V. DISCUSSION
Traditional medicines are standardized herbal preparations consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases. Several important modern drugs are extracted directly from plants. Some drugs are the synthesized copies of chemicals found naturally in plants and this has resulted in the development of herbal medicines using the traditional healing systems (Tyler, 2000). Plants and their secondary metabolite constituents have a historical background in modern and certain system of traditional medicine. The chemical principles from the natural sources have contributed significantly to the development of new drugs from medicinal plants such as atropine, codeine, digoxin, morphine, quinine and vincristine. Herbal drug is a chief constituent of traditional medicine and a common constituent in ayurveda, homeopathy, naturopathy and other medicine systems (Abhishek et al. 2006).

Herbs are considered as safe because of their natural origin. Herbal medicines contain underground or aerial plants part as their active ingredient and are collected mainly from the wild (Catherine et al. 1998). The existence of traditional medicine depends upon the plant species diversity of that region and the related knowledge of their use as herbal medicine. India has one of the richest plant medical traditions in the world, practicing all forms of traditional medicine. India is ranked at 8th position in world Biodiversity with 47,000 species of plants (Hamilton, 2003). Compared with well-defined synthetic drugs, herbal medicines exhibit some marked differences. Its empirical use in folk medicine is a very important characteristic, but the active principles are unknown and the availability of the quality raw material is problematic. Even though the standardisation, stability and quality control are feasible, it is difficult to carry out and in most of the cases it is not strictly followed. The herbal medicines are more suitable for chronic treatments and have a wide range of therapeutic uses. Since it costs less with minimum or no side effects, herbal medicine still occupies a larger portion in the health area.

It has been estimated that only 6 percent of all described species have been analysed chemically and only a small fraction analysed pharmacologically (Choudhary, 2002). Some alkaloids like ephedrine are found in some species of Ephedra. This has been isolated, characterised and its mechanism of action is understood (Calixto, 2000) whereas for many other commonly used products, such information is incomplete or unavailable. This is mainly due to complexity of herbal
and botanical preparations since they are not pure compounds and also may be due to the combination of several active principles in some herbal preparations leading to the beneficial effects. A group of experts constituted by WHO have reported that ‘A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore or improve health’ (WHO, 1998). Even though the rich experience obtained from their traditional use over the years cannot be ignored, still most of the herbal medicines need to be studied scientifically.

In terms of the volume of medicinal plants exported, India ranks second in the world. Hence there is an enormous scope for India to emerge as a major player in the global herb based medicines and products by developing its Research and Development capability.

In the present investigation, two *Garcinia sp.* i.e. *Garcinia indica* and *Garcinia gummi-gutta* were studied in Agumbe and Nagara range of Western Ghats. These two species were evaluated for the variation in morphometric characters, Protein and HCA content, phytochemical constituents, antioxidant, wound healing, antimicrobial proprieties in addition to molecular characterization by RAPD. The results of the study undertaken are discussed in the proceeding pages.

**A. Variability studies**

Variability in quantitative characters is very useful in tree breeding programme. The variability present in a population gives scope for the selection of elite genotypes. Existence and expression of variation is essential for the improvement and adoptability of a species to diverse environmental situations. Quantitative characters are controlled by polygenes and they are highly influenced by the environmental conditions.

**1. Variability in sex forms of two species**

*Garcinia indica* and *Garcinia gummi-gutta* belonging to Clusiaceae family are sub-dioecious in nature. Both male and bisexual trees exist separately. The different sex forms in the two species were studied at the two ranges and two different sex forms were found only in *Garcinia gummi-gutta* sp. but were absent in *Garcinia indica* sp. in both the locations. The absence of male flowers and in turn the male
trees in *Garcinia indica* is confirmed in ‘Indian Medicinal plants: A compendium of 500 species’ (1995). Male and bisexual trees were observed in *Garcinia gummi-gutta* in both the locations.

2. Variability in flowering and fruit set

Flowering and fruit initiation was studied in the two ranges. Early flowering of both the species was observed in Nagar range compared to Agumbe range. This may be due to the difference in altitude and other environmental conditions. Nagar range comprising of the chakra dam area is placed at low altitude (562 to 573 m above MSL) compared to Agumbe (628 to 632m). Nagar receives high rainfall, onset of monsoon is early and peak during June, July and August compared to Agumbe range. Because of its proximity to sea, humidity and temperature is relatively more in Nagar range which might promote early flowering. Agumbe, even though regarded as chirapunji of South, receives around 2800-2900 mm annual rainfall compared to 3200-3300 mm rainfall of Nagar (Shimoga District Statistics, 2011).

During the period of investigation, a white type was observed in *Garcinia indica* in Nagar range only and that too two trees were found in the entire area and no such type was observed in Agumbe range. Even though there is a mention of presence of white type fruit in the Indian Medicinal plants Compendium (1995), no reports is available on the characteristic of this white fruit; its phytochemistry and its wound healing and other pharmacological properties. The presence of white fruit tree is not reported even from other traditional spots of Western Ghats, Maharashtra and Goa where *Garcinia indica* is found growing naturally.

Hence the white type was included in the study and observed for different characters as a part of the study. The colour of the juice, TSS content, acidity, flower characters, leaf characters and wound healing properties were differing from that of normal red fruit type. It may be a variant of the red type but not distributed widely in the natural habitat. Further detailed studies targeting this white type are required in this aspect.

Like many other perennials, *Garcinia gummi-gutta* yield fluctuates over the years while *Garcinia indica* maintains the yield. Normally, a fully grown established tree of *G. gummi-gutta* yields 300 to 400 kg fruits annually while *Garcinia indica* tree
yield 200 to 350 kg. *Garcinia indica* fruits are red, less sour compared to *Garcinia gummi-gutta* and hence damage by monkey and other animals in *Garcinia indica* is more resulting in less harvest per tree in naturally grown habitat. Observation over four years of study has indicated that there was no much variation in the per tree yield of *Garcinia indica* in both Agumbe and Nagara ranges, while *Garcinia gummi-gutta* has shown lot of variation.

During the first year of study, good bunches of fruits were visible in *Garcinia gummi-gutta*. During next consecutive years, about 10-20 percent and up to 40 percent reduction in fruit set was observed. Usually male tree shows profuse flowering than the bisexual tree. Even in male trees, the number of flower reduced drastically over the 4 years and it appears that a cycle of 4-5 years may be ruling in *Garcinia gummi-gutta* species. This has to be ascertained by further studies.

It was observed in *Garcinia indica* that damage by monkey and other animals in white fruits is comparatively less than in red type and it may be due to the fact that white fruits has very less TSS (4.2%) compared to red type (14%) and also higher total acidity (2.23 me/100g) compared to red fruits (2.18 me/100g).

3. Variability in morphometric characters

Owing to the forest tree species to its timber value, much work was concentrated on improvement of timber species and fewer efforts are gone into the selection of fruit yielding trees (Kushalappa, 1986).

3.1 ANOVA

Wide variability was observed for morphometric characters in *Garcinia indica* in the selected two ranges. It was highly significant for tree height, height up to first branch and number of branches in both the ranges. In each range, five locations were selected comprising of five candidate trees per location within each location. Within each location, the extent of variability was less and between locations in each range observed variability. Canopy width and diameter at breast height (dbh) have shown little variability in Agumbe range while appreciable amount of variability was observed in Nagara range. When these characters were viewed between the two ranges, the variation for tree height and height up to first branch were not significant.
It may be because the trees were almost of similar age group. Variation for diameter at breast height and number of branches was marginally significant while for canopy width was highly significant. These results are in agreement with studies of Kallaje (2000) and Korikantimath and Desai (2005).

In *Garcinia gummi-gutta*, all the tree characters were significantly differing within each location and also between locations in each range. The total variability is indicated in terms of probability values. Common observation in both the ranges is that *Garcinia gummi-gutta* trees were closely placed and congregating around each other while *Garcinia indica* trees were comparably scattered. This may also attribute to the damage of fruits by animals.

Variability of tree characters of *Garcinia gummi-gutta* between the ranges is in similar line with that of *G. indica*. Tree height and diameter at breast height were not significantly differing between Agumbe and Nagar range while canopy width, number of branches and height up to first branch has number significant variability. This is in confirmation with the results of Muthulashkhi et al. (1999).

### 3.2 Variability studies using principal component analysis

Principal component analysis helps in providing evidence of the phenotype relationships within the plant population under natural and artificial selections. The analysis helps in isolating the genotypes that are distantly related and bringing together the closely related genotypes.

The geographical diversity is positively associated with genetic diversity. The extent of genetic divergence existing is crucial for a productive heterosis breeding programme. The extent of heterosis has been related to genetic diversity of parents in a number of species (Hagberg, 1952).

#### 3.2.1 *Garcinia indica* of Nagar range

In *Garcinia indica* at Nagar range, the first principal component has accounted for 69 percent of total variance and the next three components accounted for 29.20 percent. The four components put together accounted for 98.2 percent variance. When it is plotted on a graph with two major components on X and Y-axis, 8 clusters are formed as shown in Table 5.1 along with one genotype no.22 remaining.
Table 5.1 Clusters formed and genotypes in *Garcinia indica* at Nagara range

<table>
<thead>
<tr>
<th>Cluster No.</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5,21,23,24,25</td>
</tr>
<tr>
<td>II</td>
<td>6,7,8,9,10</td>
</tr>
<tr>
<td>III</td>
<td>2,3,4</td>
</tr>
<tr>
<td>IV</td>
<td>12,13,16</td>
</tr>
<tr>
<td>V</td>
<td>11,15</td>
</tr>
<tr>
<td>VI</td>
<td>17,19</td>
</tr>
<tr>
<td>VII</td>
<td>1,20</td>
</tr>
<tr>
<td>VIII</td>
<td>14,18</td>
</tr>
<tr>
<td>Solitary</td>
<td>22</td>
</tr>
</tbody>
</table>

solitary. It reveals that genotypes 6,7,8,9 and 10 are so closely plotted that the genetic divergence among them is narrow especially with respect to component 1 and 2. In the dendrogram (Fig 4.7), genotype 8 and 10 are very close to each other, the extent of variability is slightly higher between 6 and 9 and these two clusters are placed at a distance in the distance matrix.

The extent of additional diversity may be attributing to 3rd, 4th and 5th factor which is not revealed in the principal component plot.

Similarly in case of genotype 2 and 20, there is less diversity when all the five components are considered while in the Principle component plot, the two are distantly placed and will not fall under the same cluster. The major diverging factors between the two genotypes are factor 3, 4 and 5.

3.2.2 *Garcinia indica* at Agumbe range

In *Garcinia indica* at Agumbe range, the first component explained 70.4 percent of the total variance while the next three components attributed to 27.4 percent of total variance. When this quantum of variability expressed is plotted against X and Y axis representing component 1 and 2 respectively five major clusters were formed as shown in Table 5.2 with solitary genotypes 8, 18, 2 and 17 scattered; not falling in any cluster.
Table 5.2 Clusters formed and genotypes in *Garcinia indica* at Agumbe range

<table>
<thead>
<tr>
<th>Cluster No.</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4,5,9,12,20,21,23</td>
</tr>
<tr>
<td>II</td>
<td>3,11,14,15,24,25</td>
</tr>
<tr>
<td>III</td>
<td>6,7,16,19</td>
</tr>
<tr>
<td>IV</td>
<td>10,13</td>
</tr>
<tr>
<td>V</td>
<td>1,22</td>
</tr>
<tr>
<td>Solitary</td>
<td>2,8,17,18</td>
</tr>
</tbody>
</table>

In the dendrogram at Fig 4.10, genotypes 11 and 24 are nearest at a distance of 0.004 units. Here all the five components are considered and is true when only the first and second component is considered as the two genotypes falls in same cluster as shown in Fig 4.8. Genotype 8 is solitary and in accordance with both dendrogram and principal component plot, not including in any cluster. Genotype 2 and 16 are placed wide apart at a distance 0.021 units (Table 4.9) and similarly the two are placed in different clusters.

Whereas genotypes 16 is grouped in cluster 3 (Table 5.2) along with genotypes 6, 7 and 16, 19 while the distance between the two groups is more as seen in dendrogram. This may be attributed to the 3rd, 4th and 5th factor variability bringing genotypes 16 closer to the cluster.

These findings are in agreement with results of Hilling and Fezzoni (1987) where they analysed 16 cultivars of sour cherry using principal component analysis. It revealed that genetically related cultivars tended to cluster suggesting that there was a significant genetic component to the underlying pattern of morphological variation and has also contributed the patterns. Selective forces may also have contributed to the patterns of morphological variation.

3.2.3 *Garcinia gummi-gutta* of Agumbe range

In this case, no component has accounted for more than 50 percent of the total variability. Tree height has contributed 49.7 percent variability while the next three components shared 48.5 percent variability, indicating no major role by these components in contrary to that of *Garcinia indica*. 
Table 5.3 clusters formed and genotypes in *Garcinia gummi-gutta* at Agumbe range

<table>
<thead>
<tr>
<th>Cluster No.</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12,14,15,16,17,18,19,20</td>
</tr>
<tr>
<td>II</td>
<td>4,5,6,9,10</td>
</tr>
<tr>
<td>III</td>
<td>21,22,23,24</td>
</tr>
<tr>
<td>IV</td>
<td>7,8,25</td>
</tr>
<tr>
<td>V</td>
<td>1,2,3</td>
</tr>
<tr>
<td>vi</td>
<td>11.12</td>
</tr>
</tbody>
</table>

While plotting of these genotypes against the first two factors, 6 clusters are arbitrarily formed (Fig 4.11) and listed in Table 5.3, genotype 11 and 13 are closely placed at a distance of 0.019 units (Table 4.13) and is in confirmatory with plotting (Fig. 4.13). Genotype 1 and 2 has no much diversity, are at a distance of 0.033 units and clustering with genotype 3 forming cluster no. V. Genotypes 7 and 25 are not showing much diversity when component I and II are considered, but the distance matrix place them apart. Similarly, genotypes 24 and 25 have no much diversity in dendrogram, but placed in different clusters in the plotting. This may be due to the 3rd and 4th factor which have an almost the equal share put together to that of the 2nd component.

### 3.2.4 *Garcinia gummi-gutta* of Nagara range

Contribution of individual component variability to the total variability was found to be similar to that of Agumbe range. The first four components accounted for 97 percent of total variability when these variability was plotted on the graph, four clusters and two solitary genotypes could be arbitrated and listed in Table 5.4.

Table 5.4 clusters formed and genotypes in *Garcinia gummi-gutta* at Nagara range

<table>
<thead>
<tr>
<th>Cluster No.</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.16.17.18.19.20.21.22.23.24.25</td>
</tr>
<tr>
<td>II</td>
<td>11.12.13.14.15</td>
</tr>
<tr>
<td>III</td>
<td>6.7.8.9.10</td>
</tr>
<tr>
<td>IV</td>
<td>2.4</td>
</tr>
<tr>
<td>Solitary</td>
<td>1,5</td>
</tr>
</tbody>
</table>

As evidenced in dendrogram (Fig 4.13) and PCA plot (Fig 4.11), genotypes 1 and 5 are solitary, not getting included in any of the clusters formed. Genotypes 2 and
4 appear to be nearer in Principal component plot, but are distant in the dendrogram and can be concluded that canopy width and dbh diversity have played major role distancing the two genotypes at 0.073 units. Genotypes 16 and 25 are hard to separate in the plotting (Fig 4.11) while 16 and 20 are closely spaced at minimum distance of 0.020 units (Table 4.11). The principal components tree height and height up to first branch are almost similar in these two genotypes, height up to first branch are almost similar in these two genotypes, but placing them apart in clustering is due to the other two components. As discussed earlier, positioning of the *gummi-gutta* genotypes in each location was closer to each other, thereby the genotypes has similar soil type, received same amount of rainfall, dew and precipitation and were exposed to similar environmental conditions. Also because of the same age group, the variability of individual components was significant. However genetic diversity present in individual genotype has resulted in the shown or expressed variability in interaction with external factors.

These findings are in agreement with results of Gangaprasad (1993) in his studies on 282 tamarind genotypes across regions of Southern Karnataka. He studied the variability for 15 characters comprising pod yield, trunk length, pulp yield, seed yield, tartaric acid yield, pod weight, Number of seeds per pod, Trunk radius, Pod length, Pulp weight, seed weight, pod thickness, pod width and number of primary branches. He concluded that pod yield was found to contribute more towards divergence in all the regions followed by pulp yield and seed yield.

**4 Leaf character**

On visual observation, there appears to be difference in length and width of leaf between *G. indica* red and white type. But statistically leaf length variability is significant and not the leaf width. Leaf of *G. indica* white is lengthier compared to the red type.

The leaf length and width of both the species was compared between trees of different locations and significant variability was observed within the species. Usually there should not be noted difference within the spices and the variability observed may be due to environmental effect.
5 Flower characters

As *Garcinia indica* and *Garcinia gummi-gutta* are sub dioecious in nature, they produce male and bisexual flowers and the flower character differs. Bisexual flowers possess stamen (androecium) and pistil (gynoecium) while male flowers are staminate. Apart from this, flower size also differs. The bisexual flowers are bigger in size as it has to accommodate ovary and ovules whereas staminate flower possess rudimentary or no pistil. As already discussed, male and bisexual and flowers are noticed only in *Garcinia gummi-gutta* while only bisexual flowers were found in *Garcinia indica* in both the ranges.

In *Garcinia indica*, bisexual flowers were sessile in white type whereas the normally found red type exhibited pedicellate flowers. Being pedicellate, the total length of the flower found to be more and flower width is very less. The difference was highly significant when statistically analysed.

The white type, if to be considered as a mutant of red type, need to think about the possibility of mutation to such a higher degree, to bring about changes in the total flower character. If it cannot be considered as a mutant, then it needs to nomenclated under a different sub-species. Such difference is also conspicuous in leaf texture as the white type is smooth while red type has rough leaves. Observed the difference in rind character also as the fruit rind is slightly thin and on peeling, retains a light white tinge which otherwise found red tinge in red type rinds. All these aspects need further investigation.

The male and bisexual flower of *Garcinia gummi-gutta* was comparable. Flower width was more in bisexual flower because of above said reasons. The total flower length is made up of head length and petiole length. Petiole length in both bisexual and male flowers was found to be similar while contribution of head length in bisexual flowers was found to be similar while contribution of head length. This may also be due to inclusion of ovary space. Muthulakshmi et al. (1999) while studying 15 genotypes of male and bisexual trees in *Garcinia gummi-gutta*, has not reported any differences in leaf or floral characters.
6. Variability in fruit characters

Fruits of *Garcinia indica* white and red type are morphologically differing in fruit colour and size as described earlier. The differences were highly significant with that of Agumbe red fruits while it was significantly differing in most of the fruit traits with that of red fruits of Nagara range. When the red fruits of Agumbe and Nagara range were compared, the fruits of Agumbe were bigger in size compared to Nagara fruits and is reflected through fruit weight (34.88 g and 28.86 g respectively), fruit width (4.17 cm and 4.04 cm respectively), and fruit diameter (14.13 cm and 13.02 cm respectively). Similar variation in number of fruit and fruit yield in *Garcinia indica* was reported by Muthulakshmi *et al.* (2000) and Sawant *et al.* (1999). Kallaje (2000) reported variability in morphological and yield attributing characters in *Garcinia indica*.

On analysing the protein content of *G. indica* red and white, protein content of white type is less compared to red fruit while total acidity is more in white type. Total soluble sugar of white type is significantly less and helps in protecting the fruits from animal damage. Because of high acid content, the fruit juice is sour and might contribute to the digestive and pharmaceutical properties.

B. Phytochemical analysis

Yield of different extracts and their colour is given in the previous chapter. The qualitative analysis reveals that all three leaf and bark extracts were found positive for presence of carbohydrates. Bark samples of all the three found to contain tannins. The bark and leaves of all the three samples found to contain alkaloids. Flavonoids were present in all the leaf samples and Phytosterols were found to be present in leaf and bark extracts of *Garcinia gummi-gutta*, *Garcinia indica* red and leaf extract of *Garcinia indica* white. Alkaloids are the secondary metabolites and many of them are toxic to other organisms. The presence of alkaloid will determine its use in medicine because of its pharmacological effect. The alkaloids invoke a bitter taste and possess antibacterial activity. Flavonoids are the most important plant pigments, the most common group of polyphenolic compounds in the human diet which have low toxicity compared to other active plant compounds like alkaloids. Flavonoids are known for
their antioxidant activity in vitro (Mukherjee et al. 2006). Flavonoids reported to exhibit anti-carcinogenic activity similar to the alkaloids.

The presence of these secondary metabolites in bark and leaves reveals the potentiality for various pharmacological activities. *Garcinia gummi-gutta* has emerged as a potent source compared to the other two and hence finds place in the pharmacological utility. This is in agreement with the studies conducted by Kagbo (2010).

**Physico-chemical analysis**

Acid soluble, water soluble and acid insoluble ash content was determined in three different extracts and the total ash content was highest in *Garcinia indica* (red) followed by white and *gummi-gutta*. Water soluble and acid soluble ash was highest in red (2.28 and 7.42 respectively). Sulphated ash value was highest in red (0.97%) followed by *Garcinia gummi-gutta* (0.85%) and white type (0.59%).

Fluorescence analysis was done under three different wavelength-visible light, short wavelength (254 nm) and long wavelength (360 nm) for all the three samples with different solvents. Leaf samples of *Garcinia indica* red have exhibited dark green colour in visible and long wavelength of 360 nm with sulphuric acid. Dark brown colour was obtained with sodium hydroxide and water under visible light and long wave length while it was dark green under short wavelength of 254 nm. With water, it exhibited light straw, light green and straw colour at visible light, long and short wavelength respectively.

Similarly *Garcinia indica* white leaf samples on treating with sulphuric acid have exhibited dark green and greenish black colour under different wavelengths of light. On treatment with sodium hydroxide and water, the samples have shown brown, dark brown and dark green under visible light, long and short wavelength light respectively. Straw colour was observed under visible light and at 254 nm light when treated with Hydrochloric acid while it was light green at 360 nm.

*Garcinia gummi-gutta* leaf samples on testing with sulphuric acid have shown dark brown colour at all wavelengths. The colour varied from light straw, light green to dark green on treating with sodium hydroxide, methanol and hydrochloric acid.
DISCUSSION

The study involving morphological and biophysical identification is the oldest, simplest and cheapest of all methods, thus to be preferred when its use is feasible along with the other parameters like ash value, extractive value and qualitative chemical tests serve as source of information. Hence, the studies on above parameters are useful tools to determine the purity of plants and to avoid adulteration in the process of commercialization of raw material. Further, the determination of the amount of any organic and inorganic constituents which may be present in any plant to which its value or therapeutic activity is attributed to proximate values like extractive and ash values (Remington, 1980). It is a good indicator of previous extraction of water-soluble salts in the drug or in correct preparation.

In view of establishing the identity and characterizing the plants for their purity, almost all the medicinal plants have been subjected to evaluation and such measures are indispensable for characterization of germplasm resources or for any prospective pharmacological screening, further drug discovery and development. Hence there are umpteen number of investigations undertaken utilizing several medicinal plants viz. Coleus forskohlii (Shrivastava et al. 2002), Actaea racemosa L. (Applequist, 2003), Uncaria tomentosa and Uncaria guianensis (Gattuso et al. 2004), Maytenus ilicifolia (Duarte and Debur, 2005), Giselia pharnacioides (Musa et al. 2006), Crateva nurvala (Sikarwar, 2009), Annona squamosa (Sharma et al. 2009), Holoptelea integrifolia (Padmaja, 2009), Polygonum nepalense (Rakesh et al. 2011), Ficus hispida (Ravichandra et al. 2011) and Xanthium strumarium (Bhogaonkar et al. 2012).

Estimation HCA by HPLC

The quantity of HCA present in the dried rinds of Garcinia indica red, Garcinia indica white and Garcinia gummi-gutta was calculated based on the area under the major curves at particular retention time as indicated above and the result revealed the presence of 12.92, 13.22 and 15.29 per cent w/w HCA content respectively. It is evident that the unexplored white type of Garcinia indica possess slightly higher content of HCA than its counterpart red type. The determination of HCA is important in view of its role in obesity management which is discussed below.
HCA and its role in obesity management

Obesity or increase in body weight is becoming a major health problem which may lead to chronic disease and disability like type 2 diabetes, cardiovascular disease, hypertension and stroke. Especially childhood obesity is already an epidemic in some parts and is on the rise in other parts of the world. According to an estimate, about 22 million children under five years of age are overweight globally and it has an enormous financial burden on the health service with direct costs being estimated at £500 million per year (Anonymous, NAO, 2001).

Two different types of obesity treatment drugs have been advocated to induce weight loss. One of these is orlistat which acts in the lumen of the stomach and small intestine by forming a covalent bond with the active serine residue sites of gastric and pancreatic lipases (Ballinger and Peikin, 2002 and Drew et al. 2007). The other is sibutramine (Reductil) which is an anorectic or appetite suppressant (Lean, 2001 and Tziomalos et al. 2009). Both the drugs have side effects.

Some natural biomaterials possessing multi-functional anti-obesity activities have been discovered. Green tea and *Garcinia cambogia* are good examples. Researchers originally found green tea possessed higher anti-oxidant activity than anti-obesity activity, owing to its high concentration of catechins, including epicatechin, ECG, and EGCG. Subsequent research proved the antiobesity activity of catechins resulted from the combined actions of appetite reduction, greater lipolytic activity and energy expenditure, and less lipogenic activity and adipocyte differentiation (Thielecke and Boschmann, 2009).

*Garcinia indica* and *Garcinia cambogia* are widely known for their anti-obesity activity (Kim et al. 2006a). Its commercially-available extract is derived from the dried fruit rinds. Its main active ingredient is (-)-hydroxycitric acid (Kim et al. 2004a). HCA prevents the metabolism of carbohydrates into fats by inhibiting lipogenesis, burning excess fats, and suppressing appetite (Kim et al. 2006a). *Garcinia cambogia* extract has also displayed multifunctional anti-obesity effects. Research has shown that it inhibits adipocyte differentiation, reduces fatty acid synthesis, lipogenesis, and epididymal fat accumulation through reducing ATP-citrate lyase activity, and suppresses appetite (Kim et al. 2006a). It has been on the market for over 10 years with no adverse side-effects (Saito et al. 2005).
C. IN VITRO ANTIOXIDANT STUDIES

Molecular oxygen is an essential component for all living organisms, but the formation of various reactive intermediates of molecular oxygen called free radicals leads to a process termed as ‘oxidation’. These free radicals are highly reactive, unstable and can therefore cause oxidative destructive processes, wherein it breaks down and damages various biomolecules such as lipids, polysaccharides, proteins, nucleic acids etc. by giving out or accepting single electron (Halliwell and Gutteridge, 1999).

Extensive experimental and epidemiological studies support the involvement of oxidative stress in pathogenesis and progression of many diseases. Endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical induced damages of tissues. Antioxidant compounds in plants play an important role as a health-protecting factor. 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. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller et al. (2000 a and b).

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like 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. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity in Machilus odoratissima (Amit subedhi et al. 2012) and Convolvulus arvensis (Elzaawely et al. 2012).

In the present investigation, various in vitro antioxidant assays have been used to monitor and compare the antioxidant activity of chloroform leaf extracts of Garcinia indica and Gummi-gutta and the results are discussed below.
1. DPPH radical scavenging assay

This is a widely used technically simple and rapid method of antioxidant assays (Brand-Williams et al. 1995). In the present study, it was demonstrated that the dose dependant DPPH radical scavenging activity wherein *Garcinia gummi-gutta* has exhibited comparatively higher antioxidant activity with lower IC$_{50}$ values of $24.96 \pm 0.02$ followed by *Garcinia indica* white $(31.25 \pm 0.94)$ and *Garcinia indica* red $(39.05 \pm 0.48)$. This is due to the fact that the antioxidants present in these plants on interaction with DPPH, either transferred electron or hydrogen atom to DPPH, thereby neutralizing its free radical character, which gives rise to un-coloured methanol solutions. Similar results were obtained by several investigators by using the DPPH assay to study antioxidant capacity of the medicinal herbs and dietary plants for their relative antioxidant property (Cai et al. 2004, Onder et al. 2008, Singh et al. 2009, Sheetal and Jamuna, 2009, Tomczyk et al. 2010, Abirami et al. 2011, Sindhu et al. 2010, Mishra et al. 2010, Bora and Sharma, 2011, Deepika et al. 2011, Rajamanikandan et al. 2011, Dejan et al. 2011, Amit subedhi et al. 2012 and Elzaawely et al. 2012).

2. Scavenging of Hydrogen peroxide assay

Hydrogen peroxide non reactive, but sometimes it can be toxic to living cells, because in living cell it is converted into free radical called hydroxyl radicals ($^\cdot$OH), react with biomolecules, cause tissue damage and cell death. In this assay, among the three different plants under study, *Garcinia gummi-gutta* has exhibited highest hydrogen peroxide scavenging activity followed by *Garcinia indica* white and *Garcinia indica* red in terms of their IC50 values exhibiting their scavenging activities. Further, the scavenging of hydrogen peroxide was proved to be concentration dependent. Similar observations of scavenging of hydrogen peroxide was made by several researchers (Nagai et al. 2005, Sakanaka et al. 2005, Balasundram et al. 2006, Liu et al. 2008, Alpinar et al. 2009 and Rahmat Ali Khan et al. 2012).

3. REDUCING POWER ASSAY

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free
radical chain, by donating a hydrogen atom. Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. _Garcinia gummi-gutta_ has exhibited highest reducing power activity at all the concentrations tested indicating its potential antioxidant abilities. Among the two types of _Garcinia indica_, white has registered better reductive abilities over the red type at variable concentrations showing their efficacy as excellent reductants due to which reduction of the Fe^{3+} ferricyanide complex to the ferrous form. The results are in conformation with observations of Cai _et al._ 2004, Mathew and Abraham, 2006, Velavan _et al._ 2007, Onder _et al._ 2008, Singh _et al._ 2009, Mishra _et al._ 2010, Deepika _et al._ 2011, Abirami _et al._ 2011, Bora and Dejan _et al._ 2011 and Amit subedhi _et al._ 2012.

4. Total Phenolic Assay (TPA)

Despite the undefined chemical nature of FCR, the total phenol assay by FCR is convenient, simple, and reproducible (Huang, _et al._ 2005). The total phenolic assay of the three leaf extracts were compared with standard catechol and the results shown that _Garcinia gummi-gutta_ has exhibited highest total phenolic content at 100 µg concentration followed by _Garcinia indica_ white and _Garcinia indica_ red. Several investigators have reported the use of total phenol assay and antioxidant capacity assays (e.g. DPPH, reducing power assay, hydroxyl radical assay _etc._) and often found excellent linear correlations between the two assays (Gheldof and Engeseth, 2002, De Beer _et al._ 2003, Madhujith _et al._ 2006, Shahidi _et al._ 2006, Stratil _et al._ 2006, Velavan _et al._ 2007; Onder, _et al._ 2008; Sheetal and Jamuna, 2009, Abirami _et al._ 2011, Sindhu _et al._ 2010, Mishra _et al._ 2010, Bora and Sharma, 2011, Deepika _et al._ 2011, Dejan _et al._ 2011 and Amit subedhi _et al._ 2012). Thus, the evident total phenolic concentration in the methanolic leaf extract could forecast its antioxidant property.

5. Total antioxidant capacity

The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (Prieto, _et al._1999). All the three extracts registered potent total antioxidant capacity wherein _Garcinia gummi-gutta_ has recorded highest total antioxidant capacity at 100 µg concentration

The medicinal plants associated with antioxidant activity contain phytoconstituents *viz.* alkaloids, sterols, phenolic compounds (flavonoids), coumarins, glycosides, *etc.* has been known for their antioxidant property. Antioxidant activity of phenolic compounds was correlated to their chemical structures. Structure-activity relationship of some phenolic compounds (*e.g.* flavonoids, phenolic acids and tannins) has been studied (Rice-Evans *et al.* 1996, Lien *et al.* 1999 and Son and Lewis, 2002). In general, free radical scavenging and antioxidant activity of phenolics (*e.g.* flavonoids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolics and is also affected by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH) *etc.* Therefore the antioxidant activity of the tested samples can be attributed to these phytochemicals. Further, high content of acids *viz.* HCA, citric acid tartaric acid and compounds such as garcinol could be the major contributing factor to their antioxidant capabilities.

**D. WOUND HEALING ACTIVITY**

Wound healing may be defined as the interaction of a complex series of phenomena that eventuates in the resurfacing, reconstitution and proportionate restoration of tensile strength of wounded skin (Goslen, 1989). Many research works on animal model have been showed that wound healing process involves four phases of haemostasis, inflammation, proliferation or granulation and remodelling or maturation.

In I phase, the platelet cell play an important role, which acts as the utility worker sealing off the damaged blood vessels. Haemostasis occurs within minutes of the initial injury unless there are underlying clotting disorders. Clinically inflammation, the second stage of wound healing presents as erythema, swelling and warmth often associated with pain. The cellular elements important in the
inflammatory phase of wound healing are the polymorphonuclear leukocyte (PMN) and the monocyte or macrophage. The granulation stage is characterized clinically by the presence of pebbled red tissue in the wound base and involves replacement of dermal tissues and sometimes sub-dermal tissues in deeper wounds as well as contraction of the wound. In remodelling or maturation phase, the healing process involves remodelling the dermal tissues to produce greater tensile strength. The principle cell involved in this process is the fibroblast.

A critical outcome of the wound repair process is restoration of the mechanical properties of tissue strength. Measurement of wound strength provides highly quantifiable estimates of the efficacy of the aggregate healing process. Determination of various individual components of the phases of healing can provide important insights about events operative during repair. However, if sufficient wound strength is not attained, the net effect may be wound failure. Breaking strength is simply the load required to break a wound. In the beginning a wound will be having little breaking strength because the clot will alone will be holding the edges together. Thereafter breaking strength increases rapidly as collagen deposition increases and cross linkages are formed between the collagen fibres. In the incision wound model, the animals treated with extract of *Garcinia indica* white has proved superiority in terms of wound healing efficiency by registering highest mean values and percent tensile strength than the other two plant extracts and the effect is comparable to the reference standard on 10th post wounding day. All the test extracts have recorded significant tensile strength indicating their potency in wound healing. The order of effectiveness of extracts is *Garcinia indica* white > *Garcinia indica* red > *Garcinia gummi-gutta*. Further, the promotion of wound healing was noticed to be dose dependent which is positively correlated to the concentration of the extract.

It is evident that phytoconstituents can significantly improve the quality of wound healing and scar formation (Kapoor *et al.* 2004). Flavonoids because of their antioxidant property, accelerates the wound healing process and could be a potential therapeutic tool in the treatment of vascular lesions (Mensah *et al.* 2001), triterpenoids, flavonoids (Tsuchiya *et al.*1996), saponins (Gulcin *et al.* 2004) *etc.* are known to promote the wound healing process due to their antioxidant and antimicrobial activities. Earlier reports reveal that the plant contains many active
constituents such as glucosides, terpenoids, flavonoids *etc.* which are known for their antioxidant property, the present study reveals that the plants possess significant wound healing promoting activity which may be attributed to the active constituents present in the extracts supporting their folklore medicinal claims.


E. ANTI MICROBIAL STUDIES

Many plants have been used because of their antimicrobial traits and have been investigated by a number of researchers worldwide (Ncube, 2008). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment (Iwu, 1999). Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases (Tanaka *et al.* 2006) especially in the light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents. Over the past several years, intensive efforts have been made to discover clinically useful antimicrobial drugs, which have been reviewed by many researchers. (Rasadah and Houghton, 1998; Blondeau, 1999; Jacoby, 1999 and Cowan, 1999). This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor *et al.* 2001).

The antimicrobial susceptibility test (AST) is an essential technique in many disciplines of science. It is used in pathology to determine resistance of microbial strains to antimicrobials, and in ethnopharmacology research, it is used to determine the efficacy of novel antimicrobials against microorganisms, essentially those of medical importance. The test is the first step towards new anti-infective drug
development. There are various AST methods that are employed by researchers and these could lead to variations in results obtained (Lampinen, 2005).

In the present investigation, antimicrobial activity of the three samples was evaluated against four bacterial species (*Escherichia coli*, *Micrococcus luteus*, *Salmonella typhi* and *Staphylococcus aureus*) by MIC (Minimal Inhibitory concentration). Minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. All the three samples under study inhibited the growth of the bacteria at 125 μg/ml concentration of the extract and exhibited no differences in terms of MIC values indicating positive antibacterial efficiency of the test samples. With regard to antifungal screening, *Garcinia indica* red proved potent antifungal effect by registering MIC of 500μg/ml. While *Garcinia indica* white and *Garcinia gummi-gutta* recorded MIC values of 1000 and >1000 μg/ml respectively against *Candida albicans*. However all the plant extracts required greater than 1000 μg/ml MIC against *Aspergillus niger* indicating the fairly resistance of the latter against the extracts.

The antimicrobial activity could be attributed to the presence of phytoconstituents *viz.* flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins which have multiple biological effects, including antioxidant, wound healing *etc.* which are toxic to the microorganisms. Flavonoids, phenolic compounds in particular of plant are important for the plant growth and defense against infection and injury. These compounds while exhibiting antioxidant property are usually also act as good antimicrobial agents (Kahkonen *et al.* 1999; Cos *et al.* 2001; McGaw, *et al.* 2002; Kotze and Eloff, 2006 and Paramesha, *et al.* 2009). The underlying mechanisms could be enzyme inhibition by oxidation (Cowan, 1999). Thus the present antimicrobial activity effects of *Garcinia indica* white, *Garcinia indica* red and *Garcinia gummi-gutta* might be because of these constituents. The secondary metabolites and their efficacy as antimicrobial agents have been documented by several investigators. The sites and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms.
with evidence that increased hydroxylation results in increased toxicity (Geissman, 1963). Some authors have found that highly oxidized phenols possess more inhibitory activity (Scalbert, 1991). The mechanisms thought to be responsible for phenolics toxicity to microorganisms include enzyme inhibition by the oxidized compounds possibly through reaction with sulphhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987). The activity of flavonoids is probably due to its ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. The lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al. 1996). A wide range of antiinfective actions, have been assigned to tannins. One of their molecular actions is to complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Haslam, 1996 and Stern et al. 1996). Thus their mode of antimicrobial action is related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins etc. They also form complex with polysaccharides (Ya et al. 1988). The mechanism of action of terpenes is speculated to involve membrane disruption by the lipophilic compounds. Alkaloids are also found to have microbicidal effect. The mechanism of action of highly aromatic planar quaternary alkaloids is attributed to their ability to intercalate with DNA (Phillipson and Neill, 1987).

Maridass et al. (2010) using the leaf extract of Garcinia gummi-gutta observed significant antibacterial activity against Bacillus subtilis, Klebsiella pneumoneae, Aeromonas hydrophila, Pseudomonas aeruginosa, Salmonella typhii, 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. Studies also conducted in Cocos nucifera (Rajiv et al. 2011), in Candida species (Amit kumar eta al. 2011), in different medicinal plants (Chitra et al. 2012) and in four medicinal plants collected from Dharapuram (Natchimuthu et al. 2012).

F. MOLECULAR CHARACTERIZATION BY RAPD

Selection, domestication and cultivation of plants are based on the genetic diversity of the species. Therefore, information on distribution, preservation, variation and relations is extremely important for several purposes. Studies of genetic diversity
in plants have greatly enhanced our understanding of modes of speciation, adaptation, and population dynamics. Such studies have important applications in in situ and ex situ conservation strategies as well as in plant breeding (Bussell, 1999; Nybom & Bartish, 2000). Genetic diversity of a species depends on factors like phyletic group, life form, geographic range, regional distribution, breeding system, seed dispersal mechanism, mode of reproduction and succession status (Hamrick & Godt, 1989; Bhat et al. 1999). Several techniques, including morphological, biochemical and molecular methods, have been used to measure genetic diversity in plant species. The most widely used PCR-based DNA marker systems are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and recently simple sequence repeats (SSRs) or microsatellites (Staub et al. 1996, Gupta and Varshney, 2000, Kumaraswamy, 2009). RAPD stands for random amplification of polymorphic DNA. It is a type of PCR reaction, but the segments of DNA that are amplified are random.

In the present investigation, RAPD 1 and RAPD 3 have shown polymorphism. RAPD 1 has shown polymorphism for male and bisexual *Garcinia gummi-gutta* (lane 3 and 4 with 1 and 5). But RAPD 3 and RAPD 4 have not shown clear polymorphism between the four groups. None of the primers could signify and differentiate *Garcinia indica* white from red. SB primers could able to produce bands but not effective enough to differentiate between red and white *indica* or between male and bisexual *gummi-gutta* genotypes. RAPD 4 could slightly show polymorphism for male and bisexual *Garcinia gummi-gutta* species, but it needs further studies. Clustering of genotypes indicates that the genotype 1 and 2 of *Garcinia gummi-gutta* are closely related and this group formed a cluster with genotype 4 (0.79 co-efficient). Genotype 7 and 9 are closely related and this group in turn with genotype 9 (*Garcinia indica* red) at 0.62 coefficient. Genotypes 1 and 2 (*Garcinia indica* red) are distantly related to genotype 8 (*Garcinia indica* white). However *Garcinia indica* white genotypes 6 and 8 stands apart from the other genotypes. This study provides the preliminary information on the molecular basis of polymorphism detected as RAPD markers in different types of *Garcinia sp*. The DNA amplification products, which represent one allele per locus, could result from changes in either the sequence of the primer binding site or changes which alter the size and prevent the successful amplification of target DNA.
Random amplified polymorphic DNA (RAPD) markers were used by many researchers to evaluate the somaclonal variation/genetic variability of micropropagated plants such as *Picea glauca* (Isabel, 1996); *Hordeum spontaneum* (Breiman, 1987); Sahasrabudhe and Deodhar (2010) have reported reliable and fast method of isolation of genomic DNA.

Thus the present study utilizing the two species of *Garcinia* mainly in their natural habitats of Western Ghats yielded important information about their morphological features, yield and also important variability that existed between them in a region wise manner. The study also generated important information about their HCA content in these types as it is important compound of clinical significance in obesity management. The programme also resulted in evaluation of antioxidant, wound healing, antimicrobial potentialities in these plants. The major discovery of the present research is however is the identification of individual plants of *Garcinia indica* white type which has proven distinct in variable features for many of the parameters tested, both morphological, biochemical as well as medicinal value. Moreover a preliminary RAPD analysis also stands testimony to its distinctness over the red type of the same species. Any further study involving white type of *Garcinia indica* is highly encouraged and also warrants its appropriate nomenclature to define it is an ecotype, variety or a sublevel of a species *etc.*