SUMMARY

Medicinal plants constitute an important source of new candidates for therapeutic compounds, in regards to the chemical diversity found in several species. There is an increase in interest to explore the secret of traditional herbal remedies based on information collected from traditional practitioners in different parts of the world. Chemical and biological investigations of folk medicinal plants with the reputation of being curative have provided the world with many of clinical drugs of today. As medicinal plants receive increased scientific and commercial attention, there is an increasing pressure on the wild plant populations from which most medicinal plants are harvested. Over harvesting has placed many medicinal species at risk of extinction. Commercial exploitation has also sometimes led to traditional medicine becoming unavailable to the indigenous peoples who have relied on them for centuries or millennia. For all of these reasons, the study and conservation of medicinal plant species has become increasingly urgent.

_Hypocharis radicata_ (Asteraceae) is an edible perennial herb with many medicinal uses, distributed in high hills of Nilgiris, the Western Ghats at 2000m above msl. The traditional uses claim that this species is a potential folk medicine, due to which it is being exploited severely and diminished in population size in Nilgiris. In addition, the traditional medicinal usage is not validated so far. Therefore for better management of this species, data on current availability status is required. In light of this fact, the present study was carried out to know the current ecological status of _H. radicata_ along with their associated plant species inhabiting at Kattabettu, Nilgiris, the Western Ghats, India. Phytochemical and biological activities were studied by using established scientific method to justify the traditional usage of this species for medicinal purposes. Further, propagation strategies of this plant through _in vitro_ regeneration and synthetic seed production were devised by employing tissue culture technology.

The floristic analysis in Kattabettu shola margins showed the presence of 43 plant species in that area which includes, 2 grasses (4.65%), 30 forbs (69.76%), 3 shrubs (6.97%), 6 climbers (13.95%) and 2 trees (4.65%) which are included in 27 plant families, the Poaceae member, _Cyanodon dactylon_ was represented by the high number of 2,480 individuals. On the other hand, the Oleaceae member, _Jasminum syzygium_
has only one individual. The data on quantitative ecological characters *viz.*, frequency, density, basal cover and importance value index exhibited that in addition to *Jasminum syimbriflorum* the study species, *Hypochaeris radicata* also perpetuated poorly in the study area which indicates that these species have no major functional role in the community metabolism.

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance to make the best and judicious use of available natural wealth. The preliminary phytochemical analysis has been conducted in various alcoholic and aqueous extracts of leaf and root parts of the study species, *H. radicata*. The study revealed that most of the secondary metabolites were present only in the methanolic extract. The result of the gravimetric and spectrometric studies reported that the quantity of secondary metabolites identified (total alkaloids, total phenolics, total flavonoids, total tannins, total saponins and ascorbic acid) was varied across the solvent extracts analyzed. Quantification studies showed that the methanolic extract of leaf and root parts contained significantly higher content of secondary metabolites than that of the other alcoholic and aqueous solvent extracts. As the methanolic extract of studied parts contained rich variety of secondary metabolites, this extract was subjected for further phytochemical studies.

The TLC and HPTLC finger printing studies confirms the presence of secondary metabolites of medicinal importance *viz.*, alkaloids, flavonoids, glycosides, saponins and terpenoids in methanolic leaf and root extracts. Mostly the high polar alcoholic solvents have been determined to be the more suitable mobile phases for the separation of secondary metabolites in the crude plant extract. GC-MS analysis of methanolic leaf and root extract of *H. radicata* revealed the presence of 11 and 9 compounds respectively. The major chemical constituents in leaf and root extracts are phytol, acetate (19.22%), hexadecanoic acid, methyl ester (17.37%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (16.74%) and phytol (13.60%), and (3β)-11-oxolanosta-8,24-dien-3-yl acetate (43.86%) and 1-benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-(3-oxo-1-butenyl) perhydro-, methyl ester (30.31%) respectively. Interestingly, it is known that these are the first reported compounds in the study species, *H. radicata* by the present investigation.

Isolation and structure elucidation of methanolic leaf and root extracts of *H. radicata* revealed the presence of active known compounds *viz.*, confertin
(sesquiterpenoid) and scopoletin (coumarin) respectively. The structures of the known compounds were established by comparison of their spectroscopic data (\(^1\)HNMR, \(^{13}\)CNMR, IR and MS). Further, the activity of the isolated compounds was predicted by using computer programme, PASS. The Pa (probability to be active) and Pi (probability to be inactive) values of confertin was ranging between 0.942 and 0.704 (Pa), and 0.004 and 0.016 (Pi). The predicted values exhibited the activities like antineoplast (0.942Pa-0.004Pi), antiprotozoal (Lieshmania) (0.908Pa-0.003Pi), antieczematic (0.842Pa-0.011Pi) and cardiovascular analeptic (0.825Pa-0.004Pi) for the compound, confertin. The prediction of PASS for scopoletin showed the activities like antimutagenic (0.890Pa-0.002Pi), spasmolytic and urinary (0.872Pa-0.004Pi), antiseptic (0.853Pa-0.004Pi), cardiovascular analeptic (0.784Pa-0.005Pi) and antiseborrheic (0.791Pa-0.021Pi).

Recently many medicinal plants have been evaluated for their biological activity in order to rationalize their use in traditional medicine. To confirm the biological properties of the study species, *H. radicata*, studies on antimicrobial, antioxidant, acute oral toxicity and antiinflammatory activities were made. The antimicrobial activity of various alcoholic and aqueous leaf and root extracts of *H. radicata* against 15 bacterial and 9 fungal pathogenic microbes clearly showed that the methanolic root extract had more pronounced activity than that of the leaf part. MIC was also determined for methanolic leaf and root extracts of the study species against these pathogenic microbes which was determined to be ranged between 200 and 700μg/mL and they were compared with positive and negative controls. Based on the overall performance, it was known that the methanolic leaf and root extracts of *H. radicata* may be a solution for infectious diseases.

The *in vitro* antioxidant activities *viz.*, reducing power activity, DPPH\(^\cdot\), NO\(^\cdot\), ABTS\(^{++}\) radical scavenging activity, β-carotene bleaching assay and antihaemolytic activity were evaluated to know the free radical scavenging capacity of various alcoholic and aqueous extracts of leaf and root parts of *H. radicata* compared with natural (rutin and quercetin) and synthetic (BHA and BHT) antioxidants. The results of the study revealed that the methanolic leaf and root parts possessed significant antioxidant activity in all the assays tested. Therefore, the species, *H. radicata* can be used potentially as a source of natural antioxidant. Due to the higher biological activity registered by
methanolic extract, further studies on acute oral toxicity and anti-inflammatory activity were made in this solvent for both parts of the study species, *H. radicata*.

The acute oral toxicity study showed that the oral administration of the methanolic leaf and root extracts of *H. radicata* at the dose from 100 to 3000mg/kg b.w. did not produce significant changes in behaviour of animals up to 14 days. It indicates that the methanolic extract is nontoxic to mice up to an oral dose of 3000mg/kg b.w. Therefore, the biological evaluation was carried out using two different dose (150 and 300mg/kg b.w.) levels. In carrageenan induced rat paw oedema, administration of indomethacin at 10mg/kg b.w. and methanolic leaf and root extracts at the doses of 150 and 300mg/kg b.w. reduced the inflammation significantly \((d_{p}<0.001)\) from 2hrs onwards compared to control group. However, the methanolic root extract at 300mg/kg b.w. showed higher effect (85.16%) near to normal rats. The results of haematological parameters have showed that oral administration of methanolic leaf and root extracts at the doses of 150 and 300mg/kg b.w. exhibited significant effect \((d_{p}<0.001)\) towards normal levels.

When compared with the normal rats, the levels of serum NO• was found to be higher in carrageenan intoxicated rats \((d_{p}<0.001)\). Among the test groups, the higher dose of the methanolic root extract (300mg/kg b.w.) exhibited better reduction \((d_{p}<0.001)\) than the leaf extract and it was comparable to that of the standard group treated with indomethacin. Estimation of serum protein on carrageenan induced rats exhibited significant \((d_{p}<0.001)\) decline in protein content when compared to Group I normal rats. A significant restoration \((d_{p}<0.001)\) of protein levels was noticed in oral administration of the methanolic root extract at the high dose (300mg/kg b.w.) than the leaf extract which was comparable to the Group VII. The antioxidant potential of the methanolic leaf and root extracts of *H. radicata* was evaluated using antioxidant enzymes *viz.*, SOD, CAT, GPx, GST and G6PD, and non-enzymic antioxidants *viz.*, GST and Vitamin C on the samples of spleen, thymus and hind paw. The Group II animals showed decrease in levels \((d_{p}<0.001)\) up on carrageenan induction. The methanolic leaf and root extracts at the doses of 150 and 300mg/kg b.w. retrieved antioxidant activity towards normal rats. Interestingly, the methanolic root high dose had better renovation \((d_{p}<0.001)\) than the leaf extract and it was comparable to Group VII.

The control groups reported to have increased \((d_{p}<0.001)\) LPO and HPO levels in serum, spleen, thymus and hind paw samples. It also revealed a significant decrease in
LPO and HPO levels by treatment with methanolic extracts which suggests their protective effect. However, the treatment with root high dose methanolic extract showed significant decrease ($p<0.001$) in LPO and HPO levels. The in vivo antiinflammatory activity is further supported by histopathological studies. It revealed the protective nature of the methanolic extract of *H. radicata* against carrageenan induction.

Owing to lower population size, for the study species, *H. radicata*, in vitro regeneration studies were made by employing tissue culture technology. Direct organogenesis studies showed that high number of shoots was obtained from cotyledonary leaf explant in the MS medium fortified with BAP at 2.0 mg/L (100%). Rooting percentage (98.48%) was better while subcultured onto the MS medium contained NAA at 1.0mg/L. Rooted plantlets were established in pots with 93.33% survival rate in the hardening medium containing garden soil, sand and vermicompost (1:1:1 by volume). Indirect organogenesis by employing in vitro derived leaf explants showed effective callus formation (98.83%) in MS medium containing BAP and NAA at 3.0 and 1.0mg/L respectively. Higher shoot formation (94.25%) and rooting (87.65%) were also observed in this same standardized medium. The acclimatization of plantlets was higher (92.22% survivability) in the hardening medium composed by autoclaved garden soil, sand and vermicompost in the ratio of 1:1:1 by volume.

Synthetic seed production was made successfully by the encapsulation of in vitro derived leaf, root and callus explants of *H. radicata* by encapsulating different concentrations of sodium alginate hydrogel (1-6%) containing MS medium. An encapsulation matrix of 3% sodium alginate with 100mM of CaCl$_2$·2H$_2$O was found to be most suitable for the formation of ideal beads. Among the three explants attempted, higher conversion frequency (86.64%) was observed for the leaf explants encapsulated beads followed by the callus encapsulated beads (63.12%) in the standardized MS medium supplemented with BAP at 2.0mg/L. Based on the better response, the in vitro derived leaf and callus explants encapsulated beads were taken for further subculturing experiments. The shoot emergence from in vitro derived explant encapsulated beads is directly depending upon storage period and temperature. It has been observed that 4 months old in vitro derived leaf, root and callus explants encapsulated beads recorded higher emergence of shoots which was ranging between 10.33 and 82.52%. The suitable temperature for storage of in vitro derived leaf, root and callus explants encapsulated beads was determined to be 25°C up to 2, 4 and 6 months of storage respectively.
After storage in proper conditions, the synthetic seeds prepared from *in vitro* derived leaf and callus segments were cultured onto the MS medium with different growth regulators to determine the optimum combinations and concentrations of growth regulators for effective regeneration. The conversion frequencies of encapsulated leaf and callus beads into multiple shoots were higher (88.55 and 63.87% respectively) in the MS medium fortified with BAP at 2.0mg/L. The regenerated shoots from *in vitro* derived leaf and callus explants encapsulated beads were rooted well (73.54 and 59.40% respectively) on MS medium fortified with NAA at 1.0mg/L. The *in vitro* regenerated plantlets were acclimatized successfully by using garden soil, sand and vermicompost (1:1:1 v/v/v) by obtaining higher survivability rate of 87.77% for leaf encapsulated beads and 70.00% for callus encapsulated beads.

By considering the wide medicinal uses and less population size, priority must be given to this species to enhance the individuals and hence the population adequately in the sholas of Kattabettu region and hence to conserve the species effectively. The studies on phytochemical investigation confirm the occurrence of bioactive secondary metabolites in *H. radicata* and also the investigation on therapeutic properties scientifically supports the traditional use of this crude plant drug for the treatment of diseases practiced by the traditional healers of Nilgiris, the Western Ghats. The standardized protocol developed in *in vitro* regeneration technique is not only useful for its mass scale propagation but also conservation of germplasm.