SUMMARY AND CONCLUSIONS

The Rayalaseema (Chittoor) possess rich and diversified plant wealth due to varied eco-climatic conditions and undulated topography. The availability of abundant natural resources might have fetched the adivasi tribes for their settlement in interior forests in the area. In spite of the rapid urbanization and fast penetration of western culture most of these tribes are still practicing their traditions of their ancestors. However, their ancestral traditions have been threatened by indiscriminate biotic interference in their habitats in the form of deforestation, development of dams and roads, implementations of New Forest Policies, etc. With this, the valuable well-protected guarded secrets regarding the application of crude drugs for different ailments, hidden in tribal communities are being disappeared. In the light of this, the present work was taken up to conduct extensive and intensive explorations in and around the tribal habitations and collected the information regarding human ailments.

The present study provides information on therapeutic properties of *L. nepetifolia* crude drug used for different human ailments. The extensive exploration studies yielded a good number of *L. nepetifolia* parts used by different tribes viz., sugalis, yanadis and yerukalas, etc. The first hand information was collected with regard to folklore medicine pertaining to discussion of *L. nepetifolia* belonging to Lamiaceae family of higher plants. Among them, the following disease viz., night blindness, scabies, cough, cold, wound, cuts, paralysis, asthma, necrosis, convulsions, malaria, eczyma, piles, burning sensation, post natal breast pain, skin problems, diarrhoea, diabetes, joint pains, veterinary diseases, fever, skin diseases, jaundice, ring worms, itches, scalds and relieve dandruff were reported for medicobotanical
properties pertaining to different human ailments \[1, 2, 3, 4, \text{and} 5\]. Information on the remaining human ailments was hitherto not reported, hence, new to the science.

In the traditional systems of medicine there is considerable variation in the identity of the various source plants of the individual drug selected for use. Based on the tribal information leaves are the important and most common parts used for external diseases in the form of powder/paste. The pharmacological characters reported in this study can serve as a valuable source of information and provide suitable diagnostic tool for the standardization as well as adulterant identification of powdered crude drugs in future. It will also be immense use in carrying out further research and revalidation of its use.

Based on effective use by the local tribal’s \textit{L. nepetiifolia} was selected for experimental analysis. The preliminary phytochemical analysis was conducted on the crude extracts obtained from different polar solvents like petroleum ether, ethyl acetate, methanol and water. The alkaloids, coumarins, flavonoids, flavones, phenols, saponins, steroids, terpenoids, proteins, tannins, lignins, volatile oils and carbohydrates are the common compounds in the test species. Maximum number of phytochemical constituents was found in leaf extracts of \textit{L. nepetiifolia} followed by inflorescence and root, while minimum were noticed in stem. The comprehensive data is provided to indicate the distribution of various groups of compounds in the respective solvent extracts. The part wise analysis revealed that the maximum chemical compounds are found in leaves followed by inflorescence and root, while the minimum chemical compounds are found in stem.

The presence of various secondary metabolites such as alkaloids, coumarins, fatty acids, flavonoids, saponins, steroids, triterpenoids, tannins and volatile oils may confirm the different medicinal properties of plants \[6,7\]. Alkaloids, which are
reported to have dramatic physiological activities and act mainly on controlling nervous system, were observed in *L. nepetifolia* screened. Carotenoids having nutritional importance as vitamin A [8, 9 and 10] were observed. Coumarins, reported to have anticoagulation, estrogenic, vasodilation, antibacterial and antihelmintic properties were found [7]. Flavonoids having antiviral, anti-inflammatory and cytotoxic activities and used in the treatment of capillary fragility, retinal haemorrhage, hypertension, diabetic retinopathy, rheumatic fever and arthritis [11] were observed. Saponins, well known for their expectorant, spasmolytic and antitissue activities [12] were observed. Steroids and Triterpenoids, which are known for anti-inflammatory, lipolytic and anti-cholesteremic activities [13], were recorded. Gallic tannins, which are well documented for the astringent, cytotoxic and antineoplastic activities and used in diarrhoea, haemorrhage, wounds healing and deep burns were observed [14].

During the course of study *L. nepetifolia* crude drug was screened for antimicrobial activity. Three different solvents viz., petroleum ether, ethyl acetate and methanol were used for preparation of crude drugs. The alcoholic extracts exhibited inhibitory activity against most of the organisms. A majority of the plant extracts of *L. nepetifolia* (Leaf, Inflorescence, Root and stem) have shown significant inhibitory activity against test pathogenic microorganisms.

The observation made in phytochemical studies coupled with medicinal uses mentioned by traditional herbal healers provide valuable information for further biological screening and antioxidant property based fractions of each plant extract. The screening for antimicrobial and antifungal activity of crude extracts of plants was carried out for their inhibitory effects individually on active cultures of nine different pathogenic microorganisms. The nine organisms employed were *Bacillus cereus, B. 
subtilis, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium and Candida albicans. All the organisms are pathogenic and the diseases caused by them are candidiasis, a micotic disease by Candida albicans, moderate to severe gastroenteritis by Escherichia coli, nasal infection by Staphylococcus aureus, conjunctivitis and irridocheroiditis by Bacillus cereus, inflammatory lesions by Pseudomonas aeruginosa, typhoid fever by Salmonella typhimurium. Out of nine organisms, viz., Bacillus cereus, B. subtilis, Micrococcus luteus, and Staphylococcus aureus are Gram (+ve), Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Salmonella typhimurium are Gram (-ve) bacteria while Candida albicans is filamentous fungus.

Secondary metabolites like alkaloids, anthraquinones, aubcins, coumarins, dihydrochalcones, emodins, gallic-tannins, reducing compounds and polyoses are known to affect physiological activities. Anthraquinones, coumarins, gallic-tannins, and volatile oils are known to have antimicrobial activity [15, 16]. Alkaloids, coumarins, flavonoids, phenols, steroids and triterpenoids were present in most of the samples, screened for the study. Individual (or) combination of several compounds may be one of the reasons for uncertain antibacterial activity.

It is increasingly being realized that many of today’s diseases are due to the “oxidative stress” that results from an imbalance between formation and neutralization of pro-oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases [17, 18]. Many synthetic
drugs protect against oxidative damage but they also have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicine [19]. Plants are rich sources of natural antioxidants, which play a vital role in the prevention or progression of the degenerative diseases [20]. Plant compounds, mainly derived from natural sources that showed potential antioxidant activity includes carotenoids, coumarins, flavonoids, tannins etc. Plant phenolics constitute one of the major group of compounds acting as primary antioxidants or free radical terminators [21].

Phenolics are aromatic secondary metabolites, widespread throughout the plant kingdom. The phenols contain hydroxyls that are responsible for the radical scavenging redox properties [22]. Tannins are antioxidants often characterized by reducing power [23]. Plant derived antioxidants especially polyphenols and flavonoids have ascribed been to various properties like anticancer, antidiabetic, antiageing and prevention of cardiovascular diseases [24]. Poly phenolic compounds like flavonoids have been labeled as high level natural antioxidants based on their abilities to scavenge free radicals and active oxygen species [25]. In the present study different parts with different solvent extracts of *L. nepetiifolia* were subjected for *in vitro* antioxidant activity and free radical scavenging activity. All the extracts (petroleum ether, ethyl acetate, alcohol and water) in the present study exhibited different extent of antioxidant activity and free radical scavenging activity. Very little or undocumented antioxidant activities were observed in the stem extracts of *L. nepetiifolia*. Results suggest that the above mentioned crude drug samples serve as good candidates for further evaluation of their bioefficacies, active constituents and biological mechanisms *in vitro* as well as *in vivo* on antioxidation or chemoprevention.
effects. Possible applications of the selected target plant extract as medicinal supplements for human healthcare are also under evaluation.

Helminthiasis is a world wide and one of the common diseases of all ages especially in third world countries. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs; infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation.

In the present study, two crude drug samples of Leonotis nepetiifolia leaf and inflorescence were assessed for anthelmintic activity against Pheretima posthuma, which is well accepted assay for the preliminary screening of crude extracts for potential anthelmintic activity. All the extracts were used at different concentrations (25, 50 and 75mg/ml), in order to determine the time taken for paralysis and complete death of the worms. The results can lead to discovery of new anthelmintic drugs from L. nepetiifolia.

Crown gall is a plant neoplastic disease induced by a Gram-negative bacterium Agrobacterium tumefaciens [26, 27]. Agrobacterium tumefaciens infection symptoms resemble tumor in mammalian cells. The tumor starts when bacterial cell transfer part of the Ti (Tumor inducing) plasmid to the infected plant cell genome [28]. The validity of potato disc bioassay is predicted on the observation that certain tumorigenic mechanisms are similar on plants and animals like multiplying rapidly without apoptosis [29].

In the present study, two crude drug samples L. nepetiifolia leaf and inflorescence were assessed for antitumor activity using the potato disc assay, which is well accepted assay for the preliminary screening of crude extracts for potential
anticancer and antitumor activity. All the extracts were used at different concentrations (10 ppm, 100 ppm and 1000 ppm) and exhibited significant percentage of tumor inhibition (ID$_{50}$ values) in potato disc assay. The results can lead to discovery of new anticancer drugs from $L.$ nepetiifolia.

Diabetes mellitus is a major endocrine disorder characterized by elevated blood glucose levels resulting from absence of inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action. In fact, diabetes nowadays a global problem affecting nearly 10% of population all over the world, comes to 150 million people [30]. According to World Health Organization (WHO) report the number of cases of diabetes mellitus was 171 million in 2000, which may increase to 360 million in year 2030. By the year 2025, 75% of diabetes will reside in developing countries [31].

In the present study, aqueous leaf extracts of $L.$ nepetiifolia were assessed for antidiabetic activity which is well accepted assay for the preliminary screening of crude extracts for potential antidiabetic activity in order to know the body weight, blood glucose levels and serum lipid proteins. The extracts show significant antidiabetic activity. The results can lead to discovery of new anticancer drugs from $L.$ nepetiifolia.

The overall results show that the effective crude extracts, which exhibited significant biological activity even at low concentrations, were carefully analyzed and subjected for further fractionation and characterization. Based on the preliminary investigations the following plant with different parts were selected for fractionation and characterization of active principle using spectral studies like HPTLC and LC-MS analysis on the organic solvent extracts of $L.$ nepetiifolia leaf, inflorescence and root
resulted 8, 7 and 6 compounds respectively including earlier studies and information from Natural product dictionary library, IICT, Hyderabad.

In the present study the analysis of methanolic leaf extracts revealed the presence of leaf extracts the following compounds were identified viz., albizoin, isotrityrosine, ferrioxamine A, leonotin, heptaphylline, ezomycin, marrubiin and premarrubiin. The fractions are being subjected for characterisation of compounds using NMR spectral studies. Since the reported compounds have significant biological properties viz., which are used in protein cross linking agent, anthelmintic activity, iron transport, antiplasmodial, antimicrobial and antibiotic activity (Sanduja et al., 1985; Bickel et al., 1993). In inflorescence affinoside B, protostreptovaricin, monamidocin, rotenalone, majapolone, nepetaefolin and nepetaefolinol were reported, which needs further confirmation with spectral analysis. The compounds are used in reverse transcriptase inhibitor, receptor antagonist, platelet aggregation inhibitor, antiscabies, antimicrobial and cytotoxic activity (Deshmukh et al., 1976; Begum et al., 2008). In root betrachamine, slagenin A, desmodianone A, arglicin, clausne D, cetocycline were observed. They are used in cell regulation modulator, sphingosine burst, antiplatelet aggregation, cytotoxic and antimicrobial activity (Wardens et al., 1999; Mitscher, et al., 1983)

The present study revealed the comprehensive data on ethnobotany, preliminary phytochemical screening followed by fractionation of potential fractions and biological screening with reference to crude drugs used for different human ailments. This helps to promote the future investigations on locally available medicinal plants and capture the biological and cultural data of local people. Further, the data can be used for intensive studies in order to evaluate active principle, which is biologically active against the causative principle or pathogenic microorganisms. It
is apparent through this review that the pace of natural product research and level of global interest in this particular area has raised dramatically in the past few years. This trend is projected to continue for the interface between chemistry and biology becomes more blurred and the public demand rises for cost effective medications and biological agents from sustainable natural resources.

The salient observations from the present study are

- The comprehensive data on *Leonotis nepetiifolia* crude drugs used for different human diseases present in Chittoor district of Andhra Pradesh was provided, which is hither to not reported.
- The voucher herbarium specimens were prepared using standard methods and deposited at Sri Krishnadevaraya University Herbarium (SKU), Anantapur.
- The preliminary phytochemical screening was conducted for *Leonotis nepetiifolia* using standard methods and distribution of various groups of secondary metabolites were analysed.
- The phytochemical diversity among the various parts of the test species was statistically analyzed and indicated the phytochemical richness, which will be helpful for further isolation and characterization.
- The antimicrobial evaluation was conducted using standard methods and recorded potential fractions along with MIC values.
- The total flavonoid content of different parts and various solvent extracts of *Leonotis nepetiifolia* were reported first time.
- The total phenol content of different parts and various solvent extracts of *Leonotis nepetiifolia* were conducted first time and supported with the biological properties.
The antioxidant activity of different parts and various solvent extracts of *Leonotis nepetiifolia* were reported first time and the present observations substantiate the folk claims.

The free radical (DPPH) scavenging activity of different parts and various solvent extracts of *Leonotis nepetiifolia* were conducted first time.

The anthelmintic activity of different parts of various solvent extracts of *Leonotis nepetiifolia* was reported first time.

The potato disc tumor assay of test species *Leonotis nepetiifolia* yielded significant antitumor activity which supports the folk claim on the use of test species as anticancerous drug.

The aqueous extracts of *Leonotis nepetiifolia* showed significant antidiabetic activity.

HPTLC and LC-MS analysis was conducted on *Leonotis nepetiifolia* and enumerated the active compounds, which helps to understand the molecular basis of therapeutic activity and also substantiate the folk claims.

The majority of the objectives were fulfilled with sufficient data, which was substantiated by the experimental evidences. However, the fractionated samples of *Leonotis nepetiifolia* require further investigations especially NMR spectral studies in order to establish the exact molecular basis for the therapeutic properties. The attempts in this direction are being carried out in collaboration with IICT, Hyderabad and the results are in progress, and this may be helpful to the pharmaceutical industry for manufacturing of safe, easily accessible biomedicine to cater the needs of the world, especially the developing countries.