EVALUATION AND SCREENING OF GENETIC VARIABILITY IN THREE MEDICINAL PLANTS OF TIRUNELVELI HILLS IN SOUTH INDIA

Thesis submitted to

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DOCTOR OF PHILOSOPHY IN BOTANY - BIOTECHNOLOGY

By

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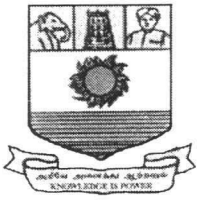

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CERTIFICATE

This is to certify that this thesis entitled **"EVALUATION AND SCREENING OF GENETIC VARIABILITY IN THREE MEDICINAL PLANTS OF TIRUNELVELI HILLS IN SOUTH INDIA"** Submitted by **Mrs. G. S. REKHA to MANONMANIAM SUNDARANAR UNIVERSITY** is a bonafide research work carried out by her, for the degree of **DOCTOR OF PHILOSOPHY IN BOTANY - BIOTECHNOLOGY** under my guidance and supervision.

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DECLARATION

I hereby declare that the thesis entitled **"EVALUATION AND SCREENING OF GENETIC VARIABILITY IN THREE MEDICINAL PLANTS OF TIRUNELVELI HILLS IN SOUTH INDIA"** submitted by me for the Degree of Doctor of Philosophy in Botany-Biotechnology to Manonmaniam Sundaranar University, is the result of my original and independent research work carried out with the guidance of Dr. A. John De Britto, Reader, Plant Molecular Research Unit, St. Xavier's College (Autonomous), Palayamkottai-627002, Tamil Nadu and it has not been submitted for the award of any other degree, diploma, associateship, fellowship of any University or Institution.

Place: Palayamkottai
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G. S. Rekha

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Acronyms

%	Percentage
µl	Microliter
°C	degree centigrade
AFLP	Amplified Fragment Length Polymorphism
cm	Centimeter
CTAB	Cetyl Trimethyl Ammonium Bromide
CW	Cell Wall
D	Distance
Df	Dilution factor
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
g	Gram
GDA	Genetic Data Analysis
H_2O	Water
HPLC	High Performance Liquid Chromatography
ISSR	Inter Simple Sequence Repeats
IUCN	International Union Code and Nomenclature
Km	Kilometer
min	Minute
ml	Milliliter
mm	Millimeter
ng	Nanogram
NOR	Nuclear Organising Regions
Obs.Het	Observed Heterozygosity
Obs.Hom	Observed Homozygosity
OD	Optical Density

PCR	Polymerase Chain Reaction
pH	Potential Hydrogen
Polymorp	Polymorphism
Pop Gene	Population Genetic Analysis
Pop	Population
PVP	Poly Vinyl Pyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA.
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rpm	retention per minute
SD	Standard Deviation
SSR	Simple Sequence Repeats
TBE	Tris Boric EDTA
TE	Tris EDTA
UV	Ultraviolet
VNTR	Variable Number Tandem Repeats

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INTRODUCTION

Diversity simply means "different or unlike". Biological diversity or biodiversity means the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

Biodiversity is of vital importance to human kind from many points of view. Diversity is essential for proper functioning of food chain in an ecosystem, which in turn is essential for the survival of human kind, which has adjusted him to the biodiversity. Biodiversity provides the basic material for food, clothing, medicines, housing and major industries. It is of scientific importance for protecting and maintaining soil and regulating climate. Ecosystems clean our air and water, provide pollinator for crops and help to control diseases.

India is one of the mega biodiversity centers in the world and has two of the world's 18 'biodiversity hotspots' located in the Western Ghats and in the Eastern Himalayas. The forest cover in these areas is very dense and diverse and of pristine beauty, and incredible biodiversity. This rich biological diversity is matched equally by rich cultural diversity and health traditions. Over 7000 species out of an estimated 17,000 higher angiosperms recorded from India are reportedly used for medicinal purposes (Groombridge, 1992).

The Indian subcontinent is endowed with an amazing array of herbal plants, which constitute the main resource base of the health care system in the country. Nearly $4/5^{th}$ of the medicinal plants mentioned in the major pharmacopeias are available in the natural state in India. This rich biological diversity is also matched with equally rich health-care system. It is estimated that 40% of the 16, 000 recorded flowering plants in India have medicinal value. Only 10% of these plants are known to the scientific world and exploited for their use in drug and pharmaceutical industries. There is an ever-mounting interest on the part of various pharmaceutical companies world over in bioprospecting the rich biodiversity of the tropical countries, presumably with the aim of locating and isolating promising pharmaceutical products.

India's rich vegetation wealth and diversity is undoubtedly due to the immense variety of the climatic and altitudinal variations coupled with varied ecological habitats. There are almost rainless areas to the highest rainfall area in India. The altitude varies from the sea level to the highest mountain ranges in India. The habitat types vary from the humid tropical Western Ghats to the hot desert of Rajasthan; from cold desert of Ladakh and Ice Mountains of the Himalayas to the long, warm coastline stretches of Peninsular India. The extreme diversity of the habitats has resulted in such luxuriance and variety of flora and fauna that almost all types of forests, ranging from scrub forest to the tropical evergreen rain forest, coastal mangrove to the temperate and alpine flora occur in this region.

Biodiversity prospecting particularly on medicinal plants of the Himalayan and Western Ghats flora has immense prospects for development of high valued drugs. Recent developments in molecular biology have made it possible to scan the biodiversity for molecules with potential for commercial application. Also, now it is possible to transfer the useful genes from any plant or animal to the required organism. Therefore, we have to not only conserve the biodiversity but safeguard the total gene-pool of India against any unauthorized exploitation by foreigners.

The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. Mention of the medicinal virtues of plants in India has been made even in the epics like Ramayana and Mahabharatha. Nearly 2500 species of plants are used in one way or other by some of these systems. In addition to these traditional systems, there also exists in India a vast knowledge of tribal and folk medicine, which utilize around 7500 species of plants as medicinal. Some of the ethnobotanically important species have also provided leads for production of modern drugs by pharmaceutical companies. It is estimated that in India 90% of the prescriptions contain plant products. Ayurvedic and other traditional system of Indian medicines fully depend on wild plants for preparation of drugs.

Medicinal plants and herbs form a significant part of the rich heritage of our country. Most of them form part of the daily intake of food and have therapeutic and curative properties. Vegetables and fruits, spices and condiments, grains, legumes and pulses all have nutritive and medicinal value. The only difference is that

sometimes the peel, seeds and kernels, flowers and leaves of some of the fruits and vegetables may not be consumed as food but only as medicine and vice versa. There are more than 6000 medicinal plants and herbs used in our country. Unfortunately many are extinct and many more are under extinction. There is a drastic need to conserve the herbal wealth.

The Indian region supports almost all types of habitats for luxuriant growth of medicinal plants. Over 8000 species reported to be medicinal are found in different ecosystems in the country. Again, these species are represented by numerous subtypes or populations depending upon the climatic and edaphic conditions. While on one side we have not been able to evaluate a vast majority of these medicinal plants for identifying the elite types, many species are facing the threat of extinction due to several anthropogenic reasons.

The Western Ghats comprise the mountain range that runs along the western coast of India, from the Vindhya-Satpura ranges in the north comprising Tirunelveli and Kanyakumari jungles to the southern tip. There is a great variety of vegetation all along the Ghats, scrub jungles, grassland along the lower altitudes, dry and moist deciduous forests, and semi-evergreen and evergreen forests. There are two main centers of diversity, the Agashyamalai hills and the Silent Valley. The entire Western Ghats is known for its biodiversity, richness and endemism of different species. The plant species known to be present in the Western Ghats are about 4500 species out of which 35 percent are endemic.

The basic characteristic of life is its unlimited diversity. No two individuals sexually reproducing populations are the same. The underlying factors in this diversity is genetic, though other factors like age, sex, immune system, etc., do play their part in bringing about the observed differences. In order to prevent depletion of biodiversity due to man-made efforts or otherwise, it is necessary to understand how the diversity of life particularly in the genetic level is maintained under natural conditions. It is equally necessary to know how the terminal extinction of species takes place under natural conditions. Based on this knowledge one can suggest appropriate strategies and policies for the conservation of biodiversity.

In the wake of the current pace of habitat loss and depletion of plant genetic resources in the tropics, it is essential for the developing countries which are rich in biological diversity, to evolve strategies and conscious efforts to scrutinize their resources and identify the variants of economic value for conservation and utilization.

In general, medicinal plants in the tropics are characterized by low population densities, greater inter population and intra specific variations. Anthropogenic pressures such as habitat degradation and over harvesting are largely responsible for genetic depletion and variety of nearly 80 out of 300 medicinal plant species in southern India. New means and mechanisms are to be worked out for long-term conservation and sustainable utilization of economically important medicinal plants.

There are thousands of new and little known medicinal plants deserves comprehensive scientific standardization and evaluation. Knowing the superiority is crucial and useful for research and development purpose, consumption purpose, commercial purpose and sometimes for the plant identification purpose. Some of the selected medicinal species are extensively cultivated in various parts of Tamilnadu and used by industries for preparation of drugs. They are also cultivated for export to many countries. There is a competition between supply and demand. At the same time because of many factors such as climatic, edaphic and methods of cultivation batch-to-batch variation has been reported in all these species. Variability within the species and between varieties has also been reported.

Intra-specific variation exhibited by the medicinal plants is largely grouped under two categories: phenotypic and genotypic, besides variation in medicinal plants is often noticed at biochemical level, which is due to the synthesis and accumulation of a wide variety of chemicals which are often plant-specific. These compounds are high – value low –volume secondary metabolites.

Against an increasingly complex and interesting array of variations observed in plants, these variations are now grouped into two categories – genetic and epigenetic. Genetic variations are strictly heritable. They occur mostly due to the alterations in the DNA base sequence, the genetic material, may affect both phenotypic and chemical characteristics of a medicinal plant.

Epigenetic variations, on the other hand, are mostly induced by the environment in which the plants are growing. These are also partially affected by the developmental efforts. Epigenetic variations in medicinal plants usually comprise morphological, chemical as well as physiological variations. Therefore, a great deal of information on the morphological, biochemical, physiological and genetic variation is necessary before an observed pattern of variation is interpreted. It is also true that beneath these intra specific variations, there exists a fixed spectrum of unchangeable characteristics, thereby making the species unique. The different types of variations observed in medicinal plants can be better understood through the following types of variants.

Morphological variants: Morphological variants are the most common of the variants. They are the easiest to identify. The variations are usually observed in the plant height, number of branches, root morphology, multiplicity, number, shape, size, variegation and pigmentation of leaves and fragrance and colour of flowers.

Chemical variants: Most, if not all, of the medicinal compounds of plant origin are secondary metabolites, which confer on the plants the ability to tolerate, adopt, adjust and depend under conditions of abiotic and biotic stresses. These phytochemical constituents may also truly reflect the genetic diversity or epigenetic responses or both.

Genetic variants: the morphological and chemical variations observed in certain medicinal plants may have a genetic root. Genetic variants are ascertained by

isozyme techniques and Random Amplified Polymorphic DNA (RAPD) assays. Changes in the isozyme levels of the crude enzyme preparations of the leaf extracts reveal the presence of genetic variants.

Importance of genetic diversity:

Genetic diversity is the sum of genetic information contained in the genes of medicinal plants, animals and micro-organisms. Each species is the store house of an immense amount of genetic information in the form of traits, characteristics etc. The number of genes are more than 30,0000 in many flowering plants. Each species consists of many organisms and virtually no two members of the same are genetically identical. Genetic differentiation within species occurs as a result of sexual reproduction, in which genetic differences between individuals are combined in their offspring to produce new combination of genes or from mutations causing changes in the DNA.

Genetic variability within a population can sometimes allow a species to adapt to a changing environment, it leads to long term survival of a species, it comes to the rescue of a species at crucial situations by lending genes that impart resistance, surveillance and higher productivity.

Genetic variability paves more and more avenues for crop improvement and hybridization. Species with little or no genetic variability will have greater tendency to go extinct when a new disease, a new predator, or some other change occurs in the environment.

Consequences of low genetic variability are inbreeding depression, loss of evolutionary flexibility, and outbreeding depression all of which lead to weak, sterile individuals. Plant genetic resources and their conservation are the basic requirements for any plant improvement program.

Genetic diversity measurements are important for considering conservation of particular species. A decline in genetic variation can undermine the ability of an organism to respond to natural selection and consequently limits its evolutionary potential. Small populations are often subject to the loss of alleles through genetic drift, or random fluctuations in allele frequency. Thus any study on genetic diversity has to address the above issues.

Molecular techniques provide tools for studying inter and intra specific variation may shed light on the role of migration in the evolutionary dynamics of these plants, which are very important for conservation measures. Among the various molecular techniques, DNA markers are preferable, as developmental or environmental effects do not influence them. RAPD is one such DNA based marker system, which could be used for screening by DNA fingerprinting. The RAPD markers, which are practically unlimited in number and portray variation in the whole genome analyzed, are cost effective and easy to do with large number of samples.

Molecular markers:

Molecular marker is a DNA sequence that is readily detected and whose inheritance can easily be monitored. The use of molecular marker is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purpose. Application of these markers for genetic studies has been so much diverse. Main uses include,

- ❖ assessment of genetic variability and characterization of germplasm.
- ❖ identification and fingerprinting of genotypes.
- ❖ estimation of genetic distances between population,
- ❖ detection of monogenic and qualitative trait loci (QTL).
- ❖ marker-assisted selection.
- ❖ identification of sequences of useful candidate genes, etc.

There are many types of molecular markers such as RAPD, RFLP, AFLP, ISSR, VNTR etc.

RFLPs are non-PCR based and co-dominant markers that enable the heterozygotes to be differentiated from homozygotes at a species or population level. However, it directly identifies a genotype or cultivar in any tissue at any developmental stage in a given environment. The RFLPs are detected by using the unique sequences of DNA called genomic probe and RFLP map is constructed.

AFLP is a combination of both RFLP and RAPD which is very sensitive in detecting polymorphism through out the genome. Therefore, it is superior to RAPD and distinguishes heterozygotes to homozygotes. It is applied universally due to its reproducibility at high level. The basis of its working is PCR amplification of genomic restriction fragments that is produced by restriction enzymes.

Variable number of tandem repeats (VNTR) has also been recently emphasized that polyallelic markers will be very useful for mapping both the simple Mendelian traits as well as polygenic traits in segregating populations. The most widespread of these polyallelic markers are the minisatellites or variable number of tandem repeat loci, which are uncovered by locus specific probes and exhibit highly polyallelic fragment length variation. An interesting and common example of VNTRs involves the number of tandem repeat loci associated with rDNA concentrated at nuclear organizing regions (NORs) of specific chromosomes of an organism.

Microsatellites or simple sequence repeats (SSRs) are more ameanable and can easily be cloned and characterized. Due to the variation in the number of repeat units, they show considerable stable polymorphism. Therefore, microsatellites are the ideal markers to consider high resolution molecular maps.

Although RFLP, AFLP etc are a source of readily obtainable genetic information which is easily reproduced, they often do not show polymorphisms

which are necessary to determine variation within a group of genetically similar individuals. Moreover, the RFLP which detects DNA polymorphisms through restriction enzyme digestion, compared with DNA hybridization is in general, time consuming, expensive, laborious and requires large amount of equipments.

In contrast, low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable. It has further advantages over other systems of genetic documentation, because it has a universal set of primers. No preliminary work such as probe isolation, filter preparation or nucleotide sequencing is necessary (Williams *et al.*, 1990).

RAPD markers are dominant markers and hence it cannot distinguise between the heterozygous individuals possessing a specific allele. RAPD is being used for phylogenetic studies for species and sub species identification. With RAPD, polymorphism can be detected in closely related organisms.

RAPD markers typically have high overall variability and so can be useful for detecting genetic differences within species (Williams *et al.*, 1990 ; Parker et al 1998, Sunnucks 2000). This technique has been used to investigate intraspecific genetic variation in several fungi (Fegan *et al.*,1993 ; Moore *et al.*, 2001). RAPD analysis is a DNA finger printing technique used to detect genomic polymorphisms. In the RAPD method, genomic DNA is PCR - amplified under low stringency conditions using a single short oligonucleotide primers of arbitrary sequence. The low - stringency PCR conditions allow the primer to anneal to

multiple sites on the genome, resulting in an array of amplified DNA fragments. Polymorphisms between individuals or closely related strains are detected as differences in banding patterns on an agarose gel electrophoresis.

RAPD analysis has been widely used in numerous applications including gene mapping, detection of strain diversity, population analysis, epidemiology and the demonstration of phylogenetic and taxonomic relationships. Its popularity arises from its ability to quickly detect polymorphisms at a number of different loci using nanogram quantities of genomic DNA.

RAPD markers are also used for characterization, estimation of genetic relatedness and determination of genetic diversity of plant germplasm. These markers are able to distinguish between genotypes but limited to comparisons of populations from a few sources.

Chromatography is a technique of separation of substances according to their partition coefficients. The various chromatographic techniques are paper chromatography, column chromatography, gas chromatography, Thin layer chromatography, Gas liquid chromatography, High Performance Liquid chromatography (HPLC) etc.

HPLC (High Performance Liquid Chromatography) is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. It is a specialized and versatile form of chromatography that, depending on the particular combination of packing material

and solvent, can incorporate the principles of partition, ion-exchange exclusion or affinity chromatography.

HPLC is used for chemistry and biochemistry research analyzing complex mixtures, purifying chemical compounds, developing processes for synthesizing chemical compounds, isolating natural products, or predicting physical properties. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation. In addition, it is used for analyzing air and water pollutants, for monitoring materials that may jeopardize occupational safety or health, and for monitoring pesticide levels in the environment. Federal and state regulatory agencies use HPLC to survey food and drug products, for identifying confiscated narcotics or to check for adherence to label claims.

Significance of the chosen medicinal plants:

Acalypha indica Linn. (Plate.1) comes under the family Euphorbiaceae, it is an erect, annual herb. Leaves long, petiolated, ovate, acute, cuncate at base, crenate, serrate, glabrous, thin both male and female flowers in axillary spikes, fruits-capsule. The whole plant is used as medicine. The leaves and roots are used to treat skin diseases, constipation, ulcers and bronchitis. The herb is said to possess diuretic, carminative and emetic properties. A decoction of the herb is used as a safe and speedy laxative, and also to cure tooth and earache. Fresh juice of the leaves is applied with oil, salt or lime in rheumatoid arthritis and to cure scabies and other

Plate - 1

Andrographis paniculata **(Burm.f.)Nees**

Acalypha indica **Linn.**

Gymnema sylvestre **(Retz)R.Br.**

skin affections. The powdered leaves are used for bedsores and infected wounds (Varier, 1994).

The plant contains a cyanogenetic glucoside and two alkaloids *viz.*, acalyphine and triacetonamine. The other constituents are n-octasosanol, beta-sitosterol, kaempferol, quebrachitol tannin and resin and essential oil.

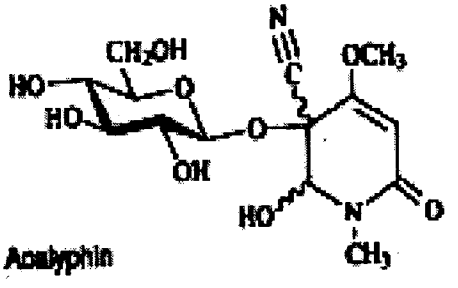


Fig. 1. Structure of Acalyphin.

Andrographis paniculata (Burm.f.) Nees (plate.1) comes under the family Acanthaceae. It is an erect, branched annual herb, 0.3m to 0.9m in height with quadrangular stem, leaves-simple, lanceolate, acute at both ends. Flowers-small, pale but blotched and spotted with brown and purple colour. Fruits - linear capsule, acute at both ends, seeds-numerous. The whole plant is used as medicine. It is useful in hyperpiesia, burning sensation, wounds, ulcer, chronic fever, malarial and intermittent fevers, inflammations, cough, bronchitis, skin diseases, leprosy, pruritus, intestinal worms, dyspepsia, flatulence, colic, diarrhoea, dysentery, haemorrhoids (Varier, 1994).

The plant contains flavanoids and andrographolides. Two flavanoids, identified as 5, 7, tetramethoxyflavanone and 5-hydroxy-7, trimethoxyflavone, as well as several other flavanoids, andrographolide diterpenoids and polyphenols, were obtained from the phytochemical investigation of the whole plant.

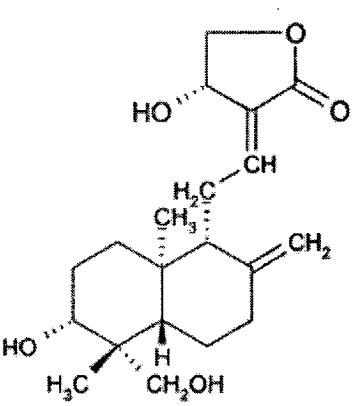


Fig. 2. Structure of Andrographolide

Gymnema sylvestre (Retz.)R.Br. (Plate.1) belongs to the family Asclepiadaceae, it is a large, woody, much branched climber. Leaves-simple, opposite, elliptic or ovate, more or less pubescent on both sides, base rounded or cordate. Flowers-small, yellow in umbellate cymes. Fruits-slender, follicles up to 7.5 cm long. The whole plant is used as medicine. It is useful in inflammations, hepatosplenomegaly, dyspepsia, constipation, jaundice, haemorrhoids, strangury, renal and vesical calculi, helminthiasis, cardiopathy, cough, asthma, bronchitis, intermittent fever, and amenorrhoea (Varier, 1994).

The plant contains Gymnemic acid (GA), (+) quercitol, lupeol, ß-amyrin, stigmasterol etc. GA I, II, III and IV are anti sweet substance from the leaves of G. sylvestris. A second series of Gymnemic acid V-VII has also been reported. GA VII is the 3-O-glucuronide of gymnemagenin and GA V is the O-3-glycuronyl- 22, 21-bis-O-tigloyl substitution pattern. GA VIII-IX are also esters of saponin, have an oxoglycoside moiety attached to the glucuronic acid residue. Gurmarin, another constituent of the leaves, and gymnemic acid have been shown to block sweet taste in humans. Some researchers have suggested gymnemic acid as one possible candidate responsible for antidiabetic activity.

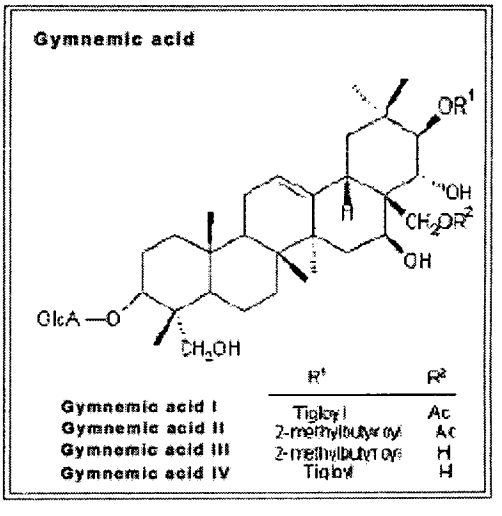


Fig. 3. Structure of Gymnemic acid.

Area of the Study:

Tirunelveli hills are situated in the southernmost end of the Western Ghats, which comes under Tirunelveli and Kanyakumari districts of Tamilnadu. It is between 77° 10 – 77° 40 E and 8° 25 – 8° 53 N. The elevation varies from 50-1869

m. The forest tracks of Tirunelveli hills including Thirukurungudi, Kalakad, Manjoli, Papanasam, Mundanthurai, Valayar, Ainthalaipotigai, Nagapotigai, Agasthiamalai, Sivasilum, Courtallum, Puliarai and Sivagiri hills have undulating topography with inhospitable terrains and undoubtedly the floristic diversity of this region is of ancient lineage (Map.1).

Tirunelveli hills are characterized by numerous folds and extension engulfing small, narrow valleys. The elevation varies from 50 to 1869 M and the various peak from Sivagiri (north) to Mahendragiri (south) have been noted. They include Kallimalai (1000m) Agasthiyamalai peak (1869m), Ainthalaipothigai (1600m), Neterikal (1500m), Kakachi (1500m), Naraikada (1775m), Kailasaparvatham (1770m). The vegetation is floristically rich when compared to other regions of the Western ghats and represent several unique habitats *viz.*, southern tropical dry deciduous forests (200-400m), grassland at low altitude (below 500m), southern tropical moist deciduous forest (400-600m), southern tropical semi ever green forest(600-800m), southern tropical wet ever green forests(800-1500m), sub tropical montane forest (above 1500m), and grassland at high altitude (above 1000m).

Regarding temperature, the major gradient is linked to altitude, the range in monthly means of temperature increases from the south to the north as well as from the east to the west on account of the monsoon type of rainfall. The annual rainfall varies from 100 – 4366 mm. In general, the climate is pleasantly cool above 500m.A

significant feature of Tirunelveli hills flora is that it is the epitome of the Tamil nadu flora and confluence flora from Sri Lanka (Manickam, 2003).

The present study aims at the following:

a) Identification and collection of the chosen medicinal plants which exhibit phenotypic plasticity (***Acalypha indica*** Linn., ***Andrographis paniculata*** (Burm.f.) Wall.ex. Nees, ***Gymnema sylvestre*** (Retz.) R.Br.).

b) Analysis of batch-to-batch variation, caused by phytogeographical and genetic parameters.

c) Analysis of intraspecific variations in the chosen medicinal plants through RAPD-PCR fingerprint.

d) Quantification of chemical constituents present in the chosen plants by HPLC.

e) Selection of superior genotypes in each species of the chosen medicinal plants.

REVIEW OF LITERATURE

Nowadays molecular techniques have been used to monitor DNA sequence variation in and among the species and create new sources of genetic variation. In recent years different marker systems such as RAPD, RFLP, STS (Sequence Tagged Site), AFLP, SSR or microsatellites, single nucleotide polymorphisms (SNPs) and others have been developed to analyse genetic variability in plants.

Protein electrophoresis is the oldest among molecular techniques used for studying genetic variation in organisms. Allozymes electrophoresis is an indirect method to assess nuclear DNA variability. The effective use of allozymes, like other markers, depends on their selective neutrality. Iso electric focussing (IEF) based on Iso Electric Point of a protein has also been used as a protein based marker in species differentiation and population studies.

Aswati *et al.*, (2004) employed RAPD to study the genetic diversity and interrelationships among 12 domesticated and 3 wild mulberry sps. They used 19 random primers and generated 125 discrete markers ranging from 500-3000 bp in size. 119 of these were polymorphic (92%) with an average of 6.26 markers per primer. Nowadays molecular techniques have been used to monitor DNA sequence variation in and among the species and create new sources of genetic variation. In recent years different marker systems such as RAPD, RFLP, STS (Sequence Tagged

Sites), AFLP, SSR or microsatellites, single nucleotide polymorphisms (SNPs) and others have been developed to analyse genetic variability in plants.

Chatterjie *et al.,* (2004) studied the morphological and molecular variation of ***Morus laevigata*** in India using RAPD. They investigated the variability occurring for 12 morpho-biochemical parameters and RAPD profiles generated with 13 selected RAPD primers, and used accessions from 6 different zones. Their analysis revealed high degree of genotypic similarity of collection from Himalayan foot hill (West Bengal) with those Andaman Islands. Specific accessions from Central India and South India also revealed genotypic similarities with specific accessions from North –East India.

Xu *et al.,* (2002) did a preliminary RAPD-PCR analysis of Cimicifuge species and other botanicals used for women's health, (***Trifolium*** species) and it was found that accessions of cultivated ***Trifolium*** species collected from the same field at different times, produced identical profiles. Accessions of Cimicifuge species collected from different geographical areas produced similar but non identical DNA profiles ;however species – specific DNA fragments were identified.

Level and pattern of variation in the nuclear genome of ***Arabiodopsis thaliana*** were studied by sequencing, microsatellite and AFLP analyses. Despite selfing nature of this plant species, a high level of DNA variation was found. In three of the five genes sequenced, dimorphic variation and intragenic recombination were detected. The results suggested that recombination could not be ignored as a

mechanism influencing the pattern of DNA variation in the evolutionary history of ***A.thaliana*** (Blattner *et al.*, 1999).

Fico *et al.,* (2003) used RAPD markers to characterize 8 populations of ***Helleborus*** growing in different parts of Italy. This has clarified the systematic position of the genus.

Singh *et al.,* (2006) reported that Random Amplified Polymorphic DNA (RAPD) technique was used as a tool for assessing genetic diversity and species relationships among 28 accessions of egg plant representing 5 species. Twenty-eight samples of egg plants were collected from different parts of the country. A total of 144 polymorphic amplified products were obtained from 14 decamer primers, which discriminated all the accessions. The value of Jaccard's coefficient ranged from 0.05 to 0.82. The similarity result indicates presence of high level of genetic diversity in egg plants and a dendrogram constructed by UPGMA method shows that ***S.incanum*** is closest to ***S.melongena*** followed by ***S. nigrum***.

Genetic structure and variability were examined in the only three extant populations of the narrow-endemic tree ***Antirhea aromatica*** (Rubiaceae, Guettardeae), an endangered species of the tropical forest of eastern Mexico. The results indicated that the populations evaluated have high genetic variability, compared with other endemic and geographically narrowly distributed plant species, in areas with high levels of environmental heterogeneity (e.g. tropical forests) (Jorge and Gonzalo, 2004).

The Mauritius species are used in traditional pharmacopoeia for their expectorant properties, and most of them are heavily threatened. Molecular genetic relationships between representatives of eight endangered endemic ***Psiadia*** species from Mauritius, conserved in Le Mondrain Reserve, and ***P. dentata*** (Cass.) DC, endemic from Reunion Island, was studied. RAPD analysis revealed a relatively high intra-specific variability in accordance with the outcrossing mode of reproduction of ***Psiadia*** species. A molecular germplasm database for ***Psiadia*** species was established, which will allow further characterisation of new samples being introduced in Le Mondrain Reserve for conservation purpose (Pascale *et al.*, 2003).

Walisch *et al.,* (2005) studied the genetic population structure of the rare rock plant ***Saxifraga rosacea*** ssp. ***sponhemica*** that occurs in naturally isolated populations in several European countries. There was strong genetic differentiation (RAPD patterns) among countries (15% of total variation), among populations within countries (21%) and between transects within populations (11%) (all $P < 0.001$).

Neem (***Azadirachta indica***) is an important tropical tree species with a number of medicinal and biopesticidal properties. The extent of intra-population genetic variation was evaluated in neem accessions growing in district Kanpur (UP), India, along with two exotic accessions. Two PCR-based markers namely, AFLP and SAMPL were employed to measure the genetic variation. The fingerprints corresponding to both the AFLP and SAMPL markers revealed high levels of heterozygosity, indicating that neem is predominantly an out-crossing species. Based

on AFLP and SAMPL analysis, it is concluded that neem maintains high levels of genetic variation at intra-population level (Singh *et al.*, 2002).

Ester *et al.,* (2001) investigated the levels and patterns of genetic diversity in 162 individuals from 17 ***Digitalis minor*** populations across the entire geographic range using random amplified polymorphic DNA (RAPD) markers.

Random amplified polymorphic DNA (RAPD) markers were used to measure genetic diversity in germplasm collection of Kentucky bluegrass (***Poa pratensis*** L.). Total of 20 populations (local varieties and cultivars) were collected from Slovakia, Czech Republic and Ukraine. From 12 tested primers, a 23 bands (in average 12 bands per population) was detected by two 10-mer RAPD primers. Cluster analysis UPGMA was used to illustrate the diversity of genetic material. This analysis detected 4 populations (South Ukraine, 63/718, 63/721 and Maly Slizovnik) with high genetic diversity (Beo and Brindza, 2005).

Morphological and genetic diversity among ***Acacia aroma, A. macracantha, A. caven,*** and ***A. furcatispina*** were studied with morphometric, isozymal, and RAPD approaches (Paola *et al.*, 2002).

According to Christopher and Margaret (2001) ***Aconitum noveboracense***, a rare, herbaceous perennial, is restricted to recently unglaciated areas in Iowa, Wisconsin, Ohio, and New York, and federally classified as a threatened species. They characterized genetic variation within and among the ***Aconitum*** populations in question using isozymes and randomly amplified polymorphic DNA (RAPDs).

Isozymes indicate a high degree of similarity among all populations and a high level of genetic diversity in Black Hills populations.

Nutmeg (***Myristica fragrans***) is an important tree species, value for nutmeg, the kernel of the seed and mace, the dried outer covering of the seed. The analysis of variability in nutmeg revealed that exploitable genetic variability is limited. The nutmeg populations have evolved from a narrow gene pool of original introduction. Hence to enhance the variability, introduction of nutmeg is a must for crop improvement (Krishnamoorthy *et al.*, 2000).

The technique has been applied to anti malarial plant ***Artemesia annua*** to measure the gene diversity in the present day population derived from the founder accessions in India (Sangwan and Sangwan, 2000).

Kelmegh (***Andrographis paniculata Nees***) belonging to family Acanthaceae, is one of the most important herbs used in Indian traditional Ayurvedic and homeopathic system of medicine. Andrographolide and related compounds were investigated for their pharmaceutical properties and all showed varying degree of antipyretic, anti malarial and anti-inflammatory activity. Genetic improvement for quantitative traits in Kalmegh can be achieved through a clear understanding of the nature and amount of variability present in genotypes and the extent to which the desirable traits are heritable (Misra *et al.*, 2000).

According to Lavania, (2002) the basic unit of conservation in vogue is the morpho-species, where the role of genetics is concerned with the assessment of diversity for single loci or random DNA sequences.

Ten polyembryonic and monoembryonic cultivars each traditionally grown in the west coast of southern India were used to determine the genetic relatedness among them using RAPD markers. DNA isolation and RAPD analysis were carried out using 19 random primers, which amplified 153 polymorphic and 33 monomorphic markers. Dendrogram analysis of RAPD and chloroplast DNA, RFLP data clearly grouped the cultivars into two based on embryo types (Ravishankar *et al.*, 2004).

RAPD genetic markers were used to address the genetic diversity and the distribution of variation in 20 breeding populations in Red clover (***Trifolium sps***). Genetic distances were calculated for all possible pair wise combinations. A high level of polymorphism was found and the proportion of polymorphic loci across populations was 74.2%. A population derived from a non-certified seed lot displayed a higher proportion of polymorphic loci than its respective certified seed lot. Gene diversity values and population genetics parameters suggested that the populations analyzed were diverse. An analysis of molecular variance (AMOVA) revealed that the largest proportion of variation (80.4%) resided within population level. A dendrogram based on genetic distances divided the breeding populations analyzed into three distinct groups (Ulloa *et al.*, 2003).

To quantify the genetic diversity within the species of ***Coccinella septempunctata*** and monitor the spatial foraging, populations were sampled from Belgium and analyzed for RAPD DNA variation. Twenty decamer primers generated more than hundred polymorphic RAPD bands and pairwise distances were calculated between populations according to Nei and Li, then used to construct a radial neighbour-joining dendrogram and examine intra- and inter-population variance coefficients, by analysis of molecular variation (AMOVA) (Haubruge *et al.*, 2002).

Isobe *et al.,* (2003) constructed a genetic linkage map of *red clover* (***Trifolium pratense*** L., 2n=2 x=14) using RFLP markers from cDNA probes of a backcrossed mapping population, and investigated the transferability of the markers to other red clover germplasm. The map contains 157 RFLP markers and one morphological marker on seven linkage groups. The result indicated that RFLP markers on the present map were transferable to the genome analysis of other red clover germplasm. This was the first report to construct a linkage map of ***Trifolium*** species; it should provide fundamental and useful genetic information relevant to the breeding of red clover and genus ***Trifolium.***

RAPD markers were used to measure genetic diversity of ***Coelonema draboides*** (Brassicaceae) by Chen *et al.,* (2005). They sampled 90 individuals in 30 populations. A total of 186 amplified bands were scored from the 14 RAPD primers, with a mean of 13.3 amplified bands per primer, and 87% (161 bands) polymorphic bands (PPB) was found. Analysis of molecular variance (AMOVA) showed that a

large proportion of genetic variation (84.2%) resided among individuals within populations, while only 15.8% resided among populations. The species showed higher genetic diversity between individuals than other endemic and endangered plants. The RAPDs provide a useful tool for assessing genetic diversity of rare, endemic species and for resolving relationships among populations. The results showed that the genetic diversity of this species is high, possibly allowing it to adapt more easily to environmental variations.

According to Aga *et al.,* (2003) genetic diversity within the forest ***Coffea arabica*** L. gene pool in Ethiopia has not been extensively examined with molecular markers. In this study, a total of 75 polymorphic RAPD bands generated by twelve random primers were used to assess genetic diversity among 144 genotypes representing 16 ***C. arabica*** populations. The number of polymorphic bands detected with each primer ranged from 2 to 9 with a mean of 6.25 bands per primer. Banding patterns ranged in percentage polymorphism from 37% to 73% with an overall mean of 56% for the populations analyzed. The amount of genetic variation among populations estimated by Shannon-Weaver diversity index was (H = 0.30). The within and between populations differentiation values were 0.65 and 0.35, respectively. Genetic differentiations within and between zones of sample collection sites were 0.80 and 0.20, respectively.

DNA-based fingerprinting technologies including random amplified polymorphic DNA (RAPD) and universally primed PCR (UP-PCR), a novel method for studying genetic variation, were employed as genetic markers for assessing genetic diversity and relationships in timothy (***Phleum pratense*** L.). This study sought to identify the genetic background of the genotypes used in timothy breeding. Thirty eight genotypes from fifteen countries were used as test materials. RAPD and UP-PCR dendrograms based on 132 (from 3 primers) and 44 highly reproducible bands, respectively, were analyzed. The electrophoretic gels showed that the PCR products were informative and polymorphic. Different geographic genotype groups were distinguished according to the combined RADP and UP-PCR results. The results demonstrate that methods based on molecular fingerprinting can be used for timothy identification (Guo *et al.*, 2003).

Random Amplified Polymorphic DNA (RAPD) markers were used to measure genetic diversity within and divergence among species of ***Dendroseris*** (Asteraceae: Lactuceae), (Elizabeth *et al.*, 2000). For five of the species, RAPD band diversities ranged from 0.003 to 0.022 within species; >90% of total diversity was among species and <10% within them. ***Dendroseris*** represents an example where RAPD markers, because of their greater variability, provide a useful alternative to allozymes for assessing diversity in rare species endemic to oceanic islands and for resolving relationships among the species.

Molecular markers have been used in breeding programs in an attempt to evaluate the genetic bases of populations involved in breeding programs, identify hybrids and parental lines, etc. The aims of this study were to evaluate genetic variability of a base population of ***Eucalyptus urophylla*** using RAPD, to assess the genetic base of populations and to construct a molecular data bank. The base population consisted of 61 individuals of Flores and Timor provenance and a local commercial variety. Seventy polymorphic loci were analyzed. The mean genetic similarity was 0.3168 for the base population and the commercial variety showed the lowest similarity (0.2885). Crosses based on genetic distance were proposed (Leite *et al*, 2002).

Random Amplified Polymorphic DNA (RAPD) markers were used to measure genetic diversity of ***Changium smyrnioides*** Wolff (Apiaceae), an endangered medicinal plant, collected from five populations along the Yangzi River. A total of 92 amplified bands were scored from the 13 RAPD primers, and a mean of 7.1 amplified bands per primer and 69% (64 bands) percentages of polymorphic bands (*PPB*) was found. The Shannon's index was used to partition genetic diversity. Genetic diversity estimates indicated that 51.2% of total diversity was among populations and 48.8% within populations. The species shows higher genetic diversity between populations than other endangered plants (Chengxin Fu *et al.*, 2003).

To evaluate genetic differences of ***Posidonia oceanica*** (L.) and ***P. oceanica*** were analyzed by PCR technique and compared using random amplified polymorphic DNA (RAPD) markers. Results were associated to known differences in phenology. Cluster analysis also indicated that previously described phenological differences among ***P. oceanica*** populations in different sectors of the Mediterranean are not mere phenotypic responses to different climatic and hydrological conditions but may well have a genetic basis (Micheli *et al.*, 2005).

Forty-two avocado accessions maintained at the Chiayi Agricultural Experimental Station (Chiayi, Taiwan) were evaluated for genetic diversity using random amplified polymorphic DNA (RAPD) markers. A total of 107 polymorphic bands were detected for genetic diversity analysis upon polymerase chain reaction (PCR) amplification of 21 octamer primers, an average of five scorable bands per primer. These primers were considered highly informative because they amplified at least one polymorphic band that distinguished between accessions. Jaccard's coefficient was applied to calculate genetic similarity, and UPGMA cluster analysis to generate the dendrogram (Tsu-Liang Chang *et al.*, 2003).

Ghanavati *et al.*, (2005) studied the genetic diversity of 54 populations from 22 species of ***Medicago*** collected from Iranian natural habitat. DNA was extracted from bulked leaf samples of each population and used for RAPD. RAPD markers produced by 11 UBC primers was analyzed according to Maximum Parsimony method. Using PAUP software, a phylogenetic tree with 5 main clusters was generated. The result indicates that genetic diversity correlates with geographical

distribution of wide spread annual medic species in Iran. Results showed RAPD is applicable as a complementary tool in taxonomic identification of ***Medicago*** at both species and population levels.

This study covered interspecific genetic diversity of ***Alliums*** commonly grown in the Philippines such as ***Allium ascalonicum, A. cepa, A. chinense, A. fistulosum, A. odorum*** and ***A. sativum***. Eight morphological characters were used to construct a dendogram. The RAPD analysis yielded 64 loci all of which are polymorphic. This studyis useful for the selection breeding and species conservation of Philippine ***Alliums*** (Panes *et al.*, 2005).

Hasnaoui *et al.,* (2005) studied the molecular diversity of pomegranate cultivars using PCR-RAPD markers. Four random primers generated 29 RAPD loci among them 24 are polymorphic. A similarity matrix was constructed on the basis of the presence or absence of bands. Among pomegranate cultivars the mean of genetic similarity is 0.516. The polymorphisms in PCR amplification products were subjected to the unweighted pair group method for arithmetic average (UPGMA) and plotted in a phenogram. Cluster analysis identified two main clusters.

Acharya *et al.,* (2005) studied the interrelationship of five medicinally important species of ***Typhonium*** (Araceae) including ***T. venosum***, which was previously under the genus ***Sauromatum***, was inferred by analysis of RAPD. DNA from pooled leaf samples was isolated and RAPD analysis was performed using 20 decamer oligonucleotide primers. Out of a total of 245 bands amplified, 12 were

found to be monomorphic while 233 bands were polymorphic including 86 species specific bands. The genetic similarities were analyzed from the dendrogram constructed by the pooled RAPD data using a similarity index. The dendrogram showed two distinct clades, one containing ***T. roxburgii, T. trilobatum, T. venosum*** and the other containing the remainder two species, i.e., ***T. diversifolium*** and ***T. flagelliforme***. Both the clusters showed a common node approx. at 23.7% level of similarity of 31.2% was observed between ***T. venosum*** and ***T. trilobatum*** In view of its close genetic similarity with other members of ***Typhonium,*** transfer of ***Sauromatum venosum*** to the genus ***Typhonium*** and merger of the two genera was supported.

Wang *et al.,* (2000) studied different ***Amomum villosum Lour*** species and some of their adulterants of Zingiberaceae using RAPD. The PCR indicates favourable differentiation of the reaction. The ***Amomum villosum Lour.*** Species have the similar DNA fingerprints while it is obviously differ from adulterants. There are 12.17 percent of primers which appear polymorphism. They constructed the tree of the molecular evolution through NJ software, the hereditary distance of the graph shows different relationships between ***Amomum villosum Lour.*** species and their adulterants. The result created by software is analogous to the traditional methods.

According to Li *et al.,* (2004) the development of an HPLC-PDA method for the simultaneous determination of bioactive diterpenoids, andrographolide, isoandrographolide, neoandrographolide, and 14-deoxy-11, 12-

didehydroandrographolide in plant materials and commercial products of ***Andrographis paniculata***. Separations were achieved using a conventional C 18 column with PDA detection at 200-400 nm for UV spectrum and 225 nm for quantification. The mobile phase consists of water and acetonitrile with acetonitrile varying from 20% to 50% over 40 min. The quantification was performed using external standards. The method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), inter-day and intra-day reproducibility, and recovery.

Pholphana *et al.,* (2004) reported that Simple and rapid methods have been developed for the extraction and simultaneous determination of the three active diterpenoids, andrographolide (AP(1)), 14-deoxy-11,12-didehydroandrographolide (AP(3)) and neoandrographolide (AP(4)), from various samples of ***Andrographis paniculata*** (Burm.f) Nees by Pholphana *et al.,* (2004) Methanol extracts from the dried leaves, stems and crude products were analysed by isocratic HPLC using a methanol and water mobile phase with monitoring at 220 run. There was a large variation of the three active diterpenoids in different ***A. paniculata*** products obtained from Thai markets. The results indicated that the amounts of these active compounds consumed, based on the recommended daily doses, from materials obtained from the different suppliers will be different. In addition, the stability of these three active compounds was also examined in dry herbs stored at room temperature. The results showed that andrographolide was more stable than the others. In contrast, the content of 14-deoxy-11, 12- didehydroandrographolide increased and the neoandrographolide content fluctuated during storage time. The combination of

different levels of these compounds in the source materials and the changes during storage could have a significant effect on the efficacy of this traditional herbal medicine in clinical treatment.

A reverse-phase high performance liquid chromatographic method is developed for the determination of andrographolide, a characteristic diterpene lactone in commercial ***Andrographis*** (***Andrographis paniculata***) products (Li *et al.*, 2002).

Cheung *et al.,* (2001) reported that the development of a micellar electrokinetic chromatographic (MEKC) method for simultaneous determination of andrographolide, deoxyandrographolide and neoandrographolide in ethanol extracts of ***Andrographis paniculata***. The method was validated with good correlation coefficients obtained (0.9986-0.9989) while relative standard deviation (RSD) of migration time was between 1.14 and 2.42. It is concluded that this method could be used for speedy and accurate qualitative and quantitative analysis of bioactive diterpenoids in ***andrographis*** herb and its derived products.

According to Saxena *et al.,* (2000) a rapid and simple high-performance thin-layer chromatographic (HPTLC) method has been developed for the simultaneous quantitative estimation of the biologically active diterpenoids, 14-deoxy-11,12-didehydroandrographolide, andrographolide, neoandrographolide and andrographiside in ***Andrographis paniculata***. The assay combines the isolation and separation of andrographolide derivatives on silica gel 60 F-254 HPTLC plates with

spot visualization and scanning at 540 nm, Methanol was found to be the most appropriate solvent for the exhaustive extraction of andrographolide derivatives.

Srivastava *et al.,* (2004) has been made to develop a method by which to determine the chemical fingerprint of ***Andrographis paniculata*** (Acanthaceae). High-performance thin layer chromatography (HPTLC) was used to analyse hexane, chloroform, methanol and water extracts of leaves of ***A. paniculata***. The analyses showed that andrographolide and neoandrographolide are absent in the hexane extract but are present in greater amounts in the methanol extract as compared with the other extracts. These chromatograms may serve as a chemical fingerprint of the drug ***A. paniculata*** for quality control purposes and in the preparation of formulations based on the drug.

Liu *et al.,* (1992) reported that the structure of gymnemagenin (3-beta, 16-beta, 21-beta, 22-alpha, 23, 28-hexahydroxy-olean-12-ene), the sapogenin of the antisweet principles of ***Gymnema sylvestre***, was established by X-ray analysis of the 3-beta, 23; 21-beta, 22-alpha-di-O-isopropylidene derivative. On the basis of this result, the structure of deacylgymnemic acid was elucidated as the 3-O-beta-glucuronide from the carbon-13 nuclear magnetic resonance spectra. Five antisweet principles, gymnemic acid-III, -IV, -V, -VIII, and -IX, were isolated in pure states from the hot water extract of leaves of Gymnema sylvestre. Of these, three (GA-III, -IV, and -V) were known, while two (GA-VIII and -IX) were new compounds. The structures of GA-VIII and -IX were elucidated as 3'-O-beta-D-arabino-2-hexulopyranosyl gymnemic acid-III and -IV, respectively.

MATERIALS AND METHODS

MATERIALS:

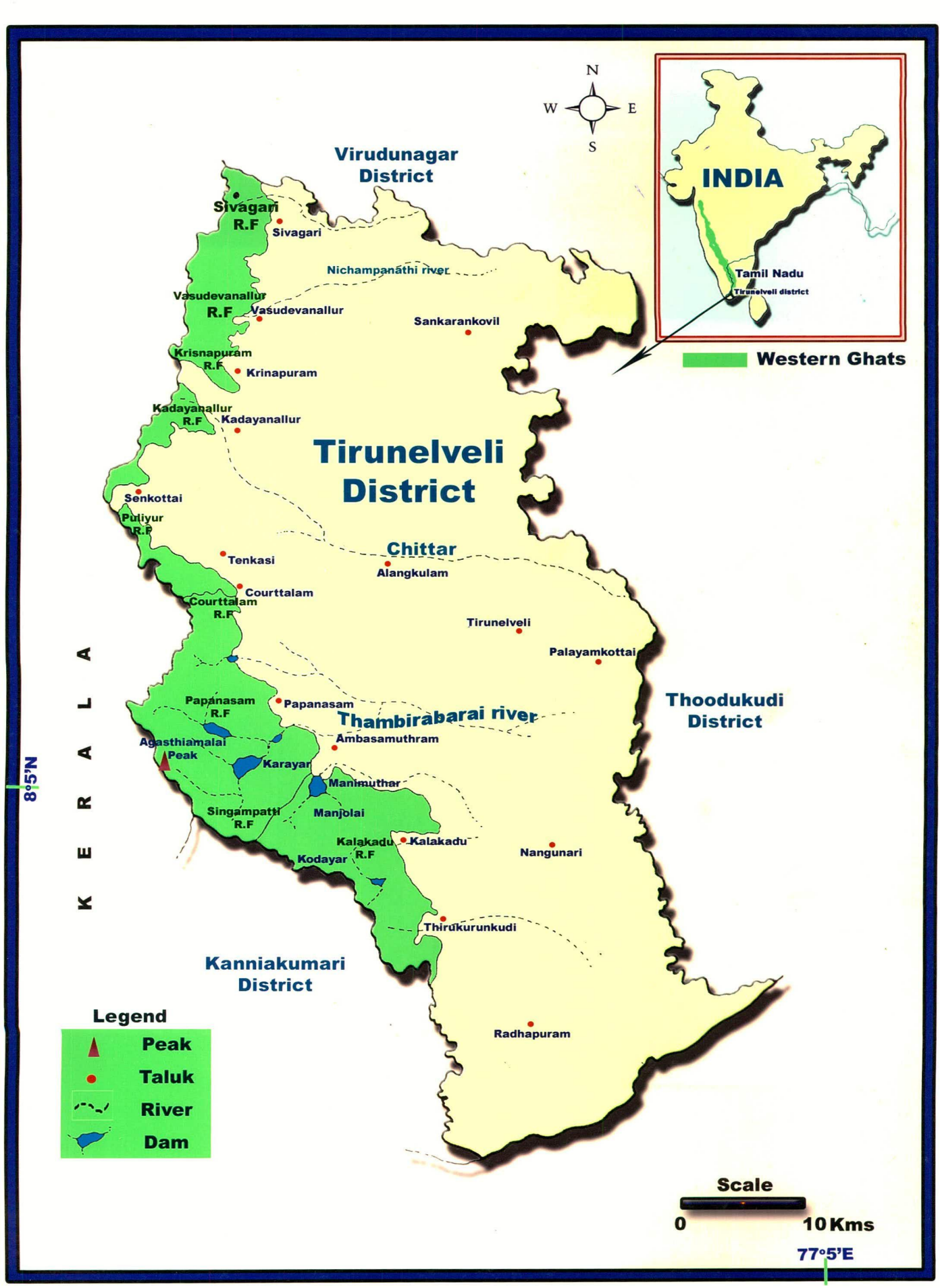
The following medicinal plants have been collected for the study.

1. ***Acalypha indica*** Linn.
2. ***Andrographis paniculata*** (Burm.f.) Nees.
3. ***Gymnema sylvestre*** (Retz.)R.Br.

Collection:

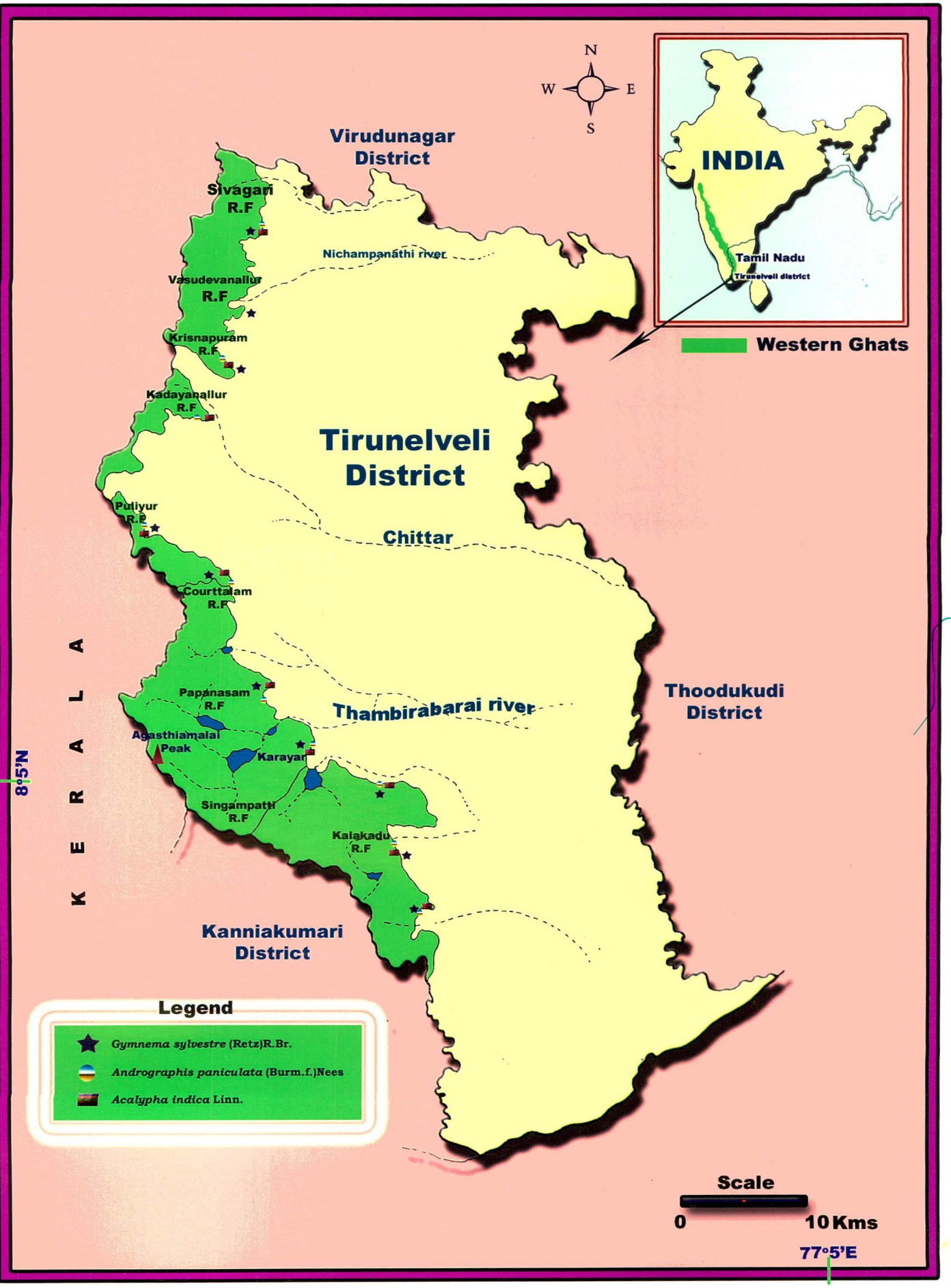
The tender leaves of these three plants were randomly collected from ten different places of Tirunelveli hills. The distance between two populations is 15KM. Each population consisted of ten plants within a radius of 5M.

The field trips were undertaken often in many places like Courtallum, Papanasam, Kalakad, Tirukurungudi, Manimuthar, Ambasamuthram, Kadayanalloor, Krishna puram, Vasudevanalloor, Sivagiri, Puliyur, Karayar, Chenkottai, Ainthalipothigai, Nagapothigai, Agasthiamalai etc. (Map. 2. Area of collection).



ROUTE Map : Tirunelveli Hills

Route



Area of Collection

Table.1. Areas of collection and Accession ID of ***Acalypha indica***

Sl. No	Area	Accession ID
1	Thirugurungudi	1
2	Kalakad	2
3	Manimuthar	7
4	Ambasamuthram	4
5	Papanasam	8
6	Courtallum	10
7	Kadayanalloor	3
8	Krishnapuram	5
9	Vasudevanalloor	6
10	Sivagiri	9

Table.2. Areas of collection and Accession ID of ***Andrographis paniculata***

Sl. No	Area	Accession ID
1	Kalakad	1
2	Karayar	5
3	Manimuthar	6
4	Agasthiyamalai	2
5	PapanasamRF	9
6	Courtallum	10
7	Chenkottai	3
8	PuliyurRF	4
9	Kadayanalloor	7
10	Vasudevanalloor	8

Table.3. Areas of collection and Accession ID of ***Gymnema sylvestre***

Sl. No	Area	Accession ID
1	Thirugurungudi	1
2	Manimuthar	2
3	Karayar	7
4	Papanasam	4
5	PapanasamRF	8
6	CourtallumRF	10
7	PuliyurRF	3
8	Krishna puramRF	5
9	VasudevanalloorRF	6
10	SivagiriRF	9

METHODS:

The collected plant materials were taken to the lab and stored in -70 ^{0}C in deep freezer. Then DNA was extracted from these plants.

DNA isolation:

Genomic DNA was isolated from the collected plants by CTAB (modified) technique of Doyle and Doyle (1987).

Procedure:

1. Plant tissue was frozen in LN2 and ground with isolation buffer (CTAB).
2. The extract was collected and kept in the water bath for 30 minutes at 60°C.
3. 5ml of chloroform:iso-amyl alcohol (24:1) was added.
4. It was spun at 5000 rpm for 10 minutes.
5. The supernatant was collected and equal volume of isopropanol was added.
6. It was spun at 5000 rpm for 10 minutes.
7. The supernatant was collected and 500µl of 70% ethanol was added.
8. It was kept for 30 minutes and spun at maximum speed for 5 minutes.
9. The supernatant was discarded and the pellet was allowed to dry.
10. 400µl of TE buffer and 1µl of RNase were added.
11. It was kept under 37°C for 30 minutes for incubation.
12. DNA was re-Precipitated by adding 80µl ethanol.
13. It was spun at 15,000rpm for 5 minutes.
14. Pellet was collected.
15. Pellet (DNA) was dissolved by adding 400µl TE buffer and it was stored at - 20°C.

By using this method DNA was isolated from these 10 plants of each populations and the DNA was pooled.

Quantification of DNA:

The quantification of DNA was performed in UV-Visible spectrophotometer, in order to findout the purity as well as the quantity of the DNA. The purity of DNA was calculated by using the formula,

Purity of DNA =OD at 260nm/OD at 280nm

The amount of DNA was calculated by using the formula ,

Amount of DNA =OD at 260nm × 50 × Df/1000

1 OD =50µg of DNA.

If the OD between 260nm and 280nm is 1.8, it is pure DNA. If the OD is below 1.8 it is contaminated with protein, if it is above 1.8 it is contaminated with RNA. The OD between 1.7 to 1.8 was taken to this study.

RAPD Analysis:

For RAPD analysis Williams *et al.,* (1990) (modified) method was followed. The reaction was performed by 50ng of DNA, 15 picomoles of a single decamer random primer, PCR mixture and water to a total volume of 25µl in 2.5ml PCR tubes. The reaction mixtures taken in PCR tubes for amplification are tabulated as follows.

Table.4. Reaction mixtures for PCR

	Amount needed	**Amount taken**
Buffer	10 µM Tris Hcl	2.5 µl
$MgCl_2$	2.0 mM	2.5 µl
DNTP	2.0mM	0.5 µl
Taq	0.2 µl	0.2 µl
primers	15 picomoles	1.2 µl

All the reaction mixtures (5.7µl) were taken in an eppendorf tube and added 1.2µl of primer and 50ng of genomic DNA solution. The final volume of the PCR mixture was made up to 25µl by using sterile double distilled water. The amplification was carried out in a thermal cycler programmed as given below.

Table.5. PCR cycles

Activity	**Temperature (^{0}C)**	**Time**	**Number of cycles**
Initial Denaturation	94	5 Min	One
Denaturing	94	1 Min	35
Annealing	37	1 Min	
Extension	72	1 Min	
Final Extension	72	5 Min	One
Storage	4	For ever	

The primers used and their sequences are tabulated as follows.

Table.6. Primers used and their sequences for ***Acalypha indica***

Sl. No	Primer name	Primer sequences (5' to 3')
1	OPX-3	TGGCGCAGTG
2	OPX-12	TCGCCAGCCA
3	OPX-18	GACTAGGTGG
4	OPX-19	TGGCAAGGCA
5	OPX-20	CCCAGCTAGA

Table.7. Primers used and their sequences for ***Andrographis paniculata***

Sl. No	Primer name	Primer sequences (5 to 3)
1	OPX-3	TGGCGCAGTG
2	OPX-4	CCGCTACCGA
3	OPX-6	ACGCCAGAGG
4	OPX-12	TCGCCAGCCA
5	OPX-19	TGGCAAGGCA

Table.8. Primers used and their sequences for ***Gymnema sylvestre***

Sl. No	Primer name	Primer sequences (5 to 3)
1	OPX-3	TGGCGCAGTG
2	OPX-4	CCGCTACCGA
3	OPX-6	ACGCCAGAGG
4	OPX-12	TCGCCAGCCA
5	OPX-19	TGGCAAGGCA

The amplified DNA fragments were separated by running 1.2% agarose gel and documented in gel documentation system.

Casting of agarose gel:

The volume of the agarose gel was working out with the following formula.

Volume of a gel preparation= Length × breadth × width of the gel plate

= 10 × 5 × 1

= 50 ml

Procedure for visualization of DNA and RAPD bands:

1. UV transport electrophoretic tray was kept in a clean and dry tray gel setting platform. After levelling and tightening the tray, the comb was placed on the tray.
2. 400 mg of agarose was added to a flask containing 45ml of sterile double distilled water. Then it was heated for a few minutes to melt. After cooling

5ml of 10×TBE buffer was added to it. Then 5 µl of Ethidium bromide was added as a stainer.

3. After mixing, the gel casting solution was placed on the electrophoretic tray.
4. After solidification the comb was removed from the tray and was placed in electrophoresis tank containing approximately 500ml of 1×TBE buffer to immense the gel to a depth of about 1mm.
5. Then load the DNA sample into the well. (5ml of DNA sample+5µl dye+5µl of 1×TBE buffer).
6. Electrophoretic tank was connected to the electric lead.
7. Observe the migration of DNA from cathode to anode.
8. Voltage of the tank was maintained at 5v/cm.
9. Gel was run, till the tracing dye reached upto the other end of the gel.
10. After switching off the electric current gel tray was taken out of the electrophoresis tank and observed under gel documentation system.

Analysis of RAPD profiles:

PCR amplification was repeated atleast two to three times for RAPD profiling and only reproducibie, well-marked amplified fragments were counted. For quantification of diversity/similarity, pair wise comparisons of banding patterns were made by calculating indices of similarity (S) using the matching co-efficient method of Nei and Li (1979); AB = 2 × Number of shared bands / Total number of bands. Based on average matrices for all primers a dendrogram was obtained by using an Unweighed Pair Group Method (UPGMA) analysis for genetic distances.

For RAPD finger printing, all samples were compared within the species collected from 10 different places. Data scored manually based on the presence and absence of bands. Comparisons were carried out between the samples amplified by the same primer in a pair wise manner. The similarity index between individuals was calculated following the method of Nei and Li (1979).

Estimation of genetic distance:

Estimation of genetic distance within the samples was calculated using the Jaccard's distance co-efficient (Sneath & Sokal, 1973). This co-efficient calculates the percentage of the number of polymorphic markers between the samples. Dendrograms were produced using the software Popgene package version 1.31. The percentage of polymorphism also calculated by the same software.

Then the five populations, which showed the highest polymorphism, were selected for further HPLC. Leaves from the plants of these five populations were collected and shade dried and powdered.

HPLC Analysis:

Determination of acalyphin in *Acalypha indica* methanol extracts:

Chemicals : Acetonitrile, HPLC gradient grade and caffeine, Sigma reference standard was from Sigma (St. Louis, MO, USA). Other chemicals were obtained from local distributors and were of analytical grade.

Sample preparation : Dry plant (5 samples of ***Acalypha indica***, 5 g) had been extracted individually with 250 ml of methanol using Soxhlet apparatus for eight hours. After extraction, the extracts were evaporated to a residue with constant weight. Residues were stored prior to the analyses in dark at 4 °C. The samples (approx 3 mg) were dissolved in 50% ACN, an internal standard was added and then samples were centrifuged at 2000 x g for 4 min, and applied onto an HPLC column.

HPLC conditions:

HPLC analyses were carried out on a Waters Cap LC system with QTOF MS/MS detector using a C18 reversed phase column at 20 °C. The following gradient of A and B (Solvent A = 5.7 mM CH3COOH + 5 % ACN; Solvent B = ACN (acetonitrile)) with the flow 6 µl/min was applied.

Time (min)	Percent A	Percent B
0.00	90.0	10.0
5.00	90.0	10.0
25.00	10.0	90.0
40.00	10.0	90.0
45.00	90.0	10.0
60.00	90.0	10.0

MS spectrum was measured in ES+ mode in the range 50 – 1000 m/z with selected mass 195 and 361. The fragmentation of the parent ion was monitored as well.

Quantification: Acalyphin quantification was based on the analysis of kvazimolecular ion m/z = 361.12 with an internal standard caffeine.

Determination of Andrographolide in *Andrographis paniculata* methanol extracts:

5 gm of dried powdered leaf material of each sample was extracted with methanol in Soxhlet apparatus for eight hours. After extraction, the crude extract was evaporated by vacuum evaporation to dryness and the weight of the extract was measured.

1 mg of the extract was dissolved in 1 ml of methanol. This was diluted to 0.1 mg/ml by the mobile phase and 20 ml of the sample was injected into the system. Analysis was carried out on a Shimadzu Class VP HPLC system (Shimadzu Corp., Tokyo, Japan) using 250/4 Purospher STAR RP-18e column with guard column 4/4 containing the same sorbent (Merck, Darmstadt, Germany). The HPLC system is equipped with SPD-M10Avp UV detector, SCL-10Avp system controller, a GT-104 degassing unit, an FCV-10AL low- pressure gradient flow control valve, an LC-10AT pump, an SIL-10Advp auto injector with 500ml loop, a CTO-10AC column oven, an SPD-M10Avp diode array detector. Samples (20 ml) were eluted in isocratic mode (0.5ml/min) with mobile phase consisting of methanol and water (55:45;v/v). The column temperature was 25°C. Elution was monitored at 223 nm and the retention time for standard Andrographolide was plotted against concentration ranging from 1 to 100 mg/ml. Results were measured by taking mean SD values from duplicate samples.

Determination of gymnemic acids in *Gymnema sylvestre* methanol extracts:

Chemicals : Acetonitrile, HPLC gradient grade and caffeine, Sigma reference standard was from Sigma (St. Louis, MO, USA). Other chemicals were obtained from local distributors and were of analytical grade.

Sample preparation : Dry plant (5 samples of ***Gymnema sylvestre***, 5 g) had been extracted individually with 250 ml of methanol using Soxhlet apparatus for eight hours. After extraction, the extracts were evaporated to a residue with constant weight. Residues were stored prior to the analyses in dark at 4 °C. The samples (approx 3 mg) were dissolved in 50% ACN, an internal standard was added and then samples were centrifuged at 2000 x g for 4 min, and applied onto an HPLC column.

HPLC conditions:

HPLC analyses were carried out on a Waters Cap LC system with QTOF MS/MS detector using a C18 reversed phase column at 20 °C. The following gradient of A and B (Solvent A = 5.7 mM CH3COOH + 5 % ACN; Solvent B = ACN (acetonitrile)) with the flow 6 µl/min was applied:

Time (min)	Percent A	Percent B
0.00	90.0	10.0
5.00	90.0	10.0
25.00	10.0	90.0
40.00	10.0	90.0
45.00	90.0	10.0
60.00	90.0	10.0

MS spectrum was measured in ES+ mode in the range 50 – 1000 m/z with selected mass m/z 836.5 and 723.5. The fragmentation of the parent ion was monitored as well.

Following m/z were studied in great detail for the determination of gymnemic acids.

Gymnemic acid is not only a chemical individuum but the mixture of several very complicated compounds.

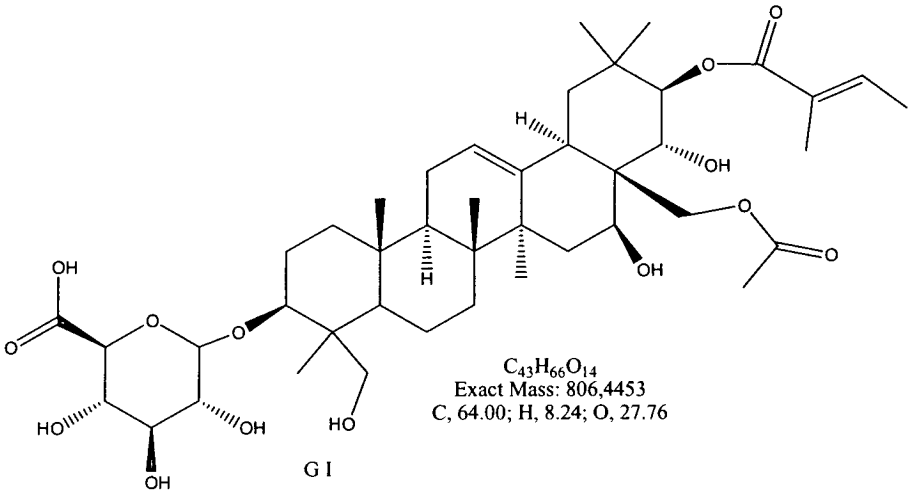


Fig.4. Gymnemic acid I

Because of the standard of gymnemic acids was not available and the published m/z for gymnemic acids were not found or the fragmentation of the parent ion was not typical for the structure of gymnemic acids, it was not possible to do the quantification of gymnemic acids in the plant samples.

Gymnemic acid I:

ESI+: Predicted value m/z = 807.5 [M+H]+

Putative adduct with sodium m/z = 829.4 [M+Na]+

Putative adduct with potassium m/z = 846.4 [M+K]+

ESI-: Deprotonization on carboxygroup m/z = 805.4 [M-H]-

Gymnemic acid II:

ESI+: Predicted value m/z = 809.5 [M+H]+

Putative adduct with sodium m/z = 831.4 [M+Na]+

Putative adduct with potassium m/z =848.4 [M+K]+

ESI-: Deprotonization on carboxygroup m/z = 807.4 [M-H]-

Gymnemic acid III:

ESI+: Predicted value m/z = 767.5 [M+H]+

Putative adduct with sodium m/z = 789.4 [M+Na]+

Putative adduct with potassium m/z =805.4 [M+K]+

ESI-: Deprotonization on carboxygroup m/z = 765.4 [M-H]-

Gymnemic acid IV:

ESI+: Predicted value m/z = 765.5 [M+H]+

Putative adduct with sodium m/z = 787.4 [M+Na]+

Putative adduct with potassium m/z =803.4 [M+K]+

ESI-: Deprotonization on carboxygroup m/z = 763.4 [M-H]-

RESULTS AND DISCUSSION

RESULTS:

Biodiversity is the variety and differences among living organisms from all sources. India is one of the mega biodiversity centres in the world and it is also rich in medicinal plants. The entire Western Ghats is known for its biodiversity, richness and endemism of different species. It comprises of 4500 species, out of which 35 % are endemic. Herbal medicine is one of the oldest medicine known to mankind. Majority of the rural and tribal populations in developing countries depend on traditional medicine for their health care. Forests have long been regarded as one of the important source for herbal medicine. Recently, the medicinal plant research gained more attention because of their efficacy and efficiency.

In general, medicinal plants in the tropics are characterized by low population densities, greater inter- population and intra- specific variation. Anthropogenic pressures such as habitat degradation and over harvesting are largely responsible for genetic depletion and variety of nearly 80 out of 300 medicinal plant species in southern India. New means and mechanism are to be worked out for long-term conversion and sustainable utilization of rare and economically important medicinal plants.

Genetic diversity refers to the variation at the level of individual genes (polymorphisms), and provides a mechanism for populations to adapt to their ever-

changing environment. The more variation, the better the chance that at least some of the individuals will have an allelic variant that is suited for the new environment, and will produce offspring with the variant that will in turn reproduce and continue the population into subsequent generations. In order to prevent depletion of biodiversity due to man- made efforts or otherwise, it is necessary to understand how the diversity of life particularly in the genetic level is maintained under natural condition. Based on this knowledge one can suggest appropriate strategies and policies for the conservation of biodiversity. An understanding of the diversity of genes responsible for individual species adaptations and responses to their environment (intra-specific diversity) is a foundation for understanding almost all ecological and evolutionary processes. Further analysis is necessary to find out the individual polymorphism in each population and the population which shows more heterozygosity may be considered to be the best and this data may be correlated with other features of the population and the superior population can be identified. Thus, genetic variability studies among genotypes would be ideal for the selection of the superior genotypes.

Hence, in order to select the superior genotype in three medicinal plants of Tirunelveli hills ***Acalypha indica Linn.***, ***Andrographis paniculata (Burm.f.)Nees,*** and ***Gymnema sylvestre R.Br.***, an investigation has been done.

There are many molecular markers used to determine the genetic variability in plants. RAPD is one of the molecular markers. RAPD analysis is a DNA fingerprinting technique used to detect genomic polymorphisms. In RAPD method,

genomic DNA is PCR amplified under low-stringency conditions using a single short oligonucleotide primer of arbitrary sequence.

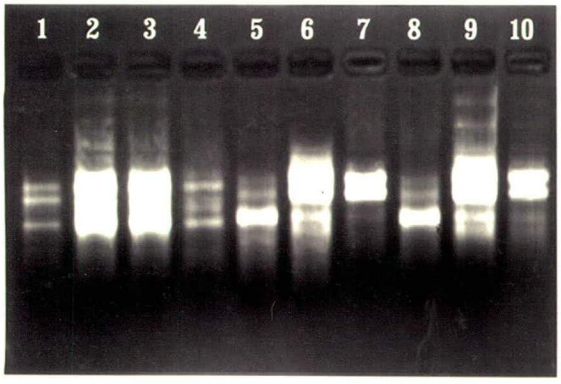
The tender leaves of the chosen plants were collected from ten different accessions and DNA extraction was done by CTAB method. By using different random primers DNA amplification was done. Then, based on the primary data (presence or absence of bands), pair wise genetic identity and genetic distance between samples were calculated using Popgene package version 1.31. Dendrogram was constructed and analysed.

The whole plant of ***Acalypha indica*** Linn. is used as medicine. Mostly it is used to treat skin diseases, constipation, ulcers, bronchitis, diuretic, carminative, rheumatoid, scabies, arthritis, wound etc. The plant contains a cyanogenetic glucoside and two alkaloids *viz*, acalyphin and triacetonamine. The other constituents are n-octasosanol, beta-sitosterol, kaempferol, quebrachitol, tannin and resin and essential oil.

The five primers used to analyze genetic variation in ***Acalypha indica*** produced 59 polymorphic bands (Plate.2). The same type of bands occurred at different frequencies in all populations. The genetic distance between the population ranged from 0.1214 to 0.8286 and the genetic identity ranged from 0.6000 and 0.8857 (Table.9). The overall observed and effective number of alleles is about 1.68 and 1.39 respectively. Nei (1978) overall gene diversity is 0.2366.

Plate 2: *Acalypha indica* Linn.

OPX - 3



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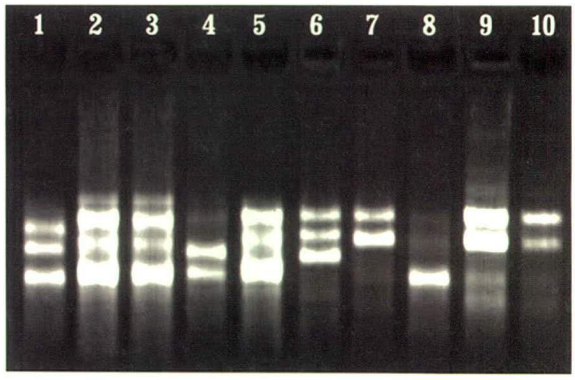

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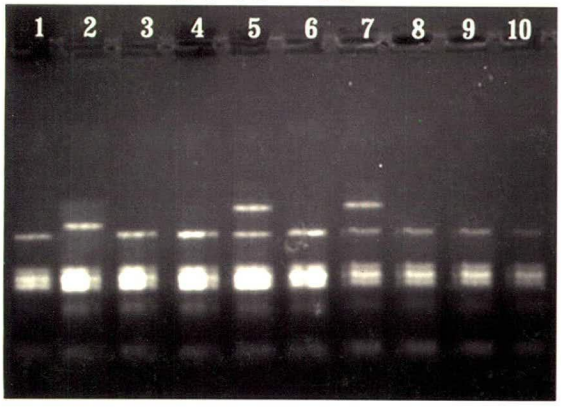

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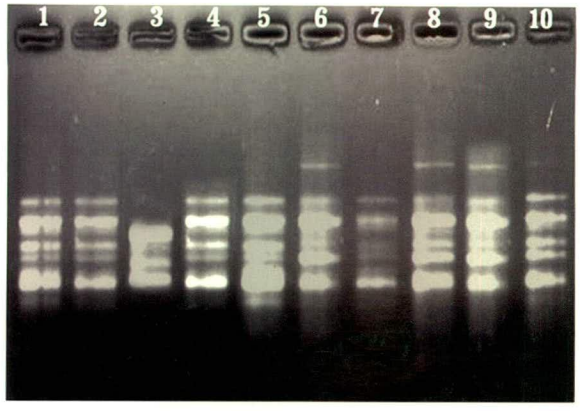

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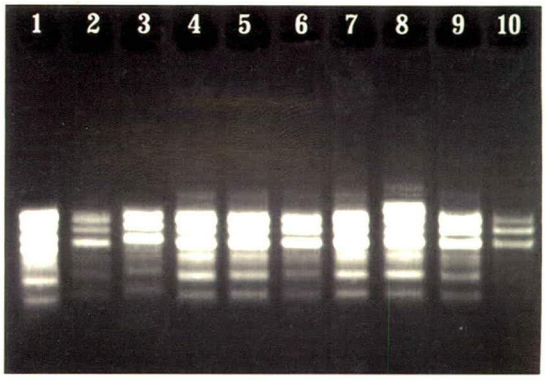

Table.9. Nei's Unbiased Measures of Genetic Identity and Genetic distance in *A. indica*

Pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.6571	0.6571	0.8286	0.8286	0.6571	0.7143	0.7143	0.6571	0.7143
2	0.4199	****	0.8857	0.6000	0.7143	0.7714	0.6571	0.6571	0.7714	0.6571
3	0.4199	0.1214	****	0.6000	0.7143	0.7714	0.6571	0.6571	0.7714	0.6571
4	0.1881	0.5108	0.5108	****	0.8857	0.7143	0.7714	0.8286	0.6571	0.7143
5	0.1881	0.3365	0.3365	0.1214	****	0.7714	0.8286	-.8286	0.7714	0.7143
6	0.4199	0.2595	0.2595	0.3365	0.2595	****	0.7714	0.7714	0.7714	0.7143
7	0.3365	0.4199	0.2595	0.2595	0.3365	0.2595	****	0.8286	0.7714	0.8286
8	0.3365	0.4199	0.4199	0.1881	0.1881	0.2595	0.1881	****	0.8286	0.7143
9	0.4199	0.2595	0.2595	0.4199	0.2595	0.2595	0.2595	0.1881	****	0.7143
10	0.3365	0.4199	0.4199	0.3365	0.3365	0.3365	0.1881	0.3365	0.3365	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

The dendrogram of ***Acalypha indica*** (Fig.5) produced three clusters. Cluster 2 was the largest cluster among the three, containing population 7, 8, 9 and 10. Here population 7, 8 and 9 are closely together than population 10. In the first cluster population 1 form a separate clade and population 4 and population 5 are closely together. In the third cluster population 2 and population 3 are close together and population 6 form a separate clade. It is understood that there is considerable amount of genetic variability between the 10 populations of ***Acalypha indica.***

Then the number of polymorphic loci and percentage of polymorphism (Table.11) was calculated by using the software Popgene package version1.31. Among these ten populations, populations 1, 4, 7, 8, 10 (Thirugurungudi, Ambasamuthram, Manimuthar, Papanasam and Courtallum) showed highest polymorphism. Among these five, percentage of polymorphism was higher in population 10. The five populations, which showed the highest polymorphism, were selected for further HPLC. Leaves from the plants of these five populations were collected and shade dried and powdered.

Quantification of Acalyphin:

Acalyphin quantification (Table.12) was based on the analysis of kvazimolecular ion m/z = 361.12 with an internal standard caffeine.

Among these five, (1, 4, 7, 8, and 10) population 10 which was collected from Courtallum, showed the highest amount of Acalyphin (3.25µg ± 0.20µg), followed by pop 8, which was collected from Papanasam (2.28µg ± 0.49µg).

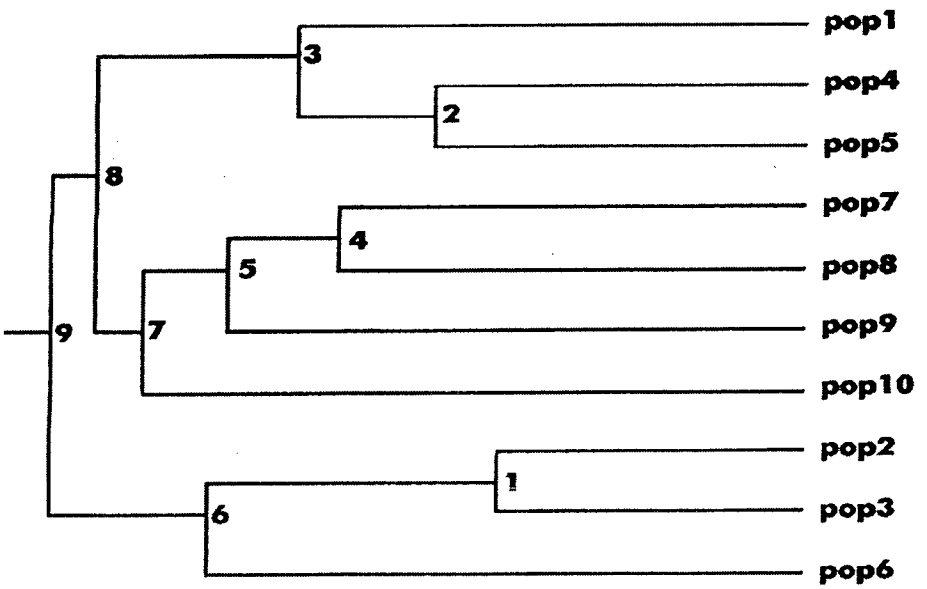

Fig. 5 : UPGMA dendrogram of ***Acalypha indica*** based on Nei's Genetic distance derived from RAPD data.

Table.10. Distance Between And population in ***Acalypha indica***

Between	**And**	**Length**
9	8	3.31930
8	3	5.61926
3	Pop 1	9.40261
3	2	3.33457
2	Pop 4	6.06804
2	Pop 5	6.06804
8	7	0.67192
7	5	3.16086
5	4	1.78647
4	Pop 7	9.40261
4	Pop 8	9.40261
5	Pop 9	11.18909
7	Pop 10	14.34995
9	6	5.36561
6	1	6.90752
1	Pop 2	6.06804
1	Pop 3	6.06804
6	Pop 6	12.97556

Table.11. Number of polymorphic loci and percentage of polymorphism in ***Acalypha indica***

Population no	**Number of polymorphic loci**	**Percentage of polymorphic loci**
1	14	40.00
2	8	22.86
3	10	28.57
4	12	34.29
5	8	22.86
6	8	22.86
7	10	28.57
8	10	28.57
9	6	17.14
10	16	45.71

Table.12. Quantification of acalyphin in ***Acalypha indica*** dry plant samples carried out with MS/MS detection

Sample	**Weight for extraction (g)**	**Residuum after evaporation (g)**	**Determined w (%) in residuum**	**Amount of acalyphin in 1 g of dry plant (µg/g)**
AI -1 Thirugurungudi	5	2.99	0.188	1.80 ± 0.23
AI - II Ambasamuthram	5	2.96	0.083	0.95 ± 0.14
AI - III Manimuthar	5	2.13	0.070	1.03 ± 0.33
AI - IV Papanasam	5	1.23	0.126	2.28 ± 0.49
AI - V Courtallum	5	2.00	0.190	3.25 ± 0.20

Results are expressed as mean ± SD, n = 2.

AI ****Acalypha indica***

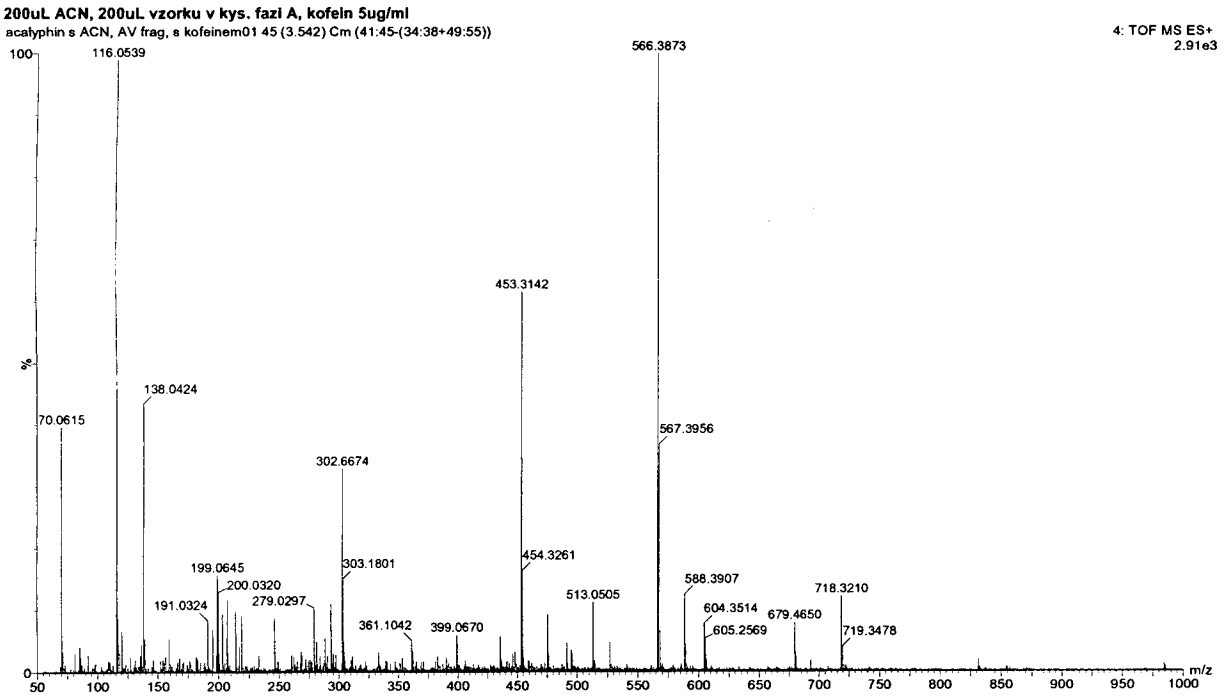

Fig. 8. MS spectrum of acalyphin, measured in the range 50 – 1000 m/z.

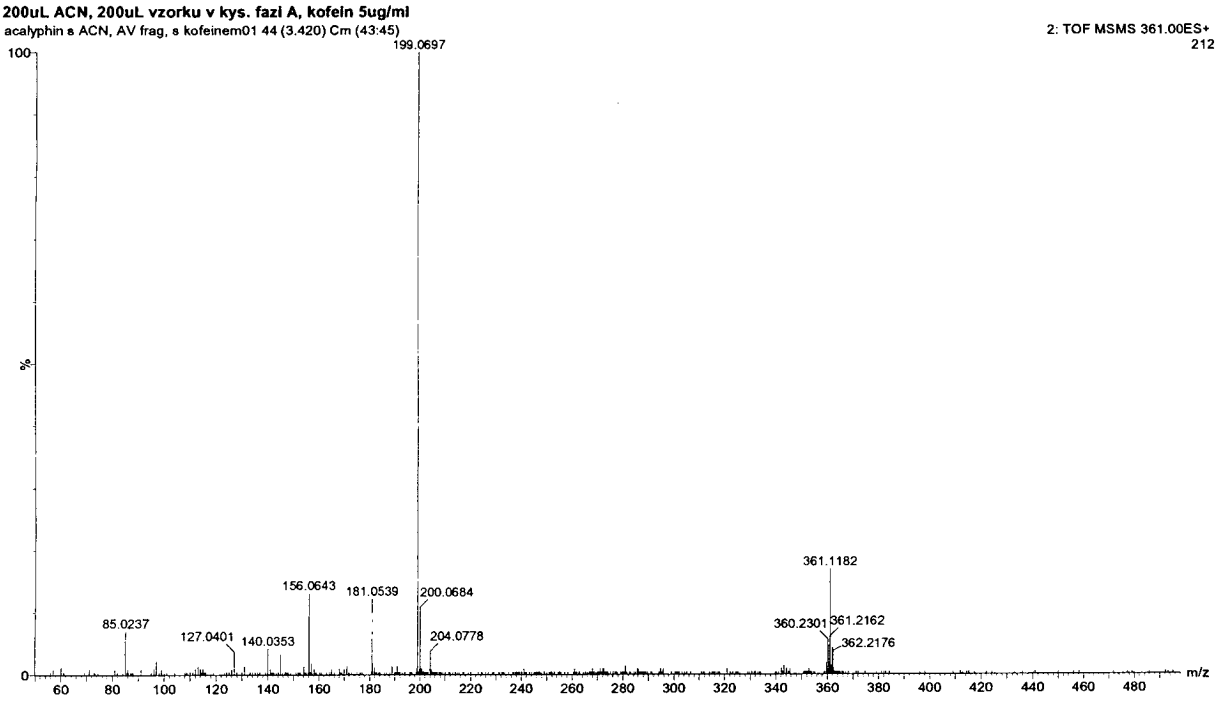

Fig. 9. MS spectrum of acalyphin, measured in the range 50 – 500 m/z

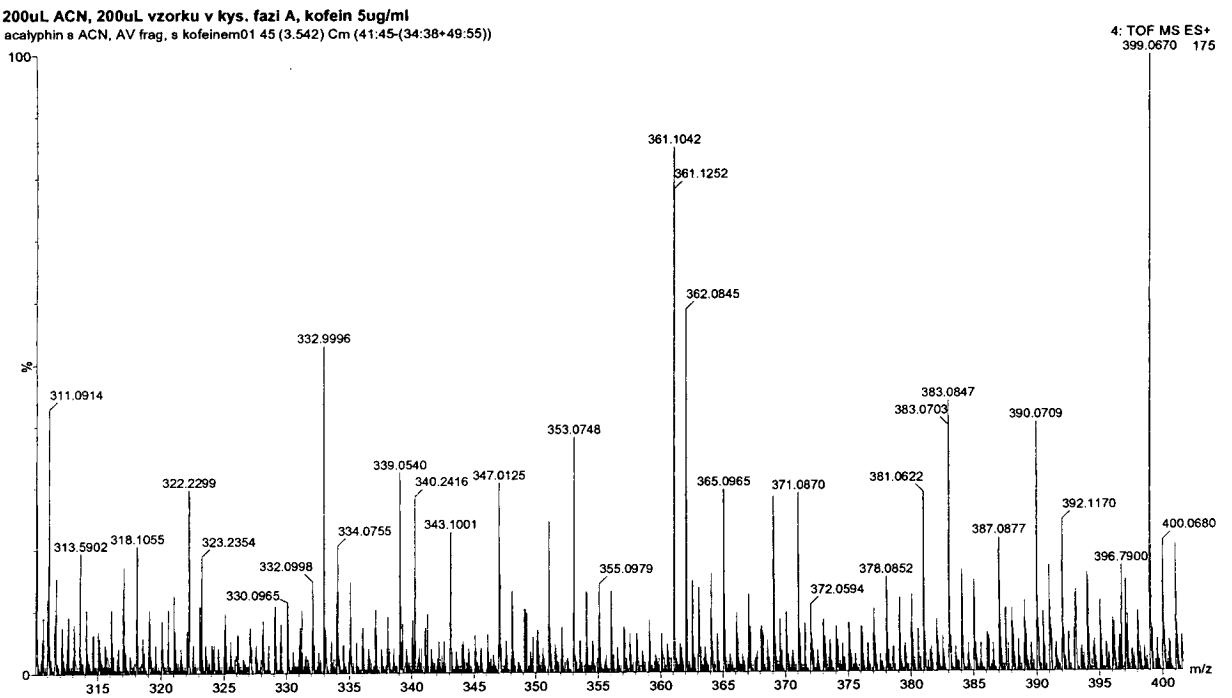

Fig. 10. MS spectrum of acalyphin, measured in the range 310 – 400 m/z.

The whole plant of ***Andrographis paniculata*** (Burm.f.) Nees is used as medicine. It is useful in hyperpiesia, burning sensation, wounds, ulcer, chronic fever, malarial and intermittent fevers, inflammations, cough, bronchitis, skin diseases, leprosy, pruritus, intestinal worms, dyspepsia, flutulence, colic, diarrhoea, dysentery, haemorrhoids. The plant contains flavanoids and andrographolides. Two flavanoids, identified as 5, 7, tetramethoxyflavanone and 5-hydroxy-7, trimethoxyflavone, as well as several other flavanoids, andrographolide diterpenoids and polyphenols, were obtained from the phytochemical investigation of the whole plant.

The five primers used to analyze genetic variation in ***Andrographis paniculata*** produced 51 polymorphic bands (Plate.3). The same type of bands occurred at different frequencies in all populations. The genetic distance between the population ranged from 0.1724 to 0.9310 and the genetic identity ranged from 0.6897 to 0.9655 (Table.13). The overall observed and effective number of alleles is about 1.65 and 1.26 respectively. Nei (1978) overall gene diversity is 0.1724.

The dendrogram of ***Andrographis paniculata*** (Fig.6) produced three clusters. Here population 1 and population 3 are ungrouped. The largest cluster consists of 4 sub groups. Here population 2 and 7, population 4 and 6, population 8 and 5 and population 9 and 10 are closely together. From this it is understood that there is considerable amount of genetic variability between the ten populations of ***Andrographis paniculata.***

Plate 3: *Andrographis paniculata* (Burm.f.)Nees.

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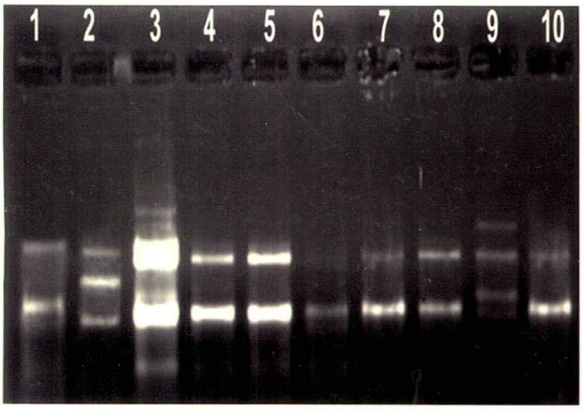

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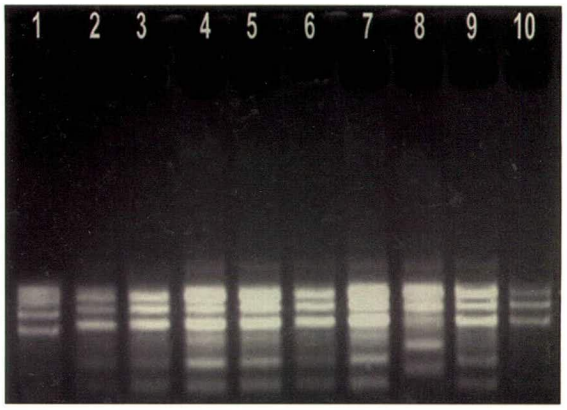

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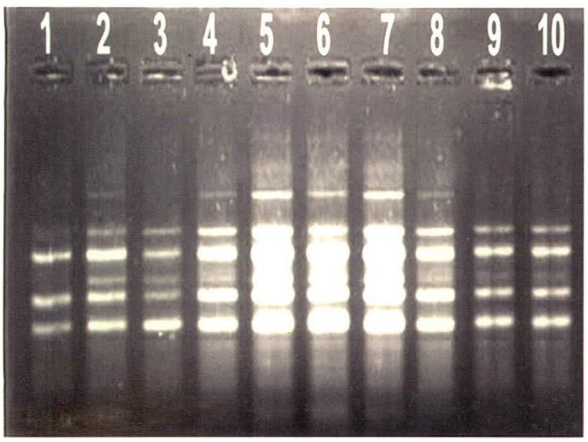

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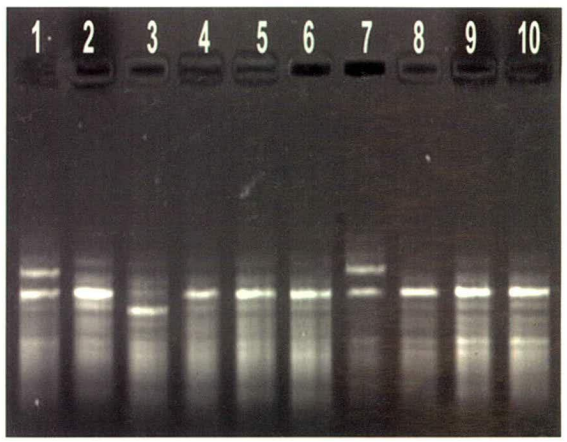

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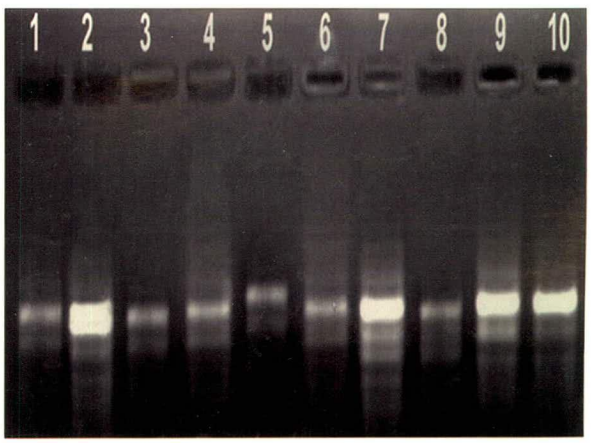


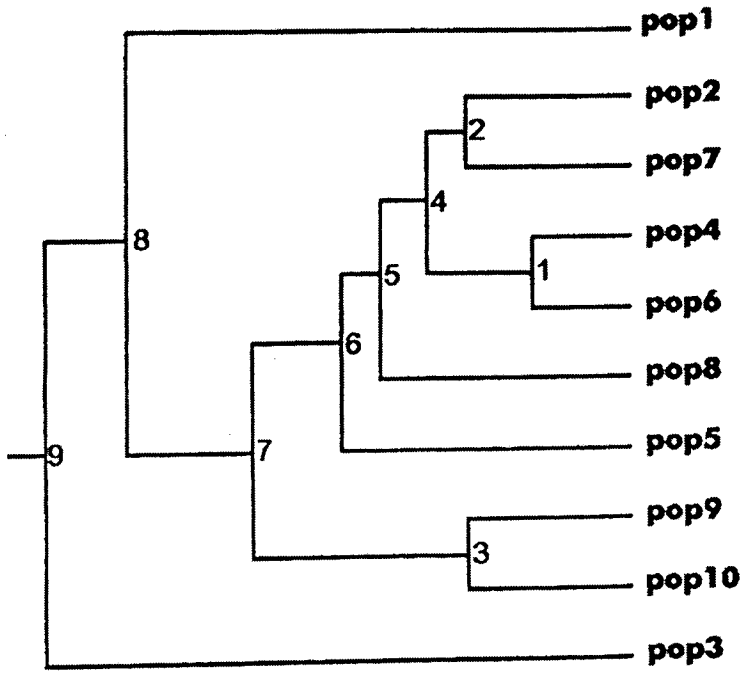


Fig.6. UPGMA dendrogram of ***Andrographis paniculata*** based on Nei's Genetic distance derived from RAPD data.

Table.13. Nei's Unbiased Measures of Genetic Identity and Genetic distance in *A. paniculata*

Pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.6897	0.6552	0.7586	0.6897	0.7241	0.7586	0.7241	0.7586	0.8276
2	0.3716	****	0.6897	0.9310	0.8621	0.8966	0.9310	0.8966	0.7931	0.8621
3	0.4299	0.3716	****	0.7586	0.6897	0.7241	0.6897	0.6552	0.6897	0.6897
4	0.2763	0.0715	0.2763	****	0.9310	0.9655	0.9310	0.8966	0.7931	0.8621
5	0.3716	0.1484	0.3716	0.0715	****	0.8966	0.8621	0.8276	0.7241	0.7941
6	0.3228	0.1092	0.3228	0.0351	0.1092	****	0.8966	0.8621	0.7586	0.8276
7	0.2763	0.0715	0.3716	0.0715	0.1484	0.1092	****	0.8966	0.7931	0.8621
8	0.3228	0.1092	0.4229	0.1092	0.1892	0.1484	0.1092	****	0.8276	0.8966
9	0.2763	0.2318	0.3716	0.2318	0.3228	0.2763	0.2318	0.1892	****	0.9310
10	0.1892	0.1484	0.3716	0.1484	02318	0.1892	0.1484	0.1092	0.0715	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Table.16. Distance between And population in ***Andragraphis paniculata***

Between	And	Length
9	8	3.30582
8	Pop 1	15.04173
8	7	4.79516
7	6	3.57917
6	5	0.71718
5	4	1.43377
4	2	0.94351
2	Pop 2	3.57295
2	Pop 7	3.57295
4	1	2.76189
1	Pop 4	1.75457
1	Pop 6	1.75457
5	Pop 8	.95022
6	Pop 5	6.66740
7	3	6.67362
3	Pop 9	3.57295
3	Pop 10	3.57295
9	Pop 3	18.34755

Then the number of polymorphic loci and percentage of polymorphism (Table.15) was calculated by using the software Popgene package version 1.31. Among these ten populations, populations 1, 5, 6, 9, and 10, (Kalakad, Karayar, Courtallum, Papanasam RF and Manimuthar) showed highest polymorphism. The five populations, which showed the highest polymorphism, were selected for further HPLC. Leaves from the plants of these five populations were collected and shade dried and powdered. Among these five, percentage of polymorphism is higher in population 1.

Quantification of Andrographolide:

Andrographolide quantification (Table.16) was based on the analysis of kvazimolecular ion m/z = 361.12 with an internal standard caffeine.

Among these five samples (1, 5, 6, 9, 10) population 10, which was collected from Courtallum, showed the highest amount of Andrographolide (15.090µg) followed by population 9, which was collected from Papanasam RF (11.077µg).

The whole plant of ***Gymnema sylvestre*** (Retz.)R.Br. is used as medicine. It is useful in inflammations, hepatosplenomegaly, dyspepsia, constipation, jaundice, haemorrhoids, strangury, renal and vesical calculi, helminthiasis, cardiopathy, cough, asthma, bronchitis, intermittent fever, and amenorrhoea. The plant contains Gymnemic acid (GA), (+) quercitol, lupeol, ß-amyrin, stigmasterol etc. GA I, II, III and IV are anti sweet substance from the leaves of ***G. sylvestre.*** A second series of

Table.15. Number of polymorphic loci and percentage of polymorphism in *Andrographis paniculata*

Population no	Number of polymorphic loci	Percentage of polymorphic loci
1	14	48.28
2	9	31.03
3	8	27.59
4	10	34.48
5	11	37.93
6	12	41.38
7	9	31.03
8	10	34.48
9	11	37.93
10	13	44.83

Table.16. Quantification of andrographolide in ***Andrographis paniculata*** dry plant samples carried out with MS/MS detection.

Sample	**Weight for extraction (g)**	**Residuum after evaporation (g)**	**Determined w (%) in residuum**	**Amount of andrographolide in 1 g of dry plant (µg/g)**
AP1 (Papanasam RF)	5	1.57	0.993	11.077
AP II (Karayar)	5	1.20	1.000	10.058
AP III (Courtallum)	5	1.47	0.994	15.090
AP IV (Kalakad)	5	1.84	0.987	3.039
AP V (Manimuthar)	5	1.94	0.841	3.567

AP **Andrographis paniculata*

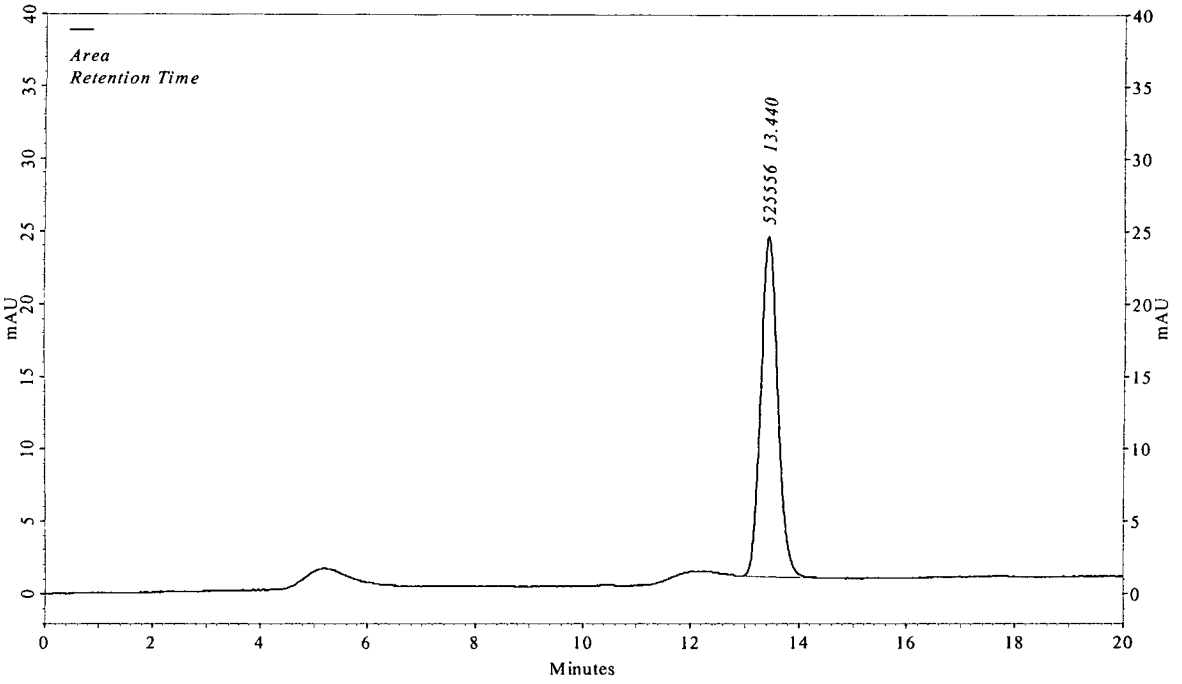
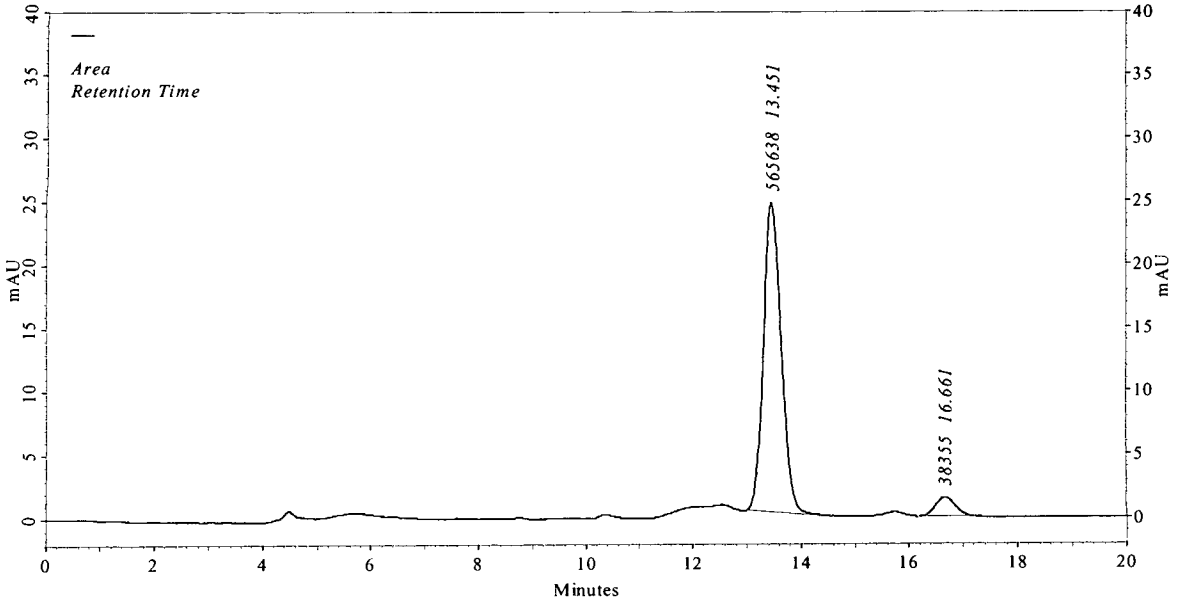
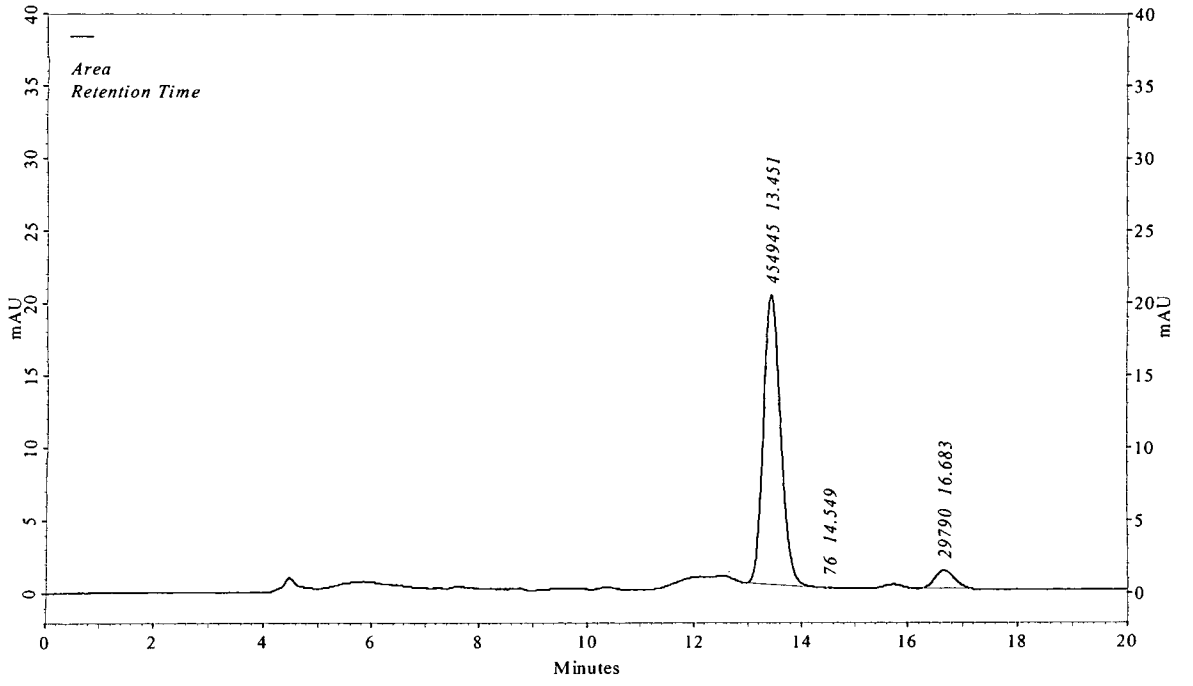


Fig. 11. Chromatogram of Andrographolide in ***A.paniculata*** standard.



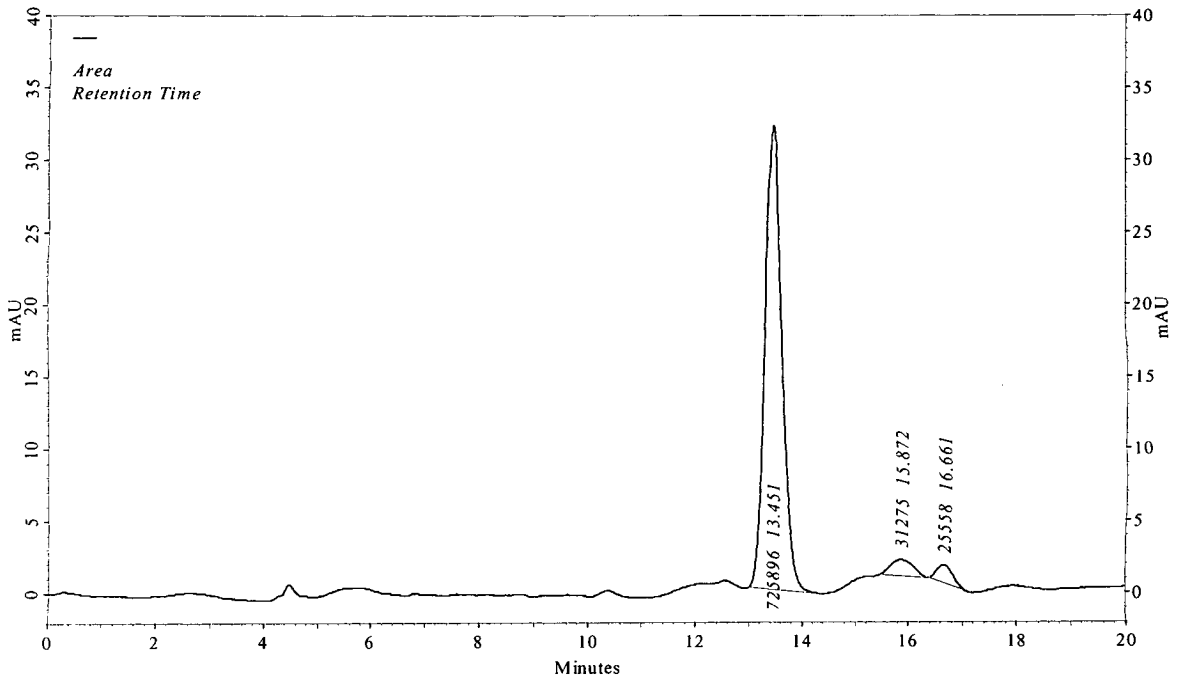
aprox. 0.1 mg/ml mobile phase

Fig. 12. Chromatogram of Andrographolide in population 1 of ***A.paniculata***



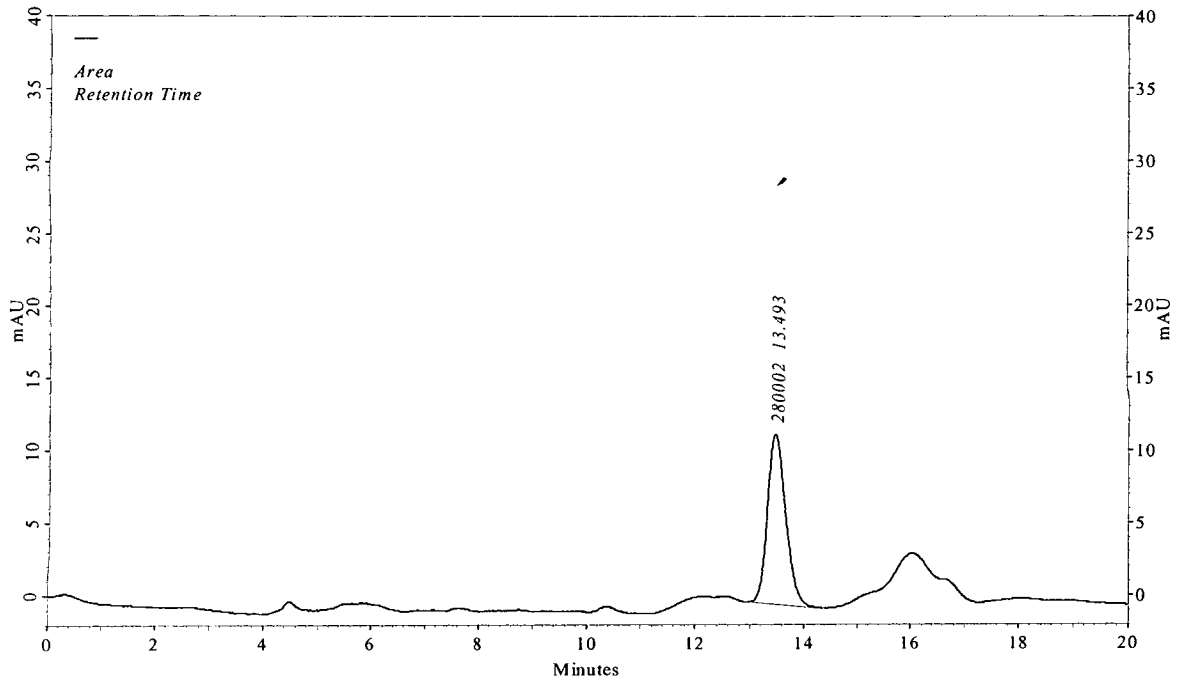
approx. 0.1 mg/ml mobile phase

Fig. 13. Chromatogram of Andrographolide in population 2 of ***A.paniculata***



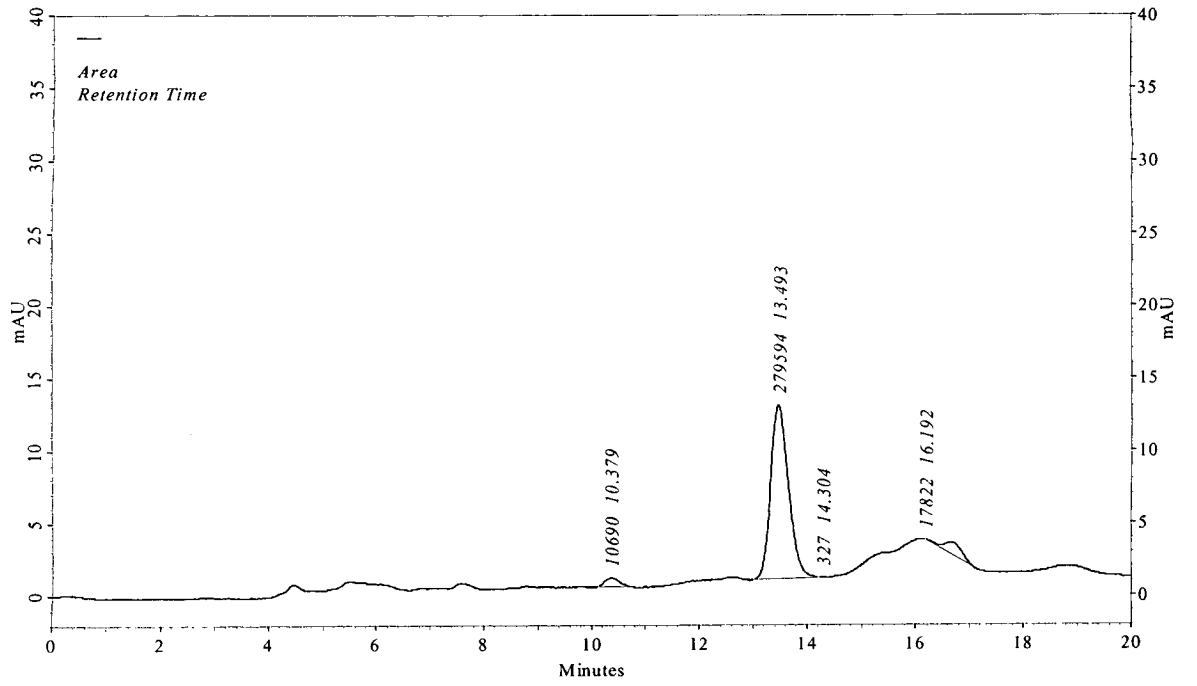
approx. 0.1 mg/ml mobile phase

Fig. 14. Chromatogram of Andrographolide in population 3 of ***A.paniculata***



approx. 0.1 mg/ml mobile phase

Fig. 15. Chromatogram of Andrographolide in population 4 of ***A.paniculata***



approx. 0.1 mg/ml mobile phase

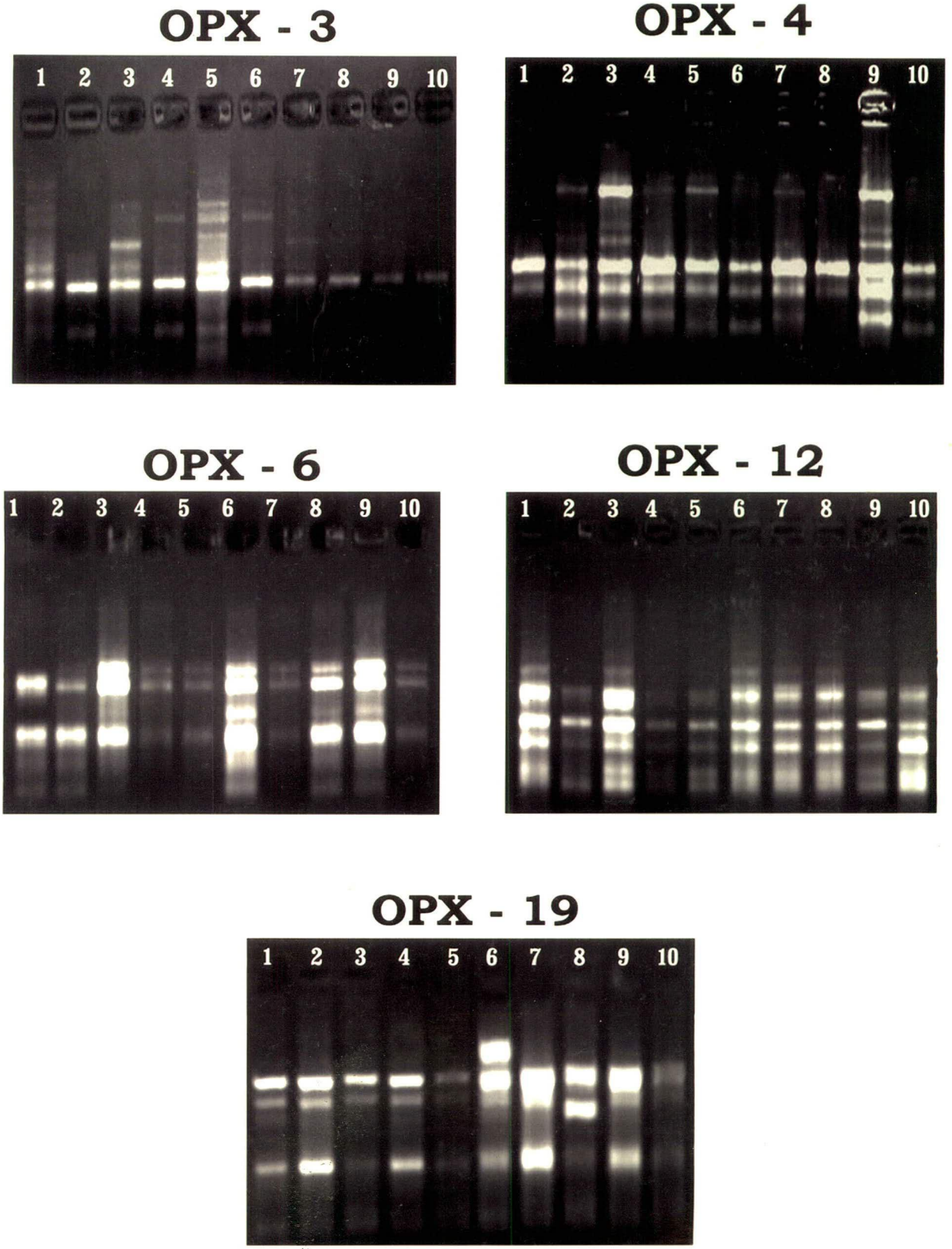
Fig. 16. Chromatogram of Andrographolide in population 5 of ***A.paniculata***

Gymnemic acid V-VII has also been reported. GA VII is the 3-O-glucuronide of gymnemagenin and GA V is the O-3-glycuronyl- 22, 21-bis-O-tigloyl substitution pattern. GA VIII-IX are also esters of saponin, have an oxoglycoside moiety attached to the glucuronic acid residue. Gurmarin, another constituent of the leaves, and gymnemic acid have been shown to block sweet taste in humans. Some researchers have suggested gymnemic acid as one possible candidate responsible for antidiabetic activity.

The five primers used to analyse genetic variation in ***Gymnema sylvestre*** produced 56 polymorphic bands (Plate.4). The same type of bands occurred at different frequencies in all populations. The genetic distance between the population ranged from 0.6562 to 0.8750 and the genetic identity ranged from 0.1335 to 0.8438 (Table.17). The overall observed and effective number of alleles is about 1.59 and 1.36 respectively. Nei (1978) overall gene diversity is 0.2169.

The dendrogram of ***Gymnema sylvestre*** (Fig.7) produced three clusters. Population 5 did not group with any cluster. Cluster 2 was the largest cluster among the 3, containing population 2, 4 , 6, 3, 9, 7, and 8. Here population 2 and 4, 3 and 9 and population 7 and 8 are closely together. Population 6 did not group with any other clade in this cluster. In the first cluster population 1 and population 10 are closely together. In the third cluster population 7 and population 8 are close together. From this it is clear that there is considerable amount of genetic variability between the 10 populations of ***Gymnema sylvestre***.

Plate 4: *Gymnema sylvestre* (Retz.) R.Br.



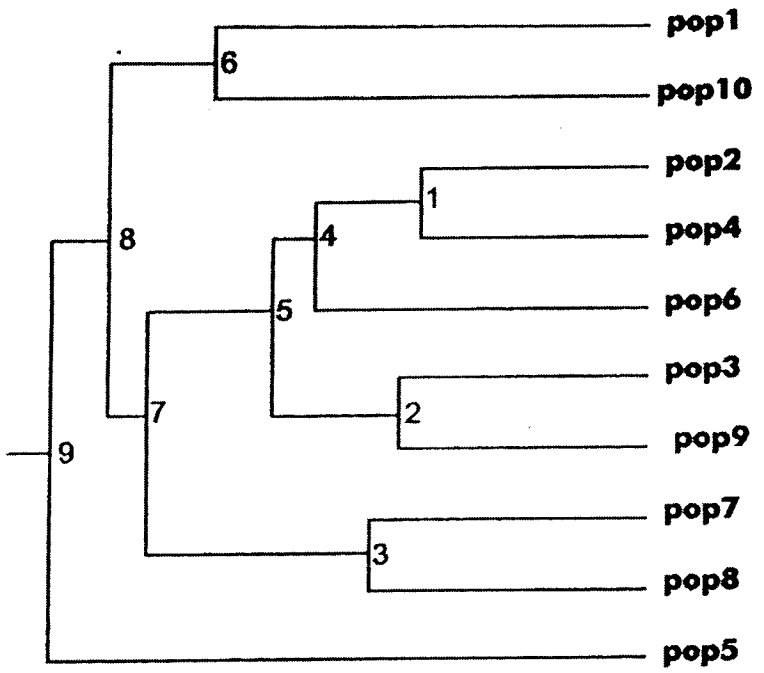


Fig. 7: UPGMA dendrogram of ***Gymnema sylvestre*** based on Nei's Genetic distance derived from RAPD data.

Table.17. Nei's Unbiased Measures of Genetic Identity and Genetic distance in *G. sylvestre*

Pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.8125	0.7500	0.6875	0.6562	0.7500	0.6562	0.7500	0.6875	0.7812
2	0.2076	****	0.8125	0.8750	0.7188	0.8125	0.7188	0.7500	0.8750	0.7812
3	0.2877	0.2076	****	0.7500	0.7188	0.8125	0.7188	0.7500	0.8750	0.6562
4	0.3747	0.1335	0.2877	****	0.8438	0.8125	0.7188	0.7500	0.8750	0.6562
5	0.4212	0.3302	0.3302	0.3302	****	0.7188	0.7500	0.5938	0.6562	0.7500
6	0.2877	0.2076	0.2076	0.2076	0.3302	****	0.7188	0.8125	0.8125	0.7188
7	0.4212	0.3302	0.3302	0.2469	0.2877	0.3302	****	0.8438	0.7188	0.8125
8	0.2877	0.2877	0.2877	0.3747	0.5213	0.2076	0.1699	****	0.8125	0.7188
9	0.3747	0.1335	0.1335	0.2076	0.4212	0.2076	0.3302	0.2076	****	0.7812
10	0.2469	0.2469	0.4212	0.1699	0.2877	0.3302	0.2076	0.3302	0.2469	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Table.18. Distance Between And population in ***Gymnema sylvestre***

Between	**And**	**Length**
9	8	2.24119
8	6	2.63643
6	Pop1	12.34300
6	Pop 10	12.34300
8	7	0.31362
7	5	4.23439
5	4	0.04946
4	1	3.70540
1	Pop 2	6.67657
1	Pop 4	6.67657
4	Pop 6	10.38197
5	2	3.75485
2	Pop 3	6.67657
2	Pop 9	6.65657
7	3	6.17086
3	Pop 7	8.49495
3	Pop 8	8.49495
9	Pop 5	17.22062

Then number of polymorphic loci and percentage of polymorphism (Table.19) was calculated by using the software Popgene package version 1.31. Among these ten populations, populations 1, 2, 7, 8, and 10, (Thirugurungudi, Manimuthar, Karayar, Papanasam RF and Courtallum RF) showed highest polymorphism. Among these five, percentage of polymorphism is higher in population 10.

The five populations which showed the highest polymorphism were selected for HPLC analysis. Since the Gymnemic acid is a complex mixture of chemical compounds, quantification of Gymnemic acid was not possible, though an attempt has been made. Hence, the genotype, which showed, highest DNA polymorphism, i.e., population 10, seems to be the superior genotype based on the results obtained from molecular analysis.

The plant contains Gymnemic acid (GA), (+) quercitol, lupeol, β-amyrin, tigmasterol etc. GA I, II, III and IV are anti sweet substance from the leaves of ***G. sylvestre.*** They all contain a glucuronic acid moiety, and the gymnemagenin aglycone esterified at position C-21 and C-28. A second series of Gymnemic acid V-VII has also been reported. GA VII is the 3-O-glucuronide of gymnemagenin and GA V is the O-3-glycuronyl- 22, 21-bis-O-tigloyl substitution pattern. GA VIII-IX are also esters of saponin, have an oxoglycoside moiety attached to the glucuronic acid residue. Gurmarin, another constituent of the leaves, and gymnemic acid have been shown to block sweet taste in humans. Some researchers have suggested gymnemic acid as one possible candidate responsible for antidiabetic activity.

Table.19. Number of polymorphic loci and percentage of polymorphism in *Gymnema sylvestre*

Population no	Number of polymorphic loci	Percentage of polymorphic loci
1	14	43.75
2	12	37.50
3	6	18.75
4	12	37.50
5	9	28.12
6	8	25.00
7	13	40.62
8	12	37.50
9	10	31.25
10	17	53.01

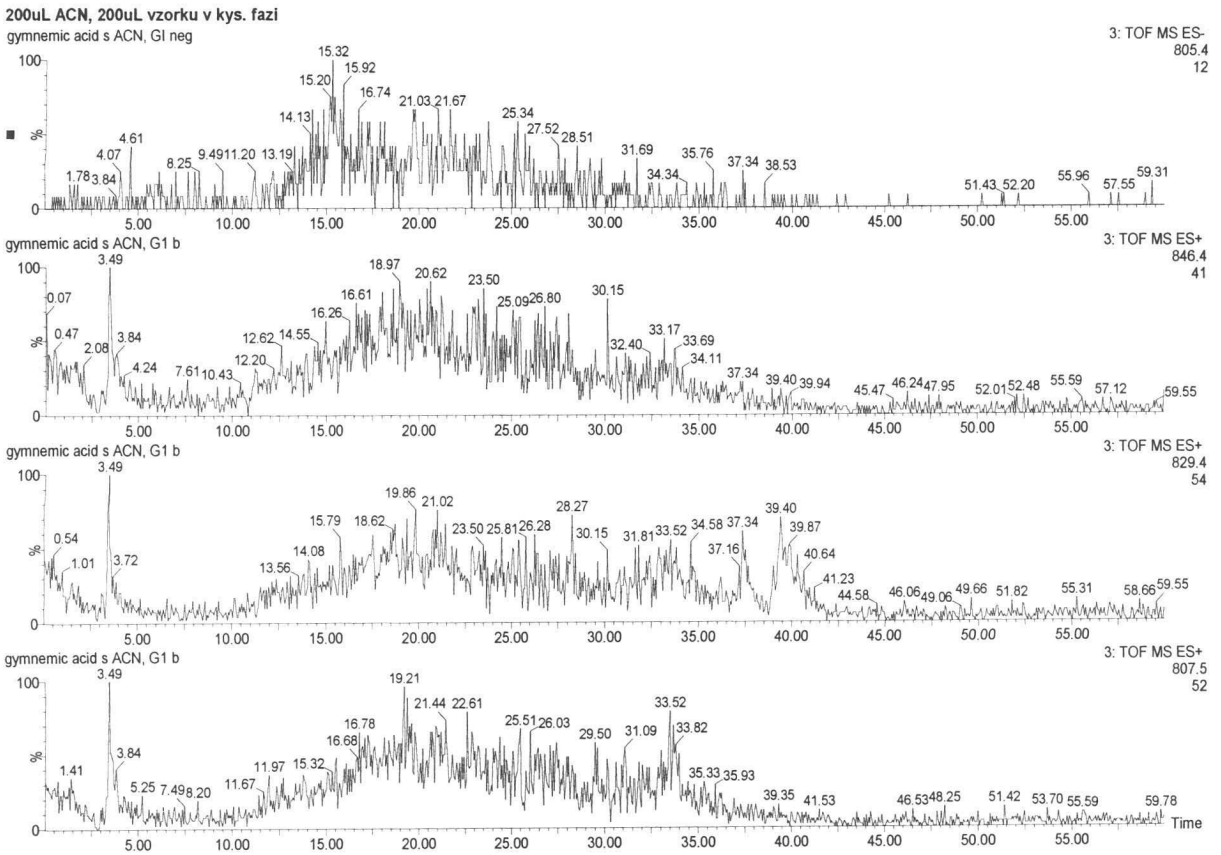


Fig. 17. Chromatogram for gymnemic acid 1

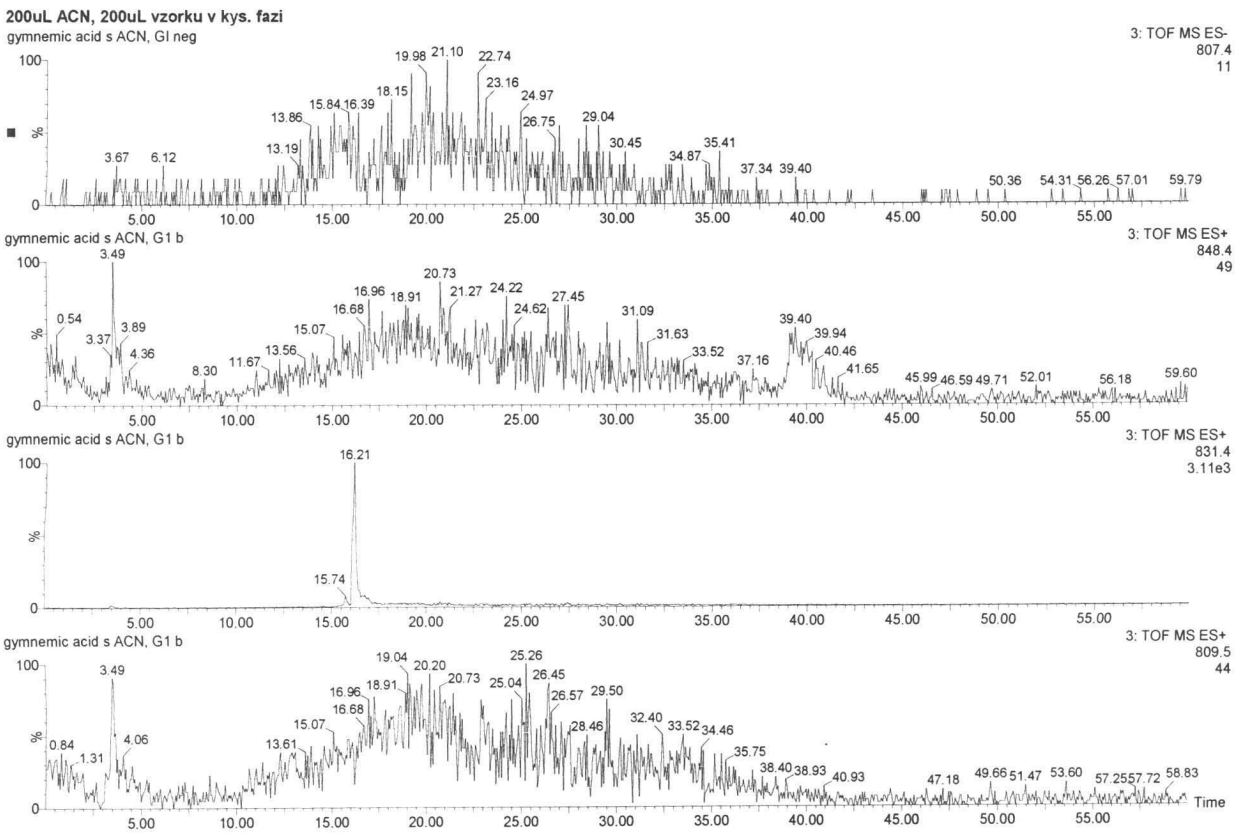


Fig. 18. Chromatogram for gymnemic acid II

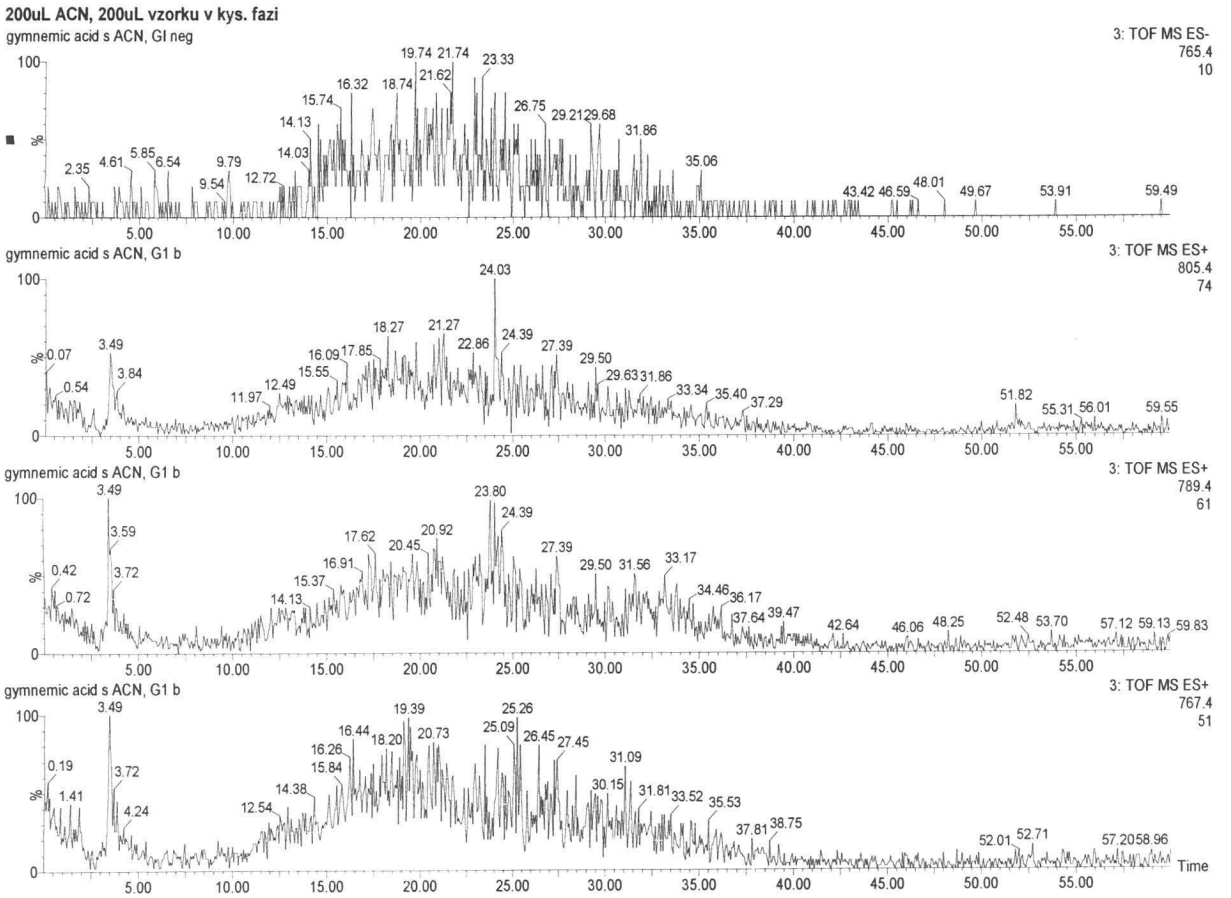


Fig. 19. Chromatogram for gymnemic acid III

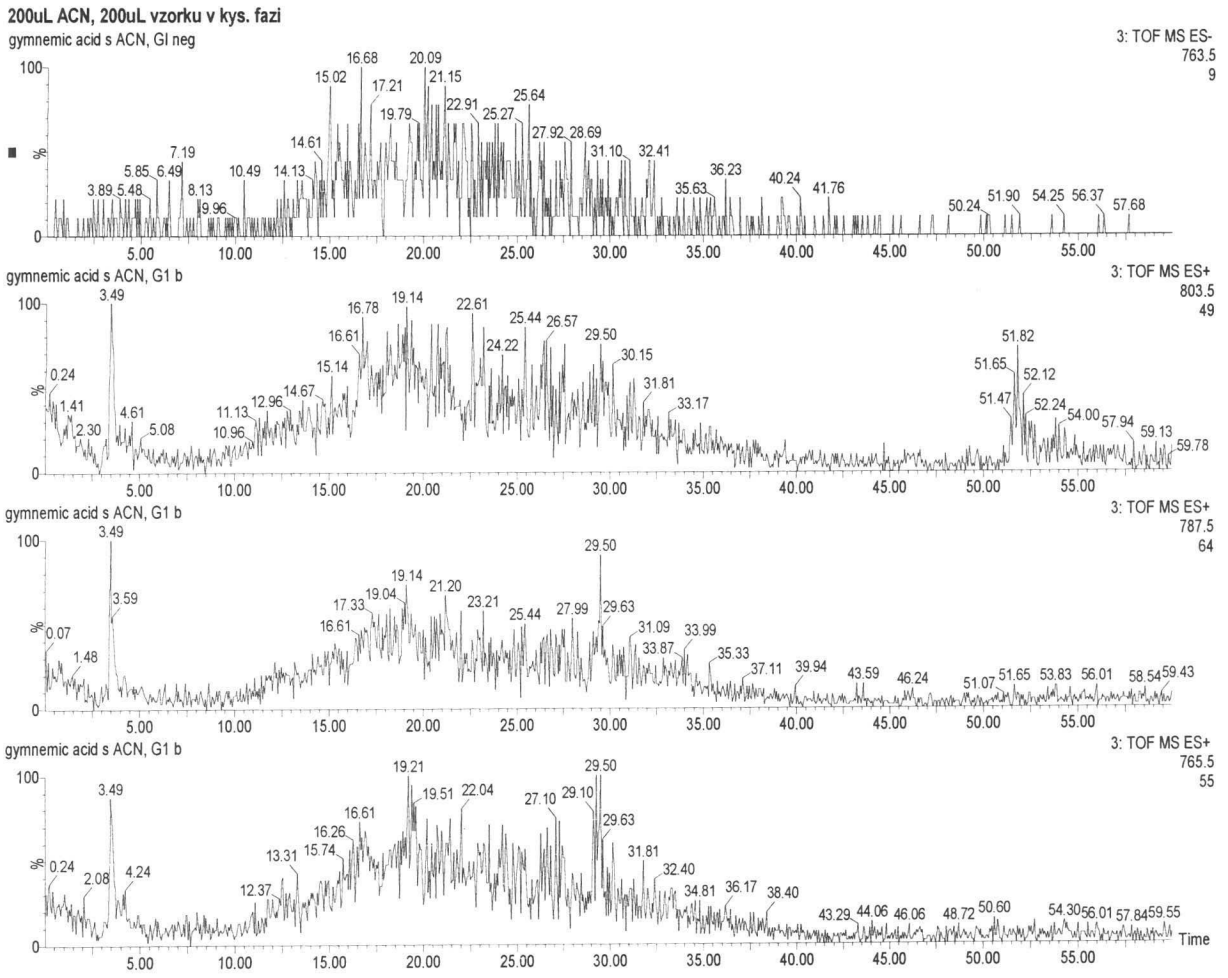


Fig. 20. Chromatogram for gymnemic acid IV

Similar type of work (RAPD) has been reported earlier by many researchers in many species.

The dendrogram generated by UPGMA distinguished ***Withania somnifera*** from ***W. coagulans*** and formed two major clusters. The dendrogram further separated ***W.somnifera*** into three subclasses corresponding to Kashmiri and Nagori groups and an intermediate type (Nagi *et al.*, 2000).

Genetic diversity of 54 populations from 22 species of ***Medicago*** collected from Iranian natural habitat was studied by Ghanavati and Mozafari (2005). RAPD markers produced by 11 UBC primers was analyzed. Populations of each species were classified in subclusters, indicating that RAPD was capable of identifying genetic diversity at the species level. Populations of one species grown under similar climatic conditions were grouped together under the cluster of that species. This indicates that genetic diversity correlates with geographical distribution of wide spread annual medic species in Iran. Results showed RAPD is applicable as a complementary tool in taxonomic identification of ***Medicago*** at both species and population levels.

Roberto *et al.*, (2001) studied that morphological, chemical and genetic differences of 12 tree basil (***Ocimum gratissimum*** L.) accessions to determine whether volatile oils and flavonoids can be used as taxonomical markers and to examine the relationship between RAPDs to these chemical markers. A distinct essential oil and flavone chemotype was found in an accession genetically more

distant from the other two groups when analyzed by molecular markers. Cluster analysis of RAPD markers showed that there are three groups that are distinct genetically and highly correlated (r = 0.814) to volatile oil constituents.

Genetic stability of plants derived from cryopreserved shoot tips was evaluated using molecular, morphological and biochemical methods. The diosgenin contents of cryopreserved-derived plants, analyzed using high performance liquid chromatography, were found to be same as those of control plants. Thus, with the experimental conditions tested, the ***Dioscorea floribunda*** plants derived after cryopreservation were found to be genetically stable at the molecular, phenotypic and biosynthetic levels (Sangeeta *et al.*, 2002).

Fifteen wild-growing plants of ***Digitalis obscura*** collected in three different regions were characterized according to their capacities to biosynthesize cardenolides and to proliferate in vitro. Great genotype-dependent variability was found in both parameters (Isabel *et al.*, 1996).

Attempts were made to develop molecular markers linked to genes determining the bisbolol and chamzulene content being of special importance for the oil quality of chamomile. The identification of closely linked molecular markers for these traits which follow a monogenic recessive mode of inheritance would facilitate pre flowering marker based selection procedures thereby enhancing the process of breeding ***camomile*** with high bisabolol and chamazulene content (Wagner *et al.*, 2003).

Lal *et al.,* (2000) reported that plant genetic resources and their conservation are the basic requirements for any plant improvement program, specially that concerning curry neem. Conservation of diversity of curry neem plant has assumed importance for genetic improvement. The development of improved varieties of curry neem with respect to high yield of essential oil of superior better quality and other medicinal constituents has been possible through existing germplasm evaluation, selection and hybridization techniques.

The collection of ***Phyllanthus amarus*** was made from various parts of India to determine the extent of genetic variability using analysis at DNA level. RAPD profiling of 33 collections from different locations was generated (Jain *et al.*, 2003).

Three albanian ecotypes of ***Oreganum vulgare*** were undergone RAPD analysis and GC analysis. Genomic DNA was isolated from leaves of individual plants and PCR amplifications were carried out. The composition of the essential oils was completed via GC. Based on the results of the RAPD analysis, essential oils composition, and their attitude toward the "in vitro" propagation conditions it was concluded that these ecotypes differ from each other and that they can not be grouped into groups of similarity (Bacu *et al.*, 2005).

Similar type of work (HPLC) has been reported earlier by many researchers in many species.

The bioactive diterpenes andrographolide and neoandrographolide from the leaves of ***Andrographis paniculata*** nees (Acanthaceae) were successfully separated by counter-current chromatography. A single 280-min separation yielded 189 mg of 99.9% andrographolide and 9.5 mg of 98.5% neoandrographolide applying water-methanol-ethyl acetate-n-hexane (2.5 : 2.5 : 4 : 1) solvent system (Qizhen *et al.*, 2003).

Kelmegh (***Andrographis paniculata Nees***) belonging to family Acanthaceae, is one of the most important herbs used in Indian traditional Ayurvedic and homeopathic system of medicine. Andrographolide and related compounds were investigated for their pharmaceutical properties and all showed varying degree of antipyretic, anti malarial and anti-inflammatory activity (Misra *et al.*, 2000).

Genetic stability of plants derived from cryopreserved shoot tips was evaluated using molecular, morphological and biochemical methods. The diosgenin contents of cryopreserved-derived plants, analyzed using high performance liquid chromatography, were found to be same as those of control plants. Thus, with the experimental conditions tested, the ***Dioscorea floribunda*** plants derived after cryopreservation were found to be genetically stable at the molecular, phenotypic and biosynthetic levels (Sangeeta *et al.*, 2002).

According to Li *et al.,* (2005) the development of an HPLC-PDA method for the simultaneous determination of bioactive diterpenoids, andrographolide, isoandrographolide, neoandrographolide, and 14-deoxy-11, 12-

didehydroandrographolide in plant materials and commercial products of ***Andrographis paniculata***.

A reverse-phase high performance liquid chromatographic method is developed for the determination of andrographolide, a characteristic diterpene lactone in commercial ***Andrographis*** (***Andrographis paniculata***) products (Li *et al.*, 2002).

A method of the quantitative analysis was established for the determination of deacylgymnemic acid (DAGA) in the alkaline hydrolysate of the sample containing gymnemic acids, which are ingredients of ***Gymnema sylvestre*** R. BR. leaves, by means of high-performance liquid chromatography. This method was used for comparing the contents of gymnemic acids in various samples (Suzuki *et al.*, 1993).

Liu *et al.,* (1992) reported that the structure of gymnemagenin (3-beta, 16-beta, 21-beta, 22-alpha, 23, 28-hexahydroxy-olean-12-ene), the sapogenin of the antisweet principles of ***Gymnema sylvestre***, was established by X-ray analysis of the 3-beta, 23; 21-beta, 22-alpha-di-O-isopropylidene derivative. On the basis of this result, the structure of deacylgymnemic acid was elucidated as the 3-O-beta-glucuronide from the carbon-13 nuclear magnetic resonance spectra. Five antisweet principles, gymnemic acid-III, -IV, -V, -VIII, and -IX, were isolated in pure states from the hot water extract of leaves of ***Gymnema sylvestre***. Of these, three (GA-III, -IV, and -V) were known, while two (GA-VIII and -IX) were new compounds. The

structures of GA-VIII and -IX were elucidated as 3'-O-beta-D-arabino-2-hexulopyranosyl gymnemic acid-III and -IV, respectively.

DISCUSSION:

The basic characteristic of life is its unlimited diversity. Plants also have morphological as well as genetic variations. Genetic variations are strictly heritable. They occur mostly due to the alterations in the DNA based sequence, the genetic material, may affect both phenotypic and chemical characteristics of plants. Variation in genes is necessary to allow organisms to adapt to ever-changing environments. However, it is actually the variation in alleles that is critical. Alleles are different versions of the same gene that are expressed as different phenotypes. New alleles appear in a population by the random and natural process of mutation, and the frequency of occurrence of an allele changes regularly as a result of mutation, genetic drift and selection.

Every diploid individual has two copies (two alleles) of each gene, one inherited from each parent. If an individual has two different versions of a particular gene, the individual is said to be heterozygous for that gene; if the two alleles are the same, the individual is homozygous. Since a population needs variation, the measure of the amount of heterozygosity across all genes can be used as a general indicator of the amount of genetic variability and genetic health of a population.

Variations that enable individuals to produce more offspring are considered to be "most fit". These variations become more frequent with each generation. Speciation occurs when individuals become genetically isolated from other groups by conditions that prevent inter-breeding. Many traits show high heritability, that is,

similarity between parents and offspring; this evidence suggests that some variation for that trait is genetically based. Environmental influences on individuals can also produce important variation, but that variation is not observed to be inherited and cannot drive evolution or adaptation. Phenotypic variation equals genetic variation plus environmental variation.

Genetic variability among all species is important to maintain since it represents the 'blueprint' for all of the living things on earth. It is important to point out that the genetic variation that a population of organisms possesses is the fuel that allows them to be able to change or evolve in response to changing environmental conditions.

Genetic variations within the population can sometimes allow a species to adapt to a changing environment. Genetic variability leads to long-term survival of species. It comes to the rescue of a species at crucial situations by lending genes that impart resistance, surveillance and higher productivity. It paves more and more avenues for crop improvement and hybridization. Species with little or no genetic variability will have greater tendency to go extinct, when a new disease, a new predator, or some other changes occur in the environment. Consequences of low genetic variability are inbreeding depression, loss of evolutionary flexibility, and out breeding depression, all of which lend to weak, sterile individuals.

Genetic variation is a factor in competition among individuals in real ecological communities. Traits with a genetic basis such as flower size are key

factors in competition among individuals. Since organisms make energetic or life history tradeoffs among traits (for example, allocating energy between growth and reproduction), genetic variability is an important factor in how populations function (Koyama and Kira 1956; Thompson and Plowright 1980; Fowler 1981; Gurevitch 1986; Goldberg 1987; Manning and Barbour 1988; Weldon *et al.*, 1988; Grace and Tilman 1990; Tilman and Wedin 1991; Pantastico-Caldas and Venable 1993; Wilson and Tilman 1993; Delph et al., 1998).

In addition to its adaptive value at the population level, genetic variation (or its lack) within individuals can affect their survival and performance. When both copies of a gene (in a diploid organism) are identical (*i.e.*, when an individual is homozygous at that gene or locus), the expression of that gene may include traits that are less beneficial to survival or reproduction in particular circumstances. This may lead to physiological or behavioral problems of genetic origin, such as malformed physical structure, poor biochemical balance, improper organ formation and function, altered social behavior, and susceptibility to disease (Chai and Chen Kang, 1976).

In one species more than one form occurs in the same area, and these forms may be adapted to different environment in which their adaptations are maintained by natural selection. Such species are said to show polymorphism. In transient polymorphism, a genetically controlled condition is in the process of spreading through the population and may ultimately lead to uniformity. In balanced polymorphism, on the other hand, two or more forms are maintained at reasonable

frequencies in the same populations. In one such condition, the heterozygote is favoured compared with either of the homozygotes, and this maintains a high degree of genetic diversity in the species (Narain, 2000).

Comparisons of the genetic distances generated by different molecular markers in diversity studies have been reported by several authors (Hahn *et al.*, 1995, Russell *et al.*, 1997, Yang *et al.*, 1996) and have revealed only moderate agreement between genetic distance estimates made using RFLP and RAPD markers. Pejic *et al.*, (1998) compared different molecular markers to assess the genetic similarities between maize inbred lines and found great differences in the RAPD similarity cluetering pattern.

In the present study, genetic variability was investigated using RAPD-PCR marker and the population which showed high percentage of polymorphism were selected. These populations were chosen for further phytochemical analysis. The active principle in these plants have been quantified by HPLC analysis. The population which exhibited both high percentage of polymorphism and high amount of active principle was considered to be the superior genotype.

SUMMARY AND CONCLUSION

SUMMARY:

Biodiversity is the variety of all the genes, species and ecosystems, which are found on our planet. It provides humanity with the cornucopia of goods and services, from food, energy and materials to the genes, which protect our crops and cure our diseases. The loss of the earth's biological diversity is one of the most pressing environmental and developmental issues. Sustainability highlights the idea that the current use of natural resources should not diminish the options of future generations, and maintaining biodiversity is clearly one of the requirements for meeting this goal.

India is the country of immense biotic wealth and as many species were used as medicinal purposes. Most of which are being exploited recklessly for the extraction of drugs. It will be prudent to study species of indigenous medicinal plants at genetic and molecular levels for efficient conservation and management of genetic diversity. Study of inter and intra- specific variation at the molecular level provides an efficient tool for taxonomic and evolutionary studies and for devising strategies to protect genetic diversity of species. Genetic variability also can be exploited to select useful genotypes that could be utilized as cultivars to avoid batch to batch variation in extraction of standard drugs. Recent global emphasis on exploitation of herbal resources and instances of patenting of developing – country plants by developed

countries emphasize the need to genetic databases on indigenous medicinal plants which can be used for future references.

Genetic variation within and among individuals is important to, among other issues, the survival, adaptation to changing environments, and reintroduction to the wild populations under captive breeding. Maintaining genetic variations is, therefore, one of the primary goals in managing the conservation of populations of endangered species.

Hence, the main objective of this study is to analyse the intraspecific genetic variations present in the chosen medicinal plants, ***Acalypha indica Linn, Andrographis paniculata (Burm.f.) Nees*** and ***Gymnema sylvestre R, Br.*** and to quantify the active principles in these plants and to select the superior genotypes in each species.

There are many molecular markers used to determine the genetic variability in plants. They are RAPD, RFLP, AFLP, ISSR, VNTR etc. RAPD has been used in this study.

Genetic variation in a population is measured by the heterozygosity or the degree of polymorphism by the phenotypic, genetic and environmental variance or any other statistical measure of dispersion. It is important to point out the genetic variation that a population of organisms possesses is the fuel that allows them to be

able to change or evolve in response to changing environmental conditions. Molecular markers are the tools, which reveal the mystery of genomes.

RAPD analysis was done to determine the intra-specific variability in the chosen three plants, ***Acalypha indica, Andrographis paniculata*** and ***Gymnema sylvestre***. The plants were collected from 10 different accessions (Courtallum, Papanasam, Kalakad, Tirukurungudi, Manimuthar, Ambasamuthram, Kadayanalloor, Krishnapuram, Vasudevanalloor, Sivagiri, puliyur, Karayar, Chenkottai, Ainthalipothigai, Nagapothigai and Agasthiamalai). Then DNA was extracted by CTAB method and using different random primers, DNA amplification was done. Based on the primary data (presence or absence of bands), pair wise genetic identity and genetic distance between samples were calculated and dendrogram was constructed. Then number of polymorphic loci and percentage of polymorphism was calculated. The five populations, which had the highest polymorphism, were selected and the active principle of these plants were quantified by HPLC. The population which exhibited both high percentage of polymorphism and high amount of active principle was considered to be the superior genotype. In ***Acalypha indica*** population 10, in ***Andrographis paniculata*** population 1 and in ***Gymnema sylvestre*** also population 10 which were collected from Courtallum seem to be the superior genotypes.

CONCLUSION:

Thus, in the present study, superior genotypes in the three most important medicinal plants such as ***Acalypha indica, Andrographis paniculata*** and ***Gymnema sylvestre*** distributed in Tirunelveli hills in Tamilnadu have been identified by both molecular and phytochemical markers.

This type of study will be useful to extract the active principle in each medicinal plant for medicinal purposes. Such genotypes can be collected and micropropagated through tissue culture and the active principle can be extracted in large quantities for various medicinal uses.

Such superior genotypes may be introduced into different areas of the forest as one of the methods of superior species recovery programme.

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LIST OF PAPERS PUBLISHED

- **Rekha G.S.,** John De Britto A., Nirmal Kumar N and Petchimuthu K. 2005. Randam amplified polymorphic DNA markers reveal genetic variation in ***Alpinia calcarata*** **Roscoe** in Kanyakumari District in Tamilnadu. Proceedings of the National Seminar on Bioprospecting of Bioresources. 8-10 December, 2005, St.Xavier's College (Autonomous), Palayamkottai.

- Nirmal Kumar N., John De Britto A., Petchimuthu K. and **Rekha G S.** 2005. Ascertaining the genetic variability of ***Leucas aspera*** (Willd.) Link in Tirunelveli hills in Tamilnadu in India. Proceedings of the National Seminar on Bioprospecting of Bioresources. 8-10 December, 2005, St.Xavier's College (Autonomous), Palayamkottai.

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RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS REVEAL GENETIC VARIATION IN *ALPINIA CALCARATA* ROSCOE IN KANYAKUMARI DISTRICT IN TAMILNADU.

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ABSTRACT

Alpinia calcarata Roscoe commonly known as Indian ginger belonging to the family Zingiberaceae , is one of the most important medicinal herbs used in Indian medicine for asthma, rheumatism, etc. The distribution of genetic variation as revealed by RAPD markers was examined in Kanyakumari District. It was observed that this medicinal plant posseses a considerable amount of genetic variation. In order to ascertain the genetic variability, plants were collected from different populations at various localities and Random Amplified Polymorphic DNA (RAPD) fingerprints were analyzed by Polymerase Chain Reaction (PCR) of genomic DNA using random primers. The RAPD fragments were scored for presence/absence, to calculate Jaccard's similarity index. Clustering based on similarity index was done following unweighted pair group with arithmetic mean method.

INTRODUCTION

Alpinia is the largest, most widespread and taxonomically complex genus in the Zingiberaceae with 230 species occurring throughout tropical and subtropical Asia. (Kress et al, 1998)*Alpinia calcarata* is one of the important species in the genus ***Alpinia*** , is commonly known as Indian ginger (snap ginger) is a perennial herb with ginger-scented rhizome. It is cultivated in plains and hills. (Mathew ., 1981). The rhizomes of ***Alpinia calcarata*** is sometimes substituted for the genuine drug. The oil obtained from ***Alpinia calcarata*** rhizome contains methyl cinnamate (48%), cineol (20-30%), camphor and probably d-pinene. A hydrocarbon is also reported to be present. The oil is carminative and in moderate doses has an anti-spasmatic action on involuntary muscle tissue, inhibiting excessive peristaltic movement of the intestine. It also possesses bacteriocidal properties and also it is used in perfumery industry. The drug is much used in rheumatism and bronchial catarrh (asthma), cough and digestive problems. It's chief use is for clearing the voice.(Wealth of India, 1985).

Genetic variability studies become important these days and many reports have been published. The collection of ***Phyllanthus amarus*** was made from various parts of India to determine the extent of genetic variability using analysis at DNA level. RAPD profiling of 33 collections. Analysis through UPGMA reavealed upto 65% variation among these accession from the Southern part of the country (Jain et al.,2003).

Genetic structure and variability were examined in the only three extent populations of the narrow-endemic tree ***Antirhea aromatica*** (Rubiaceae) ,an endangered species of the tropical forest of Eastern Mexico. The results indicated that the populations evaluated have genetic variability compared with other endemic and geographically narrowly distributed plant species, in areas with high levels of environmental heterogeneity (eg:Tropical forests) (Jorge and Gonzalo., 2004).

Ten polyembryonic and monoembryonic cultivars each traditionally grown in the West coast of Southern India were used to determine the genetic relatedness among them using RAPD markers. DNA isolation and RAPD analysis were carried out using 19 random primars, which amplified 153 polymorphic and 33 monomorphic markers. Dendrogram analysis of RAPD and chloroplast DNA ,RFLP data clearly grouped the cultivars into two based on embryo types (Ravishankar et al., 2004). According to Arambewela et al., (2004) the rhizomes of ***Alpinia calcarata*** possesses several bio-activities and are used in tradicinal medicine of Srilanka.

Kong Ly et al.,(2000), reported that four new labdane-type diterpenoids, calcarations A-D (1-4),along with six known labdane – type diterpenoids, a known elemane-type sesquiterpenoid, and a known coumarin were isolated from the rhizomes of ***Alpinia calcarata***.

In Asia, especially in China (Wu and Larsen, 2000) alpinas are *Alpinia galanga* (L.) Willd.

The area of the study Kanyakumari District is rich in biodiversity and has age old traditional medicinal practices. The Western Ghats is extended in many parts of the District.Five populations have been collected from different parts of the Western Ghats of this area. Hence an investigation has been done on the genetic variability of ***Alpinia calcarata*** distributed in Kanyakumari District in Tamil Nadu in South India.

MATERIALS AND METHODS

Samples of Alpinia calcarata were collected from Five different accessions of Kanya kumari District in South India.The collected plant materials were taken to the lab and stored in -70^0 c until DNA isolation and RAPD analysis was carried out.

TABLE 1. AREAS OF COLLECTION AND ACCESSION ID

S. No	Area	ID
1	Mothira malai	Population 1
2	Kallar view point	Population 2
3	Kothayar lower dam hills	Population 3
4	Kodutharai hills	Population 4
5	Petchiparai beat	Population 5

Isolation of DNA

Individual young fresh leaves were collected from plants, washed with distilled water and weighted for DNA extraction.The tissue sample was placed in a porcelain mortar chilled with false liquid Nitrogen and was ground with a pestle to a fine powder.Total genomic DNA was extracted using the CTAB technique (slightly modified) of Doyle and Doyle_(1987).The A_{260}/A_{280} reading of DNA ranged from 1.5 to 1.9. Amplification reactions of the RAPD method were conducted in a 25µl volume reactions mixture containing 10mM Tris-cl P^H 8.3, 50 Kcl, 15Mm $Mgcl_2$, 0.001% gelatin, 100µM each of DATP,DCTP,DGTP AND DTTP. 0.2µM of random primers, 25ng of genomic DNA and 0.5 units of Taq polymerase (Williams et al., 1990). Amplification was performed in eppendorf thermal cycler for 40 cycles of 1 minute at 94°C ,1 minute at 36°C, 2 minutes at 72°C and a final extension for 7 minutes at 72°C using the fastest available transitions between each temperature.Amplification products were analyzed by electrophoresis in 1.4% agarose gels and detected by staining with Ethidium Bromide.

Preliminary screening with 8 RAPD primers from Operon Technologies was conducted. In the final analyses, 5 RAPD primers were used (Table 2) which all produced reproducible banding patterns. Reproducibility was conformed by comparing duplicate reactions for most samples.

Table:2: Oligonucleotides used as random primers in *Alpinia calcarata* and their sequences.

No	Primer	Sequence(5 to 3)	No of polymorphic bands
1	OPX-4	CCGCTACCGA	3
2	OPX-12	TCGCCAGCCA	2
3	OPX-18	GACTAGGTGG	4
4	OPX-19	TGGCAAGGCA	6
5	OPX-20	CCCAGCTAGA	7

Data analysis

Based on the primary data (presence or absence of bands) pairwise genetic distances between samples were calculated using Popgene Package version 1.31.

RESULTS AND DISCUSSION

RAPD analysis has been widely used in numerous applications including genetic variability, gene mapping , detection of strain diversity, population analysis, epidemiology and the demonstration of phylogenetic and taxonomic relationships. Although population studies of several medicinal plant species have been examined genetically since 1990 using RAPD technique, the amount of genetic data concerning ***Alpinia calcarata*** is relatively limited. These studies in genetic variation can undermine the ability of the plant species to respond to the natural selection.This study facilitates to identify the variants of ***Alpinia calcarata*** and for selection of superior genotypes.

According to Lavania (2002) the basic unit of conservation in vogue is the morpho-species, where the role of genetics is concerned with the assessment of diversity for single loci or random DNA sequences. The five primers used to analyze genetic variation in *Alpinia calcarata* produced 22 polymorphic bands (loci) (table-3). The same type of bands occurred at different frequencies in all populations.The genetic distance between the population ranged from 0.3185_ to 0.8938_ and the genetic identity ranged from 0.4091 and 0.7273 (Table-3). The overall observed and effective number of alleles is about 1.78 and 1.58 respectively. Nei (1978) overall gene diversity is 0.3273.

TABLE:3-Nei's Unbiased Measures Of Genetic Identity and Genetic Distance.

Pop id	1	2	3	4	5
1	****	0.6818	0.4091	0.6818	0.5000
2	0.3830	****	0.7273	0.6364	0.4545
3	0.8938	0.3185	****	0.5455	0.6364
4	0.3830	0.4520	0.6061	****	0.6364
5	0.6931I	0.7885	0.4520	0.4520	****

The populations showed differences by their own genetic distance. The results suggests that there is remarkable genetic variability within the populations distributed in different locations of kanyakumari district.

RAPD and genetic variability studies are useful to distinguish taxa especially the medicinal plants.For example, *Withania somnifera* is an important medicinal plant, and it's anticancerous properties have been attributed to various classes of Withaolide compounds.

There is apparent lack of improved varieties of ***Kalmegh (Andrographis paniculata*** Nees) to make upon existing and or induced variability, genetic improvement for quantitative traits in *Kalmegh* can be achieved through a clear understanding of the nature and amount of variability present in genotypes and the extent to which the desirable traits are heritable.(Misra *et al.*, 2000).

This type of molecular systematic study is essential in such medicinal plants like *Alpinia calcarata* supported by phytochemical studies and phytochemical markers will be helpful to understand the nature of distribution and variability among them and this is important for the identification and the selection of superior genotypes and for further exploitation.

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ASCERTAINING THE GENETIC VARIABILITY OF *LEUCAS ASPERA* (WILLD.) LINK IN TIRUNELVELI HILLS IN TAMILNADU IN INDIA.

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ABSTRACT

Leucas aspera (Willd.) Link belonging to the family Lamiaceae is one of the most important herbs used in Indian traditional Ayurvedic and homeopathic systems of medicine for treatment of acrid, anti-inflammatory, antipyretic, expectorant, antibacterial, antifungal dyspepsia, verminosis, chronic skin eruptions, psoriasis, cough and catarrh in children, intermittent fevers and ulcers. The juice of the leaves is highly recommendable as eye drop in encephalopathy due to worm infestation in children and is useful as a nasal drop in catarrh and caphalalgia. Genetic improvement for quantitative traits in *Leucas aspera* can be achieved through a clear understanding of the nature and amount of variability present in genotypes and the extent to which the desirable traits are heritable. In order to ascertain the genetic variability, plants were collected from different populations at various localities and RAPD fingerprints were analyed by PCR of genomic DNA using random primers. The RAPD fragments were scored for presence/absence to calculate Jaccard's similarity index. Clustering based on similarity index was done unweighted pair group with arithmetic mean methods and a dendrogram was constructed and analyzed.

INTRODUCTION

Leucas aspera in an erect, annual herb, 50-90 cm in height, occurring in gardens, waste place and roadside area and also through out the India. It is collected from wild sources, the fresh (or) dried herbs *mainly* leaves and roots are woody. The herb is said to be possess diuretic, and also for the intermittent fevers and ulcers. The juice of the leaves is highly recommendable as eye drop is encephalopathy due to worm Infestation in children. Hence *Leucas Aspera* was selected for the genetic variation study.

RAPD markers were used to compare genetic differentiation with in the *Leucas aspera* species. The utility of RAPD markers in estimating genetic divergence has been demonstrated in several studies, which have reported close correspondence between RAPD and other molecular data sets (Zhikumu et al., 2001 and Timro et al., 2004). Because RAPD detect multiple loci per primer in many cases and can conveniently generate a large set of genetic markers, they have become increasingly common for analyzing genetic differentiation with in the species. Although population studies of several medicinal plants species have been examined genetically since the early 1990 using RAPD technique, the amount of genetic data concerning. *Leucas aspera* is relatively limited. These studies in genetic variation can undermine the ability of the plant species to respond to the natural selection. The small populations are often to the loss of alleles, through genetic drift, (or) random fluctuations in allele frequency. Hence

genetic diversity measurements are Important for selection of superior genotypes with respect to longevity of that particular species. This study facilitates to identify the variants of *Leucas aspera* and for the selection of superior genotypes.

MATERIALS AND METHODS

Plant Materials : Samples of *Leucas aspera* were collected from off road side, (Table 1) in Tirunelveli District in TN Ten plant specimens from each accession were collected. The collected plant materials were transferred to plastic bags for transport from field to laboratory. Permanent storage was at 70°c until DNA Isolation RAPD analyzed was carried out

Table 1. Collected area and the accession ID.

Collected Area	Accession ID	Collected Area	Accession ID
Sivagiri	Population 1	Alangulum	Population 6
Sankarankoil	Population 2	Ambai	Population 7
Shencottai	Population 3	Nellai	Population 8
Tenkasi	Population 4	Palai	Population 9
V.K. Pudur	Population 5	Nanguneri	Population 10

DNA Method

Individual young top fresh leaves were removed from plants, washed with sterile distilled water. The tissue sample, was placed in a porcelain mortar chilled with liquid nitrogen and was ground with a pestle to a fine powder. Total genomic DNA was extracted using the CTAB technique of Doyle and Doyle (1987). The $A_{□□}/A_{□□}$ reading of DNA ranged from 1.5 to 1.9.

Amplification reactions of the RAPD method were conducted in a 25 1 volume reactions mixture containing 10 mm Tris-CI pH 8.3,50 mMKCI, 15 $mMgCI_{□}$ 0.001% gelatin, 100 Meachp of d ATP, dGTP, and dTTP, 0.2 M of random primers, 25 ng of genomic DNA and 0.5 units of Tag Polymerase (William et al., 1990). Amplification was performed in eppendorf thermal cyclear for 45 cycles of 1 min 94°c at 36°c 2 min at 72□ c and a final extension for 7 minutes at 72°c using the fastest available transitions between each temperature. Amplification product were analyzed by electrophoresis in 1.4% agarose gets and detected by staining with Ethidium Bromide. Preliminary screening with 9 RAPD primers from operon Technologies was conducted. In the final analysis, 5 RAPd primers were used (Table 2) which all produced reproducible banding patterns.

Table 2. Oligonucleotides used as random primers in *Leucas aspera*

Primer	Sequence (5' to 3)	No. of bands
OBP-13	TTCCGCCACC	11
OBP-8	TGGCGCAGIG	17
OBP-5	CCGCTACCGA	05
OBP-11	TGGCAAGGCA	16
OBP-20	CCCAGCTAGA	09

Data analysis

Based on the primary data (presence (or) absence of Lands), per wise genetic distance between samples were calculated using pop gene package version 1.31.

RESULTS AND DISCUSSION

Nei's Unbiased Measures of Genetic Identity and Genetic distance

POP ID	1	2	3	4	5	6	7	8	9	10
1	****	0.7872	0.6809	0.6809	0.7447	0.5319	0.6809	0.5106	0.5745	0.6383
2	0.2392	****	0.7660	0.8085	0.8298	0.7021	0.7660	0.5957	0.6596	0.6809
3	0.3844	0.2666	****	0.7021	0.7234	0.6383	0.6596	0.5319	0.5532	0.6170
4	0.3844	0.2126	0.3536	****	0.8085	0.5532	0.7447	0.6170	0.5532	0.6170
5	0.2948	0.1866	0.3238	0.2126	****	0.6170	0.8085	0.3683	0.6170	0.6809
6	0.6313	0.3536	0.4490	0.5921	0.4829	****	0.5957	0.6809	0.6596	0.6809
7	0.3844	0.2666	0.4162	0.2948	0.2126	0.5179	****	0.7021	0.5957	0.7872
8	0.6721	0.4162	0.6313	0.4829	0.4490	0.3844	0.3536	****	0.7234	0.7447
9	0.5543	0.4162	0.5921	0.5921	0.4829	0.4162	0.5179	0.3238	****	0.7234
10	0.4490	0.3844	0.4829	0.4829	0.3844	0.3844	0.2392	0.2948	0.3238	****

The polymorphic bands (loci). The same type of band, occurred at different frequencies in all population. There was many additional bands neglected which ere irreproducible. The genetic distance between the population ranged from 0.3 and the genetic identity ranged from

The overall observed and effective number of alleles in about 2 and 1.21 respectively Nei(1978) over all gene diversity in 0.5018.

In the dengrogram based on UPGMA obtained, the populations were highly differenentiated by their own genetic distance. The absence of origin of genetic variation with in the species and the clustering the result of different accession suggest that *Leucas aspera* undergo major part of genetic variation by environmental factors. By analyzing the RAPD data they are inherited as dominants although

the banding patterns cannot be used to analyze gene diversity Esselman *et al.*, (2004). This information could be very valuable in the management of genetic resources in this economically important species .

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PRELIMINARY PHYTOCHEMICAL STUDIES ON A MEDICINAL PLANT *MIMOSA PUDICA* L. (MIMOSACEAE)

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ABSTRACT

A preliminary phytochemical analysis was carried out in *Mimosa pudica* L. a very useful and much exploited medicinal plant of Tirunelveli Hills in Tamil Nadu. The air – dried leaf powder was extracted with polar and non-polar solvents such as Petroleum ether (40-60°C), Benzene, Chloroform, Ethanol, and Distilled Water. Physico-chemical parameters, fluorescence analysis and phytochemical analysis were carried out. The preliminary study will be helpful to study the active principles using modern techniques in the later part of this work.

INTRODUCTION

The quality control of medicinal plant materials, which are used worldwide as folk medicine or raw materials for the pharmaceutical industry, has always been one of the main concerns of the World Health Organization (WHO). Therefore, WHO organized the meetings of expert from various countries to establish internationally accepted guidelines for assessing the quality of medicinal plants so that they can be used by the regulatory agency in each country to set up the national quality specifications of medicinal plant materials. The quality control of medicinal plant materials that all parties involved in the production of herbal medicine need to understand in order to manufacture good quality, effective and safe products.

Mimosa pudica L. is belong to the family Mimosaceae, widely distributed in throughout India and probably native of South America (Mathew, 1981). *Mimosa pudica* L. is commonly known as Sensitive plant in Tamil name "Thottaar Sinungi", which is used to cured piles, diabetes and healing of wound. Root decoction used in gravel and other urinary complaints (Agarwal, 1997 and Yoganarasimhan, 1996). Leaf juice is used to dressings for sinus, sores, piles, fistula, wounds, cuts and paste applied on hydrocele and as cure for swelling of leg of cattle (Vaidyaratnampsvariers, 1994, Pal. D.C and Jain.S.K, 1998). Ayurvedic medicinal uses of whole plants are cure diarrhoea, dyspnoea, leprosy, urine disorders, healing of wounds, oedema, burning sensation Yoganarasimhan (1996). He also reported that the medicinal uses of Siddha medicine to cure certain diseases like diabetes, sinus, wound, eye diseases, diseases of vatam, urinary calculi (Yoganarasimhan, 1996). Leaf paste was given early in the morning along with milk for diabetes. Garland made from the roots was tied around the neck of the child for whooping cough (Vedavathy et al. 1997).

Mucilage, galactose and mannose were isolated from seed. Phytochromes and adrenaline were identified in the leaf extract (Yoganarasimhan, 1996 and Shankar Gopal Joshi, 2003). The present study of preliminary phytochemical and pharmacognostical aspects of *Mimosa pudica* L. was investigated.

MATERIALS AND METHODS

Plant materials of *Mimosa pudica* L.was collected from Kalakkad Mundanthurai Tiger Reserve forest, Papanasam (Lower dam) during the month of April 2005. Plant specimens were identified using the flora of Tamil Nadu Carnatic. The leaves of *Mimosa pudica* L. were shade- dried and powdered. About 50g of this powder were extracted with Petroleum ether, benzene, chloroform, alcohol and water in a Soxhlet apparatus separately. The extraction process was performed for 5hr.The behavior of the powdered treated with different chemical reagents and fluorescence character was observed in ordinary and under UV light based on the method described by Kokoski et al., (1958). Determination of physicochemical parameters such as extractive values, ash value was followed by the methods of African Pharmacopoeia (1986) and Anonymous (1966). Preliminary phytochemical screening was performed by according to Brindha *et al.*, (1981) method.

RESULTS AND DISCUSSION

Macroscopic characters

Spreading herb; branchlets glabrescent (or) hispid, Pickles short, erect or curved to 4mm. Leaves to 4cm. Pinnae 2 or 3 pairs 4.5-6cm; leaflets 14-20 pairs elliptic- oblong Overlapping, hispid; Base truncate-obtuse margin ciliate; apex acute, Petiole to 3cm, rachis and petiole hispid, stipules, lanceolate, stipules linear. Flower head axillary 2 or 3 in a cluster, oblong - globose, peduncle bracteoles, linear. Flowers 0.6m across, bisexual; calyx tube 4 lobed; Petals 4, pink; stamens 4, filaments to 6mm. Ovary subsessile, globose. Pods clustered flat, slightly undulate jointed, bristly along margins, apex obtuse, horned seeds 2-5 compressed ovoid.

The moisture content is 11.28%; total ash is 5.51%, water soluble ash is4.32 % and Acid insoluble ash is 1.93%. The maximum percentage of alcohol extract yield was 5.12% (w/w) and the minimum percentage of chloroform extract yield was 2.78% (w/w). Most of the extracts are orange, light green under UV light and brown, green under ordinary light except the water extract, which is yellow in ordinary light and brown under UV light. Regarding the chemical constituents the secondary metabolites such as Steroids, Triterpenoids, Reducing sugars, Sugars, Alkaloids, Phenolic compounds, Flavonoids, Catechins, Saponins, and Tannins have been reported from the different extracts of the leaves.

Many workers did similar kind of studies earlier. Raveendra Retnam and John De Britto (1998) dealt with the preliminary phytochemical screening of results obtained for bioactive constituents of amino acid, sugar and flavonoids of medicinal plants. Roychandhary and Nandi (1998) studied microscopical characters on the leaf samples obtained from different plant sources. Muthulakshmi and Indra

Ponjeyanthi (2001) studied macroscopic and microscopic character of two species and it shows their distinctness. Thus this type of preliminary phytochemical analysis is the first step towards understanding the nature of bioactive principles in medicinal plants and this type of study will be helpful for further detailed study.

Table 1: Physical constants

Physical constants	Values
Moisture	11.28
Total ash	5.51
Water soluble ash	4.32
Acid insoluble ash	1.93

Table 2: Extractive values of leaves

Solvents	Extractive values (%)
Petroleum ether	3.89
Chloroform	2.78
Acetone	4.12
Ethanol	5.12
Distilled water	4.79

Table -3: Color and fluorescence analysis of different extracts of leaf of *Mimosa pudica* L.

S.No	Particulars of treatment	Day light	Under UV light	
			365nm	255nm
1	Powder as such	Pale green	Black	Light green
2	Powder + 1*N* NaoH	Brown	Dark brown	Green
3	Powder + HCL	Light brown	Brown	Green
4	Powder + H_2SO4	Dark brown	Dark brown	Dark brown
5	Powder + HNO_3	Pale green	Brown	Light green
6	**Extracts**			
a.	Ethanol	Dark green	Orange	Light green
b.	Benzene	Light brown	Orange	Light green
c.	Chloroform	Light brown	Orange	Light green
d.	Petroleum ether (40-60 C)	Light Yellow	Orange	Green
e.	Water	Yellow	Brown	Green

Table 4: Preliminary phytochemical screening of leaf of *Mimosa pudica* L.

S.No	Phytochemicals	Extract(s)				
		Ethanol	Benzene	Chloroform	Petroleum ether (40-60 C)	Aqueous
1	Steroids	+	+	+	+	-
2	Triterpenoids	+	-	-	-	-
3	Reducing sugars	-	-	+	-	-
4	Sugars	+	+	+	+	+
5	Alkaloids	+	-	-	-	+
6	Phenolic compounds	+	+	+	+	+
7	Flavonoids	+	+	+	-	-
8	Catechins	+	+	+	-	+
9	Saponins	+	+	+	+	+
10	Tannins	-	-	-	-	+
11	Anthroquinones	-	-	-	-	-
12	Amino acids	-	-	-	-	-

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Wrightria tinctoria, Anogeissus pendula, Anogeissus latifolia and *H. integrefolia* etc. have very less importance value index. Half area of middle zone is covered by open forest, 27% by degraded forest and rest is dense forest. Average biomass density of open forest is 50.57 t ha^{-1} while it is 100.23 t ha^{-1} for dens forest. In upper zone 50% area is under open forest. Here dominant species includes *Dendrocalamus* Spp., *D. melanoxylon, M. tomentosa, Terminalia tomentosa, Wrightia tinctoria, A. marmelos.* And *Albizzia lebbeck, B. monosperma, A. pendula* are less abundant in the upper elevation. Upper zone have less forest cover due to less soil depth, excessive water run off and high wind velocity. Association index of sample plots shows that lower and middle zone are more similar than middle and upper. Higher plant diversity among woody plants was noticed in middle (300msl to 600msl) altitude zone while lower zone (blow 300msl.) and upper zone (above 600msl.) were on second and third position respectively. Highest biomass of the forest was found in the middle zone due to high abundant and frequency of the species.

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PI-05 DETERMING THE GENETIC VARIABILITY IN DIOSCOREA ALATA L. IN TIRUNELVELI HILLS IN TAMILNADU

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Dioscorea alata commonly known as "Winged Yam" belonging to the family Dioscoreaceae is one of the most important medicinal herbs used in Indian medicine for asthma, rheumatism, and food for cattles. The distribution of genetic variation as revealed by RAPD markers was examined in population of this species from Tirunelveli district. It was observed that this medicinal plant possesses a considerable degree of genetic variation. Random Amplified Polymorphic DNA (RAPD) fingerprints were analyzed by polymerase chain reaction (PCR) of genomic DNA using random primers. The RAPD fragments were scored for presence/absence, to calculate Jaccard's similarity index. Clustering based on similarity index was done following unweighted pair group with arithmetic mean method and a dendrogram was constructed and analyzed. The five primers used to analyze genetic variation in this plant produced 35 polymorphic bands (loci). The same type of bands occurred at different frequencies in all populations. The genetic distance between the population ranged from 0.2157 to 07623 and the genetic identity ranged from 0.3231 and 0.6275. The overall observed and effective number of alleles is about 2 and 1.58 respectively. The overall genetic diversity according to Nei's index is 0.4022. Considerable amount of genetic variability is observed. Reasons for such variability is discussed.

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