II. REVIEW OF LITERATURE
Herbal medicine is an integral part of "traditional medicine" (TM). Traditional medicines are diverse health practices, approaches, knowledge and beliefs that incorporate plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises which are applied singularly or in combination to treat, diagnose, maintain well-being and to prevent illness (WHO, 2008). Herbal medicines consist of plants and their parts as the ingredients and that includes seeds, berries, roots, leaves, bark or flowers (Chrlích, 2010). In the developed countries, TM has been adapted outside its indigenous culture to ‘Complementary’ or ‘Alternative’ medicine (WHO, 2008).

At the global level, people developed unique indigenous healing traditions adapted and defined by their culture, beliefs and environment, which satisfied the health needs of their communities over centuries (WHO, 2008). The increasing widespread use of TM has prompted the WHO to promote the integration of TM and CAM into the national health care systems of some countries and to encourage the development of national policy and regulations as essential indicators of the level of integration of such medicine within a national health care system (WHO, 2011).

Local Communities in the Asian, African and Latin American countries have a history of dependence on traditional remedies. In India, out of 4,752 communities, as many as 3,226 communities (around 70% of the community population) are dependent on traditional plant based medicine (Gadgil and Rao, 1998).

The search and use of drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are looking for phytochemicals for the treatment of various diseases. According to the World Health Organization, approximately 25 percent of modern drugs used in the United States have been derived from plants.

Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. More than two thirds of the world's plant species (around 35,000) having the medicinal value come from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants (Meskin, 2002).
Phytochemicals

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function (Meskin, 2002). For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs. Examples are inulin from the roots of dahlia, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove (Meskin, 2002). Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs.

1. **Alkaloids** are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in ethnogenic rituals. Examples are the local anaesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimetic galatamine; the spasmyloyis agent atropine; the vasodilator vincamine; the anti-arrhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

2. **Polyphenols** (also known as phenolics or polyphenols) are compounds contain phenol rings. The anthocyanins that give grapes their purple colour, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics.
3. Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore.

4. Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. (The name "Terpene" is derived from the word "turpentine"). Terpenes are major biosynthetic building blocks within almost every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavour additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural Terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavours used in food additives. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the red, yellow and oranges of pumpkin, corn and tomatoes respectively.

Therapeutic use of medicinal plants

Salicylic acid is a precursor of aspirin that was originally derived from white willow bark and the meadow sweet plant (Filipendula ulmaria L) (Raskin, 1992). Vincristine is an anticancer drug derived from periwinkle (Cantharnthus roseus Linn. G. Donn.) (Elhardallou, 2011).

In folklore medicine in Nigeria, Rauwolfia vomitoria (Afzel) is used for treating hypertension, stroke, insomnia and convulsion while Ocimum gratissimum L.
is used for treating diarrheal diseases. The seeds of *Citrus parasidi* are effective in treating urinary tract infections that are resistant to the conventional antibiotics (Oyelami *et al.* 2005); pure honey healed infected wounds faster than eusol (Okeniyi *et al.* 2007); dried seeds of *Carica papaya* is effective in the treatment of intestinal parasitoids; the analgesic and inflammatory effects of *Garcinia kola* is known to enhance its use for osteoarthritis treatment (Oyelami *et al.* 2009) and *Aloe vera* gel is as effective as benzyl benzoate in the treatment of scabies. Similarly, in South Africa, plant extracts with muscle relaxant properties are used by traditional birth attendants to assist in child deliveries (Klouceck *et al.* 2005).

Whole plant of *Achillea millefolium* (Biranjasipha) is useful as carminative and a simulative tonic that helps to expel gas from the stomach and intestine. It is reported to have healing and soothing effect on the mucous membrane and the aqueous extracts are used for hair thinning (Subhose and Narian, 2005). Root and leaf of *Adhatoda vasica* are used as expectorant in asthma, bronchitis, cough and dysmenorrheal. Similarly the popular Bilwa (*Aegle marmelos*) fruits are used against diarrhoea, gastritis and adult onset diabetes (Chopra *et al.* 2002).

Chopra *et al.* (2002) reported that the roots of Vacha (*Acorus calamus*) can be used for memory loss, anxiety, bronchitis, mental fatigue sinusitis, tension, head ache and joint pains.

Ayurvedic pharmacopoeia describes the use of *Asparagus racemosus* roots to increase muscle strength, stomach, lungs and sexual organs, to increase the breast milk secretion during lactation and in male impotence. The bulbs of garlic (*Allium sativum*) are carminative (Ariga and Seki, 2005), anti-hypertensive (Lee *et al.* 2005), stimulant in fevers, cough febrifuge and used to cure skin diseases (Maries and Fransworth, 1995). Brahmi is very useful as anti-ageing agent, used for bronchitis, cough, nervous exhaustion and epilepsy. It also improves memory and generalized fatigue (Chopra *et al.* 2002).

Punarnava (*Boerhaavia diffusa*) roots are diuretic, laxative, anti-inflammatory and used in asthma, bronchitis and anaemia (Subhose and Narian, 2005). The whole plant of *Centella asiatica* is useful to promote memory power and to reduce blood pressure and anxiety (Samy *et al.* 2008). Leaves of tejpatra (*Cinnamomum iners*) are useful in scorpion sting. Chicory is a powerful hepatic stimulant that increases bile
secretion, promotes digestion and enhances the action of liver glycogen, free radical induced DNA damage (Samy et al. 2008).

Guggul is a resin, the major ingredient in joint care and immune care (Kokate et al. 2005), increase white blood cell count and found to possess strong immune-modulating properties, it lowers cholesterol (Evans, 2006) and triglycerides, used as adjuvant for many therapies (Samy et al. 2008). The stigmas of Kunkuma (Crocus sativus) are carotenoid pigments having antioxidant properties, are the natural source of two B vitamins, riboflavin and thiamine (Subhose and Narian, 2005).

Datura plant is useful in whooping cough, muscle spasm, asthma and painful menstruation whereas Bhringaraj is useful in liver disorders, skin and hair care, improve complexion, calms the mind and strengthen spleen (Ayurvedic Pharmacopoeia, 1997). Emblica officinalis is administered for increasing RBC count (Kandya, 2005), anaemia asthma, bronchitis and stomach problems (Jose and Kuttan, 2000). Meshashringi is reported as sugar destroyer, has been shown in vitro to have a glycolytic action and reduces the strength of glucose solution. It increases insulin production and regeneration of pancreas cells-the site of insulin production. Piper longum (Pipali) is a powerful stimulant for both the digestive and the respiratory systems and has showed a rejuvenating effect on lungs (Samy et al. 2008).

Black pepper is one of the most renowned culinary spices containing an alkaloid piperine aiding in digestive process (Subhose and Narian, 2005). Phyllanthus amarus is useful in chronic liver disorders, jaundice, viral hepatitis and urinary tract infection (Nadig et al. 2004). Sarpagandha roots are used for high blood pressure, insomnia, sedative and are the source of reserpine, an anti-hypertensive drug used since 1970. Rubia cardifolia is considered as the best blood purifying herb (Chopra et al. 2002).

Guduchi is a rich source of natural vitamin C that has now been proved to be effective in inhibiting the growth of bacteria and in building up the immune resistance (Deshmukh and Usha, 1994). It possesses anti-cancer (Sultana et al. 1995), antimarial (Najib et al. 1999), anti-periodic, anti-allergic, anti-spasmodic, anti-inflammatory and anti-oxidant properties (Singh et al. 2003). The daily used ginger improves digestion and prevents nausea (vomiting and unsettled stomach).
Haldi (*Curcuma longa*) is an aromatic stimulant, carminative, blood purifier and useful in wound healing (Gul *et al.* 2004) whereas Methi is a carminative aphrodisiac, and used as a cooling drink (Nadig *et al.* 2004). Similarly several other plants like Castor, Ashoka, Babool, Ganja, Jira, Lal chandan, Jamun and Rose are used for therapeutic purpose (Ayurvedic pharmacopoeia, 1993).

*Aconitum ferox* is used as a cardiac stimulant (Agarwal *et al.* 2005), anti-rheumatic and anti-inflammatory (Ali Mohd, 2006) whereas neem is used as anthelmintic (Agarwal *et al.* 2005), astringent, antiseptic, purgative, emollient and antiplaque (Evans 2006). Saffron is used as colouring and flavouring agent, anti-spasmodic and stimulant (Kokate *et al.* 2005) and as an anti-tumour drug (Fikrat, 2002).

Kokum is used as an anti-obesity, hypolipidemic (Datta *et al.* 2002) anti-fungal (Oluyemi *et al.* 2007) and anti-ulcer agent (Mackeen *et al.* 2002).

Holi basil (*Ocimum tenuiflorum*) is an aromatic, stimulant tonic (Evans, 2006): it also possesses anti-oxidant, anti-inflammatory (Khan and Balick, 2001) and anti-diabetic properties (Samy *et al.* 2008). Similarly Ashwagandha is used as an anti-rheumatic, sedative, diuretic (Kandya, 2005), anti-inflammatory, anti-stress, anti-tumour (Anbalagan and Sadique 1999 and Archana and Namasiyavan 1999), rejuvenator, hypotensive and hemopoietic agent (Mishra *et al.* 2000).

The dried rind of *Garcinia gummi-gutta* is used as flavouring agent in curries instead of tamarind and lime in Konkan, Goa and SriLanka- especially in the preparation of fish and non-vegetarian curries (Thomas, 1965). Combogin, a toxic resin, has been obtained from *Garcinia* (Kirtikar and Basu, 1987).

**Safety concern**

Despite the widespread use of herbal medicines globally and their reported benefits, they are not completely harmless. The indiscriminate, irresponsible or non-regulated use of several herbal medicines may put the health of their users at risk of toxicity (Bury and Fullinfaw 1987 and Oshikoya *et al.* 2007). Also, there is limited scientific evidence from studies done to evaluate the safety and effectiveness of traditional medicine products and practices. Adverse reactions have been reported to herbal medicines when used alone or concurrently with conventional or orthodox
medicines. Despite the international diversity and adoption of traditional medicine in different cultures and regions, there is no parallel advance in international standards and methods for its evaluation. National policies and regulations also are lacking for traditional medicine in many countries and where these are available; it is difficult to fully regulate traditional medicine products, practices and practitioners due to variations in definitions and categorizations of traditional medicine therapies. Lack of knowledge of how to sustain and preserve the plant populations and how to use them for medicinal purposes is a potential threat to traditional medicine sustenance.

Selection of elite lines

Phenotype selection of superior lines within the available population forms the basic part of most of the tree improvement programme. Early work was mainly restricted to the basic aspects of tree improvement such as reproductive biology, cytology, phenology etc. Genetic variability within and among tree population is essential to derive their potentiality. The variability studies are helpful in analyzing and comparing the superior and inferior genotypes for each character which provides a benchmark for advanced breeding programme.

In case of forest trees, tree improvement through the application of genetic and silvicultural practices is required to hasten the process of modifying the selected elite lines so as to meet the required need. In tree species, fruit yield, seed yield and availability of essential parts like leaves per tree and economic parts determine the elite character. There are several micro and macro-environmental factors that influences the economic yield of a tree. Many of the yield attributing characters determines the quality and quantity of each tree yield.

Dogra (1981) provided genetic potentiality of different Indian tree species and opined that selection of superior phenotype through multigenerational improvement programme should be from indigenous species than from exotic lines, which are adapted to marginal lands in India. Khurana and Khosla (1982) studied the natural stands of Populus ciliata distributed in 25 provinces, grouped them into ecological blocks. The selected trees were analyzed for six characters viz. height, diameter, clear bole, taper, specific gravity and fibre length. Kushalappa (1986) reported that more studies were attempted for selection of candidate trees of timber species than fruit
yielding species. Beniwal and Singh (1990) selected the candidate plus trees of commercially important species in natural virgin forests and plantations of Arunachal Pradesh. They considered cylindrical and straight pole, good vigour, light crown with thin branches and self pruning ability characters during selection. Singh (1992) made selection of Chilgoza pine trees from different parts of Himachal Pradesh considering tree height, dbh, crown size, number of cones and 100 seed weight.

**Variability for economic traits**

Uniformity of the selected population is highly desirable, which is not expressed in most of the tree species. In these species, some of the traits like height, clear bole, dbh, crown depth, crown diameter, branch angle, fruit and seed yield, fruit characters are even though genetically controlled, there found lot of variability in tree species in general and *Garcinia* species in particular. It may be attributed by the ecological, soil and environmental factors. The variability observed in *Garcinia indica* and *Garcinia gummi-gutta* in particular and other related tree species are documented.

Zobel and Talbert (1984) concluded that intra and inter-population variability contribute more than ninety percent variations observed in most of the tree species. The Wealth of India (1956) describes *Garcinia combogia* as a small or medium sized tree with rounded crown and horizontal or drooping branches, leaves are dark green, simple, opposite: flowers are 4-merous, polygamous in fascicles. Thomas (1965) reports that *Garcinia combogia* is a medium sized dioecious tree generally attaining a height of 18 m.

*Garcinia mangostana*, simply known as ‘the mangosteen’ is a tropical evergreen tree growing to a height of 7 to 25 m (Parmar and Kaushal, 1982). Its fruits are referred to in Thailand as the “Queen of fruits’ as a result of its delicious taste. It is cultivated principally in Indonesia, Malaysia, the Philippines and Thailand. The purple ripe fruits consist of 6-8 seeds, and have a white and juicy pulp (Farnsworth and Bunyapraphatsara, 1992).

Korimanthimath and Desai (2005) reported abundant variation in naturally spread kokum trees in Goa and Konkan region. Tree habit varied from tall and conical to dome shaped and spreading type whereas the branching pattern varied from erect,
spreading to drooping type. Tree height varied from 6-12m, Early and Late types: Fruit yield varied between 50 and 350 kg per tree; Fruit size varied from 21 g to 85 g. Fruit shape also had shown variation (Round, oblong and oval) and the rind thickness varied between 0.2-0.8mm. In each fruit, the number of segments varied (4 to 8) and also found variation in TSS of fruit juice (6 to 12 degree Brix). As reported by Joshi et al. (2004), anthocyanin pigmentation varied from 7.87 mg/100 g to 17.03mg/g.

Kaekar et al. (1986) observed significant variations in *Tecomelia undulata* for diameter at base, at breast height and number of branches. Similarly Keiding et al. (1986) observed lot of variation in height and trunk diameter in teak. Manojkumar et al. (1996) reported high heritability with high genetic advance for leaf breadth while low heritability (0.207) and low genetic advance (15.68%) for basal diameter in a cloned bank comprising of 22 trees of sandal.

Muthulakshmi et al. (1999) studied 25-30 years old 15 well differentiated male and 15 bisexual *Garcinia gummi-gutta* trees for morphological variations. They observed variability for plant height, plant spread, collar girth, height at first branching, canopy shape, branching habit, colour of young flush, bark and feeder roots, and leaf characters during March-April 1998. No significant differences in morphological characteristics were observed between male and bisexual trees, except for emerging flush colour. It was light green in male trees and pinkish in bisexual trees. Again when they (Muthulakshmi et al. 2000) studied the variability in several homesteads of Kerala, they observed wide variability for vegetative, floral, fruiting and biochemical characters of the fruits. The results indicated that dome shaped trees were high yielding because of huge canopy, more interception of sunlight and more fruiting branches. Variation in fresh fruit yield was observed between 200 kg to 500-600 kg per annum. Occurrence of Gamboge, a physiological disorder, was observed in fruits harvested during the rainy season and the early flowered trees were almost free of the disorder. Kallaje (2000) conducted improvement studies in five ranges of natural population of *Garcinia indica*. He studied variability in morphological and yield attributing characters along with PCV and GCV and reported lot of variation in different ranges. In heritability studies, broad sense heritability of 0.847 was highest for tree parameters whereas fruit diameter (0.902) and seed thickness (0.750) have shown highest values for yield attributing characters.
Muthulakshmi et al. (2000) observed lot of variation in number of fruits and
fruit yield in the trees grown in homesteads of Kerala. Medium yielding trees
produced up to 200 kg fresh fruit per annum while high yielding trees
(Ac.40,42,43B,45A and 46) yielded up to 500-600 kg fruit per annum. Sawant et al.
(1999) evaluated 38 high yielding and early *Garcinia indica* types at Ratnagiri
District in Maharashtra. One genotype (S8) exhibited higher yield consistently for
seven years with short harvesting period (78 days) and minimum number of three
harvests. The fruit had the highest average width (4.15 cm), average circumference
(13.15 cm), average weight (34.45g) and average rind thickness (4.45mm). The fruits
also had the longest shelf life of 15 days.

Chacko and Pillai (1997) reported the seed characters in *Garcinia gummi-
gutta* (L). Generally they observed poor seed germination and they classified the seed
based on cotyledon colour as light cream, honey dew, new marigold, cherry and
damaged. They observed increase in seed germination from 50 percent to 90 percent
on removal of seed coat. Seeds with a moisture content of 41.65 percent were viable,
but failed to germinate on drying to 33.4 percent.

Mathew and George (1995) reported the dormancy and method of breaking it
in *Garcinia gummi-gutta*. They reported that freshly harvested seeds subjected to
germination took 13 months to germinate, but achieved 78 percent germination.
Germination percent declined as seed storage period increased. Removing the seed
coat reduced the time taken to germinate from 48-52 weeks to 4-5 weeks.

**Anti-obesity drugs for weight management**

Two different types of obesity-treatment drugs are currently available on the
market. One of these is orlistat (Xenical), that reduces intestinal fat absorption by
inhibiting pancreatic lipase (Ballinger and Peikin, 2002; Hutton and Fergusson, 2004;
Thurairajah et al. 2005 and Drew et al. 2007). The other is sibutramine (Reductil),
which is an anorectic, or appetite suppressant (Lean, 2001; Poston and Foreyt, 2004
and Tziomalos et al. 2009). Both drugs have side-effects, including increased blood
pressure, dry mouth, constipation, headache, and insomnia (Thurairajah et al. 2005;
A number of anti-obesity drugs are currently undergoing clinical development, including centrally-acting drugs (e.g. radafaxine and oleoyl-estrone), drugs targeting peripheral episodic satiety signals (e.g. rimonabant and APD356), drugs blocking fat absorption (e.g. cetilistat and AOD9604), and human growth hormone fragments (Halford, 2006 and Melnikova and Wages, 2006).

At present, because of dissatisfaction with high costs and potentially hazardous side-effects, the potential of natural products for treating obesity is under exploration, and this may be an excellent alternative strategy for developing future effective, safe antiobesity drugs (Park et al. 2005; Nakayama et al. 2007 and Mayer et al. 2009). A variety of natural products, including crude extracts and isolated compounds from plants, can induce body weight reduction and prevent diet-induced obesity. Therefore, they have been widely used in treating obesity (Moro and Basile, 2000; Han et al. 2005a and Rayalam et al. 2008).

A wealth of information indicates numerous bioactive components from nature that are potentially useful in obesity treatments. A good example of such is the polyphenols. These show strong anti-obesity activity and include apigenin, genistein, and the catechins (Wolfram et al. 2006; Rayalam et al. 2008 and Thielecke and Boschmann, 2009).

A growing body of evidence indicates that natural products having anti-obesity effects can be arranged into five categories based on their distinct mechanisms (1) decreased lipid absorption
(2) decreased energy intake
(3) increased energy expenditure
(4) decreased pre-adipocyte differentiation and proliferation, or
(5) decreased lipogenesis and increased lipolysis.

**HCA as natural appetite suppressant**

Natural (-)-hydroxycitric acid (HCA), prepared from *Garcinia cambogia*, is a potential natural appetite suppressant. Currently, it is commercial available under the names HCA-SX and Super CitriMax™ (Ohia et al. 2002). This phytochemical acts by increasing the release/availability of 5-hydroxytryptamine and/or serotonin; the latter
is a neurotransmitter implicated in the regulation of eating behaviour and appetite control (Ohia et al. 2002).


A study compared 42 adults taking 1.2 g/day HCA for 12 weeks with 47 adults taking placebo on weight loss and appetite suppression. Both groups lost weight, with the HCA group showing a significantly greater reduction (3.7± 3.1 kg vs. 2.4 ± 2.9 kg). However, there was no difference in appetite variables and the study did not support a satiety effect of HCA (Mattess and Bormann, 2000). The lack of effect of HCA on satiety was supported in a separate trial on 11 overweight males receiving 500 mg HCA per day for two weeks (Kovacs et al. 2001). Furthermore, satiety was unaffected in 24 adults receiving 900 mg/day HCA for two weeks compared with placebo, while 24-hour energy intake was decreased by 15–30 percent (Westerterp Plantega and Kovacs, 2002).

Although research has identified several active constituents possessing appetite-suppressive capabilities (*e.g.* glycosides, saponin, and flavonoids), the ways in which they work to suppress appetite are unclear; they are thought to amplify signalling in the basal hypothalamus’s energy-sensing function. The endogenous mechanisms of appetite regulation by these materials depend on the plant type. For example, *Hoordia gordonii* extract increased ATP content in the hypothalamic neurons regulating food intake in the rat brain (MacLean and Luo, 2004)

List of different plants and their part containing the active ingredient employed for appetite suppression is shown below.
### Table 2.1. Different medicinal plant parts showing appetite-repression activity

<table>
<thead>
<tr>
<th>Source</th>
<th>Active component</th>
<th>Experimental methods&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Major activity&lt;sup&gt;b&lt;/sup&gt;</th>
<th>References&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Panax ginseng</em> (root)</td>
<td>Crude saponins</td>
<td>200 mg/kg, SD rats with HFD, 3 weeks</td>
<td>37% decrease in body weight gain</td>
<td>Kim <em>et al.</em> (2005)</td>
</tr>
<tr>
<td><em>Garcinia cambogia</em></td>
<td>(-)-Hydroxycitric acid (HCA)</td>
<td>154 nmol HCA/kg, Zucker obese rats, 92 days</td>
<td>8% decrease in body weight gain</td>
<td>Saito <em>et al.</em> (2005), Heymsfield <em>et al.</em> (1998), Ohia <em>et al.</em> (2002)</td>
</tr>
<tr>
<td><em>Camellia sinensis</em> (leaf)</td>
<td>( )-Epigallocatechin gallate (EGCG)</td>
<td>1) 82 mg/kg SD rats (7 days), 2) 81 mg/kg lean Zucker rats (8 days), 3) 92 mg/kg obese Zucker rats (4 days)</td>
<td>1) 53% decrease in weight gain, 2) 32% decrease in weight gain, 3) 11% decrease in weight gain</td>
<td>Kao <em>et al.</em> (2000), Moon <em>et al</em> (2007), Dulloo <em>et al.</em> (1999), Nagao <em>et al.</em> (2005); Wolfram <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><em>Caralluma fimbriata</em> (cactus)</td>
<td>Crude ethanolic extract (pregnane glycosides)</td>
<td>1 g/day, overweight adult Indian men and women, 60 days</td>
<td>2.5% decrease in body weight gain</td>
<td>Kuriyan <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Cox lachrymajobi var.mayeun</em> (seed)</td>
<td>Crude aqueous extract</td>
<td>500 mg/kg, SD rats with HFD,4 weeks</td>
<td>36%* decrease in body weight gain</td>
<td>Kim <em>et al.</em> (2006a)</td>
</tr>
<tr>
<td><em>Hoodia gordonii</em> and <em>H. pilifera</em></td>
<td>Steroidal glycoside (P57AS3)</td>
<td>Intracerebroventricular injection, 24 h</td>
<td>40–60% reduction in food intake</td>
<td>MacLean and Luo (2004), van Heerden (2008), van Heerden <em>et al.</em> (2007), Lee and Balick (2007)</td>
</tr>
<tr>
<td><em>Phaseolus vulgaris</em> and <em>Robinia pseudoacacia</em></td>
<td>Lectins</td>
<td>100 mg/kg, Harlan–Wistar rats, 16 h</td>
<td>8.25-fold* decrease in food intake</td>
<td>Baintner <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><em>Pinus koraiensis</em> (pine nut)</td>
<td>Pine nut fatty acids</td>
<td>3 g, obese women, 4 h</td>
<td>60% increase in cholecystokinin-8(satiety hormone) secretion</td>
<td>Saito <em>et al.</em> (2005)</td>
</tr>
</tbody>
</table>

<sup>a</sup> (treated dose, subjects, duration of treatment): The mg/kg indicates a dose (mg) based on the experimental animal's body weight (kg), and% refers to proportion of the diet.

<sup>b</sup> The author recalculated values with an asterisk (*) from the original data (shown as figures) in each reference.

<sup>c</sup> Data from the first reference cited.
Effect of (-)-HCA on Fatty acid synthesis

(-)-HCA being a potent inhibitor of ATP citrate lyase, it limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis. In a cell-free system, consisting of particle-free cytoplasm and mitochondria prepared from rat liver, (-)-HCA was shown to inhibit fatty acid synthesis from citrate and also from \(^{14}\)C-alanine by measuring the incorporation of \(3\) H\(_2\)O and \(^{14}\)C (Watson and Lewenstein, 1970). Sullivan et.al (1972) studied the effect of stereo-isomers of hydroxyl citrate on the rate of lipogenesis on rat liver and found that only (-)-HCA significantly decreased the conversion of \(^{14}\)C citrate into lipid in the hi-speed supernatant and the conversion of \(^{14}\)C alanine into lipid \textit{in vivo}. Beynen and Geelen (1982) observed the inhibition of fatty acid synthesis from glucose by (-)-HCA in isolated hepatocytes. (-)-Hydroxycitrate markedly reduced the tritiated water incorporation into fatty acid by lung tissue slices (Evans and Scholz, 1977).

Early studies on obese rats and mice indicated that HCA decreased food intake and body weight gain. Body lipid levels decreased but body protein levels remained unchanged. In contrast, citrate had no such effect (Sullivan and Triscari, 1977). In genetically obese Zucker rats, administration of HCA in the diet for 39 days reduced food intake and body weight but had no effect on percentage of body fat (Greenwood et al. 1981).

Sheehan and Yeh (1984) observed that (-)-HCA inhibited fatty acid synthesis in neonatal rat lung, from glucose, pyruvate and \(\beta\)-hydroxybutyrate by 88 percent, 70 percent and 60 percent respectively, but had no effect on that from acetoacetate. Hood et al. (1985) have shown that (-)-HCA reduced the synthesis of fatty acids from lactate and glucose in bovine adipose tissue and rat adipose tissue respectively. Inhibition of lipogenesis in rat brain slices is also observed by Patel and Owen (1976) and Sterling and O’Neill (1978).

(-)-HCA promoting Glycogenesis and Lipid oxidation

Sullivan et.al (1974) reported an increase in the rate of \textit{in vivo} hepatic glycogen synthesis with the administration of (-)-HCA. They proposed that (-)-HCA acted primarily through its affect on the appetite, possibly involving increase in glycogen level.
Shara et al. (2004) studying on the physico-chemical properties of (-)-HCA, have demonstrated the efficacy of 60 percent Calcium-Potassium salt of HCA derived from *Garcinia cambogia* (HCA-SX, Super citrimax) on weight management. They concluded that HCA-SX promotes fat oxidation, enhances serotonin release and availability in the brain cortex, normalizes lipid profiles and lowers serum leptin levels in obese subjects. They evaluated the dose and time-dependent effects in Sprague-Daley rats and concluded that 90 days treatment of HCA-SX resulted in reduction in body weight without any changes in major organs.

Preuss et al. (2004b) evaluated the efficacy of (-)-HCA alone (HCA-SX) and in combination with niacin-bound chromium (NBC) on moderately obese human subjects and revealed that HCA-SX had shown to reduce appetite, inhibit fat synthesis and decrease body weight by 5-6 percent without stimulating the central nervous system. Again Preuss et.al (2004a) conducted a pilot study in Elluru, India administering the treatment of HCA coupled with 30 min/day walk. At the end of eight weeks, observed decrease in body weight (6.3%), food intake (4%), total cholesterol (6.3%) and triglycerides (8.6%). Soni et al. (2004) suggested that intake of HCA up to 2800 mg/day is safe for human consumption.

**Antioxidant activity**

Free radicals are atomic or molecular species with unpaired electrons. They are highly reactive and unstable as compared to similar ions. Free radicals play an important role in many biological processes including metabolic pathways, cell signaling, immune response, and a variety of pathophysiological conditions. Free radicals are generated in the biological environment as a result of reactions associated with common biochemical pathways involving oxygen metabolism. Thus, their universal presence and their role as critical mediators of normal and pathophysiology have resulted in considerable development of techniques that can detect these radicals (Rao et al. 2010). Some of the free radicals and other important oxidants found in living organisms are shown in the Table 2.2.

Reactive oxygen species are produced continuously in the human body as a consequence of normal metabolic processes. If free radicals are not inactivated, their chemical reactivity can damage all types of cellular macromolecules, including
Table 2.2 Some important reactive oxygen species in living organisms

<table>
<thead>
<tr>
<th>Free radicals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl radical</td>
<td>OH</td>
</tr>
<tr>
<td>Superoxide radical</td>
<td>O2^-</td>
</tr>
<tr>
<td>Nitric oxide radical</td>
<td>NO</td>
</tr>
<tr>
<td>Lipid peroxyl radical</td>
<td>LOO^-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non radicals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>H2O2</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>^1O2</td>
</tr>
<tr>
<td>Hypochlorous acid</td>
<td>HOCl</td>
</tr>
<tr>
<td>Ozone</td>
<td>O3</td>
</tr>
</tbody>
</table>

proteins, carbohydrates, lipids, and nucleic acids. Figure 2.1 illustrates some of the types of damage that can result from the actions of free radicals. Several of these effects have been implicated in the causation of degenerative diseases. For example, destructive effects on proteins may play a role in the causation of cataracts, effects on DNA are involved in cancer causation, and effects on lipids apparently contribute to the causation of atherosclerosis (Lillian Langseth, 1995).

![Fig 2.1 Types of damages due to the action of free radicals](Lillian Langseth, 1995)
Antioxidants inhibit oxidation either by inhibiting pro-oxidants thereby preventing the formation of free radical species or by retarding the rate of reaction of oxidative species with their biological targets, thereby slowing down the free radical chain propagation (Kohen and Nyska, 2002). If the free radical chain is allowed to propagate, it can follow multiple pathways leading to a vast number of end products.

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. The major characteristic of an antioxidant is its ability to trap free radicals viz. phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in grains, oil seeds, fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported by Miller and Rigelhof et al. (2000a).

Methods

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of natural products. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. The different types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the superoxide anion radical (O$_2^-$), the hydroxyl radical (OH), or the peroxyl radical (ROO$_2$).

The various methods used to measure antioxidant activity of plant extracts can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. Antioxidant activity methods using free radical traps are relatively straightforward to perform. The ABTS [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (Miller and Rigelhof et al. 2000a &b) has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics. Prior et al. (1998) has used the Oxygen Radical Absorbance
Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2, 2’-azobis (2-amidinopropane) dihydrochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound, B-phycoerythrin or R-phycoerythrin. Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified employing Folins reagent.

Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of natural products would be to reference to a common standard Trolox, Vitamin C etc.

There are several examples of isolation and extraction of antioxidants from plant materials viz. Herbal Cigarettes, tea, and capsules, (Siegel, 1976); Paetta indica and Osbeckia octandra (Thabrew et al. 1987); Chrysanthemum morifolium (Duh, 1999); Mutisia friesiana (Asteraceae) and Sanicula graveolens, (Vituro et al. 1999); Salvia reflexa (Malencic et al. 2000); Rubus idaeus, Rubus occidentalis, and Fragaria ananassa (Shiow and Hsin-Shan, 2000); Cordyceps sinensis (Li et al. 2001); Olive extracts (McDonald et al. 2001); Cetraria islandica, (Gulcin et al. 2002); Pluchea indica, (Sen et al. 2002); Allium cepa, Illicium religiosum, Fagopyrum esculentum, Origanum officinalis, Rosmarinus officinalis, Pyrus pyrifolia, Acanthopanax senticosus, Eugenia caryophyllata and Erigeron annuus (Young and Kyong, 2003); Ardisia compressa (Sonia and de Mejia, 2004); Aframomum danielli, Allium cepa, Allium sativa, Capsicum frutescens, Citrus sinensis, Curcuma longa, Justicia flava, Ocimum gratissimum, Piper guineense (Odukoya et al. 2005); Fagopyrum esculentum (Ting and Chi-Tang, 2005); Cytisus scoparius (Raja Sundararajan et al. 2006); Rhodiola sacra, Polygonum multiflorum and P. multiflorum (Chi-Chun et al. 2006); Zanthoxylum piperitum (Yamazaki et al. 2007), Coleus Blumei, Orthosiphon Stamineus, Ocimum basilicum and Mentha arvensis (Zuraini Zakaria et al. 2008); Caryya cathayensis (Chenggang Zhu et al. 2008), Boerhaavia diffusa (Rachh et al. 2009), Thymus vulgaris and Lavendula multifida (Ramchoun, 2009), Calotropis procera, Gmelina arborea Kigelia pinnata, , Potentilla species (Tomczyk et al. 2010), Carotenoid lutein (Sindhu et al. 2010), Oxyxylum indicum (Mishra et al. 2010), Artemisia absinthium (Bora and Sharma, 2011), Enicostemma littorale (Abirami et al. 2011).

Mullika et al. (2007) demonstrated that out of nineteen Thai medicinal plants tested for antioxidant property, *Garcinia mangostana* possessed the most significant antioxidant activity and reduced the reactive oxygen species production. The antioxidant activity evaluation using *in vitro* methods conducted by Kamil et al. (2010) revealed significant antioxidant activity for *Garcinia indica* extract. The observed activity may be mainly due to the presence of phytochemicals like garcinol and hydroxycitric acid (HCA) apart from citric acid, maleic acid, polyphenols, carbohydrates, anthocyanin pigments and ascorbic acid.

In another study to estimate the total phenolic and flavonoid content, and to evaluate *in-vitro* antioxidant activity of Methanolic fruit extract of *Garcinia indica*, the raw, dry fruit powder was extracted with 99.9 percent methanol. Phytochemical test showed that extract contains higher level of total phenol and flavonoids. Total phenolic compound in methanolic fruit extract of *Garcinia indica* was found to be 0.348 mg/g of extract calculated as gallic acid equivalent ($R^2 = 0.985$) and total flavonoids compound was found to be 137.27 µg/g of extract calculated as quercetin equivalent ($r^2=0.997$). The extract was screened for its potential antioxidant activities using tests such as hydroxyl radical-scavenging activity, reducing power activity, and hydrogen peroxide-scavenging activity. The *in-vitro* antioxidant assay showed *Garcinia indica* posses potent antioxidant activity when compared with reference compound ascorbic acid. *Garcinia indica* could be useful for preparation of nutraceuticals as potent antioxidant to treat various human diseases and its complications (Tushendra Singh et al. 2011).

**Antioxidative Properties of Garcinol**

The antioxidative ability of Garcinol has been investigated in *in vitro* and *in vivo* model systems (Yamaguchi et al. 2000). Using a hypoxanthine/xanthine oxidase system, Garcinol was shown to retard superoxide anion to nearly the same amount as DL-alpha-tocopherol, an established anti-oxidant, while its ability to quell hydroxyl radicals in the Fenton reaction system was even better than that of alpha-tocopherol.
In addition, the authors also explored the antioxidative power of Garcinol \textit{in vivo} using an indometacin induced rat model for acute ulceration. Oral administration of Garcinol prevented acute ulceration in these rats, suggesting its potential as an antiulcer drug. Yamaguchi \textit{et al.} (2000) also analyzed the chelating activity, free radical scavenging activity and anti-glycation activity in addition to anti-oxidative activity and opined that garcinol might be a potent anti-oxidant and a glycation inhibitor under specified conditions. Sang \textit{et al.} (2002) studying on \textit{Garcinia indica} fruit rind purified the Garcinol, a poly-isoprenylated benzophenone and studied its anti-oxidant actions. He isolated four reaction products with peroxy radicals and identified. In another study, antioxidative and neuroprotective properties of Garcinol in rat cortical neuron cultures was observed. This was suggested to occur via prevention of nitric oxide (NO) accumulation in lipopolysaccharide treated astrocytes (Liao \textit{et al.} 2005).

The examples above help to establish the antioxidative effect of Garcinol \textit{in vitro} and \textit{in vivo} model systems. In terms of its structure, the phenolic hydroxyl groups coupled with the $\beta$-diketone moiety in Garcinol may, via formation of resonance stabilized intermediates, help to prevent the free radical species from propagating and thereby limiting further oxidative-stress-related damage down the road.

\textbf{Wound healing activity}

Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissue (Chithra \textit{et al.} 1998) or the ‘wound’ refers to any opening in tissue due to either internal or external factor which results in cell death and cell injury. The word ‘healing’ means replacement of destroyed tissue by living tissue. Wound may be caused by trauma-either accidental or surgical, by physical, chemical and microbial agents or by ischaemia, which leads to infarction.

Healing is the interaction of a complex cascade of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured skin. Healing is a systematic process, traditionally explained in terms of 3 classic phases: inflammation, proliferation, and maturation. A clot forms and inflammatory cells debride injured tissue during the inflammatory phase. Epithelialization,
fibroplasia, and angiogenesis occur during the proliferative phase. Meanwhile, granulation tissue forms and the wound begin to contract. Finally, during the maturation phase, collagen forms tight cross-links to the other collagen and with protein molecules, increasing the tensile strength of the scar (Iba, 2004).

In the traditional systems of medicine, various plants have been used to promote wound healing. Many investigators reported the wound healing effect of the various plant extracts such as *Aloe vera* (Udupa *et al.*1994); *Trigonella foenum graecum* (Taranalli and Kuppast, 1996); *Hypericum mysoreNSE* (Mukherjee and Suresh, 2000); *Nelumbo nucifera* (Mukherjee *et al.* 2000); *Ginkgo biloba* (Bairy and Rao, 2001); *Gmelina arborea* Roxb (Shirwaikar *et al.* 2002); *Bryophyllum pinnatum* (Mahamood and Patil, 2002); *Terminalia arjuna* (Madhura and Sushma, 2003); *Eucalyptus globulus* (Kusum *et al.* 2004 and Hukkeri *et al.* 2006); *Diospyros cordifolia* (Mankani *et al.* 2004); *Saussurea lappa* (Ganachari *et al.* 2005); *Plagiochasma appendiculatum* (Meenakshi *et al.* 2006); *Madhu qhrita* (Charde *et al.* 2006); *Embelia ribes* (Kumara Swamy *et al.* 2007); *Lycopodium serratum* (Manjunatha *et al.* 2007) and *Ocimum sanctum* (Somasekhar Shetty *et al.* 2008); *Abutilon indicum* (Roshan *et al.* 2008); *Lantana camara* (Nayak *et al.* 2008), *Rubia cordifolia* (Karodi *et al.* 2009), *Sabutilon indicum* (Ganga suresha *et al.* 2011) and *Ocimum basilicum* (Renu solanki *et al.* 2012).

*Garcinia mangostana* fruit rind has been in use in Thai folk medicine for the treatment of skin infections (Mahabusarakam *et al.* 1986) and wounds (Pongphasuk *et al.* 2003). Jirat *et al.* (2008) studying on the effect of topical administration of *Centella asiatica* and *Garcinia mangostana* fruit rind extraction in diabetic rats revealed that size of the wounds in diabetes condition were bigger than those of normal rats at the same time of window treatment. *Garcinia* extract was more effective over *Centella* extract, neomycin and control. Increase in collagen was observed, which in an index of improvement of wound healing. Nainwal *et al.* (2010) studied the antioxidant potential and wound healing activity of the aqueous extract of fruits of *Garcinia mangostana*. The results indicated that there was a decrease in the epithelialisation period along with a visibly decreased scar area compared to Nitrofurazone ointment and control, indicating the stimulated wound contraction.
Antimicrobial activity

Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns.

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly diverting their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004).

The plants possess innumerable number of secondary metabolites which are usually produced under stress conditions and often in response to infections. These secondary metabolites possess profound antimicrobial potency. Many workers have isolated different types of active constituents and studied for their antimicrobial potency. Alkaloids (Burdick, 1971); phenolic compounds (Mason and Wasserman, 1987); tannins (Scalbert, 1991); flavanones and flavonoids (Panilio et al. 1992); sesquiterpenes (Topcu et al. 1993); anthroquinone (Kazmi, 1994); flavonoid glycosides (Hasan and Ahmad, 1996); triterpene acid glycosides (Kirmizigul et al. 1996); diterpenes and triterpenes (Akbar and Malik, 2002). These active constituents isolated from medicinal plants showed significant antimicrobial effect (Cowan, 1999).

The efficacy of plant extracts against microorganisms is of considerable interest among various investigators. Many plant species has shown antimicrobial activities like Mitracarpus scaber (Ekpendu et al. 1994); Landolphia owrrience (Ebi and Ofoefule, 1997); Enantia polycarpa (Ajali, 2000); Ricinus communis (Parameswari and Tulasi Latha, 2001); Bixa orellina (Castello et al.2002); Melissa officinalis (Mimica-Dukie, 2004); Solanum stramoenifolium Jacq., S. seaforthianum Andr. and S. violaceum Ortg (Manjunatha et al. 2004); Eupatorium glandulosum (Sasikumar et al. 2005); Quercus infectoria (Basri and Fan, 2005) Bacopa monnieri
(Ghosh et al. 2006), Althaea officinalis, Mentha longifolia, Melissa officinalis and Rosa damascene (Bassam Abu-Shanab et al. 2006); weeds of Euphorbia family Euphorbia tirucalli (Asha et al. 2009), Carthamus tinctorious (Paramesha et al. 2009). Cocos nucifera (Rajiv et al. 2011), in Vitex negundo (leaf), Adathoda vasica (leaf), Azadirachta indica (leaf), Mentha piperita (leaf) and Curcuma longa (rhizome) (Amit kumar et al. 2011), in Peumus boldus, Agathosma betulina, Echinacea angustifolia, Humulus lupulus, Glycyrrhiza glabra, Mahonia aquifolium, Usnea barbata and Anemopsis californica (Chitra et al. 2012) and in Aegle marmelos, Albizia amara, Cassia auriculata (Caesalpinoideae), Cissus quadrangularis (Natchimuthu et al. 2012).

Maridass et al. (2010) using the leaf extract of Garcinia gummi-gutta observed significant antibacterial activity against Bacillus subtilis, Klebsiella pneumonaeae, Aeromonas hydrophila, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Streptococcus pyogenes. Varalakshmi et al. (2010) in their studies on Garcinia indica fruit rinds reported that extract has both antifungal and antibacterial properties and has a potential for use as a biopreservative in food applications.

**Molecular markers and RAPD analysis**

Herbal medicinal products may vary in composition and properties, unlike conventional pharmaceutical products, which are usually prepared from synthetic, chemically pure materials by means of reproducible manufacturing techniques and procedures. Correct identification and quality assurance of the starting material is, therefore, an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy (Straus, 2002).

Molecular markers generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids. DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors (Chan, 2003).

DNA can be extracted from fresh or dried organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection (Powell et al. 1996). Various DNA-based methods for species
characterization and adulteration detection in medicinal plants; agricultural crops and genetically modified (GM) foods have been published.

**Types of DNA markers used in plant genome analysis**

Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR)-based methods and sequencing-based methods (Joshi et al. 1999).

**Hybridization-based methods**

Hybridization-based methods include restriction fragment length polymorphism (RFLP) and variable number tandem repeats. Labelled probes such as random genomic clones, cDNA clones, probes for microsatellite and minisatellite (Jeffrey et al. 1985) sequences are hybridized to filters containing DNA, which has been digested with restriction enzymes. Polymorphisms are detected by presence or absence of bands upon hybridization.

**PCR-based methods**

PCR-based markers involve *in vitro* amplification of particular DNA sequences or loci, with the help of specific or arbitrary oligonucleotide primers and the thermostable DNA polymerase enzyme. PCR-based techniques where random primers are used, include random amplified polymorphic DNA (RAPD), arbitrarily primed PCR (AP–PCR) 30 and DNA amplification fingerprinting (DAF).

Inter simple sequence repeats (ISSRs) polymorphism is a specific primer-based polymorphism detection system, where a terminally anchored primer specific to a particular simple sequence repeat (SSR) is used to amplify the DNA between two opposed SSRs of the same type.

Polymorphism occurs whenever one genome is missing in one of the SSRs or has a deletion or insertion that modifies the distance between the repeats. A recent approach known as amplified fragment length polymorphism (AFLP) (Vos et al. 1995) is a technique that is based on the detection of genomic restriction fragments by PCR amplification. Adaptors are ligated to the ends of restriction fragments followed by amplification with adaptor-homologous primers. AFLP has the capacity to detect thousands of independent loci and can be used for DNAs of any origin or complexity (Kumar, 1999).
Isozyme genetic markers are efficient tools to study genetic variations within and between populations of less known wild species as well as for studies on spatial distribution of genetic variation. A study was conducted by Parthasarathy et al. (2010) with four important isozyme markers namely, peroxidase, polyphenol oxidase, esterase and superoxide dismutase in *Garcinia gummigutta* population collected from Western Ghats in South India. The cluster analysis of the marker bands showed that most of the population from similar geographic locations was the first one to group them, though a significant pattern was not noticed. The mean percentage of polymorphic loci was 52.5 percent. Total heterozygocity was 0.97 which is consistent with the average of tropical tree species.

**Random Amplification of Polymorphic DNA (RAPD)**

It is a type of PCR reaction, but the segments of DNA that are amplified are random. RAPD markers are decamer (10 nucleotide length) DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way (Kala et al. 2006).

**Genetic variation/genotyping**

It has been well documented that geographical conditions affect the active constituents of the medicinal plant and hence their activity profiles (Oleszek et al. 2002). Many researchers have studied geographical variation at the genetic level. Estimates of genetic diversity are also important in designing crop improvement programmes for management of germplasm and evolving conservation strategies. RAPD-based molecular markers have been found to be useful in differentiating different accessions of *Taxus wallichiana*, neem, *Juniperus communis* L., *Codonopsis pilosula, Allium schoenoprasum* L., *Andrographis paniculata* (Padmesh et al. 1999).

Along with authentication of species identity, prediction of the concentration of active phytochemicals may be required for quality control in the use of plant materials for pharmaceutical purposes. Identification of DNA markers that can correlate DNA fingerprinting data with quantity of selected phytochemical markers associated with that particular plant would have extensive applications in quality control of raw materials.
AFLP analysis has been found to be useful in predicting phytochemical markers in cultivated *Echinacea purpurea* (Baum *et al.* 2001) germplasm and some related wild species. RAPD fingerprint has been developed to support the chemotypic differences in oil quality of three different genotypes of *Pelargonium graveolens* (Shasany *et al.* 2002) and flavonoid composition of *Aconitum* (Fico *et al.* 2003) species.

DNA profiling has been used to detect the phylogenetic relationship among *Acorus calamus* chemotypes differing in their essential-oil composition. *Artemisia annua*, a source of anti-malarial compound artemisinin, shows variation in artemisinin content all over India. These chemotype variants of *A. annua* L. have been characterized using RAPD markers. This study also revealed existence of high levels of genetic variation in the Indian population despite geographical isolation and opens out a possibility of further genetic improvement for superior artemisinin content. An attempt has also been made to study variation in essential-oil components and interspecific variations using RAPD technique (Sangawan *et al.* 2003).

Morphological, chemical and genetic differences in twelve basil (*Ocimum gratissimum* L.) accessions were studied to determine whether volatile oil and flavonoids can be used as taxonomical markers and to examine the relation between RAPDs and these chemical markers (Vieira *et al.* 2001).

Sahasrabudhe and Deodhar (2010) have reported a fast, reliable and less expensive method of genomic DNA isolation from leaves of *Garcinia indica*. They were able to isolate pure and sufficient amount of DNA, which proved to be amenable to RAPD analysis. A preliminary study of variation within *Garcinia indica* species was carried out with nine plants with twenty decamers. Out of twenty, six primers showed polymorphism while three had monomorphic banding pattern.

RAPD markers have been used for identification and DNA fingerprinting of the date palm varieties, although the exhibited polymorphism was low (Sedra *et al.* 1998) in comparison with other cultivated species (Koller *et al.* 1993; Akkak, 1996). Moreover, RAPD analysis is not particularly robust because the results are influenced by experimental conditions, often making reproduction of results between labs difficult or impossible (Lowe *et al.* 1996). This technique has been used for cultivar
genotyping (Ben-Abdallah et al. 2000; Trifi et al. 2000) and for analyses of phylogenetic relationships and genetic diversity (El-Tarras et al. 2007). Using this technique, most of the examined Saudi grown cultivars were observed to have a narrow genetic base; that is, more than 50% genetic similarity (Al-Moshileh et al. 2004; El-Tarras et al. 2007), except Barhi which exhibited only 34% genetic similarity in the study by Al-Khalifah and Askari (2003). Using the experimental conditions and the particular collection of varieties in their study, Sukkari Asfar (genetic similarity 66-85%) clustered with other Saudi cultivars (Ajwa, Rothanah and Nabtet Ali) that exhibited a narrow genetic base (66 - 96.3% similarity). It is worth mentioning that Barhi and Sukkari are Iraqi cultivars, while Sukkari Asfar cv. is a Saudi-grown biotype of Sukakari (Ghaleb, 2008).

RAPD markers were used to detect somaclonal variation in TC-derived plant from four date palm varieties. RAPD markers were used by Eshraghi et al. (2005) to analyze the genetic stability of somatic embryogenesis-derived regenerates and mother plant in the Iranian-grown date palm cultivar Khinaizi.