6. SUMMARY AND CONCLUSION

Medicinal plants (MPs) played a significant role in various ancient traditional systems of medication such as Ayurvedic and Unanic medicines in India. Today, MPs still play an important role in developing countries in Asia, both in preventive and curative treatments, despite advances in western medicine. The high cost of modern medicines (mostly imported), their unavailability in remote areas and, most importantly, the serious side effects of certain, have resulted in a significant return to traditional medicine.

Since large portions of pharmaceutical drugs are derived from MPs, the demand for these raw materials is steadily rising. But the scientific report on the use of such plants in the field of medicine is scarcely available. However, *Gisekia pharnaceoides*, of *molluginaceae* family, a wild plant commonly found in India and Africa is known to be used in folklore medicine in the treatment of asthma, as purgative, diarrhoea, prevention of swelling, warts, and helmenthiasis. This is also used as a vegetable in the interior rural parts of India and also in Africa.

However, the pharmaceutical or nutritional value of the plant has not been reported so far. Medicinal value of the plants is generally determined by the presence of biologically active compounds. Hence in this study, the phytochemical analyses of the different extracts of *Gisekia pharnaceoides* and their application in various pharmacological activities have been investigated. Besides this, nutritional value of the whole plant was also determined. The
results obtained on the various biological activities of the plant of this investigation are discussed with a view of proposing *Gisekia pharnaceoides* as a neutraceutically important plant.

Pharmacognostic investigation of the fresh, whole plant of *Gisekia pharnaceoides* was carried out to determine its anatomical structure, micro and macroscopical characters. The cytochemical analysis of the anatomical sections using polychromatic stain, the toluidine blue, revealed the presence of cellulose, calcium oxalate, lignin mucilage and protein bodies in the plant. The drug powder of the plant was subjected to successive extraction using petroleum ether followed by chloroform and then methanol.

Qualitative phytochemical screening of the plant revealed the presence of carbohydrates and phenolic compounds including flavanoids, and tannins in all the three extracts of this study; whereas, the alkaloids, proteins and amino acids are detected only in methanolic extract (GPM), in addition to the other compounds stated above.

Further studies on the isolation and purification yielded dotriacontane and kaempferol in the extracts of petroleum ether and methanol respectively, as characterized by TLC, UV, IR, $^1$H-NMR, $^{13}$C-NMR and LC-MS. However the chloroform extract did not yield any particular compound in an appreciable quantity.

As part of biological value, determination of the shade dried plant powder was assessed for its nutritive value. The various nutritive parameters
determined were, the crude protein, fat, crude fibre, carbohydrates and ash. Apart from this, the herbal powder was also checked for its vitamins and mineral content. The vitamins such as vitamin A, B1, B2 and C were found to be present. The minerals including calcium, potassium, sodium, magnesium, zinc, iron etc were also detected in the sample.

Prior to the evaluation of various pharmacological activities, acute toxicity study was carried out by OECD-423 guidelines (oral acute toxic class method). Wistar rats of either sex were selected by random sampling technique. Petroleum ether, chloroform and methanolic extracts of *Gisekia pharnaceoides* were administered orally at the dose of 2000 mg/kg body weight, in 1% suspension. The test animals did not show any symptoms of toxicity, nor was observed any abnormal behaviour; and no mortality was seen. Hence in the subsequent pharmacological studies, a dosage of 250 or 500 mg of crude extract per kg body weight was used.

The anti inflammatory activity of the different crude extracts of *Gisekia pharnaceoides* viz. petroleum ether (GPP), chloroform (GPC) and methanol (GPM) were determined by following carageenan induced paw oedema model. Based on the observation of the preliminary studies of the three extracts the GPM showed higher percentage of anti inflammatory activity (72%) in terms of paw oedema inhibition, followed by GPP (66%) and GPC (60%). Diclofenac sodium at a dose of 5 mg/kg p.o was used as reference drug in this experiment.
The analgesic activity of these extracts was also determined by following tail immersion method. The analgesic activity was studied by following two-dose system (250 and 500 mg/kg body weight per oral) as was followed in anti inflammatory experiment. The GPP produced 4.55 and 4.65 sec basal reaction time for lower and higher doses respectively. Whereas, GPC produced 4.82 and 5.327 sec and the GPM produced 5.08 and 5.83 sec respectively .The reference drug morphine (5 kg/mg) produced 6.83 sec basal reaction time. All the above data presented are maximum inhibition values at 90 min after the drug treatment in all the groups.

Since the plant extract possesses anti inflammatory and analgesic property, it was subsequently tested for the free radical scavenging property as well. Accordingly, the crude extracts were used against the free radicals such as hydroxyl radical, nitric oxide radical, super oxide radical, ABTS$^+$ radical and the DPPH that were produce in vitro, and observed for the combating property of crude extracts to minimise the reactivity of the active radicals. Vitamins E was used as the reference antioxidant, for comparison study. Of the three extracts studied, the GPM showed more antioxidant property than the other two extracts. The free radical scavenging ability is represented as follows: GPM > GPC > GPP.

Detoxification of reactive oxygen species by the free radical scavenging system is one of the important events necessary to promote wound healing. Since *Gisekia pharmaceoides* has been demonstrated to possess this property, it was used in the wound healing study to evaluate its efficacy. The
plant extracts namely GPP, GPC or GPM were used individually for topical application on cutaneous wounds and were compared with that of the soframycin (reference) treated wounds. The percent wound contraction was calculated periodically on day 5, 10 and 15. The period of epithelialization was found to be 16.15, 17.86, 14.12 days respectively for GPP, GPC and GPM. The healing pattern was also examined histologically. Compared to other treatment groups, the GPM treated group showed a better-oriented and uniformly accumulated collagen fibers with prominent fibrillar bundles in their re-epithelialised tissue.

The plant extract was also studied for its efficiency to strengthen the defence mechanism in-vitro, in conditions like cutaneous exision wound, where the process of healing begins with inflammation process followed by other cellular events. To know the antioxidant status, the plant extract-treated epithelialised tissue after healing, were analysed estimated for their antioxidant content. The antioxidants quantified were super oxide dismutase, catalase, glutathione peroxidase and vitamin C. The extent of lipid peroxidation, which is an index of ROS level and is inversely proportional to the antioxidant levels, was also measured and presented in this thesis.

Since the plant, was found to contain variety of compounds, as understood from the preliminary phytochemical analyses, it was also used for the study of anthelmintic activity. Earthworm, a nematode was used to assay the in-vitro anthelmintic activity of the extracts of *Gisekia pharmaceoides*. The petroleum ether extract of the plant, being rich in tannin content (as
Tannins are highly soluble or extractable in low polar solvents like petroleum ether) showed increased anthelmintic activity compared to other solvent extracts.

In conclusion, the plant selected for this study was confirmed as *Gisekia pharnaceoides* from its pharmacognostic data. Preliminary phytochemical screening showed that the plant contains flavanoids, tannins, carbohydrates and glycosides as major compounds. Studies on the characterization of isolated compounds confirmed the presence of kaempferol (a flavanoid) as predominant compound. This flavanoid seem to be responsible for various biological properties such as anti inflammatory, analgesic, free radical scavenging (*in vitro*), and promoter of wound healing and *in vivo* antioxidant status. Tannin rich fraction was seen as a potential anthelmintic agent. Apart from various pharmacological activities, the plant also posses a high nutritional value as it contains appreciable level of protein, carbohydrate, fibre, vitamins and minerals as well. Since it has no toxicity, the plant can also be used as a source of medicine and food and hence, a good nutraceutical herb for mankind. Further research on this plant can be carried out in the area of cancer therapy, as the plant extract exhibits a high degree of free radical scavenging property.