V. DISCUSSION
DISSCUSSION

One of the phenomena of the last three decades has been the huge increase in use of ‘herbal products’. These can be defined as plants, parts of plants or extracts from plants that are used in healthcare or in combating disease (Mukherjee and Houghton, 2009a). Ethnomedicine may be defined broadly as the use of plants by humans as medicines whereas Traditional medicine is a broad term used to define any non-Western medical practice. Ethnopharmacology is a highly (Newman, 2007) diversified approach to drug discovery involving the observation, description, and experimental investigation of indigenous drugs and their biologic activities. It is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines (anthropology, archaeology, history, and linguistics) that contribute to the discovery of natural products with biologic activity.

In recent times, there have been increased waves of interest in the field of research in natural products chemistry. This level of interest can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural products as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, such as chromatographic techniques - TLC, column chromatography HPTLC, HPLC and GC etc. are invaluable for identifying and isolation of phytopharmaceuticals. Adsorption chromatography has proved particularly important in the isolation and purification of vitamins, hormones, many alkaloids, cardiac glycosides anthraquinones etc. It is commonly employed as a 'clean-up' technique for the removal of unwanted materials from plant extracts prior to assay. Further, use of physical techniques to establish structures of new compounds and to identify known compounds in plant sources ultraviolet, infrared, mass and nuclear magnetic resonance spectroscopy together with X-ray crystallographic and optical rotatory
dispersion methods have all played a significant role in these developments. Various modifications of mass spectrometry (MS) have become of increasing importance for the structural characterization and determination of the active constituents of plants; these include electron ionization MS, chemical ionization MS, field desorption MS, fast atom bombardment MS and electrospray ionization MS (Trease and Evans, 2005). These improved techniques to isolate, purify, and structurally characterize active constituents (Soumya, 2009) are contributing significantly in solving the demand for supply of complex natural products (Clark, 1996). The R & D thrust in the pharmaceutical sector is focused on development of new innovative/indigenous plant based drugs through investigation of leads from the traditional system of medicine (Patwardhan, 2004).

It is somewhat ironic that this ‘return to nature’, as far as medicinal substances are concerned, has occurred at a time when medicine has become increasingly technologically sophisticated, both in the equipment and products used for diagnosis and treatment, and also in the design and research into the mechanisms underlying disease. However, it should not be forgotten that these advances in medicine and therapy are easily available to only a minority in the world as a whole. In many places, mainly in developing countries, but also in pockets in every affluent society, herbal products are the major, if not the only, source of medication, for economic or geographical reasons.

Several different reasons have been put forward for the resurgence of interest in and use of herbal products. These include a reaction against the serious side-effects sometimes observed when orthodox drugs are used, especially the more potent ones; the inability of Western medicine to treat some diseases satisfactorily, especially chronic conditions such as eczema and arthritis, and the generally mistaken idea that ‘natural’ must be better or safe (Mukherjee and Houghton, 2009b).
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Herbal medicinal products occupy a significant place in consumer consciousness in the developed world and are important in healthcare in most developing countries. The value of natural products in this regard can be assessed from: (i) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semisynthetic and total synthetic modification, (ii) the number of diseases treated or prevented by these substances, and (iii) their frequency of use in the treatment of disease. The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more “druglikeness and biological friendliness than totally synthetic molecules,” (Koehn, 2005) making them good candidates for drug development (Balunas, 2005 and Drahl, 2005). Analysis of the sources of new and approved drugs during the period 1981 to 2002 reveals that natural products play a highly significant role in the drug discovery and development process (Jones, 2006). Review of all approved agents during the time frame of more than 25 years from 1981 to 2006 for all diseases worldwide and from 1950 (earliest so far identified) to 2006 for all approved antitumor drugs worldwide reveals the utility of natural products as sources of novel structures, but not necessarily the final drug entity, is still alive and well (Newman, 2007). It is often noted that 25% of all drugs prescribed today come from plants (Farnsworth, 1976 and Raskin, 2004). This estimate suggests that plant-derived drugs make up a significant segment of natural product–based pharmaceuticals. Out of many families of secondary metabolites, or compounds on which the growth of a plant is not dependent, nitrogen-containing alkaloids have contributed the largest number of drugs to the modern pharmacopoeia, ranging in effects from anticholinergics to analgesics and from antiparasitics to anticholinesterases to antineoplastics (Raskin, 2002). Although not as plentiful as alkaloids in the modern pharmacopoeia, terpenoids (including steroids) have made an equally important contribution to human health. They range from Na+/K+ pump-
inhibiting cardiac glycosides (Dewick, 2001), to antineoplastic (Cragg, 1998) to antimalarial (Abdin, 2003), to anti-inflammatory (Goldbach-Mansky, 2006 and Kupchan, 1972). The goals of using plants as sources of therapeutic agents are, a) to isolate bioactive compounds for direct use as drugs, b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, c) to use agents as pharmacologic tools, and d) to use the whole plant or part of it as a herbal remedy.

Despite several problems, one cannot discount the past importance of plants as sources of structurally novel drugs and it provides a great opportunity to the scientists in the field of Natural Product Chemistry, Pharmacognosy, Pharmacology, Ethnobotany and other related fields of life science to come together and work in the direction of getting new drugs from natural sources, especially from Plants for betterment of mankind. Nature is the best combinatorial chemist and till now natural products compounds discovered from medicinal plants (and their analogues thereof) have provided numerous clinically useful drugs. In spite of the various challenges encountered in the medicinal plant based drug discovery, natural products isolated from plants will still remain an essential component in the search for new medicines. In this regard, the results of the pharmacognostic, phytochemical and pharmacological experiments carried out utilizing two different plants *Azima tetracantha* and *Cocculus hirsutus* are discussed in the proceeding pages.

1. PHARMACOGNOSTIC STUDY

The definition and practice of pharmacognosy have been evolving since the term was first introduced about 200 years ago (Kinghorn, 2001 and Samuelsson, 2004), as drug use from medicinal plants has progressed from the formulation of crude drugs to the isolation of active compounds in drug discovery. As practiced today, pharmacognosy involves the broad study of
natural products from various sources including plants, bacteria, fungi, and marine organisms. Pharmacognosy includes both the study of botanical dietary supplements, including herbal remedies (Tyler, 1999 and Cardellina, 2002), as well as the search for single compound drug leads that may proceed through further development before considered as approved medicines. Drug discovery from medicinal plants is most frequently associated with the second of these two endeavors.

Pharmacognosy is closely related to botany and plant chemistry and indeed, both originated from the earlier scientific studies of medicinal plants. As the late as the beginning of the 20th century, the subject had developed mainly in the botanical side, being concerned with the description and identification of drugs both in the whole state and in porodler, and with their history, commerce, collection, preparation, and storage. Such branches of pharmacognosy are still of fundamental importance, particularly for pharmacopoeial identification and quality control purposes, but rapid development in other areas has enormously expanded the subject (Trease and Evans, 2005a).

The plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. Large numbers of plants are constantly being exposed for their pharmacognostic value. Accurate identification of botanicals for use in herbal medicines is both a fundamental and regulatory requirement. Botanical identification is the primary means by which a plant is accurately identified and must be done by someone with the requisite skills, ideally a formally trained botanist. From earliest times until the mid-19th century much attention was given to plant identification, specifically so that adulterations could be avoided. The very early works suffered botanically because of the relative infancy of botany as a science, but the later works were drawn with exquisite detail, providing medical professionals with a relatively high degree of accuracy to ensure the
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identity of their materia medica (Mukerjee and Houghton, 2009b). The increasing demand for herbal medicines both in the developing and developed countries inevitably led to maintaining the quality and purity of the herbal raw materials and finished products. The standardization problem relating to the herbal drugs arises from the complex composition of drugs that are used in the form of whole plant, plant parts or extracts obtained there from. To ensure reproducible quality of an herbal remedy, proper control of the starting material is utmost essential. Usually plants cannot be identified to species using only rhizomes, roots or barks, which for many medicinal plants are the parts found in market. As a result, the development of evermore sophisticated or molecular methods to be employed in quality control has become necessary, especially in such morphologically problematic species.

Such trials require complete characterisation of the raw materials and finished product of the herbal drug being investigated to ensure its identity, purity, quality, consistency, and reproducibility. Without such knowledge, the findings of any clinical trial will be subject to question as well as be irreproducible. Testing methodologies that are applied to medicinal plants include botanical, macroscopic, microscopic, chemical and molecular methods of testing, which provide analytical tools that can be used for full botanical product characterisation.

The Pharmacognostic study of *Azima tetracantha* and *Cocculus hirsutus* under investigation constitute an important feature of organoleptic evaluation. The leaves of *Azima tetracantha* are petiolate, entire and elliptic to oblong in shape. The surface is smooth leathery, sharp-tipped or spiny at the apex and are about 4.86 cm long to 3.31 cm wide. The stem of the plant is low spinous highly branched bush, woody below but with green herbaceous, almost quadrangular.
The microscopic examination of transverse section of the leaf shows upper and lower epidermis with cuticle, the upper epidermis is multilayered featuring the xerophytic nature. The stomata are hypostomatic and actinocytic type of cellular arrangements are seen. The mesophyll has upper palisade and lower spongy parenchyma. Collateral closed cambium is seen in the vascular tissues, which can be stained with phloroglucinol and hydrochloric acid representing the presence of lignin. The microscopy of stem shows outer single layered epidermis followed by outer cortex containing parenchymatous starch sheath and sclerenchymatous cells with bundle sheath. Cambium breaks with number of strips. Pith at the center is parenchymatous.

Leaf surface data of *Azima tetracantha* leaf were found to show stomatal index of 11.53, stomatal number 08.70, palisade ratio 04.48, vein islet number 04.30 and vein termination number 04.40. The leaf powder extractive values of petroleum ether, benzene, chloroform, alcoholic and aqueous were 1.55, 1.25, 1.15, 9.95 and 10.5% respectively. The determination of extractive values with a range of solvents gives information about extractable non polar and polar as well as total extractable plant constituents. The total ash value, acid insoluble ash value and water-soluble ash value was 8.2, 0.16 and 2.09% respectively.

Similarly *Cocculus hirsutus* leaves are alternate, dorsiventral, petiolate, exstuplate, ovate, ovate to oblong or sub lanceolate and base varies from truncate to cordate. Apex is pointed, mucronate or rarely blunt. Apices of lateral lobes are invariably mucronate. Margin is entire, rarely slightly wavy. Both the surfaces of the lamina are tomentose and bear small grey anteriorly directed trichomes, size ranging from 1.2 to 8.8 cm in length and 0.8 to 4 cm in breadth.

The microscopic examination of transverse section shows the epidermal cells appear rectangular and are filled with chloroplast and some
crystalline substances. Palisade and spongy parenchyma form mesophyll. Palisade cells are rectangular and arranged in a single row except near the midrib where two or three layers of such cells are present. Cells of spongy parenchyma are thin walled and vary between elongated to rounded forms. They are arranged roughly in two rows enclosing air space between them. Distributed amongst them are certain elongated excreatory sacs filled with brown substances. The vascular bundles of larger veins are enclosed in parenchymatous bundle sheaths. Outer cells of this sheath tend to become sclerenchymatous. Bundle sheath is enclosed by thin walled hexagonal parenchymatous cells and is arranged in eight to ten layers. Smaller veinlets have also a thin zone of bundle sheath around them. Individual vascular bundles are collateral. The stomata are hypostomatic and anomocytic (ranunculacious) type of cellular arrangements is seen.

The leaf surface data are as follows; stomatal index 07.15; stomatal number 05.60, palisade ratio 08.85, vein islet number 04.40 and vein termination number 02.70. Powders of leaf showed extractive values of 1.26, 1.08, 2.30, 10.08 and 6.35 for petroleum ether, benzene, chloroform, alcoholic and aqueous extracts respectively.

The total ash value, acid insoluble ash value and water-soluble ash value was found to be 7.90, 0.75 and 1.01 respectively.

Pharmacognostic study involving morphological and organoleptic identification is the oldest, simplest and cheapest of all methods, thus to be preferred when its use is feasible along with the other parameters like ash value, extractive value and qualitative chemical tests serve as source of information. Hence, the studies on pharmacognostic parameters are useful tools to determine the purity of plants and to avoid adulteration in the process of commercialization of raw material. The numerical values, stomatal number, stomatal index, palisade ratio, vein islet and vein termination
numbers (Wallis, 1965) which are used for identification purposes are particularly useful in determining the purity and botanical sources of certain drugs of vegetable origin because these values are based upon the microscopic structure of leaves, they are fairly constant for the leaf of any particular plant and are used as reliable character by which the botanical origin of a leaf can be established, especially when concerned with closely related species.

The determination of the amount of any organic and inorganic constituents which may be present in any plant to which its value or therapeutic activity is attributed to proximate values like extractive and ash values (Remingtons, 1980). It is a good indicator of previous extraction of water-soluble salts in the drug or in correct preparation.

In view of establishing the identity and characterizing the plants for their purity, almost all the medicinal plants have been subjected to pharmacognostic evaluation and such measures are indespensible for any prospective pharmacological screening, further drug discovery and development. Hence there are umpteen number of investigations under taken utilizing several medicinal plants viz. Coleus forskohlii (Shrivastava et al. 2002); Actaea racemosa L. (Applequist, 2003), Uncaria tomentosa and Uncaria guianensis (Gattuso et al. 2004), Maytenus ilicifolia, (Duarte and Debur, 2005), Pithecellobium dulce (Shanmugakumar et al. 2006), Gisekia pharnacioides (Musa et al. 2006), Crateva nurvala (Sikarwar, 2009), Annona squamosa Linn. (Sharma et al. 2009) and Holoptelea integrifolia (Padmaja, 2009).

2. PHYTOCHEMICAL INVESTIGATION

In spite of tremendous developments in the field of allopathy during the 20th century, plants still remain as one of the major source of drugs in modern as well as traditional systems of medicine throughout the world. The
therapeutic properties of the medicinal plants are due to the occurrence of active principles, which has to be extracted and screened for medicinal properties. Natural products have played an important role as new chemical entities (NCEs) approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived. Another 20% of NCEs during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products (Newman et al. 2003). Combining these categories, research on natural products accounts for approximately 48% of the NCEs reported from 1981–2002. Natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereocenters that can be challenging synthetically (Clardy and Walsh, 2004; Nicolaou and Snyder, 2004; Peterson and Overman, 2004 and Koehn and Carter, 2005). Many structural features common to natural products (e.g. chiral centers, aromatic rings, complex ring systems, degree of molecule saturation, and number and ratio of hetero atoms) have been shown to be highly relevant to drug discovery efforts (Lee and Schneider, 2001; Feher and Schmidt, 2003; Clardy and Walsh, 2004; Piggott and Karuso, 2004 and Koehn and Carter, 2005). Further more, drugs derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists.

The present research programme on phytochemical investigations of *Azima tetracantha* and *Cocculus hirsutus* have yielded interesting results. The phytochemical experiments of the alcoholic extract of *Azima tetracantha* leaf led to the isolation of five compounds 1, 2, 3, 4 and 5 and were identified as β-sitosterol, friedelin, 16β - Hydroxy betulinic acid, taraxerol and rutin respectively. A report on isolation of β-sitosterol, friedelin and lupeol was carried out by Venkatarao and Prasadrao, (1978). While, isolation of 16β - Hydroxy betulinic acid, taraxerol and rutin in this plant form the first time report.
Similarly, phytochemical investigation of the alcoholic extract of *Cocculus hirsutus* resulted in isolation of four compounds A, B, C and D and identified as stigmasterol, alpha amyrin, Urs-12-ene-3beta-22 beta-diol and beta-sitosterol glycoside respectively. All the above mentioned compounds are reported for the first time. The constituents isolated were characterized by spectral data and are discussed below.

**Compound 1:** showed IR spectrum of absorption band for hydroxyl group at the region of 3299.05 cm$^{-1}$ and trisubstituted double bond at the regions 2949.12 and 1463.87 cm$^{-1}$. The $^1$H NMR spectrum revealed the presence of six tertiary methyl groups at $\delta$ 0.7 to 1.0701, along with a multiplet at $\delta$ 1.5529 to 2.1656 which was assigned to methine protons. In the electron impact mass spectrum of compound, besides molecular ion peak at m/z 414, the major fragment ion peaks were recorded at m/z 397 (M-Me)$^+$ . Other abundant ion peaks were observed at m/z 329 (M-C$_5$H$_7$-H$_2$O)$^+$ , and 303 (M-C$_7$H$_9$-H$_2$O)$^+$ which were characteristic for sterol with double bond at C-5 (Nargis Akhtar, 1992). The presence of ion peaks at m/z 273 and 255 corresponded to (M-side chain) and (M side chain-H$_2$O)$^+$ respectively. Therefore, compound 1 was identified as stigma-5-en-3-ol or 24-ethylcholest-5-en-3-ol which is commonly known as beta-sitosterol. This compound is widely distributed in plants and considered as the most common sterol of higher plant. There are some reports on the isolation of beta-sitosterol from the rhizomes of *Alpinia pinnanensis* (Giang et al. 2005). Lim et al. (2005) isolated this phytosterols from the thorns of *Gleditsia sinensis* and also from many other plant species (Waffo et al. 2006).

The betasitosterol is reported to posseses analgesic (Villaseñor, 2002) and antibacterial (Kabouche, 2005) properties.
Compound 2: The IR (KBr) spectra of the compound 2 revealed band at 1716.10 cm\(^{-1}\) due to C=O stretching; 2927.25 cm\(^{-1}\) due to C-H stretching of CH\(_3\), an absorption at 1389.46 cm\(^{-1}\) due to C-H deformation in gem dimethyl.

The \(^1\)H-NMR (CDCl\(_3\)) spectrum of compound 2 showed the presence 3H at \(\delta\) 0.7265. The resonance in the region \(\delta\) 1.1822 and 1.5333 corresponding to the protons of terminal methyl groups. Further the molecular weight of the Compound 2 was established based on the Fab-mass spectrometry. The molecular ion peak at 426 [M\(^+\)] corresponds to the molecular weight of the compound \(i.e.\) 426.72. Based on the above data and by comparing with spectral data reported in the literature (Kamaya et al. 1990), the compound 2 was identified as friedelin. It is a triterpenoid present in plants. Anjaneyulu et al. (1965) isolated friedelin from G. tiliaefolia; Moiteiro et al. (2001) isolated friedelin form the cork extracts of Quercus suber; Huerta-Reyes et al. (2004) from the leaves of Calophyllum brasiliense; Abe et al. (2004) from the leaves of Garcinia intermedia. The compound has shown fungicidal and bactericidal and insecticidal activities (Moiteiro, 2006).

Compound 3: The infrared spectrum revealed the presence of a hydroxyl group in the regions 3423.23 cm\(^{-1}\) and carbonyl at 1682.07 cm\(^{-1}\). The \(^1\)HNMR spectrum showed five tertiary methyls \(\delta\) 1.2636(Me-23), 1.0823 cm\(^{-1}\) (Me-24), 0.8890 cm\(^{-1}\) (Me-25), 1.1021 cm\(^{-1}\) (Me-26), 1.1723 cm\(^{-1}\) (Me-27) and one vinylic methyl at \(\delta\) 1.7968 cm\(^{-1}\) (Me-30), two protons of an isopropenyl moiety at \(\delta\)4.8926 cm\(^{-1}\). Based on spectral data; the compound was identified as 16\(\beta\) - Hydroxy betulinic acid (Ye Y, 1998). 16\(\beta\) - Hydroxy betulinic acid is a derivative of betulinic acid. It is reported that betulinic acid and their derivatives have antibacterial activity (Perumal, 2005).

Compound 4: The infrared spectrum revealed the presence of a hydroxyl group in the regions 3483.21 cm\(^{-1}\). The \(^1\)H NMR spectrum of compound 4 contained resonances corresponding to eight methyl groups in the region \(\delta\)
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0.8523 to δ 1.1639, all as singlets. The ethylenic proton (H-15) resonated as a double doublet at δ 5.65 and the signal of one exchangeable hydroxy group was observed as a doublet at δ 3.4970. According to the fact that naturally occurring polyoxygenated triterpenoids in general contain an oxygen function at position 3 the single hydroxy group was placed at that position (Agarwal and Rastogi, 1974). The characteristic double doublet signal of the single olefinic proton in the $^1$H NMR spectrum suggested the compound 4 to be a Δ$^{14}$-pentacyclic triterpenoid (Ageta and Arai, 1983). The mass spectrum indicated a molecular ion at $m/z$ 427 [M$^+$ +1] confirming to the molecular formula C$_{30}$H$_{50}$O. From the above evidence compound 4 was designated as taraxerol.

There are some similar reports on isolation of taraxerol from the stem bark extracts using column chromatography with stepwise gradient elution technique. For example, Byung-Sun et al. (2004) isolated taraxerol from the stem bark of Styrax japonica. In their study they chromatographed methanolic extract on silica gel column with a stepwise gradient elution of chloroform and methanol to yield the respective fractions. The initial fractions eluted with chloroform yielded taraxerol. Lie-Chwen et al. (2001) isolated taraxerol using the same method as mentioned above. They isolated taraxerol form the ethanolic extract of stem bark of Ventilago leiocarpa. Further, Shankar and Krishna (2006) and Raja Naika et al. (2007) have isolated taraxerol from the petroleum ether extract of the leaves of Embelia ribes and Naravelia zeylanica respectively. Taraxerol is proven to have antibacterial activity.

**Compound 5**: The IR spectrum showed absorption peak at 3421.4 cm$^{-1}$ (hydroxyl group) 1658.5 cm$^{-1}$ (carboxyl group) and 1598.3 cm$^{-1}$ (C=C). The glycoside on acid hydrolysis afforded aglycone quercetin and sugars-rhamnose and glucose. In $^1$H NMR spectra, the glucose and rhamnosyl signals observed were in consistent with 3, 5, 7, 3', 4' penta substituted flavonoid glycoside. A doublet at δ 1.13 of H-3 proton of rhamnosyl methyl, a multiplet
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at δ 3.2 - δ 3.9 for ten protons can be ascribed for rhamnosyl glucose protons. The LC-MS spectrums displayed the molecular ion peak at m/z 610[M⁺] corresponding to the molecular formula (C_{27}H_{30}O_{16}). Its identity as rutin was further confirmed by m.p, IR, ^1H NMR, mass spectra, hydrolysis study and by co-chromatography with an authentic sample (Sigma Chemical Co., USA).

Flavonoids were reported as active substances for treating hepatitis induced by chemical materials (Khalid et al. 2002) and virus (Kang et al. 2006) in vitro and in vivo. It has been well reported previously that Quercetin and its glycosides in herbal medicine are capable of scavenging peroxidations and protecting hepatocytes. Several flavonoids were separated from effect of *Cistus laurifolius* L. leaf extracts and investigated their effects on acetaminophen induced hepatotoxicity in mice (Kupeli et al. 2006). According to the study, the isolated flavonoids possessed potent hepatoprotective activities and were conjectured as the main active compounds in *Cistus laurifolius* L. Similar results occurred in vitro studies, which evaluated the hepatoprotective activity of isolated flavonoids from *Equisetum arvense*, indicating that flavonoids had the bioactivity of superoxide and free radical scavenging (Oh et al. 2004).

The qualitative analysis of *Cocculus hirsutus* gave positive tests for steroids, triterpenoids and tannins. Triterpenoids steroids and alkaloids were found to be present in chloroform extract. Carbohydrates, tannins, triterpenoids steroids saponins flavonoids and alkaloids were found to be present in ethanolic extract. The aqueous extract gave positive results for carbohydrates, saponins and tannins.

**Compound A:** was shown to be hydroxyl group as indicated by IR spectrum (3351.63 cm⁻¹). The ^1H NMR spectrum of the compound completely corresponded to the data for stigmasterol as reported by Bernard and Tokes, (1977). It showed peaks for two tertiary (δ 0.80 and 0.70), three secondary (δ 0.92, 0.831 and 0.837) and a primary (δ 0.85) methyl group. It further showed
two multiplets at $\delta$ 5.14 (1H) and 5.12 (2H) for three olefinic protons and another multiplet at $\delta$ 2.28 for carbynylic proton. All these physical and spectral data fully confirmed the identity of compound A as Stigmasterol. The molecular formula was established as C$_{29}$H$_{48}$O by Fast Atomic Bombardment mass spectrometry which showed [M] peak at m/z 412. The mass spectrum showed characteristic fragmentation pattern of $\Delta^5$, $\Delta^{22}$ sterol. The spectral characteristics of stigmasterol were similar with the reports of Nargis Akhtar (1992). There are some reports on the isolation of this phytosterols from the other plant species viz. Calotropis gigantea (Rowshanul, 2007), Polyalthia debilis (Supaluk, 2009) and Teucrium manghuaens, (Guihao, 2009). This constituent is known to possess anti-inflammatory antitumour (Yoshimasa, 2006), antihypercholesterolemic (Chandler, 1979) and its activities are almost similar to $\beta$-sitosterol.

**Compound B:** m.p 179-181 °C. It gave a characteristic colour reaction for triterpenoids. The IR (KBr) spectrum exhibited strong absorption at 3444.04 cm$^{-1}$(OH), 2948.83, 2891.32 cm$^{-1}$ (C-H) and 1639.19 cm$^{-1}$ (C=C). FAB mass spectrum displayed the molecular ion peak m/z 426[M$^+$] corresponding to the molecular formula (C$_{30}$H$_{50}$O). The $^1$HNMR spectrum of this compound exhibited the presence of methyl group at 0.8023 (H-24), $\delta$ 0.8897 (C-29H, C-30H), $\delta$ 0.8943 (C-28H), $\delta$ 0.9989 (C-23H), $\delta$ 0.9597 (C-25H), $\delta$ 1.0827 (C-26H), $\delta$ 1.1617 (C-27H) $\delta$ 1.0827 (C-26H) $\delta$ 1.1617 (C-27H) $\delta$ 3.8536 (C-H), $\delta$ 5.3500 (12-H). Therefore the structure of compound B was confirmed as alpha amyrin. It has analgesic (Michel et al. 2005), antiarthritic (Kweifio-Okai, 1994), antilipoxygenase (Kweifio-Okai, 1992) and anti-inflammatory (Safayhi, 1997) activities.

**Compound C:** m.p 194-196 °C It gave a characteristic colour reaction for triterpenoids. The molecular formula was established as C$_{30}$H$_{50}$O$_2$ from FAB-mass spectrum. The FAB-mass spectrum displayed the characteristic retro-Diels-Alder fragment peak at m/z 234 indicating a C-12/C-13 double bond
which suggested an ursane structure substituted by two hydroxyl groups, one located at A/B rings and another on D/E rings. These results are in good agreement with the IR spectrum, which showed absorption bands assigned to the hydroxyl groups (3434.22 cm\(^{-1}\)) and double bond (1642.07 cm\(^{-1}\)). The \(^1\)HNMR spectrum displayed eight methyl signals including six methyl singlets and two methyl doublets confirming an ursane-type triterpenoid structure. In addition, the presence of the olifenic triplet at \(\delta\) 5.1616 and two hydroxyl bearing methane protons exhibiting two double doublets at \(\delta\) 3.2216 and \(\delta\) 3.443. On the basis of the HNMR spectrum of the compound, a beta-configuration of two hydroxyl groups was assigned according to the fact that their respective germinal protons appeared as dd thus confirming axial orientation. Therefore the structure of compound C is identified as Urs-12-ene-3beta-22 beta-diol. It is an ursolic acid derivative and they have known action like anti-infective (Kowalewski, 1976); antineoplastic (Trumbull, 1976) agents, and as cyclooxygenase inhibitors.

**Compound D:** m.p. 279-28\(^0\) C. It gave a characteristic colour reaction for steroidal glycoside. The IR (KBr) spectrum exhibited strong absorption at 3420.23 cm\(^{-1}\) (OH), 2925.83, 2823.08 cm\(^{-1}\) (C-H) 1672.07 cm\(^{-1}\) (C=C). FAB- Mass spectrum displayed the molecular ion peak at m/z 577 [M\(^+\) +1] corresponding to the molecular formula (C\(_{35}\)H\(_{61}\)O\(_6\)). The mass of the aglycone (\(\beta\)-sitosterol) was determined m/z 415 [M- ] indicating the presence of \(\beta\)-sitosterol and glucose unit. The \(^1\)HNMR spectrum signals exhibited the presence of methyl signals at \(\delta\) 0.6824 (H-18), \(\delta\) 0.8524 (H-26) \(\delta\) 0.8843 (H-2, 29), \(\delta\) 0.9597 (H-19), \(\delta\) 1.0233 (H-21) ppm. A signal present at \(\delta\) 4.5870 ppm was assigned to H-6 the signal of the proton of anomeric carbon of glucose unit was found as doublet at 5.2542 ppm. Therefore the structure of compound D was confirmed as \(\beta\)-sitosterol glycoside. It has been documented to possess antiinflammatory, analgesic and anthelmintic activities (Irene, 2002).
3. PHARMACOLOGICAL STUDIES
HEPATOPROTECTIVE ACTIVITY

Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. It is also involved in metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents like paracetamol, alcohol and CCl₄ etc. has been well documented (Pandey and Shrivastava, 1990; Tripathi, 1991 and Wagner, 1986).

Among different hepatotoxins the hepatic damage caused by CCl₄ simulates the human hepatitis leading to cirrhosis (Recknagel, 1983) and it is a model system of toxic injury. Hence, CCl₄ was used as a tool to induce toxic hepatitis in the experimental models. Chronic hepatic diseases stand as one of the foremost health troubles worldwide, with liver cirrhosis and drug induced liver injury accounting ninth leading cause of death in western and developing countries. Therapies developed along the principles of western medicine are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating liver diseases with plant-derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. Infact, one of the important and well-documented uses of plant-products is their use as hepatoprotective agents (Agarwal, 2001). A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multiingredient plant formulations (Handa et al. 1986). Inspite of the tremendous advances made, no significant and safe hepatoprotective
agents is available in modern therapeutics. Therefore, due importance has been given globally to develop plant-based hepatoprotective drugs effective against a variety of liver disorders (Mohamed, 2010).

However, the exact mechanism by which plant based products protect the liver has not been studied extensively. Some studies conducted on hepatoprotective plants revealed that, the activity is because of inhibition of free radical-damage to the cells (Sultana, et al. 2005). The involvement of free radicals such as superoxide anions and hydroxyl radicals and, other reactive oxygen species like hydrogen peroxide in various diseases has been established. The metabolism of certain pesticides, drugs, cigarette smoke and various other pollutants generate a number of reactive oxygen species and free radicals in the biological system. These radicals cause depletion of antioxidant enzymes and induce lipid peroxidation in the liver. Some studies show that the herbs may promote the antioxidant defense system to prevent CCl₄ induced hepatic damage (Koul and Kapil, 1999).

Elevation of serum markers are a known effect of CCl₄ toxicity and used as biochemical parameters of liver damage (Sturgill, 1997). The toxicity produced by CCl₄ is mediated through free radical mechanism. CCl₄ induced hepatic damage is due to its cytochrome P450 enzyme system catalysed hepatic conversion into highly reactive trichloromethyl radical, which upon reaction with oxygen radical gives trichloromethyl peroxide radical. This radical forms covalent bond with sulfahydryl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl₄ (Cotran et al. 1994 and Kaplowitz et al. 1986), by encouraging the auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane, thus altering the permeability of the liver cell membranes and hepatic tissue distruption (Handa
and Sharma 1990). Hepatocellular necrosis leads to high level of serum markers in the blood. From the results of the present investigation it was evident that the alcoholic extract of *Azima tetracantha* and *Cocculus hirsutus* at two different doses were able to reduce the elevated biochemical parameters due to the hepatotoxin administration. The reduced levels of total proteins and albumin in CCl₄ induced hepatotoxicity is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P₄₅₀ leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Recknagel, 1967). Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew, *et al.* 1987). Reduction of raised bilirubin level suggests the stability of the biliary function during injury with CCl₄. Estimations of serum bilirubin is the most sensitive test because it confirms the intensity of the hepatic damage determinations in serum and is used for the diagnosis, differentiation and follow up of jaundice. The protein and albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis (Elliot and Strunin, 1993). Further more, serum proteins are affected both quantitatively and qualitatively in liver diseases and such changing levels of serum protein thus provide valuable indices of severity, progress, and prognosis in hepatic disease (Kagan, 1943 and Henry, 1986). The protective effect exhibited by the extracts was comparable to standard drug silymarin.

The histopathological observations in CCl₄ rats showed severe necrosis, with disappearance of nuclei. This could be due to the formation of highly reactive radicals because of oxidative threat caused by trichloromethyl radicals. All these changes were very much reduced histopathologically in rats treated with alcoholic extracts of both the plants. Thus, administration of
alcoholic extracts of leaves revealed hepatoprotective activity of *Azima tetracantha* and *Cocculus hirsutus* leaves against the toxic effect of CCl₄, which was also supported by histological studies.


The present study reveals that the ethanol stem bark extract possess significant hepatoprotective activity which may be attributed to the individual or combined effects of active constituents present in it. Several phytoconstituents *viz.* triterpenes, alkaloids, flavonoids, flavones, glycosides, *etc.* have been found effective in the hepatoprotection against CCl₄ induced hepatic toxicity (Baek, 1996; Vijyan, 2003 and Chin, 2004). Several phytoconstituents *viz.* triterpenes (Tran, 2001), alkaloids (Vijyan, 2003), flavonoid (Baek, 2005), flavon glycosides (Singab, 2005 and Chin, 2004) *etc.* have been found effective in the hepatoprotection against CCl₄ induced hepatic toxicity.

**ANTIOXIDANT ACTIVITY**

Molecular oxygen is an essential component for all living organisms, but the formation of various reactive intermediates of molecular oxygen called free radicals leads to a process termed as ‘oxidation’. These free radicals are highly reactive, unstable and can therefore causes oxidative destructive processes, wherein it breaks down and damages various biomolecules such as lipids, polysaccharides, proteins, nucleic acids *etc.* by giving out or accepting single electron (Halliwell and Gutteridge, 1999).
Extensive experimental and epidemiological studies support the involvement of oxidative stress in pathogenesis and progression of many diseases. It is well known that oxygen sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS can trigger a host of disorders in biological systems. Oxidative stress is an outcome of imbalance between ROS production and antioxidant defenses, which in turn evoke a series of events deregulating the cellular functions (Bandyopadhyay, 1999). Endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical induced damages of tissues.

Antioxidant compounds in plants play an important role as a health-protecting factor. There are a number of clinical studies suggesting that the antioxidants in grains, oil seeds, fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller, et al. (2000a and b).

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity (Aruoma and Cuppett, 1997).
Discussion

Among the various mechanisms involved in hepatotoxic effect of carbon tetrachloride, one is oxidative damage through free radical generation (DeLeve and Kaplowitz, 1995; Farrel, 1998) and antioxidant property is claimed to be one of the mechanism of hepatoprotective effect of indigenous substances. They can react with reactive lipids including cholesterol, unsaturated fatty acids, and glycolipids, leading to lipid peroxidation (Girotti, 1998). Lipid peroxides are commonly found in oxidative stress-mediated liver injury (Jayatilleke & Shaw, 1998; Sadrzadeh et al. 1994). In order to probe the possible mechanism by which active constituents prevent hepatic damage caused by CCl₄ and to examine the presence of oxidative stress in CCl₄ treated rat livers, investigation on lipid peroxidation and glutathione were carried in the liver homogenate. CCl₄ is capable of generating highly reactive free radicals, inhibiting glutathione (GSH) synthesis, producing glutathione loss from the tissue, increasing malondialdehyde (MDA) levels and impairing antioxidant defense systems in humans and experimental animals.

In the present investigation, various in vivo antioxidant assays have been used to monitor and compare the antioxidant activity of alcoholic extracts of *Azima tetracantha* and *Cocculus hirsutus* and the results are discussed below.

Administration of CCl₄ caused decrease in levels of GSH, total thiol and catalase whereas, MDA level was increased in rats. However, pretreatment of alcoholic extracts, of *Azima tetracantha* and *Cocculus hirsutus* has shown significant (P<0.01) protective activities for GSH; for total thiols and for catalase activity at 250 and 500 mg doses in a dose dependent manner. However in regard to MDA, effect of alcoholic extracts revealed statistically significant (P<0.01) reduction in both *Azima tetracantha* and *Cocculus hirsutus* when compared to CCl₄ intoxicated animals. It is apparent from the data that the effectiveness of the extracts. Further, among the two doses of alcoholic extracts, 500 mg was proved to be
Discussion

more prominent as evident by its significant antioxidant features and comparable to the effect of Silymarin.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance and Greenstein, 1992). Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide as a result of oxidative stress caused by CCl₄. The CAT activity was restored to normal after pretreatment of alcoholic extracts of *Azima tetracantha* and *Cocculus hirsutus* evidently showing the antioxidant property of the extracts against oxygen free radicals.

Glutathione (GSH) content in the liver plays a primary role in the protection against trichloromethyl radical-induced liver damage (Recknagel *et al.* 1989 and Campo *et al.* 2001). GSH is widely distributed in cells and is an important constituent of intracellular protective mechanisms against a number of noxious stimuli, including oxidative stress. It is widely known that deficiency of GSH within living organisms can lead to tissue disorder and injury. Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species and maintains membrane protein thiols. Also it is a substrate for glutathione peroxidase (Prakash, 2001). Decreased level of GSH is associated with an enhanced lipid peroxidation in CCl₄ treated rats. The present study has demonstrated that by using CCl₄ induced rats, which is known model for both hepatic GSH depletion and injury, administration of *Azima tetracantha* and *Cocculus hirsutus* extracts increased the level GSH in a dose dependent manner.
Reduced thiols have long been reported to be essential for recycling of antioxidants like vitamin E and vitamin C (Constantinescu, 1993). Administration of thiol compounds such as glutathione, cysteine and methionine have been shown to protect against oxidative stress in humans and animals. Treatment with test extracts resulted in increased level of total tissue sulphydryl (thiol) group compared to the untreated rats.

MDA is the major oxidation product of peroxidized poly-unsaturated fatty acids and its elevation is an important indicator of lipid peroxidation (Halliwell, 1993) leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Malondialdehyde (MDA) formed as a result of lipid peroxidation induced by CCl₄ in liver, is an important parameter to assess the oxidative stress generated in liver. In the present investigation, a significant elevation in the levels of end product of lipid peroxidation, MDA in the liver of rats treated with CCl₄ was observed when compared to normal control. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. On the other hand pretreatment with alcoholic extracts of *Azima tetracantha* and *Cocculus hirsutus* as well as silymarin significantly (P<0.01) reversed changes caused by CCl₄. Hence, the mechanism of hepatoprotection of this extracts and constituents may be due to their antioxidant effect.

Therefore the reduced lipid peroxidation with simultaneous significant increase in GSH, total thiol and CAT content of liver suggested antioxidant activity of alcoholic extract of *Azima tetracantha* and *Cocculus hirsutus* leaves and silymarin and thus it can be concluded that possible mechanism of hepatoprotection of leaves may be due to its antioxidant activity.

The phytochemical screening of the extracts has revealed the presence of triterpenoids, steroids, tannins, alkaloids and flavonoids in both the plants.
Several phytoconstituent antioxidants *viz.* triterpenes, alkaloids, flavonoid, flavon glycosides *etc.* have been found effective in the hepatoprotection against CCl₄ induced hepatic toxicity (Baek, 1996; Vijyan, 2003 and Chin, 2004). The present study provides the scientific basis on the positive correlative effect of antioxidant and hepatoprotective activities of ethanolic extracts of *Azima tetracantha* and *Cocculus hirsutus* leaves.

There are several examples of isolation and extraction of antioxidants from plant materials *viz.* *Cordyceps sinensis* (Li et al. 2001), *Pluchea indica*, (Sen et al. 2002); *Erigeron annuus* (Young and Kyong, 2003); *Ardisia compressa* (Sonia and de Mejia, 2004); *Piper guineense* (Odukoya et al. 2005); *Cytisus scoparius* (Raja Sundararajan et al. 2006); *Lycium barbarum* (Li et al., 2007); *Zanthoxylum piperitum* (Yamazaki et al. 2007), *Carya cathayensis* (Chenggang Zhu et al. 2008) and *Sphaeranthus indicus* (Brijesh, 2009).

**ANTIPYRETIC ACTIVITY**

A fever is considered one of the body's immune mechanisms to attempt a neutralization of a perceived threat inside the body, be it bacterial or viral. It is caused by an elevation in the thermoregulatory set-point, causing typical body temperature to rise, and effector mechanisms are enacted as a result.

PGE2 release comes from the arachidonic acid pathway. This pathway is mediated by the enzymes phospholipase A2 (PLA2), cyclooxygenase-2 (COX-2), and prostaglandin E2 synthase. These enzymes ultimately mediate the synthesis and release of PGE2. PGE2 is the ultimate mediator of the febrile response. The set-point temperature of the body will remain elevated until PGE2 is no longer present. PGE2 acts on neurons in the preoptic area (POA) through the prostaglandin E receptor 3 (EP3). EP3-expressing neurons in the POA innervate the dorsomedial hypothalamus (DMH), the rostral raphe pallidus nucleus in the medulla oblongata (rRPa) and the paraventricular
nucleus (PVN) of the hypothalamus. Fever signals sent to the DMH and rRPa lead to stimulation of the sympathetic output system, which evokes non-shivering thermogenesis to produce body heat and skin vasoconstriction to decrease heat loss from the body surface. It is presumed that the innervation from the POA to the PVN mediates the neuroendocrine effects of fever through the pathway involving pituitary gland and various endocrine organs. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory centre at a lower temperature (Howard, 1993).

In the present investigation antipyretic activity was studied by adopting yeast induced pyrexia in rats using paracetamol as standard. A rise in temperature was recorded from 18th h after yeast injection at 19, 20, 21 and 22nd h in yeast induced group of rats. The effect of alcoholic extracts of *Azima tetracantha* at 250 mg dose showed significant reduction in pyrexia at 21 and 22 h while at 500mg, the pyrexia has reduced significantly (P<0.01) at all the experimental intervals indicating dose efficiency. Similarly alcoholic extracts of *Cocculus hirsutus* have also shown significant reduction in pyrexia at 250 mg dose at 20, 21 and 22nd h of rectal temperatures. In higher dose of 500 mg, the temperature reduced at 19, 20, 21 and 22nd h (P<0.01) and the effects are comparable to the standard drug Paracetamol.

Thus, alcoholic extracts of *Azima tetracantha* and *Cocculus hirsutus* leaves produced an efficent decrease in the body temperature in hyperthermic rats. The cause of this decrease may be central and/or peripheral in origin. Clinically available antipyretic drugs, such as paracetamol and the non-steroidal antiinflammatory drugs are able to lower the body temperature only in feverish patients. Neuroleptic drugs and other central depressants can also reduce the normal body temperature (Ali, 1995). In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus (Clark, 1975 and Zeil,
The possible mechanism of action may be by inhibition of prostaglandin synthesis as in case of paracetamol (Chandrashekar et al. 2002). Also, there are several mediators or multi-processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis (Akio et al. 1988). Similar kind of antipyretic activity was reported by several workers in various plants viz. *Nelumbo nucifera* (Mukherjee et al. 1996 and Senha et al. 2000), *Mallotus peltatus* (Geist) Muell.Arg. var acuminatus (Chattopadhyay et al. 2002), *Ocimum suave, Ocimum lamiifolium* (Eyasan, 2003), *Ventilago harmandiana* Pierre (Ampai, 2004), *Garcinia hanburyi* Hook f (Ampai et al. 2007), *Cleome rutidosperma* (Anindya et al. 2007), *Sargassum fulvellum, Sargassum thunbergii* (Kang, 2008), *Corchorus capsularis* L. (Zakaria, 2009), *Piper nigrum* and *Nyctanthes arbor-tristis* (Ghiware and Nesari, 2010).

**ANTI-INFLAMMATORY ACTIVITY**

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbial agents and is the body's effort to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair (Mary, 1997). Upon interaction of foreign pathogens with innate immune cells like macrophage or monocytes, inflammatory immune response is triggered. A series of pro-inflammatory mediators, specialized cytokines, prostaglandins, chemokines are produced as a result in a way to amplify the inflammatory response (Beg, 2002).

In the present study, administration of carrageenan to the control group of rats showed a rise in paw volume at different time intervals viz. 1, 2 and 3h. However oral administration of alcoholic extracts of *Azima tetracantha* at 250 mg dose exhibited significant reduction in inflammation induced by carrageenan at 1h 2h and 3h manifesting a percent reduction of 26.92, 40.62 and 44.00. The reduction in paw volume was more prominent at 500 mg dose by recording superior mean values and percent inhibition of 0.34 ± 0.01
Discussion

(34.61%), 0.34 ± 0.03 (46.87%) and 0.24 ± 0.04 ml (52.00%) at 1, 2 and 3h respectively. Similarly alcoholic extracts of Cocculus hirsutus also exhibited a significant \((P<0.01)\) dose dependent anti inflammatory activity by reducing the paw volume. Further, the percentage of inhibition was found to be 37.50 and 30.00\% at 250 mg and 26.92, 43.75 and 48.00\% at 500 mg dosage. It is also evident from the data that the anti-inflammatory activity was positively correlated to time intervals exhibiting highest inhibition of paw volume at 3 h at all the treatment under study.

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Seibert, 1994). The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Vinegar, 1969). The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also to histamine and serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The second phase is sustained by prostaglandins released and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages component (Crunkhorn, 1971 and Brito, 1988) and play a major role in the development of the second phase of inflammatory reaction. In the present investigation, alcohol extract at the employed dose showed effect at 1st, 2nd and 3rd h onwards. In general, the anti-inflammatory activity is more evident at the later phases of time interval. Therefore, it can be inferred that the inhibitory effect of alcoholic extracts on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. The phytochemical screening shows the presence of flavonoids in alcoholic extracts in both the plants and is known to inhibit prostaglandin synthetase (Ramaswamy, 1985). Similar investigations on plants as anti-inflammatory
agents has been carried out by various workers *viz.* *Curcuma amada* (Mujumdar et al. 2000); *Goniothalamus andersonii* (Shigeo et al. 2001); *Clitoria fairchildiana* (Pereira da Silva and Paz Parente, 2002); *Calendula officinalis, Hypericum perforatum, Plantago lanceolata* and *Glycyrrhiza glabra* (Herold et al. 2003); *Alchornea cordifolia* (Mavar et al. 2004); *Vitex negundo* (Rasadah et al. 2005) *Bacopa monniera* (Shabana, 2006); *Andrographis paniculata* (Sheeja et al. 2006); *Ruta graveolens* (Ratheesh and Helen, 2007); *Putranjiva roxburghii* (Wantana, 2009) and *Magnolia ovate* (Candida, 2009).

**ANALGESIC ACTIVITY**

Pain, even though is an unpleasant sensation, is mainly a protective mechanism for the body (Kanodia, 2008). It is a consequence of complex neurochemical processes in the central and peripheral nervous systems. Typically, it is a direct response to an event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause or it can also occur as a consequence of brain or nerve injury. Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively.

The nonopioid analgesics relieve pain without interacting with opioid receptors, reduce elevated body temperature, possess anti-inflammatory property and are non-addicting drugs. These effects are achieved with doses that do not produce significant depression of CNS. The NSAIDs can be classified mainly into two groups, namely, non-selective COX inhibitors (acetyl salicylic acid, paracetamol, phenylbutazone, diclofenac, ibuprofen, piroxicam etc) and selective COX-2 inhibitors (nimesulide, meloxicam, celecoxib, rofecoxib). Though these drugs have different chemical structures, they produce qualitatively similar actions. During inflammation, pain and fever, arachidonic acid is liberated from phospholipid fraction of the cell
Discussion

membrane. This acid is then converted via cyclo-oxygenase (COX-1 and COX-2) pathways to prostaglandins. These prostaglandins sensitize blood vessels to the effects of inflammatory mediators that increase permeability. The prostaglandins particularly PGE and PGI produce hyperanalgesia associated with inflammation. They sensitize the chemical receptors of the afferent pain endings to other mediators such as bradykinin and histamine. Further, release of prostaglandins in the CNS may lower the threshold of the central pain circuits.

Opioid are drugs which have morphine like action viz. relief of pain and depression of the CNS. The opioid drugs produce their effects by combining with opioid receptors, which are widely distributed in the CNS and other tissues. The opioid receptors have been classified into mu, delta, kappa (K₁ and K₂) and sigma types. The vast majority of opioid drugs used as analgesics are agonists at mu receptors. The major drawbacks of these opioid analgesics are the development of tolerance and physical as well as psychological dependence.

In the present investigation, for analgesic effect of alcoholic extracts of Azima tetracantha on tail flicking in rats at different time intervals viz. 0, 30, 60, 90 and 120 min, rats treated with alcoholic extracts at 250 and 500 mg/kg dose recorded significant (P<0.01) increase over the control in reaction time while, alcoholic extracts of Cocculus hirsutus at 250 mg concentration elevated the reaction time significantly (P<0.01) at 90 and 120 min only whereas, at 500 mg dosage the reaction time was significant at all the different time intervals indicating dose dependent analgesic effect. It is interesting to note that the activities of both the plant extracts are more effective in later phases of treatment. Further, the Azima tetracantha has more effective analgesic property compared to Cocculus hirsutus. Notably the alcoholic extract of the latter @ 500mg/kg at 120 min has recorded higher reaction time than the paracetamol indicating its analgesic potential.
Discussion

Pain is a complex event, centrally modulated \textit{via} a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti, 1983; Headley, 1985; Pasero, 1999 and Wigdor, 1987) The phytochemical screening of the extracts has revealed the presence of triterpenoids, steroids tannins, alkaloids and flavonoids. Analgesic effects of flavonoids steroids and tannins have been well documented (Das, 1989). Further, the higher efficacy of alcoholic extracts of \textit{Azima tetracantha} could be attributed to the presence of piperdine alkaloids \textit{viz.} azimine, azcarpaine and carpaine in alleviation of pain (Parmar, 1997). The present work corroborates investigations carried out in several medicinal plants \textit{viz.} \textit{Wilbrandia ebracteata} (Gonzalez, 2002); \textit{Sida acuta}; \textit{Stylosanthes fruticosa}, \textit{Toona ciliata}, \textit{Bougainvilla spectabilis}, \textit{Ficus glomerata} and \textit{Polyalthia longifolia} (Malairajan, 2006); \textit{Mahonia oiwakensis}, (Jung Chao 2009); \textit{Argyreia speciosa}, (Bachhav, 2009); \textit{Citrus decumana} (Shailja,2009) for analgesic activities.

CARDIOVASCULAR ACTIVITY

The heart has been one of the most widely studied organs of the body because heart disease is the world's leading cause of death (Birmingham, 2002). The World Health Organisation estimates that this disease is responsible for the deaths of approximately 30,000 people each day (Middlemiss & Watson, 1994).

Despite of incredible advances in the diagnosis and treatment of cardiovascular diseases, the incidence, and mortality resulting from these diseases continue to escalate. Cardiovascular disease (CVD) encompasses the entire spectrum of heart disease, ranging from coronary artery disease (CAD), stroke, hypertension, elevated cholesterol, angina and acute myocardial infarction. Each year, around 8 million people die from heart attacks and many millions more suffer from, and eventually succumb to heart diseases. Global figures are rising, yet calculations suggest that this number could be
slashed by around 50 per cent. In United States, heart failure afflicts more than 4.5 million patients and approximately 4,000,000 new cases are diagnosed annually. Both the incidence and prevalence of heart failure increase dramatically with age. The prevalence of heart failure nearly doubles with each decade of life after the age of 50. Heart failure represents a significant cause of mortality in the senior population. Consequently, heart failure has become one of the most expensive health problems around the world (WHO, 2000). Knowledge about the physiology, structure and molecular biology of cardiac ion channels/ion transporters has grown substantially in recent times. The fruitful combination of herbal drugs with state-of-the art has advanced the treatment and understanding of many cardiovascular disorders (Miller, 1996).

Natural products have featured prominently in the clinical treatment of cardiovascular diseases and in providing lead compounds for the development of more efficient drugs. Digoxin, a steroid glycoside from *Digitalis lanata*, is the oldest of the commonly used compounds in the treatment of heart disease; its clinical history dates back to 1785 (Repke et al. 1995). Apart from its use in congestive heart failure, it also finds use in the treatment of arrhythmia. Quinidine, a stereoisomer of the antimalarial quinine, found in cinchona bark, is an adequate antiarrhythmic agent, but has recently been replaced by pacemakers and newer drugs. Slow heart beat (bradycardia) is treated with atropine, a metabolite of the solanaceous plant *Atropa belladonna*, or isoproterenol (Emilio, 1998). Many new drugs have been developed over the last three decades in attempts to improve on those in clinical use. The phosphodiesterase inhibitors which were meant to supplement or replace digitalis compounds as cardiotonic drugs have not yielded the results which they seemed to promise (Repke et al. 1995). None of the digitalis, diuretics and ACE drugs, when used alone, satisfy all the criteria for optimally managing heart failure. The discovery of natural compounds which may be useful, directly or as lead compounds, in the treatment of this collection of
diseases is a long-standing and continuing research challenge and the
modelling and synthesis of more efficient drugs remains a desirable goal of
the medicinal chemist. One of the problems encountered in screening and
identifying compounds with promising cardiovascular activities is related to
the availability of suitable, efficient and rapid bioassay systems. In recent
years, a number of simple inexpensive 'bench-top' bioassays have been
introduced for the rapid screening of biological activities of plant extracts and
fractions. The animal or organ-based assays, e.g. the Langendorff isolated
heart, have long been used and still retain significance as secondary assays for
the determination of the cardiotonic effect of a compound.

The Langendorff-perfused isolated mouse heart offers a high
throughput and potentially reliable model for the analysis of contractile
function and responses to ischaemic insult (Melissa, 2010).

The administration of single dose of 0.2ml alcoholic extract of Azima
tetracantha (1mg/ml) induced significant decrease in heart rate to 80.50
(26.8% of negative chronotropic effect) from normal 110.00 BPM and force
of contraction was increased to 11.25 (55% positive inotropic effect) from
7.25 (normal), whereas Coronary Perfusion Rate was decreased (9.12 ml/min)
from initial 14.08 ml/min to an extent of 35.22%. Further increase in dose to
0.6 ml after achieving normal heart rate decreased the heart rate to 41.00
(62.72% of negative chronotropic effect) but increased the force of
contraction to 13.00 mm. (79.31% positive inotropic effect) while, CPR was
reduced to 5.35 ml/min to an extent of 62.00% indicating dose dependent
cardiotonic activity. Similarly, alcoholic extract of Cocculus hirsutus at 0.2
and 0.6ml doses reduced the heart rate (78.50 and 40.75 BPM) reducing by
28.6 and 62.9% of negative chronotropic effect; coronary flow rate was
reduced to 8.27ml and 5.18 ml/min reducing by 41% and 63.2%; increase in
force of contraction was noted at 12.25 and 15.00 mm showing an elevation
to the extent of 68.96 and 106 % of positive inotropic effect respectively. The
results validates that both the plants possess negative chronotropic and positive inotropic effect on isolated rats’ heart in a dose dependent manner. A close observation of the data reveals the superiority of the plant *Cocculus hirsutus* for cardiovascular activity as it has shown better mean values for the parameters under study.

The above results show that the extracts of *Azima tetracantha* and *Cocculus hirsutus* leaves mediates an initial, but transient, positive inotropic effect followed by an immediate decrease accrued simultaneously with decreased chronotropism and coronary perfusion rate. All induced effects lasted less than 10 min, after which the isolated rat heart recovered fully and return to the original levels of rate and contraction and as wells as coronary flow rate. All these responses are similar to those associated with digitalis effect like digoxin (Dec, 2003). The direct slowing of sinoatrial rate and atrioventricular conduction that is produced by test extract elicited the decrease in coronary blood flow and decreased heart rate. The increased myocardial contractility by increasing inward calcium flux in the heart during the action potential; they may also alter the intracellular movements of calcium by influencing the sarcoplasmic reticulum.

The extracts of *Azima tetracantha* and *Cocculus hirsutus* leaves may increase contraction of the cardiac sarcomere by increasing the free calcium concentration in the vicinity of the contractile proteins during systole. The increase in calcium concentration may be the the result of a two-step process: first, an increase of intracellular sodium concentration because of Na+/K+ ATPase inhibition and second, a relative reduction of calcium expulsion from the cell by the sodium-calcium exchanger by the increase in intracellular sodium. The increased cytoplasmic calcium is sequestered by the SERCA (Sarco/Endoplasmic Reticulum Ca$$^{2+}$$-ATPase), in the SR for later release. The net result of the action of *Azima tetracantha* and *Cocculus hirsutus* leaves may be due to the type of cardiac glycosides which have a distinctive increase

ANTHELMINTIC ACTIVITY

Helminthiasis is one of the major problems of livestock production throughout the world, particularly in tropical and subtropical areas. The World Health Assembly, in a number of resolutions has emphasized the need to the use of natural products with therapeutically proven efficacy particularly in patients residing in tribal areas who are very much prone to the attack of several infections due to lack of knowledge about proper sanitation and can affect most populations with major economic and social consequences (Ghosh et al. 2006). Further, helminthes also affect millions of livestock resulting in considerable economic losses in domestic and farm yard animals because of limited availability and affordability of modern medicines. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance (Coles, 1999). The helminthes which infect the intestine are cestodes e.g. Tape worm (Taenia solium), nematodes eg. Hookworm (Ancylostoma duodenale), round worm (Ascaris lumbricoids) and termatodes or flukes (Schistosoma mansoi and S. hematobilium). The disease originated from parasitic infections causing
severe morbidity includes lymphatic filariasis, onchocerciasis and schistosomiasis. Ideally an anthelmintic agent should have broad spectrum of action, high percentage of cure with a single therapeutic dose, free from toxicity to the host and should be cost effective. None of the synthetic drug available meets this requirement. Even most common drugs like piperazine salts have been shown to have side effects like nausea, intestinal disturbances and giddiness (Liu, 1996), resistance of the parasites to existing drugs (Walter, 1985) and their high cost warrants the search for newer anthelmintic molecules. Therefore, many investigators are focusing researches on the alternatives to the chemical control of helminthes particularly herbal drugs. There are various plants which are reported as anthelmintic such as Pongamia glabra, Enhyra fluctuans, Mimusops elongi, Mentha piperita, Lantana camara, Picrolemma sprucei, Trachyspermum ammi, Nigella sativa, Azadirachta indica, Clitoria termatea, Terminalia chebula etc. (Patil et al. 2009).

Considerable research in this regard has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects. For example, plants that contain condensed tannins, a class of phenolic secondary metabolites, have these effects (Jalalpure et al. 2003). Search for anthelmintic factor in plants therefore remains a potential area of investigations.

Most of the screening reported are in vitro studies using some worm samples like Pheretima posthuma, Ascaris galli, A. lumbricoides etc. Adult Indian earth worm, P. posthoma has been used as test worm in most of the anthelmintic screening, as it shows anatomical resemblance with the intestinal roundworm parasite of human beings (Vidyarthi, 1967; Vigar, 1984; Thorn, 1977 and Chatterjee, 1967). Because of easy availability, earthworms and A. galli worms are used as suitable models for screening of anthelmintic drug. These in vitro screenings are important as they give basis for further in vivo studies (Ravindra, 2008).
The present study revealed that the Petroleum ether and alcoholic extracts of *Azima tetracantha* and *Cocculus hirsutus* on Indian earthworm *Pheretima posthuma* and fowl roundworm, *Ascaris galli* at different concentration viz. 0.1, 0.5 and 1% possess potent anthelmintic property in a dose dependent manner for the parameters studied viz. paralysis and death which is quite comparable with standard anthelmintic drug in both organisms. Among the extracts of *Azima tetracantha* and *Cocculus hirsutus* pet ether has shown the maximum reduction in time for paralysis, followed by alcohol, chloroform and aqueous. Similarly, in regard to the time taken to death (minutes), among the extracts, pet ether has shown the maximum reduction in time followed by alcohol, chloroform. Aqueous extract did not show significant activity for this parameter v/s standard at any of the concentration tested. Comparison of the results on the effect of different extracts of leaves of the two plants *Azima tetracantha* and *Cocculus hirsutus* for anthelmintic activity reveals that petroleum ether and alcoholic extracts of both plants are more potent than other extracts. The result indicates a negative correlation between time and concentration of the extracts. Further, among the two plants under study alcoholic extract of *Cocculus hirsutus* is found to be more potent than *Azima tetracantha* irrespective of type of extract, dosage and worms for both the parameters of the activity.

The major effect of anthelmintic compounds could be due to decrease in motility, paralytic action, damage to the mucopolysaccharide membrane and on the neuromusculature of helminthes worms. The metabolic pathways in general and carbohydrate pathways in particular and neuromuscular coordination are the major targets. (Dhar, 1965). It is due to the presence of active principles in the plant extracts (Ghosh, *et al.* 2007). It acts as potent anthelminthic, because the extracts of the plant contains flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins. Specifically, tannins and alkaloids present in the extracts may be attributed to profound anthelmintic activity (Athnasiadou *et al.* 2001; Dash, *et al.* 2002;
Discussion

Shivkar, and Kumar 2003; Mali et al. 2007; Khadatkar et al. 2008; Paramesha et al. 2009; Parvathi et al. 2009; Asha, et al.2009 ; Deore and Khadabade, 2010). Tannins are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death (Thompson, 1995). The anthelmintic activity of the various extracts may be attributed to similar reasons.

ANTIMICROBIAL ACTIVITY

Many plants have been used because of their antimicrobial traits and have been investigated by a number of researchers worldwide (Ncube, 2008). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment (Iwu, 1999). Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases (Tanaka et al. 2006) especially in the light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents. Bacteria have evolved numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the rise. This resistance is conferred by multidrug resistance pumps (MDRs), membrane translocases that extrude structurally unrelated toxins from the cell. These protect microbial cells from both synthetic and natural antimicrobials (Stermitz et al. 2000). Secondary metabolites resemble endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to their recognition in potential target sites (Parekh et al. 2005). The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments and could help curb the problem of these multi-drug resistant organisms. Moreover, the synergistic effects of extracts with antimicrobial
activity in association with antibiotics can provide effective therapy against drug resistant bacteria.

Over the past several years, intensive efforts have been made to discover clinically useful antimicrobial drugs, which have been reviewed by many researchers. (Rasadah and Houghton, 1998; Blondeau, 1999; Jacoby, 1999 and Cowan, 1999). This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor et al. 2001).

The antimicrobial susceptibility test (AST) is an essential technique in many disciplines of science. It is used in pathology to determine resistance of microbial strains to antimicrobials, and in ethnopharmacology research, it is used to determine the efficacy of novel antimicrobials against microorganisms, essentially those of medical importance. The test is the first step towards new anti-infective drug development. There are various AST methods that are employed by researchers and these could lead to variations in results obtained (Lampinen, 2005).

i. **Antibacterial activity**

In the present investigation, among the various extracts *viz.* petroleum ether, chloroform, alcoholic and aqueous extracts of *Azima tetracantha* and *Cocculus hirsutus* only chloroform and alcoholic extracts showed encouraging results. Both the chloroform and alcoholic extracts exhibited potent antibacterial activity in a concentration dependent manner against the test organisms at concentrations of 50 and 100 μg/ml and are comparable with the standard drug Streptomycin and Procaine penicillin. Both the test extracts have exhibited broad spectrum efficacy by recording remarkable zone of inhibition against all the tested bacteria *viz.* *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia* and *E. coli*. Among the two extracts studied, alcoholic extract was found to be more effective in
terms of antibacterial activity. The sensitivity order of bacteria in general was found to be *Pseudomonas aeruginosa* > *Escherichia coli* > *Staphylococcus aureus* > *Bacillus subtilis* > *Klebsiella pneumonia*. Further, among the two plants, *Cocculus hirsutus* has demonstrated higher mean values for clear zone of inhibition indicating potential antibacterial property.

**ii. Antifungal activity**

The screening of various plant extracts of *Azima tetracantha* and *Cocculus hirsutus* for their plausible antifungal effects against test organisms viz. *Candida albicans* and *Aspergillus niger* were noticed only in alcoholic extract of the several tested in *Azima tetracantha* while chloroform and alcoholic extracts of *Cocculus hirsutus* have shown zone of inhibition in a dose dependent manner indicating the variation in the quality and quantity of phytoconstituents. *Candida albicans* exhibited 12 and 14 mm at 50 and 100 \( \mu \text{g/ml} \) respectively, while *Aspergillus niger* has shown 12 and 16 mm of clear zone at similar concentrations. However in *Cocculus hirsutus*, even though, chloroform extract has inhibited the growth of *Candida albicans* and *Aspergillus niger* at 50 and 100 \( \mu \text{g/ml} \) concentrations the alcoholic extract recorded higher inhibition against same organisms indicating better efficacy of the latter. Further, the *Candida albicans* was found comparatively resistant than *Aspergillus niger* by virtue of their lesser zone of inhibition. It can be also inferred that the *Cocculus hirsutus* is marginally better for its antifungal activity.

The antimicrobial activity could be attributed to the presence of phytoconstituents viz. flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins which have multiple biological effects, including antioxidant, wound healing etc. which are toxic to the microorganisms. Flavonoids, phenolic compounds in particular of plant are important for the plant growth and defense against infection and injury. These compounds while exhibiting antioxidant property are usually also act as good
antimicrobial agents (Kahkonen et al. 1999; Cos et al. 2001; McGaw, et al.
mechanisms could be enzyme inhibition by oxidation (Cowan, 1999). Further,
the variation in antimicrobial sensitivity may be due to the differences in the
chemical nature of the cell wall and cell membrane of each micro organism
(Bal-Tembe et al. 1996). Thus the present antimicrobial activity effects of
*Azima tetracantha* and *Cocculus hirsutus* might be because of these
constituents. The secondary metabolites and their efficacy as antimicrobial
agents have been documented by several investigators. The sites and number
of hydroxyl groups on the phenol group are thought to be related to their
relative toxicity to microorganisms with evidence that increased
hydroxylation results in increased toxicity (Geissman, 1963). Some authors
have found that highly oxidized phenols possess more inhibitory activity
(Scalbert, 1991). The mechanisms thought to be responsible for phenolics
toxicity to microorganisms include enzyme inhibition by the oxidized
compounds possibly through reaction with sulfhydryl groups or through more
nonspecific interactions with the proteins (Mason and Wasserman, 1987). The
activity of flavonoids is probably due to its ability to complex with
extracellular and soluble proteins and to complex with bacterial cell walls.
The lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya,
et. al. 1996). A wide range of antiinfective actions, have been assigned to
tannins. One of their molecular actions is to complex with proteins through
nonspecific forces such as hydrogen bonding and hydrophobic effects, as well
as by covalent bond formation (Haslam, 1996 and Stern, et. al. 1996). Thus
their mode of antimicrobial action is related to their ability to inactivate
microbial adhesins, enzymes, cell envelope transport proteins etc. They also
form complex with polysaccharides (Ya, et. al. 1988). The mechanism of
action of terpenes is speculated to involve membrane disruption by the
lipophilic compounds. Alkaloids are also found to have microbicidal effect.
The mechanism of action of highly aromatic planar quaternary alkaloids is
attributed to their ability to intercalate with DNA (Phillipson and Neill, 1987).