VI. SUMMARY
1. There has been a virtual explosion of interest in all kinds of alternative therapies, in particular those, which have been around for thousands of years. Naturally, in an illness where there is no "cure" and in which pharmaceutical drugs typically produce side effects, people look for other remedies. The use of herbal heritage has become a part of general health care by the tribes since time immemorial. The use of modern medicines of synthetic origin is believed to impart dramatic results in a short span in the therapeutic field has a hidden drawback of serious afterward consequences on the health on prolonged treatment due to various pathological, pharmacological and chrono-pathological parameters of mankind.

In spite of overwhelming influence of modern medicine and tremendous advances made on the production of synthetic drugs, traditional medicaments designated now a days as herbal drugs in different places in literature, have retained their place in therapy. The World Health Assembly emphasized the need to ensure quality control of medicinal plants with appropriate moral techniques and suitable standards, as it is estimated that about 80 percent of the people living in developing countries mainly depend upon herbal drugs for their preliminary health care needs. The World Health Organization, to achieve their goal of "health for all" has recommended to all member countries to actively promote native medicines of their country as well as to initiate steps to conserve and/or to cultivate medicinal plants so that genuine raw materials become rapidly available to a large section of population.

2. Medicinal plant based drug industries are progressing very fast in India but associated with a number of problems. Most alarming problem the industry has started facing and will face in future is the demanding supply of plant material from natural resources. Many of the ancestor plants are highly endangered and urgently need to be maintained in their native habitats. Surveying, monitoring and collecting material for propagation from populations of these species is to be thought of seriously.

3. Hydroxycitric acid is found to be one of the active components in various weight-loss formulations and appetite-suppressor products. It is obtained from the rind of the fruits of *Garcinia indica* and *Garcinia combogia*. After harvesting and drying, the rind contains up to 30 percent by weight of HCA. Hydroxycitric acid is an extremely
popular dietary supplement. *Garcinia indica* and *Garcinia gummi-gutta* are rich source of HCA. These two species formed the materials for the present study.

4. *Garcinia indica* is a moderate dioecious evergreen tree with spreading or drooping branches. It is seen throughout the Western Ghats in evergreen forest. Bark is dark brown and smooth, leaves are opposite on long petioles and reddish young leaves turn bright green later. Flowers are small, sub-diecious and the fruit ripens as the size of a small orange, dull red or red in colour, depression at the apex. Pericarp is thick and soft, entirely filled with a firm juicy dark purplish-red pulp. In literature, there is a mention of white type which is believed to have medicinal use. Therefore present study also explores the White type of *Garcinia indica* along with differentiating characters from that of normally existing red type.

*Garcinia indica* is used as an acidulant, fruit is antihelmintic, cardio tonic and is used in treatment of piles, dysentery, tumours, pains and heart complaints. The seeds contain 23-26 percent oil; remain solid at room temperature and yields valuable edible oil known as ‘Kokum butter’. The fruit rind is a rich source of Garcinol, Isogarcinol and (-)-hydroxycitric acid-an anti-obesity compound. Kokum rind is a natural colorant for acid foods. The fruit rind extract has exhibited the antimicrobial and cytotoxic effect, has anti-oxidant property and HCA present in fruit rind has been reported to reduce cholesterol synthesis, food consumption and weight gain.

5. *Garcinia gummi-gutta* is distributed throughout the Western Ghats in evergreen and lower shola forests of Western Ghats. It is a medium sized evergreen diecious tree, the flowers are yellowish white and fleshy. Staminate flowers are smaller in size while pistillate or dioecious flowers are larger in size. It flowers in February-March and the fruits are harvested in July-August. This species is categorized as ‘near threatened globally’. As per the IUCN classification, it is in the ‘endangered’ category.

The fruits have excellent therapeutic value and the dried rind is a popular fruit spice used in curries in place of tamarind or lime, the fruits are rich in non-volatile acids. The dried rind of *Garcinia gummi-gutta* which is of commercial value contains citric acid, tartaric acid, phosphoric acid and reducing sugars. Leaf extract found to contain alkaloid, terpenoid, steroid, catechin and Phenol, which in turn responsible for
anti-bacterial activity. Leaf extracts of *Garcinia gummi-gutta* have shown significant antibacterial activity.

6. The selected two potential species are over-exploited and hence were selected to study morphometric variability in two ranges of Western Ghats namely Agumbe and Nagar range, to identify the female trees, qualitative analysis for phytoconstituents, estimation of Protein and HCA (Hydroxycitric acid) content in fruits, molecular characterization of elite lines of two species by RAPD analysis and to assess the therapeutic properties like antioxidant activity, wound healing and antimicrobial activities.

7. The experimental material for the present investigation consisted of 40-45 years old trees of Garcinia Species-*Garcinia indica* and *Garcinia gummi-gutta*. The study was taken up in two ranges of Western ghat region of Karnataka-Agumbe and Nagar range. The trees selected were grown as natural wild trees in the Western ghat and few trees were selected from the private homesteads. Five sites were selected in each range for both the species and in each site; observations were recorded from five representative trees for tree height, height up to first branch, diameter at breast height, canopy width and number of branches for morphological study. Similarly leaf characters, flower characters, fruit and seed characters were studied for the said two species in addition to *Garcinia indica* white type. The data was analysed through ANOVA, Principal component analysis, correlation and cluster analysis.

8. *Garcinia indica* and *Garcinia gummi-gutta* are sub-dioecious in nature. Both male and bisexual trees exist separately. The different sex forms in the two species were studied in the two ranges and two different sex forms were found only in *Garcinia gummi-gutta*, but were absent in *Garcinia indica* in both the locations. During the course of investigation, it was observed a substantial fluctuation or variation in the fruit set and fruit yield of *Garcinia gummi-gutta* over the years while stable fruit set in *Garcinia indica*.

9. 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. Within each location, the extent of variability was less and between locations in each range observed variability. In
Garcinia gummi-gutta, all the tree characters were significantly differing within each location and also between locations in each range.

Principal component analysis for morphometric characters of the two species at Nagara and Agumbe ranges has revealed that some genotypes were plotted very close to each other indicating narrow genetic diversity between them. The distance matrix provides information on how closely or distantly the genotypes are related with respect to the two principal components. Garcinia indica genotypes 8 and 10 of Nagara range were found to be very close and on plotting on the PCA diagram, they form one cluster. Similarly in Garcinia indica at Agumbe range, tree height and height up to first branch have accounted for 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 with solitary genotypes 8, 18, 2 and 17 scattered; not falling in any cluster.

10. The leaf characters were studied in the two species. 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 was significant and leaf width was non-significant. Leaf of G. indica white is lengthier compared to the red type.

11. 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 male and bisexual flowers of Garcinia gummi-gutta were comparable. 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 more. Flower width was more in bisexual flower.

12. 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, fruit width and fruit diameter.

Garcinia indica white fruits were collected at Nagara while G. indica red fruits were collected from both Nagara and Agumbe range. The fruit is also a distinguishing
feature in Red and white type of *G. indica*. In red type, fruits were bigger in size and red to dark red in colour whereas in white type, the fruits were comparably smaller and orange to light red colour. Fruit juice of red type was deep red in colour whereas that of whit type was white to cream colour. On analysing the protein content of *G. indica* red and white, protein content of white type was less compared to red fruit while total acidity was more in white type. Total soluble sugar of white type was significantly less.

13. The presence of carbohydrates was evaluated through Molisch’s test and found that both bark and leaf samples contained carbohydrates. Test for Glycosides was carried out by Modified Borntrager’s test and Keller-Killiani test, for Saponins by Foam test and for Alkaloids by Mayer’s test and Dragendorff’s test. The bark and leaves of all the three samples found to contain alkaloids in Mayer’s test while *Garcinia indica* red and *Garcinia indica* white were positive in Dragendorff’s test. Flavonoids were present in all the leaf samples while *Garcinia gummi-gutta* bark sample has shown the presence of flavonoids as confirmed by alkaline reagent test. 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.

14. 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. Sulphated ash value was highest in red followed by *Garcinia gummi-gutta* and white type. Fluorescence analysis was done under three different wavelength-visible light, short wavelength (254 nm) and long wavelength (360 mm) for all the three samples with different solvents.

15. Extracted organic acids in fresh fruits and dried rinds of *Garcinia indica* and *Garcinia gummi-gutta* were utilised to determine the HCA content by High-performance liquid chromatography. 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 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* contained
slightly higher content of HCA than its counterpart red type. The determination of HCA is important in view of its role in obesity management.

16. Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods and 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. In the present research programme, in vitro antioxidant activity was determined in leaf extracts of *Garcinia indica* (red and white) and *Gummi-gutta* using DPPH, scavenging of Hydrogen peroxide, Reducing power activity, total phenol activity and total antioxidant activity assays.

The leaf extracts were treated with different reagents in respective assays and the absorbance was measured. A standard curve was prepared based on the absorbance value and equivalents of the test samples were arrived.

In DPPH radical scavenging assay, it was demonstrated that the dose dependant DPPH radical scavenging activity wherein *Garcinia gummi-gutta* has exhibited comparatively higher antioxidant activity with lower IC$_{50}$ value followed by *Garcinia indica* white and *Garcinia indica* red.

In Scavenging of Hydrogen peroxide assay, among the three different plants studied, *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.

For Reducing power assay again *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.

Total Phenolic Assay is simple and reproducible. The total phenolic assay of the three leaf extracts were compared with standard catechol and the results have shown that *Garcinia gummi-gutta* has exhibited highest total phenolic content at 100 µg concentration followed by *Garcinia indica* white and *Garcinia indica* red.

Total antioxidant capacity of the samples were tested and result indicated that all the three extracts registered potent total antioxidant capacity wherein *Garcinia*
**gummi-gutta** has recorded highest total antioxidant capacity at 100 µg concentration followed by *Garcinia indica* white and *Garcinia indica* red.

17. The test extracts were subjected to evaluate wound healing activity by adopting incision wound model in Albino wistar rats. The animals were divided into nine groups with n=6 per group. Group I served as negetive control and Group II served as vehicle control. Group III to VIII involved three test samples at two concentrations viz. 5 percent and 10 percent. Group IX was treated with standard Nitrofurazone. The result of the incision wound healing efficiency was recorded. *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 incision 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.

18. 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). 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 to exhibit potent antifungal effect by registering MIC of 500 µg/ml. while *Garcinia indica* white and *Garcinia gummi-gutta* recorded MIC values of 1000 µg/ml 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.

19. In molecular marker studies involving RAPD markers, RAPD 1 and RAPD 3 have shown polymorphism. RAPD 1 has shown polymorphism for male and bisexual *Garcinia gummi-gutta*. 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 coefficient). 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.

20. The prominent part of the present investigation is the identification and description of *Garcinia indica* white type and its characters which is distinguishable from that of naturally existing red type. The leaf length of *Garcinia indica* (white) was almost same as that of red but is was statistically differing at 1 percent level of significance. Leaf width was numerically significant but statistically non-significant. On evaluating flower characters, flowers of *Garcinia indica* red were pedicellate and comparably long similar to the flowers of *Garcinia gummi-gutta* whereas white type produced sessile flower and bigger in size.

Fruit and the juice colour were distinguishable between the two. In red type, fruits were bigger in size and red to dark red in colour with persistent calyx while white type fruits were comparably smaller and orange to light red colour. Fruit juice of red type is deep red in colour whereas white type is white to cream colour.

Total soluble sugar content of fruit juice of *Garcinia indica* (red) was high compared to the white type. On the contrary, acid value was less in red type. The protein content of *Garcinia indica* white was less compared to the red type.

HCA content was comparably high in white type compared to red type. In different assays of antioxidant studies, *G. indica* (white) has exhibited better performance than the red type. In wound healing activity, white type has outscored red type proving its potentiality in pharmaceuticals.
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 amongst them in a region wise manner. The study also generated important information about their HCA content in these types as it is an important compound of clinical significance in obesity management. The programme also resulted in evaluation of antioxidant, wound healing, antimicrobial potentialities in these plants and establishes some of their traditional claims. The major discovery of the present research is however is the identification of individual plants of *Garcinia indica* white type which has proven distinct with variable features for many of the parameters tested *viz.* 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 and definition as to whether it is an ecotype, variety or a sublevel of a species *etc.*