeric position of each chromosome was determined, from

which arm lengths of each were measured and centromeric indices were calculated. On the basis of arm ratio and centromeric indices, homologous chromosomes were identified and classified according to Abraham and Prasad (1983). The images were reloaded to Photoshop and karyograms as well as idiograms generated.

Karyotype formula was expressed depending upon the length of chromosome, position of the centromere and presence or absence of the secondary constriction.

Disparity index (DI) of the chromosomes were calculated with the method of Mohanty *et al*. (1991) by using the formula,

$$DI = \frac{\text{Longest chromosome} - \text{shortest chromosome}}{\text{Longest chromosome} + \text{shortest chromosome}} \times 100$$

The variation coefficient (VC) among the chromosome complements was determined after Verma (1980) as follows,

$$VC = \frac{\text{Standard Deviation}}{\text{Mean Length of chromosomes}} \times 100$$

The total Forma percentage (TF %) or mean centromeric index value was calculated after Huziwara (1992) by the formula,

$$TF\ \% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} X\ \ 100$$

All the numerical data were prepared after comparing at least five well spread metaphase plates.

Table 2. Details of chromosome nomenclature in relation to centromere location based on arm ratios and centromeric indices (Abraham and Prasad, 1983)

Nomenclature	Notation	R_1 s/l	R_2 l/s	I_1 100 s/c	I_2 100 l/c
Median	M nm	1.00	1.00	50.00	50.00
Nearly median	nsm(-)	0.99 to 0.61	1.01 to 1.63	49.99 to 38.01	50.01 to 61.99
Nearly submedian	SM	0.60 to 0.34	1.64 to 2.99	38.00 to 25.01	62. 00 to 74. 99
Submedian	nsm(+)	0.33	3.00	25.00	75.00
Nearly submedian	nst(-)	0.32 to 0.23	3.01 to 4.26	24.99 to 18.20	75. 01 to 81.80
Nearly subterminal	ST	0.22 to 0.15	4.27 to 6.99	18.19 to 12.51	81.81 to 87. 49
Subterminal	nst(+)	0.14	7.00	12.50	87.50
Nearly subterminal	nt	0.13 to 0.07	7.01 to 14.38	12.49 to 5.01	87.51 to 94.99
Nearly terminal	T	0.06 to 0.01	14. 39 to 19.99	5.00 to 0.01	95.00 to 99.99
Terminal		0.00	α	0.00	100.00

b. ANTIOXIDANT AND ANTI-INFLAMMATORY ASSAYS

It is worthwhile to investigate the various biological potentialities of plants so as to provide scientific justification to their use in indigenous healthcare systems. The studies on overall antioxidant and anti-inflammatory activities of species that have long been used as traditional remedies could be supportive in order to validate their uses as remedies for oxidation and inflammation related disorders. Moreover, such studies can also point out the necessity of further investigations on such least explored species with a view of identifying their curative and defensive abilities possibly leading to new remedies for various chronic disorders.

i. PREPARATION OF ROOT METHANOLIC EXTRACTS

Roots of the six species of plants *u*sed in the study were collected thoroughly washed and shade dried. The dried roots were chopped into small pieces and powdered. 50 g each of the dried powder was extracted with 500 ml of 100% methanol for 6 h in Soxhlet apparatus. The extract so obtained was then cooled, filtered and evaporated to dryness in a water bath by complete removal of methanol. The concentrated extracts were stored in amber coloured bottles at 4^{oC} in a refrigerator.

ii. DRUG PREPARATION FOR *IN VITRO* STUDIES

Stock solutions of the drugs were prepared by dissolving 10 mg of methanolic extract of the six plants under study in 200 µl dimethyl sulphoxide (DMSO) and made up to 1 ml with distilled water. Various dilutions of all the stock solutions of the six plant extracts were then prepared in distilled water.

iii. ANTIOXIDANT ASSAYS

Concentrations of the methanolic extracts ranging from 10 to 140 µg/ml were used for the *in vitro* antioxidant assays. Triplicate determinations were made for all the experiments.

DPPH radical scavenging assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity was determined by the method proposed by Coruh *et al*. (2007). Commercially available stable free radical DPPH, soluble in methanol was used for the assay. 100 µl of the extract solution in methanol at various concentrations was mixed with 1 ml of 100 µM freshly prepared solution of DPPH in methanol. The reaction mixture was incubated at room temperature in the dark for 20 min and thereafter its absorbance was measured at 515 nm against the blank. For the control, 1 ml of DPPH solution in methanol was mixed with 100 µl methanol and the absorbance of the solution was recorded after 20 min as earlier. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition of DPPH radical, which was calculated as follows:

$$\text{Percentage inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the blank control (DPPH radical solution without test sample) and A_{sample} is the absorbance of the test sample.

ABTS radical scavenging assay

In this assay, the extract was allowed to react with $ABTS^{+}$, a stable free radical derived from 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) or ABTS (Long and Halliwell, 2001). The assay relies on the antioxidant capability of the samples to inhibit the oxidation of ABTS to $ABTS^{+}$ radical

cation. Ammonium persulphate (2.45 mM final concentration) was added to a stock solution of ABTS in deionized water (7 mM). The reaction mixture was left to stand at room temperature overnight (12~16 h) in the dark before use. ABTS and persulphate react with each other leading to the incomplete oxidation of ABTS to generate ABTS radical. The resultant intensely coloured $ABTS^{+}$ solution (stable for 2 days) was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.7 at 734 nm. Different concentrations of the extract in 10 µl were added to 1 ml of ABTS radical solution. Decrease in absorbance was measured by a spectrophotometer at 6 min after initial mixing, using PBS as reference. A control reaction was carried out by taking the absorbance of ABTS solution without the test sample. The ABTS radical-scavenging activity of the samples was expressed as follows:

$$\text{Percentage inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the blank control (ABTS radical solution without test sample) and A_{sample} is the absorbance of the test sample.

Superoxide radical scavenging assay

Superoxide radical (O_2^{-}) scavenging activity was estimated by the riboflavin photoreduction method as described by Mc Cord and Fridovich (1969). The scavenging activity of the extracts on superoxide anion radicals was determined by light induced superoxide generation with riboflavin and subsequent reduction of nitroblue tetrazolium (NBT). The reaction mixture contained EDTA (6 µM) containing NaCN (3 µg), riboflavin (2 µM), NBT (50 µM), phosphate buffer (67 mM, pH 7.8) and various concentrations of the extract in a final volume of 3 ml. The tubes containing the reaction mixture were uniformly illuminated under incandescent lamp for 15 min. The optical

density was measured at 530 nm before and after illumination. The percentage inhibition was evaluated by comparing the absorbance value of the control tubes and experimental tubes as per the formula given below:

$$\% \text{ of inhibition} = \frac{\text{OD of the control} - \text{OD of the treated}}{\text{OD of the control}} \times 100$$

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the test material was measured by studying the competition between deoxyribose and test compounds for hydroxyl radicals generated from the Fe^{3+}/Ascorbate/EDTA/H_2O_2 system (Fenton reaction) as described by Elizabeth and Rao (1990). The hydroxyl radicals attack deoxyribose eventually resulting in the formation of thiobarbituric acid (TBA) reacting substances. The reaction mixture containing deoxyribose (2.8 mM), ferric chloride (0.1 mM), EDTA (0.1 mM), H_2O_2 (1.0 mM), ascorbate (0.1 mM), KH_2PO_4 - KOH buffer (20 mM, pH 7.4) and various concentrations of the extract in a final volume of 1 ml. The reaction mixture was incubated for 60 min at 37^{o}C. The thiobarbituric acid reacting substances formed was estimated by TBA method of Ohkawa *et al*. (1979). The hydroxyl radical scavenging activity was determined by comparing absorbance value of control with that of treatments. The percentage inhibition of hydroxyl radicals in the reaction mixture is given by the formula,

$$\% \text{ of inhibition} = \frac{\text{OD of the control} - \text{OD of the treated}}{\text{OD of the control}} 100$$

Lipid peroxidation assay

Lipid peroxidation was induced in rat liver homogenate by the method described by Bishayee and Balasubramanian (1971) in the presence of different concentrations of the test material and estimated by thiobarbituric acid reactive substances by the method of Ohkawa *et al*. (1979). Different

concentrations of the test material was incubated with 0.1 ml of rat liver homogenate (25%) containing 30 mM KCl, Tris-HCl buffer (40 mM, pH 7.0), ascorbic acid (0.06 mM) and ferrous ion (0.16 mM) in a total volume of 0.5 ml at 37^{o}C for 1 h. At the end of the incubation period, 0.4 ml of the reaction mixture was treated with 0.2 ml SDS (8.1%), 1.5 ml TBA (0.8%) and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4 ml by adding distilled water and kept in a water bath at 95^{o}C for 1h. After cooling, 1 ml distilled water and 5 ml butanol-pyridine mixture (15:1 v/v) was added. After vigorous shaking, the tubes were centrifuged and the upper layer containing the chromophore was read at 532 nm. The percentage of inhibition of lipid peroxidation was calculated as before.

$$\% \text{ of inhibition} = \frac{\text{OD of the control} - \text{OD of the treated}}{\text{OD of the control}} \times 100$$

Statistical analysis

Each data represents the arithmetic mean ± standard error (SE) of three independent experiments. For statistical analysis, one-way analysis of variance (ANOVA) was conducted employing Tukey–Kramer HSD test (Sokal and Rohlf, 1981) to analyze significant differences between means. Results with $P < 0.05$ were considered to be statistically significant. The 50% inhibitory concentration (IC_{50} value) for each plant extract was determined by performing linear regression analysis. All statistical analyses were carried out by using the computer software SPSS 20.0 for Windows.

iv. ANTI-INFLAMMATORY ASSAYS

Drug preparation and administration for *in vivo* studies

The stock solutions of the drug for *in vivo* studies were prepared by suspending the methanol extracts of the six plants of Acanthaceae in 10 ml of 1% gum acacia. It was done by dissolving 100 mg of the extracts in a

minimum known volume of methanol and was poured into a beaker containing 100 mg gum acacia dissolved in 10 ml phosphate buffered saline (PBS) by boiling. After boiling, the stock solutions were measured to confirm that the volume of methanol added was eliminated by evaporation. From the stock solution the required doses for each experiment (100 and 250 mg/kg body weight) were made by dilution in PBS. The drug was administered orally to Swiss Albino mice (8-10 week old and weighing 20-25 g). The standard group was administered with diclofenac intraperitoneally.

Experimental animals

Swiss Albino mice of either sexes belonging to the age group of 8-10 weeks, which weighed 20-25 g were purchased from the Small Animal Breeding Station, College of Veterinary and Animal Science, Mannuthy, Thrissur. The animals were maintained under sterilized environmental conditions (22-28^{o}C, 60-70% relative humidity, 12 h dark/light cycle) and fed with standard rat feed (Sai Feeds, India) and water *ad libitum*. All animal experiments of the present study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) (Lic No. 149/1999) and were conducted strictly according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA) constituted by the Animal Welfare Division, Government of India.

Evaluation of anti-inflammatory activities of the extracts

Carrageenan induced acute inflammation

The anti-inflammatory activities of the extracts were evaluated by the carrageenan-induced paw edema test in mice (Adeyemi *et al.*, 2002). Swiss Albino mice were divided into 4 groups comprising of 5 animals in each group. In all groups, acute inflammation was induced by injection of 0.02 ml freshly prepared 1% suspension of carrageenan in normal saline on the sub-

plantar region of the right hind paw of mice (Winter *et al*., 1962). Group I remained as control for carrageenan which received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle, i.e., 1% gum acacia. Group II was the positive control, administered with the standard reference drug diclofenac (10 mg/kg body weight (b. wt) intraperitoneally. Group III and IV received 100 and 250 mg/kg b. wt of extract orally 1 h before carrageenan injection. The paw thickness was measured 1 h before and at 0, 1, 2, 3, 4, 5 and 24 h intervals after carrageenan administration using digital calliper. The percentage of inhibition of paw thickness was calculated using the formula given below:

$$\%\ \text{inhibition of paw thickness} = \left[\frac{(tC_n - tC_0) - (tT_n - tT_0)}{(tC_n - tC_0)}\right] \times 100$$

where, tC_0 = paw thickness of control animal before induction; tC_n = paw thickness of control animal at particular time point; tT_0 = paw thickness of treated animal before induction and tT_n paw thickness at particular time point of treated animal.

Formalin induced chronic inflammation

The anti-inflammatory activity of the various extracts against formalin induced chronic inflammation was evaluated (Roy *et al*., 1982). Single dose of 0.02 ml of freshly prepared 1% formalin was used to induce chronic inflammation in mice. The animals were divided into 4 groups of 5 animals each. Group I was kept as untreated vehicle control and received 1% gum acacia in PBS for 6 days orally. Group II was treated with standard drug diclofenac 10 mg/kg b. wt for the same period intraperitoneally. Group III and IV were treated with the extracts in 1% gum acacia in PBS at 100 mg/kg b. wt and 250 mg/kg b. wt respectively for 6 days orally. Drug treatment was

started 1 h prior to formalin injection and continued for 6 consecutive days (Chau, 1989). The thickness of the paw was measured before injection and every day for six days after formalin injection using a digital calliper. The percentage inhibition was calculated using the same formula given earlier:

$$\% \text{ inhibition of paw thickness} = \left[\frac{(tC_n - tC_0) - (tT_n - tT_0)}{(tC_n - tC_0)}\right] \times 100$$

where tC_0 = paw thickness of control animal before induction; tC_n = paw thickness of control animal at particular time point; tT_0 = paw thickness of treated animal before induction and tT_n = paw thickness at particular time point of treated animal.

Statistical analysis

The experimental results are expressed as mean ± standard error (SE) of five scorings. Statistical comparisons between different groups were made by conducting one-way ANOVA followed by Dunnett's test (Dunnett, 1955). Differences with $p < 0.5$ between the experimental groups and control group were considered statistically significant. All statistical analyses were performed by using the computer software SPSS 20.0 for Windows.

B. PHYTOCHEMICAL ASSAYS

The phytochemical research approach is considered effective in discovering bioactive profile of plants of therapeutic importance (Masih and Singh, 2012).

1. PREPARATION OF METHANOLIC EXTRACTS

One gram of the dried powdered drug of the roots was extracted using 25 ml methanol for 6 h in a Soxhlet extractor. The extract was then cooled, filtered and concentrated to dryness in a vacuum evaporator. The extract was then

dissolved in 10 ml methanol (HPLC Grade, Merck). It was then filtered through 0.20 mm membrane filter. This extract was used for the analysis.

2. PHYTOCHEMICAL ASSAYS OF METHANOLIC EXTRACTS

Methanolic root extracts of the source plants of 'Sahachara' were screened for the presence of various bioactive phytochemical compounds. Specific qualitative and quantitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods.

a. HIGH PERFORMACE THIN LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS

Standard preparation: 0.1 mg/ml standard lupeol solution was prepared in methanol (Analytical Grade; Merck (India) Limited).

HPTLC analysis of the extracts was performed on silica gel 60 F_{254} TLC plates (10 x 10 cm; Merck, Darmstadt, Germany). 4µl of the samples and standard lupeol were applied to the plates as sharp bands by means of CAMAG Linomat V sample applicator with a bandwidth of 6 mm. The distance between the tracks was 5 mm. Plates were developed in a TLC chamber previously saturated with the solvent system, toluene: ethyl acetate in 8:2 ratio. Development distance was 90 mm. The developed plate was then dried and derivatized using Anisaldehyde-Sulphuric acid (A-S) reagent. Detection was performed with a CAMAG TLC Scanner 3 at 366 nm. R_f values of major bands were determined. Using the spot data, paired affinity indices (PAI) were calculated using the formula:

$$\text{PAI} = \frac{\text{No. of spots common to A \& B}}{\text{Total no. of spots}} \text{ X } 100$$

b. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

The Gas chromatography – mass spectrometry (GC-MS) analyses were performed on a Varian model CP-3800 GC interfaced with a Varian Saturn 2200 Ion Trap Mass Spectrometer (ITMS) operating at 70eV and 250˚C, equipped with a CP-1177 Split/Splitless capillary injector and Combi PAL autosampler. A cross linked FactorFour capillary column, VF 5ms with 30 m x 0.25 mm ID and 0.25 µm film thickness was utilized. Helium was used as the carrier gas at a flow rate of 1 ml/min. Injection volume was 1µl. The split ratio was 1:20. The temperature programme for the chromatographic analysis was set at 60^{o}C for 1 min (initial) and then heated up at a rate of 3˚C/min to 280˚C. Run time was 40 min. Quantification was performed using percentage peak area calculations and identification of individual components was done using the NIST MS Search. The relative concentration of each compound in the methanolic extract was quantified based on the peak area integrated by the analysis programme.

c. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS) ANALYSIS

The experiment was carried out using an Agilent 1260 Infinity Bio-inert Quaternary LC equipped with an Agilent 6120 Series Quadrupole MS System with Agilent MassHunter Workstation software. The chromatographic separation was achieved on a reverse-phase Agilent Zorbax-C18 110 Ȝ analytical column (100 x 4.6 mm ID., 3.5 µm) operated at 40°C. Elution was achieved with a gradient mobile phase consisting of deionized water (A), methanol (B) and 0.05% formic acid (C) at a flow rate of 0.7 mL/min. The gradient system used was as follows: Mobile phase C was held constant throughout the run and a linear gradient of B from 0% to 4% from 5 to 10 min, increased to 9%–10% for 18 min, and then held at 49% for 7 min. From

25 to 28 min it was 89% and then from 28 to 32 min 99% and held at the same composition till 40 min. Mode of injection was ALS (Automatic liquid sampling). Electrospray ionization (ESI) was used as the interface and was operated in positive selected ion monitoring (SIM) mode. The analytes were monitored by tandem-mass spectrometry with positive electrospray ionization. The injection volume was 10 µl. The probe temperature was set at 500°C and needle voltage was set at 20 V. The cone voltage was set at 50 V for all Selected Ion Monitoring (SIM) scans.

d. DETERMINATION OF PHENOLICS AND FLAVONOIDS

Phenolic and flavonoid compounds are widely dispersed over the plant kingdom and are generally accepted as contributing towards the beneficial pharmacological properties of several plant species. They are proven helpful in preventing the deleterious consequences of oxidative stress by virtue of their manifold protective biochemical functions. The analysis of total phenolics and flavonoids of indigenous plant species would be helpful in identifying potent sources of such health promoting phytochemicals.

i. ESTIMATION OF TOTAL PHENOLIC CONTENT

Total phenolic content of the root extracts was determined using Folin–Ciocalteu reagent based assay as described by Oueslati *et al*. (2012) using gallic acid as a standard. An aliquot of diluted sample extract was added to 0.5 ml of distilled water and 0.125 ml of the Folin–Ciocalteu reagent (1 N). The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 ml of 7% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 3 ml and mixed thoroughly, and held for 90 min at ambient temperature in dark. After incubation, the absorbance at 760 nm was recorded against blank, *i.e.*, distilled water. Total phenolic content of the extracts was expressed as milligrams of gallic acid equivalents per gram of dry weight

(mg GAE/g DW) of the extract through the calibration curve with gallic acid. All samples were analysed in triplicates.

ii. ESTIMATION OF TOTAL FLAVONOID CONTENT

The aluminium chloride colorimetric method with some modifications was used to measure the total flavonoid content of all plant extracts (Oueslati *et al*., 2012). Quercetin was used as the standard for estimation of total flavonoids. An aliquot of diluted sample or standard solution of quercetin was added to 75 µl of $NaNO_2$ solution (7%), and mixed for 6 min, before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml of 1 M NaOH solution was added. The final volume was adjusted to 2.5 ml, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm against a reagent blank of methanol. Total flavonoid content was expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW), through the calibration curve of quercetin. All samples were analysed in triplicates.

RESULTS

The current study, which is designed to carry out the cytogenetic analysis, antioxidant and anti-inflammatory assays as well as phytochemical characterization of the six taxa of Acanthaceae (Plate 1) used as the source plants of an important Ayurvedic drug 'Sahachara', brought to light the following cytogenetical and phytochemical findings.

A. CYTOGENETICAL ANALYSIS

In the present investigation, cytogenetical analysis comprised karyomorphological analysis together with the antioxidant and anti-inflammatory assays. Karyomorphological analysis revealed the existence of distinct cytotypes in all the taxa as revealed by their specific karyomorphometrical details (Plates 2-7; Tables 3-9).

Antioxidant assays revealed the specific mechanism of action of the root methanolic extracts of the six taxa used as the source plants of 'Sahachara' in preventing the oxidation of cellular and genetic components (Figs 7-11; Tables 10-15).

Anti-inflammatory assays brought to light the specific ability of the root methanolic extracts of these six members of Acanthaceae in effecting the cessation of inflammation and thereby checking/limiting cellular and genetic damage in the test organism (Figs 12-23; Tables 16-27).

a. KARYOMORPHOLOGICAL ANALYSIS

Analysis of the chromosome constitution of the six taxa of Acanthaceae used as the source plants of the drug 'Sahachara' was performed by mitotic squash preparations. The root tip meristems were examined for this purpose. The present study revealed a detailed karyomorphological analysis of

the plants investigated. Chromosome numbers of *Ecbolium viride* and *Strobilanthes ciliatus* are reported for the first time. Though chromosome numbers have already been reported in the rest of the species, karyomorphometrical data has been rather lacking in most of them. Results were confirmed by screening several samples collected from different locations and subsequently grown in the net house of Department of Botany, University of Calicut.

The observed somatic chromosome numbers of the plants were found to range from 2n = 28 to 2n = 40 and are as follows. Chromosome complement analysis revealed 2n = 40 each for *Barleria cristata* (Plate 2) and *B. prionitis* (Plate 3), 2n = 36 in *Ecbolium viride* (Plate 4), 2n = 34 in *Justicia betonica* (Plate 5), 2n = 28 in *Strobilanthes ciliatus* (Plate 6) and 2n = 32 in *S. heyneanus* (Plate7). Except those of *B. cristata* and *B. prionitis*, the karyotypes of all others were characterized by the presence of comparatively smaller chromosomes. All the plants possessed a majority of chromosomes with nearly median primary constriction. Very few chromosomes could be observed with nearly submedian primary constriction (Tables 3-8). The karyotype of *E. viride* showed only chromosomes with nearly median primary constriction. The chromosomes with secondary constriction were found to range from 4-6 in the different plants (Table 9).

The cytological analysis of the plants revealed that the total chromosome length of different plants investigated ranged from 138.9312 µm to 22.9642 µm. The highest value of average chromosome length noted in the study is 3.4733 µm for *B. cristata* and the lowest value is 0.6754 µm found in *J. betonica*. The chromosome length varied from 4.9287 µm to 0.4814 µm. the disparity index ranges from 38.77 to 26.89, the variation coefficient from 22.77 to 17.71 and the total forma percentage from 44.52 to 41.49 (Table 9).

The detailed karyotypic descriptions of chromosome length and other karyotypic features among the different plants are shown in Tables 3-8. Summarized data of the karyomorphometric features is given in Table 9. Photomicrographs, karyograms and idiograms of mitotic metaphase stages of the six taxa are also given (Plates 2-7).

b. ANTIOXIDANT AND ANTI-INFLAMMATORY ASSAYS

i. ANTIOXIDANT ASSAYS

The antioxidant activities of the crude methanolic extracts of roots of the six taxa of Acanthaceae, used as the source plants of the drug 'Sahachara' in Ayurveda were determined by five different *in vitro* assays. The extracts were evaluated for their free radical scavenging activities against DPPH radical, ABTS radical, superoxide radical, hydroxyl radical and inhibition of lipid peroxidation. The methanolic extracts showed potent antioxidant activity at concentrations ranging from 20-140 µg/ml (Figs 7, 9-11; Tables 11, 13-15). In the case of ABTS radical scavenging assay, potent activity was shown at concentrations ranging from 10-70 µg/ml (Fig. 8; Table 12). Analysis was performed in triplicate and the IC_{50} values of the different extracts were calculated.

DPPH radical scavenging activity

Being a stable organic free radical, DPPH is frequently used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form (Blois, 1958). Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. In the present study, all extracts were found to be effective scavengers against DPPH radical and their activities increased in a

concentration dependent manner (Fig. 7; Table 11). *Barleria prionitis* was found to be the most efficient scavenger of DPPH radical, with an inhibition of 47.70 ± 0.25% at the concentration of 20 µg/ml (Table 11). It also revealed an IC_{50} of 31.06 ± 1.28 µg/ml, followed by *B. cristata* (IC_{50} = 34.46 ± 0.89 µg/ml) (Table 10). The IC_{50} values for the inhibition of DPPH radicals calculated for the different extracts are as follows, *S. ciliatus* (37.38 ± 1.11 µg/ml), *E. viride* (74.90 ± 1.62 µg/ml), *J. betonica* (91.57 ± 1.39 ug/ml) and *S. heyneanus* (103.92 ± 1.55 µg/ml).

ABTS radical scavenging activity

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating and chain-breaking antioxidants (Leong and Shui, 2002). Methanolic extracts of six taxa of Acanthaceae studied exhibited excellent scavenging activity against ABTS radicals generated in the system (Fig. 8; Table 12), that increased in a dose-dependent manner. *B. prionitis* displayed high ABTS radical scavenging activity with an inhibition of 45.86 ± 0.74% at a concentration of 10 µg/ml (Table 12) and shows an IC_{50} value of 12.89 ± 3.46 µg/ml (Table 10). The lowest activity was shown by *J. betonica* with an IC_{50} value of 86.20 ± 2.11 µg/ml. The antioxidant potentialities of the other plants also was quite good with IC_{50} values of 13.99 ± 2.91, 18.65 ± 2.95, 57.30 ± 2.91 and 71.58 ± 1.28 µg/ml respectively for *B. cristata*, *S. ciliatus*, *E. viride* and *S. heyneanus*.

Superoxide radical scavenging activity

All the extracts showed effective scavenging capacities for the superoxide radicals generated by the riboflavin photoreduction method that showed positive correlation with concentration. The percentage inhibition of the various extracts at different concentrations is summarised in Fig. 9 and Table 13. The activity showed positive correlation with concentration.

The highest inhibition of 34.16 ± 0.95% at a concentration of 20 µg/ml was shown by the extract of *B. cristata* (Table 13). Among the various extracts, *B. prionitis* and *B. cristata* were better radical scavengers (IC_{50} = 44.54 ± 2.90 and 55.23 ± 1.81 µg/ml respectively) (Table 10). The IC_{50} values for the other extracts are *S. ciliatus* (61.76 ± 2.68 µg/ml), *E. viride* (81.51 ± 2.38 µg/ml), *S. heyneanus* (84.17 ± 1.77 µg/ml) and *J. betonica* (110.34 ± 1.72 µg/ml).

Hydroxyl radical scavenging activity

All the extracts exhibited significant inhibitory effects on degradation of deoxyribose induced by hydroxyl radicals generated by Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fig. 10; Table 14) in a dose dependent manner. *Strobilanthes ciliatus* was the most efficient scavenger, with an inhibition of up to 40.77 ± 1.06% at a concentration of 20 µg/ml (IC_{50} = 54.58 ± 0.94 µg/ml). The least active was *J. betonica*, which could cause only 9.53 ± 0.70% inhibition at the same concentration (IC_{50} = 117.49 ± 1.30 µg/ml). *B. prionitis* and *B. cristata* were also found to be fairly effective with IC_{50} values of 70.10 ± 1.73 and 74.84 ± 2.13 µg/ml respectively (Table 10). The IC_{50} values for *S. heyneanus* and *E. viride* were reported to be 105.58 ± 2.60 and 108.3 ± 0.94 µg/ml respectively, showing moderate activity. As in most of the other assays, the minimum activity was shown by *J. betonica* with an IC_{50} value of 117.49 ± 1.30 µg/ml.

Inhibition of lipid peroxidation

The root methanolic extracts of all the six taxa caused significant inhibition of lipid peroxidation induced by Fe^{2+} in rat liver homogenate (Fig. 11; Table 15). Among the plants studied, *B. cristata* and *B. prionitis* showed promising results when compared to others. At the dose of 20 µg/ml, the highest inhibition of lipid peroxidation (33.77 ± 0.56%) was shown by *B. cristata* (IC_{50} = 55.59 ± 1.44 ug/ml) which was followed by *B. prionitis*

(IC_{50} = 69.12 ± 3.04 µg/ml). Scavenging activities of *E. viride*, *S. ciliatus* and *S. heyneanus* are more or less comparable with IC_{50} values of 72.80 ± 1.71 ug/ml, 72.82 ± 1.55 ug/ml and 73.85 ± 1.18 ug/ml respectively. *J. betonica* (IC_{50} = 95.76 ± 1.11 ug/ml) was found to be the least active one (Table 10).

Results obtained from the antioxidant assays clearly prove that though all the six taxa are found to be having positive effects, promising results are shown by *B. prionitis*, *B. cristata*, *S. ciliatus* and *E. viride*. The IC_{50} values of *S. heyneanus* and *J. betonica* appear to be considerably high when compared to the others, suggesting lower antioxidant potential.

ii. ANTI-INFLAMMATORY ASSAYS

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat/mouse after injection of a phlogistic agent. The anti-inflammatory activititiy of the crude methanolic extracts of roots of the six taxa of Acanthaceae, used as the source plants of the drug 'Sahachara' in Ayurveda has been determined by two *in vivo* models of inflammation, *viz*., carrageenan induced (acute) and formalin induced (chronic) paw edema in Swiss Albino mice. Treatment groups such as solvent control and positive control included in both the assays were common for all the six plants investigated. A statistically significant reduction in the edema volume was shown by all the six taxa of Acanthaceae in carrageenan as well as formalin induced paw edema models (Figs 12-23; Tables 16-27).

Carrageenan induced acute inflammation

Carrageenan-induced rat paw is the most suitable experimental model to evaluate the anti-edematous effect of natural products (Perianayagam *et al.*, 2006). The sub-plantar injection of carrageenan into the mice hind paw

elicited a local inflammatory response that was maximal at the 3rd h after carrageenan injection. The paw edema was found to decrease then and was quantified by measuring changes in thickness of footpad.

A notable increase in paw edema volume was observed in animals in the control group from 1st to 5th h, with a maximum edema paw volume of 3.71 ± 0.12 mm observed in the control mice, 3 h after the carrageenan injection while the diclofenac treated (10 mg/kg b. wt p.o.) groups showed significant ($p < 0.001$) inhibition of paw edema throughout the period of study with a maximum inhibition of 70.81% at the 3rd h (Figs 12, 14, 16, 18, 20, 22; Tables 16, 18, 20, 22, 24, 26). The swelling of paw was nearly completely reduced in diclofenac administered mice during the 5th h.

Oral administration of animals with root methanolic extracts of all the six taxa at doses of 100 and 250 mg/kg b. wt caused potent inhibition of carrageenan induced inflammation right from the 1st h to 5th h. The extracts of *B. prionitis* (Table 18) and *S. ciliatus* (Table 24) at the dose of 250 mg/kg b. wt were found to cause significant reduction in edema, after 3rd and 4th h of carrageenan injection. The lower dose of these extracts (100 mg/kg b. wt) could also bring about a considerable decrease in paw swelling. The extracts of *B. cristata* and *E. viride* at the dose of 250 mg/kg b. wt induced moderate inhibition of paw edema (Tables 16, 20).

In the control group, the paw thickness increased by 1.37 ± 0.08 mm at the 3 h after the injection of carrageenan. The inflammatory response to sub-plantar carrageenan injection was significantly reduced especially by *B. prionitis*, *S. ciliatus* and *E. viride* extracts at doses of 100 mg/kg and 250 mg/kg b. wt given orally 1 h prior to carrageenan, but the time course of their anti-edema effects varied slightly. As the control animals showed a maximum difference in paw thickness at the 3rd h, the inhibition of edema response was most marked at the 3rd h. Compared to the control animals, significant

reduction in paw swelling was evident in all the treated groups that was notable right from the 2nd h of injection.

The anti-inflammatory effects of higher doses of the extracts of *B. prionitis* and *S. ciliatus* were comparable to that of the standard group administered with diclofenac (10 mg/kg b. wt) as a non-steroidal anti-inflammatory drug (70.81%) ($p < 0.001$). The percentage inhibition of inflammation for *B. prionitis* was 62.77% ($p < 0.001$) for 100 mg/kg and 72.26% ($p < 0.001$) for the dose of 250 mg/kg b. wt) (Fig. 14; Table 18) and that for *S. ciliatus* was 55.47% ($p < 0.01$) and 72.99% ($p < 0.001$) for 100mg/kg and 250 mg/kg b. wt (Fig. 20; Table 24) respectively. The marked decrease in paw edema volume caused by the high dose (250 mg/kg b. wt) treatment of both these extracts was even greater than that observed for the diclofenac treated group.

Though comparatively lesser, the anti-inflammatory effects of the other plants were also commendable. The extract of *E. viride* brought about marked inhibition of edema that was practically highly significant when compared to the control. Treatment with *E. viride* extract at doses of 100 mg/kg and 250 mg/kg b. wt resulted in considerable inhibition of paw swelling, *i.e.*, 49.64% ($p < 0.01$) and 63.50% ($p < 0.001$) respectively (Fig. 16; Table 20). The methanolic extract of *B. cristata* also showed good inhibitory effect that was moderately significant. The extract could bring about a reduction in inflammatory response at the doses of 100 mg/kg and 250 mg/kg b. wt with the percentages of inhibition of 49.63% ($p < 0.01$) and 54.64% ($p < 0.001$) respectively at the 3rd h following carrageenan injection (Fig. 12; Table 16). The inhibitory effects of *S. heyneanus* closely follow that of *B. cristata* with 37.22% ($p < 0.01$) and 53.28% ($p < 0.01$) inhibition of the paw edema at the doses of 100 mg/kg and 250 mg/kg b. wt respectively (Fig. 22; Table 26).

The least reduction in edema and so the least anti-inflammatory activity was shown by *J. betonica* extract. The percentage of inhibition produced by the lower dose of the extract was not statistically significant (34.31%), whereas the higher dose could bring about a considerable decrease (45.25%; $p < 0.01$) in the volume of paw edema (Fig. 18; Table 22).

Formalin induced chronic inflammation

Formalin-induced paw edema is one of the most effective and widely used experimental models for assessing chronic inflammation. The sub-plantar injection of formalin in Swiss albino mice produced profound inflammation (swelling and erythema) in the hind paw of mice and was quantified by measuring changes in thickness of footpad consecutively for six days. The effect of the various extracts on formalin induced inflammation was expressed as percentage inhibition of the extracts in the control, standard and different treated groups calculated on the 6^{th} day.

In the formalin induced paw edema test for chronic inflammation, paw edema was induced by formalin injection on the first day and the extracts were administered orally at doses of 100 mg/kg and 250 mg/kg b. wt daily for six days. As a consequence of the inflammatory response, the paw edema volume was increased in control animals, the intensity of which was consistent throughout the period of study. The difference in paw edema volume in the control group on the 6^{th} day was 1.83 ± 0.06 mm, whereas in the standard group, *i.e.*, the diclofenac treated group, the difference in edema volume was much lesser (0.86 ± 0.06 mm; $p < 0.001$) displaying 53.01% of inhibition (Figs 13, 15, 17, 19, 21, 23; Tables 17, 19, 21, 23, 25, 27). On the 6^{th} day there was significant ($p < 0.001$) reduction in paw edema of diclofenac (10 mg/kg b. wt) treated animals in comparison to the untreated control group.

The present investigation demonstrates that significant inhibition of paw edema volume upon the administration of all the six extracts in a dose and time dependent manner. The results obtained from the formalin induced paw edema analysis of the methanolic extracts of six taxa of Acanthaceae were in tune with that obtained from the carrageenan induced acute paw edema test. The anti-inflammatory effects of both the doses (100 and 250 mg/kg b. wt) of most of the extracts started 2-3 days after formalin injection.

The high percentage inhibition of paw swelling recorded in mice pre-treated with the extracts of *B. prionitis* and *S. ciliatus* clearly demonstrates that these two plants are highly potent in modulating the various inflammatory responses in formalin induced edema as well. The percentage reduction in paw edema shows significant differences in the groups treated with both the low and high doses, *i.e*., 100 and 250 mg/kg b. wt of these plant extracts when compared with the control group. At doses of 100 and 250 mg/kg b. wt, *B. prionitis* produced 39.34% ($p < 0.01$) and 51.91% ($p < 0.001$) inhibition on the 6^{th} day. (Fig. 15; Table 19). The mice treated with extract of *S. ciliatus* too showed notable reduction in paw edema induced by formalin with percentage inhibition 37.70% ($p < 0.01$) and 49.73% ($p < 0.001$) respectively at the doses 100 and 250 mg/kg b. wt, on the 6^{th} day (Fig. 21; Table 25).

Similar to the carrageenan induced paw edema test, the anti-inflammtory activites against formalin induced paw edema of *B. prionitis* and *S. ciliatus* were followed by *B. cristata* and *E. viride* extracts, which registered moderate inhibitory effects. *B. cristata* extract at the doses of 100 mg/kg and 250 mg/kg significantly reduced the paw thickness by 31.14% ($p < 0.05$) and 45.90% ($p < 0.001$) when compared with the control group (Fig. 13; Table 17). The extract of *E. viride* also could decrease formalin induced inflammation to a great extent with percentage inhibition of 32.24% ($p <$

0.01%) and 43.17% ($p < 0.001$) respectively in 100 mg/kg and 250 mg/kg treated groups (Fig. 17; Table 21).

Though moderately active, the anti-inflammatory activities of *S. heyneanus* and *J. betonica* were lesser when compared to that of the other plants. *S. heyneanus* showed statistically significant reduction in paw thickness by 30.05% ($p < 0.05$) and 39.34% ($p < 0.01$) respectively for the low and high doses of treatments (Fig. 23; Table 27). The group that was subjected to low dose treatment with *J. betonica* showed no significant difference in the anti-inflammatory activity (23.50%) when compared with the control. The higher concentration of the extract (250 mg/kg b. wt) showed 34.43% ($p < 0.01$) reduction in paw thickness (Fig. 19; Table 23).

The results of the present study clearly illustrate that Swiss Albino mice treated with methanolic extracts of *B. prionitis*, *S. ciliatus*, *E. viride* and *B. cristata* had promising results in anti-inflammatory assays. The effects of *B. prionitis* and *S. ciliatus* extracts are more pronounced and were comparable to the standard drug, diclofenac. Both the species were found to be very potent in inhibiting inflammation as the mice pretreated with the lower as well as the higher doses of these extracts showed a very negligible increase in paw edema after the injection of the respective phlogistic agents in both acute and chronic models of inflammation. The maximum anti-inflammatory effect was seen in mice administered with *S. ciliatus* extract (250 mg/kg b. wt) on carrageenan induced paw edema test and in mice administered with *B. prionitis* extract (250 mg/kg b. wt) on formalin induced paw edema test. In both anti-inflammatory tests, the lowest activity was observed in *J. betonica* (100 mg/kg b. wt).

B. PHYTOCHEMICAL ASSAYS

a. HIGH PERFORMACE THIN LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS

The HPTLC studies on the methanolic extracts of species used under the name 'Sahachara' in Ayurveda, using lupeol as the marker compound revealed major significant spots which appeared at about R_f 0.63, 0.48 and 0.38 and other less evident ones (Plates 8-11; Table 28). The HPTLC photographic plate represents the detailed chemical profile of these six plants (Plates 8-10). The 3D space visualisation of the chromatograms of the six extracts and the lupeol marker is displayed in Plate 11. The peak densitogram display of standard lupeol (Fig. 24) and those of each extract showing the peak of lupeol are also given (Figs 25a-c).

HPTLC studies indicated relatively good amount of variability among the different species that are used as 'Sahachara', with regard to spot number and pattern. Out of the six taxa, *J. betonica* had the maximum number of spots *i.e*., 13, while *B. cristata* and *S. heyneanus* had the miniumum number, 8. A unique fingerprint profile with specific as well as distinguishing bands has been revealed for each of the species, which is distinctly different from those of the others. By comparing the spot data, an apparent idea about the relative similarity and differences between the different tracks in the HPTLC profile also has been made. The data of the HPTLC fingerprint patterns of the different species are presented in Table 28. The profiles generated in HPTLC analysis at 366 nm before derivatization, after derivatization with A-S reagent at daylight and 366 nm are given in Plates 8, 9 and 10 respectively.

Lupeol is a pharmacologically active triterpenoid with immense anti-inflammatory properties and is reported to possess beneficial effects against a range of disorders (Saleem, 2009). Several plants used in folk medicine to treat inflammatory diseases have been shown to contain lupeol as one of their

active principles (Gallo and Sarachine, 2009). The presence of lupeol has been previously reported from *S. ciliatus* and *B. prionitis* and is regarded as one of the active ingredients of the above plants (Venkatachalapathi and Ravi, 2012; Kosmulalage *et al.*, 2007). Hence in the present study, lupeol was used as the phytochemical marker for HPTLC profiling. A phytochemical marker is any chemical constituent in a medicinal plant that can be used to verify its potency or identity. Standard lupeol is run along with the samples to evaluate its presence in the different extracts. The results of the HPTLC analysis revealed the presence of lupeol at R_f 0.63 after spraying with anisaldehyde sulphuric acid reagent and viewing in day light and at UV 366 nm (Fig. 24). The identity of the peak of lupeol in the sample solutions was confirmed by comparing the retention factor (R_f) value of the samples with that of the standard solution of lupeol having the same R_f (Figs 25a-c). The methanolic extracts of all the taxa showed the presence of lupeol. The HPTLC fingerprint reveals that the relative intensity of the band corresponding to standard lupeol varies in the different plants. The difference in the size of the lupeol peak is very evident in the individual chromatograms of different extracts given in Figs 25a-c. The most prominent peak was found in the chromatogram of *S. ciliatus*. The chromatogram of *B. prionitis* also showed rather intense peak for lupeol. The size of the peak was found to be the minimum in *J. betonica*.

The data from the HPTLC studies of the root methanolic extracts was used to calculate the paired affinity indices (PAI) for the different species. The PAI values represent the percentage chemical similarity between any two species. The PAI between different species are given in Table 29. The results obtained from the HPTLC analysis showed that there is only moderate similarity between the different species that are used as 'Sahachara'. Maxiumum similarity of the spots was found between *B. cristata* and *B. prionitis* as well as between *S. ciliatus* and *S. heyneanus* (70%). *E. viride*

and *J. betonica* share the least similarity among the different extracts (19.04%).

b. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

The chemical compositions of root methanolic extracts of six taxa of Acanthaceae used as the source plants of the Ayurvedic drug 'Sahachara' were revealed by GC-MS. A wide spectrum of chemical compounds was detected in the methanolic extracts from the roots of these plants. The components identified by GC-MS analysis with their percentage composition are listed (Table 30). The gas chromatograms of the different extracts are shown in Figs 26-31 and the mass spectra of the compounds identified in the GC-MS analysis are shown in Figs 32 (i-xvi). The total number of chemical components detected by GC-MS was found to be 63 representing about 91.14-99.18% of the methanolic extracts. The compounds from the six methanolic extracts could be assigned to seven different classes: phenols (9.49-60.35%), terpenoids (23.27-58.50%), polyphenols (0.00-18.32%), phytosterols (0.52-12.50%), fatty acid esters (0.00-16.89%), coumarins (0.00-7.75%), Furans (0.00-11.58%) and other miscellaneous compounds (1.40-14.53%) (Table 31).

Sixteen compounds were detected from the methanolic extract of *B. cristata* corresponding to 94.94% of total extract. The major components identified were estragole (51.10%), β-eudesmol (7.41%), lupeol (6.34%), canthaxanthin (5.08%), desaspidinol (4.72%) and squalene (4.14%). The methanolic extract of *B. prionitis* showed nineteen compounds accounting for 98.1% of the total extract with desaspidinol (35.20%), hydrocinnamic acid (13.66%), lupeol (6.44%), 5-quinolinol (4.95), lupenone (4.57%) and betulinic acid (3.95%) as the main constituents. Twenty four compounds were detected from the root extract of *E. viride* corresponding to 91.14% of

the total (Table 30). The extract contained lupeyl acetate (18.4%), isoeugenyl acetate (9.12%), α-spinasterol (8.69%), lupeol (5.73%), τ-cadinol (5.53%), estragole (4.79%) and betulinic acid (4.76%) as the major components (Table 30).

The root methanolic extract of *J. betonica* shows 28 compounds accounting for 97.44% of the total extract with 5-hydroxy methyl furfural (11.58%), lupeyl acetate (8.25%), 2-tert-Butyl-4-methoxyphenol (7.60%), geranyl linalool (6.62%), isoeugenyl acetate (4.63%), 2-Acetyl-4-methylphenol (4.15%) as its main constituents. Sixteen compounds amounting to 99.18% were identified from the root methanolic extract of *S. ciliatus*. The main constituents in the extract were lupeol (38.70%), syringol (26.61%), phytol (5.33%), desaspidinol (3.76%), betulinic acid (3.66%), sclareol (3.42%), α-spinasterol (3.42%), lupeyl acetate (3.31%) and β-sitosterol (3.15%). In *S. heyneanus*, twenty six compounds were identified corresponding to 95.82% of the total extract. The extract contained syringol (15.34%), lupeol (12.25%), α-spinasterol (10.39%), 4-methyl-2-tert-octylphenol (7.12%) and 3-methoxycatechol (4.35%) as the major components (Table 30).

The composition analysis of methanolic extracts of the six taxa of Acanthaceae by GC-MS revealed that phenols predominated in *B. cristata, B. prionitis* and *S. heyneanus* while terpenoids form the major fraction in *E. viride*, *J. betonica* and *S. ciliatus*. Though terpenoids predominate in *S. ciliatus*, phenols are also abundantly present. Substantial amounts of terpenoids were found in *B. cristata*, *B. prionitis* and *S. heyneanus* also. Polyphenoles have been detected in all the extracts except *E. viride*, the maximum content of polyphenols was shown by *B. prionitis.* Fatty acid esters are reported from *B. cristata*, *B. prionitis*, *E. viride*, *J. betonica* and *S. heyneanus*. All the extracts showed the presence of phytosterols in varying

amounts. Moderate to lesser amounts of other two classes of compounds, *viz.*, coumarins and furans have also been seen in different plants (Table 31).

c. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS) ANALYSIS

The chemical constitutions of the methanolic extracts of six species of Acanthaceae used as the source plants of the drug 'Sahachara' in Ayurveda revealed by LC-MS are presented in Tables 32-37. The respective liquid chromatograms of the different samples are shown in Figs 33-38. A wide spectrum of phytochemicals was detected in the methanolic extracts obtained from the roots of these plants. The compounds from the methanolic extracts could be assigned to six general classes of compounds: phenolics, terpenoids, flavonoids, fatty acids, esters, steroids and others.

The LC-MS analysis of the methanolic extract of *B. cristata* revealed seventeen compounds, out of which phenolic compounds predominate (Table 32). Flavonoids and fatty acids are also present in considerable amounts. The major components identified are methyleugenol (25.16%), arachidonic acid (10.81%), quercetin (10.75%), coumaric acid (10.42%), farnesyl butanoate (5.54%), β-sitosterol (5.23%), cinnamic acid (5.10%) and luteone (4.18%) (Table 32). The LC-MS analysis of *B. prionitis* shows fifteen compounds (Table 33) with a high proportion of phenolics, flavonoids and terpenoids. Among these catechin (20.67%) and betulinic acid (20.14%) are the main constituents. The other active ingredients were barlerin (13.07%), β-sitosterol (10.42%), α-tocopherol (7.92%) and lupeol (4.93%) (Table 33). Seventeen compounds were detected from the methanolic extract of *E. viride*. Phenolic compounds constitute the principal category of components in *E. viride*. The other classes of compounds like flavonoids, terpenoids and fatty acids are also seen in lesser amounts. The major constituents are coumaric acid (11.79%), wogonin (11.52%), myricetin (11.07%), catechin (8.92%), ascorbic acid

(7.73%) and linoleic acid (6.90%). Stigmasterol (4.79%), cinnamic acid (4.42%) and hydronaphthoquinone (4.15%) are also present in significant quantities (Table 34).

The methanolic extract of *J. betonica* revealed twenty two compounds of which terpenoids and steroids are the major classes of compounds. The main constituents identified are spinasterol (23.62%), ursolic acid (22.93%), cannabidiol (8.88%), betulinic acid (6.26%), sinapine (6.06%) and coumaric acid (4.78%) (Table 35). The LC-MS analysis of the methanolic extract of *S. ciliatus* revealed thirteen compounds of which terpenoids, phenolics, flavonoids and steroids are present in higher amounts. The chief constituents are lupeol (15.35%), asiatic acid (15.12%), coumaric acid (10.42%), cannabidiol (9.59%), ethyl-isoallocholate (8.27%) and sinapine (6.97%) (Table 36). Fifteen compounds were identified from the methanolic extract of *S. heyneanus*. Terpenoids form the major class of compounds. The main constituents include asiatic acid (24.61%), ursolic acid (19.10%), quercetin (7.74%), arachidonic acid (7.61%), cinnamic acid (6.59) and coumaric acid (6.41%) (Table 37).

d. DETERMINATION OF PHENOLICS AND FLAVONOIDS

Methanolic extracts of six species of Acanthaceae that are used as the source plants of the Ayurvedic drug 'Sahachara' were examined for their total phenolic and flavonoid content (TPC and TFC respectively).

TPC of extracts and ethyl acetate fractions was measured by Folin-Ciocalteu (F-C) reagent in terms of gallic acid equivalent (GAE) (standard curve equation: $y = 0.03x - 0.08$, $R^2 = 1$; Fig. 39). A linear calibration curve of gallic acid, in the range of 10-30 µg/ml with coefficient of determination (R^2) value of 1.00, was obtained (Fig. 39). The TPC values of the various extracts ranged from 3.14 ± 0.22 to 22.64 ± 1.93 mg GAE/g DW of the extract (Table

38). The methanolic extract of *B. prionitis* revealed the highest total phenolic content 22.64 ± 1.93 mg GAE/g DW, approximately 8 fold more than the methanol extract of *J. betonica* with 3.14 ± 0.22 mg GAE/g DW. The TPC of the methanolic extract of *B. cristata* (19.34 ± 0.40 mg GAE/g DW) was also quiet high, indicating considerable amount of phenolics. The TPC of *S. ciliatus* (15.91 ± 0.70 mg GAE/g DW) and that of *E. viride* (15.16 ± 1.28 mg GAE/g DW) were comparable as there was no significant difference ($P > 0.05$) between the two. The extract of *S. heyneanus* also displayed good TPC value of 10.80 ± 0.60 mg GAE/g DW.

The total flavonoid contents, that were measured by aluminium chloride colorimetric technique in terms of quercetin equivalent (QE) (the standard curve equation: $y = 0.004x + 0.017$, $R^2 = 0.991$; Fig. 40). The result showed that *S. ciliatus* had the highest TFC value among the samples (6.36 ± 0.46 QE/g DW) (Table 38). Total flavonoids in *B. prionitis* extract was also quite high (5.84 ± 0.26 mg QE/g DW) and is comparable to that of *S. ciliatus* with no significant difference ($p < 0.05$). The flavonoid content of the other plant extracts in the descending order were 3.80 ± 0.31, 3.72 ± 0.21, 3.63 ± 0.11 and 1.89 ± 0.30 mg QE/g DW respectively for *B. cristata*, *E. viride*, *S. heyneanus* and *J. betonica* (Table 38). The flavonoid contents of the first three plants are more or less comparable ($p < 0.05$; Tukey–Kramer HSD test).

DISCUSSION

The present work includes an attempt to study the chromosome constitution of six taxa of Acanthaceae (Plate 1) that are used as the source plants of a well known Ayurvedic drug 'Sahachara', commonly used against inflammatory disorders and the antioxidant and anti-inflammatory analyses of their root methanolic extracts. Efforts have also been done to identify the phytochemical constituents that are responsible for the biological activities observed. The study also envisaged development of chemical fingerprints of the taxa through HPTLC, GC-MS and LC-MS, through by which the taxa could be easily identified and discriminated from one another. The results obtained were analyzed and are discussed hereunder.

A. CYTOGENETICAL ANALYSIS

In angiosperms, the species of several families, both dicotyledons and monocotyledons are found to exhibit a direct relationship between their phylogeny and cytogenetical constitution. The chromosomes being the carriers of heredity, both structural and numerical changes in them can influence the genetic-evolutionary process. A detailed cytogenetical analysis can serve as a useful tool to understand systematic relationships and for tracing the direction of evolution (Love and Love, 1975).

Some of the major karyotype characteristics of considerable evolutionary and taxonomic significance are (1) differences in the absolute chromosome size, (2) differences in the position of the centromere, (3) differences in total chromatin length (4) differences in karyotype formula and (5) number as well as position of satellites.

a. KARYOMORPHOLOGICAL ANALYSIS

Detailed chromosomal analyses were conducted in *Barleria cristata*, *B. prionitis*, *E. viride*, *J. betonica*, *Strobilanthes ciliatus* and *S. heyneanus*. Chromosomal survey, which primarily is the determination of chromosome number and meiotic behaviour, is an important step in understanding the cytogenetic constitution of species, relationship among taxa and to provide a base for the future improvement programmes (Gill and Singhal, 1998). Chromosome number counts have provided indispensable information on genetic discontinuities within and among species (Semple *et al*., 1989). As intraspecific cytological variations can occur in plant species (Ebert *et al*., 1996; Murray and Young, 2001), cytological attributes need to be taken into account when conservation strategies are planned for restoration of depleted populations (Murray and Young, 2001). Detailed karyotype information has shown to yield useful and dependable data for tracing the direction of karyotype variation and for assessing the nature and magnitude of chromosome structural alterations within and between species in plant families (Sreekumari and Mathew, 1992).

In the present investigation, chromosome numbers determined for the different plants are 2n = 40 (*B. cristata* and *B. prionitis*), 2n = 28 (*S. ciliatus*), 2n = 32 (*S. heyneanus*), 2n = 36 (*E. viride*) and 2n = 34 (*J. betonica*) (Plates 2-7). The details of karyotypes of the taxa examined are summarised in Tables 3-8. The respective karyograms and idiograms are given in Plates 2-7. The mitotic and meiotic chromosome counts of *B. cristata*, *B. prionitis* and *J. betonica* and the meiotic chromosome number of *S. heyneanus* have been reported by earlier workers. However, most of the earlier cytological studies were confined to mere chromosome counts with only scanty information on karyomorphology. The results of the present study confirm the findings of the earlier reports and represent a detailed karyomorphological investigation of

the plants pertaining to chromosome length and other karyomorphometric attributes.

When examining the chromosomes of Acanthaceae their small size and the diversity of their numbers among taxa are readily apparent (Grant, 1955). The small size of somatic chromosomes generally made studies of chromosome morphology of Acanthaceae rather impractical. According to Daniel *et al*. (1990), the somatic chromosomes of Acanthaceae are often clumped, making accurate chromosome number determinations difficult. In the present study, the small size of chromosomes was clearly noticeable in the karyotypes of *J. betonica*, *E. viride*, *S. ciliatus* and *S. heyneanus*.

Most of the chromosomes observed in the study were rod shaped, apparently with narrow constrictions. The size of the chromosomes showed gradual reduction from the largest one to the smallest one in all the plants investigated (Plates 2-7). Data with respect to the length of chromosomes, short arm and long arm lengths was accurately measured through analysis of the digitalized image. Average chromosome length (ACL) and total chromosome length (TCL) were found to be much higher in *B. prionitis* and *B. cristata*, when compared to the other taxa. The differences in the chromosome length and volume may be attributed to different content of protein and DNA, along with differential spiralization and condensation of chromosomes. Chromosomal differences reflect the fundamental changes in morphological, physiological and biological characters that result from different gene action and expression (Sharma and Sen, 2002). The alteration of a chromosome segment may affect several genes and thereby leads to several sets of phenotypic characteristics (Zhao *et al*., 2005).

Forma percentage or centromeric index is used to classify chromosomes based on centromere. Derived parameters like disparity index (DI) and coefficient of variation (VC) are used to trace the symmetry of the

karyotype. The size variations of the chromosomes are reflected in disparity indices (DI). Higher the variation in the size of the chromosomes, disparity index tends to achieve high values. Variation coefficient reflects the distribution of chromosomes of different size around a mean value. High variation coefficient therefore reflects the heterogeneity of the karyotype. These derived parameters along with other quantitative data are described in detail for all the plants investigated (Tables 3-8).

Though the six members of Acanthaceae studied exhibit differences in karyomorphometric parameters, the predominance of chromosomes with nearly median centromeres and the higher values of TF% suggest that the karyotypes are cytologically more primitive than advanced. Among the six taxa studied, the karyotypes of *B. cristata* and *B. prionitis* seem to be comparatively more advanced because of their heterogenous chromosome complements and fairly high variation coefficient (VC) values. But the large sized chromosome is a rather primitive character. The karyotypes of *S. ciliatus*, *S. heyneanus*, *E. viride* and *J. betonica* are characterized by the small size of the chromosomes (Plates 4-7).

Barleria cristata

Chromosome number was found to be 2n = 40 and three pairs of chromosomes with secondary constriction were detected (Plate 2). Earlier workers reveal the existence of meiotic chromosome number 2n = 20 (Sharma, 1970; Vasudevan, 1976; Saggoo, 1983; Saggoo and Bir, 1986; Daniel and Chuang, 1989; Khaton and Ali, 1993) and 2n = 40 (Devi and Mathew, 1991) in *B. cristata*. Hence the somatic chromosome number of 2n = 40 in *B. cristata* is confirmed.

Among the 40 chromosomes observed in *B. cristata*, 34 chromosomes are with nearly median centromeres and 6 with nearly submedian centromeres

(Table 3). The chromosome length ranges between 4.9287 µm to 2.1745 µm. TCL was found to be the maximum in *B. cristata* (138.9312 µm). DI and VC values were the highest observed (38.77 and 22.77 respectively) in the study whereas the TF value was somewhat low (42.54) when compared to *B. prionitis*. The ACL was found to be 3.4733 µm.

B. prionitis

Chromosome number was found to be the same as that of *B. cristata*, *i.e*., 2n = 40 (Plate 3). Meiotic studies conducted by Vasudevan (1976), Saggoo (1983) as well as Saggoo and Bir (1986) reveal 2n = 20 and mitotic analyses by Sarkar *et al*. (1980) and also by Devi and Mathew (1982, 1991) disclose 2n = 40 in *B. prionitis*. Moreover, n = 20 and 2n = 40 are the widespread chromosome counts found in the genus *Barleria* (Table 1). So it seems probable that during karyotype evolution of *Barleria*, the chromosome counts with 2n = 40 and n = 20 might have become the principal and recognized chromosome complement in the genus.

In the present study, *B. prionitis* showed a high TCL of 129.3066 µm. Two pairs of chromosomes with secondary constriction were observed. ACL was found to be 3.2327 µm. The range of chromosome length (RCL) was identified between 4.9330 µm to 2.2728 µm. DI and VC values were quite high with 36.92 and 22.56 respectively. The TF% value was calculated as 43.04. The karyotype of *B. prionitis* consists of 36 chromosomes with nearly median centromeres and 4 chromosomes with nearly submedian centromeres (Table 4).

Ecbolium viride

The somatic chromosome number of *E. viride* was found to be 2n = 36 (Plate 4). The existence of gametic chromosome number of n = 18 in *E. lineanum* (Sharma, 1970) and *E. syringifolium* (Daniel, 2006) and somatic

chromosome number of 2n = 36 in *E. lineanum* (Devi and Mathew, 1982) reveals that this number prevails in the genuc *Ecbolium*. Thus there is every possibility for the occurrence of 2n = 36 in *E. viride*, which seems to be a new report.

The karyotype of *E. viride* has two pairs of chromosomes with secondary constriction. The TCL (45.0734 µm) is much lower than that of *B. prionitis* and *B. cristata*. The ACL is 1.252 µm. The chromosomes are relatively small sized with the size ranging from 1.7630 µm to 0.9209 µm. The DI and VC values are 31.38 and 18.66 respectively. The TF% value calculated was the highest recorded (44.52) in the study. Chromosomes with nearly submedian centromeres were altogether absent and all of them were found to be with nearly median centromeres (Table 5).

Justicia betonica

Chromosome number was 2n = 34 showing two pairs of chromosomes with secondary constriction (Plate 5). Barring one previous report of 2n = 28 (Subramanian and Govindarajan, 1980), the prevalent mitotic chromosome number (Bir and Saggoo, 1979; Ranganath, 1981; Devi and Mathew, 1982; Krishnappa and Basavaraj, 1982; Govindarajan and Subramanian, 1983) and meiotic chromosome number (Bir and Saggoo, 1979; 1981; Ranganath, 1981; Saggoo, 1983; Saggoo and Bir, 1986; Daniel and Chuang, 1998; Daniel, 2000; Daniel *et al*., 2000) in *J. betonica* is 2n = 34 and n = 17 respectively. Apart from four species *viz*., *J. adhatoda* (Daniel and Chuang, 1998), *J. carnea* (Daniel and Chuang, 1989), *J. extensa* (Daniel and Chuang, 1998) and *J. trinervia* (Ranganath, 1981; Krishnappa and Basavaraj, 1982) this chromosome number is uncommon among the 87 species of *Justicia* that has been cytologically worked out (Table 1).

The shortest chromosomes reported in the present study are observed in *J. betonica* (size ranges from 1.0294 µm to 0.4814 µm). TCL was only 22.9642 µm. The ACL was 0.6754, the least value reported in the study. The DI and VC values were high and were calculated as 36.27 and 22.28 respectively. The TF value was also shown to be fairly high (44.11). The karyotype consists of 32 chromosomes with nearly median centromeres and a single pair of chromosomes with nearly submedian centromeres (Table 6).

Strobilanthes ciliatus

Chromosome number was found to be 2n = 28 and two pairs of chromosomes with secondary constriction were observed (Table 6). Previously, chromosome counts were not made in *S. ciliatus* and hence the somatic chromosome number of 2n = 28 is a novel report. Out of the 30 reported taxa of *Strobilanthes* that had been investigated cytologically, only one plant *viz*., *S. callosus* (Saggoo and Bir, 1982) is with the meiotic chromosome count of n = 14 (Table 1).

The chromosomes of *S. ciliatus* are smaller when compared to *B. cristata* and *B. prionitis* with a TCL of 40.8232 µm. The ACL was 1.458 µm. DI and VC values were calculated as 30.81 and 20.28 respectively. The TF value was 42.40. The chromosome complement comprises 26 chromosomes with nearly median centromeres and 2 chromosomes with nearly submedian centromeres (Table 7).

S. heyneanus

The karyotype shows a diploid chromosome number of 2n = 32, four of which were found to be having secondary constriction (Plate 7). An earlier study conducted by Devi and Mathew (1997) thus confirms the present chromosome count of 2n = 32 in *S. heyneanus*. Majority of earlier workers reports the existence of the same chromosome count of 2n = 32 and n =16 in

other species of *Strobilanthes* (Table 1). Thus it seems probable that this chromosome complement is a dominant and established one in the karyotype evolution of *Strobilanthes*.

In the present study, RCL was between 1.1058 µm and 0.6371 µm in *S. heyneanus*. The ACL (0.863 µm) was less than that of *S. ciliatus*. The TCL was estimated to be 27.6154 µm which is quite low when compared to *S. ciliatus*. DI was calculated as 26.89, which is very low when compared with other taxa. The lowest VC value among the studied taxa was found in this plant (17.71). The TF value (%) calculated was the lowest among the six plants (41.49). In the chromosome complememt, twenty eight chromosomes possess nearly median centromere while four show nearly submedian centromere (Table 8).

The cytogenetical study revealed a wide spectrum of chromosomal data among the six taxa studied, in the parameters *viz*., total chromosome length, average chromosome length, range of chromosome length, disparity index, variation coefficient and TF value (%). The TCL of different plants investigated ranges from 138.9312 µm to 22.9642 µm (Table 9). The highest value for ACL is 3.4733 µm, and the lowest is 0.6754 µm. The range of chromosome length varied between 4.9330 – 2.2728 µm to 1.0294 – 0.4814 µm. The DI value ranged from 26.89 to 38.77. All the plants showed moderately high TF (%) values, ranging from 41.49 to 44.52 and the VC values were found to be ranging from 17.71 to 22.77. All the karyotypes invariably possess a majority of chromosomes with nearly median primary constriction.

Karyotype analysis swings around the concept that symmetrical karyotypes are more primitive than asymmetrical ones, shorter chromosomes are advanced than longer ones, chromosomes with median centromere and arms of equal length are more primitive than chromosomes with arms of

unequal length and higher basic chromosome numbers are evolved from lower ones.

Among the six taxa investigated, *B. cristata* showed highest DI value (38.77) and *S. heyneanus* showed lowest DI value (26.89) (Table 9). Normally a low DI value corresponds to the homogeneity of chromosomes and a high disparity index value corresponds to the heterogenous assemblage of chromosomes (Mohanty *et al*., 1991). The comparatively higher values of DI estimated also in *B. prionitis* and *J. betonica* suggest the heterogeneity of the respective chromosome complements. The karyotypes that are heterogenous both cytologically and genetically are considered important in the evolution of the species. The karyotype homogeneity of *S. ciliatus* and *S. heyneanus* was confirmed by a low DI value (30.81 and 26.89 respectively) (Table 9). In addition to these, both the karyotypes show high TF% values. An increase in the mean centromeric index value represents a highly symmetrical karyotype, which is often considered as a primitive feature (Sharma and Sharma, 1984; Vasil'eva *et al*., 1985). Therefore, both *S. ciliatus* and *S. heyneanus* seem to possess primitive karyotypes with fairly symmetrical chromosome complements.

Variation coefficient (VC) is the most reliable karyomorphological parameter because it is calculated considering the length of the chromosomes. Moreover, the lower value of the variation coefficient as well suggests the homogeneity and primitiveness of the karyotypes (Stebbins, 1959). The maximum VC value is showed by three plants, viz., *B. cristata*, *B. prionitis* and *J. betonica* and the minimum is in *S. heyneanus*. The highest content of chromatin material was found in *B. cristata*. Though it is a primitive feature, the karyotype of *B. cristata* shows rather high DI and VC values. The karyotype of *S. ciliatus* shows a lower chromosome number and was characterized by a moderately higher TF% value (Table 9).

All the karyotypes in general, are characterized by the gradual decrease in chromatin length. A decrease in chromatin length is one of the factors responsible for evolution of higher plants (Sharma and Sharma, 1984). Most of the chromosomes are nearly metacentric with very few nearly submetacentric ones. Excessive number of nearly metacentric chromosomes reveals a primitive evolutionary status (Levitsky, 1931). Advancing evolution leads to sub-terminal and terminal centromeres. Low VC values denote primitive homogenous nature of the karyotypes (Verma, 1980). Change of the centromere from median to sub-median and increased size difference between different chromosomes of the same set are the two basic processes responsible for karyotype speciation and this is often considered as a potential factor in the evolution of species at the diploid level (Mathew and Thomas, 1974).

Majority of the chromosomes of the karyotypes belong to the nearly median group which suggests that the plants hold comparatively primitive position by revealing a more symmetrical karyotype. The moderately higher values of TF % in general also support the element of symmetry in these karyotypes. A TF% (mean centromeric index) of 50 implies that all the chromosomes have median centromeres and is thus an indicator of absolute symmetry of the karyotype. A TF% of zero implies that all the chromosomes possess terminal centromeres and the karyotype exhibits complete asymmetry (Kapoor, 1977). The comparatively higher TF% values and nearly median centromeric position of majority of chromosomes and the lower value of the variation coefficients indicate that all the taxa included in the study possess more or less primitive karyotype features.

Though the chromosomes of *B. prionitis* and *B. cristata* are much bigger in size compared to those of the others, yet they showed the greatest size difference between the different chromosomes. Sharma (1991) reported that a karyotype which is characterized by great size difference among the

chromosomes is an asymmetric one. The least difference in size of different chromosomes is noted in *J. betonica.* This species shows somewhat symmetric karyotype. The chromosomes of *J. betonica* are extremely small and homogeneous, with nearly median to nearly sub-median centromeres. Moreover, the lower range of chromosome length, low variation coefficient, and high total forma percentage (Table 9) values confirm karyotype symmetry.

A decrease in total chromosome length (TCL) and average chromosome length (ACL) found in *S. ciliatus*, *S. heyneanus*, *J. betonica* and *E. viride* are considered as higher karyomorphometrical characteristics. Reduction in chromosome length in the karyotype is a highly advanced evolutionary feature (Sharma and Sharma, 1984). Even though *E. viride* shows advanced karyotype features, a low DI value of 31.38 and high TF% value of 44.52 reveal its karyotype homogeneity. Both advanced and primitive features denote that the taxon is actively involved in karyotype evolution.

Basic chromosome number is one of the most widely used characters in biosystematic studies for formulating phylogenetic speculations and hence can be considered as a dependable and stable marker of the direction of evolution (Jones, 1978). Basic chromosome numbers are considered to be having tremendous taxonomic and evolutionary significance. Basic chromosome numbers varying from 5 to 9 are considered as primary base numbers in ancestral forms of angiosperms. Basic chromosome numbers have been implied in the determination of systematic position of a taxon at higher taxonomic levels (Raven, 1975). These basic numbers may often lead to polyploidy and aneuploidy.

Previous studies reveal the existence of diverse primary and secondary basic chromosome numbers in the family. Grant (1955) proposed the base

numbers of 5, 6, 7, 8, 9, 10 and 11 in Acanthaceae, with most species studied occurring in the series, x = 7, 8 or 9. Polyploidy and more commonly aneuploidy were responsible for the diversity of chromosome numbers in the family (Grant, 1955). Daniel *et al*. (1984) also was of the opinion that polyploidy appears to be frequent in the family and aneuploidy may also have been important in the origin of some genera. The basic number x = 7 seems to be primitive for the family, from which the widespread x = 14 would have been derived (Piovano and Bernardello, 1991). Daniel and Chuang (1993) also proposed that the primitive basic number of x = 7 is the probable ancestral base number for the family.

According to Govindarajan and Subramanian (1983), the Acanthaceae show a wide range of basic numbers from x = 8 to x = 34 and the commonest basic number in the family is x = 16. The basic chromosome number, x = 8 is assumed to be the original basic number of the family and that x = 16 is derived by polyploidy. They suggested that evolution of an aneuploid series (x = 13, 15, 17, 18, 19, 20, 21, 22, 25, 26, 28, 30 and 34) from the original number x = 8, followed by polyploidy might well have given rise to the wide range of basic numbers now observed in the family. Basic numbers 17 to 34 may have arisen by diphasic amphidiploidy, by combination of any two basic numbers or by gain or loss of chromosomes following polyploidy. Mehra and Bawa (1969) believed that basic numbers higher than x = 13, are secondary polyploids derived by compounding of lower numbers of related or extinct taxa. This is evidenced by the study of frequency of diploid chromosome numbers in various species of Acanthaceae they studied. The constancy of chromosome numbers within many genera of Acanthaceae and between or among morphologically similar genera often suggests relatedness of the taxa.

Barlerieae are the most primitive subtribe among the *Justicieae* (Acanthaceae) (Dutta and Maity, 1970). Though chromosome complements

of n = 12, 15, 16, 17, 18, 19, 20 and 21 have been reported for *Barleria* species (Grant, 1955; Moore, 1973; Goldblatt, 1985, 1988), however, most counts have been either n = 20 or 2n = 40. In the present study, counts of 2n = 40 for *B. cristata* and *B. prionitis* agree with most of the previous reports of the same or related species. Gosavi *et al*. (2011) reports that the karyotypes of most of the Barlerias are fairly symmetrical and their somatic complements being predominated by m-type chromosomes. A perusal of the literature shows that among the Indian Barlerias, 2n = 40 is the most common somatic chromosome complement, though 2n = 32, 36 and 44 have also been reported. All these counts can be descending aneuploid derivations of the n = 20 condition (Ranganath and Krishnappa, 1990). De (1966) suggested that all the known numbers in the genus are derived from alterations of the basic number of x = 15. As 2n = 40 is found in most of the species of *Barleria* studied so far (Table 2), it is, however, premature at present to draw a definite conclusion on the basic number of the genus when the cytological information available is meagre. Besides, some of the earliest counts need to be confirmed employing recent techniques.

According to Devi and Mathew (1991), the probable basic chromosome number could be x = 10 among species of *Barleria*. All the known species hence could be tetraploids based on x = 10. In the present investigation, karyotypes of both these plants were found to be fairly asymmetrical and unspecialised. However, each taxon was distinct in respect of the relative positions of the centromere, length of the individual chromosomes and the total length of haploid set. The karyotype of *B. prionitis* was shown to be more symmetrical with most of the chromosomes showing more or less equal arms when compared to that of *B. cristata*. The highest number of nearly sub-metacentric chromosomes was noted in *B. cristata*; *i.e*., 6 pairs. The presence of relatively long chromosomes and a relatively high basic number of x = 20 makes *Barleria* a distinct taxon among Acanthaceae

(Ranganath and Krishnappa, 1990). Daniel and Chuang (1989, 1998) summarized all the previously reported chromosome numbers in *Barleria* and noted the prevalence of n = 20 among species of the genus and the likelihood of x = 20 as a basic number in the genus. In the present investigation, the chromosome number of 2n = 40 estimated in the karyotypes of *B. cristata* and *B. prionitis* might have derived from the secondary basic chromosome number of x_2 = 20, which in turn might have originated from the primary basic number of x_1 = 10 by protoautoploidy, as evidenced by earlier reports.

The chromosome complement count of 2n = 36 revealed for *E. viride* is the first report for the species and it agrees with most of the previous counts for the genus. The chromosome number 2n = 36 of *E. viride* agrees with the view of Daniel (2006) that x = 18 is the basic chromosome number of *Ecbolium* and might have originated from the primary base number x_1 = 9. Doubling of the primary base number due to protoautoploidy leads to the formation of the secondary basic number x_2 = 18 from which the normal chromosome number of 2n = 36 might have originated. In the present investigation, the evolution of somatic chromosome number in the two species of *Barleria* and *E. viride* seems to be following the same pattern of protoautoploidy from the primary basic chromosome number, even though their base numbers are different.

According to Govindarajan and Subramanian (1983), both euploidy and aneuploidy might have played an important role in the origin and evolution of the species of *Strobilanthes.* The normal chromosome number of *S. ciliatus*, 2n = 28 seems to be apparently originated by the doubling of the primary basic number of x_1 = 7, which has been suggested to be commonest in the family. According to Fernandes and Leitao (1984), the secondary base number, x_2 = 16 in *S. heyneanus* might have arisen from the primary base number x_1 = 8, through autopolyploidy.

Govindarajan and Subramanian (1983) have reported the somatic chromosome number of *J. betonica* as 2n = 34. The results of the present study are in agreement with this report. The chromosome number of 2n = 34 in *J. betonica* could arise in various ways. Daniel *et al*. (1984) suggested that although a wide range of basic chromosome numbers has been reported for species of *Justicia,* x = 14 appears to be the most common number. A basic chromosome number of x = 7 would appear to be highly probable for *Justicia*, as suggested by Piovano and Bernardello (1991) as well as Daniel (2000). The other possible base numbers of *Justicia*, according to Daniel (2000) include 6 (although not much known in the genus or family), 9 and 11. Whatever be the basic number, given the diversity of numbers now known for the genus, it appears evident that both dysploidy and euploidy could have been important components of evolution within the genus. A polybasic origin also is highly probable by the joining of two primary base numbers $x_1 = 8$ and $x_1 = 9$ leading to the formation of the secondary number $x_2 = 17$ by amphiploidy (Sharma and Sharma, 1984), doubling of $x_2 = 17$ forms cells with 2n = 34 chromosomes (Grant, 1981). According to Jones (1978) and Evans (1988), amphidiploid origin of basic chromosome number is a primitive condition. A chance for the origin of $x_2 = 17$ through a 'polyploid lift' from $x_1 = 8$ or by a 'polyploid drop' from $x_1 = 9$ is another possibility (Jones, 1978). Daniel (2000) discussed the diversity, frequency and taxonomic distribution of chromosome numbers reported for the genus *Justicia*. He noted that x = 7 would appear to be the basic chromosome number for the genus, and that n = 14 is by far the most commonly reported. It also suggests that both aneuploidy/dysploidy and euploidy have accompanied evolutionary diversification of the genus.

The base number x = 7 has been suggested as the original basic chromosome number for the family (Grant, 1955). According to Piovano and Bernardello (1991), the base number of x = 7 with x = 14 in the greatest

number of genera seems to indicate that the basic primitive number for the family Acanthaceae could be x = 7, from which the widespread x = 14, as well as the other base numbers, could have been derived. When compared with the probable basic number of x = 7 for the family, most of the counts reported here are relatively high. The high basic numbers suggested for most genera undoubtedly result from ancient polyploidizations. Given the variation in chromosome numbers reported within the numerous species and genera (Table 1), it is likely that chromosomal alterations might have been responsible for the proliferation in numbers of taxa in this large family. Long (1970) also has suggested that aneuploidy may be responsible for the large number of genera and species in the family, Acanthaceae.

According to Daniel *et al*. (1984), the presence of a prominent basic chromosome number for most genera is evident. In spite of an array of numbers in some large genera (e.g., *Justicia* and *Barleria*), a single basic number predominates. The heterogeneity of chromosome numbers in *Justicia* may be indicative of a polyphyletic assemblage or may simply indicate evolution of chromosome number after the origin of the genus. This variability is also typical of many other genera (Grant, 1955) and of the family Acanthaceae as a whole (Piovano and Bernardello, 1991).

Structural rearrangements of chromosomes happen during karyotypic evolution which keeps the chromosome number constant but induce variation in terms of karyotype organization, karyotype symmetry, average chromosome length *etc*. Changes in chromosome length may arise due to difference in the DNA and protein content and also by translocations and deletions. The data obtained in the present study are significant for effective breeding programmes, genetic diversity analysis and elucidation of the phylogeny and the species origin of the respective plants.

Karyomorphological studies are of considerable importance in order to throw light on the phylogenetic relationships among taxa of flowering plants (Iwatsubo and Naruhashi, 1991). Like many other large and predominately tropical families of flowering plants, the Acanthaceae remain poorly known cytologically (Daniel, 2000). The karyotypic variations observed among the plants here could not be correlated or interpreted in a regular evolutionary order as they belong to four different genera. Among the two species of *Barleria*, more primitive features were exhibited by *B. prionitis* when compared to *B. cristata*. In the case of *Strobilanthes*, more advanced characters were shown by *S. heyneanus* when compoared to *S. ciliatus*. In the present investigation, majority of karyomorphological features reveal the homogeneity of all the taxa investigated. The karyomorphological diversity found in the six taxa of Acanthaceae can be best explained by assuming that these plants are still in an active phase of karyotype evolution and their evolutionary status appears to be enigmatic. The counts for *E. viride* and *S. ciliatus* are the first reported chromosome numbers for these taxa. As detailed cytogenetic studies had not been attempted previously on any of these plants, the aim of this work was to provide wholesome and complete cytological information on the taxa that would contribute to the better understanding of the chromosomal constitution of the different taxa that are used as the source plants of the drug 'Sahachara'.

b. ANTIOXIDANT AND ANTI-INFLAMMATORY ASSAYS

i. ANTIOXIDANT ASSAYS

Free radicals have been implicated in the pathological processes of various serious diseases such as cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation (Juan and Chou, 2010). Reactive Oxygen species (ROS) are continuously produced during regular physiological processes, have very

short half life and hence are very reactive, causing cellular injuries leading to the accumulation of lipid peroxides in biological membranes, inducing oxidative damage in crucial biomolecules and form the main responsible elements for numerous degenerative diseases (Ray and Hussain, 2002; Yadav *et al*., 2012). Overproduction of ROS and free radicals may result in oxidative stress resulting in a number of pathological conditions, affecting components of the electron transport chain, ultimately leading to cell death.

According to Winrow *et al*. (1993), the inflammatory process is often associated with free radical damage and oxidative stress. Narmadha and Devaki (2012a) suggested that free radicals play an important role in the etiology of many autoimmune disorders like rheumatoid arthritis. The generation of oxygen free radicals is known to be involved in the development of several systemic inflammatory response syndromes. In addition to their actions as noxious mediators generated by inflammatory cells, these molecules play also a crucial role contributing to the onset and progression of inflammation in distant organs (Closa and Folch-Puy, 2004). Reactive free radicals are produced during the process of inflammation by the mast cells, neutrophils and macrophages and this production of ROS contributes to expand the inflammatory response itself (Harput *et al*., 2011; Noufou *et al*., 2012).

Antioxidants are free radical scavengers (FRS) which function as inhibitors at both initiation and propagation stages of degenerative diseases and consequently play an important role in the protection of human body against oxidative damages by blocking the chain initiated by high energy molecules (Govindarajan *et al*., 2005; Cespecles *et al*., 2008). According to Stangl *et al*. (2006), the active principles found in several antioxidant herbs are known to react directly with ROS and thus can reduce their destructing activity. The proper and judicious use of herbs is often been successful in the

treatment of many adverse health conditions caused by free radicals and is essentially ascribed to their radical scavenging activities. Although many promising synthetic antioxidants are currently used against various human ailments, their pro-oxidant or cytotoxic nature at higher concentration prevents them from long term use.

Plant extracts with high level of polyphenolic compounds show good antioxidant activity in *in vitro* systems (Shukla *et al*., 2012). There are increasing evidences that natural antioxidants present in spices, herbs and medicinal plants may be useful in preventing the deleterious consequences of oxidative stress. Many plant species in Acanthaceae have been investigated for their antioxidant properties and their active constituents have been suggested to be contributing to their radical scavenging ability (Ilango *et al*., 2009; Thangavel and Gupta, 2010).

Having in mind the significance of oxidative stress and inflammatory process in the development of many severe chronic diseases, it would be worthwhile to investigate the antioxidant and anti-inflammatory activities of these plant species which are used as the source plants of the drug 'Sahachara' and also to probe the correlation between these activities. The models for scavenging free radicals are widely used to estimate the antioxidant properties in a relatively short time with high reliability (Reische *et al*., 2002). Ikram *et al*. (2009) suggested that it is important to use different assays, instead of relying on a single one to assess and compare the antioxidant capacity. The antioxidant potential of the root methanolic extracts of six taxa of Acanthaceae was evaluated employing a variety of *in vitro* methods that includes assessing their ability to scavenge various free radicals and to inhibit lipid peroxidation *in vitro*.

The effective concentration for radical scavenging activity (IC_{50}) is the concentration of the extract at which the antioxidant activity is 50%, *i.e*., the

amount of each extract needed for 50% scavenging of various radicals and for causing 50% inhibition of lipid peroxidation. A lower IC_{50} value corresponds to higher antioxidant activity of the extracts and vice versa. All the methanolic extracts showed moderate to good antioxidant capacities against different reactive oxygen species, although with different efficiencies (Figs 7-11; Tables 10-15). The scavenging effect of extracts increased with increasing concentration. The potent antioxidant activities displayed by the various extracts shows their ability to protect various biomolecules from oxidative damages. Of the plants investigated, *B. prionitis*, *B. cristata* and *S. ciliatus* seem to be most promising.

DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable, synthetic radical that does not disintegrate in water, methanol or ethanol (Aksoy *et al*., 2013). The model system for scavenging DPPH free radicals is an easy, rapid, and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Othman *et al*., 2007). The DPPH method is one of the most preferred antioxidant assays because it is highly reliable and does not require a special reaction and device.

DPPH shows a strong absorption band at 517 nm in visible spectroscopy because of the odd electron. As this electron becomes paired off in the presence of a free radical scavenger, the absorption diminishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (de Oliveira *et al*., 2009). In the presence of an antioxidant agent, a solution of DPPH radicals prepared in methanol is converted into its reduced form, DPPH-H (diphenylhydrazine) molecules.

In the present study, all extracts were found to be effective scavengers against DPPH radical and their activities increased in a dose dependent

manner (Fig. 7; Table 11). The root methanolic extract of *B. prionitis* exhibited the highest DPPH antiradical properties, with an IC_{50} of 31.06 ± 1.28 µg/ml, followed by *B. cristata* and *S. ciliatus*. The extract of *S. heyneanus* displayed the weakest IC_{50} of DPPH radical scavenging activity (103.92 ± 1.55 µg/ml) (Table 10). Maximum DPPH is scavenged by *B. prionitis* (84.73 ± 1.31%) and *B. cristata* (85.97 ± 1.05%) at a concentration of 140 µg/ml (Table 11). Free radical scavengers donate an electron to the free radical, causing the disappearance of DPPH radical's absorbance. The disappearance of DPPH radical is directly proportional to the amount of antioxidants present in the reaction mixture.

ABTS radical scavenging assay

The peroxidase substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), forming a relatively stable radical cation ($ABTS^{\bullet+}$) upon one-electron oxidation has become a popular substrate for estimation of total antioxidant capacity (Tirzitis and Bartosz, 2010). Bleaching of a pre-formed solution of ABTS radical has been extensively used to evaluate the antioxidant capacity of complex mixtures and individual compounds. The radical is generated directly in a stable form prior to reaction with putative antioxidants. The pre-formed radical monocation ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with persulfate and is reduced in the presence of hydrogen-donating antioxidants to an extent depending on the activity and concentration of the antioxidant as well as the duration of the reaction. Antioxidant activity can be determined by the decolorization of the $ABTS^{\bullet+}$, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm.

ABTS radical cation is reactive towards most antioxidants (Walker and Everette, 2009) and is applicable for both lipophilic and hydrophilic antioxidants (Aghdam *et al.*, 2011). It is more reactive than DPPH radical,

and unlike the reactions with DPPH which involves H atom transfer, ABTS radical scavenging activity involves an electron transfer process (Srikanth *et al*., 2010).

The free radical scavenging activity of the extracts show a higher efficiency in ABTS assay with much lower IC_{50} values (Table 10). The dose dependent inhibition of ABTS radicals by various extracts is shown in (Fig. 8; Table 12). As in the case of DPPH radical scavenging, the highest activity was noted in *B. prionitis*, *B. cristata* and *S. ciliatus* extracts which could produce more than 90% radical inhibition at the dose of 70 µg/ml (Table 12). The most effective scavenger was *B. prionitis* extract with an IC_{50} of 12.89 ± 3.46 µg/ml. The extract of *B. cristata* also was an efficient scavenger with an IC_{50} of 13.99 ± 2.91 µg/ml. The IC_{50} of *S. ciliatus* extract was also quite low when compared to the other extracts, *i.e*., 18.65 ± 2.95 µg/ml (Table 10). The root methanolic extract of *J. betonica* registered the lowest activity with the highest IC_{50} value of 86.20 ± 2.11 µg/ml.

Superoxide radical scavenging assay

Molecular O_2 is reduced to O_2^- (superoxide) when it absorbs an excited electron. Superoxide is known to denature enzymes, oxidize lipids and fragment DNA (Smirnoff, 1993). Superoxide radicals are generated during the normal physiological processes mainly in mitochondria and reacts with sensitive and critical cellular targets. Despite its involvement in many pathological processes, superoxide itself is considered as a weak oxidant. But it can give rise to the more toxic hydroxyl radicals, singlet oxygen and hydrogen peroxide damaging biomacromolecules directly or indirectly with severe consequences (Lee *et al*., 2004).

Superoxide dismutase is a group of enzymatic antioxidants that dismutase the two otherwise repulsive superoxide radicals to hydrogen

peroxide (Jojo, 2007). It is considered as a stress protein synthesized in response to oxidative stress, found virtually in all aerobic organisms. In higher plants, superoxide dismutase enzymes (SODs) act as antioxidants and protect cellular components from being oxidized by ROS (Cao *et al*., 2008). SODs catalyze the production of O_2 and H_2O_2 from superoxide, which results in less harmful reactants, thus protecting the cell from superoxide toxicity.

In vitro superoxide radical scavenging activity is measured by riboflavin/light/NBT (nitro blue tetrazolium) reduction method which is the most popular one. Superoxide anions were generated by the auto oxidation of riboflavin in presence of light and assayed by reduction of NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture.

Results of the present study showed that incubation of extracts of six different taxa of Acanthaceae could scavenge the superoxide radicals generated in the system. Addition of the various extracts showed decrease in absorbance corresponding to their concentration (Fig. 9). Maximum inhibition was noted in *B. prionitis* extract at 140 µg/ml concentration (94.21 ± 0.89%), followed by *B. cristata*, *S. ciliatus*, *E. viride*, *S. heyneanus* and *J. betonica* in the descending order (Fig. 9; Table 13). The superoxide radical scavenging activity displayed by the extracts was absolutely similar to that observed for ABTS radical. The IC_{50} for inhibition of superoxide radicals of *B. prionitis*, *B. cristata* and *S. ciliatus* were calculated as 44.54 ± 2.90, 55.23 ± 1.81 and 61.76 ± 2.68 µg/ml respectively (Table 10).

Hydroxyl radical scavenging assay

The hydroxyl radical is an extremely reactive species capable of reacting with and damaging all surrounding biomolecules (Tirzitis and Bartosz, 2010). It is widely implicated as the most potent oxidant and the

major damaging species in the free radical pathology (Sheeja *et al*., 2011). Halliwell and Gutteridge (2006) stated that generation of hydroxyl radicals is crucial for the irreversible damage inflicted by oxidative stress. They are the most reactive ones, reacting with every non-selective compound in its way and almost every biological membrane causing lipid peroxidation.

Ferric EDTA when incubated with H_2O_2 and ascorbic acid at pH 7.4 leads to the formation of hydroxyl radicals in free solution by the Fenton reaction and can be detected by their ability to degrade deoxyribose into fragments that on heating with thiobarbituric acid (TBA) and low pH form a pink chromogen (Aruoma *et al*., 1989). According to Babu *et al*. (2001), hydroxyl radical scavenging capacity of an extract is directly related to its anti-oxidant activity. In the present study all the extracts inhibited hydroxyl radical mediated deoxyribose degradation in a concentration dependent manner (Fig. 10; Table 14). *S. ciliatus* was found to be the most powerful scavenger of the hydroxyl radical (IC_{50} value 54.58 ± 0.94 µg/ml), with an inhibition of up to 78.83 ± 0.63% at a concentration of 140µg/ml (Table 14). It is worth mentioning that *S. ciliatus* showed an inhibition of 40.77 ± 1.06% at a concentration as low as 20µg/ml (Table 14). This was followed by *B. prionitis* and *B. cristata* with IC_{50} values of 70.10 ± 1.73 and 74.84 ± 2.13 µg/ml respectively (Table 10). The weakest scavenger was found to be *J. betonica* that produced 57.72 ± 1.72 % inhibition at 140 µg/ml (Table 14).

Lipid peroxidation assay

Lipid peroxidation is a key process in many pathological events induced by oxidative stress. The unsaturated fatty acids in cell membrane on oxidation lead to the formation and proliferation of lipid peroxides. The oxygen uptake, structural rearrangements of unsaturated fatty acids and eventual damage of membrane lipids lead to production of malondialdehyde,

which contributes to carcinogenesis, mutagenesis and cell toxicity (Miyake and Shibamoto, 1997).

Malonaldehde, resulting from the peroxidation of biological membranes is a major reactive aldehyde. It can react with TBA and produce a red coloured product. Addition of Fe^{2+}/ascorbate to the liver causes increase in lipid peroxidation. The antioxidant fractions present in the various extracts could very well lower malonaldehyde production and thus can prevent cell damage caused by peroxidation of membrane lipids.

The various extracts have showed good ability to inhibit lipid peroxidation at all concentrations (Fig. 11; Table 15). The extracts of *B. cristata* and *B. prionitis* were found to be superior to the others in inhibiting lipid peroxidation. Methanolic extract of *B. cristata* (IC_{50} 55.59 ± 1.44 µg/ml) was the most efficient in inhibiting lipid peroxidation, while the weakest inhibition and hence the least antioxidant activity was shown by *J. betonica* (IC_{50} 95.76 ± 1.11 µg/ml) (Table 10). The effect of *B. prionitis* (IC_{50} 69.12 ± 3.04 µg/ml) follows that of *B. cristata*. The inhibitions produced by *S. ciliatus* (IC_{50} 72.82 ± 1.55 µg/ml), *E. viride* (IC_{50} 72.80 ± 1.71 µg/ml) and *S. heyneanus* (IC_{50} 73.85 ± 1.18 µg/ml) were more or less comparable.

The data of the present study shows that root methanolic extracts of the six taxa of Acanthaceae, used as source plants of the Ayurvedic drug 'Sahachara' displayed strong *in vitro* antioxidant activities as measured by their scavenging abilities against DPPH, ABTS, superoxide and hydroxyl radicals and inhibition of lipid peroxidation (Figs 7-11; Tables 10-15). Significant free radical scavenging activities were shown by all root extracts, though those of *J. betonica* and *S. heyneanus* were comparatively lower. *B. prionitis* was revealed to be the most effective scavenger of free radicals in ABTS, DPPH and superoxide radical assays. Maximum inhibition of lipid peroxidation was recorded in the reaction mixture incubated with *B. cristata*

extract. Hydroxyl radical activity was found to be the maximum in *S. ciliatus* followed by *B. prionitis* and *B. cristata*. The minimum antioxidant activities were shown by *J. betonica* in all the assays except in DPPH radical scavenging assay where the lowest IC_{50} value was exhibited by *S. heyneanus*.

The free radical scavenging activities of acanthaceous plants have been reported by a number of authors. According to Sawadogo *et al.* (2006), members of Acanthaceae are good sources of antioxidants and suggest their use in the treatment of cardiovascular and anti-inflammatory diseases. Amoo *et al*. (2011) studied the antioxidant effects of three species of *Barleria*, *viz*., *B. prionitis*, *B. greenii* and *B. albostellata*. Many authors like Jimenez *et al.* (2009), Marathakam *et al*. (2012), Suriyavathana *et al*. (2011), Medapa *et al*. (2011), Muslim *et al*. (2010) and Chetan *et al*. (2011) have studied the free radical scavenging activities of aerial parts of several acanthaceous plants and reported positive correlation between the content of phenolics and flavonoids with antioxidant activities. In most of the studies, the alcoholic extracts showed better results than other solvent extracts including aqueous extract. It has been suggested that the extracts of acanthaceous plants like *B. lupulina*, *Clinacanthus nutans*, *B. prionitis*, *B. cristata* and *J. wynadensis* are potential scavengers of free radicals which may be responsible for their anti-inflammatory and other beneficial pharmacological effects (Wanikiat *et al.*, 2008; Khobragade and Bhande, 2012; Gambhire *et al*., 2009b; Medapa *et al*., 2011). Thangavel and Gupta (2010) recently isolated anti-inflammatory compounds from *Andrographis stenophylla* through activity guided fractionation. Ata *et al*. (2009) investigated the enzyme inhibitory and free radical scavenging activities of the chemical constituents of *B. prionitis* and concluded that barlerinoside showed higher potential of antioxidant property with an IC_{50} value of 0.41 mg/ml.

In the light of the antioxidant studies conducted using the above five different assays, it can be concluded that the prominent antioxidant potential exhibited by *B. prionitis*, *B. cristata* and *S. ciliatus* as well as the moderate antioxidant effects shown by other species may be due to terpenoids, flavonoids, phenols and sterols present in the methonolic extracts. Many studies in the literature present positive correlations between the quantity of phenolic and flavonoid constituents and free radical scavenging effects, suggesting that phenolic and flavonoid content of the plant contribute directly to the antioxidant activity (Lesjak *et al*., 2011; Rao *et al*., 2010; Nabavi *et al*., 2009). Yoshida and Niki (2003) have shown the antioxidant effects of the phytosterols like sitosterol, stigmasterol, and campesterol against lipid peroxidation. Vivacons and Moreno (2005) also have suggested the preventive effects, at least in part of phytosterols like sitosterol on the development of diseases due to ROS.

Shahidi and Marian (2003) reported that differences in antioxidant activities of plant extracts could be due to different qualitative and quantitative composition of their phenolic constituents. The phenolic compounds are reported to eliminate radicals due to their hydroxyl groups which contribute directly to the antioxidant effect of system (Soobrattee *et al*., 2005; Hossain *et al*., 2011). According to Hagerman *et al*. (1998), high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging activity by phenolics than their specific functional groups. de Oliveira *et al*. (2009) also has suggested that polyphenolic compounds possess their scavenging effects by virtue of their chelating of redox-active metal ions, inactivation of lipid free radical chains and also by prevention of hydroperoxide conversion into reactive oxyradicals.

The free radicals such as ROS are produced during the process of inflammation by the mast cells, neutrophils and macrophages. This production of ROS contributes to expand the inflammatory response (Harput *et al*., 2011). Hence, free radical scavenging activity of the methanolic extracts may be one of the basic reasons for the anti-inflammatory activity of these extracts. The observed *in vitro* activities suggest that the investigated plant extracts could exert protective effects against oxidative and free radical injuries occurring in different pathological conditions. With this respect, the most promising plants in the present investigation appear to be *B. prionitis, B. cristata* and *S. ciliatus* as revealed by their low IC_{50} values.

ii. ANTI-INFLAMMATORY ASSAYS

Inflammation, which is functionally a protective response, can be considered as a complex series of events that develop when the body is injured either by mechanical or chemical agents by a self-destructive (autoimmune) process and the cardinal signs of inflammation include swelling, redness, heat, pain and altered function (Murugan *et al*., 2012). Inflammation is an important physiological reaction during which leukocytes infiltrate at the site of injury and release mediators that help in decreasing the inflammatory response, aiming to perform the dual function of limiting damage and promoting tissue repair (Kim *et al*., 2009). Inflammation involves macrophage and lymphocyte activation along with the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 as well as inflammatory mediators such as NO and prostaglandin E2 (PGE2), that are synthesized by inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (O'Shea and Murray, 2008).

There are two fundamental types of inflammation: acute and chronic. Acute inflammation is a short term process which is characterized by a vascular response and initial recruitment of polymorphonuclear granulocytes,

typically neutrophils, followed by monocytes, which differentiate locally into macrophages. This leads to a co-ordinate activation of various signalling pathways that regulate expression of both pro- and anti- inflammatory mediators. Briefly, after a tissue is injured, there is an increase of blood flow into the area, followed by an increased permeability of the capillaries that permit plasma proteins and leukocytes to leave the circulation. If the inflammatory process persists, chronic inflammation takes place. Chronic inflammation indeed can be defined as a prolonged, deregulated and maladaptive response that results in cellular destruction, and is associated with many chronic human diseases. The hallmark of chronic inflammation is the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, and plasma cells). These cells are recruited from the circulation by the steady release of chemotactic factors (de las Heras and Hortelano, 2009).

However, excessive or persistent inflammation causes a variety of pathological conditions and so has become the focus of global scientific research (Fawole *et al*., 2010; Adedapo *et al*., 2008). Sometimes, it seems to produce events that are quite serious and become chronic like occurrence of rheumatoid arthritis and hay fever which may be life threatening (Singh *et al*., 2008a). According to Li *et al*. (2003), chronic inflammatory diseases remain one of the world's major health problems. Apart from rheumatoid arthritis, inflammation processes are implicated in many other degenerative diseases also, such as shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma and cancer (Polya, 2003; Iwalewa *et al*., 2007).

Most clinically important medicines for treatment of inflammation-related diseases belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics. These have potent activity; yet long-term administration is required for treatment of chronic diseases. The use of non-

steroidal anti-inflammatory drugs (NSAIDs) is limited by a high occurrence of intestinal side effects and mucosal erosions that can progress into ulcers (Sawadogo *et al*., 2011). These conventional drugs either are too expensive or toxic or are not commonly available to the rural folks that constitute the major populace of the world. Apart from the NSAIDs various corticosteroids too are primarily used as anti-inflammatory agents but these steroidal agents also have severe adverse effects (Beg *et al*., 2011).

One fifth of the world's elderly suffer with arthritis and yet the issues they face get little attention and remedy other than some symptomatic relief from the pain. Arthritis is the result of extreme joint inflammation; it is a chronic and disabling autoimmune disease that progresses very rapidly causing swelling and tissue damage in the joints. Herbal drugs and its isolated constituents can play vital role in the management of arthritis (Murugananthan *et al*., 2013). According to Bang *et al*. (2009), there are a number of herbs that work synergistically to reduce chronic joint inflammation, such as in osteoarthritis and rheumatoid arthritis. The six taxa of the present investigation have long been used in the indigenous system of medicine and are well known folk remedies for arthritic and rheumatic disorders among the local people. The research into plants with alleged folkloric use as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-inflammatory drugs (Gupta *et al*., 2006).

The most widely used primary tests to screen anti-inflammatory property measure the ability of a compound to reduce local edema induced in the mouse/rat paw by injection of an irritant agent (Winter *et al*., 1962). Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow. Anti-inflammatory activity of the extracts

was examined by employing two widely used anti-inflammatory models: carrageenan-induced acute and formalin-induced chronic paw edema in Swiss Albino mice. The oral administration of the extracts could significantly reduce the paw edema in a dose-dependent manner in both the inflammation models, hence corroborating the previous observations on the anti inflammatory properties of the source plants (Figs. 12-23; Tables 16-27). Among the six taxa, *B. prionitis* and *S. ciliatus* demonstrated maximum activity and hence they prove to be highly potent anti-inflammatory agents.

Carrageenan induced acute inflammation

Carrageenan-induced rat paw edema is a widely used *in-vivo* model to predict the value of anti-inflammatory agents, which act through inhibiting the mediators of acute inflammation. Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase of the inflammation is due to the release of histamine, serotonin and similar substances and the later phase is associated with the activation of kinin-like substances, *i.e.*, prostaglandins, proteases and lysosome (Thomazzi *et al*., 2010). The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells produced by tissue macrophages (Georgewill and Georgewill, 2010). The first phase begins immediately after injection of carrageenan and diminishes in 2 h. The second phase begins at the end of first phase and remains through 3^{rd} h up to 5^{th} h.

The sub plantar injection of carrageenan produced a local edema in rats that increased progressively around 1-2 h; the degree of paw swelling was maximal at the 3^{rd} h after injection of carrageenan. However, after the 5^{th} h, edema decreased but remained obvious. Among the six taxa studied, the anti-inflammatory activitiy of *S. ciliatus* was the most pronounced followed by *B. prionitis*, *E. viride*, *B. cristata*, *S. heyneanus* and *J. betonica* in the decreasing order compared to the control group (Figs. 12, 14, 16, 18, 20, 22;

Tables 16, 18, 20, 22, 24, 26). Treatment with the methanol extracts of *S. ciliatus* and *B. prionitis* revealed significant reduction in paw volume after carrageenan injection, in both the phases (1-5 h). The results of the study reveal that the extracts have non-selective inhibititory effect on the synthesis and/or the release of the mediators of inflammation, especially the cyclo-oxygenase products. Suppression of the 1st phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the 2nd phase may be explained by an inhibition of cyclo-oxygenase. According to Just *et al*. (1998), the significant inhibition caused by the extracts at 250 mg/kg b. wt dose towards the later phases of the experiment probably involve arachidonic acid dependent neutrophil mobilization.

In the carragenin-induced edema, doses of *S. ciliatus* extract exerted the maximum effect (Fig. 20; Table 24), followed by *B. prionitis* (Fig. 14; Table 18) both at early as well as late stages of inflammation indicating the potency of *S. ciliatus* and *B. prionitis* to inhibit probably histamine, serotonin and kinins that are involved in the early stage of carragenin induced edema. The extracts that were found to be further inhibiting later stage of the edema may be due to its ability to inhibit prostaglandin which is known to mediate the second phase of carrageenan induced inflammation.

The inflammatory effect induced by carrageenan is associated with free radicals and in a number of pathophysiological conditions associated with inflammation or oxidant stress, these ROS have been proposed to mediate cell damage. From the results of the present study it can be concluded that the probable mechanism of anti-inflammatory action of the extracts may be mostly due to the influence on the second phase of inflammation, the cyclooxygenase (COX) pathway as is evident by their maximal inhibition of inflammation at the end of the third hour after the treatment with carrageenan (Figs 12, 14, 16, 18, 20, 22).

The inhibitory effect of *B. prionitis* and *S. ciliatus* could be seen right from the very beginning, immediately after the injection of carrageenan, even before the inhibitory effect of standard diclofenac is noted (Figs 14, 20; Tables 18, 24). Exudation which is a consequence of increased vascular permeability is considered a major feature of acute inflammation that results in edema. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemically induced vascular permeability can cause an immediate reaction and its early inhibition by the extracts suggests that administration of the extracts of *S. ciliatus* and *B. prionitis* could effectively suppress the exudative phase of acute inflammation induced by carrageenan.

Formalin induced chronic inflammation

Inhibition of formalin induced paw edema in mice is one of the most suitable test procedures to investigate anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Subcutaneous injection of formaldehyde into hind paw of mice produces localized but persisting inflammation. According to Wheelmer-Aceto and Cowan (1991) the effect of formalin is biphasic, an early neurogenic component, followed by a later tissue mediated response. Chronic inflammation which is characterised by infiltration of damaged tissue by mononuclear cells such as macrophages, lymphocytes and plasma cells, together with tissue destruction arises if there is continuous release of pro-inflammatory mediators (Serhan *et al.*, 2007). The activating inflammatory cells act as potential endogenous sources of a variety of ROS, including superoxide anion, hydrogen peroxide and nitric oxide which can produce tissue damage and are the main culprits of cellular transformation in chronic inflammation (Kundu, and Surh, 2005).

The root methanolic extract of *B. prionitis* showed the highest inhibition of chronic inflammation and a notable reduction in edematous paw volume was observed by the 4^{th} day, which was quite comparable with that of the standard drug diclofenac (Fig. 15; Table 19). The anti-edematous response shown by the different extracts against formalin are more or less similar to their anti-inflammatory activities shown against carrageenan (Figs 13, 15, 17, 19, 21, 23; Tables 17, 19, 21, 23, 25, 27). The maximum anti-inflammatory potential was shown by *B. prionitis* extract with a highly significant ($p < 0.001$) decrease in paw volume till 24 h at both the doses, which is suggestive of its long lasting action. The activity of *S. ciliatus* also was quite high, comparable to that of *B. prionitis*. The lowest anti-inflammatory effects were shown by *S. heyneanus* (Fig. 23; Table 27) and *J. betonica* (Fig. 19; Table 23), but the anti-edematous effect shown by them at 250 mg/kg b. wt was statistically significant.

In carrageenan induced acute inflammation, the percentage decrease in the edema volume at 250 mg/kg b. wt was in the order of *S. ciliatus* > *B. prionitis* > *E. viride* > *B. cristata* > *S. heyneanus* > *J. betonica* and in formalin induced chronic inflammation, it was in the order of *B. prionitis* > *S. ciliatus* > *B. cristata* > *E. viride* > *S. heyneanus* > *J. betonica*. No significant differences were observed between *S. cliatus*, *B. prionitis*, *B. cristata* and *E. viride* with regard to their ability to decrease paw edma volume at the dose of 250 mg/kg b. wt in both the assays. Their effects were well comparable with the standard drug diclofenac (10 mg/kg b. wt) also. But *S. ciliatus* (55.47% and 37.70%) and *B. prionitis* (62.77 and 39.34%) gave the most promising inhibitions even at the low dose (100 mg/kg b. wt) respectively for carrageenan and formalin models of inflammation respectively (Tables 24, 18). The extracts of *S. heneanus* and *J. betonica* displayed minimum activities with the latter being the least effective in suppressing inflammation. The inhibitions produced by *J. betonica* at the

lower concentration were not significant when compared to the control (Tables 22, 23). Yet significant decrease ($p < 0.01$) in paw volume was observed at its higher concentration. The responses of *B. cristata* and *E. viride* to inflammation in both the models were moderate.

There are quite a few reports on the anti-inflammatory activities of acanthaceous plants. Evaluation of anti-inflmmatory properties of the aqueous and alcoholic extracts of *B. cristata* leaves has been carried out by Gambhire *et al*. (2009a, 2009b, 2009c). The studies were conducted using petroleum ether, chloroform and methanol fractions of aerial parts of *B. cristata*. The methanol fraction was revealed to be the most effective in suppressing inflammation. Amoo *et al*. (2009) suggested that the anti-inflammatory effect of *Barleria* species is mediated by the inhibition of the cyclooxygenase enzymes. The experiments conducted by Maji *et al*. (2011) validated the anti-inflammatory activity of *B. prionitis* whole plant extract and also provide evidence for its membrane stabilization and mast cell protection activity. Sing *et al*. (2013) reported the anti-inflammatory property of the whole plant of *B. prionitis*. Lalitha and Sethuraman (2010) reported anti-inflammatory activity of ethyl acetate fraction of roots of *E. viride*, determined by carrageenan-induced paw edema and cotton pellet granuloma models in rats.

Though there are not many reports on the anti-inflammatory properties on species of *Strobilanthes*, there are still quite a few. The protective efficacy of *Strobilanthes callosus* against acute and chronic inflammation has been assayed by Kumar *et al*. (2013). Ho et al. (2003) found that the methanol extract of *Strobilanthes cusia* leaf reduced the paw edema induced by carrageenan in rats to a significant extent. *In vitro* anti-inflammatory and anti-osteoarthritic effect of ethanolic extracts of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus* was reported by Desu *et al*. (2011a). Though there

are numerous reports on the anti-inflammatory properties of various other *Justicia* species, no reports are available regarding the anti-inflammatory activity of *J. betonica*.

Khadse and Kakde (2011) suggested that inhibition of prostaglandin synthesis may be the major mechanism behind the anti-edematous effect shown by aqueous extract fractions of *Barleria prionitis* roots. Wanikiat *et al*. (2008), based on the anti-inflammatory activities of members of Acanthaceae, stated that the powerful anti-inflammatory properties of the extracts are associated with reduced neutrophil migration and so mediated, in part, by inhibition of neutrophil responsiveness. The extracts did not affect neutrophil viability, but treatment of neutrophils with the extracts dose-dependently inhibited N-formyl-methionyl-leucyl-phenylalanine (fMLP) induced chemotaxis, superoxide anion generation, and myeloperoxidase and elastase release.

According to Winrow *et al*. (1993) and Gutteridge (1995), the mechanism of inflammation injury is attributed, in part, to release of ROS from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. ROS affect virtually every step of the development of inflammation and propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor, and interferon. Joint fluid analysis in patients with osteoarthritis showed lower concentrations of the SOD suggesting an increase in oxidative derivatives contributing to damage (Regan *et al*., 2008). Thus free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation (Jiang *et al*., 2013; Geronikaki and Gavalas, 2006). In the present investigation also, the results obtained from the anti-inflammatory assays were more or less in tune

with the results observed for the antioxidant assays. As there is good correlation between the antioxidant and anti-inflammatory activities, it can be concluded that the free radical scavenging property of the extracts must be contributing significantly to their anti-inflammatory property as well. The best anti-inflammatory activities were exhibited by *B. prionitis, S. ciliatus, B. cristata* and *E. viride* which were found to have the highest antioxidant effect. This result suggests that the anti-inflammatory activity could be possibly attributed to the free radical scavenging activity of the extracts.

Considering the production of arachidonic acid metabolites via COX-2 enzyme, that is the main responsible factor for both early and delayed phases of the carrageenan induced inflammation, it can be concluded that the anti-inflammatory activities of the extracts may probably be related to the inhibition of the synthesis or release of COX-2 products. Hamsa and Kuttan, (2011) also assumed that the anti-inflammatory property of plant extracts result mainly from the inhibition of some key enzymes and mediators such as iNOS, COX-2 and proinflammatory cytokines.

Tumour Necrosis Factor (TNF)-α is a multifunctional cytokine that mediates key roles in acute and chronic inflammation. It has the capability to induce the expression of other proinflammatory cytokines, such as interleukin-1 (IL-1) and several chemokines leading to complications like septic shock, rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease (Palladino *et al*., 2003). *In vivo* and *in vitro* studies support the concept that plant-derived compounds can exert their effects through the modulation of the cytokines system (Calixto *et al*., 2004). Similarly, histamine is an important inflammation mediator, a potent vasodilator substance that increases the vascular permeability (Vasudevan *et al*., 2007). Since the extracts effectively suppressed the edema at various stages, it shows that the anti-inflammatory action of the extracts is the result from inhibiting

the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins.

According to Arslan *et al*. (2010) and Govindappa *et al*. (2011), triterpenoids, polyphenols and flavonoids contribute significantly to the antioxidant and anti-inflammatory activities of plant extracts. Anti-inflammatory activity of many plants has been attributed to their high sterol and triterpene (Paduch *et al*., 2007) as well as to the glycosides or steroids present in the extract (Rosa *et al*., 1971). These compounds are known to possess anti-inflammatory, analgesic and antipyretic effects due to their inhibitory effect on enzymes involved in the synthesis of inflammatory mediators (Oweyele *et al*., 2005). Singh *et al*. (2002) observed the remarkable anti-inflammatory property of the terpenoid fraction isolated from *S. callosus* in carrageenan-induced paw edema test. Agarwal and Rangari (2003b) reported anti-inflammatory and antiarthritic activities of lupeol and 19 α-H-lupeol isolated respectively from the roots of *S. callosus* and *S. ixiocephala* in both acute and chronic models of inflammation.

Recent studies conducted by Warokar *et al*. (2010) suggested the role played by several polyphenolic compounds in acting as potent cyclo-oxygenase inhibitors. The anti-inflammatory properties of flavonoids also have been extensively studied and beneficial effects have been demonstrated in many animal models (Talhouk *et a*l., 2007). The anti-inflammatory activities demonstrated by the extracts hence may be related to their phenolic and/or flavonoid content. These components in the extracts could be serving as natural antioxidants to protect cells against inflammatory damage.

As the extracts could significantly inhibit the free radicals, it suggests that the oral administration of the extracts might inhibit the release of free radicals during inflammation and contributes to reinforce the anti-inflammatory effects. Among the six extracts used in the study, the

antioxidant and anti-inflammatory activities of *S. ciliatus* and *B. prionitis* were better and stronger than the others. No significant difference was found in their potency to inhibit the paw edema induced by carrageenan as well as formalin. The main active compounds revealed to be present in these extracts like lupeol, catechin *etc*. either singly or in combination with other compounds might be contributing to these activities. Possible synergestic actions of other components in the extracts should also be taken into account. The study is thus a confirmation for the use of the plants by the traditional healers to treat inflammatory diseases.

The studied pharmacological activities clearly indicate that the roots of both *B. prionitis* and *S. ciliatus* can be used as the source plants of 'Sahachara', as these plants showed the most promising activities in the various antioxidant and anti-inflammatory assays which demonstrate them highly effective in the treatment of chronic inflammatory diseases.

B. PHYTOCHEMICAL ASSAYS

The traditional medicinal system is facing a serious problem of standardization as the same name being used for herbal drugs of diverse botanical origin and vice versa. This causes considerable confusion and there is an urgent need for identification and standardization of the real source of the drug. The full potential of the herbal market is mainly not realised due to lack of knowledge of the chemical composition of most herbal products. According to Heyman and Meyer (2012), the growth potential of the herbal medicine industry can only be achieved if the composition of herbal medicine is standardised to ensure proper quality control and accountability.

With this background, the present study attempts to carry out phytochemical studies of the six taxa used as 'Sahachara' by GC-MS and LC-MS analyses and development of HPTLC fingerprint profile of their

methanolic extracts. The estimation of total phenolic and flavonoid content of the root methanolic extracts of the six plants also has been carried out.

a. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS

Evaluation of a crude drug is an integral part of establishing its correct identity. Phytochemical evaluation is one of the tools for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques. In addition to morphological markers, anatomical, cytological, biochemical and molecular markers are also recently being used for characterization. Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products (Xie *et al*., 2006). A chemical fingerprint is a unique pattern that indicates the presence of multiple chemical markers within a sample. Correct botanical identification and authentication of crude plant materials is an important part of standardization and quality control of traditional drugs and chemical markers play a crucial role for the same (Firenzuoli and Gori, 2007).

Chromatographic fingerprint uses chromatographic techniques to construct specific patterns for recognition of herbal drugs. The developed fingerprint pattern of components can then be used to determine the absence or presence of markers of interest. High performance thin layer chromatography (HPTLC) is an immensely valuable tool for chromatographic fingerprinting which can be visualized and stored as electronic images (Mariswamy *et al*., 2012). Although HPTLC technique has a few limitations, such as the limited developing distance and lower plate efficiency, it is still an effective tool for quality evaluation of herbal drugs due to its simplicity, precision, reproducibility, low operating cost and short analysis time. HPTLC

is a widely accepted technique and has been successfully utilized in the development of chromatographic fingerprints for many botanical drugs (Chen *et al*., 2006b; Anandjiwala *et al*., 2007; Qian *et al*., 2007). The other advantages of HPTLC are the simplicity of the sample preparation and the possibility of analyzing several samples of herbal products simultaneously in a short time. In addition, HPTLC could provide adequate information and parameters for comprehensive identification and differentiation of two closely related herbal medicines (Rathee *et al*., 2011). HPTLC methodology workable at microgram and nanogram scale requires far less solvent consumption than the HPLC method.

The HPTLC profile of six taxa of Acanthaceae, *viz*., *B. cristata, B. prionitis, E. viride, J. betonica, S. ciliatus* and *S. heyneanus,* used as the source plants of an important Ayurvedic drug 'Sahachara' has been established in the present study. A rapid, simple and valid HPTLC method has been undertaken for the phytochemical profiling of root extracts of the six taxa. The identification and documentation of the bands produced by the individual extracts was carried out by comparison of their R_f values. Chromatographic fingerprinting can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern (Rajkumar and Sinha, 2010). The generated HPTLC fingerprint (Plates 8, 9, 10) may be helpful in the identification and differentiation of the different source plants known as 'Sahachara" from each other and also from other crude drugs and adulterants.

The HPTLC analysis of methanol extracts of the six taxa gave major as well as minor (less prominent) spots. The HPTLC studies were conducted using lupeol as the marker compound to evaluate its presence in the different extracts. The R_f (midvalue) of the standard lupeol was found to be 0.63 (Table 28). The peaks with R_f ranging between 0.61 to 0.63 are considered to

be indicative of lupeol. All the six plants investigated in the present study showed the presence of lupeol. The different species showed varying number of peaks ranging from 8 to 13 (Plates 8, 9, 10; Table 28). The root methanolic extract of *J. betonica* showed the maximum number of peaks, *i.e*., 13, followed by *E. viride* with 12 peaks. Extracts of *B. prionitis* and *S. ciliatus* showed 9 peaks each. The least number of peaks were found in *B. cristata* and *S. heyneanus* (8 each).

In HPTLC, the sample in question is separated into its unique biochemical spectrum, the colour bands of which are then scanned in order to obtain the fingerprint. A two-dimensional spectrographic image analysis of the chromatographic plate using a computerized densitometer can provide a useful profile of the sample under test. The HPTLC analysis of the root extracts indicated good difference among the taxa that are used as 'Sahachara'. Though HPTLC fingerprint revealed a good number of peaks, only few were common to these taxa.

The TLC plate after development was derivatized with anisaldehyde sulphuric acid reagent. As already mentioned, HPTLC fingerprints revealed variability with regard to spot number and pattern in all the six taxa examined (Table 28). The bands at R_f 0.13 and 0.63 were common to all the plants. Five taxa, *viz*., *B. cristata*, *B. prionitis*, *J. betonica*, *S. ciliatus* and *S. heyneanus* shared the band at R_f 0.19. The band at R_f 0.09 was common to *B. cristata*, *B. prionitis*, *E. viride* and *J. betonica.* The band at R_f 0.48 was present in *E. viride*, *J. betonica*, *S. ciliatus* and *S. heyneanus*. The band at R_f 0.70 was shared by *B. cristata*, *B. prionitis*, *E. viride* and *S. ciliatus* (Table 28). Root extracts of *B. cristata* showed a unique band at R_f 0.37. Extract of *B. prionitis* showed a unique band at R_f 0.50, whereas *B. cristata* and *B. prionitis* shared two common and specific bands at R_f 0.08 and 0.51. The extract of *E. viride* showed six unique bands at R_f 0.12, 0.24, 0.52, 0.53, 0.66 and 0.68. Seven

unique bands at R_f 0.04, 0.14, 0.16, 0.25, 0.40, 0.49, and 0.64 were observed in *J. betonica*. Extracts of *S. ciliatus* and *S. heyneanus* displayed one unique band each at R_f 0.07 and 0.33 respectively (Table 28). The rest of their bands have been shared with the other plants.

The band at R_f 0.64 and R_f 0.49 were unique to *J. betonica*. These bands could be visible after derivatization and the spot at R_f 0.64 was very prominent and dark brown in colour whereas the band at R_f 0.49 was light brown and less prominent. The plant *J. betonica* could be easily distinguished from the others by the presence of these two bands after the plates have been derivatized (Plate 9). Similarly *S. ciliatus* is unique by the characteristic red coloured bands that were visible under UV 366 nm before derivatisation (Plate 8). It also showed an intense purple spot at R_f 0.48 after derivatization in visible light (Plate 9). The extract of *B. prionitis* showed an intense yellow spot at R_f 0.50 which was highly characteristic and was absent in all other taxa. This unique yellow spot of *B. prionitis* was visible only under UV 366 before derivatization (Plate 8).

The HPTLC profile of the leaf ethanolic extract of *B. cristata* has been generated in a previous study by Narmadha and Devaki (2012b). They prepared separate profiles for alkaloids, flavonoids and phenols in the extract by running the extracts along with standard compounds. Their study included the leaf extracts only. The fingerprints generated from the root methanolic extracts in the current study prove their assumption that fingerprint analysis by HPTLC can be an effective and powerful tool for linking the chemical constituent profile of the plants with botanical identity.

Because common bands and differentiating bands can be useful for identification and authentication of herbal drugs, the HPTLC profile of root methanolic extracts of the six plants of Acanthaceae studied could play an important role in correct identification and authentication of the crude drug

'Sahachara', as besides the two common bands at R_f 0.13 and 0.63, obtained for all the six taxa, there were many other bands characteristic to individual samples by which they can easily be identified and discriminated.

Marker compound means chemical constituents within a medicinal plant that can be used to verify its identity or potency. Ideally, chemical markers should be unique components that contribute to the therapeutic effects of an herbal medicine. As only a small number of chemical compounds were shown to have clear pharmacological actions, other chemical components are also used as markers. It is very difficult to identify correct marker compounds for all traditional medicinals, because some have unknown active constituents and others have multiple active constituents. Hence it is very important to obtain reliable chromatographic fingerprints of a herbal medicine that represent pharmacologically active and chemically characteristic component of the particular herbal drug (Chothani *et al*., 2012; Patil and Shettigar, 2010). l p

Lupeol is a pharmacologically active pentacyclic triterpenoid, reported to possess many beneficial effects as a therapeutic and preventive agent for a range of disorders (Manjula *et al*., 2013). It has been extensively studied for its inhibitory effects on inflammation and reported to have a wide spectrum of medicinal properties that include prominent antioxidant and anticancer activities along with its immense anti-inflammatory potential (Chaturvedi *et al*., 2008; Saleem, 2009). Lupeol is a multi-target agent with immense anti-inflammatory potential targeting several key molecular pathways (Saleem, 2009). Geetha and Varalakshmi (2001) have reported that lupeol can decrease paw swelling in rats by 39%, compared to 35% for the standardized control compound indomethacin. Lupeol is also a potentia hytochemical in controlling arthritis also (Geetha and Varalakshmi, 1999).

Several plants employed in folk medicine to treat inflammatory and arthritic symptoms have been shown to contain lupeol as one of their active principles. Since lupeol was reported earlier from some of the taxa of present investigation (Venkatachalapathi and Ravi, 2012; Kosmulalage *et al.*, 2007; Reneela and Sripathi, 2010) and as no other marker compounds have so far been reported from any of these taxa, lupeol was used as the marker compound for the HPTLC analysis of the root extracts.

All the samples investigated, showed the peak at R_f 0.63 which corresponds to lupeol indicating its presence in all the taxa. But the comparative thickness of the lupeol band in the different tracks corresponding to various taxa was found to vary (Figs 25a, 25b, 25c). This indicates that the relative amount of lupeol varies in the different extracts. A very dense band of lupeol was shown by the track corresponding to *S. ciliatus,* showing it to be one of the major active ingredients. A previous study conducted by Reneela and Sripathi (2010) reported lupeol to be the major constituent of *S. ciliatus*. The results of the present study thus confirm that lupeol might be one of the active compounds conferring this important medicinal plant its apparent pharmacological properties. *S. ciliatus* could be easily identified from others with the help of this exclusive thick band of lupeol in the chromatogram. The chromatogram of *B. prionitis* also showed a fairly thick band of lupeol at R_f 0.63, though not as prominent as that of *S. ciliatus*. Similar bands at the same R_f value, corresponding to lupeol were noted in other plants also. With regard to the width of lupeol band, *B. cristata*, *S. heyneanus* and *E. viride* are comparable, showing moderate thickness. Hence all the plants investigated showed fair to good amount of lupeol except *J. betonica* where the band specific to lupeol at R_f 0.63 was characteristically very narrow, accounting for its low levels of anti-inflammatory potential displayed in the current study.

The Paired Affinity Index (PAI) values calculated based on HPTLC data showed moderate to low chemical similarity among the taxa (Table 29). Highest affinity was observed between *B. cristata* and *B. prionitis* as well as between *S. ciliatus* and *S. heyneanus*, *i.e*., 70% each. The rest of the taxa showed very low similarity percentages in between, varying from 19.04% to 38. 46%. The data from the HPTLC analysis showed relatively low chemical affinity between *B. prionitis* and *S. ciliatus*. However, both these plants displayed maximum activity in antioxidant and anti-inflammatory assays conducted, indicating that both are chemically quite different, though their pharmacological properties are comparable. Even though *B. prionitis* and *S. ciliatus* showed a PAI value of only 38.46, they exhibit average similarity among them, when compared to the values obtained for the other taxa. The lowest PAI value and hence the lowest similarity was noticed between *E. viride* and *J. betonica* (19.04%), even when both showed the highest number of unique spots among the plants studied (Table 29). Though good difference in spot number and pattern was observed, yet the different plants exhibited few common or shared bands (Table 28). The low PAI values obtained reflect the relative average to low affinities between the taxa.

By means of data analysis system and optimized experimental conditions, HPTLC is a feasible technique for development of chromatographic fingerprint to characterize complex herbal extracts just like HPLC and GC (Chen *et al*., 2006b). Furthermore, the colourful picture like HPTLC image provides extra intuitive parameters of visible colour and or fluorescence and, unlike HPLC and GC, HPTLC can simultaneously determine different samples on the same plate. The unique feature of the image of HPTLC, coupled with the digital scanning profile is attractive to the herbal analysts to construct herbal chromatographic fingerprints that can be used in routine quality control of herbal materials and also is suitable for rapid

screening of large number of plant samples. Moreover, this analysis can be performed without any special sample pre-treatment also.

The developed HPTLC chromatograms of root methanolic extracts of *B. cristata*, *B. prionitis*, *E. viride*, *J. betonica*, *S. ciliatus* and *S. heyneanus* may be treated as chromatographic fingerprints and could be used efficiently for the quality assessment of the drug, 'Sahachara'. Besides being specific and precise, HPTLC as a tool is easy to carry out and cost effective. Such an HPTLC fingerprinting can be effective in differentiating the taxa and hence act as biochemical markers for these medicinally important plants in the pharmaceutical industry and plant systematic studies. The developed HPTLC fingerprint provides information about the relative similarities among the source plants and can therefore be successfully applied to identify the source plant in the raw drug form.

The overall quality of a herbal medicine may be affected by many factors, including seasonal changes, harvesting time, cultivation sites, post-harvesting processing, adulterants or substitutes of raw materials, and procedures in extraction and preparation. HPTLC fingerprint has better resolution and can be done with reasonable accuracy in a shorter time. The optimised chromatographic fingerprint is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such database for further studies.

b. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

GC-MS instruments have long been used for identification of large number of components present in natural and biological systems (Binit *et al.*, 2010). GC and GC-MS are unanimously accepted methods for the analysis of

volatile constituents of herbal medicines, due to their sensitivity, stability and high efficiency (Nikam *et al*., 2012). The hyphenation with MS moreover provides reliable information for the qualitative analysis of the complex constituents (Teo *et al*., 2008).

The GC-MS analysis of the methanolic extracts of the six taxa revealed variation in their chemical composition (Figs 26-31; Tables 30, 31). A wide range of chemical constituents was detected in the methanolic extracts obtained from the roots of these plants (Figs 32 (i-xvi). A total of 63 compounds were identified representing about 91.14 – 99.18% of the methanolic extracts. The chemical constituents from the essential oils could be assigned to eight different classes, *viz*., phenols, terpenoids, polyphenols, phytosterols, fatty acid esters, coumarins, furans and miscellaneous (Table 31). Previous reports on the GC-MS analysis of the extracts of the investigated taxa were found to be rare, barring few reports on related species.

The methanolic extract of *Barleria cristata* was characterized up to 94.94% and it comprises of sixteen compounds (Tables 30, 31). The major components identified were estragole (51.10%), β-eudesmol (7.41%), lupeol (6.34%), canthaxanthin (5.08%), desaspidinol (4.72%) and squalene (4.14%) (Table 30). The GC-MS data of the present study reveals that phenols (60.35%) predominated in *B. cristata* and terpenoids form the next major fraction (24.65%), while coumarins and furans could not be detected in the species (Table 31). The methanolic extract of *Barleria prionitis* shows nineteen compounds accounting for 98.1% of the total extract with desaspidinol (35.20%), hydrocinnamic acid (13.66%), lupeol (6.44%), 5-quinolinol (4.95%), lupenone (4.57%) and betulinic acid (3.95%) as its main constituents (Table 30). About 36.72% of the detected components constitute phenols. Terpenoids form the next fraction (25.22%), followed by 18.32% polyphenols (Table 31). Phytosterols and fatty acid esters also

constitute minor fractions of the extract of *B. prionitis*. In this species also furans were found to be absent whereas it also shows small amount of coumarins (1.44%).

A recent study conducted by Nidhi and Kumar (2013) which analyzed the methanolic extract of *B. prionitis* reported the presence of 27 biologically active components and some of the constituents could be found in common. Earlier workers like Sriram and Sasikumar (2012), Natarajan *et al*. (2011) and Rao *et al*. (1999) had reported GC-MS studies on different species of *Barleria* like *Barleria montana* and *Barleria longiflora*. Sriram and Sasikumar (2012) carried out phytochemical screening and GC-MS analysis of the ethanol extract of the leaves of *B. montana* and revealed the presence 26 compounds which included coumarin, sterol, quinone, flavonoids, alkaloids, terpenoids and tannins. Natarajan *et al*. (2011) isolated a total of ten biological compounds from the leaves of *B. montana* by using GC-MS analysis. Rao *et* al. (2010) carried out phytochemical examination of *B. longiflora* that included GC-MS analysis of stem and root extracts and reported the presence of quinones, terpenes and a mixture of various sterols.

The GC-MS characterization of methanolic extract of *Ecbolium viride* has revealed twenty four compounds corresponding to 91.14% of the total (Table 30, 31). The extract contained lupeyl acetate (18.4%), isoeugenyl acetate (9.12%), α-spinasterol (8.69%), lupeol (5.73%), τ-cadinol (5.53%), estragole (4.79%) and betulinic acid (4.76%) as the major components (Table 30). The major fraction of essential oil was terpenoid compounds (43.17%), followed by fatty acid esters (16.89%) and phytosterols (11.66%). Phenols also constitute 9.49% of the extract (Table 31). Chaudhuri *et al*. (2011) investigated the phytochemical composition of *Ecbolium linneanum* by GC and reported the presence of alkaloids, terpenoids, amines, acids and ethers.

Evaluation of methanolic extract of *Justicia betonica* revealed the presence of twenty eight compounds amounting to 97.44% of the total extract with 5-hydroxymethylfurfural (11.58%), lupeyl acetate (8.25%), 2-tert-butyl-4-methoxyphenol (7.6%), geranyl linalool (6.62%) and isoeugenyl acetate (4.63%) as its main constituents (Table 30). Terpenoids (23.27%), fatty acid esters (17.44%) and phenols (16.29%) were the predominant compounds in *J. betonica* (Table 31). In addition, furans (11.58%) were also detected in *J. betonica*. Though there are no previously published reports regarding the GC-MS analysis of *J. betonica*, few reports are available on other species. de Vries *et al*. (1988) analysed the extracts of *Justicia pectoralis* by GC-MS. Ponnamma and Manjunath (2012) investigated the phytocompounds present in the methanolic extract of *Justicia wynaadensis* by GC-MS analysis and identified twenty four compounds among which dihydrocoumarin, phytol and palmitic acid were found as the major components. Pino (2011) analyzed the volatile constituents from leaves of *J. pectoralis* var. *tipo* by GC-MS. Thirty-two compounds were identified, of which the most prominent were nonanal, 1-octen-3-ol and coumarin.

The methanolic extract of *Strobilanthes ciliatus* was characterized by the presence of sixteen compounds that constitute 99.18% of the extract. The main constituents detected in the extract were lupeol (38.7%), syringol (26.61%) and phytol (5.33%) (Table 30). Terpenoids constitute the major part (58.5%), followed by phenols (30.37%). Lesser amounts of phytosterols (6.57%) are also detected in the extract. In *S. heyneanus* the twenty six compounds that were identified amounts to 95.82% of the total extract. The extract contained syringol (15.34%), lupeol (12.25%), α-spinasterol (10.39%), 4-methyl-2-tert-octylphenol (7.12%) and 3-methoxycatechol (4.35%) as the major components (Table 30). The main groups of compounds that could be detected in the extract were phenols (27.45%) and terpenoids (25.78%). This

was followed by phytosterols (12.50%) and minor amounts of polyphenols (4.46%) and fatty acid esters (4.27%) (Table 31).

Previous reports on the phytochemical analysis of *Strobilanthes* species were found to be rare. The essential oil from *S. callosus* from Central India was analyzed by a combination of GC, GC/MS and NMR (Weyerstahl *et al*., 1992). Koay *et al*. (2013) carried out phytochemical studies on the extracts of *Strobilanthes crispus* leaves using IR, GC-MS, MS, ^{1}H and ^{13}C NMR techniques and identified several terpenes, fatty acids, fatty esters, ketones *etc*. Rahmat *et al*. (2006) investigated the chemical constituents of essential oil from *S. crispus* by GC-MS analysis. The analysis revealed the presence of 28 components among which the main constituents were phytol, 2-undecanone, α-cadinol, megastigmatrienone, 2,3-dihydrobenzofuran and eugenol.

c. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS) ANALYSIS

LC-MS technologies are extremely important for characterization and quantification of herbal medicines because full characterization of these products is a desirable goal (Wang *et al*., 1997). It is ideal for the analysis of complex mixtures of compounds which are commonly found in biological matrices such as plant tissues. Herbal drugs, singly or in combinations, contain countless number of components in complex mixes in which no single active constituent is responsible for the overall efficacy rather the complex interactions of numerous ingredients in combination contributes towards the therapeutic efficacy.

One of the advantages of LC-MS is that separation and structural elucidation of compounds can be achieved in a continuous manner without the need for purification or derivatization steps (Kallenbach *et al*., 2009).

Though GC-MS and LC-MS have been found suitable for the determination of phenolic acids, LC-MS is a good choice for the semi-quantitative analysis of phenolic acids, while GC-MS is a useful method for the accurate quantification of low molecular weight phenolic acids (Kivilompolo *et al*., 2007). A major drawback of GC is the need for volatility, as it limits the range of compounds that can be analyzed.

Methanolic extracts of six taxa of Acanthaceae, used as the source plants of the Ayurvedic drug 'Sahachara' were subjected to LC-MS analysis and revealed the existence of several potential chemical principles (Figs 33-38). Previous reports on the LC-MS analysis of the investigated taxa or related taxa was found to be rare, barring the reports on species of *Justicia* (Calderon *et al*., 2013) and *Strobilanthes crispus* (Hanisa *et al*., 2012).

The methanolic extract of *B. cristata* which was explored through LC-MS analysis disclosed seventeen phytochemicals, among which phenolic compounds exhibit dominance, followed by flavonoids, fatty acids, tepenoids and phytosterols (Table 32). Methyleugenol, a phenolic compound was found to be the most abundant among the constituents that were identified (25.16%). The other major compounds detected were quercetin, which is a flavonoid (10.75%), the fatty acid arachidonic acid (10.81%) and the phenolic compound coumaric acid (10.42%). Cinnamic acid (5.10%), β-sitosterol (5.23%) and farnesyl butanoate (5.54%) were the other major components (Table 32).

The phytochemical constitution of *B. prionitis* detected through LC-MS reports the presence of fifteen phytochemical components (Table 33). LC-MS ascertains flavonoids as the major class of compounds present in the methanolic extract of *B. prionitis* followed by terpenoids. Apigenin, luteolin, catechin and luteone seems to be the prominent flavonoids present in the methanolic extract of *B. prionitis*, as revealed by LC-MS analysis. The

analysis exposes catechin (20.76%) as well as betulininic acid (20.14%) in sizeable amounts, barlerin (13.07%), β-sitosterol (10.42%) and α-tocopherol (7.92%) in moderate amounts and lupeol (4.93%), apigenin (3.38%), conessine (3.23%), myricetin (2.81%) and luteolin (2.16%) in lesser amounts (Table 33).

Ecbolium viride methanolic extract which was explored through LC-MS analysis disclosed seventeen phytochemicals, among which phenolic compounds exhibited dominance, followed by flavonoids and terpenoids. Moderate amounts of fatty acids have also been detected (Table 34). Coumaric acid (11.79%), wogonin (11.52%) and myricetin (11.07%) were found to be the probable compounds in *E. viride* as revealed by LC-MS analysis. Ascorbic acid (7.73%), catechin (8.92%) and quercetin (8.01%) were also detected in moderate amounts. The fatty acid linoleic acid (6.90%), cinnamic acid (4.42%) and stigmasterol (4.79%) were seen in lesser amounts. Compounds like vanillic acid (3.76%), oleic acid (3.16%), 3β-hydroxymanool (3.34%), taxifolin (3.67%) and chlorogenic acid (2.17%) were detected in minor amounts.

Phytochemical assessment of the root methanolic extract of *Justicia betonica* exposed the highest number of compounds detected among all the taxa. Out of the 22 compounds, phytosterols and terpenoid compounds formed the major portion, followed by lesser amounts of phenolics and small amounts of flavonoids, esters, fatty acids and phenols (Table 35). When compared to the other taxa, the proportion of phenolic compounds was much lesser in *J. betonica*. The chief constituents in *J. betonica* as revealed by LC-MS were spinasterol (23.62%) and ursolic acid (22.93%). The sesquiterpenoid, cannabidiol was found in good amounts (8.88%). Another major compound detected was sinapine (6.06%). The fatty acid, arachidonic acid as well as the phenolic compound, coumaric acid also was detected in

moderate amounts (5.45% and 4.78% respectively). The rest of the compounds were all present in minor amounts.

LC-MS analysis of the methanolic extract of *Strobilanthes ciliatus* revealed thirteen compounds, the lowest number noted among the six taxa. The major classes of compounds detected were terpenoids, followed by phenols. Appreciable amounts of flavonoids were also detected. The triterpenoid compounds lupeol (15.35%) and asiatic acid (15.12%) were found to be present in highest quantities. Coumaric acid (10.42%), cannabidiol (9.59%), sinapine (6.97%) and gallic acid (6.29%) were found in moderate amounts. The other important compounds detected were quercetin (4.24%), chlorogenic acid (4.61%), cinnamic acid (5.10%) and α-tocopherol (6.71%). Luteolin (3.51%) and spinasterol (3.82%) were also detected in lesser amounts (Table 36).

S. heyneanus extract revealed the presence of fifteen compounds (Table 37) out of which terpenoids were found to be most dominant, followed by flavonoids and phenolic compounds. Among the terpenoid compounds, asiatic acid (24.61%) and lupeol (5.33%) were of considerable biological importance. Similarly the presence of the terpenoid, ursolic acid (19.1%), the phenolic compounds like cinnamic acid (6.59%) and coumaric acid (6.41%) and the flavonoids *viz*., quercetin (7.74%) and taxifolin (3.11%) were also detected. The minor and trace phytochemicals detected from the methanolic extracts of *S. heyneanus* include luteone, diosmetin, farnesyl butanoate, geranyl linalool, ellagic acid *etc*. (Table 37).

d. ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF COMPONENTS OF THE ROOT METHANOLIC EXTRACTS

The strategic screening programme for phytochemicals and pharmacological activities of plants used in traditional medicine has provided scientific evidence for their rational use in prevention and treatment of infections and oxidative stress related diseases. Many plant derived compounds have been shown to possess significant antioxidant, anti-inflammatory and other therapeutic effects that they represent potential molecules for the development of new drugs, which could be targeted against the treatment of chronic inflammatory states.

According to Preethi *et al*. (2009), plant extracts containing flavonoids, terpenoids, coumarins *etc* show promising anti-inflammatory activity by modulating the activity of pro-inflammatory cytokines as well as by inhibiting the expression of COX-2. Phenolics, flavonoids and tepenoids are potent antioxidants and their chemo-preventive properties are generally believed to be due to their ability to scavenge endogenous ROS. They interact with important cellular targets, mainly by controlling the gene expression of relevant key pro-inflammatory substances involved in the genesis and maintenance of inflammation (Calixto *et al*., 2003).

The root methanolic extracts of the six taxa, used as the source plants of the drug ‘Sahachara’ and which could elicit varying degrees of antioxidant and anti-inflammatory activities contain a wide range of biologically active phytoconstituents, well known for their antioxidant, anti-inflammatory and other pharmacological properties, that act either in isolation or in combination with other constituents.

The highest activities, in all the assays were displayed by extracts of *Barleria prionitis* and *Strobilanthes ciliatus*. The activities of *B. cristata*, *Ecbolium viride* and *S. heyneanus* were found to be moderate, yet good. The extract of *J. betonica* seems to be the least potent with the lowest antioxidant and anti-inflammatory activities among the six taxa studied. Plant polyphenols, terpenoids, flavonoids and sterols were the major classes of compounds detected from the phytochemical analysis of the different extracts. The protective effect of plant polyphenols against oxidative as well as inflammatory damages of the tissue has well been documented in various assay models. Sergent *et al*. (2010) proposed phenolic compounds as an alternative natural approach to prevent or treat chronic inflammatory diseases as they are considered to possess marked anti-inflammatory effects. The pharmacological actions of phenolic antioxidants stem mainly from their free radical scavenging and metal chelating properties as well as their effects on cell signalling pathways and on gene expression.

The next category of compounds adding to the therapeutic properties of the extracts can be flavonoids. Flavonoids are broadly distributed in plants and have been reported to have marked *in vitro* and *in vivo* antioxidant and anti-inflammatory properties (Soetan, 2008). Studies of Ismail *et al*. (2004) suggested the direct correlation between total antioxidant activity and flavonoid content. Apart from the above classes of compounds, terpenoids are also detected in substantial amounts in the extracts, contributing to the observed biological activities. Terpenoids are one of the most extensive and varied structural compounds occurring in nature, displaying a wide range of biological and pharmacological activities. De las Heras and Hortelano (2009) suggested the clinical potential of terpenoids to inhibit cell signaling pathways involved in the inflammatory response of the body such as Nuclear Factor (NF)-κB.

Lupeol was found to be one of the biologically active major compounds present invariably in all the extracts. Lupeol exhibits a broad spectrum of biological activities and can be used as a chemopreventive to avoid several diseases (Gallo and Sarachine, 2009). Lupeol is a multi-target agent with immense anti-inflammatory potential targeting key molecular pathways. Fernandez *et al*. (2001) stated that the application of the pentacyclic triterpenes could decrease edema formation and the effect is likely to involve decreased prostanoid production. It is noteworthy that lupeol at its effective therapeutic doses exhibits no toxicity to normal cells and tissues (Wal *et al*., 2011). Lupeol showed significant inhibitory activity against intracellular ROS generation (Jin *et al*., 2012). Roots of *Strobilanthes callosus* and *S. ixiocephala* have long been used for inflammatory disorders in folk medicines and their medicinal property is thought to be conferred by lupeol (Agarwal and Rangari, 2003b). Several plants used in indigenous systems of medicine to treat inflammatory diseases are shown to contain lupeol as one of their active principles. According to Gallo and Sarachine (2009), lupeol has a different mode of action in comparison with the conventional NSAIDs that are non-specific COX inhibitors and cause peptic ulceration as side effect.

Substantial amounts of biologically active phytosterols and fatty acids are also found to be present in the extracts. Muslim *et al*. (2010) reported the presence of phytosterols such as alpha-sitosterol, campesterol, phytol and stigmasterol in the methanolic and aqueous extracts of *S. crispus* and suggested that these might contribute to the antioxidant property. The therapeutic potential of spinasterol against neurodegenerative diseases has been reported by Jeong *et al*. (2010). Therapeutic activities of fatty acids like anti-inflammatory, antioxidant, free radical scavenging, anti-hyperlipidemic activities have been suggested by many workers (Beg *et al*., 2011; Singh *et al*. 2008b). Several fatty acids, including linoleic acid, myristic acid, palmitic

acid and stearic acid have been found to exhibit anti-inflammatory properties by inhibiting COX-1 and COX-2 activities (Ringbom *et al*., 2001).

Majority of the antioxidants including quercetin and luteolin act by affecting the NF-κB pathway and inhibiting NO production. Quercetin suppresses COX-2 expression by inhibiting tyrosine kinases important for induction of COX-2 gene expression (Lee *et al*., 1998). Quercetin has a higher reduction potential, so can decrease oxidative stress and increase antioxidant enzyme activity (Zhang *et al*., 2011).

Reports on the antioxidant and anti-inflammatory nature of phytochemicals are many such as catechins (Babu and Liu, 2008; Lotito *et al*., 1999; Medvidovic-Kosanovic *et al*., 2010; Nakanishi *et al*., 2010), luteolin (Dillard and German, 2000; Calixto *et al*., 2003), apigenin (Lee *et al*., 2007; Begum and Prasad, 2012), wogonin (Chi *et al*., 2001; Yang *et al*., 2013), gallic acid (Jiang *et al*., 2011; Deng and Fang, 2012), chlorogenic acid (Li *et al*., 2009; Kang *et al*., 2010), α-tocopherol (Onyeike *et al*., 2010), β-sitosterol (Dillard and German, 2000; Nirmal *et al*., 2012), gallic acid, myricetin, quercetin (Soobrattee *et al*., 2005), ferulic acid (Balasubashini *et al*., 2004) and betulinic acid (Recio *et al*., 1995; Moghaddam *et al*., 2012).

The other phytochemicals reported to have reputed antioxidant and anti-inflammatory activities include quinolinol compounds (Chobot *et al*., 2011), asiatic acid (Ramachandran and Saravanan, 2013), coumaric acid (Pragasam *et al*., 2013), methyl eugenol (Miguel, 2010; Choi *et al*., 2010b); cinnamic acid (Sova, 2012), lupeyl acetate (Lucetti *et al*. 2010), spinasterol (Jeong *et al*., 2010), ascorbic acid (Rizzo *et al*., 2008) and myricetin (Choi *et al*., 2010a; Moser, 2008).

The analysis of the phytochemical constitution of the extracts revealed that the methanolic extracts of the taxa used in the present investigation

contain the aforesaid compounds, which may exert the multifaceted action on the free radicals and hence is contributing to the high antioxidant and anti-inflammatory potentials observed in the study. The variation in the activity may be due to the difference in the relative proportion of active components and the type of the constituents present in the respective taxa. Interaction with other elements of the mixture is another factor that may also contribute to free radical scavenging activity (Castro *et al*., 2006; Jimenez *et al*., 2009). The relatively high antioxidant and free radical scavenging activity of extracts containing low phenolic content suggests that the type of phenolics is determinant for these activities rather than their amounts (Conforti *et al*., 2008). Thus it can be concluded that the combined action of the active ingredients present in the various extracts through their free radical scavenging and inhibition of mediators of inflammation especially cytokines and prostaglandins may be conferring the anti-inflammatory activity (Preethi *et al*., 2009).

Catechin along with betulinic acid, β-sitosterol, α-tocopherol and lupeol might be the probable reason for the high antioxidant and anti-inflammatory potential of *B. prionitis*. The LC-MS data revealed that betulinic acid, catechin, barlein and β-sitosterol were the major components in *B. prionitis* extract. The GC-MS analysis detected desaspidinol, hydrocinnamic acid, lupeol, 5-quinolinol and lupenone in *B. prionitis*. The pharmacologically active compounds detected with the help of both GC-MS and LC-MS assays in *S. ciliatus* include lupeol, coumaric acid, cannabidiol, asiatic acid, gallic acid, syringol, phytol, betulinic acid, β-sitosterol *etc*. The high anti-inflammatory activity shown by *S. ciliatus* may be caused by the presence of larger proportion of lupeol. Methyleugenol, estragole, quercetin, coumaric acid, cinnamic acid, arachidonic acid, lupeol, β-sitosterol *etc*. were the biologically active components obtained by the phytochemical analyses of the methanolic extract of *B. cristata*. In *E. viride* lupeyl acetate, isoeugenyl

acetate, α-spinasterol, lupeol, coumaric acid, myricetin, wogonin, betulinic acid, ascorbic acid, catechin, quercetin *etc*. were found to be present as revealed by GC-MS and LC-MS analyses, which might be responsible for the fairly good antioxidant and anti-inflammatory activities observed in the study.

e. DETERMINATION OF TOTAL PHENOLICS AND FLAVONOIDS

Phenolic compounds are plant substances which possess in common an aromatic ring bearing one or more hydroxyl groups. There are about 8000 naturally occurring plant phenolics and about half of this number is flavonoids (Harborne and Herbert, 1993). Plant polyphenols and flavonoids have drawn increasing attention due to their potent antioxidant properties (Okpuzar *et al*., 2009) and their marked effects in the prevention of various oxidative stress associated diseases. Hence the present study also attempted an assessment of total phenolic and flavonoid content of the root extracts of the six taxa studied.

The Folin–Ciocalteu assay for total phenolic content gives a general measure of phenolic content (Samappito and Butkhup, 2010) and is used routinely in characterizing and standardizing botanical samples. The method is based on oxidation of phenolics by a molybdotungstate in Folin–Ciocalteu reagent to yield a coloured product with λ_{max} 745 – 750 nm. Total flavonoid content was determined in the sample extracts by reaction with sodium nitrite, followed by the development of coloured flavonoid-aluminium complex formation using aluminium chloride in alkaline condition which can be monitored spectrophotometrically at maximum wavelength of 510 nm.

There have been many reports on members of Acanthaceae being rich sources of polyphenolic compounds (Sawadogo *et al*., 2006; Ahmad *et al*., 2013). Though there are reports on the total phenolic and flavonoid content of

the related species of the taxa investigated, only very few studies have been made on the taxa under study, barring the reports of Jaiswal *et al*. (2010a), Chetan *et al*. (2011), Narmadha and Devaki (2012a) and Babu *et al*. (2011). In the above studies also, the phenolic and flavonoid estimations of the leaf or the whole plant alone has been carried out, with little attempts made on the analysis of the root extracts.

The content of total phenolics and flavonoids of the different extracts are summarized in Table 38 and expressed as mg gallic acid equivalent (GAE) and mg quercetin equivalent (QE)/g DW of the extracts, respectively. The total phenolic content was expressed by reference to the standard curve ($y = 0.030x – 0.080$, $R^2 = 1$; Fig 39). The amount of total phenolic compound was calculated quite high in the root extract of *B. prionitis* (22.64 ± 1.93 mg/g of gallic acid equivalent). Extract of *B. cristata* roots showed the next highest total phenolic content (19.34 ± 0.40 mg GAE/DW) and antioxidant activity. The extracts of *S. ciliatus* (15.91 ± 0.70) and *E. viride* (15.16 ± 1.28) also showed relatively high phenolic content. However, the extract of *S. heyneanus* showed an average value of phenolic content (10.8 ± 0.60). The total phenolic content of *J. betonica* was significantly lowest and hardly accountable for the presence of phenolics when compared to that of the other taxa (Table 38).

The total flavonoid content was expressed as quercetin equivalents by reference to standard curve ($y = 0.004x + 0.017$, $R2 = 0.991$; Fig. 40). Similar to phenolics, the content of total flavonoids also was found to vary, with *S. ciliatus* (6.36 ± 0.46 mg QE/g DW) extract containing the highest flavonoid content among the various extracts. This was followed by *B. prionitis*, *B. cristata*, *E. viride*, *S. heyneanus* and *J. betonica* in the decreasing order. The methanolic root extract of *B. prionitis* has also shown good flavonoid content. The total flavonoid content of *B. cristata*, *E. viride* and *S. heyneanus*

was moderate and does not show significant differences among one another. In one gram each of the above three extracts, more than 3.5 mg QE of flavonoids was detected (Table 38).

These findings show the richness of crude extracts of *B. prionitis* on phenolic content which was roughly 2-fold and 8-fold higher than those of *S. heyneanus* and *J. betonica* extracts respectively ($p < 0.05$). The best antioxidant activities were exhibited by *B. prionitis*, *B. cristata*, *S. ciliatus* and *S. heyneanus* which were found to have the highest phenolic content also.

Natural phenolics have been reported to inhibit transcription factors closely linked to inflammation like NF-κB (Tsoyi *et al*., 2008), pro-inflammatory cytokines release (Karlsen *et al.*, 2007) and enzymes such as COX-2 (Hou *et al*., 2007), lipoxygenases (LOX) (Hong *et al*., 2001) and inducible nitric oxide synthase (iNOS) (Pergola *et al*., 2006), which mediate inflammatory processes. Polyphenolic compounds seem to have an important role in lipid oxidation stabilization also (Gursoy *et al*., 2009). Hagerman *et al*. (1998) suggested that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by phenolics than their specific functional groups.

The flavonoid compounds perform antioxidant function through suppressing ROS formation, scavenging ROS and regulating or protecting antioxidant defenses (Zhang *et al*., 2010). COX is an enzyme that plays an important role as inflammatory mediator and is involved in the release of arachidonic acid, which is a precursor for biosynthesis of prostaglandins and prostacyclin. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain and this is the mechanism of action of well known class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and ibuprofen. Many flavonoids have shown to inhibit the COX pathway and blocking the release of arachidonic acid. Another

mechanism by which flavonoids act is their suggested ability to inhibit neutrophil degranulation (Nijveldt *et al.*, 2001).

Data in the literature about the relation between concentration of phenolic compounds and antioxidant activity are contradictory. According to Plazonic *et al*. (2009), the protective role of flavonoids and phenolic acids in carcinogenesis, inflammation, atherosclerosis and thrombosis appear to stem from their high antioxidant capacity. More recently, several researchers have shown a correlation between total phenolic content and antioxidant activity (Plazonic *et al*., 2009; Wojdylo *et al*., 2007). While some authors have observed high correlation, others find no direct correlation or only a very weak one (Czapecka *et al*., 2005; Wong *et al*., 2006). Gursoy *et al*. (2009) explained this poor correlation might be because of the antioxidant action being raised by other biologically active substances (Gursoy *et al*., 2009). According to Thangavel and Gupta (2010), though phenolic compounds may contribute directly to antioxidant free radical scavenging activity, there are reports indicating that terpenoid compounds also contribute to antioxidant activity. Stratil *et al*. (2007) suggested that we cannot exclude the presence of additional antioxidant components in the extracts that could contribute to these differences because some non-phenolic compounds can also reduce the Folin reagent. The free radical scavenging potential seemed to be dependent not only on the amount, but also on the type of the flavonoids or phenolics present in the extract.

In the current study, however, the scavenging properties displayed by the different extracts appear to be almost relative to the total phenolic and flavonoid content. The extracts which had high phenolic and flavonoid content showed good percentage of inhibition. The high inhibition values shown by *B. prionitis* extract in antioxidant and anti-inflammatory assays might be due to the high concentration of phenolic compounds present in the

extract. The root methanolic extracts of *J. betonica* exhibited minimum percentage of inhibition in the assays. This might be because of the lower level of phenolic and flavonoid content. In any way, due to their unique action mechanisms and significant *in vivo* activity, phenolics and flavonoids are considered to be reasonable candidates for the development of new anti-inflammatory drugs that could exert a chemo-preventive role towards cardiovascular and degenerative diseases.

The highlights of the present investigations are:

- The chromosome complements of six members of Acanthaceae, *viz*., *Barleria cristata* (2n = 40), *B. prionitis* (2n = 40), *Ecbolium viride* (2n = 36), *Justcia betonica* (2n = 34), *Strobilanthes ciliatus* (2n = 28) and *S. heyneanus* (2n = 32), used as the source plants of an important Ayrvedic drug 'Sahachara' was revealed.
- The karyomorphometric features like total chromosome length, average chromosome length, range of chromosome length, nature of primary constriction, number of chromosomes with secondary constriction, disparity index, variation coefficient and total forma percentage of the six taxa exhibited variations. The karyotype formulae of all the six taxa were established.
- The antioxidant and anti-inflammatory activities of the root methanolic extracts of the taxa were assessed using different assay systems *in vitro* and *in vivo*. The methanolic extracts of all the six taxa showed significant, but varying degrees of pharmacological activities in all the assays used.
- The different antioxidant assays used in the study *i.e*., ABTS, DPPH, superoxide and hydroxyl radical scavenging and inhibition of lipid peroxidation revealed that all the selected taxa could very well inhibit the various free radicals and lipid peroxidation. The maximum

antioxidant activity was shown by *B. prionitis* followed by *B. cristata* and *S. ciliatus*. The lowest antioxidant effect was shown by *J. betonica*.

- The methanolic extracts of all the six taxa were screened for anti-inflammatory property using carrageenan induced (acute inflammation) and formalin induced (chronic inflammation) paw edema models in Swiss Albino mice. A statistically significant reduction in the edema volume was shown by all the six taxa. The extracts of *B. prionitis* and *S. ciliatus* were demonstrated to be highly effective in bringing down inflammation in both acute and chronic models. Moderate activity was shown by *B. cristata* and *E. viride*. This was followed by *S. heynenaus* and *J. betonica*.

- Chemical analysis of the root methanolic extracts using HPTLC, GC-MS and LC-MS techniques revealed chemical complexity, variability as well as affinity among the taxa. All the six taxa were chemically characterized.
- Chemical fingerprints have been generated for each taxon using HPTLC that can be used for identification and quality evaluation of the plants used as 'Sahachara'. HPTLC chromatograms revealed the highly unique profiles generated for each taxon. Using a simple HPTLC analysis of the root extracts, the plants that are used as 'Sahachara' can be easily distinguished from each other.
- Lupeol was used as the marker compound in the analysis, which is considered as one of the pharmacologically important ingredients of the taxa investigated. HPTLC profiles showed the presence of lupeol band in all the chromatograms, with the most prominent band in *S. ciliatus*.
- GC-MS analysis of the methanolic extracts of the six taxa detected 63 components of which phenols, terpenoids, polyphenols, phytosterols,

fatty acid esters and organic acids form the major classes. Phenols predominated in *B. cristata*, *B. prionitis* and *S. heyneanus* while terpenoids form the major fraction in *E. viride, J. betonica* and *S. ciliatus*.

- LC-MS analysis of the extracts identified many biologically active phytochemicals in the root methanolic extracts of these plants which could be assigned to the five major classes of compounds: phenolics, terpenoids, flavonoids, fatty acids and fatty acid esters.

- The measurement of total phenolic and flavonoid content of the various extracts was also undertaken during the study. The phenolic and flavonoid content was found to vary, which could account for the distinct antioxidant and anti-inflammatory activities of the different samples. The total phenolic content of *B. prionitis* extract was quite high, followed by *B. cristata* and *S. ciliatus*. The methanolic extract of *S. ciliatus* revealed the highest total flavonoid content, followed by *B. prionitis.* Methanolic extract of *J. betonica* showed the least content of total phenolics and flavonoids among all the extracts.

As a conclusion of the present investigation, the karyomorphometric analysis of the taxa investigated revealed their chromosome constitution, chromosome architecture and karyotype formulae. A densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile for each taxon. Most of the taxa, which are used as the source plants of 'Sahachara' exhibited excellent antioxidant and anti-inflammatory activity. However, *B. prionitis* as well as *S. ciliatus* were found to be outstanding in reducing both acute as well as chronic inflammation with relatively the highest content of phenolics and flavonids which might be the probable reason for the manifestation of their prominent effects. This potency may be

exploited for their chemoprotective effects in the treatment of various inflammatory disorders with an element of oxidative stress in their pathology.

The outcome of the study thus suggests that it is appropriate to use the roots of both *B. prionitis* and *S. ciliatus* as the source of 'Sahachara' as these plants showed the most promising activities in the antioxidant and anti-inflammatory assays and the highest content of active phytoconstituents, that could make them highly effective in the treatment of chronic diseases like rheumatism that result from the damaging effects of free radicals and inflammation.

In the changing global scenario the interest towards plants with medicinal value is increasing substantially in the primary healthcare system, both in the developed and developing countries. Future research should concentrate to isolate and characterize the active chemical principles in these taxa. Efforts can then be made to find out the probable mechanism of observed activities followed by clinical drug experiments.

SUMMARY

A large proportion of the world relies on the traditional systems of medicines to meet their primary health care needs. Herbal preparations with known efficacy in traditional system of medicine must be proved through scientific experiments and tested both in animals and humans. The strategic screening programme for phytochemicals and pharmacological activities of plants used in traditional medicine has provided scientific evidence for their rational use. Recent studies emphasize that recurrent inflammations associated with oxidative stress form the underlying cause of various chronic diseases. The strategy for reducing inflammation and oxidation status by the use of natural products thus could lead to effective prevention or treatment of these diseases.

The Acanthaceae family is an important source of several therapeutic drugs and many of its members are extensively used as folklore medicines worldwide. 'Sahachara' is a well known Ayurvedic drug used against rheumatism and neurological disorders. The root is the official part that forms the major ingredient of several important Ayurvedic preparations. A great deal of adulteration and substitution is met with many of the commercially available raw Ayurvedic drugs owing to the confusion caused by the different local names these plants are known by. The correct identity of the species to be used as 'Sahachara' is controversial as a variety of herbs of the family Acanthaceae are being used as the plant source of the drug 'Sahachara'.

The present investigation is a preliminary effort to characterize the cytological constitution by a detailed karyomorphometric analysis and to evaluate the antioxidant and anti-inflammatory potentials of six taxa of Acanthaceae that are commonly used as the source plant of 'Sahachara', *viz*., *Barleria cristata*, *B. prionitis*, *Ecbolium viride*, *Justicia betonica*,

Strobilanthes ciliatus and *S. heyneanus*. The present study also aims to investigate their major active chemical principles that are responsible for these activities and to quantify the total phenolics and flavonoids in their root methanolic extracts. The study also attempts to provide chemical fingerprinting of root methanolic extracts of different source plants by HPTLC with a view to discriminate the different source plants that might help their differentiation from one another.

A. METHODOLOGY

Mitotic observations of the root tip cells of the above plants have been carried out. Young healthy root tips were collected from all the six taxa and mitotic studies were conducted with the help of improved techniques. Karyograms were generated from the photomicrographs with the aid of computer-based programs such as Adobe Photoshop, AutoCAD and Microsoft Excel. On the basis of arm ratio and centromeric indices, homologous chromosomes were identified. Karyotype formula was calculated depending upon the length of the chromosome, position of centromere and presence or absence of secondary constriction.

The antioxidant activity of the crude methanolic extracts of roots of the taxa was determined by five *in vitro* assays. The extracts were evaluated for their free radical scavenging activities against DPPH radical, ABTS radical, superoxide radical, hydroxyl radical and inhibition of lipid peroxidation. The methanolic extracts of all the six taxa were screened for anti-inflammatory activity using carrageenan induced acute inflammation and formalin induced chronic inflammation models in Swiss Albino mice.

In order to identify the active chemical compounds behind the antioxidant and anti-inflammatory activities, chemical profiling of the methanolic extracts were carried out with the help of GC-MS and LC-MS

analysis. HPTLC analysis of the methanolic extracts was also performed using lupeol as the marker compound. The determination of the total phenolic and flavonoid contents of the extracts was also carried out respectively by Folin–Ciocalteu and aluminium chloride colourimetric methods.

B. UPSHOTS OF THE WORK

Detailed chromosomal analyses were conducted for the first time in *B. cristata*, *B. prionitis*, *E. viride*, *J. betonica*, *S. ciliatus* and *S. heyneanus*. The six taxa studied exhibit distinct karyomorphological features. The chromosome counts of the different plants were as follows, *B. cristata* (2n = 40), *B. prionitis* (2n = 40), *S. ciliatus* (2n = 28), *S. heyneanus* (2n = 32), *E. viride* (2n = 36) and *J. betonica* (2n = 34). The number of chromosomes with secondary constriction range from two to six. The karyotype formulas of the different plants were found to be 34nm + 6 nsm(-), 36nm + 4 nsm(-), 26 nm + 2 nsm(-), 28 nm + 4 nsm(-), 36 nm and 32 nm + 2 nsm(-) respectively for *B. cristata*, *B. prionitis*, *S. ciliatus*, *S. heyneanus*, *E. viride* and *J. betonica*. The karyotypes were characterized by the presence of small chromosomes in general, except those of *B. prionitis* and *B. cristata*. The karyomorphometrical characters point towards the more primitive status of the karyotypes. The variations displayed by these karyotypes could not be correlated or interpreted in an evolutionary order with respect to the six plants. Among the 6 taxa, *B. cristata* and *B. prionitis* showed the most asymmetric karyotypes.

Experiments conducted to investigate the antioxidant and anti-inflammatory nature of the different taxa yielded positive results. The oral administration of the methanolic extracts (100 mg/kg and 250 mg/kg body weight) of all the taxa inhibited the formation of different free radicals as well as lipid peroxidation. ABTS and DPPH radical scavenging activity was found to be maximum in *B. prionitis*, followed by *B. cristata, S. ciliatus, E. viride* and the least activity was noticed in *S. heyneanus* and *J. betonica*. *B. prionitis*

was the most potent in scavenging superoxide and hydroxyl radicals also; the radical scavenging properties of the other extracts too are in accordance with the results obtained for the other radicals. Maximum inhibition of lipid peroxidation was shown by *B. cristata*, followed by *B. prionitis and S. ciliatus.* The results demonstrate that promising results are shown by extracts of *B. prionitis*, *S. ciliatus*, *B. cristata* and *E. viride*, as revealed by their low IC_{50} values.

The oral administration of the different extracts could produce a significant reduction in paw edema volume in the treated animals both at the low as well as high doses of the extract given. The results obtained for the anti-inflammatory assays were in agreement with that obtained for antioxidant assays. Among the six taxa studied, *B. prionitis* and *S. ciliatus* were found to possess maximum activity in the carrageenan induced acute inflammation as well as in formalin induced chronic inflammation models. The percentage inhibition of paw edema at the higher dose was comparable to the response of the standard group (diclofenac 10 mg/kg b. wt). This was followed by *B. cristata*, *E. viride* and *S. heynenaus* which exhibited moderate inhibition. The least reduction in edema and so the least potency was shown by *J. betonica* extract in both the models used.

A densitometric HPTLC analysis of the methanolic extracts of taxa used as 'Sahachara' was performed for the development of characteristic fingerprint profile, which makes their identification and characterization possible due to the improved reproducibility of HPTLC. The HPTLC studies using lupeol as the marker compound revealed major significant spots and other less evident ones. HPTLC studies indicated relatively good amount of variability among the different species used as 'Sahachara', with regard to spot number and pattern. The fingerprints of the different extracts have been established and the generated profiles are revealed to be highly unique for

each taxon which may be used as marker for identification. The methanolic extracts of all the taxa showed the presence of lupeol. The data from the HPTLC studies were used to calculate the paired affinity indices (PAI) for the different species. The results obtained from the HPTLC analysis showed that there is only moderate similarity between the different species that are used as 'Sahachara'.

Exploration of methanolic extracts of the six plants by GC-MS analysis revealed 63 components in which the major classes were phenols, terpenoids, polyphenols, phytosterols, fatty acid esters, coumarins and furans. Phenols dominated in *B. cristata*, *B. prionitis* and *S. heyneanus* while terpenoids form the major fraction in *E. viride*, *J. betonica* and *S. ciliatus*. Substantial amount of phenols too is detected in *S. ciliatus* along with terpenoids. Relatively higher amounts of terpenoids were found in *B. cristata*, *B. prionitis* and *S. heyneanus*. Polyphenols have been detected in most of the extracts and the maximum content of polyphenols was shown by *B. prionitis.* Fatty acid esters are reported from *B. cristata*, *B. prionitis*, *E. viride*, *J. betonica* and *S. heyneanus*. All the extracts showed the presence of phytosterols in varying amounts. Lesser amounts of coumarins and furans were also observed in some plants.

The several compounds detected by LC-MS in the root methanolic extracts fall in five major classes: phenolics, terpenoids, flavonoids, fatty acids, esters and steroids. The phytochemical analysis through LC-MS analysis revealed that the major active components in the methanolic extracts of the six taxa were; methyleugenol, arachidonic acid, quercetin and coumaric acid in *B. cristata*; catechin, betulinic acid, barlerin and β-sitosterol in *B. prionitis*; coumaric acid, wogonin and myrcetin in *E. viride*; spinasterol and ursolic acid in *J. betonica*; lupeol, asiatic acid and coumaric acid in *S. ciliatus* and in the case of *S. heyneanus*, asiatic acid and ursolic acid

predominated. These components either singly or in combination are responsible for the pharmacological effects displayed by the potential taxa.

Methanolic extracts of the roots of these taxa were also examined for their total phenolic and flavonoid content, which was found to vary among the different extracts. The methanolic extract of *B. prionitis* revealed the highest total phenolic content. The total phenolic content of *B. cristata* also was quite high, followed by those of *S. ciliatus, E. viride and S. heyneanus.* The extract of *S. ciliatus* showed the highest flavonoid content among the six taxa. It is followed by *B. prionitis*, *B. cristata*, *E. viride*, *S. heyneanu*s. Methanolic extract of *J. betonica* showed the least content of total phenolics and flavonoids.

C. CONCLUSION

Analysis of the results of the present study together with the previous reports proved that the extracts of the six taxa used as the source plants of the drug 'Sahachara' or some of its major components along with minor ones act as potential active principles involved in the scavenging of reactive oxygen species and free radicals involved in the generation and maintenance of the various inflammatory mediators. As inflammatory processes are involved in the pathogenesis of the most common chronic non-communicable diseases, these antioxidant and anti-inflammatory agents could be fundamental in prevention of carcinogenicity, cardiovascular and neurodegenerative changes.

The study confirms that all the taxa are good sources of antioxidants and support their role in treating inflammatory diseases. Both *B. prionitis* and *S. ciliatus* exhibited best scavenging effects and also are rich sources of highly active chemical principles contributing to their potent anti-inflammatory responses, substantiating their use in the preparation of Ayurvedic formulations of which the drug 'Sahachara' is an important

constituent. The use of *B. cristata* and *E. viride* as the source of 'Sahachara' can be justified because of their moderate protecting effects against oxidative damages and inflammations. But as the chemical composition of the different plants shows more differences than similarities, any attempts of using them as 'Sahachara' should be done only after detailed scrutiny and analysis, with a view not to alter the curing properties of the drug to any great extent.

The outcome of the study thus establishes that it is appropriate to use both the roots of *B. prionitis* and *S. ciliatus* as the source of 'Sahachara' as these plants showed the most promising activities in the antioxidant and anti-inflammatory activities that could make them highly effective in the treatment of chronic diseases like rheumatism that result from the damaging effects of free radicals, having an inflammatory response in their pathology. However, further detailed studies with respect to the other pharmacological actions of these plants and isolation and characterizion of their active principles and finding out their synergistic mechanisms of action are also essential.

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Table 16. Effect of *Barleria cristata* root methanolic extract on carrageenan induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume after 3rd h (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.34 ± 0.05	3.71 ± 0.12	1.37 ± 0.08	---------
Diclofenac (10 mg/kg b. wt)	2.39 ± 0.8	2.79 ± 0.04	0.40 ± 0.07***	70.81
B. cristata (100 mg/kg b. wt)	2.78 ± 0.23	3.47 ± 0.05	0.69 ± 0.21**	49.63
B. cristata (250 mg/kg b. wt)	2.51 ± 0.11	3.07 ± 0.14	0.56 ± 0.06***	54.64

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 17. Effect of *B. cristata* root methanolic extract on formalin induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume on 6th day (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.04 ± 0.04	3.87 ± 0.03	1.83 ± 0.06	---------
Diclofenac (10 mg/kg b. wt)	2.31 ± 0.01	3.17 ± 0.04	0.86 ± 0.06***	53.01
B. cristata (100 mg/kg b. wt)	2.46 ± 0.06	3.72 ± 0.07	1.26 ± 0.09*	31.14
B. cristata (250 mg/kg b. wt)	2.53 ± 0.08	3.52 ± 0.07	0.99 ± 0.10***	45.90

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 18. Effect of *Barleria prionitis* root methanolic extract on carrageenan induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume after 3rd h (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.34 ± 0.05	3.71 ± 0.12	1.37 ± 0.08	---------
Diclofenac (10 mg/kg b. wt)	2.39 ± 0.8	2.79 ± 0.04	0.40 ± 0.07***	70.81
B. prionitis (100 mg/kg b. wt)	2.45 ± 0.16	2.96 ± 0.14	0.51 ± 0.23***	62.77
B. prionitis (250 mg/kg b. wt)	2.51 ± 0.08	2.89 ± 0.20	0.38 ± 0.18***	72.26

Data represent the mean ± SE (n=5); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control (One way ANOVA, Dunnett's test).

Table 19. Effect of *B. prionitis* root methanolic extract on formalin induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume on 6th day (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.04 ± 0.04	3.87 ± 0.03	1.83 ± 0.06	---------
Diclofenac (10 mg/kg b. wt)	2.31 ± 0.01	3.17 ± 0.04	0.86 ± 0.06***	53.01
B. prionitis (100 mg/kg b. wt)	2.42 ± 0.03	3.53 ± 0.10	1.11 ± 0.10**	39.34
B. prionitis (250 mg/kg b. wt)	2.48 ± 0.06	3.36 ± 0.19	0.88 ± 0.15***	51.91

Data represent the mean ± SE (n=5); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control (One way ANOVA, Dunnett's test).

Table 24. Effect of *Strobilanthes ciliatus* root methanolic extract on carrageenan induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume after 3rd h (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.34 ± 0.05	3.71 ± 0.12	1.37 ± 0.08	---------
Diclofenac (10 mg/kg b. wt)	2.39 ± 0.8	2.79 ± 0.04	0.40 ± 0.07***	70.81
S. ciliatus (100 mg/kg b. wt)	2.33 ± 0.11	2.94 ± 0.13	0.61 ± 0.06**	55.47
S. ciliatus (250 mg/kg b. wt)	2.33 ± 0.08	2.70 ± 0.09	0.37 ± 0.13***	72.99

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 25. Effect of *S. ciliatus* root methanolic extract on formalin induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume on 6th day (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.04 ± 0.04	3.87 ± 0.03	1.83 ± 0.06	---------
Diclofenac (10 mg/kg b. wt)	2.31 ± 0.01	3.17 ± 0.04	0.86 ± 0.06***	53.01
S. ciliatus (100 mg/kg b. wt)	1.72 ± 0.04	2.86 ± 0.23	1.14 ± 0.24**	37.70
S. ciliatus (250 mg/kg b. wt)	1.70 ± 0.07	2.62 ± 0.05	0.92 ± 0.10***	49.73

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 26. Effect of *Strobilanthes heyneanus* root methanolic extract on carrageenan induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume after 3rd h (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.34 ± 0.05	3.71 ± 0.12	1.37 ± 0.08	---------
Diclofenac (10 mg/kg b. wt)	2.39 ± 0.8	2.79 ± 0.04	0.40 ± 0.07***	70.81
S. heyneanus (100 mg/kg b. wt)	2.37 ± 0.06	3.23 ± 0.07	0.86 ± 0.11**	37.22
S. heyneanus (250 mg/kg b. wt)	2.40 ± 0.03	3.04 ± 0.17	0.64 ± 0.17**	53.28

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 27. Effect of *S. heyneanus* root methanolic extract on formalin induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume on 6th day (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.04 ± 0.04	3.87 ± 0.03	1.83 ± 0.06	---------
Diclofenac (10 mg/kg b. wt)	2.31 ± 0.01	3.17 ± 0.04	0.86 ± 0.06***	53.01
S. heyneanus (100 mg/kg b. wt)	2.37 ± 0.09	3.66 ± 0.05	1.28 ± 0.11*	30.05
S. heyneanus (250 mg/kg b. wt)	2.53 ± 0.07	3.64 ± 0.03	1.11 ± 0.08**	39.34

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 20. Effect of *Ecbolium viride* root methanolic extract on carrageenan induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume after 3rd h (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.34 ± 0.05	3.71 ± 0.12	1.37 ± 0.08	---------
Diclofenac (10 mg/kg b. wt)	2.39 ± 0.80	2.79 ± 0.04	0.40 ± 0.07**	70.81
E. viride (100 mg/kg b. wt)	2.18 ± 0.10	2.87 ± 0.11	0.69 ± 0.06**	49.64
E. viride (250 mg/kg b. wt)	2.14 ± 0.04	2.64 ± 0.04	0.50 ± 0.06***	63.50

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 21. Effect of *E. viride* root methanolic extract on formalin induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume on 6th day (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.04 ± 0.04	3.87 ± 0.03	1.83 ± 0.06	---------
Diclofenac (10 mg/kg b. wt)	2.31 ± 0.01	3.17 ± 0.04	0.86 ± 0.06***	53.01
E. viride (100 mg/kg b. wt)	2.24 ± 0.04	3.48 ± 0.13	1.24 ± 0.13**	32.24
E. viride (250 mg/kg b. wt)	2.23 ± 0.07	3.27 ± 0.13	1.04 ± 0.10***	43.17

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 22. Effect of *Justicia betonica* root methanolic extract on carrageenan induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume after 3rd h (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.34 ± 0.05	3.71 ± 0.12	1.37 ± 0.08	---------
Diclofenac (10 mg/kg b. wt)	2.39 ± 0.80	2.79 ± 0.04	0.40 ± 0.07***	70.81
J. betonica (100 mg/kg b. wt)	2.21 ± 0.06	3.11 ± 0.06	0.90 ± 0.07	34.31
J. betonica (250 mg/kg b. wt)	2.20 ± 0.05	2.95 ± 0.06	0.75 ± 0.07**	45.25

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 23. Effect of *J. betonica* root methanolic extract on formalin induced paw edema in mice

Treatment groups	Initial paw edema volume (mm)	Paw edema volume on 6th day (mm)	Difference in paw edema volume (mm)	Inhibition (%)
Control	2.04 ± 0.04	3.87 ± 0.03	1.83 ± 0.06	---------
Diclofenac (10 mg/kg b. wt)	2.31 ± 0.01	3.17 ± 0.04	0.86 ± 0.06***	53.01
J. betonica (100 mg/kg b. wt)	2.14 ± 0.06	3.54 ± 0.04	1.40± 0.06	23.50
J. betonica (250 mg/kg b. wt)	2.13 ± 0.07	3.33 ± 0.18	1.20 ± 0.20**	34.43

Data represent the mean ± SE (n=5); *p < 0.05, **p < 0.01, ***p < 0.001 compared to control (One way ANOVA, Dunnett's test).

Table 10. *In vitro* antioxidant activities of root methanolic extracts of the six taxa used as 'Sahachara'

Plants	IC_{50} (µg/ml)				
	DPPH radical scavenging assay	**ABTS radical scavenging assay**	**Superoxide radical scavenging assay**	**Hydroxyl radical scavenging assay**	**Lipid peroxidation assay**
B. cristata	34.46 ± 0.89	13.99 ± 2.91	55.23 ± 1.81	74.84 ± 2.13	55.59 ± 1.44
B. prionitis	31.06 ± 1.28	12.89 ± 3.46	44.54 ± 2.90	70.10 ± 1.73	69.12 ± 3.04
E. viride	74.90 ± 1.62	57.30 ± 2.91	81.51 ± 2.38	108.3 ± 0.94	72.80 ± 1.71
J. betonica	91.57 ± 1.39	86.20 ± 2.11	110.34 ± 1.72	117.49 ± 1.30	95.76 ± 1.11
S. ciliatus	37.38 ± 1.11	18.65 ± 2.95	61.76 ± 2.68	54.58 ± 0.94	72.82 ± 1.55
S. heyneanus	103.92 ± 1.55	71.58 ± 1.28	84.17 ± 1.77	105.58 ± 2.60	73.85 ± 1.18

IC_{50} - Concentration of the samples causing 50% inhibition of radicals
Values are expressed as mean ± standard error (SE) (n=3)

Table 13. Scavenging effects of root methanolic extracts of the six taxa used as 'Sahachara' on Superoxide radical *in vitro* at different concentrations

Plants	% Inhibition of extracts against Superoxide radical at different concentrations (µg/ml)						
	20	40	60	80	100	120	140
B. cristata	34.16 ± 0.95 ^{d}	49.02 ± 0.65 ^{d}	52.40 ± 0.78 ^{c}	76.32 ± 1.23 ^{d}	81.86 ± 0.27 ^{d}	83.90 ± 1.09 ^{c}	88.12 ± 0.88 ^{d}
B. prionitis	27.79 ± 0.98 ^{c}	42.52 ± 1.16 ^{c}	54.71 ± 1.25 ^{c}	69.01 ± 1.74 ^{c}	71.53 ± 0.45 ^{c}	80.98 ± 1.18 ^{c}	94.21 ± 0.89 ^{e}
E. viride	19.81 ± 0.66 $^{a\,b}$	30.94 ± 0.32 ^{a}	35.50 ± 0.75 ^{a}	58.27 ± 1.50 ^{b}	61.84 ± 0.98 ^{b}	63.40 ± 1.71 ^{b}	75.48 ± 0.82 ^{b}
J. betonica	14.93 ± 1.11 ^{a}	28.69 ± 1.05 ^{a}	32.42 ± 0.74 ^{a}	44.43 ± 1.59 ^{a}	48.63 ± 1.03 ^{a}	51.44 ± 1.26 ^{a}	57.71 ± 0.38 ^{a}
S. ciliatus	21.09 ± 1.70 ^{b}	39.59 ± 0.49 $^{b\,c}$	56.56 ± 1.18 ^{c}	63.22 ± 0.89 $^{b\,c}$	70.91 ± 1.55 ^{c}	79.18 ± 1.42 ^{c}	83.67 ± 0.82 ^{c}
S. heyneanus	31.09 ± 1.28 $^{c\,d}$	37.86 ± 0.79 ^{b}	41.80 ± 1.16 ^{b}	46.25 ± 1.87 ^{a}	49.35 ± 0.68 ^{a}	62.63 ± 1.41 ^{b}	71.84 ± 0.85 ^{b}

Values are expressed as mean ± standard error (SE) (n=3)

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test).

Table 15. Inhibitory effects of root methanolic extracts of the six taxa used as 'Sahachara' on lipid peroxidation *in vitro* at different concentrations

Plants	% Inhibition of extracts on lipid peroxidation at different concentrations (µg/ml)						
	20	40	60	80	100	120	140
B. cristata	33.77 ± 0.56 ^{d}	46.84 ± 0.35 ^{c}	54.06 ± 0.73 ^{c}	60.27 ± 0.86 $^{c\,d}$	65.21 ± 0.56 ^{c}	70.81 ± 0.63 ^{c}	74.43 ± 1.24 $^{b\,c}$
B. prionitis	19.21 ± 1.23 ^{a}	33.71 ± 0.43 ^{a}	54.77 ± 1.05 ^{c}	61.59 ± 0.40 ^{d}	63.89 ± 1.23 ^{c}	76.40 ± 0.65 ^{d}	76.98 ± 0.42 ^{c}
E. viride	27.21 ± 0.43 $^{b\,c}$	38.21 ± 1.68 ^{b}	42.32 ± 0.85 ^{b}	58.61 ± 0.37 $^{c\,d}$	61.73 ± 0.82 $^{b\,c}$	69.60 ± 0.47 ^{c}	71.90 ± 0.16 ^{b}
J. betonica	22.71 ± 0.91 ^{a}	31.64 ± 0.94 ^{a}	34.92 ± 1.16 ^{a}	47.66 ± 0.56 ^{a}	50.97 ± 0.14 ^{a}	59.76 ± 0.30 ^{a}	63.73 ± 1.01 ^{a}
S. ciliatus	26.84 ± 0.86 ^{b}	32.74 ± 0.50 ^{a}	44.93 ± 0.28 ^{b}	58.12 ± 0.92 ^{c}	62.30 ± 0.99 ^{c}	71.97 ± 0.48 ^{c}	74.62 ± 0.57 $^{b\,c}$
S. heyneanus	30.74 ± 0.42 $^{c\,d}$	42.98 ± 0.89 ^{c}	45.45 ± 0.34 ^{b}	53.77 ± 0.66 ^{b}	58.13 ± 0.36 ^{b}	63.82 ± 1.34 ^{b}	67.63 ± 1.04 ^{a}

Values are expressed as mean ± standard error (SE) (n=3)

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test).

Table 14. Scavenging effects of root methanolic extracts of the six taxa used as 'Sahachara' on Hydroxyl radical *in vitro* at different concentrations

Plants	% Inhibition of extracts against Hydroxyl radical at different concentrations (µg/ml)						
	20	40	60	80	100	120	140
B. cristata	22.02 ± 1.00 ^{b}	30.82 ± 0.18 ^{b}	46.08 ± 0.90 ^{d}	57.39 ± 0.74 ^{d}	71.48 ± 0.70 ^{d}	76.65 ± 0.40 ^{c}	82.24 ± 0.16 ^{d}
B. prionitis	18.57 ± 0.59 ^{b}	30.86 ± 1.09 ^{b}	48.73 ± 0.63 ^{d}	52.70 ± 0.47 ^{c}	67.08 ± 2.21 $^{c\,d}$	72.93 ± 0.97 $^{b\,c}$	77.23 ± 0.79 ^{c}
E. viride	21.76 ± 0.55 ^{b}	27.72 ± 0.60 ^{b}	30.75 ± 0.52 ^{b}	39.42 ± 0.55 ^{a}	48.03 ± 0.78 $^{a\,b}$	53.22 ± 1.26 ^{a}	62.17 ± 0.52 ^{b}
J. betonica	9.53 ± 0.70 ^{a}	16.28 ± 1.08 ^{a}	24.78 ± 0.29 ^{a}	39.75 ± 0.83 ^{a}	43.40 ± 0.59 ^{a}	49.95 ± 0.52 ^{a}	57.72 ± 1.72 ^{a}
S. ciliatus	40.77 ± 1.06 ^{c}	42.36 ± 0.77 ^{c}	51.99 ± 0.67 ^{e}	59.63 ± 0.90 ^{d}	63.42 ± 0.33 ^{c}	71.06 ± 0.37 ^{b}	78.83 ± 0.63 $^{c\,d}$
S. heyneanus	9.26 ± 0.55 ^{a}	17.55 ± 0.78 ^{a}	37.19 ± 0.51 ^{c}	44.84 ± 1.43 ^{b}	51.81 ± 1.07 ^{b}	53.83 ± 1.47 ^{a}	59.59 ± 1.16 $^{a\,b}$

Values are expressed as mean ± standard error (SE) (n=3)

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test).

Table 11. Scavenging effects of root methanolic extracts of taxa used as 'Sahachara' on DPPH radical *in vitro* at different concentrations

Plants	% Inhibition of extracts against DPPH radical at different concentrations (µg/ml)						
	20	40	60	80	100	120	140
B. cristata	43.95 ± 0.36 ^{d}	52.24 ± 0.35 ^{c}	59.89 ± 0.62 ^{e}	63.34 ± 0.62 ^{d}	75.68 ± 0.63 ^{f}	78.66 ± 0.81 ^{d}	85.97 ± 1.05 ^{c}
B. prionitis	47.70 ± 0.25 ^{d}	54.75 ± 0.22 ^{c}	56.81 ± 0.29 ^{d}	63.69 ± 0.63 ^{d}	65.85 ± 0.91 $^{c\,d}$	76.18 ± 0.89 $^{c\,d}$	84.73 ± 1.31 ^{c}
E. viride	22.15 ± 0.58 ^{a}	34.56 ± 0.28 ^{a}	45.85 ± 0.22 ^{c}	55.88 ± 0.49 ^{c}	64.39 ± 0.56 ^{c}	68.69 ± 0.40 ^{b}	73.85 ± 0.35 ^{b}
J. betonica	24.99 ± 0.18 ^{b}	38.57 ± 1.03 ^{b}	39.95 ± 0.72 ^{b}	48.07 ± 1.09 ^{b}	54.53 ± 0.23 ^{b}	58.27 ± 1.02 ^{a}	61.92 ± 0.81 ^{a}
S. ciliatus	43.39 ± 0.25 ^{a}	51.48 ± 0.83 ^{c}	55.29 ± 1.04 ^{d}	63.75 ± 1.29 ^{d}	68.24 ± 0.79 ^{e}	73.58 ± 0.88 ^{c}	74.70 ± 0.47 ^{b}
S. heyneanus	29.56 ± 0.35 ^{c}	33.25 ± 1.12 ^{a}	36.64 ± 0.42 ^{a}	40.60 ± 1.33 ^{a}	44.34 ± 0.39 ^{a}	58.04 ± 1.08 ^{a}	61.58 ± 0.67 ^{a}

Values are expressed as mean ± standard error (SE) (n=3)
Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test).

Table 12. Scavenging effects of root methanolic extracts of the six taxa used as 'Sahachara' on ABTS radical *in vitro* at different concentrations

Plants	% Inhibition of extracts against ABTS radical at different concentrations (µg/ml)						
	10	20	30	40	50	60	70
B. cristata	43.58 ± 1.26 ^{c}	64.87 ± 0.17 ^{e}	70.55 ± 1.45 ^{c}	85.11 ± 0.54 ^{e}	90.80 ± 0.68 ^{d}	93.04 ± 0.31 ^{c}	96.66 ± 0.53 ^{c}
B. prionitis	45.86 ± 0.74 $^{c\,d}$	68.56 ± 0.62 ^{f}	81.47 ± 0.67 ^{d}	85.38 ± 0.30 ^{e}	91.47 ± 0.45 ^{d}	94.41 ± 0.32 ^{c}	96.06 ± 0.78 ^{c}
E. viride	28.80 ± 0.28 ^{a}	42.65 ± 0.58 ^{c}	44.68 ± 1.14 ^{b}	70.70 ± 0.48 ^{c}	76.32 ± 0.48 ^{c}	79.39 ± 0.63 ^{b}	84.59 ± 2.01 ^{b}
J. betonica	31.97 ± 0.37 ^{b}	33.76 ± 0.43 ^{b}	38.59 ± 1.03 ^{a}	44.44 ± 0.69 ^{a}	48.29 ± 0.32 ^{a}	65.16 ± 0.44 ^{a}	72.82 ± 0.71 ^{a}
S. ciliatus	47.97 ± 0.24 ^{d}	51.57 ± 0.76 ^{d}	73.76 ± 0.53 ^{c}	81.59 ± 0.35 ^{d}	91.17 ± 0.29 ^{d}	92.79 ± 0.99 ^{c}	94.33 ± 0.28 ^{c}
S. heyneanus	22.76 ± 0.45 ^{a}	30.74 ± 0.56 ^{a}	42.67 ± 0.50 $^{a\,b}$	58.79 ± 0.20 ^{b}	62.10 ± 0.90 ^{b}	77.06 ± 1.26 ^{b}	84.46 ± 0.64 ^{b}

Values are expressed as mean ± standard error (SE) (n=3)

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test).

***Barleria cristata* with 2n = 40 chromosomes**

Somatic chromosome number	:	40
Karyotype formula	:	34 nm + 6 nsm(-)
Chromosomes with secondary constriction	:	6
Range of Chromosome Length (RCL)	:	4.9287 µm to 2.1745 µm
Total Chromosome Length (TCL)	:	138.9312 µm :
Average Chromosome Length (ACL)	:	3.4733 µm
Disparity Index (DI)	:	38.77
Variation Coefficient (VC)	:	22.77
TF value (%)	:	42.54

Table 3. Detailed karyomorphometric data of *B. cristata* with 2n = 40

No. of Chr.	c (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of PC
2*	4.9287	1.7626	3.1666	0.5567	1.7962	35.76	64.24	nsm(-)
2*	4.7575	1.908	2.8495	0.6695	1.4934	40.1041	59.8949	nm
2	4.5834	2.0537	2.5297	0.8118	1.2317	44.8073	55.1926	nm
2	4.3754	2.0119	2.3635	0.8512	1.1747	45.9820	54.0179	nm
2*	4.0420	1.8620	2.1800	0.8541	1.1708	46.0663	53.9337	nm
2	4.0338	1.4217	2.6121	0.5442	1.8373	35.2446	64.7553	nsm(-)
2	3.8313	1.8500	1.9813	0.9337	1.0709	48.2864	51.7135	nm
2	3.7566	1.2289	2.5277	0.4861	2.0568	32.7130	67.2869	nsm(-)
2	3.5138	1.4544	2.0594	0.7062	1.4159	41.3910	58.6089	nm
2	3.3825	1.3129	2.0696	0.6343	1.5763	38.8144	61.1855	nm
2	3.2769	1.5966	1.6803	0.9501	1.0524	48.7288	51.2771	nm
2	3.2580	1.2936	1.9644	0.6585	1.5185	39.7053	60.2946	nm
2	3.0231	1.3884	1.6347	0.8493	1.1773	45.9263	54.0736	nm
2	2.9920	1.4481	1.5439	0.9379	1.0661	48.3990	51.6009	nm
2	2.8546	1.1383	1.7163	0.6632	1.5077	39.8759	60.1240	nm
2	2.7732	1.3867	1.3865	1.0001	0.9998	50.0036	49.9963	nm
2	2.7192	1.2790	1.4402	0.8880	1.1260	47.0358	52.9641	nm
2	2.7084	1.1145	1.5939	0.6992	1.4301	41.1497	58.8502	nm
2	2.4807	1.0536	1.4271	0.7382	1.3544	42.4718	57.5281	nm
2	2.1745	0.9878	1.1867	0.8323	1.2013	45.4265	54.5734	nm

Chr. – Chromosome, c – total length of chromosome, l – long arm length, s – short arm length, R_1 – arm ratio 1, R_2 – arm ratio 2, I_1 – centromeric index 1, I_2 – centromeric index 2, PC – primary constriction

***Chromosomes with secondary constriction**

***Barleria prionitis* with 2n = 40 chromosomes**

Somatic chromosome number	:	40
Karyotype formula	:	36 nm + 4 nsm(-)
Chromosomes with secondary constriction	:	4
Range of Chromosome Length (RCL)	:	4.9330 µm to 2.2728 µm
Total Chromosome Length (TCL)	:	129.3066 µm
Average Chromosome Length (ACL)	:	3.2327 µm
Disparity Index (DI)	:	36.92
Variation Coefficient (VC)	:	22.56
TF value (%)	:	43.04

Table 4. Detailed karyomorphometric data of *B. prionitis* with 2n = 40

No. of Chr.	c (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of PC
2*	4.9330	2.2863	2.6467	0.8638	1.1563	46.3470	53.6529	nm
2*	4.6165	2.1295	2.487	0.8562	1.6788	46.1280	53.8719	nm
2	4.021	1.8576	2.1634	0.8586	1.1646	46.1974	53.8025	nm
2	3.8313	1.6574	2.1739	0.7624	1.3116	43.2594	56.7405	nm
2	3.7729	1.7824	1.9905	0.8954	1.1167	47.2421	52.2692	nm
2	3.6819	1.7574	1.9245	0.9131	1.0950	47.7307	52.2692	nm
2	3.3629	1.5474	1,8155	0.8523	1.1732	46.0138	53.9861	nm
2	3.2900	1.3971	1.8929	0.7380	1.3548	42.4650	57.5349	nm
2	3.2763	0.8857	2.3906	0.3704	2.6991	27.0334	72.9664	nsm(-)
2	3.2150	1.1515	2.0610	0.5587	1.7898	35.8443	64.1556	nsm(-)
2	2.9882	1.2663	1.7219	0.7354	1.3597	42.3766	57.6233	nm
2	2.9621	1.4644	1.4977	0.9777	1.0227	49.4379	50.5621	nm
2	2.9878	1.3181	1.6697	0.7894	1.2667	44.1160	55.8839	nm
2	2.7457	1.1421	1.6036	0.7122	1.4040	41.5959	58.4040	nm
2	2.6480	1.2455	1.4025	0.8880	1.1260	47.0355	52.9645	nm
2	2.5872	1.0327	1.5545	0.6643	1.5052	39.9157	60.0842	nm
2	2.5534	1.1261	1.4273	0.7889	1.2674	44.1019	55.8980	nm
2	2.5352	0.7570	1.7782	0.4257	2.3490	29.8595	70.1404	nm
2	2.3721	1.0255	1.3466	0.7614	1.3131	43.2317	56.7682	nm
2	2.2728	0.9988	1.2740	0.7839	1.2755	43.9457	56.0542	nm

Chr. – Chromosome, c – total length of chromosome, l – long arm length, s – short arm length, R_1 – arm ratio 1, R_2 – arm ratio 2, I_1 – centromeric index 1, I_2 – centromeric index 2, PC – primary constriction

***Chromosomes with secondary constriction**

***Ecbolium viride* with 2n = 36 chromosomes**

Normal somatic chromosome number	:	36
Karyotype formula	:	36 nm
Chromosomes with secondary constriction	:	4
Range of Chromosome Length (RCL)	:	1.7630 µm to 0.9209 µm
Total Chromosome Length (TCL)	:	45.0734 µm
Average Chromosome Length (ACL)	:	1.252 µm
Disparity Index (DI)	:	31.38
Variation Coefficient (VC)	:	18.66
TF value (%)	:	44.52

Table 5. Detailed karyomorphometric data of *E. viride* with 2n = 36

No. of Chr.	c (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of PC
2*	1.7630	0.7352	1.0341	0.7109	1.4065	41.5531	58.4468	nm
2*	1.6774	0.7254	0.9520	0.7619	1.3123	43.2455	56.7545	nm
2	1.5236	0.5893	0.9343	0.6307	1.5854	38.6781	61.3218	nm
2	1.4207	0.6144	0.8063	0.7619	1.3123	43.2462	56.7537	nm
2	1.3767	0.6593	0.7174	0.9190	1.0881	47.8898	52.1101	nm
2	1.3420	0.6315	0.7105	0.8888	1.1250	47.0563	52.9433	nm
2	1.3252	0.6548	0.6704	0.9767	1.0238	49.4114	50.5885	nm
2	1.2647	0.6164	0.6483	0.9507	1.0517	48.7388	51.2611	nm
2	1.1974	0.5924	0.6050	0.9791	1.0212	49.4738	50.5261	nm
2	1.1773	0.4853	0.6920	0.7013	1.4259	41.2214	58.7785	nm
2	1.1518	0.5138	0.6380	0.8053	1.2417	44.6084	55.3915	nm
2	1.1293	0.4657	0.6636	0.7017	1.4249	41.2379	58.7620	nm
2	1.0689	0.4580	0.6109	0.7497	1.3338	42.8477	57.1522	nm
2	1.1011	0.4708	0.6303	0.7469	1.3387	42.7572	57.2427	nm
2	1.0792	0.5301	0.5491	0.9653	1.0358	49.1197	50.8802	nm
2	1.0286	0.4634	0.5652	0.8198	1.2196	45.0515	54.9484	nm
2	0.9889	0.4323	0.5566	0.7766	1.2875	43.7152	56.2847	nm
2	0.9209	0.3955	0.5254	0.7527	1.3284	42.9471	57.0528	nm

Chr. – Chromosome, c – total length of chromosome, l – long arm length, s – short arm length, R_1 – arm ratio 1, R_2 – arm ratio 2, I_1 – centromeric index 1, I_2 – centromeric index 2, PC – primary constriction

***Chromosomes with secondary constriction**

***Justicia betonica* with 2n = 34 chromosomes**

Normal somatic chromosome number	:	34
Karyotype formula	:	32 nm + 2 nsm(-)
Chromosomes with secondary constriction	:	4
Range of Chromosome Length (RCL)	:	1.0294 µm to 0.4814 µm
Total Chromosome Length (TCL)	:	22.9642 µm
Average Chromosome Length (ACL)	:	0.6754 µm
Disparity Index (DI)	:	36.27
Variation Coefficient (VC)	:	22.28
TF value (%)	:	44.11

Table 6. Detailed karyomorphometric data of *J. betonica* with 2n = 34

No. of Chr.	c (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of PC
2*	1.0294	0.4443	0.5851	0.7593	1.3169	43.1610	56.8389	nm
2	0.9003	0.4018	0.4985	0.8060	1.2406	44.6295	55.3704	nm
2*	0.8718	0.3444	0.5274	0.6530	1.5313	39.5044	60.4955	nm
2	0.7988	0.3929	0.4059	0.9679	1.0330	49.1862	50.8137	nm
2	0.7257	0.3451	0.3806	0.9067	1.1028	47.5540	52.4459	nm
2	0.6899	0.2684	0.4215	0.6367	1.5704	38.9041	61.0958	nm
2	0.6718	0.2903	0.3815	0.7609	1.3141	43.2122	56.7877	nm
2	0.6672	0.3123	0.4549	0.8799	1.1364	46.8075	53.1924	nm
2	0.6527	0.2954	0.3573	0.8267	1.2095	45.2581	54.7418	nm
2	0.6440	0.2950	0.3490	0.8452	1.1830	45.8074	54.1925	nm
2	0.6239	0.2625	0.3614	0.7263	1.3767	42.0740	57.9259	nm
2	0.5858	0.2223	0.3635	0.6111	1.6351	37.9481	62.0518	nsm(-)
2	0.5578	0.2483	0.3095	0.8022	1.2464	44.5141	55.4858	nm
2	0.5374	0.2552	0.2822	0.9043	1.1057	47.4879	52.5121	nm
2	0.5296	0.2333	0.2484	0.2963	0.7873	44.0521	55.9478	nm
2	0.5146	0.2199	0.2947	0.7461	1.3401	42.7322	57.2677	nm
2	0.4814	0.2330	0.2484	0.9380	1.0660	48.4005	51.5995	nm

Chr. – Chromosome, c – total length of chromosome, l – long arm length, s – short arm length, R_1 – arm ratio 1, R_2 – arm ratio 2, I_1 – centromeric index 1, I_2 – centromeric index 2, PC – primary constriction

***Chromosomes with secondary constriction**

***Strobilanthes ciliatus* with 2n = 28 chromosomes**

Normal somatic chromosome number	:	28
Karyotype formula	:	26 nm + 2 nsm(-)
Chromosomes with secondary constriction	:	4
Range of Chromosome Length (RCL)	:	1.9178 µm to 1.0145 µm
Total Chromosome Length (TCL)	:	40.8232 µm
Average Chromosome Length (ACL)	:	1.458µm
Disparity Index (DI)	:	30.81
Variation Coefficient (VC)	:	20.28
TF value (%)	:	42.40

Table 7. Detailed karyomorphometric data of *S. ciliatus* with 2n = 28

No. of Chr.	c (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of PC
2	1.9178	0.7951	1.1227	0.7082	1.4120	41.4589	58.5410	nm
2*	1.8469	0.7310	1.1159	0.6550	1.5265	39.5794	60.4201	nm
2	1.8307	0.7299	1.1008	0.6630	1.5081	39.8700	60.1300	nm
2*	1.7884	0.8586	0.9288	0.9254	1.0805	48.0653	51.9346	nm
2	1.6235	0.6300	0.9935	0.6341	1.5769	38.8050	61.8677	nm
2	1.4788	0.5639	0.9149	0.6163	1.6224	38.1322	61.8677	nm
2	1.3813	0.6660	0.7153	0.9310	1.0740	48.2154	51.7845	nm
2	1.3492	0.4958	0.8534	0.5809	1.7212	36.7477	63.2523	nsm(-)
2	1.3057	0.6044	0.7013	0.8618	1.1603	46.2893	53.7106	nm
2	1.2996	0.5413	0.7583	0.7138	1.4008	41.6512	58.3487	nm
2	1.2710	0.5254	0.7456	0.7046	1.1419	41.3375	58.6624	nm
2	1.2318	0.5888	0.6430	0.9157	1.0920	47.7997	52.2003	nm
2	1.0724	0.4981	0.5743	0.8673	1.1529	46.4472	53.5527	nm
2	1.0145	0.4254	0.5891	0.7221	1.3848	41.9319	58.0680	nm

Chr. – Chromosome, c – total length of chromosome, l – long arm length, s – short arm length, R_1 – arm ratio 1, R_2 – arm ratio 2, I_1 – centromeric index 1, I_2 – centromeric index 2, PC – primary constriction

***Chromosomes with secondary constriction**

***Strobilanthes heyneanus* with 2n = 32 chromosomes**

Normal somatic chromosome number	:	32
Karyotype formula	:	28 nm + 4 nsm(-)
Chromosomes with secondary constriction	:	4
Range of Chromosome Length (RCL)	:	1.1058 µm to 0.6371 µm
Total Chromosome Length (TCL)	:	27.6154 µm
Average Chromosome Length (ACL)	:	0.863 µm
Disparity Index (DI)	:	26.89
Variation Coefficient (VC)	:	17.71
TF value (%)	:	41.49

Table 8. Detailed karyomorphometric data of *S. heyneanus* with 2n = 32

No. of Chr.	c (µm)	s (µm)	l (µm)	R_1 (s/l)	R_2 (l/s)	I_1 (s/c%)	I_2 (l/c%)	Nature of PC
2*	1.1058	0.4703	0.6355	0.7400	1.3512	42.5302	57.4697	nm
2	1.0638	0.4074	0.6564	0.6206	1.6111	38.2966	61.7033	nm
2*	1.0473	0.3982	0.6491	0.6134	1.6300	38.0215	61.9784	nm
2	0.9984	0.4978	0.5006	0.9944	1.0056	49.8597	50.1402	nm
2	0.9847	0.4565	0.5282	0.8642	1.1570	46.4592	53.6407	nm
2	0.9477	0.3798	0.5679	0.6687	1.4952	40.0759	59.9240	nm
2	0.9150	0.3899	0.5251	0.7425	1.3467	42.6120	57.3879	nm
2	0.8946	0.3441	0.5505	0.6250	1.5998	38.4641	61.5358	nm
2	0.8632	0.3600	0.5032	0.7154	1.3977	41.7052	58.2947	nm
2	0.7884	0.2869	0.5015	0.5720	1.7479	36.3901	63.6098	nsm(-)
2	0.7445	0.3098	0.4347	0.7126	1.4031	41.61182	58.3881	nm
2	0.7333	0.2953	0.4380	0.6742	1.4832	40.2700	59.7299	nm
2	0.7153	0.2673	0.4480	0.5966	1.6760	37.3689	62.6310	nsm(-)
2	0.6855	0.2994	0.3861	0.7754	1.2895	43.6761	56.3238	nm
2	0.6831	0.2883	0.3948	0.7302	1.3694	42.2046	57.7953	nm
2	0.6371	0.2774	0.3597	0.7711	1.2966	43.5410	56.4589	nm

Chr. – Chromosome, c – total length of chromosome, l – long arm length, s – short arm length, R_1 – arm ratio 1, R_2 – arm ratio 2, I_1 – centromeric index 1, I_2 – centromeric index 2, PC – primary constriction

***Chromosomes with secondary constriction**

Table 9. Summary of karyomorphometric features of the six taxa used as the source plants of the drug 'Sahachara' in Ayurveda

Karyotype	No. of Chr. with sec. constr.	TCL (µm)	ACL (µm)	RCL (µm)	DI	VC	TF %	Karyotype formula
B. cristata (2n = 40)	6	138.9312	3.4733	4.9287 – 2.1745	38.77	22.77	42.54	34 nm + 6 nsm(-)
B. prionitis (2n = 40)	4	129.3066	3.2327	4.9330 – 2.2728	36.92	22.56	43.04	36 nm + 4 nsm(-)
E. viride (2n = 36)	4	45.0734	1.2520	1.7630 – 0.9209	31.38	18.66	44.52	36 nm
J. betonica (2n = 34)	4	22.9642	0.6754	1.0294 – 0.4814	36.27	22.28	44.11	32 nm + 2 nsm(-)
S. ciliatus (2n = 28)	4	40.8232	1.4580	1.9178 – 1.0145	30.81	20.28	42.40	26 nm + 2 nsm(-)
S. heyneanus (2n = 32)	4	27.6154	0.8630	1.1058 – 0.6371	26.89	17.71	41.49	28 nm + 4 nsm(-)

Chr. – Chromosome, sec. - secondary, constr. - constriction

Table 32. Phytochemical constituents of root methanolic extract of *Barleria cristata* revealed through LC-MS

Sl. No.	RT	Chemical constituents	Class of compounds	Molecular formula	Molecular mass (g/mol)	Peak area (%)
1	1.069	Cinnamic acid	Phenolic compound	$C_9H_8O_2$	148.16	5.10
2	1.328	Coumaric acid	Phenolic compound	$C_9H_8O_3$	164.16	10.42
3	11.584	Methyleugenol	Phenolic compound	$C_{11}H_{14}O_2$	178.22	25.16
4	13.713	3-Acetylcoumarin	Coumarin	$C_{11}H_8O_3$	188.17	3.05
5	15.010	Ferulic acid	Phenolic compound	$C_{10}H_{10}O_4$	194.18	1.84
6	16.061	Caryophyllene oxide	Sesquiterpenoid	$C_{15}H_{24}O$	220.35	1.59
7	16.808	Myristic acid	Fatty acid	$C_{14}H_{28}O_2$	228.37	3.76
8	17.294	Farnesyl acetate	Ester	$C_{17}H_{28}O_2$	264.4	1.92
9	26.700	Farnesyl butanoate	Ester	$C_{19}H_{32}O_2$	292.45	5.54
10	26.931	Ellagic acid	Phenolic compound	$C_{14}H_6O_8$	302.19	2.31
11	27.589	3β-Hydroxy-manool	Diterpenoid	$C_{20}H_{34}O_2$	306.48	1.78
12	28.273	Quercetin	Flavonoid	$C_{15}H_{10}O_7$	302.23	10.75
13	28.600	Arachidonic acid	Fatty acid	$C_{20}H_{32}O_2$	304.46	10.81
14	29.429	Myricetin	Phenolic compound	$C_{15}H_{10}O_8$	318.23	3.12
15	30.549	Luteone	Flavonoid	$C_{20}H_{18}O_6$	354.35	4.18
16	31.252	Lupeol	Triterpenoid	$C_{30}H_{50}O$	426.72	3.44
17	31.980	β-Sitosterol	Steroid	$C_{29}H_{50}O$	414.71	5.23

Table 33. Phytochemical constituents of root methanolic extract of *Barleria prionitis* revealed through LC-MS

Sl. No.	RT	Chemical constituents	Class of compounds	Molecular formula	Molecular mass (g/mol)	Peak area (%)
1	1.328	Coumaric acid	Phenolic compound	$C_9H_8O_3$	164.16	1.53
2	17.742	Apigenin	Flavonoid	$C_{15}H_{10}O_5$	270.24	3.38
3	17.992	Salicin	Phenolic compound	$C_{13}H_{18}O_7$	286.26	3.43
4	18.328	Luteolin	Flavonoid	$C_{15}H_{10}O_6$	288.24	2.16
5	22.199	Catechin	Flavonoid	$C_{15}H_{14}O_6$	290.27	20.67
6	29.429	Myricetin	Phenolic compound	$C_{15}H_{10}O_8$	318.23	2.81
7	29.901	Crocetin	Tetraterpenoid	$C_{20}H_{24}O_4$	328.4	1.54
8	30.549	Luteone	Flavonoid	$C_{20}H_{18}O_6$	354.35	2.50
9	30.634	Conessine	Steroid alkaloid	$C_{24}H_{40}N_2$	356.58	3.23
10	31.066	Diferulic acid	Phenolic compound	$C_{20}H_{18}0_8$	386.35	2.27
11	31.252	Lupeol	Triterpenoid	$C_{30}H_{50}O$	426.72	4.93
12	31.980	β-Sitosterol	Steroid	$C_{29}H_{50}O$	414.71	10.42
13	32.157	Betulinic acid	Triterpenoid	$C_{30}H_{48}O_3$	456.70	20.14
14	32.317	Barlerin	Ester	$C_{19}H_{28}O_{12}$	448.41	13.07
15	32.406	α-Tocopherol	Phenol	$C_{29}H_{50}O_2$	430.71	7.92

Table 34. Phytochemical constituents of root methanolic extract of *Ecbolium viride* revealed through LC-MS

Sl. No.	RT	Chemical constituents	Class of compounds	Molecular formula	Molecular mass (g/mol)	Peak area (%)
1	1.069	Cinnamic acid	Phenolic compound	$C_9H_8O_2$	148.16	4.42
2	1.328	Coumaric acid	Phenolic compound	$C_9H_8O_3$	164.16	11.79
3	8.559	Hydronaphthoquinone	Quinone compound	$C_{10}H_8O_2$	160.12	4.15
4	9.259	Vanillic acid	Phenolic compound	$C_8H_8O_4$	168.14	3.76
5	10.771	Ascorbic acid	Phenolic compound	$C_6H_8O_6$	176.12	7.73
6	22.199	Catechin	Flavonoid	$C_{15}H_{14}O_6$	290.27	8.92
7	22.825	Linoleic acid	Fatty acid	$C_{18}H_{32}O_2$	280.45	6.90
8	22.985	Oleic acid	Fatty acid	$C_{18}H_{34}O_2$	282.46	3.16
9	23.101	Wogonin	Phenolic compound	$C_{16}H_{12}O_5$	284.26	11.52
10	26.406	(13*E*)-labda-7,13-dien-15-ol	Diterpenoid	$C_{20}H_{34}O$	290.48	2.96
11	27.589	3β-Hydroxy-manool	Diterpenoid	$C_{20}H_{34}O_2$	306.48	3.34
12	28.180	Taxifolin	Flavonoid	$C_{15}H_{12}O_7$	304.25	3.67
13	28.273	Quercetin	Flavonoid	$C_{15}H_{10}O_7$	302.25	8.01
14	29.429	Myricetin	Phenolic compound	$C_{15}H_{10}O_8$	318.23	11.07
15	30.777	Chlorogenic acid	Phenolic compound	$C_{16}H_{18}0_9$	354.31	2.17
16	31.066	Diferulic acid	Phenolic compound	$C_{20}H_{18}0_8$	386.35	1.64
17	31.475	Stigmasterol	Steroid	$C_{29}H_{48}O$	412.69	4.79

Table 35. Phytochemical constituents of root methanolic extract of *Justicia betonica* revealed through LC-MS

Sl. No.	RT	Chemical constituents	Class of compounds	Molecular formula	Molecular mass (g/mol)	Peak area (%)
1	1.069	Cinnamic acid	Phenolic compound	$C_9H_8O_2$	148.16	2.33
2	1.328	Coumaric acid	Phenolic compound	$C_9H_8O_3$	164.16	4.78
3	16.808	Myristic acid	Fatty acid	$C_{14}H_{28}O_2$	228.37	1.67
4	17.134	Reservatol	Phenol	$C_{14}H_{12}O_3$	228.24	2.67
5	17.294	Farnesyl acetate	Ester	$C_{17}H_{28}O_2$	264.40	2.47
6	17.620	Naringenin	Flavonoid	$C_{15}H_{12}O_5$	272.25	0.96
7	17.742	Apigenin	Flavonoid	$C_{15}H_{10}O_5$	270.24	0.33
8	17.992	Salicin	Phenolic compound	$C_{13}H_{18}O_7$	286.26	0.78
9	20.712	Eriodictyol	Flavonoid	$C_{15}H_{12}O_6$	288.25	0.85
10	25.536	Geranyl linalool	Diterpenoid	$C_{20}H_{34}O$	290.48	1.28
11	28.417	Cannabidiol	Sesquiterpenoid	$C_{21}H_{30}O_2$	314.46	8.88
12	28.600	Arachidonic acid	Fatty acid	$C_{20}H_{32}O_2$	304.46	5.45
13	28.806	Sinapine	Alkaloidal amine	$C_{16}H_{24}NO_5$	310.37	6.06
14	29.429	Myricetin	Phenolic compound	$C_{15}H_{10}O_8$	318.23	1.59
15	29.901	Crocetin	Tetraterpenoid	$C_{20}H_{24}O_4$	328.40	0.7
16	30.549	Luteone	Flavonoid	$C_{20}H_{18}O_6$	354.35	0.86
17	30.777	Chlorogenic acid	Phenolic compound	$C_{16}H_{18}O_9$	354.31	2.31
18	31.475	lupeol	Triterpenoid	$C_{30}H_{50}O$	426.72	2.35
19	31.980	β-Sitosterol	Steroid	$C_{29}H_{50}O$	414.71	0.87
20	32.157	Betulinic acid	Triterpenoid	$C_{30}H_{48}O_3$	456.70	6.26
21	33.943	Spinasterol	Steroid	$C_{29}H_{48}O$	412.69	23.62
22	34.525	Ursolic acid	Triterpenoid	$C_{30}H_{48}O_3$	456.70	22.93

Table 36. Phytochemical constituents of root methanolic extract of *Stribilanthus ciliatus* revealed through LC-MS

Sl. No.	RT	Chemical constituents	Class of compounds	Molecular formula	Molecular mass (g/mol)	Peak area (%)
1	1.069	Cinnamic acid	Phenolic compound	$C_9H_8O_2$	148.16	5.10
2	1.328	Coumaric acid	Phenolic compound	$C_9H_8O_3$	164.16	10.42
3	1.658	Gallic acid	Phenolic compound	$C_7H_6O_5$	170.12	6.29
4	18.328	Luteolin	Flavonoid	$C_{15}H_{10}0_6$	288.24	3.51
5	28.273	Quercetin	Flavonoid	$C_{15}H_{10}O_7$	302.23	4.24
6	28.417	Cannabidiol	Sesquiterpenoid	$C_{21}H_{30}O_2$	314.46	9.59
7	28.806	Sinapine	Alkaloidal amine	$C_{16}H_{24}NO_5$	310.37	6.97
8	30.777	Chlorogenic acid	Phenolic compound	$C_{16}H_{18}O_9$	354.31	4.61
9	31.252	Lupeol	Triterpenoid	$C_{30}H_{50}O$	426.72	15.35
10	32.406	α-Tocopherol	Phenol	$C_{29}H_{50}O_2$	430.71	6.71
11	32.517	Ethyl iso-allocholate	Steroid	$C_{26}H_{44}O_5$	436.62	8.27
12	33.943	Spinasterol	Steroid	$C_{29}H_{48}O$	412.69	3.82
13	34.717	Asiatic acid	Triterpenoid	$C_{30}H_{48}O_5$	488.70	15.12

Table 37. Phytochemical constituents of root methanolic extract of *Strobilanthes heyneanus* revealed through LC-MS

Sl. No.	RT	Chemical constituents	Class of compounds	Molecular formula	Molecular mass (g/mol)	Peak area (%)
1	1.069	Cinnamic acid	Phenolic compound	$C_9H_8O_2$	148.16	6.59
2	1.328	Coumaric acid	Phenolic compound	$C_9H_8O_3$	164.16	6.41
3	25.536	Geranyl linalool	Diterpenoid	$C_{20}H_{34}O$	290.48	2.65
4	26.147	Vernolic acid	Fatty acid	$C_{18}H_{32}O_3$	296.44	4.08
5	26.406	(13*E*)-labda-7,13-dien-15-ol	Diterpenoid	$C_{20}H_{34}O$	290.48	3.97
6	26.700	Farnesyl butanoate	Ester	$C_{19}H_{32}O_2$	292.45	1.60
7	26.752	Diosmetin	Flavonoid	$C_{16}H_{12}O_6$	300.26	4.13
8	26.931	Ellagic acid	Phenolic compound	$C_{14}H_6O_8$	302.19	1.27
9	28.180	Taxifolin	Flavonoid	$C_{15}H_{12}O_7$	304.25	3.11
10	28.273	Quercetin	Flavonoid	$C_{15}H_{10}O_7$	302.23	7.74
11	28.600	Arachidonic acid	Fatty acid	$C_{20}H_{32}O_2$	304.46	7.61
12	30.549	Luteone	Flavonoid	$C_{20}H_{18}O_6$	354.35	1.80
13	31.252	Lupeol	Triterpenoid	$C_{30}H_{50}O$	426.72	5.33
14	34.525	Ursolic acid	Triterpenoid	$C_{30}H_{48}O_3$	456.70	19.1
15	34.717	Asiatic acid	Triterpenoid	$C_{30}H_{48}O_5$	488.70	24.61

Table 30. Phytochemical constituents of methanolic extracts of the six taxa used as 'Sahachara' revealed through GC-MS analysis

Sl No.	RT	Constituents	Peak area (%)					
			B. cristata	*B. prionitis*	*E. viride*	*J. betonica*	*S. ciliatus*	*S. heyneanus*
1	1.695	Estragole	51.1	-	4.79	-	-	-
2	4.236	m-Guaiacol	1.69	1.52	-	-	-	-
3	6.839	Syringol	-	-	-	-	26.61	15.34
4	7.414	5-Hydroxymethylfurfural	-	-	-	11.58	-	3.21
5	8.585	Hydrocinnamic acid	1.68	13.66	-	-	2.34	-
6	8.594	2-Acetyl-4-methylphenol	-	-	-	4.15	-	-
7	8.613	3-Methoxycatechol	-	-	-	-	0.36	4.35
8	8.634	4-Vinylguaiacol	-	-	-	0.84	-	-
9	9.565	5-Quinolinol	-	-	-	-	-	2.39
10	9.665	3,5-Dimethoxyphenol	3.14	4.95	-	-	-	-
11	9.705	4-Chromanol	-	-	-	2.07	-	-
12	10.958	3-Acetylcoumarin	-	1.44	-	-	-	3.64
13	10.979	Methyl p-coumarate	-	-	0.69	1.67	-	-
14	11.220	Isoeugenyl acetate	1.84	-	9.12	4.63	-	-

Sl No.	RT	Constituents	Peak area (%)					
			B. cristata	*B. prionitis*	*E. viride*	*J. betonica*	*S. ciliatus*	*S. heyneanus*
15	11.907	Ferulic acid	-	2.31	-	-	-	-
16	11.994	2-Hydroxycinnamic acid	-	2.35	-	1.92	-	-
17	12.858	Coniferol	-	-	-	0.67	-	2.42
18	13.124	Hydronaphthoquinone	-	-	-	-	0.28	4.16
19	13.172	Methyl p-hydroxycinnamate	-	-	-	-	-	2.04
20	13.502	Caryophyllene oxide	-	3.62	-	-	-	-
21	13.702	2-Tert-butyl-4-methoxyphenol	-	-	0.74	7.6	-	4.06
22	13.904	1-Benzoylpiperidine	2.15	-	1.64	-	-	-
23	15.140	4-Acetoxycinnamic acid	-	-	-	4.09	-	-
24	15.188	β-Eudesmol	7.41	-	-	0.48	-	-
25	15.204	2-Chloro-4-methylquinoline	-	-	1.63	-	-	1.02
26	15.953	Myristic acid	-	-	0.93	-	-	-
27	16.017	p-Methoxybenzylidine p-aminophenol	-	-	-	1.63	-	0.93
28	16.300	p-Dodecylphenol	0.90	-	-	-	-	-
29	16.623	Tridecanedioic acid	-	-	-	-	0.76	1.13
30	16.861	Desaspidinol	4.72	35.20	3.96	-	3.76	-
31	20.349	6-Nitrocoumarin	-	-	-	1.88	-	-

Sl No.	RT	Constituents	Peak area (%)					
			B. cristata	*B. prionitis*	*E. viride*	*J. betonica*	*S. ciliatus*	*S. heyneanus*
32	20.693	2,6-Di-tert-butylphenol	1.94	-	-	-	-	-
33	20.743	Nerolidol	1.68	-	1.69	-	-	-
34	21.185	τ-Cadinol	-	-	5.53	-	-	-
35	21. 451	α- Terpinyl acetate	-	-	-	-	-	2.81
36	22.693	4-Methyl-2-tert-octylphenol	-	-	-	-	-	7.12
37	22.890	Methyl tridecanoate	-	1.97	-	-	-	-
38	23.438	Methyl isoheptadecanoate	-	-	-	4.2	-	-
39	24.431	Methyl palmitate	-	1.00	2.95	-	-	1.34
40	24.982	1-Eicosanol	-	0.91	2.91	-	-	-
41	25.864	Geranyl linalool	-	-	-	6.62	0.92	-
42	26.024	Methyl linoleate	-	-	-	1.47	-	-
43	27.349	Methyl oleate	-	1.48	-	-	-	2.93
44	27.362	Ethyl linoleate	-	-	-	3.34	-	-
45	27.743	E-8-Methyl-9-tetradecen-1-ol acetate	-	-	-	4.52	-	1.48
46	28.153	trans-Farnesol	-	3.80	1.16	0.79	-	-
47	28.286	Octyl 4-methoxycinnamate	-	-	1.78	-	-	-
48	28.464	Eicosane	-	-	2.13	1.64	-	-

Sl No.	RT	Constituents	Peak area (%)					
			B. cristata	***B. prionitis***	***E. viride***	***J. betonica***	***S. ciliatus***	***S. heyneanus***
49	29.175	Phytol	-	-	2.04	-	5.33	3.20
50	29.746	Coumafuryl	-	-	-	4.2	-	-
51	29.915	Succinic acid, butyl 2-hexyl ester	-	-	-	3.8	-	-
52	29.934	Sclareol	-	-	0.82	-	3.42	1.39
53	30.266	Tricosane	-	-	-	4.51	-	-
54	32.957	Squalene	4.14	-	-	-	2.82	2.31
55	36.042	β-Sitosterol	0.52	3.05	2.97	1.57	3.15	2.11
56	37.318	Betulinic acid	-	3.95	4.76	-	3.66	-
57	38.839	Lupenone	-	4.57	-	-	0.34	1.09
58	39.563	γ-Tocopherol	-	-	-	3.55	-	-
59	40.696	α-Monopalmitin	0.61	-	3.04	-	-	-
60	48.725	α-Spinasterol	-	3.04	8.69	2.19	3.42	10.39
61	52.757	Lupeol	6.34	6.44	5.73	3.58	38.70	12.25
62	55.095	Lupeyl acetate	-	-	18.4	8.25	3.31	2.39
63	58.667	Canthaxanthin	5.08	2.84	3.04	-	-	0.32
		Total	94.94	98.10	91.14	97.44	99.18	95.82

Table 31. Percentage composition of various classes of compounds detected in the methanolic extracts of the six taxa used as 'Sahachara' revealed through GC-MS

Sl. No.	Class of Compounds	*B. cristata* (%)	*B. prionitis* (%)	*E. viride* (%)	*J. betonica* (%)	*S. ciliatus* (%)	*S. heyneanus* (%)
1	Phenols	60.35	36.72	9.49	16.29	30.37	27.45
2	Terpenoids	24.65	25.22	43.17	23.27	58.5	25.78
3	Polyphenols	1.68	18.32	-	6.68	2.34	4.46
4	Phytosterols	0.52	6.09	11.66	3.76	6.57	12.50
5	Fatty acid esters	2.45	4.45	16.89	17.44	-	4.27
6	Coumarins	-	1.44	0.69	7.75	-	3.64
7	Furans	-	-	-	11.58	-	3.21
8	Miscellaneous	5.29	5.86	9.24	10.67	1.40	14.53
9	Undetected	5.06	1.90	8.86	2.56	0.82	4.16
	Total	100.00	100.00	100.00	100.00	100.00	100.00

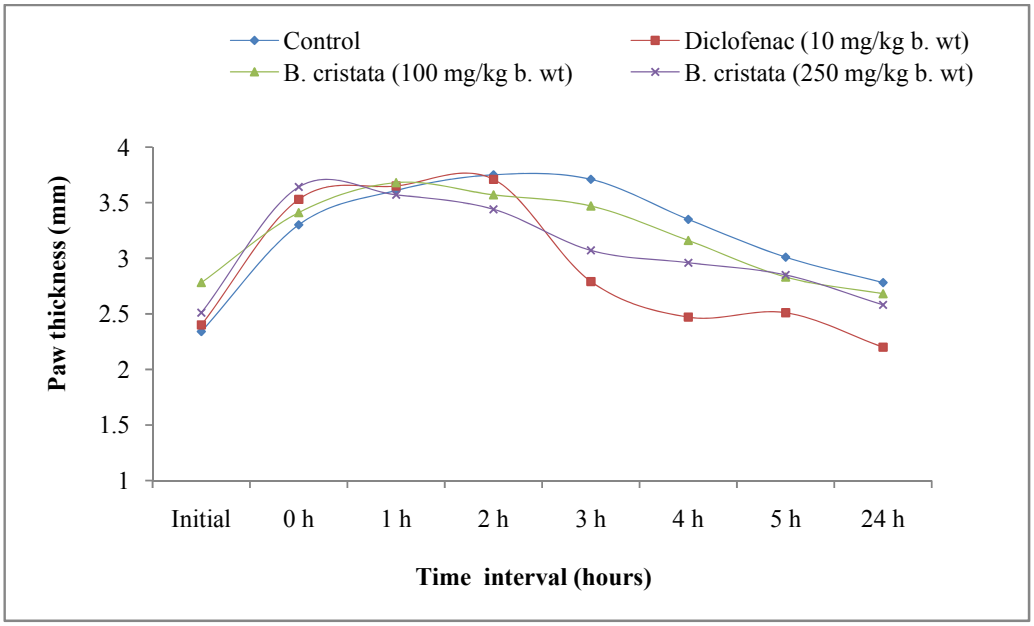

Fig. 12 Graph showing the effect of *Barleria cristata* extract on carrageenan induced inflammation

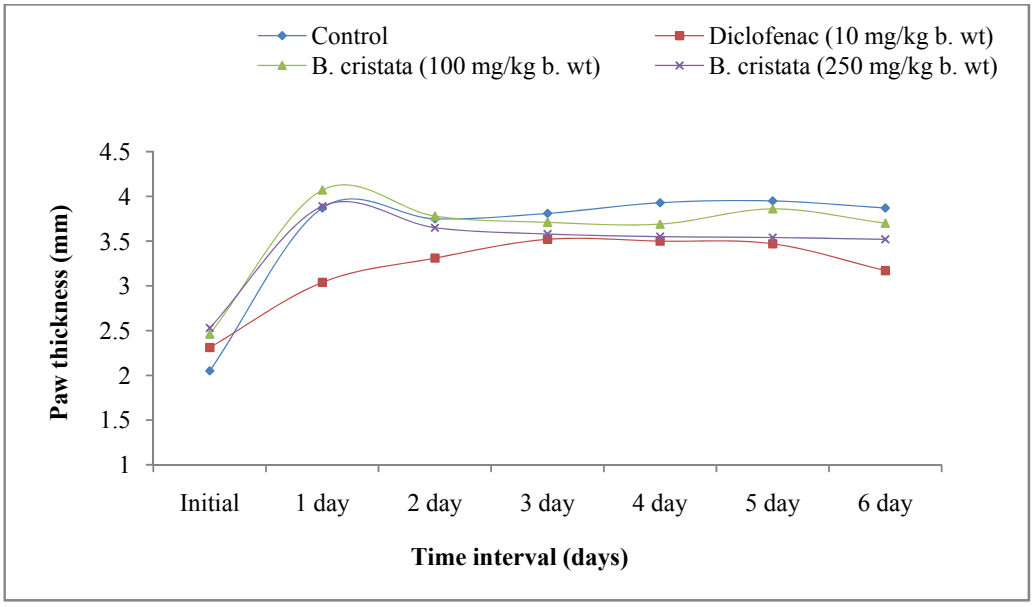

Fig. 13 Graph showing the effect of *B. cristata* extract on formalin induced inflammation

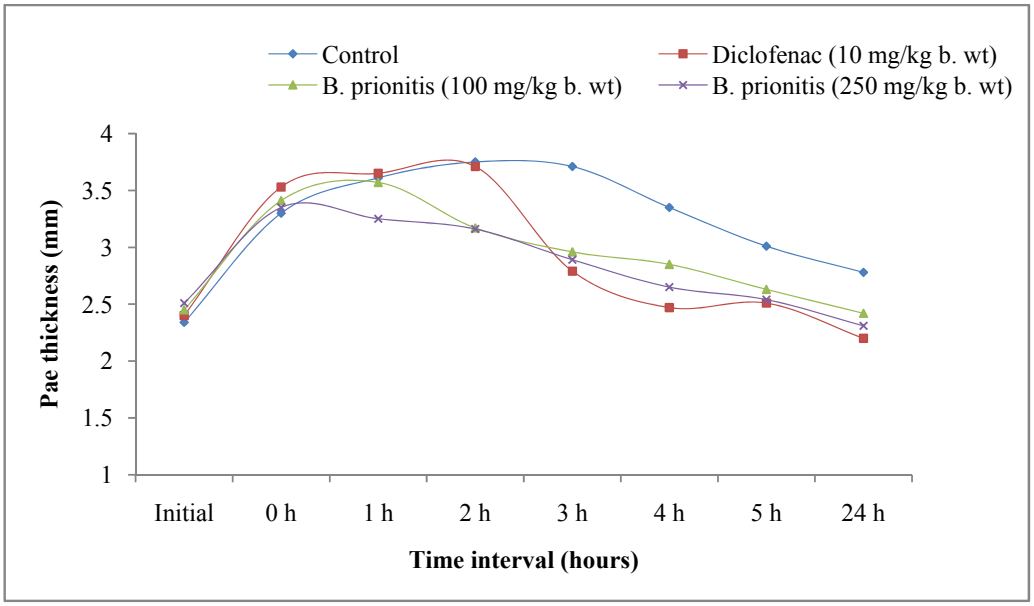

Fig. 14 Graph showing the effect of *Barleria prionitis* extract on carrageenan induced inflammation

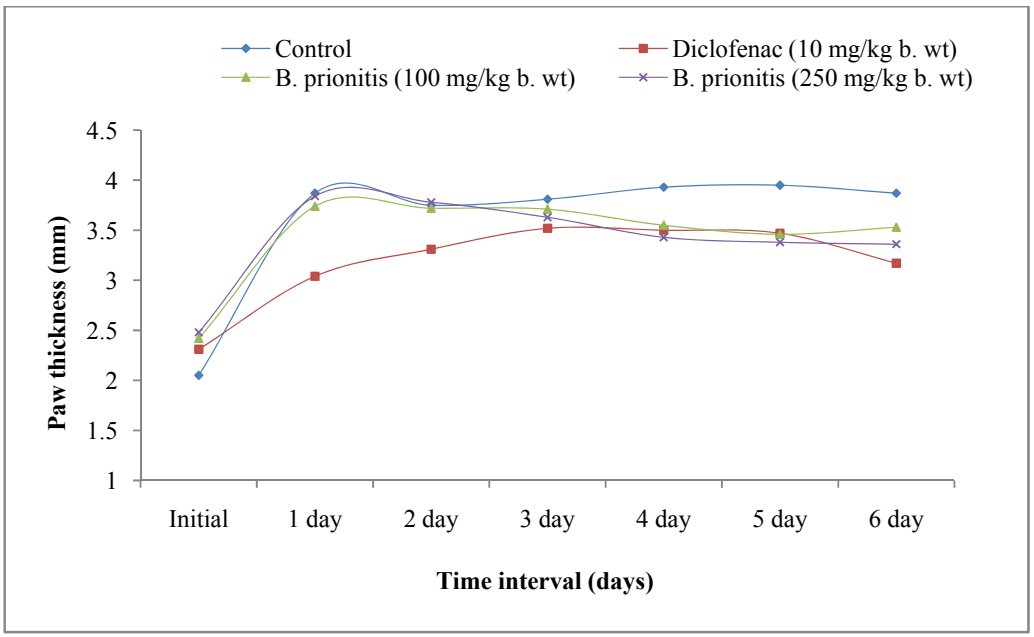

Fig. 15 Graph showing the effect of *B. prionitis* extract on formalin induced inflammation

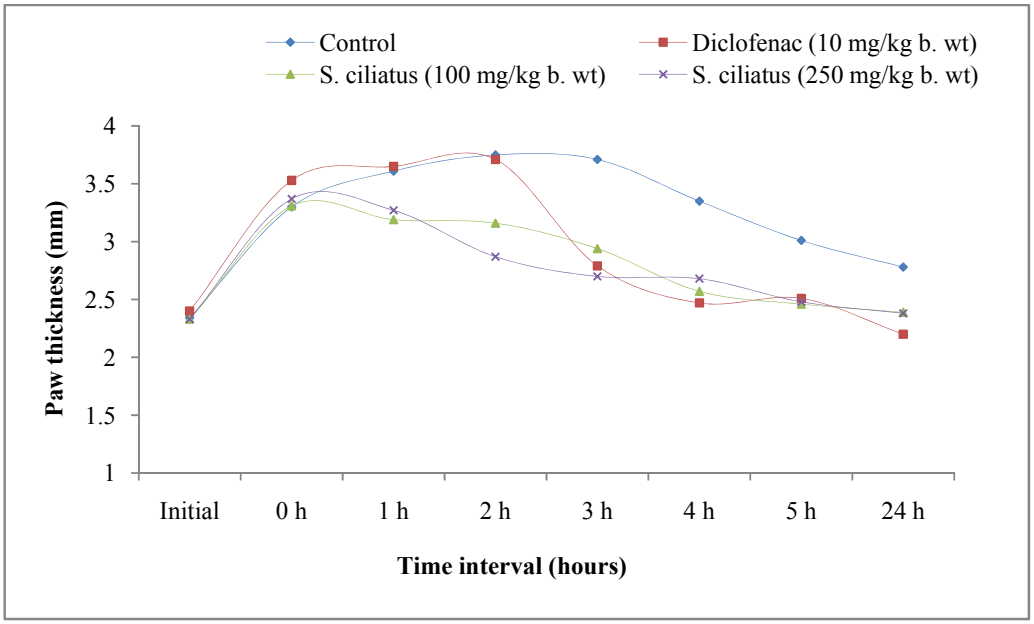

Fig. 20 Graph showing the effect of *Strobilanthes ciliatus* extract on carrageenan induced inflammation

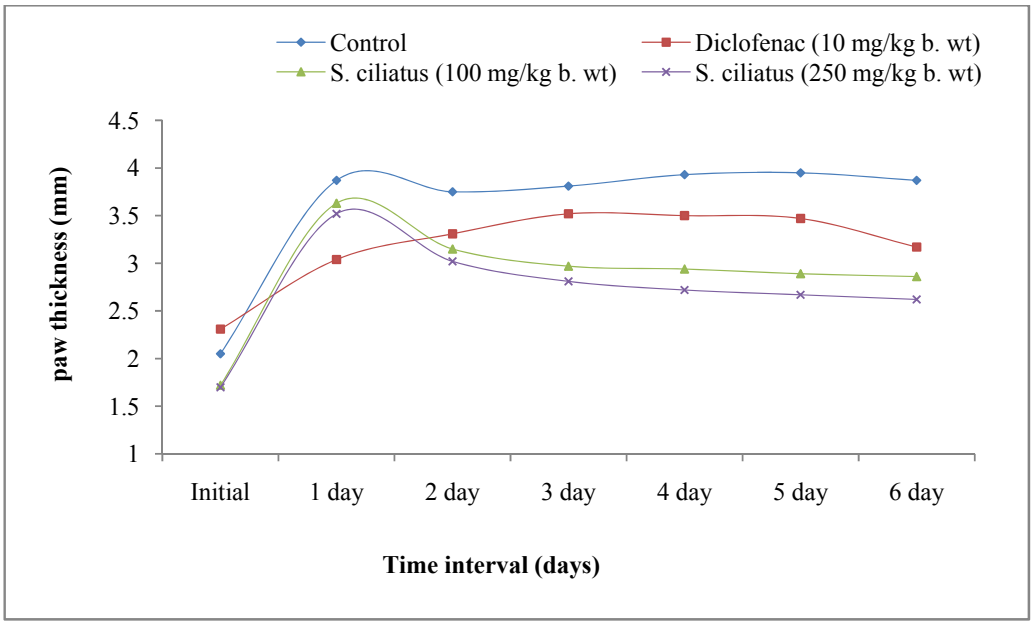

Fig. 21 Graph showing the effect of *S. ciliatus* extract on formalin induced inflammation

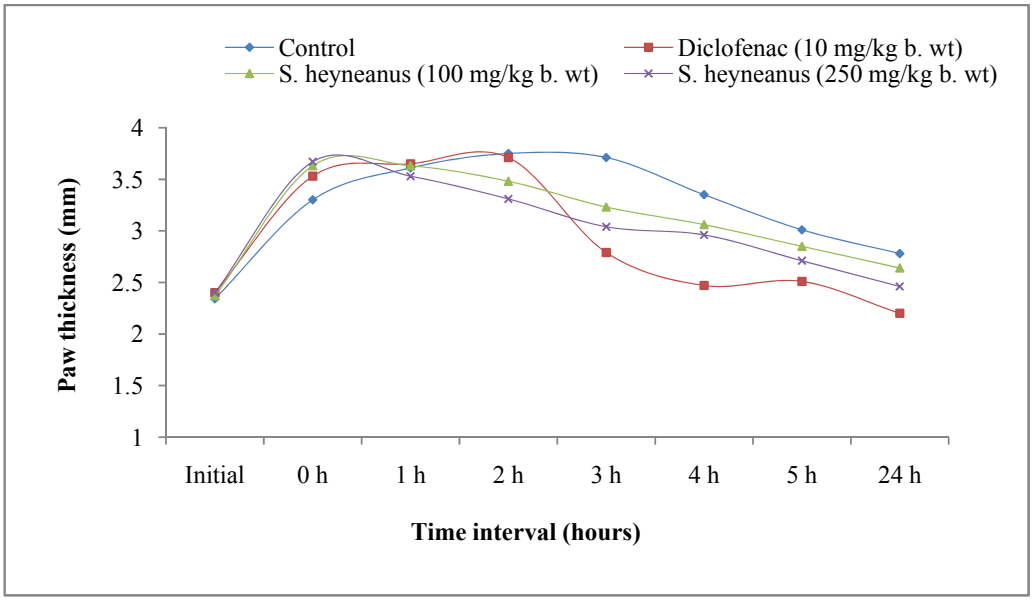

Fig. 22 Graph showing the effect of *Strobilanthes heyneanus* extract on carrageenan induced inflammation

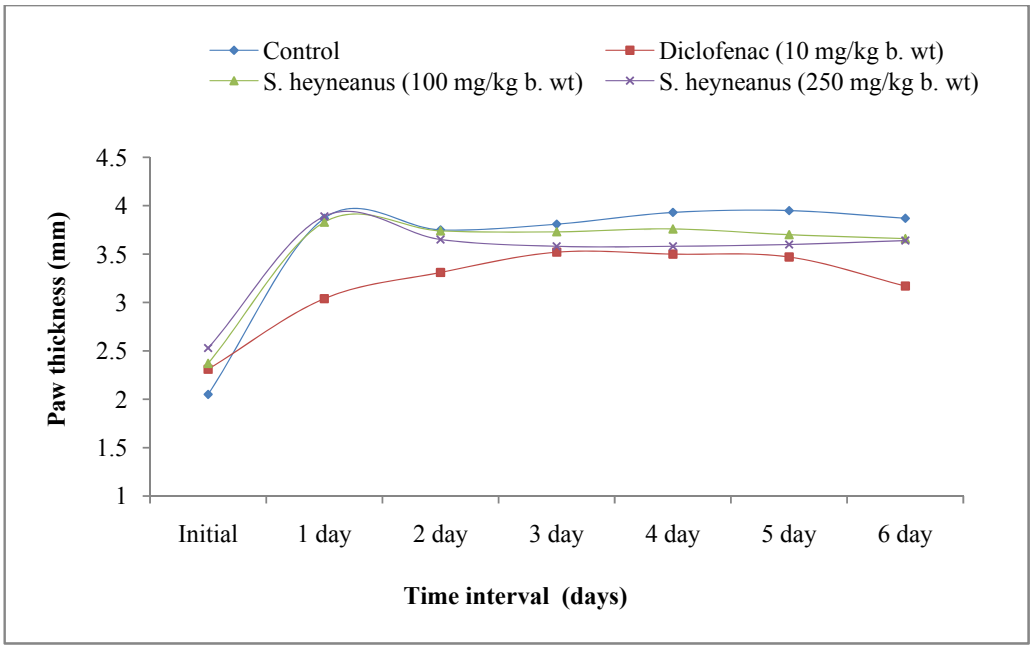

Fig. 23 Graph showing the effect of *S. heyneanus* extract on formalin induced inflammation

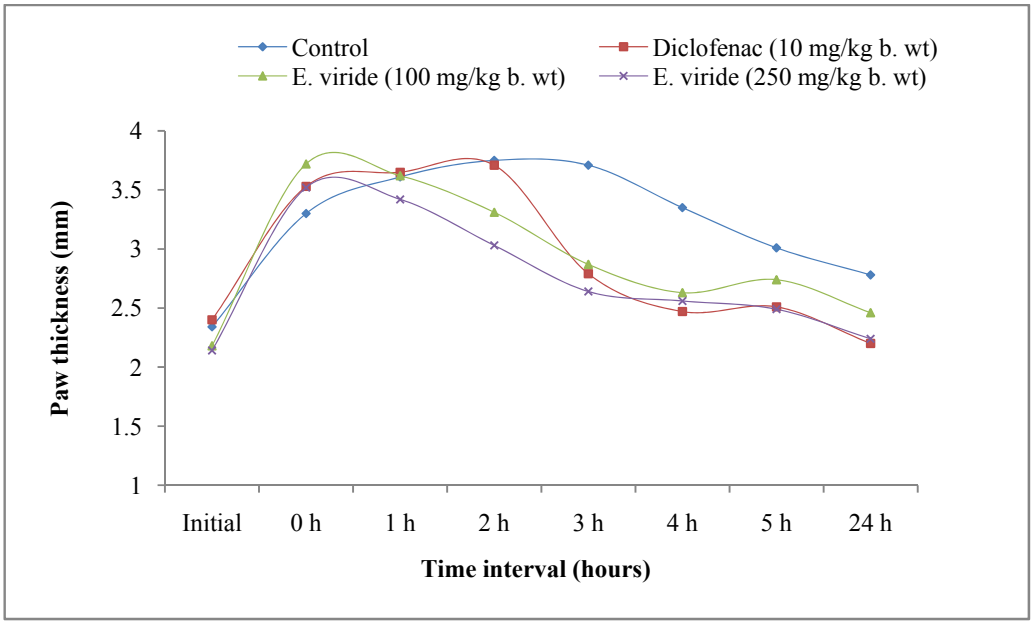

Fig. 16 Graph showing the effect of *Ecbolium viride* extract on carrageenan induced inflammation

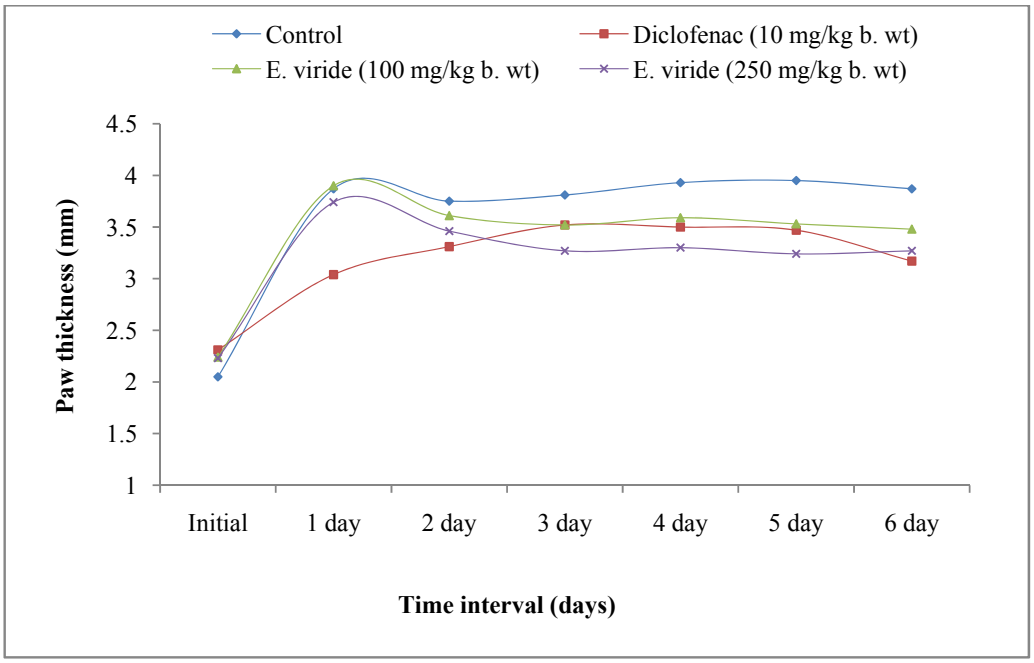

Fig. 17 Graph showing the effect of *E. viride* extract on formalin induced inflammation

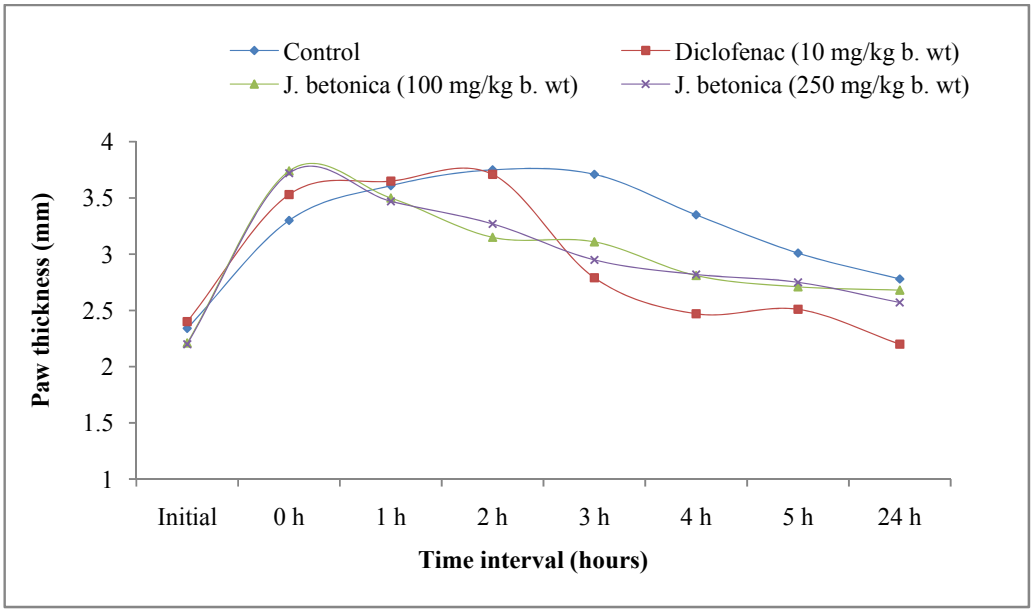

Fig. 18 Graph showing the effect of *Justicia betonica* extract on carrageenan induced inflammation

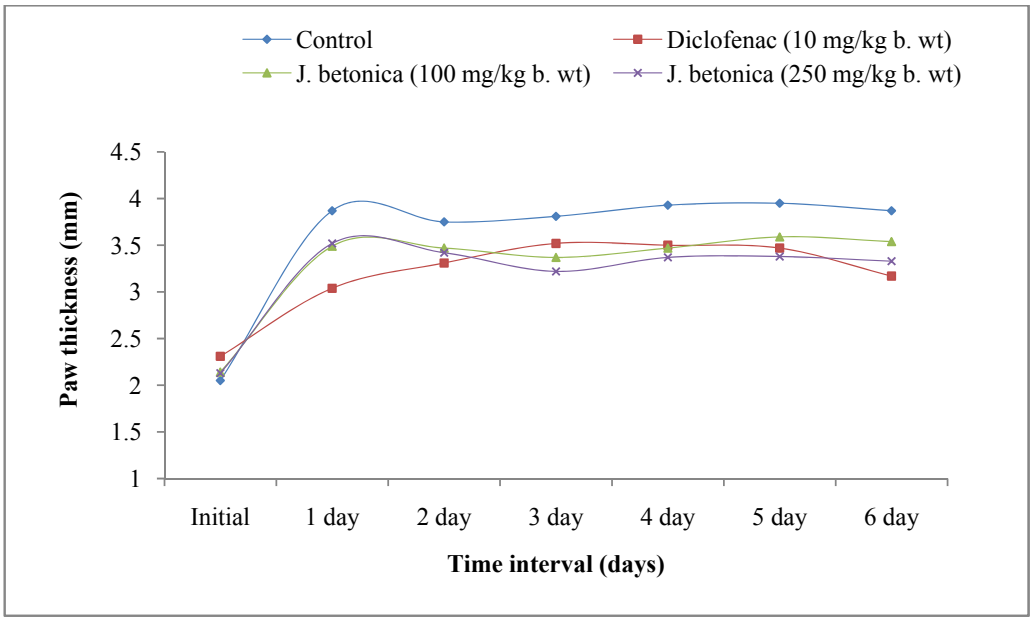

Fig. 19 Graph showing the effect of *J. betonica* extract on formalin induced inflammation

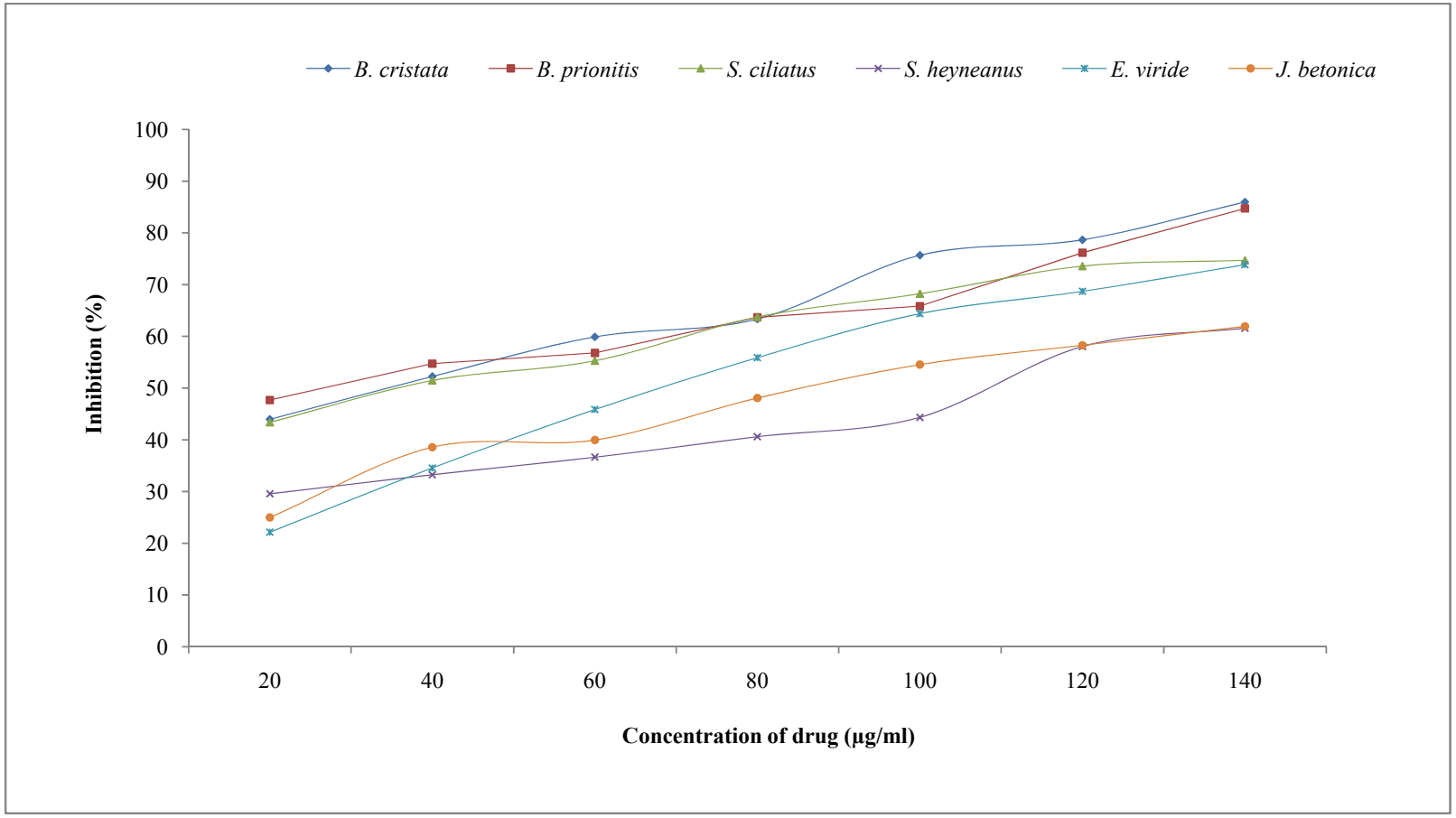

Fig. 9 Graph showing the *in vitro* superoxide radical scavenging activity of the extracts of six taxa of Acanthaceae

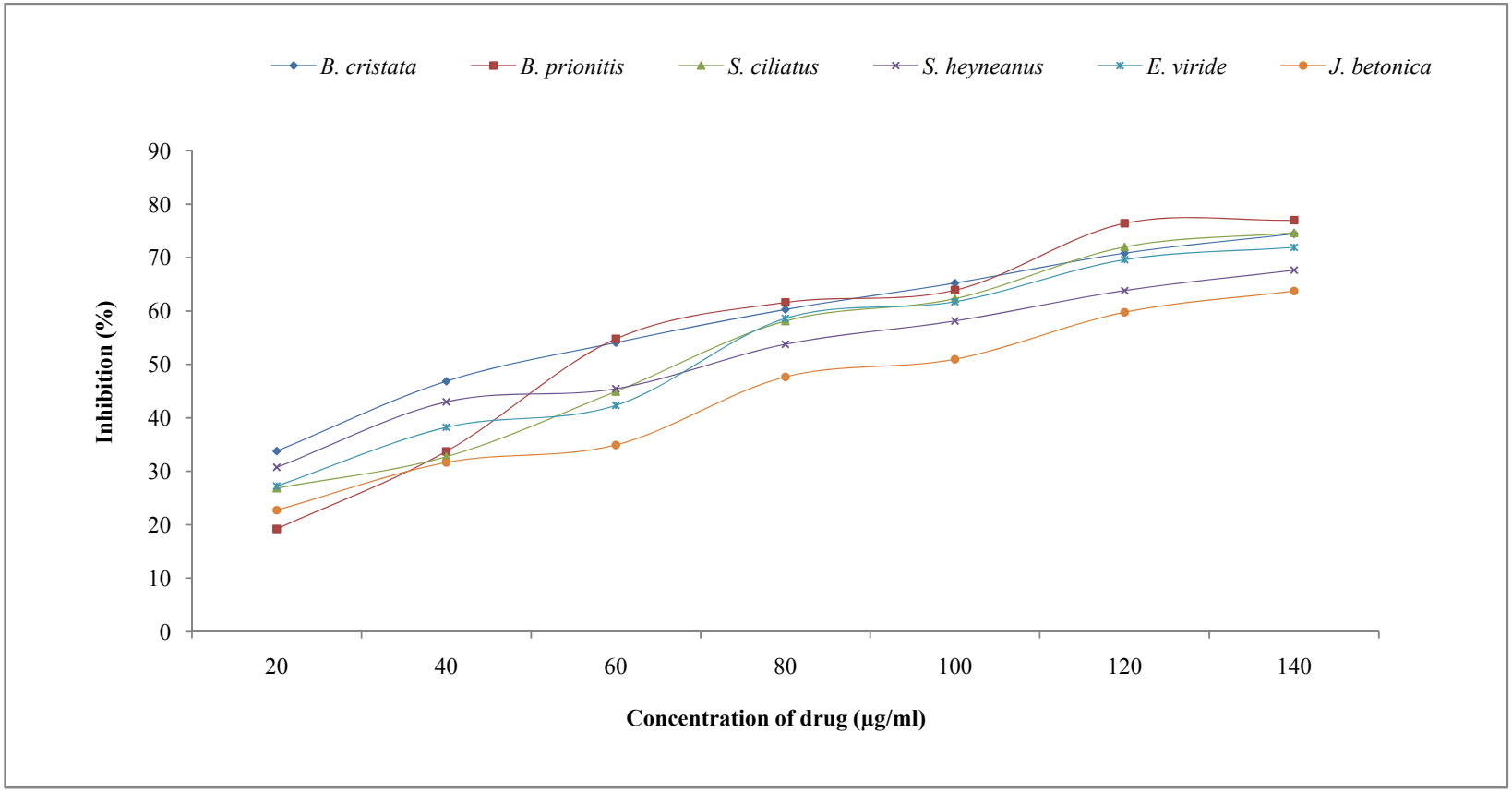

Fig. 11 Graph showing the *in vitro* inhibition of lipid peroxidation by the extracts of six taxa of Acanthaceae

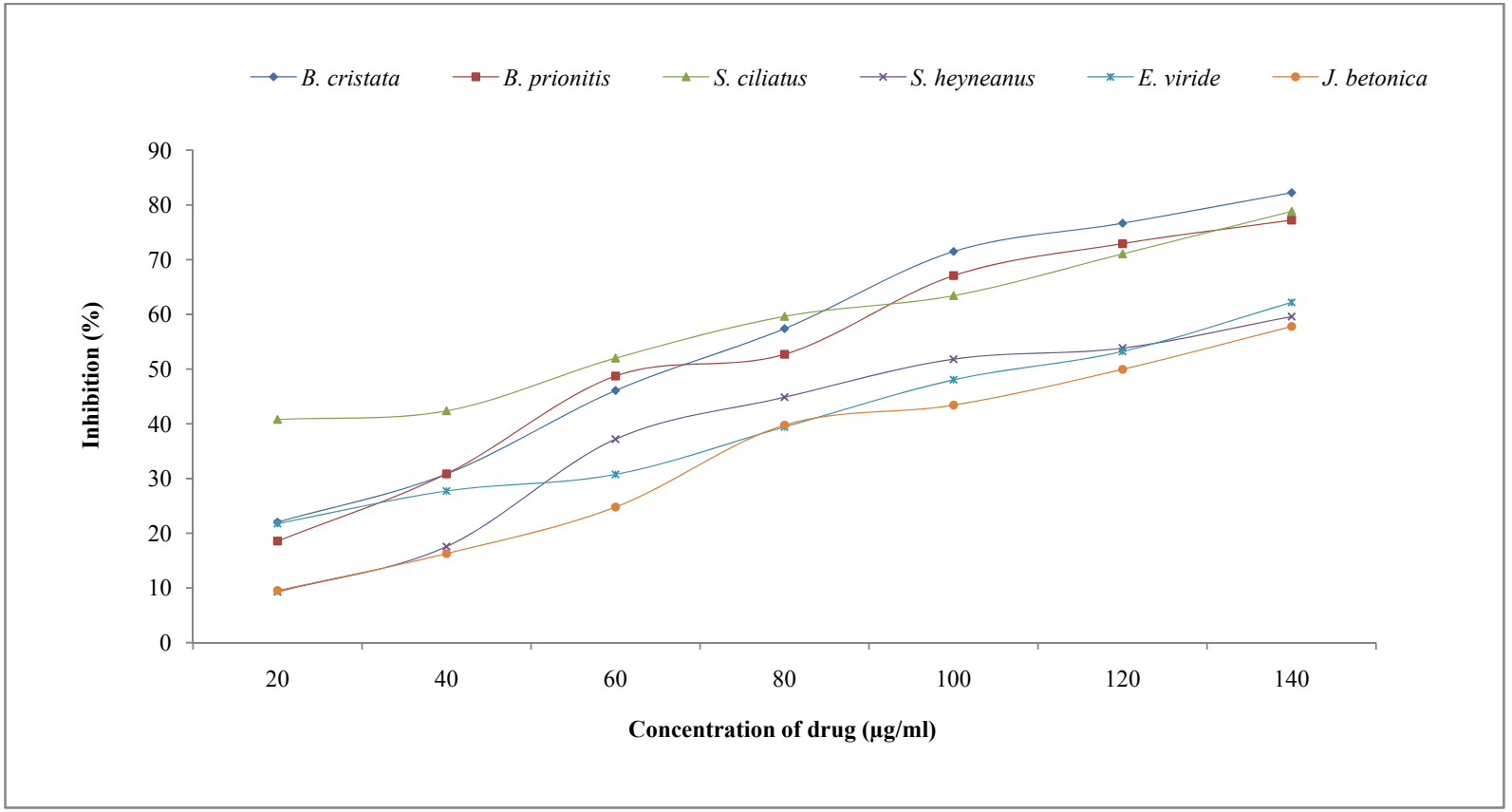

Fig. 10 Graph showing the *in vitro* hydroxyl radical scavenging activity of the extracts of six taxa of Acanthaceae

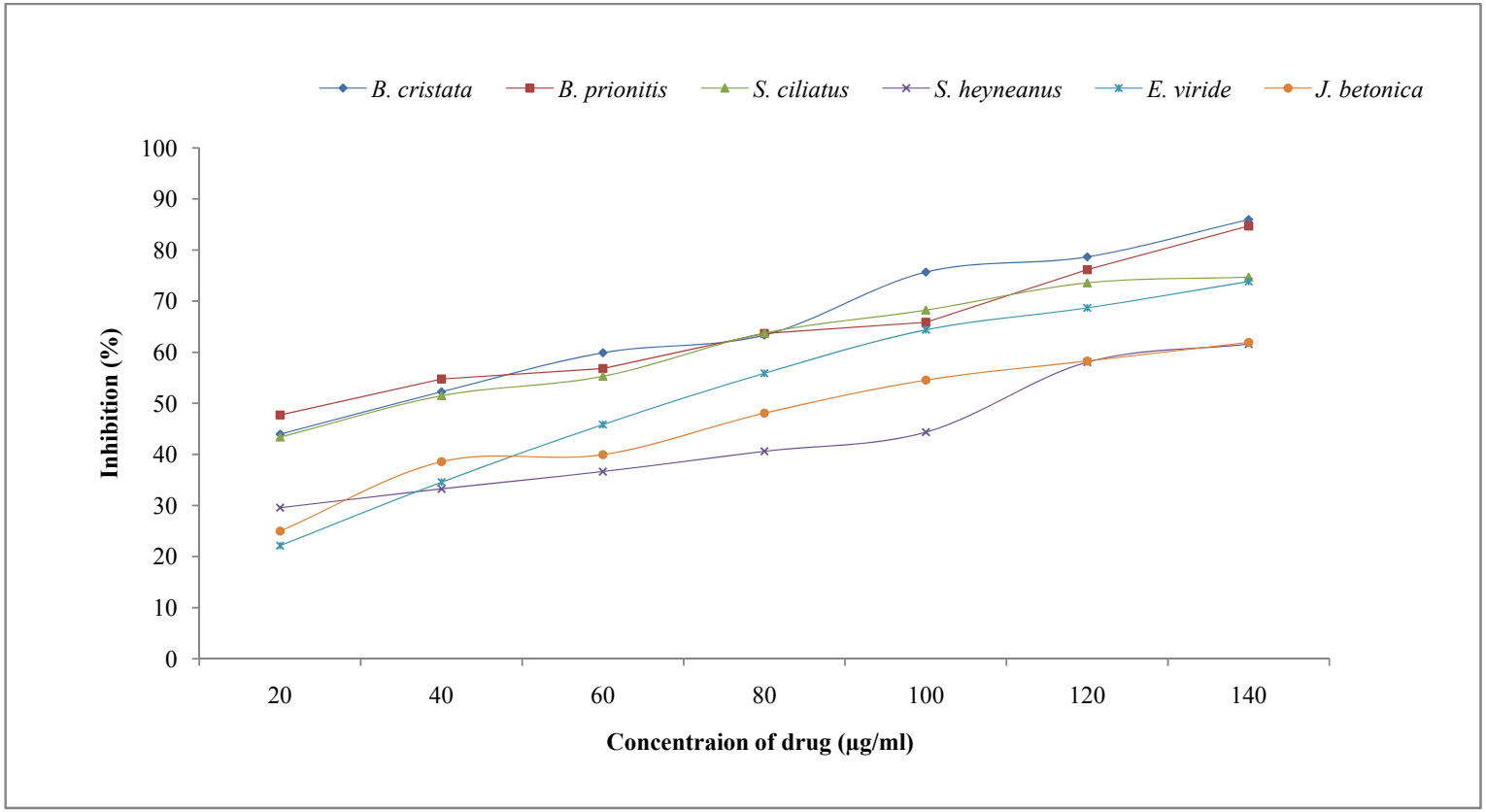

Fig 7 Graph showing the *in vitro* DPPH radical scavenging activity of the extracts of six taxa of Acanthaceae

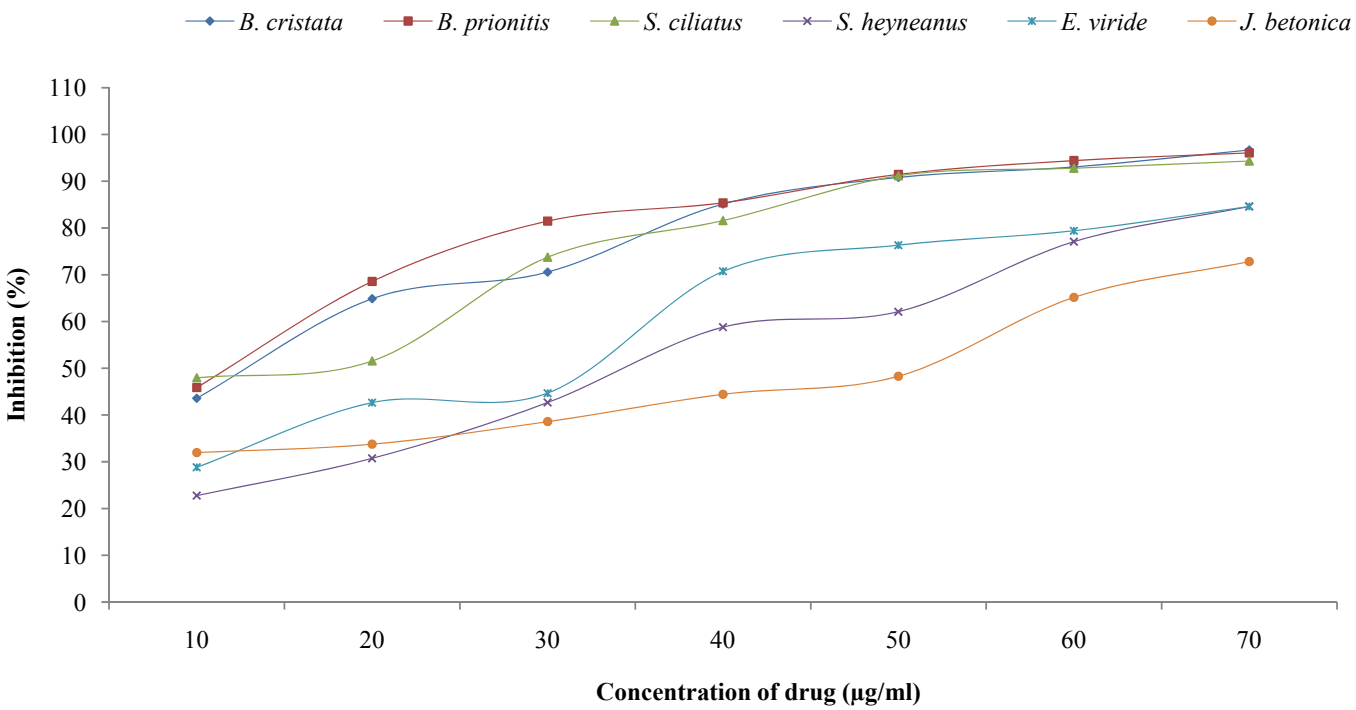

Fig. 8 Graph showing the *in vitro* ABTS radical scavenging activity of the extracts of six taxa of Acanthaceae

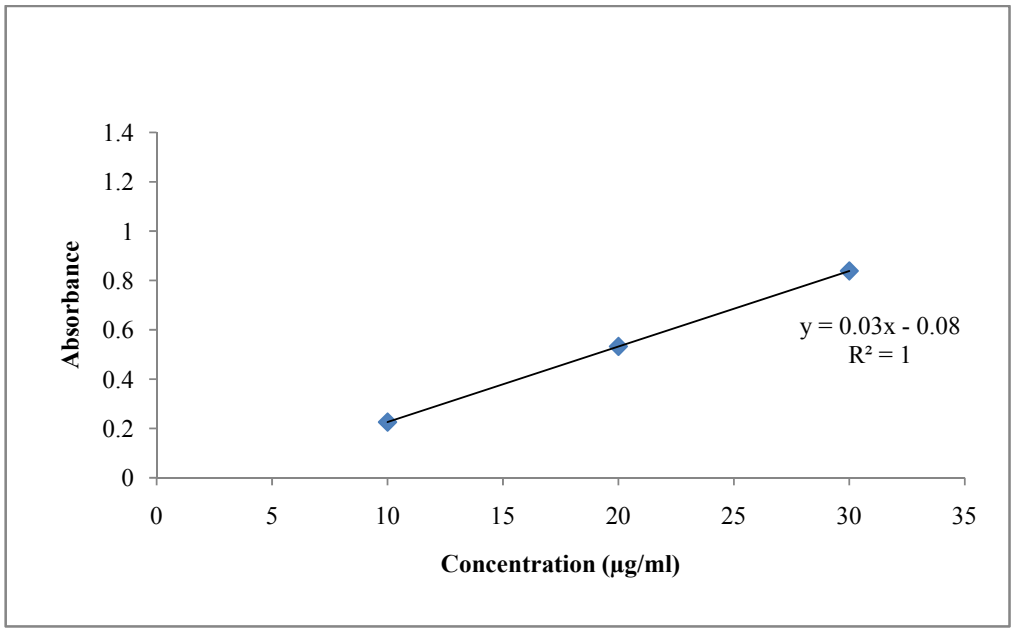

Fig. 39 Standard calibration curve for the determination of total phenolic content

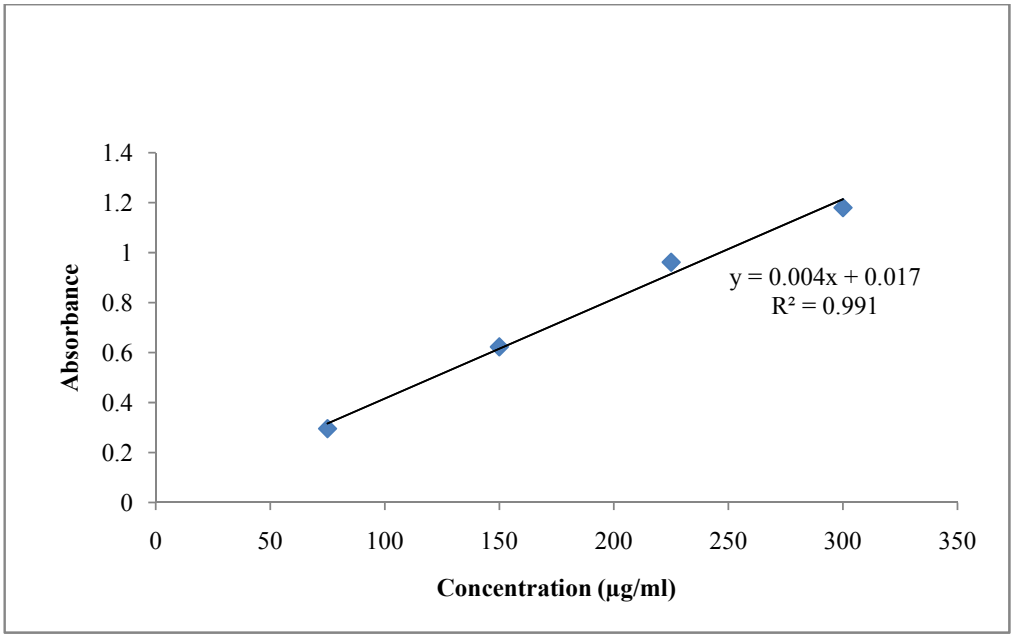

Fig. 40 Standard calibration curve for the determination of total flavonoid content

Table 28. Data showing presence/absence of bands in the HPTLC chromatogram of the six taxa used as 'Sahachara'

R_f	*B. cristata*	*B. prionitis*	*E. viride*	*J. betonica*	*S. ciliatus*	*S. heyneanus*
0.04	-	-	-	+	-	-
0.07	-	-	-	-	+	-
0.08	+	+	-	-	-	-
0.09	+	+	+	+	-	-
0.10	-	-	-	+	+	+
0.12	-	-	+	-	-	-
0.13	+	+	+	+	+	+
0.14	-	-	-	+	-	-
0.16	-	-	-	+	-	-
0.19	+	+		+	+	+
0.24	-	-	+	-	-	-
0.25	-	-	-	+	-	-
0.33	-	-	-	-	-	+
0.37	+	-	-	-	-	-
0.38	-	+	-	-	+	+
0.39	-	-	+	-	+	+
0.40	-	-	-	+	-	-
0.48	-	-	+	+	+	+
0.49	-	-	-	+	-	-
0.50	-	+	-	-	-	-
0.51	+	+	-	-	-	-
0.52	-	-	+	-	-	-
0.53	-	-	+	-	-	-
0.63	+	+	+	+	+	+
0.64	-	-	-	+	-	-
0.66	-	-	+	-	-	-
0.68	-	-	+	-	-	-
0.70	+	+	+	-	+	-
Total bands	8	9	12	13	9	8

Table 29. Paired Affinity index (PAI) from HPTLC data of the six taxa used as 'Sahachara'

	1BC	**2BP**	**3EV**	**4JB**	**5SC**	**6SH**
1BC	100.00	70.00	25.00	23.53	30.76	23.07
2BP		100.00	23.52	22.32	38.46	30.79
3EV			100.00	19.04	31.25	25.00
4JB				100.00	23.52	29.47
5SC					100.00	70.00
6SH						100.00

1. BC - *B. cristata*
2. BP - *B. prionitis*
3. EV - *E. viride*
4. JB - *J. betonica*
5. SC - *S. ciliatus*
6. SH - *S. heyneanus*

Table 38. Total phenolic and flavonoid contents of root methanolic extracts of the six taxa used as 'Sahachara'

Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg QE/g DW)
B. cristata	19.34 ± 0.40 [d]	3.80 ± 0.31[b]
B. prionitis	22.64 ± 1.93 [d]	5.84 ± 0.26 [c]
E. viride	15.16 ± 1.28 [c]	3.72 ± 0.21 [b]
J. betonica	3.14 ± 0.22 [a]	1.89 ± 0.30 [a]
S. ciliatus	15.91 ± 0.70 [c]	6.36 ± 0.46 [c]
S. heyneanus	10.80 ± 0.60 [b]	3.63 ± 0.11[b]

GAE – Gallic acid equivalents; QE – Quercetin equivalents

Values are expressed as mean ± standard deviation (n=3)

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test)