Chapter -5

5. SUMMARY AND CONCLUSION

A number of plant based traditional systems of medicines have been in use in India and also in many parts of the world for ages. In the past decade there has been renewed attention and interest witnessed in the use of traditional medicines globally. Many medicinal plants classified as 'Rasayana' in Ayurveda are believed to be useful in strengthening the immune system of an individual and some of these herbs are also used as adjuvants. The plants which fall under category of adjuvants are believed to act as tonic, adaptogenic, aphrodisiac, immunomodulator's, healing agents in injuries and also bioactivity enhancers. Many drugs under category of rasayanas of Ayurveda have been used for this purpose like Ashwagandh, Shatavari, Guduchi, Pepper, Amla, Neem, Chitrak etc.

In India Ayurvedic and other plant-derived drug products that are used either as active ingredients or as adjuvants hold paramount importance as alternative medicines but, their standardization (phytochemical and pharmacological) poses a great challenge to the practitioners as well as to the consumers. This problem multiplies many more folds especially when these plants are known by similar vernacular names, leading to controversy in their identification. A number of Indian medicinal plants used extensively in Ayurvedic or herbal medicines fall under controversial category. This is due to uncertain parameters for identity and lack of scientific information on activity. The present studies pertain to one such similar plant known as "*Brahmadandi*", of Indian system of medicine having two different plants as its sources.

'Brahmadandi', commonly available and sold in different markets of India consists of the roots of *Echinops echinatus* Roxb (Compositae) at one place, while in some place aerial parts of *Tricholepis glaberrima* DC (Compositae) are supplied. The drug is popular as an aphrodisiac and used in the treatment of male infertility. However convincing scientific data to ensure its identity, quality, safety and efficacy are still unavailable.

Keeping in view the extensive use of Brahmadandi and its controversial nature it was proposed to undertake, first, the systematic investigations on both the plants, in order to evolve certain parameters for their standardization and secondly, to investigate upon their biological activities so as to justify their traditional claims.

The studies performed are described in four chapters consisting of:

1. Introduction, current status of the traditional medicine, importance of standardization, selection of the plants and their profile, and the research envisaged.
2. Development of the standardization parameters for the selected plants.
3. Evaluation of the selected plants for the biological activity.
4. Identification and quantification of compounds from the bioactive fraction using HPTLC.

World health organization in a number of resolutions emphasized the need to ensure the quality of herbs and herbal formulations by using modern techniques and has also drafted the guidelines for the assessment of herbal medicines. These guidelines define basic criteria for the evaluation of quality, safety and efficacy of herbal medicines to assist national regulatory authorities, scientific organizations, and manufacturers to undertake an assessment of the documentation, of submissions and/or the dossiers in respect of such products. Therefore phytochemical standardization using sophisticated analytical techniques along with assessment of its activity is necessary to ensure quality and efficacy of herbal drugs and this can be done at two levels (1) standardization by chemical/biomarker compound analysis and standardization by fingerprint techniques/ chemoprofiling (2) standardization by assessment of the anticipated activity.

A comparative profile consisting of distinguishing parameters for both the plant materials was also prepared so as to provide means of correct identification of individual plant material. The parameters include preliminary phytochemical screening, studies on morphological, microscopical, physicochemical constants, fluorescence studies, finger print profiles, estimation of phenolic content, heavy metal content, etc. The preliminary phytochemical studies showed the presence of high non polar content in *E. echinatus* where as *T. glaberrima* showed a lower yield of petroleum ether extract indicating the low content of non polar constituents. Chemical tests showed that the non polar compounds in *E .echinatus* are terpenoids whereas chemical tests on *T. glaberrima* showed the presence alkaloids as the chief constituents.

Although the whole herbs can easily be differentiated by morphological and floral arrangements, some important morphological features are documented for both the plants. The plant *E. echinatus* was found to be much branched and rigid, having sessile leaves of 7.5-12.5 cm long, a round inflorescence and long roots (30-50 cm long). Whereas, *T. glaberrima* is an erect plant with small sessile leaves (2.5-6 cm long) having ovoid inflorescence and 20-30 cm long roots. The results of morphological determination pose unacceptability, because the plant materials are either available in mutilated form or sold without definite structure of the organ and hence microscopic evaluation remains indispensable and cost effective tool of conventional analytical pharmacognosy for the identification of medicinal herbs. The important microscopic features of the roots, stems and leaves have therefore been documented for both *E. echinatus* and *T. glaberrima* in the present studies.

Microscopic studies on the roots of *E. echinatus* showed the presence of an endodermis having casparian thickening, a bilayered medullary rays and parenchyma inclusions in the xylem cylinder whereas these features were not found in the roots of *T. glaberrima*. Periderm was clearly differentiated into phellogen and cortex in the roots of *T. glaberrima* but was not differentiated in the roots of *E. echinatus*.

Transverse section (T.S) of stems of *E. echinatus* was circular in outline whereas that of *T. glaberrima* was having ridges and furrows. Presence of leaf trace bundles and cluster crystals of calcium oxalate were the distinct features in case of T.S of *E. echinatus*, whereas presence of a clear chlorenchyma layer is the distinct feature of the T.S of *T. glaberrima*.

T.S of the leaves of *E. echinatus* showed the presence of 3-5 vascular bundles in the midrib while in case of *T. glaberrima* a single centrally located vascular bundle was seen.

Powder microscopy of *E. echinatus* roots showed presence of cork cells, vessel elements and tracheids whereas that of *T. glaberrima* showed presence of cortical parenchyma, vessel elements, parenchyma cells showing plasmodesmata and non lignified fibers in bundle.

Determination of physicochemical constants indicated a higher ash value in *T. glaberrima* (10% W/w) than in *E. echinatus* (6.29% w/w). A higher alcohol and water soluble extractive values in case of *T. glaberrima* (9.6 and 13.2% w/w respectively) than that of *E. echinatus* (6.8 and 10.8% w/w respectively) were found.

During the recent past, HPTLC finger print profile has emerged out as a powerful tool. The inclusion of the profile in modern pharmaceutical herbal monographs has become a standard practice for authentication of herbal drugs especially when the active principles are not known.

Preliminary phytochemical screening showed the presence of terpenoids, phytosterols and phenolic compounds in *E. echinatus* and alkaloids, terpenoids and phenolic compounds in *T. glaberrima*, all of these can be extracted using methanol as solvent. Therefore a total methanol extract was prepared from both the plant materials and was used for finger print studies. The characteristic TLC finger print profiles of the important chemical constituents of methanol extract of the roots of *E. echinatus* and aerial parts of *T. glaberrima* were generated using three different solvent systems of varying polarity. A complete TLC finger print profile of the resolved compounds comprising of the typical spectra, R_f value, UV absorption maxima and the percentage proportion of the individual components in the extract are recorded and documented. The reported data may be of great value as a reference standard for evaluation of both the plant materials especially the finger print pattern of terpenoids for the roots of *E. echinatus* and that of alkaloids for aerial parts of *T. glaberrima*.

Determination of inorganic elements, including the trace elements and heavy metals has gained outstanding importance in the life sciences. The recent reports on the potential deleterious effects of some Ayurvedic medicines, due to presence of unacceptable levels of metals and metalloids such as Lead, Arsenic, Mercury etc, and their possible chronic toxicological effects, have caused much concern not only among the herbal practitioners, but also among large population who still depend on the medicinal plants for their healthcare needs. Thus it has become necessary that all the medicinal plant materials should be assessed for the content of heavy metals and other inorganic elements. The plant materials under study viz., roots of *E. echinatus* and aerial parts of *T. glaberrima* were found to be devoid of arsenic and cadmium but were found to contain traces of lead (11.4 and 21.6 ppm respectively) which are within the limits of WHO specifications for heavy metal content.

DNA fingerprinting of herbal drugs, though still in its early years, seems to be a promising tool for the authentication of medicinal plant species and for ensuring better quality herbs and nutraceuticals. In the present studies an attempt has been made to isolate the genomic DNA of the both the selected plants *E. echinatus* and *T. glaberrima* and to record the fingerprint of the intact genomic DNA. However further studies using RAPD (random amplified polymorphic DNA), PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphisms), are necessary for differentiating the plants within the species or other morphologically similar oriental medicines.

Biological screening of medicinal plants is of vital importance, not only to provide a scientific basis for their continued usage but also to validate their traditional utilization. Additionally, these studies aid to correlate the activity with some components in the plant. Thus biological screening along with chemical profiling provides additional means of standardization of a plant drug.

The studies were aimed to screen methanol extract of roots of *E. echinatus* and aerial parts of *T. glaberrima* on reproductive system of male rats and thereby to justify their use in the traditional systems of medicine.

The data obtained reveal that oral administration methanol extract of the roots of *E. echinatus* exhibits inhibitory effects on the functions of male reproductive system and may act as an antifertility agent. Whereas, oral administration of the crude methanol extract of the aerial parts of *T. glaberrima* effectively facilitated several components of the copulatory behavior and thus promoting the sexual activity.

Treatment with the methanol extract of the roots of *E. echinatus* showed a decrease in the weight of the reproductive organs without affecting the overall body weight and regular sexual interest of the animals. Estimation of the circulatory levels of serum testosterone showed a significant decrease ($P < 0.001$) in its concentration in the animals treated with *E. echinatus* and subsequently a significant decrease ($P < 0.05$) in the sperm count.

From the results it was observed that the plant *E. echinatus* exhibits inhibitory effects on the male reproductive functions. WHO also encourages for studies on the search of new leads from plant origin which can act as antifertility agents.

Therefore the terpenoid rich fraction of petroleum ether extract (TRFPE) of *E. echinatus* was prepared and evaluated for its effects on the male reproductive parameters. The results of the study, after an acute treatment with two different doses of TRFPE at 30 and 60 mg/kg body weight, exhibited a significant decrease ($P < 0.01$) in serum testosterone levels and cauda epididymal sperm concentration. The results were further supported by histological examination of the testis which showed a significant reduction ($P < 0.01$) in the seminiferous tubular diameter and germinal epithelial cell thickness.

The results of the studies on *T. glaberrima* showed that the methanol extract of *T. glaberrima* enhanced the activity in sexually active rats. A significant increase in the weight of reproductive organs and overall body weight was observed. The serum testosterone levels and epididymal sperm count were though not statistically significant but an increase in the values was noticed. Histomorphology of the testis showed a better proliferation of the testicular tissues and thereby a significant increase in STD and GECT values ($P < 0.001$) was observed compared to the animals in control group.

Plants shows aphrodisiac properties by various mechanisms and from the data obtained in the present studies, *T. glaberrima* can fall under the category of aphrodisiacs that increase the sexual desire, improve the quality and stimulate the production of semen.

According to the traditional literature *E. echinatus* is used as abortifacient and to hasten the process of delivery in females, and is used externally on male genitals to improve the poor sexual vitality. But there exists considerable evidence that the plant is having potent anti-androgenic and anti-spermatogenic properties in male when administered orally. The present studies also showed that the plant exhibits inhibitory effects on the male reproductive functions when administered internally.

Whereas the *T. glaberrima* has been reported to be used as sex tonic and is considered as 'poor man's sex tonic' by many traditional healers. Results of the present study supports this phrase, as the methanol extract when administered internally facilitated the male reproductive functions effectively and thereby exhibiting the aphrodisiac properties.

Thus it may be substantiated that although the two plants, *E. echinatus* and *T. glaberrima*, are known by common trade name 'Brahmadandi' in the Indian market, exhibit opposite pharmacological activities when administered internally and hence this may not be considered as substitute of one for another as source.

The studies on the chemical constituents of *E. echinatus*, revealed the presence of rich terpenoid content in the roots and terpenoid rich fraction of petroleum ether extract (TRFPE) exhibited an effective and reversible inhibition of the functioning of male reproductive parameters. Therefore the separation of the possible active compounds from TRFPE was undertaken and two compounds (EEPE-1 and EEPE-2) were separated. The compound EEPE-1 was identified as lupeol based on its spectral studies and comparing the results with that reported earlier for the authentic sample of lupeol. The compound EEPE-2 could be expected to be a derivative of lupeol since most of its physical properties and spectral data were similar but due to very low yield of the compound further investigations could not be undertaken.

A quantitative HPTLC assay method for lupeol content in roots of *E. echinatus* and aerial parts of *T. glaberrima* was developed and validated. Lupeol content in TRFPE was determined using the proposed method and the presence of high lupeol content was observed (38.3% w/w). This can be used as chemical marker for the routine quality control of the commercial samples of Brahmadandi.

Thus, it can be concluded that the pharmacognostical studies carried out on both the plants, can serve as valuable tool and provide suitable standards for the identification of the plant materials. Phytochemical screening and the TLC profile of detected phytoconstituents may also serve as reference standards. Studies on the biological activity of *E. echinatus* and *T. glaberrima* provide a scientific basis to justify their usage in the traditional system of medicine independently, but also clarify that both cannot serve simultaneously as the source of "Brahmadandi".