SUMMARY

There is unprecedented demand for natural medicines, green health products, pharmaceuticals, food supplements, cosmetics and herbal pesticides which bring out alarming loss of plant biodiversity. The sustainable utilization, management and conservation of medicinal plants are influenced by number of factors, largely of socio-economic problems and lack of adequate knowledge on the ecology of these species of medicinal properties and other economic importance. Unsustainable harvesting of the raw material from the wild by untrained and poor collectors mostly using primitive methods and lack of awareness about the real value of the resources are other two important factors leading to resource depletion. Rural people derive a substantial portion of their income and products for their basic health care needs from the wild medicinal plants. Medicinal plants-based drug industries and enterprises, which run into thousands, presently shows more than 85% of their materials from the wild as they are inexpensive and believed to be of higher potency. There is great need to reduce pressure on the in-situ sources by diversifying the production sites of these important plants.

In developing countries like India, many plant species being used in traditional health care systems are not studied scientifically to prove their healing effects. However, approaches in this prescribed area are most needed to use the plant resources effectively in pharmacological industries. Acacia caesia (Mimosaceae) and Acalypha fruticosa (Euphorbiaceae) are the two important medicinal shrubs practiced by traditional healers in western districts of Tamil Nadu, India for various ailments. The former species is used for the treatment of skin diseases and stomach and tooth problems, and the later species is prescribed for digestive troubles like dyspepsia, colic, diarrhea etc. Both these species are generally distributed in the foot hills of Western Ghats at the altitude of around 450 ms above msl in stony or rocky habitats.

From our early studies, it is investigated that the wilds of these two species are exploited severely in the western districts of Tamil Nadu like Coimbatore, Tirupur, Nilgiris etc by the traditional medical practitioners due to their medicinal values. Seed germination percentage was also reported to be lower, less than 30 % only for these two
species. Hence, establishment of in vitro regeneration strategies by employing tissue culture technology is most essential not only to conserve the wilds of these species but also to meet the demand as well.

In light of these facts, in the present study, an attempt has been made to in vitro culture of these two species, Acacia caesia and Acalypha fruticosa by using leaf, node and internodal explants. The study revealed that the leaf explant responded well for callus formation followed by nodal explant in both the study species while culturing onto the MS medium supplemented with certain growth hormones in appropriate concentrations. The MS medium supplemented with TDZ and NAA at 1.5 and 0.3 mg/l respectively produced high amount of callus (83%) from the leaf segments of Acacia caesia. The other species, Acalypha fruticosa responded well for callus formation (87%) in the MS medium fortified with BAP and NAA at 2.0 and 1.2 mg/l respectively from the leaf segments. The subsequent subculturing for shoot and root formations was most successful on the MS medium fortified with appropriate concentrations of certain growth regulators. For the species, Acacia caesia, the shoot formation was highly effective (85%) in the MS medium containing IBA and TDZ at 2.0 and 0.5 mg/l respectively from the leaf derived callus, while in the other species, Acalypha fruticosa, the leaf derived callus responded well for shoot formation (90%) in the MS medium containing BAP and GA3 at 2.0 and 0.5 mg/l respectively. For the species, Acacia caesia, the leaf callus derived shoots, rooted effectively (72%) in the MS medium containing the auxin, IBA and Kn at 2.0 and 0.4 mg/l respectively while the other species, Acalypha fruticosa, the supplementation of MS medium with IBA and IAA at 1.0 and 0.2 mg/l respectively was found to be more effective (78%) after subculturing the leaf derived callus. Hardening experiments were also successfully completed by having higher acclimatization level in suitable potting medium. For the species, Acacia caesia, the leaf callus derived in vitro rooted shoots responded well (84%) in the hardening medium containing garden soil, sand and vermicompost in the ratio of 1:1:1 by volume. For the other species, Acalypha fruticosa, the survivability rate of leaf callus derived plantlet was higher (88%) in the hardening medium containing garden soil, sand and vermicompost (1:1:1 by volume) under greenhouse conditions. Among the two species, Acalypha fruticosa responded well than Acacia caesia in all aspects of in vitro regeneration.
Synthetic seed production was made successfully by the encapsulation of *in vitro* node and somatic embryos in *Acacia caesia* and encapsulation of *in vitro* leaf derived callus and node in the other species, *Acalypha fruticosa*. The study revealed that the node encapsulated beads of the species, *Acacia caesia* and leaf derived callus encapsulated beads of the species, *Acalypha fruticosa* are most successful in terms of better germination, higher survivability and longer storage period. For the species, *Acacia caesia*, among the two *in vitro* explants, the conversion frequency was highest for nodal segments (78%) when immersed with 4% sodium alginate. In the same concentration of sodium alginate, the *in vitro* leaf derived callus segments registered higher conversion frequency (79%) for the other study species, *Acalypha fruticosa*.

The shoot emergence from the encapsulated beads is directly depending upon storage period and temperature in both the species. It has been observed that 4 months old *in vitro* node explant encapsulated beads recorded higher emergence of shoots ranged between 45.24 and 75.00% in the species, *Acacia caesia*. On the other hand, it has been noted that 4 months old *in vitro* leaf callus encapsulated beads produced higher emergence of shoots between 46.00 and 77.34% in the study species, *Acalypha fruticosa*. The suitable temperature for the storage of encapsulated beads in 2, 4 and 6 months durations is 25°C for both the species. The *in vitro* nodal segment encapsulated synthetic seeds of the study species, *Acacia caesia* showed higher amount of shooting (77%) in the MS medium contained TDZ and NAA at 1.0 and 0.5 mg/l respectively and for the other species, *Acalypha fruticosa*, *in vitro* leaf derived callus encapsulated beads exhibited higher amount of shooting (80%) in the MS medium contained BAP and Kn at 2.0 and 0.5 mg/l respectively.

The rooting character of *in vitro* nodal explant encapsulated synthetic seed for the study species, *Acacia caesia* was significantly higher (70% rooting frequency) in the MS medium supplemented with IBA alone at 1.5 mg/l. For the *in vitro* leaf derived callus encapsulated synthetic seeds of other study species, *Acalypha fruticosa*, the rooting frequency was significantly higher (78%) while the shoots were subcultured onto the MS medium with IBA and IAA at 2.0 and 0.2 mg/l respectively. For the *in vitro* nodal segments encapsulated beads derived plantlets of the study species, *Acacia caesia*, the survivability rate was significantly higher (64%) in the hardening medium composed by garden soil, sand and vermicompost in the ratio of 1:1:1 by volume. For
the other species, *Acalypha fruticosa*, the hardening medium with garden soil combined with sand and vermicompost in the ratio of 1:1:1 by volume recorded higher percentage (68%) of plantlet survivability in the *in vitro* leaf derived callus encapsulated beads under greenhouse conditions.

To know the biological activity of the two study species, antimicrobial, antioxidant and anti-inflammatory studies were carried out. For antimicrobial studies, 10 bacterial strains viz., *Bacillus subtilis*, *B. thuriengensis*, *Micrococcus* sp., *Lactobacillus* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *P. stutzeri*, *Serratia* sp. and *Moraxetta* sp. and 10 fungal species viz., *Aspergillus niger*, *A. flavus*, *A. baumanii*, *Fusarium oxysporum*, *F. solani*, *Mucor rouxii*, *Alternaria alternata*, *Candida albicans*, *Cladosporium* sp. and *Rhizopus* sp. were tested against the alcoholic extracts of leaf, stem bark and root parts of the two study species by following disc diffusion method. The results of the study report that generally, of the three alcoholic solvents, ethyl acetate and methanol extracts of leaf, stem bark and root parts of both the study species effectively controlled the growth of microbial colonies. The results of the minimum inhibitory concentration (MIC) method showed that the methanolic and ethyl acetate extracts of the leaf, stem bark and root parts of *Acacia caesia* at all concentrations attempted have effectively controlled the growth of the two bacteria viz., *Bacillus subtilis* and *Pseudomonas aeruginosa* and at higher concentrations, the two other bacteria viz., *Escherichia coli* and *Serratia* sp. have also been controlled prominently. Among the four fungi, two viz., *Fusarium solani* and *Mucor rouxii* at all concentrations of the plant extracts and other two fungi, *Rhizopus* sp. and *Aspergillus niger* at higher concentrations were controlled effectively. For the other species, *Acalypha fruticosa*, higher concentration of methanolic leaf extract and ethyl acetate stem bark and root extracts arrested effectively the growth of the colonies of four bacteria viz., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia* sp. and four fungi viz., *Fusarium solani*, *Mucor rouxii*, *Rhizopus* sp. and *Aspergillus niger*.

The minimum inhibitory concentration (MIC) studies showed that for the both study species the leaf part has more effective than that of the other parts attempted in terms of antimicrobial properties. Hence further studies on *in vitro* antioxidant and anti-inflammatory activities were carried out only by using methanol extract of leaf part of
both the study species. The *in vitro* antioxidant studies viz., total antioxidant activity, DPPH free radical scavenging activity, hydroxyl radical scavenging activity and metal chelating activity were conducted. The results showed that these activities were enhanced with the increase in the concentration of leaf extracts of both the study species. The higher concentration of 300 µg/mL was found to be optimum for these activities.

For anti-inflammatory activities, in both the study species, *Acacia caesia* and *Acalypha fruticosa*, the *in vitro* studies such as protein denaturation and proteinase inhibition and *in vivo* studies such as effect of leaf extract on the reduction of paw thickness of carrageenan induced rats and the activity of enzymic antioxidants [superoxide dismutase activity (SOD), catalase activity (CAT) and glutathione peroxidase (GPx)] and non enzymic antioxidants (total reduced glutathione (TRG) and vitamin C) were carried out. For all these cases, the leaf extract of both study species at the concentration of 400 µg/mL was determined to be the optimum. The anti-inflammatory activity of the leaf extract of both the study species was also confirmed with the help of certain biomarkers like lipid peroxidation (LPO), nitric oxide (NO) and hydro peroxide (HPO).

All these investigations on therapeutic properties confirmed the medicinal usage of the two study species practiced by the traditional healers in the western districts of Tamil Nadu, India. To meet the demand and protect the wild, the standardized protocol described for multiplication in the present study by employing tissue culture technology can be followed.