# MORPHOLOGICAL AND PHYTOCHEMICAI INVESTIGATIONS ON ST. JOHN'S WORT (*Hypericum* spp.), A POTENTIAL SOURCE OF ANTI-HIV COMPOUNDS


By

**V. GANAPATHI**

**THESIS**

*Submitted in partial fulfilment of the requirement for the degree of*

Master of Science in Horticulture

*Faculty of Agriculture*
*Kerala Agricultural University*

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE
KAU P. O., THRISSUR - 680 656
KERALA, INDIA

**2003**

## DECLARATION

I hereby declare that this thesis entitled **"Morphological and Phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

**V. Ganapathi**

## CERTIFICATE

Certified that this thesis entitled **"Morphological and Phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds"** is a record of research work done independently by **Mr. V. Ganapathi** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to him.

**Dr.N.Mini Raj**
(Chairman, AdvisoryCommittee)
Assistant Professor (SS)
Departmentof Plantation Crops and Spices
College of Horticulture
Vellanikkara

Vellanikkara

## CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mr.V.Ganapathi,** a candidate for the degree of Master of Science in Horticulture with major in Plantation Crops and Spices, agree that the thesis entitled **"Morphological and Phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds"** may be submitted by **Mr. V. Ganapathi,** in partial fulfilment of the requirement for the degree.

**Dr. N. Mini Raj**
(Chairman, Advisory Committee)
Assistant Professor (Senior Scale)
Dept. of Plantation Crops and Spices
College of Horticulture
Vellanikkara

**Dr. E.V. Nybe**
(Member, Advisory Committee)
Associate Professor and Head
Dept. of Plantation Crops and Spices
College of Horticulture
Vellanikkara

**Dr. K. Vasanthakumar**
(Member, Advisory Committee)
Associate Professor and Head
Cardamom Research Station
Pampadumpara

**Dr. A. Augustin**
Member, Advisory Committee)
Associate Professor
AICRP on M & AP
College of Horticulture
Vellanikkara

**External Examiner** 22/3/0

# ACKNOWLEDGEMENT

*It is with great pleasure I place here on record my heartfelt gratitude and sincere thanks to Dr. N. Mini Raj, Assistant Professor (Senior Scale), Department of Plantation Crops and Spices and Chairman of my advisory committee for her expert guidance, valuable suggestions, constant encouragement and above all, the extreme patience, understanding and wholehearted co-operation rendered throughout the course of my study. I consider myself very fortunate in having the privilege of being guided by her.*

*My heartfelt thanks are expressed to Dr. E. V. Nybe, Associate Professor and Head, Department of Plantation Crops and Spices and member of my advisory committee, for his valuable suggestions and relentless support throughout the endeavour.*

*No words can truly express my profound gratitude and indebtedness to Dr. A. Augustin, Associate Professor (Biochemistry), AICRP on Medicinal and Aromatic Plants and member of my advisory committee for his ardent interest, valuable suggestions and ever willing help which helped a lot for the improvement of this work.*

*It is my pleasant privilege to acknowledge Dr. K. Vasanthakumar, Associate Professor and Head, Cardamom Research Station, Pampadumpara and member of my advisory committee for his expert counsel, and constructive criticism.*

*I take this opportunity to express my gratitude to Dr. P. C. Rajendran and Dr. V. S. Sujatha for rendering all sorts of help and co-operation in availing the micro photographic facilities.*

*I express my heartful gratitude to Dr. Alice Kurian, Dr. M. Asha sankar, Dr. M. R. Shylaja, Mrs. Lissamma Joseph and Mrs. B. Suma, Scientists, Department of Plantation Crops and Spices, for their unbounded support offered at different stages of the study.*

*My profound sense of gratitude to Mr. P. Anoop, Assistant Professor, College of Forestry for rendering all sorts of help and co-operation in availing the facilities of wood microtome.*

*Words cannot express my gratitude to Dr. med. Christoph M. Schempp, Universty of Hautlinik, Freiburg who provided me the standard hypericin used in the study.*

*I am extremely thankful to Dr. E. Bombardelli, Italy, Dr. Grassim M. Kitanov, Medical university of Sofia, Bulgaria and Prof. Anna Maria Pagni, University of Pisa, Italy for their kindness to supply the relevant literature and for sharing their views about the study.*

*I also express my sincere thanks to my friend Joy Scaria, PhD Scholar, for his timely help in literature collection. I duly acknowledge the valuable help rendered by my friends Bala subramaniyan and Prasanna in literature collection.*

*My profound sense of gratitude to Dr. Sasidharan, KFRI and Mr. Ratheesh, MSSRF, Kalpetta in the species identification.*

*I find it difficult to translate into words the help rendered by Balakrishnan Master, and his family during the survey work in Vattavada.*

*I duly acknowledge the valuable help and service rendered by Mr. Abdul Razack, Librarian, College of Horticulture and his colleagues throughout my study.*

*My sincere thanks are also due to Mr. Roy, Lab assistant, Department of plant breeding and Genetics for his service in anatomical work.*

*My sincere gratitude to Mr. Murugan, Mr. Arularasan and Mr. Israel Thomas for their constant encouragement and motivation throughout the study period.*

*I wish to place on record the help rendered to me in pursuit of my study by my seniors Mr. karuppaiyan, Mr. Vallal kannan, Dr. Arunachalam, Mr. V. M. Chandrasekaran and Mr. Dinesh Babu. I sincerely acknowledge the help rendered by Mr. Mani, Technical officer, NBPGR Regional Station , Vellanikkara in plant collection and preservation. I owe thanks to my friends Shankar, Arul swaminathan, Muthukannan, Ramesh, Vezhavendan, Karthikeyan, Boopathi, Rajasekar, Sundararasu, Kamalakannan, Sambasivam, Venkatesh, Pradeep, Chandrahaasan, Jinnappa, Ranav, Gopinath, and Bhavani who all extended a helping hand at each and every juncture of my work.*

*Words cannot express my gratitude to my friends Nagarajan and Manikandan for their wholehearted support and help given in times of need not only for this work but also from my graduation onwards. I duly acknowledge the valuable help rendered by Sahalai Karthikeyan, Ravi Shankar, Kalimuthu, Suresh and Yusuf.*

*My sincere thanks are also due to my friends Manimala, Binu, Manjusha, Mini, Sujatha, Vanisree, Usha vani, Roshni and Vineetha and my juniors Hena, Smilu and Lekshmy for their constant encouragement during the study.*

*The award of junior fellowship of the KAU is thankfully acknowledged.*

*My sincere thanks to Mr. Manoj, Mr. Princeson and Mrs. Bindu, Research Assistants, Department of Plantation Crops and Spices for their affection and support.*

*My sincere thanks are also due to Mr. Sree Kumar of CPBMB, for his help rendered in photographic work. It was Mr. Joy who typed the manuscript neatly and promptly. I acknowledge him.*

*I duly acknowledge the valuable help and service rendered by Mr. Chandrasekharan, Biochemistry lab assistant, Mrs. Drowpathy and Mrs. Aiysha.*

*I am extremely thankful to Santhosh and Jeo, Students' computer club for their valuable help during the preparation of the thesis.*

*I am forever beholden to my parents and family members for their moral support and inspiring encouragement, which helped me to undertake the strenuous toil successfully.*

*GOD ALMIGHTY has bestowed me the opportunity, health and confidence to undertake the work successfully. I bow my head before HIM.*

*V. Ganapathi*

*Dedicated to my beloved Parents*

CONTENTS

# List of Tables

# LIST OF PLATES

# LIST OF FIGURES

# *Introduction*

# 1. INTRODUCTION

Plants have been a major source of therapeutic agents for alleviation or cure of human diseases since time immemorial. They are extensively utilized throughout the world in two distinct areas of health management viz., traditional and modern systems of medicine. In India, a number of traditional systems of medicine such as Ayurveda, Siddha, Unani, Homoeopathy and Naturapathy are practised for the total health care. These systems of medicine are mainly dependant upon plants (Kumar *et al.*, 1997). At present about 1000 single drugs and 8000 compound formulations of recognized merits are *in vogue* in Ayurveda. Similarly, other systems of medicine prevailing in India viz., Unani, Amchi and Siddha utilize as many as 700, 600 and 600 medicinal plants respectively (Singh *et al.*, 2000). Apart from these, over 75,000 species of plants are estimated to be used by 4635 ethnic communities for human and veterinary health care.

At present about 130 clinically useful prescription drugs of known chemical structure derived from about 100 species of higher plants are used in modern system of medicine. A recent study indicates that the herbal drug market continues to grow at the rate of 15 per cent annually. As per the estimates of World Health Organization, the current global market for medicinal herbs and herbal products is about 62 billion US dollars and will hit the market by 2005 at the level of five trillion US dollars. India is exporting herbal materials and medicines to the tune of Rs.550 crores annually.

In spite of tremendous advances made in the modern system of medicine, still there are a large number of diseases for which suitable synthetic drugs are not available, but effective plant based counterparts are available. Indian Council of Medical Research has identified 20 such diseases and six have been identified as thrust areas for in-depth research. These include tropical diseases like malaria, filaria,

viral infections like HIV and opportunistic infections caused by myobacteria, chronic diseases like arthritis, liver disorders etc. Hence there is need to prioritize plants needing investigation.

*Hypericum* species were known to ancient communities as useful medicinal plants. *Hypericum perforatum* (St. John's Wort) has been mentioned in Ayurveda and is known as '*Bassant*'. The primary ancient medical herbalists including Hippocrates, Pliny, Dioscorides, Theophrastus and Galen wrote about the wound healing property of *Hypericum* and it's usage for the treatment of neuralgic conditions such as sciatica and hip pain. The importance of *Hypericum perforatum* as a phytopharmaceutical especially for the treatment of mild depression, has significantly increased in the last few years. This is evidenced by the fact that the market for St. John's Wort has exceeded $ 210 million in the U.S. alone and $ 570 million worldwide (Grunwald, 1999). Hypericin, an anthraquinone derivative from *Hypericum perforatum*, is typically used as the measure of extract potency, due to its contribution for antidepressant activity. In the European countries *Hypericum* species are widely used in the folk medicine for a number of ailments. In Homoeopathy, hypericum tincture is a well-reputed medicine for the treatment of compound fractures, gun shot wounds, hypersensitivity, neuralgia, etc.

Human Immunodeficiency Virus (HIV) infection and Acquired Immuno Deficiency Syndrome (AIDS) have now become the number one killer in Africa and several Asiatic countries. Ever since the identification of HIV as the causative agent for AIDS, the search for safe and effective treatment of HIV infection has become a major focus of the scientific community all over the world. Several plant-based chemicals are undergoing rigorous screening tests in pharmaceutical laboratories throughout the world for anti-HIV activity.

Considering the rapid spread of HIV in a developing country like India and the high cost of modern imported allopathic drugs, it is the need of the country to have an affordable indigenous medicine. *Hypericum perforatum* is under extensive investigation as a potential anti-AIDS drug plant as hypericin and pseudohypericin exhibited inhibitory effects against a wide spectrum of viruses including the retrovirus HIV (Bombardelli and Morazzoni, 1995). There have been stray attempts on screening of *Hypericum* spp. for anti-HIV property. Many of our indigenous *Hypericum* spp. have not been explored fully for their pharmacological properties. In this context, the present study on "Morphological and Phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds" was taken up at the Department of Plantation Crops and Spices College of Horticulture, Vellanikkara during 2000-2002 with the following objectives:

1. To study the natural habitat of *Hypericum* spp. in the high ranges of Idukki District.
2. To record the morphological and anatomical variability in *Hypericum* spp.
3. To find out the various phytoconstituents of *Hypericum* and their dynamics inside the plant system.

# *Review of Literature*

# 2.REVIEW OF LITERATURE

The genus *Hypericum* has been known to the ancient communities as useful medicinal plants. The primary ancient medical herbalists wrote about the medicinal properties of St. John's Wort, noting its use in wound healing and for the treatment of neuralgic conditions such as sciatica and hip pain (Bombardelli and Morazzoni, 1995). *Hypericum perforatum* has been mentioned in *Ayurveda* and is known as *Bassant* (Satyavati *et al.*, 1987). Many workers have attempted taxonomical and phytochemical studies of the genus *Hypericum*. Literature pertaining to morphological, anatomical and biochemical characters and therapeutic uses of *Hypericum* are presented in this chapter.

## 2.1 TAXONOMIC HISTORY OF *Hypericum*

The name *Hypericum* is ancient and may have several derivations. *Yperikon* was first mentioned by Euryphon, a Greek doctor during 288 BC (Pickering, 1879). Fyson (1915) gave two suggestions regarding the name *Hypericum*. The first is HUPER means 'under' and 'EIKON' means 'image' because the stamens stand like a figure, in the centre of the flower. The second suggestion is that 'HUPER' means under and 'ERIKE' means heath.

*Hypericum* belongs to the family Guttiferae (Rendle, 1925) or Clusiaceae (Upton *et al.*, 1997). Some taxonomists classified the genus *Hypericum* under separate family, Hypericaceae (Fyson, 1915; Gamble, 1957; Lawrence, 1951). The genus encompasses approximately 400 species, of which ten morphologically and chemically distinct species grow in central Europe (Hoelzl, 1993).

Hypericaceae can be distinguished from its related families by the combination of pellucid or black dotted opposite leaves, the fasicled stamens, the 3-5 loculed

ovary and essentially distinct styles. Studies by Vestal (1937) have shown that members of family Hypericaceae are advanced over the Guttiferae and that within the family the tropical woody genera are the more primitive.

Hooker (1875) reported that *Hypericum* genus consists of herbs, shrubs or small trees. He classified the 19 species available in the then British India into four sections viz.,

(i) Androsaemineae,Spach: Sepals five, unequal petal deciduous. Stamens pentadelphous at the base. Ovary five celled (commonly shrubs with a few and large flowers). *Hypericum mysorense* belongs to this section.

(ii) Hyperineae, Spach: Sepals five, connate at the base, equal or unequal. Petals persistent. Stamens are triadelphous at the base. Ovary three celled.

(iii) Brathydineae, Spach: Sepals five. Petals persistent. Stamens are connate at the base. Ovary single celled.

(iv) Elodeineae, Spach: Stamens definite (9), cohesing for one third of their length into three bundles separated by (entire) hypogynous scales.

## 2.2 CHARACTERIZATION BASED ON MORPHOLOGICAL CHARACTERS

### 2.2.1 *Hypericum* (St. John's Wort)

Hooker (1875) described 19 species of *Hypericum* seen in British India.

*H. mysorense*, Heyne, Wight.

A glabrous shrub four-six feet high; young branches four angled. Leaves one-two inches, tapering to an amplexicaul base, with slender ascending veins and pellucid striae. Cymes terminal, three flowered, flowers two-two and half inch in diameter. Sepals 1.25cm long and acute. Petals obliquely oblanceolate, twice the length of the stamens. Styles twice as long as the ovary. Capsule about half inch length. Distributed throughout the hill from Konkan to Pulneys at altitudes 3000-4000 feet.

*H. japonicum* Thumb.

Annual, stems one to fifteen inches in height, tufted or prostrate, glabrous four angled. Leaves half inch or less, stem clasping, oval or ovate, pellucid –punctate. Flowers 0.25-inch diameter, yellow. Bracts and sepals linear –lanceolate, entire, acute, glandular and pellucid –punctate at the apex. Petals equaling the calyx. Styles one third the length of the ovary. Capsule 1/6 inch, seeds ribbed and transversely striate.

It is distributed in temperate and sub tropical Himalayas, from Sikkim to Garwhal at an altitude of 2000-5000 feet. It is also seen in Assam, Silhet, Burma, Eastern and Western Peninsula and Ceylon.

Fyson (1932) described the genus *Hypericum* and formulated the key for identifying five species occurring in South Indian hill stations.

*Hypericum* is perhaps the easiest of all genera of Hypericineae family to recognize, for the bright yellow flowers have numerous stiff straight stamens in bundles alternating with the petals, and the leaves contain oil glands which against the light glow as translucent dots or streaks.

Plants mostly small, either herbs or shrubs, never trees; leaves opposite, sessile, entire. Flowers typically in cymes of three (the middle one opening first and terminating the axis), but also solitary or panicled. sepals five, petals five, yellow, over-lapping each other in bud and twisted. Stamens numerous, united into one, three or five groups, alternating with glands. Ovary superior with a corresponding number of cells and of styles. Fruit is a capsule which splits open into its constituent cells, leaving the placentas attached to the central axis or the edges of the valves.

KEY TO THE SPECIES

Shrubs - Stamens in five groups; styles five ... **b**
Herb– Stamens in three groups; ovary three celled ... *H. humifusum*
Herbs- Ovary 1 celled . . . **c**

Branches stiff; leaves decussate: styles slender longer than the ovary ... *H. mysorense*
Branches drooping, the bush round-topped; leaves bifarious; styles stout, shorter than the ovary . . . *H. hookerianum*

Bracts and sepals fringed with long-stalked glands; seeds dotted but not ribbed .... *H. wightianum*
Bracts and all parts devoid of stalked glands, but fringed with small dots just inside the margin; seeds ribbed . . . *H. japonicum.*

*Hypericum mysorense* Heyne(common St. John's Wort).

A shrub three to eight feet high; twigs four angled, green or reddish. Leaves stiffly decussate, sessile, narrow elliptic lanceolate with strong midrib. Flowers at the ends of the braches solitary or in threes, of rich yellow colour, three to four inches across, mostly facing upwards. Sepals lanceolate ½ inch by 1/8 inch. Petals obovate-oblong, limp and flat when fully out and therefore widely separated, reddish on the back of the parts exposed in bud. Stamens slender and numerous, in five bundles: anthers globular. Ovary five celled, with five styles longer than ovary. Fruit is a rich crimson colour, egg shaped and pointed capsule, surrounded by the five styles.

It is the commonest species abundant everywhere in Nilgiri slopes.

*H. japonicum* Thumb. (Marsh St. John's Wort)

A very delicate herb, growing in marshy and damp places, in tufts a few inches high; stems straight, upto eight inches; branched or not, four angled. Leaves half inch or less, oval or ovate quite entire, clasping the stem at the base, very slender; glands in the leaf as round pellucid dots. Flowers at the ends of the main stem and its branches, in comparatively large loose cymes; bracts and sepals quite entire, without any stalked glands. Flowers ¼ inch diameter pale yellow. Stamens, all free or all equally united, not in bundles. Ovary one-celled, with three parietal placentas and three very short (1/20 inch) styles. Capsule 1/6 inch, red. Seeds flat short oblong, with about seven longitudinal ribs and numerous transverse striations.

Very common in moist places or shallow standing water. Generally distributed in Anamalayis, Ganjam and Poonachi hills. Widely distributed in eastern temperate climates, Himalayas, Khasi hills, Assam and Burma.

Gamble (1957) also characterized the five *Hypericum* species occurring in the Madras Presidency. Nair and Henry (1983) reported the distribution of seven *Hypericum* species in Tamil Nadu. Among them, *H. androsaemum* and *H. patulum* were not reported by Fyson (1932) and Gamble (1957). Mitich (1994) has given the nomenclature, appearance, habitat and distribution of *H. perforatum* grown in the wastelands of United States. More recently, Sivarajan and Mathew (1997) described the *H. mysorense* found in Nilambur, Kerala, India.

*Hypericum mysorense*

Shrubs; branchlets quadrangular, leaves opposite-decussate, amplexicaule, ovate-acute; flowers in terminal cymes; pedicel 0.7 cm long; calyx persistent, five lobed, lobes ovate-lanceolate, 0.8 cm long, reflexed; petals five, bright yellow, 2.5 cm long, obovate-obtuse; stamens numerous, filaments connate at the base; styles five; capsule conical, 1.5 cm long; five valved seed numerous.

Barker and Cheek (1994) described *Hypericum buckleyi*, an endemic species. According to Robson (1995), *H. uralum* is grouped under the section Ascyreia. It belongs to a complex of species which is very difficult to classify satisfactorily due to variability of its members and which includes *H. patulum* and the three subspecies of *H. henryi*. Martonfi *et al.* (1996) explained the morphological and nomenclatural features of the natural pentaploid hybrid *H. maculatum* subsp. *maculatum* x *H. perforatum*. Robson (1998) described the *H. subsessile* belonging to Ascyreia, the basic Asian section of *Hypericum*. Bhellum and Mangotra (1998) reported four species of *Hypericum* (*H. dyeri, H. napaulense, H. oblongifolium* and *H. perforatum*) from Jammu and Kashmir. They also described the ecological notes, details of flowering and key to the identification of species. Singh (1999) described the *H. perforatum* and he also presented distinguishing morphological characters of *H. dyeri, H. oblongifolium* and *H. elodeoides*.

Kumar *et al.* (2000) gave the botanical description of *Hypericum perforatum*, having well known antidepressant activity.

*H. perforatum*, is glabrous throughout, green or some times glaucescent; the stems are erect, branched at the top and 30 to 100 cm long, the leaves are oval or elliptic, oblong to linear, sub obtuse, revolute-marginated with numerous pellucid black glandular dots. The flowers are numerous, forming a broadly paniculate, almost corymbose inflorescence, 7-11 cm long and 5-11 cm broad. The bracts are lanceolate, 0.5 cm long and acute. The calyx is deeply parted, five mm long and about two-three times shorter than corolla; sepals are lanceolate 4-5 mm long, one mm broad, as long as ovary, acute or acuminate, with black glandular dots. The petals are oblong to oblong elliptic, in equilateral, 1.2 to 1.5 cm long, 0.5 to 0.6 cm broad, with numerous black glandular dots and lines on margin of upper part, surface with numerous yellow glandular dots. The stamens are numerous, in three bundles; the ovaries ovoid, three-five mm long; there are three styles. The seed is one mm long, cylindrical, brown, minutely pitted longitudinally.

Baroni *et al.* (2000) explained the secretary structures in *Hypericum richeri.* Secretary glands are as black dots in emerging leaves and nodular structure composed by a cluster of cells.

### 2.2.2 Morphological variation within species

Campbell *et al.* (1992) observed variation in the leaf size of *Hypericum perforatum.* The species had a range of leaf size from broad to narrow. Broad-leaved plants were shorter, early flowering, had larger capsules, thicker stems than narrow leaved plants. Wide variations for full flowering, height and shoots per plant were observed by Oravec *et al.* (1996) from the native population of *H. perforatum* growing in Italy. Pluhar *et al.* (2000) evaluated morphological variability among and within populations of *H. perforatum* of different origins. Morphological diversity among population was measurable in differences of flower length, plant height, different leaf types and plant habit. The greatest morphological heterogeneity was found in accessions of wild origin.

Osinska *et al.* (2000) evaluated the morphological, developmental and chemical variability of 11 species of *Hypericum* using *H. perforatum* cultivar Topaz as standard. Distinct differences were noted between species in plant habit, mass of raw material and hypericin content. It was suggested that *H. elegans* and *H. maculatum* could be considered as medicinal raw material.

Differences in the morphological characters viz., light and dark leaf gland density, leaf area, leaf length/width ratio and stem height were observed by Walker *et al.* (2001) in the *H. perforatum* collections of north western United States.

### 2.2.3 Other crops

Vadukkoot (1996) evaluated morpho-anatomical variations in *Ocimum* spp. by selecting four species viz., *Ocimum tenuiflorum, O. gratissimum, O. basilicum* and *O. canum*. She compared various morphological observations of leaf, stem, inflorescence, floral and fruit characters between the species.

Cytomorphological investigations in *Piper* spp. were done by Anand (1997). She prepared brief descriptions of eight *Piper* spp. based on salient observations on morphology. Various morphological observations according to the descriptor included leaf colour, leaf shape, leaf tip, stem characters like colour, branching pattern etc. Based on the morphological comparisons, a key for identification of eight species studied has been proposed. Muthulakshmi (1998) conducted surveys to study the variability in kodampuli (*Garcinia cambogia* Desr.). She analysed the variability in vegetative, floral and fruit characters. Wide variations were observed in the characters like shape of the tree and branching pattern, leaf characters, floral characters and fruit characters.

Manjusha (2001) evaluated morphological and histochemical variations in 52 accessions of *Adhatoda beddomei, A. zeylanica* and intermediate type of the two species. Based on the vegetative characters viz., plant height, number of branches, leaf area and petiole length, the accessions were grouped into three clusters. Accessions in cluster II showed the characters of *A. zeylanica*, cluster III resembled *A. beddomei* and cluster I showed characters intermediate to both the species.

## 2.3 THERAPEUTIC USES OF *Hypericum* spp.

*Hypericum* species have been used as medicinal plants since ancient times. *H. perforatum* was considered as diuretic, emmenagogue and as antimalarial, it was

particularly recommended for the treatment of burns and scalds. The plant was widely used in the folk medicine in European countries as a soothing agent, antimicrobial, antiphlogistic, in the treatment of inflammation of bronchi and urinogenital tract, hemorrhoid treatment, a healing agent and in the treatment of traumas, various kinds of ulcers and cancers and other general illnesses (Barbagallo and Chisari, 1987; Brantner *et al.*, 1994; Bombardelli and Morazzoni, 1995).

### 2.3.1 Antiviral and anti-retroviral action of *Hypericum* spp.

Tang *et al.* (1990) proved the anti-retroviral activity of hypericin, isolated from St. John's Wort against Friend Leukemia Virus (FLV) and radiation leukemia virus in mice. Wood *et al.* (1990) reported that the hypericin and pseudohypercin from *Hypericum perforatum*, inhibit a variety of encapsulated viruses including herpes simplex virus type 1 and type 2. Hudson *et al.* (1991) reported that the plant pigment hypericin is having antiviral activity against murine cytomegalovirus (MCMV), Sindbis Virus and Human Immunodeficiency Virus type 1 (HIV-1). It is also active against vesicular stomatis virus, herpes simplex virus types 1 and 2, Parainfluenza virus and vaccinia virus in ***in vitro*** condition (Andersen *et al.*, 1991).

Naphthodianthrones, hypericin and pseudohypercin extracted from *Hypericum* species are selected as anti-HIV agents by employing antiviral screening methods (Vlietinck *et al.*, 1991). Moraleda *et al.* (1993) found that hypericin from *Hypericum* species is effective against a number of hepatitis B virus family, duck hepatitis B virus in the ***in vitro*** conditions.

In an open pilot study, 18 patients with Acquired Immunodeficiency Syndrome (AIDS) were treated with an intravenous *H. perforatum* preparation (Hyperforat, 2x2 ml weekly) plus additional *Hypericum* tablets of undefined dosage. Sixteen out of 18 patients with good study compliance showed increasing counts of absolute $CD_4$

values over a 40-month period. Also observed were improvements in $CD_4/CD_8$ ratios in the majority of the patients. In addition, only two of the 16 patients experienced an opportunistic infection during the 40-month observation period. The other 14 of the patients remained clinically stable (Steinbeck and Wernet, 1993).

Weber *et al.* (1994) found that hypericin was most effective against herpes simplex virus-1 (HSV-1) as a virucidal agent. Hypericin is currently in early clinical trial in the USA as an antiviral agent (Bombardelli and Morazzoni, 1995). The extract from *Hypericum cordifolium, H. uralum* are showing activity against herpes simplex virus, Sindbis virus and poliovirus *in vitro* (Taylor, *et al.*, 1996). Vlietinck *et al.* (1998) reported that the naphthadianthrones, hypericin and pseudohypericin inhibit the life cycle of Human Immunodeficiency Virus (HIV). Jing *et al.* (1998) found that hypericin from *Hypericum perforatum* and *H. triquetrifolium* are showing activity against several retroviruses, including Human Immunodeficiency Virus (HIV). Axarlis *et al.* (1998) found that the *H. perforatum* extract showed potent antiviral activity against Human Cyto Megalo Virus (HCMV). Schmitt *et al.* (2001) observed that the extracts from *H. connatum, H. caprifolium, H. polyanthemum* are active against Feline Immunodeficiency Virus (FIV).

### 2.3.2 Therapeutic use of *H. mysorense*

Mukherjee and Suresh (2000) evaluated the wound healing potential of *Hypericum mysorense*. They used the dried leaf and stem extracts of *H. mysorense* in the form of an ointment. Ointments from both extracts showed significant effect on wound contraction, closure time, tensile strength and regeneration of tissues at the wound site. All these effects were comparable to those of a standard drug, nitrofurazone ointment.

## 2.4 ANATOMY AND HISTOCHEMISTRY

Leaf, stem morphology and histology of *Hypericum ascyron, H. sampsoni, H. erectum, H. perforatum* var. *angustifolium* and *Triadenum japonicum* were studied by Kuginuki and Namba (1993). The market samples of the crude drug "otogiriso" were compared with the above four species. But none of the four species examined was present in any sample.

Fornasiero *et al.* (1998) investigated the anatomical, ultrastructural and cytochemical characteristics of *Hypericum perforatum* leaf nodules to determine the localization of active compounds. The secretary structures for bioactive compounds in *H. perforatum* are distributed throughout the leaves and consist of translucent, schizogenous oil cavities and black nodules. Nodules are differentiated during early leaf growth and are initially formed at the edges and tips of young emerging leaves. The pigments accumulating in the enlarging secretary cells and leading to the formation of the black globules are probably the anthraquinone hypericin and its derivatives. Ultra structural observations revealed a degenerative process in the nodule cells, which were filled with a granular, compact substance, suggesting that these initial secretary cells can be considered dead and are denied to become reservoirs of secreted products.

Wenzhe and Zhenghai (1999) observed three different kinds of internal secretary structures in *Hypericum perforatum*. These are secretary cell globules (black dots), secretary cavities (translucent dots) and secretary ducts (translucent streaks). The secretary cell globule, which occurred in flowers, leaves and stems, consisted of a core of large secretary cells surrounded by two layers of flattened sheath cells. The secretary cavities were present throughout the lamina and the secretary ducts throughout the flower, both comprising one or two layers of flattened cells surrounding an oil chamber or duct. Histochemical and fluorescence microscopy

revealed that hypericin accumulated in the secretary cell globules. Microscopic and ultra structural observations further demonstrated that hypericin accumulated in the large central vacuole of mature secretary cells.

Fornasiero *et al.* (2000) observed only one type of secretary gland in *Hypericum richeri* consisting of black dots, which are present even in early emerging leaves. In the fully expanded leaves, the nodular structure appears to be composed by a cluster of cells. These become unfunctional and disassembled towards the end of their development and are used only as reservoirs of secretion products.

Ciccarelli *et al.* (2001a) characterized the translucent glands and secretary canals in *Hypericum perforatum*. Translucent glands are spherical glands consisting of a sub epidermal cavity delimited by two layers of cells. Secretary canals are categorized as types A, B and C, all contained active compounds and lipids. The translucent glands or glandular pockets were present within the lamina of the leaf, close to the lower surface. Type A canals were present in all floral (with the exception of stamens) and vegetative parts of the plant. Type B canals were numerous and alternated with the veins in the sepals and petals. Type C canals were located in the ovary and style.

Ciccarelli *et al.* (2001b) characterized the black nodules of *H. perforatum*. Black nodules present on both floral and vegetative parts consist of a cluster of irregularly shaped cells surrounded by a single or double layered sheath. Histochemical tests showed that the nodules are negative for the presence of lipids, essential oil, sesquiterpene lactones, steroids and proteins and positive for the pectin - like substances, tannins and alkaloids. The inflorescence is the richest in nodules and is therefore, the best site for the extraction of secondary metabolites.

Elisabetta *et al.* (2002) observed the development of secretary nodules in the leaves of *Hypericum perforatum*. Generally, young nodules show meristematic features. During development, cellular components degenerate and secrete materials accumulated first in vacuoles and then in a periplasmic space and cell walls. Finally, the cells were filled with black material. The outer flat cells surrounding the nodules appeared morphologically different from the inner cells and showed unusual vesicles in the periplasmic spaces.

## 2.5 PHYTOCHEMISTRY OF *Hypericum* spp.

Extensive phytochemical investigations in the plant have made possible identification of several naphthodianthrones (hypericin, pseudohypericin, protohyperincin, protopseudohypericin and cyclohypericin), phloroglucinols (hyperforin, adhyperforin and hyperoside), flavonoids, tannins, xanthones, rutin and essential oil which are found to have several pharmacological activities in man.

### 2.5.1 Naphthodianthrone

Southwell and Campbell (1991) extracted *Hypericum perforatum* using soxhlet apparatus and subsequently determined the hypericin in different varieties by spectrometric methods. The leaves of the broadleaved varieties contained from 370 to 580 ppm compared with 1040 to 1630 ppm for the narrow leaved varieties. Within an individual broad leaved specimen, main stem contained 40, side stem 120, bottom leaf 290, top leaf 380, capsules 730 and flowers 2150 ppm of hypericin.

Kartnig and Gobel (1992) found out the solvent system and dipping reagents in thin-layer chromatography for the quantification of hypericin and pseudohypericin. Using thin-layer chromatography method, hypericin can be quantified within the range 5-50 ng and pseudohypericin within the range 20-200 ng.

Giuseppe *et al.* (1996) accurately determined the hypericin down to 0.7 $mgkg^{-1}$ in high performance liquid chromatography (HPLC). They used RP C-18 column thermostated at 50°C. The determination of the naphthodianthrone constituents in extracts of dried blossoms of *Hypericum perforatum* by combined HPLC electrospray, mass spectroscopy was described by Georgios *et al.* (1997).

Stochmal and Gruszczyk (1998) developed the solid phase extraction procedure for *Hypericum perforatum*. They found that the total hypericin concentration ranged from 1.16-9.34 mg $g^{-1}$of dry weight and the ratio of hypericin and pseudohypericin from 1.36 to 3.46 by analyzing 20 samples. HPLC and TLC with fluorescence detection of hypericin and pseudohypericin in the hydroalcoholic extracts of drug *Hyperici Herba* and *Hypericum* callus were done by Mulinacci *et al.* (1999) and Rani *et al.* (2001). The online separation and structure elucidation of naphthodianthrones and flavonoids and other constituents of an extract from *H. perforatum* using HPLC coupled online with ultraviolet-visible, nuclear magnetic resonnance (NMR) and mass spectrometry were done by Hansen *et al.*(1999). Balogh and Li (1999) described the HPLC method with photodiode-array and mass spectrometry (MS) detection for hypericin in commercial extract of the *Hypericum perforatum*. The chromatograms showed the peak for hypericin at 588 nm.

The hypericin and tannin contents were estimated in *Hypericum perforatum* and *H. maculatum* by Kireeva *et al.* (1999) during the different growth stages of the plant. Hypericin content was the highest in both the species during the massive flower bud formation stage (1.20% in *H. perforatum* and 1.06% in *H. maculatum*). Lozykowska *et al.* (1999) investigated the hypericin content in *Hypericum perforatm* cultivar Topaz at the yellow bud, flowering and fruiting stages. Highest hypericin was found at the flowering stage. Andrej *et al.* (1999) analysed six *Hypericum* species (*H. perforatum, H. hirsutum, H. maculatum, H. tetrapterum, H. montanum* and *H.*

*humifusum*) for the content of ten substances (rutin, hyperoside, isoquercetin, quercetin, quercitrin, biapigenin, amentoflavone, pseudohypericin, hypericin and hyperforin). The highest content of many of these substances were found in the flowers of *H. perforatum.*

Tekelova *et al.* (2000) employed HPLC method for the analysis of the content of secondary metabolites in different flower ontogenesis phases. The content of dianthrones, hypericin and pseudohypericin, quercetin and their derivatives (hyperoside, quercitrin, biapigenin) and hyperforin increased from the first bud phases (0.29%, 0.80% and 0.47% respectively) to flowers just opened (1.04%, 4.23% and 6.60% respectively). The content of dianthrones and quercetin glycosides then decreased (in unripe fruits 0.11% and 0.08% respectively), whereas the amounts of hyperforin increased to 8.07 per cent in fruits.

Gray *et al.* (2000) found hypericin and hyperforin by liquid chromatography. Reyes and Koda (2001) and Ari *et al.* (2002) also quantified hypericin and hyperforin through HPLC. Zotou and Loukou (2001) determined the hypericin and pseudohypericin by isocratic reversed phase liquid chromatography and HPLC.

Poutaraud *et al.* (2001) reported that the flowering top of *Hypericum perforatum* contains a share of approximately 30 per cent hypercins in the form of protopseudohypericin and protohypericin buds (48%), flowers (30%), leaves (17%). Kitanov (2001) found hypericin and pseudohypericin in 27 of the 36 evaluated species of *Hypericum,* belonging to 17 sections of the genus. He determined the amount of hypericin and pseudohypericin by TLC and spectrophotometric methods. Sirvent *et al.* (2002) analysed *Hypericum perforatum* plant samples in HPLC. They found that total individual plant concentrations were 0.003-0.125 per cent dry weight hypericin and 0.0019 0.8458 per cent dry weight pseudohypericin. Jurgenliemk and

Nahrstedt (2002) detected 22 phenolic compounds including hypericin and pseudohypericin by HPLC in the *H. perforatum* extract.

### 2.5.2 Hyperforin (Phloroglucinols)

Two closely related phloroglucinol derivatives, hyperforin and adhyperforin were reported from the *Hypericum perforatum* by Bystrov *et al.* (1975). Maisenbacher and Kovar (1992a) reported that the amount of hyperforin was two per cent in flowers and 4.5 per cent in the unripe fruits, while the amount of adhyperforin was 0.2 per cent in flowers and 1.6 percentage in unripe fruits. Maisenbacher and Kovar (1992 b) identified and quantitatively determined the hyperforin by TLC and HPLC after solid phase extraction.

Rocha *et al.*, (1996) isolated three new phloroglucinols (hyperbrasilols B and C and isssotypexbrasil) from a petrol extract of the leaves andd flowers of *Hypericum brasiliense.* They also found that all these 3 phloroglucinols were active against *Bacillus subtilis.* Orth *et al.*, (1999) determined the identity and purity of hyperforin by HPTLC, HPLC with diode array and UV detection, Fourier-transformed infra red and NMR Spectroscopy and Liquid chromatography coupled with positive-ion electro spray-ionization tandem mass spectrometry. Verotta *et al.* (1999) isolated furohyperforin, an oxygen analogue of the prenylated phloroglucinol hyperforin from the aerial parts of *Hypericum perforatum.* Furohyperforin showed about a tenth of the activity of hyperforin while screening for antidepressant activity.

Verotta *et al.* (2000) isolated three oxygenated analogues of the prenylated phloroglucinol hyperforin from the aerial parts of *Hypericum perforatum.* Winkelmann *et al.* (2000) isolated five new tricyclic phloroglucinol derivatives, named ialibinones A-E from the petroleum ether extract of aerial parts of *Hypericum papuanum.* Ialibinones A-D showed activity against *Bacillus cereus, Staphylococcus*

*epidemis* and *Micrococcus luteus.* Bilia *et al.* (2001) demonstrated the efficiency of two-dimensional homonuclear and 1H-1H correlated spectroscopy and two-dimensional reverse heteronuclear shift correlation spectroscopy in the evaluation of flavonols, phloroglucinols and naphthodianthrones with particular regard to the very unstable phloroglucinols, present in the extract of St. John's Wort.

### 2.5.3 Flavanoids

Flavanoids present in the hypericum include, flavanol glycosides Viz., rutin, quercitrin, isoquercitrin, hyperin/hyperoside and aglycones, viz., Kaempferol, luteolin, myricetin and quercetin.

Dogance and Okseuz (1989) studied the constituents of *Hypericum adenotrichum.* They reported that the ethanolic extract of the plant (400 g) had yielded quercetin (153 g) and quercetin-3-D-galactoside (350 mg). Saker *et al.* (1991) reported eight phenolic compounds from the aerial parts of *Hypericum montbretii,*viz., quercetin, myricetin, (+) - catechin, quercitrin, isiquercitrin, hyperoside, biapigenin and chlorogenic acid. Qingli *et al.* (1998) isolated 2 new flavonoids, 7,8-(2,2"-dimethylpyrano)-5, 3`, 4`-trihydroxy-3- mehoxyflavone and (2R, 3R) dihydroquercetin -3,7-0 alpha-L-dirhamnoside, together with a known flavanoids from the aerial parts of *Hypericum japonicum.*

Andrade *et al.* (1998) developed simple accurate reversed-phase HPLC-Diode array detector (DAD) procedure for the determination of 19 phenolic compounds (flavonoids, phenolic acids and coumarins) in seven medicinal plants including *Hypericum androsaemum.* Dias *et al.* (1999) developed a new HPLC-DAD method for the separation and identification of the main phenolics from *H. perforatum* and *H. androsaemum.* They found that flavonoids, viz., those related to quercetin were the major metabolites in methanolic extract of the plants. Pluhar *et al.* (2000) compared

chemical constituents in the *Hyperieum perforatum* accessions, they found that the flavonoid content including hyperacid, rutin and quercitrin reached 17-39 mg $g^{-1}$ in the first and 15-20 mg $g^{-1}$ in the second year.

Kumar, *et al.* (2000) reviewed the chemistry and biological properties of *Hypericum perforatum.* They reported that the flavonoid present in the *H. perfortum* include flavonol glycosides, viz., rutin (0.3%), quercitrin (0.3-0.524%), isoquercitrin (0.3%), hyperin or hyperoside (9.7-1.1%) and aglycones viz., kaempferol, luteolin, myricetin and quercetin (2%). Dogrukol *et al.* (2001) determined the rutin in the ethanolic extract of *H. perforatum* by capillary zone electrophoresis method. The amount of rutin was found as 0.21 per cent.

### 2.5.4 Other compounds

Kumar *et al.* (2000) reviewed the chemistry of *H. perforatum*. They reported the presence of various group of chemicals such as flavones, pranthrocyanidins, phenyl propanes, xanthones, carotenoids, fatty acids, amino acids and vitamin C.

### 2.5.5 Essential oil

Maisenbacher and Kovar (1992 b) analysed the 'Hyperici oleum' (St. John's Wort oil) in TLC and HPLC and identified the hyperforin, xanthones and flavonoids. The action of light during preparation of the oil lead to rise in the content of flavonoids. Weyerstahl *et al.* (1995) reported that the essential oil of Indian *Hypericum perforatum* leaves had consisted of 74 . per cent monoterpere hydrocarbons, seven per cent oxygenated monoterperess, 10 per cent sesquinterpene hydrocarbons and 1.5 per cent oxygenated sesquioterpene. Cakir *et al.* (1997) analysed the volatile oils of *H. perforatum* and *H. scabrum* by gas chromatography. The oil of *H. scabrum* contained alpha-pinene (71.6%), beta-caryophyllene (4.8%),

myrcene (3.8%), cadalene (3.4%) and beta-pinene (2.9%), the oil of *H.perforatum* contained alpha-pinene (61.7%), 3-carene (7.5%), beta-caryophyllene (5.5%), myrcene (3.6%), cadalene (3.2%) and other components. Twenty-nine and 27 terpenoid compounds were identified in the volatile oils of *H.scabrum* and *H.perforatum* respectively.

Bertoli *et al.* (2000) studied the fruit and leaf oils of *Hypericum hircinum* by gas chromatography. The major components identified in these oil were nonane (35.5%, 19.3%), alpha-gurjunene (10.7%, 0.3%), limonene (12.7%, 2.5%) and caryophyllene oxide (15.3%, 0.3%). Sajjadi *et al.* (2001) identified 23 components in the oil of *Hyperium degonbadanicum* leaves and flowers. The major constituents were alpha- pinene (34.7%), beta-pinene (32.1%), limonene (12.1%) and camphene (6.6%). Couladis *et al.* (2001) identified fifty constituents in the essential oil of the aerial parts of *H. perforatum,* collected from two different locations in Greece. The monoterpene hydrocarbons had the highest contribution. The major constituents were alpha-pinene, n-nonane, and delta-cadinene. Gudzic *et al.* (2001) compared the essential oil of *H. perforatum* collected from same location in Yugoslavia. Eighteen components were common for both species. The main components of *H. olympicum* oil were anethole (30.7%) and beta-farnesene (12.4%) and for *H. perforatum* oil there were beta-caryophyllene (14.2%) and 2-methyl-octane (13.1%).

## 2.6 EFFECT OF GROWTH STAGE AND ENVIRONMENT ON THE PRODUCTION OF SECONDARY METABOLITES IN *Hypericum* spp.

Jensen *et al.* (1995) reported that the mean hypericin content in vegetative growth of 11 Nova scotia *Hypericum perforatum* biotypes was 195 mμg per gram dry weight, whereas levels in biotypes from British Columbia and Australia were at least two and three fold higher respectively. Levels in field-collected plants were lowest in midsummer (160-280 mμg per dry weight). They also reported that in a controlled

environment study, hypericin levels increased linearly with increasing temperature Omidbaigi and Aziz (2000) reported that the hypericin and essential oil contents were higher at full bloom than before flowering and fruit set stage. Southwell and Bourke (2001) observed the seasonal variation in the hypericin content of narrow and broad leaf biotypes of *H. perforatum*. The hypericin concentration in the broad leaf biotype was lowest during winter (more than 100 ppm) and highest during summer (300 ppm). The narrow leaf biotype exhibited the lowest hypericin/pseudohypericin concentration during winter (less than 100 ppm) and the highest during summer (5000 ppm). They also found that the second year cycle recorded higher summer hypericin concentrations for both biotypes due to higher rainfall lower average daily sunlight hours and a lower average daily maximum temperature compared with the first season. Loren *et al.* (2001) found that the mean floral concentrations of hypericin (0.06%) and pseudohypericin (0.29%)were highest during anthesis coinciding with July and August sampling dates, whereas the mean leaf concentrations (0.04% and 0.19% respecectively) were highest in August.

Tekelova *et al*, (2000) analysed the *H. perforatum* in different plant development stages for dianthrones, hyperforin and flavonoids. They documented the quantitative changes in the hypericin content as follows: 0.32% (May15), 0.047% (June 8), 0.06% (June12), 0.1% (June19), 0.098% (June24), 0.089% (June29), 0.076% (July 7) and 0.041% (July 17). The date of sampling is given in the bracket.

Kitanov (2000) studied the accumulation dynamics and total content of hypericin in *H. perforatum* and *H. maculatum* by spectroscopic method. In both the species, the highest amount of hypericin was found during the budding and blossoming stage. There was no correlation between the content of hypericin and altitude of harvesting and regional origin of the plants.

# *Materials and Methods*

# 3.MATERIALS AND METHODS

The study entitled "Morphological and phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds" was conducted simultaneously in the Devikulam block of Idukki district and the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 2000 to 2002.The study consisted of three parts.

A) Evaluation of morphological variation of *Hypericum spp.* in its natural habitat and analysis of natural habitat characteristics.

B) Anatomy and histochemistry of *Hypericum* spp.

C) Biochemical analysis for qualitative and quantitative estimation of phytochemical constituents.

The details regarding the experimental materials and the methodology adopted for conducting various aspects of the study are presented in this chapter.

## 3.1 STUDY AREA

### 3.1.1 Devikulam block

Devikulam block situated in Western Ghats of Idukki district at an altitude range of 3500 to 8500 feet was the study area. Geographically, the block is divided into four parts. They are tall mountains, steep slopes, medium type mountains, valleys and plains. Tall mountains comprise of nearly 25 per cent of the total geographical area of the block. Major geographical area is covered by medium type mountains. Plantation crops like tea, coffee and cardamom are extensively cultivated in these areas.

Theni district of Tamil Nadu (East), Adimali block (West), Coimbatore district of Tamil Nadu (North) and Nedumkandam and Adimali Panchayats (South) are boundaries of the block. There are six panchayats viz., Marayur, Munnar, Kanthallur, Vattavada, Chinnakkanal and Santhanpara under this block.

The species is found abundant in Vattavada panchayat and hence Vattavada region was chosen for detailed habitat analysis.

### 3.1.2 Climate

The area receives more than 60 per cent precipitation during the southwest monsoon (June-August) while the North East monsoon (September-November) and summer (February-May) rainfall are contributing 30 and 10 per cent respectively. The mean daily maximum temperature rises during January to May but drops abruptly with the onset of monsoon in June. Winter starts by November and reaches it's peak in December and lasts until February. Frost is a frequent phenomenon during winter nights and the radiant heat loss from the surface, results in the fall of temperature and nearly reaches freezing point. The day temperature during this period goes to 23-25°C. The monthly average of weather parameters during the study period are given in Table 1.

### 3.1.3 Geology, rock and soil

The underlying rocks in the area are of Archaean igneous origin, consisting of granite and gneiss. The crystalline rock consists of minerals like silica, feldspars, muscovite and biotite with small amounts of accessory Ferro magnesium minerals. The soil is a relic of a much thick soil cover that developed formerly under pseudo-dynamic conditions prevailing from late Jurassic to early Tertiary times. It is composed of different layers of black to dark grey coloured, granular, friable, sandy loam interspersed with a little gravel (Balakrishnan, 2001).

### 3.1.4 Vegetation

The natural forest in the study area comprises four types of vegetation. They are

(i) Shola forest (Southern montane wet temperate forest)

**Table 1. Mean monthly weather data for 2001-2002 recorded at Eravikulam National Park, Munnar.**

| Month | Temperature° C | | Relative humidity (%) | | Rainfall (mm) |
|---|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum | |
| anuary | 12.0 | 24.0 | 62.5 | 85.0 | 19.0 |
| ebruary | 12.0 | 24.0 | 61.0 | 53.4 | 16.4 |
| larch | 14.0 | 25.0 | 58.0 | 79.0 | 0.0 |
| pril | 15.0 | 25.0 | 54.0 | 76.5 | 14.0 |
| lay | 15.0 | 24.0 | 54.0 | 77.5 | 91.8 |
| une | 14.0 | 19.0 | 72.0 | 86.5 | 685.8 |
| uly | 14.0 | 19.0 | 73.0 | 89.0 | 389.9 |
| ugust | 14.0 | 17.0 | 71.0 | 88.0 | 817.8 |
| eptember | 14.0 | 21.0 | 77.0 | 89.0 | 191.7 |
| ctober | 14.0 | 25.0 | 70.0 | 83.0 | 94.1 |
| lovember | 13.0 | 25.0 | 68.0 | 79.0 | 11.5 |
| ecember | 12.0 | 25.0 | 66.0 | 78.0 | 7.2 |
| anuary | 11.0 | 24.0 | 66.0 | 84.0 | 0.0 |
| ebruary | 12.0 | 25.0 | 62.0 | 82.0 | 14.1 |
| larch | 14.0 | 24.0 | 61.0 | 77.0 | 3.4 |
| pril | 14.0 | 21.0 | 59.0 | 78.0 | 36.3 |
| lay | 15.0 | 24.0 | 56.5 | 77.0 | 31.8 |

(ii) Grass land (Southern montane wet temperate grassland)

(iii) Transition forest (Southern sub tropical hill forest) and

(iv) Evergreen forest (Southern wet coast evergreen forest)

Shola forest is generally confined to the valleys, glens, hollows and depressions. They are evergreen forests characterized by stunted trees with dense crown, thick more or less dosed canopy and small coriacious leaves (Champion and Seth, 1968).

### 3.1.5 Survey of *Hypericum*

A reconnaissance survey was conducted in the Vattavada panchayat to locate the natural habitats of *Hypericum*. After this, periodic trips were made to the area to study the distribution pattern of *Hypericum*. Observations like season and extent of occurrence, habit, habitat characters, parts used and local name were recorded. Phenological observations like flowering and fruiting were noted. Herbarium was prepared and identified at Kerala Forest Research institute, Peechi. The survey was conducted for one calendar year starting from August 2001 to August 2002.

## 3.2 NATURAL HABITAT ANALYSIS

### 3.2.1 Choice of species

Since *Hypericum mysorense* is the only available *Hypericum* species in the study area, it was selected for detailed habitat analysis. During the course of investigation, *Hypericum* spp. were also located in the Western Ghat region of Wayanad district. Apart from *Hypericum mysorense, Hypericum japonicum* was also located and this species was also included in the study. A comparison was also made with the *Hypericum mysorense* available in the Wayanad region.

### 3.2.2 Community structure analysis

Plants growing together have mutual relationships among themselves and with the environment. Such a group of plants in an area forms a stand. Several similar stands represent a community. Quadrat method was employed to determine the analytical characters of community.

#### 3.2.2.1 Quadrat method

Natural habitats of the chosen species were located visually and seven different locations were selected at random to represent the whole Vattavada panchayat based on altitude, slope, nature of soil, stand composition, density and variability of stands. Permanent plots were laid out in all the ten locations by quadrat method.

A right-angled triangle of sides 0.3, 0.4 and 0.5 m was laid out on the ground with the help of a rope. The triangle was marked on the ground with three iron pegs on the corners. By extending the horizontal and vertical sides of the triangle a square of size 1 m x 1 m was made. The quadrat was marked with iron pegs on the ground and outlined by laying coloured nylon ropes to the pegs.

### 3.2.3 Site characteristics

Preparatory to enumeration, based on general visual observations, a record of the site characteristics of the plots was prepared. Elevation, slope, dominant species and other details were noted in the field book. Incidence of fire, cattle grazing, damage by wild animals and such other interventions were recorded as and when noticed. The seven habitats selected for the study and their characteristics are furnished hereunder.

1.Pazhathottam – 1

Grass land, altitude 2100 ± 150 m, flat terrain, soil dark brown, fully exposed to sun, more grazing.

2. Pazhathottam - 2

Gentle slope, altitude 2100 ± 100 m, southern aspect, rocks absent, soil dark brown, fully exposed to sun, more grazing, high fern density. Human interventions for eucalyptus plantations, plot was burnt in the previous season.

3. Irukkanai

Gentle slope, altitude 2100 ± 100 m, South East aspect, black soil, canopy cover 25 per cent, grazing less, moderate fern density.

4. Kottakompuru

Steepy slope, altitude 2100 ± 120 m, southern aspect gravelly red soil, rocky, grazing is rare, fern density high.

5. Vattavada

Moderate slope, altitude 2100 ± 50 m, Western aspect, rocks absent, soil brown, canopy coverage 30 per cent, no grazing.

6. Sudalai

Moderate slope, altitude 2100 ± 50 m, south west aspect, dry, friable black soil, canopy cover 50 per cent, litter cover 80 per cent, no grazing, less disturbed area, less density of ferns.

7. Koviloor

Slopy, rocks absent, altitude 2100 ± 75 m, gravelly red soil, fully exposed to sun, moderate slope, North West aspect.

## 3.3 STUDY OF MORPHOLOGICAL CHARACTERS

The experimental plants of *Hypericum mysorense* in each quadrat were tagged with the help of wooden poles and aluminium tags. The morphological characters of the plant were noted.

### 3.3.1 Biometrical observations

The following biometrical observations were recorded in *Hypericum mysorense*.

Height of the plant (cm)

Number of primary branches (cm)

Internodal length (cm)

Height of the first branch from ground level (cm)

Leaf length (cm)

Leaf width (cm)

Sepal length (cm)

Sepal width (cm)

Petal length (cm)

Petal width (cm)

Style length (cm)

Stamen length (cm)

Number of stamen

The following biometrical observations were recorded in *H. japonicum*

Height of the plant (cm)

Number of primary branches

Internodal length (cm)

Height of the first branch from ground level (cm)

Leaf length (cm)

Leaf width (cm)

Petal length (cm)

Petal width (cm)

Number of stamen

### 3.3.2 Qualitative characters

The following qualitative observations were recorded in *H. mysorense* and *H.japonicum.*

Habit

Angleness in tender stem

Angleness in matured stem

Colour of tender stem

Colour of matured stem

Leaf arrangement

Petiole

Leaf colour

Venation

Translucent glands on leaf

Black glands on leaf

Shape of leaf tip

Shape of leaf base

Leaf Margin

Pubescence on leaf

Pubescence on stem

Black glands on petal

Season of flowering

Stigma

Stamen bundles

No. of carpels per fruit

Fruit colour

## 3.4 PHYTOSOCIOLOGICAL ANALYSIS

The data from the quadrat method were subjected to phytosociological analysis as detailed below:

### 3.4.1 Frequency

Frequency expresses the distribution or dispersion of various species in a community. From this, percentage frequency was calculated as follows:

$$\text{Percentage frequency} = \frac{\text{No. of sampling units in which the species occurred}}{\text{Total no. of units studied}} \times 100$$

After determining the percentage frequency of each species, depending on their frequency value they are distributed in Raunkiaers frequency classes as fallows:

| Frequency (%) | Frequency Class |
|---|---|
| 0-20 | A |
| 21-40 | B |
| 41-60 | C |
| 61-80 | D |
| 81-100 | E |

### 3.4.2 Density

The term density represents the numerical strength of species in the community.

$$\text{Density} = \frac{\text{Total no. of individuals of a species}}{\text{Total no. of quadrats studied}}$$

### 3.4.3 Abundance

Abundance is described as the number of individuals per quadrat of occurrence.

$$\text{Abundance} = \frac{\text{Total no. of individuals of a species}}{\text{No. of quadrats of occurrence}}$$

## 3.5 PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

Moisture content of the soil was found out by standard procedure of oven drying. The methods adopted for chemical analysis are given in Table 2.

## 3.6 ANATOMICAL STUDIES AND HISTOCHEMICAL STAINING

The leaves of *Hypericum mysorense* and *H. japonicum* were observed under stereomicroscope to see the glands. Cross sections of leaf, stem and root of *Hypericum mysorense* and *H. japonicum* were taken. The specimen was kept tightly between two layers of thermocool and by moving the blade from one side to other sections were made. Among the sections, a finer one was selected and placed on a clean slide. The woody stem and root of *H. mysorense* were taken using Leica® wood microtome. The section was flooded with a few drops of an aqueous solution of 0.1 per cent toluidine blue solution for a minute. The stain was removed by using a piece of filter paper. The section was washed with a few drops of water and then the water was removed using a filter paper. A drop of pure glycerol was added over the sections and the cover slip was applied.

Toluidine blue is a cationic dye that binds to negatively charged groups. The aqueous solution of this dye is blue, but different colours are generated when the dye binds with different anionic groups in the cell. The colour of various chemical compounds in the cell when stained with toluidine blue is given below :

Pectin - red or reddish purple

Lignin - blue

Other phenolic compounds - Green to blue green (Yeung, 1998)

## 3.7 BIOCHEMICAL ANALYSIS

Biochemical analyses were carried out with the plant samples collected from Munnar and Wayanad areas. Literature on the phytoconstituents, their extraction medium and estimation with respect to the select species were nil or

**Table 2. Details of analytical methods used to study physical and chemical properties of soil.**

| Character | Method | Reference |
|---|---|---|
| Soil reaction (pH) | Soil water suspension of 1:2.5 and read in the pH meter | Hesse (1971) |
| Electrical conductivity | Soil water suspension of 1:2.5 and read in Conductivity Bridge | Jackson (1958) |
| Available N | Alkaline permanganate method | Subbiah and Asija (1956) |
| Available $P_2O_5$ | Bray No.1 Extract method | Watnabe and Olsen (1965) |
| Available $K_2O$ | Neutral Normal Ammonium acetate extract method using flame photometer | Jackson (1958) |

limited and all the methods had to be standardized first. Hence, habitat wise analysis of each species could not be undertaken. Composite samples were taken instead. Each sample was replicated five times and average worked out.

### 3.7.1 Preparation of sample

Leaf, stem and roots of *Hypericum mysorense* were collected and fresh extacts were used for the estimation of starch, total sugar, protein and total free amino acids. In the case of *H. japonicum* since it is a small herb, the stem and leaves were pooled together for the analyses. The crude extract of *Hypericum perforatum*, commercially available as Eleve®, manufactured by Universal Medicare Ltd., Mumbai was also used for the chromatographic analysis and quantification of hypericin. The stem and root samples of *H. mysorense* were finely chopped and dried under shade. In the case of *H. japonicum*, the root portion was removed and the above ground portion was made into small pieces and then dried under shade. The leaf samples of *H. mysorense* were dried as such under shade. During the flowering season, the buds, flowers and developing fruits were also included along with the leaf samples, in *H. mysorense* and with leaf and stem composite sample of *H. japonicum*. The samples of seed maturity stage include, the developing and matured fruits.

### 3.7.2 Estimation of total extractable matter

Two grams of finely powdered sample was taken in a filter paper thimble and soxhleted using 140 ml of methanol until the solvent became colourless. After extraction, the solvent was evaporated in the evaporating chamber and the per cent recovery of total extractable matter calculated. The experiment was repeated three times with each sample. The extract was stored for qualitative and quantitative analyses of hypericin.

### 3.7.3 Estimation of total sugars

The content of total sugars was estimated by slightly modifying the Phenol-Sulphuric acid method given by Sadasivam and Manickam (1992). Hundred milligrams of fresh sample was homogenised with mortar and pestle and exhaustively extracted with methanol until the extract was colourless. The supernatant solution was collected and made up to 100 ml with methanol. From that 0.2, 0.4, 0.6, 0.8 and one ml were taken and made up to seven ml in different test tubes. To that aliquots, one ml of five per cent phenol and five ml of concentrated $H_2SO_4$ were added. Red colour was developed and read at 490 nm.

### 3.7.4 Estimation of starch

Three hundred milligrams of fresh sample was used for the starch estimation. Starch content was estimated by Anthrone method (Sadasivam and Manickam, 1992).

### 3.7.5 Estimation of total free amino acids

The total free amino acid content was estimated by the method given by Sadasivam and Manickam (1992) fresh samples using 80 per cent methanol as solvent.

### 3.7.6 Estimation of protein

The protein content was estimated by Lowry's method suggested by Sadasivam and Manickam (1992) using Tris -HCl buffer.

### 3.7.7 Qualitative tests for anthraquinones

Qualitative test for anthraquinone was done by Borntrager reaction as suggested by Robinson (1967). Borntrager reaction was used for the identification of anthraquinone derivatives. The plant material was boiled in dilute. aqueous potassium hydroxide for a few minutes. This not only hydrolyses glycosides but also oxidises anthrones or anthranols to anthroquinones. The alkaline solution is cooled, acidified and extracted with benzene. When the benzene phase is

separated and shaken with dilute alkali, the benzene loses its yellow colour and the alkaline phase becomes red if quinones are present. The test is not only specific for anthraquinones; naphthoquinones also give a positive reaction.

### 3.7.8 Detection of hypericin by chromatograms

Thin layer chromatographic methods were followed as described by Harborne (1973).

#### 3.7.8.1 Preparation of gel plate

Thin layer chromatography was employed for detecting hypericin. Sixty grams of silica gel G or silica gel $GF_{254}$ 160-250 mesh size was taken in a flat bottomed flask and mixed with 120 ml distilled water and the slurry was spread on gel plates of 20 x 20 cm size with an applicator to provide 0.25 mm thick gel layer. The plates were allowed to set for 10 minutes at room temperature and then placed in hot air over maintained at 120-150°C for an hour to dry and activate the silica gel and stored.

#### 3.7.8.2 Sample application

The crude methanol extracts of *Hypericum mysorense* (leaf, stem, root) *H. japonicum* (aerial portion) and *H. perforatum* extract along with standard hypericin were used. Five microlitres of the samples were applied using a capillary tube on the silica gel plate at two cm from the base with two cm distance between each spots along with standard hypericin.

The plate was transferred to a chromatographic glass chamber, saturated with the solvent systems as given in the Table. 3. The chamber was closed with a lid and the solvent was allowed to run up to two-third portion of the plate. The plates were then taken out and observed under UV light at 254 or 366 nm.

**Table.3. Solvent systems for developing chromatograms of *Hypericum* spp.**

| Solvent system | Proportion | Grade of silica gel | Spray reagent | Visualisation of spots |
|---|---|---|---|---|
| Ethyl acetate – formic acid –glacial acetic acid - water | 10:1.1:1.1:2.6 | GF254 | Pyridine 10%v/v in ethanol | UV 254 nm |
| Toluene - ethyl acetate – formic acid | 50:40:10 | G | 0.5N KOH in ethanol | UV366 |
| Ethyl acetate – formic acid | 50:6 | G | 0.5N KOH in ethanol | UV366 |

### 3.7.8.3 Detection of hypericin

For the detection of hypericin, pyridine 10 per cent v/v in ethanol or 0.5 N KOH in ethanol was sprayed on chromatographic plates and visualised under UV high (365 nm and 254 nm). The standard hypericin gave a pinkish red spot with the appropriate solvent system. Colours of characteristic main zones were described, Rf values were calculated and compared with that of the standard hypericin.

$$\text{Rf value} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}$$

### 3.7.9 Quantification of hypericin from commercial extract of *Hypericum perforatum* (Eleve®)

### 3.7.9.1 Preparation of standard hypericin solution

Five milligrams of the standard hypericin was dissolved completely in a small quantity of pyridine and the volume made upto 10 ml. From this primary standard solution serial dilutions at concentrations of 10, 20, 30, 40 and 50 µg per

ml were prepared. The lambda max ($\lambda_{max}$) of hypericin was identified as 589 in spectronic® Genesys® 5 instrument using the above dilution. The absorbances of 10, 20, 30, 40 and 50 µg per ml standard hypericin were recorded at 589 nm, against methanol as a reagent blank. A standard curve of hypericin were plotted using absorbance (nm) Vs concentration of the hypericin.

The methanol extract of the commercial *Hypericum perforatum* formulation that gave positive test for the presence of hypericin was used for the quantification. The gelatin coat of the commercial formulation was cut open and the extract was squeezed out. The extract was defatted with dichloromethane and the hypericin was extracted with acetone. The solvent was evaporated and the residue was dissolved in methanol. Absorbance of the final solution was measured in spectrophotometer at 589 nm using methanol as reagent blank.

### 3.8 Statistical analysis

The biometrical data of *Hypericum mysorense* recorded at Idukki and Wayanad districts were subjected to statistical analysis using MSTATC package. Analysis of variance was performed following the procedure by Panse and Sukhatme (1978).

# *Results*

# 4. RESULTS

The results of the studies on "Morphological and phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds" are presented in this chapter.

## 4.1 SURVEY OF THE AREA

*Hypericum* sp. was located by reconnaissance survey in the Vattavada panchayat of the Devikulam block of Idukki district. Herbarium was prepared for the located species and was identified at Kerala Forest Research Institute as *Hypericum mysorense* (Plate 1A). This plant is locally known as 'Ponnavaran' in the study area. The leaves are widely used for applying on cutwounds, as a paste by the local people.

## 4.2 NATURAL HABITAT ANALYSIS

### 4.2.1 Physico-chemical properties of soil

Data pertaining to the physico-chemical properties of soil in different habitats are presented in Table 4. Soil pH ranged from 4.30 to 5.52. The electrical conductivity (EC) of the soil ranged between 0.053 to 0.089 $dSm^{-1}$. The available major nutrients varied with the habitat. The available nitrogen ranged from 0.89 to 1.12 per cent and the phosphorus from 0.18 to 0.29 per cent. The available soil potassium had the range from 0.034 to 0.051 per cent.

### 4.2.2 Vegetation studies and phytosociological parameters

Phytosociological studies of the species noted in the quadrat study at pazhathottam 1 are presented in Table 5. From the Table it is evident that *Chrysopogon zeylanicus*, a type of lawn grass was the dominant species in the

**Table 4. Physico- chemical properties of soils in different habitats**

| Habitat | pH | EC($dSm^{-1}$) | Moisture (%) | Av.N(%) | Av. P(%) | Av.K(%) |
|---|---|---|---|---|---|---|
| Pazhathottam 1 | 4.40 | 0.053 | 0.92 | 0.18 | 0.046 | 14.23 |
| Irukkanai | 4.60 | 0.059 | 0.89 | 0.23 | 0.039 | 12.71 |
| Kottakompuru | 4.72 | 0.062 | 0.95 | 0.26 | 0.042 | 8.93 |
| Vattavada | 5.34 | 0.079 | 1.12 | 0.26 | 0.034 | 11.28 |
| Pazhathottam 2 | 4.30 | 0.059 | 0.89 | 0.21 | 0.051 | 13.74 |
| Koviloor | 5.21 | 0.074 | 1.03 | 0.29 | 0.041 | 9.29 |
| Sudalai | 5.52 | 0.089 | 0.93 | 0.25 | 0.045 | 10.27 |

habitat. *Chrysopogon* and *Pteridium*, a fern,were having the high frequency values The frequency values of, *Hypericum mysorense* and *Andropogon lividis* were found to be the same.

Irukkanai

From the Table. 6 it is clear that the *Chrysopogon* had the high density with 40 per cent frequency. The density, abundance and frequency values of *Eucalyptus grandis* and *Strobilanthus* sp. were found to be same.

Kottakompuru

Table.7 shows that Pteridium had the highest frequency followed by *Andropogon* and *Hypericum*. The highest density and abundance values were noted for *Chrysopogon* in this location.

Vattavada

The density value was the highest in *Chrysopogon* (98). This was followed by *Pteridium* (1.9) and *Hypericum mysorense* (1.1). Same trend was observed for the character abundance. *Pteridium* had the highest value (60%) followed by *Hypericum* (50%) and *Chrysopogon* (40%). *Lantana camara* and *Andropogon lividis* had the same value for all the three characters Table 8.

Pazhathottam 2

The phytosociological parameters of the study area pazhathottam 2 (Table. 9) revealed that the density and the frequency were the highest for *Chrysopogon* with 221.7 and 80 per cent respectively. This was followed by *Pteridium* (density value 3.9, frequency 80%) and *Hypericum mysorense* (density value 1.2, frequency 40%).

**Table 5. Phytosociological parameters of *Hypericum mysorense* at Pazhathottam 1**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 15 | 5 | 1.5 | 3 | 50 | C |
| *Pteridium aquilinum* | 19 | 7 | 1.9 | 2.7 | 70 | D |
| *Eucalyptus grandis* | 0 | 0 | 0 | 0 | 0 | A |
| *Lantana camara* | 2 | 2 | 0.2 | 1 | 20 | A |
| *Strobilanthus* spp | 3 | 3 | 0.3 | 1 | 30 | B |
| *Andropogon lividis* | 7 | 5 | 0.7 | 1.4 | 50 | C |
| *Chrysopogon zeylanicus* | 1740 | 7 | 174 | 348 | 70 | D |

**Table 6. Phytosociological parameters of *Hypericum mysorense* at Irukkanai**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 17 | 6 | 1.7 | 2.8 | 60 | C |
| *Pteridium aquilinum* | 24 | 7 | 2.4 | 3.4 | 70 | D |
| *Eucalyptus grandis* | 1 | 1 | 0.1 | 1 | 10 | A |
| *Lantana camara* | 2 | 2 | 0.2 | 1 | 20 | B |
| *Strobilanthus* spp | 1 | 1 | 0.1 | 1 | 10 | A |
| *Andropogon lividis* | 4 | 2 | 0.4 | 2 | 20 | B |
| *Chrysopogon zeylanicus* | 982 | 4 | 98.2 | 245.5 | 40 | C |

**Table 7. Phytosociological parameters of *Hypericum mysorense* at Kottakompuru**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 11 | 5 | 1.1 | 2.2 | 50 | C |
| *Pteridium aquilinum* | 37 | 8 | 3.7 | 4.6 | 80 | D |
| *Eucalyptus grandis* | 0 | 0 | 0 | 0 | 0 | A |
| *Lantana camara* | 1 | 1 | 0.1 | 1 | 10 | A |
| *Strobilanthus* spp | 0 | 0 | 0 | 0 | 0 | A |
| *Andropogon lividis* | 7 | 6 | 0.7 | 1.2 | 60 | C |
| *Chrysopogon zeylanicus* | 230 | 2 | 23 | 115 | 20 | A |

**Table 8. Phytosociological parameters of *Hypericum mysorense* at Vattavada**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 11 | 5 | 1.1 | 2.2 | 50 | C |
| *Pteridium aquilinum* | 19 | 6 | 1.9 | 3.2 | 60 | C |
| *Eucalyptus grandis* | 1 | 1 | 0.1 | 1 | 10 | A |
| *Lantana camara* | 2 | 2 | 0.2 | 1 | 20 | A |
| *Strobilanthus* spp | 2 | 2 | 0.2 | 1 | 20 | A |
| *Andropogon lividis* | 4 | 2 | 0.4 | 2 | 20 | A |
| *Chrysopogon zeylanicus* | 980 | 4 | 98 | 245 | 40 | B |

**Table 9. Phytosociological parameters of *Hypericum mysorense* at Pazhathottam 2**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 12 | 4 | 1.2 | 3 | 40 | B |
| *Pteridium aquilinum* | 39 | 8 | 3.9 | 4.9 | 80 | D |
| *Eucalyptus grandis* | 1 | 1 | 0.1 | 1 | 10 | A |
| *Lantana camara* | 2 | 2 | 0.2 | 1 | 20 | A |
| *Strobilanthus* spp | 2 | 1 | 0.2 | 2 | 10 | A |
| *Andropogon lividis* | 6 | 2 | 0.6 | 3 | 20 | A |
| *Chrysopogon zeylanicus* | 2217 | 8 | 221.7 | 221.7 | 80 | D |

Koviloor

Table.10 shows that the density (89) and abundance (178.8) were the highest in *Chrysopogon*. This was followed by *Andropogon* (1.6) and *Hypericum* (0.9) for density and *Hypericum* (3) and *Andropogon* (2.3) for abundance. The abundance value of *Pteridium* and *Andropogon* were the same. *Andropogon* had the highest frequency (70%) followed by *Chrysopogon* (50%). *Hypericum*, *Pteridium* and *Lantana* had the same frequency value (30%).

Sudalai

Table.11 reveals that the density value was highest in *Chrysopogon* (118), followed by *Hypericum* (1.3) and *Andropogon* (1.2). Abundance was highest in *Chrysopogon* (236), followed by Hypericum (2.2). The rest of the species other than *Andropogon* showed the maximum frequency (70%) followed by *Hypericum* (60%) and *Chrysopogon* (50%). Among the species studied, *Eucalyptus* had the lowest value for density (0.1) and frequency (10%).

## 4.3 STUDY OF MORPHOLOGICAL CHARACTERS

### 4.3.1 Biometrical observations

#### 4.3.1.1 Biometrical observations on *Hypericum mysorense* (Idukki district)

The data on biometrical observations of *H. mysorense* recorded at different sites of Vattavada in Idukki district are presented in Table.12. The height of the plant ranged from 104.47 cm to 147 cm with 21.54 per cent co-efficient of variation. The character showed a significant difference over the grand mean (124.04 cm) at Pazhathotam 1. Number of primary branches in the species ranged from 3.07 and 4.00 and this character showed a significant difference over the grand mean (3.38) at Pazhathotam 1. The internodal length varied from 0.34 cm to 0.70 cm with 26.69 per cent coefficient of variation. The internodal length significantly differed from the grand mean (0.44) at Pazhathotam 1. The height at the first branch from the ground

**Table 10. Phytosociological parameters of *Hypericum mysorense* at Koviloor**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 9 | 3 | 0.9 | 3 | 30 | B |
| *Pteridium aquilinum* | 7 | 3 | 0.7 | 2.3 | 30 | B |
| *Eucalyptus grandis* | 2 | 2 | 0.2 | 1 | 20 | A |
| *Lantana camara* | 3 | 2 | 0.3 | 1.5 | 20 | A |
| *Strobilanthus* spp | 4 | 2 | 0.4 | 2 | 20 | A |
| *Andropogon lividis* | 16 | 7 | 1.6 | 2.3 | 70 | D |
| *Chrysopogon zeylanicus* | 894 | 5 | 89 | 178.8 | 50 | C |

**Table 11. Phytosociological parameters of *Hypericum mysorense* at Sudalai**

| Species | Total No.of individuals in ten quadrats | No.of quadrats in which the species occurred | Density | Abundance | Percentage freqency | Frequency class |
|---|---|---|---|---|---|---|
| *Hypericum mysorense* | 13 | 6 | 1.3 | 2.2 | 60 | C |
| *Pteridium aquilinum* | 11 | 4 | 1.1 | 2.8 | 40 | B |
| *Eucalyptus grandis* | 1 | 1 | 0.1 | 1 | 10 | A |
| *Lantana camara* | 4 | 4 | 0.4 | 1 | 40 | B |
| *Strobilanthus* spp | 1 | 1 | 0.1 | 1 | 10 | A |
| *Andropogon lividis* | 12 | 7 | 1.2 | 0.6 | 70 | C |
| *Chrysopogon zeylanicus* | 1180 | 5 | 118 | 236 | 50 | B |

**Table 12. Biometrical observations in *Hypericum mysorense* at different locations of Vattavada panchayat of Idukki district**

| Characters/Place | Pazha thottam1 | Irukkanai | Kottaa kombru | Vattavada | Pazha thottam2 | Koviloor | Sudalai | Grand Mean | CV % | CD |
|---|---|---|---|---|---|---|---|---|---|---|
| Height of the plant(cm) | 147.73 | 112.33 | 104.47 | 127.73 | 127.20 | 118.80 | 130.00 | 124.04 | 21.54 | 16.20 |
| No. of .primary branches | 4.00 | 3.80 | 3.33 | 3.33 | 3.09 | 3.07 | 3.07 | 3.38 | 29.80 | 0.61 |
| Internodal length(cm) | 0.70 | 0.51 | 0.38 | 0.37 | 0.37 | 0.38 | 0.34 | 0.44 | 26.69 | 0.07 |
| First branch ht(cm) | 48.33 | 61.20 | 55.18 | 45.77 | 44.65 | 39.12 | 33.45 | 46.81 | 28.72 | 8.15 |
| Leaf length(cm) | 3.48 | 2.81 | 2.86 | 2.88 | 2.39 | 2.60 | 2.74 | 2.82 | 18.23 | 0.31 |
| Leaf width(cm) | 1.10 | 0.63 | 0.55 | 0.59 | 0.46 | 0.66 | 0.66 | 0.66 | 28.91 | 0.12 |
| Sepal length(cm) | 0.94 | 0.96 | 0.87 | 0.92 | 0.85 | 0.91 | 0.89 | 0.91 | 11.95 | 0.07 |
| Sepal width(cm) | 0.33 | 0.35 | 0.34 | 0.33 | 0.33 | 0.35 | 0.33 | 0.34 | 14.29 | 0.03 |
| Petal length(cm) | 3.41 | 3.40 | 3.08 | 3.40 | 3.32 | 3.39 | 3.43 | 3.35 | 4.92 | 0.10 |
| Petal width(cm) | 1.78 | 1.70 | 1.50 | 1.74 | 1.60 | 1.50 | 1.55 | 1.62 | 6.76 | 0.07 |
| Style length(cm) | 1.83 | 1.80 | 1.81 | 1.85 | 1.83 | 1.87 | 1.87 | 1.84 | 4.59 | 0.05 |
| Stamen length(cm) | 1.36 | 1.36 | 1.33 | 1.37 | 1.35 | 1.33 | 1.37 | 1.35 | 4.28 | 0.03 |
| No. of stamen | 41.80 | 42.60 | 42.13 | 42.33 | 42.16 | 41.73 | 41.60 | 42.05 | 2.85 | 0.08 |

level ranged from 33.45 cm to 61.20 cm. The character exhibited significant difference at the places Pazhathotam 1 (48.33 cm), Irukkanai (61.20 cm) and Kottakombru (55.18 cm) over the grand mean (46.81 cm). Leaf length ranged from 2.39 cm to 3.48 cm and it showed a significant difference over the grand mean (2.82 cm) at Pazhathotam 1. For the leaf width also significant difference was observed at Pazhathotam 1 over the grand mean (0.66 cm). Sepal length ranged from 0.87 cm to 0.96 cm. For sepal length and sepal width no significant differences were observed in all the seven study areas over the grand mean. Petal length ranged from 3.32 cm to 3.43 cm. For petal length significant difference over the grand mean was observed in none of the locations. Style length and stamen length ranged from 1.80 cm to 1.87 cm and 1.33 cm to 1.37 cm respectively. Both style length and stamen length had no significant difference in any of the location over their respective grand means. Number of stamens in the species ranged from 41.60 to 42.60 with the grand mean of 42.05. Number of days from flowering to fruiting ranged from 89.87 (Kaviloor) to 92.00 (Sudali). In none of the places the value was significant over the grand mean.

*Hypericum mysorense* growing at Irukkanai were showing significant difference for the number of stamen (42.60) over the grand mean (42.05). The characters viz., number of stigma, number of stamen bundles and number of locules in fruit were same in all the locations studied.

#### 4.3.1.2 Biometrical observations on *Hypericum mysorense* (Wayanad district)

The data on biometrical observations of *H. mysorense* recorded at Kalpetta in Wayanad district are presented in Table 13. For the character, height of the plant, significant difference was observed between the locations Chembra 1 and Chembra 2. Significant difference was observed between Puthurvayal and Chembra 1 for the character-number of primary branches. Internodal length showed significant difference over the grand mean (90.5 cm) at Puthurvayal. There was no significant

**Table 13. Biometrical observations in *Hypericum mysorense* at different location of Kalpetta**

| Characters/Place | Puthurvayal | Chembra 1 | Chembra2 | Grand Mean | CV % | CD |
|---|---|---|---|---|---|---|
| Height of the plant(cm) | 118.40 | 128.49 | 144.13 | 130.34 | 18.99 | 15.37 |
| No. of primary branches | 3.13 | 3.60 | 3.20 | 3.31 | 20.91 | 0.43 |
| Internodal length(cm) | 0.38 | 0.38 | 0.73 | 0.50 | 17.81 | 0.05 |
| First branch height(cm) | 41.91 | 42.32 | 38.24 | 40.82 | 16.43 | 4.17 |
| Leaf length(cm) | 2.65 | 2.70 | 2.82 | 2.72 | 5.35 | 0.09 |
| Leaf width(cm) | 0.69 | 0.71 | 0.73 | 0.71 | 13.86 | 0.06 |
| Sepal length(cm) | 0.92 | 0.92 | 0.94 | 0.93 | 8.56 | 0.05 |
| Sepal width(cm) | 0.36 | 0.35 | 0.35 | 0.35 | 13.67 | 0.03 |
| Petal length(cm) | 3.41 | 3.45 | 3.42 | 3.43 | 1.88 | 0.04 |
| Petal width(cm) | 1.51 | 1.49 | 1.79 | 1.60 | 5.21 | 0.05 |
| Style length(cm) | 1.86 | 1.83 | 1.79 | 1.83 | 3.47 | 0.04 |
| Stamen length(cm) | 1.33 | 1.35 | 1.35 | 1.34 | 3.54 | 0.03 |
| No. of stamen | 41.73 | 42.00 | 41.80 | 41.84 | 2.76 | 0.72 |

difference for the height of first branch from ground level. Significant difference was observed for leaf length over the grand mean (2.72 cm) at Chembra 2. No significant difference was observed for the characters viz., leaf width, sepal length, sepal width and petal length over their respective grand means.

Petal width varied from 1.49 cm to 1.79 cm and it showed significant difference over the grand mean (1.60 cm) at Chembra 2. The characters viz., style length, stamen length and number of stamen did not have any significant difference over their respective grand means.

#### 4.3.1.3 Biometrical observations on *Hypericum japonicum* (Wayanad district)

Table.14 shows the biometrical observations recorded in *Hypericum japonicum* (Plate. 1B)at Kalpetta of Wayanad district. Height of the plant ranged from 19.27 cm to 22.56 cm and no significant difference was observed for the character in the study locations. Significant difference was observed for the number of branches between the locations Puthurvayal and Chembra 1. Chembra 2 showed significant difference for the height of first branch over the grand mean (2.52 cm). Internodal length, leaf length, leaf width, petal length, petel width and number of stamens did not differ significantly.

### 4.3.2 Qualitative characters of *Hypericum spp.*

#### 4.3.2.1 Qualitative characters of *Hypericum mysorense*

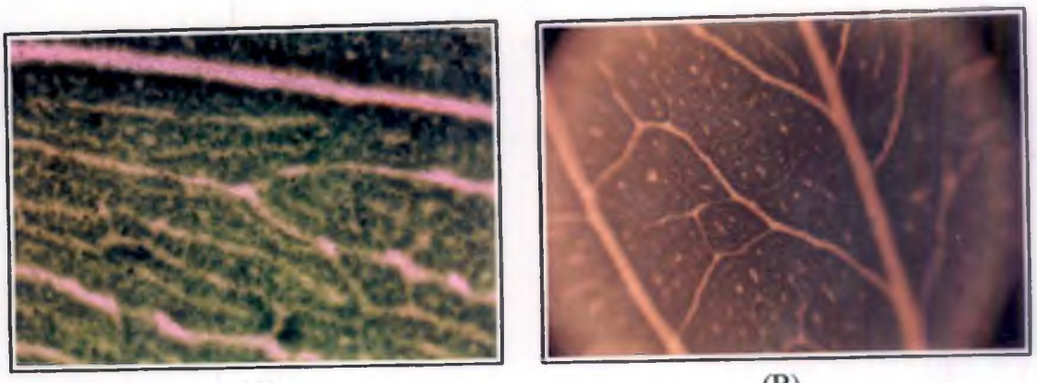
The qualitative characters of *Hypericum mysorense* located at Idukki and Wayanad districts are presented in Table 15. *H. mysorense* plants found in various locations of Idukki and Wayanad districts did not differ in any of the qualitative characters studied. Based on the observations, the species *H. mysorense* could be described as follows. *H. mysorense* is a branching shrub; deeply rooted, tap root; stem

**Table 14. Biometrical observations in *Hypericum japonicum* at different locations of Wayanad district**

| Characters/place | Puthurvayal | Chembra1 | Chembra 2 | Grand Mean | CV % | CD |
|---|---|---|---|---|---|---|
| Height of the plant (cm) | 19.27 | 19.57 | 22.56 | 20.47 | 38.06 | 4.84 |
| No. primary branches | 2.33 | 2.07 | 2.47 | 2.29 | 80.65 | 1.15 |
| Internodal length (cm) | 0.35 | 0.34 | 0.53 | 0.41 | 16.53 | 0.36 |
| First branch height (cm) | 2.46 | 2.23 | 2.88 | 2.52 | 47.82 | 0.31 |
| Leaf length (cm) | 0.81 | 0.81 | 0.81 | 0.81 | 8.70 | 0.04 |
| Leaf width (cm) | 0.36 | 0.34 | 0.34 | 0.35 | 15.67 | 0.03 |
| Petal Length (cm) | 0.37 | 0.36 | 0.37 | 0.37 | 16.59 | 0.04 |
| Petal width (cm) | 0.11 | 0.11 | 0.11 | 0.11 | 24.27 | 0.02 |
| No. of stamen | 12.87 | 13.27 | 13.33 | 13.16 | 7.70 | 0.63 |

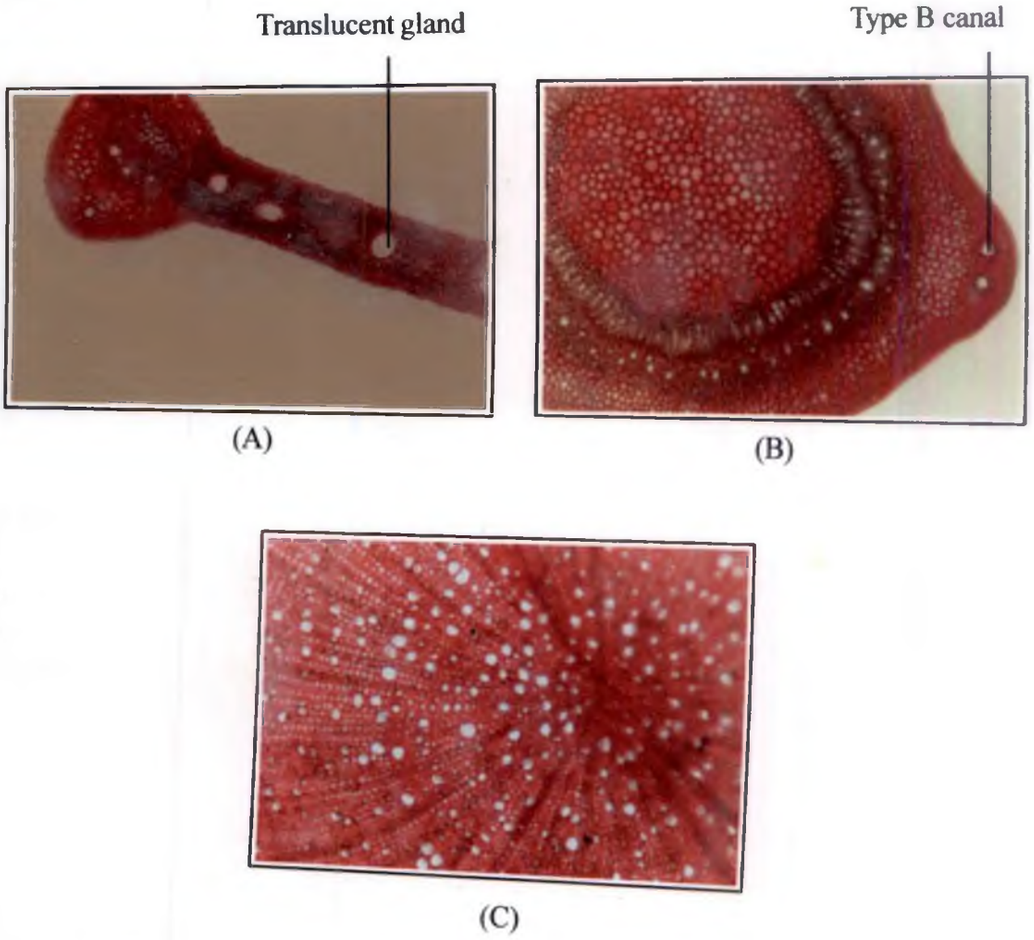
**Table 15. Qualitative characters recorded in *Hypericum mysorense* located in Idukki and Wayanad districts**

| Characters | Description |
|---|---|
| Habit | Shrub |
| Angleness in tender stem | Quadrangle |
| Angleness in matured stem | Round |
| Colour of tender stem | Green |
| Colour of matured stem | Red |
| Leaf Arrangement | Decussate |
| Petiole | Sessile |
| Leaf colour | Dark green |
| Venation | Pinnate |
| Translucent glands on Leaf | Present |
| Black glands on leaf | Absent |
| Shape of leaf tip | Acuminate |
| Shape of leaf base | Sessile |
| Leaf Margin | Entire |
| Pubescence on leaf | Absent |
| Pubescence on stem | Absent |
| Black glands on petal | Absent |
| Season of flowering | Feb-April |
| Stigma | Five |
| Stamen bundles | Five |
| No.of carpels per fruit | Five |
| Fruit colour | Dark brown |



(A) (B)

Plate 5. Variation in translucent glands of *H. mysorense* : (A) Streaks (B) Pellucid dots




(A) (B)

(C)

Plate 6. Anatomy of *H. mysorense* : (A) Leaf (B) Stem (C) Root

acetic acid : water (10:1.1:1.1:2.6) solvent system : (A) preflowering stage (B)
stage (C) seed maturation stage

quadrangular (tender) or round (mature), green tender stem on maturity turns to reddish; leaves exstipulate, sessile, decussate, dark green oblong lanceolate, entire, acuminate, prominent light green midrib; peak flowering during February-April, sparse flowering during August-September. Inflorescence terminal cyme, flowers yellow, medium size, sepals- five, green, imbricate; petals- five, yellow, free; stamens are numerous, in bundles (five); anther- yellow, globular; ovary superior, pentacarpellary; style long, five, yellow; fruit, capsule, septicidal dehiscence (Plate 2).

#### 4.3.2.2 Qualitative characters of *Hypericum japonicum*

The qualitative characters of *H. japonicum* are presented in Table 16. Based on the observations, the species *H. japonicum* could be described as follows: Annual prostrate herb, commonly found in dampy soil; tap root with numerous secondary branches; stem quadrangular green, having delicate branches. Leaves decusste, green, lanceolate, three nerved, numerous intervenal punctate glands, entire, acute, sessile, clasping the stem. Inflorescence terminal cyme with long pedicel, flower small, yellow, bracteolate, hermaphrodite, sepals five, free, green; petals- five, yellow, free, petal equaling sepals in size. Stamen 12-16, free at top, united at the base, anther globular; ovary superior, tricarpellary, parietal placentation; style short; fruit red capsule (Plate.3).

## 4.4 SECRETORY STRUCTURES IN THE LEAF

The secretory glands in the leaves of *H. mysorense* and *H. japonicum* collected from the two locations of Western Ghats are shown in Plate 5 and 8. The secretory structures appear as continuous streaks on the leaf of *H. mysorense* collected from Idukki district whereas they appear as punctate dots in the plants of Wayanad region (Plate 5). However, in *H. japonicum* the glands appeared as punctate dots (Plate 8).

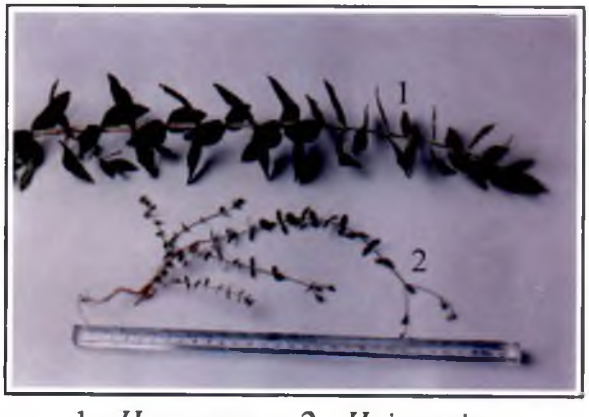
(A) (B)

Plate 1. *Hypericum spp.* in the natural habitat : (A) *Hypericum mysorense* (B) *Hypericum japonicum*

Plate 2. Flowers of *H. mysorense*


1 - Bud, 2 - Flower 3 - Fruit

Plate 3. Flowers of *H. japonicum*




1 - *H. mysorense* 2 - *H. japonicum*

Plate 4. Comparison of *Hypericum spp.*

**Table 16. Qualitative characters of *Hypericum japonicum***

| Characters | Description |
|---|---|
| Habit | Herb |
| Angleness in tender stem | Quadrangle |
| Angleness in matured stem | Quadrangle |
| Colour of tender stem | Light green |
| Colour of matured stem | Dark green |
| Leaf Arrangement | Decussate |
| Petiole | Sessile |
| Leaf colour | Light green |
| Venation | Three nerved |
| Translucent glands on Leaf | Present (Round pellucid dot) |
| Black glands on leaf | Absent |
| Shape of leaf tip | Acuminate |
| Shape of leaf base | Sessile |
| Leaf Margin | Entire |
| Pubescence on leaf | Absent |
| Pubescence on stem | Absent |
| Black glands on petal | Absent |
| Season of flowering | Sep-Oct |
| Stigma | Three |
| Stamen | Free(top)<br>United (base) |
| No. of locules per fruit | Three |
| Fruit colour | Pinkish Red |

The pith region in the periphery and the xylem vessels are positive for lignin test (blue).

### 4.5.3 Root

The cross section of *H. mysorense* root is shown in Plate 6C. The epidermis is uniseriate. The cortex region is wide and consists of parenchymatous cells. The vascular cylinder occupies the central portion of the root, which is surrounded by endodermis. The primary vascular tissue is surrounded by a region of cells termed as pericycle. In the primary body of the root, the pericycle is bordered directly on its inner surface by pholem and xylem strands. The xylem strands extending from the epidermis to the centre.

The greenish epidermis gave positive result for phenolic compounds. The bluish cortex cells gave positive response for lignin (Table 17).

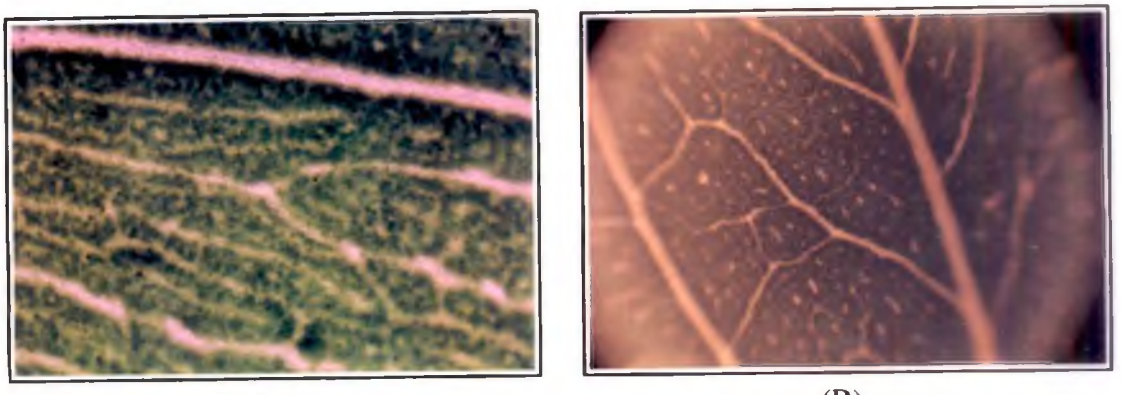
### 4.5.4 Fruit

The cross section of the fruit clearly revealed the five carpels. The fruit did not contain any glands.

## 4.6 ANATOMY AND HISTOCHEMISTRY OF *Hypericum japonicum*

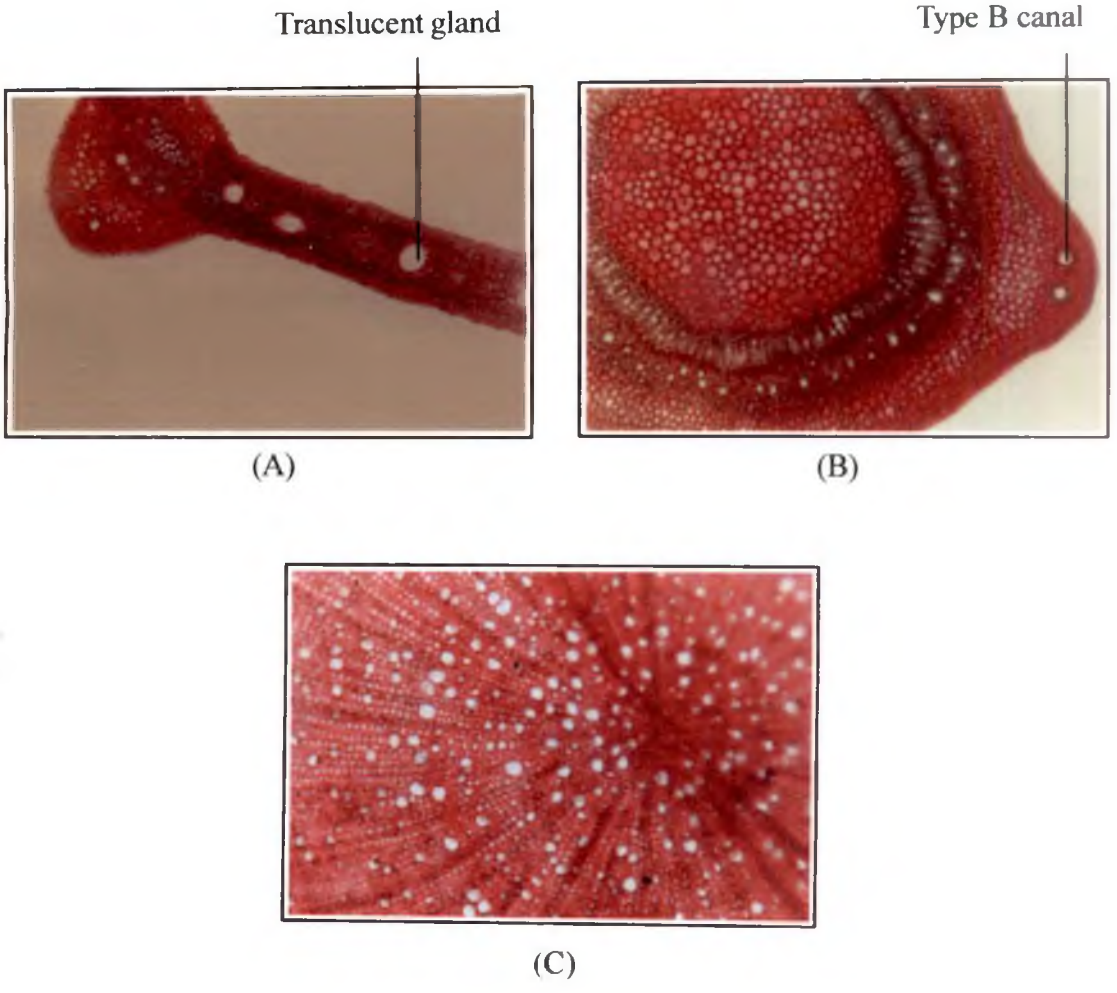
### 4.6.1 Leaf

The epidermal cells are large, arranged in single layer, mostly elongated. There are no trichomes or hairy structures in the epidermis. The mesophyll is divided into palisade and spongy tissues, although in some areas they are relatively undifferentiated. The adaxial palisade mesophyll cells are anticlinally elongated and with a few intercellular air spaces between them. The palisade tissues are arranged in one or two layers thick (Plate 8B).



(A) (B)

Plate 5. Variation in translucent glands of *H. mysorense* : (A) Streaks (B) Pellucid dots




(A) (B)

(C)

Plate 6. Anatomy of *H. mysorense* : (A) Leaf (B) Stem (C) Root

**Table 17. Histochemistry of *Hypericum mysorense***

| Chemicals tested | Leaf | Stem | Root |
|---|---|---|---|
| Lignin | + | + | + |
| Pectin | - | - | - |
| Phenolic compounds | + | - | + |

**Table 18. Histochemistry of *Hypericum japonicum***

| Chemicals tested | Leaf | Stem | Root |
|---|---|---|---|
| Lignin | + | + | + |
| Pectin | - | - | - |
| Phenolic compounds | + | + | + |

The vascular bundles were surrounded by single layered thin walled paranchymatous bundle sheath cells. Spherical or oblong translucent glands are located in the mesophyll tissues delimited by the vascular bundles (veins). The gland consists of a sub-epidermal cavity delimited by two layers of cells. The internal layer consists of very flattened, thin walled cells. The external layer consists of thicker walled parenchymatous cells. The translucent glands are situated close to the abaxial epidermis.

The vascular bundles and the epidermal cells were stained and appeared blue. This indicated that these tissues are positive for lignin (Table 18). The mesophyll tissues gave positive results for phenolic compounds (Plate 8B).

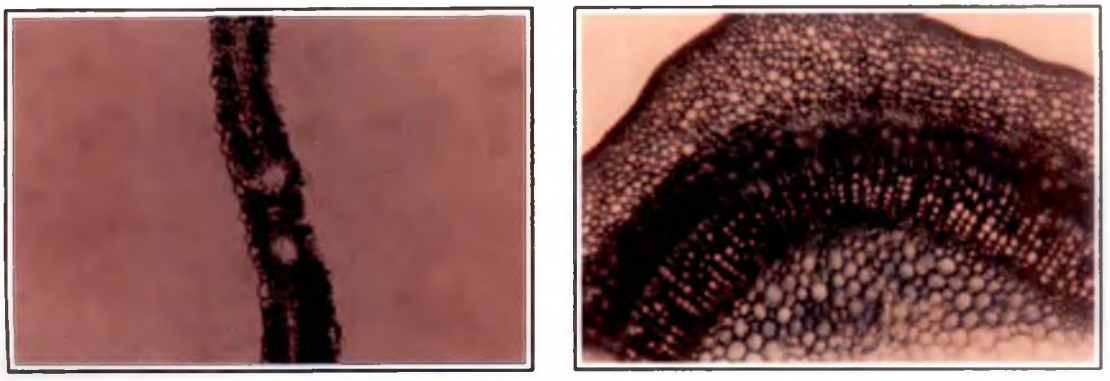
### 4.6.2 Stem

The cross section of *Hypericum japonicum* stem is shown in Plate 9A. The typical quadrangular nature of the stem is clearly visible in the plate. The cortex region is having ground tissue between the vascular tissue and the epidermis. The stem ground tissue is basically parenchymatous not interspersed with fibres. The cambium is not well developed. The xylem vessels are well developed but the phloem vessels are having small thin layered cells. The ground tissue near the epidermal region is having secretory glands which is delimited by two layers of cells.

The xylem vessels are positive for lignin and the ground tissues near the epidermis are positive for the phenolic compounds (Table 18).

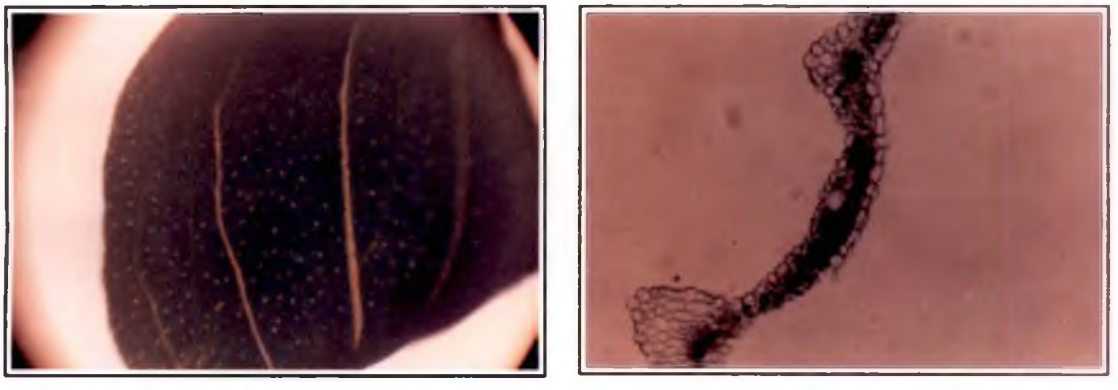
### 4.6.3 Root

The cross section of *H. japonicum* root is shown in Plate 9B. The epidermis is uniseriate. The cortex consists mainly of parenchymatous cells. The innermost layer of the cortex constitutes the endodermis. The vascular cylinder occupies the central portion of the root, and it is delimited by endodermis. The xylem strands are



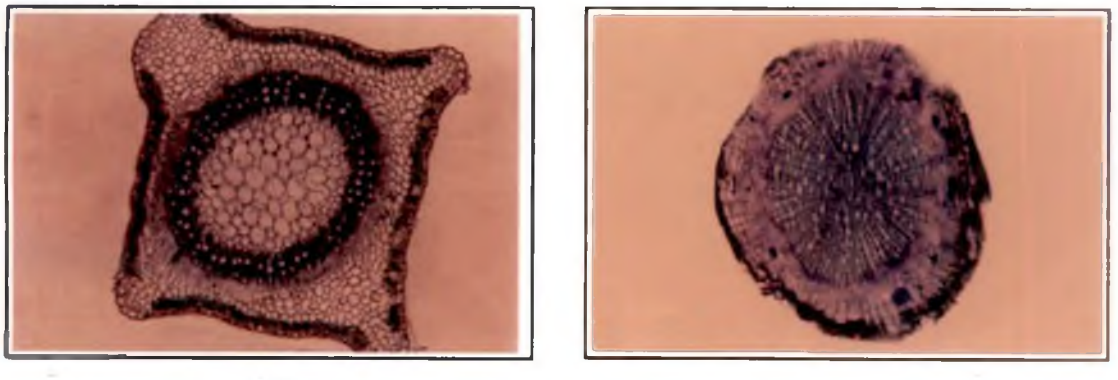
(A) (B)

Plate 7. Histochemistry of *H. mysorense* : (A) Leaf (B) Stem



(A) (B)

Plate 8. Translucent gland of *H. japonicum* : (A) Pellucid dots (B) Leaf cross section



(A) (B)

Plate 9. Anatomy and histochemistry of *H.japonicum* : (A) Stem (B) Root

numerous extending from endodermis to the centre and they are polyarch. There are no glands observed in the root.

The greenish epidermis gave positive result for phenolic compounds. The bluish cortex gave positive response for lignin (Table 18).

### 4.6.4 Fruit

The cross section of the fruit showed that it contains three carpels. The fruit wall and any other portion did not have any secretory glands.

## 4.7 BIOCHEMICAL ANALYSIS

### 4.7.1 Estimation of total extractable matter

Results of the soxhlet extraction of *Hypericum mysorense* using methanol are presented in Table 19. The per cent total extractable matter was highest at flowering stage for all the plant parts. The value varied widely from 18.75 to 31.72 per cent for the leaf at different growth stages. The lowest values were noted during pre flowering stage for leaf and stem parts. In root, the lowest value was noted during the seed formation stage.

In *Hypericum japonicum,* the per cent total extractable matter was 21.33 during the flowering stage for the pooled sample containing leaves, flowers and stem.

### 4.7.2 Estimation of starch and total sugars

The content of primary metabolites such as starch and total sugars in *Hypericum mysorense* and *H. japonicum* are presented in the Table 20. Maximum starch value (7.80 per cent) was noted in the roots of *H. mysorense*. The stem of this species had a lower starch value and the leaf the least. The samples of *H. japonicum* (leaf + stem) had lesser starch content than the *H. mysorense* leaf. The root of *H. mysorense* had the highest total sugar followed by the stem and leaf. The amount of

**Table 19. Total extractable matter of *Hypericum mysorense* at different growth stages**

| Stage of the plant | Total extractable matter (%) | | |
|---|---|---|---|
| | Leaf | Stem | Root |
| Preflowering | 18.75 | 11.70 | 11.77 |
| Flowering | 31.72 | 17.20 | 19.02 |
| Seed developing | 21.77 | 14.33 | 11.42 |

**Table 20. Starch and total sugars contents in *Hypericum* spp.**

| Plant part | Starch (%) | Total sugars(%) |
|---|---|---|
| *H.mysorense* | | |
| Leaf | 4.43 | 9.20 |
| Stem | 5.03 | 13.68 |
| Root | 7.80 | 17.25 |
| *H.japonicum* | | |
| (Leaf+stem) | 3.45 | 9.94 |

**Table 21. Total free amino acids (TFAA) and protein contents in *Hypericum* spp.**

| Plant part | TFAA (%) | Protein (%) |
|---|---|---|
| *H.mysorense* | | |
| Leaf | 0.17 | 4.20 |
| Stem | 0.60 | 0.654 |
| Root | 0.11 | 1.64 |
| *H.japonicum* | | |
| (Leaf+stem) | 0.037 | 2.31 |

total sugar in the leaves of *H. japonicum* (9.94 per cent) was slightly higher than the leaves of *H. mysorense* (9.20 per cent).

### 4.7.3 Estimation of total free amino acids and protein

The total free amino acid content was highest (0.60 %) in the stem samples of *Hypericum mysorense* and the value was the least for *H. japonicum* (Table 21). The protein content was highest (4.20) in the leaves of *H. mysorense* and the least value was noted for stem samples of *H. mysorense*. The protein value for *H. japonicum* samples is 2.31 per cent.

### 4.7.4 Qualitative tests for anthraquinone

In the tests for anthraquinone, the benzene layer did not develop yellow colour and hence the plant materials (*Hypericum mysorense* and *H. japonicum*) gave negative results in the Borntrager reaction. There was no indication of the presence of anthraquinone in the plant materials.

### 4.7.5 Detection of hypericin by chromatograms

#### 4.7.5.1 TLC spotting pattern of *Hypericum spp.* in ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6) solvent system

4.7.5.1.1 Flowering stage of *Hypericum mysorense*

On employing the solvent system ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6) on silica gel $GF_{254}$, it was observed that the spots of *Hypericum perforatum* extract (Eleve®) positively responded (Plate ) when visualised under UV light at 254 nm. Eleve®, extract of *H. perforatum* sample gave dark spots in the fluorescent background that corresponds to standard hypericin confirming the presence of hypericin. The Rf value for hypericin in this solvent system is 0.55. The

**Table 22. TLC spotting pattern of *Hypericum* spp. in ethyl acetate: formic acid : glacial acetic acid :water (10:1.1:1.1:2.6) solvent system**

| Sample | Rf value | | | |
|---|---|---|---|---|
| | 0.55 | 0.72 | 0.80 | 0.98 |
| Standard hypericin | + | - | - | - |
| *H.mysorense* Leaf | - | + | + | + |
| Stem | - | - | - | + |
| Root | - | - | + | + |
| *H.japonicum* Leaf+stem+flower | - | + | + | + |
| Eleve® (*H.perforatum*) | + | + | + | + |

+ Present, - absent

leaf, stem and root samples of *Hypericum mysorense* collected during the flowering stage and aerial parts (leaf+stem) of *H. japonicum* did not show the spots corresponding to the spot of standard hypericin (Table 22 and Plate 10B).

4.7.5.1.2 Pre-flowering stage of *Hypericum mysorense*

The chromatograms of the leaf, stem and root samples of *Hypericum mysorense* collected during the pre-flowering stage are similar to that of the flowering stage while employing the solvent system ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6) on silica gel $GF_{254}$ (Plate 10A).

4.7.5.1.3 Seed maturation stage of *Hypericum mysorense*

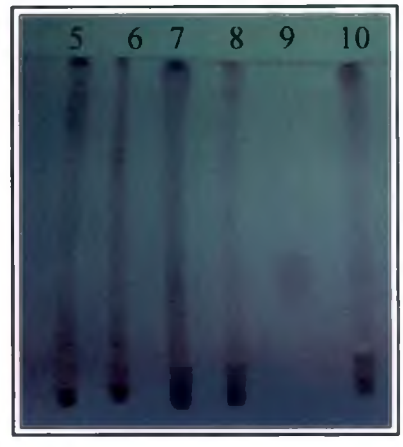
The chromatograms done using the leaf, stem, and root samples of *Hypericum mysorense* during the seed maturation stage are similar to that of the chromatograms of the flowering stage (Plate 10C).

**4.7.5.2 TLC spotting pattern of *Hypericum* spp. in toluene: ethyl acetate: formic acid (50:40:10) solvent system**

4.7.5.2.1 Flowering stage of *Hypericum mysorense*

Result of qualitative detection of hypericin in different parts of *Hypericum* spp. during flowering stage in toluene:ethyl acetate:formic acid (50:40:10) solvent are presented in the Table 23 . The standard hypericin produced red colour spot having the Rf value of 0.63. Only the *H. perforatum* extract produced the red spot coinciding with the standard which confirmed the presence of hypericin. It also produced an unseparated mass of red spot having the Rf value of 0.57 and a red spot with Rf value of 0.98. The leaf of *H. mysorense* gave three dark spots among them the spot with Rf value of 0.86 was unique to that sample. The stem of the species produced four


(A)




(B)


(C)

1, 5, 12 - Leaf. 2, 6, 13 - Stem. 3, 7, 14 - Root. 10 - Eleve®. 4, 9, 11 - Standard hypericin. 8 - *H. japonicum* (except 8 and 10 all samples are *H. mysorense*)

Plate 10. TLC spotting pattern of *Hypericum spp.* in ethyl acetate : formic acid : glacial acetic acid : water (10:1.1:1.1:2.6) solvent system : (A) preflowering stage (B) flowering stage (C) seed maturation stage

**Table 23. TLC spotting pattern of *Hypericum* spp. in toluene: ethyl acetate: formic acid (50:40:10) solvent system**

| Sample | Rf value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.43 | 0.51 | 0.57 | 0.63 | 0.75 | 0.78 | 0.86 | 0.88 | 0.98 |
| Standard hypericin | - | - | - | * | - | - | - | - | - |
| *H.mysorense* Leaf | - | - | + | - | + | - | + | - | + |
| Stem | - | ▫ | - | - | ▫ | ▫ | - | ▫ | - |
| Root | + | - | ▫ | - | ▫ | ▫ | - | ▫ | + |
| *H.japonicum* Leaf +Stem | - | + | + | - | - | - | - | - | + |
| Eleve® (*H.perforatum*) | - | - | * | * | - | - | - | - | - |

* Red spot, + brown spot, ▫ flouresent spot, - no spot

fluorescent spots. The same four spots (Rf value 0.75, 0.51, 0.78, 0.88) in the stem were also observed in the root along with two other black spots with Rf values of 0.43 and 0.98.

The spotting pattern of *H. japonicum* was different from the *H. mysorense* leaf and stem. Since the sample of *H. japonicum* contained both leaf and stem it showed coinciding spot with *H. mysorense* leaf (0.98) and *H. mysorense* stem (0.51). In *H. japonicum*, the spot with a Rf value of 0.57 was also noted. All these spots were dark brown in colour (Plate 11B).

4.7.5.2.2 Pre-flowering stage of *Hypericum mysorense*

The results of the qualitative detection of hypericin in different parts of *Hypericum mysorense* collected during the pre-flowering stage were same as in the flowering stage (Plate 11 A), in the solvent system viz., toluene: ethyl acetate: formic acid (50:40:10).

4.7.5.2.3 Seed maturation stage

The leaf, stem, root samples of *H. mysorense* collected during the seed maturation stage did not show any variation from the samples of flowering stage in the solvent system (Plate 11 C), toluene: ethyl acetate: formic acid (50:40:10).

**4.7.5.3 TLC spotting pattern of *Hypericum* spp. in ethyl acetate: formic acid (50:6) solvent system**

4.7.5.3.1 Flowering stage of *Hypericum mysorense*

The spotting pattern of *Hypericum* spp. in ethyl acetate: formic acid (50:6) solvent system are presented in the Table 24. The leaf sample of *H. mysorense* produced two spots with the Rf values of 0.57 and 0.99. Among these, the spot with the Rf value of 0.57 was unique to the sample. Stem sample produced two spots of


(A)


(B)


(C)

1, 5, 11 - Leaf. 2, 6, 12 - Stem. 3, 7, 13 - Root. 10 - Eleve®. 4, 8, - Standard hypericin. 9 - *H. japonicum* (except 9 and 10 all samples are *H. mysorense*)

Plate 11. TLC spotting pattern of *Hypericum spp.* in toluene : ethyl acetate : formic acid (50:40:10) solvent system : (A) preflowering stage (B) flowering stage (C) seed maturation stage

**Table 24. TLC spotting pattern of *Hypericum* spp. in ethyl acetate: formic acid (50:6) solvent system**

| Sample | Rf value | | | | |
|---|---|---|---|---|---|
| | 0.57 | 0.76 | 0.86 | 0.98 | 0.99 |
| Standard hypericin | - | - | - | * | - |
| *H.mysorense* Leaf | + | - | - | - | + |
| Stem | - | - | + | - | ▫ |
| Root | - | + | - | - | ▫ |
| *H.japonicum* Leaf +Stem | - | + | - | - | + |
| Eleve® (*H.perforatum*) | - | - | - | * | * |

* Red spot, + brown spot, ▫ fluorescent spot, - no spot

which one with 0.76 Rf value was unique to that sample. The spot with the Rf value of 0.86 was unique to the root sample. The spot near the solvent front (Rf value 0.99) was uniform in all the *Hypericum* spp.

The extract of *H. perforatum* gave red spot at the Rf value of 0.98, corresponding to the standard hypericin (Plate 12B). It also produced an unseparated mass of red spots with an Rf value of 0.99. *Hypericum japonicum* produced two spots with Rf value of 0.86 and 0.99, with dark brown colour.

4.7.5.3.2 Pre-flowering stage of *Hypericum mysorense*

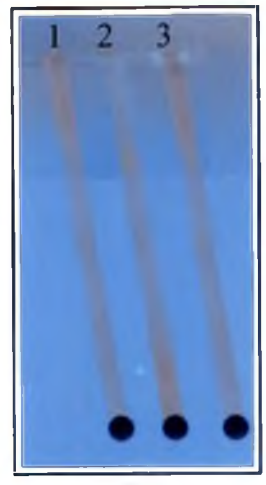
The spotting pattern of leaf, stem and root of *H. mysorense* collected during the pre-flowering stage were similar to the spotting pattern of samples of flowering stage (Plate 12 A), in ethyl acetate: formic acid (50:6) solvent system.

4.7.5.3.3 Seed maturation stage

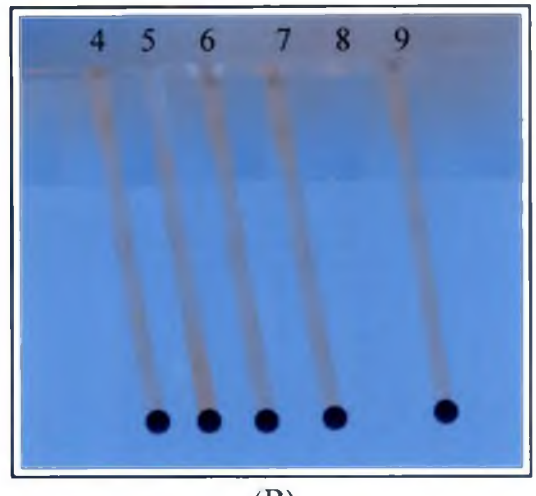
There was no variation observed in the chromatograms employed using leaf, stem, root of *H. mysorense* in seed maturation stage and flowering stage (Plate 12C), in the solvent system ethyl acetate: formic acid (50:6).

### 4.7.6 Quantification of hypericin from the commercial extract of *Hypericum perforatum* (Eleve®)

The commercial extract of *Hypericum perforatum* (Eleve®) that gave positive response in the chromatograms were used for quantification. The per cent hypericin ranged from 0.22 to 0.25 in that sample.




(A)




(B)


(C)

1, 4, 11 - Leaf. 2, 5, 12 - Stem. 3, 6, 13 - Root. 7 - Eleve®. 8, 10 - Standard hypericin. 9 - *H. japonicum* (except 7 and 9 all samples are *H. mysorense*)

Plate 12. TLC spotting pattern of *Hypericum spp.* in ethyl acetate : formic acid : (50:6) solvent system : (A) preflowering stage (B) flowering stage (C) seed maturation stage

# *Discussion*

# 5. DISCUSSION

The results of the study entitled "Morphological and phytochemica investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HI\ compounds" are discussed in this chapter.

## 5.1 SURVEY OF THE AREA

### 5.1.1 Folk use of *Hypericum*

In folk medicine, the traditional use of plants is eco-system and ethni community specific, with each community having developed its own traditiona remedies based on plants and other natural resources found in the surrounding forest (Thayil, 1997). In the present study, an attempt has been made to document the loca medicinal uses of *Hypericum mysorense* by the people of Vattavada. The leaf juice o *H. mysorense* is widely used for treating wounds. This effect has been confirmed b' Mukherjee and Suresh (2000) using the oinment produced from the extract of *H mysorense* leaves in different experimental models of wounds in rats. According t( them the effect produced by the *H. mysorense* extract oinment, in terms of woun( contracting ability, wound closure time, regeneration of tissues at the wound site an( tensil strength of the wound were comparable to those of the standard dru( nitrofurazone oinment.

## 5.2 NATURAL HABITAT ANALYSIS

### 5.2.1 Climate and soil properties

The edaphic factor of the habitat was characterised by sandy loam soi with acidic pH, with low to moderate moisture retention capacity. The fertility statu: of the soil was low in N and K and medium in P. The climatic factor of the habita

revealed that this region enjoys typical warm and humid tropical climate, receiving above average rainfall of the state. The altitude of the study area ranges between 1800 m to 2100 m above mean sea level, which is comparable with Nilgiris and Palani hills from where also *H. mysorense* was reported by Fyson (1932). At this altitude, *Hypericum mysorense* was distributed from areas receiving high light intensity (grass lands) to partially shaded areas such as beneath the trees, which indicates that this species may not be exact for light requirement.

### 5.2.2 Vegetation studies and phytosociological parameters

Each community is characterised by its species diversity, growth forms, structure, dominance, successional trend etc. To study the details of these aspects of any community, a number of characters are taken into consideration. These are then used to express the characteristics of a community. Among these characteristics density, abundance and per cent frequency are the important analytical quantitative characters which are used to give the idea of distribution and degree of competition of any species in a community.

Density gives the idea of degree of competition among the species in the natural habitat. The density of *Hypericum mysorense* in the study areas of Idukki district ranged from 0.9 to 1.7. *Chrysopogon zeylanicus* is the dominant species in the community comprising *H. mysorense*. Since *C. zeylanicus* is a grass, the number of total individuals in the unit area was the highest. *Eucalyptus grandis* had the lowest density among all the companion species in the community. Since *Eucalyptus* is a tree species, the area occupied by the individuals of the species is high. Hence the density was lowest for *Eucalyptus*.

Percentage frequency gives the idea of distribution of any species in the natural habitat. When the frequency value is correlated with the density, it will give correct

idea about the distribution of the species. The percentage frequency of *H. mysorense* ranged from 30 to 60. In the location Irukkanai, the species had the highest density and percentage frequency, which indicates that the distribution of *H. mysorense* was the highest in this location.In Pazhathottam 2, density and frequency of *Chrysopogon* were high. The soil type in this location is highly suitable for the growth of *Chrysopogon*.

*Hypericum mysorense* was found along with grasses like *Chrysopogon zeylanicus* and *Andropogon lividis*, ferns like *Pteridium aquilinum*, perennial shrub like *Strobilanthus* and trees like *Eucalyptus grandis* in the study area as a community. The climatic requirement and soil suitability of *H. mysorense* were similar to that of the above said companion species especially with *Pteridium*. It would be an useful indication for plant explorer to locate *H. mysorense*. The density of *H. mysorense* (0.9-1.7) and its abundance (2.2-3.0) values were from equal to three times lower than that of *Pteridium*.

The per cent frequency of *H. mysorense* in the study area were 30 to 60 falling under the frequency class B or C. In the habitat *Pteridium*, *Andropogon* and *Chrysopogon* also had the same frequency classes, which indicates a similar distribution as that of *H. mysorense*.

## 5.3 STUDY OF MORPHOLOGICAL CHARACTERS

### 5.3.1 Biometrical observations

#### 5.3.1.1 Biometrical observations on *Hypericum mysorense* of Vattavada in Idukki district

Among the seven locations, *H. mysorense* growing at Pazhathottam 1 was significantly different from the plants in the rest of the area for the characters *viz.*, height of the plant, number of primary branches, internodal length, leaf length and

leaf width. Height at the first branch from the ground level showed a wide variation from 33.45 cm (Sudalai) to 61.20 cm (Irukkanai). Significant difference over the grand mean for this character was observed at Pazhathottam 1, Irukkanai and Kottakkompuru.

The floral characters like sepal length, sepal width, petal length, style length and stamen length did not show any significant variation from the grand mean. This may be due to additive gene action with less environmental influence. However, the floral characters *viz.*, petal width, number of stamen and number of days from flowering to fruiting showed differences over their respective grand means in one or more locations. These characters may be controlled by polygenes, more influenced by environment.

Hooker (1875) described *H. mysorense* as a glabrous shrub 4-6 feet high, young branches four angled, leaves 1-2 inches, tapering to an amplexicaul base, with slender ascending veins and pellucid striae. Cymes terminal, sepals acute, petal obliquely oblanceolate. Styles twice as long as the ovary, capsule about half an inch length. This description agreed with the observations recorded in the study.

The leaf and floral biometrical characters of *H. mysorense* also agreed with the description by Fyson (1932), Gamble (1957), Nair and Henry (1983) and Sivarajan and Mathew (1997).

### 5.3.1.2 Biometrical observations on *Hypericum mysorense* of Kalpetta in Wayanad district

The biometrical characters of *Hypericum mysorense* agreed with the description given by Hooker (1875), Fyson (1932), Gamble (1957), Nair and Henry (1983) and Sivarajan and Mathew (1997).

### 5.3.1.3 Variation for biometrical characters in *Hypericum mysorense*

The results on various biometrical data recorded at different locations of Idukki and Wayanad districts showed variation for all the characters under study. However, the variations were not statistically significant between districts. Neverthless, within a district significant differences between locations were observed for vegetative characters like height of the plant, number of primary branches, internodal length and leaf length over their respective grand means, as evident from the high co-efficient of variations. In Vattavada, the plants at Pazhathottam 1 out performed for these characters. Pazhathottam 1 is the location having highest altitude among all habitats with flat grasslands and human interventions. That could be the reason for the maximum growth of the species here. Similarly in Kalpetta, plants at Chembra 2 out performed for the above characters.

As regard to floral traits like sepal length, sepal width, petal length, petal width, style length, stamen length and numbers of stamen per flower no variations between and within districts, could be noted. These characters showed low co-efficient of variation.

### 5.3.1.4 Variation for biometrical characters in *Hypericum japonicum*

Morphometric traits like height of the plant, number of primary branches, internodal length, height at first branch from ground level, leaf length, leaf width, petal length, petal width and number of stamen per flower recorded at Puthurvayal, Chembra 1 and Chembra 2 of Wayanad district did not exhibit significant differences over the locations over grand mean, for all the characters except height of first branch from ground level. However, barring leaf length and number of stamen per flower, all

these characters recorded high co-efficient of variation which might be due to inter plant variations (with high standard deviation) within locations.

The biometrical characters of *Hypericum japonicum* agreed with the description given by Hooker (1875), Fyson (1932).

### 5.3.2 Qualitative characters of *H. mysorense* and *H. japonicum*

*Hypericum mysorense* found at various locations of Idukki and Wayanad districts did not differ in any of the qualitative characters studied. Data on qualitative characters recorded from these regions also confirm with that of the description given by Hooker (1875), Fyson (1932) and Sivarajan and Mathew (1997) except for secretory glands in the leaf. While Hooker (1875) mentioned the presence of "Pellucid striae" in the leaf, samples of Idukki district had "Pellucid streaks" and Wayanad samples exhibited "Pellucid dots". Therefore, the presence of either pellucid dotted or pellucid streak glands may be considered as a common character in *H. mysorense*.

Likewise, the qualitative characters recorded in *H. japonicum* were similar to the description given by Hooker (1875), Fyson (1932), Mathew (1983) and Kumar (1993). It was clear that the qualitative characters did not exhibit variation between locations in Wayanad district as they are governed by major genes.

From the interspecific differences observed between *H. mysorense* and *H. japonicum*, it becomes clear that *H. japonicum* was common in moist places or shallow standing water whereas *H. mysorense* was common in drained areas. *H. mysorense* is a shrub having red round stem , while *H. japonicum* is a herb with dark green quadrangular stem at the matured stage. The venation in *H. mysorense* is pinnately netted while *H. japonicum* has three nerves. Peak flowering season for *H.*

*mysorense* was from February to April with sparse flowering noted during August-September. In *H. japonicum* flowering was noticed during September to November. Since the exploration and collection was made during September to November there were no details about the flowering of *H. japonicum* in other seasons. Pinkish red fruits with brown seeds were noticed in *H. japonicum* while the fruit in *H. mysorense* was dark brown with brown to black seeds.

## 5.4 ANATOMY OF *Hypericum* spp.

It is well known that plants secrete various chemical substances. These products of metabolism are frequently deposited as forms that can readily be seen under the microscope and when this is so they can very often be utilized by systematic anatomists as diagnostic characteristics. The secreted materials may be deposited in cells of intercellular spaces and they also occur in more elaborate structures such as canals lined with a secretory epithelium. Besides being deposited in cells, cavities and canals, which are embedded in tissues of various plant organs, chemical substances are also secreted in glandular trichomes and other structures which arise from the plant surface.

Anatomical and histochemical studies in medicinal and aromatic plants are imperative to identify the species, to know the special type of anatomical structures, which will throw light on the type and content of secondary metabolities. *Hypericum* L. is characterised by the presence of different types of secretory structures including translucent glands, black nodules and secretory canals (Ciccarelli *et al.*, 2001b). Not all of these structures are present in all species of the genus and their presence and/or frequency vary among plant organs and are also important in the recognition of different taxa (Robson, 1981). Robson (1981) also found that occurrence of black glands is an accurate indication of the presence of hypericin. No such previous report was available in this regard on indigenous species like *H. mysorense* and *H.*

*japonicum*. Salient findings on the anatomy of leaf, stem and root are discussed below.

### 5.4.1 Leaf

The result of the present study revealed the presence of translucent type of spherical glands on interveinal portion of *H. mysorense* and *H. japonicum* leaves. These observations are in confirmity with Ciccarelli *et al.* (2001 a) who observed translucent glands in *H. perforatum* leaves. Translucent glands are spherical or oblong glands consisting of a sub-epidermal cavity delimited by two layers of cells. The internal layer consists of very flattened, thin walled secretory cells. The external layer consists of thick walled parenchymatous cells. Although taxonomists like Hooker (1875) and Fyson (1932) noted the presence of translucent glands in the leaves of *H. mysorense* and *H. japonicum* and Mathew (1983) and Kumar (1993) in *H. japonicum*, detailed anatomical studies were not conducted. This study is a pioneer attempt in this regard.

### 5.4.2 Stem

Elongated, pale, translucent canals were observed in the cortex (below epidermis). These glands are having an inner layer of flattened cells and outer layer of thick walled parenchymatous cells. Such anatomical structure in *H. perforatum* was designated as "type B" secretory canal by Ciccarelli *et al.* (2001a) They described "type B" canals as numerous, elongated, translucent structures located in stem, sepals and petals of *H. perforatum*. In the stem of *H. japonicum* no secretory structures was found.

### 5.4.3 Root

Ciccarelli *et al.* (2001a) reported secretory canals with wide lumen delimited by four polygonal cells in the roots of *H. perforatum*. This was designated

as "type A" canals. However, in the present study none of the secretory canals or glands was observed in the roots of *H. mysorense* and *H. japonicum*.

Anatomical studies of the fruits of *H. mysorense* and *H. japonicum* did not reveal any secretory glands.

## 5.5 HISTOCHEMISTRY OF *Hypericum* spp.

Histochemical staining is a quick and cost saving method to know the presence or absence of primary or secondary metabolites in plant cells and tissues by suitable staining. Lignin is the hydrophobic cementing material of the cell wall that makes the wall impervious to water. It permeates and fills the spaces existing in the fibrous skeleton of cellulose and stiffens and protects the cell wall. Staining with toluidine blue of the hand sectioned tissues of *H. mysorense*, indicated the presence of lignin in vascular bundle and epidermal cells of leaf, vessels and pith of stem and cortical cells of root, the same holds good for *H. japonicum* also. Hence, the above-mentioned tissues may be hardier in nature compared to the other tissues in the organs.

Phenolics form another group of natural products contributing significantly to the medicinal values of a number of plants. Quinones (benzo, napththo, anthra), lignans, coumarins, flavonoids and tannins are the major groups of phenolics exhibiting marked pharmacological activity. In *H. mysorense*, presence of phenolic compounds was noted in leaf mesophyll cells and root epidermis. However, tender stem did not show any indication of the presence of phenolics whereas lesser content was observed in mature stem as evident from faint staining. In *H. japonicum* phenolic compounds were present in leaf mesophyll cells, ground tissues (stem) and root epidermal cells.

Positive results of phenolics might be considered as an indication for the presence of anthrone or anthraquinone derivatives, which fall in the phenolics group.

## 5.6 BIOCHEMICAL ANALYSIS

### 5.6.1 Total extractable matter

In *Hypericum mysorense*, leaf contained maximum extractable matter in all the growth stages. Related species *viz.*, *H. perforatum* and *H. maculatum* yielded maximum extractables during the vegetative phase (Kireeva *et al.* 1999). The leaf, stem and root had the highest amount of extractable matter during the flowering stage. This may be due to the high level of metabolic activity in all the plant parts during the flowering stage. The degradation and conversion of large molecules to smaller one take place at this stage, which may result in higher quantity of extractables.

### 5.6.2 Variation pattern in primary and secondary metabolites

Soluble sugars are the initial products in photosynthesis. Plants always maintain equilibrium of soluble sugars in the source and whenever the concentration exceeds it is either converted to the polysaccharide, starch or inter-converted to other primary products or translocated to other organs for the synthesis of secondary products. Once it is in the form of starch, further transformations are slow or limited. The amount of starch and total sugars were highest in roots followed by stem and leaf.

Generally, sugars are produced in the leaves and accumulated in the roots. Since sugar is the base for the synthesis of starch, the quantity of starch may have direct relation with it. In the stem and root of *H. mysorense*, the pattern of sugar and starch content was the same. Similarly, the leaf sample of both *Hypericum* species had same pattern for sugar and starch contents. The leaf of both the species had low total sugar and high starch (Table.25). It may be due to the greater conversion of sugar into starch. The total extractables were high in the leaf of *H. mysorense* and low

**Table 25. Pattern of variation in primary and secondary metabolites in *Hypericum* spp.**

| Primary metabolites and extractable matter | *Hypericum mysorense* | | | *Hypericum. japonicum* Leaf+Stem |
|---|---|---|---|---|
| | Leaf | Stem | Root | |
| Total sugars | L | H | H | L |
| Starch | H | H | H | H |
| TFAA | L | H | L | L |
| Protein | H | L | L | H |
| Total extractable matter | H | L | L | L |

H - high quantity, L – low quantity

in other parts and in *H. japonicum* (Figure.1). Though the leaf of *H. mysorense* and aerial parts of *H. japonicum* had the same trend in total sugar content (9.20 and 9.94 per cent respectively), they differed in the total extractables (31.72 and 21.33 per cent at flowering stage). Hence, it could be presumed that the dynamics of conversion of sugar to secondary metabolites in these species do not follow a definite pattern.

Amino acids are the precursors of many secondary metabolites. Kudesia and Jetley (1995) reported that the precursors for the biosynthesis of alkaloids are amino acids such as ornithine, lysine, phenyl alanine, tyrosine and tryphtophan.

Leaf of *H. mysorense* had minimum quantity of free amino acids with a high quantity of protein whereas the stem had higher quantity of amino acids and lower level of protein than the leaf (Figure 2). The same pattern was noted in the sample comprising of aerial parts of *H. japonicum*. However, the reverse trend was noted in the stem of *H. mysorense*. In the root of the species both free amino acids and protein were very low (Table.25). Hence, any type of correlation between the quantity of total free amino acids, protein and secondary metabolites (total extractable matter) could not be made. Hence, it may be assumed that there will be no relation between the quantity of free amino acids and secondary metabolites, which indicate, that there might not be any conversion of free amino acids into secondary metabolites in these species. These total free amino acids may be responsible for the pharmacological properties reported for the species except the anti –HIV action.

Amino acids like glutamine, leucine, lysine, ornithine, proline, threonine, GABA, scopoletin and umbelliferone were reported in *H. perforatum* by Kumar *et al.* (2000). Among these amino acids GABA is having sedative property.

### 5.6.3 Detection of hypericin by chromatograms

Standard hypericin produced a dark spot with an Rf value of 0.55 that is shown by commercial extract of *H. perforatum* Eleve$^{(R)}$ capsule along with an additional

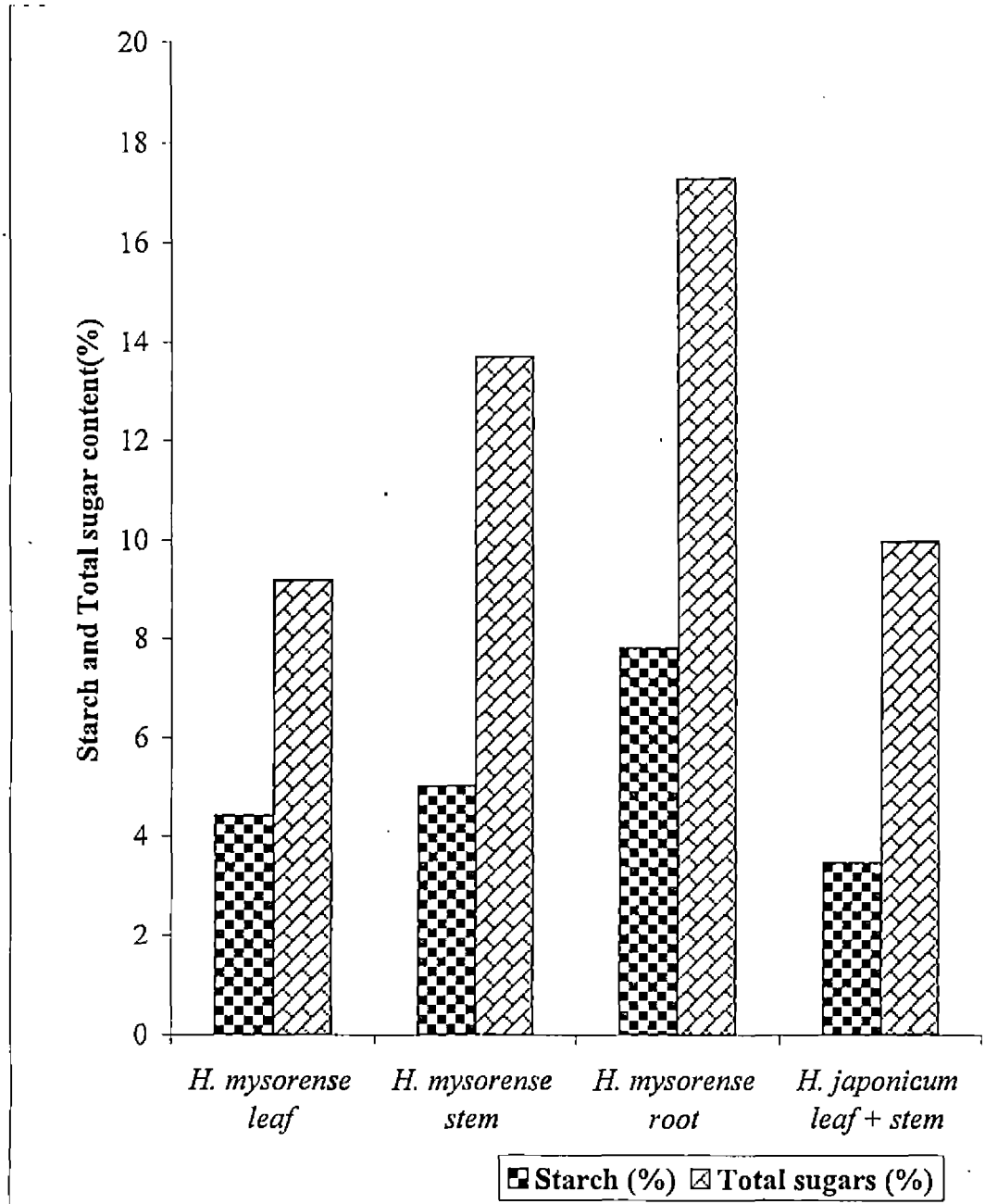



**Fig. 1. Starch and total sugar content in *Hypericum* spp**

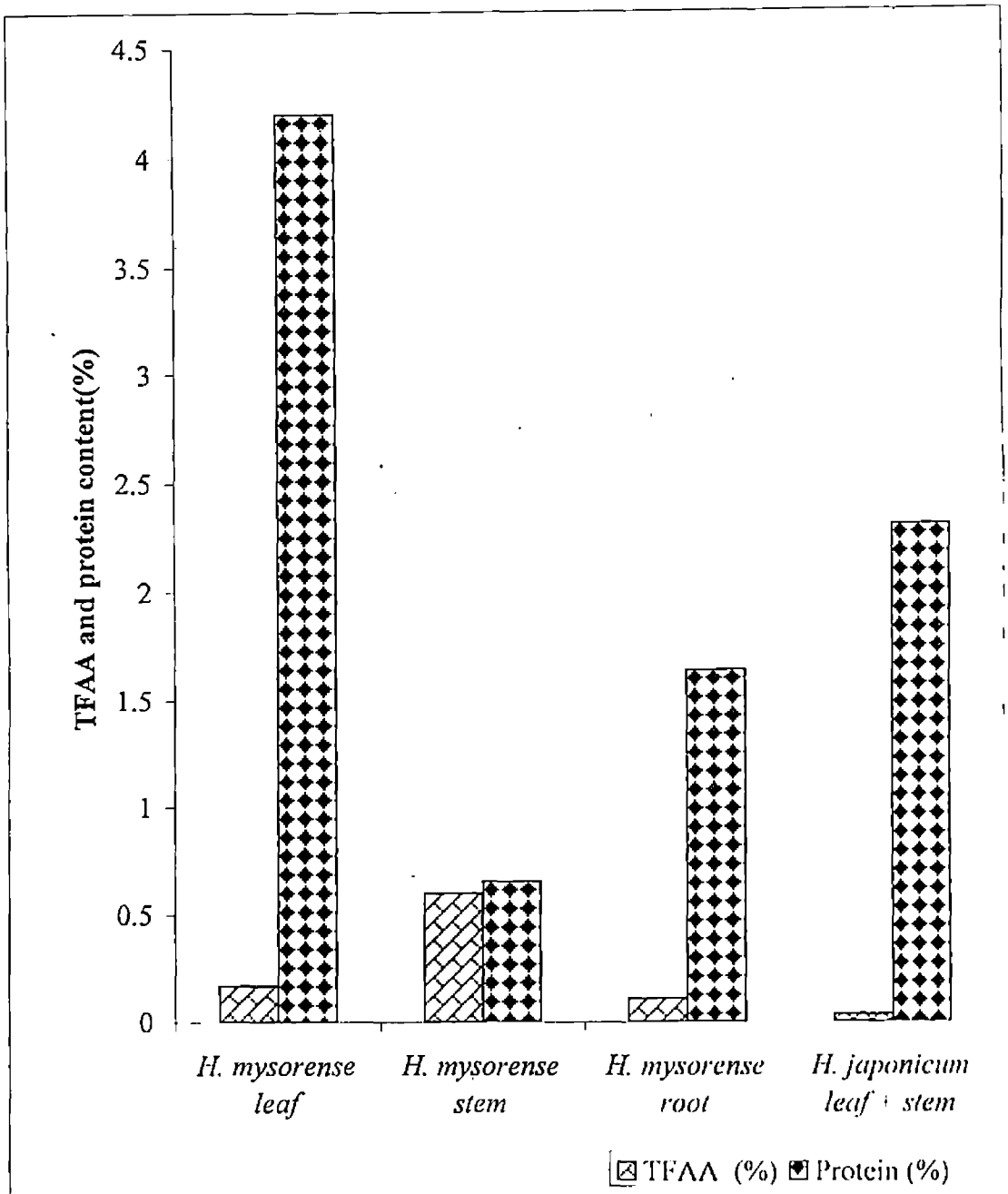



**Fig. 2. Total free amino acids (TFAA) and protein content in *Hypericum* spp.**

spot of 0.80 Rf value. Leaf, stem and root of *Hypericum mysorense* and *H. japonicum* showed a maximum of three spots of Rf values 0.72, 0.80 and 0.98 with a colour similar to that of hypericin standard. It is an indication of compounds of hypericin group or other anthrone derivatives that may express similar properties in the biological system. Leaf of *H. mysorense* and aerial part (Leaf + stem) of *H. japonicum* showed the maximum number of spots, whereas the stem had only one spot and the root had two spots. This variation may be explained as the difference in the production of hypericin like compounds by different plant organs. The above details were observed in silica gel GF plates using ethyl acetate: glacial acetic acid: formic acid: water (10:1.1:1.1:2.6) solvent system.

Similarly, the study was conducted with another solvent system toluene: ethyl acetate: formic acid (50:40:10) and fluorescent spots were recorded in the similar pattern as mentioned above. The number of spots and Rf values were different, but the standard and Eleve$^{(R)}$ capsule showed red fluorescent spots. The capsule showed three red fluorescent spots whereas the standard had only one spot with an Rf value of 0.63. Even though the fluorescent and dark brown spots were expressed in different Rf values in the solvent system, the trend of spot development indicated a supporting hypothesis of the first solvent system already explained.

It is interesting to note that the leaf sample of both the species and commercial extract of *H. perforatum* (Eleve®) had a common spot with the Rf value of 0.57. However, the spot was red in Eleve and brown in the leaf sample of *H. mysorense* and *H. japonicum*. This indicate the presence of a common anthrone derivatives in *H. mysorense* and *H. japonicum* which may be related to the pseudo hypericin or other anthrone derivatives present in *H .perforatum* (Eleve®). This is further supported by the spot of hypericin (Rf value=0.63), which is adjacent spot to the above-mentioned ones. Hence, it may be suggested that the compound giving spot with Rf value of

0.57 may be recorded as a precursor or an intermediary compound in the pathway of hypericin synthesis. Kitanov (2001) explained similar anthronoid derivative in the species belonging to the tribe 'Hypericeae' (*Hypericum* L.). Results of the present study will be further confirmed from the taxonomical inclusion of *H. mysorense* and *H. japonicum* in the tribe 'Hypericeae' (Hooker, 1875). Hence, it may be concluded that the spot with Rf value 0.57 in the leaves of *H. mysorense* and *H. japonicum* may be an anthronoid derivative.

In ethyl acetate: formic acid (50:6) solvent system, samples expressed similar trend with less number of spots. In this solvent system, the leaf samples of *H. mysorense*, samples from aerial parts of *H. japonicum* and commercial extract of *H. perforatum* (Eleve®) had the common spot with an Rf value of 0.99. It is an indication that the solvent system had some influence in the separation of secondary metabolites. Among the above three systems, toluene: ethyl acetate: formic acid (50:40:10) seems to be good in extraction.

Based on the results of the present investigation, the similarities and differences among the indigenous *Hypericum* spp. (*H. mysorense* and *H. japonicum*)(Plate4) and the exotic widely known anti-HIV source *H. perforatum* could be summarized in Table26.

Even though the samples of the indigenous *Hypericum spp.* ( *H. mysorense* and *H. japonicum* ) were negative for hypericin, the target anti-HIV compound, they were indicative for the presence of anthronoid derivatives. These anthronoid derivatives could be the precursors or intermediary compounds for the hypericin synthesis in the plant metabolism. These compounds need further analysis for identification, characterization and for confirming their anti-HIV properties.

**Table. 26 Comparison study among the indigenous *Hypericum spp.* and exotic *H. perforatum***

| Characters | *H. mysorense* | *H. japonicum* | *H. perforatum* * |
|---|---|---|---|
| Habitat | High altitude | Medium altitude | High altitude |
| Habit | Shrub | Prostrate herb | Shrub |
| Leaf arrangement | Decussate | Decussate | Decussate |
| Leaf size | Large | Small | Large |
| Translucent glands on leaf | Dotted or streaks | Dotted | Dotted |
| Secretory canals in stem | Type B canals | Absent | Type A &B canals |
| Secretory canals in root | Absent | Absent | Type A canals |
| Flower colour | Yellow | Yellow | Yellow |
| Flower size | Large | Small | Large |
| Stamen | United, five bundles | Free at top; united at base | United, three bundles |
| Stigma | Five | Three | Five |
| Ovary | Pentacarpellary | Tricarpellary | Tricarpellary |
| Black glands | Absent | Absent | Noticed in leaf, stem, sepal, petal, stamen, ovary |
| Hypericin | Absent | Absent | Present |

* as described by Bombardelli and Morazzoni (1995)

# *Summary*

# 6. SUMMARY

The scientific community is curiously searching for plant -based chemicals for curing many devasting diseases. Ever since the identification of hypericin from *Hypericum perforatum* as a potential drug against HIV, a thorough study of the genus *Hypericum* is under way. In tune with this, the study entitled "Morphological and phytochemical investigations on St. John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds" was undertaken with major thrust on native flora (*Hypericum* spp.) of Kerala. The salient results of the present study are summarised and presented hereunder:

From the survey, two species of *Hypericum* viz., *H. mysorense* and *H. japonicum* were identified. *H. mysorense* grows naturally as a weed at an altitude range of 800-1200 m above MSL. This species was located both in Idukki and Wayanad districts of Kerala. In Idukki, it was abundant in the high altitude grasslands. The preferred habitats were characterised by 12°-25° C while the relative humidity was 54 - 84 per cent.

*H. mysorense* occurred along with *Chrysopogon zeylanicus, Andropogon lividis* (grasses), *Pteridium aquilinum* (fern), *Strobilanthus* sp (shrub) and *Eucalyptus grandis* (tree) in the natural habitat as a community. Among the above-mentioned plants, *Chrysopogon zeylanicus* was the dominant species. The per cent frequency of *H.mysorense* was categorised as B and C, which reveals the status of the plant in the natural habitat, as *seldom present* and *often present.*

Among the morphological characters, barring height at first branching from the ground level, the remaining vegetative characters did not show significant differences over the study area of Idukki district. Similarly, excepting floral characters like petal width, number of stamen and number of days from flowering to fruiting, rest of the floral characters of *H. mysorense* did not exhibit significant differences over the study area.

In *H. japonicum,* no significant variation was observed except for the character height of the first branch in the study area of Wayanad district. In both the species, translucent secretory glands could be observed in the interveinal region of the leaf. In *H. japonicum,* translucent glands were as pellucid dots, while in *H. mysorense* the glands were either as "pellucid dots " (Wayanad district) or "pellucid streaks " (Idukki district).

Anatomical studies in *H. japonicum* and *H. mysorense* leaves showed spherical or oblong translucent glands in the spaces delimited by the vascular bundles (veins). The gland consisted of a sub-epidermal cavity delimited by two layers of cells. The internal layer consists of very flattened, thin walled cells. The external layer consists of thicker walled parenchymatous cells. The translucent glands were situated close to the abaxial epidermis.

Elongated, pale, translucent glands were observed in the cortex of the *H. mysorense* stem. The gland has an inner layer of flattened cells and an outer layer of thick walled parenchymatous cells.

Histochemical tests indicated the presence of lignin in all the plant parts of *H. japonicum* and *H. mysorense.* All the parts of *H. japonicum* had phenolic compounds while there was no indication for the presence of these compounds in the stem of *H. mysorense.*

In *H. mysorense,* total extractable matter was highest during flowering stage in all the plant parts. Among the plant parts, leaves had the highest extractable matter in all the growth stages of the plant. Maximum quantity of starch and total sugars were found in the root of *H. mysorense.* Samples of aerial part of *H. japonicum* and leaf of *H. mysorense* had the least starch and total sugar content respectively.

Stem samples of *H. mysorense* had high quantity of total free amino acids while the aerial portions of *H. japonicum* had the least. Protein content was maximum in the leaves and the least in the stem of *H. mysorense.*

Samples prepared from the leaf, stem and root of *H. mysorense* and aerial parts of *H. japonicum* (leaf + stem) did not give positive results in the qualitative test for anthraquinone.

None of the samples from both the *Hypericum* species showed corresponding spots to that of standard hypericin in any of the solvent systems. No variation could be observed in the spotting pattern when the samples from different growth stages of the plant were used. The commercial extract of *H. perforatum* Eleve®, manufactured by Universal Medicare Ltd., Mumbai produced corresponding spots to the standard hypericin in all the three solvent systems. The hypericin spot was fluorescent red in toluene: ethyl acetate: formic acid (50:40:10) and ethyl acetate: formic acid (50:6) solvent systems with Rf values of 0.63 and 0.98 respectively. The spot for hypericin appeared as dark in fluorescent background with the Rf value of 0.55 when ethyl acetate: formic acid: glacial acetic acid: water (10: 1.1: 1.1: 2.6) solvent system was employed.

Among the three solvent systems employed for the identification of hypericin, toluene: ethyl acetate: formic acid (50:40:10) solvent system gave good separation and hence produced more number of spots.

The hypericin content of Eleve®, commercial extract of *H. perforatum* was 0.22-0.25 per cent.

Even though the samples of the indigenous *Hypericum* spp. (*H. mysorense* and *H. japonicum* ) were negative for hypericin, the target anti-HIV compound, they were indicative for the presence of anthronoid derivatives. These anthronoid derivatives could be the precursors or intermediary compounds for the hypericin synthesis in the plant metabolism. These compounds need further analysis for their identification and characterization for anti-HIV properties.

# References

# REFERENCES

Anand, A. 1997. Cyto-morphological investigations in *Piper* spp. M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, p.98

Andersen, D.O.,Weber,N.D.,Wood,S.G.,Hughes,B.G., Murray, B.K. and North, J.A. 1991. *In vitro* virucidal activity of selected anthraquinones and anthraquinone derivatives. *Antiviral Res.*16(2): 185-196

*Andrade, P. B., Seabra, R. M., Valentao, P. and Areias, F. 1998. Simultaneous determination of flavonoids, phenolic acids and coumarins in seven medicinal species by HPLC diode-array detector. *J. Liq. Chromatogr. Rel. Tech.* 21(18). 2813-2820

Andrej, V., Samo, K., Theodor, K. and Barbara,H.1999.Quantitative phytochemical analyses of six *Hypericum* species growing in Solvenia. *Pl. Med.* 65(4): 388-390

*Ari, T., Jouko, U., Anju, H. and Jorma, J. 2002. Determination of naphthodianthrones and phloroghicinols from *Hypericum perforatum* extracts by liquid chromatography tandem mass spectrometry. *Rapid Commun. Mass Spectromet.* 16(5): 396-402

Axarlis, S., Mentis, A., Demetzos, C. and Malamas, M. 1998. Antiviral *in vitro* activity of *Hypericum perforatum* L. extract on the human cytomegalo virus (HCMV). *Phytother. Res.* 12(7): 507-511

Balakrishnan, M. 2001. *Eravikulam National Park Management Plan* (2001-2010), Eravikulam National Park, Munnar, p. 237

*Balogh, M.P. and Li, J.B. 1999. HPLC analysis of hypericin with photodiode array and MS detection: The advantages of multispectral techniques.. *Liq. Chromatogr. gas Chromatogr.* 17(6): 556-562

Barbagallo, C. and Chisari, G. 1987. Antimicrobial activity of three *Hypericum* species. *Fitoterapia* 58: 175-183

Barker, C. and Cheek, M. 1994. *Hypericum buckleyi. Kew Mag.* 11(2): 65-69

Baroni, F.R., Maffi, L., Benvenuti, S. and Bianchi, A. 2000. Morphological and phytochemical features of secretory structures in *Hypericum richeri* (Clusiaceae). *Nordiac J. Bot.* 20(4): 427-434

Bertoli, A., Pistelli, L., Morelli, I., Spinelli, G. and Menichini, F. 2000. Constituents of *Hypericum hircinum* oils. *J. Essential oil Res.* 13(1): 43-44

Bhellum, B.L. and Mangotra, R. 1998. A contribution to flora of District Doda of Jammu and Khashmir state - Family Hypericaceae Juss. *Indian J. For.* 21(4) 371-372

Bilia, A.R., Bergonzi, M.C., Mazzi, G. and Vincieri, F.F. 2001. Analysis of plant complex matrics by use of nuclear magnetic resonance spectroscopy: St. John's Wort extract. *J. agric. Fd. Chem.* 49(5): 2115-2124

Bombardelli, E. and Morazzoni, P. 1995. *Hypericum perforatum. Fitoterapia* 66(1): 43-67

Brantner, A.R., Loggia, S.D., Sosa and Kartoing, T.H. 1994. Study of antiphloistic activity of *Hypericum perforatum* L. *Sci. pharm.* 62: 97-98

Bystrov, N.S., Chernov, B.K., Dobrynin, V.N. and Kolosov, M.N. 1975. The structure of hyperforin. *Tetrachedran Lett.* 32: 2791

*Cakir, A., Duru, M.E., Harmandar, M., Ciriminna, R., Passannanti, S. and Piozzi, F. 1997. Comparison of the volatile oils of *Hypericum scabrum* L. and *Hypericum perforatum* L. from Turkey. *Flav. Frag. J.* 12(4): 285-287

Campbell, M.H., May, C.E., Southwell, I.A., Tomlinson, J.D. and Michael, P.W. 1992. Variation and varietal determination in *Hypericum perforatum* L. in New South Wales. *Pl. Prot. Q.* 7(2): 43-45

Champion, H.G. and Seth, S.K. 1968. *A revised survey of the forest types of India* Government of India Press, New Delhi, p.328

Ciccarelli, D., Andreucci, A.C. and Pagni, A.M. 2001a. Translucent glands and secretory canals in *Hypericum perforatum* L. (Hypericaceae): Morphological, anatomical and histochemical studies during the course of ontogenesis. *Ann. Bot.* 88(4): 637-644

Ciccarelli, C., Andreucci, A.C. and Pagni, A.M. 2001b. The black nodules of *Hypericum perforatum* L. subsp. *perforatum*: morphological anatomical and histochemical studies during the course of ontogenesis. *Isreal J. Pl. Sci.* 49(1): 33-40

*Couladis, M., Baziou, P., Petrakis, P.V. and Harvala, C. 2001. Essential oil composition of *Hypericum perfoliatum* L. growing in different locations in Greece. *Flav. Frag. J.* 16(3): 204-206

Dias, A.C.P., Seabra, R.M., Andrade, P.B. and Ferreira, M.F. 1999. The development and evaluation of an HPLC-DAD method for the analysis of the phenolic fractions from *in vivo* and *in vitro* biomass of *Hypericum* species. *J. Liq. Chromatogr. Rel. Tech.* 22(2): 215-217

Dogance, S. and Okseuz., S. 1989. Constituents of *Hypericum adenotrichum Fitoterapia* 60(1): 93

Dogrukol, A.D., Krimer, N., Tuncel, M. and Enenin, H.Y.A. 2001. Determination of rutin in *Hypericum perforatum* extract by capillary electrophoresis. *Analyt. Lett.* 34(2): 85-91

Elisabetta, O., Alberto, R., Anna, G., Mauro, B., Maurizio, C. and Giuseppe, P. 2002. Ultrastructural studies on the developing secretory nodules of *Hypericum perforatum. Flora Jena* 197(2): 92-102

Fornasiero, R.B., Binachi, A. and Pinetti, A. 1998. Anatomical and ultrastructural observations in *Hypericum perforatum* L. leaves. *J. Herb Spice Med. Pl.* 5(4): 21-33

Fornasiero, R.B., Maffi, L., Benvenuti, S. and Bianchi, A. 2000. Morphological and phytochemical features of secretory structures in *Hypericum richeri* (Clusiaceae). *Nordiac J. Bot.* 20(4): 427-434

Fyson, P.F. 1915. *The Flora of South Indian Hills.* Vol-I. Reprint 1995. Bishan Singh Mahendra Pal Singh, Dehradun, p.428

Fyson, P.F. 1932. *The Flora of the Presidency of Madras*, Vol-I. Bisher Singh Mahendrapal Singh, Dehradun, p364

Gamble, J.S. 1957. *The Flora of Presidency of Madras*, Vol-I. Bishen Singh Mahendrapal Singh, Dehradun, p.496

Georgios, P., Ralf, L., Annette, W., Thilo, S. and Peter, Z.K. 1997. Determination of naphthodianthrones in plant extracts from *Hypericum perforatum* L. by liquid chromatography, electrospray mass spectrometry. *J. Chromatogr.* 69(2): 309-316

Giuseppe, M., Francesco, L. and Paolina. 1996. High performance liquid chromatographic determination of the biologically active principle hypericin in phytotherapeutic vegetable extracts and alcoholic beverages. *J. Chromatogr.* 68(2): 336-339

Gray, D. E., Rottinghaus, G. E., Garrett, H. E. G. and Pallardy, S. G. 2000. Simultaneous determination of the predominant hyperforins and hypericins in St. Jhon's Wort (*Hypericum perforatum* L.) by liquid chromatography. *J. AOAC int.* 83(4): 944-949

Grunwald, 1999. The world market for hypericum products. *Nutraceuticals Wld.* 6: 22-25

*Gudzic, B., Dordevic, S., Palic, R. and Stojanovic, G. 2001. Essential oils of *Hypericum olympicum* L. and *Hypericum perforatum* L. *Flav. Frag. J.* 16(3): 201-203

Hansen, S.H., Jensen, A.G., Cornett, C., Bjorndottoir, I., Taylor, S., Wright, B. and Wilson, I.D. 1999. High performance liquid chromatography on line coupled to high field NMR and mass spectrometry for structure elucidation of constituents of *Hypericum perforatum* L. *Analyt. Chem.* 71(2): 5235-5241

Harborne, J.B. 1973. *Phytochemical Methods.* Chapman and Hill Ltd., London, p.228

Hesse, P.R. 1971. *A Textbook of Soil Chemical Analysis.* John Murray Publishers Ltd., London, p.520

*Hoelzl, J. 1993. Inhaltsstoffe wirkmechanismen des johanniskratutes. *Phytotherapy* 14: 255-259

Hooker, J.D. 1875. *Flora of British India.* Vol. I. The secretary of state for India in council, Delhi, p.510

Hudson, J.B., Lopez, B.I. and Towers, G.H.N. 1991. Antiviral activities of hypericin. *Antiviral Res.* 15(2): 101-112

Jackson, 1958. *Soil Chemical Analysis.* Indian Pvt. Ltd., New Delhi, p.498

Jensen, K.I.N., Gaul, S.O., Specht, E.G. and Doohan, D.J. 1995. Hypericin content of Nova scotiabiotypes of *Hypericum perforatum* L. *Can. J. Pl. Sci.* 75(4): 923-926

Jing, Z., Zhiping, Z., Hongshan, C. and Xianghong, C. 1998. Studies on synthesis and anti-HIV RT activity of hypericin and ethyl hypericin. *Acta Pharm. Sinica* 33(1): 67-71

Jurgenliemk,G.and Nahrstedt, A. 2002. Phenolic compounds from *Hypericum perforatum. Pl. Med.* 68(2): 88-91

Kartnig, T. and Gobel, I. 1992. Determination of hypericin and pseudohypericin by thinlayer chromatography densiometry. *J. Chromatogr.* 65(2): 423-426

Kireeva, T.B., Sharanov, U.L. and Letchamo, W. 1999. Biochemical and ecophysiological studies on *Hypericum* spp. *Perspective on New Crops and New Uses* (ed. Janick, J.). ASHS, Alexandria, pp.467-468

Kitanov, G. M. 2000.The dynamics and content of hypericin in *Hypericum perforatum* L. *and Hypericum maculatum* Crantz. growing in Bulgaria. *Acta Pharm.* 50(1): 65-68

Kitanov, G. M.2001. Hypericin and pseudohypericin in some *Hypericum* species. *Biochem. Syst. and Ecol.* 29: 171-178

Kuginuki, F. and Namba, T. 1993. Pharmacognostical studies on traditional medicine in Japan. Otogiriso: anatomical features of leaves and stems of *Hypericum* and *Triadenum* species (Hypericaceae). *J. Jap. Bot.* 68(4): 224-235

Kumar, N. A.1993. Floristic study of Pathanamthitta district - Kerala. Ph.D thesis, University of Calicut, Calicut, p.817

Kumar, S., Janardar, R., Shah, N. C. and Vinay, R. 1997. *Indian Medicinal and Aromatic Plants Facing Genetic Erosion*, Central Institute of Medicinal and Aromatic plants, Lucknow, p.420

Kumar, V., Singh, P.N., Muruganandam, A.V. and Battacharya, S.K. 2000. *Hypericum perforatum:* Nature's mood stabilizer. *Indian J. Exp. Biol.* 38(11): 1077-1085

Lawrence, G.H.M. 1951. *Taxonomy of Vascular Plants.* Indian Reprint 1967. Oxford IBH Publishing Company, New Delhi, p.358

Loren, W., Tara, S., Donna, G. and Nan, V. 2001. Regional differences in hypericin and pseudohypericin concentrations and five morphological traits among *Hypericum perforatum* plants in the northwestern United States. *Can. J. Bot.* 79(10): 1248-1255

*Lozykowska, S.K., Dabrowska, J. and Zygmunt, B. 1999. Content of active substance in the herb of St. John's wort cv. Topaz in different growth phases. *Herba polonica* 45(3): 169-172

Maisenbacher, P. and Kovar, K.A. 1992a. Adhyperforin: A homologue of hyperforin for *Hypericum perforatum*. *Pl. Med.* 58(3): 291-294

Maisenbacher, P. and Kovar, K.A. 1992b. Analysis and stability of Hyperici oleum. *Pl. Med.* 58(4): 351-354

Manjusha, C.M. 2001. Morphological and biochemical variations in Adatoda types (*Adhatoda spp.*). M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, p.111

*Martonfi, P., Repeak, M. and Mitikova, L. 1996. *Hypericum maculatum*. Crantz subsp. *maculantum* x *H. perforatum* L. (Hypericaceae): Corroboration of natural hybridization by secondary metabolite analysis. *Folia Geobot. Phytotaxon.* 31(2): 245-250

Mathew, K.M. 1983. *The Flora of Tamilnadu Carnatic* Vol.I. The Rapinat Herbarium, St. Joseph college, Thiruchirappalli, p.526

Mitich, L.W. 1994. Common St. John's Wort. *Weed Technol.* 8(3): 658-661

Moraleda, G., Wu, T.T., Jilbert, A.R., Aldrich, C.E., Condreay, L.D., Larsen, S.H., Tang, J.C., Colacino, J.M. and Mason, W.S. 1993. Inhibition of duck hepatitis B virus replication by hypericin. *Antiviral Res.* 17(3): 235-247

Mukherjee, P.K. and Suresh, B. 2000. Studies on *in vivo* wound healing activity of leaf extract of *Hypericum mysorense* with different wound models in rats. *Nat. Prod. Sci.* 6(2): 73-78

Mulinacci, N., Bardazzi, C., Romani, A., Pinnelli, P., Vincieri, F.F. and Costantini, A. 1999. HPLC-DAD and TLC-densiometry for quantification of hypericin in *Hypericum perforatum* L. extracts. *Chromatographia* 49(3-4): 197-201

Muthulakshmi, P. 1998. Variability analysis in *Garcinia cambogia* Desr. (Malabar Tarmarind). M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, p.130

Nair, N.G. and Henry. 1983. *Flora of Tamil Nadu. Vol-I.* Botanical Survey of India, Coimbatore, p.482

Omidbaigi, R. and Aziz, M. 2000. Effect of harvest on hypericin and essential oil content of *Hypericum perforatum* L. *Iran agric. Res.* 19(2): 155-164

Oravec, V., Oravec, V.J., Martonfi, P. and Repcak, M. 1996. Variability pattern in different cultivated *Hypericum perforatum* types. *Atti del convegno internazionale* 3: 631-634

*Orth, H.C.J., Rent, C. and Schmidt, P.C. 1999. Isolation, purity analysis and stability of hyperforin as a standard material from *Hypericum perforatum* L. *J. Pharm. Pharmacol.* 51(2): 193-200

Osinska, E., Weglarz, Z., Verdonck, O., Mathe, A., Relf, P.D., Matsuo, E., Groening, G.D. and Rammeloo, J. 2000. Morphological, developmental and chemical variability of *Hypericum* genus. *Acta Hort.* 523: 82-85

Panse, V.G. and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers.* ICAR, New Delhi, p.273

*Pickering, C. 1879. *Chronological History of Plants.* Little Brown and Co., Boston, p.569

Pluhar, Z., Rehak, O. and Nemath, E. 2000. Comparative investigation on *Hypericum perforatum* L. population of different origin. *Int. J. hort. Sci.* 6(1): 56-60

Poutaraud, A., Gregorio, F.D., Tin, V.C.T. and Girardin, P. 2001. Effect of light on hypericins content in fresh flowering top parts and in an extract of St. John's wort (*Hypericum perforatum*). *Pl. Med.* 66(3): 254-259

Qingli, W., Shengping, W., Lijun, D., Shuming, Z., Junshan, Y. and Peigen, X. 1998. Chromone glycosides and flavonoids from *Hypericum japonicum*. *Phytochemistry* 49(5): 1417-1420

Rani, N.S., Balaji, K. and Ciddi, V. 2001. Production of hypericin from tissue culture of *Hypericum perforatum*. *Indian J. Pharm. Sci.* 62(5): 431-433

Rendle, A.B. 1925. *The Classification of Flowering Plants.* Vol II. Indian Reprint 1979. Vikash Publishing House Pvt. Ltd., Ghaziabad, U.P p.385

Reyes, G.C. and Koda, R.T. 2001. Development of a simple rapid and responsible HPLC assay for the simultaneous determination of hypericins and stabilized hyperforin in commercial St. John's Wort preparations. *J. Pharm. Biomed. Anal.* 26(5-6): 959-965

Robinson, T. 1967. *The Organic Constituents of Higher Plants.* Burgess publishing company, Minneapolis, p.538

Robson, N. 1995. *Hypericum uralum. New plantsman* 2(4): 242-245

*Robson, N.K.B.1981.Studies in the genus *Hypericum L.* (Guttiferae)-Characters of the genus. *Bull. Br. Mus.* 8: 55-226

Robson, N.K.B. 1998. *Hypericum subsessile. Curtis's Bot. Mag.* 15(2): 92-95

Rocha, L., Marston, A., Potterat, O., Kaplan, M.A.C. and Hostettmann, K. 1996. More Phloroglucinols from *Hypericum brasiliense. Phytochemistry* 42(1): 185-188

Sadasivam, S. and Manickam,P. 1992.*Biochemical Methods.* New Age International Publishers and Tamil Nadu Agricultural University, Coimbatore, p.256

*Sajjadi, S. E., Rahimnezahad, M. R., Mehregan, I. and Poorassar, A. 2001. Constituents of essential oil of *Hypericum dogonbadanicum* Assadi. *J. Essential oil Res.* 13(1): 43-44

Saker, M.K., Ezer, N. and Ergelshowe, R.1991. Constituents of *Hypericum montbretti*. *Int. J. Pharmacognosy* 29(3): 228-230

Satyavati, G.V., Gupta, A.K. and Tandon, N. 1987. *Medicinal Plants of India*. Vol. II. Indian Council of Medical Research, New Delhi, p.648

Schmitt, A.C., Ravazzolo, A.P. and Poser, G.L. 2001. Investigation of some *Hypericum* species native to southern Brazil for antiviral activity. *J. Ethnopharmac.* 42(3): 239-245

Singh, H.B. 1999. Identity of crude drug - *Hypericum perforatum* L. *Biodiversity conservation and utilisation of spices, medicinal and aromatic plants* (eds Sasikumar, B., Krishnamoorthy, B., Rema, R., Ravindran, P.N. and Peter, K.V.). Proceedings of the National Symposium on Spices, Medicinal and Aromatic Plants. Biodiversity, Conservation and Utilization, August 10-12, 1998. Indian Institute of Spices Research, Calicut, pp.296-302

Singh, J., Srivastava, R.K., Singh, A.K. and Kumar, S. 2000. Futuristic scenario in production and trade of major medicinal plants in India. *J. Med. Arom. Pl. Sci.* 22 & 23(4A & 1A): 564-571

Sirvent, T.M., Walker, L., Vance, N. and Gibson, D.M. 2002. Variation in hypericins from wild populations of *Hypericum perforatum* L. in the pacific northwest of the U.S.A. *Econ. Bot.* 56(1): 41-48

Sivarajan,V.V. and Mathew, P.1997. *Flora of Nilambur*. Bishen Singh Mahendrapal Singh, Dehradun, p.427

Southwell, I.A. and Bourke, C.A. 2001. Seasonal variation in hypericin content of *Hypericum perforatum* L. *Phytochemistry* 56(5): 437-441

Southwell, I.A. and Campbell, M.H. 1991. Hypericin content variation in Australia. *Phytochemistry* 30(2): 475-478

Steinbeck, K.A. and Wernet, P. 1993. Sueccessful longterm treatment over 40 months of HIV patients with intravenous hypericin. International conference on AIDS, 6-11 June 1993. Germany. *Abstract* : B124

Stochmal, A. and Gruszyk, M. 1998. Solid phase extraction and HPLC determination of hypericin in *Hypericum perforatum* L. *Herba Polonica* 44(4): 315-323

Subbiah, B.V. and Asija, G.L.A. 1956. A rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci.* 25: 259-260

Tekelova, D., Repcak, M., Zemkova, E. and Toth, J. 2000. Quantitative changes of dianthrones, hypericin, flavonoids content in the flower ontogenesis of *Hypericum perforatum. Pl. Med.* 66: 778-780

Tang, J., Colacino, J.M., Larsen, S.H. and Spitzer, W. 1990. Virucidal activity of hypericin against enveloped and non-enveloped DNA and RNA viruses. *Antiviral Res.* 14(6): 313-326

Taylor, R.S.L., Manandhar, N.P., Hudson, J.E. and Towers, G.H.N. 1996. Antiviral activities of Nepalese medicinal plants. *J. Ethnopharmac.* 37(3): 157-163

Thayil,S. 1997. The nattivaidya's craft. *Amruth.* 1(8): 3-8

Upton, R.A., Graft, A., Williamson, E., Bunting, D., Gatherum, D.M., Walker, E.B., Butterweck, U.,Lieflander, U.,Nahrsstedt,A,winterhoff,H. and Cott,J.1997. *St. John's Wort, Hypericum perforatum: Quality Control, Analytical and Therapeutic Monograph.* American Herbal Pharmacopeia, Santa Cruz, New York, p. 128

Vadukkoot, L.J. 1996. Evaluation of morpho-anatomical variations in *Ocimum* spp. M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, p.88

Verotta, L., Appendino, G., Belloro, E., Jakupovic, J. and Bombardelli, E. 1999. Furohyperforin, a prenylated phloroglucinol from St. John's wort (*Hypericum perforatum*). *J. nat. Prod.* 62(5): 770-772

Verotta, L., Appendino, G., Jakupovic, J. and Bombardelli, E. 2000. Hyperforin analogues from St. John's Wort (*Hypericum perforatum*). *J. Nat. Prod.* 63(3): 412-415

Vestal, P.A. 1937. The significance of comparative anatomy in establishing the relationship of the Hypericaceae to the Guttiferae and their allies. *Phillip. J. Sci.* 64: 199-256

Vlietinck, A.J., Berghe, D.A. and Vanden. 1991. Can ethnopharmacology contribute to the development of antiviral drugs? *J. Ethnopharmac.* 32(3): 141-153

Vlietinck, A.J., Bruyne, T., Apers, S. and Pieters, L.A. 1998. Plant derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infections. *Pl. Med.* 64(2): 97-109

Walker, L., Sirvent, T., Gibson, D. and Vance, N. 2001. Regional differences in hypericin and pseudohypericin concentrations and five morphological traits

among *Hypericum perforatum* plants in northwestern United States. *Can. J. Bot.* 79(10): 1248-1255

Watnabe, P.S. and Olsen, S.R.(1965).Test of an ascorbic acid method for determining phosphate in water and ammonium bicarbonate extracts from soil. *Proc. Soil Sci. Am.* 29: 677-678

'Weber, N.D., Murray, B.K., North, J.A. and Wood, S.G. 1994. The antiviral agent hypericin has *in vitro* activity against HSV-1 through non-specific association with viral and cellular membranes. *Antiviral Chem. Chemother.* 5(2): 83-90

Wenzhe, L. and Zhenghai, H. 1999. The secretary structure of *Hypericum perforatum* and its relation to hypericin accumulation. *Acta Bot. Sinica* 41(4): 369-372

'Weyerstahl, P., Splittgerber, U., Marschall, H. and Kaul, V.K. 1995. Constituents of the leaf essential oil of *Hypericum perforatum* L. from India. *Flav. Frag. J.* 10(6): 365-370

Winkelmann, K., Heilmann, J., Zerbe, O., Rali, T. and Sticher, O. 2000. New phloroghicinol derivatives from *Hypericum papuanum. J. nat. Prod.* 63(1): 104-108

Wood, S., Huffman, J., Weber, N., Anderson, D., North, A.J., Murray, B.K., Sidwell, R. and Hughes, B. 1990. Antiviral activity of naturally occurring anthraquinones and anthraquinone derivatives. *Pl. Med.* 56(8): 651-659

Yeung, E.C. 1998. A beginner's guide to the study of plant structure. Test studies for laboratory teaching (ed. Karchar, S.J.) *Proceedings of the 19th Workshop of the*

*Association for Biology Laboratory Education* 1998. Association for Biology Laboratory Education Toronto, pp. 125-149

Zotou, A. and Loukou, Z. 2001.Determination of hypericin and pseudohypericin in extracts from *Hypericum perforatum* L. and pharmaceutical preparations by liquid chromatography-fluorescence detection. *Chromatographia* 54(3): 218-224

* Original not seen

# MORPHOLOGICAL AND PHYTOCHEMICAL INVESTIGATIONS ON ST. JOHN'S WORT (*Hypericum* spp.), A POTENTIAL SOURCE OF ANTI-HIV COMPOUNDS

By

**V. GANAPATHI**

## ABSTRACT OF THE THESIS

*Submitted in partial fulfilment of the requirement for the degree of*

Master of Science in Horticulture

*Faculty of Agriculture*
*Kerala Agricultural University*

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE
KAU P. O., THRISSUR - 680 656
KERALA, INDIA

**2003**

## ABSTRACT

Renowned laboratories throughout the world have been making intense search to detect and isolate potential chemicals from plants for curing AIDS. *Hypericum* is an important genera yielding hypericin,a potential anti -HIV chemical. Hypericin has been reported from many exotic *Hypericum* species. Investigations on the presence of hypericin in the indigenous flora (*H. mysorense* and *H. japonicum*) are lacking. Hence the present study entitled "Morphological and Phytochemical investigations on St.John's Wort (*Hypericum* spp.), a potential source of anti-HIV compounds" was constituted.

Two species of *Hypericum* were located in Kerala; *H. mysorense* at the high ranges of Idukki and Wayanad districts and *H. japonicum* in Wayanad district. The natural habitat characters of *H. mysorense* were studied at Vattavada in Idukki. Phytosociological parameters such as density, abundance and per cent frequency were determined by quatrat studies. *H. mysorense* was often present or seldom present along with *Pteridium aquilinum, Eucalyptus grandis, Lantana camara, Strobilanthus spp, Andropogon lividis* and *Chrysopogon zeylanicus* in the high altitude regions of Vattavada panchayat of Idukki district. *Chrysopogon zeylanicus* is the dominant species in the eco system. *H. mysorense* is a shrub with stiff branches while *H. japonicum* is a prostrate herb growing in marshy lands. Translucent glands were either streaks or dots in *H. mysorense* while it was pellucid dots in *H. japonicum*. Stem anatomy of *H. mysorense* revealed presence of "type B" secretory canals whereas it was absent in *H. japonicum*.

Histochemical tests indicated the presence of lignin in the leaf,stem and root of *H. mysorense* and *H. japonicum*. Leaf, stem and root of *H. japonicum* had phenolic compounds while there was no indication for the presence of these compounds in the stem of *H. mysorense*.The primary metabolites like starch, total sugars,protein and total free amino acids on leaf, stem and root were quantified. Qualitative test for anthraquinone was negative in both the species.When tested

using TLC for the presence of hypericin ,no spots corresponding to Rf value of standard hypericin were observed in *H. mysorense* and *H. japonicum* samples. Commercial extract of *H. perforatum* (Eleve®,) had 0.22 to 0.25 per cent hypericin. Even though the samples of the indigenous *Hypericum spp.* (*H. mysorense* and *H. japonicum* ) were negative for hypericin - the target anti-HIV compound, they were indicative for the presence of anthronoid derivatives. These anthronoid derivatives could be the precursors or intermediary compounds for the hypericin synthesis in the plant metabolism. These compounds need further analysis for identification and characterization for anti-HIV property.