Chapter - V

Discussion
5. DISCUSSION

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). 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. However, even if we only consider the impact of the discovery of the penicillin, obtained from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors.

Examples of important drugs obtained from plants are digoxin from Digitalis spp., quinine and quinidine from Cinchona spp., vincristine and vinblastine from Catharanthus roseus, atropine from Atropa belladonna and morphine and codeine from Papaver somniferum. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong Shu, 1998). The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and
In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Williamson et al., 1996). In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank et al., 1982; Vulto and Smet, 1988; Mentz and Schenkel, 1989). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient, abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world’s population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that “natural” products are harmless. However, the use of these substances is not always authorized by legal authorities dealing with efficacy and safety procedures, and many published papers point to the lack of quality in the production, trade and prescription of phytomedicinal products. It is estimated that, in 1997, the world market for over-the-counter phytomedicinal products was US$ 10 billion, with an annual growth of 6.5% (Soldati, 1997).

The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries (Vulto and Smet, 1998; OMS, 1991). Eastern countries, such as China and India, have a well-established herbal medicine industry and Latin American countries have been investing in research programs in medicinal plants and the standardization and regulation of phytomedicinal products, following the example of European countries, such as France and Germany. In Germany, 50% of phytomedicinal products are sold on medical prescription, the cost being refunded by health insurance (Gruenwald, 1997). In North America, where phytomedicinal products are sold as “health foods” (Brevoort, 1997; Calixto, 2000), consumers and professionals have struggled to change this by gathering information about the efficacy and safety of these products, and new guidelines for their registration are now part of FDA policy (Israelsen, 1997). In 1997, the North American market for products of plant origin reached US$ 2 billion (Brevoort, 1997). Thus, in the modern social context and economic view of health services, the needs of the pharmaceutical market and the
recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs (Elisabethsky, 1987; Calixto, 1996) have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programmes worldwide. The NCI (National Cancer Institute, USA) has tested more than 50,000 plant samples for anti-HIV activity and 33,000 samples for anti-tumour activity. In 1993, the International Program of Co-operation for Biodiversity (IPCB) was launched in order to promote natural products in Latin America and Africa, linking universities, industries and governments in a multidisciplinary programme for the sustained development and preservation of the environment (Rouhi, 1997).

Large pharmaceutical companies, such as Merck, CIBA, Glaxo, Boehringer and Syntex, now have specific departments dedicated to the study of new drugs from natural sources (Reid et al., 1993). However, the potential use of higher plants as a source of new drugs is still poorly explored. Of the estimated 250,000–500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties; in most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that 5000 species have been studied for medical use (Payne et al., 1991). Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for anti-tumour activity, but even these were not screened for other pharmacological activities (Hamburger and Hostettman, 1991).

5.A. Phytochemical Investigation

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolics compounds (Hill, 1952), followed by sesquiterpenes, diterpenes, triterpene saponins, triterpene aglycones, flavonoids, sterols, coumarins, quinine's and
monoterpenes. It is imperative that ethnobotanical researches and phytochemical tests lead to some patentable and industrially exploitable compounds for drug development.

The shade dried stem bark of *Erythrina mysorensis* was subjected for determination of physicochemical parameters such as total ash content, acid insoluble ash, water soluble ash, loss on drying and % moisture content were determined and the values are shown in Table-2 and also percentage of successive extractives were calculated and results are depicted in Table-1. The phytochemical studies reveals the presence of alkaloids, carbohydrates, flavonoids, tri terpenoids, steroids and phenolic compounds and tannins in different extracts of stem bark of *Erythrina mysorensis*. Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kaliko’nen *et al.*, 1999). Herbs are used in many domains, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics (Djeridane *et al.*, 2006). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, Anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential (Aaby, Hvattum, & Skrede, 2004; Luo, Cai, Sun, & Corke, 2004). Hence total phenolic content is most important for the purpose of evaluation of crude drugs for its pharmacological activity.

The quantitative analysis was conducted to estimate the concentration of polyphenols in extracts whose presence was qualitatively analyzed. The method is based on the principles that polyphenols when react with Folin reagent develops blue colour chromogen in alkaline media, which can be measured at 750nm. Polyphenols concentration in extracts are calculated using standard curve prepared with gallic acid. The total phenolic content of pet. ether, chloroform, ethanol and aqueous extracts of *Erythrina mysorensis* stem bark were found to be 6.5, 16.5, 23.4 and 4.3mg gallic acid equivalent per gram of extracts respectively. Ethanol extract has highest phenolic content (23.4% w/w) among all other extracts studied.

Active constituents which are phenolic characters have been reported in the related species belonging to the genus *Erythrina* by several workers. Phytochemical investigations
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on the stem bark extract from *Erythrina senegalensis* demonstrated the presence of three
diprenylated isoflavonoids identified as 2′ 3-dihydro-2′-hydroxyosajin, osajin and 6, 8-
diprenylgenistein (Jean Hubert Donfack *et al.* 2008) Rahaman (2010) reported three
isoflavones, namely, scandenone, 4′,5,7-trihydroxy-8-prenylisoflavone and 4′,5,7-
trihydroxy-8-methylisoflavone from *Erythrina variegata* L.

Kumar (2010) reported that *Erythrina indica* contains several phenolic metabolites,
such as pterocarpsans, isoflavones, flavanones and chalcones, some of which displayed
antiplasmodial activity, antimycobacterial activity (Khaomek *et al.* 2004) and cytotoxic
activity against various cancer cell lines. It also contains alkaloids like N-
norprotosmomenine, protosmomenine, erysodienone, 3-erythroidine, erysopine,
erthraline, erythramine, erysodine, erysotrine, eryrhatine, N,N-dimethyltryptophan,
hyperphorine along with sterols like campesterol, β-sitosterol, β-amyrin. The isoflavones
named as indicamines D and E together with 11 known compounds including 6 isoflavones
like genistein, wighteone, alpinum isoflavones, dimethyl alpinum isoflavone, 8-pre
erythrinin ‘C’ and eryseneagalensein E and one Erythrinassinate B. Flavonoids include
apigenin, genkwanin, iso-vitexin, swertisin, saponarin, 5-Oglucosylswertisin and 5-O-
glucosylisoswertisin. Glucoside swertiamarin, a triterpene betulin have also been isolated.
The alcohol insoluble portion of the unsaponifiable matter has yielded n-hexosamol,
heptacosine, nonacosane. The non saponifiable matter of the petroleum ether extract has
yielded myristic, stearic and oleic acids. New 3-phenylcoumarin, indicamine A, has been
isolated from the root bark of the African medicinal plant *Erythrina indica*, together with
three known compounds, robustic acid, daidzein, and 8-prenyldaidzein. The structure of
the new compound was characterized, as 4-hydroxy-5-methoxy-3-(4′-methoxyphenyl)-2′-3-
(1-methylethenyl)dihydrofurano[4′,′,5′,′,6,7] coumarin by means of extensive
spectroscopic analyses. The compounds were found to be devoid of *in vitro* antibacterial
activity. *E indica* and *E variegata* bark peelings are used as padding in certain storage
bins. Ito (1999) isolated five oxy-erythrinan alkaloids with insecticidal properties,
erthrinine, 11-hydroxyerysotrine, erysotramidine, erytharine, crystamidine and a dibenz
{d, f} azonine type alkaloid, erybidine, from *Erythrina* plants. So there are also several
other reports suggesting the presence of phenols in a variety of plant species belonging to
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various genera. All these observations clearly substantiate the phenomenon of the presence of phenolic compounds certainly attributes to the pharmacological efficacy of the plants. Hence, further studies related to the evaluation of pharmacological properties of this plant have been carried out by employing various parameters as mentioned in the objectives.

5.B. Isolation and characterization of the bioactive constituents

The phytochemical investigation of the stem bark of *Erythrina mysorensis* lead to the isolation of stearic acid, palmitic acid, β-sitosterol, stigmasterol, lupeol, β-Hydroxy Chalcone derivative and quercetin from the petroleum ether, chloroform and ethanol extracts.

**Compound I**: m.p. 63-64°C. The compound was isolated as white crystals. The IR peak at 3444 33 cm\(^{-1}\) indicates the presence of OH group, peak at 2922 75 cm\(^{-1}\) indicates C-H stretching in CH\(_3\), peak at 2871 32 cm\(^{-1}\) indicates C-H stretching in CH\(_2\), and peak at 1725 21 cm\(^{-1}\) indicates C=O stretching. The \(^1\)H NMR spectra in the range of δ 0.8286 to 0.8964 indicates the terminal methyl protons, a multiplet was observed in the range of δ 1.2634 to 1.3262 which corresponds to twenty six protons of CH\(_2\) a triplet was observed in the range of δ 2.4300 to 2.4626 which corresponds to two protons of CH\(_2\), a singlet was observed at δ 11.6826 which corresponds to one proton of OH group. The mass spectra showed the molecular ion peak at m/z 256 corresponding to the molecular formula C\(_{16}\)H\(_{32}\)O\(_2\), suggesting that this compound is ‘Palmitic acid’.

**Compound II**: m.p. 69-70°C. The compound is white coloured solid. The IR peak at 3412 61 cm\(^{-1}\) indicates the presence of OH group, peak at 2916 57 cm\(^{-1}\) indicates C-H stretching in CH\(_3\), peak at 2848 51 cm\(^{-1}\) indicates C-H stretching in CH\(_2\), and peak at 1703 52 cm\(^{-1}\) indicates C=O stretching. The \(^1\)H NMR spectra in the range of δ 0.8843 to 0.9184 suggests the terminal methyl protons, a multiplet was observed in the range of δ 1.2786 to 1.3238 which corresponds to twenty eight protons of CH\(_2\) group, \(^1\)H NMR signal in the range of δ 1.6333 to 1.6695 corresponds to two protons of CH\(_2\) group, a triplet was observed in the range of δ 2.3474 to 2.3850 which corresponds to two protons of CH\(_2\), a singlet was observed at δ 11.5410 which corresponds to one proton of OH group. The mass
spectra showed the molecular ion peak at m/z 283.4 corresponding to the molecular formula C\textsubscript{18}H\textsubscript{36}O\textsubscript{2} suggesting that this compound is 'stearic acid'.

**Compound III:** m.p. 137 - 138°C. The compound is white to cream colour solid. The IR peak at 3441.41 cm\(^{-1}\) indicates the presence of OH group, peak at 2935.42 cm\(^{-1}\) indicates C-H stretching in CH\(_3\), and peak at 1640.15 cm\(^{-1}\) indicates C=C stretching, peak at 1463.33 cm\(^{-1}\) indicates C-H deformation in CH\(_3\). In \(^1\)H NMR, a multiplet was observed in the range of \(\delta\) 0.6482 to 1.0110 which corresponds to eighteen protons of six methyl groups, a multiplet was observed in the range of \(\delta\) 1.076 to 1.5622 which corresponds to twenty two protons of eleven CH\(_2\) groups, a multiplet was observed in the range of \(\delta\) 1.8356 to 2.2871 which corresponds to four protons of four CH groups, a multiplet ranging from \(\delta\) 3.515 to 3.538 corresponds to one proton of CH group, a doublet was observed at \(\delta\) 5.022 which corresponds to two protons of CH\(_2\) group, a quadret at \(\delta\) 5.15 corresponds to two protons of CH\(_2\) group and a singlet was observed at \(\delta\) 5.34 which corresponds to one proton of OH group. The mass spectra showed the molecular ion peak at m/z 414 corresponding to the molecular formula C\textsubscript{29}H\textsubscript{50}O suggesting that this compound is '\(\beta\)-sitosterol'.

**Compound IV:** m.p. 168 - 170°C. The compound is purple white crystals. It gave a characteristic green colour in Liebermann-Burchard's test and red colour in Salkowski's test. The IR peak at 3454.80 cm\(^{-1}\) indicated the presence of OH group, peak at 2919.30 cm\(^{-1}\) indicates C-H stretching in CH\(_3\), peak at 2850.47 cm\(^{-1}\) indicate C-H stretching in CH\(_2\), and peak at 1641.03 cm\(^{-1}\) indicates C=C stretching and IR peak at 1462.76 cm\(^{-1}\) indicates C-H deformation in gem dimethyl and peak at 1045.73 cm\(^{-1}\) indicates C-O stretching of secondary alcohol. \(^1\)HNMR spectra at \(\delta\) 0.8288 - 0.8534 indicated terminal methyl protons, \(\delta\) 0.8626-0.8961 indicated CH\(_2\) protons and \(\delta\) 4.6866 indicated the presence of OH at C-3. The QI-MS spectra showed the molecular ion peak at m/z 412.79 which corresponding to the molecular formula C\textsubscript{29}H\textsubscript{48}O, clearly suggesting that this compound is 'stigmasterol'.

**Compound V:** m.p. 213 - 215°C. The compound isolated as white colour crystals. In the IR spectra the broad peak at 3423.44 cm\(^{-1}\) indicated the presence of OH, IR peak at 2929 cm\(^{-1}\) and 2850 cm\(^{-1}\) indicating the presence of C-H stretching in CH\(_3\) and CH\(_2\)
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respectively, IR peak at 1458.14 cm\(^{-1}\) represents CH deformation in gem dimethyl, and IR peak at 880.00 represents exocyclic CH\(_2\). In \(^1\)HNMR a multiplet was observed in the range of \(\delta\) 0.7605 to 0.9213 corresponding to twenty one protons of seven methyl groups, a multiplet was observed in the range of \(\delta\) 0.9436 to 1.6811 corresponding to twenty protons of ten CH\(_2\) groups, a singlet was observed at \(\delta\) 2.1735 corresponding to one proton of CH, a multiplet was observed in the range of \(\delta\) 3.1645 to 3.2051 corresponding to one proton of CH group, a triplet was observed in the range of \(\delta\) 4.2024 to 4.2321 corresponding to one proton of CH group, a multiplet was observed in the range of \(\delta\) 4.5604 to 4.5704 corresponding to one proton of CH group, a doublet was observed in the range of \(\delta\) 4.6840 to 4.6898 which corresponds to one proton of CH group, a doublet was observed in the range of \(\delta\) 1.6823 to 1.6908 corresponding to one proton of CH group. The mass spectra showed the molecular ion peak at m/z 427.4 corresponding to the molecular formula C\(_{30}\)H\(_{50}\)O\(_2\) suggesting that this compound is 'lupeol'.

**Compound VI:** m.p. 60 - 63°C. The compound was yellowish white solid. It showed positive response to Shinoda test. The IR spectrum exhibited strong absorption at 3425 cm\(^{-1}\) (br, OH), 2917 cm\(^{-1}\) (Ar-H str) and characteristic peak at 1690 cm\(^{-1}\) (\(\alpha,\beta\)-unsaturated ketone) and 1353 cm\(^{-1}\) indicating the presence of C-H bending in \(-C(CH_2)\) gemdimethyl group. IR peak at 2848 cm\(^{-1}\) and 1238 cm\(^{-1}\) indicates C-H str in CH\(_3\) and C-O-C str in OCH\(_3\). The \(^1\)HNMR spectra of this compound exhibited the presence of characteristic gem-dimethyl at \(\delta\) 1.2. The \(^1\)HNMR spectra also showed aromatic protons at \(\delta\) 6.9(d), 6.73(d), 7.4(m), 7.6(d) and 8.76(d) and 8.41(s) for methoxy protons. The Ql-MS spectra showed the molecular ion peak at 336[M+] corresponding to the molecular formula C\(_{21}\)H\(_{20}\)O\(_4\). Thus, the compound isolated is '\(\beta\)-Hydroxy Chalcone derivative'.

**Compound VII:** m.p. 95 - 99°C. The compound is found to be yellowish solid. It showed violet color to neutral ferric chloride reagent confirms presence of phenolic OH groups in the compound. The IR spectrum exhibited strong absorption at 3425 cm\(^{-1}\) (br, OH), 2917 cm\(^{-1}\) (Ar-H str) and characteristic peak at 1690 cm\(^{-1}\) (\(\alpha,\beta\)-unsaturated ketone). \(^1\)HNMR spectra of this compound exhibited the presence of characteristic aromatic peaks at \(\delta\) 6.1 to 7.7 corresponds to five aromatic protons and broad hump at \(\delta\) 8.8, 10.4 and 12.4
corresponds to four hydroxy groups respectively. The QI-MS spectra showed the molecular ion peak at 302 \([M+]\) corresponding to the molecular formula \(C_{15}H_{10}O_7\) suggesting that this compound is 'quercetin'.

Similar observations have been made by several workers in the related species belonging to the genus *Erythrina*. Kafuku, 1934 examined the seeds of *E. indica* indicated the presence of an oil (11.9%, yield) exhibiting unsaponifiable matter 0.49%. The oil contained behenic, arachidic, palmitic and lignoceric, oleic and linoleic acids. Fatty acids obtained from the seeds of *Erythrina crista galli* seeds contain oleic, linoleic, palmitic, stearic, arachidic myristic and eicosenoic acid (Chem Abstr, 1946, 40, 1051, Hilditch, 1947, 188) Pathak et al., 1956, reported that examination of the acid components of the seeds of *E. indica* revealed the presence of palmitic 8.2%, stearic 8.0%, arachidic 4.3%, behenic 13.3%, hexadecenoic 3.1%, oleic, 45.6%, linoleic 7.1%, eicosenoic 9.8%, and lignoceric 0.6%. Bhakuni et al., 1959 investigated the alcoholic extract of *E. indica* bark indicated the presence of docosyl alcohol, \(\beta\)-sitosterol, \(\gamma\)-sitosterol, \(\delta\)-sitosterol, and 2,4-dinitrophenylhydrazone. Khaomek et al., 2004 reported that *Erythrina indica* contains sterols like campesterol, \(\beta\)-sitosterol, \(\beta\)-amyrin. Nkengfack et al., 2001, carriedout the bioassay-directed fractionation of the \(\text{CH}_2\text{Cl}_2\)-MeOH (1:1) extract of the stem bark of *Erythrina indica*, has resulted in the isolation of two new isoflavone derivatives together with 11 known compounds including six isoflavones, two pentacyclic triterpenes (oleanolic acid and erythrodiol) and two phytosterols (stigmasterol and its 3-O-β-d-glucopyranoside) Rahman et al., 2007 isolated five compounds from the \(n\)-hexane and chloroform soluble fractions of a methanol extract of the stem bark of *Erythrina variegata*. The structures of the isolated compounds were elucidated as alpinum isoflavone (1), epilupeol (2), 6-hydroxysterigmat (3), 3β, 28-dihydroxyolean-12-ene (4) and stigmasterol (5) by extensive spectroscopic studies. Rukachaisiriku et al., 2007 investigated the hexane and \(\text{CH}_2\text{Cl}_2\) extracts of *Erythrina stricta* roots and *E. subumbraens* stems led to the isolation of lupeol, reported Long Cu et al., 20008 isolated four new chalcones 1-4, named abyssmones A-D, from the stem bark of the plant *Erythrina abyssinica* and their structures were elucidated on the basis of spectroscopic analyses. INNOK et al., 2009
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reported that the EtOAc extract of *E. fusca* bark was separated by extensive column chromatography to give new isomeric flavanones, named fuscaflavanones and chalcone.

In plants, more than 40 sterols have been identified, of which β-sitosterol, stigmasterol, and campesterol are the most common (Hicks *et al.* 2001). The compound β-sitosterol is a very common chemical constituent of medicinal plants, which possesses valuable biological activity (Buckingham, 1998). Three sterols cholesterol, stigmasterol, and β-sitosterol were found in all species (Ledwani *et al.* 2010).


5. C. Pharmacological investigations

1. Anti-oxidant activity

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors (Cerutti, 1991). *In-vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intercellular signaling or synthesis of biologically important compounds (Halliwell, 1997). However, ROS may also be very damaging, they can attack lipids in cell membranes and also attack DNA, inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity and also cause DNA mutation leading to cancer (Petta, 2000, Cerutti, 1994). A potent scavenger of these species may serve as a possible preventive intervention for free radical-mediated diseases (Ames *et al.*, 1995). Recent studies showed that a number of plant products including polyphenolic substances (e.g., flavonoids and tannins) and various plants or herb extracts exert antioxidant actions (Yokozawa *et al.*, 1998).
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The 1, 1-diphenyl-2-hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixtures such as different solvent extracts of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow color after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity was increased with increasing percentage of the free radical inhibition. DPPH is relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution looses colour stoichiometrically depending upon the number of electrons taken up (Blois, 1958).

The results of the present study clearly indicate that ethanol extract of stem bark of *Erythrina mysorensis* possess the significant antioxidant property compared to other extracts. The reports reveal that the decrease in the concentration of DPPH radical due to scavenging ability of the ethanolic extract. There is a 50% decrease of the DPPH radical at a 26.21 µg/ml ethanol extract concentration. Whereas chloroform extract shown moderate 47.58 µg/ml, pet ether extract less 82.73 µg/ml and aqueous extract did not show antioxidant property. The ethanol extract of stem bark shows the presence of more phenolic compounds such as terpenoids and flavonoids compared to other extracts. Therefore, it exhibited good antioxidant activity.

The above result substantiated by the earlier report by Moon *et al.*, 2010 examined the methanolic extracts of *E. indica* bark for DPPH free radical scavenging activity, reducing power and nitric oxide scavenging activity, as well as the total phenolic content. *E. indica* was found to exhibit potent antioxidant activity. The antioxidant activity of ethanol, chloroform and ethyl acetate extract of leaves and stem of *Erythrina indica* Lam was studied and the activity was compared with DPPH and BHT as standard. This study suggests that the *Erythrina indica* plants could be pharmacologically exploited for antioxidant properties (Patil *et al.* 2011). The leaves of the plant *Erythrina stricta* are found to possess rich antioxidant activity (Asokkumar *et al.* 2008).
Sakat and Juvekar (2010), examined the aqueous and methanolic extracts of the leaves of *E. indica*, using in vitro methods such as 1,1-Diphenyl-2-Picrylhydrazyl nitric oxide radical scavenging activity, and inhibition of lipid peroxidation by thiobarbituric acid reactive substances (TBARS) method on isolated rat liver tissues. According to the study, the presence of flavonoids and polyphenolics, may be responsible for the good antioxidant activity of *E. indica*.

Aquil *et al.*, 2006 studied the methanolic crude extracts of 12 traditionally used Indian medicinal plants for their antioxidant and free radical scavenging properties using α-tocopherol and butylated hydroxy toluene (BHT) as standard antioxidants. Antioxidant activity was measured by ferric thiocyanate (FTC) assay and compared with the thiobarbituric acid (TBA) method. Free radical scavenging activity was evaluated using diphenyl picryl hydrazyl (DPPH) radicals. The overall antioxidant activity of *Lawsonia inermis* was the strongest, followed in descending order by *Ocimum sanctum*, *Cichorium intybus*, *Piper cubeba*, *Punica granatum*, *Allium sativum*, *Delonix regia*, *Terminalia chebula*, *Terminalia bellerica*, *Mangifera indica*, *Camellia sinensis*, and *Trigonella foenum-graecum*. Seven plants, namely *Terminalia chebula*, *Mangifera indica*, *Terminalia bellerica*, *Punica granatum*, *Ocimum sanctum*, *Cichorium intybus*, and *Camellia sinensis* showed potent free radical scavenging activity with the DPPH method. Phytochemical analysis of plant extracts indicated the presence of major phytocompounds, including phenolics, alkaloids, glycosides, flavonoids, and tannins. The phenolic concentrations in the above plants ranged from 28.66 to 169.67 mg/g of dry plant extract. A fair correlation between antioxidant/free radical scavenging activity and phenolic content has been observed among 9 plants, however, in 3 plants (*Piper cubeba*, *Lawsonia inermis* and *Trigonella foenum-graecum*), no such relationship has been observed. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

Experiments were conducted to evaluate the direct super oxide scavenging activity of the extracts. A chemical super oxide anion generating system (NADH/PMS/NBT) was widely used to determine the scavenging activity of the compounds. It is well known that...
superoxide anions damage biomolecules directly or indirectly by forming \( \text{H}_2\text{O}_2 \), \( \cdot\text{OH} \), peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. (Yen and Duh, 1994.) In the PMS/NADH-NBT (phenazine methosulphate/nicotinamide adenine dinucleotide-nitroblue tetrazolium) system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Upon addition of various concentrations of extracts, as well as butylated hydroxyl toluene (standard) in the above coupling the reaction exhibited a decrease in the absorbance.

The superoxide scavenging activity of ethanolic extract of *Erythrina mysorensis* was increased markedly with the increase in concentrations (Table-16). Thus, higher inhibitory effects of the stem bark ethanol extract on superoxide anion formation noted herein possibly renders them as promising antioxidants. The half inhibition concentration (IC\(_{50}\)) of ethanol extract was 26.84µg. These results suggested that ethanol extract has a potent superoxide radical scavenging effects, whereas other extracts have shown moderate superoxide scavenging activity.

The above results may be due to the presence of active component quercetin, a flavonoid which is isolated from the ethanolic extract. Where, quercetin prevents oxidative injury and cell death (Larocca et al., 1995) by several mechanisms, including scavenging oxygen radicals (Cox et al., 2000), inhibiting xanthine oxidase (Chang et al., 1993), lipid peroxidation, and chelating metal ions (Chen et al., 1990).

Earlier reports of Igbinosa (2011) also suggested similar type of activity in *Jatropha curcas*. All the solvents extracts of *J. curcas* have exhibited higher superoxide radical scavenging activity when compared with BHT. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated (Liu, 1997). The scavenging activity of this radical by the *Erythrina mysorensis* extract suggests its potent scavenger of superoxide radical.
In addition to radical scavenging activity and the superoxide scavenging activity, the stem bark extracts were also evaluated for their ability to protect biomembrane from oxidative damage. Initiation of the lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex or through OH radical by Fenton's reaction. Ethanolic extract of *Erythrina mysorensis* inhibited FeSO₄/ascorbate induced lipid peroxidation in young and aged rat brain mitochondria in a dose dependent manner. The inhibition could be caused by the absence of ferryl-perferryl complex or by scavenging the OH radical or the superoxide radicals or by changing the Fe³⁺ Fe²⁺ or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself. The mitochondria of aged rats showed an elevated level of MDA content as compared to young rats. This result indicated that aging is associated with increased production of free radicals by mitochondria (Wei, 1998). Addition of 10-100 µg/ml of ethanolic extract of stem bark to rat brain mitochondria significantly reduced MDA formation in a dose dependent manner. The result shows that ethanolic extract has the capacity to prevent oxidative deterioration of mitochondrial membrane lipids. The percentage of inhibition of ethanolic extract with maximum inhibition being 50% at 26.14 µg/ml. The other extracts showed slightly moderate antioxidant activity. The beneficial effect of ethanol extract of *Erythrina mysorensis* on radical scavenging activity, the superoxide scavenging activity and lipid peroxidation could be attributed to its phenolic content (Nagulendran, 2007).

Fruits and vegetables are primary food sources providing essential nutrients for sustaining life. They also contain a variety of phytochemicals such as phenolics and flavonoids, which provide important health benefits (Kaye, 2003). Hence, regular consumption of fruits and vegetables is associated with reduced risks of chronic diseases, such as cancers and cardiovascular diseases (Dragsted, 1993). A free radical is any atom or molecule that has a single unpaired electron in an outer shell. Free radical-induced oxidative stress has been associated with several toxic cellular processes including oxidation damage to protein and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation that may lead to carcinogenesis (Poulsen, 1998). Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating free radicals. They are compounds that when added to lipids and lipid containing foods, can
increase their shelf-life by retarding the process of lipid peroxidation. Also, they have been widely used as food additives to avoid food degradation, and they play an important role in preventing many lifestyle-related diseases and aging, being closely related to the formation of reactive oxygen species (ROS) and to lipid peroxidation (Gulcin, 2004). Production of ROS during normal cell metabolism is a normal and necessary process that provides important physiological functions. An imbalance between ROS production and antioxidant defences results in oxidative stress which has been recognized as playing a prominent role in the causation of several age-related and chronic diseases, neurodegenerative and cardiovascular diseases. Intake of sufficient amounts of antioxidants is necessary to prevent free radical-induced oxidative stress. It has been reported that most of the antioxidant capacity of fruits and vegetables may come from total phenolics, anthocyanins, and flavonoids (Gulcin, 2010, Harput, 2011).

The relative activity of ethanol extract has been calculated under different in vitro model conditions and all the models of in vitro antioxidant assays, the ethanol extract exhibited strong free radical scavenging ability. It might be due to the presence of high amount of phenolic compounds, terpenoids, steroids, glycosides and flavonoids in extract. Structurally, the characterized compounds viz. β-hydroxy chalcones and quercetin are possessing α, β-unsaturated and glucoketone responsible for free radical scavenging activity (Archita Bapna et al., 2008, Singhal Manmohan et al., 2011). The presence of α-β-unsaturated keto group in chalcones is found to be responsible for their biological activity (Baviskar et al., 2007, Chadwick et al., 2004, Kitanaka S et al., 2005). Chalcones and anthocyanidines represent essential groups of natural products which possess wide range of pharmacological activity such as antibacterial, antitumor, anticancer, antitubercular, anti-inflammatory, antioxidant and anti-malarial properties (Okwu, 2010).

Highly reactive free radicals and oxygen species are present in biological system from a wide variety of sources, they are formed during normal metabolism and exposure to environmental conditions such as infections, polluting agents, ionizing and non-ionizing radiations. When these radicals are not destroyed by body's defence mechanism, they oxidize nucleic acids, proteins, lipids or DNA that can initiate degenerative diseases.
Antioxidants protect cells against the damaging effects of free radicals. Some of α, β-unsaturated ketones have been reported as good antioxidants. (Herencia et al., 2002).

The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. Quercetin is able to react with ROS and chelate ROS producing metal ions, both of which allow for decreased oxidative DNA damage. Preventing this DNA damage is believed to be the general mechanism by which quercetin is able to prevent tumorigenesis. In particular, it is known that quercetin's hydroxyl groups have electron accepting capacity when they are in the semiquinone state and that its catechol group is the structure that confers the ability to chelate metal ions. The addition of sugar molecules to form quercetin glycosides can obstruct both of its antioxidant activities. Therefore, the aglycosylated form is usually of higher antioxidant potency than the glycoside form, depending on where the sugar molecule is attached.

2. Anti-inflammatory activity

The present pharmacological screening revealed that stem bark of *Erythrina mysorensis* have a potential anti-inflammatory effect which is evidenced by the significant reduction in paw oedema. Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa, 2002).

Inflammation, clinically, causes, as shown by Cornelius Celsus of Rome 2000 years ago, rubor (redness), calor (heat), dolor (pain) of the affected region (Chaudhary, 2001) and is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells (Denko, 1992). It is defensive mechanism of the body.
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to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation, however, if runs unchecked, leads to onset of diseases such as vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis (Henson, Murphy, 1989). Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-oedematous effect of natural products (Sertie et al., 1990) and this is believed to be triphasic, the first phase (1hr after carrageenan challenge) involves the release of serotonin and histamine from mast cells, the second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products (Vinegar et al., 1969). The metabolites of arachidonic acid formed via the cyclooxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators, prostaglandins (products of the cyclooxygenase pathway) especially prostaglandin E2 is known to cause or enhance the cardinal signs of inflammation, similarly, leukotriene B4 (product of lipoxygenase pathway) is a mediator of leukocyte activation in the inflammatory cascade (Rosa, 1971).

The most widely used primary test to screen new anti-inflammatory agents to measure the ability of a compound is to reduce local edema induced in the rat paw by injection of an irritant agent Carrageenan (Winter et al., 1962). In the present study the crude extracts of Erythrina mysorensis have been used to check the Anti-inflammatory property in laboratory animals. The results have shown that the significant inhibitory activity (< 0.01) by the petroleum ether and ethanol extract of Erythrina mysorensis (200 and 400 mg/kg) over a period of 5 h in carrageenan-induced inflammation was quite similar to that exhibited by the group treated with diclofenac sodium. The highest percentage inhibition activity was found in the dose of 400 mg/kg of both petroleum ether and ethanol extracts with the mean percentage inhibition of 62.1 and 61.3 respectively after 2 hours of extract administration. Similarly, isolated constituents of Erythrina mysorensis lupeol, beta hydroxyl chalcone and quercetin also exhibited highest percentage of inhibition activity (< 0.01) at a dose of 10 mg/kg.b.w. comparable with standard diclofenac sodium for over a period of 5 hrs.
The phytochemical studies carried out by earlier workers have clearly suggested that the genus *Erythrina* is very rich in flavonoids and triterpenoids (Amer *et al*., 1991). These compounds from various plants are known to possess anti-inflammatory property. The present results also suggest the strong anti-inflammatory activity of pet. ether and ethanol extracts. Further, the three compounds isolated from the crude extracts have also shown the anti-inflammatory effect. Interestingly, among the extracts chloroform is comparatively weak in showing this effect. Various flavonoids (e.g., quercetin, apigenin, tea catechins) have also been shown to have anti-inflammatory activity by inhibiting cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (Marchand, 2002), which is related to antioxidant activity (Miller, 1996). Flavonoids also inhibit cytosolic and tyrosine kinase (Kang *et al*., 2009; Middleton *et al*., 2000) and also inhibit neutrophil degranulation (Middleton *et al*., 2000). However, one of the isolated compounds ‘lupeol’ which has been isolated from chloroform extract could exert strong anti-inflammatory activity. Therefore, it is believed that lupeol when it is isolated from the crude extract (in the pure form) may show its property effectively, than when it exists along with other compounds. It is also known that the lupeol inhibit carrageenan induced edema, which indicates that it could inhibit different aspects of chemical mediators of inflammation (histamine, serotonin, bradykinin and prostaglandins). Moreover, the lupeol seems to have a protective effect, which may contribute to its anti-inflammatory effect (Vinger *et al*., 1987; Di Rosa, 1974).

Similarly, the anti-inflammatory effect shown by pet. ether extract may be because of the presence of fatty acids such as palmitic acid and stearic acid which are known anti-inflammatory compounds (Jia, 1996). Similar observations have been made by many workers in the related species of *Erythrina*, thus showing the anti-inflammatory properties.

The anti-inflammatory activity was evaluated on egg-albumin induced paw oedema in rats as a model of acute inflammation. The stem bark extract of *E. senegalensis* exhibited significant (P<0.05) analgesic and anti-inflammatory effects which have been observed by Saidu *et al*., (2000).
The anti-inflammatory activity of the hydroalcoholic extracts from *Erythrina velutina* and *E. mulungu* in the carrageenan- and dextran-induced mice hind paw edema models have been studied. In the present work, the extracts were administered orally in male mice at the doses of 200 or 400 mg/kg. In the carrageenan-induced test, only *Em* showed anti-inflammatory activity, decreasing the paw edema. No effect was observed with *Ev* in this model. On the other hand, in the dextran model, *Ev* demonstrated anti-inflammatory effect, showing decrease of the paw edema. *Em* (200 or 400 mg/kg) presented anti-inflammatory effect after administration of dextran, as compared to control (Vasconcelos et al. 2011).

Njamen et al. 2003 reported that Erycristagallin, a pterocarpene isolated from *Erythrina mildbraedii*, was tested in the carrageenan-induced mouse paw oedema model, the ethyl acetate extract obtained from *E. mildbraedii* showed anti-inflammatory activity, and erycristagallin was isolated as the active principle. As with other phenolics, the anti-inflammatory activity of erycristagallin may be based on its capacity to inhibit the arachidonic acid metabolism via the 5-lipoxygenase pathway.

Lupeol isolated from other unrelated species has also shown a similar activity of anti inflammation. Al-Rehaily (2001) has reported the pharmacological activities of the acetonitrile (McCN), hexane extracts and isolated pure terpenoidal compound lupeol from the leaves of *Teclea nobilis*, Delile (TN), on inflammation induced by carrageenan and implantation of cotton pellets in rats they studied the nociceptive response using writhing and tail flick tests and the antipyretic activity in yeast-induced fever in mice. Oral administration of TN extracts at doses of 150 and 300 mg/kg and lupeol 5 and 10 mg/kg showed a significant anti-inflammatory activity in rats. The extracts of TN and lupeol significantly decreased the number of contractions and stretchings induced by acetic acid and heat-induced pain in mice. Their results suggest that the *Teclea nobilis* extracts and lupeol possesses anti-inflammatory, analgesic and antipyretic activities.

Investigation of *D. rubra* extracts lupeol has been isolated and reported to have antimicrobial, anti-inflammatory and anti-cancer activities (Prachayasittikul et al. 2010).
Both lupeol and 19 a-H- lupeol isolated from *Strobilanthes callosus* and *Strobilanthes ixioccephala* exhibit significant antiinflammatory activity and antiarthritic activities respectively (Agarwal et al. 2003). In another study, β-sitosterol isolated from leaves of *Rhus sylvestris* was found to have ability to block inflammatory cytokine secretion in the murine RAW264.7 macrophage cell line (Ding et al., 2009), where β-sitosterol isolated from pet ether extract of *E. mysorensis*

Quercetin, kaempferol, morin, myricetin and rutin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, antiallergic, and antiviral as well as an anticancer activity (Robak et al. 1988), where quercetin is isolated from the ethanolic extract.

Pharmacological actions of quercetin is including cardio-protection, cataract prevention, anticancer activity, anti-ulcer effects, anti-inflammatory, anti-allergic, antiviral and antibacterial activities (Bronner and Landry, 1985, Stavric, 1994) Guardia et al., reported quercetin and hesperedin given at a daily dose of 80 mg/kg inhibit both acute and chronic phase of inflammation. Kaempferol, quercetin, myricetin, fisetin are reported to possess COX and LOX inhibitory activities (Tapas et al. 2008).

Thus, the present results of Anti-inflammatory activity of *Erythrina mysorensis* substantiate the earlier findings that the plant extracts are rich in flavonoids having the ability to exert Anti-inflammatory effects. Similarly the isolated constituents such as lupeol, quercetin and chalcone derivative also possess this ability. Pharmacological investigations also revealed that palmitic acid has Anti-inflammatory effect (Jia, 1996), which is isolated from pet ether extract. However, the other four isolates which are belonging to the very common group of biomolecules 'lipids' have not been subjected for the present activity as there is ample evidence that these compounds do possess the anti-inflammatory activity.

3. **Wound Healing Activity**

Wound healing is the primary response to tissue injury with different phases like contraction, granulation, epithelization and collagenation which is mainly achieved by
connective tissue matrix synthesis (Piercee and Mustoer, 1995, Biswas and Mukherjee, 2003). Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area.

This centripetal movement of wound margin is believed to be due to the activity of myofibroblast (Gabellaiani et al., 1972). The wound healing activity study presented a multiscale modeling framework which allows to study the effect of different factors on wound healing, such as contraction of cutaneous wound, its tensile strength, collagen alignment, the hydroxyproline content and the strength of the granuloma tissue and scar formation during dermal wound healing process. So, in order to determine the above mentioned parameters, the three different models were used in this study to examine the wound healing effect of various extracts and isolated constituents of *Erythrina mysorensis* stem bark on various phases of wound healing, which run concurrently, but independent of each other. The nitrofurazone is used as standard drug to assess the activity. In this study, in excision wound model, the petroleum ether and ethanolic extract of *Erythrina mysorensis* stem bark (0.5% w/w) ointment has produced significant increase in mean % (**<0.01**) wound contraction after 4th, 8th and 12th day of treatment as compared with control group and it was on par with that of standard reference drug. In case of chloroform and aqueous extracts treated and in the control animals complete wound contraction occurred on 25th, 26th and 27th days respectively.

In the animals treated with the isolated constituent lupeol the complete wound contraction observed on 16th day. For other isolated constituents β-hydroxy chalcones and quercetin the complete wound contraction occurred on 21st day. This suggests that it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area.

The wound breaking strength is determined by the rate of collagen synthesis and more so by the maturation process where there is covalent binding of collagen fibrils through inter and intra molecular cross linking (Shanbhag et al., 2006). In the present study a significant increase (**<0.01**) in tensile strength on 12th day was observed in test group treated with petroleum ether (632.37±5.50 gm) and ethanolic extract (620.81±2.50 gm) as
compared with control group (506.87±3.80 gm). Treatment with other two extracts of *Erythrina mysorensis* exhibited less significant increase (< 0.05) in the tissue breaking strength comparatively lesser to petroleum ether and ethanol extracts.

In the incision wound model the isolated constituent lupeol was found to be more effective in increasing the breaking strength (615.54±6.10gm.) as similar to that of standard drug nitrofurazone (639.99±7.02gm.) whereas, the other isolated 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.

Granulation tissue which is formed in the final part of the proliferative phase is primarily made up of fibroblasts, collagen, oedema and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggests higher protein content (Devipriya and Shyamladevi, 1999). 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 (< 0.01) 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.

Collagen, the major protein of the extracellular matrix, is the component that ultimately liberates free hydroxyproline and its peptides (Nayak, 2006). The measurement of hydroxyproline can also be used as an index for collagen turnover. Increase in hydroxyproline content indicates increased collagen synthesis which in turn leads to enhanced wound healing. The hydroxyproline content in wounds treated with petroleum ether and ethanolic extract of *Erythrina mysorensis* bark, and isolated constituent lupeol was found to be higher than that in the wounded control animals.
Increase in skin breaking strength and tissue breaking strength in incision and dead space wound model respectively indicated enhanced collagen maturation. Increase in the granulation tissue dry weight and hydroxyproline content indicated the high collagen turnover which may be due to the activity of some phytoconstituents like flavonoids which are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity.

Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, by increasing the circulation, by preventing the cell damage and by promoting the DNA synthesis (Getie, 2002).

Histological studies of the tissue obtained from the petroleum ether extract treated (Fig.70e) group showed significant increase in collagen deposition, few macrophages, tissue edema and more fibroblasts. It was more or less equal to the animals treated with nitrofurazone (Fig.70 b). The ethanol extract (Fig.1f) treated group of animals showed similar kind of effect but to a lesser extent. The chloroform and aqueous extract (Fig.70d) treated group of animals showed lesser collagen fiber. The histological studies of the granulation tissue of the control group of animals (Fig.70a) and petroleum ether treated groups (Fig.70c) showed more aggregation of macrophages with lesser collagen fiber. The wound healing was more significant in the ethanol extract treated group of animals than the other extracts under study.

Histopathological examination of the sections of the granulation tissues of the animals treated with lupeol shown lesser monocytes, fibroblasts and increased collagen deposition. Comparatively lesser collagen formation was observed in the animals treated with other isolated constituents β-hydroxy chalcones and quercetin.

Shetty et al., 2006 reported the possible mechanism of action of cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressive to the repair and remolding of damaged tissue. Wound healing, complex sequences of events involve 4 phases. (i) coagulation which prevents
blood loss (ii) Inflammation and debridement of wound (iii) Epithelial repair, including proliferation, mobilization, migration and differentiation (iv) Tissue remolding and collagen deposition Any agent which accelerates the above process(es) can be termed as promoter of wound healing Dermal reconstitution begins approximately 3 to 4 days after injury, characterized clinically by granulation tissue formation, which includes new blood vessel formation, or angiogenesis, and the accumulation of fibroblasts and ground matrices, named fibroplasia The provisional extracellular matrix that is formed in part by the fibrin clot, which is rich in fibronectin, promotes granulation tissue formation by providing scaffolding and contact guidance for cells to migrate into wound spaces and for angiogenesis and fibroplasia to occur in an effort to replace the wounded dermal tissue

Hence, the wound healing promoting activity of *Erythrina mysorensis* stem bark may also be attributed to the above mechanism and due to their antioxidant activity In addition to this phytochemical screening has revealed the presence of flavonoids in *Erythrina mysorensis* stem bark which are known free radical scavengers This might be one of the reasons for the wound healing activity of *Erythrina mysorensis* stem bark

Phytochemical analysis has demonstrated the presence of terpenes in plants from the *Erythrina* genus (Serragiotto *et al* 1981, Nkengfack *et al* 1997), flavonoids, especially, isoflavones, pterocarpanes, flavanones and isoflavanones (Chacha *et al*., 2005) Previous studies suggest that, wound healing by some of the phytoconstituents like flavonoids, which are known to reduce lipid peroxidation are not only preventing or slowing the onset of cell necrosis but also involved in the improvement of vascularity Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers and the circulation, by preventing the cell damage and by promoting the DNA synthesis (Getie, 2002) Therefore, in the present study the ethanol extract has shown potent wound healing activity Interestingly, the flavonoids quercetin and chalcone derivative have been isolated from the ethanol extract of *Erythrina mysorensis* also exhibited potent wound healing ability

The earlier workers shown that triterpenoids are known to promote wound healing process mainly due to their astringent and antimicrobial property, which seems to be
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responsible for wound contraction and increased rate of epithelization (Shenoy et al., 2009). Therefore, in the present study the isolated constituent lupeol from the chloroform extract has shown potent wound healing activity. Surprisingly, when it is present with other phytoconstituents in chloroform extract, it has not exerted potent activity. Similar type of effect is observed in Anti-inflammatory activity also. Hence, the probable inability of producing Anti-inflammatory and wound healing activity by lupeol when it is present with other constituents in the crude extract need to be explored. It is presumed that the antagonistic effect of several compounds in the crude extract could be one of the reasons. As a result when the compound is isolated and when it is in the pure form then it has got the ability to exert its natural effect.

Similarly, the pet. ether extract has shown wound healing ability. This may be due to the presence of sterols present in the pet. ether extract. The phytochemical analysis of leaf extract of *Hyptis suaveolens* carried out by Shenoy et al., (2009) has revealed the presence of sterols, alkaloids, flavanoids and tannins. When this extract was subjected for wound healing effects it showed an increased collagen turnover suggesting the role of sterols in wound healing process.

In the present study, the stigmasterol and β-sitosterol were isolated from the pet. ether extract, have not been subjected for the evaluation of wound healing activity due to the fact that in most of the cases sterols possess excellent anti-inflammatory and wound-healing properties. Therefore, with this presumption the wound healing activity of sterols has not been carried out.

4. Anti-microbial Activity

The results of the present study revealed that only chloroform and ethanolic extracts possessed antibacterial activity compared to pet. ether and aqueous extracts. The effect of the extract was however found to be lower than the reference drugs studied; Benzyl penicillin 100mg/ml (22, 22mm), Streptomycin 100mg/ml (16, 24 mm). In the chloroform extract, maximum zone of inhibition was recorded by *E. coli*, Gram –ve bacteria at the concentrations of 50, 100 and 200mg/ml, 10, 12 and 22 mm. respectively,
and more sensitive followed by *Pseudomonas aeruginosa* 10, 14 and 20 mm respectively. In ethanolic extract maximum zone of inhibition was recorded by *Staph Aureus*, Gram +ve bacteria at the concentrations of 50, 100 and 200 mg/ml, 10, 14 and 16 mm respectively, followed by *Bacillus subtilis*, Gram +ve bacteria 10, 12 and 16 mm respectively.

Phytochemical screening of the extracts in this study revealed that *Erythrina mysorensis* bark contained some active chemical compounds (saponins, tannins, glycosides, phenols and alkaloids) (Table-3) Secondary metabolites in plants confers them protection against bacterial, fungal and pesticidal attacks and thus are responsible for the exertion of antimicrobial activity against some microorganisms (Marjorie, 1999) 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) both of which linked the antibacterial properties of plants to the presence of secondary metabolites The flavonoids were found to have antimicrobial activity (Gokhale, 2000) The chloroform and ethanol extracts exerted antimicrobial activity against all the test bacteria (*Bacillus subtilis*, *S aureus*, *E coli*, and *P aeruginosa*) and fungi (*A niger*, and *C albicans*), while the aqueous extracts did not exert any activity on all organisms tested These organisms are responsible for various types of infections, urinary tract infections (*S aureus* and *E coli*), dysentery (*E coli*), wound infections (*P aeruginosa*), while candidiasis is caused by *C albicans*. Use of the solvents accounted for increased extraction of the biologically active constituents thus displaying wider zone diameter of inhibition Though the chloroform extracts demonstrated higher activities than the ethanol extracts, their activities were still higher than those of the other extracts Different solvents have different polarities hence different degrees in solubility for the various phytoconstituents (Marjorie, 1999), thus accounting for this disparity in activity between the solvents used The study also showed that the activity of the extracts is concentration dependent This reduction in the activity at a higher concentration may be because only small amount of the active ingredient is required to inhibit the growth of the organisms, this is because only small amount is needed to attack a specific site in the organism and high concentration will cause accumulation and blockage of sensitive site thereby causes the reduction (Youman *et al.*, 1967) 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 (Hugo and Russel, 1998, Prescott et al., 2002)

Earlier workers have suggested that the secondary metabolites present in plants confers them protection against pathogenic microbes and thus are responsible for the exertion of antimicrobial activity against some microorganisms (Majorie, 1999). Particularly, flavonoids were found to have antimicrobial activity (Gokhale, 2000). The present results suggest the antimicrobial activity of chloroform and ethanol extracts. However, the response showed by the extracts is moderate when compared to the standard drugs which are mostly antibiotics. It is believed that the flavonoids- quercetin and chalcone derivative which are isolated from ethanol extract exerted the antimicrobial activity. Quercetin has been reported to completely inhibit growth of Staphylococcus aureus (Tapas et al., 2008). Earlier workers have reported the antimicrobial activity of lupeol (Prachayasittkul et al., 2010, Ragasa et al., 2005). In the present result chloroform extract which has shown antimicrobial activity may be due to the presence of lupeol. Similar, results have been made by many workers in the related species of Erythrina, thus showing the antimicrobial activity. In view of the results from crude extracts and the earlier findings, the antimicrobial property of isolated compounds has not been carried out.

Flavonoids and pterocarpsans have been isolated from E glauca Willd and E lysistemon Hutch. Flavonoids (isoflavones and flavanones) has been reported with antibacterial and antifungal properties (Linuma et al., 1984; Maillard et al., 1987). Bhale et al., 1979 examined activity of the seed oil of E ndica against ten bacterial and ten fungal strains. The oil was found to be more active than penicillin and streptomycin, and potent activity was observed against the other tested bacterial and fungal strains. Chacha et al., 2005 isolated, two isoflavones and a flavanone from the stem wood of Erythrina latissima and these compounds showed antimicrobial activity against Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Candida mycoderma. Tanaka et al., have isolated, characterized and studied the antibacterial properties of a new isoflavonoid from Erythrina poepiggiana against methicillin-resistant Staphylococcus aureus. The results shown that this new compound could be a potent phytotherapeutic agent for treating MRSA infections. The chloroform extract of the stem bark of Erythrina burttii showed
antifungal and antibacterial activities using the disk diffusion method. Flavonoids were identified as the active principles. Activities were observed against fungi and Gram(+) bacteria, but the Gram(-) bacteria \textit{Escherichia coli} was resistant. (Yenesew \textit{et al.}, 2005).

5. \textbf{Anti-tumour Activity}

Cell growth and cell multiplication process is known as cell division. It must be extremely controlled that all the cells in the body should grow at the right place, and for all the organs and tissues to function properly. When the cells divide too quickly, consequences can be disastrous. Uncontrolled cell division may have many causes, to form any type of cell. But usually results from defects or damage from one or more of the genes involved in cell division. When those genes were damaged (mutated) on some way, for instance on exposure to cigarette smoke or ultraviolet radiation, the cell may start dividing uncontrollably. Those defective cells might multiply to form a lump of abnormal tissue called a cancer.

Cancer is a general term applied for series of malignant diseases that may affect different parts of the body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The main forms of treatment for cancer in humans are surgery, radiation and drugs (cancer chemotherapeutic agents). Cancer chemotherapeutic agents can often provide temporary relief of symptoms, prolongation of life, and occasionally cures. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have more side effects. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side effects while undergoing treatment. Synthesis of modifications of known drug continues as an important aspect of research. However, a vast amount of synthetic work has given relatively small improvements over the prototype drugs. There is a continued need for new prototype, new
templates to use in the design of potential chemotherapeutic agents: natural products are providing such templates. Recent studies of tumor-inhibiting compound of plant origin have yielded an impressive array of novel structures. Many of these structures are extremely complex, and it is most unlikely that such compounds would have been synthesized in empirical approaches to new drugs (Tyler, 1994).

In the present study to evaluate the Anti-tumour activity of *Erythrina mysorensis* and for its isolated constituents, Ehrlich Ascitic Carcinoma (EAC) mouse model has been employed. Ehrlich tumour is rapidly growing carcinoma with very aggressive behavior (Segura *et al.*, 2000). It is able to grow in almost all strains of the mice The Ehrlich Ascitic tumour implantation induces *per se* a local inflammatory reaction, with increase in vascular permeability which results in an intensive oedema formation, cellular migration and a progressive ascitic fluid formation (Fecchio *et al.*, 1990). The ascetic fluid is essential for tumour growth, since it constitutes a direct nutritional source for tumour cells.

The results obtained in the present Anti-tumour study showed that the packed cell volume and the number of viable EAC tumour cells in peritoneal cavity were significantly (*< 0.001*) reduced in animals treated with ethanol and chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone and quercetin (Isolated constituents) when compared to the tumour control animals. These results indicate either direct cytotoxic effects on tumour cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition. (Table-22)

The reliable criteria for evaluating an anticancer drug are prolongation of lifespan of the animal and decrease in WBC count of blood. Results have shown an increase in lifespan accompanied by a reduction in WBC count in ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) treated mice. They exerted significant (*< 0.001*) effect on increasing the life span of ascities tumour bearing animals, inhibited the increased body weight due to tumour burden and also found to reduce the viable EAC cells in animal models when compared to the EAC control. These results clearly demonstrate the Anti-tumour effect of ethanol,
chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) against EAC.

The anaemia encountered in tumour bearing mice is mainly due to reduction in RBC and hemoglobin and this may occur either due to iron deficiency or hemolytic or myelopathic conditions (Hogland, 1982). Treatment with ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) restored the hemoglobin content, RBC and WBC cell count to normal values. This indicates that ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) possesses protective effect on the haematopoietic system. A regular and rapid increase in Ascitic fluid volume was observed in EAC bearing mice. Ascetic fluid is the direct nutritional source for tumour growth because it meets the nutritional requirements of tumor cells (Shimizu, 2004). Ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) treatment reduced the number of viable cancer cell count and increased the lifespan. It may be suggested that ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) can reduce the nutritional fluid volume and thereby arrests tumour growth and increases the life span.

It was reported that the presence of tumours in the human body or in experimental animals is known to affect many functions of the liver. The significantly elevated levels of AST, ALT and ALP in serum of tumour inoculated animals indicated liver damage and loss of functional integrity of cell membranes (Gupta, 2004). In the present study significant (< 0.001) reversal of these changes towards the normal conditions by ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) treatment in most of the cases demonstrated antioxidant nature of Erythrina mysorensis extract. The antioxidant nature of Erythrina mysorensis extract was also evident by the in vitro studies. Preliminary phytochemical analysis of this extract showed the presence of phenolic contents is known to possess strong antioxidant
properties. Reduction in the levels of these towards the respective normal values in liver is an indication of hepatic tissue damage caused by tumour inoculation (Lee, 2004).

The earlier reports indicate that the triterpenoids, flavonoids and glycosides possess potent Anti-tumour properties (Kintzios, 2006, Viswanatha, 2010, Kannan, 2010, Kumar, 2010, Melinda, 2010). Accordingly, in the present study as well the chloroform and ethanol extracts have exhibited potent Anti-tumour properties. Interestingly, triterpenoid and flavonoids are isolated from the chloroform and ethanol extracts i.e. lupeol from chloroform extract and quercetin, chalcone derivative from ethanol extract. Several workers have also suggested that chalcones (Stoll et al., 2001), quercetin (Sharififar et al. 2009), lupeol (Prachayasittikul et al. 2010) have Anti-tumour properties.

Quercetin, a flavonol is a potent antioxidant. It has all the right structural features for free radical scavenging activity. Quercetin is able to react with ROS and chelate ROS producing metal ions, both of which allow for decreased oxidative damage preventing this. DNA damage is believed to be the general mechanism by which quercetin is able to prevent tumorigenesis.

Some interesting findings were reported for a series of 2',5'-dihydroxychalcones. Most of the chalcones exhibited cytotoxic activity against a variety of tumour cell lines (B16 murine melanoma, HCT 116 human colon cancer, A31 human epidermoid carcinoma), as well as a non-tumour endothelial cell line (HUVEC human umbilical venous endothelial cells) in the low micromolar range (Nam et al., 2003). The formation of new blood vessels from endothelial cells (angiogenesis) is a prerequisite for solid tumour growth and inhibition of angiogenesis would limit the growth and proliferation of cancer tumours. Thus, this report suggests that chalcones may be angiogenesis inhibitors (Nam et al., 2003).

Lupeol was able to inhibit the lyase activity of DNA polymerase b with an IC50 value of 6.4 μM (Chaturvedula et al., 2004b). Inhibitors of this lyase might be expected to
sensitize cancer cells to DNA-damaging agents and to potentiate their cytotoxicity, being regarded as promising adjuvant drugs to anticancer therapy (Sobol et al., 2000).

Similar observations were made by several workers in the related species of *Erythrina* plants, thus showing the Anti-tumour property.

Ethanol extract of *Erythrina variegata* was investigated for anticancer and *in vitro* cytotoxic activities against transplantable tumors and human cell line. *In vitro* cytotoxicity studies were carried out using Hela and NIH3T3 cells by MTT assay and *in vivo* antitumor activity with Dalton's Ascites lymphoma (DLA). Results showed significant antitumor and cytotoxics effects of EEEV against DLA and human Cancer Cell line which support the ethno medicinal use of EEEV in cancer therapy (Baskar et al., 2012).

Quercetin, kaempferol, morin, myricetin and rutin by acting as antioxidants exhibited several beneficial effects, such as Anti-inflammatory, antiallergic, antiviral as well as an anticancer activity (Robak et al., 1988).

Quercetin inhibit protein tyrosine kinase which is also involved in cell proliferation (Havsteen, 2002; Ramos, 2007; Lamson et al., 2000). Finally, apigenin, luteolin (fig XIX) and quercetin have been shown to cause cell cycle arrest and apoptosis by a p53-dependent mechanism (Rang et al., 2007).

Ghasemzadeh et al., studies have shown that some flavonoids components such as quercetin had anticancer activities and were able to inhibit cancer cell growth (Shariffifar et al., 2009). The investigation of *D. rubra* extracts lupeol has been isolated and reported to have antimicrobial, Anti-inflammatory and anti cancer activities (Prachayasittikul et al., 2010).

Pharmacological actions of quercetin is reported including cardio-protection, cataract prevention, anticancer activity, anti-ulcer effects, anti-inflammatory, anti-allergic, antiviral and antibacterial activities (Bronner and Landrrry, 1985; Stavric, 1994).
Discussion

Recent reports documenting the potential of chalcones interfering at the transcription level by inhibiting the p53-MDM2 interaction (Stoll et al., 2001; Kumar et al., 2003). Chalcones were shown to bind to the tryptophan pocket of p53 binding site of MDM2 (mouse double minute 2) oncogene and to promote dissociation of the p53/MDM2 complex. The MDM2 oncogene is over-expressed in human breast cancer. It inhibits the tumour suppressor protein p53 by binding to the p53 transactivation site, leading to disorganization of the cell cycle. Thus, disruption of the p53/MDM2 complex is considered an attractive target in cancer therapy.

Preliminary phytochemical screening indicated the presence of triterpenoids, flavonoids and glycosides in EASM. 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 (Sousa, 2007, Lotito, 2006). The Anti-tumour properties of ethanol, chloroform extracts (400mg/kg.b.w.) and with lupeol, β-hydroxy chalcone, quercetin (Isolated constituents) may be due to these compounds.

Increased lipid peroxidations would cause degeneration of tissues and lipid peroxide formed in the primary site would be transferred through circulation and provoke damage by propagating the process of lipid peroxidations (Sinclair, 1990). MDA, the end product of lipid peroxidations was reported to higher in carcinomatous tissue than in non-disease organs (Yagi, 1997), and their levels were correlated with advanced clinical stages and the impairment is related to tumor progression (Ahmed, 1999). Moreover, it has been claimed that MDA acts as a tumor promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes (Seven, 1999). Glutathione, a potent inhibitor of neoplastic process, plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process (Sinclair, 1990).

Kumarappan (2007) has shown that the phytochemical and dietary antioxidants are known to decrease the risk of many diseases such as cancer and cardiovascular diseases. In
Discussion

their study polyphenolic extract (PPE) of leaves of *Ichnocarpus frutescens* was evaluated for antitumor activity *in vivo*. Murine Ehrlich ascites carcinoma (EAC) model was used to assess PPE antitumor activity *in vivo*. PPE cytotoxicity was determined *in vitro* in U-937 monocytoid leukemia and K-562 erythroleukemia cell lines. PPE also have been assessed for the free radical scavenging activity against superoxide and nitric oxide radicals. Acute oral toxicity was performed by acute toxic classic method. The total phenolics content was quantified by the Folin-Ciocalteu method. Results of *in vivo* study showed a significant decrease in tumor volume, viable tumor cell count and a significant increase of life span in the PPE treated group compared to untreated one: the life span of PPE treated animals increased by 53.41% (50 mg PPE/kg) and 73.95% (100 mg PPE/kg). PPE (5, 10 and 20 μg/ml) effectively inhibits *in vitro* proliferation of U-937 and K-562 cell lines. PPE exhibited pronounced radical scavenging activity with an inhibitory concentration (IC50) value of 167.46 μg/ml and 158.52 μg/ml against superoxide and nitric oxide radicals, respectively. PPE of *Ichnocarpus frutescens* possesses strong free radical scavenging activity and anti-tumor activity *in vitro* and *in vivo*.

The present results of anti cancerous activity of *Erythrina mysorensis* substantiate the earlier findings that the plant extracts rich in flavonoids exerts both antioxidant and anti cancerous effects. Similarly the isolated constituents such as lupeol, quercetin and β-hydroxy chalcone, also possess these abilities.

6. Anticonvulsant Activity

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterized by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons. The current therapeutic treatment of epilepsy with modern antiepileptic drugs [AEDs] is associated with side-effects, dose related and chronic toxicity. Approximately 30% of the patients continue to have seizures with current AED therapy. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles (Kaushik et al., 2009).
Anticonvulsant activity was performed by using following different methods.

a. Maximal electro shocks (MES) induced convulsions method
b. Pentylenetetrazole (PTZ) induced seizures
c. Locomotor activity

**MES Induced Convulsions Method:**

The maximal electro shocks (MES) is a standard procedure employed to evaluate the testing materials ability to protect against Hind Limb Extension (HLE) in MES. Toman et al., (1914) reported that the seizure pattern in MES for all laboratory animals and man are similar except for time scale. In MES-induced convulsion animals are represented with grandmal type of epilepsy. It has often been suggested that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension phase can be prevented either by drugs that inhibit voltage-dependent Na+ channels, such as phenytoin, valproate, feblamate and lamotrigine or by drugs that block glutaminergic excitation mediated by the N-methyl-D-Aspartate (NMDA) receptor such as feblamate. The chloroform extract of *Erythrina mysorensis* and isolated constituent lupeol exhibited a significant (p<0.001) anti-epileptic activity in MES induced seizures in rats. The extract exhibited maximum protection at 400 mg/kg, p.o. and lupeol (10mg/kg) that was comparable to that of standard phenytoin (90 mg/kg, i.p.). Phenytoin, a standard AED that suppresses HLE is effective in the therapy of generalized tonic-clonic and partial seizures. It limits the repetitive firing of action potentials and this effect is mediated either inhibit voltage-dependent Na+ channels or act as a NMDA antagonist. Protection against HLE in the MES predicts anticonvulsant activity of antiepileptic 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. Since, the chloroform extract and lupeol isolated constituent of *Erythrina mysorensis* showed anti-epileptic activity (< 0.001) in the MES, it may act through any of the above-mentioned mechanisms. (Thomas et al., 2001). Other extracts of *E. mysorensis* at different doses and
also other isolated constituents did not exhibit significant effect as compared to standard drug.

PTZ Induced Seizures:

Pentylenetetrazole (PTZ) is a most frequently used substance as well as an acute experimental model in the preliminary screening to test potential anticonvulsant drugs and it is produced petitmal type epilepsy. The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the GABA receptor complex. Several biochemical hypotheses have been advanced involving the inhibitory GABAergic system and the system of the excitatory amino acid glutamate and aspartate (Sander et al., 1990). Chloroform extract of *Erythrina mysorensis* bark and isolated constituent lupeol (10mg/kg) also exhibited significant (< 0.001) and delayed onset of clonic-tonic actions and protection from PTZ induced mortality and may be interfering with GABAergic mechanism to exert its anticonvulsant effect. Therefore, 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 accordance with the pharmacological effects of benzodiazepine and highlights the relevance of the putative anti-epileptic effects of chloroform extract. Petroleum ether, ethanol, aqueous extracts of *E. mysorensis* at different doses and also other isolated constituents did not possess any significant effect as compared to standard drug.

Locomotor Activity:

Locomotor activity is considered as an index of alertness, and a decrease 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 of Maharudra, (2010). The results revealed that the chloroform extract of *Erythrina mysorensis* contains biologically active substance(s) that might be acting centrally through the inhibition of dopaminergic pathway or a system linked to this pathway to mediate the potentiation of the cataleptic scores. The anti-psychotic potential of these extracts need further investigation since drug used in the treatment of various psychosis abolished apomorphine-induced stereotyped behavior and increasing the
catalepsy effect of HAL. The Phytochemical tests of the extract revealed the presence of flavonoids, phytosterols, tannins, phenolic compounds, proteins, fatty acids, carbohydrates, glycosides, stearic acid and palmitic acid. Based on the present state of knowledge of the chemical constituents of the extract, it is possible to attribute with certainty its anti-epileptic and anti-psychotic effects to one or several active principles among those detected in the screening. However, flavonoids, phytosterols, phenolic compounds, citric acid, aminoacids, tannins, fatty acid, are reported in many neuro-pharmacological activities and in different experimental seizure models.

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.

It is suggested that triterpenoids have CNS depressant activity (Al-Rehaily, 2001). Therefore, in the present study result, it recommends that strong antiepileptic activity of chloroform extract. Interestingly, the lupeol which is isolated in the present study exerted the potent antiepileptic activity.

The effect of most of anti-epileptic agents is to enhance the response to GABA (Gamma Aminobutyric Acid), by facilitating the opening of GABA-activated chloride channels. GABA<sub>\alpha</sub> receptors were involved in epilepsy and their direct activation would have an anti-epileptic effect. It is well documented that PTZ-induced convulsions are produced due to diminution of GABA level in brain (Adnaik *et al.*, 2009). Therefore, 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 accordance with the pharmacological effects of benzodiazepine and highlights the relevance of the putative anti-epileptic effects of chloroform extract.

The chloroform extract inhibited locomotor activity to a lesser extent than diazepam, and thus has a better profile for an anti-epileptic effect. The extract has able to
induced motor depressant effect, indicating significant skeletal muscle relaxant and sedative effect of this plant.

The present result can be substantiated by the reports of the antiepileptic activity in related species of *Erythrina* plants.

*Erythrina crista-galli* aqueous and organic extracts of the leaves were tested by the Hippocratic screening test, spontaneous locomotor activity and potentiation of Pentobarbital sleeping time test. Two extracts from all assayed resulted in a statistically significant depression in CNS (Etcheverry *et al.*, 2003). Dantas *et al.* showed that the crude extract of *Erythrina velutina* at lower doses interferes with mnemonic process for different tasks, while at higher doses, the sedative and neuromuscular blocking actions are the main effects. The ethanol extract of *Erythrina indica* leaves has been proved to possess anticonvulsant activity (Nadkarni, 2000).

Al-Rehaily (2001) demonstrated the pharmacological activities of the acetonitrile (MeCN), hexane extracts and isolated pure terpenoidal compound Lupeol from the leaves of *Teclea nobilis*, Delile (*TN*), on inflammation induced by carrageenan and implantation of cotton pellets in rats, the nociceptive response using writhing and tail flick tests and the antipyretic activity in yeast-induced fever were examined in mice. Oral administration of *TN* extracts at doses of 150 and 300 mg/kg and lupeol 5 and 10 mg/kg showed a significant anti-inflammatory activity in rats. The extracts of *TN* and lupeol significantly decreased the number of contractions and stretchings induced by acetic acid and heat-induced pain in mice. The antipyretic effect of extracts and lupeol was also found to be significant. The behavioral observation of animals showed that the hexane extract and lupeol caused CNS depressant activity and did not produce any toxic or lethal effects in animals at various dose levels. The results suggest that the *Teclea nobilis* extracts and lupeol possesses anti-inflammatory, CNS depressant, analgesic and antipyretic activities.

7. **Antianxiety Activity**

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed it is a universal
human emotion, closely allied with appropriate fear presumably serving psychobiological
adaptive purposes (Ross et al., 2006). Anxiety is a normal emotional behaviour. When it is
severe and/or chronic, however, it becomes pathological and can precipitate or aggravate
cardiovascular and psychiatric disorders. Although many drugs are available in allopathic
medicine to treat anxiety disorders, they produce various systemic side effects or exhibit
tolerance upon chronic use. Many plant products have been claimed to be free from side
effects and less toxic than synthetic drugs (Pari et al., 1999).

Antianxiety activity was evaluated by using following different methods.
a. Elevated plus maze test (EPMT)
b. Open field test (OFT)
c. Roto rod test (RRT)

**Elevated plus maze test:**

The fear of height induces anxiety in the animals when placed on the EPM. 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. It is based on the spontaneous or
natural aversive stimuli, i.e., height, unprotected opening and novelty. In EPM Test, the
open arms are more fear provoking than the closed arms. The number of entries and time
spent in closed arms reflects the safety of closed arms with relative fearfulness of open
arms. The reduction in the entries and time spent in open arms indicate high level of fear or
anxiety. The decrease in aversion to open arms is the result of anxiolytic effect, expressed
by the time spent and entries in the open arm (Gopala, et al., 2008). Anxiolytic agents
increase the motor activity, which manifests as increase in the time spent by the animal in
the open arms of EPM. In etiology of most anxiety disorders in various studies have shown
the involvement of GABAergic, serotonergic neurotransmission and in treatment of
anxiety. The adrenergic and dopaminergic systems have also been documented to play a
role in anxiety. Diazepam used clinically as a standard anxiolytic agent, it also employed in
behavioral pharmacology as a reference compound for inducing anxiolytic-like effects.
The anxiolytic action of diazepam is to enhance the response to GABA by facilitating the
Discussion

Opening of GABA-activated chloride channel. GABAA receptors were involved in anxiety and their direct activation would have an anxiolytic effect (Vogel & Vogel, 2002).

Statistical analysis of plus-maze data revealed that, the classic anxiolytic agent diazepam at the dose of 1 mg/kg of body weight significantly increased the activity of mice in open arms. Similarly, chloroform and ethanol extracts of E. mysorensis, at the doses of 200 and 400 mg/kg and isolated constituents lupeol and quercetin (10 mg/kg) significantly increased (< 0.001) 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 (Nishikava et al., 2004, Millan et al., 1997).

Open field test:

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, which occurs due to the separation of animals from its social group and large size of open field apparatus with relative to natural environment where they housed. In such situation animal shows fearful behavior which is identified by its peripheral movement (near the wall) and reduction in its fearless activity such as rearing in the apparatus. Anxiolytic treatments reduce such fearful behavior of animals in open field. Statistical analysis of the data obtained from these 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). The effect shows significant increase (< 0.001) 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.

Rotorod test

Rotorod test was first introduced by Dunhan and Miya as a screening test to assay the neurotoxicity of anticonvulsant and later was reported to predict motor dysfunction.
produced by centrally acting drugs to determine possible alterations in the motor coordination ability of the animal, often caused by the use of sedative and antipsychotic drugs. The monoamines, dopamine, serotonin (5-HT), nor adrenaline and adrenaline in the frontal cortex play crucial roles in the processes involved in the control of mood, cognition and motor behavior function that are compromised in anxiety as well as in depression. In this test the difference in the fall of time from the rotating rod between the vehicle and extract treated groups is taken as an index of 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 (< 0.001) 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 anxiolytic effects of chloroform and ethanolic extract of *E. mysorensis* may be related to their saponins, triterpenoids, tannins and flavonoid content. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavonoids, saponins and tannins possess activity against many CNS disorders (Bhattacharya SK, 1997).

From the previous reports it is learnt that flavonoids and terpenes proved to be active as anxiolytic agents (Ribeiro *et al.*, 2006). In the present study results show that chloroform and ethanol extracts have exerted the anxiolytic activity. This could be due to the presence of lupeol in chloroform extract, quercetin and chalcone derivative in ethanol extract. Interestingly, these isolated constituents quercetin, chalcone derivative and lupeol exhibited the potent anxiolytic property.

Similarly, aqueous-alcoholic extract of *Erythrina veluntina* wild (Fabaceae) grounded stem bark (50-100 mg/kg, p.o.) having flavonoids and terpenes proved to be active as anxiolytic in rats tested employing elevated T-maze (Ribeiro *et al.*, 2006). The ethanol extract of *Erythrina indica* leaves has been proved to possess anticonvulsant activity (Nadkarni, 2000). The *Erythrina Mulungu* Mart. Ex Benth. (Fabaceae) extract (50 mg/kg, p.o.) showed anxiolytic activity in rats employing light/dark transition model.
Alteration of GABAergic transmission has been proposed to be responsible for observed anxiolysis (Onusic et al., 2003).

The results of the study suggested that alcoholic extract of *Erythrina variegata* at doses of 400 and 800 mg/kg exhibited both anxiolytic activity. (Gummalla et al. 2008)

The anxiolytic effects of chloroform and ethanolic extract of *O. sanctum* may be related to their saponins, triterpenoids, tannins and flavonoid content. Studies of *Erythrina valutina* showed that the anxiolytic effect of stem bark is due to flavonoids and terpenes (Ribeiro, 2006). Crude saponins present in Panax giseng are also responsible for antianxiety activity (Carr, 2006). Steroidal saponins, tannins and flavonoids of *Euphorbia nariifolia* produces anxiolysis in mice and rats in EPM (Bigonia, 2005). *Passiflora* species as an anxiolytic/sedative have been conducted and it was noticed that this species possesses significant activity. Pharmacological effect of passion flower is caused, basically, by flavonoid and alkaloid activity (Bourin, 1997). Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea* (Wolfman, 1994). This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors (Medina, 1997; Griebel, 1997; Paladini, 1999).

Putative activity of hydroalcoholic and aqueous infusion extracts of *Echium amoenum* L. was investigated in mice using the rotarod model of motor coordination and the elevated plus maze model of anxiety. The extracts were administered intraperitoneally (*i.p.*) once, one hour before performing the tests. Preliminary phytochemical study of the plant, with standard procedures, showed that it contains saponins, flavonoids, unsaturated terpenoids and sterols. There was no evidence of tannins, alkaloids and cyanogenic glycosides. The hydroalcoholic extract of *Echium amoenum* in the dose range employed (125, 250 and 500 mg/kg) had no significant effect on motor coordination while the aqueous extract (62.5, 125, 250 and 500 mg/kg) disrupted motor coordination significantly. Intraperitoneal injection of aqueous extract (5, 10, 20, 30, 62.5, 80 and 125 mg/kg) showed a significant dose-dependent increase in time spent in open arm (OAT) with no significant change in open arm entries (OAE), closed arm entries (CAE) and total arm entries (TAE). The anxiolytic effect was most evident in 125 mg/kg group. It is almost evident that the
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extract produces its anxiolytic effect in the doses in which no change in motor activity is observable. Comparison of the dose response curve with the anxiolytic dose response of diazepam (0.25, 0.5, 1.0, and 2.0 mg/kg) in the same setting showed that the maximal efficacy of the extract is significantly lower than diazepam. Because of different maximal efficacies we were not able to calculate extract/diazepam potency ratio but it does not seem to be more than 1/100. It is concluded that single administration of aqueous extract of *Echium amoenum* L. produces a significant but mild to moderate anxiolytic effect.

For the present study the methodology and results obtained for anti-anxiety activity of *Erythrina mysorensis* were substantiated by the earlier above mentioned reports.

8. Anthelmintic Property

The earthworm *Pheretima posthuma* is one of the most important soil invertebrate in promoting soil fertility. Its feeding and burrowing activities break down organic matter and release nutrients and improve aeration, drainage, and aggregation of soil. Anatomy and physiology of *Pheretima posthuma* is similar to helminths (Edwards, 1992). Therefore, it was used in order to investigate the activity of *Erythrina mysorensis*. Preliminary phytochemical studies on *Erythrina mysorensis* revealed the presence of flavanoid glycosides, steroids, carbohydrates, alkaloids, tannins, proteins and flavanoids. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. From the result both ethanolic and aqueous extract of the stem bark of *Erythrina mysorensis* show an anthelmintic activity when compared to the standard drug. Each crude extract at the concentration of 0.1, 0.5 and 1.0% produced 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 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, 1995). Chemically tannins are polyphenolic compounds (Smith, 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 could be possible that tannins contained in the extracts of *Erythrina mysorensis* produced similar effects.

In the present study it is shown that the ethanolic and aqueous extracts have the anthelmintic property. It has been suggested by Niezen, (1995) that tannins of plants are responsible to produce anthelmintic property. It is suggested that quercetin is also responsible for anthelmintic activity (Lassi and Kareem, 2011). Further, it is suggested that the anthelmintic property of the ethanolic extract may be because of quercetin, which is confirmed by the isolation of the same in the present study. Possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (Athanasiadou, 2001) or glycoprotein on the cuticle of the parasite and causes death (Thompson, 1995; Mali *et al.*, 2008). In view of the results from crude extracts and the earlier findings, the antimicrobial property of isolated compounds has not been carried out.

The results of anti-helminthetic property are in agreement with the earlier findings suggesting that the presence of tannins in extracts could exert anthelmintic activity.

Similar, results have been made by many workers in the related species of *Erythrina*, thus showing the anthelmintic activity. Jesupillai (2011) reported that the ethanol, chloroform and ethyl acetate extracts of leaves of *Erythrina indica* showed significant anthelmintic activity at the concentration 50 mg/ml and 100mg/ml against *Pheritima posthuma*. Activity was found to be increased with dose (shortest time of paralysis and death was observed at 100 mg/ml) and the activity was comparable to the well known anthelmintic agent Piperazine citrate. Stem bark decoctions of the *Erythrina arborescens* shown anthelmintic activity, Manandhar (1995).

A study carried out by Kosalge *et al.*, (2009) shown that *Erythrina variegate* extract exhibited more potent activity at all concentrations against earthworms (*Ecinia faeted*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascardia galli*).
Comparison of the results as shown in table 48, between the extracts and isolated constituents for their pharmacological activities clearly indicate that pharmacological activities exhibited by ethanol and chloroform extracts of *Erythrina mysorensis* is mainly due to the presence of isolated constituents which are found to be flavanones and triterpenoids in chemical nature. Certainly, all pharmacological actions shown by ethanol and chloroform extracts and isolated constituents can be unambiguously attributed to the antioxidant abilities. Free radicals are reactive molecules involved in many physiological processes such as atherosclerosis, ischemic heart diseases, aging, inflammation, diabetes, cancer, immunosuppression, neuro degenerative diseases (Sumanth, 2006, Jadhav, 2002).

Concerns over the safety of synthetic antioxidants shifted the global interest towards the explorations of antioxidant compounds extracted from several plant species have been reported to possess strong antioxidant activities. Phenolic compounds ubiquitously present in plants, and when plants are consumed as foods, these phytochemicals contributes to the intake of natural antioxidants in the diets of human as well as animals. Out of all the phenolics, the flavonoids belongs to a large family of compounds with a common diphenyl propane structure \((C_6C_3C_6)\) with different degree of hydroxylation, oxidation and substitution. These compounds commonly occur in plants and reported to be most diverse and efficient as antioxidants (Sharma, 2009). The qualitative phytochemical study of *Erythrina mysorensis* (Fam: Fabaceae) indicates the presence of carbohydrates, saponins, steroids, glycosides, phenolic compounds such as tannins and flavonoids. Antioxidant may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease (Gutteridge, 1985). Furthermore, human observational studies also provide very strong support, showing, on one hand that oxidant stress increases clinical progression of disease (Khanzode *et al.*, 2004) and, on the other, a diet rich in antioxidants containing foods reduces the risk of many diseases (Steinmmetz and Poter, 1996).

The overall results of this investigation using the crude extracts and isolated constituents from the ethanol and chloroform extracts clearly indicate the presence of pharmacological properties. Further, the ethanol and chloroform extracts and their isolated constituents viz., lupeol, \(\beta\)-hydroxy chalcone and quercetin have also proved to be stronger.
Discussion

enough to exhibit antibacterial, anticonvulsant, antianxiety and Anti-tumour properties. Thus, all these observations made provide the supportive scientific evidence to suggest that this plant *Erythrina mysorensis* G, (Fam Fabaceae) possess all the qualities of a medicinal plant. Further, confirming the ethno-medical claim of the traditional medicine. Hence, it is hoped that, these studies could certainly pave the way for further investigations leading to the designing and authentification of the herbal drugs most suitably required for CNS disorders and tumours also.

Table-48. Comparison between the Pharmacological Properties of Extracts and Isolated Constituents of *Erythrina mysorensis* G., Stem Bark.

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Pharmacological activities</th>
<th>Different extracts of stem bark <em>Erythrina mysorensis</em></th>
<th>Isolated constituents from</th>
<th>CHCl₃ ext</th>
<th>Et. OH ext.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pet. ether ext. CHCl₃ ext Et. OH ext. Aq. ext.</td>
<td>Luepol β-Hydroxy chalcone Quercetin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>In vitro</em> Antioxidant activity</td>
<td>-ve +ve ++++ve -ve</td>
<td>--- --- ---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>In vivo</em> Anti-inflammatory activity</td>
<td>+++ve +ve ++++ve +ve</td>
<td>+++ve ++++ve ++++ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Wound healing activity</td>
<td>++++ve -ve ++++ve -ve</td>
<td>+++ve +ve +++ase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Antibacterial activity</td>
<td>-ve ++++ve +ve -ve</td>
<td>--- --- ---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Antifungal activity</td>
<td>-ve -ve ++++ve +ve</td>
<td>--- --- ---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Anti-tumour activity</td>
<td>-ve ++++ve ++++ve -ve</td>
<td>+++ve ++++ve ++++ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Anticonvulsant activity</td>
<td>-ve ++++ve -ve -ve</td>
<td>+++ve +ve +ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Antianxiety activity</td>
<td>-ve ++++ve ++++ve -ve</td>
<td>+++ve +ve ++++ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Anthelmintic activity</td>
<td>++++ve ++++ve ++++ve -ve</td>
<td>--- --- ---</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-ve=not potent, +ve=less potent, ++ve=Potent, ++++ve=more potent