STUDIES ON VARIABILITY, CONSERVATION AND PROPAGATION OF *DASAMULA* GROUP OF PLANTS

Thesis submitted in part fulfillment of requirements for the Degree of Doctor of Philosophy in Botany of the University of Calicut

By

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KERALA, INDIA
2005

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CERTIFICATE

Certified that this thesis entitled **"STUDIES ON VARIABILITY, CONSERVATION AND PROPAGATION OF *DASAMULA* GROUP OF PLANTS"** embodies the results of a piece of bona fide research work carried out as part fulfillment of requirements for the degree of **Doctor of Philosophy** in **Botany** of the **University of Calicut** by **Mr. A.V.Raghu** under my guidance and supervision and that no part of the thesis has been submitted for any other degree.

I further certify that such helps or sources of information availed of in this connection, have been duly acknowledged.

Calicut University,
20 December 2005.

(K.V.MOHANAN)

DECLARATION

I, A.V. Raghu, hereby declare that this thesis entitled **"Studies on variability, conservation and propagation of *dasamula* group of plants"** being submitted in partial fulfillment of the requirements of Ph.D. Degree in Botany embodies the results of a bonafide work done by me under the guidance of Dr.K.V.Mohanan, Reader & Research Guide, Genetics and Plant Breeding Division, Department of Botany, University of Calicut and no part of it has been submitted for any other degree.

Calicut University
20 December, 2005.

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ACKNOWLEDGEMENT

I express my sincere and profound gratitude to Dr.K.V.Mohanan, Genetics and Plant Breeding Division, Department of Botany, University of Calicut, Kerala, India for the guidance and intellectual support extended to me for the successful completion of this work.

My sincere thanks are due to Dr. P.V.Madhusoodanan, Professor and Head of the Department of Botany and Dr.S.Nandakumar, Professor and former Head of the Department of Botany, University of Calicut for providing the necessary facilities to carry out my work.

I am deeply indebted to Dr.Indira Balachandran, Project Director and Head, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala, India for permitting me to carry out this work, to use the field and laboratory facilities of CMPR and also for the constant encouragement and valuable advices during the experimental work and data analysis.

I use this occasion to remember with deep gratitude, the advices and suggestions made by Dr.P.N. Ravindran, Director (Rtd), Indian Institute of Spices Research, Calicut, Kerala and presently Visiting Scientist, CMPR.

Words of thanks would not suffice the graciousness extended by Dr.Geetha S. Pillai, Dr.A.B.Remashree, and Dr. P.S.Udayan, Senior Scientists

of CMPR because but for their constant interest in this task, it could not have been completed successfully.

The help rendered by Mr. K.P.Unnikrishnan, Scientist, CMPR in the phytochemical analysis of the materials is acknowledged with heartfelt thanks.

Heartfelt thanks are also due to, Mr.K.V.Thushar, Mr. Satheesh George, Mr. Gerald Martin, Mr. Sudhakar Raja, K.M.Harinarayanan and Ms.A.Jayanthi, Scientists of CMPR, for their valuable assistance, immense enthusiasm and help during the course of this work.

My sincere thanks are also due to Mr. K.M.Hashim, Research Assistant, CMPR and all other staff members of CMPR and also to the staff members of the Estate Department of Arya Vaidya Sala for their valuable helps in connection with the present work.

I express my sincere thanks to Dr.A.K.Pradeep, Curator, Calicut University Herbarium for valuable discussions regarding plant morphology.

The helps rendered by the teachers, librarian and the non teaching staff of the Department of Botany, University of Calicut are also acknowledged gratefully.

Deep sense of thanks is due to Ms. R. Uma Maheswari, Research Scholar (Genetics and Plant Breeding), Department of Botany, University of Calicut for her valuable helps and suggestions in the compilation of this thesis.

My heartfelt thanks are due to Ms. M. Jayasree, Teacher Fellow and Ms. C.B.Mini, Research Scholar (Genetics and Plant Breeding), Department of Botany, University of Calicut for valuable suggestions and motivation throughout the study.

A.V.Raghu

PREFACE

Dasamula, is a well known group of ayurvedic drugs very efficient in the case of a wide range of health problems caused by *vata* and *kapha*. The ten plants include five tree species known as *bruhat panchamula* and five herbaceous plants known as *laghu panchamula*. All the ten species show different levels of morphological and phytochemical variability. However, the extent of such variability has not been analysed properly and rigorous efforts have not been made for the identification of the most suitable materials for propagation and commercial exploitation. All the species face different levels of threat in their natural habitats due to destructive collection of the plants for drug purposes and also due to rapid decline of their habitats, thus making *ex situ* conservation and development of more effective propagation techniques including *in vitro* propagation highly necessary.

The present study has been under taken so as to assess the morphological and phytochemical variability among the ten species, to conserve them *ex situ* and to develop reliable propagation techniques for them.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

A.V. Raghu “Studies on variability, conservation and propagation of Dasamula group of plants” Thesis. Department of Botany , University of Calicut, 2005

INTRODUCTION

Chapter I
INTRODUCTION

India has an impressive wealth of medicinal plants, almost all of them native to the country. More than 7000 species are reportedly used for medicinal purposes, most of them being exploited recklessly for the extraction of drugs (Groombridge, 1992). It will be prudent to study the science and status of indigenous medicinal plants for evolving efficient strategies for conservation and management. Medicinal plants are viewed as a possible bridge between sustainable development, affordable health care and conservation of the vital biodiversity. At present, most of the herbal preparations are made from non-standardized plant products. Medicinal plants are the oldest known health care products. Their importance is still growing although it varies depending on the ethnological, medical and historical background of each country. Medicinal plants are also important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also when they are used as basic materials for the synthesis of drugs or as models for pharmacologically active compounds. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of mankind. The importance of plant based medicine was waxed and waned during the last 200 years. Advancements made in synthetic chemistry along with the discovery of antibiotics and corticosteroids and their

artificial synthetics caused rapid decline of plant based medicines particularly in the developed nations during the 20th century. The developing nations depend on the other hand mostly on plants for their medicine. In recent times, however, due to the increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, there has been a renewal of interest in the use of plants and plant based drugs throughout the world. According to WHO, the resurgence of public interest in traditional medicine is on the increase because of a sweeping green wave and a large number of plant drugs are now sold in the 'health food shops' all over the world including the developed world (Pushpangadan and Nair, 1997). The preventive and promotive aspects of the Eastern traditional systems of medicine particularly that of India and China are finding increased popularity and acceptance throughout the world and scope for developing plant based drugs assumes greater significance at a time when modern medicine has only limited means to provide cure for the dreadful diseases like cancer, AIDS, arthritis, liver diseases and other diseases of unknown etiology.

All human cultures from ancient times to the present day have used plants as source of medicines. The greater part of traditional therapy involves the use of plant extracts or their active principles. The number of individuals using medicinal plants is large and on the increase, even among young people. It is not just in developing countries that medicinal plants are important.

Today many medicinal plants face extinction or severe genetic loss, but detailed information is lacking. For most of the endangered medicinal plant species no conservation action has been taken. For example, there is very little material of them in gene banks. Also, too much emphasis has been put on the potential for discovering new wonder drugs, and too little on the many problems involved in the use of traditional medicines by local populations. For most countries, there is not even a complete inventory of medicinal plants. Much of the knowledge on their use is held by traditional societies, whose very existence is now under threat. Little of this information has been recorded in a systematic manner. Besides the identification and selection of medicinal plants for use in health services, there is the potential that plants hold as an inexhaustible reservoir for the identification and isolation of useful chemical compounds for diseases such as AIDS, for which there is yet no known cure.

In terms of life forms, medicinal plants are equally distributed across habits *viz.*, trees, shrubs and herbs. Roughly, one third of the known medicinal plants are trees and equal proportion shrubs and the remaining one third herbs, epiphytes and climbers. Very small proportions of the medicinal plants are lower plants like lichens, ferns, and algae.

Preliminary analysis of the distribution pattern shows that medicinal plants are distributed across diverse habitats and landscape elements. Around 70 per cent of India's medicinal plants are found in the tropical zone,

mostly in the forests of the Western and Eastern Ghats, the Vindhyas, Chotta Nagpur plateau, Aravalis, the Terai region in the foothills of the Himalayas and the North East. Less than 30 per cent of these medicinal plants are confined to the temperate and colder zones although species of great medicinal value occur in some of these habitats. A quick analysis of the available data shows that the proportion of medicinal plants recorded in the dry and moist deciduous tropical forests is higher as compared to those recorded in the tropical evergreen forests.

The history of the relationship between products from living plants and healing medications goes back to the very beginnings of medicine itself, to the ancient civilizations of India and Egypt, followed by the Chinese and later the Greeks and Romans.

Ayurveda has long been the main system of health care in India, although western medicine has become more widespread, especially in urban areas. About 70 percent of India's population live in rural areas; about two thirds of rural people still use ayurveda and medicinal plants to meet their primary health care needs. Ayurveda and variations of it have also been practiced for centuries in Pakistan, Nepal, Bangladesh, Sri Lanka, and Tibet. Currently, about 5,000 products are included in the "pharmacy" of ayurvedic treatments. Historically, plant compounds have been grouped into different categories according to their effects. For example, some compounds are thought to heal, some to relieve pain and some to promote vitality. Ayurverdic plants have been grouped in

to *dasamula, dasapuspa, tribhala, trikatu,* etc. which are used for different purposes.

Dasamula (ten roots) is one of the best known drug group of classic ayurveda. The drugs include *vilva, kasmari, syonakah, patala, agnimanthah, prsniparni, saliparni, brhatidvayam* (two plants) and *goksurah.* It is a special combination of ten plants with the ability to act on a wide range of health problems, those caused by both *vata* and *kapha.* The ten plants include five herbaceous plants known as *laghu panchamula* and five trees known as *bruhat panchamula* (Ghate, 1999). *Desmodium gangeticum, Pseudarthria viscida, Solanum melongena* var. *insanum, Solanum violaceum* and *Tribulus terrestris* are used as the source of *laghu panchamula* and *Aegle marmelos, Gmelina arborea, Oroxylum indicum, Stereospermum colais* and *Premna corymbosa* as the source of *bruhat panchamula* in South India (Sivarajan and Balachandran, 1994). Many of these plants are redlisted.

As per the text *Sarngadhara Samhita, dasamula* nourishes the lean, stimulates vitality and gives progeny to the childless. It is an excellent tonic for convalescence and general debility. It is used in the form of *asava, arishta, avaleha, churna, ghruta, kwath* and *taila.* It is also used as the basic ingredient in many other preparations. *Dasamula* is a popular health tonic, beneficial to gastric, respiratory, cardiovascular, nervous and urino genital systems of the body. It is digestive, carminative, stomachic, antispasmodic, analgesic, expectorant, cardiac,

antirheumatic, antihelmintic, restorative and is a rapid cell rejuvenating tonic (Ghate, 1999).

In the case of *dasamula* destructive collection of drugs is severe since roots are the sources of active compounds. Hence, research towards rapid propagation of the materials, genetic improvement of the species for higher production of the active components and standardizing protocols for the production of the compounds under *in vitro* culture conditions is essential.

The commercial demand of *dasamula* is presently fulfilled by wholesale suppliers through rural and tribal collectors residing in or near forest areas. Due to depletion of natural flora and increased demands, need for systematic cultivation and large scale multiplication has been realized recently and hence taken up for studies.

There is an urgent need to conserve the genetic diversity of *dasamula* group of plants since they may become extinct if the reckless exploitation continues. Conservation can be effected both *in situ* and *ex situ*. However, *in situ* conservation is not practical beyond an extent nowadays since the natural habitats of most of the plants are rapidly declining.

Ex situ conservation can be used for several purposes such as rescuing threatened genetic resources; producing material for reintroduction, reinforcement, habitat restoration and management and conservation education and research; bulking up germplasm for storage

in various forms of *ex situ* facilities; supplying material for various purposes; reducing the pressure of wild collections and providing materials for developing new propagation techniques.

Gene banks play an active role in medicinal plant conservation, propagation, improvement and other researches. The gene banks work hand in hand with the local communities to document and use indigenous and local knowledge. The collections are easily accessible and available for local initiatives in primary health care particularly in developing countries. They are also available as a resource for screening programmes for pharmaceutical companies and to provide material for those assessing the value and safety of particular herbal medicines. Germplasm banks can provide the expertise to improve the agronomy of cultivated medicinal plants and initiate cultivation of new species. Most medicinal plants are at present grown as unimproved wild plants, and so tend to be very variable. Effective plant breeding programmes are needed to select the superior genotypes available. Germplasm banks have an important role in the development of a gene pool of wild stock plants, which can contribute to breeding programmes.

The best way to ensure the availability of medicinal plants is to propagate them using advanced techniques. In the case of rare, endangered or over exploited plants, propagation and cultivation is the only way to provide material without further endangering the survival of those species. Cultivation has pharmacological advantages over

wild collection. Wild collected plants normally vary in quality and composition, due to environmental and genetic differences. In cultivation, this variation and the resulting uncertainty of the therapeutic benefit is much reduced. The plants can be grown in areas of similar climate and soil, they can be irrigated to increase yields and they can be harvested at the right time. Cultivation also greatly reduces the possibility of misidentification and adulteration.

The present study is an effort to collect and conserve *dasamula* group of plants from different geographical regions, to study their variability based on vegetative and phytochemical characters and also to standardize propagation techniques. Seed propagation has been successfully attempted in nine species namely *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Stereospermum colais*, *Desmodium gangeticum*, *Pseudarthria viscida*, *Solanum melongena* var. *insanum*, *Solanum violaceum* and *Tribulus terrestris* and vegetative propagation in *Premna corymbosa*. *In vitro* propagation has been successfully attempted in *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Solanum violaceum* and *Tribulus terrestris*.

REVIEW OF LITERATURE

A.V. Raghu “Studies on variability, conservation and propagation of Dasamula group of plants” Thesis. Department of Botany , University of Calicut, 2005

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

Dasamula is a very significant group of ayurvedic plants used in a number of medicinal formulations. The word literally means ten roots. Five of the species are trees (*bruhat panchamula*) and the other five shrubs or herbs (*laghu panchamula*) (Ghate, 1999). The present investigations have been designed to study the variability, conservation and propagation of the *dasamula* group of plants and hence the literature relevant to the above aspects is reviewed below.

Sinha (1995) studied the importance of faith and tradition in the conservation of biodiversity in India. This study reported that several members of India's flora and fauna had been identified with particular personalities of the Hindu pantheon and are worshipped. Such beliefs and practices continue in one form or another in certain sections of Indian society, particularly the rural folk and the aboriginal tribes living in the forests. Nature worship is a form of tribal belief and the faith of these laymen has helped to conserve many natural ecosystems in India.

Very few works have been carried out on *dasamula* group on this line collectively. Ghate and Sathe (1998) have carried out conservation studies on *bruhat panchamula* germplasm based on collection and nursery evaluation. Effects of seed pretreatments (soaking in water, hot water treatment + soaking with 100 ppm GA_3 or

H_2SO_4) on seed germination and of plant growth regulators (IAA or IBA, at 4000 ppm) on stem cuttings have been studied by them. *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Stereospermum chelonoides* and *Stereospermum colais* showed 80-85%, 90-95%, 80-90%, 45-50% and 10-20% seed germination respectively under controlled conditions. Seeds of *Premna obtusifolia* did not germinate. Effects of seed pretreatments were insignificant except in the case of *Stereospermum chelonoides*, which showed 5% increase in germination using GA_3. Hardwood cuttings of *Gmelina arborea*, *Oroxylum indicum* and *Premna obtusifolia* showed 6%, 80% and 73% rooting, respectively, following a quick dip in IBA (4000 ppm). Vegetative propagation using stem cuttings from 1 to 3 year old seedlings was successful in four species: *Aegle marmelos* (100%), *Gmelina arborea* (55%), *Oroxylum indicum* (57-100%) and *Stereospermum colais* (60-100%) using stem cuts of juveniles.

Yasodha *et al.* (2004) have pointed out the importance of biotechnological research in the tree species of *dasamula*. This review provides an account of the present status of medicinal importance and biotechnological research in the five tree species of *dasamula* namely *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Premna integrifolia* [*P. serratifolia*] and *Stereospermum suaveolens*, found in India. In *dasamula* tree species, where the roots are used in the preparation of ayurvedic formulations, the destruction of plants is severe and planting of seedlings of these species is almost

negligible. Clonal propagation through macro and micropropagation techniques is practiced on a limited scale for *Gmelina arborea* and *Aegle marmelos*. Research activities are negligible in species like *Oroxylum indicum, Premna integrifolia and Stereospermum suaveolens*. Research towards either genetically improving the species for higher production of the active components or increasing the production of the compounds under *in vitro* culture conditions is essential and it is practiced in several medicinal plant species. The application of *in vitro* culture for the propagation of *dasamula* tree species will help in the sustainable availability of propagules and plant products. Relevant studies on the ten species of plants used as members of *dasamula* in South India are reviewed below under separate heads.

2.1. *Aegle marmelos*

Aegle marmelos is the source plant of *vilva*. It is an armed tree with axillary straight spines single or paired and three foliolate alternate leaves (Sivarajan and Balachandran, 1994). The North Indian and South Indian varieties show some morphological differences. The North Indian variety is taller with larger leaves and fruits (Warrier, *et al.* 2001). Mehra and Khosla (1973) has reported that the large fruited forms are tetraploid.

Mathura *et al.* studied bael (*Aegle marmelos* Corr.) diversity in India in 1992. Twenty four genotypes, including three wild types, having a wide range of variation in morphological and quantitative traits were identified

1996. Seeds soaked in water for 24 h before sowing and sown directly in polyethylene bags showed the best germination percentage (75%); the next best germination was in seeds soaked for 24 h and sown in nursery beds (49%). A study of variation in fruit set, retention and yield of 10 eight year old budded trees of bael (*Aegle marmelos*) clones were conducted during 1995 and 1996, in Pantnagar, Uttar Pradesh, India by Jaiswal and Misra (1998). The results showed significant variation in fruit set, retention and yield of various clones.

Effect of growth regulators, acid and mechanical scarification on germination of *Aegle marmelos* was reported by Nayak and Sen in 1999. Among the various treatments, water soaking resulted in the highest percentage of germination (80%) which was closely followed by conc. H_2SO_4 treatment for 20 min (76%). Although water soaking resulted in the highest percentage of ultimate germination, initiation and completion of germination took longer time when compared to conc. H_2SO_4 treatment.

Effect of plant bioregulators (GA and IBA) and potassium nitrate on seedling quality of bael was studied by Misra and Jaiswal (2001). Seedling growth in terms of height, number of branches, leaves, secondary roots, and dry weight of leaves, stem and top, was found to be the maximum with foliar sprays of GA at 1000 ppm, while the number of fibrous roots per seedling, length of tap root and dry weight of roots were found to be the maximum with foliar sprays of IBA (1000 ppm). Shoot root ratio was

the maximum with GA (1000 ppm) and minimum with IBA (1000 ppm).

The comparative efficiency of grafting methods (whip, splice or cleft grafting) on survival and growth was investigated for *Aegle marmelos* grown under West Bengal conditions, India, during the monsoon by Maiti *et al.* (1999). Whip grafting was found to be the best method with 70% success and the best shoot growth. A comparative study of propagation methods of this plant using seeds and grafts has been carried out by Sumy *et al.* (2000).

Effect of sodicity on seed germination and seedling growth of bael was studied by Pandey and Pathak (1988). Percentage of seed germination, days for germination and seedling establishment and growth increased significantly with decrease in sodicity. Media requirements for seed germination and seedling establishment of bael were studied by Chattopadhyay and Mahanta in 1989. It was found to be the highest (100%) in well decomposed cow dung, next highest (72.5%) in sand + cow dung and the lowest (15%) in soil alone. A month after sowing, seedling growth was also best in cow dung. Twin seedlings in *Aegle marmelos* was noted by Yadav *et al.* in 1990. Out of a batch of 1000 seeds, 2 produced twin seedlings, which grew for two months before they died. The seedlings were apparently the result of polyembryony which has not been reported in this species previously.

Hore and Sen observed the effect of seed treatment on longevity of *Aegle marmelos* seeds in 1985. Three days after extraction the seeds were treated in several ways, then dried back to their original weight and stored in alkathene bags at room temperature for up to 132 days. Some seeds were sown 12 days after treatment and others at 132 days. Germination of seeds sown 12 days after treatment was greater than 88%, except in seeds treated in boiling water for 25 minutes (3.3%).

Moti *et al.* (1976) has reported patch budding as the most effective type of budding in bael (*Aegle marmelos*). Propagation of bael by budding was attempted by Singh *et al.*, in 1976 and found that bud take amounted to 100% with patch budding in June or July. Scion growth, assessed 90 days later, was the greatest with July budding.

Effect of growth regulators, invigoration and etiolation on rooting of air layers of bael was studied by Mukherjee *et al.* in 1986. Fifteen year old trees were invigorated by heading back some of the thick branches at 2.75 m above the ground during April, and some emerging young shoots were later wrapped in black moist cloth and alkathene as an etiolation treatment. Air layers were prepared in the 2nd week of August and some were treated with IBA at 5000 or 10 000 ppm, NAA at 5000 or 10000 or IBA + NAA, each at 5000 ppm. Invigoration, etiolation and treatment with IBA at 10000 ppm gave maximum rooting (90%) and good tree survival (77.7%).

Micropropagation of *Aegle marmelos* was achieved by using callus cultures initiated from stem explants on MS medium supplemented with different concentrations of kinetin, 2,4-D and NAA. Meristemoids developed in the callus when subcultured on medium supplemented with 1 mg kinetin with 5 mg NAA/litre. In the presence of BA, alone or in combination with NAA, these calluses showed shoot development. Multiple shoot induction from nodal explants was achieved on MS medium augmented with different concentrations of BA, kinetin and NAA. The shoot buds that developed from nodal explants were the most numerous in the medium supplemented with kinetin and NAA. Rhizogenesis of shoots was achieved in the presence of IAA (Varghese *et al.*, 1993).

A protocol for organogenesis from leaf explants of *in vitro* grown seedlings of *Aegle marmelos* was developed by Islam *et al.* in 1993. Adventitious buds were initiated on MS medium containing various concentrations and combinations of BA and IAA. The maximum frequency of shoot organogenesis (61.2%) and the highest number of shoots per explant (38.4) were obtained when 1.5 mg BA and 0.5 mg IAA/litre were used. Twenty day old seedlings proved to be the optimum source of explants. Shoots elongated on the same basal medium without auxin and supplemented with 0.5 mg BA/litre. Elongated shoots

were rooted on half strength MS medium supplemented with 0.1 mg IBA/litre.

A protocol for organogenesis from nucellar explants excised from fertilized ovules of immature fruits was developed by Hossain *et al.* in 1993. Adventitious buds were initiated on MS medium containing various combinations of BA, NAA, IAA and gibberellic acid. Medium containing 4.4 μM BA and 2.7 μM NAA produced the greatest number of adventitious buds per explant. Shoots were elongated by transferring explants with shoot buds to medium with a low concentration of BA (0.44 μM). Rooting of *in vitro* regenerated shoots was obtained in half strength MS medium with 4.9 μM IBA.

A protocol for *in vitro* plant regeneration from excised cotyledons of *Aegle marmelos* was standardized by Islam *et al.* in 1994. Cotyledon explants formed callus and shoot buds on supplemented MS agar medium. The highest frequency of explants forming adventitious buds and the maximum number of shoots per explant was obtained with cotyledons 110-150 days old. BA with IAA or GA gave better results than BA alone. Shoots were elongated by transferring explants with shoot buds to the same basal medium with 1.0 mg kinetin and 0.1 mg IAA per litre.

Hossain *et al.* (1994) achieved production of plantlets from *Aegle marmelos* nucellar callus. Slow growing calluses were induced from nucellar explants

excised from 90-120 day old developing fruits collected from a 40 year old tree on MS medium containing 40 g sucrose, 400 mg casein hydrolysate, 5 mg NAA and 1 mg kinetin/litre. Regeneration of shoots from 3 month old callus was achieved using the basal medium with 1-5 mg BA and 0.1 mg NAA/litre. Addition of 1 mg GA/litre favoured shoot growth. Callus derived shoots produced roots and developed into plantlets when transferred to half-strength MS medium supplemented with 0.5 mg IBA and 0.5 mg NAA/litre. Cotyledons from *Aegle marmelos* seedlings of various ages were cultured on MS medium supplemented with different combinations of growth regulators and regeneration was demonstrated by Hossain *et al* (1994). The optimum seedling age was 10 days for shoot induction response and BA was superior to kinetin, isopentenyladenine or zeatin. The optimum cytokinin (BA) concentration for bud induction was 2 mg/litre. The addition of 0.2 mg IAA/litre improved shoot regeneration efficiency. The proximal part of the cotyledon had the highest regeneration potential. Adventitious shoots were elongated on MS medium containing 0.5 mg kinetin and 0.1 mg GA/litre. Approximately 25% of regenerated shoots were induced to differentiate roots on half-strength MS medium with 0.5 mg IBA/litre.

Successful regeneration of plantlets from callus cultures derived from zygotic embryos is described for this tree by Islam *et al* in 1995. Murashige and Skoog basal medium with various combinations of cytokinins (BA and kinetin) and auxins (IAA and NAA) for the different regeneration stages (callus induction and differentiation,

regeneration stages (callus induction and differentiation, shoot formation, rooting) were used in this study.

Hazarika *et al.* (1996) studied the morphogenetic response of *in vitro* grown shoots of *Aegle marmelos* cultured in MS medium supplemented with BAP (0, 0.25, 0.5, 0.75, 1.0, 1.25 or 1.5 mg/litre). The highest shoot number and weight was observed after 8 weeks of culture on a medium supplemented with BAP at 0.5 mg/litre. Shoots were rooted on half strength MS medium supplemented with IBA at 0.5 mg/litre and successfully transferred to soil.

Ex vitro acclimatization of microshoots of *Aegle marmelos* was reported by Hazarika *et al.* in 1996. A protocol for acclimatization and rooting of microshoots was developed *ex vitro* in this study. Shoots (2 cm long) were obtained from proliferating cultures grown in BA supplemented media. Shoots were pulsed with either IBA or NAA (10 ppm for 2 min) before being placed to root in sterile soil rite or soil rite with sand for 6 weeks. Plant growth regulator treated cuttings rooted better than untreated cuttings (63.58-79.46% compared with 18.12-21.62% rooting). Pulsing shoots with IBA promoted early rooting in soil rite. Plants were acclimatized successfully without any transplant shock in the soil.

Ajithkumar and Seeni (1998) achieved rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos*. Bud break was dependent on cytokinin

supply, but the synergistic combination of BAP (2.0 mg/l) and IAA (1.0 mg/l) induced formation of shoots in 48% of explants after 7 weeks of culture. Shoot cuttings (3.0- 5.2 cm) were best rooted in half strength MS medium with 0.5 mg/l IAA (70%) or 10.0 mg/l IBA (90%).

Lal *et al.* (2003) developed an efficient clonal propagation system for the shoot tip culture of bael. Shoot tips were excised from 25 day old *in vitro* raised plantlets of bael and cultured vertically on Knop's medium supplemented with benzyladenine, kinetin and NAA. Treatments comprised: benzyladenine (at 0, 0.25, 0.50, 1.00 and 2.00 mg/litre), kinetin (at 0, 0.25, 0.50, 1.00 and 2.00 mg/litre) and/or NAA (at 0 or 0.5 mg/litre). 100% shoot regeneration was recorded on all media. Solitary shoot per explant was observed on all media tried within 2-5 days of inoculation. Full strength Knop's medium supplemented with 15 mg IBA/litre produced maximum rooting (43.89%).

2.2. *Gmelina arborea*

Gmelina arborea is the source plant of the drug *kasmari.* The plant is a moderate sized unarmed deciduous tree with simple opposite leaves (Sivarajan and Balachandran, 1994). Das (1970) has provided detailed information on distribution, botany, silviculture and management, principal enemies, wood properties and utilization of *Gmelina arborea.* Jackson (1974) reviewed nursery techniques and compared the merits of the two types of planting stock raised, viz. stump plants and

plants raised in polypots. Dvorak (2004) has reviewed the global opportunities and challenges of *Gmelina arborea* as a timber tree. *Gmelina* occurs naturally in 11 countries in tropical and subtropical regions of Asia. Approximately 700,000 ha of *Gmelina* have now been established in plantations, small woodlots and agro forestry settings in west-central and eastern Africa, south east Asia, the South Pacific, and northern Latin America. It is expected that planting areas will expand to 800000 ha by 2020.

Ng (1975) briefly reports, the occurrence of natural grafts between roots of neighbouring trees of the same species, for *Gmelina arborea.*

Suitable months for planting *Gmelina arborea* stumps of different ages were experimented by Zamora and Agpaoa (1976). Stump plants of different ages with 2 inches of shoot and 6 inches of taproot were planted at monthly intervals from May to Sept. The survival rate was best with 7-10-month old stumps planted in May-July, growth in height was the best with planting in May-June and it was not influenced by age of planting material. Various propagation trials were performed in Bangladesh in *Gmelina arborea* by Rahman (1977). Bud grafts were successful in this species, better results being obtained in April-May than in Nov.-Dec. The rooting response of stem cuttings was increased by treatment with IAA or IBA at 10 and 100 ppm. Better rooting was obtained in March than in November.

Survival of seeds and cuttings of *Gmelina* under different slope exposures was studied by Florido and Lim Suan (1977). The average rate of survival was 20.8% for cuttings and 18.2% for seeds. Cuttings rooted 2 or 3 days after planting; seeds started germinating 15 days after sowing. Field grafting of *Gmelina arborea* was studied by Zabala in 1977. Vegetative propagation by cuttings using growth hormones was first reported by Florido in 1978. IAA, NAA and IBA were applied at 0, 250, 500 and 750 ppm. NAA induced more root formation for all treatments. A higher concentration of the hormones enhanced root initiation and development.

Vegetative propagation of *Gmelina arborea* by stem cuttings was studied by Zakaria and Ong (1982). Rooting success in cuttings of two year old seedlings was nearly doubled by treatment with 100 ppm of IBA. The success was up to 96% in cuttings with secondary thickening and was significantly lower in cuttings from the upper part of the shoot. Cleft grafting and patch budding techniques were developed by Arya and Haque in 1982 at the Forest Genetics Nursery, Dehra Dun. Small two year old plants were used as stocks and scions and buds collected from seven year old trees and grafting done in February, March and April, 1979. All grafts were successful with those made in February and March becoming the best in graft take.

Rooting of cuttings of *Gmelina arborea* by hormone treatment (Hamsawi and Srivastava, 1985) and the effect of source, hormone treatment and position of the cutting

on rooting (Tang and Srivastava, 1985) have been investigated. In the first study cuttings from one year old seedlings were treated with IBA at 500-2000 ppm or IBA + NAA at 500 + 500 ppm before rooting in sand under mist. Treatment increased rooting success from 72% in controls to a maximum of 92% with IBA + NAA. 10 weeks after treatment, plant height, growth, dry weight and root/shoot dry matter ratio were significantly greater in treated plants than in controls, the best plants being those treated with IBA + NAA, followed by those treated with 1000 ppm IBA. In the second study, cuttings were taken from the base, middle or top of the stem of seedlings about 1 year old derived from plus-tree seed or mass collection in Malaysia. Rooting success increased significantly towards the base of the stem. Stimulation of root growth occurred through moderate moisture stress also. Best result of hormone treatment was obtained with IBA + NAA at 250 + 250 ppm.

An effect of temperature on germination of *Gmelina arborea* was studied by Ng and Hellum (1985). Seeds were germinated in the laboratory at constant temperature. No germination occurred below 17°C or above 40.5°C. Maximum germination capacity (77.5%) was observed at 28.7°C and minimum time interval (2.4 days) at 32°C. A study was made on the effect of rootstock age (7-18 months) and environmental conditions on the survival of *G. arborea* grafts by Oduwaiye *et al.* (1985). Results showed that there was no significant difference in the case of rootstock ages, but environmental conditions contributed significantly to survival. A humid polythene

tent gave the best conditions, followed by partial shade from surrounding trees, and planting in an open field.

Effect of source, hormone treatment, media and frequency of misting on rooting of cuttings of *Gmelina arborea* was studied by Sandum *et al.* (1986) in Malaysia. Stem cuttings were made from two year old plants and branch cuttings from ten month old stumps and fifteen month old plants raised from cuttings. The cuttings were treated with combination of 250 ppm IBA + 250 ppm NAA, or Seradix (active constituent IBA), or not treated (control). Treated cuttings were grown in pure sand or mixtures of sand and soil (3:1 and 1:1) in a mist chamber, where they were subjected to three misting frequencies (6, 4 or 3 daily at 2, 3 or 4 hour intervals respectively for 30 seconds). Growth of the cuttings was measured after 50 days. Stem cuttings from two year old seedlings gave a better rooting response (41.5%) than branch cuttings from 10 month old stumps (19.0%). None of the cuttings from branches of 15 month old plants raised from cuttings rooted. Root and shoot development (number and length) was also best in the stem cuttings. The combination of IBA + NAA promoted better rooting (23.3%), more roots (2.1 per cutting), and a longer dominant root (average length 12.4 cm), than Seradix (commercial rooting hormone) treatment. Percent rooting was better in mixtures of sand and soil than in pure sand, with the average length of dominant roots per cutting being the best in the 1:1 sand/soil mixture. There was no definite trend in rooting response with misting treatment, although the 3 per day

treatment was best in terms of root development (average length of root per cutting).

The importance of stump size in establishing plantations of *Gmelina arborea* was observed by Willson (1987). Trials were undertaken in this study to determine the best size of stump planting stock for the establishment of vigorous plantations. Four stump size classes based on root collar diameter were tested. The results showed that smaller sizes give the best results. Osonubi and Osundina (1987) studied comparison of the responses to flooding of seedlings and cutting of *Gmelina*. Rooted *Gmelina arborea* cuttings and eight week old seedlings of the same stock in a greenhouse were flooded for twenty days or watered and drained normally. Stomatal conductance and xylem pressure potentials were recorded every 2 days up to 28 days between 1200 and 1400 h. Other plants were harvested every four days to measure leaf area and shoot and root dry weight. Flooding resulted in reduction in stomatal conductance, xylem pressure potential and dry matter accumulation in both the groups of plants. In seedlings, flooding induced the formation of hypertrophied lenticels, stem hypertrophy and the production of short, thick adventitious roots, while in cuttings only thin roots and numerous smaller lenticels were induced. For eight days after the flooding treatment ended, the flooded seedlings grew faster than control seedlings, while in cuttings post-flooding growth was similar to that of control plants. It is suggested that seedlings may perform better than cuttings in very wet or saturated soils. Surendran reported vegetative propagation in *Gmelina* through single

node cuttings in 1990. Cuttings were prepared from mature branches (brown wood, with a dormant bud) and leafy shoots (green wood) of a three year old tree of *Gmelina arborea* at the Forestry Research Station at Mettupalayam, Tamil Nadu. Rooting ranged from 33.3 to 73.3% in the brown wood cuttings, with the best rooting occurring after IBA treatment (73.3% at 100 ppm and 60.0% at 1000 ppm). Most green wood cuttings did not root at all. This failure is attributed to the immaturity of axillary buds and attack by soil borne pathogens in the mist chamber. The best propagation technique in *Gmelina arborea* was found to be patch budding by Beniwal and Singh, 1990.

Kinako and Ogbonnaya (1990) studied the propagule size and seedling growth relationships in Gmelina arborea. The initial two week growth period showed a positive ($r = 0.529$) and significant ($p < 0.05$) correlation between propagule size and seedling growth, but the trend was only transitory for height measurements and was not significant at 4 and 6 weeks. There was a marked positive relation between propagule size and the number of emerging seedlings. All propagules gave good quality seedlings, irrespective of size.

Akbar (1994) studied the effect of IBA on cutting survival rate in *Gmelina* and observed that IBA application failed to increase survival percentage. In 1998 Chung and Yang reported vegetative propagation of *Gmelina arborea* by rooted cuttings from juvenile tissues.

Sharma *et al.* (2000) took trials on pretreatment of *Gmelina arborea* seeds to obtain better germination and seedling vigour. Fresh seeds of *Gmelina arborea* were used to study the germination behaviour with the following pretreatments: cold water for 24 h (T_1); boiled water for 24 h (T_2); 10% H_2SO_4 for 10 minutes (T_3); 20% H_2SO_4 for 10 minutes (T_4); 40% H_2SO_4 for 10 minutes (T_5); and 60% H_2SO_4 for 10 minutes (T_6). Among the various pretreatments, 20% H_2SO_4 for 10 minutes was the best treatment to stimulate the germination (97%) and plant production (87%) of seeds. Seed germination and plant production in control were 66 and 40 respectively. Karoshi *et al.* (2001) reports the occurrence of albino seedlings in *Gmelina arborea*. Root and shoot length, and number of roots of normal green plant and an albino 25 days after sowing were compared. Out of the 12500 seeds sown, only one seed produced an albino seedling.

Mindawati and Rohayat investigated the effect of fruit colour (maturity level) of *Gmelina arborea* on germination and seedling growth in 1994. Seeds from yellow and yellow greenish fruits (the more mature ones) germinated best, but colour/maturity of fruits did not influence seedling growth. Chandra and Gandhi (1995) studied rooting of cuttings of *Gmelina arborea*. Coppice shoots (12-15 cm long) of *Gmelina arborea* with only two pairs of leaves were successfully rooted under mist at 24°C and a relative humidity of 90% in coarse sand as growing medium. Rooting was 55.2% in cuttings dipped in IBA

powder at 5000 ppm, and 30.4% in untreated cuttings. Rooting started after 15 days, and fibrous roots developed after 25 days.

In 1993 Agboola *et al.* reported that there was no effect of orientation and soil types on germination of *Gmelina* seeds. The effect of different methods of extraction of *Gmelina arborea* seeds on germination was investigated by Ogunnika and Kadeba in 1993. The highest percentage of germination was recorded in freshly extracted seeds, followed in decreasing order by seeds extracted after soaking in running water, heaping, and standing in water. There was no germination in unprocessed fruits and this was attributed to the non removal of the fruit pericarp, which might have inhibited germination.

A review of propagation programs for *Gmelina arborea* was made by Romero in 2004. This tree can be propagated by seedlings, rooted cuttings, and grafting. Small tree planting programs with limited budgets tend to propagate it by seedlings, and larger or industrial plantation projects with breeding programmes propagate it by rooted cuttings.

The role of heterosis in genetic improvement of *Gmelina arborea* in North East India was studied by Singh and Deori in 1988. Assessments were made of seed setting (yield) and seed weight in the F_1 hybrids and the parents, and germination percentage and seedling height growth were measured in the nursery for all. Majority of

crosses showed positive heterosis for all characters studied; the highest incidence of negative heterosis was for seed weight and seedling height. Selfing produced the poorest performance for all characters.

The possibility of tree selection and breeding for genetic improvement of wood properties of *Gmelina arborea* was studied and reported by Akachuku (1984) in Africa. Samples were collected from plantations in Nigeria and their density, fibre length and fibre proportion measured. There were considerable differences between trees of the same age in wood density, fibre length and proportion and tree size, at the same spacing in the same environment. The magnitude of within tree variations in these properties was also different in trees within the same plot. Some individuals combined comparatively high density, fibre length and proportion with fast growth rate and uniform wood. There was sufficient phenotypic variation to encourage work on the genetic improvement of these properties. It is recommended that screening for tree form and volume should be followed by selection for desirable wood properties.

Yang *et al.* in 1992 reported micropropagation of *Gmelina arborea.* Nodes, internodes, leaf pieces and cotyledons were cultured on MS media with NAA and BA at different concentrations. The best combination for multiple shoot production was node culture on MS medium with 0.01 mg NAA and 5 mg BA per litre (3.5 shoots/node regenerated on average). Addition of 0.2% activated charcoal promoted shoot elongation. In order to

produce plantlets, shoots were excised at 0.5 and 1.5 cm and induced to root on MS medium with IBA concentrations; 71% of shoots successfully rooted on medium with 4 mg IBA/litre. Excised shoots were also rooted on growth medium consisting of vermiculite, peat and perlite (2:2:1). Though only 63.9% of shoots were induced to root *ex vitro*, and shoots shorter than 1 cm hardly rooted, the method appeared useful for mass plantlet production for clonal reforestation.

Mohan (1992) studied the response of fertilizer on germination, growth and biomass production of *Gmelina arborea* in nursery stage. Germination was greater in the N 150 kg/ha treatment (39-53%) than in the other N treatments (24-35%) and maximum in the N 150 kg/ha: P 0 kg/ha (53%) and N 150 kg/ha: P 75 kg/ha (48%) treatments. Growth and biomass production were the greatest in the N 150 kg/ha: P 75 kg/ha treatment. Rosales *et al.*, (1992) studied rooting percentage in a controlled environment and initial growth after planting in relation to position of cutting within a shoot, concentration and method of applying IBA and rooting substrate for *Gmelina arborea.* Chuan *et al.* (1993) reported *in vitro* clonal propagation and cell suspension culture of *Gmelina arborea.*

Micropropagation of *Gmelina arborea* was reported by Kannan and Jasrai (1996). In this study multiple shoots were obtained from single node explants of mature *Gmelina arborea*, a valuable timber tree of South East

Asia, on MS medium supplemented with BA. Seven to nine shoots were formed when *in vitro* derived single node explants were subcultured on MS medium supplemented with 1.1 μM BA. For root initiation, cut ends of microshoots were pulsed for 5 min with 246 μM IBA and transferred to a plastic cup containing sterile vermiculite. The shoots were covered with polyethylene and maintained in a culture room. After hardening, plantlets were transferred to earthen pots containing a mixture of garden soil: compost before being established in the field.

The effect of seed size (small or large) on germination and seedling growth was investigated by Agboola (1996) in *Gmelina arborea.* Rate of germination was faster in small sized seeds. Total dry weight of seedlings of *Gmelina arborea* raised from large sized seeds was 2-3 times more than those from small-sized seeds. The relative growth rate of seedlings of *Gmelina arborea* raised from small sized seeds was more than that from large sized seeds. Hendromono *et al.* investigated the morphological quality and growth of *Gmelina arborea* seedlings in containers sprayed by 'Kocide 80 AS' in 1996. In this experiment the effect of Kocide 80 AS on the morphological quality and shoot and root growth of *Gmelina arborea* seedlings was investigated.

Charomaini (1998) studied pre-sowing treatment of *Gmelina arborea* seeds in assisting germination. The most effective treatments were immersion in water at room temperature (27°C) for 1 or 2 days which promoted

respectively 62.2% and 51.06% germination beginning seven days after sowing. Untreated (control) seeds gave 44.4% germination. Seed viability was reduced after immersion in hot water.

Parthiban *et al.* (1999) used double nodal branch cuttings for vegetative propagation *Gmelina arborea* using stem cuttings. The stem cuttings were treated with IBA dips (1000, 2000, 3000 or 4000 ppm) and rooted in a mist chamber. The cuttings were maintained at relative humidity of 70-80%. IBA at 2000 and 3000 ppm was effective in rooting of the cuttings.

Micropropagation of *Gmelina arborea* through axillary bud culture was reported by Thirunavoukkarasu and Debata (1998). Axillary buds collected from actively growing young shoots of the epicormic and crown regions of a six year old elite tree of *Gmelina arborea* were used to initiate shoot cultures. Explants from the epicormic region produced 2.7± 0.1 shootlets/explant when cultured on McCown's medium for woody plants supplemented with 2% sucrose. Explants cultured on media supplemented with different concentrations of BA (0.25-0.5 mg/litre) produced comparatively less shootlets. Higher concentrations of BA (1.0-1.5 mg/litre) in combination with NAA (0.5 mg/litre) resulted in very few or no shootlets and profuse callusing of the explants. Explants from the crown region did not show any multiplication symptoms and gradually blackened. About 80% of the *in vitro* regenerated shoots rooted on IBA (1.5 mg/litre)

supplemented McCown's medium within 7-9 days of culture. Plantlets were established in sterilized soil in a humidity chamber and transferred to field conditions with 100% survival.

Seasonal variation in antioxidant enzymes and the sprouting response of *Gmelina arborea* nodal sectors cultured *in vitro* was studied by Thakar and Bhargava (1999). Nodal sector explants of *Gmelina arborea* showed seasonal variation in the sprouting of axillary buds *in vitro*. Explants from mature trees showed only 20% sprouting in summer, while those from seedlings and young trees showed over 85% sprouting in this season. In winter, there was a significant decrease in the sprouting response of explants from young and mature trees but not in that of seedling explants. Attempts were made to correlate the sprouting response of the explants with their antioxidant status. Activities of three enzymes, *viz.* ascorbate peroxidase, superoxide dismutase and guaiacol-dependent peroxidase, which form a part of the antioxidant defence system of plants, were studied in the excised nodal sectors before and after placing them on culture medium. Prior to culture, higher activities of ascorbate peroxidase, superoxide dismutase and guaiacol-dependent peroxidase were observed during winter than during summer. During the culture period, ascorbate peroxidase, superoxide dismutase and guaiacol-dependent peroxidase showed variation depending on the season in which the explants were isolated, and the age of the donor. Axillary bud sprouting *in vitro* appeared to depend more on the

physiological state of the donor, than on the oxidative stress generated during culture.

In 1999, Gamboa and Abdelnour reported a method for *in vitro* propagation of *Gmelina*. Seeds were isolated from previously surface disinfected endocarps. Immersion in 96% sulfuric acid for 10 min, followed by immersion in distilled and autoclaved water for 48 h was the best scarification method. During the multiplication phase, the effects of different concentrations of BAP (0-1.0 mg/litre) and inorganic salts (reduced and normal) in the culture media were evaluated. The best results were obtained with cultivation of nodes of *in vitro* germinated plantlets on MS medium with normal salt concentration and 0.5 mg/litre BAP.

Clonal micropropagation of *Gmelina arborea* was reported by Melendez and Contreras in 2000. Several assays were conducted to perform an *in vitro* clonal propagation using *Gmelina arborea*. Axillary buds from 1 to 2 year old plants were used as explants. These buds were previously sterilized and cultured on a half strength ionic MS medium, adding (mg/litre): myo-inositol 100; thiamine 0.10; nicotinic acid 0.5; piridoxin 0.5; glycine 2.0; sucrose 30000; and agar (B&T) 1.1%. TDZ (0.05-0.1), BA (0.1-0.25) and NAA (0.1-0.2) were used as plant growth regulators separately or combined. Adventitious buds, between 2-7 by explants, were obtained after 6 weeks of culture. Rooting was induced on medium containing NAA 0.1 mg/litre. Furthermore, *Gmelina* plants were

transferred to sterile substrate composed of black ground: sand (1:1) for hardening.

Naik *et al.* reported the micropropagation of *Gmelina* arborea in 2003. Axillary shoot elongation, formation of multiple shoots and rooting of shoots were compared in nodal segment cultures of *Gmelina* from seedlings obtained from six provenances, over several subcultures. Provenance dependent variation was observed with respect to these parameters. In addition, a subculture dependent decrease was observed in multiple shoot formation and root induction. Seventy percent of the rooted plantlets were successfully hardened and transferred to soil. A transient decrease in photochemical efficiency was observed during the early stages of hardening, whereas ribulose-1,5-bisphosphate carboxylase levels increased gradually as the plants acclimatized to photo autotrophic growth.

Cerdas (2004) reported micropropagation of clones from controlled crosses of *Gmelina arborea* in Costa Rica. Seeds of 10 families of *Gmelina* obtained from controlled crosses, were established *in vitro* using nodal segments. Significant differences were observed in the bud induction rate and in the rooting percentages of families and genotypes.

2.3. *Oroxylum indicum*

Oroxylum indicum is the source plant of *syonakah*. The plant is a tree with large opposite bipinnate leaves

(Sivarajan and Balachandran, 1994). A brief description of the biology, ecology and artificial regeneration of this species was provided by Nambiar in 1982. Collection and nursery evaluation of *Oroxylum indicum* was carried out by Ghate and Sathe in 1998. Effects of seed pretreatments (soaking in water, hot water treatment + soaking in 100 ppm GA_3 or H_2SO_4) on seed germination and of plant growth regulators (IAA or IBA, at 4000 ppm) on stem cuttings were studied. *Oroxylum indicum* showed 80-90% seed germination under controlled conditions. Effects of seed pretreatments were insignificant which showed 5% increase in germination using GA_3. Hardwood cuttings of *Oroxylum indicum* showed 80% rooting, following a quick dip in IBA (4000 ppm). Vegetative propagation using stem cuttings from 1 to 3 year old seedlings was successful in this species.

According to Wealth of India the tree reproduces naturally by seeds which germinate in the beginning of the rainy season; moderate shade is necessary in the early stages. Artificial reproduction may be done by sowing the seeds in the nursery during March-April and transplanting the seedlings in the 1st or 2nd rainy season. The tree can also be propagated by transplanting root suckers which are produced in great profusion, often forming a dense growth round the parent stem. The rate of growth of the tree is reported to be fast with a mean annual increment of 4 cm - 6.4 cm (Anonymous, 1991). Sumy *et al.* (2000) studied the propagation methods of this plant using seeds and root suckers.

Polyembryony in *Oroxylum indicum* was first reported by Sharma in 2004. Importance of biotechnological research in tree species of *dasamula* was reviewed by Yasodha *et al.* in 2004. This review provides an account of the present status of medicinal importance and biotechnological research in the five *dasamula* tree species including *Oroxylum indicum.* They reported that Research activities are negligible in species like *Oroxylum indicum.*

Flavonoids of the stem bark of *Oroxylum indicum* were reported by Subramanian and Nair (1972). The stem bark contained chrysin, oroxylin-A, baicalein, scutellarein, baicalein-7-glucuronide and scutellarein-7-rutinoside.

In vitro propagation of *Oroxylum* was reported by Dalal and Rai (2004) using cotyledonary nodes. Among the different types of cytokinins used for culture establishment, BA exhibited the best response with higher concentrations (8.87 µM or above) for inducing multiple shoots. Inclusion of IAA (2.85 µM) into BA supplemented medium triggered a high frequency of response as well as a proliferation of shoots. The best medium for proliferation was MS with BA (8.87 µM) and IAA (2.85 µM). However incorporation of GA was mandatory to enhance shoot elongation. Repeated subculturing of cotyledonary node and *in vitro* developed nodal segments in MS medium with BA (4.44 µM) at 4-week intervals resulted in continuous mass multiplication of shoots without any evidence of decline. Root induction was best when MS strength was

reduced to one quarter and combined with NAA (2.69 μM) and IAA (5.71 μM) with high survival rate.

2.4. *Stereospermum colais*

Stereospermum colais is used as the drug source of *patala* in Kerala. However, *Stereospermum suaveolens* is being used as the drug source in some other parts of India. *Stereospermum colais* is the white flowered species and *Stereospermum suaveolens* is red flowered (Sivarajan and Balachandran, 1994).

A study was conducted (Ilorkar and Garad, 2000) in Navegaon National Park in Maharashtra, India, to assess the regeneration status of tree species occurring in the forest area. Ratios between seedlings and trees showed poor regeneration of *Stereospermum suaveolens* (*Stereospermum chelonoides*) at 25:0. Sumy *et al.* (2000) studied the propagation methods of *Stereospermum suaveolens* using seeds. The Wealth of India (Anonymous, 2003) has reported this plant as propagated through seeds and suckers. This can be carried out by direct line sowing and by transplanting the nursery raised seedlings. For nursery purposes fresh seeds should be sown during April-May and transplanting during the second rainy season.

2.5. *Premna corymbosa*

Premna corymbosa is used as the drug source of *agnimanthah*· However, Ayurvedic Formulary of India suggests *Clerodendrum phlomides* as the real source of the

drug and *Premna corymbosa* as the substitute (Sivarajan and Balachandran, 1994). Chunekar (1982) opines that these two can be used as substitutes for each other as they have similar properties. *Premna corymbosa* is a small sized tree with simple opposite and aromatic leaves (Sivarajan and Balachandran, 1994).

Chen *et al* (1988) has studied the propagation and cultivation of *Premna microphylla*. In experiments with *Premna microphylla* for pectin production, propagation by seed was unsuccessful but propagation from hardwood cuttings was 77.6% successful. Sumy *et al.* (2000) also have studied the propagation methods of *Premna corymbosa* vegetatively through cuttings.

Effect of auxins on the rooting and survival of air layers in *Premna mucronata* was studied by Bhutani in 2003. Some 4 to 5 year old *Premna mucronata* trees were treated with 0.5 or 1.0% talc IBA, NAA or IAA and air layered in July or August. After 1 and 2 weeks, rooting percentage, number of primary roots per cutting, number of new leaves per cutting and survival percentage was higher when air layering was carried out in July than in August. IBA at 1.0% resulted in the highest rooting percentage, and number of primary roots and new leaves per cutting. Treatment with 0.5 and 1.0% IBA resulted in 100% survival of plants air layered in July.

2.6. *Desmodium gangeticum*

Desmodium gangeticum is used as the drug source of *prsniparni* in Kerala. However, some materia medicas consider *Uraria lagopodioides* and *Uraria picta* as the drug source of *prsniparni*. *Desmodium gangeticum* is a sub erect diffusely branched undershrub with alternate unifoliolate leaves (Sivarajan and Balachandran, 1994).

A comparison of the germination characters of *Desmodium* species was studied by Datta *et al.* in 1987. In a comparison of germination patterns in four species of *Desmodium* namely *Desmodium gyrans* with the thickest seed coat had the greatest water uptake after soaking for 20 h. *Desmodium gangeticum* with the thinnest seed coat absorbed more water than *Desmodium cephalotes* or *Desmodium pulchellum* at 4-20 h. Germination speed and percentage and hypocotyl length was the greatest in *Desmodium gyrans* and root length the greatest in *Desmodium cephalotes*. In *Desmodium gangeticum*, seed germination increased with increasing dry storage duration at 20°C and 50°C. In *Desmodium cephalotes* and *Desmodium gyrans*, germination at 20°C gradually decreased and ceased altogether with high temperature exposure (50°C), but not in *Desmodium gangeticum*. Pretreatment for 4 h at 70C° before transfer to room temp. (24-29°C) gave germination rates of 70.0- 97.5% for all species except *Desmodium pulchellum* (5.0-12.5%).

The effects of different agrotechniques (*e.g.*, shading, harvesting date, soil pH) on the chemical composition and

quality of the drug from *Desmodium gangeticum* plants grown on sodic soil (pH 8.6-10) were investigated by Prakash *et al.* in 2001. The roots were extracted with ethanol or 50% ethanol: water. The leaves were analyzed for ash, protein, carotenoid (provitamin-A), vitamin C, tannins, phenolics, anthocyanins, sugars, nitrate and oxalate. The total ethanol and 50% ethanol: water extracts showed that the shaded plants produced higher amounts (4.91% and 14.27%, respectively) of total extract compared to the plants grown in the open (2.89% and 11.23%, respectively). Similarly, the wild plants collected from shade treatment also had 5.17% and 11.97% total ethanol and 50% ethanol: water extractives, respectively, compared with the plants grown in the open (4.46% and 10.20%, respectively). Cultivation at different pH indicated that pH 8 to 9 provided a good quality drug. Soil mixtures prepared from 2:1:1 and 3:1:1 local soil: sand: farmyard manure to study the effects of soil texture were suitable for cultivation and gave comparatively higher amounts of 50% ethanol: water extractives (11.26% and 12.22%, respectively). Analysis of chemical constituents suggested that the plants should be harvested in summer or winter. The highest antioxidant and nutrient contents were observed in the middle order leaves harvested in summer. Sumy *et al.* (2000) studied the propagation methods of this plant using seeds.

Studies on the antioxidant activities of *Desmodium gangeticum* was carried out by Govindarajan *et al.* in 2003. Plant extract was studied for its scavenging activity

against diphenyl picryl hydrazyl (DPPH), nitric oxide, ferryl-bipyridyl and hypochlorous acid, and for its effects on lipid peroxidation. Nitric oxide was generated using sodium nitroprusside and was studied using Griess reagent. To study the iron-chelating capacity of the extract, the percentage ferryl bipyridyl inhibition was also studied. Hypochlorous acid scavenging activity was evaluated by measuring the inhibition of 5-thio-2-nitrobenzoic acid oxidation. The results indicated that *Desmodium gangeticum* extract had potent antioxidant activity.

2.7. *Pseudarthria viscida*

Kerala physicians have by and large accepted *Pseudarthria viscida* as the drug source of *saliparni.* However, some publications equate it with the two species of *Uraria,* namely *Uraria lagopodioides* and *Uraria picta. Pseudarthria viscida* is a viscid pubescent semierect perennial undershrub with slender branches and alternate three foliolate leaves (Sivarajan and Balachandran, 1994).

Sumy *et al.* (2000) studied the propagation methods of this plant using seeds and cuttings. Branches consisting of two nodes from the base of a mature plant are selected for planting.

2.8. *Solanum melongena* var. *insanum*

Solanum melongena var. *insanum* and *Solanum violaceum* are considered as the source plants of the drugs coming under *brhatidvayam* in Kerala. However, other

species of *Solanum* like *Solanum virginianum* are also considered as drug source of *brhati* somewhere. *Solanum melongena* var. *insanum* is a prickly pubescent undershrub with simple alternate leaves (Sivarajan and Balachandran, 1994).

Viability of seeds of the semi-wild *Solanum melongena* var. *insanum* declined rapidly over 120 days in warm (30^{0}C) humid storage. Seeds dried over silica gel (moisture content near 7%) and stored at 18 to 20 degrees showed no change in germination rate, which remained at 92-98%. Tetrazolium test successfully distinguished between dead and dormant seed lots (Mohamed *et al.*, 1988).

2.9. *Solanum violaceum*

Solanum violaceum is the second member of *brhatidvayam.* The plant is an armed shrub with simple alternate leaves (Sivarajan and Balachandran, 1994). Enzyme treatment as an aid in the study of seed surface structures of *Solanum* species was used by Lester and Durrands in 1984. Enzyme treatment of seeds before scanning electron microscopy showed that the seed surface of *Solanum violaceum* from India is distinct.

Isshiki *et al.* in 1998 studied RFLP of a PCR amplified region of chloroplast DNA in egg plant (*Solanum melongena*) and related *Solanum* species. Variations of restriction patterns among the species were recognized after digesting the amplified products with seven

restriction enzymes TaqI, AluI, RsaI, StyI, AseI, HinfI and XbaI. The restriction patterns divided the examined nine species into five clusters. The study demonstrated the availability of the PCR-RFLP analysis of ctDNA for assessing taxonomic relationships and identifying cytoplasmic parentage of interspecific hybrids in egg plant and related *Solanum* species.

2.10. *Tribulus terrestris*

Tribulus terrestris is the source plant of the drug *goksurah.* However, *Pedalium murex* is also used as the source plant of this drug somewhere. *Tribulus terrestris* is a hirsute prostrate herb with opposite pinnate leaves. A soil profile analysis for *Tribulus* fruit and seed was made by Goeden and Ricker in 1973. The incidence of *Tribulus terrestris* carpels and viable seeds recovered from soil samples varied inversely and linearly with depth down to 24 cm. Soil type and texture and method of tillage influenced the gradients of carpel distribution and their rate and degree of vertical migration. Evidence for enforced, induced and innate dormancy in *Tribulus terrestris* seed was presented. Most seeds germinated from depths of <4 cm in cultivated sandy loam and clay loam soils. The intra-carpel pattern of seed germination was such that the seeds located nearest to the stylar end of 2, 3 and 4 seeded carpels tended to germinate first with the other seeds tending to follow in order of their position in the carpel from the stylar to the receptacular end. However, all possible combinations of intra carpel germination patterns occurred under field conditions.

Ernst and Tolsma (1988) studied the dormancy and germination of *Tribulus terrestris*. This laboratory study showed that dormancy of seeds lasted for 3-6 years. Sumy *et al.* (2000) studied the propagation methods of this plant by using seeds.

Erhun and Sofowara (1986) reported callus induction and detection of metabolites in *Tribulus terrestris*. Callus cultures were induced from leaf and stem portions on Murashige and Skoog medium containing $2.5x10^{-6}$ μM 2,4-D and $2.0x10^{-6}$ μM kinetin. Steroidal sapogenins such as diosgenin and hecogenin, used in the synthesis of medicinal steroids, are known to occur in their glycosylated forms in *Tribulus terrestris*, although the yields are uneconomic. Lignin, saponins, flavonoids spirosta-3,5-dienes, and free and glycosylated steroidal sapogenins were detected in the cultures. Free sapogenins were not present in the explant sources.

Louveaux *et al.*, in 1998 studied the variability in flavonoid compounds of four *Tribulus* species. This study determined how variability in the phenolic metabolism of four closely related *Tribulus* taxa might affect palatability.

Variation in populations of *Tribulus terrestris* in terms of burr morphology was observed by Scott and Morrison (1996). Variation in burr morphology was investigated as part of a study to identify the origin of the widespread weed and potential biological control.

Tissue culture studies of *Tribulus terrestris* was initiated by Ali *et al.* in 1997. Direct regeneration of shoots and roots through juvenile explants has been described in this study. Cotyledonary leaves along with epicotyl segment from young seedlings were cultured on MS medium containing various concentrations of auxin with cytokinin and glutamine. A combination of 0.2 mg/l NAA, 0.5 mg/l BAP and 50 mg/l glutamine induced high frequency of shoot and root differentiation in ten weeks. Callus also could be induced on the above medium from the cut end of radical segments. Morphogenic response such as shoot and root differentiation was recorded at regular intervals. Somatic embryogenesis and plant regeneration from stem explants of *Tribulus terrestris* has been reported by Mohan *et al.*, 2000. MS medium supplemented with 0.5 mg/l 2,4-D + 0.5 mg/l kinetin was best for both callus induction and callus maintenance. MS + 0.5 mg/l IBA + 2.0 mg/l BA favoured the formation of friable embryogenic callus in 80% of the cultures, whereas addition of 500 mg/l casein hydrolysate to this medium promoted the development of somatic embryos. Addition of 2 mg/l silver nitrate to this medium induced synchronous development of somatic embryos. Cotyledonary stage somatic embryos transferred to MS basal medium developed into plantlets and on medium with 0.1 mg/l NAA + 2.0 mg/l IBA developed a vigorous tap root system.

The relationship between two morphological traits (mean length of the main four stems and taproot width) and biomass (total, vegetative and reproductive) were

studied by Mas and Verdu (2001) in a population of *Tribulus terrestris* covering a wide range of plant sizes. The best relationship was obtained between the mean length of the main four stems and the total biomass. The fitted model provides a feasible estimation of growth rates using a non-destructive sampling methodology.

Determination of steroidal saponins in *Tribulus terrestris* by reversed phase high performance liquid chromatography and evaporative light scattering detection was carried out by Ganzera *et al.*, in 2001. This paper describes the first analytical method suitable for the determination of steroidal saponins in *Tribulus terrestris*. A separation by high performance liquid chromatography (HPLC) was achieved by using a reversed-phase (RP-18) column, evaporative light scattering (ELS) detection, and a water/acetonitrile gradient as the mobile phase. The marker compound, protodioscin, was detected at a concentration as low as 10.0 μg/mL. Several different samples of plant material were successfully analyzed, and depending on origin and plant part used for extraction, significant differences in the composition of the saponins were observed.

MATERIALS AND METHOD

A.V. Raghu “Studies on variability, conservation and propagation of Dasamula group of plants” Thesis. Department of Botany , University of Calicut, 2005

MATERIALS AND METHODS

Chapter III
MATERIALS AND METHOD

3.1. Materials used

The present study is an investigation in to the variability and correlation of characters, genetic distance between the accessions and conservation and propagation of the members of an important ayurvedic group *dasamula* (group of ten roots), which enters into the composition of many ayurvedic formulations. The ten species namely *Aegle marmelos* (*vilva*), *Gmelina arborea* (*kasmari*), *Oroxylum indicum* (*syonakah*), *Stereospermum colais* (*patala*), *Premna corymbosa* (*agnimanthah*), *Pseudarthria viscida* (*saliparni*), *Desmodium gangeticum* (*prsniparni*), *Solanum melongena* var. *insanum* and *Solanum violaceum* (*brhatidvayam*) and *Tribulus terrestris* (*goksurah*) are used in South India as *dasamula* (Sivarajan and Balachandran, 1994) and hence they have been used as study materials presently. The materials used and the methodology followed are described below under appropriate heads.

3.1.1. *Aegle marmelos* (Linn.) Corr. (Family: Rutaceae)

Aegle marmelos, the source plant of the drug *vilva* is a spiny tree sparsely distributed through out India on the plains and in the hilly tracts up to 1300 m elevation (Fig. 3.1). The plant is a medium sized armed deciduous tree with straight, sharp, axillary thorns and yellowish brown shallowly furrowed corky bark. Leaves are alternate, three

Fig. 3.1. ***Aegle marmelos*** **A. Habit; B. Flower; C. Fruits**

foliate, leaflets elliptic, lanceolate or oblong-obovate obtuse, terminal one 4.5 cm x 2.5 cm, lateral ones smaller, glabrous, margin sub crenulate; flowers white, sweet scented in axillary panicles; calyx tube cupular, lobes 4 or 5; petals 5, white, oblong, thick, gland-dotted, spreading; stamens many, inserted around the disc; ovary ovoid, 10-celled with many ovules; berry ovoid, 8 cm x 6 cm, woody; seeds many. It is useful in diarrhoea, dysentery, dyspepsia, stomachalgia, vitiated conditions of *vata*, seminal weakness, uropathy, vomiting, intermittent fever, swellings and gastric irritability. The leaves are astringent, laxative, febrefuge and expectorant and are useful in ophthalmia, deafness, inflammations, diabetis and asthamatic complaints. Tender fruits are bitter, astringent, antilaxative, digestive and they promote digestion and strength, overcomes *vata*, colic and diarrhoea. The ripe fruits are astringent, sweet, aromatic, cooling, febrifuge, laxative and tonic and are good for the heart and brain in dyspepsia (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.2. *Gmelina arborea* Roxb. (Family: Verbenaceae)

Gmelina arborea, the source plant of *kasmari* is an unarmed moderate sized deciduous tree, 15-20 m in height with whitish grey corky lenticellate bark (Fig. 3.2). Leaves are simple, opposite, long-petioled, broadly ovate-acuminate, glabrous, green above, soft, fulvous tomentose beneath; flowers showy, yellow tingled with brown out side, in dense terminal pedunculate panicles; calyx campanulate, pubescent out side; corolla tube short;

Fig. 3.2. ***Gmelina arborea*** **A. Habit; B. Flowers; C. Fruits**

fruits fleshy ovoid drupes, orange yellow when ripe; seeds hard and oblong. The whole plant is used in medicine. It is astringent, bitter, digestive, cardiotonic, diuretic, laxative and pulmonary and nervine tonic. It promotes digestive power, improves memory, overcomes giddiness and is useful in burning sensation, fever, thirst, emaciation, heart diseases, nervous disorders and piles. The roots are acrid, bitter, sweet, stomachic, tonic, laxative, galactagogue and antihelmintic. The flowers are sweet, refrigerant, bitter, astringent and acrid, and are used in treating leprosy and skin diseases. The fruits are acrid, sour, sweet, refrigerant, bitter, astringent, aphrodisiac, trichogenous, alterant and tonic. They are used for promoting the growth of hair and for anaemia, leprosy, ulcers, constipation, leucorrhoea and colitis (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.3. *Oroxylum indicum* (L.) Vent. (Family: Bignoniaceae)

Oroxylum indicum the source plant of the drug *syonakah* is a medium sized deciduous tree with soft light brown bark with corky lenticels (Fig. 3.3). Leaves are very large, 90-180 cm long, 2-3 pinnate with 5 or more pairs of primary pinnae, rachis very short, cylindrical, swollen at the junction of the branches, leaflets 2-3 pairs, ovate or elliptic acuminate, glabrous; flowers large, pale purple, in long terminal racemes; calyx large, leathery, oblong, campanulate, truncate; corolla large, fleshy; fruits flat capsules, up to 1 m long, tapering to both ends, woody; seeds many, flat winged all round except at the base. The fresh root bark is soft and juicy and cream yellow to grey

Fig. 3.3. *Oroxylum indicum* A. Habit; B. Flower; C. Fruits

in colour. It is sweet, later becoming bitter. On drying, the bark shrinks, adheres closely to the wood and becomes faintly fissured. Roots, leaves, fruits, seeds and bark are the useful part of this plant. The roots are sweet, astringent, bitter, acrid, refrigerant, anti-inflammatory, anodyne, aphrodisiac, expectorant, appetizing, carminative, digestive, antihelmintic, constipating, diaphoretic, diuretic, antiarthritic, febrifuge and tonic. They are useful in vitiated conditions of *vata* and *kapha,* in inflammations, dropsy, sprains, neuralgia, hiccough, cough, asthma, bronchitis, anorexia, dyspepsia, flatulence, colic, helminthiasis, diarrhoea, dysentery, gout, vomiting, leucoderma, wounds, rheumatoid arthritis and fever. The leaves are stomachic and anodyne and are useful in stomachalgia, flatulence, cephalalgia, ulcers, splenomegaly and vitiated conditions of *vata.* The tender fruits are expectorant, carminative and stomachic, and are useful in cough, bronchitis, dyspepsia, flatulence, colic and leucoderma (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.4. *Stereospermum colais* (Dillwyn) Mabb. (Family: Bignoniaceae)

Stereospermum colais, the source plant of *patala* is a large deciduous tree (Fig. 3.4). The branches and leaves are pubescent. Leaves are opposite, imparipinnate; leaflets 7-9, 7-15 cm x 4-7 cm, thin, coriaceous, obovate to lanceolate, acute or rounded, often unequal sided at base, acute to caudate-acuminate at apex, entire or shortly serrate; flowers fragrant, yellow with reddish veins in lax terminal cymose panicles; calyx campanulate, glabrous;

Fig. 3.4. ***Stereospermum colais*** **A. Habit; B. Flowers; C. Fruits**

corolla bilabiate; stamens 4, filaments with a tuft of wooly hairs; capsule pendulous, 4-angled or ribbed, 30-40 cm x 1 cm. Roots, leaves, flowers, fruits and seeds are the useful parts of the plant. The roots are bitter, astringent, arid, anodyne, appetizing, constipating, diuretic, lithontriptic, expectorant, cardiotonic, aphrodisiac, anti-inflammatory, antibacterial, febrifuge and tonic. They are useful in vitiated conditions of *vata*, dyspepsia, diarrhoea and renal and vesical calculi (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.5. *Premna corymbosa* Rottl. (Family: Verbenaceae)

Premna corymbosa, the source plant of the drug *agnimanthah* is a small sized tree (Fig. 3.5); leaves highly aromatic, simple, opposite, elliptic-ovate, acute, 5-9 cm x 3-6 cm, irregularly toothed, thin, coriaceous, dark green and shining above, dull below; flowers small, greenish white in many flowered, terminal, short-peduncled, corymbiform, cymose panicles; calyx cupular, persistent, becoming slightly larger and saucer shaped in fruit; corolla obliquely funnel shaped, 4 or 5 lobed; stamens 4, filaments hairy at base; ovary 2 or 4 celled, 4 ovuled, style linear, ending in a shortly bifid stigma; fruit globose drupe, 4 mm across, black when ripe. Root, root bark and leaves are used in medicine. *Agnimanthah* is reported to be acrid, bitter, astringent, cardiotonic, carminative, laxative, stomachic and tonic. It improves digestive power and is useful in constipation, fever, heart diseases, neurological diseases and rheumatism. It overcomes *kapha* and *vata* disorders, anaemia, piles, oedema, poison,

Fig. 3.5. ***Premna corymbosa*** **A. Habit; B. Flowers**

and abdominal diseases. Traditionally, this drug is highly valued for its anti-inflammatory property (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.6. *Desmodium gangeticum* (Linn.) DC. (Family: Papilionaceae)

Desmodium gangeticum, the drug source of *prsniparni* is a sub erect, diffusely branched undershrub, 90-120 cm in height with a short woody stem and numerous prostrate branches with soft grey hairs (Fig. 3.6); leaves unifoliolate, alternate, stipulate, leaflet ovate-acute, 14 cm x 10 cm; flowers small, pink in terminal elongate racemes; calyx campanulate, hairy outside, with triangular teeth; corolla papilionaceous, exserted; stamens diadelphous; ovary sessile, many ovuled, style filiform, incurved, stigma capitate; fruit compressed, moniliform, 6-8 seeded. The drug is a good cardiotonic, useful in the treatment of cardiac disorders. It is hot, sweet, diuretic, laxative and nervine tonic. It overcomes corruption of *tridosa*, burning sensation, fever, cough, difficult breathing, dysentery, thirst and vomiting and is useful in *vatarakta*, insanity and ulcers (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.7. *Pseudarthria viscida* (Linn.) W. & A. (Family: Papilionaceae)

Pseudarthria viscida, the drug source of *saliparni*, is a perennial viscid pubescent semierect and diffuse undershrub, 60-120 cm long with slender branches, more or less clothed with whitish hairs (Fig. 3.7); leaves three

Fig. 3.6. ***Desmodium gangeticum*** **A. Habit; B. Flowers; C. Fruits**

Fig. 3.7. *Pseudarthria viscida* A. Habit; B. Flowers; C. Fruits

foliate, terminal leaflet rhomboid-ovate, acute, 8 cm x 8 cm, laterals obliquely ovate-acute or rhombiform, subcoriaceous; flowers small, pinkish white in long terminal branched racemes; calyx 2 lipped, campanulate, hairy outside, 4-toothed; corolla exserted, stamens diadelphous; ovary subsessile with many ovules. Style incurved, stigma capitate; fruit densely viscid-hairy, flat, linear-oblong, one celled legume; seeds 4-6, compressed, brownish black. *Saliparni* is bitter, hot, tonic, aphrodisiac and promoter of body tissues. It is strength giving and it overcomes intermittent fever, *vata*, urinary diseases, tumours, oedema, burning sensation, difficult breathing and toxic conditions (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.8. *Solanum melongena* Linn. var. ***insanum* (Linn.) Prain.** (Family: Solanaceae)

Solanum melongena var. *insanum* and *Solanum violaceum* are being used as the source of the drug *brhati*, which are described as *brhatidvayam* sometimes. However, in *dasamula*, both the plants have been included. *Solanum melongena* var. *insanum* (Fig. 3.8) is a much branched, very prickly, grey-pubescent undershrub; leaves simple, alternate, ovate or elliptic-ovate, acute, 7-12 cm x 6-8 cm, oblique at base, irregularly lobed, stellate-pubescent, prickly along the nerves; flowers purple, 1-4, extra-axillary; calyx lobes 5, lanceolate, thick, stellate-pubescent; corolla rotate, deeply five cleft, lobes 5, triangular; stamens 5, free, epipetalous; ovary villous; berry oblong-globose, 3 cm across, fruiting calyx enlarging.

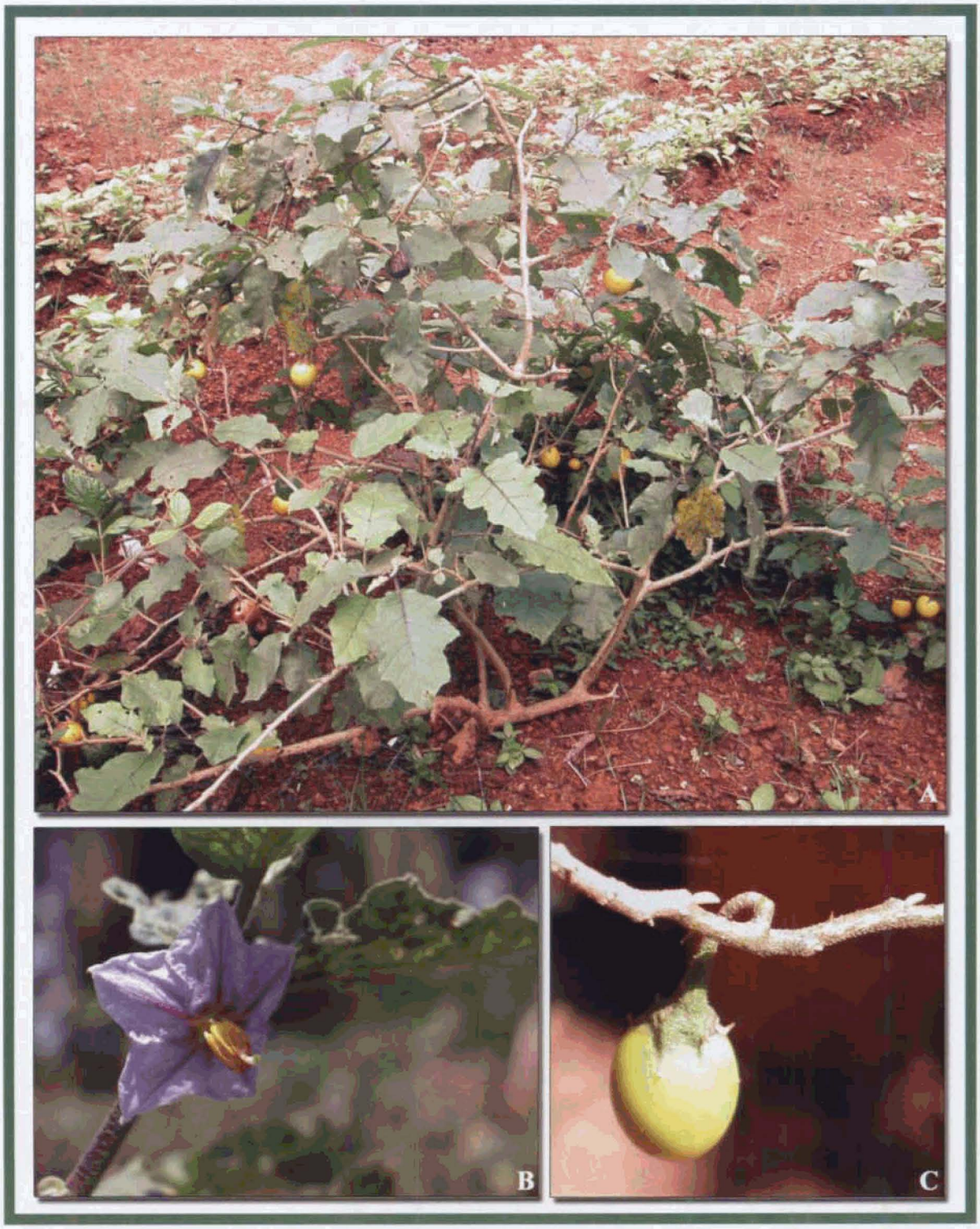


Fig. 3.8. *Solanum melongena* var. *insanum* A. Habit; B. Flower; C. Fruit

Root, fruits and leaves are the useful parts. *Brhati* is reported to be constipating, digestive, acrid and bitter. It helps vitiated tridosas and cures dyspepsia, fever, respiratory and cardiac disorders, skin ailments, vomiting, ulcers and poisonous affections (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

3.1.9. *Solanum violaceum* Ortega (Family: Solanaceae)

Solanum violaceum, the second member of *brhatidvayam* is an armed shrub (Fig. 3.9); leaves simple, alternate or sub-opposite in unequal pairs, greyish green, ovate or oblong, sinuately lobed, 9-3 cm x 6-9 cm, base oblique or unequal sided, stellately wooly or downy beneath, prickly along the mid nerve; flowers pale purple, regular in 8-10 flowered, extra-axillary racemes; calyx cupular, lobes 5, triangular, thick, prickly; corolla rotate, deeply 5 cleft; stamens 5, free, filaments very short, anthers oblong-lanceolate; ovary glabrous, style stellately pubescent; berry globose, smooth, light green, variegated with dark green when young and orange-yellow when ripe. Root, fruits and leaves are the useful parts. *Brhati* is reported to be constipating, digestive, acrid and bitter. It helps vitiated tridosas and cures dyspepsia, fever, respiratory and cardiac disorders, skin ailments, vomiting, ulcers and poisonous affections (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994).

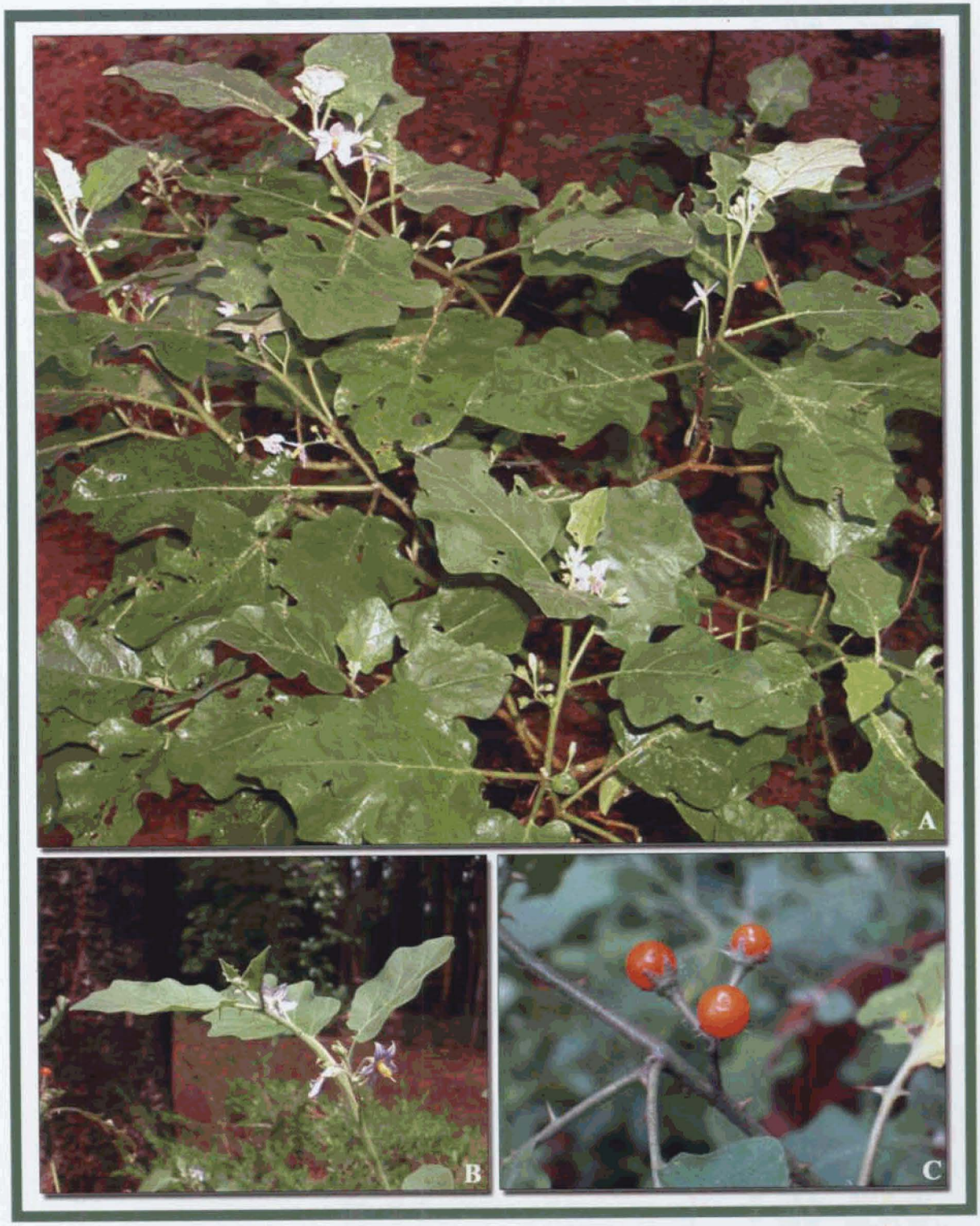


Fig. 3.9. *Solanum violaceum* A. Habit; B. Flowers; C. Fruits

3.1.10. *Tribulus terrestris* Linn. (Family: Zygophyllaceae)

Tribulus terrestris, the source plant of the drug *goksurah* is an annual or perennial prostrate herb with many slender, spreading branches up to 90 cm in length, commonly found throughout India, up to an altitude of 5,400 m (Fig. 3.10). Leaves are simple, pinnate, opposite, leaflets 4-7 pairs, almost sessile or with very short petioles, oblong, entire, 1.7 cm x 0.5 cm, villous; flowers bright yellow, solitary, extraaxillary; sepals 5, free, linear-acute; petals 4, free, golden yellow, obovate, rounded at apex; stamens 10, inserted at the base of an annular lobed disc, filaments free; ovary sessile, hairy, 5-celled, style short, stigma 5 lobed; fruit 5 angled, spinous, tuberulate, schizocarpic, separating into 5 cocci, each with a pair of spines on them; seeds several in each coccus with transverse partitions between them. Flowers and fruits are seen almost throughout the year. Flowering starts within 20-35 days and the fruit matures in 14 days after the formation of seed (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1994; Anonymous, 2003).

Fig. 3.10. ***Tribulus terrestris*** **A. Habit; B. Flower; C. Fruit**

3.2 Method of study

3.2.1. Morphological variability

The study on morphological variability has been carried out on the basis of morphological differences between the different accessions collected from different sources and maintained in the medicinal plant germplasm of Arya Vaidya Sala, Kottakkal, Kerala, India. The accessions have been collected from the states of Kerala, Karnataka and Tamil Nadu and maintained *ex situ* under natural farm conditions with organic manuring and plant protection measures (Table 3.1). Experimental observations were carried out from September 2003 to February 2005.

Table 3.1. Sources of the different accessions of *dasamula* group of plants studied.

Sl. No.	Accessions	Place of collection
1	***Aegle marmelos***	
1	AM 01	Ayurvedic Research Institute, Thiruvananthapuram, Kerala
2	AM 02	Viduthalaipuram, Trichi, Tamil Nadu
3	AM 03	Herbal garden, Arya Vaidya Sala (AVS), Kottakkal, Kerala
4	AM 04	Estate(AVS), Anoli, Malappuram, Kerala
5	AM 05	Estate (AVS), Peruvangad, Malappuram, Kerala
6	AM 06	Estate (AVS), Kanjirapuzha,

		Palakkad, Kerala
7	AM 07	Estate (AVS), Kanjikode, Palakkad, Kerala
8	AM 08	Pathiripala, Palakkad, Kerala
9	AM 09	Pattambi, Palakkad, Kerala
10	AM 10	Payannur, Kannur, Kerala
2	***Gmelina arborea***	
1	GA 01	Muniyal Herbs & Ayurvedic Remedies, Manipal, Karnataka
2	GA 02	Kerala Agriculture University Research Station, Odakkali, Ernakulam, Kerala
3	GA 03	Bird-K, Tumkur, Karnataka
4	GA 04	Manimala, Wayanad, Kerala
5	GA 05	Nagarjuna Herbals, Idukki, Kerala
6	GA 06	Sirumalai, Dindigul, Tamil Nadu
7	GA 07	Amasapuram, Theni, Tamil Nadu
8	GA 08	Herbal garden, AVS, Kottakkal, Kerala
9	GA 09	Estate(AVS), Anoli, Malappuram, Kerala
10	GA 10	Estate (AVS), Peruvangad, Malappuram, Kerala
3	***Oroxylum indicum***	
1	OI 01	Matannur, Kannur, Kerala
2	OI 02	Shanthimala, Kasaragod, Kerala
3	OI 03	Kerala Ayurvedic Pharmacy Ltd., Aluva, Ernakulam, Kerala
4	OI 04	Arya Vaidya Pharmacy, Kanjikode, Palakkad, Kerala

5	OI 05	Herbal garden, AVS, Kottakkal, Kerala
6	OI 06	Estate(AVS), Anoli, Malappuram, Kerala
7	OI 07	Estate (AVS), Peruvangad, Malappuram, Kerala
8	OI 08	Pattambi, Palakkad, Kerala
9	OI 09	Cheruvathur, Kasaragod, Kerala
10	OI 10	Kerala Agricultural University, Mannuthy, Thrissur, Kerala
4	***Stereospermum colais***	
1	SC 01	Sikkupara, Namakkal, Tamil Nadu
2	SC 02	Charmady, Dakshin Kanara, Karnataka
3	SC 03	Uppukunnu, Idukki, Kerala
4	SC 04	Nilakkal, Pathanamthitta, Kerala
5	SC 05	Kallar, Thiruvananthapuram, Kerala
6	SC 06	Ayyanarkovil, Virudhunagar, Tamil Nadu
7	SC 07	Topslip, Coimbatore, Tamil Nadu
8	SC 08	Herbal garden, AVS, Kottakkal, Kerala
9	SC 09	Estate(AVS), Anoli, Malappuram, Kerala
10	SC 10	Estate (AVS), Peruvangad, Malappuram, Kerala
5	***Premna corymbosa***	
1	PC 01	Kerala Ayurvedic Pharmacy Ltd, Aluva, Ernakulam, Kerala

2	PC 02	Nagarjuna Herbals, Idukki, Kerala
3	PC 03	Matannur, Kannur, Kerala
4	PC 04	Arya Vaidya Pharmacy, Kanjikode, Palakkad, Kerala
5	PC 05	Herbal garden, AVS, Kottakkal, Kerala
6	PC 06	Estate(AVS), Anoli, Malappuram, Kerala
7	PC 07	Estate (AVS), Peruvangad, Malappuram, Kerala
8	PC 08	Estate (AVS), Kanjirapuzha, Palakkad, Kerala
9	PC 09	Mezhathur, Palakkad, Kerala
10	PC 10	Kerala Agricultural University, Mannuthy, Thrissur, Kerala
6	***Desmodium gangeticum***	
1	DG 01	Vellanimala, Thrissur, Kerala
2	DG 02	Kodamadi, Tirunelveli, Tamil Nadu
3	DG 03	Nedumkayam, Malappuram, Kerala
4	DG 04	Shanthimala, Kasaragod, Kerala
5	DG 05	Shevayur, Virudhunagar, Tamil Nadu
6	DG 06	Kerala Agricultural University Research Station, Odakkali, Ernakulam, Kerala
7	DG 07	Herbal garden, AVS, Kottakkal, Kerala
8	DG 08	Estate(AVS), Anoli, Malappuram, Kerala

9	DG 09	Estate (AVS), Peruvangad, Malappuram, Kerala
10	DG 10	Estate (AVS), Kanjikode, Palakkad, Kerala
7	***Pseudarthria viscida***	
1	PV 01	Attayar, Thiruvananthapuram, Kerala
2	PV 02	Charmady, Dakshin Kanara, Karnataka
3	PV 03	Savandurga, Bangalore Rural, Karnataka
4	PV 04	Jendamukku, Thrissur, Kerala
5	PV 05	Moozhiyar, Pathanamthitta, Kerala
6	PV 06	Anjuruli, Idukki, Kerala
7	PV 07	Sirumalai, Dindigul, Tamil Nadu
8	PV 08	Ambayathode, Kannur, Kerala
9	PV 09	Manipal, Udupi, Karnataka
10	PV 10	Herbal garden, AVS, Kottakkal, Kerala
8	***Solanum melongena* var. *insanum***	
1	SM 01	Moozhiyar, Pathanamthitta, Kerala
2	SM 02	Herbal garden, AVS, Kottakkal, Kerala
3	SM 03	Estate(AVS), Anoli, Malappuram, Kerala
4	SM 04	Estate (AVS), Peruvangad, Malappuram, Kerala
5	SM 05	Estate (AVS), Kanjirapuzha,

		Palakkad, Kerala
6	SM 06	Estate (AVS), Kanjikode, Palakkad, Kerala
7	SM 07	Kerala Agricultural University, Mannuthy, Thrissur, Kerala
8	SM 08	Vatakara, Calicut, Kerala
9	SM 09	Mezhathur, Thrissur, Kerala
10	SM 10	Payannur, Kannur, Kerala
9	***Solanum violaceum***	
1	SV 01	Mattanur, Kannur, Kerala
2	SV 02	Seethathode, Pathanamthitta, Kerala
3	SV 03	Nagarjuna Herbals, Idukki, Kerala
4	SV 04	Paturaikkal, Thrissur, Kerala
5	SV 05	Valancheri, Malappuram, Kerala
6	SV 06	Valambur, Malappuram, Kerala
7	SV 07	Herbal garden, AVS, Kottakkal, Kerala
8	SV 08	Estate(AVS), Anoli, Malappuram, Kerala
9	SV 09	Estate (AVS), Peruvangad, Malappuram, Kerala
10	SV 10	Estate (AVS), Kanjirapuzha, Palakkad, Kerala
10	***Tribulus terrestris***	
1	TT 01	Herbal garden, AVS, Kottakkal, Kerala
2	TT 02	Centre for Medicinal Plants Research, AVS, Kottakkal, Kerala
3	TT 03	Estate(AVS), Anoli, Malappuram,

		Kerala
4	TT 04	Walayar, Palakkad, Kerala
5	TT 05	Etimada, Coimbatore, Tamil Nadu
6	TT 06	Madukkarai, Coimbatore, Tamil Nadu
7	TT 07	Mankarai, Coimbatore, Tamil Nadu
8	TT 08	Anakkatti, Coimbatore, Tamil Nadu
9	TT 09	Agali, Palakkad, Kerala
10	TT 10	Coimbatore, Tamil Nadu

Morphological variations within the species were analyzed based on observations on ten accessions of each species maintained *ex situ* in the germplasm collections of Arya Vaidya Sala, Kottakkal. Morphological variability among the accessions of the different genotypes as listed in table 3.1 was analyzed based on the characters listed in Table 3.2 with the help of mean, range, standard deviation, standard error and coefficient of variation (Khan, 2000).

Table 3.2. Morphological characters of the different species of *dasamula* plants studied.

Sl. No	Characters
1	***Aegle marmelos***
1	Terminal leaflet length (cm)
2	Terminal leaflet breadth (cm)
3	TLL / TLB
4	Terminal leaflet area (cm^2)

5	Petiole length (cm)
6	Terminal leaf let petiolule length (mm)
7	Leaflet number
8	First leaflet length (cm)
9	First leaflet breadth (cm)
10	Second leaflet length (cm)
11	Second leaflet breadth (cm)
12	Stabilized internodal length (cm)
2	***Gmelina arborea***
1	Leaf length (cm)
2	Leaf breadth (cm)
3	LL / LB
4	Leaf area (cm^2)
5	Petiole length (cm)
6	Stabilized internodal length (cm)
3	***Oroxylum indicum***
1	Terminal leaflet length (cm)
2	Terminal leaflet breadth (cm)
3	TLL / TLB
4	Terminal leaflet area (cm^2)
5	Leaflet number
6	Petiole length (cm)
7	Stabilized internodal length (cm)
4	***Stereospermum colais***
1	Terminal leaflet length (cm)
2	Terminal leaflet breadth (cm)
3	TLL / TLB
4	Terminal leaflet area (cm^2)
5	Petiole length (cm)

6	Leaflet number
7	First leaflet length (cm)
8	First leaflet breadth (cm)
9	Second leaflet length (cm)
10	Second leaflet breadth (cm)
11	Stabilized internodal length (cm)
12	First branch length (cm)
5	***Premna corymbosa***
1	Leaf length (cm)
2	Leaf breadth (cm)
3	LL / LB
4	Leaf area (cm^2)
5	Petiole length (cm)
6	Stabilized internodal length (cm)
7	First branch length (cm)
6	***Desmodium gangeticum***
1	Leaf length (cm)
2	Leaf breadth (cm)
3	LL / LB
4	Leaf area (cm^2)
5	Petiole length (cm)
6	Stabilized internodal length (cm)
7	First branch length
8	Inflorescence length (cm)
9	Pod length (cm)
7	***Pseudarthria viscida***
1	Terminal leaflet length (cm)
2	Terminal leaflet breadth (cm)
3	TLL / TLB

4	Terminal leaflet area (cm^2)
5	Petiole length (cm)
6	Leaflet number
7	First leaflet length (cm)
8	First leaflet breadth (cm)
9	Second leaflet length (cm)
10	Second leaflet breadth (cm)
11	Stabilized internodal length (cm)
12	First branch length
13	Inflorescence length (cm)
14	Pod length (cm)
8	***Solanum melongena* var. *insanum***
1	Leaf length (cm)
2	Leaf breadth (cm)
3	LL / LB
4	Leaf area (cm^2)
5	Petiole length (cm)
6	Stabilized internodal length (cm)
7	First branch length (cm)
9	***Solanum violaceum***
1	Leaf length (cm)
2	Leaf breadth (cm)
3	LL / LB
4	Leaf area (cm^2)
5	Petiole length (cm)
6	Stabilized internodal length (cm)
7	First branch length (cm)
8	Inflorescence length (cm)
9	Pedicel length (cm)

10	***Tribulus terrestris***
1	Leaf length (cm)
2	Leaf breadth (cm)
3	LL / LB
4	Leaf area (cm^2)
5	Petiole length (cm)
6	Leaflet number
7	Stabilized internodal length (cm)
8	First branch length (cm)

Leaf area/ leaf let area was calculated by multiplying leaf length x leaf breadth with a conversion factor worked out graphically for each species under study (Table 3.3).

Table 3.3. Conversion factors for leaf area/ leaflet area calculated in the case of the ten species of *dasamula* plants studied.

Sl. No.	Plant species	Conversion factor for leaf area
1	*Aegle marmelos*	0.69 (terminal leaf let)
2	*Gmelina arborea*	0.70
3	*Oroxylum indicum*	0.65(terminal leaf let)
4	*Stereospermum colais*	0.64 (terminal leaf let)
5	*Premna corymbosa*	0.66
6	*Desmodium gangeticum*	0.83
7	*Pseudarthria viscida*	0.66 (terminal leaf let)
8	*Solanum melongena* var. *insanum*	0.71
9	*Solanum violaceum*	0.70
10	*Tribulus terrestris*	0.71

3.2.2. Phytochemical variability

The ten species of plants selected for the present study were subjected to analysis of phytochemical variability using five accessions each collected from different geographical and agroclimatic regions (Table 3.4).

Table 3.4. Details of the accessions selected for phytochemical study.

Plant species	Accessions*				
Aegle marmelos	AM 01	AM 02	AM 03	AM 08	AM 10
Gmelina arborea	GA 01	GA 04	GA 06	GA 07	GA 08
Oroxylum indicum	OI 01	OI 02	OI 03	OI 04	OI 05
Stereospermum colais	SC 01	SC 02	SC 03	SC 06	SC 08
Premna corymbosa	PC 01	PC 02	PC 03	PC 05	PC 10
Desmodium gangeticum	DG 01	DG 02	DG 03	DG 05	DG 07
Pseudarthria viscida	PV 01	PV 02	PV 03	PV 07	PV 10
Solanum melongena var. *insanum*	SM 01	SM 02	SM 06	SM 07	SM 08
Solanum violaceum	SV 01	SV 02	SV 03	SV 07	SV 10
Tribulus terrestris	TT 01	TT 02	TT 06	TT 08	TT 09

* The details of the accessions are given in Table 3.1.

3.2.2.1. Extraction

Leaves with some shoot parts were used as samples for phytochemical analysis. The samples were dried in shade, powdered and 10 g each of the dried samples were reflux condensed using methanol (3 x 100 ml) for 8 hours at 50°C. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator below 50°C. 10 mg each of the concentrated extracts were dissolved in 10 ml methanol and used for HPLC analysis.

3.2.2.2. HPLC Analysis

The Shimadzu HPLC system consisting of LC-10ATVP pump, a rheodyne injector, SPD M10AVP photodiode array detector and CLASS-VP 6.12 SP5 integration software was used for the analysis. The stationary phase was Phenomenex Luna C 18 (250 x 4.6 mm) column with 5 µ particle size and a guard column. The mobile phase was passed through 0.45 µ PVDF filter, degassed and used. The column was equilibrated with the mobile phase for one hour and then pumped with a back pressure of 200 kg/cm^2. The injection volume was 20 µl and the chromatograms were run for 20– 40 min. The injector and the detector were flushed with the mobile phase before analysis. The details of mobile phase, flow rate, scanning wavelength and time of run for each plant are given in Table 3.5.

Table 3.5. Details of mobile phase, flow rate, scanning wave length and time of run for the selected species.

Plant species	Solvent system	Flow rate (ml/ min.)	Time of run (min.)	Scanning wave length in nm
Aegle marmelos	MeOH: water (7:3)	1	40	265
Gmelina arborea	MeOH (100%)	1	30	210
Oroxylum indicum	MeOH: water (7:3)	1	40	200
Stereospermum colais	MeOH: ACN (95:5)	0.9	20	224
Premna corymbosa	MeOH: water (9:1)	1	45	210
Desmodium gangeticum	MeOH: water (85:15)	1	40	210
Pseudarthria viscida	MeOH: GAA (85:15)	1	45	254
Solanum melongena var. *insanum*	ACN: water (85:15)	1	20	254
Solanum violaceum	ACN: water (85:15)	1	30	300
Tribulus terrestris	MeOH: GAA (7:3)	1	35	265

The chromatographic patterns of the various accessions were compared and the paired affinity indices (PAI) were computed. The PAI between A and B is calculated by,

$$PAI = \frac{\text{Number of spots similar to A \& B}}{\text{Number of similar spots + number of dissimilar spots in A \& B}} \times 100$$

where A and B stand for any two accessions (Ravindran *et al.*, 1992). PAI is a measure of chemical affinity between any two accessions.

3.2.3. Correlation of characters

Characters of organisms, especially polygenic characters show different degrees of interrelationships. Correlation analysis is used to analyze such relationships between characters. Correlation of the characters in the case of the different species studied has been worked out using correlation coefficient and its significance using t test (Rangaswamy, 1995).

3.2.4. Cluster analysis

Though statistically significant variability can be observed between different genotypes of a plant species, they can be grouped into different clusters of genetically closer genotypes based on genetic divergence studies. Ten accessions each of the ten species collected from different parts of South India and conserved in the gene bank of Arya Vaidya Sala, Kottakkal constituted the materials for the study. Materials of uniform age were observed in all

cases. Observations on morphological characters were subjected to cluster analysis using the soft ware STATISTICA, following UPGMA procedure (Unweighted Pair Group Mathematical Average procedure) (Sneath and Sokal, 1973).

3.2.5. Conservation and propagation

Plants used in the indigenous systems of medicine are usually being collected from the wild and most of the species face acute threat of rarity. Hence, steps to enrich their natural habitats and also to conserve them *ex situ* are very important. An effort was made presently to conserve the ten species of *dasamula* group of plants *ex situ* in the experimental gardens of Arya Vaidya Sala, Kottakkal. The details of accessions collected are presented in Table 3.1. The plants have been catalogued and maintained under organic farming conditions so as to use them as source materials for further multiplication and development of appropriate propagation techniques. Techniques for developing effective methods of propagation in the case of the different species of *dasamula* group of plants have been tried presently as described below.

3.2.5.1. Seed propagation

Seed propagation was successfully tried in the species like *Aegle marmelos, Oroxylum indicum, Gmelina arborea, Stereospermum colais, Desmodium gangeticum, Pseudarthria viscida, Solanum melongena var. insanum, Solanum violaceum* and *Tribulus terrestris.* The ripened fruits of selected plants were collected from the field plots

of the estates of Arya Vaidya Sala, Kottakkal, Kerala. *Aegle marmelos* fruits are globose woody berries with yellowish rind, 13-14 chambered, seeds numerous, embedded in yellowish brown, fleshy and gummy pulp. The ripe fruits were collected during March-May and the mucilage of seeds was removed completely by thorough washing. The fruits of *Oroxylum indicum* are pods containing 4-8 seeds respectively in each mericarp. Ripe pods were collected from December to February, before dehiscence. The fruits of *Gmelina arborea* are fleshy ovoid drupes, orange yellow when ripe. Seeds are hard and oblong. The fruits were harvested during April-June and seeds separated by removing the pulp. In *Stereospermum colais* the fruits are crisped crenate pods about 20 cm long. The fruits were collected during June-August. Fruits were plucked from the trees before dehiscence and immediately sown after collection. In *Desmodium gangeticum* and *Pseudarthria viscida* the fruits are pubescent pods containing single and four seeds respectively in each mericarp. The ripe pods of *Desmodium gangeticum* and *Pseudarthria viscida* were harvested in Feb-April. In *Solanum* species fruits are globose, orange red berries containing minutely pitted seeds. The ripe fruits were harvested in March-April. In *Tribulus terrestris* fruits consist of 5-12 woody cocci, each with 2 pairs of hard, sharp, divaricate spines, one pair longer than the other containing several seeds. Fruits were harvested in December. The experiments were conducted during different seasons of the year 2004 depending upon the flowering cycles of the species. The fruits were

depulped and the extracted seeds were shade dried for two days in all the species.

Direct sowing in nursery bed (T_1), sowing in nursery bed after pre-sowing treatment of cold water soak for 16 hours (T_2) and sowing in glass petridish with absorbent cotton (T_3) were the different treatments used for germination studies. In nursery model sand beds under green house conditions were used as sowing medium and in petridish method the materials were kept under normal laboratory conditions. Watering was done daily or as per the requirements of the seeds. Artificial lighting was not provided in the experiments. Germination was noted when radicle emerged. Germination that led to the production of normal seedlings was recorded and expressed as percentage.

3.2.5.2. Vegetative propagation

Vegetative propagation through air layering and stem cuttings was attempted in *Premna corymbosa* due to the rare occurrence of flowering and seed production in the species.

3.2.5.2.1. Stem cuttings

Healthy and fresh stem cuttings were collected from three year old *Premna corymbosa* plants. One or two node stem cuttings were taken and washed thoroughly and were planted in polythene bags filled with soil + sand (1:1) mixture in the month of August, 2003. The number of days taken for initial sprouting was recorded and all the decayed or diseased cuttings were removed regularly.

Hundred cuttings were used for the purpose and the number of plants that survived hardening calculated as survival percentage.

3.2.5.2.2. Air layering

The study was carried out in the Botanical Garden of the University of Calicut, Kerala, during the year 2003 using 3-year old uniform sized plants. The experiment was initiated during the first week of August when the atmospheric temperature and relative humidity were 23^0-26^{0}C and 80% respectively. Secondary branches (1-2 cm in diameter) were selected randomly from 3-year old trees and 2.0-2.5 cm length of bark was removed cylindrically at a distance of 25-30 cm from shoot tip. In this experiment, 50 shoots were air layered and no root promoting chemicals were applied. A mixture of coconut husk and moistened sand was placed around the debarked portion of the shoot and was wrapped with a 150 gauge thick polyethylene sheet which was tied at both ends with plastic thread to reduce loss of moisture. The layers were moistened occasionally as and when required and separated when sufficient number of roots developed.

3.2.5.3. *In vitro* propagation

Tissue culture studies of selected species *viz.*, *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Solanum violaceum*, and *Tribulus terrestris* were carried out in this study.

3.2.5.3.1. Plant material, culture conditions and initiation of cultures

Mature nodes procured from twelve to fifteen year old plants grown in the herbal garden of the Arya Vaidya Sala, Kottakkal were used as explant source for culture initiation in tree species like *Aegle marmelos* and *Oroxylum indicum*. In *Tribulus terrestris*, mature nodes, in *Gmelina arborea* nodes and in *Solanum violaceum* leaves taken from one year old seedlings were used as explants for culture establishment.

The explants were thoroughly washed with 0.1% (w/v) $HgCl_2$ for 3-8 minutes depending upon the species and then washed with distilled water mixed with two drops of Tween 20 and then with distilled water for 4 -5 times. These were then taken to the laminar air flow chamber and again treated with 0.1% (w/v) $HgCl_2$ for 2 minutes and washed with sterile double distilled water for 4 -5 times.

In *Aegle marmelos*, *Stereospermum colais*, *Gmelina arborea* and *Solanum violaceum* Murashige and Skoog's (1962) basal medium with 3% (w/v) sucrose, 0.7% agar (HiMedia, Bombay, India) and supplemented with hormones like BA and kinetin at different concentrations were used for the experiments. In *Tribulus terrestris* Woody Plant Medium (Lloyd and Mc Cown, 1981) supplemented with BA with 3% w/v sucrose and 7% agar was used. In *Oroxylum indicum* modified Woody Plant Medium (WPM salts + MS vitamins without amino acid (Glycine) (Stapfer and Heuser, 1985) supplemented with BA with 3% w/v

sucrose and 1% gel rite were used for culture. The pH of the medium was adjusted to 5.8 using 0.1 M NaOH prior to the addition of gelling substance. The culture medium was autoclaved at 120°C and 1 kg cm^{-2} pressure for 20 minutes. The tubes were incubated at 24°C ± 2°C under a photoperiod of 10/12 hr light and dark with cool white fluorescent lamps (Philips India Ltd., Mumbai), 35-40 $\mu m^{-2}s^{-1}$ as light source.

3.2.5.3.2. Shoot multiplication and rooting

Multiplication experiments were carried out after four weeks of shoot culture initiation. Regenerated shoots having more than two nodes were excised from the primary cultures, cut into single node segments and subcultured on the same basal media (MS/WPM) used for culture establishment. The carbon source and gelling agent were also similar to the culture initiation media. The growth hormone BA was used alone in *Aegle marmelos*, *Gmelina arborea*, *Solanum violaceum* and *Tribulus terrestris* and in combination with kinetin in *Oroxylum indicum* for multiplication experiments. About 2-3 cm long *in vitro* grown shoots were used for rooting. The auxin IBA alone was used for rooting in *Gmelina arborea* and *Solanum violaceum*. In *Aegle marmelos* shoots were subjected to pulse treatment with Napthoxy Acetic Acid and IBA and then with Chlorogenic Acid for rooting. The species like *Tribulus terrestris* showed rooting in half strength Woody Plant Medium with 0.5 mg/l IBA. In *Oroxylum indicum* roots were produced on half strength Woody Plant Medium with IBA and IAA. E*x vitro* rooting

was achieved in *Oroxylum indicum* by using coir pith + sand medium. The cultures were maintained under similar incubation conditions as described earlier for shoot initiation.

3.2.5.3.3. Acclimatization

Rooted shoots were carefully taken out of the medium and washed thoroughly in running tap water to remove all traces of medium attached to the roots without damaging the roots. The plantlets were planted in 5 cm thermocol cups containing a mixture of sand and soil in the ratio 1:1. The cups were covered with polythene bags to maintain humidity and kept in the shade house and observed for further growth and establishment. After 20–25 days the established plants were transplanted to polybags and then to pots containing garden soil and farmyard manure.

Twelve cultures were used per treatment and the experiments were repeated three times. All cultures were examined periodically and visual observations of any morphological changes were recorded.

RESULTS AND DISCUSSION

A.V. Raghu “Studies on variability, conservation and propagation of Dasamula group of plants” Thesis. Department of Botany , University of Calicut, 2005

RESULTS AND DISCUSSION

Chapter IV
RESULTS AND DISCUSSION

The present experiments were carried out so as to study the extent of variability among the ten species of plants used as the drug source of the ayurvedic drug group *dasamula*. Since there are some controversies regarding the drug sources, the plants being used in South India have been used for the present study (Sivarajan and Balachandran, 1994). Analysis of variability and correlation of characters, study of genetic distance between the accessions and studies on conservation and propagation of the different species coming in the group have been attempted presently as described elsewhere. The major observations are presented and discussed below under appropriate heads.

4.1. Morphological variability

4.1.1. *Aegle marmelos* (Linn.) Corr.

Aegle marmelos (Fig.3.1) is the source plant of *vilva* (Sivarajan and Balachandran, 1994). Morphological variability among the ten accessions of *Aegle marmelos* studied has been presented in Table 4.1. Leaves were trifoliate with terminal leaflet length of 10.46 (±0.45) cm, terminal leaflet breadth of 5.95 (±0.45) cm, TLL/TLB ratio of 1.83 (±0.12) and terminal leaflet area of 44.04 (±4.56) cm^2. Mean petiole length was 4.0 (±0.30) cm, terminal leaflet petiolule length 3.65 (±0.16) mm and stabilized internodal length 5.02 (±0.26). Among the quantitative characters studied, terminal leaflet area showed maximum variability as evidenced by the coefficient of variation.

Table 4.1. Morphological variations in quantitative characters in the case of *Aegle marmelos*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Terminal leaflet length (cm)	10.46	0.45	8.46-12.5	1.44	13.76
2	Terminal leaflet breadth (cm)	5.95	0.45	3.15-6.96	1.44	24.20
3	TLL / TLB	1.83	0.12	1.44-2.68	1.83	20.76
4	Terminal leaflet area (cm^2)	44.04	4.56	18.38-60.03	14.43	32.76
5	Petiole length (cm)	4.0	0.30	2.03-4.8	0.96	24.0
6	Terminal leaf let petiolule length (mm)	3.65	0.16	3.3-5.0	0.53	14.52
7	Leaflet No.	3.0	0	0	0	0
8	I^{st} leaflet length (cm)	5.75	0.34	4.36-7.93	1.07	18.60
9	I^{st} leaflet breadth (cm)	3.42	0.16	2.6-3.8	0.50	14.61
10	II^{nd} leaflet length (cm)	6.09	0.39	4.43-8.7	1.24	20.36
11	II^{nd} leaflet breadth (cm)	3.60	0.17	2.73-4.23	0.55	15.27
12	Stabilized internodal length (cm)	5.02	0.26	3.5-5.8	0.83	16.53

Aegle marmelos has been reported to show very high variability between South Indian and North Indian varieties mainly at morphological levels. It has been also reported that plants with larger habit and fruits are tetraploid (Mehra and Khosla, 1973). However such prominent differences have not been reported when South Indian accessions are considered alone. The present study has indicated 32.76 percent of variation in terminal leaflet area, 24 percent of variation in petiole length and 16.53 percent variation in internodal length in the case of the ten accessions studied.

4.1.2. *Gmelina arborea*

Gmelina arborea Roxb. (Fig.3.2) is the source plant of *kasmari* (Sivarajan and Balachandran, 1994). The plants are moderate sized deciduous trees with simple opposite leaves and showy yellow tingled flowers with brown outside in terminal panicles.

Leaf length varied from 11.5 to 18.5 cm, leaf breadth from 8.7 to 16.2 cm, leaf area from 71.64 to 209.79 cm^2, petiole length from 10.1 to 12.7 cm and stabilized internodal length from 8 to 26 cm (Table 4.2). Mean leaf length was 13.57 (±0.83) cm, leaf breadth 11.14 (±0.85) cm, leaf area from 45.82 (±15.98) cm^2, petiole length 11.15 (±0.33) cm^2 and stabilized internodal length 26.21 (±1.60) cm. Among the six quantitative characters studied presently maximum variability was shown by leaf area followed by stabilized internodal length (Table 4.2).

Table 4.2. Morphological variations in quantitative characters in the case of *Gmelina arborea.*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Leaf length (cm)	13.57	0.83	11.5-18.5	2.65	19.52
2	Leaf breadth (cm)	11.14	0.85	8.7-16.2	2.70	24.23
3	LL / LB	1.22	0.01	1.14-1.3	0.06	4.91
4	Leaf area (cm^2)	45.82	15.98	71.64-209.79	50.52	45.82
5	Petiole length (cm)	11.15	0.33	10.1-12.7	1.06	9.50
6	Stabilized internodal length	26.21	1.60	8.0-26.0	5.06	26.21

4.1.3. *Oroxylum indicum*

Oroxylum indicum (L.) Vent. (Fig.3.3) is the source plant of the ayurvedic drug *syonakah* (Sivarajan and Balachandran, 1994). It is a medium sized tree with large 2-3 pinnate leaves, large pale purple flowers in terminal racemes, flat capsular fruits and many round flat winged seeds.

Among the seven quantitative morphological characters studied presently, terminal leaflet area showed the maximum variability (Table.4.3). Mean length of

terminal leaflet was 11.9 (±0.75) cm, mean breadth 8.94 (±0.58) cm and mean area 71.62 (±8.95) cm^2. Mean petiole length was 38 (±1.76) cm and stabilized internodal length 8.3 (±0.47) cm. Terminal leaflet length varied from 8.7 to 15.2 cm, terminal leaflet breadth from 6.4 to 12.1 cm, terminal leaflet area from 36.19 to 108.94 cm^2, petiole length from 32 to 46 cm and stabilized internodal length from 6 to 11 cm.

Table 4.3. Morphological variations in quantitative characters in the case of *Oroxylum indicum*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Terminal leaflet length (cm)	11.9	0.75	8.7-15.2	2.37	19.9
2	Terminal leaflet breadth (cm)	8.94	0.58	6.4-12.1	1.85	20.69
3	TLL / TLB	1.333	0.02	1.2-1.43	0.07	5.26
4	Terminal leaf area (cm^2)	71.62	8.95	36.19-108.94	28.28	39.48
5	Leaflet No.	7.0	0	-	0	0
6	Petiole length (cm)	38.0	1.76	32.0-46.0	5.58	14.68
7	Stabilized internodal length (cm)	8.3	0.47	6.0-11.0	1.49	17.95

4.1.4. *Stereospermum colais*

Stereospermum colais (Dillwyn) Mabb. (Fig. 3.4) is the source plant of the drug *patala* (Sivarajan and Balachandran, 1994). It is a large deciduous tree with pubescent branches and leaves. Leaves are opposite and imparipinnate. Flowers are yellow and fragrant in lax terminal cymose panicles and fruits pendulous capsules.

The present study of variability based on twelve morphological characters revealed maximum variability in terminal leaflet area and minimum variability in leaflet number (Table 4.4). Mean petiole length was found to be 9.25 (±0.15) cm and it showed a range of 8.66 to 10 cm. Mean internodal length was 10.6 (±0.39) cm which showed a range of 9 to 12 cm.

Table 4.4. Morphological variations in quantitative characters in the case of *Stereospermum colais*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Terminal leaflet length (cm)	13.5	0.82	10.25-15.46	2.16	16.42
2	Terminal leaflet breadth (cm)	5.40	0.36	4.2-6.98	1.14	21.11
3	TLL /TLB	2.47	0.13	2.12-3.24	0.42	17.0
4	Terminal leaflet	46.33	4.72	27.55-63.65	14.93	32.22

	area (cm^2)					
5	Petiole length (cm)	9.25	0.15	8.66-10.0	0.46	4.97
6	Leaflet No.	11	0	-	0	0
7	I^{st} leaflet length (cm)	7.82	0.31	6.67-9.4	0.98	12.53
8	I^{st} leaflet breadth (cm)	3.65	0.12	3.16-4.56	0.38	10.41
9	II^{nd} leaflet length (cm)	7.76	0.31	6.6-9.3	0.98	12.62
10	II^{nd} leaflet breadth (cm)	3.58	0.12	3.12-4.5	0.38	10.61
11	Stabilized internodal length (cm)	10.6	0.39	9.0-12.0	1.26	11.88
12	1^{st} branch length (cm)	153.0	11.4	68.0-182.0	35.22	23.01

4.1.5. *Premna corymbosa*

Premna corymbosa Rottl. (Fig.3.5) is the source plant of *agnimanthah* (Sivarajan and Balachandran, 1994). It is a small tree with highly aromatic simple opposite leaves and small greenish white flowers in many flowered cymose panicles; fruits are globose drupes about 4 mm in diameter and black when ripe.

The study of variability of seven vegetative morphological characters showed that maximum variability was shown by leaf area (Table 4.5). Mean leaf length was 4.57 (±0.15) cm, leaf breadth 2.54 (±0.07) cm,

leaf area 7.72 (±0.49) cm^2, petiole length 1.50 (±0.01) cm and internodal length 5.48 (±0.14) cm. Leaf length ranged from 3.9 to 5.3 cm, leaf breadth from 2.1 to 2.8 cm, leaf area from 5.40 to 9.79 cm^2, petiole length from 1.40 to 1.57 cm and internodal length from 4.5 to 6.2 cm.

Table 4.5. Morphological variations in quantitative characters in the case of *Premna corymbosa.*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Leaf length (cm)	4.57	0.15	3.9-5.3	0.50	10.94
2	Leaf breadth (cm)	2.54	0.07	2.1-2.8	0.25	9.84
3	LL / LB	1.80	0.03	1.51-1.81	0.10	5.55
4	Leaf area (cm^2)	7.72	0.49	5.40-9.79	1.54	19.94
5	Petiole length (cm)	1.50	0.01	1.4-1.57	0.06	4.0
6	Stabilized internodal length (cm)	5.48	0.14	4.5-6.2	0.47	8.57
7	1st branch length (cm)	21.2	1.01	16.0-26.0	3.22	15.18

4.1.6. *Desmodium gangeticum*

Desmodium gangeticum (Linn.) DC. (Fig. 3.6), the drug source of *prsniparni* (Sivarajan and Balachandran, 1994) is a suberect diffusely branched undershrub 90-120 cm in

height and unifoliate alternate leaves, small pink flowers in terminal elongate racemes. Fruits are leguminous and 6-8 seeded.

A study of variability in quantitative morphological characters has shown maximum variability in leaf area followed by leaf length, inflorescence length and pod length (Table 4.6). Mean leaf length was found to be 6.47 (±0.58) cm, leaf breadth 5.24 (±0.29) cm, leaf area 29.22 (±4.41) cm^2, petiole length 2.09 (±0.10) cm, internodal length 2.63 (±0.09) cm, inflorescence length 23.26 (±2.01) cm and pod length 2.08 (±0.14) cm.

Table 4.6. Morphological variations in quantitative characters in the case of *Desmodium gangeticum*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Leaf length (cm)	6.47	0.58	4.7-11.0	1.85	28.59
2	Leaf breadth (cm)	5.24	0.29	4.46-6.74	0.93	17.74
3	LL / LB	1.13	0.05	1.06-1.28	0.16	14.15
4	Leaf area (cm^2)	29.22	4.41	17.39-60.80	13.96	47.77
5	Petiole length (cm)	2.09	0.10	1.8-2.37	0.32	15.31
6	Stabilized internodal length (cm)	2.63	0.09	2.36-3.2	0.30	11.4

7	1st branch length (cm)	40.1	1.09	35.0-42.0	3.45	8.60
8	Inflorescence length (cm)	23.26	2.01	12.2-28.67	6.36	27.34
9	Pod length (cm)	2.08	0.14	1.03-2.64	0.46	22.11

4.1.7. *Pseudarthria viscida*

Pseudarthria viscida (Linn.) W. & A. (Fig. 3.7) is the source plant of *saliparni* in South India (Sivarajan and Balachandran, 1994). It is a perennial semierect undershrub which is 60 to 120 cm in height. Leaves are three foliate; flowers small pinkish white in long terminal branched racemes. Fruits are leguminous and seeds 4-6 in number, compressed and brownish black.

The study of variability of vegetative morphometric characters showed that maximum variability is shown by terminal leaflet area (Table 4.7). Terminal leaflet length was 4.87 (±0.59) cm, terminal leaflet breadth 4.08 (±0.49) cm, terminal leaflet area 4.80 (±3.82) cm^2, petiole length 3.81 (±0.39) cm, stabilized internodal length 5.16 (±0.38) cm, inflorescence length 7.16 (±1.01) cm and pod length 1.39 (±0.03) cm.

Table 4.7. Morphological variations in quantitative characters in the case of *Pseudarthria viscida*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Terminal leaflet length (cm)	4.87	0.59	2.8-7.83	1.87	38.39
2	Terminal leaflet breadth (cm)	4.08	0.49	2.1-5.5	1.55	37.99
3	TLL / TLB	1.19	0.02	1.12-1.27	0.07	5.88
4	Terminal leaflet area (cm^2)	14.80	3.42	3.50-35.96	10.82	73.10
5	Petiole length (cm)	3.81	0.39	2.4-6.43	1.24	32.54
6	Leaflet No.	3.0	0	-	0	0
7	I^{st} leaflet length (cm)	3.61	0.51	1.16-5.9	1.62	44.87
8	I^{st} leaflet breadth (cm)	2.79	0.37	0.96-4.6	1.17	41.93
9	II^{nd} leaflet length (cm)	3.53	0.50	1.16-5.56	1.61	45.6
10	II^{nd} leaflet breadth (cm)	2.73	0.36	0.95-4.5	1.15	42.12
11	Stabilized internodal length (cm)	5.16	0.38	2.5-6.4	1.21	23.44

12	1st branch length (cm)	46.1	4.61	32.0-72.0	14.57	31.6
13	Inflorescence length (cm)	7.16	1.01	7.3-18.0	3.20	44.69
14	Pod length (cm)	1.39	0.025	1.3-1.53	0.08	5.75

4.1.8. *Solanum melongena* var *insanum*

Solanum melongena var *insanum* (Linn.) Pran. (Fig. 3.8) is the drug source of one of the drugs of *brhatidvayam* in South India (Sivarajan and Balachandran, 1994). It is a prickly pubescent undershrub with simple alternate leaves and extra axillary purple flowers. Fruits are oblong globose berries.

The study on morphological variations based on seven quantitative characters showed that variability in most of the cases is not very prominent (Table 4.8). Mean leaf length was 9.68 (±0.17) cm, leaf breadth 5.79 (±0.07) cm, leaf area 39.86 (±1.09) cm^2, petiole length 2.76 (±0.05) cm and stabilized internodal length 8.5 (±0.29) cm.

Table 4.8. Morphological variations in quantitative characters in the case of *Solanum melongena* var. *insanum*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Leaf length (cm)	9.68	0.14	9.0-10.5	0.46	4.75
2	Leaf breadth (cm)	5.79	0.07	5.5-6.2	0.23	3.97

3	LL / LB	1.67	0.01	1.63-1.67	0.03	1.79
4	Leaf area (cm^2)	39.86	1.09	35.14-46.22	3.46	8.68
5	Petiole length (cm)	2.76	0.57	2.5-3.0	0.15	5.43
6	Stabilized internodal length (cm)	8.5	0.29	8.0-10.0	0.94	11.05
7	1st branch length (cm)	25.8	0.97	35.14-46.22	3.08	11.93

4.1.9. *Solanum violaceum*

Solanum violaceum Ortega (Fig. 3.9) is used as the second member of *brhatidvayam* in South India (Sivarajan and Balachandran, 1994). It is a shrub with simple alternatre or sub opposite leaves, purple flowers in extra axillary racemes and smooth globose berries.

The study of morphological variability among the ten accessions of *Solanum violaceum* based on nine morphological characters revealed maximum variability in the case of leaf area (Table 4.9). Mean leaf length was found to be 6.34 (±0.25) cm, leaf breadth 4.56(±0.21) cm, leaf area 20.43 (±16.9) cm2, petiole length 4.4 (±0.080 cm, internodal length 13.8 (±0.49) cm, inflorescence length 2.95 (±0.130 cm and petiole length 1.49 (±0.06) cm.

Table 4.9. Morphological variations in quantitative characters in the case of *Solanum violaceum.*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Leaf length (cm)	6.34	0.25	4.9-7.5	0.82	12.93
2	Leaf breadth (cm)	4.56	0.21	3.2-4.9	0.69	15.13
3	LL / LB	1.40	0.01	1.33-1.54	0.06	4.28
4	Leaf area (cm^2)	20.43	16.9	10.97-29.4	5.35	26.18
5	Petiole length (cm)	4.4	0.08	4.1-4.7	0.28	6.36
6	Stabilized internodal length (cm)	13.8	0.49	11.0-16.0	1.55	11.23
7	1st branch length (cm)	32.3	1.26	26.0-39.0	4.0	12.38
8	Inflorescence length (cm)	2.95	0.13	2.2-3.5	0.42	14.23
9	Pedicel length (cm)	1.49	0.06	1.1-1.7	0.20	13.42

4.1.10. *Tribulus terrestris*

Tribulus terrestris Linn. (Fig. 3.10) is the source plant of the drug *goksurah* (Sivarajan and Balachandran, 1994). It is an annual or perennial prostrate herb with many slender spreading branches upto 90 cm in length,

simple pinnate opposite leaves, bright yellow solitary extra axillary flowers and schizocarpic five celled fruits.

Study of variability of quantitative morphological characters based on eight parameters revealed maximum variability in the case of leaf area (Table.4.10). Leaf length varied from 2.6 to 4.8 cm, leaf breadth 1.57 to 3.74 cm, leaf area 2.99 to 12.80 cm^2, petiole length 0.54 to 1.1 cm and internodal length 4.3 to 5.6 cm. Mean leaf length was 3.58 (±0.23) cm, leaf breadth 2.59 (±0.2) cm, leaf area 6.90 (±0.99) cm^2, petiole length 0.88 (±0.06) cm and internodal length 4.83 (±0.13) cm.

Table 4.10. Morphological variations in quantitative characters in the case of *Tribulus terrestris*

Sl. No.	Characters	Mean	SE	Range	SD	CV
1	Leaf length (cm)	3.58	0.23	2.6-4.8	0.74	20.67
2	Leaf breadth (cm)	2.59	0.22	1.57-3.74	0.71	27.41
3	LL / LB	1.41	0.04	1.25-1.7	0.13	9.21
4	Leaf area (cm^2)	6.90	0.99	2.99-12.80	3.14	45.50
5	Leaflet No.	12.0	0	-	0	0
6	Petiole length (cm)	0.88	0.06	0.54-1.1	0.19	21.59
7	Stabilized internodal	4.83	0.13	4.3-5.6	0.42	8.69

	length (cm)					
8	1st branch length (cm)	26.9	2.04	18.0-37.0	6.47	24.05

Morphological variability of characters shows the genotypic differences between the different accessions of the species studied. It can be used as an index to analyze the genotypic differences present within the species and such differences indicate the genetic diversity of the species that has accumulated in the course of evolution of the species in different populations. Such diversities, since they have originated in different habitats and have contributed significantly towards the adaptability and divergence of the species, can be considered as valuable sources of genes and genotypes for the selection of superior accessions leading to their use in conservation, propagation and their commercial exploitation. Similar studies have been carried out in medicinal plants (Misra *et al.*, 1998), rice (Shobha, 1993), tea (Ramasubramanian, 2005), coffee (Nikhila *et al.*, 2002; Raghu *et al.*, 2003), cardamom (Radhakrishnan *et al.*, 2005), vanilla (Umamaheswari and Mohanan, 2004) etc. by earlier workers and such studies have helped in the identification of superior genotypes of the corresponding plants.

4.2. Phytochemical variability

The number of peaks appeared in the HPLC chromatogram in each species were arranged pattern wise to study the paired affinity index (PAI) (Tables 4.11 to 4.20). Almost identical Rt values were checked by

Table 4.11. HPLC peak patterns of *Aegle marmelos*.

Accessions	Rt values																		
	2.49	2.56	2.67	2.83	2.94	3.23	3.55	3.97	4.15	4.64	5.37	5.96	6.03	6.26	6.90	7.30	7.27	7.37	9.47
AM 01	*			*		*	*		*	*		*			*		*		
AM 02		*			*	*	*	*		*	*	*			*		*		
AM 03		*				*			*	*	*		*		*			*	
AM 08		*				*	*	*		*		*		*	*		*		*
AM 10			*			*	*	*		*	*		*		*	*			

9.57	9.64	10.81	11.33	11.56	11.79	12.49	12.59	13.47	13.57	13.74	14.37	14.56	14.36	15.24	15.37	15.50
*						*			*		*				*	
*			*			*			*						*	
	*	*		*		*				*		*				*
			*	*		*		*			*		*	*		
	*				*		*			*		*				*

16.23	16.34	16.53	19.15	19.21	20.15	20.33	21.83	22.88	23.68	24.77	26.13	28.73	29.09	29.24	30.19	32.80
	*			*	*		*					*				
	*			*					*	*	*		*		*	
		*	*			*		*						*		*
*												*				
		*														

Table 4.12. HPLC peak patterns of *Gmelina arborea*

Accessions	Rt values														
	1.63	2.01	2.44	2.65	2.85	3.24	3.39	3.63	3.90	4.03	4.12	4.33	4.44	4.53	4.73
GA 01				*	*		*	*	*		*			*	
GA 04			*	*				*	*						
GA 06	*	*		*				*	*		*	*			
GA 07			*		*	*		*	*	*			*		
GA 08				*		*		*	*	*					*

5.02	5.13	5.47	5.69	5.81	6.01	6.18	6.67	6.78	6.99	7.09	7.23	7.33	7.96	8.18
	*		*			*		*					*	
				*		*							*	
						*						*		
	*	*			*				*		*			*
*		*			*		*			*	*			

8.42	8.55	8.94	9.03	9.17	9.29	9.40	9.53	9.67	9.89	10.23	10.23	10.73	11.00	11.15	11.32
	*				*					*					*
			*					*				*			
				*				*	*			*		*	
*		*					*					*	*		
	*					*		*		*				*	

11.59	11.68	11.78	11.99	12.15	12.31	12.55	12.66	12.71	13.26	13.37	13.60	13.97	14.54	14.73	14.94
										*			*		
	*		*	*		*			*			*			*
								*			*		*		
						*			*					*	
*		*			*		*				*		*		

15.08	15.55	16.09	16.42	17.34	17.43	18.04	20.39	20.97	22.69	22.97	25.11	29.88	29.96
*	*		*	*		*	*			*			
		*		*	*	*						*	*
								*	*		*		

Table 4.13. HPLC peak patterns of *Oroxylum indicum*

Accessions	Rt value													
	2.26	2.60	2.86	2.98	3.29	3.34	3.50	3.79	4.09	4.11	4.93	5.27	5.49	5.62
OI 01	*	*					*		*		*			*
OI 02	*	*					*		*		*			*
OI 03						*				*	*	*		
OI 04			*		*			*					*	
OI 05	*	*		*			*		*		*			*

6.20	6.56	6.92	7.68	7.74	8.15	9.18	9.30	10.24	10.73	10.97	11.64	12.39	12.51	13.06
*		*		*	*		*		*			*		
*		*	*		*			*	*				*	
*	*				*									
*				*	*	*				*				*
*		*		*			*	*	*		*	*		

13.44	13.51	13.60	14.45	14.58	14.66	16.36	16.45	16.68	17.60	18.43	18.63	18.86	20.10	20.27
*			*			*						*	*	
	*			*			*			*			*	*
		*		*				*			*			
					*				*					
*			*			*						*	*	

21.33	21.54	21.81	22.40	23.71	24.20	24.41	24.59	25.17	25.29	25.38	28.47	28.84	29.35	29.61
			*						*	*				
*					*						*			
							*						*	
		*		*		*		*				*		
	*		*							*				*

29.78	29.93	30.63	31.02	31.48	32.34	35.62	37.83	38.42	38.60	39.95	40.14	41.48	41.76	43.97
*			*											
*	*									*	*	*		
				*			*	*					*	*
		*			*	*			*		*			
			*											

44.14	44.23	47.37
*		*
	*	

Table 4.14. HPLC peak patterns of *Stereospermum colais*

Accessions	Rt value													
	2.91	3.24	3.35	3.74	4.19	4.33	4.70	5.07	5.18	5.45	5.57	5.86	6.18	6.42
SC 01	*	*	*	*	*	*	*			*		*		*
SC 02	*		*	*		*	*		*		*			*
SC 03	*		*	*	*	*	*		*		*	*		*
SC 06	*			*		*	*		*		*	*	*	*
SC 08	*		*			*	*	*			*			

7.0	7.10	7.89	8.02	8.44	8.55	8.65	8.96	9.01	9.19	9.72	9.93	10.13	10.25	10.51	10.75
	*					*				*		*			*
	*				*				*	*		*			
*				*				*			*		*		
*			*	*					*				*		
*		*			*		*						*		

10.87	11.10	11.72	12.03	12.63	13.17	13.22	13.53	14.35	14.73	15.73	15.98
		*									
	*						*	*		*	
					*						
*						*					
			*	*				*	*		*

Table 4.15.HPLC peak patterns of *Premna corymbosa*

Accessions	Rt value													
	2.20	2.34	2.44	2.62	3.02	3.40	3.65	3.93	4.16	4.69	5.02	5.43	5.58	5.86
PC 01	*		*	*		*	*		*		*		*	
PC 02		*		*		*		*	*	*	*	*		*
PC 03				*	*	*		*	*		*	*		
PC 05				*	*	*		*	*		*			
PC 10	*		*	*		*	*		*		*		*	

6.02	6.10	6.30	6.90	7.21	8.05	8.17	8.64	8.71	8.84	9.39	9.88	9.98	10.02	10.24
	*	*	*					*		*			*	
		*		*		*			*			*		
*		*	*		*		*				*			*
*		*	*		*									*
	*	*						*					*	

10.85	11.01	11.50	12.36	13.03	13.38	13.58	13.72	14.65	15.06	15.91	16.20	16.34	16.98	17.05
	*		*			*					*			
*		*	*				*					*		
*			*		*			*		*			*	
*			*	*	*				*	*				*
	*		*			*					*			

Table 4.16. HPLC peak patterns of *Desmodium gangeticum*

Accessions	Rt values																			
	1.39	2.13	2.61	2.98	3.35	3.77	4.06	4.23	4.31	4.48	4.68	4.75	5.19	5.24	5.48	5.56	5.74	5.81	6.60	7.31	7.40
DG 01			*		*	*	*			*	*		*		*		*		*	*	
DG 02			*	*	*	*			*					*		*		*	*		*
DG 03			*		*	*		*				*		*		*	*		*	*	
DG 05			*		*	*								*		*		*	*		*
DG 07	*	*	*		*	*		*		*				*		*		*	*	*	

8.22	8.32	8.91	9.49	10.14	10.26	10.71	10.82	10.90	11.98	12.13	13.75	14.04	14.38	14.55	15.86	16.87	17.42	22.78
*		*				*			*									
	*	*	*		*			*		*		*						
		*		*			*			*				*		*		
*		*	*					*		*							*	
*		*	*				*			*	*		*		*			*

24.02	24.30	24.15	27.40	28.84	37.99
*					
	*			*	
	*			*	
	*				*
*			*		

Table 4.17. HPLC peak patterns of *Pseudarthria viscida*

Accessions															
	2.01	2.55	2.63	2.94	3.38	3.72	4.03	4.26	4.43	4.53	4.65	5.07	5.13	5.43	5.59
PV 01	*		*	*	*	*			*	*		*			*
PV 02	*		*	*	*	*			*			*		*	*
PV 03	*	*		*	*	*		*		*	*		*	*	
PV 07	*		*	*	*	*	*			*	*		*		
PV 10	*	*		*	*	*		*		*			*		

5.69	6.27	6.34	6.53	6.69	7.10	7.21	7.80	7.95	8.30	9.03	9.15	9.38	9.87	10.23	10.52
			*		*					*				*	
	*		*		*		*		*		*			*	
*		*		*		*		*				*	*		*
*				*	*			*				*			*
*			*				*		*			*			*

11.45	11.73	13.06	13.78	13.89	13.95	14.14	16.71	17.42	22.35	22.49	23.07	23.33	27.13	33.60
*		*	*				*		*					*
*				*			*			*			*	
	*					*					*			
		*						*			*			
	*				*							*		

Table 4.18. HPLC peak patterns of *Solanum melongena* var. *insanum*

Accession	Retention time													
	2.02	2.89	3.21	3.32	3.54	3.89	3.99	4.41	4.68	4.92	5.16	5.35	5.54	5.97
SM 01	*	*		*	*		*	*		*		*	*	*
SM 02	*		*	*		*	*	*	*		*		*	*
SM 06		*	*		*	*		*		*	*		*	
SM 07	*		*	*		*		*		*	*		*	
SM 08	*		*	*	*		*		*		*	*		*

6.29	6.57	6.83	7.07	7.38	7.85	7.98	8.16	8.54	8.69	9.51	9.96	10.89	11.03	11.51
*		*	*	*			*		*	*	*	*		*
	*	*		*		*	*			*		*	*	
	*	*		*			*		*	*		*		*
*	*	*		*	*	*		*		*	*		*	*
*		*	*		*		*		*			*		*

12.19	12.68	13.00	13.25	13.49	13.64	14.17	14.56	14.99	15.24	15.96
*		*			*	*		*		*
		*	*	*			*			
	*		*		*				*	*
*		*		*	*	*		*		
	*		*			*	*		*	

Table 4.19. HPLC peak patterns of *Solanum violaceum*

Accessions	Retention time													
	2.65	3.23	3.43	3.62	4.07	4.24	4.46	4.83	5.08	5.64	5.87	6.21	6.51	6.84
SV 01	*		*	*	*		*		*		*		*	
SV 02	*		*	*	*		*	*		*		*		*
SV 03	*	*		*	*	*	*		*		*		*	
SV 07		*		*	*	*	*		*					*
SV 10	*		*		*		*			*		*		*

7.10	7.37	7.65	7.90	8.31	8.56	8.75	9.35	9.56	9.72	10.26	10.56	10.75	10.23	10.75
	*		*			*			*			*		
	*		*		*	*		*	*		*			
*		*		*		*				*			*	*
*	*		*		*		*		*		*	*		*
		*		*		*		*			*		*	

11.12	11.36	11.58	12.42	13.02	13.64	13.82	13.98	16.96	17.35	19.73
	*			*				*		*
*			*	*					*	
	*	*			*	*				
*			*				*	*		
	*			*		*	*			*

Table 4.20. HPLC peak patterns of *Tribulus terrestris*

Accessions	Retention time													
	2.10	2.60	3.16	3.27	3.46	4.12	4.25	4.65	5.15	5.38	5.45	5.93	6.05	6.13
TT 01	*	*	*			*		*		*		*		
TT02	*	*	*		*	*		*		*		*		
TT06	*	*		*	*	*		*	*		*	*		
TT 08	*	*		*		*		*	*		*		*	
TT 09	*	*	*				*	*			*			*

6.35	6.54	7.51	7.67	7.74	7.88	8.30	8.59	8.79	9.46	9.66	11.57	11.85	12.10	12.43
					*						*			*
	*				*		*		*		*			*
*		*		*				*		*		*		
	*											*		
			*			*				*			*	

12.77	13.00	14.74	15.09	15.22	15.73	18.30	18.77	18.86	19.34
		*				*			
	*	*				*			
*			*				*		
*				*				*	
	*				*				*

spectrum analysis and classified on its basis. The intra specific paired affinity indices of each species analyzed as detailed elsewhere are discussed below under appropriate heads.

4.2.1. *Aegle marmelos*

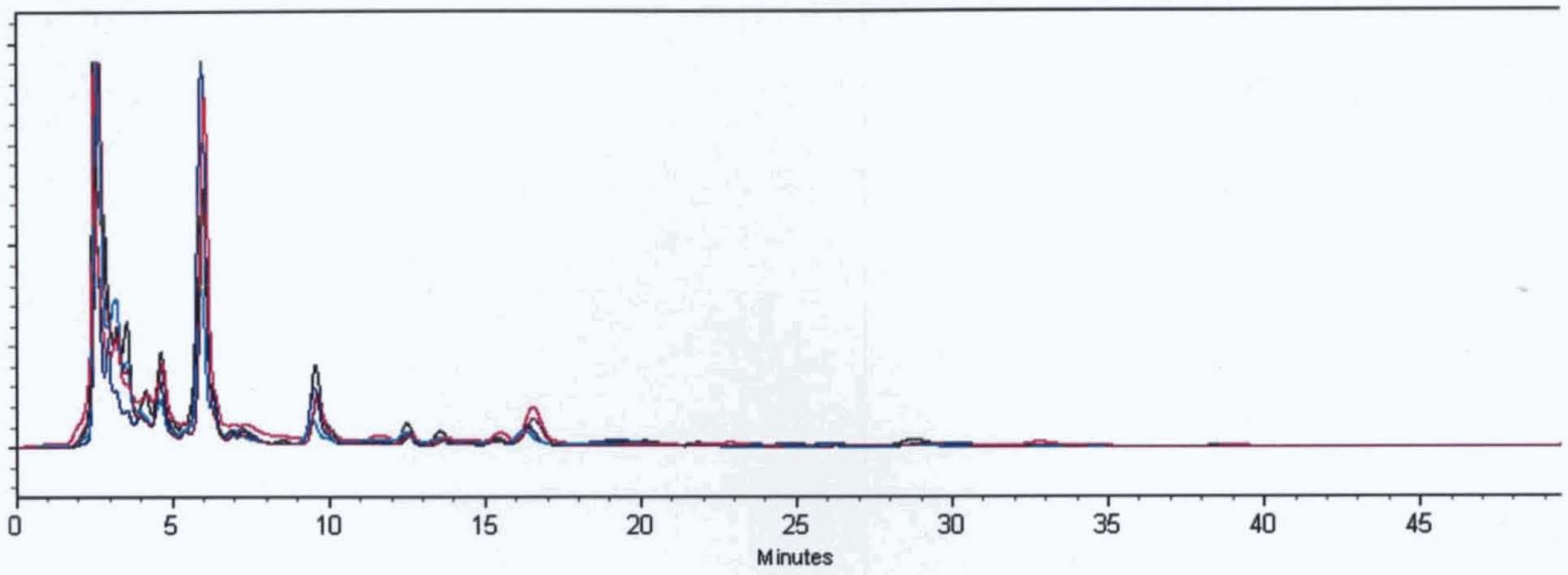
The HPLC peak patterns in the case of *Aegle marmelos* are presented in Table 4.11 and Fig. 4.1. Based on the data in Table 4.11, paired affinity indices were computed for the taxon (Table 4.21).

Table 4.21. Paired affinity indices (PAI) of the different accessions of *Aegle marmelos*

Accessions	AM 01	AM 02	AM 03	AM 08	AM 10
AM 01	100				
AM 02	41.37	100			
AM 03	14.28	16.21	100		
AM 08	28.57	33.33	17.64	100	
AM 10	12.90	17.35	40.00	16.22	100

Among the accessions used for phytochemical study, the accession from Ayurvedic Research Institute, Poojappura, Thiruvananthapuram, Kerala (AM 01) showed the highest chemical affinity (41.37%) with the accession from Viduthalaipuram, Trichi, Tamil Nadu (AM 02). Next to this, 40% affinity was observed between the accession from Herbal Garden, AVS, Kerala (AM 03) and the accession from Payannur, Kannur, Kerala (AM 10). The accessions AM 01 and AM 10 showed the lowest affinity (12.90%) among the accessions studied.

Fig. 4.1. HPLC chromatogram of 5 accessions of *Aegle marmelos*



4.2.2. *Gmelina arborea*

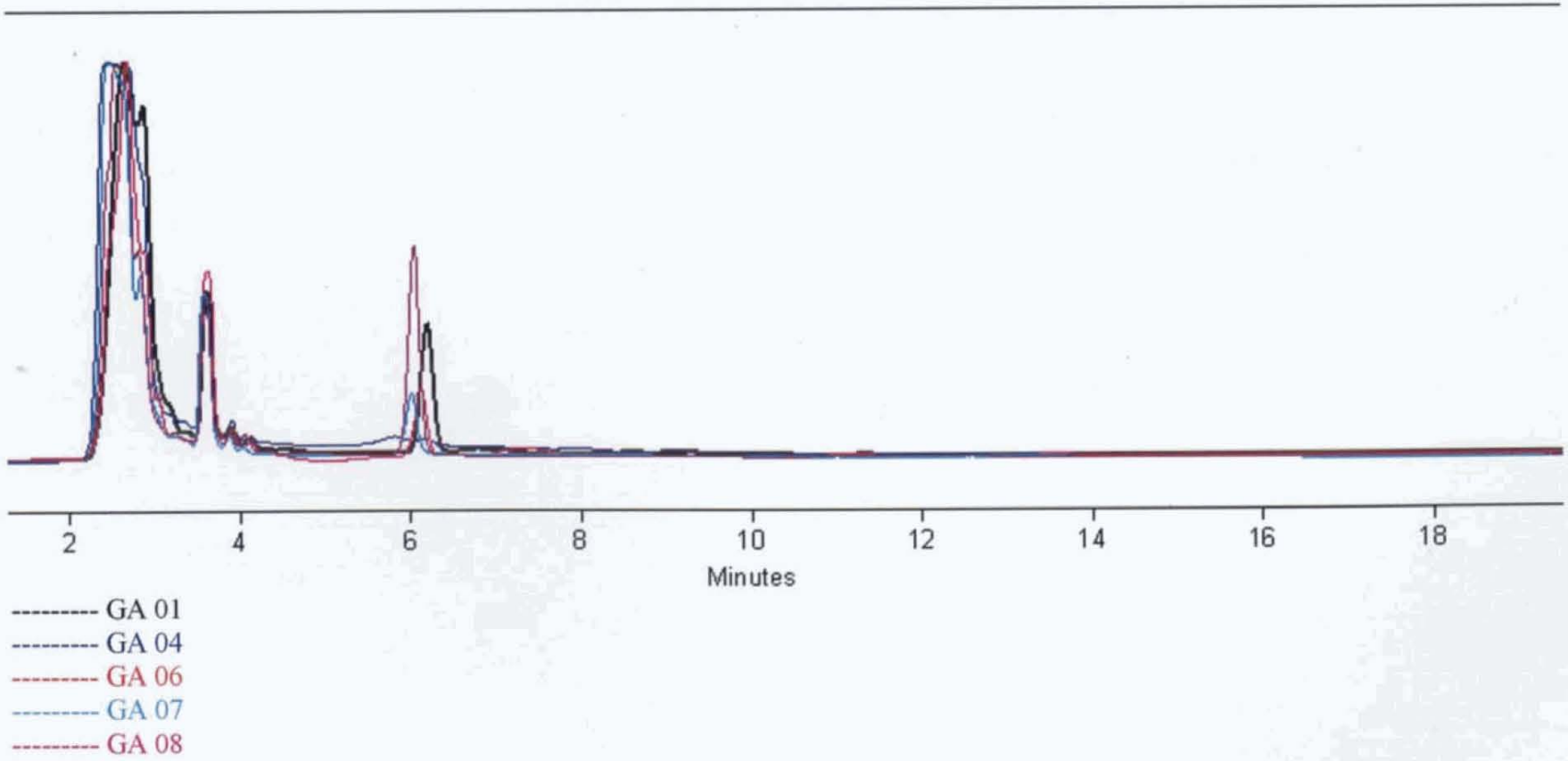
The HPLC peak patterns of this species are represented in Table 4.12 and Fig. 4.2. Based on the data in Table 4.12, PAI were computed for the species (Table 4.22).

Table 4.22. Paired affinity indices (PAI) of the different accessions of *Gmelina arborea*

Accessions	GA 01	GA 04	GA 06	GA 07	GA 08
GA 01	100				
GA 04	17.03	100			
GA 06	15.38	16.21	100		
GA 07	9.52	18.96	7.81	100	
GA 08	14.28	9.52	20.58	18.91	100

Among the accessions used for the present study, the accession from Sirumalai, Dindigul, Tamil Nadu (GA 06) showed the highest chemical affinity (20.58%) with the accession from Herbal Garden, AVS, Kerala (GA 08). Next, 18.96 % of affinity was shown by the accessions from Manimala, Wayanad, Kerala (GA 04) and Amasapuram, Theni, Tamil Nadu (GA 07). The accession GA 07 and GA 06 showed the lowest affinity (7.81%) among the species.

Fig. 4.2. HPLC chromatogram of 5 accessions of *Gmelina arborea*



4.2.3. *Oroxylum indicum*

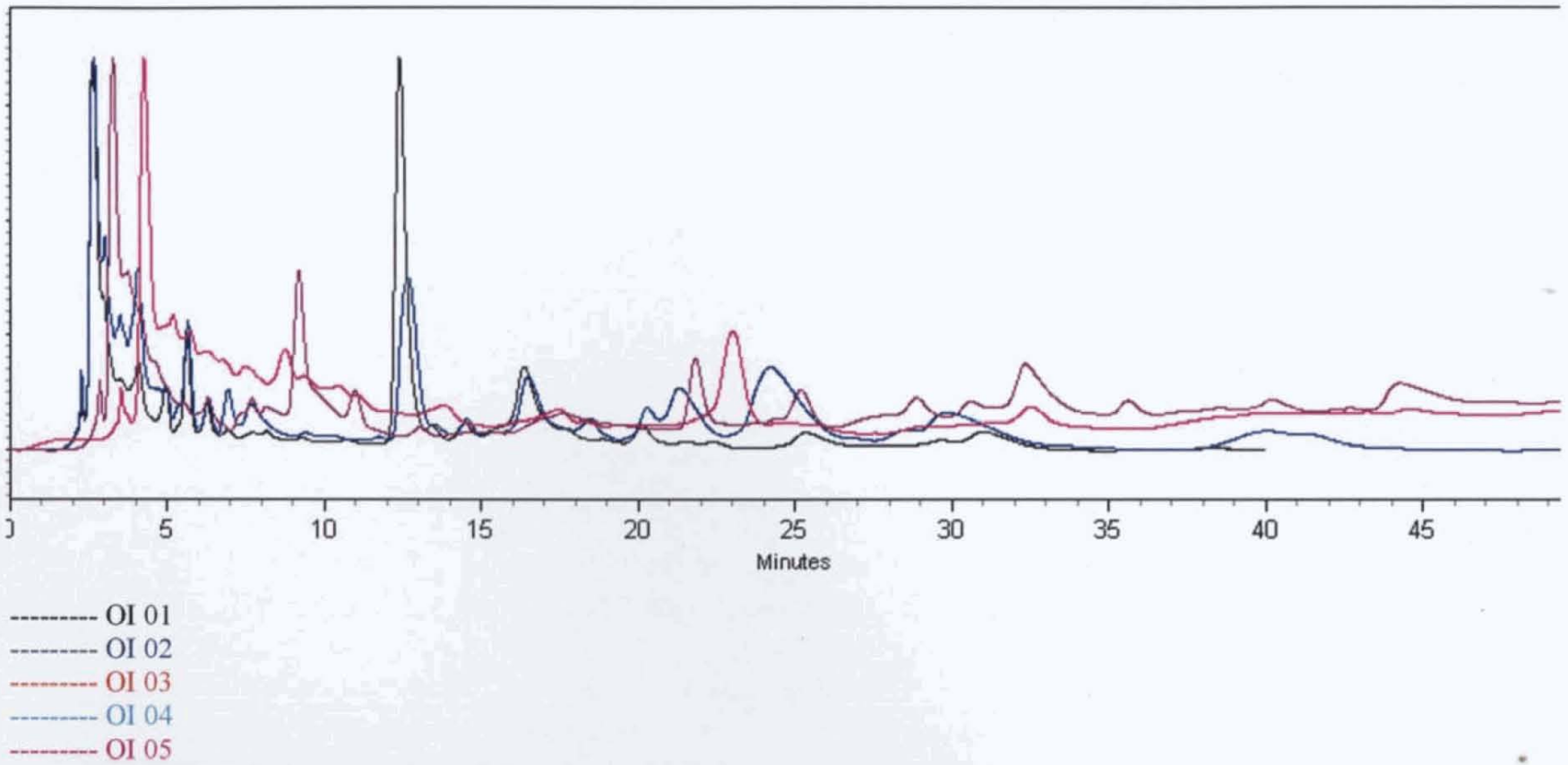
The HPLC peak patterns in the case of Oroxymum indicum are presented in Table 4.13 and Fig. 4.3. Based on the data in Table 4.13, PAI were computed for the species (Table 4.23).

Table 4.23. Paired affinity indices (PAI) of the different accessions of *Oroxylum indicum*

Accessions	OI 01	OI 02	OI 03	OI 04	OI 05
OI 01	100				
OI 02	31.59	100			
OI 03	7.31	9.30	100		
OI 04	10.0	5.71	4.88	100	
OI 05	71.42	27.5	4.65	4.34	100

Among the accessions used for the present study, the accession from Mattannur, Kannur, Kerala (OI 01) showed the highest chemical affinity (71.42%) with the accession from Herbal Garden, AVS, Kerala (OI 05). Next to this, 31.59% affinity was showed by the accessions from Mattannur, Kannur, Kerala (OI 01) and Santhimala, Kasargode, Kerala (OI 02). The accessions from Arya Vaidya Pharmacy, Kanjikode, Palakkad, Kerala (OI 04) and Herbal Garden, AVS, Kerala (OI 05) showed the lowest affinity (4.34 %) among the species.

Fig. 4.3. HPLC chromatogram of 5 accessions of *Oroxylum indicum*



4.2.4. *Stereospermum colais*

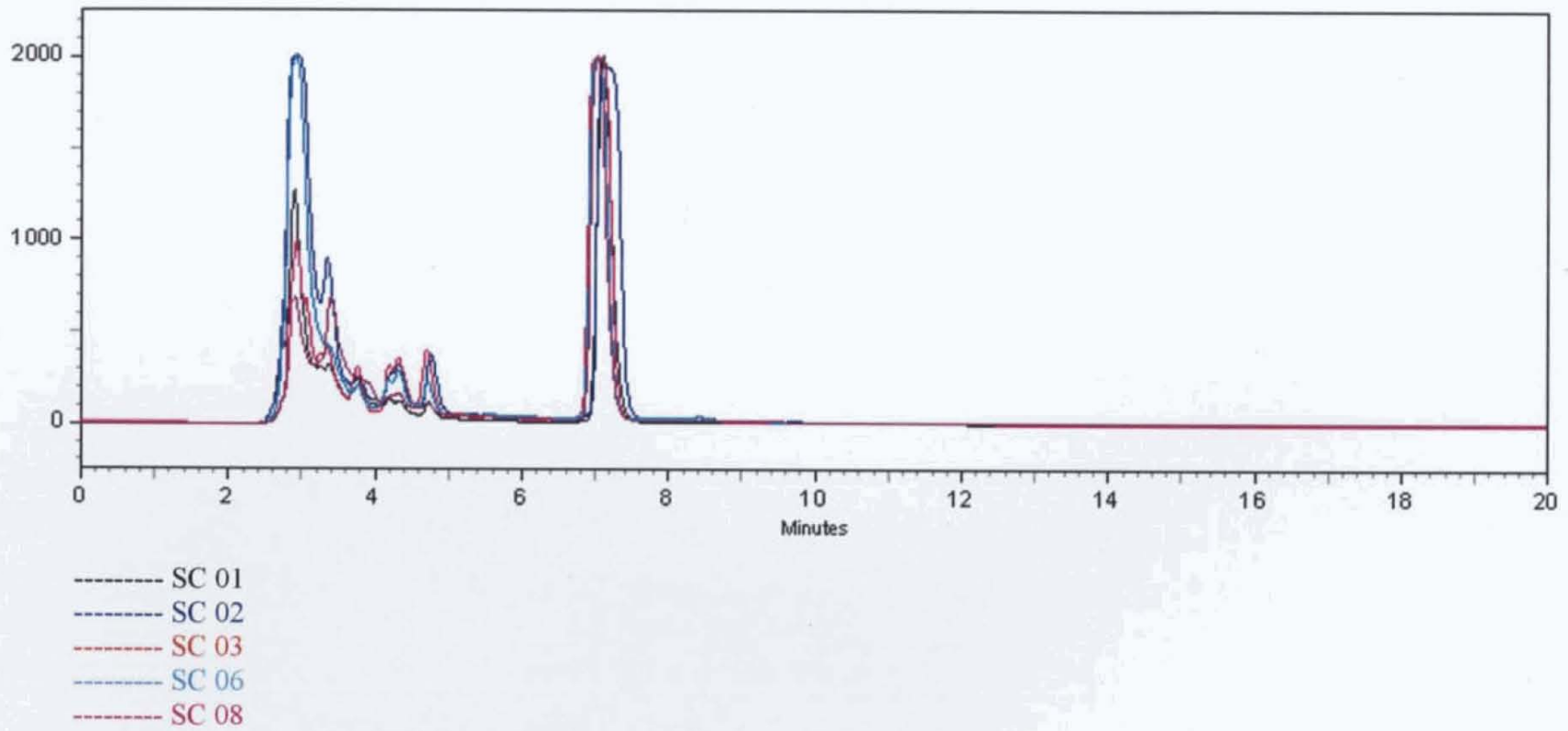
The HPLC peak patterns of this species are presented in Table 4.14 and Fig. 4.4. Based on the data in Table 4.14, PAI were computed for the species (Table 4.24).

Table 4.24. Paired affinity indices (PAI) of the different accessions of *Stereospermum colais*

Accessions	SC 01	SC 02	SC 03	SC 06	SC 08
SC 01	100				
SC 02	37.5	100			
SC 03	33.33	32.0	100		
SC 06	24.0	33.33	52.38	100	
SC 08	14.28	28.0	23.08	19.23	100

Among the accessions used for the study, accession from Uppukunnu, Idukki, Kerala (SC 03) showed the highest chemical affinity (52.38%) with the accession from Ayyanarkovil, Virudhunagar, Tamil Nadu (SC 06). Next to this, 37.5 % affinity was observed between the accessions from Sikkupara, Namakkal, Tamil Nadu (SC 01) and Charmady, Dakshin Kanara, Karnataka (SC 02). SC 01 and SC 08 (Herbal Garden, AVS, Kerala) showed the lowest affinity (14.28 %) among the species.

Fig. 4.4. HPLC chromatogram of 5 accessions of *Stereospermum colais*



4.2.5. *Premna corymbosa*

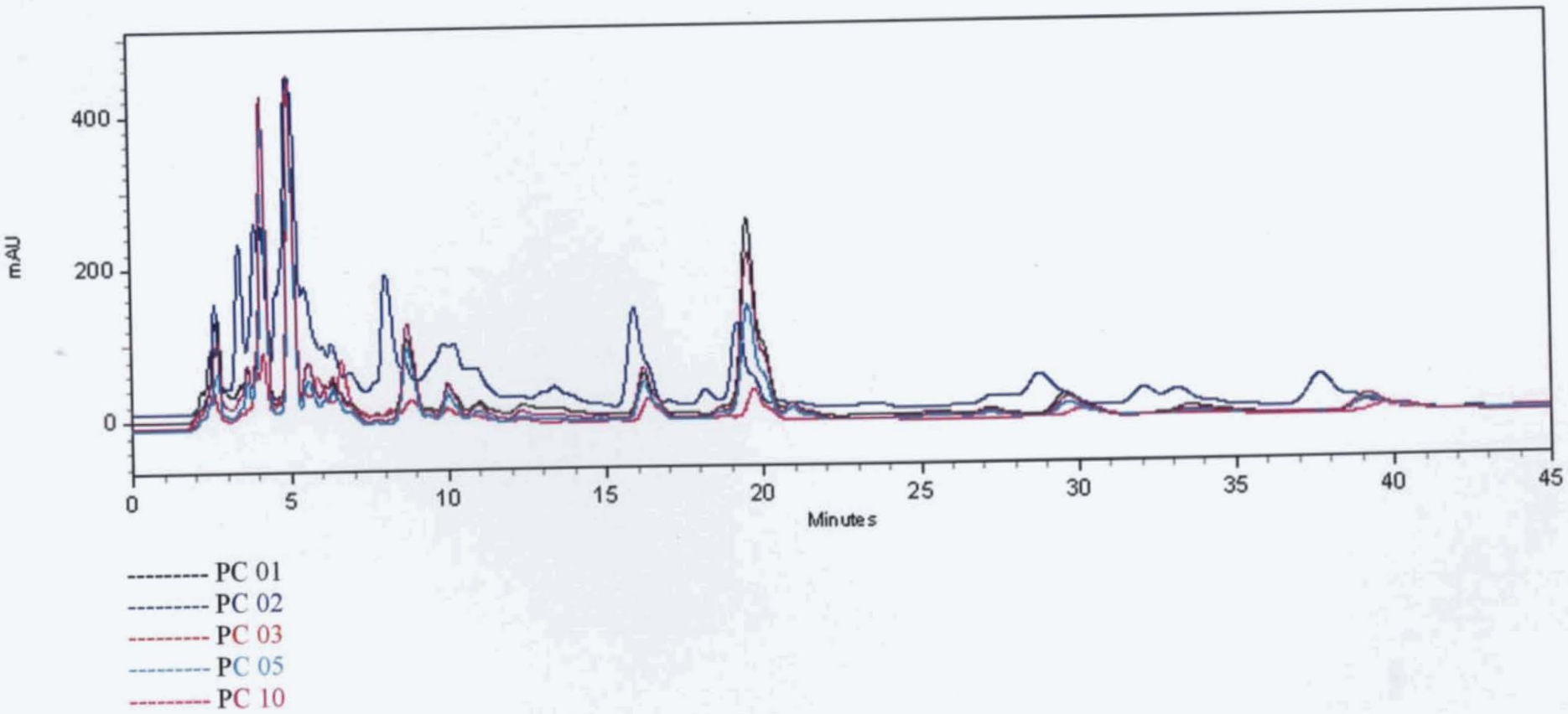
The HPLC peak patterns of this species are represented in Table 4.15 and Fig. 4.5. Based on the data in Table 4.15, PAI were computed for the species (Table 4.25).

Table 4.25. Paired affinity indices (PAI) of the different accessions of *Premna corymbosa*.

Accessions	PC 01	PC 02	PC 03	PC 05	PC 10
PC 01	100				
PC 02	15.21	100			
PC 03	16.0	16.0	100		
PC 05	15.62	18.86	56.81	100	
PC 10	74.07	16.36	11.76	11.32	100

Among the accessions used for the study, the accession from KAPL, Aluva, Kerala (PC 01) showed the highest chemical affinity (74.07%) with the accession from Kerala Agricultural University, Thrissur, Kerala (PC 10). Next to this, 56.81% affinity was observed between the accession from Mattanur, Kannur, Kerala (PC 03) and Herbal garden, AVS, Kerala (PC 05). The accessions from Herbal garden, AVS, Kerala (PC 05) and Kerala Agricultural University, Thrissur, Kerala (PC 10) showed the lowest affinity (11.32 %) among the species.

Fig. 4.5. HPLC chromatogram of 5 accessions of *Premna corymbosa*



4.2.6. *Desmodium gangeticum*

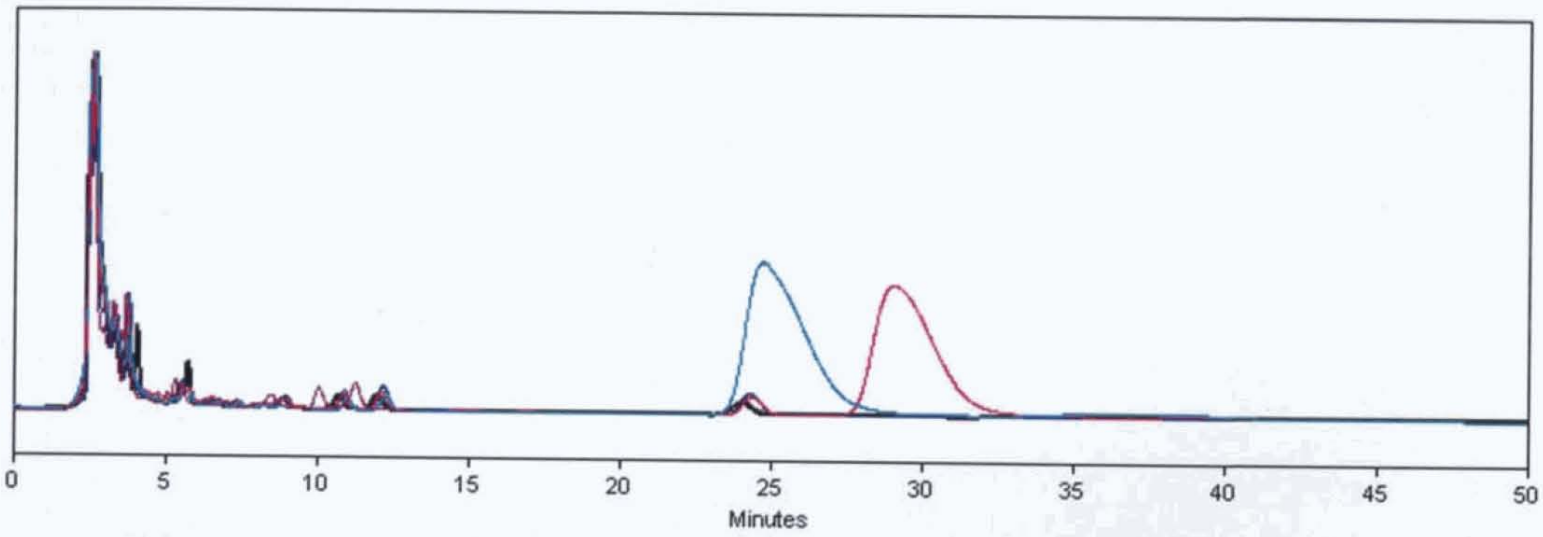
The HPLC peak patterns of this species are presented in Table 4.16 and Fig. 4.6. Based on the data in Table 4.16, PAI were computed for the species (Table 4.26).

Table 4.26. Paired affinity indices (PAI) of the different accessions of *Desmodium gangeticum*

Accessions	DG 01	DG 02	DG 03	DG 05	DG 07
DG 01	100				
DG 02	16.66	100			
DG 03	25.93	37.03	100		
DG 05	43.33	57.14	36.0	100	
DG 07	27.58	31.25	37.93	39.28	100

Among the accessions used for the study, the accession from Kodamadi, Thirunelveli, Tamil Nadu (DG 02) showed the highest chemical affinity (57.14%) with the accession from Shevayur, Virudhunagar, Tamil Nadu (DG 05). Next to this, 43.33% affinity was shown by the accessions from Shevayur, Virudhunagar, Tamil Nadu (DG 05) and Vellanimala, Thrissur, Kerala (DG 01). The accessions DG 01 and DG 02 showed the lowest affinity (16.66 %) among the accessions.

Fig. 4.6. HPLC chromatogram of 5 accessions of *Desmodium gangeticum*



4.2.7. *Pseudarthria viscida*

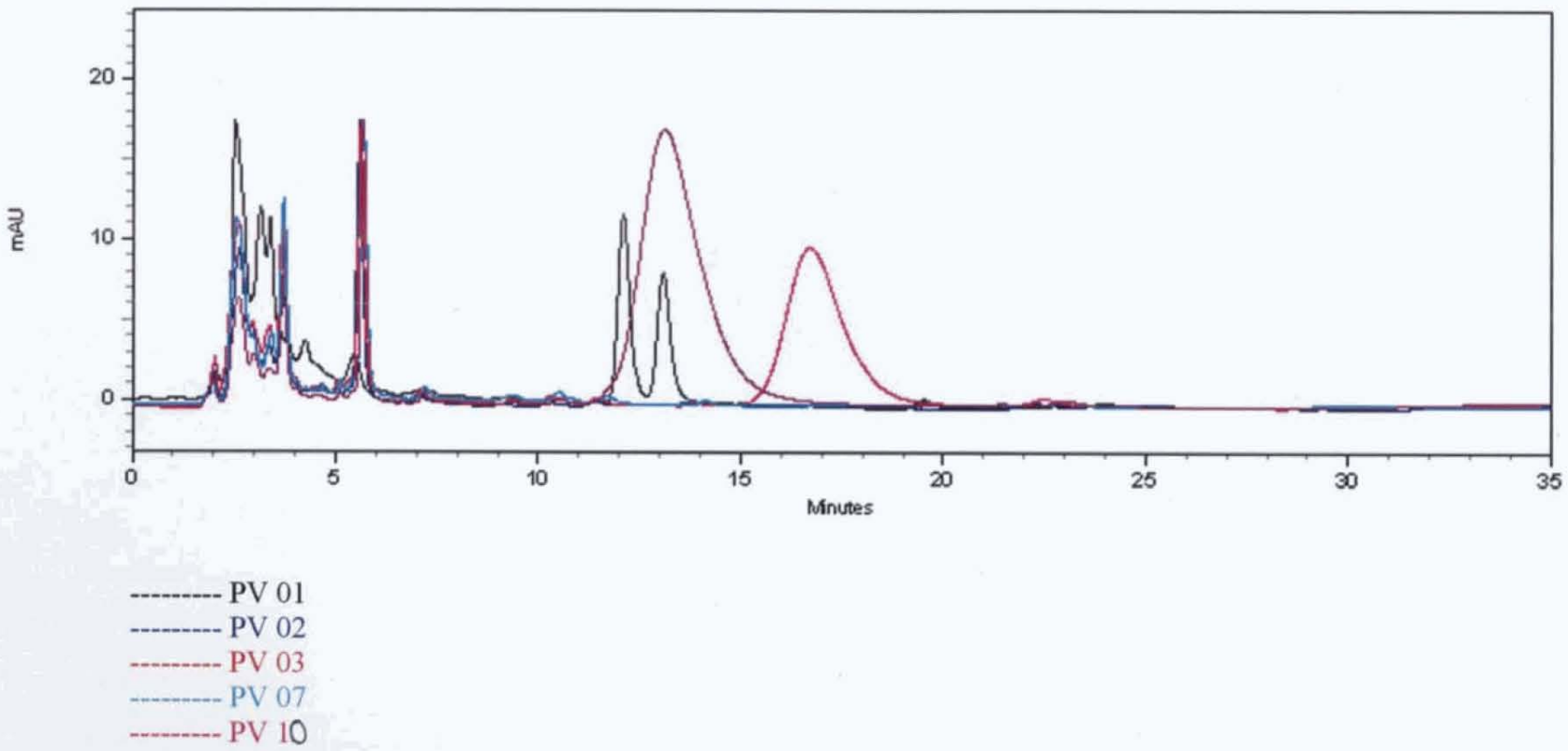
The HPLC peak patterns in the case of this species are presented in Table 4.17 and Fig. 4.7. Based on the data in Table 4.17, PAI were computed for the species (Table 4.27).

Table 4.27. Paired affinity index (PAI) of the different accessions of *Pseudarthria viscida*

Accessions	PV 01	PV 02	PV 03	PV 07	PV 10
PV 01	100				
PV 02	48.14	100			
PV 03	15.15	13.51	100		
PV 07	25.92	18.75	50.0	100	
PV 10	23.32	25.80	46.15	41.66	100

Among the accessions used for the study, the accession from Savandurga, Bangalore, Karnataka (PV 03) showed the highest chemical affinity (50.0%) with the accession from Sirumalai, Dindigul, Tamil Nadu (PV 07). Next to this, 48.14% affinity was observed between the accessions from Attayar, Thiruvananthapuram, Kerala (PV 01) and Charmady, Dakshin Kanara, Karnataka (PV 02). The accessions from Charmady, Dakshin Kanara, Karnataka (PV 02) and Savandurga, Bangalore, Karnataka (PV 03) showed the lowest affinity (13.51 %) among the accessions.

Fig. 4.7. HPLC chromatogram of 5 accessions of *Pseudarthria viscida*



4.2.8. *Solanum melongena* var. *insanum*

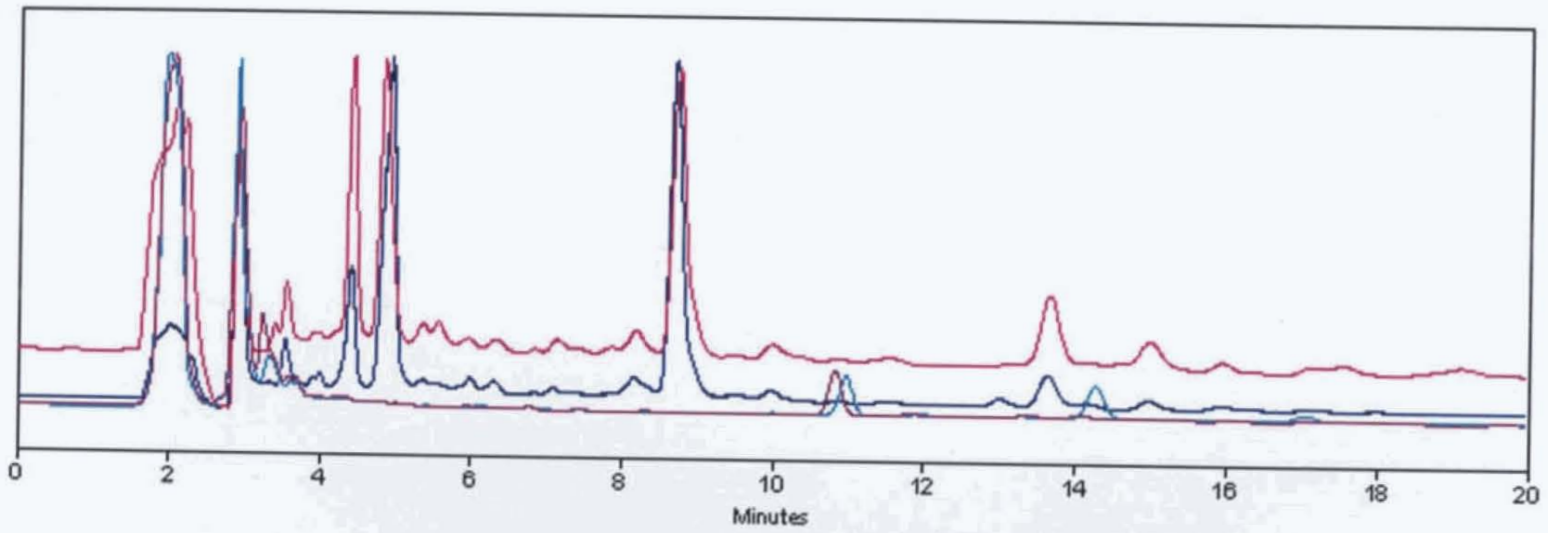
The HPLC peak patterns of this species are represented in Table 4.18 and Fig. 4.8. Based on the data in Table 4.8, PAI were computed for the species (Table 4.28).

Table 4.28. Paired affinity indices (PAI) of the different accessions of *Solanum melongena* var. *insanum*

Accessions	SM 01	SM 02	SM 06	SM 07	SM 08
SM 01	100				
SM 02	33.33	100			
SM 06	42.42	38.70	100		
SM 07	45.45	45.27	35.29	100	
SM 08	41.17	35.48	34.47	23.68	100

Among the accessions used for the study, the accession from Moozhiyoor, Pathanamthita, Kerala (SM 01) showed the highest chemical affinity (45.45%) with the accession from Kerala Agricultural University, Thrissur, Kerala (SM 07). Next to this, 45.27% affinity was observed between the accessions from Herbal Garden, AVS, Kottakkal, Kerala (SM 02) and Kerala Agricultural University, Thrissur, Kerala (SM 07). The accessions from Kerala Agricultural University, Thrissur, Kerala (SM 07) and Vadakara, Kozhikode, Kerala (SM 08) showed the lowest affinity (23.68 %) among the species.

Fig. 4.8. HPLC chromatogram of 5 accessions of *Solanum melongena* var. *insanum*



4.2.9. *Soalnum violaceum*

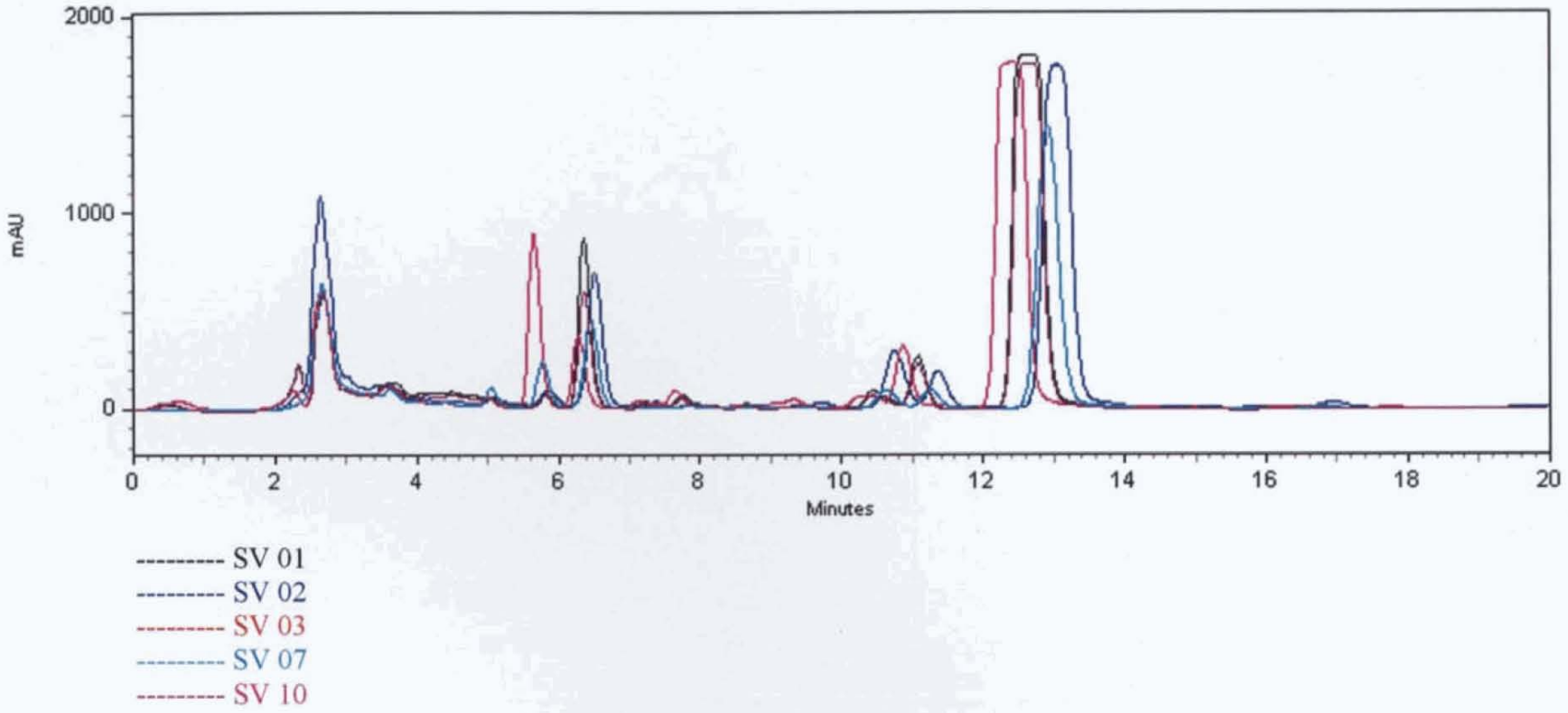
The HPLC peak patterns in the case of this species are presented in Table 4.19 and Fig. 4.9. Based on the data in Table 4.19, PAI were computed for the species (Table 4.29).

Table 4.29. Paired affinity index (PAI) of the different accessions of *Solanum violaceum*

Accessions	SV 01	SV 02	SV 03	SV 07	SV 10
SV 01	100				
SV 02	37.03	100			
SV 03	32.14	14.28	100		
SV 07	29.62	39.28	25.0	100	
SV 10	29.62	40.74	32.14	15.15	100

Among the accessions used for the study, the accession from Seethathode, Pathanamthita, Kerala (SV 02) showed the highest chemical affinity (40.74%) with the accession from Arya Vaidya Pharmacy (AVP), Kanjirapuzha, Palakkad (SV 10). Next to this, 39.28% affinity was observed between the accessions from Seethathode, Pathanamthita, Kerala (SV 02) and Herbal Garden, AVS, Kottakkal, Kerala (SV 07). The Accessions from Seethathode, Pathanamthita, Kerala (SV 02) and Nagarjuna, Idukki, Kerala (SV 03) showed the lowest affinity (14.28 %) among the accessions.

Fig. 4.9. HPLC chromatogram of 5 accessions of *Solanum violaceum*



4.2.10. *Tribulus terrestris*

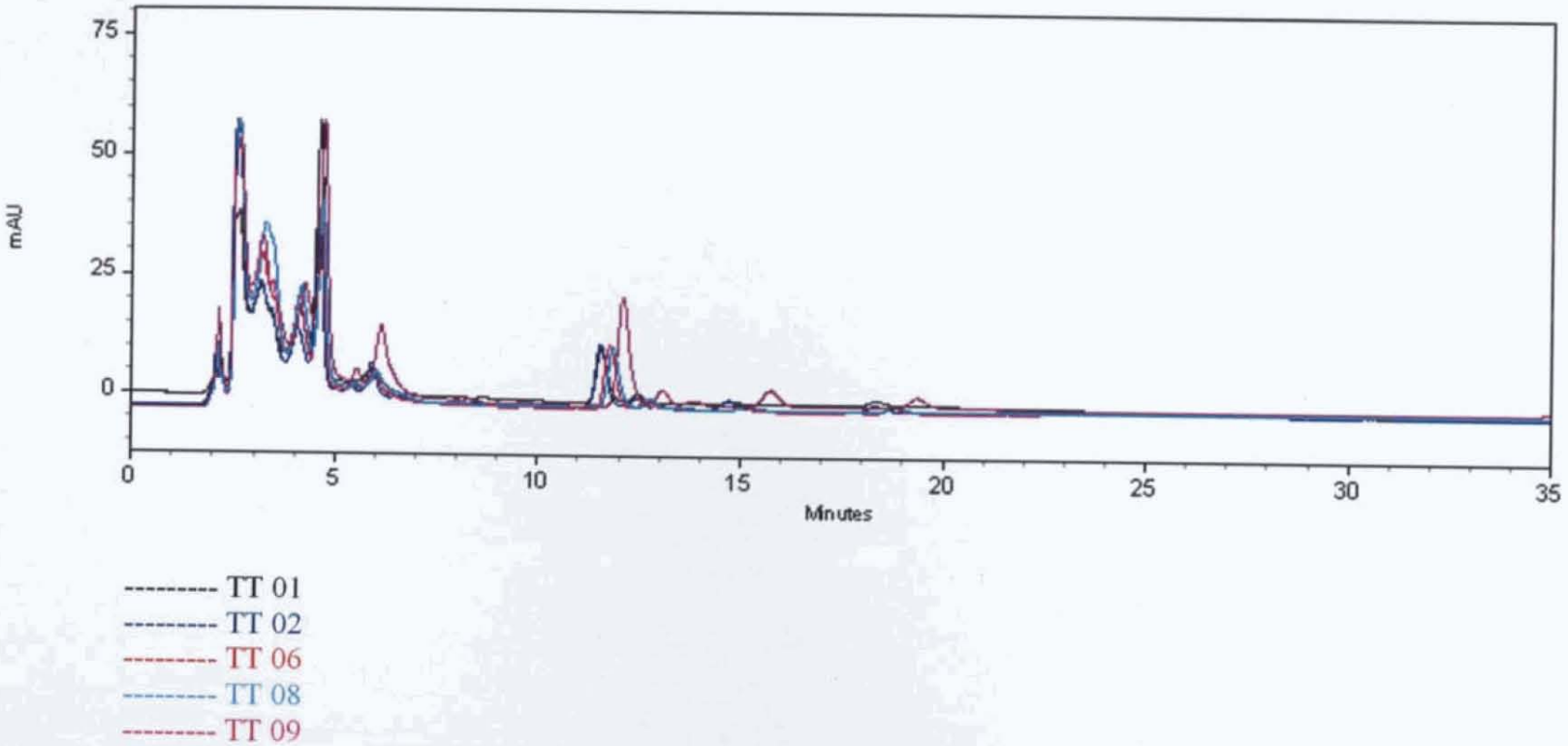
The HPLC peak patterns of this species are presented in Table 4.20 and Fig. 4.10. Based on the data in Table 4.20, PAI were computed for the species (Table 4.30).

Table 4.30. Paired affinity index (PAI) of the different accessions of *Tribulus terrestris*

Accessions	TT 01	TT 02	TT 06	TT 08	TT 09
TT 01	100				
TT 02	70.58	100			
TT 06	21.73	20.0	100		
TT 08	20.0	20.0	29.03	100	
TT 09	18.18	17.39	18.51	17.39	100

Among the accessions used for the study, the accession from Herbal Garden, AVS, Kottakkal, Kerala (TT 01) showed the highest chemical affinity (70.58 %) with the accession from Centre for Medicinal Plant Research, Arya Vaidya Sala (AVS), Kottakkal, Kerala (TT 02). Next, 29.03% affinity was observed between the accessions from Madukkarai, Coimbatore, Tamil Nadu (TT 06) and Anakkati, Palakkad, Kerala (TT 08). The accessions from Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala (AVS), Kottakkal, Kerala (TT 02) and Agali, Palakkad, Kerala (TT 09) showed the lowest affinity (17.39 %) among the species.

Fig. 4.10. HPLC chromatogram of 5 accessions of *Tribulus terrestris*



Phytochemical characters are the indicators of the chemical constitutents of plants and the study of phytochemical variation is very important in medicinal plants since medicinal property of any plant is the result of the action of such chemicals. Analysis of the phytochemical variability between accessions in the case of medicinal plants will help to identify them in terms of their differences in phytochemical constitution. More over, phytochemical differences are good indicators of their genotypic distances. Studies on phytochemical variability have been attempted by early workers in different crops like cinnamon (Ravindran *et al.*, 1992), pepper (Ravindran and Nirmal Babu, 1994), cardamom (Radhakrishnan, 2003), tea (Ramasubramanian, 2005), etc.

4.3. Correlation of characters

Biological characters are controlled by genes which may be oligogenic or polygenic in nature. Characters which show continuous distribution are polygenic in nature. Most of the plant characters related to growth, yield and productivity belong to this category. The agronomic characters of medicinal plants are no exemptions. Such characters show different levels of interrelationships between them and such relationships can be identified by correlation analysis. Presently some of the morphometric characters of the different species of plants belonging to *dasamula* group have been analysed as presented and discussed below.

4.3.1. *Aegle marmelos*

Ten leaf related morphometric characters of *Aegle marmelos* have been subjected to correlation analysis presently as shown in Table 4.31. Terminal leaflet petiole length and internodal length did not show any significant correlation with any other characters. All other characters *viz.*, terminal leaflet length, terminal leaflet breadth, terminal leaflet area, petiole length, first leaflet length, first leaflet breadth, second leaflet length and second leaflet breadth showed significant positive correlation towards each other.

Table 4.31. Correlation of quantitative morphological characters in the case of *Aegle marmelos*

Characters	Leaf length	Leaf breadth	Leaf area	Petiole length	Terminal leaf let petiolule length	Ist leaflet length	Ist leaflet breadth	IInd leaflet length	IInd leaflet breadth
Leaf length									
Leaf breadth	0.88*								
Leaf area	0.96*	0.97*							
Petiole length	0.75*	0.91*	0.85*						
Terminal leaf let petiolule length	-0.26	-0.45	-0.38	-0.64					
Ist leaflet length	0.91*	0.84*	0.90*	0.61*	-0.09				
Ist leaflet breadth	0.85*	0.93*	0.91*	0.85*	-0.33	0.85*			
IInd leaflet length	0.92*	0.81*	0.89*	0.60*	-0.14	0.99*	0.81*		
IInd leaflet breadth	0.87*	0.93*	0.92*	0.77*	-0.25	0.91*	0.98*	0.87*	
Stabilized internodal length	0.16	0.39	0.26	0.51*	-0.09	0.01	0.46	-0.07	0.36

*: significant at 5% level.

4.3.2. *Gmelina arborea*

Five leaf related morphometric characters were analysed for correlation in the case of *Gmelina arborea* (Table 4.32). Internodal length did not show any significant positive correlation with leaf length, leaf breadth, leaf area or petiole length. Leaf length, leaf breadth, leaf area and petiole length showed significant positive correlation towards each other.

Table 4.32. Correlation of quantitative morphological characters in the case of *Gmelina arborea*

Characters	Leaf length	Leaf breadth	Petiole length	Leaf area
Leaf length				
Leaf breadth	0.99*			
Petiole length	0.64*	0.72*		
Leaf area	0.99*	0.99*	0.69*	
Stabilized internodal length	-0.49	-0.57	-0.69	-0.56

*: significant at 5% level

4.3.3. *Oroxylum indicum*

Five leaf related morphometric characters of *Oroxylum indicum* were analysed presently for correlation (Table 4.33). Stabilised internodal length and petiole length did not show any significant positive correlation with other characters. Terminal leaflet length, terminal leaflet breadth and terminal leaflet area were positively correlated.

Table 4.33. Correlation of quantitative morphological characters in the case of *Oroxylum indicum*

Characters	Terminal leaflet length	Terminal leaflet breadth	Leaf area	Petiole length
Terminal leaflet length				
Terminal leaflet breadth	0.96*			
Leaf area	0.99*	0.99*		
Petiole length	0.05	-0.05	-0.01	
Stabilized internodal length	-0.36	-0.47	-0.42	0.39

*: significant at 5% level

4.3.4. *Stereospermum colais*

Ten leaf/branch related morphometric characters of *Stereospermum colais* was analysed for correlation between them (Table 4.34). Characters like first leaflet breadth, second leaflet breadth and internodal length did not show any significant positive correlation with any of the characters studied. Terminal leaflet length, terminal leaflet breadth, terminal leaflet area, petiole length and first leaflet length showed significant positive correlation with terminal leaflet length, terminal leaflet breadth, terminal leaflet area and petiole length.

Table 4.34. Correlation of quantitative morphological characters in the case of *Stereospermum colais*

Characters	Terminal leaflet length	Terminal leaflet breadth	Terminal leaflet area	Petiole length	Ist leaflet length	Ist leaflet breadth	IInd leaflet length	IInd leaflet breadth	Stabilised internode length
Terminal leaflet length									
Terminal leaflet breadth	0.62*								
Terminal leaflet area	0.84*	0.95*							
Petiole length	0.71*	0.61*	0.72*						
Ist leaflet length	0.93*	0.54*	0.75*	0.81*					
Ist leaflet breadth	-0.10	0.26	0.14	0.37	-0.17				
IInd leaflet length	0.93*	0.55*	0.76*	0.81*	0.99*	-0.17			
IInd leaflet breadth	-0.08	0.29*	0.17	0.37	-0.16	0.99*	-0.16		
Stabilized internodal length	-0.68	-0.09	-0.33	-0.22	-0.61	0.51	-0.61	0.51	
Ist branch length	0.10	0.48	0.39	0.37	0.07	0.53	0.08	0.55*	0.61*

*: significant at 5% level

4.3.5. *Premna corymbosa*

Six leaf/branch related morphometric characters were analysed for correlation in the case of *Premna corymbosa* (Table 4.35). Leaf length showed significant positive correlation with leaf breadth, leaf area and petiole length. Leaf breadth showed significant positive correlation with leaf length and leaf area. Leaf area showed significant positive correlation with leaf length and leaf breadth.

Table 4.35. Correlation of quantitative morphological characters in the case of *Premna corymbosa*

Characters	Leaf length	Leaf breadth	Petiole length	Leaf area	Stabilised internodal length
Leaf length					
Leaf breadth	0.84*				
Petiole length	0.53	0.22			
Leaf area	0.97*	0.94*	0.40		
Stabilized internodal length	-0.07	-0.04	-0.36	-0.06	
Ist branch length	-0.38	-0.20	0.10	-0.32	-0.20

*: significant at 5% level

4.3.6. *Desmodium gangeticum*

Eight quantitative morphological characters were studied for correlation in the case of *Desmodium gangeticum* (Table 4.36). Leaf length showed significant positive correlation with leaf breadth and petiole length. Leaf breadth showed significant positive correlation with leaf length and leaf area. Petiole length showed significant positive correlation with leaf length and leaf area showed significant positive correlation with leaf length, petiole length and inflorescence length. Pod length has been found to be positively correlated with inflorescence length and branch length.

Table 4.36. Correlation of quantitative morphological characters in the case of *Desmodium gangeticum*

Characters	Leaf length	Leaf breadth	Petiole length	Leaf area	Stabilised internodal length	Ist branch length	Inflores -cence length
Leaf length							
Leaf breadth	0.87*						
Petiole length	-0.96	-0.92					
Leaf area	0.99*	0.93*	-0.98				
Stabilized internodal length	-0.04	0.34	-0.01	0.07			
Ist branch length	-0.40	-0.53	0.43	-0.46	0.01		
Inflorescen ce length	-0.82	-0.99	0.90*	-0.90	-0.31	0.56*	
Pod length	-0.24	-0.21	0.30	-0.27	0.37	0.66*	0.26

*: significant at 5% level

4.3.7. *Pseudarthia viscida*

Correlation of quantitative morphological characters has been worked out in *Pseudarthia viscida* in the case of twelve charcters (Table 4.37). Terminal leaflet length was found to be positively correlated with terminal leaflet breadth, terminal leaflet area, petiole length, first leaflet length, first leaflet breadth, second leaflet length, second leaflet breadth, first branch length and pod length. Terminal leaflet breadth showed significant positive correlation with terminal leaflet length, terminal leaflet area, petiole length, first leaflet length, first leaflet breadth, second leaflet length, second leaflet breadth and first branch length. Terminal leaflet area showed significant positive correlation with terminal leaflet length, terminal leaflet breadth, petiole length, first leaflet length, first leaflet breadth, second leaflet length, second leaflet breadth and first branch length. Petiole length showed significant positive correlation with terminal leaflet length, terminal leaflet breadth, terminal leaflet area, first leaflet length, first leaflet breadth, second leaflet length, second leaflet breadth and first branch length. First leaflet length showed significant positive correlation with terminal leaflet length, terminal leaflet breadth, terminal leaflet area, petiole length, first leaflet length, second leaflet length, second leaflet breadth, first branch length and pod length. Second leaflet length showed significant positive correlation with terminal leaflet length, terminal leaflet breadth, terminal leaflet area, petiole length, first leaflet length, first leaflet breadth, second leaflet breadth, first branch length and pod length. Second leaflet breadth showed significant positive correlation with

terminal leaflet length, terminal leaflet breadth, terminal leaflet area, petiole length, first leaflet length, first leaflet breadth, second leaflet length, first branch length and pod length. First branch length showed significant positive correlation with all the characters studied except inflorescence length and pod length. Inflorescence length showed significant positive correlation with first branch length only and internodal length did not show positive correlation with any of the characters studied.

Table 4.37. Correlation of quantitative morphological characters in the case of *Pseudarthria viscida*

Characters	Terminal leaflet length	Terminal leaflet breadth	Terminal leaflet area	Petiole length	Ist leaflet length	Ist leaflet breadth	IInd leaflet length	IInd leaflet breadth	Stabilised internodal length	Ist branch length	Inflorescence length
Terminal leaflet length											
Terminal leaflet breadth	0.98*										
Terminal leaflet area	0.98*	0.99*									
Petiole length	0.95*	0.98*	0.97*								
Ist leaflet length	0.97*	0.94*	0.93*	0.93*							
Ist leaflet breadth	0.91*	0.86*	0.84*	0.81*	0.94*						
IInd leaflet length	0.97*	0.94*	0.93*	0.94*	0.99*	0.94*					
IInd leaflet breadth	0.93*	0.87*	0.86*	0.82*	0.95*	0.99*	0.95*				
Stabilised internodal length	-0.02	0.001	-0.07	0.02	0.03	0.06	0.03	0.04			
Ist branch length	0.93*	0.92*	0.93*	0.86*	0.86*	0.83*	0.87*	0.85*	-0.21		
Inflorescence length	0.40	0.32	0.34	0.17	0.31	0.52	0.31	0.52	-0.19	0.62*	
Pod length	0.56*	0.48	0.46	0.42	0.64*	0.63*	0.63*	0.63*	-0.08	0.47	0.29

*: significant at 5% level

4.3.8. *Solanum melongena* var *insanum*

Six leaf/branch related characters namely leaf length, leaf breadth, petiole length, leaf area, stabilized internodal length and first branch length were analysed for correlation in the case of *Solanum melongena* var. *insanum* (Table 4.38). All the characters under study showed significant positive correlation between them.

Table 4.38. Correlation of quantitative morphological characters in the case of *Solanum melongena* var. *insanum*

Characters	Leaf length	Leaf breadth	Petiole length	Leaf area	Stabilised internodal length
Leaf length					
Leaf breadth	0.95*				
Petiole length	0.93*	0.96*			
Leaf area	0.99*	0.99*	0.95*		
Stabilized internodal length	0.72*	0.83*	0.78*	0.79*	
Ist branch length	0.59*	0.74*	0.79*	0.66*	0.61*

*: significant at 5% level

4.3.9. *Solanum violaceum*

Eight morphometric characters, leaf length, leaf breadth, petiole length, leaf area, internodal length, first branch length, inflorescence length and pedicel length were analysed for correlation in *Solanum violaceum* (Table 4.39). Leaf length showed significant positive correlation with all the other characters studied. Leaf breadth showed significant positive correlation with leaf length, leaf area, internodal length, inflorescence length and pedicel length. Petiole length showed significant positive correlation with internodal length only. Leaf area showed significant positive correlation with leaf length, leaf breadth, internodal length and inflorescence length. Internodal length was positively correlated with all the other characters studied. First branch length showed significant positive correlation with leaf length, internodal length, inflorescence length and pedicel length. Inflorescence length was positively correlated with all the other characters studied and the pedicel length was positively correlated with leaf length, leaf breadth, internodal length, first branch length and inflorescence length.

Table 4.39. Correlation of quantitative morphological characters in the case of *Solanum violaceum*

Characters	Leaf length	Leaf breadth	Petiole length	Leaf area	Stabilised internodal length	Ist branch length	Inflorescence length
Leaf length							
Leaf breadth	0.99*						
Petiole length	0.47	0.50					
Leaf area	0.99*	0.99*	0.45				
Stabilized internodal length	0.76*	0.76*	0.77*	0.71*			
Ist branch length	0.49	0.47	0.27	0.42	0.76*		
Inflorescence length	0.67*	0.64*	0.35	0.58*	0.80*	0.80*	
Pedicel length	0.56*	0.57*	0.38	0.49	0.83*	0.93*	0.88*

*: significant at 5% level

4.3.10. *Tribulus terrestris*

Six morphometric characters *viz.*, leaf length, leaf breadth, petiole length, leaf area, internodal length and first branch length were studied for correlation analysis in the case of *Tribulus terrestris* (Table 4.40). All the characters except internodal length showed significant positive correlation towards each other.

Table 4.40. Correlation of quantitative morphological characters in the case of *Tribulus terrestris*

Characters	Leaf length	Leaf b readth	Petiole length	Leaf area	Stabilised internodal length
Leaf length					
Leaf breadth	0.98*				
Petiole length	0.76*	0.80*			
Leaf area	0.99*	0.99*	0.78*		
Stabilized internodal length	-0.33	-0.35	-0.72	-0.29	
Ist branch length	0.67*	0.73*	0.78*	0.73*	-0.17

*: significant at 5% level

Correlation of characters shows their interrelationship. Characters with significant positive correlation usually show similar trends of variation providing an opportunity for their selection jointly. This is very much important in breeding programmes by selection since the bulk of characters to be used for selection gets reduced considerably. Similar approaches have been used by earlier workers in different crops like cardamom (Radhakrishnan, 2003), tea (Ramasubramanian (2005), etc.

4.4. Cluster analysis

Clustering of genotypes based on their similarities and differences is carried out so as to group the genotypes based on the genetic distance between them. Presently cluster analysis was carried out based on the morphometric characters studied in the case of the ten species under study using the statistical software STATISTICA following UPGMA procedure (Nair *et al.*, 2005).

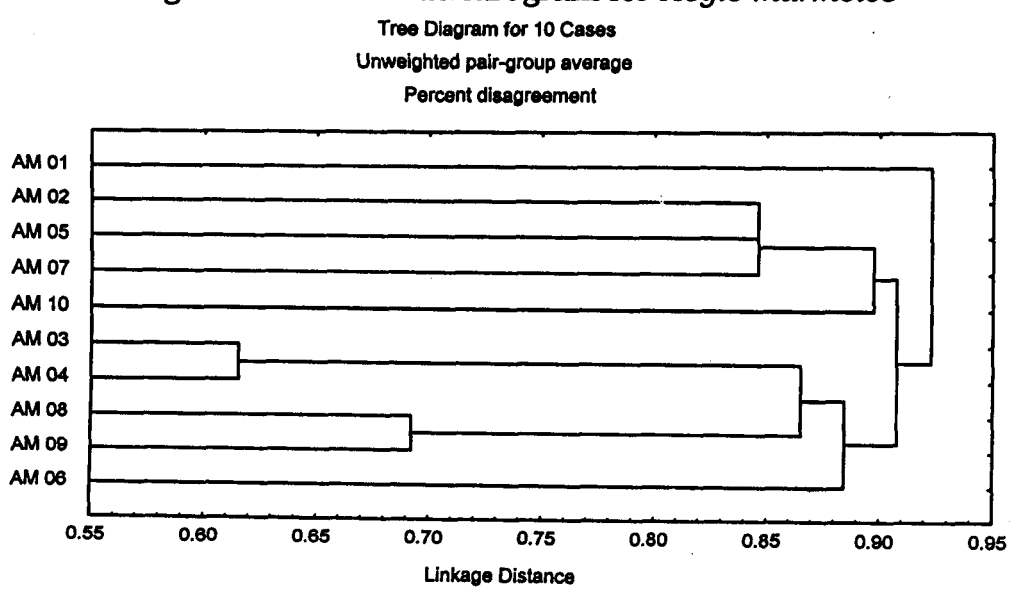
4.4.1. *Aegle marmelos*

The ten accessions of *Aegle marmelos* studied presently could be grouped into two clusters at a linkage distance of 0.92 (Table 4.41 and Fig. 4.11). The nine accessions of the larger group further segregated into two clusters at a linkage distance of 0.91. Accession number AM 03 and AM 04 were found to be the closest with a linkage distance of 0.615 followed by AM 08 and AM 09 which showed a linkage distance of 0.69.

Table 4.41. Percent disagreement between the accessions of *Aegle marmelos* studied

	AM 01	AM 02	AM 03	AM 04	AM 05	AM 06	AM 07	AM 08	AM 09	AM 10
AM 01	.00	.92	.92	.92	.92	.92	.92	.92	.92	.92
AM 02	.92	.00	.85	.92	.85	.92	.85	.92	.92	.85
AM 03	.92	.85	.00	.62	.92	.85	.92	.85	.92	.92
AM 04	.92	.92	.62	.00	.92	.92	.92	.77	.92	.92
AM 05	.92	.85	.92	.92	.00	.85	.85	.92	.85	.92
AM 06	.92	.92	.85	.92	.85	.00	.92	.85	.92	.92
AM 07	.92	.85	.92	.92	.85	.92	.00	.85	.92	.92
AM 08	.92	.92	.85	.77	.92	.85	.85	.00	.69	.92
AM 09	.92	.92	.92	.92	.85	.92	.92	.69	.00	.92
AM 10	.92	.85	.92	.92	.92	.92	.92	.92	.92	.00

Fig. 4.11. UPGMA dendrogram for *Aegle marmelos*



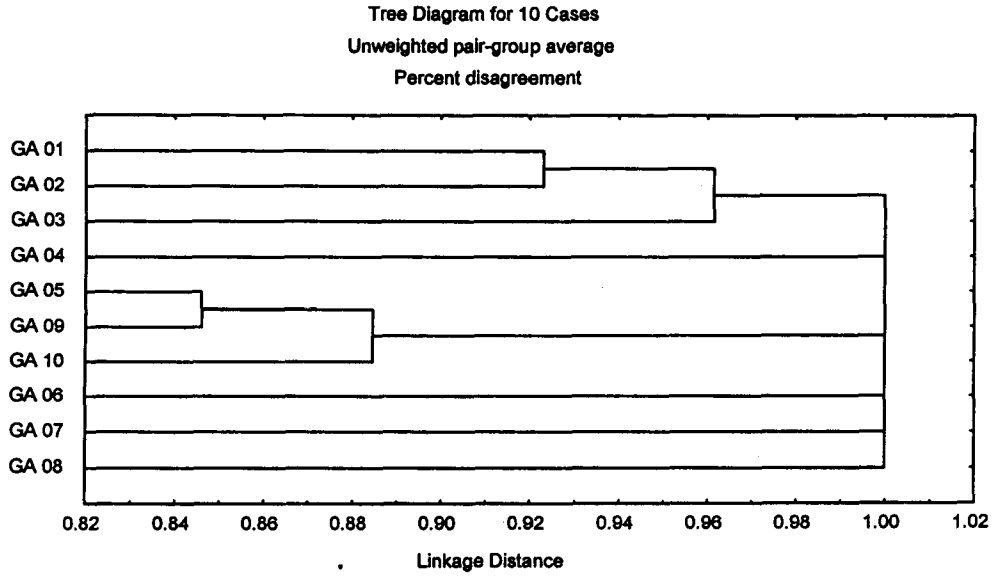
4.4.2. *Gmelina arborea*

The ten accessions of *Gmelina arborea* could be grouped into six clusters based on the present study at a linkage distance of 1. The first group was represented by the accessions GA 01, GA 02 and GA 03, second by GA 04, third by GA 05, GA 09 and GA 10, fourth by GA 06, fifth by GA 07 and the sixth by GA08 (Table 4.42 and Fig. 4.12). GA 05 and GA 09 were found to be the closest with a linkage distance of 0.85.

Table 4.42. Percent disagreement between the accessions of *Gmelina arborea* studied

	GA 01	GA 02	GA 03	GA 04	GA 05	GA 06	GA 07	GA 08	GA 09	GA 10
GA 01	.00	.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GA 02	.92	.00	.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GA 03	1.00	.92	.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GA 04	1.00	1.00	1.00	.00	1.00	1.00	1.00	1.00	1.00	1.00
GA 05	1.00	1.00	1.00	1.00	.00	1.00	1.00	1.00	.85	.92
GA 06	1.00	1.00	1.00	1.00	1.00	.00	1.00	1.00	1.00	1.00
GA 07	1.00	1.00	1.00	1.00	1.00	1.00	.00	1.00	1.00	1.00
GA 08	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.00	1.00	1.00
GA 09	1.00	1.00	1.00	1.00	.85	1.00	1.00	1.00	.00	.85
GA 10	1.00	1.00	1.00	1.00	.92	1.00	1.00	1.00	.85	.00

Fig. 4.12. UPGMA dendrogram for *Gmelina arborea*



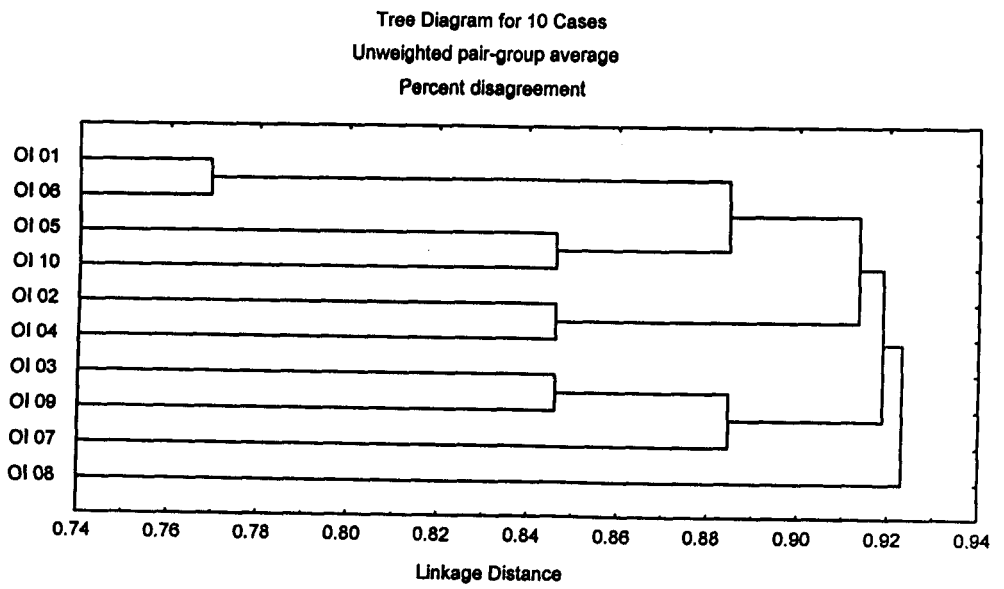
4.4.3. *Oroxylum indicum*

The accessions of *Oroxylum indicum* were grouped into two groups at 0.925 level with the accession number OI 08, forming a cluster distinct from others (Table 4.42 & Fig 4.13). The large cluster could be further classified into two at a linkage distance of 0.92. Accession number OI 01 and OI 06 were found to be the closest showing a linkage distance of 0.77.

Table 4.43. Percent disagreement between the accessions of *Oroxylum indicum* studied.

	OI 01	OI 02	OI 03	OI 04	OI 05	OI 06	OI 07	OI 08	OI 09	OI 10
OI 01	.00	.92	.92	.92	.85	.77	.92	.92	.92	.85
OI 02	.92	.00	.92	.85	.92	.92	.92	.92	.92	.85
OI 03	.92	.92	.00	.92	.92	.92	.92	.92	.85	.92
OI 04	.92	.85	.92	.00	.92	.92	.92	.92	.92	.92
OI 05	.85	.92	.92	.92	.00	.92	.92	.92	.92	.85
OI 06	.77	.92	.92	.92	.92	.00	.85	.92	.92	.92
OI 07	.92	.92	.92	.92	.92	.85	.00	.92	.85	.92
OI 08	.92	.92	.92	.92	.92	.92	.92	.00	.92	.92
OI 09	.92	.92	.85	.92	.92	.92	.85	.92	.00	.92
OI 10	.85	.85	.92	.92	.85	.92	.92	.92	.92	.00

Fig. 4.13. UPGMA dendrogram for *Oroxylum indicum*



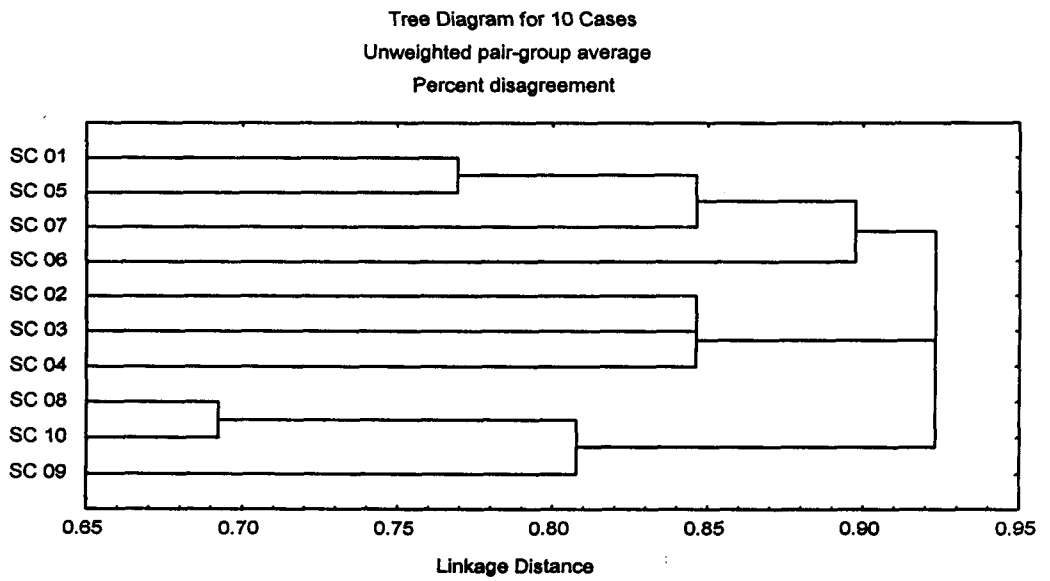
4.4.4. *Stereospermum colais*

The present study showed that the ten accessions of *Stereospermum colais* used presently could be classified into three groups at a linkage distance of 0.92. Accession numbers SC 08 and SC 10 showed maximum proximity of relationships which showed only a linkage distance of 0.71 (Table 4.44 & Fig. 4.14).

Table 4.44. Percent disagreement between the accessions of *Stereospermum colais* studied.

	SC 01	SC 02	SC 03	SC 04	SC 05	SC 06	SC 07	SC 08	SC 09	SC 10
SC 01	.00	.92	.92	.92	.77	.92	.85	.92	.92	.92
SC 02	.92	.00	.85	.85	.92	.92	.92	.92	.92	.92
SC 03	.92	.85	.00	.85	.92	.92	.92	.92	.92	.92
SC 04	.92	.85	.85	.00	.92	.92	.92	.92	.92	.92
SC 05	.77	.92	.92	.92	.00	.85	.85	.92	.92	.92
SC 06	.92	.92	.92	.92	.85	.00	.92	.92	.92	.92
SC 07	.85	.92	.92	.92	.85	.92	.00	.92	.92	.92
SC 08	.92	.92	.92	.92	.92	.92	.92	.00	.85	.69
SC 09	.92	.92	.92	.92	.92	.92	.92	.85	.00	.77
SC 10	.92	.92	.92	.92	.92	.92	.92	.69	.77	.00

Fig. 4.14. UPGMA dendrogram for *Stereospermum colais.*



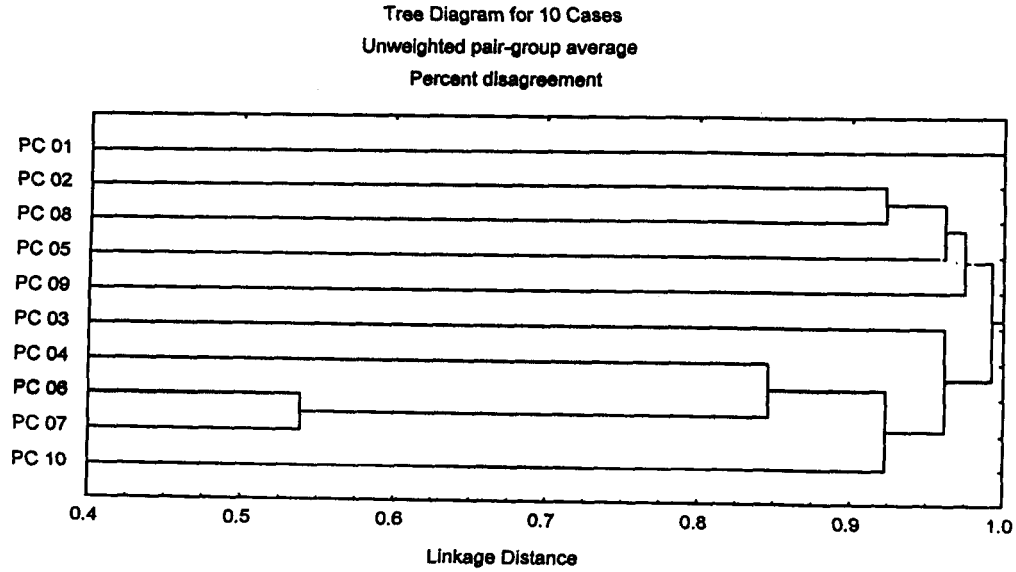
4.4.5. *Premna corymbosa*

The ten accessions of *Premna corymbosa* studied presently could be grouped into two clusters at a linkage distance of 1, the accession PC 01 forming a separate cluster and the remaining nine forming the second cluster (Table 4.45 and Fig. 4.15). Accession numbers PC 06 and PC 07 showed the highest proximity with a linkage distance of 0.525.

Table 4.45. Percent disagreement between the accessions of *Premna corymbosa* studied

	PC 01	PC 02	PC 03	PC 04	PC 05	PC 06	PC 07	PC 08	PC 09	PC 10
PC 01	.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PC 02	1.00	.00	1.00	.92	1.00	1.00	1.00	.92	1.00	1.00
PC 03	1.00	1.00	.00	1.00	1.00	.92	.92	1.00	1.00	1.00
PC 04	1.00	.92	1.00	.00	1.00	.85	.85	.92	1.00	.92
PC 05	1.00	1.00	1.00	1.00	.00	1.00	1.00	.92	1.00	1.00
PC 06	1.00	1.00	.92	.85	1.00	.00	.54	1.00	1.00	.92
PC 07	1.00	1.00	.92	.85	1.00	.54	.00	1.00	1.00	.92
PC 08	1.00	.92	1.00	.92	.92	1.00	1.00	.00	.92	1.00
PC 09	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.92	.00	1.00
PC 10	1.00	1.00	1.00	.92	1.00	.92	.92	1.00	1.00	.00

Fig. 4.15. UPGMA dendrogram for *Premna corymbosa*



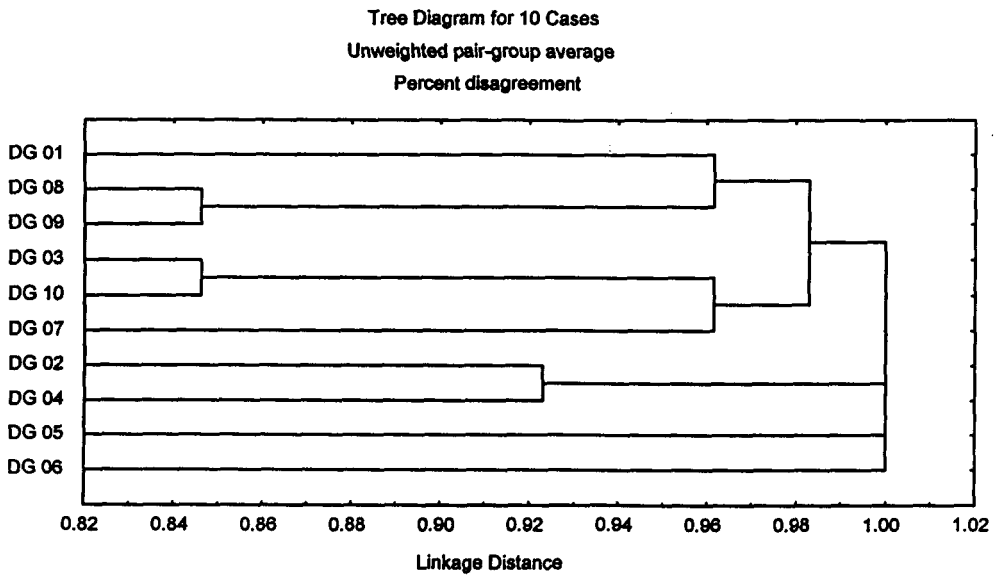
4.4.6. *Desmodium gangeticum*

The ten accessions of *Desmodium gangeticum* could be grouped into four clusters at a linkage distance of 1 (Table 4.46 and Fig. 4.16). Accession numbers DG 01, DG 03, DG 07, DG 08, DG 09 and DG 10 formed the first cluster, accession numbers DG 02 and DG 04 formed the second cluster, accession number DG 05 formed the third cluster accession number DG 06 formed the fourth cluster. Accession number DG 08 and DG 09 and DG 03 and DG 10 formed two closest groups with a linkage distance of 0.845.

Table 4.46. Percent disagreement between the accessions of *Desmodium gangeticum* studied.

	DG 01	DG 02	DG 03	DG 04	DG 05	DG 06	DG 07	DG 08	DG 09	DG 10
DG 01	.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.92	1.00
DG 02	1.00	.00	1.00	.92	1.00	1.00	1.00	1.00	1.00	1.00
DG 03	1.00	1.00	.00	1.00	1.00	1.00	1.00	.92	.92	.85
DG 04	1.00	.92	1.00	.00	1.00	1.00	1.00	1.00	1.00	1.00
DG 05	1.00	1.00	1.00	1.00	.00	1.00	1.00	1.00	1.00	1.00
DG 06	1.00	1.00	1.00	1.00	1.00	.00	1.00	1.00	1.00	1.00
DG 07	1.00	1.00	1.00	1.00	1.00	1.00	.00	1.00	1.00	.92
DG 08	1.00	1.00	.92	1.00	1.00	1.00	1.00	.00	.85	1.00
DG 09	.92	1.00	.92	1.00	1.00	1.00	1.00	.85	.00	1.00
DG 10	1.00	1.00	.85	1.00	1.00	1.00	.92	1.00	1.00	.00

Fig. 4.16. UPGMA dendrogram for *Desmodium gangeticum.*



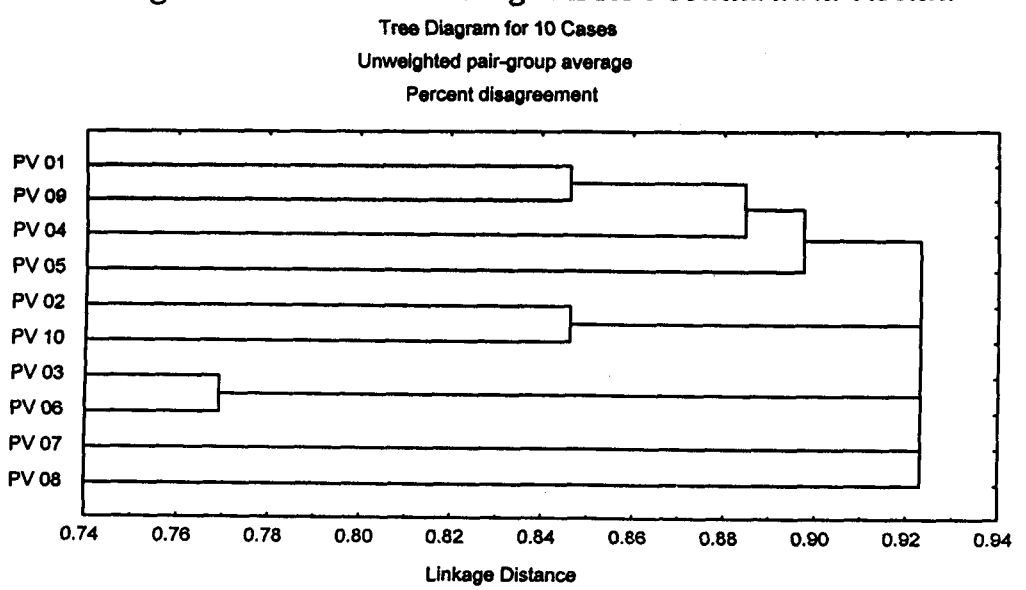
4.4.7. *Pseudarthria viscida*

The ten accessions of *Pseudarthria viscida* studied presently formed five clusters at a linkage distance of 0.925 (Table 4.47 and Fig. 4.17). Accession numbers PV 01, PV 04, PV 05 and PV 09 formed the first cluster, PV 02 and PV 10 formed the second cluster, PV 03 and PV 06 formed the third cluster, PV 07 formed the fourth cluster and PV 08 formed the fifth cluster. The closest linked accessions were PV 03 and PV 06 with a linkage distance of 0.77.

Table 4.47. Percent disagreement between the accessions of *Pseudarthria viscida* studied.

	PV 01	PV 02	PV 03	PV 04	PV 05	PV 06	PV 07	PV 08	PV 09	PV 10
PV 01	.00	.92	.92	.92	.92	.92	.92	.92	.85	.92
PV 02	.92	.00	.92	.92	.92	.92	.92	.92	.92	.85
PV 03	.92	.92	.00	.92	.92	.77	.92	.92	.92	.92
PV 04	.92	.92	.92	.00	.92	.92	.92	.92	.85	.92
PV 05	.92	.92	.92	.92	.00	.92	.92	.92	.85	.92
PV 06	.92	.92	.77	.92	.92	.00	.92	.92	.92	.92
PV 07	.92	.92	.92	.92	.92	.92	.00	.92	.92	.92
PV 08	.92	.92	.92	.92	.92	.92	.92	.00	.92	.92
PV 09	.85	.92	.92	.85	.85	.92	.92	.92	.00	.92
PV 10	.92	.85	.92	.92	.92	.92	.92	.92	.92	.00

Fig. 4.17. UPGMA dendrogram for *Pseudarthria viscida*



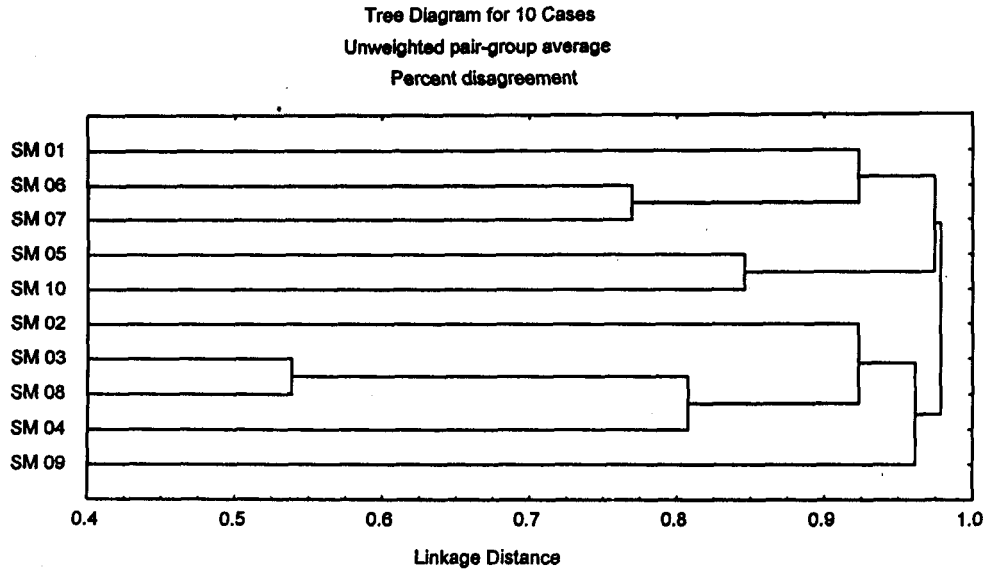
4.4.8. *Solanum melongena* var. *insanum*

The ten accessions of *Solanum melongena* var. *insanum* could be grouped into two clusters at a linkage distance of 0.75 (Table 4.48 and Fig. 4.18). The first cluster consisted of five genotypes SM 01, SM 05, SM 06, SM 07 and SM 10 and the second cluster consisted of five genotypes SM 02, SM 03, SM 04, SM 08 and SM 09. Accession numbers SM 03 and SM 08 showed the maximum proximity. Their linkage distance was 0.55 only.

Table 4.48. Percent disagreement between the accessions of *Solanum melongena* var. *insanum* studied.

	SM 01	SM 02	SM 03	SM 04	SM 05	SM 06	SM 07	SM 08	SM 09	SM 10
SM 01	.00	1.00	1.00	1.00	1.00	.92	.92	1.00	1.00	.92
SM 02	1.00	.00	.92	.92	1.00	1.00	.92	.92	1.00	1.00
SM 03	1.00	.92	.00	.77	.92	.92	1.00	.54	.92	1.00
SM 04	1.00	.92	.77	.00	1.00	1.00	1.00	.85	.92	.92
SM 05	1.00	1.00	.92	1.00	.00	.92	1.00	.92	1.00	.85
SM 06	.92	1.00	.92	1.00	.92	.00	.77	.92	.92	1.00
SM 07	.92	.92	1.00	1.00	1.00	.77	.00	1.00	1.00	1.00
SM 08	1.00	.92	.54	.85	.92	.92	1.00	.00	1.00	1.00
SM 09	1.00	1.00	.92	.92	1.00	.92	1.00	1.00	.00	1.00
SM 10	.92	1.00	1.00	.92	.85	1.00	1.00	1.00	1.00	.00

Fig. 4.18. UPGMA dendrogram for *Solanum melongena* var. *insanum.*



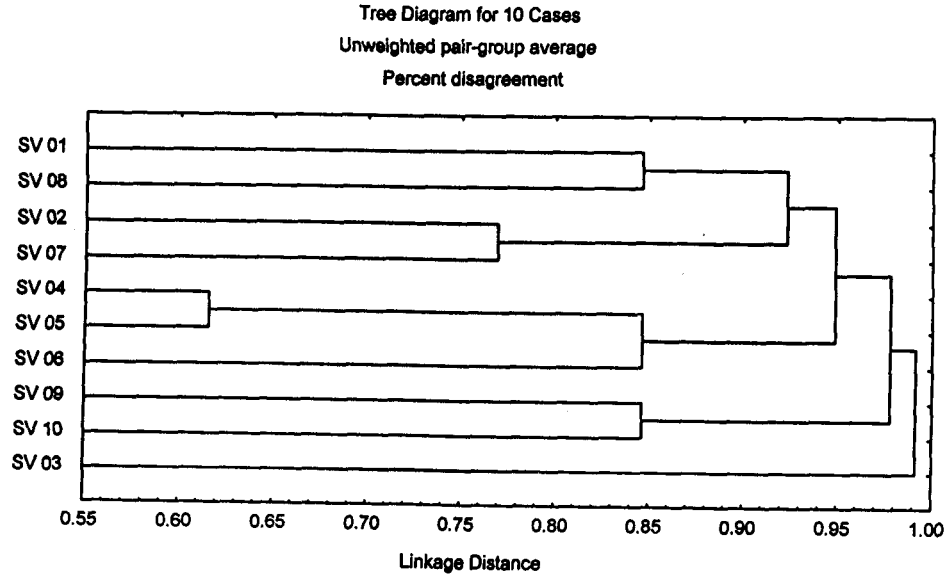
4.4.9. *Solanum violaceum*

Solanum violaceum could be grouped into two clusters, accession number SV 03 forming a separate cluster and accession numbers SV 01, SV 02, SV 04, SV 05, SV 06, SV 07, SV 08, SV 09 and SV 10 forming the second cluster at a linkage distance of 1 (Table 4.49 and Fig. 4.19). Accession numbers SV 04 and SV 05 were found to be the maximum proximal at a linkage distance of 0.62.

Table 4.49. Percent disagreement between the accessions of *Solanum violaceum* studied.

	SV 01	SV 02	SV 03	SV 04	SV 05	SV 06	SV 07	SV 08	SV 09	SV 10
SV 01	.00	1.00	1.00	1.00	1.00	1.00	.92	.85	1.00	1.00
SV 02	1.00	.00	1.00	.92	.92	1.00	.77	.85	1.00	1.00
SV 03	1.00	1.00	.00	1.00	1.00	.92	1.00	1.00	1.00	1.00
SV 04	1.00	.92	1.00	.00	.62	.92	.85	.92	1.00	1.00
SV 05	1.00	.92	1.00	.62	.00	.77	.85	.92	1.00	.92
SV 06	1.00	1.00	.92	.92	.77	.00	1.00	1.00	1.00	.92
SV 07	.92	.77	1.00	.85	.85	1.00	.00	.92	.92	.92
SV 08	.85	.85	1.00	.92	.92	1.00	.92	.00	1.00	1.00
SV 09	1.00	1.00	1.00	1.00	1.00	1.00	.92	1.00	.00	.85
SV 10	1.00	1.00	1.00	1.00	.92	.92	.92	1.00	.85	.00

Fig. 4.19. UPGMA dendrogram for *Solanum violaceum.*



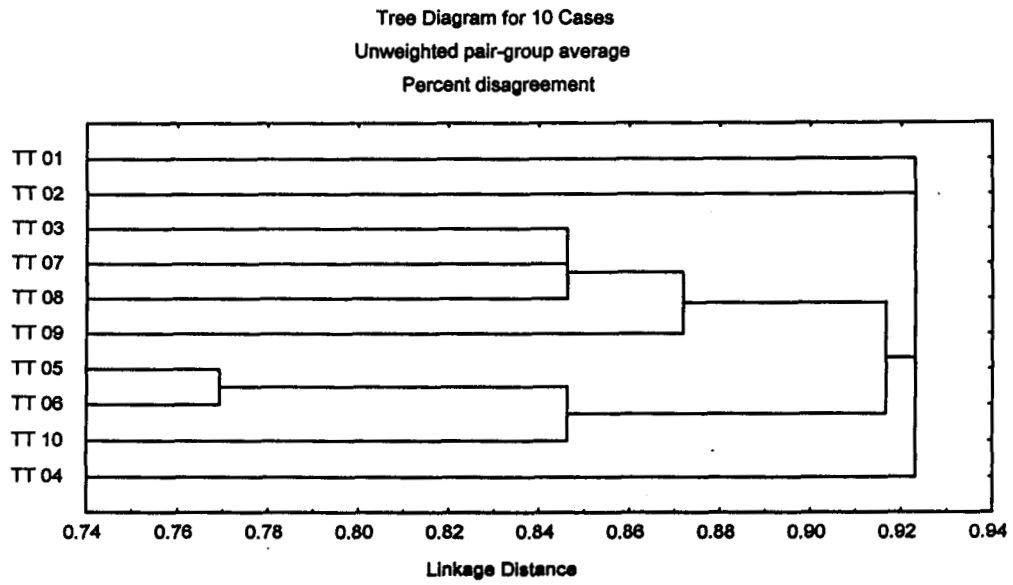
4.4.10. *Tribulus terrestris*

The ten accessions of *Tribulus terrestris* studied could be grouped into four clusters at a linkage distance of 0.925 (Table 4.50 and Fig. 4.20), accession number TR 01 forming the first cluster, TR 02 forming the second cluster, TR 03, TR 05, TR 06, TR 07, TR 08, TR 09 and TR 10 forming the third cluster and TR 04 forming the fourth cluster. TR 05 and TR 06 showed maximum proximity with a linkage distance of 0.77.

Table 4.50. Percent disagreement between the accessions of *Tribulus terrestris* studied.

	TT 01	TT 02	TT 03	TT 04	TT 05	TT 06	TT 07	TT 08	TT 09	TT 10
TT 01	.00	.92	.92	.92	.92	.92	.92	.92	.92	.92
TT 02	.92	.00	.92	.92	.92	.92	.92	.92	.92	.92
TT 03	.92	.92	.00	.92	.92	.92	.85	.85	.92	.92
TT 04	.92	.92	.92	.00	.92	.92	.92	.92	.92	.92
TT 05	.92	.92	.92	.92	.00	.77	.92	.92	.92	.85
TT 06	.92	.92	.92	.92	.77	.00	.92	.92	.92	.85
TT 07	.92	.92	.85	.92	.92	.92	.00	.85	.85	.85
TT 08	.92	.92	.85	.92	.92	.92	.85	.00	.85	.92
TT 09	.92	.92	.92	.92	.92	.92	.85	.85	.00	.92
TT 10	.92	.92	.92	.92	.85	.85	.85	.92	.92	.00

Fig. 4.20. UPGMA dendrogram for *Tribulus terrestris.*



The genetic distance between genotypes is revealed by their clustering patterns and this technique provides an efficient tool to analyze the phylogenetic distance between the accessions under study. Genotypes which are closer can be considered genetically uniform and can be used for propagation and commercial exploitation. On the other hand genotypes that are distant can be used for breeding programmes. Misra *et al.* (1990), Sodani *et al.* (1990), Indira (1994), Srivastava *et al.* (2000), Radhakrishnan (2003) and Ramasubramanian (2005) have undertaken similar studies in different crops.

4.5. Conservation and propagation

4.5.1. Conservation

The world conservation strategy was formulated in 1980 by IUCN, UNEP & WWF. It defines conservation as "the management of human use of the biodiversity so that it may yield the greatest sustainable benefit to the present generation while maintaining its potential to meet the needs and aspirations of future generations" (Anonymous, 2000). Conservation can be done *in situ* and *ex situ*. *In situ* conservation is dynamic as opposed to the semistatic nature of *ex situ* conservation. It is well established that the best and cost effective way of protecting the existing biological and genetic diversity is *in situ* or on the site conservation wherein a wild species or stock of a biological community is protected and preserved in its natural habitat. *Ex situ* conservation is the conservation outside the natural habitat by cultivating and maintaining plants in field gene banks, botanical gardens, parks and other suitable sites and through long term preservation of plant propagules in gene banks (seed bank, pollen bank, DNA library, etc.) and in plant tissue culture repositories and by cryopreservation (Anonymous, 2000).

Presently, an effort has been made to collect the ten ayurvedic plants coming under *dasamula* group from different sources and to conserve them *ex situ* under field gene bank conditions in the Experimental gardens of Arya Vaidya Sala, Kottakkal, Kerala, India (Table 3.1). Since roots form the useful part in medicine, destructive harvesting is practiced in drug collection which leads to

drastic reduction of population density of the plants in their natural habitats and this increases the importance of *ex situ* conservation.

Establishing field gene banks of plants provides ample options for long-term preservation of the genetic variability of the species. In the process, as in the case of other *ex situ* methods the genotypes are brought from an environment in which they are adapted to one in which they may not be. According to Frankel and Bennett (1970), there will be natural selection and increased opportunities for natural hybridization with alien material. The factors that will affect the population structure are climate, soil, biotic components, length of the life cycle, breeding system, competition and degree of care (Yunus, 2001).

The materials for the present study were collected from different localities of Kerala, Tamil Nadu and Karnataka states of India for the purpose during 2001 (Table 3.1). The objective of field collection is to obtain maximum diversity from minimum sample size and number. Both random and non random sampling may be used while collecting the samples. In this study non-random sampling was used for collecting these species. In this method we select only those with clear morphological characters except those associated with disease resistance and other physiological characters. The materials were planted in the National Medicinal Plant Gene Bank of Arya Vaidya Sala, Kottakkal, Kerala and maintained under standard organic maintenance (Table 4.51 and Figs. 4.21 to 4.30).

Table 4.51. Morphological characters of *dasamula* group of plants conserved *ex situ.*

Sl.No	Scientific name	Sanskrit name	Habit-at	Habit	Nature of leaf shape	Nature of leaf tip	Nature of leaf base	Pyllotaxy	Position of flower	Nature of infloresce-nce	Common method of propagat-ion
1	*Aegle marmelos*	*Vilva*	DDS	Tree	Elliptic-al	Retuse	Oblique	Alternate	Axillary	Panicles	Seed
2	*Gmelina arborea*	*Kasmari*	MMD	Tree	Deltoid	Acumina te	Cordate	Opposite	Terminal	Panicles	Seed
3	*Oroxylum indicum*	*Syonakha*	MMD	Tree	Ovate	Acumina te	Oblique	Opposite	Terminal	Racemes	Seed
4	*Stereospermum colais*	*Patala*	MMD-SEG	Tree	Elliptic-al	Arristate	Obtuse	Opposite	Terminal	Cymes	Seed
5	*Premna corymbosa*	*Agnimanth a*	DDS-Plains	Small sized Tree	Ovate	Acumina te	Cuneate	Opposite	Terminal	Cymes	Stem cuttings
6	*Desmodium gangeticum*	*Prsniparni*	MMD-SEG	Under shrub	Elliptic al	Acute	Cuneate	Alternate	Terminal	Racemes	Seed
7	*Pseudarthria viscida*	*Saliparni*	MMD-SEG	Under shrub	Rhombi c	Acute	Cuneate	Alternate	Terminal	Racemes	Seed
8	*Solanum melongena* var. *insanum*	*Brhati*	SEG-DD	Shrub	Ovate	Acute	Oblique	Alternate	Extra axillary	Racemes	Seed
9	*Solanum violaceum*	*Brhati*	SEG-DD	Shrub	Ovate	Acute	Oblique	Alternate	Extra axillary	Racemes	Seed
10	*Tribulus terrestris*	*Gokshurah*	DDS & WLP	Herb	Elliptic al	Acute	Oblique	Opposite	Extra axillary	Solitary	Seed

DDS-Dry Deciduous Scrub; MMD-Mixed Moist Deciduous; SEG- Semi Ever Green; DD-Dry Deciduous; WLP-Waste Land Plains

Fig. 4.21. *Ex-situ* conservation of *Aegle marmelos*

Fig. 4.22. *Ex-situ* conservation of *Gmelina arborea*

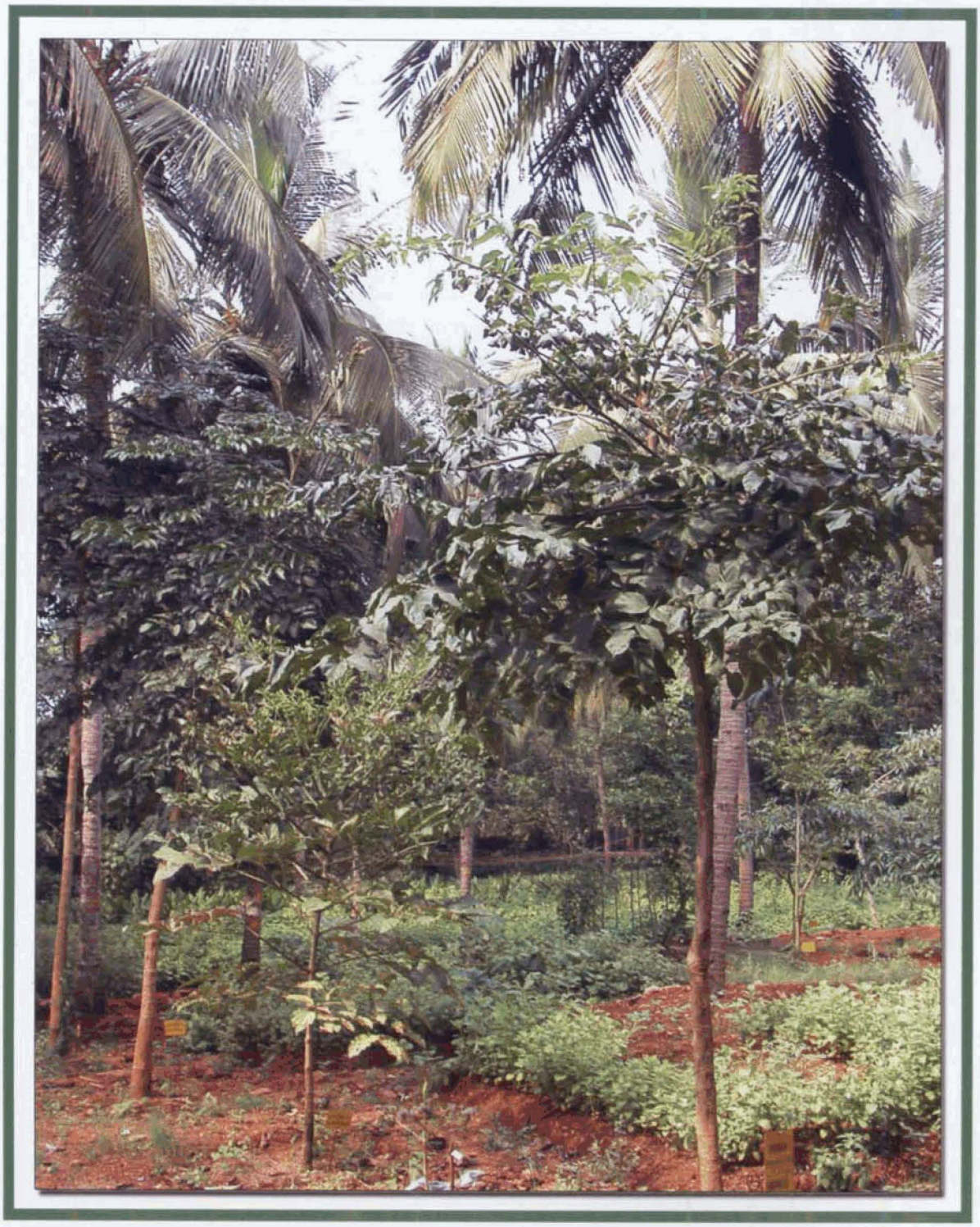


Fig. 4.23. *Ex-situ* conservation of *Oroxylum indicum*

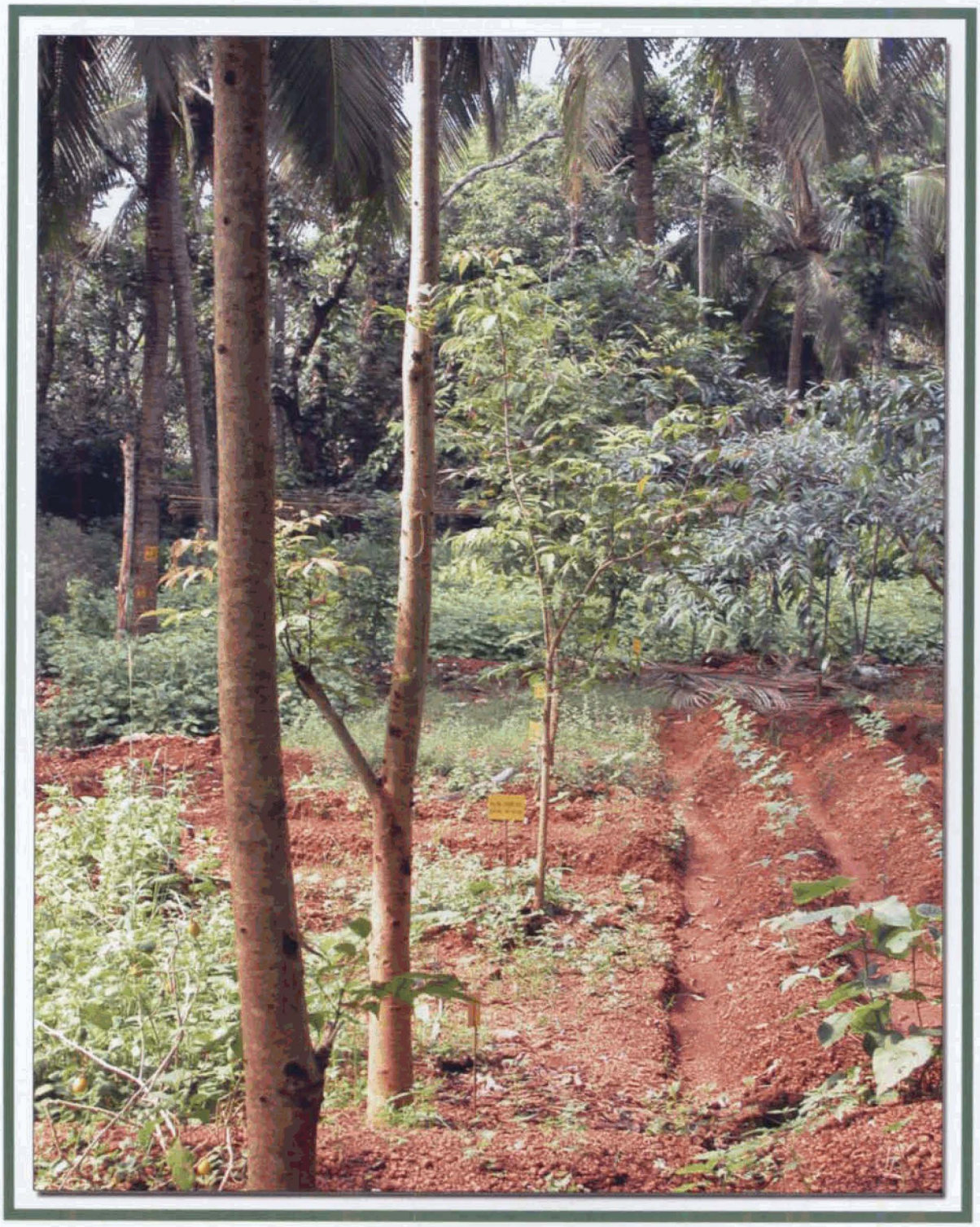


Fig. 4.24. *Ex-situ* conservation of *Stereospermum colais*

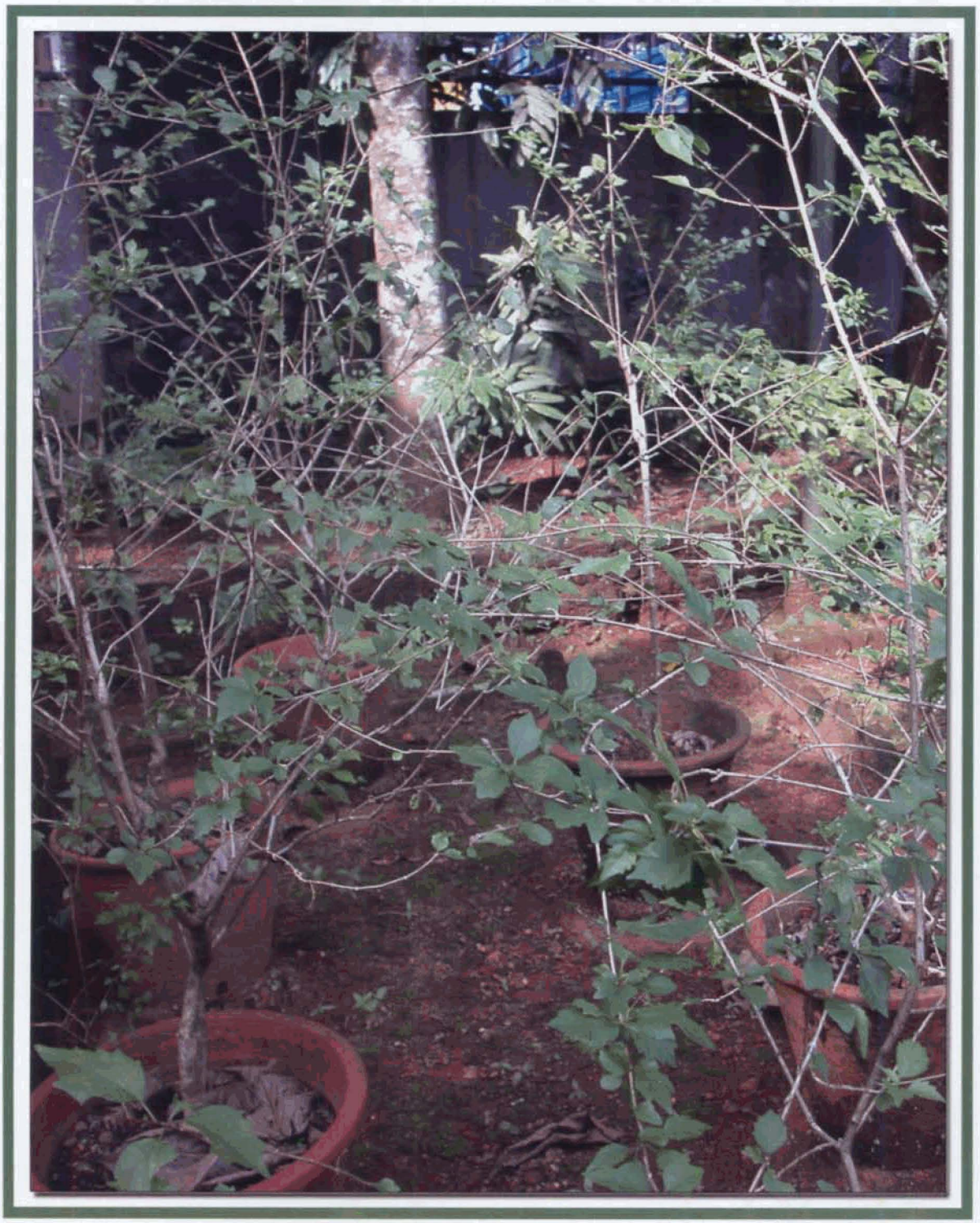


Fig. 4.25. *Ex-situ* conservation of *Premna corymbosa*

Fig. 4.26. *Ex-situ* conservation of *Desmodium gangeticum*

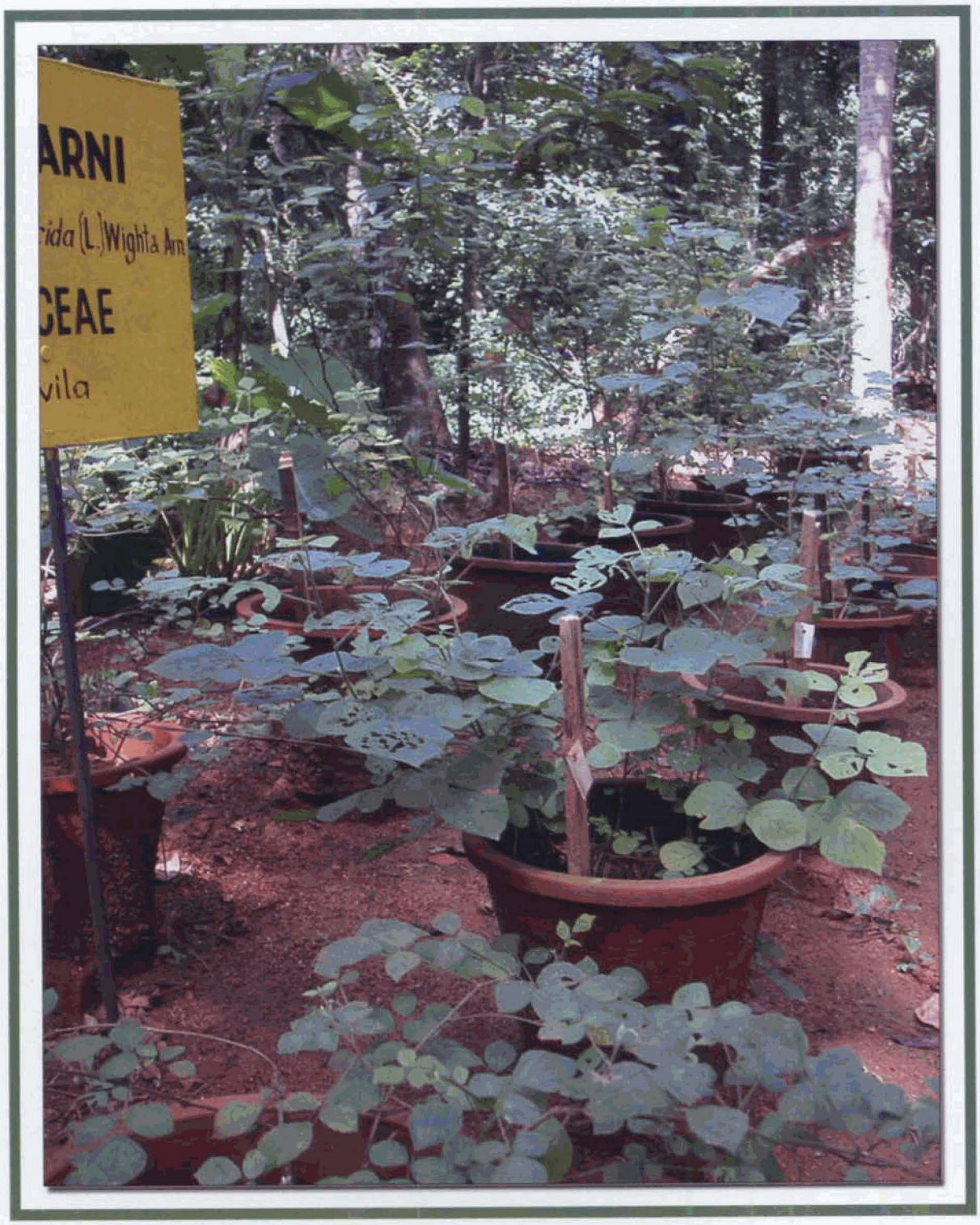


Fig. 4.27. *Ex-situ* conservation of *Pseudarthria viscida*

Fig. 4.28. *Ex-situ* conservation of *Solanum melongena* var. *insanum*

Fig. 4.29. *Ex-situ* conservation of *Solanum violaceum*

Fig. 4.30. *Ex-situ* conservation of *Tribulus terrestris*

Development of new approaches to conservation not only focused on storage, but also on rapid multiplication. Propagation has been demonstrated in all aspects of conservation and use, from germplasm exchange to multiplication, distribution and utilization. Especially *in vitro* propagation is instructive to explore the broader context of conservation and use of plant genetic resources for advanced breeding techniques.

4.5.2. Propagation

For conservation and cultivation, the first step relates to sourcing and handling of propagules or propagates. Seed propagation, vegetative propagation and *in vitro* propagation were achieved in different plants of *dasamula* group as a part of this study.

4.5.2.1. Seed propagation

Seed propagation was attempted in nine plants of *dasamula* group. The results obtained from the experiments are depicted in Table 4.52 and Figs 4.31 to 4.34. In all the above species, germination of seeds under nursery conditions showed better germination percentage when compared to petridish method. Days required for germination was also very less in nursery method. High germination percentage and early germination was observed in *Gmelina arborea* and *Stereospermum colais* with the treatment of water soaking (T_1). Romero (2004) reported that pregermination treatments with water soaking can increase the speed and germination percentage in *Gmelina* species.

Among the nine species *Stereospermum colais* showed poor germination percentage (25-30%). Ghate and Sathe (1998) also has reported poor germination frequency in this species. In our trials immediate sowing after extracting of seeds has been found to be more favorable for good germination percentage in all the species. In *Stereospermum* species mature fruits collected directly from the trees before dehiscing were suitable for achieving good germination. Germination on direct sowing on a seed bed is efficient, technically less demanding and cost effective in all these species. This is also convenient for the step by step transplantation to potted soil and the subsequent hardening of seedlings. Large scale production of these species using seed is an easy and reliable method and it will help to increase the resource availability for pharmaceutical use and at the same time help in conservation of these species.

Table 4.52. Seed germination studies on *dasamula* group of medicinal plants

Plant species	Nursery model				Petridish model	
	T_0		T_1			
	G	D	G	D	G	D
A.marmelos	80-85	20-25	80-85	18-22	20-25	25-30
O. indicum	85-90	15-20	85-90	15-20	35-40	20-25
G. arborea	70-75	20-25	75-80	20-25	15-20	25-30
S. colais	20-25	15-20	25-30	15-20	10-15	20-25
D. gangeticum	65-70	12-18	65-70	8-12	40-45	16-24
P. viscida	60-65	10-14	60-65	10-14	35-40	16-20
S. melongena var. *insanum*	80-85	12-18	80-85	10-14	35-40	14-18
S. violaceum	85-90	12-16	85-90	10-14	35-40	14-18
T. terrestris	60-65	16-20	60-65	16-20	12-14	24-28

G: Germination percentage; D: Days required for germination; T_0: Direct sowing; T_1: Pre-sowing treatment of cold water soak for 16 hours.

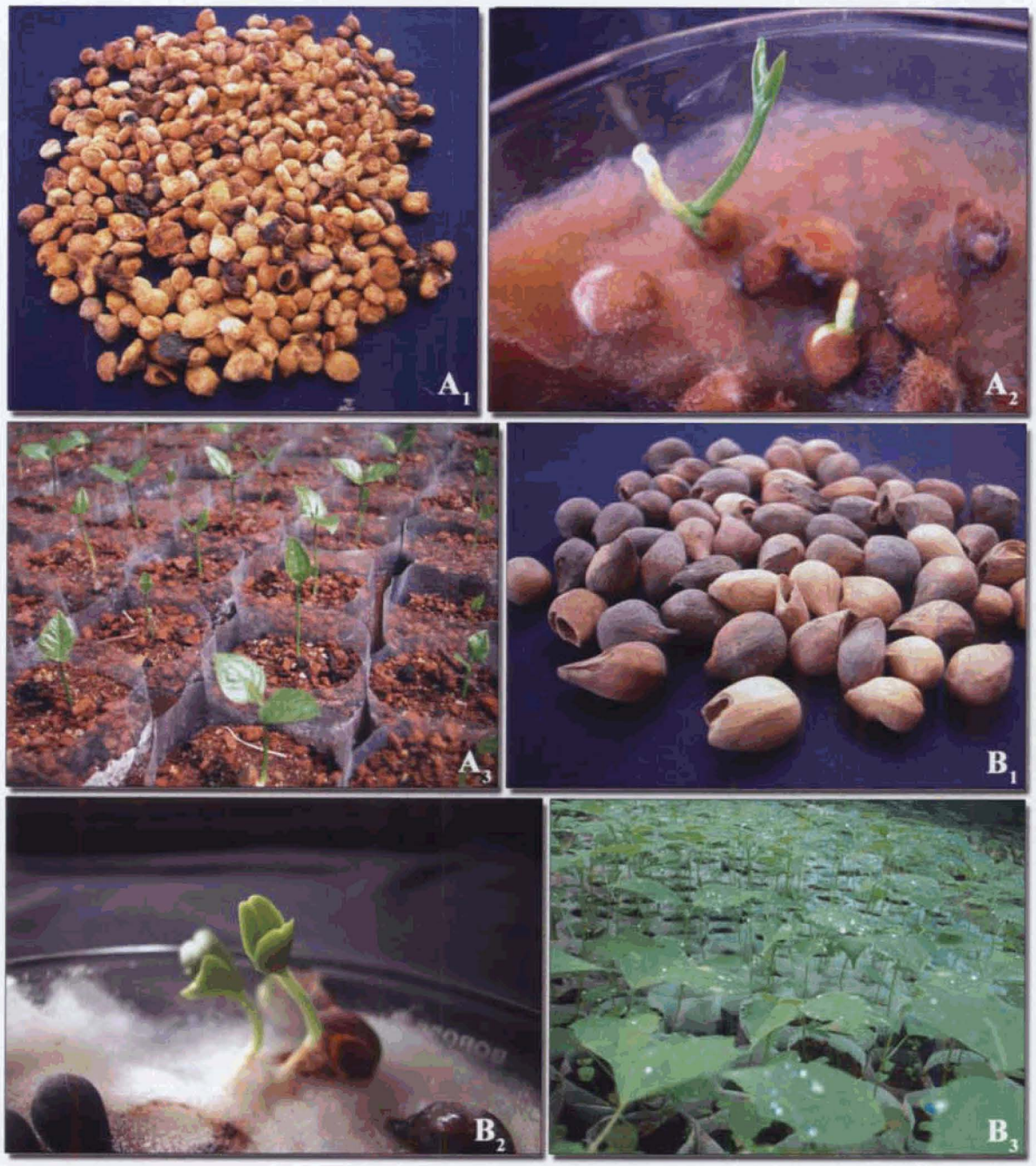


Fig. 4.31. Seed propagation studies of *Aegle marmelos* and *Gmelina arborea*

A- *Aegle marmelos* A_1- Seeds; A_2- Petriplate method; A_3-Nursery method

B- *Gmelina arborea* B_1- Seeds; B_2- Petriplate method; B_3-Nursery method

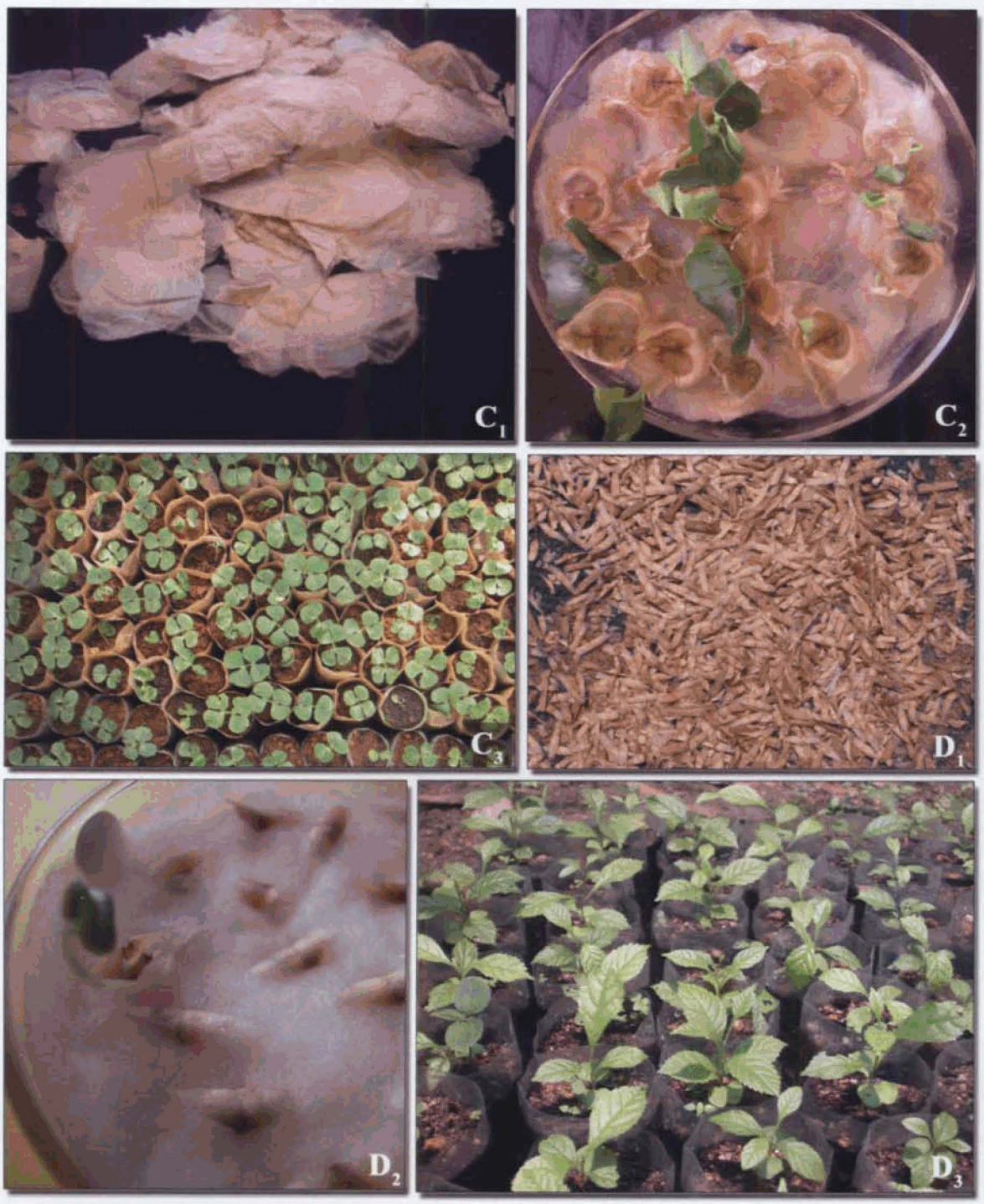


Fig. 4. 32. Seed propagation studies of *Oroxylum indicum* and *Stereospermum colais*

C- *Oroxylum indicum* C_1- Seeds; C_2- Petriplate method; C_3- Nursery method

D- *Stereospermum colais* D_1- Seeds; D_2- Petriplate method; D_3- Nursery method

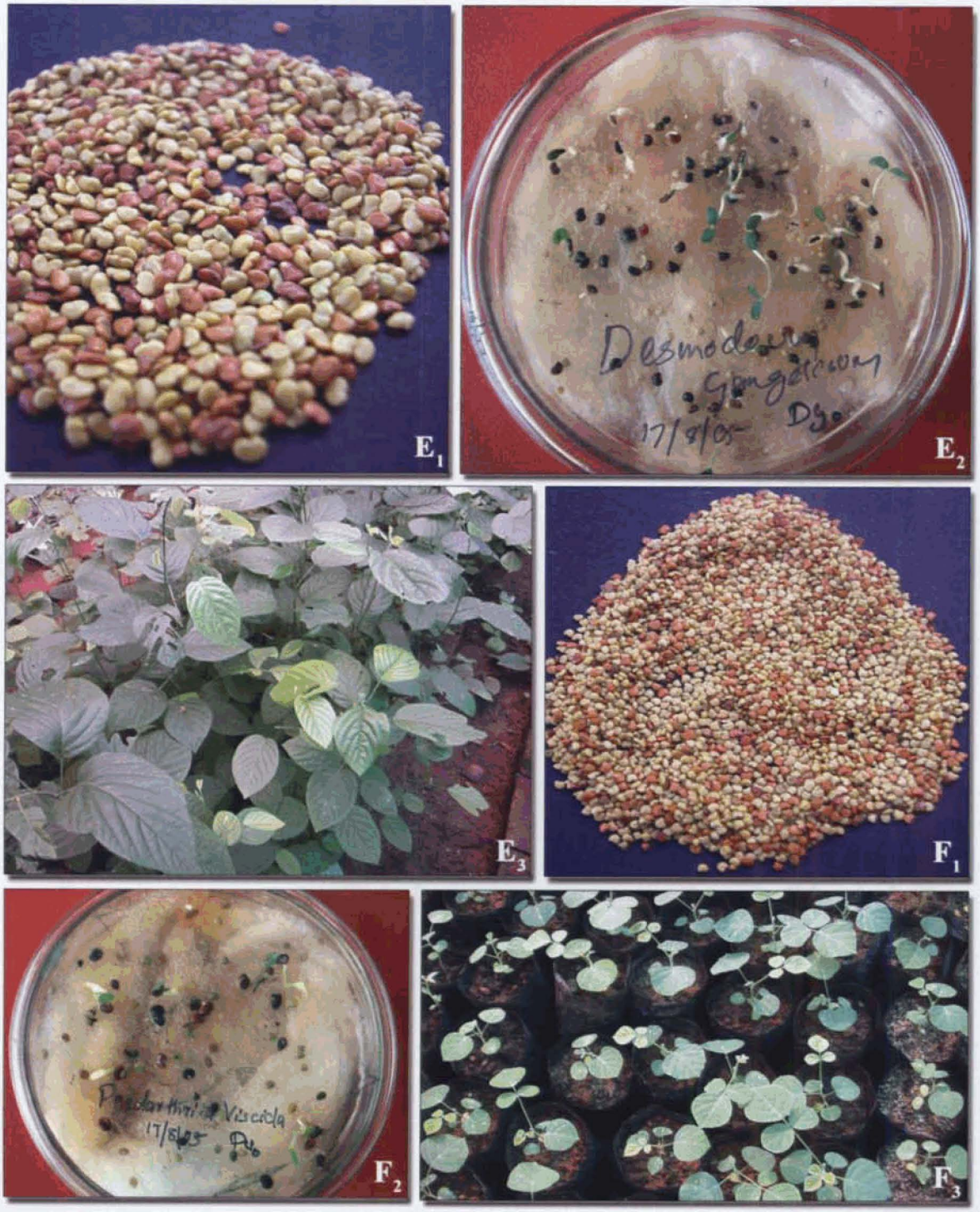


Fig. 4.33. Seed propagation studies of *Desmodium gangeticum* and *Pseudarthria viscida*

E- *Desmodium gangeticum* E_1- Seeds; E_2- Petriplate method; E_3- Nursery method

F- *Pseudarthria viscida* F_1- Seeds; F_2- Petriplate method; F_3- Nursery method

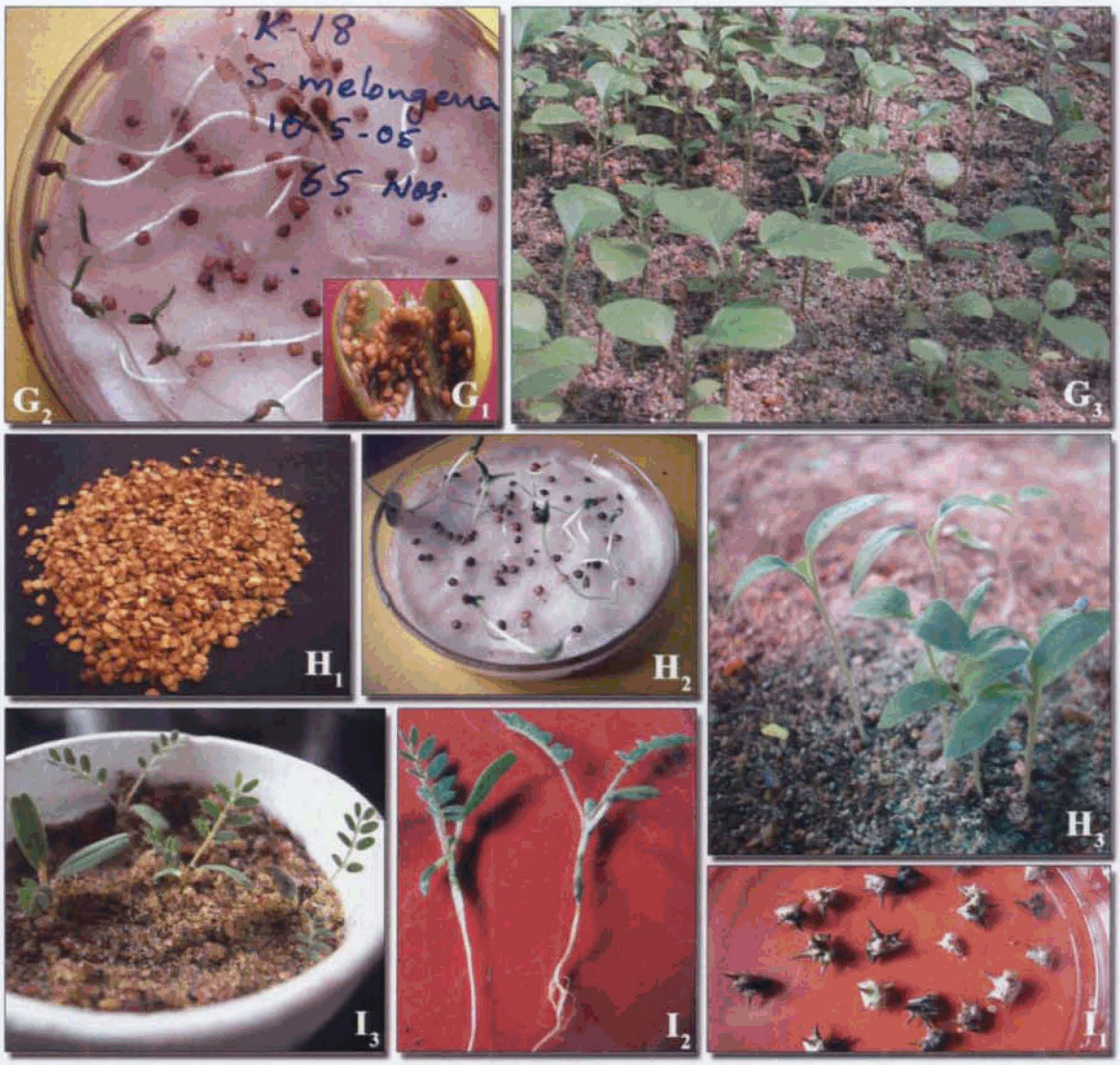


Fig. 4. 34. Seed propagation studies of *Solanum melongena* var. *insanum*, *Solanum violaceum* and *Tribulus terrestris*

G- *Solanum melongena* var. *insanum* G_1- Seeds; G_2- Petriplate method; G_3-Nursery method

H- *Solanum violaceum* H_1- Seeds; H_2- Petriplate method; H_3-Nursery method

I- *Tribulus terrestris* I_1- Seeds; I_2- Seedlings from petriplate method; I_3-Nursery method

4.5.2.2. Vegetative propagation

The goal of vegetative propagation is to reproduce progeny plants identical in genotype to a single source plant. Vegetative propagation through stem cuttings and air layering was achieved in *Premna corymbosa* since seeds are only rarely available in this species.

4.5.2.2.1. Stem cutting

Propagation by stem cuttings is an easy and cost effective method of propagation. Many new plants can be obtained in this way in a limited space from a few stock plants. It is inexpensive, rapid, and simple and does not require the special techniques necessary in grafting, budding or micropropagation. In addition to this, the parent plant is usually reproduced exactly, with no genetic change.

Stem cuttings were kept for rooting in sand soil mixture in nursery polybags and sprouting was recorded on the 12th day of the experiment. 80-90% of the stem cuttings were rooted (Fig. 4.35 A_1 & A_2). Rooting could be achieved with out using any growth regulator. Earlier, Mertia and Nagarajan (2000) and Tiwari *et al.* (1998) have standardized the macropropagation protocol using stem cuttings of some important medicinal plant species.

4.5.2.2.2. Air layering

Layering is a form of rooting of cuttings in which adventitious roots are initiated on a stem while it is still attached to the plant. The rooted stem (layer) is then

detached, transplanted, and it develops in to a separate plant on its own roots. Layering is useful to produce a relatively small number of large sized plants of a special cultivar in an outdoor environment with a minimum of propagation facilities.

Quick and higher rooting was observed in all the layers just after 15 days of layering. On an average, 20 roots per layer with an average length of 10 cm were observed on the 30th day of layering. Absence of callus was also noted on the layers (Fig. 4.35 B_1 & B_2). All the layered plants established in the nursery very well. It is therefore concluded that vegetative propagation by air layering of this tree species can be successfully undertaken without any application of auxins. Air layering of other medicinal plants has been attempted by earlier workers, but mostly using auxins to enhance root production (Anjan kumar *et al.*, 2004). According to Chauhan and Dua (1982), air layered plants have an advantage over others since the food reseves of the parent branch induce formation of a balanced and well developed rooting system. In the present study also air layering resulted in excellent rooting. Furthermore, the proposed method is convenient, practical and suitable for large scale propagation of this species.

Fig. 4.35. Vegetative propagation in *Premna corymbosa*

A_1 & A_2 - By stem cuttings

B_1 & B_2 - By air layering

4.5.2.3. *In vitro* propagation

In vitro propagation not only facilitates the agricultural production of the crop, but also underpins the use of all other biotechnologies in conservation and use. The advantages of being able to multiply a given genotype with relative ease, with a low risk of introducing or reintroducing pathogens, and with a low risk of genetic instability, need not be emphasized in this technique.

In vitro propagation was achieved presently in *Aegle marmelos* (Fig. 4.36), *Gmelina arborea* (Fig. 4.37), *Oroxylum indicum* (Fig. 4.38), *Solanum violaceum* (Fig. 4.39), *and Tribulus terrestris* (Fig. 4.40).

4.5.2.3.1. *Aegle marmelos*

Culture initiation was achieved within two weeks in the medium containing MS salts supplemented with 0.2 mg/l BA in *Aegle marmelos* (Fig. 4.36 A). The responded axillary buds were sub cultured into MS medium supplemented with the different concentrations of BA (Table 4.53). The best results were obtained (16 shoots/explant) in 0.5 mg/l BA (Fig. 4.36 B). The medium containing 0.7 mg/l BA was also found to be good for multiplication and large scale propagation. Hazarika *et al.* (1996) have also reported similar results of superiority of lower concentration of BA in the same plant. Our result is in contrast with the reports by Arumugam and Rao (1996), where the higher concenteration of cytokinin was needed for multiplication in this plant. Higher concentrations of BA produced callus from the base, resulting in the stunted growth of shoots with more or less same rate of

multiplication. Similar results were also reported in *Tridax procumbens* (Sahoo and Chand, 1998). Direct regeneration from the *in vitro* derived roots was also achieved in the same medium used for the multiplication of the nodal explant. The multiplication rate of 7.6 shoots per root explant was obtained in the media containing 0.7 mg/l BA (Fig. 4.36 C). Adventitious shoots produced from the enlarged apical regions of the root tip of intact seedlings was reported early by Islam *et al.* in 1996. Direct caulogenesis from the root explant and its multiplication in lower concentrations of BA might be due to the higher levels of endogenous growth regulators in this species.

Repeated subculturing after 6-8 cycles in the same medium resulted in underdeveloped shoots and hence the cultures transferred to ammonia free half strength MS medium could solve this problem. The multishoot clumps as such were subcultured at every 30 days intervals and large scale propagation was achieved in this crop (Fig. 4.36 D).

The plantlets of about 2-3 cm were harvested for rooting experiments. *In vitro* rooting trials attempted with different concentrations of auxins did not produce consistent rooting. This is in contrast to the earlier publications in the same plant by Ajithkumar and Seeni (1998), Arumugam and Rao (1996), Hazarika *et al.* (1996), Islam *et al.* (1994) and Varghese *et al.* (1993). Only single root formation was observed in less than 30% cultures, but number of roots did not affect the subsequent establishment of rooted plants in the field. This is in

agreement with reports by Ajithkumar and Seeni, (1998) in the same plant. One or two roots were produced (in less than 30% cultures) in half strength MS medium without any growth regulators after 10 weeks of culture (Fig. 4.36 E). This discrepancy in rooting response might be due to differential accumulation of growth regulators during different cycles of subculture. Hence an alternative method of rooting *i.e.*, pulse treatment, consisting of initial treatment in Napthoxy Acetic Acid (10 mg/l) and IBA (2 mg/l) for about 2 hours followed by Chlorogenic acid (2 mg/l) treatment for 6 minutes was tried. This treatment yielded uniform rooting (80-90%) on subsequent transfer to suitable substrate (Fig. 4.36 F). Arya *et al.* (2002) reported the rooting of plants after pulse treatment using the same auxins in *Celastrus paniculatus*. The shoots that fail to form root *in vitro* responds to this method. The type of planting substrate also played a significant role in the induction of roots from the pulse treated shoots. Thermocol cups filled with sand showed maximum rooting response within 30 days. Coir pith either alone or with sand (1:1) showed comparatively lesser rate of rooting. The cups were covered with polythene bags and kept under green house conditions for 6 weeks and then transferred to field conditions with 100% establishment (Fig. 4.36 G, H & I). The method standardised can be used for large scale planting material production and conservation of this important medicinal plant.

Table 4.53. *In vitro* response of nodal explants of *Aegle marmelos* in MS medium supplemented with cytokinin.

Growth regulators BA mg/l	Percentage response (%)	Mean* number of shoots/ explant (± SE)	Mean shoot length (cm)
0.2	36	8.0 ± 0.8	0.3 ± 0.3
0.4	68	13.4 ± 0.7	0.5 ± 0.6
0.5	79	16.3 ± 1.4	2.0 ± 0.6
0.7	75	15.8 ± 0.6	2.0 ± 0.7
0.9	69	9.8 ± 0.7	1.5 ± 0.4
1.0	38	5.2 ± 0.0	0.5 ± 0.7

*Values are Mean± SE of three independent experiments each with 12 replicates.

Observations were made after 4 weeks of the incubation.

Fig. 4. 36. A-I. *In vitro* clonal propagation of *Aegle marmelos*

A- Culture initiation on MS medium with 0.2 mg/l BA; B- Multiple shoot formation on MS with 0.5 mg/l BA; C- Multiple shoot formation on root explant; D- Large scale propagation; E- Roots produced on half strength MS medium ; F- *Ex vitro* (Pulse) rooted plants; G- Acclimatized plantlets; H & I- One year old tissue cultured plants in pot and field

4.5.2.3.2. *Gmelina arborea*

Eighty to ninety percent of the primary explants were contamination free after the disinfection procedures described in this study. Culture initiation was achieved by using nodal explants of one year old seedlings on MS medium without any growth hormones (Fig. 4.37 A). After 28 days of growth, the initiated single shoots were excised for multiplication experiments. Nodal segments excised from the primary cultures were cultured on MS medium with concentrations of BA ranging from 0.01 mg/l to 0.1 mg/l (Table 4.54). The explants responded within 18 days of incubation. They exhibited the highest percentage of response (80%) with 3.4 shoots/explant in the medium supplemented with 0.08 mg/l BA (Fig. 4.37 B & C). The regenerated shoots elongated in the same medium. BA at lower concentration was found to be ideal for multiple shoot induction in this species. This was agreeing with the results by Kannan and Jasarai (1996) and Cerdas *et al.* (2004) in the same plant. Similarly, best results for nodal segments of *Morus* were obtained with low concentration of BA without auxin (Sharma and Thorpe, 1990). BA is considered as the most effective synthetic cytokinin for stimulating axillary shoot proliferation in different plant systems (Bhojwani, 1980; Sahoo and Chand, 1998). During the multiplication stage, the new shoots when cut into segments contained one or two nodes and cultured in the same medium gave rise to new shoots. The rate of multiplication was not declined as the number of subcultures increased (every subculture was made at 4 week intervals). Similar observations have been reported

for *Picrorhiza kurroa* (Upadhyay *et al.*, 1989) and *Clitoria ternatea* (Rout, 2004).

In vitro shoots (4-5 cm long) with 2-4 nodes were transferred to root initiation medium containing half strength MS medium with various concentrations of IBA. Three or four roots with little callus were observed in the cut ends of the microshoots within 28 days in the medium containing 0.2 mg/l IBA (Fig. 4.37 D). Callusing was found to increase at higher levels of auxin used. The basal callusing nature of this plant did not affect the establishment of the plantlets during acclimatization (Fig. 4.37 E). After 10-15 days, the acclimatized plantlets were transplanted to the field.

Table 4.54. *In vitro* response of nodal explants of *Gmelina arborea* in MS medium supplemented with cytokinin.

Growth regulators BA mg/l	Percentage response (%)	Mean* number of shoots/ explant (± SE)	Mean shoot length (cm)
0.01	18	1.0 ± 0.9	0.7± 0.6
0.02	26	1.0 ± 1.7	0.6 ± 0.6
0.03	28	1.3 ± 1.4	0.6 ± 0.6
0.04	34	1.8 ± 1.2	2.2 ± 1.6
0.05	49	2.2 ± 0.6	1.5 ± 0.4
0.06	58	2.3 ± 0.7	1.8± 1.4
0.07	69	2.8 ± 0.8	2.0± 1.6
0.08	80	3.4 ± 0.4	3.8 ± 0.5
0.09	79	2.7 ± 0.9	3.2± 0.8
0.1	78	2.6 ± 0.7	3.2± 0.8

*Values are Mean± SE of three independent experiments each with 12 replicates.

Observations were made after 4 weeks of the incubation.

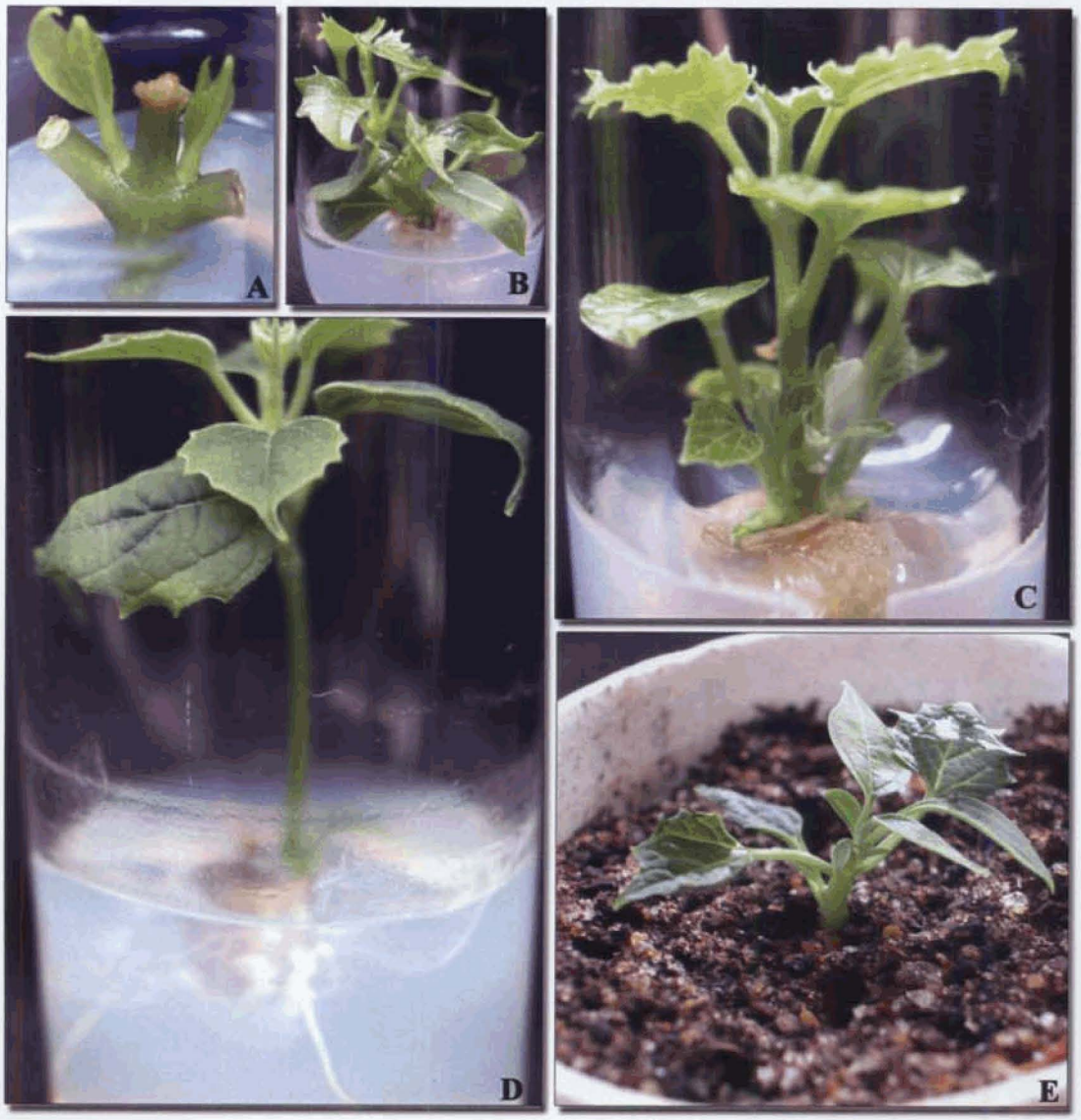


Fig. 4. 37. A-E. *In vitro* clonal propagation of *Gmelina arborea*

A- Culture initiation on MS basal medium ; B & C- Multiple shoot formation on MS with 0.08 mg/l BA; D- Roots produced on half strength MS medium with 0.2 mg/l IBA ; E- Acclimatized plantlet

4.5.2.3.3. *Oroxylum indicum*

There was no response when nodal explants were cultured on media without cytokinin. By contrast, shoot proliferation (Fig. 4.38A) and elongation were observed within 18 days of culture on Stapfer and Heuser (1985) modified WPM supplemented with varying concentrations and combinations of BA and kinetin (Table 4.55). Stapfer and Heuser modified WPM has been reported for shoot culture of several species (George, 1993). It was observed that the responce of the culture was not well in WPM and MS salts for shoot proliferation. That is why the modification of WPM was attempted in this species. In modified medium WPM salts (macro and micro nutrients) with MS vitamins were used. Amino acids were exempted in this medium. *In vitro* plant regeneration without amino acid was also obtained in apple (Caboni, 2000). Among the two cytokinins (BA and kinetin) tried alone or in combinations, BA alone was more effective for multiple shoot induction and shoot elongation. The optimal concentration of BA for establishment of maximum culture was 0.5 mg/l (Table 4.55 and Fig. 4.38 B&C). At higher concentrations of cytokinins, basal callusing was observed in the growing shoots. Cytokinin formulations were earlier shown to be critical for shoot elongation of many other plant species, including medicinal plants (Jha and Jha, 1989; Chen *et al.*, 1995; Saxena *et al.*, 1998; Rout *et al.*, 2000; Rout, 2004).

Gelrite was used as a gelling agent instead of agar in this plant. Gelrite is an attractive alternative to agar for

plant tissue culture because it produces a clear gel which assists the proper observation of cultures and their possible contamination. It has proved to be a suitable gelling agent for tissue cultures of many herbaceous and woody species and the rooting of plantlets. In most cases the results have been as good as those obtainable on agar solidified media: in many instances superior results have been obtained (George, 1993). In our results on this species also there was a considerable improvement in the shoot cultures where the gelrite was used instead of agar.

Rooting was attempted through *in vitro* and *ex vitro* means. Shoots excised with 3-4 cm length on half strength MS medium with 0.5 mg/l IBA and 0.5 IAA with out any gelling substances gave the best *in vitro* root induction (70%) within 18 days. The shoots were kept vertically by using paper bridges in this trial (Fig. 4.38E). Shoots with 4-5 cm gave rooting on half strength MS medium without any hormones (Fig. 4.38D). But the percentage of root induction (50%) in this trial was very low.

Ex vitro rooting is extensively used in commercial laboratories, because it eliminates a separate culture stage for rooting and reduces the overall cost on micropropagation. Debergh and Maene (1981) estimated that 35–75 percent of total cost of micropropagation is involved in the rooting of individual shoots *in vitro,* depending on the species. Rooting of micro cuttings *extra vitrum* eliminates one set of labour cost and overheads, as rooting and acclimatization are combined into a single

stage of micropropagation process and expensive and extensive growth room space is released for other operations. In this species, elongated shoots (3-4 cm) excised from *in vitro* cultures were rooted (Fig. 4.38F) when planted in moist sand with coir pith and kept under humid chamber in shade house for two weeks (80%). *Ex vitro* rooting is reported earlier in many species without any pre-treatment (McCown and Lloyd, 1983; Gupton, 1986; Clemente, 1991). In this species also *ex vitro* rooting was found to be more reliable. Rooted plants were successfully established with high survival rate in the field and were morphologically uniform (Fig. 4.38G&H).

Table 4.55. *In vitro* response of nodal explants of *Oroxylum indicum* in modified WPM supplemented with cytokinins.

Sl. No.	Media Composition MS +		Percentage of response (%)	Mean* number of shoots/ explant (± SE)	Mean shoot length (cm)
	KN Mg/l	BA Mg/l			
1	0.2	-	0	0 (0.0)	0
2	0.5	-	60	1.0 (±0.0)	0.5 ±1.2
3	1.0	-	60	1.8 (±0.3)	1.0 ±0.4
5	-	0.2	80	2 (±0.9)	1.5 ±0.5
6	-	0.5	90	8 (±0.2)	2 ±0.6
7	-	1.0	90	3 (±1.4)	0.8 ±0.4
8	0.2	0.2	70	2 (±0.7)	1.2 ±1.3
9	0.2	0.5	90	4 (±0.6)	1.8 ±0.8
10	0.5	0.2	90	2 (±0.4)	1.4 ±0.6
11	0.5	0.5	90	2 (±0.7)	0.5 ±0.3
12	Control		0	0	0

*Values are Mean± SE of three independent experiments each with 12 replicates.

Observations were made after 28 days of the incubation.

Fig. 4. 38. A-H. *In vitro* clonal propagation of *Oroxylum indicum*

A- Culture initiation on modified WPM with 0.2 mg/l BA; B & C- Multiple shoot formation on WPM with 0.5 mg/l BA; D- Roots produced on the half streangth WPM after 28 days of incubation; E- Roots produced on half strength MS medium with 0.5 mg/l IBA and 0.5 mg/l IAA with out any gelling substances after 18 days of incubation; F- *Ex vitro* rootied plants; G- Acclimatized plantlets; H- Six months old tissue cultured plants in the field

4.5.2.3.4. *Solanum violaceum*

Multiple shoots were induced from the leaf, internodal segments and petiole explants (Fig. 4.39). All the explants failed to respond morphogenetically to a growth regulator free medium. The initial multiplication rate varied from 5 to 18 shoots per explant depending on the type of explant and concentrations of the growth regulator (BA). The percentage of explants with regenerated shoots and the number of shoots per explants were determined after 28th days (Table 4.56).

Direct shoot formation was observed from the abaxial side of the leaf lamina without any callusing (Fig. 4.39A). Leaf explants exhibited differentiation of shoots in MS medium augmented with different concentrations of BA ranging from 1.5–3.0 mg/l after 28 days of incubation. Low concentrations of BA (0.5 and 1.0 mg/l) did not promote regeneration of shoot buds. The media containing other growth regulators such as IAA, NAA, Kinetin, etc. did not induce shoots even after culturing for a prolonged period. The non-response of similar explants on kinetin and auxin (like IAA or NAA) containing media were also reported in *Plumbago* (Das and Rout, 2002). The optimum rate of regeneration of shoot buds was obtained in the medium having 2.5 mg/l BA and 3% sucrose. The nature of the leaf also played an important role in shoot regeneration. Among the three types of leaf explants used (mature, semi mature and immature leaves), semi mature leaf showed positive response for initiation of shoot bud regeneration. Similar observation was reported in *Zizyphus jujuba* (Gu and Zhang, 2005). The average number of

shoot buds/explant increased twice within 6 weeks of the initial culture, which could be maintained for longer periods (up to 80 days) without any loss in the morphogenetic potential.

Petiole explants were highly organogenic producing direct multiple shoots, especially from the lower part of the leaf petiole (Fig. 4.39B). Petiole explants showed differentiation of shoots in MS medium supplemented with different concentrations of BA ranging from 0.5–3.0 mg/l after 28 days of incubation. The highest number of shoots per explant occurred at 2.5 mg/l BA. Further increase in the levels of BA beyond 2.5 mg/l resulted in a decrease in the extent of shoot regeneration. The lower parts of the leaf petiole showed early response and the highest rate of shoot regeneration.

Internodal segments (1.0 cm length) induced shoot buds at higher concentrations of BA (1.5 - 3.0 mg/l) (Table 4.56). Shoots were formed on the upper portion of the segments whereas the lower portion showed slight tendency of callusing (Fig. 4.39C). Maximum number of shoots was obtained in MS medium containing 3.0 mg/l BA. BA promotion of shoot bud induction has been reported by using similar explants in *Feronia limonia* and *Bacopa monnieri* (Hiregoudar *et al.* 2005; Tiwari *et al.* 1998). Direct induction of shoot buds from the internodes in the presence of cytokinin alone, without the addition of any auxin is not an often encountered phenomenon since the internodes do not have preformed meristems. This result may be attributed to the high intrinsic auxin levels

in the internodes of this plant, so that by supplementing them with high concentrations of BA it is possible to balance both the growth regulators so that the explant becomes competent for organogenesis (Christianson and Warnick, 1985).

Solanum violaceum plantlets were produced *via.* organogenesis procedure using leaf, petiole and stem explants. Among these, the shoot induction responses of the petiole explants were better than that of stem and leaf explants. On the other hand, the stem explants had poorer shoot formation than the leaf explants. The varied concentrations of BA required for maximum shoot formation in different explants may be related to different endogenous levels of cytokinin. While the less frequent shoot initiation was preceded by little callusing in inter nodal segments, direct shoot formation was observed for all other explants. Concomitant with our results, increased numbers of adventitious shoots have been reported using BA alone in *Solanum melongena* (Gleddie *et al.* 1983; Mukherjee *et al.* 1991). Regenerated shoots derived from all the systems easily elongated in MS media containing BA at 0.2 mg/l.

For rooting, the elongated shoots were transferred to half strength MS medium containing IAA with 3% (w/v) sucrose. High percentage of rooting (98.2%) was obtained in the medium containing half strength MS medium with 0.5 mg/l IAA. Similar results of superiority of IAA over other auxins for rooting have already been reported in other species of *Solanum* (Magioli *et al.,* 1998). The rooted

seedlings showed quick adaptation to the hardening mixture and to field conditions (Fig. 4.39D).

Table 4.56. Effect of BA on shoot formation from leaf, petiole and stem segments of *Solanum violaceum* cultured on MS medium.

BA (mg/l)	Segment					
	Leaf		Petiole		Internode	
0	0%*	(0)[0]	0%	(0)	0%	(0)
0.5	0%	(0)	12%	(2.3 ± 0.6)	0%	(0)
1.0	0%	(0)	26%	(3.2 ± 1.1)	0%	(0)
1.5	9%	(2.7 ± 0.8)	32%	(8.2 ± 0.9)	6%	(1.8 ± 0.7)
2.0	26%	(12.5 ± 0.7)	56%	(18.3 ± 1.1)	18%	(2.3 ± 1.1)
2.5	62%	(16.3 ± 0.9)	95%	(26.3 ± 0.9)	36%	(8.5 ± 1.3)
3.0	52%	(13.2 ± 0.7)	87%	(21.4 ± 0.6)	52%	(12.7 ± 0.8)

*Percentage of segments with regenerated shoots

[0]Average number of shoots per segment ± standard error

(Segments without regenerated shoots were excluded).

Observations were made after 28 days of culture.

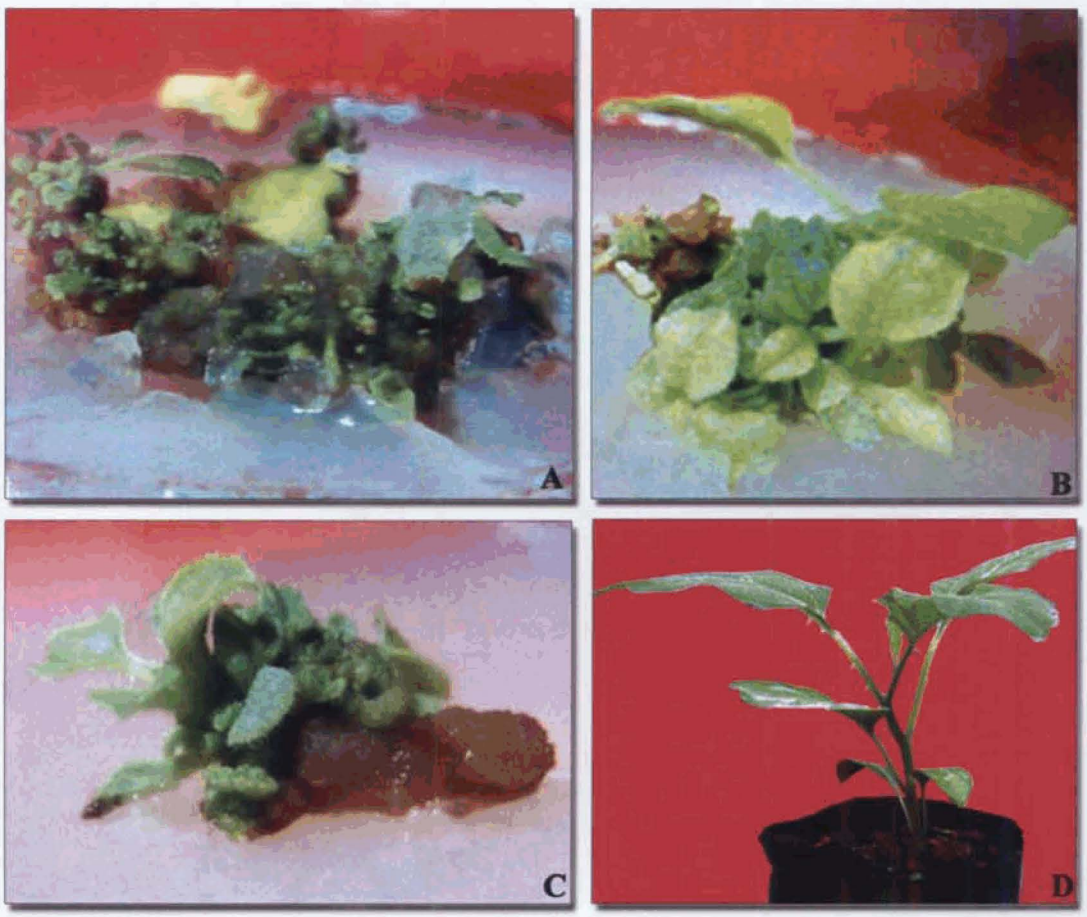


Fig. 4. 39. A-E. *In vitro* clonal propagation of *Solanum violaceum*

A- Shoots organogenesis from leaf explants in MS medium containing BA (2.5 mg/l) ; B- Shoot organogenesis from petiole explants in MS medium containing BA (2.5 mg/l); C- Multiple shoots formed from internodal explant in MS medium containing BA (3.0 mg/l) ; D- Acclimatized plantlet

4.5.2.3.5. *Tribulus terrestris*

The nodal explants responded with 70% bud break within two weeks in the media containing woody plant salts supplemented with 1.0 mg/l BA (Fig. 4.40A). Only single shoot was initiated from each explant in this medium that also prohibited the elongation of shoots. After four weeks the 2-3 nodes were excised from these cultures and subcultured for multiplication on woody plant medium (WPM) supplemented with various concentration of BA (Table 4.57). Along with this, the nodes from mature plants were also used for the multiplication experiments. Both of them responded similarly in the experimental medium but the nodes from *in vitro* responded early. MS medium supplemented with BA at a concentration of 1.0-3.0 mg/l was also tried for shoot proliferation. The explant did not show any response in this medium. After 3 weeks it became yellow and lost viability. WPM was effective for giving favorable responses and hence it was used for multiplication trials. WPM with 0.5 mg/l BA did not show good response compared with higher concentration. The higher concentrations of BA showed increased multiplication but with lower rate of shoot elongation. The suppression of shoot elongation at higher concentrations of BA was reported earlier in *Tridax procumbens* (Sahoo and Chand 1998a). From this study the maximum number (4 -5 number in average) of shoots/nodes were obtained in WPM supplemented with 6.0 mg/l BA (Fig. 4.40B&C). Further increase in BA concentration resulted in a decline in the number of shoots with callusing nature. The significance of optimum concentration of BA for inducing maximum number of shoots has been reported in *Rotula*

aquatica (Sebastian *et al.*, 2002) and *Pterocarpus santalinus and P. marsupium* (Anuradha & Pullaiah, 1999).

For root induction, *in vitro* obtained shoots with 2-3 cm were transferred to MS hormone free medium. The roots were also produced in the presence of lower concenterations of auxins (0.5 mg/l IBA) in WPM (Fig. 4.40D). This result is in contrast with the Mohan *et al* (2000) in the same species that high concentrations of auxins were very much needed for rooting. The induction of root in MS hormone free medium was also reported in *Hemidesmus indicus* (Raghu *et al.*, 2004). Thin fibrous like roots were established within 20 days of the culture. Thin nature of the root will not affect the establishment rate. The rooted plantlets were successfully transferred to polybags with 80% survival (Fig. 4.40E&F).

Table 4.57. *In vitro* response of mature nodal explants of *Tribulus terrestris* in WPM supplemented with BA.

Growth regulators BA mg/l	Percentage response	Mean* shoots/explant (± SE)	Mean length (cm)
0.5	0	0.0	0.0
1.0	34	1.0 ± 1.2	1.0 ± 1.6
2.0	44	1.3 ± 0.7	1.2± 1.4
3.0	47	1.4 ± 0.4	1.3 ± 0.5
4.0	59	2.4 ± 0.7	1.6± 0.8
5.0	62	2.1± 0.5	1.8 ± 0.6
6.0	70	4.6 ± 0.8	2.8± 0.8

*Values are Mean± SE of three independent experiments each with 12 replicates.

Observations were made after 4 weeks of the incubation.

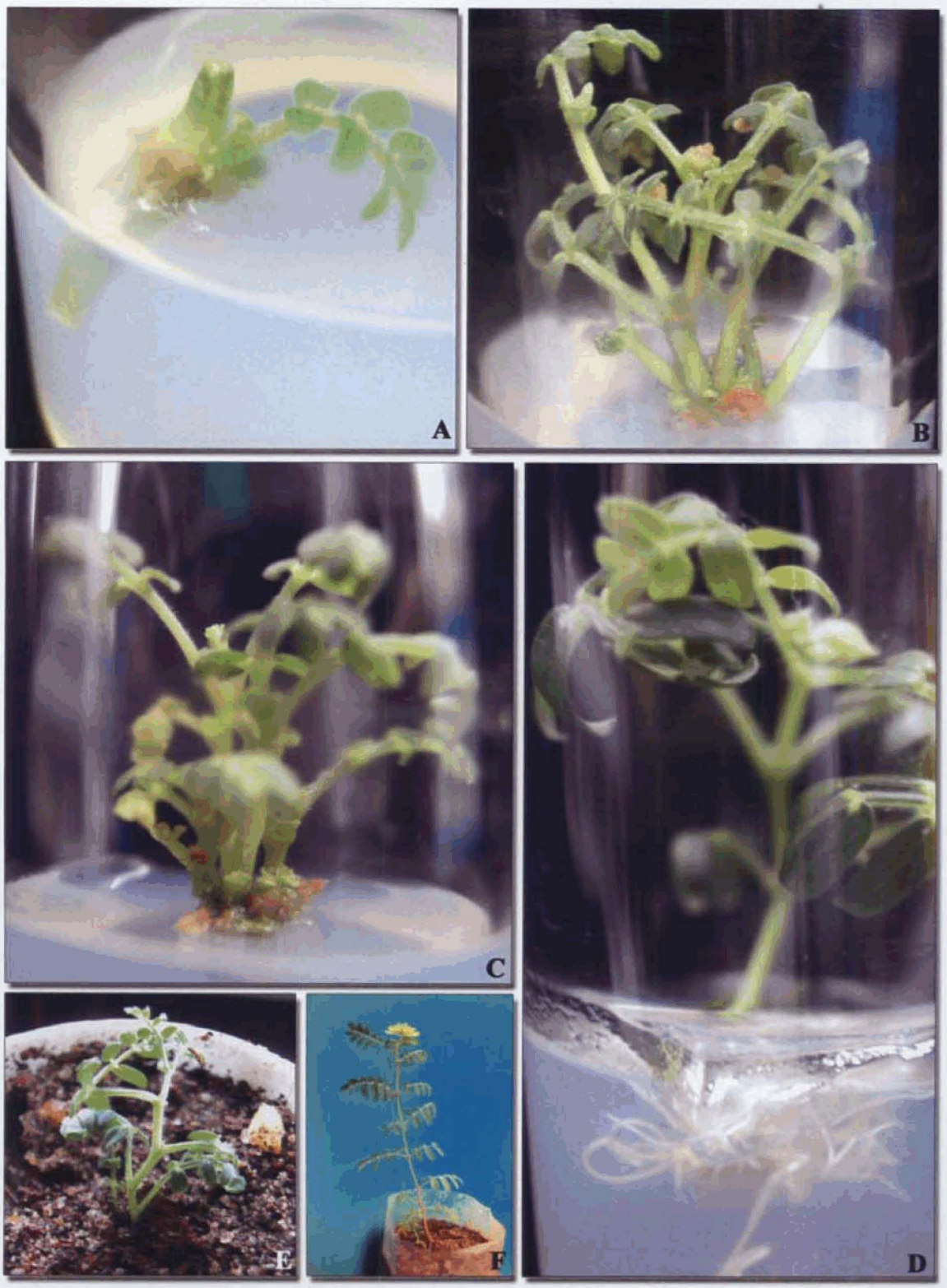


Fig. 4. 40. A-E. *In vitro* clonal propagation of *Tribulus terrestris*

A- Culture initiation on WPM with 1.0 mg/l BA ; B & C - Multiple shoot formation on WPM with 6.0 mg/l BA; D- Roots produced on half strength WPM medium with 0.5 mg/l IBA ; E- Acclimatized plantlet; F- Flowering tissue cultured plant in polybag

SUMMARY AND CONCLUSION

A.V. Raghu “Studies on variability, conservation and propagation of Dasamula group of plants” Thesis. Department of Botany , University of Calicut, 2005

SUMMARY AND CONCLUSION

Chapter V
SUMMARY AND CONCLUSION

Dasamula is one of the best known drug groups of classic ayurveda. The word means ten roots and roots are the most important drug yielding parts of these plants. The drugs include *vilva, kasmari, syonakah, patala, agnimanthah, prsniparni, saliparni, brhatidvayam* (two plants) and *goksurah.* The combination of these drugs is very efficient in the case of a wide range of health problems caused by *vata* and *kapha.* The ten plants include five tree species known as *bruhat panchamula* and five herbaceous plants known as *laghu panchamula. Aegle marmelos* (*vilva*), *Gmelina arborea* (*kasmari*), *Oroxylum indicum* (*syonakah*), *Stereospermum colais* (*patala*) and *Premna corymbosa* (*agnimanthah*) are the members of *bruhat panchamula* and *Desmodium gangeticum* (*prsniparni*), *Pseudarthria viscida* (*saliparni*), *Solanum melongena* var. *insanum* (*brhati*), *Solanum violaceum* (*brhati*) and *Tribulus terrestris* (*goksurah*) are the members of *laghu panchamula* as used in South India. The present study is an effort to analyse the variability of these ten plants at morphological and phytochemical level, to find out the interrelationship of characters by correlation analysis, to analyse the clustering pattern of the accessions studied, to conserve the species *ex situ* and to develop reliable propagation techniques for them.

Aegle marmelos showed the highest variation in terminal leaflet area among the ten accessions studied presently. Among the six quantitative characters studied

in the case of *Gmelina arborea* the maximum variability was shown by leaf area followed by Internodel length. Among the seven quantitative characters studied in *Oroxylum indicum* also, terminal leaflet area showed the maximum variability. In *Stereospermum colais* the maximum variability was shown by terminal leaflet area and the minimum variability by leaf let number. *Premna corymbosa* also showed the maximum variability in the case of leaf area. In the case of *Desmodium gangeticum* the maximum variability was shown by leaf area followed by leaf length, inflorescence length and pod length. In *Pseudarthria viscida* the maximum variability was shown by terminal leaf let area. In the case of *Solanum melongena* var. *insanum* morphological variations were not very prominent and in *Solanum violaceum* the maximum variability was shown by leaf area. In *Tribulus terrestris* also leaf area showed the maximum variability. Morphological variability of characters indicates the genotypic differences between the different accessions of the species studied. Such differences can be considered as valuable sources of gene differences that can be exploited in propagation and breeding programmes.

Phytochemical analysis with the help of HPLC in the case of the above ten species revealed differential levels of chemical affinities, very often associated with population distances and differences. Such variation can also be exploited both for commercial and plant breeding purposes.

Biological characters, especially polygenic characters show different levels of interrelationships in any species. A preliminary attempt has been made presently to work out such interrelationships in the case of some morphological characters in the ten species studied by way of correlation analysis. The study of such interrelationships may help in the identification of characters that can be used as lead characters in selection programmes.

Cluster analysis of genotypes based on their similarities and differences is carried out so as to group them based on their genetic distances. Presently, cluster analysis was carried out in the ten species under study following UPGMA procedure. The ten accessions of *Aegle marmelos* studied presently could be grouped into two clusters at a linkage distance of 0.92, nine accessions forming one cluster and the remaining one forming a separate cluster. In the case of *Gmelina arborea* the ten accessions under study could be grouped into six clusters. In *Oroxylum indicum* the accessions were grouped into two groups at 0.925 level with one accession forming a distinct cluster from others. The ten accessions of *Stereospermum colais* used presently could be classified into three clusters at a linkage distance of 0.92. In *Premna corymbosa* the ten accessions could be grouped into two clusters with a cluster of nine accessions and a second cluster of one accession. In *Desmodium gangeticum*, the ten accessions could be grouped into four clusters. In *Pseudarthria viscida* the ten accessions formed five clusters at a linkage distance of 0.925. The ten accessions of *Solanum*

melongena var. *insanum* could be grouped into two clusters at a linkage distance of 0.75. The accessions of *Solanum violaceum* could be grouped into two clusters, one cluster with a single accession and the second cluster with nine accessions. In *Tribulus terrestris* the ten accessions could be grouped into four clusters at a linkage distance of 0.925.

As in the case of most of the drug source plants *dasamula* group also face acute threat of devastation in their natural habitats thus making steps for *ex situ* conservation inevitable. Presently an effort has been made to collect these plants from different sources and to conserve them *ex situ* under field gene bank conditions.

Development of an efficient method of propagation is an acute need of the hour in the case of medicinal plants. As a part of the present experiment, reliable techniques for seed propagation have been developed in nine of the present species namely *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Stereospermum colais*, *Desmodium gangeticum*, *Pseudarthria viscida*, *Solanum melongena* var. *insanum*, *Solanum violaceum* and *Tribulus terrestris* and reliable vegetative propagation techniques in *Premna corymbosa*.

In vitro propagation techniques have been standardized in the case of five species of plants belonging to *dasamula* group namely *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Solanum violaceum* and *Tribulus terrestris*. The plantlets were hardened and

successfully established in the field. Such biotechnological interventions in the case of medicinal plants will help in the rapid propagation of such plants that face acute threat due to the increased demand as drug sources.

The present study has helped to reveal certain aspects of the diversity of *dasamula* group of plants and thus to initiate their conservation under *ex situ* conditions. It has further helped to standardize methods of *in vivo* propagation techniques in all the ten species of them and *in vitro* propagation techniques in five of them.

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