Chapter V
5. SUMMARY AND CONCLUSIONS

Medicinal plants have been in use all over the world to treat various diseases including infections, cancer, inflammation, heart diseases etc. The use of natural products for treatment of all kinds of diseases is due to their less harmful effects as compared to drugs synthesized in laboratory. In all the areas of the world, locally used herbal treatments are common and have been under investigation to get active principle of these remedies.

Indian subcontinent possess rich biodiversity hot spots, which have been recognized as major biodiversity centres in the world. The Eastern Ghats of Rayalaseema region is one among them ranging from dry deciduous to thorny shrub forests with occasional patches of evergreen forests. The study area was extensively explored with regard to ethnobotanical studies by various ethnobotanists. However, most of them dealt with general studies in which they have collected the information for common ailments and not particularly related to specific diseases. Basically most of the tribal people are suffering from different kinds of cancerous diseases (tumors, wounds and skin cancer) due to lack of hygiene drinking water, poor diets and cigarette smoking. At the same time they are depending on their nearby forests to cure their common health problems. At this juncture a little attempt was made to document the folklore claims and to evaluate the efficacy of the crude drugs by using scientific methods.

The present study provides information on therapeutic properties of crude drugs used for cancerous diseases. The extensive exploration studies yielded a good number of medicinal plants used by different tribes viz., chenchus, yerukalas, yanadis and sugalis. The first hand information was collected with regard to folklore medicine pertaining to 111 drug yielding plants belonging to 97 genera and 50 families of higher
plants (including one pteridophyte). Among them, the following species viz., *Agave americana*, *Ageratum conyzoides*, *Allium sativum*, *Andrographis paniculata*, *Anogeissus latifolia*, *Argemone mexicana*, *Azadirachta indica*, *Calotropis gigantea*, *Cassia fistula*, *Catharanthus roseus*, *Chloroxylon swietenia*, *Datura metel*, *Diospyros melanoxylon*, *Dregea volubilis*, *Elephantopus scaber*, *Euphorbia hirta*, *Heliotropium indicum*, *Jatropha gossypifolia*, *Mangifera indica*, *Moringa oleifera*, *Mucuna pruriens*, *Nerium indicum*, *Nyctanthes arbor-tristis*, *Plumbago zeylanica*, *Ricinus communis*, *Rubia cordifolia*, *Semecarpus anacardium*, *Syzygium cumini*, *Tephrosia purpurea*, *Terminalia chebula*, *Tinospora cordifolia*, *Tridax procumbens*, *Vitex leucoxylon*, *Woodfordia fruticosa*, *Wrightia tinctoria*, *Xanthium strumarium*, *Ziziphus oenoplia* were reported for medicobotanical properties pertaining to cancerous diseases (Balachandran & Govindarajan, 2005; Kumar *et al.*, 2007; Graham *et al.*, 2000). Information on the remaining crude drugs was hither to not reported, hence, new to the science. The medicinal properties of 30 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 plants of the individual drug selected for use. Based on the tribal information leaves are the important and most common parts used for external wounds and tumors 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. The microscopic features could help in laying down micro morphological standards as per WHO guidelines for authentication of the original drugs.

Efforts were made to substantiate the folklore claims with the help of phytochemical constituents and their biological activities. In this connection, 40 species were subjected for phytochemical and 35 species for antimicrobial evaluation based on the potential usage and rare distribution. The phytochemical profile of test crude drug samples revealed that the maximum chemical diversity among the test samples, which supports the statement “tropical plant posses more chemical diversity” (Harborne, 1991). 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 37 spp. (92.5%) of the 40 species screened. Anthocyanidins known to possess healing and cytotoxic properties (Jenkins et al., 1957; Fairbairo, 1959; Lewis and Elvin – Lewis, 1977) were observed in 20 spp. (50%); Anthocyanins which are known to decrease the capillary permeability in inflammatory conditions of blood vessels (Alcaraz and Jimenez, 1988) were found in 9 spp. (22.5%), Anthroquinones and volatile oils, known to exhibit antimicrobial activity (Rios et al., 1987, 1988; Diaz et al., 1988) were recorded in 11 spp. (27.5%) and 16 spp. (40%) respectively. 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 9 spp. (22.5%). Coumarins, reported to have anticoagulation, estrogenic, Vasodilation, antibacterial and antihelmintic properties (Chhabra et al., 1984) were found in 36 spp. (90%). 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 (Tripathi and Rastogi, 1981) were observed in 24 spp. (60%). Saponins, well known for their expectorant, spasmolytic and antitissue activities (Banerji et al., 1982; Stood et al., 1985; Nigam and Misra, 1989) were observed in 20 spp. (50%). Steroids and Triterpenoids, which are known for anti-inflammatory, lipolytic and anti-cholesteremic activities (Chawal et al., 1987) were recorded in 30 spp. (75%) and 24 spp. (60%) respectively. Gallic tannins which are well documented for the astringent, cytotoxic and antineoplastic activities and used in diarrhoea, haemorrhage, wounds healing and deep burns (Rama Krishna and Krishna, 1994) were observed in 13 spp. (32.5%).

The observation made in phytochemical studies coupled with medicinal uses mentioned by traditional 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 seven different pathogenic microorganisms. The seven organisms employed were Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, Salmonella typhimurium. 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 iridochoroiditis by *Bacillus cereus*, inflammatory lesions by *Pseudomonas aeruginosa*, typhoid fever by *Salmonella typhimurium*. Out of seven organisms, two organisms viz., *Bacillus cereus* 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.

During the course of study 35 crude drugs were screened for antimicrobial activity. Three different solvents viz., petroleum ether, ethyl acetate and methanol were used for preparation of crude drugs. All the three solvent extracts exhibited inhibitory activity against most of the organisms. A majority of the plant extracts (*Dodonaea viscosa*, *Nyctanthes arbor-tristis*, *Semecarpus anacardium*, *Syzigium cumini*, *Acanthuspermum hispidum*, *Aristolochia indica*, *Buchanania lanzan*, *Cassia fistula*, *Chloroxylon swietenia*, *Cochlospermum religiosum*, *Elytraria acaulis*, *Euphorbia hirta*, *Helicteres isora*, *Lannea coromandelica*, *Lepidagathis cristata*, *Moringa concanensis*, *Plumbago zeylanica*, *Rhinacanthus nasutus*, *Terminalia chebula*, *Tinospora cordifolia* and *Wrightia tinctoria*) showed less activity with petroleum ether solvent. Some extracts (*Dodonaea viscosa*, *Semecarpus anacardium*, *Euphorbia hirta*, *Lepidagathis cristata* and *Chloroxylon swietenia*) have shown significant inhibitory activity against a few test microorganisms only.

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; Diag *et al.*, 1988; Saju *et al.*, 1998). Alkaloids render important as potential drugs as anticancer agent (Atal and Kapoor, 1982). Coumarins, emodins, flavonoids, alkaloids, essential oils, 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 cancer, 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 7 species (Chloroxylon swietenia (Lf), Entada pursaetha (Sd), Hedychium coronarium (Rh), Pulicaria wightiana (Inf), Mallotus philippensis (Sd), Vitex leucoxylon (Fr & Lf ) and Vitex peduncularis (Lf) ) were subjected for in vitro antioxidant activity. All the extracts in this study exhibited different extent of antioxidant activity. Very little or undocumented antioxidant activities were observed in the plant extracts from Chloroxylon swietenia (Lf), Pulicaria wightiana (Inf), Mallotus philippensis (Sd) and Vitex peduncularis (Lf).

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 cancer chemoprevention effects. Possible applications of the selected target plant extracts as medicinal supplements for human healthcare are also under evaluation.

The brine shrimp lethality assay represents a rapid, inexpensive and simple tool
for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties (Mc Laughlin et al., 1993). The cytotoxicity of plant materials is considered to be due to the presence of antitumor compounds. Brine shrimp (*Artemia salina* L.) bioassay considered as a preliminary screening for the presence of antitumor compounds and used to determine the plant extracts toxicity (Meyer et al., 1982). Brine shrimp bioassay can be used as preliminary *in vitro* antitumor assay while it has positive correlation with 9 KB (human nasopharyngeal carcinoma) cytotoxicity and 3PS (p388) (*in vivo* murine leukemia) and can substitute both of them (Ferrigni et al., 1982; Ferrigni and Mc Laughlin, 1984; Mc Laughlin and Roger, 1998). The brine shrimp lethality bioassays on plant extracts are valuable in screening of the crude methanol extracts which indicate that further investigation is worthwhile. The present study clearly indicates that of 111 species, enumerated as crude drugs for different cancerous diseases, 23 crude drug extracts were highly lethal to brine shrimp nauplii out of the 40 crude drug extracts used in the study. From the range of doses it is also clear that *Ageratum conyzoides*, *Andrographis paniculata*, *Hedychium coronarium*, *Moringa concanensis*, *Phyllanthus reticulatus*, *Semecarpus anacardium*, *Terminalia chebula*, *Buchanania lanzan*, *Cochlospermum religiosum*, *Curculigo orchioides*, *Entada pursaetha*, *Hygrophila auriculata*, *Rhinacanthus nasutus*, *Syzygium alternifolium*, *Tylophora fasciculata*, *Vitex leucoxylon*, and *Vitex peduncularis* have potent brine shrimp lethality activity indicating that these plants contain potential bioactive compounds, which if properly and extensively studied, could provide many chemically interesting and biologically active drugs, including some with antitumor and antiproliferative properties which need to be investigated thoroughly.

Crown gall is a plant neoplastic disease induced by the Gram-negative bacterium *Agrobacterium tumefaciens* (Kahl and Schell, 1982; Lippincott and Lippincott, 1975). The *Agrobacterium tumefaciens* infection symptoms resemble tumor in mammalian cells. The tumor formation starts when bacterial cell transfer part of the Ti (tumor -- inducing) plasmid to the infected plant cell genome (Anand and Harberleim, 1977). The validity of potato disc bioassay is predicted on the observation that certain tumorigenic mechanisms are similar in plants and animals like multiplying rapidly without apoptosis (Braun, 1972).

In the present study, 7 crude drug samples (*Chloroxylon swietenia* (Lf), *Entada pursaetha* (Sd), *Hedychium coronarium* (Rh), *Pulicaria wightiana* (Inf), *Mallotus 
Philippensis (Sd), Vitex leucoxylon (Fr & Lf) and Vitex peduncularis (Lf) were assessed for antitumor activity using the potato disc assay, which is a well accepted assay for the preliminary screening of crude extracts for potential anticancer and antitumor activity. All the extracts were used at three different concentrations (10 ppm, 100 ppm and 1000 ppm) and exhibited significant percentage of tumor inhibition (ID₅₀ values) in potato disc assay. These results can lead to discovery of new anticancer drugs in future. An interesting aspect of the present study is less or no inhibition of tumor at low concentration (10 ppm) in case of ethyl acetate extract of Hedychium coronarium (Rh), methanolic extract of Vitex leucoxylon (Fr), petroleum ether extract and ethyl acetate extracts of Chloroxylon swietenia (Lf) respectively.

Agents capable of inhibiting cell proliferation including apoptosis or modulating signal transduction are currently used for the treatment of cancer (De Flora et al., 2005). Flavonoids are also potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). Phenolic compounds including flavonoids are especially promising candidates for cancer prevention (Bravo, 1998). Of all the above bioassay studies, methanolic extracts showed significant antimicrobial, antioxidant and antitumor activity. Hence, in the present study the methanolic extracts of Entada pursaetha (Sd), Hedychium coronarium (Rh) and Mallotus philippensis (Sd) were tested for the cytotoxicity and showed most effective inhibition of HEp-2 cell proliferation. The best potential was obtained at certain optimal concentrations of the extracts. The potential use of the above drugs as therapeutic agent hold great promise as the isolation of one or more cytotoxic chemicals from crude extract and judicious use of such chemicals can control the progression of cancer and also can prevent the formation of tumor in individuals who are highly susceptible to developing a tumor.

The overall results show that the effective crude extracts, which exhibited significant biological activity and antioxidant property, even at low concentrations were carefully analyzed and subjected for further fractionation and characterization. Based on the preliminary investigations the following drug yielding plants were selected for fractionation and characterization for active principle using spectral studies:

1. Chloroxylon swietenia DC. (Flindersiaceae)
2. Entada pursaetha DC. (Mimosaceae)
3. *Mallotus philippensis* (Lam.) Muell.-Arg. (Euphorbiaceae)

4. *Pulicaria wightiana* (DC.) Clarke (Asteraceae)

The investigations on the biological activities of essential oils of the above-mentioned crude drugs revealed the presence of thymol – as potential antimicrobial agent; myrtenol – soothing agent for eye; terpineole – antiseptic; nerol – anti-inflammatory agent; terpinene – immune stimulant, antibacterial and antiviral; δ - cadene – heart tonic; β – caryophyllene – antiviral; spathulenol – anti-inflammatory and antioxidant while sabenine possess antibacterial and antifungal activity (Anonymous, 2002). Carveol was shown to have antioxidant activity also (Aeschbach *et al.*, 1994). Interestingly some very little known compounds (Z – citrol, sesquiceniole, β – acoradiene, 5 – tert - butyl 1, 3 cyclo pentadiene, caryophylleneoxide, 2 ethyl, 3-6, dimethyl pyrazine) were found to possess effective therapeutic principles.
The salient observations from the present study are

➢ The comprehensive list of crude drugs (111 species belongs to 97 genera and 50 families) used for cancerous diseases present in the Rayalaseema region was provided.

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

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

➢ The preliminary phytochemical screening was conducted for 40 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.

➢ *Chloroxylon swietenia, Entada pursaetha, Hedychium coronarium, Mallotus philippensis, Moringa concansis, Pulicaria wightiana, Rhynchosia beddomei, Syzygium alternifolium, Tylophora fasciculate, Vitex leucoxylon and Vitex peduncularis* were recorded as potential antimicrobial crude drugs.

➢ The brine shrimp lethality activity of *Ageratum conyzoides, Aristolochia indica, Curculigo orchioides, Entada pursaetha, Hedychium coronarium, Mallotus philippensis, Moringa concansis, Pulicaria wightiana, Tylophora fasciculata, Vitex leucoxylon* and *Vitex peduncularis* were reported first time.

➢ The potato disc tumor assay of *Chloroxylon swietenia, Entada pursaetha, Hedychium coronarium, Mallotus philippensis, Pulicaria wightiana, Tylophora fasciculata, Vitex leucoxylon* and *Vitex peduncularis* was conducted first time.

Interestingly the majority of observations regarding cancerous diseases have substantiated the folk claims by antioxidant and bioassay evidences.