CHAPTER - 11

DISCUSSION

An herbal medicine can be defined as a medicinal product consisting of a substance produced by subjecting a plant or plants to drying, crushing or any other processes or of a mixture whose sole ingredients are two or more substances so produced, and water or some inert substances[1]. Herbs are an integral part of nature and play a vital role in humans by preventing diseases. Herbal medicine based upon the premise that plants contain natural substance that can promote health and alleviate diseases. Their increasing use in recent years is clear evidence of public interest in having alternatives to conventional medicines. Scientists have proven to humanity the effective use of some herbs. The development of modern chemistry has permitted the isolation of chemicals from medicinal herbs that have served as drugs or starting materials for the synthesis of many important drugs today[2]. The history of herbal medicine is a great help to our current state where many new diseases are being discovered. We need all the knowledge which can get from natural and herbal medicine in order to be able to find cures and alternatives to otherwise harmful medicines.

Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed[3]. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations are based on plants[4]. However, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants. The use of medicines from plants in the form of local medicine dates back to 4000-5000 B.C. While the medicinal values of these plants are due to the presence of small doses of active compounds which produces physiological actions in the human and animal body .Some of the important bioactive compounds found in medicinal plants are alkaloids, glycosides, resins, gums, mucilages etc[5]. It was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries[6].

Medicinal plants are valuable for modern medicine in four basic ways

a) They are used as sources of direct medicinal agent.
b) They serve as a raw material base for elaboration more complex semi-synthetic compounds.
c) The chemical structure derived from phytoconstituents can be used as models for new synthetic compounds.
d) Plants can be used as taxonomic markers for discovery of new therapeutic compounds[7].

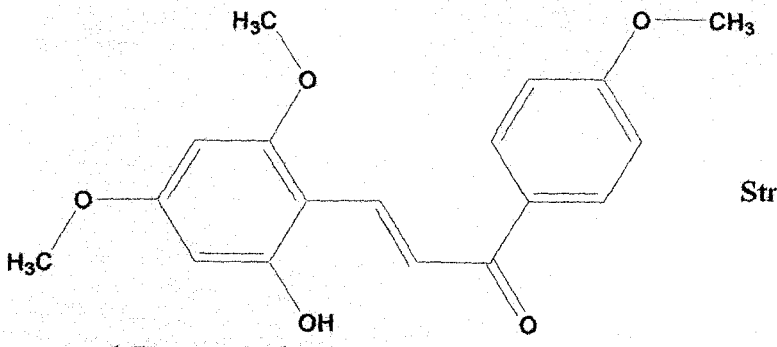
Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. These complex materials are referred to as secondary metabolites. Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect[8,9]. Antioxidants protect other molecules from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the etiology of many diseases and in food deterioration and spoilage[10,11]. Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. Most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds[12].

Present study investigated for the phytochemical, their toxicities and pharmacological activities of two well-known plant used in traditional medicines of the family *Zingiberaceae* and *Asteraceae* from Sikkim Himalayan region. Both of these plants were extensively used by the traditional healer of Sikkim for their primary health care. The present study on the plants was undertaken to evaluate the bioactivity of two plants *K. rotunda* and *E. cannabinum* both *in vitro* and *in vivo* for justification of their use as ethnomedicine and to isolate and identify the bioactive principle(s) in pure form. Standard methods were followed for the collection and processing of the plants and their useful parts. Authentication of the plants was made with the help of qualified scientists

from the BSI, Gangtok branch, Sikkim, India. The extractions of the plant parts and prescreening of the extracts were done by standard protocols and the universally accepts methodologies, as described in materials and methods.

The phytochemical study of the plants was explained in **chapter 3**. The extractions of the plant parts were made at room temperature using methanol as solvent and the collected extracts were concentrated under reduced pressure. The extracts were then concentrated and extracted with distilled water. The water-soluble component was fractionated by extracting it successively with petroleum ether, ethyl acetate. The ethyl acetate soluble fraction was subjected to chromatographic analysis[13]. By using column chromatography technique with various developing phases compound I and compound II were isolated from ethyl acetate fractions respectively. Their structures were determined with the help of spectral analysis like Ultraviolet (UV), Infrared (IR), Proton Nuclear magnetic resonance (^{1}H NMR) and Mass (MS) spectra.

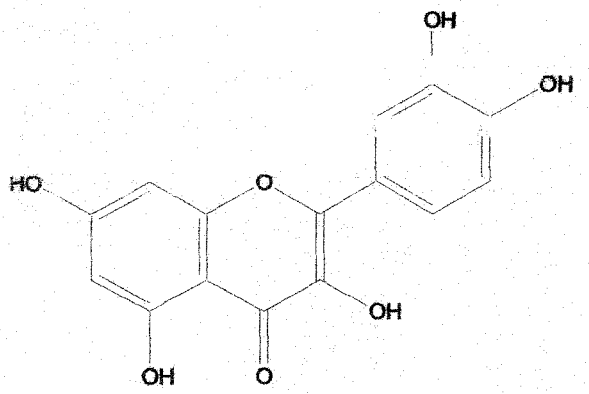
The crystalline material isolated from the *K. rotunda* yielded compound I (chalcone) a flavonoid soluble in water and methanol. The UV absorption spectrum confirmed that presence of phenolic aromatic rings in compound I. The IR spectrum confirmed the presence of hydroxyl group and aromatic ring in the compound. ^{1}H NMR spectrum confirmed that compound I was indicated the presence chalcone i.e. HC=CH–C=O. The molecular ion peak (base peak, M^+) at m/z 312.2670 (calculated for $C_{18}O_5H_{18}$, 314.1154) and the fragmentation peaks at m/z 153, m/z 165 revealed the empirical formula of $C_{18}O_5H_{18}$. The result is corresponding to the molecular formula $C_{18}O_5H_{18}$. It has been concluded that the structure of the isolated compound I was established according to combined spectral data i.e. 2- hydroxy, 4, 4′, 6- trimethoxy chalcone.



Structure of compound I

Compound II was obtained as yellow amorphous powder, partially soluble in water and methanol. The UV absorption spectrum showed two major absorption bands which are

typical for flavonols that confirmed presence of phenolic aromatic rings in compound II. The IR spectrum showed the presence of hydroxyl group and aromatic ring. The ^{1}HNMR spectrum indicated the presence of 1, 2, 3, 5- tetrasubstituted benzene ring. The molecular ion peak (base peak, M^+) at m/z 302 and the fragmentation peaks at m/z 153, m/z 285 revealed the empirical formula of $C_{15}H_{10}O_7$. The UV, ^{1}H NMR and EI-MS data led to the identification of the compound II corresponds to be molecular formula $C_{15}H_{10}O_7$ and the structure of the isolated compound II was 3′,4′,5,7- tetrahydroxy flavonol or quercetin.



Structure of compound II

All the recorded spectral data discussed in **chapter 3** conclusively prove the identity of the isolated compound I as 2-hydroxy 4,4′,6- trimethoxy chalcone and compound II as 3′,4′,5,7-tetrahydroxy flavonol confirmed one of the major bio constituent of the plant. A large number of natural flavonoids with biological activity have been identified in recent decades. One group of these products, the polyhydroxylated chalcones, exhibit antimicrobial antiviral, antitumoral and antiinflamatory activities, and applications of therapeutic effects have been reported. The increase of the bacteriostatic action due to free hydroxyl groups on the aromatic A- and B- rings which showed that the introduction of hydroxyl groups, especially in the 4- and 4′- positions enhance the bioactivity of 2-hydroxychalcone[14-21]. Chalcones are yellow phenolic pigments present in plants. Chalcone is an aromatic ketone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones. They show antibacterial, antifungal, antitumor and anti-inflammatory properties. They are also intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities. Chalcones are also intermediates in the

synthesis of flavones[22]. The flavones are characterized by a planar structure because of a double bond in the central aromatic ring. One of the best-described flavonoids, quercetin, is a member of this group. Quercetin is found in abundance in onions, apples, broccoli, and berries. The second group is the flavanones, which are mainly found in citrus fruit. An example of a flavonoid of this group is narigin. Flavonoids are mainly found in green and black tea and in red wine[23] whereas anthocyanins are found in strawberries and other berries, grapes, wine and tea. An important effect of flavonoids is the scavenging of oxygen-derived free radicals. *In vitro* experimental systems also showed that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties[24]. The aim of this review was to give an overview of the research in the field of flavonoids. The potential valuable working mechanisms of flavonoids are discussed, followed by present knowledge on the absorption, conjugation, and toxicity of these substances. Quercetin is widely distributed in the plant kingdom and is the most abundant in the flavonoid molecules. It is found in many consumed foods, including apple, onion, tea, berries and brassica vegetables as well as many seeds, nuts, flowers, roots, barks and leaves. It is reported for having many beneficial effects on human including cardiovascular protection, anticancer activity, antiulcer effects, anti-allergy activity, cataract prevention, antimicrobial activity, antioxidant activity and anti-inflammatory effects[25,26]. It is reported to inhibit metabolic enzyme systems and P-glycoprotein[27]. Recent reports indicate that quercetin and its glycoside stimulate human peripheral blood leukocyte proliferation and significantly increase the helper T-cells[28].

Determination of acute toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of unknown compound which has been included in **chapter 4** of the present studies. Fifty percent lethal dose or LD_{50} (acute oral toxicity) is performed in Swiss albino mice following standard protocol[29]. The median lethal dose (MLD) of the methanol extract of *K. rotunda* rhizome was found to be 5g/kg body weight in oral route. While the MLD of the methanol extract of *E. cannabinum* was to be 4.5g/kg body weight in oral route. The MLD of isolated compound I was found to be 213.7mg/kg body weight, where as the MLD of the isolated compound II was found to be 196.3mg/kg body weight by oral route. Therefore, the doses of 200mg/kg, 400mg/kg p.o. for extracts and 10mg/kg, 20mg/kg for isolated compounds were fixed to carry out all pharmacological experiments. These selected doses are well tolerated in the system tested

and no untoward effect was observed with the methanol extract of either *K. rotunda* or *E. cannabinum*. The study included several pharmacological activities of the methanol extracts and isolated compounds of *K. rotunda* rhizome and *E. cannabinum* leaf on the basis of their ethnomedicinal uses. The toxicity study indicates that the extract is not toxic at the tested doses.

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. In humans, oxidative stress is involved in many diseases, such as Atherosclerosis, Parkinson's disease and Alzheimer's disease and it may also be important in ageing. In chemical terms, oxidative stress is a large increase (becoming less negative) in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione[30]. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis[31]. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds including quinones into more aggressive radical species that can cause extensive cellular damage[32]. Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart[33,34]. During lipid peroxidation, the end products like MDA and 4-HNE are formed by oxidation of polyunsaturated fatty acids. The MDA can be reacted with two molecules of thiobarbituric acid to give a pinkish red colour complex which is measured at 530nm. Likewise 4-HNE is treated with DNPH solution and the absorbance was taken at 350nm. Increase in MDA and 4-HNE levels of the drug-treated group suggests the occurrence of lipid peroxidation. This process of lipid- peroxidation especially occurs in the presence of some metal ions like Fe^{2+} and other prooxidants[35]. So the decrease in MDA and 4-HNE content of tissue homogenates implies the free radical scavenging property of ascorbic

acid. The increase in MDA content with respect to control when the tissue homogenates were treated with ascorbic acid alone indicates the prooxidant effect of ascorbic acid[36]. The quantification of MDA and 4-HNE can be directly correlated with the lipid peroxidation inhibition capacity of the extracts. The TBARS formation inhibitory effect of the methanolic extract of the plants of *K. rotunda* and *E. cannabinum* were significantly higher than that of control which was discussed in **chapter 5**. The lipid peroxidation inhibition capacity of *K. rotunda* was studied by dose dependent manner and it was concluded that the antioxidant activity has inverse relationship with dose i.e. high at low dose and vice versa. The extract at 100µg/ml and 200µg/ml has significant lipid peroxidation inhibition activity in respect to 500 and 1000µg/ml. The lipid peroxidation inhibition activity of extract of *E. cannabinum* has observed in higher doses. The dose of 100µg/ml and 200µg/ml has insignificant activity whereas 500µg/ml and 1000µg/ml has moderate and significant lipid peroxidation activity respectively.

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of amino acid (hydroxyproline) is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an index for collagen turnover. In **chapter 6** the data obtained revealed that the hydroxyproline content of the granulation tissue of the animals treated with methanol extract was significantly increased when compared to the control group, indicating increased collagen turnover. Increase in breaking strength of granulation tissue of methanol extract treated animals indicated the enhanced collagen maturation by increased crosslinking. In addition, increase in dry granulation tissue weight also indicated the presence of higher protein content. The effect of methanol extracts of *K. rotunda*, *E. cannabinum* and their isolated compounds were screened on excision, incision and dead space wound models concurrently with the control and reference standard framycetin

sulphate cream treated animals. The preliminary phytochemical analysis of methanol extracts of *K. rotunda* and *E. cannabinum* revealed the presence of flavanoids and triterpenoids. Flavonoids 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 fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis. Flavonoids, triterpenoid are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation[37-39]. Thus, wound-healing property of *K. rotunda* and *E. cannabinum* may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. The results of present study indicated that the isolated compound I and II i.e. chalcone and quercetin from *K. rotunda* and *E. cannabinum* respectively promotes significant wound healing activity. This was demonstrated by a significant increase observed in the rate of wound contraction, enhanced epithelialization, increase in hydroxyproline and hexosamine content. Hydroxyproline, the main constituent of collagen serves as a marker of collagen biosynthesis at the wound site. Collagen not only confers strength and integrity to the tissue matrix but also plays an important role in homeostasis and in epithelialization at the later phase of healing. Increased hexosamine content reflects the stabilization of collagen molecules by enhancing electrostatic and ionic interactions[40]. In the present study enhanced levels of collagen and hexosamine in flavones treated rats probably provided the strength to regenerate tissue and aided healing pattern. Histological evidences further supported these results greater degree of epithelialization and fibroblastic deposition observed in flavonoid treated wounds signifies pro-healing effect of the plant material. Significant promotion of wound-healing activity was observed in methanol extracts of both the plants and its isolated compound-I and II in all the three wound models such as excision, incision and dead space wound. In excision model the percentage closure of wound area was significantly increased by the curative effect of methanol extract of tuber of *K. rotunda* and *E. cannabinum* leaf and their isolated compound in the animal group. The data revealed that the rate of wound contraction was significantly high in the animals treated with isolated compound I and II. In incision

wound model the maximum breaking strength was observed in animals treated with isolated compound-I and II followed by methanol extracts of both the plants. In dead space wound model the hydroxyl proline content was found to be more in isolated compound treated animals in case of both the plants. Histological study of the sections of the granuloma tissue of the animals treated with methanol extract and isolated product showed complete epithelialization, increased fibrosis and collagen formation with lesser macrophages, whereas in the isolated compound treated animals the healing activity was comparatively more. From these findings it was concluded that isolated compound-I and II exhibited significant wound healing activity. The extracts as well as isolated compound I and II may be suggested for treating various types of wounds in human beings.

Uncontrolled acid secretion and ulceration of stomach mucosa due to several reasons have posed serious problems to the human health all over the globe[41]. Gastric mucus plays an important role in gastric defensive mechanisms by acting as a protective barrier, mainly because of its glycoprotein content. The water stored by this glycoprotein prevents hydrogen ions from reaching the cell surface[42]. Prostaglandins play an important role in modulating the integrity of the gastric mucosa in the presence of gastric acid secretion[43]. Non steroidal anti-inflammatory drugs can damage the gastrointestinal mucosa by local injury, when surface cells are damaged and allow acid diffusion into the sub mucosa, and by systemic injury, when systemic inhibition of prostaglandin synthesis occurs, thereby reducing gastric mucus production, bicarbonate secretion, and mucosal blood flow[44,45]. NSAIDs also delay the healing of peptic ulcers, interfere with the action of growth factors, decrease epithelial cell proliferation at the ulcer margin, decrease angiogenesis in the ulcer bed, and slow the maturation of granulation tissue[46]. Phytochemical analysis revealed the presence of flavonoids in the methanolic extract. These compounds, which are important for the normal growth, development, and defense of plants[47]. also exert a gastro protective action in mammals by increasing endogenous prostaglandin levels, decreasing histamine secretion, inhibiting *Helicobacter pylori*, and scavenging oxygen-derived free radicals[48]. This gastro protection has been reported for various flavonoids including rutin, naringin, quercetin, kaempferol, sophoradin and luteolin. Rutin reduces the levels of lipoperoxides and increases the activity of the anti oxidant enzyme GSH-PX[49-53]. Then the protective action of flavonoids may be assessed by the stimulation of mucus and bicarbonate secretion and by their

inhibitory effect on the proton pump of parietal cells[54]. Recent studies found that different substances from plant sources not only afford gastro protection but also accelerate ulcer healing[55-57]. Peptic ulceration was immensely induced with different intensities in each of the ulcer models. The ulcer index for *K. rotunda* extracts (200 and 400 mg/kg) and isolated compound I (10, 20 mg/kg) in model-A were found to be statistically significant when compared with control for positive control group at 5 % level of significance ($P<0.05$). The ulcer index for *E. cannabinum* extract (200 and 400 mg/kg) and isolated compound II (10mg, 20mg/kg) in model-A were found to be statistically significant when compared with control for positive control group. Similarly the volume of gastric content, total acidity and total acidity output/100 ml of extract treated groups for *K. rotunda* and *E. cannabinum* 200 and 400 mg/kg and their isolated compound had significantly reduced the values when compared with control. All the extract treated animal parametric values were comparable with that of standard group The extracts at the dose level of 200,400 mg/kg and the isolated compound I and II at the dose level of 10, 20 mg/kg showed dose response antiulcer effect. The group 400 mg/kg for both the plant extract showed significant difference when compared with standard drug ranitidine. For another model aspirin induced (model-B) the ulcer index values of methanolic extract of *Kaempferia rotunda* and *Eupatorium cannabinum* and their isolated compounds results compared to control and ranitidine treated groups and clarified then verified and found to be highly significant when compared with control and ranitidine group at 5% level of significance. The results of the present investigation in **chapter 7** showed antigastric ulcer properties of a methanolic extracts of *K. rotunda* and *E. cannabinum* as demonstrated by all experimental gastric ulcer models. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by the different models used in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin, mucosal damage induced by nonsteroidal anti-inflammatory drugs. In effort to search curative and safe agents for the treatment of peptic ulcers in our indigenous medicinal plants for this purpose *Kaempferia rotunda* and *Eupatorium cannabinum* were selected for preliminary screening of their antiulcer in rats.

Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious physical, chemical or microbiological stimulus. It is a complex

process involving various mediators, such as prostaglandins, leukotrienes and platelet activating factor[58]. The major macrophage derived inflammatory mediators such as pro-inflammatory cytokines, tumour necrosis factor-a (TNF- a) and the reactive free radical nitric oxide (NO) synthesized by inducible NO synthase (iNOS), contribute to the development of inflammatory diseases[59]. Thus, inhibition of the excessive production of TNF-a and/or NO could be employed as criteria to evaluate potential anti-inflammatory compounds Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. To give a scientific validation to this plant, an attempt was made to study the anti-inflammatory activity.

Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that is a suitable criterion for evaluation of anti-inflammatory agents[60]. Inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents[61-64]. The development of edema in the paw of the rat has been described[65] as a biphasic event. The initial phase is attributed to the release of histamine and serotonine[66]. The second, accelerating, phase of swelling is due to release of prostaglandin like substance. The presence of prostaglandin E2 in inflammatory exudates from the injected foot can be demonstrated at three hours time period and thereafter[67]. Indomethacin is used as standard reference drug as it is reported to inhibit inflammation by its effect upon plasma exudation associated with carrageenan mediated inflammation[68]. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents and they are releated to COX inhibition, especially COX-2[69]. Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induces accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility[70]. Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility. Indigenous drug systems can be source of variety of new drugs which can provide relief in inflammation. The anti-inflammatory activity of the methanolic extract of *K. rotunda* and *E. cannabinum* against oedema showed

significant anti-inflammatory activity and the results were comparable to that of standard steroidal anti-inflammatory drug. Carrageeenan induced odema is mediated by release of histamine and 5HT followed by the prostaglandin, kinin[71] and has been frequently used to assess the anti-inflammatory effects of natural products[72]. Phytochemical analysis revealed the presence of steroids, flavonoids, tannins and saponnins. Flavonoids which have been shown to exhibit useful anti-inflammatory activity[73] .Flavonoids may act by reducing the effect of inflammatory mediators. They have been shown to inhibit the migration of leucocytes in experimental models. Hence anti-inflammatory activity of the extract might be due to the presence of flavonoids. In **chapter 8** it was revealed that the methanolic extract of *K. rotunda* and *E. cannabinum* exhibited significant ($p<0.05$) anti-inflammatory activity at doses of 200 and 400 (mg/Kg b.w.p.o.). Both the extracts exhibited inhibition in rat paw odema. The present study indicates the potential of these herbal drugs as anti-inflammatory drugs. Such drugs can be explored in various inflammatory diseases. The activity may be attributed to the inhibition of the COX-2 enzyme or inhibition of the activation of transcription factors. It can be concluded that both the extracts have potential to be explored as anti-inflammatory agents due to presence of flavonoids as active constituent.

Pain is a complex phenomenon that is modified by experience, emotion. Pain begins with the activation of receptors known as nociceptors. Once these nerve endings are stimulated, their signals are transmitted to the spinal cord, and then relayed to higher brain centers where the impulse is interpreted. it is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[74-76]. The primary purpose of an analgesic is to relieve pain. For many people, at the first sign of pain headache, migraine, muscle pain, backache, cramps, joint pains, premenstrual syndrome. Herbal pain medicine in the form of an herbal pain killer becoming a popular alternative to traditional pain-relief medication. Many people choose a type of herbal pain killer to relieve them of their pain[77]. Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are drugs, which reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and a drug like paracetamol do not influence body

temperature when it is elevated by factors such as exercise or increases in ambient temperature[78]. Paracetamol has been shown to suppress fever by inhibiting prostaglandin synthetase resulting in the blockade of synthesis of prostaglandin in the brain[79]. This study, intended to investigate the analgesic and antipyretic activities of *K. rotunda* and *E. cannabinum* by studying the effects of methanol extracts of the plants on nociception induced by immersing the tail in a cup of freshly filled water of exactly 55°C, and on fever induced by yeast. Phytochemical screening of the methanolic extract of the plant under investigation shows that it contains triterpenoid[80]. The plant-derived secondary metabolites have, over the years, greatly contributed to our current understanding of the important mechanisms related to the process of pain transmission and treatment[81]. In **chapter 9** the methanolic extracts of *K. rotunda* rhizomes and leaves of *E. cannabinum* on tail immersion method using mice was investigated. The methanolic extract of *K. rotunda* and *E. cannabinum* shows a good degree of analgesic activity at (200 and 400 mg/kg) in comparison with pentazocine (10mg/kg). The analgesic activity exhibited by the methanolic extract may be significant ($p < 0.05$) due to inhibitory effect on histamine, 5-HT and kinin like substance. On preliminary phytochemical screening the methanolic extract of *K. rotunda* and *E. cannabinum* was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception[82] .Hence, the presence of flavonoids in both the extracts may be contributory to the anti-inflammatory and analgesic activities. The plant extract, at doses of 200 and 400 mg/kg body weight, showed significant central as well as peripheral analgesic activity by oral route. Therefore, the current study indicated that the methanol extract *K. rotunda* and *E. cannabinum* have significant central and peripheral analgesic activity. Thus, the extracts could have a good analgesic activity in pain. The effect of methanol extract of *K. rotunda* and *E. cannabinum* on yeast induced pyrexia has been shown in chapter 9. Treatment with the extracts of both the plants at the doses of 200, 400mg/kg body weight and paracetamol decreased yeast induced elevation of body temperature of rats. The results thus obtained from both the standard drug treated group and extracts treated groups were compared with the control group and a significant reduction in the yeast elevated rectal temperature was observed in a dose dependent manner.

Antimicrobial therapy aims to treat infection with a drug to which the causative organism is sensitive. Antimicrobial agents can be administered on a 'best guess' basis, with a sound knowledge of the infectious disease, the most probable pathogen and the usual antibiotic sensitivity pattern of the pathogen. This is called empirical therapy, and contrasts with rational therapy when antimicrobial agents are administered after the sensitivity of the pathogen is established by culture and *in vitro* testing in the laboratory. In general, empirical therapy is undertaken in a majority of situations encountered in dentistry. Antimicrobial agents can either inhibit the growth of microbes (bacteriostatic agents) or kill micro-organisms (bactericidal agents) by a variety of mechanisms. Depending on the concentration of the antibiotic the same drug could act as a bacteriostatic or a bactericidal agent. In general, however, one or more of the following target sites are involved in the process: the cell wall, the cytoplasmic membrane, ribosomes, and nucleic acid replication sites. Large quantities of antimicrobial agents are produced and consumed globally with the simultaneous potential for exerting selective pressure for resistant bacteria. Mechanisms of antimicrobial resistance are inactivation of the drug and altered cell wall permeability: so that the drug is unable to enter the organism. Modification of the active site of the drug: modification of the enzyme or substrate with which the antimicrobial agent reacts enables the organism to function normally despite the presence of the drug[83]. The remarkable powerful and specific activity of anti-microbial drugs is due to their selectivity for specific targets that are either unique to microorganisms or much more important in terms of human use[84]. The antimicrobial compounds mainly isolated from microbes are structurally different from the compounds isolated from plant sources. Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants and quite a number of chemical compounds of plant origin have been shown to possess antimicrobial activities. In diseases of microbial origin, the plants function as a result of antimicrobial activity against the causative agents[85-91]. The antimicrobial of plant source 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. Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinines). The addition of a 3-hydroxyl group yields flavonoids. Flavonoids are also hydroxylated phenolic substances

but occur as a C_6-C_3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes[92,93]. The calculated minimum inhibitory concentration also proved to be effective. The antimicrobial activities of various plants have been reported by many researchers. Phytoconstituents present in plants namely flavonoids, alkaloids, tannins and triterpenoids are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms. In present study a variety of Gram-positive, Gram-negative bacteria were selected for the screening of antimicrobial effect of three selected plant extracts to perceive the antimicrobial spectrum as well to authenticate ethnomedicinal claims[94,95]. The sensitivity tests of the microorganisms with respect to particular concentration (plant extract and antibiotic) were determined by measuring the diameters of zone of inhibition. The methanol extracts of *K. rotunda* and *E. cannabinum* demonstrated significant *in vitro* antimicrobial activity against seven different Gram-positive and Gram-negative bacteria. The disc diffusion test also demonstrated significant degree of antibacterial activity as compared with standard drug ciprofloxacin. It was also observed in **chapter 10** that the crude methanol extract of *K. rotunda* exhibits better antibacterial potency against selected pathogenic Gram-positive and Gram-negative strains as compared to the extract of *E. cannabinum*. A total of seven bacterial strains belonging to both Gram-positive and Gram-negative were screened for synergism effect by the combination of plant extracts with standard antibiotic ciprofloxacin. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment. The present investigation therefore revealed that the methanol extract of *K. rotunda* and *E. cannabinum* have a significant degree of antimicrobial activity, which may be due to the presence of flavonoids in both the plants[96-98].

References

1. Chaudhri RD. *Herbal drug industry*. Eastern publishers, New Delhi, 1996, p 1-5.
2. Edwin E, Sheeja E, Neeraj M, Sudha V and Vinod T. Herbal medicines, *The Indian Pharmacist*, 2005, **4(38)**, 38-41.
3. Edward A. *Pathogenesis Justicia adhatoda: New, Old and Forgotten remedies*. 2001, p 210-220.
4. Shinwari MI and Khan MA. Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. *Pak. J. Forest.*, 1998. **48**, 63-90.
5. Sack RB and Froehlich JL. Berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins. *Infect Immune.*, 1982, **35**, 471-475.
6. Singh RP and Padmavathi P. Modulatory influence of *Adhatodava sica* leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid per oxidation in mice. *Mol. Cell Biochem.*, 2000, **213**, 99-109.
7. Raman N. *Phytochemical techniques*. New India publishing agency. New Delhi, 2006, p 2.
8. Halliwell B and Gutteridge JMC. Free radicals, antioxidants and human diseases: where are we now? *J. Lab. Clin. Med.*, 1992, **119**, 598-620.
9. Farombi EO, Nwamkwo JO and Emerole GO. Effect of methanolic extract of browned yam flour diet on 7,12-Dimetylbenzanthracene (DMBA) and 3-methylcholanthrene (3-MC) induced toxicity in the rat. *Proc. Fed. Afr. Soc. Biochem.*, 1998, **1**, 5-10.
10. Farombi EO. Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacol. Res.*, 2000, **42**, 75-80.
11. Koleva II, Niederlander HAG and Van Beek TA. An online HPLC method for detection of radical scavenging compounds in complex mixtures. *Anal. Chem.*, 2000, **72**, 2323-2328.
12. Hill AF. *Economic Botany*. A textbook of useful plants and plant products. 2nd edn., McGraw-Hill Book Company Inc, New York, 1952.
13. Litchfield JT and Wilcoxon F. A simplified method of evaluating dose effect experiments. *J. Pharmacol. Therpeu.*, 1949, **96**, 99-113.

14. Lorimer SD and Perry N.B. Antifungal hydroxyacetophenones from New Zeland Liverwort *Plagiochila fasciculta. Planta Medica*, 1994, **60**, 386-387.

15. Pappano NB, Centorbi OP and Ferretti FH. Determination of the responsible molecular zone for the chalcones bacteriostatic activity. *Rev. Microbiol.*, 1994, **25**, 168-174.

16. Hu C, Chen K, Shi Q, Kilkus RE, Cheng Y and Hsiung K. Anti-acids agents, Acacetin-7-O-a-D-Galactopyranoside, an anti-HIV principle from *Chrysanthemum morifolium* and a structure-activity correlation with some related flavonoids. *J. Nat. Prod.*, 1994, **57**, 42-51.

17. Mahmood N, Piacente S, Burke A, Khan A and Pizza C. Constituents of *Cuscuta reflexa* are anti-HIV agents. *Antiviral Chem. Chemother.*, 1997, **8**, 70-74.

18. Iwata S, Nishino T, Nagata N, Satomi Y, Nishino H and Shibata S. Anti-tumorigenic activities of chalcones. I. Inhibitory effects of chalcone derivatives on Pi-incorporation into phospholipids of HeLa cells promoted by 12-O-tetradecanoyl-phorbol 13-acetate (TPA). *Biol. Pharm. Bull.*, 1995, **18**, 1710-1713.

19. Makita H, Tanaka T, Fujitsuka H, Tatematsu N, Satoh K, Hara A and Mori H. Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by the dietary flavonoids chalcone, 2-hydroxychalcone and quercetin. *Cancer Res.*, 1996, **56**, 4904-4909.

20. Min B, Ahn B and Bae K. Synthesis and structure-activity relationship of cytotoxic 5,2,5′-trihydroxy-7,8-dimethoxyflavanone analogues. *Arch. Pharm.*, 1996, **19**, 543-550.

21. Williams CA, Hoult JRS, Harborne JB, Greeham J and Eagler J. A biologically active lipophilic flavonols from *Fanacetum parthenium. Phytochemistry*, 1995, **38**, 267-270.

22. O'Neil MJ. The Merck Index: An Encyclopedia of chemicals,drugs and biological.14th edn., Merk & Co., Whitehouse station, NJ, USA, 2006, p 336.

23. Geraets L, Moonen HJJ, Brauers K, Wouters KEFM, Bast A, and Hageman GJ. Dietary flavones and flavonoles are inhibitors of Poly (ADP-ribose) polymerase-1 in pulmonary epithelial cells. *J. Nutr.*, 2007, **137(10)**, 2190 – 2195.

24. Reddivari L, Vanamala J, Chintharlapalli S, Safe SH, and Miller JC. Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through activation of caspase dependent and caspase independent pathways, *Carcinogenesis*, 2007, **28(10)**, 2227-2235.

25. Anonymous. Monograph of quercetin. *Altern Med. Rev.*, 1998, **3(2)**, 140-143.

26. Dhanawat M, Singh GK and Paul A. Pharmacology and potential therapeutic uses of quercetin: A plant flavonoid. *Ind. J. Nat. Prod.*, 2005, **21(2)**, 3-11.

27. Zhang H, Wong CW, Coville PF and Wanwimolruk S. Effects of the grapefruit flavonoid on pharmacokinetics of quinine in rats. *Drug Metabol. Drug Interact.*, 2000, **17**, 351-363.

28. Bhattacharya R, Tulsawani R and Vijayaraghavan R. Effect of trolox and quercetin on sulfur mustard induced cytotoxicity in human peripheral blood lymphocytes. *Ind. J. Pharmacol.*, 2006, **38(1)**, 38-42.

29. Ghosh MN. Toxicity studies. In: Fundamentals of Experimental pharmacology, Scientific Book Agency, Calcutta, 1984, p 153-158.

30. Schafer F and Buettner G. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.*, 2001, **30 (11)**, 1191-212.

31. Lennon S, Martin S and Cotter T. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell Prolif.*, 1991, **24 (2)**, 203-214.

32. Valko M, Morris H and Cronin M. Metals, toxicity and oxidative stress. *Curr. Med. Chem.*, 2005, **12(10)**, 1161-208.

33. Lelli J, Becks L, Dabrowska M and Hinshaw D. ATP convert necrosis to apoptosis in oxidant-injured endothelial cells. *Free Radic. Biol. Med.*, 1998, **25(6)**, 694-702.

34. Lee Y and Shacter E. Oxidative stress inhibits apoptosis in human lymphoma cells. *J. Biol. Chem.*, 1999, **274(28)**, 19792-8.

35. Gutteridge JMC and Halliwell B. *Antioxidants in Nutrition, Health and Disease*. Oxford University Press, Oxford, 1994, p 12.

36. Feller DR, Hagerman LM, Newman HAI and Witiak DT. *Principles of Medicinal Chemistry*. In: Foye WO, Lemke TL, Williams DA, Eds., BI Waverly Pvt. Ltd., New Delhi, 1995, p 523.

37. Getie M, Gebre T, Reitz R and Neubert RH. Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscose*. *Pharmazie*, 2002, **57**, 320-322.

38. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T and Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant *Staphylococcus aureus*. *J Ethnopharmacol.*, 1996, **50**, 27-34.

39. Scortichini M and Pia Rossi M. Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill). *J. Appl. Bacteriol.*, 1991, **71**, 109-112.

40. Clark RAF. Wound repair: Overview and general considerations. In: Clark RA and P.M. Henson PM, Eds., The Molecular and Cellular Biology of Wound Repair. Plenum Press, New York, 1996, p 3.

41. Bandopadhyay D, Biswas K, Bandopadhyay U, Reiter RJ and Banerjee RK. Melatonin protects against stress- induced gastric lesions by scavenging the hydroxy radical. *J. Pineal Res.*, 2000, **29(3)**, 143-151.

42. Motilva V, Lopez A, Martin MJ & Arcon DL. Cytoprotective activity ofcisapride on experimental gastric mucosal lesions induced by ethanol. Role of endogenous prostaglandins. *Prostaglandins*, 1996, **52**, 63-74

43. Curtis GH, Macnaughton WK, Gall DG and Wallace JL. Intra luminal pH modulates gastric prostaglandin synthesis. *Can. J. Physiol. Pharmacol.*, 1995, **73**, 130-134.

44. Wallace JL. Prostaglandins, NSAIDs, and cytoprotection. *Gastroenterol. Clin. J. North Am.*, 1992, **21**, 631-641.

45. Bjorkman DJ. 1996. Nonsteroidal anti-inflammatory drug-induced gastrointestinal injury. *Am. J. Med.*, 1996, **101**, 25S-32S.

46. Schmassmann A. 1998. Mechanisms of ulcer healing and effects of nonsteroidal anti-inflammatory drugs. *Am. J. Med.*, 1998, **104**, 43S-51S.

47. Cody V, Middleton E and Harborne JB. 1986. Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships, Alan R (Eds.), New York, 1986, p 158.

48. Park S, Hahm KB, Oh TY, Jin JH and Choue R. Preventive effect of the flavonoid, wogonin, against ethanol-induced gastric mucosal damage in rats. *Dig. Dis. Sci.*, 2004, **49**, 384-394.

49. Lewis DA. Antiulcer Drugs from Plants. *Chemistry in Britain*, 1992, **28**, 141-144.

50. Di Carlo G, Mascolo N, Izzo AA and Capasso F. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. *Life Sci.*, 1999, **65**, 337-353.

51. Harborne JB and Williams CA. Advances in flavonoid research since 1992. *Phytochemistry*, 2000, **55**, 481-504.

52. Borrelli F and Izzo AA. The plant kingdom asa source of anti-ulcer remedies. *Phytother. Res.*, 2000, **14**, 581-591.

53. La Casa CC, Villegas I, Arcon DL, Motilva V and Calero MJ. Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *J. Ethnopharmacol*, 2000, **71**, 45-53.

54. Cristoni A, Malandrino S and Magistretti MJ. Effect of a natural flavonoid on gastric mucosal barrier. *Arzneimittel forschung*, 1989, **39**, 590-592.

55. Gracioso JS, Vilegas W, Hiruma-lima CA and Brito AR. Effects of tea from *Turneraulmi folia* L. on mouse gastric mucosa support the Turneraceae as a new source of antiulcerogenic drugs. *Biol. Pharm. Bull.*, 2002, **25**, 487-491.

56. Beil W, Birkholz C and Sewing KF. 1995. Effects off lavonoids on parietal cell acid secretion, gastric mucosal prostaglandin production and *Helicobacter pylori* growth. *Arzneimittel forschung*, 1995, **45**, 697-700.

57. Galvin G and Szabo S. Experimental gastric mucosal injury: Laboratory models reveal mechanism of pathogenesis and new therapeutic strategies. *FASEB J.*, 1992, **6**, 825–831.

58. Gryglewski RJ. Molecular mechanisms of inflammation. *Eur. J. Rheumatol. Inflamm.*, 1981, **4**, 153-159.

59. Freeman BD and Natanson C. Antiinflammatory therapies in sepsis and septic shock. *Expert Opin. Investigl. Drugs*, 2000, **9**, 1651-1663.

60. Gibaldi M. *Biopharmaceutics and Clinical Pharmacokineics*. Philadelphia, 1982, p 11-17.

61. Gibaldi M and Perrier D. *Farmacocinetica*, Barcelona, 1982, p 149-162.

62. Goodman Gilman A. The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, 1966, p 144.

63. Rothwell NJ. Eicosanoids, Thermogenesis and Thermoregulation. *Prost. Lts. Ess. Fat. Ac.*, 1992, **46**, 1-7.

64. Di Rosa M, Giroud PJ and Willoughby DA. 1971. Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageenan and turpine. *J. Pathol.*, 1971, **101**, 15-29.

65. Vinegar R, Schreiber W and Hugo R. Biphasic development of carrageenan edema in rats. *J. Pharmacol. Exp. Therapeutics*, 1969, **166**, 96-103.

66. Crunkhon, P. and Meaccock SER. Mediators of the inflammation induced in the rat paw by carrageenan. *Brit. J. Pharmacol.*, 1971, **42**, 392-402.

67. Vineagar R, Traux JF, Selph JH, Johnston PR, Vinable AH and Mckenzie KK. Pathway to carrageenan-induced inflammation in hind limb of the rat. *Fed. Proc.*, 1987, **46**, 118 -126.

68. Maria TZ and Ferreira H. Relationship between edema and plasma exudation in the rat paw carrageenan inflammation. *Infla. Res.*, 1978, **8(6)**, 606-609.

69. Di Rosa M, Giroud PJ and Willoughby DA. Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageenan and turpine. *J. Pathol.*, 1971, **101**, 15-29.

70. Meade CJ, Turner GA and Bateman PE. The role of polyphosphoinositides and their break down products in A23187 induced release of arachidonic acid from rabbit polymorphonuclear leucocytes. *Biol. Che. J.*, 1986, **23**, 425-436.

71. Castro J, Sasame H, Sussman J and Bullette P. Diverse effects of SKF525A and antioxidants on [CCl.sub.4]-induced changes in liver microsomal p-450 content and ethylmorphine metabolism. *Life science*, 1968, 7, 129.

72. Della Loggia A, Tubaro A, Dri P, Zilli C and Del Negro P. The role of flavonoids in the antiinflammatory activity of *Chamomilla recutita*. *Biol. Res.*, 1986, **213**, 481-484

73. Mukherjee PK, Mukherjee K, Bhattacharya S, Pal M and Saha BP. Studies on the anti-inflammatory of *Drymaria cordata* Willd. *Nat. Prod. Sci.*, 1998, **4(2)**, 91-94.

74. Merskey H. Classification of chronic pain, description so chronic pain and definitions of pain terms. *Pain*, 1986, **3**, 1-226.

75. Garrett N and McShane F. The pathophysiology of pain. *AANA Journal*, 1999, **67(4)**, 349-57.

76. Deal CL and Moskowitz RW. Nutraceuticals as therapeutic agents in osteoarthritis. Rheum. *Dis. Clin. North Am.*, 1999, **25(2)**, 379-95.

77. Rothner AD. A practical approach to headaches in adolescents. *Pediatr. Ann.*, 1991, **20(4)**, 200-205.

78. Goodman and Gilman. *The pharmacological Basis of Therapeutics*. 19th edn., New York, Mc Graw-Hill, 1996, p 959-975.

79. Flower R.J and Vane JR. Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). *Nature*, 1972, **240**, 410-411.

80. Joao JB, Scheidt C, Otuki M, Adair RS and Santos RS. Biological activity of plant extracts: novel analgesic drug. *Expert Opinion on Emerging Drugs*, 2001, **6(2)**, 261-279.

81. Rajnarayana K, Reddy MS, Chaluvadi MR and Krishna DR. Bioflavonoids classification, pharmacological, biochemicaleffects and therapeutic potential. *Indian J. Pharmacol.*, 2001, **33**, 2-16.

82. Trease GE and Evans WC. *Pharmacognosy*. 4th edn., ELBS Publication, Bailliere Tindall, London, 1985, p 344.

83. Samaranayake LP and Johnson NW. Guidelines for the use of antimicrobial agents to minimise development of resistance. *International Dental Journal*, 1999, **49**, 189-195.

84. Katzung BG. *Basic and clinical pharmacology*. 7th edn., Appleton & Lange, Stamford, Connecticut, U.S.A., 1998, p 62-67, 242-244.

85. Verpoorte R, Tjenatsoi A, Van-Doorne J and Svedsen AB. Medicinal plants of Suriname: Antimicrobial activity of some medicinal plants. *J. Ethnopharmacol.*, 1982, **5**, 221-226.

86. Akobundu IO and Agyakwa CN. *A handbook of West African Weeds*. International Institute of tropical Agriculture, 1987, p 240, 272, 348.

87. Rocio MC and Rion JL. (1989). A review of some antimicrobial substances isolated from medicinal plants reported in the literature 1978-1972. *Phytother. Rev.*, 1989, **3**, 117-125.

88. Almagboul AZ, Basho AK, Karim A, Salibm TA and Khalid SA. Antimicrobial activity of certain Sudanese plants used in folklore medicine. Screening for antifungal activity VI, *Fitoterapia*, 1988, **59**, 393-396.

89. Corthout B, Piefers L and Cleays M. Antiviral caffeoylester from *Spondias mornbin. Phytochemistry*, 1992, **31**, 1979-1981.

90. Misra TN, Sigh RS, Pandey NS, Prasal C and Singh BP (1992).Antifungal essential oil and a long chain alcohol from *Achyranthisaspera. Phytochemistry*, 1992, **31**, 1811-1812.

91. Hablemarian S, Gray AI and Waterman PG. A new antibacterial sesquiterpene from *Premma oligotrichia. J. Nat. Prod.*, 1993, **56**, 140-143.

92. Dixon RA, Dey PM and Lamb CJ. Phytoalexins: Enzymology and molecular biology. *Adv Enzymol.*, 1983, **55**, 1-69.

93. Taniguchi M and Kubo I. Ethnobotanical drug discovery based on medicine men's trials in the African savanna: screening of east African plants for antimicrobial activity II. *J. Nat. Prod.*, 1993, **56**, 1539–1546.

94. Lutterodt GD, Ismail A, Basheer RH and Baharudin HM. Antimicrobial effects of *Psidium guajava* extracts as one mechanism of its antidiarrhoeal action. *Malaysian J. Med. Sci.*, 1999, **6(2)**, 17-20.

95. Marjorie MC. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 1999, **12(4)**, 564-582.

96. Miles RS and Amyes SGB. *Medical Microbiology*. Livingstone, Edinburgh, New York, 1996, p 151-177.

97. Lamanna C and Shapiro IM. Western blot analysis indicated migration of cytochrome. *J. Bacteriol.*, 1943, **45**, 385-394.

98. Garrod LP, Lambert HP, Grady F and Worth PM. *Antibiotics and chemotherapy*. Livingstone, Edinburgh, New York, 1981, p 405-408.