CHAPTER-V

SUMMARY AND CONCLUSIONS
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The Eastern Ghats 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. Inspite of the rapid urbanization and fast penetration of western culture most of the 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, developmental activities of Girijan Cooperative Corporation (GCC), 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 and their ethnomedicinal remedies.

The Eastern Ghats are one of the nine Bio-diversity centres of India, possess rich vegetation and inhabited by 35 different major tribal communities. The present investigation emphasized on the exploration of rare, endemic, endangered and little known Phyllanthus species may be helpful to the government and non-government organizations to implement the suitable measures to protect the specified potential medicinal plants, which have limited distribution. This helps in minimizing the exploitation of certain
species. The awareness imports especially on conservation of such species should be created which helps in effective utilization and sustainable management of drug yielding plants. Thorough studies on ethno-medico-botanical literature revealed that little knowledge is available on use of certain Phyllanthus species and on the biological and phytochemical studies, from Eastern Ghats of Andhra Pradesh. So, an attempt was made to explore the Phyllanthus species to evaluate phytochemical and In vitro antimicrobial and other biological activities.

The present study provides information on therapeutic properties of Phyllanthus crude drugs used for different human ailments. The extensive exploration studies yielded a good number of Phyllanthus species used by different tribes viz., bagata, chenchus, gadaba, jatapu, konda reddy, kondhu, konda kammaras, koyas, savaras, sugalis, yanadis and yerukalas, etc. The first hand information was collected with regard to folklore medicine pertaining to Phyllanthus drug yielding plants belonging to Euphorbiaceae families of higher plants. Among them, the following diseases viz., Abscess, Allergy, Anaemia, Antiemetic, Asthma, Astringent, Aphrodisiac, Burns, Bone fracture, Cooling, Cough, Diuretic, Dysentery, Fever, Genito-Urinary disorders, Gonorrhoea, Gum bleeding, Head ache, Itching, Indigestion, Jaundice, Laxative, Leucorrhoea, Scorpion sting, Skin diseases, Stomach ache, Sores, Tonic, Tooth ache, Ulcers and Wounds, were reported along with their medicobotanical remedies (Kirtikar & Basu 1933-35, Rama Rao and Henry 1996, Jain 1991). Information on the remaining human ailments and their natural remedial measures as recorded in the present study was hither to not reported, hence, new to the science. The medicinal properties of 40 per cent of plant species enumerated as part of the present study were found to be new and hither to not reported, which are indicated with asterisk(*).

In the traditional systems of medicine there is considerable variation in the identity of the various source/plant parts 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 provided 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.

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Based on effective and wide usage by the local tribals, *Phyllanthus* species were selected for experimental analysis. The preliminary phytochemical analysis was conducted on the crude extracts obtained from different polar solvents like petroleum ether, methanol and water. Statistical analysis of preliminary phytochemical compounds among 15 spp revealed 30 major groups of compounds. The alkaloids, coumarins, flavonoids, flavones, phenols, saponins, steroids are the common compounds in all the test species (100 %), followed by anthocyanins, catecholic compounds, dihydrochalcones (14 spp each 93.33 %). While the emodins and iridoids were noticed in single species (6.66). Maximum number of phytochemical constituents was found in *Phyllanthus narayanaswamii*, followed by *P. amarus, P. maderaspatensis, P. reticulatus* and *P. emblica*, while minimum were noticed in *P. pinnatus*. 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 were recorded in leaves (83.33 %), followed by stem bark, shoot (66.66 %), while the minimum chemical compounds were found in root and root bark (56.6 %).

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 (Kapoor *et al.*, 1969; Chhabra *et al.*, 1993). Alkaloids which are reported to have dramatic physiological activities and act mainly on controlling nervous system were observed in all *Phyllanthus* (100%) species screened. Anthocyanidins known to possess healing and cytotoxic properties (Jenkins *et al.*, 1957; Fairbairn, 1959; Lewis and Elvin-Lewis, 1977) were observed in 2 spp. (13.33%); Anthocyanins which are known to decrease the capillary permeability in inflammatory conditions of blood vessels (Alcaraz and Jimenez, 1988) were found in 12 spp. (93.33%), Anthraquionones and volatile oils, known to exhibit antimicrobial activity (Rios *et al.*, 1987, 1988; Diaz *et al.*, 1988). Carotenoids having nutritional importance as vitamin A (Geissman, 1962, Ramwell *et al.*, 1964; Cody *et al.*, 1986; Farakas *et al.*, 1986; Nigam and Misra, 1989) were observed in 4 spp. (26.66%). Coumarins, reported to have anticoagulation, estrogenic, vasodilation, antibacterial and anthelmintic properties (Chhabra *et al.*, 1984) were found in 15 spp. (100%). Flavonoids, having antiviral, anti-inflammatory and cytotoxic activities, were used in the treatment of capillary fragility,
retinal haemorrhage, hypertension, diabetic retinopathy, rheumatic fever and arthritis (Tripathi and Rastogi, 1981) were observed in 15 spp. (100%). Saponins, well known for their expectorant, spasmylytic and antitissue activities (Banerji et al., 1982; Sood et al., 1985; Nigam and Misra, 1989) were observed in 15 spp. (100%). Steroids and Triterpenoids, which are known for anti-inflammatory, lipolytic and anti-cholesteremic activities (Chawla et al., 1987), were recorded in 15 spp. (100% each) respectively. Gallic tannins which are well documented for the astringent, cytotoxic and antineoplastic activities and used in diarrhoea, haemorrhage, wounds healing and deep burns (Ramakrishna and Krishna, 1994) were observed in 10 spp. (66.66%).

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, Bacillus 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 very common like 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, the organisms viz., Bacillus cereus, Bacillus subtilis, Micrococcus luteus, and Staphylococcus aureus are Gram (+ ve), and the remaining Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Salmonella typhimurium are Gram (- ve) bacteria while Candida albicans is filamentous fungus.

During the course of study Phyllanthus crude drugs were 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 (Phyllanthus amarus (Leaf, Shoot), P. emblica (Stem bark), P. indofischeri (Leaf), P. maderaspatensis (Leaf) P.
P. narayanaswamii (Leaf, Root), P. pinnatus (Root), P. polyphyllus (Leaf), P. reticulatus (Leaf), P. rheedia (Leaf), P. rotundifolius (Stem bark, Root bark), P. tenellus (Leaf, Root), P. urinaria (Leaf, Root) and P. virgatus (Shoot, Root) have shown significant inhibitory activity against a test microorganisms.

Secondary metabolites like alkaloids, anthraquinones, aucubins, 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 (Rios et al., 1987, 1988; Diaz et al., 1988; Saju et al., 1998). 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 (Braca et al., 2002; Maxwell, 1995). 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 (Yazdanparast et al., 2008). Plants are rich sources of natural antioxidants, which play a vital role in the prevention or progression of the degenerative diseases (Ames et al., 1993). 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 (Cook and Samman, 1996).

Phenolics are aromatic secondary metabolites, widespread throughout the plant kingdom. The phenols contain hydroxyls that are responsible for the radical scavenging redox properties (Rice-Evans et al., 1997). Tannins are antioxidants often characterized by reducing power (Mi-Yea et al., 2003). Plant derived antioxidants especially polyphenols and flavonoids have ascribed been to various properties like anticancer, antidiabetic,
antiageing and prevention of cardiovascular diseases (Dixon et al., 2005; Rice-Evans, 2004). Poly phenolic compounds like flavonoids have been labelled as “high level natural antioxidants based on their abilities to scavenge free radicals and active oxygen species (Birt et al., 2001). In the present study 2 species (Phyllanthus narayanaswamii and P. virgatus) were subjected for In vitro antioxidant activity and free radical scavenging activity. All the extracts (petroleum ether, ethyl acetate, alcohol, water and decoction) in this study exhibited different extent of antioxidant activity and free radical scavenging activity. Hither to undocumented antioxidant activities were observed in the plant extracts from P. narayanaswamii and P. virgatus. Results of the present study 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 extracts as medicinal supplements for human healthcare are also under evaluation.

Based on the preliminary investigations the species P. narayanaswamii (a rare endemic species restricted to galikonda hills in Visakhapatnam district) was selected for LC-MS analysis. The LC-MS phase separation was carried out to isolate different chemical constituents present in the alcoholic extract (leaf), which revealed Azadirachtin N, 5-Hydroxy-3, 3', 4', 6, 7-Pentamethoxy flavone and Polyoxin-G as chief component. These compounds were shown to have possessed antiviral, antiproliferative and antifungal effects and this helps to substantiate the folk claims.
The salient observations from the present study are

- The comprehensive list of *Phyllanthus* crude drugs used for different human diseases present in the Eastern Ghats of Andhra Pradesh was provided.

- The voucher herbarium specimens were prepared using standard methods and deposited at Sri Krishnadevaraya University Herbarium (SKU), Anantapur.

- 40 per cent of the folklore claims recorded during the study were found to be new and hither to unknown which were indicated with asterisk.

- The preliminary phytochemical screening was conducted for 15 *Phyllanthus* species using standard methods and distribution of various groups of secondary metabolites were analysed.

- The phytochemical diversity among 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 for 13 *Phyllanthus* species using standard methods and recorded certain potential crude drugs.

- The total flavonoid content of different parts and various solvent extracts of *Phyllanthus narayanaswamii* and *P. virgatus* were reported first time.

- The phenol content of different parts through various solvent extracts of *Phyllanthus narayanaswamii* and *P. virgatus* was conducted first time.

- The antioxidant activity of various solvent extracts of *Phyllanthus narayanaswamii* and *P. virgatus* were reported first time.

- The free radical (DPPH) scavenging activity of various solvent extracts obtained from different parts of *Phyllanthus narayanaswamii* and *P. virgatus* was conducted first time.

- Fractionation and isolation of different groups of compounds present in *Phyllanthus narayanaswamii* was conducted first time using LC-MS analysis.
The majority of the objectives were fulfilled with sufficient data which was substantiated by the experimental evidences. However, the *Phyllanthus* species (as listed above) require further investigations in order to establish the exact molecular basis for the therapeutic properties. 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.