Chapter - VI

Summary
6. Summary

Natural products have been the source of most of the active ingredients of medicines. This is widely accepted to be true when applied to drug discovery in ‘olden times’ before the advent of high-throughput screening and the post-genomic era: more than 80% of drug substances were natural products or inspired by a natural compound (Sneader, 1996). It is, however, arguably still true: comparisons of the information presented on sources of new drugs from 1981 to 2007 (Newman, 2007, Butler, 2008) indicate that almost half of the drugs approved since 1994 are based on natural products. The Industrial Revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture. Obviously, this approach was against the new modus vivendi of the industrialized western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value (Rates, 2001).

- As literature survey reveals that the presence of biologically active principles in the natural products which causes tremendous molecular diversity in the application of pharmaceutical area. *Erythrina* genus plants have been widely reported to have several medicinal properties in traditional form of medicine. The beneficial properties are anti-dysentry; cures Kapha and vata, stomachic, anthelmintic, improve appetite, cure urinary discharges, inflammation, febrifuge, ear ache, tooth ache, aphrodisiac and recommends for snake bite in sushruth samveda. The leaves are applied externally to disperse venereal buboes, and to relieve pain of the joints. The decoction of bark scrapings with lemon juice is employed to treat infertility. Traditionally different parts of the plants also employed as a nervine sedative,
collyrium in ophthalmia, anti-asthamatic, and antiepileptic, antiseptic and as an astringent. The bark is used in fever, liver ailments and rheumatism. (Telikepalli et al.). From above literature studies indicates the medicinal value of Erythrina genus plants. Hence Erythrina mysorensis G., have been selected for systematic study. In the present study the phytochemical investigation and pharmacological screening of Erythrina mysorensis G., have been carried out.

- Pharmacological experiments were designed to screen for antioxidant, anti-inflammatory, wound healing, antimicrobial, anticancer, antiepileptic, anti anxiety and anthelmintic activities, for stem bark extracts of Erythrina mysorensis G., and for isolated constituents of chloroform and ethanol extracts.

- The stem bark of Erythrina mysorensis G., were collected and subjected for successive extraction using different solvents (petroleum ether, chloroform, ethanol and water) and subsequently all extracts were subjected to the qualitative phytochemical analysis which indicated the presence of flavonoids, alkaloids, tannins, glycosides, carbohydrates and triterpenoids. Further, the ethanol extract was found to be stronger in terms of the presence of polyphenolic compounds.

- All the extracts were subjected to *invitro* antioxidant studies by using different methods, in which ethanol extract exhibited more potent antioxidant activity with lower EC$_{50}$ values in DPPH, Superoxide anion inhibition and lipid peroxide inhibition assay methods. However, chloroform extract showed a moderate effect and pet. ether, aqueous extracts comparatively exhibited less potent activity.

- The petroleum ether, chloroform and ethanol extracts were selected and subjected for isolation by employing column chromatography and structures were elucidated by IR, $^1$H NMR, and Mass spectral studies with respect to their *invitro* antioxidant studies. The characterized compounds are found to be palmitic acid, stearic acid, stigmasterol, $\beta$-sitosterol, lupeol, $\beta$-hydroxy chalcone and quercetin.
All the extracts and isolated active principles were subjected to acute toxicity study to evaluate LD_{50} value.

The anti-inflammatory activity study, in acute inflammation model, the carrageenan induced rat paw edema was significantly reduced in the animals pretreated with petroleum ether and ethanol extracts of stem bark of *Erythrina mysorensis* which was in a dose and time-dependent manner. The percentage inhibition of paw edema in petroleum ether and ethanol extract treated animals was also found to be significant when compared to control group of animals. Whereas, the rats treated with chloroform and aqueous extracts exhibited moderate anti-inflammatory activity in the percentage of inhibition of acute paw edema induced by carrageenan injection. Similarly isolated constituents of *Erythrina mysorensis* lupeol, beta hydroxyl chalcones and quercetin were also exhibited highest percentage of inhibition activity comparable with standard diclofenac sodium for over a period of 5 hrs. The significant activities of the extracts may be related to their inhibitory effect on the enzyme cyclooxygenase leading to the inhibition of mediators of inflammation such as histamine, kinin, serotonin and prostaglandin. Further, it may also be due to their inhibitory effect on lipoxygenase pathway.

The wound healing activity was assessed for the extracts and its isolated compounds in rats using three models viz., excision, incision and dead space wound. As a reference standard drug, the commercial Nitrofurazone ointment was used. Among the four crude extracts treated animals the petroleum ether and ethanolic extracts treated animals were exhibited significant epithelialization of the excision wound. In case of chloroform and aqueous extracts treated animals shown moderate wound contraction activity. In the animals treated with the isolated constituent lupeol also exhibited significant wound healing activity. The wound healing activity for other isolated constituents β-hydroxy chalcone and quercetin were not significant.

In the present study of incision wound model, a significant increase in tensile strength were observed in test groups treated with petroleum ether and ethanolic extracts as compared with control group. Treatment with other two extracts exhibited less
significant increase in the tissue breaking strength comparatively less to petroleum ether and ethanol extracts. The isolated constituent lupeol was found to be more effective in increasing the breaking strength as similar to that of standard drug nitrofurazone and other constituents were comparatively less effective. This increase in tensile strength may be either because of increase in the collagen content or due to alteration in maturation process by affecting the cross linking of collagen or improving the quality of collagen fibrils.

The effects of oral administration of stem bark extracts and isolated constituents on the dead space wound models were also assessed by the increase in the weight of the granulation tissue and its hydroxyproline content. Among these treated animals the response was shown to be best in petroleum ether and ethanolic extracts of *Erythrina mysorensis* stem bark, and isolated constituent lupeol by significant increase in dry granulation tissue weight indicating increased collagen turnover. Collagen which is the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Kumar *et al*., 2006). Other extracts and isolated constituents did not exhibit potent activity. The increase in breaking strength and tissue weight of granulation tissue indicates the enhanced collagen maturation (increased cross-linking of collagen fibers) higher protein content respectively. The hydroxyproline content in wounds treated with petroleum ether and ethanolic extracts of *Erythrina mysorensis* bark, and isolated constituent lupeol was found to be higher than that in the wounded control animals.

In histopathological studies the sections of granulation tissue of the untreated animals showed monocytes and fibroblasts. On the contrary, incomplete healing was evidenced with lesser epithelialization, fibrosis, and collagen formation. The granulation tissues section of the animals treated with petroleum ether and ethanolic extracts of *Erythrina mysorensis* bark, and lupeol showed the sign of tissue repair with increased collagen formation and less macrophage. The results of this experiment revealed that the weight of the granulation tissue and its hydroxyproline
content was high in the animals treated with petroleum ether and ethanolic extracts of *Erythrina mysorensis* bark, and lupeol. The exhibition of wound healing activities in all the three models by the petroleum ether and ethanolic extracts, isolated compound lupeol can be clearly attributed to their antioxidant abilities as these two parameters are interrelated.

The antibacterial activity test was performed by cup plate (diffusion technique) method. The chloroform and ethanol extracts exerted potent antimicrobial activity against all the test bacteria viz., *Bacillus subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* and fungi (*A. niger*, and *C. albicans*). Though the chloroform extract demonstrated higher activity than the ethanol extract, their activities were still higher than those of the two other extracts. The study also showed that the activity of the extracts is concentration dependent. Several factors ranging from concentration of antimicrobial agent, initial population density of the organisms, their growth rate and the rate of diffusion into the medium affects the activity of antimicrobials. The inhibitory activity exhibited by the secondary metabolites tends to agree with various other previous reports (Leven *et al.*, 1979; Scherbonvaski, 1971; Adebayo *et al.*, 1983; Igboke *et al.*, 2006) both of which linked the antibacterial properties of plants to the presence of secondary metabolites. The flavanoids were found to have antimicrobial activity (Gokhale, 2000).

All four extracts of *Erythrina mysorensis* bark studied for antifungal activity, the aqueous and ethanolic extracts found to possess antifungal activity for all the three doses studied. But petroleum ether and chloroform extracts shown less antifungal activity. Ethanolic extract seems to be more effective in terms of antifungal activity compared to aqueous extract. The zone of inhibition for *Candida albicans* more sensitive than *Aspergillus niger*. However, the zone of inhibition was less compared to reference standard gentamycin.

For antitumour activity of *Erythrina mysorensis* extracts and its isolated constituents on Ehrlich Ascitic Carcinoma (EAC) mouse model has been employed The result of antitumour activity reveals that, the treatment with ethanol, chloroform extracts
Summarizing, (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (isolated constituents) were inhibits the increase of the body weight and increases the life span of tumour bearing mice. A significant increase in total WBC count and decrease in haemoglobin content was observed in tumour bearing mice, when compared to normal mice. Treatment with ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (isolated constituents) caused a significant reduction in total WBC and increase in haemoglobin content, when compared to tumour control. These effects of extracts and isolated constituents are compared with standard 5-Fluorouracil drug effects.

- Tumour control animals inoculated with EAC showed a significant increase in the level of ALT, AST and ALP enzyme in serum, when compared to normal animals. The treatment with ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (isolated constituents) and 5-Fluorouracil caused a reversal of these changes towards the normal.

- In the results obtained antitumour study the packed cell volume and the number of viable EAC tumour cells in peritoneal cavity were significantly reduced in animals treated with ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (isolated constituents) when compared to the tumour control animals. Preliminary phytochemical screening indicated the presence of triterpenoids, flavonoids and glycosides in Erythrina mysorensis. These compounds are known to possess potent antitumor properties (Kintzios, 2006, Viswanatha, 2010, Kannan, 2010, Kumar, 2010, Melinda, 2010). In addition, flavonoids could also induce mechanisms that may kill cancer cells and inhibit tumor invasion (De Sousa, 2007, Lotito, 2006). The antitumour property of ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (isolated constituents) may be due to these compounds. These results indicate either direct cytotoxic effect on tumour cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition.
The histology of the liver sections of normal control mice with vehicle showed
normal liver structure with normal chords of hepatocytes. The liver sections of EAC
tumour bearing mice control mice showed altered architecture of liver tissue and the
individual hepatocytes displayed severe nuclear atypia and individual cell necrosis.
The standard 5-Fluorouracil revealed the normal architecture of the liver tissue. The
liver sections of EAC tumour bearing mice treated with ethanol and chloroform
extracts shown liver tissue with maintained architecture with less necrosis and few
mitotically active hepatocytes. However, treatment with lupeol, β-hydroxy chalcone
and quercetin exhibited significant liver protection, which is evident by the presence
of liver tissue with normal architecture and normal hepatocytes. All these results
suggest potent antitumour nature of ethanol, chloroform extracts (400mg/kg.b.w.) and
with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) and support the
antioxidant property observed earlier.

Anticonvulsant activity was performed by using following different methods.
Maximal electro shocks (MES) induced convulsions method; Pentylenetetrazole
(PTZ) induced seizures and locomotor activity. The maximal electro shocks (MES) is
one of the standard procedure employed to evaluate the testing materials ability to
protect against hindlimb extension (HLE). The chloroform extract and lupeol isolated
constituent of Erythrina mysorensis showed anti-epileptic activity in the MES, it may
act through Protection against HLE in the MES which predicts anti-epileptic drugs
that prevent the spread of the epileptic seizure from an epileptic focus during seizure
activity. Protection against HLE also indicates the ability of the testing material to
inhibit or prevent seizure discharge within the brain stem substrate.

Pentylenetetrazole (PTZ) induced seizures is a most frequently used acute
experimental model in the preliminary screening to test potential anticonvulsant drugs
and it is produces petitmal type epilepsy. Chloroform extract of Erythrina mysorensis
bark and isolated constituent lupeol (10mg/kg) also exhibited significant
anticonvulsant activity, by producing delayed onset of clonic-tonic actions and
protection from PTZ induced mortality and may be interfering with GABAergic
Summary

mechanism to exert its anticonvulsant effect. Chloroform extract might possibly be producing anti-epileptic action by increasing the level of GABA, an inhibitory neurotransmitter in the central nervous system. This is in accord with the pharmacological effects of benzodiazepine and highlights the relevance of the putative anti-epileptic effects of chloroform extract and lupeol isolated constituent. Pet ether, ethanol, aqueous extracts of *E.mysorensis* at different doses and also other isolated constituents did not possess any significant effect as compare to standard drug.

- Locomotor activity is considered as an index of alertness, and a decrease in locomotor activity indicates a sedative effect. In the present study, chloroform extract of *Erythrina mysorensis* bark and isolated constituent lupeol (10mg/kg) were found to decrease the locomotion, supporting the earlier evidence.

- Finally, the results of this study show that the chloroform extract of *Erythrina mysorensis* contain compound(s) with anti-epileptic and anti-psychotic properties. These neuro-pharmacological properties are possibly mediated via facilitation of GABA transmission as well as blockade of D2 receptors.

- Antianxiety activity was evaluated by using following different methods. Elevated plus maze test (EPMT), Open field test (OFT) and Rotarod test (RRT). The fear of height induces anxiety in the animals when placed on the EPM apparatus. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. The reduction in the entries and time spent in open arms indicate high level of fear or anxiety. Chloroform and ethanol extract of *E.mysorensis*, at the doses 200 and 400 mg/kg and isolated constituents lupeol and quercetin (10 mg/kg) significantly increased the percentage of time spent and number of entries in open arms as compared to vehicle treated group. This type of effect is observed with the drugs that act on GABA/benzodiazepine receptor complex and that stimulate glucocorticoid production and release in the
adrenal cortex, as well as with those which antagonize 5-HT1B receptor and which agonize 5-HT1A receptor.

- In open field test animals are subjected to a single five minute exposure to an unknown environment, this confrontation with the situation induces fear or anxiety like state in animals. Anxiolytic treatments reduce such fearful behavior of animals in open field. Statistical analysis of the data obtained from this experiments supported anxiolytic like activity of Chloroform and ethanol extract of *E. mysorensis* at both the doses (200 and 400 mg/kg) and 400 mg/kg and isolated constituents lupeol and quercetin (10 mg/kg) as its effect shows significantly increased in number of rearing, number of assisted rearing and number of square crossed as compared to vehicle treated group which indicate the stimulant effect on the central nervous system.

- To predict the motor coordination ability of the animals, Rotorod test was performed which helps in the understanding of skeletal muscle relaxation. The skeletal muscle relaxation together with taming or calming effect, also reduce anxiety and tension, thus in this study chloroform and ethanol extract of *E. mysorensis* at both the doses (200 and 400 mg/kg) and 400 mg/kg, isolated constituents lupeol and quercetin (10 mg/kg) and diazepam significantly reduced the fall of time of the mice from the rotating rod as compare to vehicle treated group, indicates the skeletal muscle relaxant activity.

- The extracts of *Erythrina mysorensis* were screened for anthelmintic activity by using *Ascaris galli* (Fowl roundworm) and *Pheretima posthuma* (Indian Earthworm). The ethanolic and aqueous extracts of the stem bark of *Erythrina mysorensis* exhibited potent anthelmintic activity when compared to the control. Each crude extract shown anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D). The predominant effect of Albendazole citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole Citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarisation and reduced excitability that leads to muscle relaxation and flaccid
Summary

paralysis (Martin, 1985). Phytochemical analysis of the crude extracts revealed the presence of tannins as one of the chemical constituents. Tannins were shown to produce anthelmintic activities (Niezen, et al., 1995). Chemically tannins are polyphenolic compounds. (Bate smith EC,1962) Some synthetic phenolic anthelmintics (eg) niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). It is possible that tannins contained in the extracts of *Erythrina mysorensis* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal (Athanasiadou al., 2001) or glycoprotein on the cuticle of the parasite (Thompson,1995) and cause death.

- In conclusion the present studies provide scientific evidence for the presence of several beneficial medicinal properties in the plant material *Erythrina mysorensis* belonging to the family Fabaceae. Among all the extracts, the ethanol extract has shown to possess *in vitro* antioxidant property and found to be a more potent. The phytochemical analysis has resulted in the characterization of terpenoid compound lupeol, a chalcone β-hydroxy chalcone derivative, a flavanone quercetin, two sterols stigmasterol and β-sitosterol and two acids palmatic acid, stearic acid. The petroleum ether and ethanol extracts, isolated constituent lupeol were found to possess *in vivo* wound healing activity in three wound models which are supported by biochemical and histological observations. Potent Anti-inflammatory activity was exhibited by petroleum ether, ethanol extract and lupeol isolated constituent. Chloroform extract and lupeol isolated constituent has prominent anticonvulsant activity. Chloroform and Ethanol extract of *E.mysorensis*, at the doses 200 and 400 mg/kg and isolated constituents lupeol and quercetin (10 mg/kg) has produced potent anti anxiety activity. Ethanol, chloroform extracts (400mg/kg,b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) proves the antitumour activity in EAC induced tumours in mice.

- The potent biological properties of the ethanol, chloroform and petroleum ether extracts is presumed due to the presence of β-hydroxy chalcone, quercetin, lupeol, β-
Summary

sitosterol, stigmasterol, respectively. However there could be many more compounds in the above extracts which need a thorough phytochemical characterization. When isolated compounds have also exhibited the potent biological properties with various parameters, it is opined that the plant *Erythrina mysorensis* do possess the medicinal properties. Thus studies carried out provide a supportive scientific evidence for the medicinal use of *Erythrina mysorensis* against the various diseases, thereby justifying its use in the Indian traditional system of medicine.

*E. mysorensis* has been ethnomedicinally used as a therapeutic agent for a variety of diseases, it has been illustrated. Moreover, numerous research works have proven its uses beyond the ethnomedicinal ones in experimental animals. Triterpenoids and flavonoids which were isolated from this plant may be responsible for its pharmacological activities. The road ahead is to establish specific bioactive molecules, which might be responsible for these actions. Therefore the cultivation, collection, and further pharmacological exploration of *E. mysorensis* are essential.