Pharmacognostic Studies on *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. (*Lamiaceae*)

Thesis submitted to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

BOTANY

By

K. SUBRAMONIAN

(Register Number: 3440)

DEPARTMENT OF BOTANY,

ST. XAVIER'S COLLEGE (AUTONOMOUS),

PALAYAMKOTTAI – 627 002.

MANONMANIAM SUNDARANAR UNIVERSITY,

TIRUNELVELI – 627 012

September 2014

Dr. S. RAVIKUMAR, Ph.D.,
(Co-Guide)
Professor,
School of Marine Sciences,
Alagappa University
Thondi – 623 409.

Dr. K. NATARAJAN, Ph.D.,
(Guide)
Head, (Retd.)
Department of Botany,
St. Xavier's College (Autonomous),
Palayamkottai – 627 002.

CERTIFICATE

This thesis entitled "**Pharmacognostic Studies on *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. (*Lamiaceae)***" submitted by **Mr. K. Subramonian** for the Degree of Doctor of Philosophy in Botany of Manonmaniam Sundaranar University is a record of bonafide research work done by him, and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any university / institution.

Dr. S. RAVIKUMAR, Ph.D.,
(Co-Guide)

Dr. K. NATARAJAN, Ph.D.,
(Guide)

Dr. S. RAVIKUMAR, Ph.D.,
(Co-Guide)
Professor,
School of Marine Sciences,
Alagappa University
Thondi – 623 409.

Dr. K. NATARAJAN, Ph.D.,
(Guide)
Head, (Retd.)
Department of Botany,
St. Xavier's College (Autonomous),
Palayamkottai – 627 002.

CERTIFICATE

This thesis entitled "**Pharmacognostic Studies on *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. (*Lamiaceae)***" submitted by **Mr. K. Subramonian** for the Degree of Doctor of Philosophy in Botany of Manonmaniam Sundaranar University is a record of bonafide research work done by him, and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any university / institution.

Dr. S. RAVIKUMAR, Ph.D.,
(Co-Guide)

Dr. K. NATARAJAN, Ph.D.,
(Guide)

Pharmacognostic Studies on *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. (*Lamiaceae*)

Thesis submitted to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

BOTANY

By

K. SUBRAMONIAN

(Register Number: 3440)

DEPARTMENT OF BOTANY,

ST. XAVIER'S COLLEGE (AUTONOMOUS),

PALAYAMKOTTAI – 627 002.

MANONMANIAM SUNDARANAR UNIVERSITY,

TIRUNELVELI – 627 012

September 2014

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

$	Dollar
%	Percentage
&	and
µg	microgram
µM	micromole
µm	micron
1 µ l	Microliter
2- 4 D	2 – 4 Dicholoro phenoxy acetic acid
AFLP	Amplified Fragment Length Polymorphic DNAs
ALP	Amplified Length Polymorphisms
BAP	6-Benzyl Amino Purine
BC	Before Christ
BSA	Bovine Serum Albumin
CCRAS	Council of Centre for Research in Ayurveda and Siddha
cm	centimeter
CTAB	Cetyl Trimethyl Ammonium Bromide
Df	Dilution factor
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
e.g.,	*Exempli gratia* (for example)
ELISA	Enzyme Linked Immuno Sorbent Assay
g	gram
GC	Gas Chromatography
Govt.	Government
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Liquid Chromatography
i.e.,	*id est* (= that is)
IAA	Indole Acetic Acid
IBA	Indole Butyric Acid
Km	Kilometer
Kn	Kinetin

M. H.	Muller Hinton
mg	milligram
mg/gdw	milligram / gram dry weight
mg/l	milligram / liter
min	minute
ml	milliliter
mm	millimeter
MS	Murashige and Skoog
MSL	Mean Sea Level
N.H.	National Highways
NAA	Naphthalene Acetic Acid
ng	nanogram
Nm	Nano meter
^{o}C	degree centigrade
OD	Optical Density
PCR	Polymerase Chain Reaction
Pet. ether	Petroleum ether
Pop Gene	Population Genetic analysis
RAPD	Random Amplified Polymorphic DNA
RFLP	Restricted Fragment Length Polymorphism
RH	Relative Humidity
rpm	rotation per minute
SCAR	Sequence Characterized Amplified Regions
STS	Sequence Tagged Sites
TBE	Trisboric EDTA
TE	Tris EDTA
TLC	Thin Layer Chromatography
UV	Ultra Violet
w/v	weight / volume
WHO	World Health Organization

INTRODUCTION

Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Early humans recognized their dependence on nature for sound health and treating illness. Physical evidence of the use of herbal remedies was found from some 6000 years ago in a burial site of a Neanderthal man uncovered in 1960 in a cave in Northern Iraq (Heinrich, 2000; Wickramasinghe and Bandaranayake, 2006). The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian Papyrus (Uniyal *et al.,* 2006). Another oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake (Stojanoski, 1999). The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung Circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even now-a-days such as the following: *Rhei rhisoma*, camphor, *Theae folium, Podophyllum*, the great yellow Gentian, Ginseng, Jimson weed, Cinnamon bark, and *Ephedra* (Kelly, 2009). The Ebers Papyrus, written Circa 1550 BC, represents a collection of 800 prescriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc. (Wiart, 2006). According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense (Biljana Bauer Petrovska, 2012). Led by instinct, taste, and experience, primitive men and women

treated illness by using plants, animal parts, and minerals that were not part of their usual diet.

Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal Pharmacopeias. Traditional medicine evolved over centuries, depending on local flora, culture, and religion (Sapna and Ravi, 2007). Indeed, well into the twentieth century, much of the Pharmacopeia of scientific medicine was derived from the herbal lore of native people. This knowledge of plant-based drugs developed gradually and was passed on, thus laying the foundation for many systems of traditional medicine all over the world.

Medicinal plants have played a key role in world health. They are distributed worldwide, but they are most abundant in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (Calixto, 2000). By definition, a herb is a plant or a part of a plant valued for its medicinal, aromatic, or pungent qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They are also referred to as botanicals, biomedicines, or herbal supplements. Herbal drugs range from parts of plants to isolated and purified active constituents. They may come from any part of the plant but are most commonly made from leaves, roots, bark, seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied to the skin (Bisset, 1994).

Quality control for efficacy and safety of herbal products is of paramount importance (WHO, 1981; WHO, 1999; EMEA, 2005). Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to the processes involved in maintaining the quality and validity of a manufactured product. Quality control and the standardization of herbal medicines involve several steps. The source and quality of raw materials, good agricultural practices and manufacturing processes are certainly essential for the quality control of herbal medicines and play a pivotal role in guaranteeing the quality and stability of herbal preparations (Blumenthal *et al*., 1998; WHO, 2000).

1.1. Standardization of herbal drugs

The evaluation or standardization of a crude drug involves Pharmacognostic methods such as Physico – chemical, Phytochemical and Pharmacological analysis. Pharmacognosy, which literally means studying preparation of medicines from natural sources, has been a part of medical arts and sciences since mankind first began to treat illnesses (Blondeau *et al.*, 2010). To get a proper perspective about this science, which deals with plant, animal, mineral and other natural medications, it is extremely helpful to investigate the historical aspects of this science and to recognize the pioneers of this field. The study of medications of plant origin includes the subjects of botany, chemistry and pharmacology. Botany includes taxonomy, genetics, and cultivation of plants. Chemical characterization includes the isolation, elucidation and quantification of constituents and natural products pharmacology. The science of Pharmacognosy has been progressed to phytochemistry, plant biotechnology, medical ethnobotany, ethnopharmacology, phytotherapy and marine and zoo pharmacognosy (Shinde *et al.*, 2008).

According to Willard (1996) standardization should involve the compilation of complete data on herbs such as the season in which the herb is harvested, the ripeness, the taste, smell, appearance, drying, storage, processing and fingerprinting which needs much larger spectrum of five or more active or marker constituents. Standardization is also considered as the way to deal with the regulations framed by regulatory authorities that require drug measurability and the active ingredients to be stated on product labels (Page, 2001). Quality control starts at the level of the starting material. The plant material is the most important factor in manufacturing herbal medicinal products. Plants are inevitably "irregular" because their composition may be influenced by multiple factors, such as origin, growth, harvesting, drying, and storage conditions. To ensure a consistent quality of product, however, it may be necessary to utilize the starting material from different geographical sources, this ensures compensation of annual variations that may occur despite a constant geographical source. Adulteration must also be taken into consideration when the material is purchased from commercial sources. This highlights the importance of the correct identification of an herbal drug. Consistent quality for products of herbal origin can only be assured if the starting material is defined in a rigorous and detailed manner. Besides botanical identification of plant material used, the exclusion or limitation of impurities such as other plants parts (foreign matter), microorganisms and their metabolites (aflatoxins) are important (Bauer, 1998).

To prove the consistent composition of herbal preparations, adequate analytical methods have to be applied including Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC) and Gas Chromatography (GC) (Bauer, 1998). To secure this, a number of pivotal quality standards needs to be set at the time of extract processing and the primary evaluation in pharmacological screening mode (Cos *et al.,* 2006). A vital part is the development of standardized protocols for extraction

of plant-derived crude extracts to be used in herbal medicine (Hildreth *et al.,* 2007). Adulteration in market sample is one of the greatest drawbacks in promotion of herbal products from India (Khatoon *et al.,* 1993).

1.2. Herbal Medicine in India

The roots of Indian medicine were set forth in the sacred writings called the *Vedas*, which date back as far as the 2nd century BC. It is estimated that, over 6000 plants used in India are in use in traditional, herbal and folk medicines. There are 121 most frequently used prescription drugs in different countries, on which 74% are derived from only 90 species of plants (Dubey and Tripathi, 2004).

India is one of the world's 12 biodiversity centres with the presence of over 45000 different plants species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value (Zeeshan *et al*., 2009). Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been coded in Ayurveda. The *Rig-Veda* (5000 BC) is recorded 67 medicinal plants, *Yajurveda* 81 species, *Atharvaveda* (4500-2500 BC) 290 species, *Charak Samhita* (700 BC) and *Sushrut Samhita* (200 BC) had described properties and uses of 1100 and 1270 species respectively. 7000-7500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda since ancient times. The Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, Amchi 600 and modern

medicine around 30 species. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed. In India, forest cover is disappearing at an annual rate 1.5mha/yr. What is left at present is only 8% as against a mandatory 33% of the geographical area. Many valuable medicinal plants are under the verge of extinction. *The Red Data Book of India* has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81vulnerable, 100 rare and 34 insufficiently known species (Thomas, 1997). The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant products such as gum, resins and latex. Even the allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern Pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. Diosgenin, Solasodine). Not only, the plant-derived drug offers a stable market worldwide, but also plants continue to be an important source for new drugs.

1.3. Methods of standardization

Standardization is a system that ensures a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose (Zafar *et al.,* 2005). Herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product. Moreover, many dangerous and lethal side effects have recently been reported, including direct toxic effects, allergic reactions, effects from contaminants, and interactions with herbal drugs (Svicekova *et al.*, 1993). Therapeutic activity of an herbal formulation

depends on its phytochemical constituents. The development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of marker/ bioactive compounds and other major constituents, is a major challenge to scientists. In view of the above standardization it is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of an herbal drug (Patra *et al*., 2010). The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health and to ensure reproducible quality of herbal medicine (Straus, 2002).

Standardization of herbal raw drugs include passport data of raw plant drugs, botanical authentification, microscopic and molecular examination, identification of chemical composition by various chromatographic techniques and biological activity of the whole plant (Sudha Revathy, 2012). Macroscopic and microscopic evaluation and chemical profiling of the herbal materials for quality control and standardization have been reported by various workers (WHO, 1996). Macroscopic identity of medicinal plant materials is based on sensory evaluation parameters like shape, size, colour, texture, odour and taste, while microscopy involves comparative microscopic inspection of powdered herbal drug. Further, advances in microscope technology have increased the accuracy and capabilities of microscopy as a mean of herbal crude material identification due to the implication of light and scanning electron microscopes (SEM) in herbal drug standardization (Bhutani, 2003). Furthermore, various advanced methods such as chromatographic, spectrophotometric and combination of these methods, electrophoresis, polarography, and the use of molecular biomarkers in fingerprints are currently employed in standardization of herbal drugs (Seitz *et al.,* 1991; O'Shea, 1995; Bhutani, 2003; Patel *et al.,* 2006; Mosihuzzaman and Choudhary, 2008).

Flowering plants (Angiosperms) comprise about 90% of the Kingdom Plantae. The total number of described species exceeds 234000 and many tropical species are as yet unnamed (Pullaiah, 2004). During the past 130 million years, flowering plants have colonized practically every conceivable habitat on earth, from sun-baked deserts and windswept alpine summits to fertile grasslands, freshwater marshes, dense forests and lush mountain meadows. Angiosperms are extremely useful for their seeds, fruits, and flowers. They are also used widely in medicines. *Lamiaceae* is one such a family, in which many plants have medicinal property. *Lamiaceae* or *Labiatae*, also called as the mint family, is a family of flowering plants. The family has a cosmopolitan distribution (Yuan *et al.,* 2010). The family *Lamiaceae* Lindl. consists of 258 cosmopolitan genera (Judd *et al.,* 1999), mostly distributed in the Mediterranean region and eastward into Central Asia (Cronquist, 1981). The *Lamiaceae* contains about 6,900 to 7,200 species (Venkateshappa and Sreenath, 2013). They are mainly herbs and shrubs, very fragrant and rich in medicinal properties of great worth in natural medicine and Pharmacopoeia. Medicinal constituents include the strong aromatic essential oil, tannins, saponins and organic acids. The plant has sedative, diuretic, tonic, antispasmodic and antiseptic properties. Still, several potent plants of *Lamiaceae* particularly from the rural areas are unexplored which deserve attention and research. *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. are such plants which have not been explored extensively by the scientific world so far. Literature data and folk knowledge confirm the use of plants *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth.

1.4. Significance of the selected medicinal plants

Orthosiphon is a genus of plants native to South East Asia and some parts of tropical Australia. It is an herbaceous shrub which grows to a height of 1.5 m (4.9 ft.). This plant has been used in traditional medicine for centuries to improve general health,

treatment of kidney diseases, bladder inflammation, gout, diabetes, back pain, dyspepsia, arthralgia, headache, fatigue and dysuria (Adam *et al*., 2009). Among the two selected species for investigation, *Orthosiphon comosus* Wight ex Benth. has very little previous record of use in traditional medicine. *Orthosiphon comosus* is a medium sized shrub and endemic aromatic plant to Kalakad Mundanthurai Tiger Reserve Forest (KMTR), in South India (Maridass *et al*., 2008). It is used in the treatment of back pain by the Kanis community of Karaiyar region, Kalakad Mundanthurai Tiger Reserve Forest, India (Maridass *et al*., 2008).

Considered one of the largest genera, ***Plectranthus*** L'Hér. includes ornamental (Judd *et al*., 1999), edible (Temple *et al.,* 1991; Allemann and Hammes, 2003; Allemann *et al.,* 2003) and medicinal species, *e.g. Plectranthus amboinicus* (Lour.) Spreng.(Ruiz *et al*., 1996) and *Plectranthus barbatus* Andrews (Matu and Van Staden, 2003; Pereira *et al*., 2004; Morais *et al*., 2005). Among the medicinal species of the genus, *Plectranthus wightii* Benth. is an aromatic herb employed in folk medicine for treating hepatic insufficiency and dyspepsia. Kurichia tribes residing at Vythiri taluk, Kerala, mixed the leaf paste with turmeric powder and salt is applied externally on wounds (Devi Prasad and Shyma, 2013). Its fresh leaves have a distinct odour and are taken as infusion or aqueous extract for healing purposes (Lorenzi and Matos, 2002).

1.5. Objectives

The main objectives of the present investigations are

- To study the anatomy of *Orthosiphon comosus* and *Plectranthus wightii.*
- To evaluate the fluorescence and physico – chemical properties of the *Orthosiphon comosus* and *Plectranthus wightii.*

- To carry out a preliminary phytochemical analysis of the taxa extracted in different solvents to find out their organic constituents.
- To carry out a systematic phytochemical study of the above taxa by extracting the chemical constituents of the plants with different solvents.
- To study the phytoconstituents of ethanolic extract of and *Orthosiphon comosus and Plectranthus wightii* by Gas Chramatogrophy and Mass Spectroscopy.
- To explore the antimicrobial efficacy of crude extract of *Orthosiphon comosus* and *Plectranthus wightii.*
- To investigate the effect of plant extract on *in-vitro* antioxidant potential and *in-vitro* anti-diabetic activity
- To evaluate the acute toxicity of ethanolic extract of the study plants.
- To study the Nephroprotective activity of ethanolic extract of *Orthosiphon comosus* and *Plectranthus wightii.*

It is assured that the findings will fill up the lacuna in scientific authentication of the selected plants for commercial preparation of validated drugs.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Herbal medicines prepared from plants are prone to contaminations, deterioration and variation in composition. This can be controlled by monitoring the growing conditions and harvesting time. Quality control starts at the level of part of plant harvested, absence of toxic pesticides or other contaminants. Pharmacognostic standards such as drying methods, freshness, storage, processing, extracting solvents, the whole extraction process and analytical controls used to prove the consistent composition of herbal preparations. The product quality control testing may lead to the production of standardized and therapeutically effective herbal formulations. Every country needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. The WHO has issued some guidelines for the assessment of herbal medicine (WHO, 1991) that include quality assessment of crude plant material, plant preparation and finished product. The following review focuses on the earlier reports on strategies, significance, guidelines and the research findings related to the present investigations.

2.1. The importance of *Labiatae* family

Plants of this family are used for different purposes, but we can group their uses into three main categories; a) medicinal b) ornamental and c) aromatic plants which are used as culinary herbs, vegetables and in the perfume industry.

a) Medicinal uses:

Many species are used in traditional and modern medicines and recent investigations have proved the basis of the medicinal uses. There are diverse uses of the

family members in traditional ways in different parts of the world. For instance *Ajuga chamaepitys* subsp. *tridactylites* used to treat jaundice, joint pain, gout (Brimani, 1987), *Ajuga reptans* for fever, asthma, gout (Zargari 1989 - 1992), *Calamintha graveolens* for impotence, stimulant (Aynehchi, 1986), *Calamintha acinos* for bronchitis, pertussis, dyspnoea, measles (Amini, 1997), *Lavandula dentata* for catarrah (Hooper and Field, 1937; Zargari, 1989 - 1992), malaria diarrhoea, nervous disorders (Aynehchi, 1986), *Lavandula angustifolia* for inflammation, *Mentha sylvestris* for rheumatism, dysentery, dyspepsia (Hooper and Field, 1937), *Mentha piperata* for flatulence, inflammation, dyspepsia, jaundice, throat infections diaphoretic, diuretic (Amini, 1997), *Mentha arvensis* for diuretic, stomach tonic (Zargari, 1989 - 1992), *Nepeta bracteata* for asthma, flatulence (Amin, 1991), *Ocimum basilicum* is used to treat urinary tract inflammation (Amini, 1997), chest and lung complaints (Hooper and Field, 1937), *Ocimum majorana* for rheumatism, eye pain and headache (Salami, 2002). Hot water extract of *Leucas aspera* is used orally as stimulant, anthelmintic, laxative, and diaphoretic (Balunas and Kinghorn, 2005), *Leucas aspera* and *Ocimum canum* are externally used to fumigate dwellings (Pushpangadan and Atal, 1984). The decoction *Leucas lavandulaefolia* is used orally for treatment of diarrhoea (Johns *et al.,* 1990). *Anisomeles indica*, whole plant extracts inhibit the inflammatory mediators and tumor cell proliferation (Hsieh *et al.,* 2008).

b) Aromatic and culinary uses:

Species of *Mentha, Thymus, Lavandula, Ocimum, Origanum, Melissa* and *Satureja* are also used as culinary and flavoring plants. These uses are mostly due to the presence of essential oils common to the family. The Mediterranean region has been the main center for domestication and cultivation of *Labiatae* and many cultivated species are derived from wild ancestors of this region. *Lallemantia iberica* was cultivated in Iran and

southern parts of the former USSR as an oil-seed plant (Rivera and Obon, 1992). *Mentha spicata* is used as a culinary herb and also in toothpaste and chewing gum industry. Other species of *Mentha* such as *Mentha aquatica* and *Mentha longifolia* are used as wild vegetables and culinary herbs. *Satureja hortensis* and *Satureja mutica* are used as flavoring plants. *Ocimum, Origamum* and *Melissa* species are cultivated as vegetables. Species of Thymus (Avishan) are used as culinary herbs and as tea in many parts of Iran. *Ziziphora tenuior* is a common teapot herb. Species of *Lavandula*, which contain aromatic terpenoid compounds, are also used in culinary and perfumery.

c) Ornamental uses:

Several genera of the family grow as ornamentals, including *Lavandula, Mentha, Molucella, Nepeta, Perovscia, Stachys, Teucrium, Salvia* and *Thymus*. Many species of *Stachys, Thymus* and *Satureja* are used as rock garden plants. Many of the decorative new world species of *Salvia* are cultivated throughout the world. *Nepeta cataria, Nepeta grandiflora*, and *Nepeta sibirica*, with big blue flowers are common ornamentals.

2.2. Ethnomedicinal uses of *Orthosiphon* and *Plectranthus*

Ethnobotanical studies were conducted to document new phytomedicines used and to determine their chemical constituents with a view to provide the scientific basis of the remedies in traditional medical practice and as a guide to bioprospecting of drugs. *Labiatae* are best known for the essential oils common to many members of the family. Many biologically active essential oils have been isolated from various members of this family. The family is also famous for the presence of diterpenoids in its members. These plants have been used by humans since prehistoric times. This family is one of the major sources of culinary, vegetable and medicinal plants all over the world. Species of *Mentha, Thymus, Salvia, Origanum, Coleus* and *Ocimum* are used as food flavorings, vegetables

and in cosmetic industry. Also several species of this family are used in traditional and modern medicines. *Orthosiphon stamineus* is used in several South East Asian countries especially in Indonesia, Malaysia, Thailand, Vietnam and Myanmar. It is found extending from tropical Asia to tropical Australia. It is wild but can be planted. This plant has been traditionally used for many diseases. *Orthosiphon stamineus* is also recognized in European country, such as Holland, France and England to be consumed as an herbal product. In Indonesia, this plant is under systemic cultivation. The leaves have been used for diuretic, preventing and treating rheumatism, diabetes mellitus, hypertension, tonsillitis, epilepsy, menstrual disorder, gonorrhea, syphilis, renal calculi, gallstone, acute and chronic nephritis, gout arthritis, and antipyretic (Anonymous, 1995; Isnander and Kumpulan, 2005; Arisandi *et al*., 2006).

2.3. Pharmacognostic studies in *Lamiaceae*

The World Health Organization (WHO) has been promoting traditional medicines as a source of less expensive, comprehensive medical care, especially in developing countries. Eighty percent of the world's population relies on medicinal plants for their primary health care. Such herbal medicines are easily available, cheaper, time tested and considered safer than some modern synthetic drugs (Farooqi and Sreeramu, 2001). Recently World Health Organization (WHO) introduced guidelines on research and evaluation of traditional medicines and practices. This guideline has a major objective of developing traditional medicines which ultimately leads into standardized and scientifically validated drugs. This guideline aims to ensure quality and safety of botanicals before being evaluated for its efficiency. On this background Pharmacognosy is playing a paramount role in the evolution of novel medicines taking lead from natural products (Vaibhav and Kamlesh, 2007).

The initial step in the identification and authentication of botanical materials entails classical botanical methodologies for collection and documentation of the plant at its source. It is the first step for authentication (Smille and Khan, 2010). Macroscopic identity of botanical materials is based on parameters like shape, size, color, texture, surface characteristics, powder characteristics, odor, taste and such organoleptic properties that are compared to a standard reference material (WHO, 1998).

Herbal drugs are still at the risk of quality because sources of raw material of herbal drugs are different and an herb is mixture of multi-constituents, and lack of availability of efficient analytical methods. Hence, it is necessary to set official standards to maintain quality, the first step of which must be identification of entire constituents and physicochemical parameters in herbs. Organoleptic evaluation, physicochemical parameters viz; ash values, extractive values, heavy metals, microbial counts, phytochemical screening are the primary evaluation in Pharmacognosy. The physicochemical parameters of total ash, acid insoluble ash, water soluble ash and sulphated ash were studied in the leaf of *Oreganum vulgare* and the results were 11.5%, 11%, 5%, 10.5% w/w respectively. The extractive values of ethanol extract and aqueous extract by hot extraction method were found to be 0.003% and 0.02% w/w respectively and that of by cold extraction method were found to be 0.003%, 0.02% w/w respectively (Veni Bharti and Neeru Vasudeva, 2013).

Pharmacognosy of the *Lamiaceae* species has been investigated by many researchers. The morphology and anatomy of some species have been studied (Tahir *et al.,* 1995; Ozdemir and Senel, 2001; Baran and Ozdemir, 2006). The most important features of *Lamiaceae* taxa are the presence of glandular hairs distributed in vegetative and reproductive organs (Werker, 2006). These hairs are the source of etheric oils and

their structures have been examined anatomically and micromorphologically (Hanlidou *et al.,* 1991; Vrachnakıs, 2003; Kaya *et al.,* 2007).

2.4. Anatomical studies in *Lamiaceae*

Plant anatomy is the study of internal structure of plants which originally included plant morphology namely the description of the physical form and external structure of plants. Since mid-20th century the investigations of plant anatomy are considered as a separate, distinct field, and plant anatomy refers to just the internal plant structures (Raven *et al*., 2005). The most important features of *Lamiaceae* taxa are the presence of glandular hairs distributed in vegetative and reproductive organs (Werker, 2006). Anatomical structures of the petiole are very important in family *Lamiaceae*. The taxonomic significance of the structure of trichomes is well known in the *Lamiaceae* (Metcalfe and Chalk, 1972). The morphology, distribution and frequency of glandular trichomes are used as discriminative characters at subfamilial level in the *Lamiaceae* (Ascensao *et al*., 1995). Many species of this family are aromatic and are often used as herb spices, folk medicines and fragrances (Werker *et al*., 1985).

Histology and microscopy are valuable both for powders and ungrounded drugs. The type of certain factors such as epidermal parenchyma, stomata, trichomes, fibres, vessels and calcium oxalate crystals, helps in the identification of drugs. The family is known for its fine ornamental or culinary herbs like basil, lavender, mint, oregano, rosemary, sage and thyme and is a rich source of essential oils for the flavouring and perfume industry (Wagstaff *et al*., 1998). Plant anatomy is often divided into the flower anatomy, leaf anatomy, stem anatomy, wood anatomy and root anatomy (Craig and Vassilyev, 2010). Glandular hairs are generally peltate and capitates. Peltate hairs have short stalk with many celled head. Capitate hairs have a stalk which consists of many

cells (Hanlidou *et al*., 1991). Concerning the leaf anatomy, the family exhibits predominantly diacytic stomata on both surfaces. The occurrence of diverse kinds of glandular and non-glandular trichomes is characteristic of *Lamiaceae*. The peltate trichomes have a uniform morphology. Capitates glandular and non-glandular trichomes have been diverse for this family. With reference to the chlorenchyma organization, isobilateral, dorsiventral or centric mesophylls have been frequently reported for *Lamiaceae* (Metcalfe and Chalk, 1950).

In *Orthosiphon rubicundus* Benth. the epidermis is a single layer with compactly arranged cells. The upper surface is covered with cuticle and trichomes. Trichomes are few capitate and glandular with short unicellular heads. Non-glandular trichomes are numerous multicellular and uniseriate, with an acute apex. Anatomically non-glandular trichomes are common in epidermis of all these plants stem sections, capitate glandular trichomes present in *Orthosiphon rubicundus*, peltate glandular trichomes present in *Leucas cephalotes*; both capitate and peltate trichomes are found in *Coleus forskohlii*. Diacytic stomata located towards lower epidermis of leaf in *Orthosiphon rubicundus* and *Coleus forskohlii*. Diacytic amphistomata shows in *Ocimum basilicum* and anomocytic stomata located towards lower epidermis in *Leucas cephalotes* (Venkateshappa and Sreenath, 2013).

Trichomes are considered relevant in comparative systematic investigations and morphodiagnosis (Metcalfe and Chalk, 1988). The occurrence of diverse kinds of glandular and non-glandular trichomes is characteristic of *Lamiaceae* (Metcalfe and Chalk, 1950). Concerning the leaf anatomy, the family exhibits predominantly diacytic stomata on both surfaces (Metcalfe and Chalk, 1950). *Plectranthus neochilus* shows this pattern, unlike the reported anomocytic stomata for *Plectranthus barbatus* (Scavone, 1965).

Rapisarda *et al* (2001) studied *Nepetha sibthorpii* (*Lamiaceae*) and showed the glandular hair micromorphology characteristic to be beneficial. Similarly, Bosabalids and Tsekos (1984) stressed the importance of the hair structures on *Origanum* species. Ozdemir and Senel (1999, 2001) showed the importance of the amount of vascular bundles and its arrangement within the petiole of *Salvia* species.

Microscopic determinations such as vein-islet number, veinlet termination number, palisade ratio, stomatal number, stomatal index and the size of fibres and vessels help in the identification of the plant and the differentiation of the closely allied species. Besides the ordinary light microscope, polarized and fluorescence microscopes can also be used to enhance the accuracy of authentication. Use of these microscopes expands the number of features available for use in identification. For example, it has been found that starch grains, crystals of calcium oxalate, stone cells, vessels and fibres have stable and special polariscopic characteristics (Zhao *et al.,* 1996).

Hullatti Prasenjit Bhattacharjee (2011) reported the pharmacognostical evaluation of leaves, stems and roots of *Coleus amboinicus* by using different parameters which include morpho-anatomical studies, physicochemical properties and fluorescence analysis to set the quality control parameters for the raw material. Gupta *et al* (2011) studied the pharmacognostic parameters of leaves of *Ocimum gratissimum*. Macroscopic study showed that the leaves of this plant possess dark green color with characteristic aromatic odour and bitter taste. Powder microscopy showed the presence of numerous glandular simple trichomes.

Microscopy is used to determine the structural, cellular and internal tissue features of botanicals. It is usually used to identify and differentiate two herbals that are similar (Lau *et al.,* 2004; Cheng *et al.,* 2007). Kahraman *et al* (2010) reported some anatomical

characters of *Salvia chrysophylla* such as 2–24 rowed pith rays in roots, dorsiventral leaves, obviously larger upper epidermal cells, and two to three large vascular bundles in the center and two to four small subsidiary bundles in the wings of petiole provide information of taxonomical significance. Allemann *et al* (2003) studied that the organographic and anatomical evidence of edible storage organs of *Plectranthus esculentus.*

Oznur Ergen Akcin *et al* (2011) examined and compared the anatomical structures of the petiole of seven taxa viz., *Glechoma hederacea*, O*riganum vulgare, Scutellaria salviifolia*, *Ajuga reptans, Prunella vulgaris*, *Lamium purpureum* var. *purpureum, Salvia verbenaca*, *Salvia viridis*, *Salvia virgata*, belonging to the family *Lamiaceae*.

The secondary growth of *Plectranthus neochilus* is of the ordinary type, as observed in many herbaceous dicotyledons (Esau, 1977). The rectangular transection is frequently described for *Lamiaceae* (Metcalfe and Chalk, 1950; Barroso, 1991). The presence of collenchyma in the four angles (Cronquist, 1981) is considered as a diagnostic value, according to Metcalfe and Chalk (1950). Pith rays of *Lamiaceae* family are 2 - 12 or more rowed and quite heterogeneous in structure (Metcalfe and Chalk, 1972).

2.5. Phytochemistry in *Lamiaceae*

This family contains a wide variety of chemicals. A wide range of compounds such as terpenoids, iridiods, phenolic compounds and flavonoides have been reported from the members of the family (Richardson, 1992; Zegorka and Gloiwniak, 2001; Jamzad, 2001). Some of the short chain terpenoids in essential oils are responsible for odor and taste in these plants. *Lavandula* species contain several pleasant-smelling terpenoid compounds and are used in perfumes and for deterring moth damage in stored

clothing (Richardson, 1992). Lebdanditerpenoids are found in 20 genera of the family including *Ballota, Coleus, Lagichilus, Leonotice, Marrubium* and *Sideritis*. *Coleon* compounds (tri-cyclic diterpenoids), found in leaves and inflorescence of *Plectranthus* and other genera, have some antioxidant properties (Jamzad, 2001).

Iridoides are also found in the family and have taxonomic importance. The family is also a rich source of plant species containing large amounts of phenolic acids. For example, rosmarinic acid occurs in species of the subfamily *Nepetoideae* and it is absent in the subfamily *Lamioideae* (sensu Erdetman). This compound has anti-bacterial, anti-viral, antioxidant and anti-inflammatory properties. More and more studies carried out in numerous research centers show that the complex pharmacological activity of some medicinal plants of the family is strictly connected with the presence of phenolics (Zegorka and Glowniak, 2001). Flavonoides also occur in the *Labiatae* in a variety of structural forms including flavones, flavonols, flavanones, dihydroflavonols and chalcones (Tomas-Barberan and Gil, 1992). In addition to essential oils, abietanediterpenoids and 8, 13-epoxy-labd-14-en-11-one diterpenoids are the main constituents found in *Plectranthus barbatus.* The major ethnobotanical uses are for intestinal disturbance and liver fatigue, respiratory disorders, heart diseases and certain nervous disorders. Forskolin as one of the major constituents with its unique adenylyl cyclase activation that underlies the wide range of pharmacological properties could explain the different traditional uses of *Plectranthus barbatus* (Rawiya *et al.,* 2010).

A wide range of compounds such as terpenoids, iridiods, phenolic compounds and flavonoids have been reported from the members of the family *Lamiaceae* (Zegorka and Glowniak, 2001; Lu and Yeap, 2002). Flavonoides also occur in the *Labiatae* in a variety of structural forms including flavones, flavonols, flavanones, dihydroflavonols and chalcones (Tomas-Barberan and Gil, 1992).

Egwaikhide and Gimba (2007) screened the extracts of *Plectranthus glandulosus* by using hexane, ethyl acetate and ethanol as solvents for secondary metabolites and antibacterial activity. The extracts revealed the presence of alkaloids, tannins, anthraquinones, glycosides, reducing sugars, saponins, flavonoids, steroids and terpenoids.

Smith *et al* (1996) showed the presence of coumarins, hydrolysable tannins, essential oil, (thymol 62.53%, the major component) and triterpenoids. Alkaloids, steroids, anthraquinones, flavonoids, condensed tannins, cardiac and anthraquinone glycosides are absent in fresh leaf of *Plectranthus tenuiflorus*. Essential oil, abietanediterpenoids and 8, 13-epoxy-labd-14-en-11-one diterpenoids are the main constituents found in *Plectranthus barbatus* (Alasbahi and Melzig, 2010).

Several *Plectranthus* plants have been studied chemically and a large number of abietane, phyllocladane, kaurane, clerodane and labdanediterpenoids have been isolated from them, together with oleanane, ursane and lupanetriterpenoids, aristolane and aromadendranesesquiterpenes, flavonoids and long-chain alkylphenols (Abdel-Mogib *et al.,* 2002; Rijo *et al.,* 2002; Gaspar-Marques *et al.,* 2004). Two new diterpenoids, ent-7a-acetoxy-15-beyeren-18-oic acid and (13S, 15S)-6b,7a,12a,19-tetrahydroxy-13b,16-cyclo-8-abietene-11,14-dione, have been isolated from *Plectranthus saccatus* and *Plectranthus porcatus* (Maria *et al.,* 2010).

The phytochemical analysis of essential oil of *Orthosiphon aristatus* revealed that sesquiterpenes were the main components, including β-elemene, β–caryophyllene and its oxide, β-selenene, cadinene, humulene, and α-guayene (Schut and Zwaving, 1986; Fernandes *et al.*, 2007). The chemical composition of the essential oil of *Orthosiphon diffuses* was investigated by Sadashiva *et al* (2013) and reported the major volatile

components of the oil were n-eicosane (19.5%), t-caryophyllene (18.6%), octocosane (12.2%), limonene (11.6%), β-ocimene (4.2), methyl palmitate (2.8%) and elemol (2.6%).

In five *Salvia* species, Nickavar *et al* (2007) reported the total flavonoid content which ranged from 8.58±0.99 to 53.16±1.95 mg/g DW due to the difference in species. Tannin content among different tissues of three species of *Coleus* was found to be 0.085-0.210 mg/g FW (Rasineni *et al.,* 2008). Sandhyarani *et al* (2011) reported that the flavonoid ranged from 13.30±0.684 (*Perilla frutescens*) to 26.03±0.217 (*Ocimum basilicum*). Hakkim *et al* (2008) reported the variable range of total phenolic content on dry weight basis as 42.1±3.1 in *Ocimum selloi*, 123.1±2.3 in *Ocimum americanum* and 168.2±3.2 mg/g in *Ocimum gratissimum*. More than twenty phenolic compounds were isolated from *Orthosiphon stamineus*, the most important constituents are nine lipophilic flavones, two flavonol glycosides, and nine caffeic acid derivatives (Sumaryono *et al.,* 1991). *Plectranthus barbatus* contains rosmarinic acid and also flavonoid glucuronides and diterpenoids (Fale *et al.,* 2009).

Sermakkani and Thangapandian (2012) reported that Mass spectrometry is the most sensitive and selective method for molecular analysis and can yield information on the molecular weight as well as the structure of the molecules. Combining chromatography with mass spectrometry (GC-MS) provides the advantage of both chromatography as a separation method and mass spectrometry as an identification method. In mass spectrometry, there is a range of methods to ionize compounds and then separate the ions.

Chemical composition of essential oil was analyzed by GC and GC-MS from *Salvia sclarea.* The oils comprised 21 oxygenated monoterpenes, 13 monoterpene

hydrocarbons, 19 sesquiterpene hydrocarbons, 18 oxygenated sesquiterpenes, and 7 oxygenated diterpenes (Lukasz *et al.,* 2009).

The essential oils of *Melissa officinalis* was analyzed by GC/MS (Belgin *et al.,* 2009) and oils ranged from 0.04 to 0.10%. Citronellal (36.62 to 43.78%), citral (10.10 to 17.43%), thymol (0.40 to 11.94%), and *β*-caryophyllene (5.91 to 7.27%) were recorded as major components.

The essential oil of *Mentha arvensis* was analyzed by GC-MS, the components of oil were identified by comparing their retention indices and mass spectra fragmentation patterns with those stored on the MS-computer library. The major constituents reported from essential oils of *Mentha arvensis* were: L-Menthone, Menthol, Isomenthone, Eucalyptol, Piperitone oxide, Carvone, dl-Limonene, trans-Dihydrocarvone, Germacrene-D (Sharma *et al.,* 2009).

The essential oil compositions determined by GC-MS revealed 45 components present in the three species: 29 chemical compounds in *Salvia verticillata* sample, 12 in *Salvia aethiopis* ones and 24 respectively in *Salvia nemorosa* sample (Magda *et al.,* 2012).

Edeoga *et al* (2006) investigated *Hyptis suaveolens* and three putative hybrids of *Ocimum gratissimum* (Hybrid A, B and C). All the plants contain high percentage yield of crude alkaloids and flavonoids ranging from 10.44 to 14.32 % and 9.28 to 12.54 %, respectively. Tannins and phenols were present in all plants.

Mangathayaru *et al* (2006) isolated a liquid alkaloid from the aerial parts of *Leucas aspera* (Willd) Link and identified as nicotine based alkaloid on TLC and HPTLC analysis. Phytochemical studies on *Stachys schtschegleevii* revealed the presence of

phenolic compounds (Nazemieyh *et al.,* 2006), and mono- and sesquiterpenes (Norouzi-Arasi *et al.,* 2004; Sonboli *et al.,* 2005).

2.6. Anti-microbial studies in *Lamiaceae*

Medicinal plants are found to be valuable for the treatment of infections caused by bacteria resistant to many antibiotics. Hassawi and Kharma (2006) reported that the extracts of many plants worldwide, were suitable for treating bacterial, fungal or viral infections. Brul and Coote (1999) highlighted the mechanisms of antimicrobial effects in certain plants. In addition, phenolic and aromatic compounds of medicinal plants seem to possess an essential antibacterial role (Holley and Patel, 2005). The *Lamiaceae* is one of the most diverse and a widespread family in terms of ethnomedicine and its medicinal value is mainly based on the volatile oils concentration (Sarac and Ugur, 2007).

Several studies indicate that the essential oils of the plants belonging to *Lamiaceae* family possess biological activity against several bacteria and yeast (Delamare *et al.,* 2007). Sokovic *et al* (2010) studied the antibacterial effects of several essential oils. Essential oils contain a large number of components, and it is likely that their mode of action involves several targets in the bacterial cell (Burt, 2004; Palmeira *et al.,* 2009; Astani *et al.,* 2010).

Spearmint oil showed antimicrobial activity against the broadest group of viruses, fungi and bacteria (Chao, 2000). The ethanol extract of *Anisomeles indica* exhibited antibacterial activity (Wang and Huang, 2005). The antibacterial activity of essential oil of *Satureja bachtiarica* against *Pseudomonas aeruginosa* have inhibition zone ranging from 16 to 30 mm (Abdollah *et al.,* 2013).

The methanolic extract from roots of *Ocimum basilicum* showed a good inhibition against *Staphylococcus epidermidis*, *Staphylococcus aureus, Bacillus paludis,*

Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella flaxinely and *Enterobacter aerogenes* (MIC between 10 and 80 µg/ml) (Dinanath *et al*., 2011).

In earlier studies, diterpenoids and flavonoids isolated from *Ballotain aequidens* are investigated for their activities against *Bacillus subtilis, Stapylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Candida krusei* (Citoglu *et al.,* 2004). A high aromatic compound content of the phenol rich oils (carvacrol and thymol), which are found in aerial parts of the *Thymus kotschyanus*, seems to be accounting for the strong antibacterial activity (Rasooli and Mirmostafa, 2002).

Antibacterial activities of various concentrations of (leaves, flowers and mixtures) of *Thymus vulgaris* extracted with distilled water and 90% ethanol were carried out by Al-Saimary *et al* (2011). Alcoholic extracts are more efficient on various pathogenic bacteria and mixed extracts have a highly antibacterial activity. The alcoholic extracts have a greater effects than aqueous extracts, this may indicate that the main plant compounds are dissolved or extracted in alcoholic extracts than in aqueous solutions (Al-Saimary and Baker, 2001).

Essential oil of *Ballota pseudodictamnus* has been investigated for their antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Klebsiella pnemoniae, Pseudomonas aeruginosa, Candida albicans, Candida tropicalis* and *Candida glabrata* using the dilution technique. Essential oil of the plant exhibited strong to moderate activity against all tested bacteria (MIC values 0.45-10.15 mg/mL), while it appeared inactive against the tested fungi (Couladis *et al*., 2002).

Essential oils derived from aromatic medicinal plants have been reported to exhibit significant effects against bacteria, yeasts, filamentous fungi, and viruses (Reichling *et al.,* 2009). In the past decade, a variety of essential oils have been evaluated

for their antimicrobial activity by several research workers (Nakatsu *et al.,* 2000; Cimanga *et al.,* 2002: Faleiro *et al.,* 2002). The methanolic extracts obtained from the leaves of *Ballotaace tabulosa* (L.) Bentham (*Lamiaceae*) were investigated for their antimicrobial activities against the pathogens causing complicated urine tract infections (*Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis and Candida albicans*) by disc diffusion method and microdilution method. The extracts showed strong antimicrobial activity against *Escherichia coli* with inhibition zones of 18.6 mm, with MIC's and MBC's of 32(64) mg/mL. Also, the extracts exhibited moderate activity against the other test microorganisms (Basaran *et al.,* 2012).

Sharma and Bhadange (2013) observed that, the antibacterial activity of *Pogostemon benghalensis* was found to be highest against *Salmonella typhymurium* with activity index 0.81 and its antimicrobial activity index against *Escherichia coli* and *Staphylococcus aureus* is 0.66.

Extract of *Orthosiphon stamineus* showed antibacterial activity on serotypes C and D of *Streptococcus mutans* (MIC = 7.8–23.4 mg/ml). In the presence of 5% sucrose, the potency decreased about one-half for type d but no change was found in type C (Chen *et al.,* 1989). Methanol extract at concentration of 50% inhibited *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Litseriamono cytogenes, Escherichia coli, Vibrio parahaemolyticus, Salmonella enteritidis, Salmonella typhimurium,* and *Klebsiella pneumonia* (Hossain *et al.,* 2008). A survey of the literature revealed that *Salvia* species from different places in the world have been widely tested against both Gram-positive and Gram-negative bacteria (Marino *et al.,* 2001; Longaray Delamare *et al.,* 2007; Sultanbawa *et al.,* 2009).

2.7. Pharmacological studies in *Lamiaceae*

Biological screening is necessary to provide a scientific basis for validating the traditional utilization of medicinal plants. A great number of screening programs are ongoing worldwide for new plant based bioactive molecules. Several researchers have worked on medicinal plants with activity against different ailments. Preclinical pharmacological studies and randomized clinical trials form an important part of the biological screening of medicinal plants. Preclinical studies usually serve to verify the data on mechanisms of action reported in animals or humans. However, a pharmacological effect observed *in vitro* or in animal models, for both safety and efficacy has to be reconfirmed by clinical studies and the information obtained from the preclinical studies can form the basis for further clinical trials (Lipsky and Sharp, 2001; Bleicher *et al.,* 2003; Kenakin, 2003; Knowles and Gromo, 2003; Verkman, 2004).

Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. These complex materials are referred to as secondary metabolites. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992; Farombi *et al.,* 1998).

Several Indian medicinal plants have been studied for pharmacological activity in recent years. To understand the mechanism of action, the researchers have worked at molecular levels and several significant phytochemicals have been isolated (Malhotra and Singh, 2007). Many plants, especially those belonging to the family *Lamiaceae* show strong antioxidant activity (Marinova and Yanishlieva, 1997; Triantaphyllou *et al.,* 2001).

The occurrence of type II diabetes is becoming a global epidemic which is essentially due to dramatic changes in diet and lifestyle. Diabetes is known to occur due to elevated blood glucose levels and the inability of a body's response to high blood glucose, which is caused by defects in insulin secretion, insulin action (Schulze and Hu, 2005) and several other pathological changes. Diabetes mellitus is known to be effecting people of both developed and developing countries, harming a large percentage of the world's population. Type II diabetes mellitus is reported to make up 90% - 95% of all diabetic cases. It develops when the cellular sensitivity to insulin signaling is reduced (Schulze and Hu, 2005). Type I diabetes, which is caused due to complete deficiency of insulin secretion, is due to the destruction of pancreatic beta cells. It is believed that the reason behind increase in occurrence of type II diabetes is due to environmental risk factors, and for type I diabetes there are only a few known environmental risk factors that could potentially cause the disease (Schulze and Hu, 2005). Previous studies have suggested that the adoption of Western diet (Van Dam *et al.,* 2002), along with other risk factors such as obesity and the lack of physical activity are associated with increased incidence of type II diabetes.

Lamiaceae herbs are rich sources of phenolic phytochemicals and antioxidants (Kwon *et al.,* 2006), which are highly correlated with α-glucosidase inhibitors, playing a potential role in hyperglycemia management. Plants belonging to the family *Lamiaceae* are great sources of natural antioxidants, often used as spices and aromatic herbs. Therefore phenolic phytochemicals from *Lamiaceae* family in general have potential for managing chronic oxidation linked complications, such as cardiovascular diseases and type II diabetes (Shetty, 1997; Kwon *et al.,* 2006). The antioxidant activity associated with phytochemicals is also linked potentially to lowering mortality rates of cancer in humans (Velioglu *et al.,* 1998). The antioxidant activity of phenolic compounds may not

be mainly due to phenolic content, but may be due to the redox properties, physico-chemical structure and nature of the individual phenolics (Kahkonen *et al*, 1999; Zheng and Wang, 2001; Pandancode Chandran *et al.,* 2002; Chun *et al.,* 2005). The high antioxidant activity potentially suppresses the oxidative stress caused to pancreatic beta cells, reducing the risk of diabetes (Song *et al.,* 2005; Bhandari *et al.,* 2008). Therefore, plants from *Lamiaceae* family with high phenolic antioxidants could prevent the occurrence of diabetes, applied through dietary management strategies in early stages.

Lamiaceae herb samples containing certain phenolic compounds such as rosmarinic acid, caffeic acid and rutin may have hypoglycemic effects, due to high α-glucosidase inhibitory activities. The consumption of *Lamiaceae* herbs as condiments would result in enhancement of our health, due to their potential health benefits in terms of α-glucosidase inhibition relevant to hyperglycemia linked to type II diabetes. Inclusion of these herbs in our diet would potentially reduce blood glucose concentration and lengthen the duration of carbohydrate absorption (Ye *et al.,* 2002). This would serve to be important because lowering of the blood glucose level to normal is the most important part of treating persistent hyperglycemia, which is the characteristic of diabetes (Ye *et al.,* 2002).

Herbs are a rich source of phenolic phytochemicals having high antioxidant activity (Kwon *et al.,* 2006). High phenolic antioxidants from plants are highly correlated with natural α-glucosidase inhibitors, and its presence increases the potential for preventing hyperglycemia linked to on-set of type II diabetes. Phenolic phytochemicals are associated with having potential for managing chronic oxidation linked diseases, such as cardiovascular diseases and diabetes (Shetty, 1997; Pinto and Shetty, 2010). Also, phytochemicals contain significant antioxidant capacities which have potential to lower mortality rates of cancer in human populations (Velioglu *et al.,* 1998).

High total antioxidant activity measured via DPPH assay also indicates the potential that these antioxidants might play an important part in suppressing oxidative stress caused to pancreatic beta cell function, which may reduce the incidence of type II diabetes (Song *et al.,* 2005; Bhandari *et al.,* 2008).

Lamiaceae species such as Sage and Marjoram have high α-glucosidase inhibitory activities for both ethanolic and aqueous extracts, suggesting that the inclusion of these high phenolic herbs in our diet could help reduce blood glucose levels and lengthen the duration of carbohydrate absorption (Ye *et al.,* 2002; Kwon *et al.,* 2006). Lowering of blood glucose level to normal is the most important part of treating persistent hyperglycemia, which is the characteristic of type II diabetes (Ye *et al.,* 2002).

Free radicals play an important role in developing tissue damage in several diseases such as metabolic syndrome diseases, cancer, neurodegenerative diseases and pathological disorders (Erdemoglu *et al.,* 2006). Antioxidants are known to play a crucial part in prevention of many such diseases. Antioxidants inhibit the oxidation of lipids, by inhibiting the propagation of oxidative chain reactions (Javanmardi *et al.,* 2003). Medicinal plants and herbs such as those species in the family *Lamiaceae* are an excellent source of natural antioxidants used as spices and aromatic herbs.

Devasagayam *et al* (2004) reported the extract of *Orthosiphon stamineus* showed free radicals and antioxidants in human health. Farruch and Iqbal Ahamadzafer Mehmood (2006) reported the aqueous extract of *Orthosiphon stamineus* showed antioxidant and free radical scavenging properties. Mariam and Asmawi (1999) reported the aqueous extract of *Orthosiphon stamineus* showed the hypoglycemic activity. Ohashi *et al* (2000) demonstrated the antihypertensive activities of *Orthosiphon stamineus* and showed an

inhibitory effect on the contractile responses on rat thoracic aorta smooth muscle stimulated with KCl beforehand.

Alternations in the values of creatinine levels are taken as the indication of abnormal glomerular functions and these changes in the renal correlated with the nephrotoxic effects of negative controls which affect the renal function (Donadio *et al.,* 1997). Excessive breakdown of body protein in conjugation either inadequate supply or defective utilization observed in uncontrolled diabetes may be accompanied by hypoalbuminemic (Latner, 1958). An *in-vivo* study of nephroprotective effect of aqueous extract of *Plectranthus amboinicus* on Glycerol induced Acute Renal Failure (ARF) was carried out on albino rats. The blood biochemical parameters like urea, uric acid, and creatinine were estimated along with histopathological studies of the kidney. The result showed significant nephroprotective activity of aqueous extract of *Plectranthus amboinicus* at 500 mg/kg. The presence of quercetin may be the reason for the significant nephroprotective effect (Nirmala and Periyanayagam, 2011).

Nirnoy and Muangman (1991) proved that folio *Orthosiphon* showed favourable and non favourable effect of stone prevention. Uric acid and uric acid containing stone may be prevented by the increased alkalinity of the urine. After drinking *Orthosiphon* tea may be beneficial in prevention of uric acid formation. Englert and Harnischfeger (1992) reported an aqueous extract of *Orthosiphon folium,* given orally enhance considerably ion extraction in rat to a level comparable to that obtained with furosemide. Bartanov and Bartanov (2005) reported the nephrophyte includes *Arctostephylo suvaursi, Orthosiphon stamineus*, *Poligonum aviculase, Desmodium canadenase* extracted a Nephroprotective effect that was confirmed by decreasing the creatinine and urea concentration and by dropping proteinuria, increase of GFR and diuresis compared to controls.

Orthosiphon stamineus have been reported to possess anti-inflammatory (Masuda and Masuda, 1992), antihypertensive (Ohashi *et al.,* 2000). Hypoglycemic activity (Mariam and Asmawi, 1999) and Diuretic effect (Galyuteva and Benson, 1990; Dona *et al.,* 1992).

Aqueous extracts of *Sambucus nigra* and *Arcto staphylosuva-ursi* and hydro-alcohol extracts of *Orthosiphon stamineus* and *Hieracium pilosella* were tested for their diuretic activities in rats; pharmacological evaluation revealed that they led to an increase in urine flow. Urinary sodium excretion in rats was increased with *Orthosiphon stamineus* and *Sambucus nigra* (Beaux *et al.,* 1999).

An aqueous extract of *Orthosiphon folium*, given orally, enhances considerably ion excretion in rat to a level comparable to that obtained with furosemide. No aqueretic action was observed. The increased ion excretion is not due to the potassium content of the starting material (Englert and Harnischfeger, 1992). Uric acid and uric acid containing stone may be prevented by the increased alkalinity of the urine after drinking *Orthosiphon* tea (Nirnoy and Muangman, 1991).

Diuretic activity of hydro-alcohol extract from aerial parts of *Orthosiphon stamineus* was reported. At a dose of 50 mg/kg, this extract showed similar effect with hydrochlorotiazide at a dose of 10 mg/kg (Beaux *et al.,* 1999). Nephroprotective effect of methanol extract was observed using gentamycin-induced nephrotic model in rats. Administration of methanol extract at doses of 100 and 200 mg/kg bw significantly decreased serum creatinine levels, blood urea, urinary protein and extent renal damage after 10 days administration (Kannappan *et al.,* 2010).

Premgamone *et al* (2001) reported efficacy of *Orthosiphon grandiflorus*, and the drug sodium potassium citrate (SPC) in treatment of renal calculi. Dissolution of stones

was least which was related to higher excretions of Ca and uric acid in the urine. After treatment, 90% of the initial clinical symptoms (i.e. back pain, headaches and joint pain) were relieved.

Sriplang *et al* (2007) investigated the effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin induced diabetic rats. In oral glucose tolerance test, the extract (0.2-1.0 g/kg) significantly decreased plasma glucose concentration in a dose-dependent manner in both normal and diabetic rats. Furthermore, plasma HDL cholesterol significantly increased in diabetic rats treated with the extract.

The administration of *Anisochillus dysophylloides* (AD) ethanolic extract for 21 days on the functions of hyperglycemia, hyperinsulinemia and serum hepatic marker enzymes were evaluated with STZ induced diabetic mellitus in male wistar albino rats. The results showed that a significant increase in the levels of blood glucose, urea, creatinine and glycosylated Hb and decrease in the level of plasma insulin in the diabetic control rats (Pandancode Chandran *et al.,* 2002).

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. PLANT MATERIALS

The two different species of medicinal plants selected for the present Pharmacognostical studies are *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. Mature and healthy plants were collected from different specific locations after the rainy season (February, March and April). The specimens were identified referring to the Flora of Presidency of Madras (Gamble, 1915-1936) and Flora of Tamil Nadu Carnatic (Mathew, 1983-1988). Voucher specimens of the collections are deposited at the Herbarium of Survey of Medicinal Plant Unit, CCRAS (Siddha), Govt. Siddha Medical College Campus, Palayamkottai, Tirunelveli, Tamil Nadu.

Botanical Name	:	*Orthosiphon comosus* Wight ex Benth.
Family	:	*Lamiaceae*
Voucher Number	:	2562
Collected Locality	:	Kalakad Mundanthurai Tiger Reserve Forest (KMTR), Sengaltheri, South India (Map: 1)
Altitude	:	900 MSL
Synonyms	:	*Hemizygia comosa* (Wight ex Benth.) A.J. Paton *Syncolostemon comosus* (Wight ex Benth.)D.F.Otieno

Botanical Name	:	*Plectranthus wightii* Benth.
Family	:	*Lamiaceae*
Voucher Number	:	8001
Collected locality	:	Kalakad Mundanthurai Tiger Reserve Forest (KMTR), Sengaltheri, South India (Map: 1)
Altitude	:	1100 MSL
Synonyms	:	*Isodon wightii* (Benth.) H. Hara *Rabdosia wightii* (Benth.) Hara *Plectranthus pulneyensis* Hook. F. *Plectranthus nepataefolius* Benth.
Regional Names	:	Malayalam: Murikootty, Iruvely

Orthosiphon comosus is endemic aromatic species at the Agastiyamalai peak, Kalakad Mundanthurai Tiger Reserve Forest (KMTR), in South India (Maridass *et al*., 2008).

3.2. METHODS - Pharmacognostic Studies

3.2.1. Macroscopic observation (Taxonomy)

Fresh plants collected from the wild were brought to the laboratory before desiccation. The morphological and taxonomical characters were carefully examined under stereomicroscope (Model: AE-31). The observations were recorded in technical terms.

3.2.2. Microscopic observation

Mature and healthy plants with normal plant parts were collected and the required samples of different organs *viz*., leaf, petiole, young internodes, young and old roots and young and old stems were cut and removed from the plant and then fixed in FAA (5 ml formaldehyde solution + 5 ml glacial acetic acid + 90 ml 70% ethyl alcohol). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by Sass (1940). Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60°C) until tertiary butyl alcohol solution attained super saturation. The specimens were cast into paraffin blocks by the method of Johansen (1940).

Sectioning and staining

Paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 to 12 µm. Dewaxing of the sections was done by customary procedure (Johansen, 1940). The sections were stained with toluidine blue as per the method of O' Brein *et al* (1964). Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies. Whenever necessary, the sections were also stained with safranin and fast green.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections were prepared (sections taken parallel to the surface of the leaf) by clearing of leaf with 5% sodium hydroxide or epidermal peelings by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared.

Small segments of lamina were immersed in Jeffrey's maceration fluid (10% chromic acid and concentrated nitric acid mixed in equal volumes) and kept in thermostat at 60°C for a few hours. Due to partial maceration, the epidermal peeling gets separated from each other. Then the peelings were washed thoroughly, stained with 0.5% aqueous safranin or 0.25% aqueous toluidine blue and mounted in glycerin for microscopic examination.

For the study of venation pattern, leaf bits measuring 1 cm square were boiled in alcohol to remove the chlorophyll and other pigments and then immersed in warm sodium hydroxide (5-10%) for several hours. As the leaf bits became transparent they were washed thoroughly, stained with safranin and mounted in glycerin.

Photomicrographs

Microscopic descriptions of the root stem and leaves have been carried out and the anatomical characters are presented as photomicrographs. Microphotographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit to reveal the anatomical characters. For normal observations, bright field was used. For the study of crystals, starch grains, and lignified cells polarized light was employed. Since these structures have birefringent property under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are taken from the works of Esau (1977) and Metcalf and Chalk (1979). Measurement of cells was made with micrometer. For each element, 15–20 measurements were taken and average is presented.

3.2.3. Determination of physico-chemical characters

Physico-chemical characters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, moisture content, alcohol soluble extractive values and water soluble

extractive values were determined by employing standard methods of analysis as described in *Pharmacopoeia of India* (Anonymous, 1996). The percentages of extractive values in various solvents were also determined and presented.

a) Fluorescence analysis

For analytical studies, air-dried leaf, stem, root plant powders and the extracts of the powdered plant material in various solvents such as petroleum ether, benzene, chloroform, ethanol and water were examined under ordinary white light and in ultra-violet light (UV 255nm and 365nm). The fluorescence characters were determined according to the methods of Chase and Pratt (1949). These powders were also treated with various chemical reagents *viz*., acetone, ethyl alcohol, 1N NaOH, 1N HCl, 50% H_2SO_4 and 50% HNO_3 and the changes in colour were recorded.

b) Determination of total ash

Two grams of accurately weighed plant sample was taken in a previously weighed silica dish and ignited carefully not exceeding dull red heat until the ash was free from carbon. It was then cooled and then weighed. The total percentage of ash was calculated with reference to the air-dried plant sample.

c) Determination of acid insoluble ash

The ash obtained above was boiled for five minutes with 25 ml of dilute hydrochloric acid. Insoluble matter was collected on an ashless filter paper (Whatman No. 41), washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried plant sample.

d) Determination of water soluble ash

A known weight of ash was boiled for five minutes with 25 ml of distilled water, filtered through ashless filter paper (Whatman No. 41). The insoluble matter was collected on the ashless filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter from the weight of the ash was subtracted; the difference in weight represents the water-soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried plant sample.

e) Determination of sulphated ash

Two grams of the air-dried and powdered sample was taken in a nickel crucible and moistened with concentrated sulphuric acid. It was ignited gently and moistened again with concentrated sulphuric acid and then reignited. The crucible was cooled and weighed. The percentage of sulphated ash was calculated with reference to the air-dried samples.

f) Determination of moisture content

Two grams of the fresh plant sample was weighed in a pre-weighed silica dish. It was dried in the oven at 105^0C and weighed at intervals of one hour until two successive constant weights were obtained. The loss of weight was recorded as moisture content.

g) Determination of alcohol soluble extractive value

Five grams of the air-dried, macerated and powdered plant sample was soaked with 100 ml of alcohol in a closed flask for twenty four hours, shaken frequently during first six hours and allowed to stand for eighteen hours. It was filtered rapidly taking precautions against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. Again, it was dried at 105°C and weighed. The

percentage of alcohol soluble extractive was calculated with reference to the air-dried sample.

h) Determination of water soluble extractive value

Five grams of the air - dried, macerated and powdered plant sample was soaked with 100 ml of water in a closed flask for twenty four hours. It was shaken frequently during first six hours and was allowed to stand for eighteen hours. Then it was filtered rapidly taking precautions against loss of chloroform. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. Again, it was dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air-dried sample.

i) Determination of extractive values (Successive extraction)

Ten grams of the air dried and coarsely powdered plant material was taken in a soxhelt apparatus and successively extracted with petroleum ether, benzene, chloroform, and methanol till the extracts became colourless. Each time before extracting with the next solvent, the powdered material was dried in a hot - air oven below 50^0C to remove the solvents. Each extract was concentrated by distilling off the solvent in a water bath. The extracts obtained with each solvent were weighed and the percentage extractive values were calculated with reference to the air dried sample.

3.3. Phytochemical analysis

The specimens were shade-dried at room temperature (18-20^{o}C) for a period of 3 weeks to 8 weeks depending on the water content. The completely dried materials were separately powdered by means of wearing blender. Then the powdered materials were stored in polythene bags having labels. Estimations, phytochemical tests and

chromatographic studies were carried out using the powders. The quantitative estimations were done in triplicates and the mean value was taken.

Procurement of chemicals and solvents

The chemicals and solvents used throughout the investigation were of analytical grade.

3.3.1. Preliminary Phytochemical screening

The air-dried and powdered plant materials were taken in different amber coloured bottles, extracted (by Soxhelt method) with petroleum ether, benzene, chloroform, ethanol and water, and then the solvent were filtered off. The extracts thus obtained from each plant were then subjected to qualitative tests for the identification of various plant constituents by the methods described by Trease and Evans (1985), Harborne, (1973) and Brindha *et al*. (1981) (Table: 3.1). The preliminary phytochemical screening is a qualitative chemical evaluation which indicates spectrum of chemical constituents in the chosen plant.

3.3.2. Quantitative estimations of minerals

Estimation of elements

The percentage of major elements like carbon, nitrogen, phosphorus, potassium, sodium, calcium, magnesium and sulphur was determined by the method of Anonymous (1984). The trace elements like zinc, copper, iron, manganese, boron and molybdenum were determined by the method of Williams and Twine (1960).

Estimation of major elements

2 g of the dried powder of the sample was taken in 250 ml conical flask and 12 ml triple acid mixture (nitric, sulphuric and perchloric acid in the ratio of 1:2:1) was

added. The mouth of the conical flask was covered with a funnel. The contents were digested in the flask over a sand bath till a clear solution was obtained. The solution was filtered through Whatman No.40 filter paper and the filtrate was collected in a 250 ml volumetric flask. The conical flask was washed with small increments of hot water and the washing was added to the filter paper. The residue on the filter paper was also washed with hot water till the filtrate runs free of chloride. The volumetric flask was cooled under tap water and made up to 250 ml with cold distilled water. This triple extract was used for the analysis of major constituents. The minerals (N, P, K, Na and Ca) were estimated using Flame Photometer (Spectronics Flame Photometer, India).

Estimation of trace elements

The ground plant samples were sieved with a 2 mm rubber sieve and 2 g of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3: HCl: H_2O (1:2:3) mixture and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through Whatman No. 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis. Potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum were estimated using Atomic Absorption Spectrophotometer.

3.3.3. Quantitative estimation of Phytochemicals

Estimation of lignin

Refluxing the sample material with acid detergent solution removes the water soluble and materials other than fibrous component. The left-out materials is weighed after filteration, dried, treated with 70% H_2SO4 and filtered, dried and ashed. The loss of weight on ignition gives the acid detergent lignin.

Estimation of total alkaloids

Alkaloids were determined by using the method of Harbone (1973). 5 g of the powdered sample was taken in a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The beaker has covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The final residue has been dried and weighed.

Estimation of total flavonoids

Flavonoids were determined by the method of Boham and Kocipal-Abyazan (1974). 10 g of the powdered plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a previously weighed china dish and evaporated to dryness over a water bath. The final residue was weighed.

Estimation of Carbohydrates

Principle

Carbohydrates are dehydrated by concentrated sulphuric acid to form furfural. Furfural condenses with anthrone to form a blue coloured complex, which is measured calorimetrically at 620 nm.

Reagents

Anthrone (0.2%) in concentrated H_2SO_4, perchloric acid, ethanol and D-glucose.

Sugars (Mc Creedy *et al.,* 1950)

Powdered sample (100 mg) was homogenized in a mortar. 100 ml of 80% ethanol was added to the homogenized mass and filtered. The filtrate was centrifuged at 3000 rpm for about five minutes. 1 ml aliquot was taken in a corning test tube and 4 ml of anthrone reagent was added. The contents were kept in a boiling water bath for five minutes and allowed to cool. A blue colour developed and its absorbance was read in a UV-VIS spectrophotometer at 630nm.

Starch (Sadasivam and Manickam, 1996)

The residue, after the removal of total sugars, was repeatedly washed with 80% ethanol to remove the last traces of soluble sugars. Distilled water was then added to the residue, kept in a boiling water bath for fifteen minutes, treated with 9.2 N perchloric acid ($HClO_4$) with occasional stirring and centrifuged. After collecting the supernatant liquid, the residue was again treated with 4.6 N $HClO_4$. After fifteen minutes of incubation, it was centrifuged and the supernatant liquid was collected. The pooled supernatant liquids were made up to appropriate volume depending on the starch content of the sample. Then the starch content was determined by using 0.2% anthrone in concentrated H_2SO_4 as that

of described above for sugar. The sugar and starch content of the sample was determined in terms of mg glucose equivalents based on a standard curve.

Estimation of Amino acids (Moore and Stein, 1948)

Principle

Ninhydrin, a powerful oxidizing agent reacts with amino acid to give a purple colour. The intensity of the colour is proportional to the concentration of amino acid.

Reagents

0.2 M Sodium acetate buffer (pH 5.5), ninhydrin (0.2%) in methyl cellosolve, ethanol and glycine.

Procedure

100 mg of powdered sample was ground well in a mortar with 10 ml of 80% ethanol. The extract was centrifuged at 3000 rpm for ten minutes. The supernatant was taken. To 1 ml of aliquot, 3 ml of distilled water and 1 ml of ninhydrin reagents were added and heated for fifteen minutes in a boiling water bath. The colour intensity at 550 nm was measured in a spectrophotometer (Pharma Spec-1700). The amount of free amino acids is expressed as glycine equivalents per gram dry weight.

Estimation of Protein (Lowry *et al.,* 1951)

Principle

Protein forms a coloured complex with Folin Phenol reagent. The colour is formed due to the reduction of phosphomolybdate by tyrosine and tryptophan present in protein. The absorbance was read at 650 nm using spectrophotometer (Pharma Spec-1700).

Reagents

0.2 M phosphate buffer (pH 7.2), 10% ice cold TCA, 0.2 N NaOH, alkaline sodium carbonate (2 g of Na_2CO_3 dissolved in 100 ml of 0.1 N NaOH), $CuSO_4$ in sodium potassium tartarate (500 mg of $CuSO_4$ dissolved in 100 ml of 1% sodium potassium tartarate); Alkaline copper reagent was prepared freshly by mixing 50 ml of alkaline sodium carbonate and 1 ml of $CuSO_4$ sodium potassium tartarate, commercial Folin phenol reagent diluted to 50% using distilled water.

Procedure

Extraction

100 mg of fresh material was taken and homogenized with a little amount of 0.2 M phosphate buffer (pH 7.2). The homogenate was filtered through a three layered muslin cloth and was centrifuged at 1000 rpm for 10 minutes. The supernatant was taken and an equal amount of 10% ice cold trichloroacetic acid (TCA) was added to it and left for 30 minutes at 4°C. The precipitated protein was centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and the pellet was used for protein estimation.

2 ml of 0.2 N NaOH was added to the residue and shaken well. Aliquots in duplicates were prepared each with 0.5 ml from the source and were made up to 4 ml using distilled water. To this 5.5 ml of alkaline copper reagent was added, shaken well and allowed to stand at room temperature for 10 minutes. Then 0.5 ml of Folin phenol reagent was added rapidly and mixed well. The absorbance was read at 650 nm after 30 minutes using spectrophotometer Pharma Spec-1700. Protein content was calculated by referring to standard curve of Bovine Serum Albumin (BSA) and expressed as mg/g/dry weight.

Estimation of Phenolic compounds (Bray and Thorpe, 1954)

Principle

The widest variety of more polar phenolic compounds can readily be oxidized to form a blue coloured complex with a strong oxidizing agent phosphomolybdate present in Folin-Ciocalteu reagent at a higher pH. The intensity of the colour developed is measured in a spectrophotometer (Pharma Spec-1700).

Reagents

Extraction mixture n-butanol: acetic acid: water (6:1:2), Folin–Ciocalteu reagent, 20% sodium carbonate.

Procedure

100 mg of the powdered sample was taken and homogenized with the extraction mixture. One ml of the extract was taken and 1 ml of Folin-Ciocalteu reagent, followed by 2 ml of 20% sodium carbonate was added. The content was shaken well, heated in a boiling water bath exactly for one minute and cooled in running water. The blue coloured complex was diluted with appropriate volume of distilled water and the absorbance was read at 630 nm in a spectrophotometer.

Estimation of Tannin (Mahadevan, 1982)

Principle

Tannin reduces phosphotungsto-molybdic acid in alkaline condition to produce a highly coloured blue solution. The intensity of colour is proportional to the amount of tannins present.

Reagents

Folin Denis reagent, sodium carbonate solution.

Procedure

0.5 g of powdered material was taken and transferred to a conical flask containing 75 ml water. The flask was gently heated and boiled for 30 min. The extract was centrifuged at 2000 rpm for 20 minutes and the supernatant was collected and made up to a known volume. 1 ml of sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. 5 ml Folin Denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml with distilled water. It was mixed well and the absorbance was read at 700 nm after 30 minutes. A blank was prepared with water instead of the sample.

3.3.4. Gas Chromatography-Mass Spectrometry

Mature and healthy plant was collected and dried at room temperature (25 - 30^{o}C), for about two weeks. The dried plant materials such as leaf, stem and root were ground to powder and mixed.

Soxhlet extraction

About 60 g dried sample was refluxed with 250 ml of the selected solvent for 5 hour on a steam bath. The extract was collected and concentrated.

1. GC Programme

Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25µm df

Equipment: GC Clarus 500 Perkin Elmer

Carrier gas: 1ml per min, Split: 10:1

Detector: Mass detector Turbo mass gold-Perkin Elmer

Software: Turbomass 5.2

Sample injected: 2µl

Oven temperature Programme -1

110° C-2 min hold

Up to 200° C at the rate of 10 ° C/min-No hold

Up to 280 ° C at the rate of 5° C / min-9 min hold

Injector temperature 25Of C

Total GC running time 36 minutes

2. MS Programme

Library used NIST Version-Year 2005

Inlet line temperature 200° C

Source temperature 200 ° C

Electron energy: 70 eV

Mass scan (m/z): 45-450

Solvent Delay: 0-2 min

Total MS running time: 36 min

The concentrated extract was injected into the GC/MS instrument (Hewlett Packard 5890 GC/MS with Mass Selective Detector with an HP-1 glass capillary column). The sample is volatilized at the injection port and eluted through a capillary column under increasing temperature. As the sample moves through the column, various components are separated due to their affinity for the stationary phase of the column and can be identified by retention time (the time it takes for a compound to pass through the column and gas chromatograph system). Each chemical component in a sample has a

distinct retention time measured in minutes, shown in a peak on a graph which measures abundance on the ordinate against retention time on the abscissa. The integrated peak is correlated to the concentration of the chemical. A mass selective detector breaks up each chromatographic component into fragment ions, which are shown by their abundance, with each ion represented as a vertical line in increasing molecular weight. The height of each line corresponds to the abundance of that ion. The resulting mass spectrum is unique to that chemical. This mass spectrum forms a ‘‘fingerprint’’ that can identify the compound by a computer search of mass spectra. A computer search of the mass spectra corresponding to all the chromatographic peaks for a sample should yield a statistical match for nicotine at a 12·9 min retention time value if they were present two modes of GC/MS were possible with this instrumental method. First, there is a ‘‘Scan’’ mode which looks at all the constituents of a sample, listing whatever chemical components are present.

Compound Identification

Components of the methanolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST ’98 MS computer library (Wiley).

3.4. Antimicrobial activity assay

Preparation of plant extracts

60 g of root, leaf and stem samples were air dried separately, ground and extracted successively with of 400 ml of petroleum ether (60-80^{o} C), benzene, chloroform, methanol and water. This sequence of solvents allows for leaching of all compounds based on their polarity. The individual fractions were collected and concentrated to obtain

crude extracts. For the purpose of experimental use each extract sample was dissolved in respective solvent so as to get 1/10 solution.

Microbial strains

The antimicrobial activity was tested against five different microbial strains namely *Acinetobacter baumannii*. (MTCC 2031), *Chromobacterium violaceum* (MTCC 2431), *Enterococcus faecalis* (ATCC 29212), *Proteus vulgaris* (MTCC 5412), *Staphylococcus aureus* (ATCC 29213). These bacteria were obtained from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The bacterial strains were grown in Muller Hinton (MH) agar plates at 37^0 C and maintained on nutrient agar slants, while fungi were grown at 30^0C and maintained in Saboraud glucose agar slants. Each organism was maintained in a separate culture media and was recovered for testing by sub culturing on a fresh media. Inoculums of each bacterial strain were transferred in 10 ml of Muller Hinton agar broth and incubated overnight at 37°C.

Preparation of sterile antibiotic discs

Antimicrobial activity was assayed by filter paper disc diffusion method. Whatman No. 1 filter paper of 5 mm diameter was used. These discs were sterilized before use. The extracts of the medicinal plants were added to the sterile disc. Each sterile disc was incorporated individually with 200 - 500 µl of extract of the medicinal plants using micropipette. Precautions were taken to prevent the flow of the solvent extract from the outer surface of the disc. The condensed extracts were applied to the disc.

Antibacterial assay

Antibacterial assay was conducted by the method described by Lennette (1985) with some modification. 0.5 ml of the dilute microbial culture was spread on sterile

Muller Hinton agar plates. The presoaked and dried discs were placed on the seeded plates and gently pressed down to assure contact.

Levomycin (10 µg / ml) was used as positive control and the solvents which were used to dissolve the crude extracts served as negative control. The plates were incubated at room temperature for 24 hrs. After the incubation period the inhibition zone around the discs were measured and recorded. Three replicates for each concentration were maintained.

3.5. Pharmacological Studies

Preparation of Drug

The plant material was shade dried and pulverized. Methanol extract of the coarsely powdered material was prepared by employing Soxhlet method. The extract was concentrated and stored in brown bottles for future use.

3.5.1. The *in vitro* antioxidant potential

Free radical scavenging activity on DPPH

The antioxidant activity of the samples were determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (1958).

The sample extracts at various concentrations (40-200µg) was taken and the volume was adjusted to 100 µl with methanol 0.5 ml of 0.1 ml methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Percentage radical scavenging activity of the sample was calculated as follows:

% DPPH radical scavenging activity = (control OD-sample OD / control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

3.5.2. *In vitro* Anti-diabetic activity

In vitro inhibition of α-glucosidase

The enzyme α-glucosidase inhibitory activity of the methanol extracts of two chosen plants was determined following the method of Miller (1959). Premix α-glucosidase (0.07 Units) with different concentrations (100-500μg) of extract. Then 3 mM p- nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37˚C for 30 min and the reaction was terminated by addition of 2 ml of sodium carbonate. The α- glucosidase activity was determined by measuring the pnitrophenyl release from p- nitrophenyl glucopyranoside at 400 nm after which the IC_{50} value was calculated.

In vitro inhibition of α-amylase

The α-amylase inhibition assay was adapted and modified from Giancarlo *et al* (2006). Different concentrations (100-500 μg) of samples and 1 ml enzyme solution were mixed in a tube and incubated at 25°C for 30 min. To 1 ml of this mixture, 1 ml of 0.5% starch solution was added and the tube incubated at 25°C for 3 min. Then, 1 ml of the colour reagent (DNSA) was added and the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm. Individual blanks were prepared. In this case, the colour reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. Controls were conducted in an identical fashion replacing plant extracts with 1 ml

DMSO. The inhibition percentage of α-amylase was assessed by the following formula after which IC_{50} was calculated.

% Inhibition = ((μA Control–μA Sample)/μA Control) x 100

3.5.3. The *in vivo* studies - Behavioral and toxicological effects

Acute oral toxicity study was done according to OECD guidelines 423. Swiss Albino male mice with a mass ranging from 20-30 g, breed and raised at small animals Research station, Mannuthy, Kerala, India were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 25±2˚C. All the animals had free access to tap water and the same type of food throughout the experiment. Animals were starved overnight but have free access for water *ad libitum.* In this experiment, animals were divided into 7 groups of 6 animals each. First group served as control and was treated with normal water. Group 2, 3, and 4, were treated with the single graded dose (1000, 2000 and 3000 kg b. wt. orally) of *Orthosiphon comosus* respectively. Group 5, 6, and 7 were treated with the single graded dose (1000, 2000 and 3000 mg/kg b. wt. orally) of *Plectranthus wightii* respectively. Monitoring of the parameters commenced immediately after the administration of the drug. Animals were observed at 0 hr, 1 hr, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 24 hrs and 72 hrs (with special attention given during the first four hour). Observation includes mortality and clinical signs, which includes changes in skin fur, eyes and mucous membranes. The gross behaviors like body positions, locomotion, rearing, tremors, gait were observed. The effect of plant extract on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, body weight intake were also observed.

3.5.4. The *in-vivo* Nephroprotective studies

Experimental animals

Male albino Wistar rats (150-200 g) used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark /10h light cycles; temp 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experiment use. Animals were fasted over night before the experimental schedule, but have free access for water *ad libitum*.

Gentamicin induced Nephrotoxicity (Yaman and Balikci, 2010)

Animals were randomly divided into six groups with 6 animals each. Group I animals were normal control group. Groups II-VI was intraperitoneally injected with 100 mg/kg b.w. of gentamicin for 8 consecutive days. Groups three and four were orally co-administered with methanolic extract of *Orthosiphon comosus* (200 and 400 mg/kg b.w., respectively), whereas groups V and VI were orally co-administered with methanolic extract of *Plectranthus wightii* (200 and 400 mg/kg b.w., respectively) for eight consecutive days. Gentamicin was injected exactly one hour after the treatments were given. At the end of the experimental regime, all animals were anaesthetized with ether; blood samples were collected by cardiac puncture and were allowed to clot for 20 min after which it was centrifuged at 2500 rpm for 15 min at 4°C. The separated serum samples were used for measuring the level of urea, uric acid creatinine, blood urea nitrogen (BUN) and electrolytes (Na+, Cl-, K+ ions). Urea, uric acid, creatinine and BUN were estimated using commercially available diagnostic kits whereas the electrolytes were assayed using an electrolyte analyzer.

RESULTS AND DISCUSSION

4.1. PHARMACOGNOSTIC STUDIES

Macroscopic, microscopic and analytical features of the two aromatic medicinal plants *viz.*, *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. were studied and the diagnostic characters are presented.

4.1.1. Macroscopic characters

Macroscopic characters of *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. were described using conventional technical terms. These species belong to the family *Lamiaceae* of the order *Lamiales.*

a) *Orthosiphon comosus* Wight ex Benth. (Plate: 4.1)

An erect shrub, 3-5 feet high, tomentose. Stem terete with multicellular shining glandular hairs. Branches usually opposite. Leaves crowded towards the inflorescences, subsessile, obovate, cuneate, crenato-serrate, with shining hairs on both surfaces. Racemes terminal, very long, rachis stout, somewhat 4-longed, verticillasters laxly arranged, 6-flowered. Bracts rose-coloured, upper ones larger, foliaceous obovate, 3-nerved, nearly entire, glandular, concealing the flower buds and deciduous.

Calyx ½ inch erect but deflexed when fruiting, upper lip broad, entire, concave at first and convex when fruiting, lower lip with 4subulate teeth. Corolla 11/2 in - 2 in. long pubescent, tube long, slender, inflexed towards the tip. Upper lip slightly larger than the lower, 3 lobed, mid-lobe very slightly emarginated, lower lip entire, concave. Stamens 4 free, declinate. Disc gibbous, style filiform, stigma clavate, nutlets ovoid, smooth. Flowering peak during December and February.

RESULTS AND DISCUSSION

Distribution: Found only in the Tinnevelly hills. Endemic to Kalakkad Mundanthurai hills. Nayar (1996) reported as Endemic to Peninsular India.

b) *Plectranthus wightii* Benth. (Plate: 4.2)

Subshrub 50 – 100 cm; branches hirsute. Leaves ovate 2 – 7 x 1- 5 cm, strigose on either side, base truncate, margin deeply crenate, apex acute; petiole 5 cm; cymes branched, 13 – 17 flowered; peduncle 15 – 25 cm; bracts scaly or linear; pedicel 0.5 cm. Calyx – lobes 5, sub-equal, 3 + 2, obscurely bilipped, 5 mm, strigose, without, acute. Corolla white, 5 x 3 mm across; tube 5 mm; lobes 5, bilipped, 4 + 1; upper lip recurved, 2.5 mm; lowerlip 3 mm, glandular – hairy, obtuse. Stamen 4; filaments free, 5.5 mm; anthers 0.6 mm. Ovary 0.3 mm; style 8.5 mm.

Flowering peak during December and February.

Distribution: Tamil Nadu, Kerala. Nayar (1996) reported as Endemic to Peninsular India.

4.1.2. Anatomical features

Microscopic characters of *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. were described.

a) Microscopical characters of *Orthosiphon comosus* Wight ex Benth.

Root (Plate: 4.3 & 4.4)

The root has deep and wide fissures which break the periderm into wide and thick segments (Plate: 4.3.a). The root is 2 mm thick. The periderm is 500 µm thick. It is crushed and compressed and no distinct cell layers are evident (Plate: 4.4). The secondary phloem and xylem cylinders are dense and solid with circular outline. Secondary phloem is 300 µm thick. The phloem elements are wide, angular and occur in compact radial rows (Plate: 4.4). Secondary xylem includes diffuse solitary circular vessel which are

narrow in the centre wider towards periphery (Plate: 4.3.a). The vessels are thick walled and are 30-70 µm in diameter. Xylem fibres are highly thick walled and lignified; the cell lumen is narrow. Xylem rays are distinct, straight and thin (Plate: 4.3.a & 4.4).

Stem (Plate: 4.5 & 4.6)

The stem is four angled with four thick and wide ridges and shallow wide furrows (Plate: 4.5.a). It is 2 mm thick. The epidermal layer is wavy and bears dense trichomes. The cortex consists of 6 or 7 layers of angular thin walled parenchyma cells. The vascular cylinder is four angled with four thick ridges of xylem and thin cylinder of vascular tissues in the region of furrows (Plate: 4.5.a). In the ridges, there are several lines of wide, thick walled xylem elements and thick arc of phloem situated on the outer part of the xylem. There is a thick arc of fibres alternating the phloem (Plate: 4.5.b). Inbetween the ridges, the vascular cylinder shows initiation of vascular cambium which has produced small collateral vascular bundles with sclerncychyma cap (Plate: 4.5.b). Thick stem assumes circular outline. It has thick trichome bearing epidermal layer and wide parenchymatous cortex. The outer cortical cells are angular and compact and the inner cell is circular and less compact (Plate: 4.6.b). The vascular cylinder is circular and continuous and consists of thick secondary phloem and secondary xylem (Plate: 4.6.a). Secondary phloem consists of thick cylinder, in which small clusters of sieve elements are situated. On the outer border of the phloem several small discrete masses of thick walled and lignified fibres are present (Plate: 4.6.b). Secondary xylem consists of thick, ridged cylinder of vessels and fibres. The vessels are wide and angular and thick walled and measure upto 60 µm wide. The secondary xylem also includes thick walled lignified fibres (Plate: 4.6.c). The pith is wide and includes intact thin walled parenchyma cells.

Petiole (Plate: 4.7)

The petiole is planoconvex is sectional view with flat adaxial side (Plate: 4.7.a). It is 1.1 mm thick and 2.35 mm wide. The epidermis is wavy in outline bearing dense trichomes. Four or five layers of cells inner to the epidermis are collenchymatous; remaining ground tissue is parenchymatous, the cells are large, angular or circular and compact. The vascular strand is single slightly boat shaped and thin (Plate: 4.7.a). The strand is 120 µm thick and 1.5 mm wide. The vascular strand includes several short narrow lines of xylem elements and small discrete strands of phloem (Plate: 4.7.b). The xylem elements are narrow, angular and thin walled.

Leaf (Plate: 4.8 & 4.9)

The leaf is distinctly dorsiventral and has prominent veins (Plate: 4.8.a). The midrib is planoconvex with flat adaxial side and prominently projecting thick abaxial midrib (Plate: 4.8.b). The midrib is 600 µm thick and 750 µm wide. It consists of a thick neck part and wider and thicker body part. The epidermal layer is prominent comprising thick tubular cells. The epidermal cells bear glandular and non-glandular trichomes and raised stomata (Plate: 4.8.b). The ground tissue is homogenous, parenchymatous, angular and thin walled. There is a single vascular bundle which is planoconvex; the adaxial side is flat and the abaxial side is convex. It is 100 µm thick and 250 µm wide. The bundle is collateral and possesses about six rows of xylem elements and thin layer of a few discrete phloem units (Plate: 4.8.b). Lateral vein is also projecting on the abaxial side of the lamina (Plate: 4.8.b). It is semicircular and 200 µm thick. There is a single small vascular strand which possesses a vertical row of xylem elements and a small nest of phloem elements. Marginal part of the lamina is bent and semicircular with dilated thick epidermal cells; the mesophyll tissue is as in the middle part of the lamina (Plate: 4.9.a).

Lamina is 200 µm thick. Dorsiventral side is possessing thick and wide adaxial epidermal cells and thin cylindrical with dense projecting stomata. The palisade parenchyma zone consists of a single row of short, less compact cylindrical cells and three or four layers of lobed spongy parenchyma cells.

Epidermal cells and stomata (Plate: 4.10)

The epidermal tissue was studied from paradermal section (Plate: 4.10.a). From surface view, the epidermal cells are wide and possess thin highly undulate cell walls, so that the epidermal cells appear amoeboid in outline.

The stomata are diacytic type. Each stoma has two subsidiary cells (Plate: 4.10.b). The two cells are located at two poles of the guard cells with their common cross walls. Cross walls are at right angles to the stomatal aperture (Plate: 4.10.c). The stomata are 20x30 µm in size.

Glandular epidermal trichomes (Plate: 4.11)

Glandular trichomes are abundant on the leaf. The glands are capitate type. The gland has a one celled stalk which is located on an epidermal cell. The trichome bearing epidermal cell is surrounded by seven or more epidermal cells which radiate from the central cell (Plat: 4.11.b). These radiating cells are called rosette cells (Plate: 4.11.a). The stalk cell bears a spherical multicellular body (Plate: 4.11.c).

Powder microscopic observations

The powder preparation of the plant exhibits the following inclusions.

I. **Thick masses of periderm cells** are often seen in the powder (Plate: 4.12.a). The cells are squarish to rectangular and occur in compact horizontal rows. The cells are thin walled. They are 70x100 µm in size.

II. Fibres (Plate: 4.12.b, 4.13.a, b & c)

Libriform types of xylem fibres are common in the powder. They have thick lignified walls and narrow lumen. Pits are not evident. The tips of the fibres are pointed. The fibres are 370 -800 µm long and 20 µm thick.

III. Vessel elements (Plate: 4.13 & 4.14)

The vessel elements are very frequently seen in the powder. They are long, narrow and cylindrical (Plate: 4.13.a & 4.14.b), some of them are short, wide and barrel shaped (Plate: 4.14.a & c). Some of the vessel elements have long or short tails at one or both ends. On the lateral walls elliptic, multiseriate vertical rows of bordered pits are found (Plate: 4.14). On the end walls, simple, wide, horizontal or slightly oblique perforations are seen. (Plate: 4.14.a, b & c).The vessel are 250 - 300 µm long.

iv. **Parenchyma cells** (Plate: 4.13.a)

Elongated scales like cells or polyhedral short cells of parenchyma are occasionally seen in the powder. The elongated cells have dense simple pits. These cells are 50-200 µm long and 20 µm wide. The polyhedral cells are 60x70 µm in size.

b) Microscopical characters of *Plectranthus wightii* Benth.

Root (Plate: 4.15 & 4.16)

Root measuring 1.7 mm was studied. The epidermis is preserved in certain regions only. In other regions, it is replaced by a few layers of periderm (Plate: 4.15.a). The periderm is followed by a wide zone of parenchymatous cortex (Plate: 4.15.b). The vascular cylinder is circular and dense. It is 1.2 mm in diameter. Secondary phloem surrounds the xylem cylinder (Plate: 4.15.b). It consists of large parenchyma cells and

small thick walled sieve elements. The phloem elements are seen in thick radial segments separated by wide phloem rays. Secondary xylem exhibits apparently ring porous growth rings in which the vessels in the beginning of the growth ring are wider and those towards the periphery are distinctly narrow (Plate: 4.16). The vessels are 10-30 µm wide. Xylem fibres are thick walled and lignified. They have wide or narrow lumen. Xylem rays are wide and the ray cells are also thick and lignified.

Stem (Plate: 4.17 & 4.18)

Stem is four angled and with pith tissue being totally disintegrated (Plate: 4.17.a). The corners part of the stem is 100 µm thin and the portion in between is 400 µm thick. The stem has a thick intact continuous epidermal layer of small spindle shaped cells. The cortex is homogenous and parenchymatous. It is thicker along the corners and thin inbetween the corners. The vascular cylinder is thick, and continuous all along the stem. The corner region of the stem has thicker secondary xylem and secondary phloem with thin layer of fibres located on the outer border of the phloem (Plate: 4.17.a & b). The xylem consists of short radial lines of vessels which are circular thick walled and solitary (Plate: 4.17.a & b). Thick cylinder of secondary xylem occurs in the region in between the corners. This portion includes compact parallel lines of thick walled lignified fibres and radial clusters of wide, circular thick walled vessels. The vessels are 20 µm wide.

Petiole (Plate: 4.19 & 4.20)

The petiole is semicircular in sectional view. In the proximal part, the petiole has deep wide adaxial groove (Plate: 4.19.a). In the distal part, the adaxial groove is V-shaped and there are thick short lateral wings (Plate: 4.19.b). The epidermal layer is thin and epidermal cells are small and circular. The ground tissue is homogeneous and parenchymatous, the cells are circular to angular. The petiole is 1.5 µm thick and 1.4 µm

wide. The vascular strand is either shallow cup shaped (Plate: 4.19.a) or deep bowl shaped (Plate: 4.20). It is collateral with thin long parallel lines of xylem elements and xylem fibres. Phloem occurs in small separate clusters on the abaxial part of the xylem (Plate: 4.20).

Leaf (Plate: 4.21)

The leaf consists of a thick, abaxially banding midrib and thin lamina (Plate: 4.21.a). The midrib is planoconvex comprising flat adaxial side and short stacked spherical thick abaxial midrib. The midrib is 700 µm thick and 600 µm wide. The epidermal layer is undulate. The epidermal cells are small, squarish and thick walled (Plate: 4.21.a). The ground tissue consists of wide circular, thin walled less compact homogeneous parenchyma cells. The vascular strand is single thick and semicircular. It is flat on the adaxial side and semicircular on the abaxial part. The bundle is 150x220 µm in size. The bundle includes thin, long continuous lines of xylem elements and an arc of phloem elements. The xylem elements are angular and thick walled with 15 µm wide lumen. Phloem elements are in small clusters of thick walled cells (Plate: 4.21.c).

Lamina (Plate: 4.22)

The lamina is 110 µm thick. It is dorsiventral and hypostomatic. The adaxial epidermis is thick and the cells are wide, thick and cylindrical in shape (Plate: 4.22.a). The cells are 20 µm thick. The adaxial epidermis is apostomatic. The abaxial epidermis is thin, comprises cylindrical or spindle shaped cells. It is densely stomatiferous. The mesophyll is differentiated into adaxial band of single row of cylindrical palisade cells and abaxial zone of three or four layers of lobed loosely arranged spongy parenchyma cells.

Epidermal trichomes are abundant on the abaxial epidermis. The trichomes are either nongladular or glandular type. The nonglandular type is multicellular, uniseriate and unbranched (Plate: 4.22.b). They are narrow and cylindrical or arise from thick multicellular base. The glandular trichomes are short glands with single stalk cells and spherical multicellular head (Plate: 4.22.b). The gland is capitate type.

Epidermal cells and stomata (Plate: 4.23)

The epidermal cells have thick, highly wavy anticlinal walls (Plate: 4.23.a). The stomata are diacyctic type with two subsidiary cells placed at opposite poles of the guard cells; the common walls of the subsidiary cells are at right angles to the long axis of the guard cells (Plate: 4.23.c). The guard cells are 15x30 µm in size.

Powder microscopic observation (Plate: 4.24 & 4.25)

1. Dense masses of epidermal trichomes are abundant in the powder. The trichomes are non-glandular, 3 or 4 celled uniseriate and unbranched with thin cell wall. The trichomes are mostly curved. The trichomes are 150 µm long and 20 µm wide.
2. Fibres (Plate: 4.24.b & c). The fibres are libriform type. They are thin 400 µm long and 10 µm wide, pointed at the ends. Their walls are thick and lignified. The cell lumen is narrow.
3. Vessel elements: (Plate: 4.24.a, b & c). The vessel elements are long, narrow and cylindrical. Some of the vessel elements have long or short tails. The lateral walls have elliptical, multiseriate pits. The end walls have elliptical, oblique perforations (Plate: 4.24.b & c). The vessel elements are 400 µm long.

4. Parenchyma cells: (Plate: 4.25)

Spherical and scale like parenchyma cells are seen in the powder. The scale shaped cells are upto 350 µm long and 20 µm thick. The cells have no cell inclusions. Pits are also absent.

Diagnostic anatomical characters

Trichomes are found at the surface of the aerial parts of plant for example: leaves, stem and calyx. The morphology of glandular and non-glandular trichomes was very important especially in taxonomic and ecological studies of *Labiatae* family (Seyed Mehdi Talebi *et al*., 2012). In the present study *Orthosiphon comosus* and *Plectranthus wightii* leaf epidermal cells bear both glandular and nonglandular trichomes. Glandular trichomes are abundant and are capitate type. The nonglandular type trichome is multicellular, uniseriate and unbranched in *Plectranthus wightii*. The glandular trichomes are short glands with single stalk cell and spherical multicellular head in both chosen species. The most important features of *Lamiaceae* taxa are glandular hairs distributed in vegetative and reproductive organs (Werker, 2006). These hairs are source of etheric oils and their structures have been examined anatomically and micromorphologically (Hanlidou *et al*., 1991; Vrachnakıs, 2003; Kaya *et al*., 2007). The species belonging to this family are characterised by the presence of these secretion hairs. Glandular hairs are generally peltate and capitate. Peltate hairs have short stalk with the head cell containing a lot of cells. Capitate hairs have a stalk which consists of many cells or a single cell. In addition, the head cell of capitate hairs consists of either two or single cells (Hanlidou *et al*., 1991; Ascensao *et al*., 1995). Tahir *et al* (1995) examined the morphology of the leaf surface of 13 species belonging to the *Lamiaceae* family and reported the presence of sessile glandular hairs. In the present study, the sessile hairs are not found in the two chosen species. Rapisarda *et al* (2001) studied *Nepetha sibthorpii* (*Lamiaceae*) and

showed the glandular hairs micromorphology characteristic to be beneficial. Vrachnakis (2003) reported the presence of big peltate hairs in *Origanum dictamnus* and the capitate hairs were found in different forms. In this study, capitate hairs found with one stalk cells and multicellular head. Trichomes are considered relevant in comparative systematic investigations and morphodiagnosis (Metcalfe and Chalk, 1988). The occurrence together with diverse kinds of glandular and non-glandular trichomes is characteristic of *Lamiaceae* (Metcalfe and Chalk, 1950). The peltate trichomes, capitate glandular and non-glandular trichomes have been described for the family *Lamiaceae* (Metcalfe and Chalk, 1950; Werker, 2006) and for the genus *Plectranthus madagascariensis* (Pers.) Benth. (Ascensao *et al*., 1998), *Plectranthus ornatus* Codd (Ascensao *et al*., 1999) and *Plectranthus barbatus* (Scavone, 1965).

Concerning the leaf anatomy, the family exhibits predominantly diacytic stomata on both surfaces (Metcalfe and Chalk, 1950). *Orthosiphon comosus* and *Plectranthus wightii* showed the same pattern. With reference to the chlorenchyma organization, isobilateral, dorsiventral or centric mesophyll has been frequently reported in *Lamiaceae* (Metcalfe and Chalk, 1950). In the present investigation both species exhibit dorsiventral type of mesoplyll and differentiated into adaxial band of cylindrical palisade cells and abaxial zone of loosely arranged spongy parenchyma cells. In comparison with *Orthosiphon comosus*, the *Plectranthus wightii* shows undulate squarish epidermal cells, but *Orthosiphon comosus* shows tabular epidermal cells.

The structure of petiole shows differences between genera and species. Thus, useful petiole anatomic characters are determined in designated taxonomical structures of some species (Olowokudejo, 1987; Shaheen, 2007; Eric *et al*., 2007). Anatomical structures of the petiole are very important in family *Lamiaceae* (Metcalfe and Chalk, 1972). The epidermal layer is circular in *Plectranthus wightii* and wavy in *Orthosiphon*

comosus. The ground tissue is homogenous in *Plectranthus wightii* and heterogeneous in *Orthosiphon comosus*. The results showed that within the studied taxa, there were many differences between shapes of vascular bundles. The vascular strand is boat shaped in *Orthosiphon comosus* and cup shaped vascular strand in *Plectranthus wightii*.

The rectangular transection is frequently described for *Lamiaceae* (Metcalfe and Chalk, 1950; Barroso, 1991). According to Metcalfe and Chalk (1950), the presence of collenchyma in the four angles (Cronquist, 1981), is considered as diagnostic value. In the studied species, the stem reveals quadrangular cross-section, however the arrangement of the collenchyma is not evident to the angles. In *Orthosiphon comosus* the vascular cylinder is four angled with four thick ridges of xylem and thin cylinder of vascular tissues in the region of furrows. In *Plectranthus wightii* the vascular cylinder is thick, and continuous all along the stem. The occurrence of wide interfasicular regions with distinguishable collateral bundles has been mentioned for herbaceous dicotyledons (Esau, 1977). The vascular system organization of *Orthosiphon comosus* shows initiation of vascular cambium which has produced small collateral vascular bundles with sclerencychyma cap.

This investigation shows that the assembled anatomical characters of *Orthosiphon comosus* and *Plectranthus wightii* contribute for this medicinal species identification. The two species of *Lamiaceae* family are similar in structure but they also exhibit differences. Both species have a bifacial leaf and a hypostomatic, heterogenous mesophyll. Differences occur in the number of palisade and spongy layers of cells and the types of non-glandular and glandular trichomes, diversity, structure and density on the petiole and lamina surface.

4.2. Physico Chemical analysis

4.2.1. Fluorescence analysis

This is an important pharmacognostic parameter that will reveal the nature of chromophores present in a drug source. The nature of fluorescence of drug powder serves as a means of identification of similar drugs. Fluorescence properties of the two medicinal plants *viz. Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. have been studied and the colour changes were observed. The results are presented in Tables 4.1 and 4.2. Under fluorescent light, stem, leaf and root powder showed different colours in various extracts.

The air dried leaf, stem and root powder extracts exhibit dark colour in UV light as compared with the powders treated with visible light. *Orthosiphon comosus* stem, leaf and root powders show dark green colour under short UV light when treated with 50% H_2SO_4 and 50% HNO_3. Petroleum ether extract and benzene extract of *Orthosiphon comosus* stem, leaf and root powders show orange colour under long wave length UV light. Powders treated with water exhibit fluorescent green colour under short wavelength UV light, but in visible light these powders exhibit pale yellow colour. Under visible light, stem and leaf powder exhibit light brown when treated with acetone, ethyl alcohol and 1N HCl (Table: 4.1). *Plectranthus wightii* leaf, stem and root powders treated with 50% H_2SO_4 and1N HCl under long UV light appear dark red colour, but when treated with ethyl alcohol they appear reddish orange and methanol extract of these powders exhibit yellowish red colour. Under visible light, *Plectranthus wightii* powders treated with 50% H_2SO_4 express yellowish green colour, but when treated with 50% HNO_3 exhibit reddish orange colour and treated with water appear light brown colour. Under short wavelength, all the powders treated with methanol exhibit fluorescent green colour

(Table: 4.2*).* These fluorescent characters of the selected plants can be used as a diagnostic tool for testing adulterations if any. For e.g., when Ceylon cinnamon (*Cinnamomum zeylanicum* Nees. *Lauraceae*), China cinnamon (*C. cassia* Blm.) and Saigon cinnamon (*C. loureirii* Nees.) powders are placed on the microscopic slides treated with 1N NaOH and methanol and observed under UV radiation, Ceylon cinnamon fluoresces but the China and Saigon cinnamon appear green in colour (Chase and Pratt, 1949). Drugs like Hydrastis, Calumba, Viburnum and wild Cherry bark showed brilliant effects in UV light and may be used as an aid in the identification and to detect certain adulteration (Wallis, 1985).

4.2.2. Quantitative determination

The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earthy matter. The total ash value is useful to exclude drugs, which have been coated with chalk, lime or calcium sulphate. The percentage of total ash, water soluble ash, acid insoluble ash, sulphated ash, moisture content and extractive values have been determined by standard methods (Anonymous, 1996). The residue remaining after incineration is the ash content of drugs, which simply represents inorganic salts, naturally occurring in drugs or adhering added to it as form adulteration. The percentage of extractive values in petroleum ether (40 – 60^{o}C), benzene, chloroform, methanol and water have also been determined. These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment (Kunle *et al.,* 2012).

The physico-chemical determinations of powders *Orthosiphon comosus* Wight ex Benth. and *Plectranthus wightii* Benth. are shown in the Table: 4.3. The total ash value is higher in leaf powder and lesser in stem powder. The amount of water soluble ash is

8.9% in leaf of *Orthosiphon comosus* and 6.25% in leaf powder of *Plectranthus wightii*. The value of sulphated ash is much higher in leaf samples than in stem and root samples. The acid insoluble ash is lesser in the stem samples (Table: 4.3; Fig : 4.1 & 4.2). The percentages of extractive values (successive extraction) in different solvents are varying from one another. The methanol extracts shows the maximum extractive values and chloroform extracts shows minimum extractive values. In methanol the high percent of extractive value is observed in the leaf extract of *Orthosiphon comosus* (11.5%) and low extractive value in stem sample of *Plectranthus wightii* (4.8%).

Similar to the present study in *Orthosiphon aristatus* revealed that ash content is not more than 13%w/w, acid insoluble ash content is not more than 1%w/w of each, water soluble extractive is not less than 16%w/w, ethanol soluble extractive is not less than 11%w/w, 50% ethanol soluble extractive is not less than 23%w/w (Duangpen *et al.,* 2003). Ramasubramania Raja (2012) observed total ash is not more than 12.0% and acid insoluble ash is not more than 4.0% in *Thymus vulgaris.*

4.3. Phytochemical analysis

Phytochemistry is a branch of chemistry dealing with the chemical processes associated with plant life and the chemical compounds produced by plants. The applications of the discipline can be for Pharmacognosy or the discovery of new drugs. Medicines can be either manufactured by using plant materials as raw materials or synthesized in the laboratory using chemicals as raw materials.

The plant may be considered as a biosynthetic laboratory not only for the chemical compounds such as carbohydrates, proteins and lipids but also for the secondary metabolites like glycosides, alkaloids, volatile oils, tannins etc., that exert a physiological effect. These compounds are responsible for the therapeutic effect of plants. Keeping

REFERENCES

REFERENCES

Abdel-Mogib M Albar H A and **Batterjee S M** (2002). Chemistry of the genus *Plectranthus*. *Molecules.***7:** 271 - 301.

Abdollah Ghasemi Pirbaloutil Andshohreh Dadfar and **Acta Poloniae** (2013). Chemical constituents and antibacterial activity of essential oil of *Satureja bachtiarica* (*Lamiaceae*). *Pharmaceutica N. Drug Research.***70(5):** 933 - 938.

Abou Arab A A K and **Donia M A A** (2000). Heavy metals in egyptian spices and medicinal plants and the effect of processing on their levels. *J. Agri. Food Chem.* **48(6):** 2300 - 2304.

Adam M N Somchit M and **Sulaiman R** (2009). Diuretic properties of *Orthosiphon stamineus* Benth. *J. Ethnopharmacology*. **124(1):** 154 - 158.

Ahmad Bashir Niaz Ali Shumaila Bashir and **Muhammad Iqbal Choudhary** (2009). Biological activities of aerial parts of *Tylophora hirsuta* Wall. *African J. Biotechnology*.**8 (18):** 4627 - 4631.

Akihisa T Ogihara J Kato J Yasukawa K Ukiya M Yamanouchi S and **Oishi K** (2001). Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus - 1 reverse transcriptase. *Lipids.* **36:** 507 - 512.

Alasbahi R H and **Melzig M F** (2010). *Plectranthus barbatus*: a review of phytochemistry, ethnobotanical uses and pharmacology - 1. *Planta Med.***76(7):** 653 - 661.

Allemann J Robbertse P J and **Hammes P S** (2003). Organographic and anatomical evidence that the edible storage organs of *Plectranthus esculentus* NEBr. (*Lamiaceae*) are stem tubers. *Field Crops Res.* **87:** 352 - 357.

Allemann J and **Hammes P S** (2003).Chemical composition of South African *Plectranthus esculentus* tubers. *South Afr. J. Sci.* **99:** 127 - 129.

Al-Saimary Sundus S Bakr Bassam Y Khudaier and **Yass K** (2011). Efficiency of antibacterial agents extracted from *Thymus vulgaris* L. (*Lamiaceae*). *J. Medicinal Plants Research.* **5(20):** 30.

Al-Saimary I E and **Baker S S** (2001). Extraction of antibacterial agents from *H. lasocarpium* Fisch and Mey (*Boraginaceae*). 1st conf. of the Nat. Board for Biotechnical Res. Baghdad.

Amin G (1991). *Popular Medicinal Plants of Iran*.Iranian Research Institute of Medicinal Plants, Tehran.

Amini A (1997). *Illustrated Dictionary of Therapeutic Plants and their Traditional Usage in Kurdistan*. Taqbostan Publication, Khoramabad.

Anam K Widharna R M and **Kusrini D** (2009). Alpha-glucosidase inhibitor of *Terminalia* species. *Int. J. Pharmacol*. **5:** 277 - 280.

Andrew L Kau Steven M Martin William Lyon Ericka Hayes Michael G Caparon Scott J and **Hultgren I** (2005). *Enterococcus faecalis* - Tropism for the Kidneys in the Urinary Tract of C57BL/6J Mice. *Infect. Immun.***73(4):** 2461 - 2468.

Anonymous (1984). "Official Methods of Analysis (AOAC)", 14th Edn, In: Helrich, K., Association of Official Analytical Chemists, Washington.

Anonymous (1995). Medicinal herb index in Indonesia. *Eisai Indonesia*. 263.

Anonymous (1996). *Pharmacopoeia of India* 2nd Ed. Manager of Publications, New Delhi, 947 - 948.

Arisandi Y Khasiat Tanaman and Obat (2006). *Penerbit Pustaka Buku Murah*, 196 -198.

Ascensao L N Marques and **Pais M S** (1995). Glandular trichomes on vegetative and reproductive organs of *Leonotis leonurus* (*Lamiaceae*). *Annuals of Botany.***75:** 619 - 626.

Ascensao L Figueiredo A C Barroso J G Pedro L G Schripsema J Deans S G and **Scheffer J J C** (1998). *Plectranthus madagascariensis*: morphology of the glandular trichomes, essential oil composition, and its biological activity. *Int. J. Plant Sci.* **159:** 31 - 38.

Ascensao L Marques N and **Pais M S** (1995). Glandular trichomes on vegetative and reproductive organs of *Lamiaceae*. *Annals of Botany*.**75:** 619-626.

Ascensao L Mota L and **Castro M M** (1999). Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: morphology, distribution and histochemistry. *Ann. Bot.* **84:** 437 - 447.

Astani A Reichling J and **Schnitzler P** (2010). Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. *Phytother Res.***24:** 673 - 679.

Aynehchi Y (1986). *Pharmacognosy and Medicinal Plants of Iran*. Tehran University Publication, Tehran.

Balunas M J and **Kinghorn A D** (2005). Drug discovery from medicinal plants. Life Sci. **78:** 431 - 441.

Baran P and **Ozdemir C** (2006). The morphological and anatomical characters of *Salvia napifolia* Jacq.in Turkey. *Bangladesh J. Bot.* **35(1):** 77 - 84.

Barroso G M (1991). *Sistematica de angiospermas do Brasil*. Vicosa: Universidade Federal de Vicosa.

Bartanov A I and **Bartanov E A** (2005).Nephroprotective effect of nephrophyte in drug nephropathy induced by urographin. *Euromedica Hannoeve*. 40 - 41.

Basaran Dulger Gorkem Dulger Couladis M Chinou I B Tzakou O and **Loukis A** (2012). Antimicrobial activity of the leaves of *Ballota acetabulosa* on microorganisms isolated from urinary tract infections. *Turk. J. Pharm. Sci.* **9(3):**257 - 262.

Bauer R (1998). Quality criteria and standardization of phyto-pharmaceuticals: can acceptable drug standards be achieved? *Drug Inf. J.***32:** 101 - 110.

Beaux D Fleurentin J and **Mortier F** (1999). Effect of extracts of *Orthosiphon stamineus* Benth, *Hieracium pilosella* L., *Sambucus nigra* L. and *Arctostaphylos uva-ursi* (L.) Spreng in rats. *Phytother Res.* **13(3):** 222 - 225.

Belgin Cosge Arif Ipek and **Bilal Gurbuz** (2009). GC/MS Analysis of Herbage Essential Oil from Lemon Balms (*Melissa officinalis* L.) grown in Turkey. *J. Applied Biological Sciences.* **3(2):** 136 - 139.

Benjamin J Muir T Briggs K and **Pentl B** (2001). A case of cerebral haemorrhage - can *Ginkgo biloba* be implicated? *Post grad. Med. J*. **77:** 112 - 113.

Bhandari M R N Jong-Anurakkun G Hong and **Kawabata J** (2008). α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata* Haw.). *Food Chem.* **106:** 247 - 252.

Bhutani K K (2003). Herbal medicines enigma and a challenge for science and guidelines for new initiatives. *J. Nat. Prod*. **19(1):** 3 - 8.

Biljana Bauer Petrovska (2012). Historical review of medicinal plants usage**.** *Pharmacogn Rev.* **6(11):** 1 - 5.

Birch N J and **Padgham C** (1994). Handbook on metals in clinical and analytical Chemistry. Marcel Dekker, New York.

Bisset N G (1994). *Herbal Drugs and Phytopharmaceuticals*. CRC Press, Boca Raton.

Bleicher K H Bohm H J Muller K and **Alanine A I** (2003). Hit and lead generation: Beyond high throughput screening. *Nat. Rev. Drug Discov*. **2:** 369 - 378.

Blois M S (1958). Antioxidant determinations by the use of a stable free radical. *Nature* **26**: 1199 - 1200.

Blondeau S Do Q T Scior T Bernard P and **Morin-Allory L** (2010). Reverse Pharmacognosy: another way to harness the generosity of nature. *Curr. Pharm. Des*. **16(15):** 1682 - 1696.

Blumenthal M Brusse W R Goldberg A Gruenwald J Hall T Riggins C W and **Rister R S** (1998). *The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines*. The American Botanical Council, Austin.

Boham B A and **Kocipal-Abyazan R** (1974). Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *Vaccinium calycinum*. *Pacific Scientific*. 48: 458 - 463.

Bosabalidis A M and **Tsekos I** (1984). Glandular hair formation in *Origanum* species. *Annuals of Botany.* **53:** 559 - 563.

Bray H C and **Thorpe W V** (1954). Analysis of Phenolic compounds of interest. *Methods in Biochemical Analysis*.**1**: 27 - 52.

Brimani L (1987). *Traditional Medicine and Traditional Drugs*. Gutenberg Publication, Tehran.

Brindha P Sasikala P and **Purushothaman K K** (1981). Pharmacognostic studies on Merugan Kizhangu. *Bull. Medico.Ethno. Bot. Res.***2(3):** 84 - 96.

Brul S and **Coote P** (1999). Preservative agents in foods. Mode of action and microbial resistance mechanisms. *Int. J. Food Microbiol*. **50(1):** 17.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. *Int. J. Food Microbiol*.**94:** 223 - 253.

Caccioni D L R Guizzardi M Biondi D M Rends A and **Ruberto G** (2000). Relationship between volatile components of *Citrus* fruit essential oil and antimicrobial action on *Pencillium digitatum* and *Pencilium italicum. Int. J. Food Microbiol.***88:** 170 - 175.

Calixto J B (2000). Efficacy, Safety, Quality Control, Marketing and Regulatory Guidelines for Herbal Medicines (Phytotherapeutic Agents). *Braz. J. Med. Biol. Res*.**33 (2):** 179 - 189.

Chami F Chami N Bennis S Bouchikhi T and **Remmal A** (2005). Oregano and Clove essential oil induce surface alteration of *Saccharomyces cerevisiae*. *Phytotherapy Research.***19 (5):** 405 - 408.

Chanegriba N Sabaou N Baaliouamer and **Meklari B Y** (1994). Active anti-bacterienne of antifongique de I hulie essentielle du Cypress. *Ageric Rivista Italiana Epposs.***12:** 5 - 12.

Chao S C (2000). Screening for inhibitory activity of Essential oils on selected Bacteria, Fungi and Viruses. *J. Essent. Oil Res.***12(5):** 639 - 649.

Charles P (1992). Calcium absorption and calcium bioavailability. *J. Int. Med.* **231(2):** 161 - 165.

Chase C R and **Pratt R J** (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. American Pharmaceutical Association.* **38**: 324 - 331.

Chen C P Lin C and **Tsuneo N** (1989).Screening of Taiwanese crude drugs for antibacterial activity against *Streptococcus mutans*, *J. Ethnopharmacol*. **27(3):** 285 - 295.

Cheng X X Kang T G and **Zhao Z Z** (2007). Studies on microscopic identifying of animal drugs remnant hair: identification of several species of *Cauda cervi*. *J. Nat. Med*. **57:** 163 - 171.

Chun S Vattem A D and **Shetty K** (2005). Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. *Proc. Biochem.* **40:** 809 - 816.

Chung K T Wong Y C Wei L Huang Y W and **Lin Y** (1998). Tannins and human health. *J. Pharm. Biol. a review.***6:** 421 - 464.

Cimanga K Kambu K Tona L and **Apers S** (2002). Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the democratic republic of Congo. *J. Ethnopharmacology.***79:** 213 - 220.

Citoglu G S Sever B Antus S Baitz-Gac E and **Altanlar N** (2004). Antifungal diterpenoids and flavonoids from *Ballota inaequiden.Pharm Biol*. **42:** 659 - 663.

Colson C R and **De Broe M E** (2005). Kidney injury from alternative medicines. *Adv. Chronic Kidney Dis*. **12:** 261 - 275.

Cos P Vlietinck A J Berghe D V and **Maes L** (2006). Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. *J. Ethnopharmacol.* **106(3):** 290 - 302.

Couladis M Chinou I B Tzakou O and **Loukis A** (2002). Composition and antimicrobial activity of the essential oil of *Ballota pseudodictamnus* L. Bentham. *Phytother Res.***16:** 723 - 726.

Cox S D Mann C M and **Makham J L** (2000). The mode of the essential of *Malaleuca alternifolia* (Tea tree oil). *J. Appl. Microbiol.* **88:** 170 - 175.

Craig Richard and **Vassilyev Andrey** (2010). *Plant Anatomy*. McGraw-Hill. Co., New York.

Cronquist A (1981). *An integrated system of classification of flowering plants*. New York: Columbia University.

Delamare A P L Pistorello I T M Artico Liane Serafini L A and **Echeverrigaray S** (2007). Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. *Food Chemistry*.**100:** 603 - 608.

Devasagayam T P Tilak A and **Bollork J C** (2004). Review; Free radical and antioxidants in human health. *Curr.stat. fut. prosp.***52:** 794 - 804.

Devi Prasad A G and **Shyma T B** (2013). Medicinal plants used by the tribes of Vythiri taluk, Wayanad district (Kerala state) for the treatment of human and domestic animal ailments. *J. Medicinal Plants Research*. **7(20):** 1439 - 1451.

Dharmananda S (2003). Gallnuts and the uses of Tannins in Chinese medicine – A paper delivered at Institute for Traditional Medicine, Portland, Oregon.

Dinanath D Patil Dnyandeo K Mhaske Gurumeet and **Wadhawa C** (2011). Antibacterial and Antioxidant study of *Ocimum basilicum, Labiatae* (sweet basil) *J. Advanced Pharmacy Education & Research.***2:** 104 - 112.

Dona D D Nguyen N H and **Doan H K** (1992). Studies on the individual and combined Diuretic Effects of Four Vietnamese Traditional Herbal Remedied (*Zea Mays*, *Imperatacylindrica*, Plantago major and *orthosiphon stamineus*). *J. Ethnopharmacol*. **36(3):** 225 - 231.

Donadio C Lucchesi A Tramonti G and **Bianchi C** (1997). Creatinine clearance redicted from body cell mass is a good indicator of renal function. *Kidney. Int*. **52(63):** 166 - 168.

Duangpen Pattamadilok Yenchit Techadamrongs Thidarat Boonruad and **Jaree Bansiddhi** (2003). Chemical Specification of *Orthosiphon aristatus* (Blume). Miq. *Int. J. Biomed. Pharmaceut. Sci*. **44 (3):** 189 - 200.

Dubey N K and **Tripathi P** (2004). Global promotion of herbal medicine: India's opportunity. *Curr.Sci.* **86(1):** 37 - 41.

Eastwood M A (1999). Interaction of dietary antioxidants *in vivo*: How fruit and vegetables prevent disease? *Q.J.M.* **92:** 527 - 530.

Edeoga H O Omosun G and **Uche L C** (2006). Chemical composition of *Hyptis surveolens* and *Ocimum gratissimum* hybrids from Nigeria. *Afr. J. Biotechnol.* **5(10):** 892 - 895.

Egwaikhide P A and **Gimba C E** (2007). Analysis of the Phytochemical content and Anti-microbial activity of *Plectranthus glandulosis* whole plant. *Middle-East. J. Scientific Research.***2 (3-4):** 135 - 138.

EMEA (2005). Guidelines on Quality of Herbal Medicinal Products/Traditional Medicinal Products, EMEA/CVMP/814OO Review. *European Agency for the Evaluation of Medicinal Products.* London.

Englert J and **Harnischfeger G** (1992). Diuretic action of *Orthosiphon stamineus* extract in rats. *Planta Med.* **58(3):** 237 - 238.

Erdemoglu N N Turan N Cakici I Sener B and **Aydin A** (2006). Antioxidant activities of some *Lamiaceae* plant extracts. *Phytother. Res.* **20:** 9 - 13.

Eric T J Michael V A and **Linda W E** (2007). The importance of petiole structure on inhabitability by ants in *Piper macrostachys* (*Piperaceae*). *Botanical J. Linnean Society.***153(2):** 181 - 191.

Ernst E (2003). Serious psychiatric and neurological adverse effects of herbal medicines - a systemic review. *Acta Psychiatr. Scand.* **108:** 83 - 91.

Esau K (1977). *Anatomy of seed plants*.2nd ed. John Wiley, New York.

Esmaeili A Masoudi S H Masnabadi N and **Rustaiyan A H** (2010). Chemical Constituents of the Essential oil of *Sanguisorba minor* Scop. Leaves. *Iran J. Medicinal Plants*. **9(35):** 67 - 70.

Fale P L Borges C Madeira P J A and **Ascensao L** (2009). Rosmarinic acid, scutellarein 4′-methyl ether 7-O-glucuronide and (16S)-coleon E are the main compounds responsible for the antiacetylcholinesterase and antioxidant activity in herbal tea of *Plectranthus barbatus* ("falso boldo"). *Food Chem.* **114:** 798 - 805.

Faleiro M L Miguel M G and **Ladeiro F** (2002). Antimicrobial activity of essential oils isolated from Portuguese endemic species of *Thymus*. *Letters in Applied Microbiology*.**36:** 35 - 40.

Farombi E O Nwamkwo J O and **Emerole G O** (1998). Effect of methanolic extract of browned Yam flour diet on 7, 12-Dimetylbenzanthracene (DMBA) and 3-methylcholanthrene (3-MC) induced toxicity in the rat. *Proc. Fed. Afr. Soc. Biochem. Mol. Biol.* **1**: 5 - 10.

Farooqi A A and **Sreeramu B S** (2001). Introduction In: Cultivation of Medicinal and Aromatic Crop*s University Press Delhi***:** 77 – 79.

Farruch A and **Iqbal Ahamadzafer Mehmood** (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medical plants. *Turk. J. Biol.* **30:** 177 - 183.

Fernandes E S Passos G F Medeiros R Da Cunha F M Ferreira J Campos M M Pianowski L F and **Calixto J B** (2007). Anti-inflammatory effects of compounds alpha-humulene and (-)-trans caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur. J. Pharmacol*. **569(3):** 228 - 236.

Fred-Jaiyesimi A Kio A and **Richard W** (2009). α-Amylase inhibitory effect of 3,-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf. *Food Chem*. **116:** 285 - 288.

Fuhrman B and **Aviram M** (2001). Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Curr.Opin. Lipidol*. **12:** 41 - 48.

Gaibazzi N Gelmini G P Montresor G Ca-Nel D Comini T Fracalossi C and **Long Q R S** (2002). Tachycardia secondary to *Aconitum napellus* alkaloid ingestion. *Ital. Heart J. Suppl*. **3:** 874 - 877.

Gallo M B C and **Sarachine M J** (2009).Biological activities of Lupeol. *Int. J. Biomed. Pharmaceut. Sci*. **3:** 46 - 66.

Galyuteva G I and **Benson N A** (1990). Comparative evaluation of the diuretic activity of leaves and leaf tissue culture biomass of *Orthosiphon stamineus* Benth. *Rastite 'Nye Resursy.***26(4):** 559 - 565.

Gamble J S (1915-1936). *Lamiaceae* In: *Flora of the Presidency of Madras*, Adlard and Son, London.

Gaspar-Marques C Simoes M F and **Rodrıguez B** (2004). Further labdane and kaurane diterpenoids and other constituents from *Plectranthus fruticosus*. *J. Nat. Prod.* **67:** 614 - 621.

Giancarlo S Rosa L M Nadjafi F and **Francesco M** (2006). Hypoglycaemic activity of two spices extracts: *Rhus coriaria* L. and *Bunium persicum* Boiss. *Nat. Prod. Res.* **20:** 882 - 886.

Guenther W and **Konieczynski P** (2003). Speciation of Mg, Mn and Zn in extracts of medicinal plants. *Anal. Bioanal. Chem.* **375(8):** 1067 - 1073.

Gupta R Gigras P Mohapatra H Goswami V K and **Chauhan B** (2003). Microbial α-amylases: A Biotechnological perspective Process. *Biochem*.**38:** 1599 - 1616.

Gupta V K Singh J Kumar R and **Bhanot A** (2011). Pharmacognostic and Preliminary Phytochemical Study of *Ocimum gratissimum* Linn. (Family: *Lamiaceae*). *Asian J. Plant Sciences*.**10(7):** 365.

Hakkim F L Arivazhagan G and **Boopathy R** (2008). Antioxidant property of selected *Ocimum* species and their secondary metabolite content. *J. Medicinal Plants Research*. **2(9):** 250 - 257.

Halliwell B and **Gutteridge J M C** (1992). Free radicals, antioxidants and human diseases: where are we now? *J. Lab. Clin. Med*. **119**: 598 - 620.

Hamilton E M N Whitney E N and **Sizer F S** (1994). Nutrition: Concepts and Controversies, 4th edition, West Publishing Co., St. Paul, MN, USA.

Hanlidou E S Kokkini A M Bosabalidis and **Bessiere M** (1991). Glandular trichomes and essential oil constituents of *Calamintha menthifolia* (*Lamiaceae*). *Plant Systematics and Evolution.* **177(1-2):** 17 - 26.

Hanus O Dembitsky V M and **Moussaieff A** (2006). Comparative study of volatile compounds in the fresh fruits of *Mandragora autumnalis* L. *Acta Chromatographica.* **17:** 151 - 160.

Harborne J B and **Williams C A** (2000). Advances in flavonoid research since 1992. *Phytochemistry*.**55(6):** 481 - 504.

Harborne J B (1973). *Phytochemical Methods*, 1st Edn, Chapman and Hall Ltd, London.

Harborne J B (2001). *Phytochemical Methods*, 2nd Edn, Chapman and Hall Ltd, London.

Harnafi H and **Amrani S** (2008). Spectrophotometric methods for determination of plant polyphenols content and their antioxidant activity assessment: an overview. *Pharmacognosy Reviews*. **2(3):** 20 - 22.

Hassawi D and **Kharma A** (2006). Antimicrobial activity of some medicinal plants against *Candida albicans. J. Biol. Sci.* **6:** 109 - 14.

Havsteen B H (2002). The Biochemistry and medical significance of flavonoids. *Pharmacol. Therapeutics*. **96(2-3):** 67 - 202.

Heinrich M (2000). Plant resources of south-east Asia: medicinal and poisonous plants. *Phytochemistry*. **53**: 619 - 620.

Hildreth J Hrabeta-Robinson E Applequist W Betz J and **Miller J** (2007). Standard operating procedure for the collection and preparation of voucher plant specimens for use in the nutraceutical industry. *Anal. Bioanal Chem.***389(1):** 13 - 17.

Holley R A and **Patel D** (2005). Improvement in shelf life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol*.**22:** 273 - 292.

Hollman P C M and **Katan B** (1999). Health effects and bioavailability of dietary flavonols. *Free Radic. Res.* **31:** 75 - 80.

Hooper D and **Field H** (1937). *Useful plants and drugs of Iran and Iraq*. Field Museum of Natural History, *Botanical Series*.**9:** 71 **-** 241.

Horowitz R S Feldhaus K Dart R C Ster-Mitz F R and **Beck J J** (1996). The clinical spectrum of Jin Bu Huan toxicity. *Arch. Intern. Med.* **156:** 899 - 903.

Hossack D J N (1962). *Proteus vulgaris* - Urinary tract infections in Rats; treatment with Nitrofuran Derivatives. *Br. J. Pharmacol Chemother.* **19(2):** 306 –312.

Hossain M A Ismail Z Rahman A and **Kang S C** (2008). Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. *Industrial Crops and Products*. **27(3):** 328 - 334.

Hsieh S C Fang S H Rao Y K and **Tzeng Y M** (2008). Inhibition of proinflammatory mediators and tumor cell proliferation by *Anisomeles indica* extracts. *J. Ethnopharmacol*.**118:** 65 - 70.

Huang Z R Y K Lin J and **Fang Y** (2009). Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. *Molecules.***14:**540 -554.

Hullatti Prasenjit Bhattacharjee K (2011). Pharmacognostical evaluation of different parts of *Coleus amboinicus* Lour. *Lamiaceae. Pharmacognosy J*.3(24**):** 39 - 44.

Isnandar H and **Kumpulan** (2005).1001 ramuan obat traditional Indonesia. PJ Dayang sumbi. 109 - 112.

Ivanova A Kostova I Tsvetkova I and **Najdenski H** (2001). GC-MS Investigation of *Haplophyllum suaveolens* extracts. *Comptes Rendus de l' Academie Bulgare des Sciences*. **54(6):** 35.

Jamzad Z (2001). A Phylogenic Study of *Nepeta* L. (Ph.D thesis) Birkbeck college, university of London.

Javanmardi J Stushnoff C Locke E and **Vivanco J M** (2003). Antioxidant activity of total phenolic content of Iranian *Ocimum* accessions. *Food Chem*. **83:** 547 - 550.

Jean Fotie (2008). The Antiprotozoan potential of Flavonoids, *Pharmacognosy Reviews*. **2(3):** 6 - 19.

Jensen W I and **Allen J P** (1981). Naturally occurring and experimentally induced castor bean (*Ricinus communis*) poisoning in ducks. *Avian Dis*. **5:** 184 - 194.

Johansen D A (1940). *Plant Microtechnique.* Mc Graw Hill Book Co., New York.

Johns T Kokwaro J O and **Kimanani E K** (1990).Herbal remedies of the luo of siaya district, Kenya: Establishing quantitative criteria for consensus. Econ Bot. **44:** 369 - 381.

Joshi H Jagadeesh P and **Parle M** (2008). Potential of phytochemicals in management of cognitive disorders - An update. *Pharmacognosy reviews.* **2(3):** 54 - 60.

Judd W S Campbell C S Kellogg E A and **Stevens P F** (1999). *Plant systematics - A phylogenetic approach*. Sunderland: Sinauer.

Juven B J Kanner J Schved F and **Weisslouiza H** (1994). Factors that interact with antimicrobial action of thyme essential oil and its active constituents. *Appl. Bacteriol.* **76:** 626 - 631.

Kahkonen M P A Hopia H J Vuorela J P Rauha K Pihlaja T Kujala S and **Heinonen M** (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agr. Food Chem.* **47:** 3954 - 3962.

Kahraman A F Celep and **Dogan M** (2010). Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf. (*Lamiaceae*). *South African J. Botany*.**76(2)**: 187 - 195.

Kanias G D Kilikoglou V Tsitsa E and **Loukis A** (1993). Determination and statistical analysis of trace element and active constituent concentrations in the medicinal plant *Eucalyptus camaldulensis* Dehnh.(*Eucalyptus rostratus* schlecht). *J. Radioanal. Nucl. Chem.* **169(2):** 483 - 491.

Kannappan N Madhukar A Marymmal Sindhura P and **Mannavalan R** (2010). Evaluation of nephroprotective activity of *Orthosiphon stamineus* Benth.extract using rat model. *Int. J. Pharm. Tech. Res. Service*. **2(1):** 209 - 215.

Kasahara Y Kumaki K Katagiri S Yasukawa K Yamanouchi S Takido M Akihisa T and **Tamuta C** (1994). *Carthami flos* extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytotherapy Res.* **68:** 327 - 331.

Kaya A B Demirci K H and **Baser C** (2007). Micromorphology of glandular trichomes of *Nepeta congesta* Fisch. & Mey.var.*congesta* (*Lamiaceae*) and chemical analysis of the essential oils. *South African J. Botany.***73(1):** 29 - 34.

Kelly G S (1999). Squalene and its potential clinical uses. *Altern. Med. Rev.* **4(1):** 29 - 36.

Kelly K (2009). *History of medicine* : Facts on file. New York 29 - 50.

Kenakin T (2003). Predicting therapeutic value in the lead optimization phase of drug discovery. *Nat. Rev. Drug. Discov*.**2:** 429 - 438.

Kenner D and **Requena Y** (1996). *Botanical Medicine*. A European professional perspective. Paradign publications, Brookline, Massachusetts**.**7 - 12.

Khatoon S Mehrotra S Shome U and **Mehrotra B N** (1993). Analysis of commercial 'Ratanjot' by TLC fluorescence finger printing. *Int. J. Pharmacog*. **31(4):** 269 - 277.

Kluytmans J Van Belkum A and **Verbrugh H** (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin. Microbiol. Rev.* **10(3):** 505 - 520.

Knowles J and **Gromo G** (2003). Target selection in drug discovery. *Nat. Rev. Drug Discov*. **2:** 63 - 69.

Kris-Etherton P M Hecker K D Bonanome A Coval S M Binkoski A E Hilpert K F Griel A E and **Etherton T D** (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am. J. Med*. **113:** 71 - 88.

Kulkarni M G and **Sathe P S** (2013). Phytochemical and GC-MS analysis of *Hamiltonia suaveolens* (Roxb.). *International J. Chem. Tech. Research.* **5(1):** 212 - 219.

Kunle Oluyemisi Folashade Egharevba Henry Omoregie and **Ahmadu Peter Ochogu** (2012). Standardization of herbal medicines - A review. *International J. Biodiversity and Conservation*.**4(3):** 101 - 112.

Kwan T H Tong M K Leung K T Lai C K Poon W T and **Chan Y W** (2006). Acute renal failure associated with prolonged intake of slimming pills containing anthraquinones. *Hong Kong Med. J.***12:** 394 - 347.

Kwon Y I Vattem D V and **Shetty K** (2006). Evaluation of clonal herbs of *Lamiaceae* species for management of diabetes and hypertension. *Asia Pac. J. Clin. Nutr.***15:** 107 - 118.

Lanciotti R Gianotti A Patrignani N Belleti N Guerzoni M E and **Gardini F** (2004). Use of natural aroma compounds to improve self – life of minimally processed fruits. *Trends in Food Sci. & Tech.* **15:** 201 - 208.

Latner A (1958). Carbohydrate metabolism, abnormalities of post abortive blood sugar level, clinical biochemistry, 2nd ed. W B Saunder Co. *Philadelphia.* 48.

Lau F C Shukitt-Hale B and **Joseph J A** (2005). The Beneficial effects of fruit polyphenols on brain aging. *Neurobiol.Aging.***26:** 128 - 132.

Lau P W Peng Y and **Zhao Z Z** (2004). Microscopic identification of Chinese patent medicine- Wu Zi Yan Zong. Wan. *J. Nat. Med.* **58:** 258 - 265.

Lennette E H (1985). Antibiotic susceptibility testing by a standard single disc method. In: Manual of Clinical Microbiology. Macmilan Publishers, Washington, D.C. 978.

Lipsky M S and **Sharp L K** (2001). From idea to market: The drug approval process. *J. Am. Board Fam. Pract*.**14:** 362 - 367.

Longaray Delamare A P Moschen-Pistorello I T Atti-Serafini L and **Echeverrigaray S** (2007). Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. *Food Chemistry*.**100(2):** 603 - 608.

Lorenzi H and **Matos F J A** (2002). Plantas medicinais no Brasil-nativas e exoticas. Nova Odessa: Plantarum.

Lowry O H Rosenbury N J Farr A L and **Randall R J** (1951). Protein measurement with the Folin–phenol reagent. *J. Biological Chemistry*. **193**: 262 - 275.

Lu Y and **Yeap-Foo L** (2002). Polyphenolics of Salvia - a review. *Phytochem*.**59:** 117-140.

Łukasz Kuzma Danuta Kalemba Marek Rozalski Barbara Rozalska Marzena Więckowska Szakiel Urszula Krajewska and **Halina Wysokińska** (2009). Chemical composition and Biological Activities of Essential Oil from *Salvia sclarea* plants regenerated *in vitro*. *Molecule.***14:**1438 - 1447.

Luo Q Cai Y Yan J Sun M and **Corke H** (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. *Life Sci*. **76:** 137 - 149.

Lutete T Kambu K Ntondele D Cimanga K and **Luki N** (1994). Antimicrobial activity of Tannins. *Fitoterapia.***653:** 267 - 278.

Magda Coisin Ioan Burzo Marius Stefan Elida Rosenhech and **Maria Magdalena Zamfirache** (2012). Chemical composition and antibacterial activity of Essential Oils of Three *Salvia* species, Widespread in Eastern Romania. *Biologie Vegetala.* **58(1):** 51 - 58.

Mahadevan A (1982). Estimation of Tannin. In: *Methods in Physiological Plant Pathology*. Sivakami Printers, Chennai, India.

Mai T T Thu N N Tien P G and **Chuyen N V** (2007). Alpha-glucosidaseinhibitory and antioxidant activities of Vietnamese edible plants and theirrelationships with polyphenol contents. *J. Nutritional Science and Vitaminology.* **53:** 267 - 276.

Majid A A B Sarmani S Yusoe N I Wie Y K and **Hamzah F** (1995). Trace elements in Malaysian medicinal plants. *J. Radioanal. Nucl. Chem.* **195(1):** 173 - 183.

Malhotra S and **Singh A P** (2007). A Review of Pharmacology of Phytochemicals from Indian Medicinal Plants. *The Interat. J. Alternative Medicine.* **5(1):** 1 - 7.

Mandel S Amit T Reznichenko L Weinreb O and **Youdim M B** (2006). Green tea catechins as brain-permeable, natural iron chelators antioxidants for the treatment of neurodegenerative disorders. *Mol. Nutr. Food Res.* **50:** 229 - 234.

Mangathayaru K Thirumurugan D Patel P S Pratap D V David D J and **Karthikeyan J** (2006). Isolation and Identification of Nicotine from *Leucas aspera* (Willd) Link. *Indian J. Pharm. Sci.* **68(1):** 88 - 90.

Maria Fatima Simoes A Patrıcia Rijo A Aida Duarte A Diana Barbosa A Diogo Matiasa Joana Delgado Nadia Cirilo and **Benjamın Rodrıguez** (2010). Two new diterpenoids from *Plectranthus* species. *Phytochemistry Letters*. **3:** 221 - 225.

Mariam A and **Asmawi M Z** (1999). Hypoglycaeic activity of the aqueous extract of *Orthosiphon stamineus*. *Fitoterapia.***67(5):** 465 - 468.

Maridass M Victor B Manickam V S Ghanthikumar S and **Arockium A** (2008). The Ethnobotanical uses of the aromatic oils from two Indian endemic plant species of the family *Lamiaceae*, *Pogostemon travancoricus* Bedd. and *Orthosiphon comosus* Wight ex Benth. *Ethnobotanical Leaflets*.**12:** 191 - 194.

Marino M Bersani C and **Comi G** (2001). Impedance measurement to study antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *Int. J. Food Microbiol*.**67:** 187 - 195.

Marinova E M and **Yanishlieva N V** (1997). Antioxidative activity of extracts from selected species of the family *Lamiaceae* in sunflower oil. *Food Chem*. **58:** 245 - 248.

Masuda T and **Masuda** (1992). Orthosiphol A and B, Novel diterpenoid inhibitors of TPA (12-O-tetradecanoylphorbol-13-acetate) - induced inflammation, from *Orthosiphon stamineus*. *Tetrahedron*.**48(33):** 6787 - 6792.

Mathew K M (1983-1988). *The Flora of Tamilnadu Carnatic*.**I.** The Rapinat Herbarium.St. Joseph College, Tiruchirapalli, India.

Matsuda S P Darr L B Hart E A Herrera J B McCann K E Meyer M M Pang J and **Schepmann H G** (2000). Steric bulk at cycloartenol synthase position 481 influences cyclization and deprotonation. *Org. Lett.* **2(15):** 2261 - 2263.

Matu E N and **Van Staden J** (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J. Ethnopharmacol.* **87:** 35 - 41.

Mc Creedy R M Guaggolz J Siliviera V and **Owens H S** (1950). Determination of standard amylase in vegetables. *Analytical Chemistry*.**22**: 1156 - 1158.

Mercer N S and **Davis D M** (1991). The chemistry of tannin. *Tattoos Br. Med. J*.**3 (3):** 380 - 382.

Metcalfe C R and **Chalk L** (1979). *Anatomy of the Dicotyledons*. 1. Clarendon Press. Oxford. 276.

Metcalfe C R and **Chalk L** (1950). *Anatomy of Dicotyledons - leaves, stem, and wood in relation to Taxonomy*. Oxford: Clarendon Press.

Metcalfe C R and **Chalk L** (1988). *Anatomy of the dicotyledons*. 2nd ed. Oxford: Clarendon Press.

Metcalfe C R and **Chalk L** (1972). *Anatomy of the dicotyledons*.**2:** Clarendon Press, Oxford.1041- 1053.

Miller G L (1959). Use of dinitro salicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31:** 426 - 428.

Mojarrab M A Delazar S Esnaashari F and **Heshmati Afshar** (2013). Chemical composition and general toxicity of essential oils extracted from the aerial parts of *Artemisia armeniaca* Lam. and *Artemisia incana* (L.) Druce growing in Iran. *Research in Pharmaceutical Sciences.***8 (1):** 65 - 69.

Moore S and **Stein W H** (1948). Estimation of carbohydrates. In: Colowick, S.P. and Kaplen, N.D. (ed.) *Methods in Enzymology,* Academic Press, New York. 3468.

Morais S M Dantas J D P Silva A R A and **Magalhaes E F** (2005). Plantas medicinais usad as pelos indios Tapebas doCeara. *Rev. Bras Farmacogn.***15:** 169 - 177.

Moritz F Compagnon P Kaliszczak I G Kaliszczak Y Caliskan V and **Girault C** (2005). Severe acute poisoning with homemade *Aconitum napellus* capsules: toxic kinetic and clinical data. *Clin.Toxicol*.**43:** 873 - 876.

Mosihuzzaman M and **Choudhary M I** (2008). Protocols on safety, efficacy, standardization, and documentation of herbal medicine. *Pure. Appl. Chem.* **80(10):** 2195 - 2230.

Murray B E (1990). The life and times of the *Enterococcus*. *Clin. Microbiol Rev*. **3(1):** 46 - 65.

Nakatsu T Lupo A T and **Chinn J W** (2000). Biological activity of essential oils and their constituents. *Studies in Natural Products Chemistry*.**21:** 571 - 631.

Nayar M P (1996). Hot Spots of Endemic Plants of India, Nepal and Bhutan. *Endemic to Peninsular India.* Tropical Botanical Garden Research Institute, Thiruvananthapuram. 202- 203.

Neuhouser M L (2004). Dietary flavonoids and cancer risk: evidence from human population studies. *Nutr.Cancer.***50:** 1 - 7.

Nickavar B Kamalinezad M and **Izadpanah H** (2007). *In vitro* free radical scavenging activity of five *Salvia* species. *Pak. J. Pharm Sci*. **20(4):** 291 - 294.

Nirmala Devi K and **Periyanayagam K** (2011). Nephroprotective effect of *Plectranthus amboinicus* (Lour) Spreng. on Glycerol induced acute renal failure (Arf). *Herbal Tech Industry.***8(8)**: 211 - 214.

Nirnoy M and **Muangman V** (1991). Effect of folio *Orthosiphon* on urinary stone promoters and inhibitors. *J. Med. Assoc. Thai*. **74(6):** 318 - 321.

NIST (2005).Chemistry Web Book. NIST standard reference database number 69, http://webbook.nist.gov/chemistry.

Norouzi-Arasi H Yavari I and **Alibabaeii M** (2004). Chemical constituents of the essential oil of *Stachys schtschegleevii* Sosn.from Iran. *J. Essen. Oil Res*. **16:** 231 - 232.

O' Brien T P Feder N and **Mc Cull M E** (1964). Polychromatic staining of plant cell walls by toluidine blue – O. *Protoplasma.***59:** 364 - 373.

Odhav B Jughal S and **Govinden R** (2002). Spices, oils for the control of co-occurring mycotoxins producing fungi. *European Food Res. & Technol*. **65:** 683 - 687.

Ogunlesi M Okiei W Ofor E and **Osibote A E (**2009). Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn. (*Euphorbiaceae*), A potential medication for asthma. *African J. biotech.* **8:** 7042 - 7050.

Ohashi K Bohagari J Matsubara T and **Shibuya H** (2000). Chemical structures of two new migrated pimarane – type diterpenes neoortho siphols A and B and Suppressive effects on rat thoracic aorta of chemical constituents isolated from the leaves of *Orthosiphon stamineus.* Indonesian medical plants XXIII. *Bull.* **48(3):** 433 - 435.

Ohashi K Bohgak T and **Shibuya H** (2000). Antihypertensive substances in the leaves Kumis Kucing (*Orthosiphon stamineus*) in Java Island. *Yakugaku Zasshi.* **120(5):** 474 - 482.

Olowokudejo J D (1987). Taxonomic value of petiole anatomy in the genus *Biscutella* L. (*Cruciferae*). *Bull. Jard. Bot. Nat. Belg.* **57:** 307 - 320.

Osawa T (1994). Novel natural antioxidants for utilization in food and biological systems. In: Uritani I, Garcia VV, Mendoza E M (Eds.) *Post harvest*

biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press, Japan. 241 - 251.

O'Shea T J (1995).Capillary electrophoresis/electrochemistry. *Curr. Sci.* **14(1):** 18 - 23.

Oyaizu M (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese J. Nutrition.***44:** 307 - 315.

Ozdemir C and **Senel G** (2001). The morphological, anatomical and karyological properties of *Salvia forskahlei* L. (*Lamiaceae*) in Turkey. *J. Economic & Taxonomic Botany. 2*97 - 313.

Ozdemir C and **Senel G** (1999). The morphological, anatomical and karyological properties of *Salvia sclarea* L. *Tr. J. of Botany.***23:** 7 - 18.

Oznur Ergen Akcin M Sabri Ozyurt and **Gulcan Senel** (2011). Petiole Anatomy of some *Lamiaceae* taxa. *Pak. J. Bot*. **43(3):** 1437 - 1443.

Page L R (2001). Whole herbs or standardized plant constituents? *Total Health.* **23(4):** 20.

Palmeira-De-Oliveira A Salgueiro L and **Palmeira-De-Oliveira Retal** (2009). Anti-*Candida* activity of essential oils.*Mini Rev. Med. Chem*. **9:** 1292 - 1305.

Pandancode Chandran Sajitha David Punitha Uthaman Danya Madathupatti Ramanathan Parejo I F Viladomat J Bastida A Rosas-Romero N Flerlage and **Burrilo J** (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. *J. Agri. Food Chem.* **50:** 6882 - 6890.

Parmar N S and **Ghosh M M N (**1978). Current trends in flavonoid research. *Indian J. Pharmacology.***12**: 213 - 228.

Patel P M Patel N M and **Goyal R K** (2006). Quality control of herbal products. *The Indian Pharmacist.* **5(45):** 26 - 30.

Patra K C Pareta S K Harwansh R K and **Jayaram Kumar K** (2010).Traditional approaches towards standardization of herbal medicines - A review. *J. Pharm. Sci. Technol*. **2 (11):** 372 - 379.

Pattanaik S Subramanyam V R and **Kole C** (2002). Antibacterial and antifungal activity of the essential oils *in vitro*. *Microbios*.**86(349):** 237 - 246.

Pereira R C Oliveira M T R and **Lemos G C S** (2004). Plantas utilizadas como medicinais no municipio de Campos de Goytacazes – RJ. *Rev. Bras. Farmacogn.***14:** 37 - 40.

Perez C Paul M and **Bezique P** (1990). An Antibiotic assay by the agar well diffusion method. *Alta Biomed. Group Experiences.***15:** 113.

Pinto M D S and **Shetty K** (2010). Health Benefits of Berries for Potential Management of Hyperglycemia and Hypertension. In: Flavor and Health Benefits of Small Fruits, ACS Publications, Washington, DC, USA. **8:** 121 - 137.

Porter G A and **Bennett W M** (1981). Nephrotoxic acute renal failure due to common drugs. *American j. Physiology* .**241(7):** F1 - F8.

Powel J Burden T J and **Thompson R P H** (1998). *In vitro* mineral availability from digested tea: a rich dietary source of manganese. *Analyst.* **123(8):** 1721 - 1724.

Prasad A S (1982). Clinical, Biochemical and Nutritional aspects of trace Elements. Alan R. Liss, Inc, New York.

Premgamone A Sriboonlue P Disatapornjaroen W Maskasem S Sinsupan N and **Apinives C** (2001). A long-term study on the efficacy of herbal plant, *Orthosiphon grandiflorus*, and sodium potassium citrate in renal calculi treatment. *Southeast Asian J. Trop. Med. Public Health.***32(3):** 654 - 660.

Pullaiah T (2004). Angiosperms: Origin And Evolution In: *Taxonomy of Angiosperms.* Regency Publications, New Delhi.

Pushpangadan P and **Atal C K** (1984). Ethno-medico-botanical investigations in Kerala I. Some primitive tribals of western ghats and their herbal medicine. *J Ethnopharmacol*. **11:** 59 - 77.

Rajurkar N S and **Pardeshi B M** (1997). Analysis of Some Herbal Plants from India Used in the Control of Diabetes Mellitus by NAA and AAS Techniques. *Appl. Radiat. Isot.***48(8):** 1059 - 1062.

Ramasubramania Raja R (2012). Medicinally Potential Plants of *Labiatae* (*Lamiaceae*) Family: An Overview. *Research J. Medicinal Plant.* 1 - 11.

Raphael K R Sabu M C and **Khuttan R** (2002). Hypoglycemic effect of methanol extract of *Phyllanthus amarus* Schum & Thonn on alloxan induced Diabetes mellitus in rats and its relaxation with antioxidant potential. *Indian J. Experimental Biology.***40:** 905 – 909.

Rapisarda A E Galati M Tzakou O and **Flores M** (2001). *Nepeta sibtropii* Bentham. (*Lamiaceae*) micromorphological analysis of leaves and flowers. *Farmaco.* **56(5-7):** 413 - 415.

Rasineni G K Siddavattam D and **Reddy A R** (2008). Free radical quenching activity and polyphenols in three species of *Coleus*. *J. Medicinal Plants Research.* **2(10):** 285 - 291.

Rasooli I and **Mirmostafa S A** (2002).Antibacterial properties of *Thymus pubescens* and *Thymus serpyllum* essential oils. *Fitoterapia.***73:** 183 - 279.

Raven P H Evert R F and **Eichhorn S E** (2005). *Biology of Plants*.Freeman W.H, New York. **7:**9.

Ravish Kumar M (2012). *Chromobacterium violaceum*: A rare bacterium isolated from a wound over the scalp. *Int. J. Appl Basic Med. Res.* **2(1):** 70 - 72.

Rawiya H Alasbahi Matthias F and **Melzig** (2010). *Plectranthus barbatus*: A Review of Phytochemistry, Ethnobotanical Uses and Pharmacology – Part 2. *Planta Med.* **76(8):** 753 - 765.

Reddy M B Chidambaram M V and **Bates G W** (1987). Iron Transport in Microbes, Plants and Animals. VCH, New York.

Reichling J Schnitzler P Suschke U and **Saller R** (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – An overview. *Forsch Komplementmed.* **16:** 79 - 90.

Rhabasa-Lhoret R and **Chiasson J L** (2004). Alpha-glucosidase inhibitors (3rd ed.). International text book of diabetes mellitus 1. John Wiley. UK.

Rhoda M Kariba (2001). Antibacterial activity of *Ajuge remota. Fitoterapia* **72:** 177.

Richardson P (1992). The chemistry of the *Labiatae*: An introduction and overview. In: Harley RM and Reynolds T. (Eds.) Advances in *Labiatae* Science. Botanical Garden Kew. 291- 297.

Rijo P Gaspar Marques C Simoes M F Duarte A Apreda Rojas M C Cano F H and **Rodrıguez B** (2002). Neoclerodane and labdane diterpenoids from *Plectranthus ornatus*. *J. Nat. Prod.***65:** 1387 - 1390.

Rivera Nunez D and **Obon de Gastro C** (1992).The ethnobotany of *Labiatae* of old world. In Harley, R.M. Reynolds, T., Advances in *Labiatae* Science. Royal Botanical Gardens, Kew, London. 455 - 473.

Ruiz A R Torre R A Alonso N Villaescusa A Betancourt J and **Vizoso A** (1996). Screening of medicinal plants for induction of somatic segregation activity in *Aspergillus nidulans*. *J. Ethnopharmacol.* **52:** 123 - 127.

Russel A D (1991). Mechanisms of bacterial resistance to non – antibiotics food additives are pharmaceutical preservatives. *J. Appl. Bacteriology.* **71:** 191 - 201.

Saad B Azaizeh H Abu-Hijleh G and **Said O** (2006). Safety of traditional arab herbal. Evidence based complement. *Alternat. Med.* **3:** 433 - 439.

Sadashiva C Sharanappa T P Naidoo Y Sulaimon C and **Indira Balachandran T** (2013). Chemical composition of essential oil from *Orthosiphon diffuses* Benth. *J. Medicinal Plants*. **7(4):**170 - 172.

Sadasivam S and **Manickam A** (1996). *Biochemical methods for Agricultural Sciences*. Willey Eastern Ltd., New Delhi.

Salami A (2002). *Anicent Iranian Medicine: The Traditional Medicine of Davan.* Didavar, Tehran.

Sandhyarani D Khomdram Potsangbam K Singh and **Richardson P** (2011). Polyphenolic Compounds and Free Radical Scavenging Activity in Eight *Lamiaceae.* Herbs of Manipur. *Not. Sci. Biol*. **3(2):** 108 - 113.

Sapna S and **Ravi T K** (2007). Approaches towards development and promotion of Herbal drugs. *Pharmacognosy Review*.**1(1):** 180 - 184.

Sarac N and **Ugur A** (2007). Antimicrobial activities and usage in folkloric medicine of some *Lamiaceae* species growing in Mugla, Turkey. *Eur. Asia J. Bio. Sci*. **1(4):** 28 - 34.

Sass J E (1940). *Elements of Botanical Microtechnique,* Mc Graw Hill Book Co, New York.

Savithramma N Linga Rao M and **Suhrulatha D** (2011). Screening of Medicinal Plants for Secondary Metabolites. *Middle-East J. Scientific Research.* **8(3):** 579 - 584.

Scavone O (1965). Contribuiçao ao estudo morfologico eanatomico de *Coleus barbatus* Benth. *Labiatae. Rev. Fac. Farm Bioquim Sao Paulo.***3:** 249 - 270.

Schulze M B and **Hu F B** (2005). Primary prevention of diabetes: What can be done and how much can be prevented? *Ann. Rev. Pub. Health.***26:** 445 - 467.

Schut G A and **Zwaving J H** (1986).Content and composition of the essential oil of *Orthosiphon aristatus. Planta Med.***52:** 240 - 241.

Seitz U Bonn G Oefner P and **Popp M** (1991). Isotachophoretic analysis of flavonoids and phenolcarboxylic acids of relevance to phytopharmaceutical industry. *J. Chromatogr.***559:** 499 -504.

Sermakkani M and **Thangapandian P** (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian J. Pharm Clin. Res.* **5(2):** 90 - 94.

Seyed Mehdi Talebi Asghar Rezakhanlou and **Gity Salahi Isfahani** (2012). Trichomes Plasticity in *Ziziphora tenuior* L. (*Labiatae*) in Iran. *An ecological review Annals of Biological Research*.**3 (1):** 668 - 672.

Shaheen A M (2007). Characteristics of the stem-leaf transitional zone in some species of *Caesalpinioideae* (*Legumuninosae*). *Turk. J. Bot.***31:** 297 - 310.

Shahidi F Janitha P K and **Wanasundara P D** (1992). Phenolic antioxidants CRC Critical Rev. *Food Science and Nutrition.***32 (1):** 67 - 103.

Sharma S M and **Bhadange D G** (2013).Antimicrobial potential of *Lamiaceae* members *International J. Pharma Sciences.***3(5):** 324 - 327.

Sharma Vivek Sharma Nisha Singh Harbans Srivastava K Devendra Pathaniavijaylata Singh and **Bikram Gupta C Raghbir** (2009).Comparative account on GC-MS Analysis of *Mentha arvensis* L. Corn Mint.from three different locations of North India. *Int. J. Drug Dev. & Res.* **1(1):** 1 - 9.

Shetty K (1997). Biotechnology to harness the benefits of dietary phenolics; Focus on *Lamiaceae*. *Asia Pac. J .Clin.Nutr.***6:** 162 - 171.

Shinde V Dhalwal K and **Mahadik K R** (2008). Some issue related to Pharmacognosy. *Pharmacogn.Rev.***2(3):** 1 - 5.

Sigel H (1978). Metals in Biological Systems. Marcel Dekker, New York.

Smille T J and **Khan I A** (2010). Comprehensive approach to Identifying and authenticating Botanical Products, Clinical Pharmacology and therapeutics. **87(2):** 175 - 186.

Smith R M Bahaffi S O and **Albar H A** (1996). Chemical composition of the essential oil of *Plectranthus tenuiflorus* from Saudi Arabia. *J. Essential Oil Research.* **8(4):** 447 - 448.

Smith T J (2000). Squalene: potential chemopreventive agent *Expert Opin Investig Drugs.***9(8):** 1841 - 1848.

Sokovic M Glamoclija J and **Marin P D** (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. *Molecules*.**15:** 7532 - 7546.

Sonboli A Salehi P and **Ebrahimi S N** (2005). Essential oil composition and antibacterial activity of the leaves of *Stachys schtschegleevii* from Iran. *Chem. Nat. Compds*. **41:** 171 - 174.

Song Y Manson J E Buring H Sesso D and **Liu S** (2005). Association of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: A prospective study and cross sectional analysis. *J. Am. Coll. Nutr.* **24:** 376 - 384.

Sriplang K Adisakwattana S Rungsipipat A and **Yibchok-Anun S** (2007). Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. *J. Ethnopharmacol*. **12.109(3):** 510 - 514.

Stojanoski N (1999). Development of health culture in Veles and its region from the past to the end of the 20th century.*Veles: Society of science and art.* 13 - 34.

Straus S E (2002). Herbal remedies. *New Engl. J. Med.* **347:** 2046 - 2056.

Sudha Revathy S Rathinamala R and **Murugesan M** (2012). Authentication methods for drugs used in Ayurveda, Siddha and Unani systems of medicine: an overview. *International J. Pharmaceutical Science and Research.* **3(8):** 2352 - 2361.

Sultanbawa Y Cusack A Currie M and **Davis C** (2009). An innovative microplate assay to facilitate the detection of antimicrobial activity in plant extracts. *J. Rapid Methods & Automation in Microbiology*. **17:** 519 - 534.

Sumaryono W Proksch P Wray V Witte L and **Hartmann T** (1991). Qualitative and quantitative analysis of the phenolic constituents from *Orthosiphon aristatus. Planta Medica*. **57(2):** 176 - 180.

Sureshkumar P (2013). Phytochemical assessment on various extracts of *Calotropis gigantea* (L.) R. BR. through GC-MS. *Int. J. Pharm. Bio. Sci.* **4(2):** (B) 803 - 810.

Svicekova M Havranek E and **Novak V** (1993). Determination of heavy metals in samples of herbal drugs using differential pulse polarography. *J. Pharm. Biol*. **42(2):** 68 - 70.

Tahir S Khenam S M and **Husain S Z** (1995). A micromorphological study of *Pogostemon* Desf. Species (*Lamiaceae*) from Bangladesh. *Pak. J. Bot*. **27(1):** 73 - 82.

Tassou C C Koutsoumanis K and **Nychas G J E** (2000). Inhibition of *Salmonella enteridis* and *Staphylococcus aureus* on nutrient broth by mint essential oil. *Food Res. Int.* **48:** 273 - 280.

Tchinda A T Tchuendem Khan S N Omar I Ngandeu F Nkeng P E A and **Choudhary I M** (2008). Antioxidant activity of the crude extract of the fruits of *Pycnanthus angolensis* and α-glucosidase inhibitory activity of itsconstituents. *Pharmacology.***1:** 422 - 431.

Temple V J Ojobe T O and **Onobun C E** (1991). Chemical composition of livingstone potato tubers (*Plectranthus esculentus*). *J. Sci. Food Agric.* **56:** 215 - 217.

Thomas J (1997). Medicinal and aromatic plants research in India. In UNDP. Proc. Training course on industrial exploitation of indigenous medicinal and aromatic plants. Beijing, China. 17 - 27.

Tomas Barberan F A and **Gil M L** (1992). Chemistry and natural distribution of Flavonoids in the *Labiatae.* In Harley R M Reynolds T Advances in *Labiatae* Science. Royal Botanical Gardens, Kew, London. 200 - 305.

Tomas-Barberan F A and **Gil M L** (1992). Chemistry and natural distribution of Flavonoids in the *Labiatae*. In Harley, R.M. Reynolds, T., Advances in *Labiatae* Science. Royal Botanical Gardens, Kew, London. 200 - 305.

Tona Lutete K Kumbu D Tondole N and **Manga K C** (1999). Antimicrobial activity of tannins. *Fitoterapia* **2:** 279.

Trease G E and **Evans W C** (1985). Pharmacognosy 11th Ed. Baillere Tindall Ltd., London**:** 60 - 75.

Trease G E and **Evans W C** (1983). *Text book of Pharmacognosy*. 12th Ed., Balliere, Tindall, London. 57 - 383.

Triantaphyllou K Blekas G and **Boskou D** (2001). Antioxidative properties of water extracts obtained from herbs of the species *Lamiaceae*. *Int. J. Food Sci.Nutri*.**52:** 313 - 317.

Tzong Huei Lee Shin-Hun Juang Feng Lin Hsu and **Cheng Yi Wu** (2005). Triterpene Acids from the Leaves of *Planchonella Duclitan* (Blanco) Bakhuizan. *J. Chinese Chemical Society*. **52(6):** 1275 - 1280.

Underwood E J (1977). Trace Elements in Human and Animal Nutrition.4th edition. Academic Press.New York.

Uniyal S K Singh K N Jamwal P and **Lal B** (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *J. Ethnobiol. Ethnomed.***2:** 14.

Vaibhav Shinde and **Kamlesh Dhalwal** (2007). Pharmacognosy: The changing scenario *Pharmacognosy Magazine* **1 (1):** 1 - 6.

Valabhji J McColl A J Richmond W Schachter M Rubens M B and **Elkeles R S** (2001). *Diabetes Care*.**24:** 1608 - 1613.

Van Dam R M Rimm E B Willett W C Stampfer M J and **Hu F B** (2002). Dietary patterns and risk for type 2 diabetes mellitus in U.S. men. *Annuals of Int. Med.* **136:** 201 - 209.

Vanher-Weghem L J (1998). Misuse of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). *J. Altern. Complement Med*. **4:** 9 - 13.

Vaya J Mahmood S Goldblum A Aviram M Volkova N Shaalan A Musa R and **Tamir S** (2003). Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. *Phytochemistry.***62:** 89 - 99.

Velioglu Y Mazza S G Gao L and **Oomah B D** (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agr. Food Chem.***46:** 4113 -4117.

Veni Bharti and **Neeru Vasudeva** (2013). *Oreganum vulgare* Linn. leaf: An Extensive Pharmacognostical and Phytochemical Quality Assessment. *Adv Pharm.Bull*. **3(2):** 277 - 281.

Venkateshappa S M and **Sreenath K P** (2013).Some Species of *Lamiaceae* – Comparative Anatomical Studies. *Indo American J. Pharmaceutical Research.* **3(11):** 9243 - 9254.

Venkateshappa S M and **Sreenath K P** (2013). Potential medicinal plants of *Lamiaceae*. *American International J. Research in Formal, Applied & Natural Sciences*. **3(1):** 82 - 87.

Verkman A S (2004). Drug discovery in academia. *Am. J. Physiol. Cell Physiol***. 286:** 465 -474.

Visca P Seifert H and **Towner K J** (2011). Acinetobacter infection--an emerging threat to human health. *IUBMB Life.* **63 (12):** 1048 - 1054.

Vrachnakis T (2003). Trichomes of *Origanum dictamnus* L. (*Labiatae*). *Phyton.***43(1):** 109 -133.

Wagstaff S J Hickerson L Spangler R Reeves P A and **Olmstead R G** (1998). Phylogeny in *Labiatae* s. 1., inferred from cpDNA sequences. *Pl. Syst. Evol*.**209:** 265 - 274.

Wallis T E (1985). *Text Book of Pharmacognosy*. CBS Publishers and Distributors New Delhi India: 652.

Wang Y C and **Huang T L** (2005). Screening of anti-Helicobacter pylori herbs deriving from Taiwanese folk medicinal plants. *FEMS Immunol. Med. Microbiol*. **43:** 295 - 300.

Weimann C Goransson U Ponprayoon-Claeson P Bhlin L Rimpler H and **Heinrich M** (2002). Spasmolytic effects of *Baccharis conferta* and some of its constituents. *J. Pharm. Pharmacol*. **54(1):** 99 - 104.

Werker E Ravid U and **Putievsky E** (1985). Structure of glandular hairs and identification of the main components of their secreted material in some species of the *Labiatae. Israel J.Botany* **34:** 31 - 45.

Werker E (2006). Function of essential oil-secreting glandular hairs in aromatic plants of *Lamiaceae*- a review. *Flavour and Fragrance J*. **8(5):** 249 - 255.

WHO (1981). *The International Pharmacopeia*. **2:** *Quality Specifications*, 3rd edn. World Health Organization, Geneva.

WHO (1991). Guidelines for the assessment of herbal medicines. Programme on traditional medicines. Geneva.

WHO (1996). Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva.

WHO (1998). Quality control methods for Medicinal Plant materials, World Health Organization, Geneva.

WHO (1999). *WHO Monographs on Selected Medicinal Plants*. **1:** World Health Organization, Geneva.

WHO (2000). *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*. World Health Organization, Geneva.

Wiart C (2006). *Etnopharmacology of medicinal plants.* New Jersey: Humana Press. 1 - 50.

Wickramasinghe and **Bandaranayake M** (2006).Quality control, screening, toxicity, and regulation of Herbal Drugs. In:*Modern Phytomedicine. Turning Medicinal Plants into Drugs Edited by* I Ahmad F Aqil and Owais WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Willard T (1996). Edible and medicinal plants of the Rocky Mountains. Calgary: Wild Rose College of Natural Healing.

Williams A and **Twine J** (1960). *Modern Methods of Plant Analysis,* Peach, K, Tracey, M V (Edu.), Springer Verlog, Berlin.

Yaman I and **Balikci E** (2010). Protective effects of *Nigella sativa* against gentamicin induced nephrotoxicity in rats. *Experimental and Toxicologic Pathology*. **62(2):** 183 - 190.

Yasukawa K Takido M Matsumoto T Takeuchi M and **Nakagawa S** (1991). Sterol and triterpene derivatives from plants inhibit the effects of tumour promoter and sitosterol and betulinic acid inhibits tumour formation in mouse skin two-stage carcinogenesis. *J. Oncology*. **41:** 72 - 76.

Ye F Shen Z and **Xie M** (2002). Alpha - glucosidase inhibition from a Chinese medical herb (*Ramulus mori*) in normal and diabetic rats and mice. *Phytomed.* **9:** 161 - 166.

Yi - Ming Chiang Jen - Kuan Su Yi - Hung L I U and **Yueh - Hsiung Kuo** (2001). New Cyclopropyl - Triterpenoids from the Aerial Roots of *Ficus microcarpa. Chem*. *Pharm. Bull.* **49(5):** 581 - 583.

Yuan Y W Mabberly D J Steane D A and **Olmstead R G** (2010). Further disintegration and redefinition of *Clerodendrum* (*Lamiaceae*): Implications for the understanding of the evolution of an intriguing breeding strategy. *Taxon.***59:** 125 - 133.

Zafar R Panwar R and **Sagar Bhanu P S** (2005). Herbal drug standardization: *The Indian Pharmacist*. **4(36):** 21 - 25.

Zargari A (1989-1992). *Medicinal Plants*. Tehran University Publication, Tehran.

Zbarsky V Datla K P Parkar S Rai D K Aruoma O I and **Dexter D T** (2005). Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6 - OHDA model of Parkinson's disease. *Free Radic. Res.* **39:** 1119 - 1125.

Zee-Cheng R K (1997). Anticancer research on *Loranthaceae* plants. *Drugs Future.* **22:**515 - 530.

Zeeshan Hasan S Vedant Misra Swati Singh Garvita Arora Sunita Sharma and **Sarika Sharma** (2009). Current status of herbal drugs and their future perspectives. *Biological Forum- An International Journal.* **1(1):** 12 - 17.

Zegorka G and **Glowniak K** (2001).Variation of free phenolic acids in medicinal plants belonging to the *Lamiaceae* family. *J. Pharm. Biomed. Anal*.**26:** 179 - 187.

Zhao Z Shimomura H Sashida Y Tujino R Ohamoto T and **Kazami T** (1996). Identification of traditional Chinese patent medicine by polariscope (1): Polariscopic Characteristics of starch grains and calcium oxalate crystals. *Natural Medicine*.**50:** 389 - 398.

Zheng W and **Wang S Y** (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agr. Food Chem.***49:** 5165 - 5170.

Zhu Y P (2002). Toxicology of the Chinese herb mu tong (*Aristolochia manshuriensis*). What history tells us. Adverse Drug Reaction. *Toxicol. Rev* .**21:** 171 - 177.

APPENDIX

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-1: Jan-Mar -2011 IJABPT *ISSN 0976-4550*

WOUND HEALING ACTIVITIES OF *EUGENIA JAMBOLANA* LAM. BARK EXTRACTS IN ALBINO RATS

Palanimuthu.D[1] Nandagopal S.[2] Jalaludeen MD.[1] Subramonian K[*] and A. Saravana Ganthi[4] Sankar ram S.[5]

[1] Department of Biochemistry, Annamalai University, Chidambaram, Tamil Nadu, India
[2] Dept. of Environmental Sciences, SPKCES, Manonmaniam Sundaranar University, Tamil Nadu, India
[*] Department of Botany, the MDT Hindu College, Tirunelveli, Tamil Nadu, India
[4] Department of Botany, Rani Anna Govt. College for women, Tirunelveli, Tamil Nadu, India
[5] CARE unit, St. Xavier's college, Palayamkottai, Tamilnadu, India

ABSTRACT: Wound healing is physiological process, which takes place by body's natural regenerative capacity. Due to various reasons there may be delay in healing and this prolonged healing may sometimes lead to scar formation. Currently attention has been focused on natural products to prevent infection and to promote healing. In the present study, *Eugenia jambolana* bark extracts was taken to investigate its wound healing property. Full thickness deep burn wound model in Albino rats, were used to study the healing efficiency. Formulations (10% ointment) of crude ethanolic extract of the *Eugenia jambolana* bark was applied tropically over thermal wound. It was found that ointment treated rats showed accelerated healing than the control. It was observed that 10% extract of the *Eugenia jambolana* bark has progressive effects on wound healing in the experimental groups. This study suggests that *Eugenia jambolana* bark powder could be developed as a therapeutic agent for wound healing.
Keywords: *Eugenia jambolana*, wound healing activity

INTRODUCTION

Wound healing is a complication interaction of many factors. This phenomenon has mystified early and modern man. This is evident from Archeological findings which showed that ancient man also had to use a variety of tools to deal with various injuries inflicted on him under hard conditions and during wars (Manjo, 1991). The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases (Cragg *et al.,* 1997). *Eugenia jambolana* Lam. (Syn. *Syzygium cumini* (L) Skeels or *Syzynium jambolana* DC.) belonging to the family Myrtaceae is a large evergreen tree up to 30 m high. Bark pale brown, slightly rough on old stems. Fruit is one seeded berry and blue. It is widely distributed through out India, Ceylon-Malaya and Australia and known as Jamun, Jam, Jambul in India. It has been valued in Ayurveda and Unani systems of medicine for possessing variety of therapeutic properties. Most of the plant parts of *E. jambolana* are used in traditional systems of medicine in India. According to Ayurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers (Kirtikar and Basu, 1975). It is also acts as a gargle in sore throat; spongy gums etc. and when externally used, bark shows good wound healing properties (Priyavtra Sharma and Mehta, 1969**;** Nadkarni, 1954). This study was designed to explore the healing effects of topically applied ointment prepared from *Eugenia jambolana* leaves extracts in rat intraoral wound.

MATERIALS AND METHODS

Collection of plant materials

Barks of *Eugenia jambolana* Lam .were collected in Perambular district, Tamil Nadu during the month of January 2005. The collected plant were botanically identified and dried at room temperature.

Experimental Animals

The protocol of the study was approved by the Local Ethical Committee for animal experimentation. Healthy adult Wistar albino rats (150-180g) were obtained from Venkateshwara Enterprises, Bangalore and used in wound healing model experiments. The rats were divided into four groups of six animals each. The rats were used after an acclimatization period of 7 days to the laboratory environment. Animals were housed in metal cages and provided with standard food and tap water ad libitum during the whole period of the experiment. The changes were observed on 10^{th} and 16^{th} day.

Preparation of plant extract

The collected plant parts were pulverized by a mechanical grinder, sieved through 40 mesh. The powdered materials were extracted with ethanol using soxhlet extraction apparatus. This ethanolic extract was then concentrated and dried under reduced pressure. The ethanol free semi-solid mass thus obtained was used for the experiment.

Ointment Formulation

Two types of ointment formulation were prepared from the extract 5% (w/w), 10% (w/w), where 5 g and 10 g of the extract were incorporated in 100 g of simple ointment base B.P. respectively. Nitrofurazone ointment (0.2% w/w, Smithkline – Beecham) was used as a standard drug for comparing the wound healing potential of the extract of the bark.

Incision wound

Four groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm full thickness of skin from the depilated area; the wound was left undressed to the open environment. No medication was used throughout the experiment. After the incision was made, the ointment was applied to the wound once a daily in the experimental group animals. The progressive changes in wound area were measured in mm at every 3 days interval. Progressive decrease in the wound size was monitored periodically.

Qualitative Phytochemical Evaluation

The methanolic extract of the bark was subjected to qualitative tests for the identification of the phytoconstituents present in it *viz.* alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds and tannins, proteins and free amino acids, gums and mucilage, flavanoids, lignin and saponins.

RESULTS AND DISCUSSION

In the preliminary phytochemical evaluations of the ethnolic extract of *Eugenia jambolana* Lam. bark powder showed the presence of alkaloids, phytosterol, phenolic compounds and tannins, flavanoids and lignin.

The measurements of the progress of the wound healing induced by the NFZ ointment (0.2 w/w), 5% w/w extract ointment, 10% w/w extract ointment and the control group were reported in the Table: 1. It is observed that the wound contracting ability of the extract ointments were significantly greater than that of the control, which was comparable to that of the reference standard, NFZ ointment. A better healing pattern with complete wound closure was observed in rat at 10^{th} day and 16^{th} day by 5% w/w and 10% w/w extract ointments respectively.

The process of wound healing occurs in four phases: (i) Coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition, any agent which accelerates the above process is a promoter of wound healing. Plant produces have been showed to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing, due to the presence of active terpenes, alkaloids and flavanoids (Suguna *et al*., 1996). A glycosidal mixture extract of *Centella asistica* has been reported to be responsible for enhanced repair only in incised wounds (Rosen *et al*., 1967) and in stimulating collagen in human skin fibroblast cells (Vogel and De Souza, 1991).

Tannins and anthraquinones are the major phytoconstituent present in this plant which may be responsible for wound healing action. The plant *Portulaca oleracea* containing the tannins possesses wound healing activity as that of the *E. jambolana* (Rashed, 2003). The gel of ethanolic extract of the plant *Vernonia scorpioides* possess wound healing action by improving regeneration and organization of the new tissue due to the presence of tannins (Leite, 2002). The wound healing property of the extract of the *Eugenia jambolana* bark appears to be due to the presence of its active principle which accelerative the healing process and confers breaking strength to the healed wound.

Conclusion

Wounds one visible results of individual cell death or damage, and can be classified by site, size, depth and causation – surgery, accident or circulatory failure etc. The present study was undertaken to evaluate the wound healing activity of the extract of the *Eugenia jambolana* bark in excision. In excision wound model the extract ointment showed faster epithelialization, when compared with the control. Further investigations are necessary to determine the bioactive constituents present in the extracts used for studies.

Table: 1 The preliminary phytochemical screening of the methanolic extracts of *Eugenia jambolana* Lam.

Phytoconstutient	Result
Alkaloids	+
Reducing Sugar	-
Phytosterol	+
Fixed oil & Fats	-
Phenolic compounds & Tannins	+
Proteins & Amino Acids	-
Gums & Mucilage	-
Flavonoids	+
Lignin	+
Saponins	-

* + = Present, - = Absent

Table – 2 Effect of extract of *Eugenia jambolana* Lam. bark and Nitrofurazone on excision wound model

Post wounding days	*Wound Area (mm^2)*			
	Simple ointment (Control)	Nitrofurazone ointment (0.2% w/w)	Extract ointment (5% w/w each)	Extract combination (10% w/w each)
0	530±33.6(0)	516±36.8(0)	514±21.0(0)	507±39.8(0)
2	509±18.6(3.9)	458±36.8(11.2)	372±18.8(27)	338±14.8(33)
4	465±13.8(12.2)	318±12.6*(38.3)	312±19.9*(39)	268±18.6*(47)
6	424±30.1(20.0)	270±14.7*(47.6)	245±15.3*(52)	171±19.4**(66)
8	389±14.8(26.6)	193±11.4**(62.5)	162±12.5**(68)	105±9.8**(79)
10	345±23.6(34.9)	110±8.6**(77.3)	95±9.6**(81)	68±5.9**(86)
12	269±14.3(49.2)	79±6.3**(84.6)	66±7.4**(87)	36±2.1**(92)
14	215±11.3(59.4)	36±1.6**(93.0)	37±3.5**(92)	14±1.1**(97)
16	189±14.3(64.3)	10±1.9**(98.0)	19±0.8**(96)	0.0**(100)
18	171±15.1(67.7)	0.0**(100)	0.0**(100)	0.0**(100)

Values are mean ± S.E of 6 animals in each group. Figures in parenthesis indicate percentage of wound contraction.
*p<0.01., **p<0.001 vs. respective control by students t-test

PLATE 1

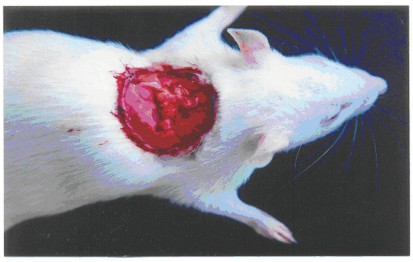

PLATE 2

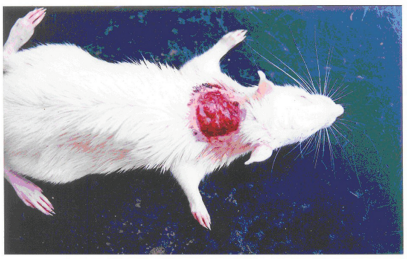

PLATE 3

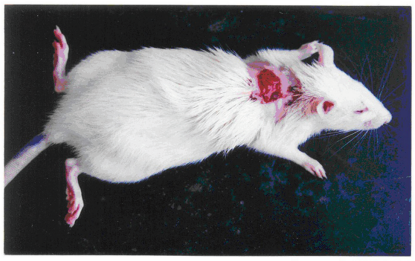

PLATE 4

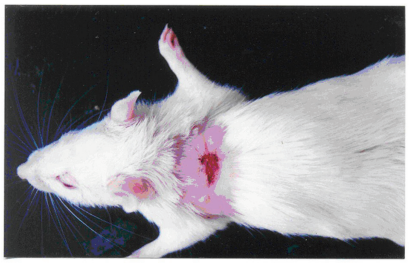

PLATE 5

PLATE 6

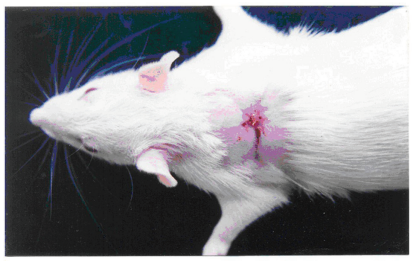

PLATE 1 : CONTROL WOUND WITH NITROFURAZONE OINMENT (initial day)

PLATE 2 : CONTROL WOUND WITH NITROFURAZONE OINMENT (day 10)

PLATE 3 : CONTROL WOUND WITH NITROFURAZONE OINMENT (day 16)

PLATE 4 : CONTROL WOUND WITH NITROFURAZONE OINMENT (day 26)

PLATE 5 : EXPERIMENTAL TREATED WITH EUGENIA JAMBOLANA BARK EXTRACT 10% W/W (day 10)

PLATE 6 : EXPERIMENTAL TREATED WITH EUGENIA JAMBOLANA BARK EXTRACT 10% W/W (day 16)

REFERENCES

1. Cragg GM, Newman DJ, et al. (1997) Natural products in drug discovery and development. J. Nat. Prod; 60: 52-60.
2. Kirtikar KR and Basu BD (1975). Eugenia Jambolana In: Indian Medicinal Plants. vol. II, Periodical Experts, New Delhi :1052-53.
3. Leite SN, Palhano G, et al. (2002) Wound healing activity and systemic effects of Vernonia scorpioides gel in Guinea pig. Fitoterapia 73: 496-500.
4. Manjo G (1991). The Healing Hand, Man and Wound in the Ancient World, Harward University Press, Cambridge, MA: 141-206.
5. Priyavtra Sharma and Mehta (1969) Eugenia Jambolana In Dravyaguna Vignyan. Part II & III, The Chowkhamba Vidyabhawan, Varansi: 586.
6. Rashed AN, et al. (2003) Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. growing in Jordan. J. Ethnopharmacology 88:131-136.
7. Rosen H et al. (1967) Effect of Asiaticoside on wound healing in rats. Exp. Med. Surg, 125: 279.
8. Suguna L and Sivakumar, P (1996) Effects of Centella asiatica extract on dermal wound healing in rats. Ind. J. Exp. Biol. 34: 1208.
9. Vogel HG and De Souza NJ (1991) Effect of terpenoids isolated from Centella asiatica, Acta theriologia, 16: 285.

****

Papers published in Journals (With ISSN)

- Paper entitled "Quantitative Biochemical analysis of ***Lentinus squrrosulus*** Mont" **Published** in "**Indian Journal Of Botanical Research**" (ISSN-0973-2233 Quarterly International Journal of Plant Science research)Volume:5, Number 1&2 March & June 2009.Page No.33-36.
- Paper entitled "Effect of different media on the mycelia growth of ***Lentinus squrrosulus*** Mont" **Published** in **"International Journal Of Plant Sciences"** (ISSN-0973-1547 Quarterly International Journal of Plant Science research) Volume:5 Issue1,January to June 2010.Page No.78-80.
- Paper entitled "Wound Healing Activities Of ***Eugenia jambolana*** .Lam Bark extracts in Albino Rats" **Published** in **International Journal Of Applied Biology and Pharmaceutical Technology** (ISSN 0976-4550)Vol.2. Issue 1 Jan-Mar.2011.
- Paper entitled "Antibacterial Effect of *Tephrosia spinosa* (L.F) Pers" **Published** in **RAC Journal Of research** (ISSN 2230-7362)Vol.2. No.5, August 2012. Page No.114-118.
- Paper entitled "Effect of Azotobacter on Seedling Growth of ***Sesbania grandiflora*** (L.)POIR For Energy Plantation" **Published** in **Sciencia Acta Xaveriana** (ISSN 0976-1152) Special Issue December2012. Page No.S216-S218.
- Paper entitled "Effect of ***Calotropis gigantea*** L. leaf Extracts On Chosen pathogenic Bacteria" **Published** in **RAC Journal of research** (ISSN :2230 – 7362) Vol. I No.6, February 2013.

Papers published in Books (With ISBN)

- Paper entitled "Herbs For Sports Injury" **Published** in "FACTS OF SPORTS MEDICINE" (ISBN-978-81-907252-3-1) Issue1, January 2010. Page No.156-160.

- Paper entitled “Rules of Disease Prevention” Published in “FACTS OF SPORTS MEDICINE” (ISBN-978-81-907252-3-1) Issue1, January 2010.Page No.160-163.
- Paper entitled “***Lentinus squarrosulus*** Mont.- A Biomass Converting Protein rich Mushroom” **Published** in the proceedings of 10th Seminar of Tamilnadu Science Council” Organised by Manonmaniam Sundaranar University, Tirunelveli-627 012.(ISBN 938062717-3), Page 165-166.
- Paper entitled “Sacred groves of Tirunelveli Corporation – ideal centre’s for biodiversity conservation” **Published** in the Proceedings Of State Level Seminar on Bio-Prospecting Of Natural Resources(ISBN 978-81-7966-299-1)) Issue 1 January 2011.
- Paper entitled “Pollination Efficiency of Apies dorsata in Brinjal, ***Solanum melongena*** L.” **Published** in the Proceedings Of State Level Seminar on Bio-Prospecting Of Natural Resources (ISBN-978-81-7966-299-1) Issue 1 January 2011.
- Paper entitled “Community Structure and Seasonal Dynamics of Pierid Butterflies of Anaikutty hills, Western Ghats” **Published** in the Proceedings Of State Level Seminar on Bio-Prospecting Of Natural Resources(ISBN 978-81-7966-299-1) Issue 1 January 2011.
- Paper entitled “Antioxidants For Sports and Fitness” **Published** in “ FACTS OF SPORTS SCIENCE” (ISBN-978-81-907252-4-8) Issue1,January 2011.Page No.315-319.
- Paper entitled ‘Traditional knowledge of the Wild Edible Plants Used by the Tribal People of Point Calimere, Nagapattinam District, Tamilnadu” **Published** in the Proceedings (ISBN: 978-81-7966-301-1.) of **International Conference** of Environmental Security For Food Health (ICESFH-2012). Page No.66-69.
- Paper entitled “Studies on the Chemical constituents of ***Cadaba indica*** Lam.(Capparaceae).” **Published** in the Proceedings of the National Seminar on Recent Trends in Bioactive Substances – 2013 (RTBASS-2013) (ISBN-978-93-90-5126-701-0) Sponsored by UGC Organized by PG Research department of Botany, Govt. Arts College, Karur. Page No.201-208.

Paper published Proceedings

- Paper entitled "Biochemical Properties Of Different Parts Of Basidiomata of ***Lentinus squrrosulus***" **published** in the Proceedings of National Conference on Bioprospecting of Bioresources held at St.Xavier's College, Palayamkottai sponsored by DBT & organized by PG &Research Department of Plant Biology & Plant Biotechnology, St.Xavier's College, Palayamkottai. Page No.60-64.
- Paper entitled " Role of Womens participation in Higher education" **published** in the Proceedings of NAAC Sponsored National conference on Higher education and students participation in Quality assurance" organized by Internal Quality Assurance Cell, The MDT Hindu College, Tirunelveli. 627010.Page No.141-142.
- Paper entitled "Biochemical Properties of ***Lentinus squarrosulus*** Mont-A Research" **Published** in the proceedings of Aiyuvu sinthanaigal organized by Ayinthamil ayuvaalar mantram Madurai.
- Paper entitled "Biochemical Analysis of Different parts of ***Lentinus squarrosulus*** Mont" Published in the proceedings of the VII All India Conference of Scott Research Forum. Volume- II .Page No. 209-211.

RAC Journal Research 2 (5) : 114 - 118, 2012

Antibacterial Effect of *Tephrosia spinosa* (L. F) Pers

Rajabudeen #E. Subramonian$ K. Saravana Ganthi A. and M. Padma Sorna Subramanian+*

Dept. of Botany, Dr. Zahir Husain College, Ilayankudi, Tamil Nadu
$Dept. of Botany, The MDT Hindu College, Tirunelveli, Tamil Nadu
*Dept. of Botany, Rani Anna Govt. College for Women, Tirunelveli, Tamil Nadu.
+ Siddha Medicinal Plants Garden, CCRS, Mettur Dam, Tamil Nadu
E-Mail: saran_gan@rediffmail.com

Abstract

The traditional medical systems of northern India such as Ayurveda and Siddha are part of a time-tested culture and honored by people still today. Many advantages of such eco-friendly traditions exist. The plants used for various therapies are readily available, are easy to transport, and have a relatively long shelf life. The aromatic and medicinal plants represent an enormous reservoir of potential microbicidal compounds that could be useful as an alternative to synthetic microbicides and are being used to develop drugs. In the present study the leaf, stem and root powder extracts of *Tephrosia spinosa* (L. f) Pers (*Fabaceae)* were tested against ten different randomly selected bacteria by disc diffusion method. It was found that the methanol extract of leaf, stem and root were strongly effective against all the chosen bacteria.

Key words: Antibacterial study, *Tephrosia spinosa*, inhibition zone

Introduction

Plants are an indispensable source of chemical compounds and plant physiologists in collaboration with chemists and biochemists have been able to isolate and characterize a myriad of chemical compounds from plants. Biological screening is necessary to provide a scientific basis for validating the traditional utilization of medicinal plants. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents, with general as well as specific antimicrobial activity. Keeping this in mind, the present study was carried out to evaluate antibacterial activity of *Tephrosia spinosa* (L. f) Pers. The *Fabaceae* family (= Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families (Polhill *et al.*, 1981; Judd *et al.*, 1999). Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of *Fabaceae* are unexplored which deserve attention and research. *Tephrosia spinosa* (L. f) Pers. is such plant which has not been explored extensively by the scientific world so far. The genus *Tephrosia* is a pantropical taxa with about four hundred species distributed throughout the world (Gillett, 1971). About twenty four species of *Tephrosia* were recorded in India (Gamble and Fischer, 1918; Saldanha and Singh, 1984). Most of the *Tephrosia* species are herbs to under shrubs and grow as weeds. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellant, larvicidal, piscicidal, antimicrobial and anticancer properties (Sarin Jagat 1976; Chen Yuh-Lin, 1978; Bentley *et al.*, 1987). *Tephrosia spinosa* (L. f) Pers. is commonly known as Mullu Kolingi in Tamil. Decoction of roots is given for rheumatism, indigestion, diarrhea and fevers (Yoganarasimhan, 2000; Useful Plants of

India, 2000). The whole plant is used to treat asthma, ulcer, diarrhea, swellings and leucorrhoea (Murugesa Medaliar, 1988). Bark decoction is used to cure enlargement of spleen (Sadasiva Pillai, 1978).

Materials and Methods

The experimental material selected for the present study is *Tephrosia spinosa* belonging to the family *Fabaceae*. The identity of the specimen was confirmed with Voucher specimen No. 4189 deposited at the Survey of Medicinal Plant Unit (SMP), Government Siddha Medical College, Palayamkottai. The Plant material was collected from Rediiyarpatti village, Tirunelveli District, Tamil Nadu.

Extracts were made from air dried samples. 60 g of the leaf, stem and root (powdered test materials) was extracted successively with of 400 ml of petroleum ether (60-80° C), benzene, chloroform and methanol. This sequence of solvents allows for leaching of all compounds based on their polarity. The individual fractions were collected and concentrated to obtain crude extracts. The above solvents were diluted and the final concentration used for bacterial bio-assay was 5-10 mg/ml of solvents. Filter paper discs of 6mm diameter of Whatman filter paper No.1 were soaked in solution for an hour and dried at room temperature.

The antibacterial activity was tested against ten randomly selected bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhii, Serratia marcescens, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus vulgaris* and *Bacillus subtilis.* The selected bacterial strains were obtained from Department of Microbiology, Sri Paramakalyani College, Alwarkuruchi.

Antibacterial assay was demonstrated by a modification of the method described by Bauer *et al.*, (1966). 0.5 ml of the dilute microbial culture was spread on sterile Muller Hinton Agar plates. The presoaked and dried discs were placed on the sterile plates and gently pressed down to assure contact.

Streptomycin 10 mg/ml was used as positive control and the respective solvents which were used to dissolve the crude extracts served as negative control. The plates were incubated at room temperature for 24 hrs. After the incubation period the inhibition zone around the discs were measured and recorded. Each test was carried out in triplicate.

Results and Discussion

The crude extracts of stem, leaf and root powders of *Tephrosia spinosa* exhibits *in-vitro* inhibition on the growth of test organisms. The results of the zones of inhibition (in mm) are summarized in Table: 1.

Stem: Stem extracts are effective against all the chosen bacterial strains. It is observed that the methanol extract is more active against selected bacteria by producing larger inhibition zones than other extracts. Benzene extracts show antibacterial activity against all the chosen bacterial cultures except *Klebsiella pnuemoniae* and *Bacillus subtilis*. Chloroform shows a minimum inhibitory zone to all the chosen microorganisms except *Klebsiella pnuemoniae* and *Enterobacter aerogenes.* The water and petroleum ether extract did not show any activity against chosen bacteria.

Leaf: In the antimicrobial test, benzene extract had least activity against *Enterobacter aerogenes* and *Serratia marcescens.* In methanol extract the

inhibition zones ranged from 6 - 9 mm. *Bacillus subtilis* is the most susceptible strains in methanol extract when tested by the disc diffusion method. Significant antibacterial effect is expressed in water extract against *Klebsiella pneumonia* (10 mm).

Root: Benzene extract shows inhibitory effect against five of the chosen strains. Chloroform extract did not show any zone against *Staphylococcus aureus* and *Streptococcus pyogenes*. Methanol extract showed inhibitory zones against all the chosen bacteria however, strong antibacterial activity was reported against *Klebsiella pneumoniae,* The water extract of root is effective against all chosen bacterial species except *Escherichia coli, Enterobacter aerogenes* and *Klebsiella pneumoniae*.

In this investigation methanol extract recorded significant antibacterial activities against all tested bacterial strains, while aqueous extract recorded medium activity and less significant results recorded in petroleum ether, benzene and chloroform extracts. It is also evident from the results that stem extracts have exhibited a high degree of activity. The results of present study reveal that the methanol extracts of *Tephrosia spinosa,* exhibited potential antibacterial activity against the tested pathogens, suggesting that methanol is the appropriate solvent for extraction of antibacterial principle (Nkere and Iroegbu, 2005). Thus methanol is recommended for the large-scale extraction of active principle (Taous *et al.,* 2005).

Saponin and tannins are reported to possess antibacterial activity (Newman *et al.,* 2000). The secondary metabolites like tannin, flavonoids and steroids showed antibacterial activities (Tona *et al.,* 1999; Rhoda, 2001). Preliminary phytochemical analysis of the methanol and water extracts of *Tephrosia spinosa* revealed the presence of saponin, tannin and flavonoid, which could be the active principle. The result of the present antibacterial study may serve as a guide in the selection of plant for further work on the isolation and elucidation of the active compounds.

Table: 1. Antibacterial activity of leaf, stem and root extracts of *Tephrosia spinosa*

S. No	Name of the bacteria	Part	Diameter of inhibition zone (mm)				
			Pet. ether	Benzene	Chloroform	Methanol	Water
1.	*Escherichia coli*	Leaf	-	-	-	8 ± 0. 64	-
		Stem	-	6 ± 0.36	6 ± 0.90	-	-
		Root	-	6± 0.08	6 ± 0.21	8 ± 0.85	-
2.	*Pseudomonas aeruginosa*	Leaf	-	-	7 ± 0.83	-	8 ± 0.56
		Stem	-	9 ± 0.55	6 ± 0.67	10 ± 0.21	-
		Root	-	8 ± 0.45	9 ± 0.67	9 ± 0.45	8 ± 0.48
3.	*Staphylococcus aureus*	Leaf	-	-	-	6 ± 0.48	8 ± 0.36
		Stem	-	-	7	7 ± 0.58	-
		Root	6 ± 0.97	-	-	10 ± 0.44	9 ± 0.29

4.	*Streptococcus pyogenes*	Leaf	-	-	-	8 ± 0.60	6 ± 0.48
		Stem	-	7 ± 0.78	8 ± 0.86	7 ± 0.69	-
		Root	-	-	-	7 ± 0. 38	6 ± 0.59
5.	*Salmonella typhii*	Leaf	-	-	-	-	-
		Stem	-	8 ± 0.98	10 ± 0.95	7 ± 0.73	-
		Root	-	-	9 ± 0.40	7 ± 038	-
6.	*Serratia marcescens*	Leaf	-	6 ± 0.04	8 ± 0.43	7 ± 0.42	-
		Stem	-	6 ± 0.00	9 ± 0.68	10 ± 0.47	-
		Root	-	-	9 ± 0.05	10 ± 0.30	7 ± 0. 27
7.	*Klebsiella pneumoniae*	Leaf	-	-	-	6 ± 0. 48	10 ± 0.53
		Stem	-	-	-	10 ± 0. 39	-
		Root	-	7 ± 0.52	8± 0.97	14 ± 0.56	-
8.	*Enterobacter aerogenes*	Leaf	7± 0.67	9 ± 0.98	7 ± 0.43	7 ± 0.49	-
		Stem	-	-	-	7 ± 0.50	-
		Root	6 ± 0.55	6 ± 0.23	7 ± 0. 39	7 ± 0.04	-
9.	*Proteus vulgaris*	Leaf	-	-	-	-	-
		Stem	-	7 ± 0.18	7 ± 0.90	10 ± 0. 93	-
		Root	-	-	9± 0.28	12 ± 0.46	10 ± 0.53
10.	*Bacillus subtilis*	Leaf	-	-	-	9 ± 0.27	7 ± 0. 40
		Stem	-	-	6 ± 0.00	7 ± 0.54	-
		Root	-	6 ± 0.37	8 ± 0.89	10 ± 0.55	8 ± 0.74

'-' : Absence of inhibition zone . Pet. ether - Petroleum ether (40 – 60^{0}c)

References

Bauer, A.W. Kirby, W.M.M. Sherries, S.C. and Tunk, M. (1966). Antibiotic susceptibility of testing by a standard single disc method. *Amer. J. Clinical Pathology* 36 pp 492 – 496.

Chen Yuh-Lin (1978). New piscicidal flavonoids from *Tephrosia obovata* Merr. *Asian. Journ. Pharm,* 3 (4): 18.

Murugasa Mudaliyar C S (1988). Gunapadam Mooligai Vaguppu IVth Ed. Sivakami vilas publications Chennai.

Gamble J S and Fischer C E C (1918). Flora of Presidency of Madras; Botanical Survey of India - Howrah – India.

Gillett J B (1971). Flora of Tropical East Africa Leguminosae Part 3 Sub family Papilionoideae (1) Crown Agents- London- U.K.

Judd W S Campbell C S Kellogg E A and P F Stevens (1999). Plant Systematics: a phylogenetic approach. Sinauer Associates, Sunderland p: 464.

Newman, D. J. Gragg, G. M. and Snader, K. M. (2000). The influence of natural products upon drug discovery *Nat. Prod. Res*. 17 pp 215 – 234. Nkere, C. K. and

Iroegbu C.U. (2005). Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida Afr. J. Biotechnol.* 4 pp 522 – 526.

Polhill R M Raven P H and C H Stirton (1981). Evolution and systematics of the Leguminosae, pp. 1-26. *In*: R. M. Polhill & P. H. Raven (eds.), *Advances in legume systematics* Part 1, Royal Botanic Gardens, Kew p 425.

Rhoda M Kariba (2001). Antibacterial activity of *Ajuge remota. Fitoterapia* 72 pp 177.

Sadasiva Pillai (1978). *Tephrosia spinosa* In: Tamil – English Dictionary vol. 5 G.D. Naidu Foundation Arakattalai p: 36 – 49.

Saldanha C J and B G Singh (1984). Leguminosae- In; Saldanha C.J. (Ed.) Flora of Karnataka vol. I p: 495-499.

Sarin Jagat P S Singh S Garg H Khanna N M and M Dhar (1976). A flavonol glycoside with anticancer activity from *Tephrosia candida. Phytochemistry* 15 (1): 232-234.

Yoganarasimhan (2000*). Tephrosia villosa* In: Medicinal plants of India. *Interline Publishing Private Ltd., Bangalore India*: p. 537.

Taous, K. Mansoor, A. Hamayun, K. and Mir, A. K. (2005).Biological activities of aerial parts of Paeonia emodiwall *Afr. J. Biotechnol.* 4 pp 1313 – 1316.

Tona Lutete, K. Kumbu, D. Ntondole and Manga, K. C. (1999). Antimicrobial activity of tannins. *Fitoterapia* 2 pp 279.