

CHAPTER - 12

SUMMARY AND CONCLUSION

The major aim of the investigation was to explore two popular ethnomedicines used by traditional healer of Sikkim in their health care system since time immemorial. The medicinal plants used by the ethnic community of Sikkim are neither systemically documented nor properly tested by scientific approaches. The systemic scientific studies of these ethnomedicines can lead to promising phytochemicals (drugs) for many health problems. In view of the above, it was thought to be much worthy to take up the present research project Studies on pharmacological screening of the bioactive molecules from some selected plants so as to explore the therapeutic values and development of some novel drugs from those indigenous plants

Chapter 1

Herbal medicine or phytotherapy is the science of using herbal remedies to treat the sick. It therefore covers everything from medicinal plants with powerful actions. A great variety of plants are used for medicinal treatments. Either the dried plant, or a specific part of it (root, leaves, fruit, flowers, seeds), is formulated into suitable preparations—compressed as tablets or made into pills, used to make infusions (teas), extracts, tinctures, etc., or mixed with excipients to make lotions, ointments, creams, etc. The active principle is extracted and purified from plant material for as long as that process remains economically viable compared with chemical synthesis. Herbal medicine has been used for centuries by people all over the world to treat disease and promote health. Both the west and the east have spent considerable time, research and energy developing the theories and applications within the field of herbal medicine. Herbs are generally easy to administer and cost-effective and when properly prescribed and used, have the advantage of being relatively free of side effects when compared to western pharmaceutical medicines. Herbal medicine is currently enjoying a revival in popularity in the west and in fact it is the primary form of medicine in many parts of the world. With the great reliance on this type of medicine, it becomes pertinent to search for potent, effective and relatively safe plant medicines as well as scientific validation of the success claims about plants already in use by traditional medicine practitioners in order to enhance their safety and efficacy. These are some of the problems making this alternative healthcare system less acceptable, especially by orthodox medicine practitioners. In Sikkim traditional healers and remedies made from plants play an important role in the health of people.

Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations are based on plants

Chapter 2

Various reports on phytochemical and pharmacological analysis of *K. rotunda* and *E. cannabinum* along with their allied species have been presented. The current updated status of natural origin used as antioxidant, wound healing, antiulcer, analgesic and antipyretics, anti-inflammatory and antimicrobials have been elaborated in the form of comprehensive review of literature. Though some reports on phytochemical and pharmacological analysis of *K. rotunda* and *E. cannabinum* exist in the literature, the present study revealed the presence of additional constituents with significant pharmacological activities.

Chapter 3

The modern methodologies for extraction and isolation of bioactive compounds from *K. rotunda* and *E. cannabinum* along with phytochemical group tests have been presented. The isolated bioactive compounds were analyzed by using physical spectroscopic methods like UV, IR, ¹HNMR, Mass and melting point determination etc. The evidence presented conclusively proved that the isolated bioactive compounds isolated were 2-hydroxy 4,4',6- trimethoxy chalcone from *K. rotunda* and 3',4',5,7-tetrahydroxy flavonol or quercetin from *E. cannabinum*.

Chapter 4

Toxicological investigation of methanol extract of *K. rotunda* and *E. cannabinum* were described. The MLD of methanol extract of *K. rotunda* rhizome was found to be 5 g/kg body weight and the MLD of the methanol extract of *E. cannabinum* leaves was to be 4.5 g/kg body weight in oral route whereas the MLD of isolated compound I and II were found to be 213.7mg/kg body weight and 196.3mg/kg body weight in oral route

respectively. The MLD values of both the extracts were found to be much higher indicating the wide safety range of action.

Chapter 5

Antioxidant activities of methanol extract of *K. rotunda* and *E. cannabinum* presented. The TBARS and 4-HNE formation inhibitory effect of the methanolic extract of the plants of *K. rotunda* and *E. cannabinum* were significantly higher than that of control. The lipid peroxidation inhibition capacity of *K. rotunda* was studied by dose dependent manner and it was concluded that the antioxidant activity has inverse relationship with dose i.e. high at low dose and vice versa. The extract at 100µg/ml and 200µg/ml has significant lipid peroxidation inhibition activity in respect to 500 and 1000µg/ml. The lipid peroxidation inhibition activity of extract of *E. cannabinum* has observed in higher doses. The dose of 100µg/ml and 200µg/ml has insignificant activity whereas 500µg/ml and 1000µg/ml has moderate and significant lipid peroxidation activity respectively

Chapter 6

Wound healing activity of methanol extract of *K. rotunda* and *E. cannabinum* as well as compound I and II has been presented. Significant promotion of wound-healing activity was observed in both methanol extracts and isolated compound in all the three wound models such as excision, incision and dead space wound.

In excision wound model, the percentage closure of wound area was significantly increased by the methanol extracts of rhizome of *K. rotunda* and leaves of *E. cannabinum* and their isolated compounds in the respective animal groups. From the result it was revealed that, *K. rotunda* extract treated animals showed significant reduction in the wound area and faster rate of epithialisation as compared to *E. cannabinum* extract. The isolated compound treated animals showed faster epithelisation of wounds than the animals treated with methanolic extracts of both the plants.

In incision wound model the breaking strength of the 10th day old restored incision wound was significantly increased in animals treated with isolated compounds and methanolic extracts of both the plants, when compared to control. The maximum breaking strength was observed in animals treated with isolated compound II in the dose of 20mg/kg bw. Significant breaking strengths were also observed in the animals treated

with isolated compound I followed by methanol extracts of rhizome of *K. rotunda* and leaves of *E. cannabinum*.

In dead space wound model, the extract of both the plants and their isolated compounds were assessed by the increase in the weight of granuloma, increase in breaking strength and hydroxylproline content of the granuloma tissue. This effect may be due to the enhancement of collagen maturation by increased cross linking of collagen fibers. The increased weight of the granuloma also revealed the presence of higher hydroxyl proline content. Among the methanolic extract and isolated compound treated groups the hydroxyl proline content was found to be more in isolated compound treated animals followed by methanolic extracts of *K. rotunda* and *E. cannabinum*.

In this study extracts as well as isolated compound significantly increased the granuloma tissue breaking strength and hydroxyproline content as compared to control. The result of the investigation provides pharmacological evidence on the folkloric use of both the plants for wound.

Chapter 7

Antiulcer activity of methanol extract of *K. rotunda* and *E. cannabinum* as well as compound I and II has been presented. Here the effect of methanolic extracts of *K. rotunda* and *E. cannabinum* and their isolated compound on pylorus ligated rat and aspirin induced ulcer model has investigated. The result of the present studies indicates that the isolated product of both the plant significantly reduces the total volume of gastric juice, free and total acidity of gastric secretion. The control animals had ulcers and hemorrhagic streaks whereas in animals administered with the methanolic extracts of *K. rotunda* and *E. cannabinum* and their isolated compound there was significant reduction in ulcer index. Thus in the present study concludes that both the above mention plant extracts and their isolated compounds possess significant antiulcer activity in different experimental models of ulcer in rats. The magnitude of activity obtained at the two dose levels used indicates high potency of anti-ulcer effect and together with the array of compounds already isolated from the plant provide impetus to continue the search for novel anti-ulcer constituents from this plant.

Chapter 8

This study was undertaken to evaluate the anti-inflammatory potential of the methanolic extract of *K. rotunda* and *E. cannabinum* on carrageenan-induced rat paw edema. The results of the present study suggest that the methanolic extract of *K. rotunda* and *E. cannabinum* at the dose levels of 200 and 400 mg/kg significantly suppressed carrageenan-induced paw edema in rats. On preliminary phytochemical screening the methanolic extract of *K. rotunda* and *E. cannabinum* was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception. Hence, the presence of flavonoids may be contributory to the antiinflammatory and analgesic activities of *K. rotunda* and *E. cannabinum*. The study indicates the potential of these herbal drugs as anti-inflammatory drugs. Such drugs can be explored in various inflammatory diseases. It can be concluded that methanolic extract of *K. rotunda* and *E. cannabinum* possess significant anti-inflammatory activity in rats. The extracts significantly reduce the increase in hind paw volume induced by carrageenan, compared to the vehicle treated control. The findings of the present work justified the use of this plant in the treatment of rheumatism and other inflammatory conditions for its anti-inflammatory action.

Chapter 9

This chapter carried out a preliminary screening for analgesic and antipyretic activities of the rhizome and leave extracts of *K. rotunda* and *E. cannabinum* plants in order to establish some of its pharmacological properties. The responses of mice to tail immersion and rats to yeast induced pyrexia method.

The present result showed that the methanol extract of *K. rotunda* and *E. cannabinum* possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats and its effect is comparable to that of paracetamol. The present study has been undertaken to investigate the analgesic potential of methanol extract of *K. rotunda* and *E. cannabinum* by tail immersion method in comparison with pentazocine.

Of the two doses, the dose of 400mg/kg is found to be more potent and efficacious towards the analgesic and antipyretic, and the activity is in dose dependent manner. These data indicate the analgesic and antipyretic potential of *K. rotunda* and *E. cannabinum*. Generally, plants showing the antipyretic effect also possess analgesic

activity. The present analgesic and antipyretic activity of *K. rotunda* and *E. cannabinum* may be attributed to the presence of alkaloids, flavonoids and tannins and triterpenoid in their extract.

Chapter 10

Evaluation has done on the potential of plant extracts and phytochemicals on standard microorganism strains as well as multi-drug resistant bacteria, which were isolated from hospitals. Moreover, the study investigated the synergistic effects of extracts with antimicrobial activity in association with antibiotics against drugs resistant bacteria. Therefore, the results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

All the experiments were performed both by using *in vitro* and *in vivo* model to establish that the plants of the Himalayan region of Sikkim investigated provide valuable proof of use of these plants by the people of this region as aids in their health care system in form of indigenous ethnomedicines since time immemorial. In conclusion two medicinally important pytoconstituents 2-hydroxy 4, 4', 6-trimethoxy chalcone (compound I) and 3', 4', 5, 7- tetrahydroxy flavonol (compound II) have been isolated from the rhizomes of *Kaempferia rotunda* and leaves of *Eupatorium cannabinum* respectively. The results of the studies performed in this thesis may contribute to the formulation of potential herbal medicine from the plants investigated.