Chapter 2

Review of Literature
Review of literature

Chlorophytm borivilianum

Chlorophytm borivilianum Santpau and Fernandes (safed musli) is an important endangered medicinal plant of Liliaceae family and holds principle place in Ayurvedic and Allopathic system of medicine. The fleshy roots (fasciculated) are widely used as a natural "sex tonic" and are an integral part of more than 100 herbal drug formulations (Oudhia, 2001a). Presently a number of health tonics (sexual tonics) are prepared from it as a traditional diet of mothers (after delivery) in the form of "Laddoos". Besides, it is a supplementary therapy for blood purification, nervous disorder and some gynecological problems (Ramawat et al., 1998). The roots of the plant contain steroidal saponins viz. neotigogenin, neohecogenin, stigmasterol and tokorogenin as secondary metabolites. In addition to saponins, it is also a rich source of over 25 alkaloids, vitamins, minerals, proteins, carbohydrates and polysaccharides etc. This species has been successfully introduced recently in the farming system, to meet the increasing demand of raw material and to stop indiscriminate exploitation of natural resources. Although this species has been brought under commercial cultivation through systematic research and evaluation of germplasm (Gupta et al., 1988), the crop still demands systematic breeding research to earn foreign exchange. Thirteen species of Chlorophytm that are found in India are collected from their natural habitat and after processing, traded as safed musli. There is a large genetic variation in the material being cultivated (Kothari et al., 2001). However, maximum diversity was observed in root shape, size and colour, respectively.

Origin of Plant

The origin of safed musli can be traced back in the oldest mountain ranges on the continent, the Aravalis from where it spreads to the near-by areas of the sub-continent. Presently it is grown in the states of Gujarat, Rajasthan, Madhya Pradesh and the Central Deccan Plateau. Although Indian forests are rich in safed musli and demand is increasing rapidly in Indian and International drug markets, foreign demand has been estimated as 300-700 tonnes annually (Bordia et al., 1995), a quantity that Indian forests cannot sustain. This has created a pressure on Indian forests and if steps for timely conservation are not taken, the Indian forests will lose this valuable plant (Oudhia, 2001b). At present
the availability of *Chlorophytum* is decreasing and obnoxious weeds like *Parthenium hysterophons* and *Lantana* are taking its place (Oudhia, 1996).

**Climate and soil**

Safed musli requires warm and humid climate for optimum plant growth and root development. Areas receiving 50-150 cm annual rainfall, the major part of which is received during rainy season (July-October), are considered suitable for its cultivation. Too high day temperature (35°C and above), and excessive rainfall and relative humidity do not favour growth and development of the root. Considering the above conditions, several areas in Uttar Pradesh, Andhra Pradesh, Madhya Pradesh, Chhattishgarh, Gujarat, Rajasthan, Jharkhand, Haryana, Maharashtra and Bihar states are considered suitable for safed musli cultivation. Well drained sandy loam and loam soils rich in organic matter are most suited. Porous soils with high organic matter content help in optimum root development. The *C. borivilianum* is highly susceptible to water logging, and water stagnation for a period of 1-2 days may cause heavy damage to the plantation. Soils with high calcium carbonate content and high pH (8.0 and above) should be discarded as the availability of several major and micro nutrient, especially iron, becomes a limiting factor in such soils to which *C. borivilianum* is highly susceptible (Aparbal et al., 2004).

**Taxonomic classification**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Phanerogames</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class/Subphylum</td>
<td>Monocotyledones</td>
</tr>
<tr>
<td>Series</td>
<td>Coronaricaceae</td>
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<tr>
<td>Family</td>
<td>Liliaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Chlorophytum</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>borivilianum</em></td>
</tr>
</tbody>
</table>

**Growth behavior**
It is annual herb and in nature propagated by seeds and tuberous roots, but encounters the problems of low seed set, viability and germination. Further the population of plant is dwindling at an alarming rate due to over exploitation from wild conditions (Bordia et al., 1995; Arora et al., 2004), resulting into an endangered species (Nayar and Shastri, 1998).

Root

Root tubers are fleshy, fascicled, cylindrical, 5-20 in number and directly originate from the stem disc devoid of any fibrous structure. The tubers are 10-25 cm x 1-2 cm in dimension.

Leaves

Radical, 6-13 in number, 13-23 cm x 1.0 -1.75 cm in size, spirally imbricate at the base, sessile, linear or ovate, acute apex, slightly narrowed at the base. Margins are wavy and parallel venation.

Inflorescence

Scape solitary, terete, 15-30 cm long, terminal, unbranched and bears flowers on above upper 3/4th of its length.

Flowers

Small, white, bracteate, pedicillate, zygomorphic, usually arranged in alternate clusters, each cluster comprising of 3 flowers; the flower clusters are dense on the upper part of the scape, bracts are linear, papery and purplish, 1.0-10.5 cm long, pedicle whitish, 6-10 mm long, jointed and kneeed at the joint.

Perianth

Tepals white, 6 in number arranged in two whorls of 3 each, linear, membranous, acute, 3-5 nerved, imbricate aestivation.
Androecium
Stamens 6, arranged in two whorls situated opposite to tepals, united to the perianth, as long as perianth, dithecus, filaments are glabrous, anthers are yellow, linear and dehisces by longitudinal slits.

Gynoecium
Tricarpellary, syncarpous, ovary superior, adnate at the base of the perianth, trilocular with axile placentation, angles obtuse, green, glabose, ovules numerous in each locule, style single, slightly longer than the stamens.

Fruit and Seeds
A loculicidal capsule, green to yellow coloured, triquertous to 3-sulcate, almost equal in length and breadth. Seeds are endospermic, onion like, black coloured, angular, each capsule bearing 14-16 seeds.

Chromosomes - 2n=4x=28 (Lavania et al., 2005)

Floral Formula: + P (3+3), A (3+3) G (3)

Identification of Chlorophytum sp. with morphological marker
The morphological comparison of the two species *C. borivilianum* and *C. arundinaceum* reveals that *C. borivilianum* supports an annual growth habit (aerial parts dry up), and *C. arundinaceum* is perennial. The inflorescence is usually unbranched in *C. borivilianum*, but branching is commonly encountered in *C. arundinaceum*. Stomata are absent on the adaxial leaf epidermis of *C. borivilianum*, but present in *C. arundinaceum*. Stamens are free and arranged away from one another in *C. borivilianum*, while they are closely packed in *C. arundinaceum*. Stamen measurements also differ between the two species (filament is about 0.78 cm and anther about 1.44 cm in *C. borivilianum* and about 0.3 and 1.02 cm in *C. arundinaceum*) respectively. Fleshy roots are colored to creamy in *C. borivilianum*, while it is creamy white in *C. arundinaceum*. Of course, the saponin profile is same in both species (Maiti and Geetha, 2005).
Identification of different species
Of safed Musli (*Chlorophytum sp.*)

- C. brevescapum
  - Flower sparse or less in number
- C. attenuatum
- C. tuberosum

C. brevescapum
- Petiolate scape 3” 6”

C. attenuatum
- 1. Not petiolate
  - 2. Scape as long as the leaves

C. tuberosum
- 1. Not petiolate
- 2. Scape longer than leaves

Tubers attached at the end of fibrous root

C. tuberosum
- *Without sepal gland
- *7-9 veins in the petals
- C. borivilianum
- Tuber sessile.
  - With special glands.
  - 3 veins

(Odhia, 2003)

Species diversity
The genus *Chlorophytum* is distributed in the tropical and subtropical regions of the world and represented by about 175 valid taxa of rhizomatous herbs (Bordia et al., 1995). This species came into the prominence around the late Eighties. In Genera Plantarum, Bentham and Hooker (1880) reported 40 species of *Chlorophytum* distributed in Asia, tropical Africa, America and Australia. Cooke (1908) also mentioned about 40 species distributed in tropical and subtropical parts of the world. The probable center of origin of...
the genus is believed to be tropical and subtropical Africa, where about 85% of the species are found. The species distributed in India are *C. heyneanum* Wall., *C. breviscapum* Dalz., *C. arundinaceum* Baker., *C. glaucum* Dalz., *C. tuberosum* Baker., *C. khastianum* Hooker., *C. attenuatum* Baker., *C. malabaricum* Baker., *C. undulatum* Wall syn. *C. nepalense* (Lindl) Baker., *C. orchidastrum* Lindl., *C. laxum* Br. and *C. borivilianum* Sant. & Fernand (Hooker, 1894; Santapau and Fernandes, 1954; Purohit et al., 1994b). Aundhe and Deokule (2001) reported 10 species from Maharashtra and classified them on the basis of their root morphology. These species are *C. borivilianum*, *C. bharuchae*, *C. orchidastrum*, *C. arundinaceum*, *C. glaucum*, *C. attenuatum*, *C. glaucoides*, *C. breviscapum*, *C. laxum*, *C. tuberosum* and *C. borivilianum* are distributed mainly in southern Rajasthan, northern Gujarat and western Madhya Pradesh in India.

The comparative chemical composition and systemic studies related to medicinal properties of different species of *Chlorophytum* have not worked out so far. Different *Chlorophytum* species (medicinal) have a specific area of occurrence in India (Bordia et al., 1995). The *C. arundinaceum* Baker has been reported to occur in all the districts of Chhota Nagpur (Haines, 1961), parts of central India (Daljeet, 1974) and foothills of north-east Himalaya in Assam, West Bengal and Bihar (Chadha, 1980). The *C. attenuatum* Baker mostly occurs in Western Ghat from Karnataka, Southward to Coimbatore (Hooker, 1894). The natural habitat of *C. borivilianum* is Southern Rajasthan, Western Madhya Pradesh, and North Gujarat.

**Present scenario**

Recently, *Chlorophytum borivilianum* was brought into cultivation, and presently, it is cultivated in about 400 ha area mainly in Andhra Pradesh, Maharashtra, Madhya Pradesh, Chhattisgarh, Rajasthan, Gujarat, Utter Pradesh etc. The processed and dry tuber yield (1.0-1.2 t/ha) (Kothari et al., 2001) is very low mainly due to non-availability of agro technology and high yielding cultivars. There is a large genetic variation in the planting material being cultivated (Kothari et al., 2001). Presently, the majority of growers sell raw tubers of safed musli in the form of planting material because of its economic advantages.
Phyllanthus amarus Seum. & Thonn

Phyllanthus amarus commonly known as Bhuiamla in India is a member of Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. This is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis, 1977) and is mainly a pan-tropical family with some species either more or less temperate.

Phyllanthin and hypophyllanthin present in Phyllanthus amarus are reported as hepatoprotective agents and protect hepatocytes against carbon tetrachloride (CCl₄) and galactosamine induced cytotoxicity in rats (Syamsundar et al., 1985). A variety of natural products have been found to inhibit unique enzymes and proteins crucial to the life cycle of HIV including efficient intervention with the reverse transcription process, but also virus binding, the integrase or protease (Vlietinck et al., 1998; De Clercq, 2000; Jung et al., 2000; Cos et al., 2004). Inhibition of HIV has been demonstrated for P. amarus among other Phyllanthus species in vitro (Cutrone et al., 1996; Ogata et al., 1992), and was previously confirmed also for drug resistant HIV strains (Notka et al., 2003). The gallotannins, especially corilagin and geraniin were very potent inhibitors and participate to a high degree in P. amarus antiviral activity (Notka et al., 2004).

Taxonomic classification of plant:

Kingdom: Plantae
Division: Angiospermae
Class: Dicotyledonae
Order: Tubiflorae
Family: Euphorbiaceae
Genus: Phyllanthus
Species: amarus Scum. & Thonn

Centre for Transgenic plant Development, Jamia Hamdard
Botanical description of plant:

Stem
Plant grows up to 10-60 cm tall, erect, stem terete, younger parts rough, cataphylls 1.5-1.9 mm long, deltoid acuminate.

Leaf
Leaf size 3.0-11.0 x 1.5-6.0 mm, elliptic oblong to obate, obtuse or minutely apiculate at apex, obtuse or slightly inequilateral at base.

Flower
Flowers axillary, proximal 2-3 axils with unisexual 1-3 male flowers. Male flowers: pedicel 1mm long, calyx 5, sub equal 0.7x 0.3mm, oblong, elliptic, apex acute, hyaline with unbranched mid rib, disc segments 5, rounded stamen 3, filament connate.
Female flowers: pedicel 10.8-1.0 mm long. Calyx lobes 5, 0.6 x 0.25mm, ovate oblong, acute at apex; disc flat deeply 5 lobed, lobes often toothed at apex, styles 3, free shallowly bifid at apex. Capsule: 1.8 mm in diameter, oblate and rounded. Seeds about 0.9 mm long, triangular with 6-7 longitudinal ribs and may transverse striations on the back.

Chromosome number: 2n =56 (Webster, 1975)

Biogeography and Ecology
The *P. amarus* is widely distributed in all tropical regions of the planet. Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas (Cabieses, 1993; Tirimana, 1987), the Philippines or India (Cabieses, 1993, Chevallier, 2000). It is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993; Nanden, 1998).

Origin and Distribution
Plants in the genus *Phyllanthus* can be found around all tropical regions of the world from Africa to Asia, South America and the West Indies. It was first identified in central
and southern India in 18th century. This genus contains about 550 to 750 species in 10-11 subgenera (Unander, 1995). This plant is a common Arabic weed of disturbed ground in southern Florida, the Bahamas, the West Indies, tropical America and is naturalized in the Old World tropics. It is usually misidentified with the closely related *Phyllanthus niruri* L. in appearance, phytochemical structure and history of use. *Phyllanthus niruri* reaches a length of 60 cm, the fruits are larger, and the seeds are dark brown and warty (Morton, 1981).

**Ethnobotanical Uses**

In many countries around the world the genus *Phyllanthus* are used in folk remedies and has a great importance in traditional medicine (Foo, 1993). Phytochemicals occur in various parts of plant and their functions are diverse which include provision of strength to plant, attraction of insects for pollination and feeding, defense against predators, provision of colour, while some are simply waste products (Ibegbulem et al., 2003). These secondary metabolites exhibit variation in biochemical and pharmacological actions in animals when ingested (Trease and Evans, 1983). This genus has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites. According to Foo and Wong (1992), in a number of countries, the aerial part of *P. amarus* is highly valued in traditional medicines for its healing properties (Table 1). It has shown to work as an antifungal, antibacterial and antiviral agent (Houghton et al., 1996). It is mainly used to treat liver diseases, asthma, bronchial infections and cardiovascular problems (Foo and Wong, 1992; Chevallier, 2000). Besides, this plant is also used in the most popular Ayurvedic formulations, Chyawanprash, which is consumed at large scale, not only in India but also throughout the world (Ratna Bhushan, 2003).

Interest in this plant has been heightened by reports of antiviral activities and its potential as a remedy for hepatitis B virus infection (Thyagarajan et al., 1988). Different plant parts are also ethnobotanically reported to have various therapeutic activities e.g. leaves as expectorant, diaphoretic and useful in Strangury and sweats, the seeds as
carminative, laxative, astringent to the bowels, tonic to the liver, diuretic, diaphoretic, useful in bronchitis, carache, griping, opthalmia and ascites (Kirtikar and Basu, 2001).

<table>
<thead>
<tr>
<th>Location</th>
<th>Ailment treated / Properties and Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahamas</td>
<td>Appetizer, ♦Colds, Fever, ♦Flatulence*, Flu, Stomachache,</td>
</tr>
<tr>
<td>Barbados</td>
<td>♦Arbortifacient</td>
</tr>
<tr>
<td>Cuba</td>
<td>Edema and Malaria</td>
</tr>
<tr>
<td>India</td>
<td>Appetizer, Asthma, Bronchial infections, Diuretic, Dyspepsia,</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Colic, Cough, Diuretic, Eye diseases (external), Kidney diseases,</td>
</tr>
<tr>
<td>Island of North</td>
<td>♦Fever, Prevention of intestinal worms</td>
</tr>
<tr>
<td>Trinidad</td>
<td>Diuretic, Venereal diseases</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Diabetes, Dysentery, Diuretic, Edema, ♦Gonorrhea, Jaundice,</td>
</tr>
<tr>
<td>Aruba</td>
<td>Blood purifier</td>
</tr>
</tbody>
</table>

Morton, 1981; Raghoenandan, 1994
♦ = Combined with other plants
* = Only roots

**Phytoconstituents of medicinal plants**

Plants are the traditional source of many active constituents used as pharmaceuticals. They are capable of synthesizing an overwhelming variety of low molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. To date about, 100,000 such compounds have been isolated from higher plants (Verpoorte, 2000). Numerous plant secondary metabolites possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors, and fragrances. In sharp contrast, metabolism of microorganisms has been successfully engineered for increased production of pharmaceuticals or novel compounds. Despite a few decades of research, plant secondary metabolism remains poorly characterized (Verpoorte and Memelink, 2002).

Speculative estimations of the total number of metabolites, which are produced within the plant kingdom, including the 'secondary' metabolites, vary considerably and the real number is likely to be in the range between 100,000 and 200,000 (Oksman Caldentey and Inze, 2004). This complexity can, therefore, be used to define plants at every level of genotype, phenotype, tissue and cell. Metabolomics can provide a useful complement to
the existing functional genomics technologies in this regard. Furthermore, it will be essential when the aim is to characterize the majority of so called ‘silent plant phenotypes’ (Weckwerth et al., 2004) or correlate function with ‘orphan genes’ (Goodacre, 2005).

The total number of plant secondary metabolites for which structures have been elucidated is around 50,000 (De Luca and St Pierre, 2000), and this is likely to be only the tip of the iceberg in terms of the chemical diversity that is represented in nature. It is estimated that around 5000 genes (25% of the total) are involved in secondary metabolism in *A. thaliana* (The Arabidopsis Genome Initiative, 2000), while around 25% of rice genes are predicted to be involved in primary or secondary metabolism collectively (Goff et al., 2002). The functions of only a very limited subset of these genes are understood. These include genes required for the synthesis of phytoprotectants such as glucosinolates and indole phytoalexin, camalexin in *A. thaliana*, and flavanone and diterpene phytoalexins in rice. Genes sharing sequence similarity with those required for alkaloid biosynthesis in species such as *Papaver somniferum*, *Berberis stolonifera* and *Catharanthus roseus* (strictosidine β-glucosidase, berberine bridge enzyme and strictosidine synthase) are present in the genomes of both *A. thaliana* and rice (Goff et al., 2002). The synthesis of secondary metabolites is often tightly regulated, and is commonly either restricted to specific plant tissues or developmental stages, or induced in response to pathogen attack or treatment with inducing agents (methyl jasmonate and other elicitors). Interference with transcriptional regulators for genes encoding biosynthetic enzymes can ‘wake up’ dormant biochemical pathways. For example, activation tagging of a gene for a transcriptional regulator of alkaloid biosynthesis in *C. roseus* circumvented the normal jasmonate requirement for pathway induction and led to constitutive synthesis of these secondary metabolites (Van der Fits and Memelink, 2000). Expressed sequence tag (EST) collections derived from plant tissues that are actively synthesizing secondary metabolites represent valuable resources for gene discovery (Lange et al., 1999; Ohlrogge and Benning, 2000; White et al., 2000; Gang et al., 2001; Brandic et al., 2002).
Review of literature

Phytochemicals of *Phyllanthus amarus*

The secondary metabolites present in *P. amarus* are alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols (Table 2). Several chemical investigations have been conducted where the structures of most of these phytochemicals were determined by UV, IR, Mass and NMR spectroscopy (Foo and Wong, 1992; Foo, 1993; Foo, 1995; Houghton et al., 1996).

**Table 2: Phytochemicals in *P. amarus***

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Phytoconstituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Securinine, Norsecurinine, Epibubbialine</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Catechin, Gallocatechin, Quercetin,</td>
</tr>
<tr>
<td>Hydrolysable tannins</td>
<td>Amarin, Amarinic acid, Amaratulone,</td>
</tr>
<tr>
<td>Major lignans</td>
<td>Phyllanthin and Hypophyllanthin</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Ellagic acid, Phenazine</td>
</tr>
</tbody>
</table>

**Phytoconstituent Saponins**

The saponins are naturally occurring, important group of surface-active secondary metabolites consisting of glycosylated triterpenes and steroids. They are mainly produced by plants and also by lower marine animals and some bacteria. These compounds are widespread in dicotyledonous plant species. Cereals and grasses, however, appear to be generally saponin deficient with the exception of oats (*Avena* sp.) (Hostettman and Marston, 1995). The process of saponin biosynthesis is not well understood for any plant species, despite the considerable interest in this important group of natural products (Haralampidis et al., 2001a). In plants, synthesis of sterols is initiated by cyclisation of 2,3-oxidosqualene to cycloartenol, mediated by the oxidosqualene cyclase enzyme cycloartenol synthase.

In cultivated crops the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as medicinal herbs or for their health promoting properties (Fenwick et al., 1991). Triterpenoid saponins have been detected in many legumes such as soyabean, beans, peas, lucerne, etc. and also in onion, tea, spinach, sugar, sugarcane beet, quinoa, liquorice, sunflower, horsechestnut, and ginseng. Steroid saponins are found in oats, capsicum peppers, aubergine, tomato seed, allium, asparagus,
yam, fenugreek, yucca and ginseng. Saponins consist of sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose, or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin), which may be triterpenoid or steroid in nature. Several reviews have been published in recent years on various aspects of plant saponins (Kensil, 1996; Barr et al., 1998; Sen et al., 1998).

Saponins of *Chlorophytum* species

*Chlorophytum* species hold a great promise for the medicinal market due to the presence of valuable saponins. Among all the species of *Chlorophytum* present in India, *C. borivilianum* produces the high yield of roots along with the highest saponin content (Bordia et al., 1995). The roots are reported to contain 42% of carbohydrates, 8-9% of proteins, 3-4% fibres and 2-17% saponin (Bordia et al., 1995). However, the saponin content was found to be affected by genotype and environment. When same accessions were collected from forest and cultivated at sandy loam soil at CTAE, Udaipur and clay loam soil of RCA, Udaipur showed varied response of genotypes to the locations, in terms of saponin content. Genotype RC-14 yielded saponin as high as 9.3%, while at the same site other genotype RC-28 yielded only 1.8% (Kaushik et al., 2005). Further, this species has not been investigated for its phytochemical constituents. In spite of its great popularity, there is no report on the characterization of saponins from this species. This emphasizes the need for evaluation of variations in saponin content and selection of ecotypes.

Steroids and triterpenoids

*Asparagus adscendens* Roxb. (Liliaceae) and *Chlorophytm borivilianum* Baker (Liliaceae) are commonly known as safed musli (white roots). *Curculigo orchioides* Gaerth (Hypoxidaceae) is reported to be known as Kali musli. The Kali musli (black roots) is distinguishable from the tuberous roots of *A. adscendens*. Because of the scarcity of the costly material of *C. arundinaceum*, the cheap material *A. adscendens* is adulterated to it. The saponin profile of both these plants differ considerable (Tandon and Shukla, 1995)
Table 3: Steroidal sapogenins and glycosides isolated from different species of *Chlorophytum*.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Phytoconstituents</th>
<th>Source</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gitogenin</td>
<td><em>C. comosum</em></td>
<td>Anti-tumour</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>2</td>
<td>Hecogenin</td>
<td><em>C. comosum</em></td>
<td>Anti-tumour</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>3</td>
<td>Tigogenin</td>
<td><em>C. comosum</em></td>
<td>Anti-tumour</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>4</td>
<td>Tigogenin</td>
<td><em>C. arundinaceum</em></td>
<td>Anti-tumour</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>5</td>
<td>Stigmasteryl</td>
<td><em>C. arundinaceum</em></td>
<td>Anti-tumour</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>6</td>
<td>Neo-hecogenin</td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Qiu et al., 2000</td>
</tr>
<tr>
<td>8</td>
<td>Neo-gitogenin</td>
<td><em>C. arundinaceum</em></td>
<td>Anti-tumour</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>9</td>
<td>Tokorogenin</td>
<td><em>C. arundinaceum</em></td>
<td>Anti-tumour</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>Glycosides</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>Chloromaloside A</td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Qiu et al., 2000</td>
</tr>
<tr>
<td>11</td>
<td>Chloromaloside B</td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>12</td>
<td>Chloromaloside C</td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>13</td>
<td>Chloromaloside D</td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>14</td>
<td>Chloromaloside E</td>
<td><em>C. malayense</em></td>
<td>Anti-tumour</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>15</td>
<td>Gitogenin glycosides</td>
<td><em>C. comosum</em></td>
<td>Anti-tumour</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>16</td>
<td>Hecogenin glycosides</td>
<td><em>C. comosum</em></td>
<td>Anti-tumour</td>
<td>Mimaki et al., 1996</td>
</tr>
</tbody>
</table>

Table 4: Phenolic compounds

<table>
<thead>
<tr>
<th>S.N</th>
<th>Phytoconstituents</th>
<th>Source/Plant parts</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,4,4-Trihydroxy-2-xylopyranosylbibenzyl</td>
<td><em>C. arundinaceum</em> (roots)</td>
<td>Tandon and Shukla., 1993</td>
</tr>
<tr>
<td>2</td>
<td>Curcuriligoside5-hydroxy-2-o-&lt;br&gt;β-D</td>
<td><em>C. archioides</em> (rhizomes, roots)</td>
<td>Kubo et al., 1993</td>
</tr>
<tr>
<td></td>
<td>Glucopyranosyl benzyl-2, 6-dimethoxy</td>
<td></td>
<td>koyyo et al.,1983</td>
</tr>
<tr>
<td></td>
<td>Benzocate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CurcuriligneA</td>
<td><em>C. archioides</em> (rhizomes, roots)</td>
<td>Xu et al., 1987</td>
</tr>
<tr>
<td>4</td>
<td>Orcinol glucoside</td>
<td><em>C. archioides</em> (rhizomes, roots)</td>
<td>Xu et al., 1986</td>
</tr>
<tr>
<td>5</td>
<td>Corchioside A</td>
<td><em>C. archioides</em> (rhizomes)</td>
<td>Garg et al., 1989</td>
</tr>
<tr>
<td>6</td>
<td>3-O-{β-D-Glucopyranosyl (1→4)-&lt;br&gt;α-L-xylopyranosyl}</td>
<td><em>C. archioides</em> (rhizomes)</td>
<td>Tiwari and Mishra., 1976</td>
</tr>
</tbody>
</table>

Centre for Transgenic plant Development, Jamia Hamdard
Table 5: Secondary metabolites in different organs of *Asparagus adscendens* and *Chlorophytum sp.*

<table>
<thead>
<tr>
<th>S.N</th>
<th>Phytoconstituents</th>
<th>Source/Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Sitosterol</td>
<td><em>A. adscendens</em> (fruits, roots)</td>
<td>Sharma et al., 1980, 1982; Garg et al., 1989</td>
</tr>
<tr>
<td>2</td>
<td>Sarsapogenin</td>
<td><em>A. adscendens</em> (fruits, roots)</td>
<td>Sharma et al., 1980, 1982;</td>
</tr>
<tr>
<td>3</td>
<td>Disogenin</td>
<td><em>A. adscendens</em> (fruits, roots)</td>
<td>Sharma et al., 1980, 1982;</td>
</tr>
<tr>
<td>4</td>
<td>Stigmasterol</td>
<td><em>A. adscendens</em> (roots)</td>
<td>Tandon and Shukla, 1992</td>
</tr>
<tr>
<td>6</td>
<td>Stigmasterol</td>
<td><em>C. arundinaceum</em> (roots)</td>
<td>Tandon and Shukla, 1992</td>
</tr>
<tr>
<td>7</td>
<td>Neo-Gitogenin</td>
<td><em>C. arundinaceum</em> (roots)</td>
<td>Tandon and Shukla, 1992</td>
</tr>
<tr>
<td>8</td>
<td>Tecorogenin</td>
<td><em>C. arundinaceum</em> (roots)</td>
<td>Tandon and Shukla, 1992</td>
</tr>
<tr>
<td>9</td>
<td>Yuccagenin</td>
<td><em>C. orchioides</em> (rhizomes)</td>
<td>Rao et al., 1978</td>
</tr>
<tr>
<td>11</td>
<td>Cycloartenol</td>
<td><em>C. orchioides</em> (rhizomes)</td>
<td>Garg et al., 1989</td>
</tr>
<tr>
<td>12</td>
<td>Curculigol(24-methyl cycloaet-7-en-3</td>
<td><em>C. orchioides</em> (rhizomes)</td>
<td>Misra et al., 1990</td>
</tr>
<tr>
<td>13</td>
<td>Curculigenin A (2β,16 β,16 [trihydroxy cycloarten-24-one)</td>
<td><em>C. orchioides</em> (rhizomes)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>14</td>
<td>Curculigenin B</td>
<td><em>C. orchioides</em> (rhizomes)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>15</td>
<td>Curculigenin C</td>
<td><em>C. orchioides</em> (rhizomes)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>16</td>
<td>β-sitosterol-β-D-glucoside</td>
<td><em>A. adscendens</em> (fruits, roots)</td>
<td>Sharma et al., 1982</td>
</tr>
<tr>
<td>17</td>
<td>AsparaninA</td>
<td><em>A. adscendens</em> (fruits)</td>
<td>Sharma et al., 1982</td>
</tr>
<tr>
<td>18</td>
<td>AsparaninB</td>
<td><em>A. adscendens</em> (fruits)</td>
<td>Sharma et al., 1982</td>
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<td>19</td>
<td>AsparaninC</td>
<td><em>A. adscendens</em> (roots)</td>
<td>Sharma et al., 1982</td>
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<tr>
<td>20</td>
<td>AsparaninD</td>
<td><em>A. adscendens</em> (roots)</td>
<td>Sharma et al., 1982</td>
</tr>
<tr>
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<td>AsparosideA</td>
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<td>Sharma et al., 1982</td>
</tr>
<tr>
<td>22</td>
<td>AsparosideB</td>
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<td>Sharma et al., 1982</td>
</tr>
<tr>
<td>23</td>
<td>AsparosideC</td>
<td><em>A. adscendens</em> (fruits)</td>
<td>Sharma et al., 1982</td>
</tr>
<tr>
<td>24</td>
<td>AsparosideD</td>
<td><em>A. adscendens</em> (fruits)</td>
<td>Sharma et al., 1982</td>
</tr>
<tr>
<td>25</td>
<td>Ascosidendin A</td>
<td><em>A. adscendens</em> (leaf)</td>
<td>Sharma and Sharma, 1984</td>
</tr>
<tr>
<td>26</td>
<td>Ascosidendin B</td>
<td><em>A. adscendens</em> (leaf)</td>
<td>Sharma and Sharma, 1984</td>
</tr>
<tr>
<td>27</td>
<td>AscosendosideA</td>
<td><em>A. adscendens</em> (leaf)</td>
<td>Sharma and Sharma, 1984</td>
</tr>
<tr>
<td>28</td>
<td>AscosendosideB</td>
<td><em>A. adscendens</em> (leaf)</td>
<td>Sharma and Sharma, 1984</td>
</tr>
<tr>
<td>29</td>
<td>3-β-(β-D-2-tetracosyl-xylopyranosyl) Stigmasterol</td>
<td><em>A. adscendens</em> (roots)</td>
<td>Tandon et al., 1990</td>
</tr>
<tr>
<td>30</td>
<td>3-β-(β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl</td>
<td><em>A. adscendens</em> (roots)</td>
<td>Tandon et al., 1990</td>
</tr>
<tr>
<td>31</td>
<td>Stigmasterol-β-D-gluco side</td>
<td><em>C. orchioides</em> (Rhizome)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>32</td>
<td>Curculigosaponin A</td>
<td><em>C. orchioides</em> (Rhizome)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>33</td>
<td>Curculigosaponin B</td>
<td><em>C. orchioides</em> (Rhizome)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>34</td>
<td>Curculigosaponin C</td>
<td><em>C. orchioides</em> (Rhizome)</td>
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<tr>
<td>35</td>
<td>Curculigosaponin D</td>
<td><em>C. orchioides</em> (Rhizome)</td>
<td>Xu et al., 1992</td>
</tr>
<tr>
<td>36</td>
<td>Curculigosaponin E</td>
<td><em>C. orchioides</em> (Rhizome)</td>
<td>Xu et al., 1992</td>
</tr>
</tbody>
</table>

Centre for Transgenic plant Development, Jamia Hamdard
Conservation of rare and endangered plants

Genetic diversity studies of rare and endangered plant population are helpful in formulating plans for management by preserving their genetic diversity and ensuring their long term survival. This is done by protecting habitat variation and controlling the introduction of new individuals. High yielding cultivars can be identified by RAPD and AFLP markers (Lata et al., 2002). Analysis of genetic diversity by AFLP marker of the critically endangered plant, Rumex rothsschildianus (Rottenberg and Parker, 2003) has been performed to devise conservation strategy to save its only two remaining populations. AFLP markers are also used for endangered Medicago citrina (Juan et al., 2004) to study variability and gene flow.

Eurycoma longifolia, traditionally used as blood coagulant during emergencies of childbirth is harvested unsustainably resulting into rapid thinning of its natural population. Osman et al (2003) identified single nucleotide polymorphism (SNP) within the genomes of E. longifolia accessions thus helping in the discrimination of populations. This information can be utilized for devising conservation programme.

Correlation of Genomics and Metabolomics

The comprehensive chemical analysis of metabolites and the computation of huge datasets are the key components of metabolomics (Bino et al., 2004; Fiehn et al., 2002; Sumner et al., 2003; Weckwerth et al., 2003). Metabolomics is principally required to determine all metabolites in a plant extract; however, no single technology for metabolomics, such as a DNA sequencer for genomics or DNA arrays for transcriptomics, is available, and such a method may never be possible. This is because
the analysis of metabolites of divergent physicochemical properties needs a wide range of chemistries and a single chemistry cannot deal with metabolomics as it can with nucleic acids and proteins. Metaphorically speaking, metabolomics is like attempting whole genome sequencing without either the Sanger method or the Maxam Gilbert method. At present, combinations of different analytical methods of high sensitivity are generally used for comprehensive non targeted chemical analysis (Kopka et al., 2004; Stitt et al., 2003).

Nutritional and abiotic stresses modulate re-programming of the transcriptome and metabolome. Thus, an integrated analysis may lead to the identification of gene functions that are modulated by these stresses. A good example of this was obtained from a study of sulfur starvation in Arabidopsis (Hirai et al., 2004). Glucosinolate related metabolites and genes involved in their metabolism were coordinately regulated by nutritional sulfur stress. Some genes involved in the formation of volatile compounds in Strawberry (Aharoni et al., 2004), rose (Guterman et al., 2002) and spider mint (Mercke et al., 2004) have been identified using DNA microarrays in combination with targeted analysis of volatile metabolites. The chemical analysis of fragrance related metabolites by GC-MS is possible and leads to a sensitive chemical analysis, which together with gene expression profiles is sufficient for gene identification. Activation tagged lines, in which a gene is over expressed by random insertion of an enhancer sequence in the genome, are good resources for gene hunting. By screening activation-tagged lines in tomato, a Myb transcription factor gene and co-regulated structural genes involved in anthocyanin formation were successfully identified (Mathews et al., 2004).

The classical biochemical approach has resulted in considerable knowledge of the genes involved in the synthesis of flavonoids (Springob et al., 2003) and terpenoid indole alkaloids (Verpoorte et al., 2002). Considering the huge chemical diversity of plants compared with those of animals and microorganisms, a major future challenge will be to explore the molecular genetic origins of chemical diversity in non-model exotic plants. Further prospect beyond conventional omics would be the comprehensive analysis of the function and activity of an array of plant metabolites, leading to the ‘phytochemical
array' concept. The phytochemical array consists of genomics, transcriptomics, proteomics, metabolomics and activity arrays of a given plant species. Such an array would allow visualization of all the connections between genes, transcripts, proteins, metabolites and their activities. Drawing links from the genome to the activity of metabolites will be necessary the high throughput discovery of the plant based pharmaceuticals and for the development of functional foods and stress resistant plants.

The ability to synthesize particular class of secondary metabolite is commonly restricted to selected plant groups, and the evolution of different pathways in distinct plant lineages is likely to have been the key for survival and for the generation of diversity at the organism level. An understanding of the evolution of secondary metabolism requires the characterization of enzymes and genes for complete pathways in a broad range of plants. However, a comprehensive picture of the in vivo combinatorial chemistry behind the myriad of compounds found in nature will depend on a detailed knowledge of enzymes,
genes and pathways required for the synthesis of secondary metabolites in other species. Information of this kind will also be important in assessing the potential of different genetic backgrounds to synthesize novel or altered compounds. Multifaceted approaches that combine biochemistry, molecular genetics and genomics with classical genetics have proved to be highly effective in the dissection of secondary metabolite biosynthetic pathways (Anne et al., 2003).

Genetic diversity and Metabolite profiling
Metabolite profiling is a fast growing technology and is useful for phenotyping and diagnostic analysis of plants. It is also rapidly becoming a key tool in functional annotation of genes and in the comprehensive understanding of the cellular response to biological conditions. Metabolomic approaches have recently been used to assess the natural variance in the metabolite content between individual plants, an approach with great potential for the improvement of the compositional quality of crops. The use of metabolite profiling in diagnostics is currently the best documented, and the application of standard statistical analysis tools is now routine to clarify the major metabolic changes caused by any given perturbation. However, in recent years, many groups have taken up the challenge of integrating metabolite profiling within broader experimental analyses to gain a deeper understanding of metabolic regulation. These studies tend to be focused on either environmental or genetic perturbations of metabolism. Metabolite profiling is being used exclusively in the studies of environmental perturbations in attempts to elucidate factors underlying the complex shifts that occur under nutrient limitation and biotic stress (Kaplan et al., 2004; Urbanczyk Wocniak and Fernie, 2005; Homzehzarghani et al., 2005; Broeckling et al., 2005; Ishizaki et al., 2005). Alternatively, forward and reverse genetics strategies, when used in conjunction with metabolite profiling, have been clearly demonstrated to aid in gene annotation and identification of candidate genes for biotechnology and/or breeding strategies.

The majority of cultivated crops carry only small fractions of the genetic variation available in related wild species and land races (Fernie et al., 2006). It has therefore, become a goal of modern plant breeding to screen wild genetic resource that could be
introduced into modern varieties to improve specific traits. Integrated genomic approach is increasingly including metabolite-based approach (Suzuki et al., 2005; Tohge et al., 2005; Andersson-Gunneras et al., 2006; Nikiforova et al., 2005; Kristensen et al., 2005; Alba et al., 2005). However, the aims of such approach are diverse, from the identification of candidate genes and the assessment of how confined the effects of a specific genetic modification are to be a comprehensive understanding of the regulation of metabolic networks. Testing of several of these candidate genes using reverse genetics approach has recently confirmed that manipulation of at least one of the genes identified by this method resulted in the anticipated change in the metabolite content. Similar approaches have recently proved successful for manipulating pyrimidine alkaloid biosynthesis in tobacco (Goossens et al., 2003) and for metabolic engineering if the production of medicinally important polyketides in *Aspergillus terreus* (Askenazi et al., 2003).

Assessing genetic diversity using molecular markers

**Genetic diversity and clonal propagation**

A variety of life history traits, such as life form, breeding system and seed dispersal mechanism, influence genetic variation and its spatial distribution within populations of plant species (Heywood, 1991), causing the genetic architecture within populations to change through time (Gillespie, 1998). The mode of reproduction is likely to have important effects on genetic variation and its spatial distribution within plant populations (Harada and Iwasa, 1996; Winkler and Stocklin, 2002). Sexual reproduction is accompanied by genetic recombination, which leads to the continuous emergence of new genotypes and thus can buffer the loss of genotypic diversity. Some models regarding the genetic structure of population with little or no sexual requirement envision a few localized genotypes, while others consider that asexual population can be genotypically as polymorphic as sexual ones for plant involving clonal propagation (Ellstrand and Roos, 1987). Asexual populations maintain higher genetic diversity at each single locus but a lower number of different genotype. Mixed clonal/sexual reproduction is nearly indistinguishable from strict sexual reproductions as long the proportion of clonal reproduction is not strongly predominant (Balloux et al., 2003). Although the genotypic...
diversity will decrease at a constant rate with increasing rates of asexual reproduction, a small number of sexual individuals per generation are sufficient to make an asexual population highly genetically variable (Stehlik and Holderegger, 2000; Balloux et al., 2003; Bengtsson, 2003). In addition, somatic mutations also account to some extent for genetic variation present in clonal populations (Lamote et al., 2002). In vegetatively reproducing plants, somatic mutations can be fixed and passed on to the succeeding ramets (Gill et al., 1995) and the mutation rates vary across the gamete. Lack of effective mechanism for long distance dispersal of seeds may also play an important role in shaping the observed genetic structure (Wallace, 2002).

Many perennial plants combine sexual reproduction through seeds with reproduction through vegetative propagation (Richards, 1986). However, the relative proportions of sexual vs. asexual progeny produced and recruited may often vary widely within a species, due to variations in ecological and/or genetic factors that limit or enhance one or other reproductive mode (Eckert, 2002). This, in turn, may directly affect the genotypic diversity within natural populations. Since vegetative reproduction yields offspring that are genetically identical to both the maternal plant and each other, the resultant patches are expected to exhibit no genotypic diversity.

Traditional approaches to measure the diversity rely upon the ability to resolve differences in morphological characters. The range of characters available may be increased by the use of electron microscopy or biochemical and physiological assays. However, various treatments to plants often result in the alteration of the morphological, anatomical, and even chemical features of the plant parts, and thus, make the traditional methods of authentication, which depend upon the analysis of these features, unreliable. During the early period of research, classical strategies including comparative anatomy, physiology and embryology were employed in genetic analysis to determine inter and intraspecies variability. In the past decade, however, molecular marker(s) have very rapidly complemented the classical strategies. Molecular marker(s) are generally referred to as biochemical constituents, including primary and secondary metabolites in plants and macromolecules, viz. proteins and deoxyribonucleic acids (DNA).
Markers can be categorized into two groups

1. Morphological markers
2. Molecular markers

Morphological marker
Morphological marker(s) have been routinely used to identify genetic diversity but major disadvantages associated with these markers are, the limited number of morphological characters available for analysis and these characters are also influenced by environmental factors. Consequently different plant genotypes cannot be distinguished (Staub and Meglic, 1993). The genetic basis of most morphological variations is generally unknown and hence, these marker(s) could not provide desirable information about the genome of the plants used in the study.

Characteristic of morphological markers:
- The phenotype of most morphological marker(s) can only be determined at the whole plant level.
- Allele frequency tends to be much lower with morphological loci.
- It is generally associated with undesirable phenotypic effect.
- Alleles at morphological loci interact in a dominant/recessive manner that limits the identification of heterozygous genotypes.
- More epistatic or pleiotropic effects are observed with morphological marker(s).

Molecular marker
Molecular markers have been widely used to characterize population genetic structure in plants. Molecular markers allow analysis of variation at the genomic level (Karp et al., 1997) and permit detection of genetic variation at the molecular level (Rani et al., 1995).

Types of molecular marker
- Biochemical marker (Storage proteins and isozymes)
- DNA based molecular marker
Biochemical marker

Isozymes (or isoenzymes) are different variants of the same enzymes having identical or similar functions and are present in the same individual. They are powerful tools to study genetic variability within and between populations of plants and animals. Isozymes have been used in taxonomic, genetic, evolutionary, and ecological studies, identification of cultivars and lines (Peirce and Brewbaker, 1973). Despite the use of DNA markers such as RAPDs, AFLPs, RFLPs, isozymes etc; are still widely employed in species delimitation and conservation (Booy and Van Raamsdonk, 1998; Chamberlain, 1998), assessment of genetic variability in species and populations (Buso et al., 1998) and gene flow studies (Gauthier et al., 1998). They are especially useful when several taxa, accessions and individuals are to be compared, as the assumption of homology is more accurate than with some DNA markers. Isozyme electrophoresis has been successfully used to identify clones and to examine the clonal structure of plant populations (Mc Neilly and Roose, 1984; Mc Clintock and waterway, 1993; Johanson et al., 1996; Lehman, 1997).

Limitations of Biochemical Markers

- Much of the genome (including much of the most polymorphic portions of it that are less subject to evolutionary restrictions) does not code for gene product, and hence remains unanalyzed.
- Different biochemical procedures are required to visualize allelic differences for enzymes having different functions.
- Many proteins undergo post transcriptional modifications and hence, can mask variations present at DNA level (e.g., differences in tri-nucleotide sequences coding for the same amino acid, introns sequences that are post transcriptionally removed from the mRNA; all these can all contribute to reduced polymorphism expression at the protein level compared to that at the DNA level.
DNA based markers

DNA-based markers have acted as versatile tools have found their own position in various fields like taxonomy, physiology, embryology, genetic engineering, etc. They are no longer looked upon as simple DNA fingerprinting markers in variability studies or as mere forensic tools. A variety of molecular assays could be used to assess the genetic diversity; each method differs in principle, application, the amount of polymorphism detected, cost and time required. These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively. DNA fingerprinting in plant can be adapted to numerous applications and uses including characterizing individual plants to clarify errors in the identification of accessions and cultivars (Saunders et al., 2001b; Chavarriaga-aguirre et al., 1998). Molecular linkage maps also being used successfully in many crop species for directed germplasm improvement. Linkage maps facilitate the identification and localization of genes controlling important traits, subsequently allowing marker assisted selection and positional cloning of genes.

Molecular markers based on DNA sequences detect more polymorphism than morphological and protein based markers and constitute a new generation of genetic marker ( Tanksley et al., 1989). Polymorphism may be defined as simultaneous occurrence within or between populations of multiple phenotypic forms of a trait attributable to the alleles of a single gene or the homologues of a single chromosome (Acquaah, 1992).

Properties desirable for ideal DNA markers

- Highly polymorphic in nature
- Co dominant inheritance (determination of homozygous and heterozygous states of diploid organisms)
- Frequent occurrence in genome
- Selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices)
- Easy access (availability)
Easy and fast assay
- High reproducibility
- Easy exchange of data between laboratories.

Types of DNA based markers
- PCR based markers
- Non PCR based markers

PCR based markers
PCR-based markers involve in vitro amplification of particular DNA sequences or loci, with specific or arbitrarily chosen oligonucleotide sequences (primers) and a thermo stable DNA polymerase enzyme. The amplified products are separated electrophoretically and banding patterns are detected by different methods such as staining and autoradiography. The most important DNA based markers used in revealing genetic diversity in plant genome are given below:

PCR based markers
Randomly Amplified Polymorphic DNA (RAPD)
These markers are very quick and easy to develop due to the arbitrary sequence of the primers (Karp et al., 1997; Hansen et al., 1998). It is a PCR based technique and resolved most of the technical obstacle owing to its cost effective and easy to perform approach (Welsh and McCleland, 1990; Williams et al., 1990). This efficient technique obviates the need to work with radioisotope and gives satisfactory results even with crude DNA preparations. RAPDs have, therefore, been extensively used in assessing genetic relationship amongst various accession of different plant species (Chalmers et al., 1992; Adams et al., 1993; Castilione et al., 1993, Russel et al., 1993; Wachira et al., 1995). RAPD has been used to identify medicinal plant tea (Camellia sinensis) (Wachira et al., 1995), dried roots of P. ginseng, P. quinquefolius, P. notoginseng and their adulterants (Shaw and But, 1995). The reproducibility of RAPDs is affected by DNA quality, primer concentration, different thermal cyclers and the brand of DNA polymerase used (Meunier and Grimont, 1993; Mac Pherson et al., 1993; Ellsworth et al., 1993). If the
Review of literature

amplification conditions (reagents and thermocycler parameters) are identical for all reactions, the results are highly reproducible. Variation in the primer concentration is one of the main sources of RAPD pattern variations (Hansen et al., 1998; Virk et al., 2000). Thus, the lack of specificity and reproducibility is the major drawbacks of RAPD.

Simple Sequence Repeat (SSR)

Microsatellites or simple sequence repeat (SSR) markers are tandemly repeated DNA sequences that occur throughout the eukaryotic genome. The length polymorphism arises from variations in the number of repeated units, probably due to DNA polymerase slippage during replication of the SSR (Eisen, 1999; Levinson and Gutman, 1987). The frequency of SSRs in plant genome is estimated as one in every 6-7 Kb, based on the information that can be found in public sequence database (Cardle et al., 2002). Thus, these are abundant resource in the genome and have a high level of allelic diversity. Consequently, they are frequently used as genetic markers in plant genetic studies (Powell et al., 1996; McCouch et al., 1997). The codominant nature and allelic polymorphism revealed by SSR markers have provided detailed information on genetic structure (Bonnin et al., 2001; Li et al., 2000) and gene flow (Konuma et al., 2000) in natural plant populations. Construction of a genomic library or SSR enriched library has been the principal means of discovering SSRs in eukaryotic genomes for which public DNA sequence data is lacking. The SSR loci differ in the number of repetitive di, tri, or tetranucleotide units present (Tautz and Renz, 1984; Tautz et al., 1986) and this length variation is detected with the polymerase chain reaction (PCR) by utilizing pairs of primers flanking each simple sequence repeats. These markers have been developed for plant species, including Soyabean (Akkayao et al., 1992; Morgante and Olivieri, 1993; Rongwen, 1995), and Rice (Wu and Tankesley, 1993; Yang et al., 1994). Rapid evolution and the properties of the replication slippage mechanism proposed for SSR polymorphism generation, it may not be suitable for estimating genetic similarities except very closely related taxa (Bowcock et al., 1994). Despite the advantage of SSR markers, their development is time consuming for plant species for which there is little DNA sequence information is available in the public database.
Inter Simple Sequence Repeat (ISSR)

Inter simple sequence repeat (ISSR) has been available since 1994 (Zietkiewicz et al., 1994) and these are semiarbitrary marker(s) amplified by PCR in the presence of one primer complementary to a target microsatellite. ISSR primers are derived from an arbitrary nucleotide sequence of di and trinucleotide repeats with a 5' or 3' anchoring sequence of a few nucleotides to prevent strand-slippage (14-22 bp). These nucleotide repeats are based on the ubiquitous presence of simple sequence repeats that are distributed throughout genomes. It is a powerful tool for investigating genetic variation within species (Gupta et al., 1994; Wolf and Liston, 1998) especially when sequence information about the study organism is limited. Amplification does not require genome sequence information and leads to multilocus and highly polymorphic patterns (Zietkiewicz et al., 1994; Nagaoka et al., 1997). Each band corresponds to a DNA sequence delimited by two-inverted microsatellites. Recent studies on genetic diversity of clonal plant species have demonstrated the great discrimination power of ISSR markers for genetic identification (Esselman et al., 1999; Camacho and Liston, 2001; Liston et al., 2004; Wang et al., 2004). The plant *Monimopetalum chinensis* which is endangered endemic species of Eastern China was assessed by ISSR marker (Xie et al., 2005). Like RAPDs, ISSRs markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers.

Restriction Fragment Length Polymorphism (RFLP)

RFLP markers were the first to provide a means to directly detect variations present at DNA level. RFLPs have been used to document genetic diversity in cultivated plants and their wild relatives (Wang et al., 1992; Diers and Osborn, 1994). The polymorphism detected by RFLP is mainly due to a variation at the restriction site, where as with AFLP, an additional number of nucleotides, apart from restriction site are screened for polymorphism (Becker et al., 1995). These markers were used for the first time in the construction of genetic maps by Botstein et al (1980). Being a codominant marker, it can detect coupling phase of DNA molecules as DNA fragments from all homologous chromosomes are detected. They are very reliable markers in linkage analysis and breeding study. This can easily determine, if a linked trait is present in a homozygous or...
heterozygous state in an individual and information is highly desirable for recessive traits (Winter and Kahl, 1995). Although highly specific, performing RFLP is quite tedious and expensive since it requires large amount of pure quality of DNA and an expertise in handling radioactivity.

Single Nucleotide Polymorphism (SNP)
DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered. The potentiality of single nucleotide polymorphism (SNPs) to identify the genetic variability has been proved (Hayashi et al., 2004). Because SNP are highly abundant, occurs frequently throughout genome and tend to be relatively stable genetically (Batley et al., 2003), their potential use as the next generation of genetic marker in a species lacking polymorphism (e.g. peanut) should be explored in future.

Cleaved amplified polymorphic sequence (CAPS)
Cleaved amplified polymorphic sequence (CAPS) is also known as PCR-RFLP marker, based on STSs derived from ESTs. These markers have several advantages. First, since analysis of restriction fragment length polymorphism is based on PCR amplification and it is much easier and less time consuming, especially for species with large genome (Wakamiya et al., 1993; Hizume et al., 2001) than analyzing alternative types of markers that require southern hybridization. Second, the primers for CAPS markers based on ESTs are more useful as genetic markers for comparative mapping study than those based on anonymous, nonfunctional sequences such as microsatellite markers, because the coding regions of functional genes are generally well conserved, not only within but also between species. Third CAPS marker is inherited mainly in a codominant manner. If STS and SCAR markers fail to reveal any polymorphism, then they can be easily converted to CAPS by employing restriction enzyme digestion (Konieczny and Ausubel, 1993). Unlike RAPD marker, the CAPS marker is a PCR based codominant marker that is reproducible and easier to manipulate in MAS (Caranta et al., 1999).
Sequence Characterized Amplified Region (SCAR)
The critical RAPD marker(s) can be converted into special STS marker(s) named sequence characterized amplified regions (Paran and Michemore, 1993). There are several advantages to the use of SCAR markers. First, SCAR markers do not require large amount of purified DNA, in contrast to hybridization methods like restriction fragment length polymorphism (RFLP). Second, with SCAR marker(s), analysis of the results is more straightforward than other PCR based markers, such as RAPD and AFLP. SCAR marker(s) can be revealed by a single PCR procedure, without subsequent digestion with a restriction enzyme. Third, genomic information can be obtained directly by analyzing PCR products (Kethidi et al., 2003). SCAR markers are more advantageous than RAPD markers because they usually detect only a single locus and are therefore, more specific. Their PCR amplification is less sensitive to reaction conditions, they are more likely to be codominant markers, and are therefore reproducible (Kethidi et al., 2003). Consequently SCAR markers offer the most practical method for screening numerous samples in a short time with minimal labour. They also offer the starting point for cloning any target gene uncovered by the marker(s) gene. These markers are based on sequencing the prominent band derived from RAPD primers and designing longer primers that will specifically bind to same loci of exact molecular weight as amplified in RAPD. SCAR marker(s) was developed for discrimination of *Artemisia princeps* and *A. argyi* from other *Artemisia* herbs (Lee et al, 2006). It also allows comparative mapping or homology studies among related species, thus making it an extremely adaptable concept in the near future.

Selective Amplification of Microsatellite Polymorphic Loci (SAMPL)
A modification of the AFLP procedure called selective amplification of microsatellite polymorphic loci (SAMPL) (Morgante and Vogel, 1994) combines the advantages of AFLP with the analysis of highly variable microsatellite regions of eukaryotic genomes. SAMPL has been reported to be more powerful than AFLPs in discriminating between closely related individuals in several plant complexes (Paglia and Morgante, 1998; Roy et al., 2002; Singh et al., 2002; Tosti and Negri, 2002) and in the rust pathogen, *Puccinia striiformis* f. sp. tritici, which is strictly clonal (Stubbs, 1985; Keiper et al., 2003). Keiper
et al. (2003) found SAMPL to be the most informative marker(s) system in assessing genetic variation among isolates of five cereal rust pathogens. The SAMPLs, RAPDs and AFLPs were used for characterization of genetic variation among 11 cowpea (Vigna unguiculata sub sp. unguiculata) land races and two commercial varieties showed marginally greater diversity indeces for SAMPL than AFLP and RAPD (Tosti and Negri, 2002). Additionally, fewer SAMPL primer combinations were needed to obtain similar discrimination when compared with AFLP and RAPD analyses. In another study, SAMPL was found to be more efficient than AFLP in differentiating closely related accessions of neem, Azadiracta indica (Singh et al., 2002).

Sequence Tagged Site (STS)
RFLP probes specifically linked to a desired trait can be converted into PCR based STS marker(s) based on nucleotide sequence of the probe giving polymorphic band pattern, to obtain specific amplicon. Using this technique, tedious hybridization procedures involved in RFLP analysis can be overcome. This approach is extremely useful for studying the relationship between various species. When these marker(s) are linked to some specific traits, for example powdery mildew resistance gene (Hartl et al., 1993) or stem rust resistance gene in barley (Oh et al., 1994), they can be easily integrated into plant breeding programmes for marker assisted selection of the trait of interest.

Expressed Sequence Tagged (EST)
Adams et al (1991) gave term ESTs for the markers obtained by partial sequencing of random cDNA clones. Once generated, they are useful in cloning specific genes of interest and synteny mapping of functional genes in various related organisms. ESTs are popularly used in full genome sequencing and mapping programmes underway for a number of organisms and for identifying active genes thus helping in identification of diagnostic markers. Moreover, an EST that appears to be unique assists to isolate new genes. EST markers are identified to a large extent for Rice, Arabidopsis, etc. wherein thousands of functional cDNA clones are being converted into EST marker (Sasaki et al., 1994).
Single Strand Conformation Polymorphism (SSCP)

This is a powerful and rapid technique for gene analysis particularly for detection of point mutations and typing of DNA polymorphism (Orita et al., 1989). It can identify heterozygosity of DNA fragments of the same molecular weight and can even detect changes of a few nucleotide bases as the mobility of the single stranded DNA changes with change in its GC content due to its conformational change. To overcome problems of reannealing and complex banding patterns, an improved technique called asymmetric-PCR SSCP was developed (Ainsworth, 1991), wherein the denaturation step was eliminated and a large-sized sample could be loaded for gel electrophoresis, making it a potential tool for high throughput DNA polymorphism. It was found useful in the detection of heritable human diseases. In plants, however, it is not well developed although its application in discriminating progenies can be exploited, once suitable primers are designed for agronomically important traits (Fukuoka, 1994).

Amplified Fragment Length Polymorphism (AFLP)

Vos et al (1995) developed AFLP (amplified fragment length polymorphism) technique to detect polymorphism. This AFLP technique provides a novel and very powerful DNA fingerprinting technique for DNAs of any origin or complexity. It has quickly become one of the widely used methods of DNA fingerprinting for crops and wild plant species (Mueller and Wolfenbarger, 1999; Ridout and Donini, 1999). In this technique, genomic DNA is restricted with two different restriction endonucleases, and a subset of the resulting fragments is amplified using forward and reverse primers each with 1–4 (usually three) additional bases. The fragments are then visualized using radioactivity, silver staining or fluorescent dyes. The resulting AFLP markers tend to show a strongly asymmetric size distribution, with a much higher proportion of smaller fragments, and this pattern does not appear to be affected by GC content or by genome size (Vekemans et al., 2002).

AFLP markers have been shown to be widely distributed across the nuclear genome in *Oryza sativa* (Maheshwaran et al., 1997; Zhu et al., 1998), *Zea mays* (Vuylsteke et al., 1999) and *Pinus taeda* (Remington et al., 1999). There is evidence, however, that AFLP
marker(s) show some clustering on genetic maps. In *Arabidopsis thaliana*, markers were found spread across different parts of the genetic map and there was evidence of some clustering around the centromeric regions of some linkage groups (Alonso-Blanco et al., 1998). At least some AFLP marker(s) consist of or include highly repetitive sequences and these may represent parts of retrotransposons in some cases (Reamon Buttner et al., 1999). AFLP has a number of advantages over pre-existing techniques of particular relevance to work on rare plants and only a small quantity of DNA (0.5 µg per restriction) is used to carry out PCR. In addition to this, the fingerprints (visualized as traces on automated platforms) are highly reproducible and consist of many markers, allowing greater discernment between closely related plants than other techniques such as random amplified polymorphic DNAs (RAPD) and microsatellites. They are more efficient in terms of time spent per marker(s) produced, but the need to use a sequencing gel to resolve the bands that the AFLP part of the analysis had to be done in a separate facility. As a result of these advantages, AFLP has now been successfully used in projects on a range of different species, including germplasm assessment in *Miscanthus* (Hodkinson et al., 2002); conservation-based studies of *Astragalus cremnophylax* (Travis et al., 1996), *Populus euphratica* (Fay et al., 1999), *Tecophilaea cyanocrocus* and *Tulipa sprengeri* (Maunder et al., 2001), *Grevillea scapigera* (Krauss et al., 2002) and *Cosmos atrosanguineus* (Wilkinson et al., 2003); studies of species delimitation in *Calopogon* sp. (Goldman et al., 2004), *Dactylorhiza* sp. (Hedren et al., 2001) and *Phylica* sp. (Richardson et al., 2003); paternity analysis in *Persoonia mollis* (Krauss, 2000); studies of hybridization in *Sorbus* sp. (Fay et al., 2002a) and *Schoenoplectus* sp. (Fay et al., 2003), respectively. The angiosperms chosen by Vos et al. (1995) have 1C-values ranging from 157 Mb (0.16 pg) in *A. thaliana* (Bennett et al., 2003) to 5440 Mb (5.55 pg) in *H. vulgare* (Bennett and Smith, 1976), and these all yielded acceptable AFLP fingerprints with either 2 + 3 or 3 + 3 selective nucleotides. The wide range in C-values observed in angiosperms will have a marked effect on the number of fragments produced in restriction digests, and using the standard AFLP technique, it was observed that species with high C-values (1C > 15 pg) tend to produce traces with large numbers of weakly amplifying and often co-migrating bands (Fay and Cowan, 2001; Fay et al., 2002a). The simplest modification is to change the number of bases in one or both of the
selective primers (Vos et al., 1995). Decreasing the number of bases can generate higher quality traces in species with smaller genomes (Vos et al., 1995). Increasing the number of bases reduces the number of fragments amplified and can generate cleaner genetic fingerprints in species with larger genomes. By using a 4-base extension on the Msel primer in conjunction with a more stringent PCR protocol rather than the standard AFLP technique, Krauss (1999) was able to generate cleaner fingerprints and assign paternity to offspring in Persooniamollis (Proteaceae; C-value unknown). Similarly, Costa et al. (2000) used a 4-base Msel primer extension with Pinus pinaster (1C = 24.35 pg), while Han et al. (1999, 2000) used a 4-base Msel primer extension together with a 4-base EcoRI primer extension in Alstroemeria spp. (1C = 22.1–39.5 pg) following a modified pre-selective PCR amplification.

The reproducibility of three popular marker(s) technologies, namely RAPDS, SSRs and AFLP, recommended AFLP as a highly repeatable assay with low error rates (Jones et al., 1997). Thus, AFLP methodology has a potential to screen a large number of genetic loci per experiment (Ellis et al., 1997). This technique is particularly useful for evaluating genetic diversity in those plant species where prior information regarding the genome is not available. The AFLP technique since it allows the simultaneous analysis of a large number of DNA fragments per gel (Pejic et al., 1998). This technique was used to analyze 132 accession of Erytheoxylum. It resulted into the identification of the four cultivated taxa, as well a feral taxon. It is a dominant marker(s) and require little template DNA, the ability to resolve without the use of radioactivity and good levels of polymorphism in many species (Beedanagari and Dove, 2005). It has been estimated that the scoring errors lie approximately in the range of 1-2%, which is consistent with that estimated in other studies (Mueller and Wolfenbarger 1999).

Authentication of Market herbs

Traditional herbal and herbo-mineral drugs have been used since the dawn of civilization to maintain and alleviate human sufferings from diseases. According to an estimate of the world health organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. From the very beginning,
herb authentication has presented a great challenge for people using them for medical purposes. The authentication of medicinal plants is a critical issue for the protection of consumers. Usage of wrong herb may be ineffective or it may worsen the condition and may even cause death. Ideally, authentication should be done from the harvesting of the plant material to the final product. Herbal drugs are normally processed parts of various plants, such as roots, stems, leaves, flowers, fruits, seeds, etc. The pharmaceutical companies are procuring materials from traders, who are getting these materials from untrained persons from rural and/or forest areas. This has given rise to widespread adulteration/substitution (Meherotra and Rawat 2000), leading to poor quality of herbal formulations. Misidentification of herbs can be non-intentional (processed plant parts are inherently difficult to distinguish) or intentional (profit-driven merchants sometimes substitute expensive herbs with less-expensive look-alike ones).

Traditionally, people authenticate herbs by their appearance, smell and/or taste; some of these methods are still skillful. Later on, herbs were authenticated by inspection under microscopes, where the shape and content of various plant cells are examined and analyzed. These methods, based on organoleptic markers or anatomical characters, are sometime imprecise. By and large analytical chromatography, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or liquid chromatography mass-spectrometry (LC-MS) has been used for herb authentication. During this decade, however, molecular markers have rapidly complemented the classical strategies (Weising, 1995). Molecular markers are generally referred to as biochemical constituents, including primary and secondary metabolites in plants and macromolecules, viz. proteins and deoxyribonucleic acids (DNA). Secondary metabolites, as markers, have been extensively used in quality control and standardization of herbal drugs, but these also suffer with few limitations. Focus is therefore, on marker based on genetic composition and hence is unique, stable, and ubiquitous to the plants. These DNA markers are not affected by age, physiological condition as well as environmental factors (Chan, 2003). Different types of DNA based markers viz., RAPD, RFLP, ISSR, AFLP etc., are employed for species discrimination of plants (Joshi et al., 1999).
Each marker has its own advantage and disadvantage, but none is universally ideal. The choice of technique is therefore, often a compromise that depends upon the nature of research pursued, the genetic resolution needed, financial constraints and the technical expertise available. In present study we will be attempting to evaluate genetic diversity by using molecular markers.