CHAPTER 11

DISCUSSION

The use of plant and animal parts for medicine has long being in existence and is widely documented in records in ancient China, India and Egypt. These ancient indigenous practices were discovered by a series of 'trial and error', which then could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies compared to conventional modern medicine (Chopra *et al.*, 1956). In recent times, herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations. A recent survey in the United States of America (USA) indicate an expected 20% annual growth in herbal medicine in the next 5 years (Saxena, 2001) with an estimated 80% of the world population living in the developing countries still relying on plants for health care. In the USA, the total number of visits to unconventional healers in 1988 was 425 million compared with 388 million visits to primary health care physicians, accounting for an estimated $13.7 billion in the unconventional market (Eisenberg *et al.*, 1993). In view of this large dependence on traditional health practices, the World Health Organisation (WHO) recognized the implicit role of herbal medicine in the Alma Mata declaration of Health for All by the Year 2000 A.D. In 1978, WHO approved the use of these natural products (Schillhorn, 1997). More than 60% of approved and pre-new drug application (NDA) candidates are either natural products or related to them, not including biologicals such as vaccines and monoclonal antibodies (Cragg *et al.*, 1997).

Nature apparently optimizes certain compounds through many centuries of evolution. Secondary metabolism has evolved in nature in response to needs and challenges of the natural environment. Nature has been continually carrying out its own version of combinatorial chemistry (Verdine, 1996) over 3 billion years during which bacteria have inhabited the earth (Holland, 1998). Combinatorial chemistry practiced by nature is much more sophisticated than that in the laboratory, yielding exotic structures rich in stereochemistry, concatenated rings, and reactive functional groups (Verdine, 1996). As a result, an amazing variety and number of products have been found in nature. The total number of natural products produced by plants has been estimated to be over 500,000 (Mendelson *et al.*, 1995). One-hundred sixty thousand natural products have been identified, a value growing by 10,000 per year (Henkel *et al.*, 1999). About 100,000 secondary metabolites of molecular weight less than 2500 have been characterized, half from microbes and the other half from plants (Fenical *et al.*, 1993; Berdy, 1995; Roessner *et al.*, 1996). More natural product research is needed to face the challenges of: unmet medical needs, remarkable diversity of structures and activities, utility as biochemical probes, novel and sensitive assay methods; improvements in isolation, purification, and characterization; and new production methods (Clark, 1996).

The traditional medicine is still the mainstay of health care and most drugs come from plants. Although many plants have long been recognized and widely used in Nepalese traditional medicine, some are relatively unexplored and not arrived to mainstream medicine (Bhattarai *et*

al., 2006). Therefore, the search on new drugs must be continued and natural products from plants, microorganisms, fungi and animals can be the source of innovative and powerful therapeutic agents for newer, safer and affordable medicines (Cooper, 2001; Lindequist *et al.,* 2005).

There are many ethno-pharmacological tradition exists in developing countries like India. Many recent entrepreneurs of botanicals have created a vast, frequent, and indiscriminate use of plants and plant extracts by millions of people, generating not only health dangers but also permanent destruction of large areas of the precious and irreplaceable primary rainforest. Proliferation of small, unregulated firms that promote and market the use of hundreds of poorly studied natural remedies. In fact, the majority of plants used in folk medicine, and phytomedicines in general, are traditionally sold over the counter, and lack adequate pharmacological, toxicological, and clinical evaluation (Liebstein, 1927). Thus it makes an urgent need for determination of the efficacy of medicinal plants and their therapeutic usefulness as well as for safety.

It is true that, in contrast to wealthy communities, the use of herbal products in developing regions is not an alternative to other effective medicines, but indeed the only option for primary health care. Therefore, implementation of good practices for production, development, and use of these compounds, as well as their scientific evaluation and separation of toxic products, are of primary importance to public health. In response to these circumstances, the World Health Organization (WHO), in the context of its Resolution WHA 31.33, has recognized the importance of medicinal plants for primary healthcare and has recommended to its Member States the use of a comprehensive approach to medicinal plants (WHA, 1978).

Three plants were selected for this research work. They are *Urtica parviflora* Roxb., family-Urticaceae, *Callicarpa arborea* Roxb., family-Verbenaceae and *Morinda citrifolia* Linn., family-Rubiaceae. These plants were subjected: (i) to evaluation for their bioactivity both *in vitro* and *in vivo*, to justify their use in ethnomedicine; (ii) to isolation and identification of the bioactive principle(s) in pure form. Standard methods were followed for the collection and processing of the plants and their useful parts. The extractions of the plant parts and prescreening of the extracts were done by standard protocols and the universally accepted methodologies, as described in Materials and Methods.

11.1 Phytochemical studies and ethno-medicinal importance

This research work includes the study of the phytochemistry, toxicity, antimicrobial potency and pharmacology of the three above said medicinal plants. These plants are in use in the traditional medicines, which is practiced by the local hill people of Sikkim Himalayan region.

Authentication of the plants was made with the help of qualified scientists of the Botanical Survey of India, Gangtok region, Sikkim, India.

The powdered plant materials were subjected to methanol extraction (70%) in a Soxhlet extractor fitted with a waterbath. The methanol extracts were concentrated, suspended in hot distilled water, cooled and the blast precipitate was filtered off. The water soluble component was fractionated by extracting it successively with petroleum ether, chloroform and acetone. The chloroform soluble fraction was subjected separately to chromatographic analysis in case of *U. parviflora* and *M. citrifolia.* Similarly, the acetone soluble fraction was taken for chromatographic analysis in *C. arborea.* The aqueous, and petroleum ether fraction did not show any positive pharmacological activities under purview of this investigation and was discarded. Using multistep column chromatography technique with various developing phases Compound I, compound II and Compound III were isolated from the above stated fractions of *U. parviflora* leaf, *C. arborea* leaf and *M. citrifolia* root respectively.

11.1.1 *Urtica parviflora* Roxb.

The word 'Urtica' came from the Latin word 'urtic' referring the pain caused in the hand or any part of the body by touching the aerial part having stings (hair). It produces an urticarial inflammatory nettle rash, accompanied by a considerable burning and itching sensation. The rashes may come out in large or small patches, remains for few minutes or several hours and may disappear quite abruptly. Urtication was practiced for the treatment of certain diseases and involved of beating the skin with a bunch of nettles. The result is erythema and whealing but after the third or fourth successive application, the skin ceases to react under fresh contact (BoDD, 2007). The stem fibers are used to make ropes, the leaves are used as fodder, and the young shoots are used as a seasoning substitute for sorrel. This plant is in use in the traditional medicine practiced by local Bungthings (Medicine Practitioners) to treat dislocation and fracture of bones, fever, cold and cough, and liver diseases (Gurung 1, 2002). About 50 species are found in northern temperate regions, a few in tropical and south temperate regions. Amongst them *U. ardens, U. parviflora, U. thunbergiana, U. fissa, U. mairei, U. dioica, U. angustifolia, U. urens, U. atrichocaulis, U. taiwaniana, U. laetevirens, U. hyperborean, U. cannabina, U. triangularis* are important species. *Urtica parviflora* is confused with *U. ardens*, and *U. dioica* but that species have denser, setulose indumentum and ovate leaf blade with the surface conspicuously wrinkled and the margin sharply doubly serrulate (Jiarui *et al.*, 2003). Cooked tender leaves of stinging nettles are eaten not only in India but in several countries (Hadjichambis *et al.*, 2007).

11.1.2 *Callicarpa arborea* Roxb.

This plant is called 'Sunga' in Lepcha and 'Guenlo' in Nepali belongs to family *Verbenaceae.* Ethnomedicinally the plant (bark) is used as tonic and carminative, applied in cutaneous diseases, rheumatism and gonorrhoea (Gurung 2, 2002), paste of the bark and leaf applied on scorpion sting area of skin (Anonymous, 1976). The literature review reveals that almost no work on pharmacological activity had been performed on this plant previously.

11.1.3 *Morinda citrifolia* Linn.

The common name of this plant is called 'Indian Mulberry' or 'Noni' and in Nepali it's called as 'Hardikath', belongs to family *Rubiaceae.* The root is cathartic. The leaf is used as febrifuge and tonic; heals wounds and ulcers. The baked fruit is given in asthma and dysentery. The leaf juice is applied to gout externally (Gurung 3, 2002). Traditionally the tender leaves and fruits are used as food. Apart from the traditional use of this plant in Sikkim, it has numerous uses in many countries (Etkin *et al.*, 2003) and it has several pharmacological activities (Wang *et al.*, 2002). People are crazy about this plant. They use it for diabetes, high blood pressure, cancer, and many other illnesses (Abbott, 1985). Noni is a traditional remedy to treat broken bones, deep cuts, bruises, sores, and wounds (Bushnell *et al.*, 1950). Morton gave numerous references for medicinal uses of Noni (Morton, 1992). In addition, Polynesians are reported to have successfully used Noni to treat breast cancer and eye problems.

11.2 Isolation and identification of compounds derived from plant extracts

The preliminary phytochemical group tests indicated the presence of amino acids, proteins, steroids and triterpenoids in *Urtica parviflora.* The column chromatography and TLC studies confirmed the chemical nature of the Compound I. The chemical nature of the isolated compound was further characterized from its physical parameters and spectral (IR, GC-MS, ^{13}C and ^{1}H NMR) data (Faizi *et al.*, 2001; Peirs *et al.*, 2006).

The white shining, needle shaped crystals isolated from *Urtica parviflora* Roxb. yielded (β-sitosterol (Compound I). The UV absorption spectrum showed strong absorption at 492 nm, which implied the presence of steroidal ring in its structure and the UV spectrum of Compound I is similar to the standard spectrum of β-sitosterol with the presence of phenolic aromatic rings. The Melting Point range is within 128.5^{0}-$129.2^{0}C$. The IR spectrum confirmed the presence of hydroxyl group and aromatic ring in the compound. The ^{13}C NMR spectrum showed C-5 and C-6 double bond carbons at δ 122.09 and 138.29 suggesting the sitosterol structure. The fragmentation ion at m/z 414 in its mass spectrum, inferred the compound is corresponding to the molecular formula $C_{29}H_{50}O$ and the compound should be + (–) β-

sitosterol. The structure of the compound was further confirmed by elemental analysis of the available literature of β-sitosterol. All the recorded spectral and elemental analysis and evidence discussed in **Chapter 3** conclusively prove the identity of the isolated compound as **β-sitosterol**. This confirmed that β-sitosterol is one of the major bioconstituent of the plant *Urtica parviflora* Roxb. Presence of this compound is not reported in the available literature.

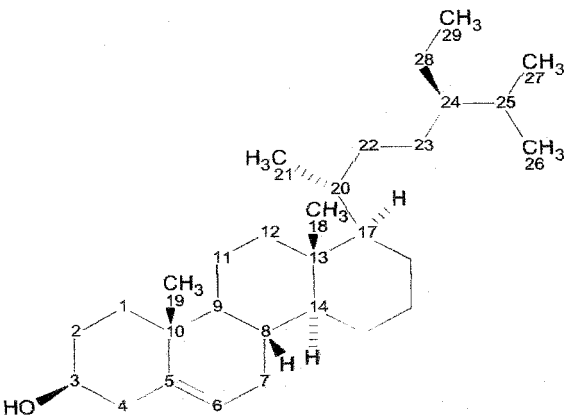

Fig 11.1 Compound I (β-sitosterol) isolated from methanol extract of U. parviflora.

In the second plant, *Callicarpa arborea* Roxb., the preliminary phytochemical group tests indicated the presence of steroids, flavonoids, proteins. It was further subjected to chemical tests, column chromatography and TLC to confirm the chemical nature of the isolated compound, which yielded a triterpenoid glycoside. The chemical nature of the isolated compound was further characterized by comparison of its physical parameters and spectral (UV, IR, Mass, ^{13}C and ^{1}H NMR) data with that of the reported values of triterpenoid glycoside (Alvarez *et al.,* 2003; Abe *et al.,* 2002 and Yoshida *et al.,* 2005).

The colourless amorphorphus powder material was isolated from *Callicarpa arborea.* The compound was melted at 139^{0}–143^{0}C.The UV absorption spectrum showed a significant absorption at 242 nm indicates that the compound is an isoprene derivative (Yu *et al.,* 2003). The IR spectrum confirmed the functional groups of OH (2928 cm^{-1}), COO (1691 cm^{-1}) of COOH, C=C of steroidal moiety; matched with the reference spectrum of Oleanolic acid. The ^{1}H and ^{13}C NMR spectra of Compound II showed that most of the signals of the aglycone were in good agreement with literature data of oleanolic acid (Kubota *et al.,* 1968). The ^{13}C NMR showed the presence of carbonyls and hydroxyl groups and the fragmentation ion at 248 (m/z) in its mass spectrum inferred the compound is having the molecular formula of $C_{30}H_{48}O_3$ and ascertained the Oleanolic acid moiety. From these data, it is concluded that the structure of the isolated triterpenoid is of **Oleanolic acid**.

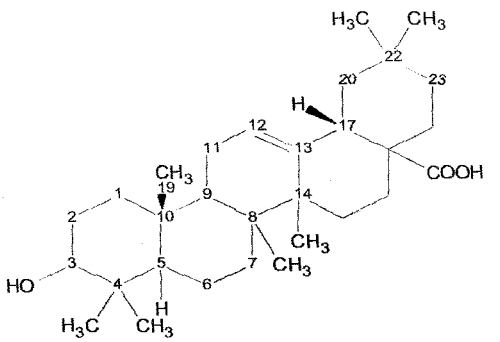

Fig 11.2 Compound II (Oleanolic acid) isolated from methanol extract of *C. arborea.*

The preliminary phytochemical group tests in the extract obtained from the plant *Morinda citrifolia* Linn. indicated the presence of alkaloids, amino acids, reducing sugars, steroids, triterpenoids and anthraquinones which was further subjected to thin layer chromatographic study and coloumn chromatographic separation of the chloroform fraction to yield an anthraquinone derivative (Compound III). The chemical nature of the isolated compound was further characterized from its physical parameters and spectral (UV, IR, MS, ^{13}C and ^{1}H NMR) data (Rajendran *et al.,* 2007; Ling *et al.,* 2002). The UV spectrum of Compound III is identical with that of anthraquinone glycoside. The IR spectrum shows the presence of absorption bands at 1633, 1614, 1035, 3572, 3636 and 2984 cm^{-1}. The IR spectrum confirmed the presence of OH, OCH_3, C=C, C=O groups in compound III. The proton and carbon NMR of Compound III matched with reference spectra as follows: C-3 at δ 26.69, C-6 δ 16.27, C-8 at δ 113.39 to 161.07. The fragmentation ion at 291 m/z inferred the compound is anthraquinone derivative i.e **1-8 dihydroxy, 3 methyl, 6 methoxy anthraquinone** (Kamiya, *et al.,* 2005; Chan *et al.,* 2005 and Wab *et al.,* 2007).

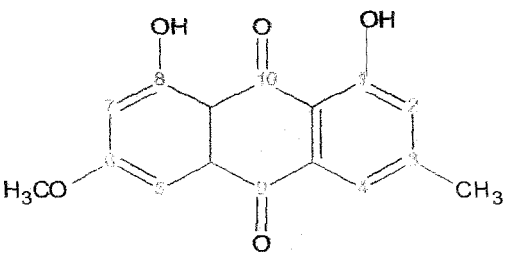

Fig 11.3 Compound III (1-8 dihydroxy, 3 methyl, 6 methoxy anthraquinone) isolated from methanol extract of *Morinda citrifolia* Linn.

11.3 Toxicity Study

The Arithmetical method of Karber, was used to evaluate the acute toxicity in adult albino rats (**Chapter 4**). The animal studies were approved by Institutional Animal Ethics Committee. In this study, ethanolic extracts were used instead of methanol extracts to avoid the toxicity of methanol itself. The LD_{50} (Median Lethal Dose) (Ghosh, 1984) of orally administered ethanolic extracts of leaves of *U.parviflora*, *C. arborea* and root bark of *M. citrifolia* was found to be 3500 mg/kg, 1666.67 mg/kg and 950 mg/kg body weight per oral respectively. *Urtica parviflora* is a commonly used plant in Sikkim which is consumed as cooked food and has low toxicity.

11.4 Hepatoprotective study

Chapter 5 of this thesis deals with the hepatoprotective activity. Liver is the principal organ of metabolism and excretion and is subject to a number of diseases which may be classed as liver cirrhosis (cell destruction and increase in fibrous tissue), acute chronic hepatitis (inflammatory disease) and hepatitis (non-inflammatory condition). The terminal events in the attack on the liver by carbon tetrachloride, which is commonly used as a liver toxicant, involved in the production of a highly reactive radical leading to lipid oxidation and the inhibition of the calcium pump of the microsome, giving rise to liver lesions. A number of plant drugs (cholagogues) are in use to treat biliary disorders and other liver disorders (Evans, 2006). In this study only *Urtica parviflora* was selected for the study. Other two plants i.e. *Callicarpa arborea* and *Morinda citrifolia* were not included because they did not showed significant anti-hepatotoxic activity. Also the ethanolic extract of *Urtica parviflora* was selected as because methanol and any other organic solvent may induce hepatotoxicity.

The results of hepatoprotective effect reveals that the administration of ethanol extract at 250 mg/kg body weight protected the liver partially in CCl_4 induced liver damage in albino rats. The histopathology showed, that the liver necrosis was controlled in rats treated with ethanol extract at 500 mg/kg body weight along with CCl_4. The histology of the livers was found normal when treated with ethanol extract and standard drug Silymarin at 750 mg/kg p.o. and 100 mg/kg body p.o. weight respectively. In these groups, the distribution of Kupffer cells and sinusoidal cells was normal with clear bile canaliculi. To elucidate the biochemical mechanism of hepatoprotective activity of the *U.parviflora* extract, the levels of ALT, AST, ALP, total protein and bilirubin were estimated. Carbon tetrachloride (CCl_4) is the extensively studied liver toxicant and its metabolite, trichloromethyl peroxy radical ($CCl_3O_2^{\cdot}$) is involved in the liver damage. The toxic chemical causes oxidative degradation in the adipose tissue resulting in infiltration of fat into the hepatocytes. The increased level of serum bilirubin reflected the depth of jaundice and the increment in the transaminases and alkaline phosphatase were clear indication of cellular leakage and the loss of cellular integrity of the cell membrane (Sarawat

et.al 1993) as because in hepatocellular injury (e.g. hepatitis), the damaged liver cells develop leaky membranes, allowing for escape of intracellular enzymes into the bloodstream (Green *et al.*, 2002; Pratt *et al.*, 2000). The major intracellular enzymes are aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The results showed that the administration of the test formulation to the animals at a dose of 750 mg/kg body weight returned the elevated enzymes level, total protein and bilirubin near to normal, than that of the animals that received only CCl_4 and found to be closer to the Silymarin (standard drug) treated animals. Oral administration of *U.parviflora* ethanol leave extract revealed significant protection against the hepatic cell damage, in a dose dependent manner up to the dose of 750 mg/kg body weight p.o. The findings of the study provide some scientific basis to the traditional use of *U.parviflora* leaves in the management of hepatic disorders. The data obtained are consistent with the literature on hepatoprotective activity of *U. parviflora* leaves through the studies of enzyme assays and histopathological examination of liver in rats (Gurung, 1999; Kar *et al.*, 2007).

11.5 Wound healing study

Wound care can be traced back to early civilizations, and many of these treatments were based on the use of herbal remedies. Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1–3% of modern drugs (Mantle *et al.*, 2001). Reports about medicinal plants affecting various phases of the wound healing process, such as coagulation, inflammation, fibroplasia, collagenation, epithelization and wound contraction are abundant in the scientific literature (Ulubelen *et al.*, 1995; Choi *et al.*, 2001; Bairy, 2002).

In the wound healing study (**Chapter 6**), three wound models were selected. They are Excision wound model, Incision wound model, and Dead space wound model. The result of excision wound model revealed that the ointments prepared from the extract of *C. arborea* and *M. citrifolia* are comparable to the standard drug Framycetin (ointment) in healing of wound. The epithelization period was also found to be less in Group V, which received MEMC orally (12.6 days) and in Group VII, which received MECA ointment (12.9 days) is similar to the standard drug (Framycetin) treated group (12.5 days). The effect of the test drugs in incision wound model showed that The breaking strength was found to be maximum in Group VIII which received ointment of MEMC (710.00 ± 4.22) and is similar to standard drug Framycetin group (Group II) (712.23 ± 2.84). The other two groups who received ointment of MEUP and MECA (Group VI and VII) also showed better breaking strength as compared to orally fed drugs groups.

Three parameters were studied in dead space wound model, namely dry granuloma weight; breaking strength and estimation of hydroxyproline. Animals of group VIII treated with ointment of MEMC showed maximum dry granuloma tissue weight (72.01 ± 1.19 mg/100g) which is much higher than that of the standard drug Framycetin treated group (62.12 ± 0.38 mg/100g).The MEUP ointment treated group (Group VI) showed the same dry granuloma tissue weight with that of the standard drug group i.e. (62.12 ± 0.38 mg/100g) as compared to the control group, which showed the lowest dry granuloma tissue weight (26.32 ± 0.41 mg/100g). The MEMC ointment treated group (Group VIII) also showed maximum breaking strength (600.13 ± 4.36g) amongst all the test groups and is comparable to standard drug treated group (Group II) which is found to be 612.13 ± 2.31g). The MEMC ointment treated group also showed maximum formation of hydroxyproline (2397.24 ± 2.01µg/100g) which is comparable to the results found in the standard drug treated group of animals (2439.61 ± 0.87µg/100g) as hydroxyproline and collagen are primarily responsible for the strength of tissues (Harkness, 1961). It reveals that the extract of the roots of *M. citrifolia* is found to be the most effective in healing of wound among the three plant drugs in this model.

The results of the histopathological examinations were recorded in five parameters i.e. Keratinization, Epithelization, Fibrosis, Collagen, and Neovascularisation. Wounds of all ages create an impression of individuality on a microscopic level as specific as a finger print. The microscopy showed that the MECA and MEMC treated groups (Groups VII and VIII) had similar stage of keratinization (4.1 ± 0.09 and 4.1 ± 0.03 respectively) which is comparable to the effect of the standard drug Framycetin in Group II (4.2±0.05). Similarly the MEMC ointment treated group showed maximum epithelization (4.2 ± 0.26) comparable to the standard drug treated group (4.3 ± 0.14). The stage of fibrosis was highest in case of *Morinda citrifolia* ointment, compared to *U. parvifolia* and *C. arborea* (4.0 ± 0.13 and 4.0 ± 0.12). The collagen formation was maximum in Group VII (4.4± 0.16), which received MECA ointment amongst the test groups and is comparable to the standard drug treated group i.e. Group II (4.5 ± 0.17). The stage of neovascularization in MECA ointment treated group was found to be 4.4±0.07 which is similar to the standard drug treated group (4.4± 0.09). The value in MEUP ointment treated group is also very appreciable i.e. 4.3±0.08, and is comparable to the standard drug treated group and is much higher than that of the control group (0.6+0.07). Thus the ointment forms of the extracts of two plants *U. parvifolia* and *C. arborea* were found to have potent wound healing activity.

It can be concluded from this study that, the three plants have significant wound healing activity. The test drugs used in this study showed variability in different parameters. The MECA 5%w/w and MEMC 5%w/w ointments have high incision breaking strength. The incision breaking strength depends upon the formation of collagen in the skin. The great bulk of

collagen is formed and gain in tensile strength of healing skin is achieved within 18 days, and the wound reached its maximal strength at the end of this time (Howes *et al.,* 1929). During fixation of wounds with formalin, it was taken care of the factor that, formalin has the potency to increase the breaking strength of wounds (Levenson *et al.,* 1965). Also there were variations in the phase of the skin cycle as well as in skin thickness observed in the study (Butcher, 1934; Chase *et al.,* 1953; Randall *et al.,* 1954; Strauss *et al.,* 1953). The highest wound healing activity showed by the ointment prepared from the methanolic root bark extract of *Morinda citrifolia* may be due to the presence of the anthraquinone moiety i.e. **1-8 dihydroxy, 3 methyl, 6 methoxy anthraquinone.**

11.6 Antioxidant study

The **Chapter 7** deals with the antioxidant study. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable dimagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption at 517 nm. DPPH radical reacts with suitable reducing agents and then by accepting an electron becomes paired off and the solution loses colour stoichometrically with the number of electrons taken up (Nagai *et al.,* 2003). Such reactivity has been widely used to test the ability of compound to act as free radical scavengers. Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. The effect of the methanol extract of *U. parviflora* and Compound I in DPPH scavenging model revealed that the extract of *U. parviflora* at 1000 µg/ml concentration showed an inhibition to DPPH reduction by 75.56 % as compared to the inhibition produced by Vitamin E (85.23 %). Compound I at 50 µg/ml showed higher inhibition of DPPH radical, 82.17% compared to the standard drug Vitamin E i.e. 85.23 % at 5 mM concentration. The percentage of activity was found to be time dependent.

The results of ferrous sulphate induced lipid peroxidation, showed that methanol extract of *U. parviflora* leaf at 1000 µg/ml concentration produced maximum percentage inhibition (71.54 %) of lipid peroxidation as compared with the standard antioxidant Vitamin E, while compound I at 50 µg/ml showed an inhibition of 80.19 % similar to the inhibition produced by Vitamin E i.e. 82.01%. The inhibition could be attributed to the prevention of ferryl-perferryl complex or by change of the Fe^{3+} / Fe^{2+} ratio or by reduction of the rate of conversion of ferrous to ferric or by chelation of iron itself or combination thereof (Braugghler *et al.,* 1986).

Superoxide radical O_2^- is highly toxic species, which is generated by numerous biological and photochemical reactions. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyse the breakdown of superoxide radical (Govindarajan *et al.,* 2003). Reduced phenazine methosulfate assay was used to measure the superoxide dismutase activity of methanol extract of *U. parviflora* and Compound I. The methanol extract showed significant

scavenging capacity of superoxide free radical at a concentration of 1000 µg/ml by 60.18 %. The Compound I at 50 µg/ml concentration showed maximum inhibition of superoxide radicals (69.01 %) as compared with standard drug Vitamin E (70.2 %) at 5mM concentration.

The results of antioxidant activity of methanol leaf extract of *C. arborea* on ferrous sulphate induced lipid peroxidation showed that the extract inhibited the lipid peroxidation in a dose dependent manner. Compound II at 50 µg/ml concentration exhibited 75.01% inhibition, as compared to the inhibition produced by vitamin E (78.26%) at 5 mM concentration. The DPPH scavenging capacity of the extract was found to be 71.22% at the maximum tested concentration (1000 µg/ml). While the Compound II at 50 µg/ml exhibited 73.65% inhibition compared with 83.59% for the standard drug vitamin E at 5mM. In the superoxide radical scavenging activity, results indicated that the scavenging capacity of the extract was 61.81% at 1000 µg/ml concentration as compared with standard drug Vitamin E (66.38 %) at 5 mM concentration. The Compound II, at 50 µg/ml exhibited similar inhibition of superoxide radicals, as compared with the standard drug (66.28%). IC_{50} was found to be 891.01µg/ml. The inhibition was proportional to the amount of the extract added.

The results of antioxidant activity of methanol root extract of *M. citrifolia* on ferrous sulphate induced lipid peroxidation showed that the extract inhibited the lipid peroxidation in a dose dependent manner. Compound III at 50 µg/ml concentration exhibited 68.38 % inhibition, as compared to the inhibition produced by vitamin E (74.61%) at 5 mM concentration. The DPPH scavenging capacity of the extract was found to be 74.67% at the maximum tested concentration (1000 µg/ml). While the Compound III at 50 µg/ml exhibited 74.81% inhibition compared with 81.34 % for the standard drug vitamin E at 5mM. In the superoxide radical scavenging activity, results indicated that the scavenging capacity of the extract was 56.63% at 1000 µg/ml concentration as compared with standard drug Vitamin E (67.33 %) at 5 mM concentration. The Compound III, at 25 µg/ml exhibited similar inhibition of superoxide radicals, as compared with the standard drug (67.18%). IC_{50} was found to be 895.6µg/ml. The inhibition was proportional to the amount of the extract added.

The methanol extracts of *U. parviflora* leaf *C. arborea* leaf and *M. citrifolia* root as well as Compound I, Compound II and Compound III showed significant antioxidant effects in concentration dependent manner in all the models tested.

11.7 Hypoglycemic study

Diabetes mellitus has a high prevalence, morbidity and mortality rate worldwide and is regarded as an incurable but controllable disease. Many synthetic drugs, plant remedies and dietary traditions are in use to minimize the suffering that it causes. The potential role of medicinal plants as hypoglycemic agents has been reviewed by several authors and is

supported by ethno botanical surveys, and the use in traditional medicines in numerous cultures (Afifi *et al.*, 2005; Grover *et al.*, 2002; Ivorra *et al.*, 1988; Jouad *et al.*, 2001; Li et al., 2004; Yeh *et al.*, 2003; Chhetri *et al.*, 2005).

The results of the antidiabetic activity (**Chapter 8**) on glucose tolerance in streptozotocin induced diabetic rat reveals that, MEUP (methanolic extract of *Urtica parviflora*) succeeded to control the rise of serum glucose level (70.6%) within 1st hour of GTT in streptozotocin induced diabetic rats, followed Compound I treated group (60.0%) and Compound II (isolated from *Callicarpa arborea*) treated group (55.2%). The serum glucose levels between '0' hour and 1 hour are generally compared in these type of studies because there is sudden rise of serum glucose level at the 1st hour after glucose loading in '0' hour. The immediate rise of serum glucose level challenges the efficacy of the test drugs to control it. The CEMC and compound III (isolated from *Morinda citrifolia*) treated groups failed to normalize the rise of serum glucose level. Thus out of the three plants studied, the plant *Urtica parviflora*, was found to have remarkable hypoglycemic activity.

The antidiabetic effect of test drugs as compared to the standard drug, Glibenclamide is very much significant. The percentage reduction of serum glucose level by the MEUP treated group (27.2) is much higher than the standard drug, Glibenclamide treated group (18.5). Also MECA treated group is slightly higher (19.2) than the standard drug treated group. The percentage reduction values are obtained when compared to diabetic control value. The result indicates high efficacy of MEUP and MECA in reducing serum glucose level in diabetic rats.

In the study of hypoglycemic activity of the plant drugs, in normal healthy rats by considering the percentage reduction when compared to the value of the control group, it is revealed that Compoud I and Compound II exhibited same percentage reduction i.e. 14.1%, followed by MEUP and MECA respectively.

It is fairly evident from the studies performed in all the three models that maximum percentage reduction of serum glucose level was found with the MEUP and Compound I, isolated from the same plant *Urtica parviflora*. Hence the plant *Urtica parviflora* is having highest hypoglycemic activity, followed by *Calicarpa arborea* and *Morinda citrifolia* in streptozotocin induced diabetes in rat.

Streptozotocin induced hyperglycemia has been described as an important experimental model to study activity of hypoglycemic agents (Szkudelski, 2001). It selectively destroys the pancreatic insulin secreting β-cells, leaving less active cell resulting in a diabetic state (Szkudelski, 2001; Kamchouing *et al.*, 1998). From this study, it can be inferred that the plant, *Urtica parviflora* and its isolated compound i.e. β-Sitosterol may have restorative activity on

insulin secreting β-cells in pancreas like the standard drug Glibenclamide, which is a sulphonylurea hypoglycemic drug, known to stimulate insulin secretion from the pancreas.

11.8 Antimicrobial study

Now a days, mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug (Cowan, 1999). Synthetic antibiotics used to control infection produces adverse toxicity to host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be prevented or antagonize with herbs (Lin *et al.*, 1989). Herbal molecules are safe, and will overcome the resistance produced by the pathogens since they are in combined form or in pooled form of more than one molecule in the protoplasm of the plant cell. Some herbs have antibacterial and antifungal properties which will be useful to clinical use (Kalemba *et al.*, 2003). Some *in vitro* studies have been conducted and proved that herbal oral liquids can be used clinically to overcome drug resistant strains and different serotype strains of infection (Lu *et al.*, 2002). Antimicrobial drugs have received immense importance in last few decades. The plant derived antimicrobials are structurally different from those, which are isolated from microbes. The antimicrobials of plant origin include flavonoids, essential oils, alkaloids, anthraquinones, triterpenoids etc. One of the main approaches for the discovery of antimicrobials from higher plants is the evaluation of the medicinal plant extracts on pathogenic microbes (Verpoorte *et al.*, 1982).

In this study, the methanol extract of *Urtica parviflora* leaf exhibited a significant antimicrobial activity against 257 stains of Gram-positive and Gram-negative bacteria including MRSC strain. All the three reference MRSC strains of bacteria were found to be sensitive between 256 and 1000 μg/ml concentration of the extract. The results of the antimicrobial spectrum of the leaf extract described in **Chapter 9** showed that, out of 257 bacteria, the growth of 168 isolates were inhibited (65.36%) at a concentration of 128–512 μg/ml. 76 isolates (29.57%) were resistant at <1000μg/ml, while remaining 13 isolates (5.05%) were resistant up to <2000 μg/ml, the highest concentration of the extract tested. The MICs tests revealed that 58 out of 63 Gram-positive bacteria were sensitive between 128 and 256 μg/ml (zone diameter 10–16 mm); while out of 179 Gram-negative isolates, 92 were sensitive between 256-512 μg/ml concentration of the extract (zone diameter 10-14 mm). Hence, it appears that the antimicrobial activity of the extracts was directed both against Gram-positive and Gram-negative bacteria. The isolated Compound I was also tested for antimicrobial activity. The result reveals that all the isolates were sensitive at 128-256 μg/ml concentration of the Compound I except the *Vibrio cholerae* 14033. It was interesting to note that all the MRSC

strains were susceptible to Compound I at concentration of 128-256 μg/ml, while they are resistant to both the standard antibiotics used.

The methanol extract of *Callicarpa arborea* leaf exhibited a significant antimicrobial activity against 257 Gram-positive and Gram-negative bacteria. The results showed that out of 257 bacteria, the growth of 221 isolates (85.99%) were inhibited by the extract at a concentration of 128 – 512 μg/ml. 35 isolates (13.61%) were resistant at <1000μg/ml, while remaining 1 isolate (0.38%) was resistant up to <2000 μg/ml, the highest concentration of the extract tested. The MICs tests revealed that 51 out of 78 Gram-positive bacteria were sensitive between 128 and 256 μg/ml (zone diameter 10–16 mm); while out of 179 Gram-negative isolates, 73 were sensitive between 128-256 μg/ml concentration of the extract (zone diameter 10-14 mm). Hence, it appears that the antimicrobial activity of the extracts was directed against both Gram-positive and Gram-negative bacteria but more sensitive to Gram-negative strains. The isolated Compound II was also tested for antimicrobial activity. The result is reveals that all the isolates were sensitive at 128-256 μg/ml concentration of the Compound II. It was noted that all the MRSC strains were resistant to compound II at concentration of 128 μg/ml, while they are resistant to both the standard antibiotics used.

The methanol extract of *Morinda citrifolia* root exhibited a significant antimicrobial activity against 257 Gram-positive and Gram-negative bacteria including multiresistant *Staphylococcus* (MRSC) strains. All the three reference MRSC strains of bacteria were found to be sensitive within 1000 μg/ml concentration of the extract. The results of the antimicrobial spectrum of the root extract showed that out of 257 bacteria, the growth of 217 isolates (85.99%) were inhibited by the extract at a concentration of 128– 512 μg/ml, 37 isolates (14.39%) were inhibited at a concentration of 1000μg/ml, while the remaining 03 isolates (1.16%) where inhibited at concentration >2000 μg/ml, the highest concentration of the extract tested. The MICs tests revealed that 64 out of 78 Gram-positive bacteria were sensitive between 128 and 256 μg/ml (zone diameter 10–16 mm); while out of 179 Gram-negative isolates, 73 were sensitive between 256-512 μg/ml concentration of the extract (zone diameter 10-14 mm). Hence, it can be inferred that the antimicrobial activity of the methanol extract was directed both against Gram-positive and Gram-negative bacteria. The isolated compound III was also tested for antimicrobial activity. The result revealed that all the isolates were sensitive at 128-512 μg/ml concentration of the Compound III except the *P. auruginosa*. It was observed that all the MRSC strains were susceptible to Compound III at a concentration of 128 μg/ml, while they were resistant to the two standard antibiotics used.

The present investigation therefore reveals that the methanol extracts of *Urtica parviflora* leaf, *Calicarpa arborea* leaf and *Morinda citrifolia* root have a significant degree of antimicrobial activity, which may be due to the presence of Compound I, Compound II and Compound III as evident by the tests. This can explain the rationale for the use of the plants in treating infections in traditional medicine.

11.9 Antiinflammatory and antipyretic study

Inflammation is a homeostatic response to pathogens and tissue injury. Inhibiting such processes may do more harm than good and may be associated with some degree of cellular and organ system toxicity. The use of anti-inflammatory agents should therefore be carefully considered, restricted to a limited time, and followed up with more appropriate therapies to address the underlying cause of inflammation (Mujumdar *et al.,* 2000).

From the result of the carrageenan induced paw edema study, presented in **Chapter 10**; it is evident that the test drugs inhibited the paw edema in dose and time dependent manner. Compound II showed percentage inhibition of 17.39 at the 1st hour, where as the standard drug Ibuprofen had percentage inhibition of 18.26 followed by MECA (16.52). In the 2nd hour maximum inhibition percentage amongst the test drugs treated groups was observed incase of Compound II treated group which was continued for 3rd (48.76) and 4th hour (61.69). It indicates the high efficacy of Compound II as compared to the standard drug Ibuprofen. In other words it can be said that Compound II at the dose of 30mg/kg b.w.p.o. has anti-inflammatory activity in carrageenan induced paw edema in rats similar to the standard drug at the dose of 100mg/kg b.w.p.o. In conclusion out of the three plants the plant *C. arborea* (contains Compound II) has got maximum anti-inflammatory activity.

There was dose dependant reduction in granuloma tissue formation in extract, isolated compouds and Indomethacin treated rats. The activity was found to be statistically significant for the dose ranges used. In this test the standard drug Indomethacin at the dose of 10 mg/kg b.w.p.o. showed maximum (51.94%) inhibition of granuloma formation followed by Compound II (38.20%) at the dose of 30 mg/kg b.w.p.o. and MECA (36.01%) at the dose of 200 mg/kg b.w.p.o. In conclusion, out of three plants the plant *C. arborea* (contains Compound II) has got maximum anti-inflammatory activity in cotton pellet induced granuloma model.

The effect of methanolic extracts of *U. parviflora, C. arborea, M. citrifolia* and their respective compounds on arachidonic acid induced inflammation in rats was found to be statistically significant for the dose ranges used. The anti-inflammatory activity of Compound II (64.06%) at the dose of 30 mg/kg b.w.p.o. is comparable to that of standard drug Phenidone (71.86%)

at the dose of 200 mg/kg b.w.i.p. followed by Compound III (63.20%) at the dose of 30 mg/kg b.w.p.o and MEMC (56.70%) at the dose of 200 mg/kg b.w.p.o..

The effect of MECA, MEMC, MEUP and their isolated compounds on yeast-induced pyrexia revealed that the rectal temperature of 38.19^{0}C at 19 hr was markedly elevated to 40.52^{0}C at the 21 hr and then slowed down for vehicle control. The results showed that the MECA at doses of 200 mg/kg b.w.p.o. caused significant lowering of the body temperature up to 4 hr following administration, as the normal mean temperature 39.20^{0}C at 19 hr was reduced to 38.00^{0}C at 23 hr. While maximum lowering of body temperature was noticed in case of its isolated compound treated group i.e. Compound II treated group, which received 30 mg/kg b.w.p.o. In this group the body temperatures slightly increased at 19hr (37.59^{0}C) from the basal temperature (37.55^{0}C) and slightly go on increasing up to 21 hr (38.46^{0}C) and then decreased to 37.79^{0}C. The compound succeeded to keep the body temperature near normal. The pattern of body temperature was different from other groups. In the paracetamol treated group the body temperature was 38.46^{0}C at 19 hr after the subcutaneous injection of yeast suspension which rose to 38.31^{0}C in the next hour and then slowed down up to 23 hr. It was found that the antipyretic effect of Compound II which is Oleanolic acid (Tang *et al.*, 2000; Jeong *et al.*, 1999) at 30 mg/kg b.w.p.o. is similar to the paracetamol group. The study revealed that MECA and compound II i.e. the plant *C. arborea* possess the maximum antipyretic activity out of the three plants studied. The statistical analysis also revealed that the body temperature differences were significant in this case.

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