CHAPTER 2

REVIEW OF LITERATURE

2.1. *Colebrookea* species.

Colebrookea belongs to family Labiatae (sub- Lamiaceae), which comprises of 100 genera and about 3000 species having considerable economic and medicinal importance. Most of the species found in India are distributed in the hill regions. The family yields many useful steroids, flavonoids and glycosides [1]. Many members of the family are used as culinary or medicinal herbs, as sources of volatile oils and in some cases for the preparation of constituents of the volatile oils such as menthol and thymol. In addition to the volatile oils, constituents of the family include diterpenoids and triterpenoids, saponins, a few pyridine and pyrollidine alkaloids, insect moulting hormones, polyphenols, tannins, coumarins and furanoids. Some of the flavonoid present in the species has been demonstrated to have CNS stimulant activity [2].

2.1.1 *Colebrookea oppositifolia* Smith [3].

The synonyms of the plant are:

Beng. – *Pansara*; Hindi – *Binda, pansara*; Kan. – *Falia, tuggigidda*; Mar. – *Bhamini, dusarika jhar*; Oriya – *Bosiki, darigopi*; Tel. – *Jolidi*, *Nep.* – Dhusure, Lepcha – Kumfyjemkung.

2.1.1.1. Distribution

The plant is found to be distributed throughout India in the hilly regions, at elevation ranging from 3000 to 5000 ft, especially in valleys and ravines, often gregarious near watercourses [3].

2.1.1.2. Features of different parts of the plant

Colebrookea oppositifolia is a densely tomentose, hoary, much branched shrub or small tree. The plant is 1.2 to 3.6 m tall, bearing oblong-elliptic leaves with smooth grey bark. Trunk stout, stems irregularly indented, bark corky, and leaves 10 – 20 cm × 3.8 – 5.0 cm. Flowers small, numerous, white, red or pale chocolate, in crowded spikes; fruits 1.2 cm, nuts, usually one, hairy [3]. The leaves are used as fodder. The wood is grayish white, moderately hard (736-738 kg/m^3) and closed grained. It is converted into charcoal for gunpowder. (Fig. 2.1)

Figure 2.1. Photograph showing the twig of *Colebrookea oppositifolia* Smith obtained from the Rangpo region of Sikkim.

2.1.1.3. Pharmaceutical and Phytochemical aspects

In folk medicine, the leaves as well as root of *Colebrookea oppositifolia* is being used to treat various ailments. Ethnobotanical studies indicate that the decoctions of *Colebrookea oppositifolia* leaves and stem bark are widely used among the tribal populations of Sikkim to treat skin infections [2], indigestion [2], diarrhoea [5], wounds and cuts [4]. Alcoholic extracts of leaves is reported to be useful in asthma [6], epilepsy [7] and helminthes [8]. It is administered either as decoction, infusion or tincture. The leaves pastes are applied to wounds and bruises, and are also used in eye diseases. The roots are used in epilepsy by the tribals of western Himalaya. The defatted alcoholic extract of the roots showed CNS excitation with increased rate of respiration and induced motor incoordination in mice [3]. Leaf decoction is also used as aphrodisiac and relief from tension (personal experience of the authors).

Phytochemical analysis of *C.oppositifolia* revealed the presence of number of flavonoids: Baicalein, tri-O-methyl [9], Chrysin [10], Colebrookia flavonoid [11], 2'–5–7– trihydroxy flavone [10], 2–hydroxy –2– 3 –7–8– tetramethoxy flavonol [12], 4'–5–6–7– tetramethoxy flavone [13], Ladanein [10], Negletin [10], Quercetin [14] and Scutellarein [9]. The bark also contains steroid, lipid, Triacontane and triacontene [8]. The alkanol and alkene (C_5 or more) isolated from plant showed remarkable Anthelminetic avtivity. Methanol: water (1:1) extract of dried aerial part of *C.oppositifolia* showed significant cytotoxic and antitumor activity [15].

2.2. *Heracleum Species* [2]

The Genus belongs to the family Umbelliferae (sub Apiaceae). The family contains about 275 genera and 2850 species, out of which *Heracleum* contains 70 species. The constituents of the family, other than volatile oil and resins, include coumarins, furocoumarins, chromonocoumarins, flavonoids, terpenes and sesquiterpenes, triterponoid saponins and acetylenic compounds. Alkaloids remain as rare constituent in the species.

2.2.1. *Heracleum nepalense* D. Don. DC [2]

The synonyms of the plant are: Nepali – *Chimphing*, Lepcha – *Sanben*.

Figure 2.2. Photograph showing the plant *Heracleum nepalense* D.Don. DC obtained from Pstangu region of Sikkim.

2.2.1.1. Distribution

This species is a forest dweller and is found in North- Eastern part of Sikkim Himalayan range at an altitude of 2000 – 3000 m. This plant is found in several parts of the Himalayan range of Sikkim, including spring banks, freshly burnt area, and association with Rhododendron. (Fig. 2.2)

2.2.1.2. Features of different parts of the plant

It is a small shrub 0.9 to 1.2m high glabrescent stem. Leaves 25 cm long uni or bipinnate; leaflets sessile oblong or ovate, irregularly toothed; flowers white, in umbels. Flowering in July and fruits are obovate. Fruiting August to.September. The dried herb is used as a fodder in winter. The seeds are being used for preparation of chatni [1].

2.2.1.3. Pharmaceutical and Phytochemical aspects

Ethnomedicinal studies indicate that the root juice is being used to treat various ailments [2]. The root juice is said to posses antidiarrhoeal, aphrodisiac and analgesic property. The plant is used in veterinary medicine. It exhibits stimulant property and increases the rate of respiration and blood pressure in goats. The essential oil from fruits showed moderately good antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Vibrio cholerae* [16]. Phytochemical analysis of the root of the plant revealed the presence of a number of Coumarin: Bergapten [17, 18, 19], Pimpinellin [17], Saphondin [17].

2.3. Effect of herbs on bioavailability and pharmacokinetics of drugs

The therapeutic effectiveness of a drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate and amount sufficient to elicit the desired pharmacologic response. This attribute of the dosage forms referred to as physiologic availability, biologic availability or simply bioavailability. For most drugs, the pharmacologic response can be related directly to the plasma levels. Thus the term **bioavailability** is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form. It is an absolute term. The concentration of drug in plasma and hence the onset of action, and the intensity and duration of response depend upon the bioavailability of drug from its dosage form. Other processes that play a role in the

therapeutic activity of a drug are distribution and elimination. In order to administer drugs optimally, knowledge is needed not only of the mechanisms of drug absorption, distribution, metabolism and excretion (*ADME*) but also the rates (kinetics) at which the processes occur. So **pharmacokinetics** is defined as the study of time course of drug *ADME* and their relationship with its therapeutic and toxic effects of the drug [20].

There is a growing awareness that herbal remedies and other phytochemicals could severely affect the disposition of drugs and chemicals. During the past five years, a few herbs that have been widely popularized, such as ginkgo, ginseng, and St. John's wort, were specifically cited as causing, or suspected of causing, interactions with drugs (Table 2.1). A possible interaction refers to the possibility that herbs may alter the bioavailability or the clinical effectiveness of drug when both are given concurrently. The net result may be an increase or a decrease in effect of one or both substances. The main causative factors in the effect of herbs on bioavailability of drugs were identified as the phytoconstituents that modulated a major of drug metabolizing enzyme system, Cytochrome P450 (CYP) known as acromyn [21]. Additionally, molecules that are substrate of P-glycoprotein efflux transporter pumps may also get affected by the interaction of herbs [22].

The cytochrome P450 (CYP) enzyme system consists of a superfamily of hemoproteins that catalyse the oxidative metabolism of a wide variety of exogeneous chemicals including drugs, carcinogens, toxins and endogenous compounds such as steroids, fatty acids and prostaglandins [22]. The CYP enzyme family plays an important role in phase-I metabolism of many drugs. The broad ranges of drugs that undergo CYP mediated oxidative biotransformation are responsible for the large number of clinically significant drug interactions during multiple drug therapy. The enzyme cytochrome P450 is derived from the proteins that have a heme group and unusual spectrum. The name cytochrome P450 is appropriated from the fact that these enzymes are characterized by a maximum absorption wavelength of 450 nm in reduced state in the presence of carbon monoxide. Naming a cytorome P450 gene included root symbol "CYP" for humans ("CYP" for mouse and Drosophilla), an Arabic numeral denoting the CYP family (e.g. CYP1, CYP2), letters A, B, C indicating subfamily (e.g. CYP3A, CYP3C) and another Arabic numeral representing the

Table 2.1. Herbs reported to have potential for drug interactions.

Herb	Source	Interactions reported or suspected
St. John's wort	*Hypericum perforatum* (Whole plant)	Warfarin (to cause bleeding); serotonin-uptake inhibitors (to cause mild serotonin syndrome); indinavir (increased bioavailability); digitoxin, theophylline, cyclosporin, phenprocoumon, and oral contraceptives (all with reduced bioavailability)
Ginseng	*Panax ginseng* (Root)	Antidepressants such as phenelzine sulfate (to cause manic episodes, headache); warfarin (to cause bleeding or to decrease effectiveness); corticosteroids (potentiation); estrogens (potentiation)
Ginkgo	*Ginkgo biloba* (Leaf)	Warfarin (to cause bleeding)
Ginger	*Zingiber officinale* (Rhizome)	Sulfaguanidine (enhance absorption)
Garlic	*Allium sativum* (Bulb)	Warfarin (to cause bleeding)
Rhubarb	*Rheum officinale* (Root)	Cardiac glycosides and antiarrhythmic agents (potentiating by reducing potassium via laxative effect)
Aloe	*Aloe ferox* (Leaf sap)	Cardiac glycosides and antiarrhythmic agents (potentiating by reducing potassium via laxative effect)
Astragalus	*Astragalus membranaceus* (Root)	Cyclosporine, azathioprine, methotrexate (to impair intended immuno-suppressive effects).
Bupleurum	*Bupleurum falcatum* (Root)	Sedatives (potentiation)
Liquorice	*Glycyrrhiza uralensis* (Root)	Corticosteroids and thiazide diuretics (potentiation); digitalis or other cardiac glycosides (increased sensitivity)

individual gene/isoenzyme/isoform (e.g. CYP3A4, CYP3A5) [23]. CYP3A4 isoenzyme is the most predominant isoenzyme in the liver and is involved in the metabolism of approximately 30-40% of drugs [24, 25].

Literature and anecdotal reports suggest that concomitant oral administration of some natural products and pharmaceuticals may affect metabolism in human and significantly increase the plasma-drug concentration [26-76].

Bailey *et.al* reported a significant pharmacokinetic and pharmacodynamic interaction of felodipine (a calcium antagonist) when the drug was given with concomitant intake of grape fruit juice; this investigation was carried out in a cohort of subjects who had a borderline hypertension [26]. As a result of the pharmacokinetic interaction, parameters such as peak plasma concentration (C_{max}) and area under plasma concentration-curve (AUC) increased approximately by 2.2 fold and 2.5 fold respectively of control treatment (water alone). In a similar fashion, the pharmacodynamic interaction resulted in a significant 2-fold decrease in diastolic blood pressure and a 2-fold increase in heart rate measures compared to the treatment. Interestingly, the concomitant administration of felodipine with orange juice to the same subjects did not alter the pharmacokinetics or pharmacodynamics of felodipine. Another contemporary work showed that other dihydropyridine substrates such as nitredipine and nisoldipine when administered with grape fruit juice exhibited increased rate and extent of absorption when compared to the respective control treatment[27].

Proppe *et.al* observed a stable creatine clearance in all but one patient, in spite of significant alternation in the pharmacokinetics of cyclosporine following intake of grape juice [28]. In a group of women patients, it has been shown that the intake of grape juice significantly inhibited the metabolism of 17 β-estradiol (hormone supplement) and as result significantly increased the bioavailable amounts of 17 β-estradiol and its metabolite, estrone. However, it appeared that this pharmacokinetic interaction between grape juice and 17 β-estradiol might not be clinically important [29].

Anderson *et.al* have reported, a 2-fold increase in the exposure of midazolam following grapefruit juice administration in patients with liver cirrhosis. In the same report, authors concluded that those liver cirrhotic patients are more dependent on the intestinal metabolism of midazolam than the normal subjects who have the normal liver function [30].

Nagy *et.al* have reported an approximately 3.2 fold increase in the availability of albendazole, when grape juice was ingested with the drug [31]. In another study, the bioavailability of sildenafil was enhanced, while the absorption was delayed when grape juice was administered with sildenafil [32]. Both C_{max} and AUC values of cisapride were tremendously increased by concomitant grape juice ingestion, however, neither T_{max} nor elimination half-life values were altered [33]. In case of saquinavir, a HIV protease inhibitor, concomitant intake of grape juice increased the bioavailability of saquinavir by approximately 1.7 fold [34].

One of the main causative factors in the grape juice effect on drug availability was identified as a group of furanocoumarins that inhibited a major drug metabolizing enzyme system, cytochrome P450. In particular, CYP3A4 appears to be the enzyme most affected, though the entire CYP system is inhibited to some extent [35, 36]. CYP is mainly found in the small intestine and liver; it is believed that the major effect of grape juice is to inhibit the small intestine CYP, thus preventing the drug from being metabolized before it enters the blood stream. In fact, the process has been described as more than simple inhibition: to a certain extent the enzyme is bound to the chemical compounds in the juice and washed away from the small intestine. Repeated administration of the juice increases its effect, rather than causing a rebound of enzyme levels [37].

Interaction between grape juice and clinically used drugs has been reported in recent years. These drugs include cyclosporine, midazolam and trizolam. All of these drugs are substrates for CYP3A4 and undergo extensive metabolism by intestinal CYP3A4 [38, 39].

Different other studies have been conducted to know the compounds in which grape juice involved in the drug interaction. Analysis of ethyl acetate extracts from grape juice

revealed the presence of several furanocoumarins of which bergamottin, the parent compound of 6', 7'-dihydroxybergamottin, is the major one and was found to be a mechanism based inactivator of CYP3A4 in human liver microsomes [40]. The other non-flavonoid components found in grape juice such as limolin and obacunone, a triterpene-derived product, also reduced microsomal testosterone 6β-hydroxylation in human liver [41].

Ping. Cheun Ho *et al.* studied the effect of flavonoids and furanocoumarin compounds found in grape juice, on activity of human liver CYP3A4. The study has reported that besides the flavonoids, other compounds found in grapefruit including furanocoumarins can produce strong inhibition of CYP3A4. The grape juice drug interactions could involve CYP3A4 inhibition by more than one component present in grape juice [42]. Other *in vivo* and *in vitro* studies have shown that flavonoids can enhance or inhibit the activities of certain P450 isozymes [43, 44, 45, 46, 47].

When predicting potential drug-herbs interactions, the role of the membrane transporter P-glycoprotein (Pgp) must also be considered [48]. Pgp, a member of the superfamily of proteins known as the ATP-binding cassette, is capable of pumping a wide range of structurally diverse, lipophilic drugs out of cells, potentially acting as a defense mechanism. This glycoprotein is well recognized because of its contribution to multiple-drug resistance during cancer chemotherapy. Pgp has been discovered in the liver, kidney (where it pumps drug out of the body through the urine), bloodbrain and bloodtestis barrier, lymphocytes, and the placenta. Pgp has also been located in the enterocytes of the small and large intestine, where its role is to carry lipophilic molecules from the enterocyte back into the intestinal lumen for elimination. Many hydrophobic drugs are either metabolized by CYP3A or pumped back into the lumen by Pgp after intestinal absorption and enterocyte uptake. Therefore, CYP3A and Pgp, acting in tandem, may decrease the oral absorption and delivery of different pharmaceuticals [49].

Abernethy *et.al* have shown that grape juice can inhibit the P-glycoprotein related efflux transport of talinolol both *in vitro* and *in vivo* situations [50]. In another study, it was

reported a decrease in the concentrations of celiprolol following grape juice administration. The authors have attributed a physical interaction of grape juice such that it interferes with phase of celiprolol. The data is well supported by the fact that itracanozole's treatment increases the plasma concentrations of celiprolol due to itracanozole's known inhibitory on CYP3A4 and P-glycoprotein [51]. Oral bioavailability of some protease inhibitor was unexpectedly increased by about 25% when the patients were preadministered with grape juice [52].

Piperine (1-piperoyl piperidine) a major component of the Piper species *Piper nigrum* L. (Piperaceae) is a potent inhibitor of drug metabolism and inhibited hepatic monooxygenase and UDP-glucuronyltransferase [53, 54].

Hiwale *et.al* studied the effect of piperine on bioavailability and pharmacokinetics of β-lactam antibiotics in rats. It has been observed that an earlier t_{max}, higher C_{max} and AUC were obtained in the mice that received both the drugs concurrently [55]. In addition piperine has also been shown to enhance the bioavailability of drugs like vascicine, spartenin, barbiturate and oxyphenbutazone, zoxazolamine, propranalol and theophylline in animal experiments [56-59].

Bano *et.al* studied the effect of piperine on pharmacokinetics of phenytoin in the healthy human volunteers and reported the significant increase in the bioavailability of the phenytoin in volunteers treated with piperine [60]. In another study, piperine was reported of the increase in bioavailability of indomethacin in rabbits, including t_{max}, C_{max} and AUC [61].

St. John's Wort (SJW), an herbal product derived from the perennial plant *Hypericum perforatum* and has gained immense popularity as an antidepressant. In recent years, there have been numerous case reports of interactions between SJW and prescription drugs metabolized by CYPs such as indinavir, cyclosprorin A, warfarin, digoxin, and theophylline [62]. Several *in vitro* and *in vivo* studies have evaluated the effects of SJW extract on the expression and activity of hepatic CYP3A4 and the drug efflux protein P-glycoprotein (Pgp) [63-67]. These data suggest that SJW induces CYP3A4 activity, which

could explain many of these drug interactions. Additionally, SJW may also induce intestinal Pgp expression and activity and influence pharmacokinetics of drugs such as indinavir and digoxin [63, 64]. A pharmacokinetic interaction between SJW and theophylline was reported, where plasma concentrations following SJW therapy were decreased [68]. Since theophylline is metabolized mainly by CYP1A2, it is possible that induction of CYP1A2 by SJW may explain the reduced theophylline concentrations and loss of therapeutic efficacy. The use of medicinal herbs has particularly increased over the past few years among specific patient populations including HIV-infected patients. St. John's wort altered pharmacokinetics of the HIV protease inhibitor, indinavir in individuals on retroviral therapy [69]. According to the Card L, St. John's wort induces CYP3A4, which metabolizes most protease inhibitors. The concomitant administration of St. John's wort with protease inhibitors could result in the induction of CYP3A4, increased metabolism, and sub therapeutic levels of the protease inhibitor.

Rosemary, *Rosmarinus officinalis* (Labiatae) is used externally to improve circulation in hypotonic circulatory disorders, rheumatic conditions, eczema, and as a poultice for poorly healing wounds. Rosemary is taken orally for dyspeptic disorders, loss of appetite, liver and gallbladder complaints, and blood pressure problems [70]. It inhibits the binding of doxorubicin and vincristine to Pgp, thereby increasing the intracellular accumulation of these chemotherapeutic agents [71]. Rosemary may thus also increase cyclosporine plasma concentrations by increasing its oral bioavailability, through inhibition of Pgp activity.

Strandell *et. al* studied the interactions of herbals and other natural remedies on cytochrome P450. They confirmed that extracts of herbal preparation were potent inhibitor of all tested metabolic enzymes [72]. In another study, it was reported that garlic inhibited the metabolic enzymes and increases the bioavailability of different pharmaceuticals [73]. Traditional Chinese medicines (TCM) are believed by many to be safe and used for self-medication without supervision. Although the risk appears to be low, certain TCM have been associated with a number of serious adverse reactions. In different studies it was reported that many Chinese medicinal herbs were inhibitors or inducers of hepatic CYP450 [74-76].

2.4. Immunostimulatory agents from herbal source

Immunostimulants or immunopotentiators are drugs leading predominantly to a non-specific stimulation of immunological defense mechanisms [77,78]. Change in the immunity leads to incurable life threatening diseases like cancer and AIDS. So, stimulation of the host immune response presents an interesting supplementary approach for conventional anti-AIDS therapy. The stimulation of immune response by using herbal products, as a possible therapeutic measure has become a subject of scientific investigations. The basic concept has, however, existed in the ancient Vedic scripture, the Ayurveda, and has been practiced in Indian traditional medicine for many centuries. One of the therapeutic strategies in Ayurveda medicine is to increase body's natural resistance to the disease causing agent rather than directly neutralizing the agent itself; in practice, this is achieved by using extracts of various plant materials called *rasayanas* (Charak Samhita, 1000 BC)[79]. This concept in modern scientific understanding would mean enhancement of immune responsiveness of an organism against a pathogen by non-specifically activating the immune system using immunostimulatory agents of plant origin. It is now being recognized that immunostimulation could provide an alternative to conventional chemotherapy for a variety of diseased conditions, especially when host's defense mechanisms have to be activated under the conditions of impaired immune response [80].

The basic function of the immune system is to protect against foreign pathogens and infectious agents. This is achieved either through innate or natural immunological mechanisms which essentially serve as a short term first line of defense or through elaborate adaptive mechanisms which are highly specific, complex and are marked by diversity and memory. In both types of immunity, cells and molecules play important roles. While in natural or innate responses the cellular players are monocytes, macrophages, polymorphonuclear phagocytes and natural killer cells, in the adaptive immunity the pivotal role is played by two classes of lymphocytes, viz., T (thymus derived) and B (Bursa- or bone marrow derived) cells and these are assisted by accessory cells such as antigen presenting cells. Further, the cellular dichotomy in adaptive immune response is also reflected in functional division of labour as the T cells serve as effectors of cell mediated immune responses such as delayed type hypersensitivity and killing of virus

infected cells and also as helpers for the production of highly specific proteins, called antibodies, by the B lymphocytes. These antibodies possess binding sites complementary to the antigen and are responsible for their removal from the system. The molecular constituents of the natural immune system are the complement proteins some of which help in opsonisation of foreign bacteria, lysozyme, defensin peptides, certain cytokines, etc [81-83]

Although extensive work has been carried out in the field of medicinal chemistry during this century, it is only in the last two decades that a number of compounds with immunostimulating activity have been identified from the plant materials. These include: alkaloids, terpenoids, quinines in one category and polysaccharides, peptides, glycoproteins and nucleotides in the other.

Upadhaya S.N. has highlighted the therapeutic potential of immunomodulatory agents from plant products. They have evaluated Indian medicinal plants for immunostimulatory activity. The author had also reviewed the Ayurvedic preventive healthcare medicines. A list of Ayurvedic medicinal plants showing immunostimulatory activity has been provided which includes agents like *Withania somnifera, Allium sativum, Azadirachta indica, Piper longum, Asparagus racemosus, Glycyrrhiza glabra, Aloe vera, Gmelina arborea* and *Tinospora cordifolia* [80].

Thatte and Dahanukar, have described how the description of ancient writings can lead to the development of new immunostimulatory agents. The experiments carried out to prove the *rasayana* concept of Ayurveda have demonstrated that *Asparagus racemosus, Tinospora cordifolia* and *Withania somnifera* protected animals against infections in normal and immunosupressed states induced by hemisplenectomy or surgery [85]. These plants also produced leucocytosis with predominant neutrophilia and prevented, to varying degrees, the leucopenia induced by cyclophosphamide. They were found to activate the polymorphonuclear and monocyte-macrophage systems. Only these *rasayanas* which produced sweet (madhur) vipaka (*Tinospora cordifolia, Asparagus racemosus, Emblica officinalis, Terminalia chebula* and *Withania somnifera*) were found to stimulate the

reticulo-endothelial system, but not these like *Acorus calamus*, *Commiphora mukul*, and *Picorrhiza kurroa*, which produced bitter (katu) vipaka [84].

Among the immunostimulant *rasayanas*, *Tinospora codiofolia* has been extensively studied by Dahanukar *et al* [85]. It has been found to activate the mononuclear cells to release cytokines like GMCSF [86] and IL-1 in a dose dependent manner [84]. Whole aqueous extract of *Tinospora codiofolia*, standardized using HPTLC, has been evaluated as an adjuvant in clinical conditions like obstructive jaundice, tuberculosis and cancer chemotherapy and has been found to increase the efficacy of conventional therapy. Active principle of *Tinospora codiofolia* were found to possess anticomplementary and immunomodulatory activities. Syringin (TC-4) and cordial (TC-7) inhibited the *in vitro* immunohaemolysis of antibody coated sheep erythrocytes by guinea pig serum by inhibiting the C-3 convertase of the classical complement pathway. The compounds also gave rise to significant increases in IgG antibodies in serum. Both humoral and cell-mediated immunity were dose dependently enhanced. Macrophage activation was reported by cordioside (TC-2), cordiofolioside A (TC-5) and cordiol (TC-7) and this activation was more pronounced with increasing incubation time [87].

The effect of *Asparagus racemosus, Tinospora cordifolia, Withania somnifera* and *Piccorrhiza kurroa* on macrophage function obtained from mice treated with the carcinogen, ochratoxin (OTA) was evaluated by Dhuley J.N. [88]. Treatment with these plants significantly attenuated the OTA induced suppression of chemostatic activity as well as IL-1 and TNF-α production by macrophages. Moreover, *Withania somnifera* potentiated macrophage chemotaxis and *Asparagus racemosus* induced excessive production of TNF-α as compared to controls.

Ray *et al* demonstrated that ovalbumin immunized mice treated with *Azadirachta indica* leaf extract had higher IgG and IgM levels and anti-ovalbumin antibody titers as compared to control (humoralresponse). *Azadirachta indica* also induced cell-mediated response as seen from the enhancement of macrophage migration inhibition and footpad thickness [89]. These finding were supported by Ansari et al. They found that *Azadirachta indica*

potentiated the antibody titres following typhoid H antigen immunization and induced delayed hypersensitivity following administration of tuberculin and DNBC to animals. In human volunteers, it stimulated humoral immunity by increasing total lymphocyte and T-cell count in 21 days [90].

Oral treatment with leaf extract of *Azadirachta indica* reversed the inhibitory of restraint stress on formation of anti-sheep RBC antibody titres in rats immunized with sheep RBC and also the increase in footpad thickness. It reversed the DDT induced suppression of antibody response and leucocyte migration inhibition in tetanous toxoid immunized rats. Restraint stress along with administration of DDT in subthreshold doses resulted in an inhibition of the immune response. *Azadirachta indica* attenuated the immunotoxicity of environmental and xenobiotic stressors [91]. In another study, the animals were treated intraperitoneally with the neem oil. Peritoneal macrophages exhibited enhanced phagocytic activity and expression of MHC class-II antigens. Neem oil treatment also induced the production of gamma interferon. Spleen cells showed a significantly higher proliferative response of Con A and tetanus toxoid TT *in vitro* compared to that of controls. Pretreatment with neem oil, however, did not augment the anti TT antibody response. These data suggested that neem oil acts as a non-specific immunostimulant for cell mediated immune mechanisms [92].

Root suspension of *Janakia arayalpathra* was found to have immunostimulatory properties in mice. It stimulated an increase in humoral antibody titres and also of antibody secreting spleen cells in the plaque forming cells assay following immunization with sheep erythrocytes. It also increased the number of peritoneal macrophages and produced an increase in delayed hypersensitivity reaction in mice [93].

The alkaloidal fraction of *Boerrhiva diffusa* significantly restored the suppressed humoral response in stressed rats as observed by Mungantiwar et. al., wherein *Boerrhiva diffusa* increased the suppressed antibody titres following immunization by sheep RBCs in rats subjected to restraints stress. It also significantly reversed the depleted adrenal cortisol

level and elevated plasma cortisol level in the stressed rats, thus appearing to have a corticosteroid sparing effect in experimental stress [94].

Immune-21, a polyherbal natural product has been shown to exhibit significant immunopotentiating and immunoprophylactic activity, both *in vitro* and *in vivo* [95]. In another study, one hundred and seventy-eight ethanolic plant extracts from the pharmacopoeia of the Tacana, an ethnic group from Bolivia, were screened for immunomodulatory activity using complement cascade inhibition and ADP-induced platelet aggregation inhibition assays. Six plants impaired both complement pathways (classical and alternative): stem bark from *Astronium urundeuvea* (Anacardiaceae), *Cochlospermum vitifolium* (Cochlospermaceae), *Terminalia amazonica* (Combretaceae), *Triplaris americana* (Polygonaceae), *Uncaria tomentosa* (Rubiaceae) and *Euterpe precatoria* (Arecaceae) roots. Inhibition of complement cascade was independent of essential ion complexation, and was not due to direct hemolytic activity on target red blood cells. For *A. urundeuvea*, *C. vitifolium*, and *T. amazonica*, anti-inflammatory activity relied on cyclo-oxygenase inhibition. Four of these species (*A. urundeuva, T. americana, U. tomentosa* and *E. precatoria*) are used traditionally to treat inflammatory conditions [96].

Jayathirtha *et. al.* made an attempt to assess the immunomodulatory activity of methanol extracts of whole plant of *Eclipta alba* and *Centella asiatica* at five dose levels, ranging from 100 to 500 mg/kg body weight., using carbon clearance, antibody titer and cyclophosphamide immunosupression parameters. In the case of *E. alba,* the phagocytic index and antibody titer increased significantly and the F ratios of the phagocytic index and WBC count were also significant. Regression analysis showed linearity in patterns of the dose response relationship, greatest in the case of the phagocytic index, moderate in the WBC count and lowest in the antibody titer. For *C. asiatica*, significant increases in the phagocytic index and total WBC count were observed and the F ratio of the phagocytic index was also significant. Regressed values revealed maximum linearity in the case of the phagocytic index, moderate linearity in the total WBC count and lowest linearity in the antibody response [97].

Joharapurkar *et al* developed a new model to screen the immunomodulatory activity of *Rubia cordifolia*. He concluded that, the minimum dose of pyrogallol, which can induce significant immunosupression, was 50 mg/kg bodyweight. Thus, pyrogallol can be used as an experimental tool to induce immunosupression while screening the immunomodulatory activity [98].

2.5. Antioxidants from herbal source

During normal biochemical reactions in our body there is a generation of reactive oxygen and nitrogen species (ROS and RNS). These are enhanced during patho-physiological conditions creating 'oxidative stress'. During this phenomenon cellular constituents get altered resulting in various diseased states. This may be effectively neutralized, by enhancing the cellular defense mechanisms, in the form of antioxidants. So, an antioxidant may be defined as any substance that when present in low concentrations compared to that of an oxidizable substrate significantly delays or inhibits oxidation of the substrate [99,100].
The generation of ROS begins with the rapid uptake of oxygen and activation of NADPH oxidase and the production of the superoxide free radicals (O_2^-) Superoxide is then rapidly converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD).

These ROS can act by either of the two oxygen dependent mechanisms resulting in the destruction of the microorganism or other foreign matter. The reactive species can also be generated by the myeloperoxidase-halide-H_2O_2 system. The neutrophil cytoplasmic granules contain the enzyme myeloperoxidase (MPO). In presence of chloride ion, which is ubiquitous, hydrogen peroxide is converted to hypochlorous acid (HOCl), a potent oxidant andantimicrobial agent [101].

The MPO dependent mechanism, though not as important as the previous one, is still essential. ROS are generated from superoxide and H_2O_2 produced via respiratory burst by Fenton (A) and/or Haber-Weiss (B) reactions.

(A) $H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$

(B) $O_2^- + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + O_2$

Reactive nitrogen species are also important. The free radical nitric oxide (NO), first described as endothelium derived relaxation factor (EDRF), is produced from arginine by nitric oxide synthatase (NOS). An inducible nitric oxide synthatase is capable of continuously producing large amount of NO. In activated cells, it acts as a killer molecules[102].

There is increasing evidence to support the involvement of free radical reactions in several human diseases. Free radical reaction is an important pathway in a wide range of unrelated biological systems. Amongst the very many ways to chemically injure and kill cells, an important class of reactions is that producing free radical intermediates which trigger a network of multifarious disturbances because of ubiquity of molecular oxygen in aerobic organisms and its ability to accept electron [103].

Oxygen derived free radicals are often mediators and/or products of normal, pathological or toxic free radical reactions. A vast amount of circumstantial evidence implicates oxygen-derived free radicals (superoxide and hydroxyl radical) and high-energy oxidants (peroxynitrile) as mediators of shock, inflammation and ischemia reperfusion injury. Active oxygen species and other free radicals have long been known to be mutagenic. Further, these agents have more recently emerged as mediators of the other phenotypic and genotypic changes that lead from mutations to neoplasia. Therefore, free radicals may contribute widely to cancer development in humans. In last decade, evidences have been accumulated that the free radical process known as lipid peroxidation plays a crucial and causative role in the pathogenesis of atherosclerosis, cancer, myocardial infraction, and aging. Participation of free radical oxidative interactions in promoting tissue injury in conditions like brain trauma, ischaemia, toxicity and also in neuro-degenerative diseases such as Parkinson's disease, Alzheimer's dementia, multiple sclerosis and lipofuscinosis are now well documented [104].

The involvement of reactive oxygen species (ROS) in the pathogenesis of several lung diseases has also been suggested. The pioneer studies on the role of free radical reactions in the genesis and the expression of cellular and tissue damage has been carried out mainly in the liver, in particular, during the last 25 years by using acute rat poisoning with carbon

tetrachloride (CCl_4) as a model system. Several scientists have demonstrated the different mechanisms by which carbon tetrachloride activation to free metabolites lead to liver degeneration and necrosis [105].

Studies in experimental models have incriminated reactive oxygen species as primary mediators in the pathogenesis of ischaemic, toxic and immunologically mediated renal injury. Diabetes mellitus is also associated with oxidative reactions, particularly those that are catalyzed by decompartmentalized transition metals, but their causative significance in diabetic tissue damage remains to be established [105].

More interestingly, free oxygen radicals are increasingly discussed as important factors involved in the phenomenon of biological aging. Higher formation rates of free radicals from senescent animals observed in isolated biological material (mainly mitochondria), accumulation of the free radical damage and changes of antioxidants capacities appear to prove correctness of this assumption. The basic idea behind this assumption is that aging results from random deleterious effects of tissue brought about by free radicals. A conclusion from the data available so far was the free radicals are very likely to contribute considerable to the development of stochastic disorders observed during the progress of aging [106].

Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzymes. In traditional South-East Asian medicine the therapeutic value of the parenchymatous leaf-gel of *Aloe vera* for inflammation-based diseases was reported. Extracts from leaf-gel contain glutathione peroxidase activity. The low molecular weight constituents of this extract inhibit the release of reactive oxygen species (ROS) by phorobol myristic acetate (PMA)- stimulated human polymorphonuclear leucocytes (PMN) [107].

The antioxidant effects of oils isolated from onion and garlic on nicotine-induced lipid peroxidation in rat tissues were studied. Lipid peroxidation was significantly increased in the tissues of nicotine treated rats. Both the garlic oil and onion oil supplementation to

nicotine treated rats increased resistance to lipid peroxidation. With garlic oil or onion oil supplementation, nicotine treated rats showed increased activities of antioxidant enzymes and increased concentrations of glutathione [108].

Kamat *et al.* have examined the antioxidant effects of crude extract as well as purified polysaccharide fraction of *Asparagus racemosus* against membrane damage induced in rat mitochondria and liver by free radicals generated during γ-irradiation. They found that these materials had potent antioxidant properties *in vitro* [109].

Selvam *et.al.* have isolated turmeric antioxidant protein (TAP) from the aquous extract of turmeric. The protein showed a concentration-dependent inhibitory effect on lipid peroxidation. Ca^{2+}-ATPase of rat brain homogenate was protected to nearly 50% of the initial activity from the lipid peroxidant induced inactivation by this protein. This protection of Ca^{2+}-ATPase activities was found to be associated with the prevention of loss of –SH groups [110]. Turmeric contains several small molecular weight components with antioxidant, medicinal and immunomodulatory activities. Natural curcuminoids, isolated from turmeric, were compared for their potential use as anti-promoters [111]. The curcuminoids inhibited lipid peroxidation besides the production of superoxides and hydroxyl radical.

The antioxidant activity of tannoid an active principles of *Emblica officinalis* consisting of emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%), was investigated on the basis of their effects on rat brain frontal cortical and striatal concentrations of the oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and lipid peroxidation in terms of thiobarbituric acid-reactive products [112].

Extract of *Tinospora cordiofolia* has been shown to inhibit the lipid peroxidation and formation of superoxide, and hydroxyl radicals *in vitro*. Administration of the extract partially reduced the elevated lipid peroxides in serum. *Tinospora* extract may be useful in reducing the chemotoxicity induced by free radical forming chemicals like

cyclophosphamides. Several glycosides with potential antioxidant activity were also isolated, as poly acetates, from the n-BuOH fraction of the *T. cordiofolia* stems [113].

Antioxidant activity of principles of *Withania somnifera*, consisting of equimolar concentrations of sitoindosides VII-X and withaferin A, was investigated for their effects on rat brain frontal cortical and striatal concentrations of superoxide dismutase, catalase and glutathione peroxidase [114]. Active glucowithanolides, administered once daily for 21 days, induced a dose-related increase in SOD, CAT and GPX activity in frontal cortex and striatum, which was statistically significant on days 14 and 21 [115].

The antioxidant properties of methanol extracts of 12 Indian medicinal plants were evaluated by two methods, namely the DPPH (1,1-diphenyl-2-picryl hydrazyl) and lipid peroxidation assay. In the later assay, seven of these extracts showed 90% or more activity compared with the standard, vitamin E and hence were studied in detail after the removal of interfering pigments. The selective pigment removal from the extracts led to an increase in free radical scavenging activity and a decrease in inhibition of lipid peroxidation [116].

Gupta *et al.* have examined the antioxidant and antitumor role of *Bahunia racemosa* against ehrlich ascites carcinoma in Swiss albino mice. They found that the methanol extract of the plant exhibited antitumor activity by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice [117].

Bafana *et.al* have studied the antiulcer and antioxidant activity of a herbomineral formulation, Pepticare. They concluded that the increase in the levels of superoxide dismutase, catalase, reduced glutathione and membrane bound enzymes like Ca^{2+} ATPase, Mg^{2+} ATPase and Na^{+} K^{+} ATPase and decrease in lipid peroxidation in both the models proved the antioxidant activity of the formulation [118]. In another study, the antioxidant and antimicrobial activities of *Bahunia racemosa* was conducted on the basis of DPPH, superoxide anion radical and hydroxyl radical scavenging activity. The results obtained in the study indicate that *Bahunia racemosa* can be a potential source of natural antioxidant and antimicrobial agents [119].

2.6. Antimicrobial agents from herbal source

Antimicrobial drugs are immensely important therapeutics discovered in the last century. Their advents have changed the outlook of the physician about the power of the drugs on disease management. Many infectious diseases once considered incurable and lethal are now amenable to treatment with a few pills. The remarkable powerful and specific activity of antimicrobial drugs is due to their selectivity for highly specific targets [120].

Antimicrobial agents can be classified on basis of the following two ways:

A. Chemical structure:

- Sulfonamides and related drugs : Sulfadiazine, Dapsone.
- Diaminopyrimidine :Trimethoprim.
- β- Lactam antibiotics : Penicillin, Cephalosporin.
- Tetracycline : Doxycycline.
- Nitrobenzene derivatives : Chloramphenicol.
- Aminoglycoside : Streptomycin, Neomycin.
- Macrolides : Erythromycin, Roxythromycin.
- Polypeptides : Polymyxin-B, Bacitracin.
- Nitrofurantoin derivatives : Nitrofurantoin, Furazolidone.
- Nitroimidazole derivative : Metronidazole, Tinidazole.
- Quinolones : Nalidixic acid, Ciprofloxacin.
- Nicotinic acid derivatives : Isoniazid, Pyrazinamide.
- Polyene antibiotics : Nystatin, Amphotericin B.
- Imidazole derivatives : Miconazole, Kitoconazole.
- Miscellaneous : Rifampicin, Griseofulvin.

B. Mechanism of action

- Inhibit cell wall synthesis : Penicillin, Cephalosporin.
- Cause leakage of cell membrane : Polymixin, Colistin.
- Inhibit protein synthesis : Tetracycline, Chloramphenicol.
- Cause misreading of mRNA code : Streptomycin.
- Interfere with DNA function : Rifampicin, Metronidazole.

- Interfere DNA synthesis : Idoxuridine, Acyclovir.
- Interfere with intermediary metabolism: Para amino salycyclic acid, Ethambutol.

Agents that inhibit bacterial cell wall synthesis are essentially nontoxic to the animal and human host, since cell walls are absent in mammalian tissues. These agents inhibit synthesis of certain active enzymes resulting disruption of bacterial walls to cause loss of viability and often cell lysis; these include the penicillins and cephalosporins [121]. Drug that affect the cell membrane bring about permeability changes in the microbial cells. The other antibiotics, which affect the cell membrane, are polymyxin B, colistimethate, Nystatin and amphotericin B [122]. These antimicrobials alter the osmotic properties of the cell resulting in leakage of important cellular constituents like ammonium ions, potassium ions and nucleotides leading to their cell death [123, 124].

The major inhibitor of protein synthetic machinery are the aminoglycosides to produce two types of changes on the bacterial cell, firstly, it binds irreversibly with 30S ribosomal subunits and interacts at the recognition region causing an inhibition of amino acyl t-RNA to bind with m-RNA and 50S ribose in order to distort the codon-anticodon interaction. Secondly, it causes cyclic polysomal blockage [125]. When it interferes with the chain elongation steps, the ribosome distortion takes place, which is seen through the steps of protein synthesis, producing a bacteriostatic effect. Due to its cyclic formation of inhibition complex caused by the 'dropping off' of ribosome no protein is synthesized and the cell undergoes death [124].

The principal inhibitors of nucleic acid synthesis are rifampcin and nalidixic acid against bacteria, and griseofulvin against fungi [126]. The other antibiotics selectively inhibiting the synthesis of nucleic acid include actinomycin, kenamycin, neomycin, novomycin, olivomycin against RNA and actidin, bruneomycin, mitomycin, sereomycin against DNA[127]. Drugs that are structurally similar to cellular metabolites and can complete with the natural substrate for incorporation in functionally important antimetabolites are sulfa drugs, para aminosalicylate, trimethoprim, ethambutol, 5-fluro-cytosine, primethamine and the antibiotic antimetabolites are leucine, furanomycin [126, 128].

The use of medicinal plants and their extracts for the cure of localized and specific human and cattle infections is an age-old practice from time immemorial. As early as 1630, Europeans used natural quinine from the bark of cinchona tree to treat malaria; a dreaded disease caused by a protozoan parasite *Plasodium* species [129]. Numerous studies have been performed throughout the globe in search of newer antimicrobial agents and most of those studies are directed towards the microbes. A number of studies showed that the antimicrobial principles could also available from marine algae and higher plants, particularly among angiosperms [130]. The antimicrobial compounds isolated from higher plants are different in chemical structures. They may be flavonoids, essential oils, alkaloids, anthraquinones, triterpenoids, etc. One approach that has been used for the discovery of antimicrobial agents from higher plants is based on the evaluation of the medicinal plant extracts. The work carried out on American and Europeans folk medicines are most important in this respect [131, 132].

The World Health Organization at its meeting on Herbal Medicine for human Health at Kuwait in 1985 specified some plants, as the remedies for varieties of skin diseases. These include *Aloe vera, Ficus carica, Azadirachata indica, Fumaria officinalis, Lausonria alba, Santalum albam* [133].

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents followed this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants [134]. However, plants used in traditional medicine are still understudied, particularly in clinical microbiology [135]. Therefore, it is a new challenge to search for the *in vitro* and *in vivo* antimicrobial activity of natural compounds from these ethnomedicinal plants on pathogenic bacteria.

Polyphenols are a group of highly hydroxylated phenolic compounds present in the extractive fraction of several plant materials. Polyphenols in plants include hydroxycoumarins, hydroxycinnamate derivatives, flavanols, flavonols, flavanones,

flavones, anthocyanins, proanthocyanidins (tannins), hydroxystilbenes, aurones, etc. Polyphenols are well documented to have microbicidal activities against a huge number of pathogenic bacteria. Oxidized polyphenols also have inhibitory activity against bacterial growth. The mechanism of polyphenols toxicity against microbes may be related to inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesions, cell envelope transport proteins, non specific interactions with carbohydrates, etc [136]. There are numerous illustrations of plant-derived drugs used as antimicrobials. Some selected examples, are presented below.

The alcoholic extract of dry nuts of *Semecarpus anacardium* (Bhallatak) showed bactericidal activity *in vitro* against three gram-negative strains (*Escherichia coli, Salmonella typhi* and *Proteus vulgaris*) and two gram-positive strains (*Staphylococcus aureus* and *Corynbacterium diphtheriae*). Subsequent studies have shown that the alcoholic extracts of different parts of the plant (leaves, twings, green fruit) also possess antibacterial properties, especially the leaf extract. No dermatoxic effect (irritant property) was observed in the mouse skin irritant assay [137].

The acetone and alcoholic extracts of the leaves of *Cassia alata* showed significant *in vitro* antibacterial activity against *Staphylococcus aureus*, coagulase positive *Staphylococcus aureus, Baciilus sublitis, Bacillus cereus, Bacillus stearothermophilus, Escherichia coli, Salmonella typhi* and *Salmonella dysentriae*. In addition, the alcoholic extract also inhibited growth of *Klebsiellae pneumoniae* whereas the acetone extract inhibited the growth of *Vibro cholerae* [138].

Kazmi *et al.* [139] described an anthraquinone from *Cassia italica*, a Pakistani tree, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphthericum*, and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*. Hypericin, an anthraquinone from St. John's wort (*Hypericum perforatum*), has received much attention in the popular press lately as an antidepressant, and Duke, P. reported the general antimicrobial properties of the plant [140].

Scalbert [141] reviewed the antimicrobial properties of tannins in 1991. He listed 33 studies, which had documented the inhibitory activities of tannins. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been found to bind cell walls of ruminal bacteria, preventing growth and protease activity [142]. Terpenenes or terpenoids are active against bacteria, fungi, viruses and protozoa [143, 144]. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Mendoza *et. al* [145] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity. Food scientists have found the terpenoids present in essential oils of plants to be useful in the control of *Listeria monocytogenes* [146].

Due to lack of ideal diffusion and evaporation from the surface it is generally difficult to assess the antibacterial properties of aromatic oils derived from plants using the agar cup methods. Hence, Agnihotri and Vaidya have developed a novel approach to study the antibacterial property of certains plants like *Eugenia caryophyllus, Thymus vulgaris, cinnamonum zeylanium* and *Cuminum ciminium.* Volatile components of the hexane extracts of these plants were tested against gram-positive and gram-negative bacteria grown on agar slants and the results were expressed as percentage inhibition of the area of the slants [147].Shirataki *et. al.* studied the medicinal significance of prenylflavanones obtained from *Sophora tomentosa* L. and *Sophora moorcroftiana* Benth. Ex Baker (Leguminosae) [148]. Further, two flavanones (YS01, YS02) and eight prenylflavanones (YS03-YS10) were investigated for both *in vitro* and *in vivo* antibacterial activity. The *in vitro* activity was shown by spot inoculation method and *in vivo* activity was carried out by determining the protection offered by Swiss albino mice against the virulent strain of *Salmonella typhimurium* NCTC 74 [149]. Owasis *et al.* evaluated the antibacterial activity of *Withania somnifera* against experimental salmonellosis in Balb/C mice and determined the bacterial load in various vital organs of the treated mice [150]. Considering the relevant literatures or depending upon the information collected from the reliable actual users of the flora in their clinical practice, the screening of herbal species for chemotherapy was felt out most essential.

References

1. Chetry, B. N. and Gurung, D. (2003). Medicinal plants from Sikkim region, 2nd ed., Dhar Hill Publishing Co. Ltd., Gangtok, Sikkim, pp. 24, 27.
2. Gurung, B. (2002). The medicinal plants of the Sikkim Himalaya, 1st ed., Subash publication, Sikkim, pp.156.
3. Annonymous, The Wealth of India, Raw material, Publication and information Directorate, CSIR, New Delhi. (2001). Vol-2, Ci-Cy, 153-154.
4. Jain, S. P. and Puri, H. S. (1984). Ethnopharmacological plant of Jaunsar-Barwar hills, Uttar pradesh, India. *J Ethnopharmacol* 12, 213-222.
5. Manadhar, N. P. (1995). Inventory of some herbal drugs of Myagdi district, Nepal. *Econ Bot* 49, 371.
6. Singh, V. (1995). Herbal folk remedies of Morni Hills, Haryana, India. *Fitoterapia* 66, 425-430.
7. Paul, S. R. (1977). Medicinal plants of Netarhat, Bihar, India. *J crude drug Res*1 79-97.
8. Ansari, S., Dobal, M. P., Tyagi, R. P., Joshi, B, C. and Barar, F. S. K. (1982). Chemical investigation and pharmacological screening of *C.oppositifolia* Smith. *Pharmazie* 37,70-74.
9. Ahmad, S.A., Siddiqui, S.A. and Zaman, A. (1974). Flavones from *Colebrookia oppositifolia*. *Ind J Chem* 12, 1327-1331.
10. Yang, F., Li Xi., Wang, H.Q. and Yang, C.R. (1996). Flavonoid glycosides from *Colebrookia oppositifolia*. *Phytochem* 42 (3), 867-869.
11. Sharma, D. (1981). A pill for Men. *India today,* 5, 98.
12. Dixit, V.P. (1982). Contraceptive-like properties of certain plant materials in male Presbytis monkeys. Contraceptive delivery systems. edn. E.S.E Hafez, MTP press Ltd, Lancaster, England, 33, 300-302.
13. Dixit, V.P. (1982). Contraceptive like properties of certain plant material in male presebytis monkeys. *Arch Androl* 9 (1), 28.

14. Mukherjee, P.K., Mukherjee, K., Hermans., lookkrbol, A.C.J., Verpoorte, R.and Suresh, B. (2001). Flavonoid content of *Eupatorium glandulosum* and *Colrbrookea oppositifolia*. *J Nat Reme* 11, 21-24.

15. Aswal, B.S., Bhakuni, D.S., Goel, A.K., Kar, K. and Mehorta, B.N. (1984). Screening of Indian plants for biological activity. *Ind J Exp Biol* 22 (6), 312-332.

16. Annonymous, The Wealth of India, Raw material, Publication and information Directorate, CSIR, New Delhi. (2001). Vol-3, D-I, 253-254.

17. Gupta, B.D., Banerjee, S.K.and Handa, K.L. (1975). Coumarins from *Heracleum wallichii* and *H. nepalense*. *Phytochem* 14, 2533-2538.

18. Pathak, M.A., Daniels, F. and Fitzpatrick, T.B. (1962). The presently known distribution of furocoumarins (Psoralens) in plants. *J Invest Dermatol* 39, 225-239.

19. Sun, H.D., Lin, Z.Q. and Nij, F.D. (1980). Study of the Chinese drugs of Umbelliferae, II, Chemical constituents of the roots of Heracleum nepalense D. Don. *Yunnan zhiwuyanjiu*, 224-227.

20. Brahmankar, D.M. and Jaiswal, S.B. (2003). Biopharmaceutics and Pharmacokinetics a treaties, 1st ed, Vallabh prakashan, New Delhi, 1, 62.

21. Chao, L.P, Hsiu, L.S. and Hou, Y.C. (2002). Flavonoids in herbs: Biological fates and potential interactions with xenobiotics. *J Food Drug Anal* 10 (4), 219-228.

22. Srinivas, N.R. (2004). Clinical implication and applications of grape fruit juice intake during oral therapy of drugs known to be metabolized by CYP 450. *Ind J Pharm Sci* 66 (5), 602-606.

23. Nelson, D.R., Koymans, L., Kamataki, T., Stegman, J.J. and Waxman, D.J. (1996). P450 superfamily: update of new sequence, gene maping, accession, numbers and nomenclature. *Pharmacogen* 6, 1-42.

24. Von Moltek, L.L., Greenblatt, D.J., Schider, J., Daun, S.X. and Wright, C.E. (1996). Midazolam hydroxylation by human liver microsomes in vitro: inhibition by fluoxetine, norfluxetine and azole antifungals. *J Clin Pharmacol* 52, 231-238.

25. Thummel, K.E. and Wilkinson, G.R. (1998). *In vitro* and *in vivo* drug interactions involving CYP3A. *Ann Rev Pharmacol toxicol* 38, 389-430.

26. Bailey, D.G., Spence, J.D., Munoz, C. and Arnold, J.M.O. (1991). Interaction of citrus juices with felodipine and nifedipine. *Lancet* 337, 854.

27. Bailey, D.G., Arnold, J.M.O., Strong, H.A., Munoz, C. and Spence, J.D. (1993). Interaction of grape fruit juice with calcium antagonist. *Clin Pharmacol Ther* 54, 589-595.

28. Proppe, D., Hoch, O., Visser, K. and McLean, A.J. (1995). Clearance of cyclosporine following intake of grapefruit juice. *Clin Pharmacol Ther* 57, 191-196.

29. Schubert, W., Culberg, G., Edgar, B. and Hedner, T. (1995). Effect of grapefruit juice on 17 ß-estradiol. *Maturitas* 20, 155.

30. Anderson, V., Pedersen, N., Larsen, N.E., Sonne, J. and Larsen, S. (2002). Grapefruit juice- midazolam interaction. *Brit J Clin Pharmacol* 54, 120-128.

31. Nagy, J., Schipper, H.G., Koopmanas, R.P., Buter, J.J., Van Boxtel, C.J. and Kagar, P.A. (2002). Drug interaction of albendazole with grapefruit juice. *Amer J Trop Med Hyg* 66, 260-267.

32. Jetter, A., Kinzi-Scippers, M., Walchner-Bonjean, M., Hering, U., Bulitta, J., Schreiner, P., Sorgel, F. and Fuhr, U. (2002). Grapefruit juice increases the oral bioavailability of sildanafil. *Clin Pharmacol Ther* 71,21-25.

33. Offman, E.M., Freeman, D.J., Dresser, G.K., Munoz, C., bend, J.R. and Bailey, D.G. (2001). Cispride-grapefruit juice interaction. *Clin Pharmacol Ther* 70, 17-25.

34. Ho, P.C., Ghose, K., saville, D. and Wanwinwimorluk, S. (2000). Interaction of grapefruit juice with protease inhibitor. *Eur J Clin Pharmacol* 56, 693-699.

35. Ameer, B. and Weintraub, R.A. (1997). Drug interactions with grapefruit juice. *Clin Pharmacokine* 33 (2), 103-121.

36. Fukuda, K. (1997). Specific CYP3A4 inhibitors in grapefruit juice: furocoumarin dimers as components of drug interaction. *Pharmacogen* 7(5), 391-396.

37. Lowin, K.S. (1997). Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 99, 2297-2298, 2545-2553.

38. Kolars, J.C., Awani, W.M., Merison, R.M. and Watkins, P.B. (1991). First pass metabolism of cyclosporin by the gut. *Lancet* 338, 1488-1490.

39. Paine, M.F., Shen, D.D., Kaunze, K.L., Perkins, J.D., Marsh, C.L., McVicar, J.P. and Thummel, K.E. (1996). First pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* 60, 14-16.

40. He, K., Iyer, K.R., Hayes, R.N., Sinz, M.W., Woolf, T.F. and Hollenberg, P.F. (1998). Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. *Chem Res Toxicol* 11, 252-259.

41. Fukuda, K., Ohata, T. and Yamazoe, Y. (1997). Grapefruit component interacting with rat and human P450 CYP3A: possible involvement of non-flavonoid component in drug intreraction. *Biol Pharm Bull* 20, 560-564.

42. Ping Chen, Ho., Dorthy, J.S. and Wanwimolruk, S. (2001). Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. *J Pharm Pharmaceut Sci* 4 (3), 217-227.

43. Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol* 32, 1141-1148.

44. Lasker, J.M., Huang, M.T. and Conney, A. H. (1984). *In vitro* and *in vivo* activation of oxidative drug metabolism by flavonoids. *J Pharmacol Exp Ther* 229, 162-170.

45. Friedman, F.K., West, D., Sugimura, T. and Gelboin, H.V. (1985). Flavone modulators of rat hepatic aryl hydrocarbon hydroxylase. *Pharmacol* 31, 203-207.

46. Triel, B.A. and Carlson, G.P. (1987). Effect of flavonone on mixed function oxidase and conjugation reactions in rats. *Xenobiotica* 17, 11-16.

47. Obrmeier, M.T., White, R.E. and Yang, C.S. (1995). Effects of biflavonoids on hepatic P450 activities. *Xenobiotica* 25, 575-584.

48. Thummel, K.E. and Wilkinson, G.R. (1998). *In vitro* and *in* vivo drug interactions involving CYP3A. *Annu Rev Pharmacol Toxicol* 38, 389-430.

49. Yu, DK. (1999). The contribution of P-glycoprotein to pharmacokinetic drug-drug interactions. *J Clin Pharmacol* 39,1203-1211.

50. Abernethy, D.R and Flockhart, D.A. (2001). Molecular basis of cardiovascular drug metabolism: Implications for predicting clinically important drug interactions. *Circulation* 101 (14), 1749-1753.

51. Lilja, J.J., Backman, J.T., Laitila, J., Lurila, H. and Neuvonen, P.J. (2003). Drug interaction and P-glycoprotein. *Clin Pharmacol Ther* 73, 192-198.

52. Huisman, M.T. and Smit, J.W. (2000). Significance of P-glycoprotein for the pharmacology and clinical use of HIV protease inhibitors. *AIDS* 14 (3), 237-242.

53. Atal, C.K., Dubey, R.K. and Singh, J. (1985). Biochemical basis of enhanced drug bioavailability by piperine-evidence is a potent inhibitor of drug metabolism. *Pharmacol Exp Ther* 232, 258-262.

54. Shobha, G., Joseph, T., Majeed, M., Rajendran, R. and Srinivas, P.S.S.R. (1998). Influence on the pharmacokinetics of curcumin in animals and human volunteers. *Plant Med* 64, 353-359.

55. Hiwale, A.R., Dhuley, J.N. and Naik, S.R. (2002). Effect of co-administration of piperine on pharmacokinetics of β-lactam antibiotics in rats. *Ind J Exp Biol* 40, 277-281.

56. Atal, C.K., Zutrshi, U. and Rao, P.G. (1981). Scientific evidence on the role of Ayurvedic herbals on bioavailability of drug. *J Ethnopharmacol* 4, 117-125.

57. Majumdar, A.M., Dhuley, J.N.,. Deshmukh, V.K., Raman, P.H. and Naik, S.R. (1990). Antiinflamattory activity of piperine. *Jap J Med Sc Biol* 43, 95-101.

58. Majumdar, A.M., Dhuley, J.N., Deshmukh, V.K., Raman, P.H., Tharat, S.L. and Naik, S.R. (1990). Effect of piperine on a phentobarbitone induced hypnosis in rats. *Ind J Exp Biol* 28, 486-491.

59. Majumdar, A.M., Dhuley, J.N., Deshmukh, V.K., Raman, P.H. and Naik, S.R. (1999). Effect of piperine on bioavailability of oxyphenbutazone. *Ind Drugs* 36, 123-127.

60. Bano, G., Amla, V. and Raina, R.K. (1987). The effect of piperine on kinetics of phenytoin in healthy human volunteers. *Planta Medica* 53, 568-569.

61. Karan, R.S., Bhargava, V.K. and Garg, S.K. (1999). Effect of Trikatu on pharmacokinetic profile of indomethacin in rabbits. *Ind J Pharmacol* 32, 160-164.

62. Izzo, A.A. and Ernst, E. (2001). Interactions between herbal medicines and prescribed drugs: a systematic review. *Drugs* 61,2163-2175.

63. Durr,D., Stieger, B., Kullal-Ublick, G.A., Rentsch, K.M., Steinert, H.C. and Meier, P.J. (2000). St. John's Wort induces intestinal P-glycoprotien/MDR1 and intestinal and hepatic CYP3A4. *Clin Pharmacol Ther* 68, 598-604.

64. Hennessy, M., Kelleher, D., Spiers, J.P., Barry, M., Kavanagh, P. and Back, D. (2002). St John's Wort increases expression of P-glycoproetin: implications for drug interactions. *Br J Clin Pharmacol* 53, 75-82.

65. Roby, C.A., Anderson, G.D., Kantor, E., Dryer, D.A. and Burstein, A.H. (2000). St. John's Wort: Effect on CYP4A3 activity. *Clin Pharmacol Thera* 67,451-457.

66. Moore, L.B., Goodwin, B., Jones, S.A., Wisley, G.B., Singh, C.J. and Wilson, T.M. (2000). St. John's Wort induces hepatic drug metabolism through activation of pregnane X receptor. *PNAS* 97, 7500-7502.

67. Wang, Z., Gorski, C., Hamman, M.A., Huang, S., Lesko, L.J. and Hall, S.D. (2001). The effects of St John's wort (hypericum perforatum) on human cytochrome P450 activity. *Clin Pharmacol Ther* 70, 317-326.

68. Nebel, A., Schneider, B.J., Baker, R.K. and Kroll, D.J. (1999). Potential metabolic interaction between St. John's Wort and theophylline. *Ann Pharmacother* 33, 502-509.

69. Piscitelli, S.C., Burstein, A.H., Chaitt, D., Alfaro, R.M. and Falloon, J. (2000). Indinavir concentrations and St. John's wort. *Lancet* 355, 547–548.

70. Fleming, T. (1998). Physicians' Desk Reference for Herbal Medicines. Medical Economics Company, Inc., pp.1101-1102.

71. Plouzek, C.A. and Ciolino, H.P. (1999). Inhibition of P-glycoprotein activity and reversal of multidrug resistance *in vitro* by rosemary extract. *Eur J Cancer* 35 (10), 1541-1545.

72. Strandell, J., Neil, A. and Carlin, G. (2004). An approach to the *in vitro* evaluation of potential for cytochrome P450 enzyme inhibition from herbals and other natural remedies. *Phytomed* 11, 98-104.

73. Foster, B.C., Foster, M.S., Vandenhoek, S., Krantis, A., Budzinski, J.W., Arnason, J.T., Gallicano, K.D. and Choudri, S. (2001). An *in vitro* evaluation of cytochrome P450 3A4 and P-glycoprotein inhibition by garlic. *J Pharmacy & Pharm Sci* 4, 176-184.

74. Liu, G.T. (1991). Effects of some compounds isolated from Chinese medicinal herbs on hepatic microsomal cytochrome P450 and their potential biological consequences. *Drug Metab Rev* 23 (3 & 4), 439- 465.

75. Kang, J.J., Chen, Y.C., Kuo, W.C., Chen, T., Cheng, Y.W., Kuo, M.L. and Ueng, T.H. (1996). Modulation of microsomal cytochrome P450 by *Scutellariae* Radix and *Gentianae scabrae* Radix in rat liver. *Am J Chin Med* 24 (1), 19-29.

76. Ishihara, K., Kushida, H., Yuzurihara, M., Wakui, Y., Yanagisawa, T., Kamei, H., Ohomori, S. and Kitada, M. (2000). Interactions of drugs and Chinese herbs: pharmacokinetic changes of tolbutamide and diazepam caused by extract of *Angelica dahurica J Pharmacol*, 52 (8), 1023-1029.

77. Wagner, H. and Proksch, A. (1985). Immunostimulatory drugs of fungi and higher plants: Economic and medicinal plant research, Vol-1, Academic press, London, pp.113-153.

78. Wagner, H. (1990). Search for plant derived natural compounds with immunostimulatory activity (recent advances), *pure & Appl Chem* 62, 1217-1222.

79. Charak Samhita. (1000 BC). (1941). Chikitsa Sansthana 26, pp.156.

80. Upadhayay, S.N. (1997). Immunomodulation, Narosa Publishing House, New Delhi, pp.141-187.

81. Kuby, J. (1997). Immunology, 3rd ed., WH Freeman & Co, New York, pp.24.

82. Janeway Jr, C. A. and Travers, P. (1994). Immunobiology: The immune system in health and disease, Current Biology Limited, New York, 59.

83. Mossman, T.R. and Coffman, R.L. (1989). Th1 and Th2 cells: different pattern of lymphokines secretion lead to different functional properties, *Annu Rev Immunol* 7, 145-153.

84. Dahanukar, S.A. and Thatte, U.M. (1997). Current status of Ayurveda in phytomedicine. *Phytomed* 4, 359-368.

85. Dahanukar, S.A., Rege, N.N. and Thatte, U.M. (1997). Adaptogens. Medicinal plants, their bioactivity, screening and evaluation- Proceeding of the international workshop, CDRI, Lucknow, December 2-5, pp.143-163.

86. Thatte, U.M., Rao S.G. and Dahanukar, S.A. (1994). *Tinospora cordiofolia* induces colony-stimulating activity in serum. *J Postgraduate Med* 40, 202-204.

87. Kapil, A., Sharma, S. (1997). Immunopotentiating compounds from *Tinospora codiofolia. J Ethnopharmacol* 58, 89-95.

88. Dhuley, J.N. (1997). Effect of some Indian herbs on macrophage functions in ochratoxin A treated mice. *J Ethnopharmacol* 58, 15-20.

89. Ray, A., Banerjee, B.D. and Sen, P. (1996). Modulation of humoral and cell-mediated immune responses by *Azadirachta indica* (Neem) in mice. *Ind J Exp Biol* 34, 698-701.

90. Ansari, K.U., Kasturi, N., Tewarson, S.L., Singh, S. and Pandey, R.C. (1997). An experimental and clinical evaluation of immunomodulating potential of *Azadirachta indica* (Neem) extract. *Ind J Clin Pharmacol Ther* 18, 82-84.

91. Ray, A., Banerjee, B.D., Koner, B.C. and Sen, P. (1997). Inflence of *Azadirachta indica* (Neem) leaf extract on the immunotoxicity of stress and xenobiotics in experimental animals. *Ind J Pharmacol* 29, 38-42.

92. Upadhaya, S.N., Dhawan, S., Garg, S. and Talwae, G.P. (1992). Immunomodulatory effects of neem (*Azadirachta indica*) oil. *Int J Immunopharmacol* 14, 1187-1193.

93. Subramoniam, A., Rajasekharan, S. and Latha, P.G. (1996). Immunomodulatory and antitumor activities of *Janakia arayalpathra* root. *Fitoterapia* 67, 140-144.

94. Mungantiwar, A.A., Nair, A.M. and Shinda, U.A. (1997). Effect of stress on plasma and adrenal cortisol levels and immune response in rats: modulation by alkaloidal fraction of *Boerhaavia diffusa*. *Fitoterapia* 68, 498-500.

95. De, P., Dasgupta, S.C. and Gomes, A. (1998). Immunopotentiating and immunoprophylactic activities of immune 21, a polyherbal product. *Ind J Pharmacol* 30, 163-168.

96. Deharo, E., Baelmans, R., Gimenez, C., Quenevo, C. and Bourdy, G. (2004). *In vitro* immunomodulatory activity of plants used by the Tacana ethnic group in Bolivia. *Phytomed* 11, 516-522.

97. Jayathirtha, M.G.and Mishra, S.H. (2004). Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asaitica*. *Phytomed* 11, 361-365.

98. Joharapurkar, A.A., Wanjari, M.M., Dixit, P.V., Zambad, S.P. and Umathe, S.N. (2004). Pyrogallol: A novel tool for screening immunomodulators. *Ind J Pharmacol* 36 (6), 355-359.

99. Devasagagayam, T.P.A., and Kesavan, P.C. (1996). Radioprotective and antioxidant action of caeffine: Mechanistic considerations. *Ind J Exp Biol* 34, 291-295.

100. Devasagayam, T.P.A. (1995). Molecular damage by reactive oxygen species: Its possible role in health and disease. Biological concepts in radiotherapy, edited by BB Singh & D Bhatacharya (Narosa Publishing House, New Delhi), pp.105.

101. Babior, B.M. (1984). Oxidants from phagocytes: agents of defense and destruction. *Blood* 64, 959-960.

102. Anggard, E. (1994). Nitric oxide: Mediator, murderer and medicine. *Lancet* 343, 199-206.

103. Roberfroid, M.B., Veihe, H.G. and Remacle, J. (1984). Free radicals in drug research: Advances in drug research, Testa, B. ed. Academic Press Ltd., London, 16, pp.1-84.

104. Buyukokuroglu, M.E., Gulcin, I., Oktay, M. and Kufrevioglu, O.I. (2001). *In vitro* antioxidant properties of dantrolene sodium. *Pharmacol Res* 44, 491-494.

105. Chesseman, K.H. and Slater, T.F. (1993). Free radicals in medicine. *Brit Med Bull* 49, 475-724.

106. Tripathy, K.D. (1995). Essentials of Medical Pharmacology, 3rd ed, Jaypee Brothers Medical Publishers, New Delhi, pp.376-378.

107. Sabeh, F., Wright, T. and Norton, S.J. (1993). Purification and characterization of a glutathione peroxidase from the *Aloe vera* plant. *Enzyme Protein* 47, 92-98.

108. Helen, A., Rajasree, S.R., Krishnakumar, K., Augusti, K.T. and Vijayammal, P.L. (1999). Antioxidant role of oils isolated from garlic (*Allium satium* Linn) and onion (*Allium cepa Linn*) on nicotine induced lipid peroxidation. *Vet Hum Toxicol* 41, 316-321.

109. Kamat, J.P., Boloor, K.K., Devsagayam, T.P.A. and Venkatachalam, S.R. (2000). Antioxidant properties of *Asparagus racemosus* against damage induced by γ-radiation in rat liver mitochondria. *J Ethnophrmacol* 71, 425-431.

110. Selvam, R., Subramanian, L., Gayathri, R. and Angayarkanni, N. (1995). The antioxidant activity of turmeric (*Curcuma longa*). *J Ethnopharmacol* 47, 59-63.

111. Ruby, A.J., Kuttan, G., Babu, K.D., Rajasekharan, K.N. and Kutan, R. (1995). Anti-tumor and antioxidant activity of natural curcuminoids, *Cancer Lett*, 109, 87-92.

112. Bhattacharya, A., Chatterjee, A., Ghosal, S. and Bhattacharya, S.K. (1999). Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla). *Ind J Exp Biol* 37, 676-670.

113. Bhattacharya, S.K., Satyan, K.S. and Chakrabarti, A. (1997). Effect of Trasina, an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase activity in hyperglycaemic rats. *Ind J Exp Biol* 35, 297-301.

114. Dhuley, J.N. (1998). Effect of ashwagandha on lipid peroxidation in stress-induced animals. *J Ethnopharmacol* 60, 173-177.

115. Bhattacharya, S.K., Satyan, K.S. and Ghosal, S. (1997). Antioxidant activity of glycowithanolides from *Withania somnifera, Ind J Exp Biol* 35, 236-242.

116. Hemant, R.J. and Bhutani, K.K. (2002). Antioxidant properties of Indian medicinal plants. *Phytother Res* 16, 771-773.

117. Gupta, M., Mazumder, U.K., Kumar, R.S. and Kumar S.T. (2004). Antitumor and antioxidant role of *Baunia racemosa* against ehrich ascites carcinoma in Swiss albino mice. *Acta Pharmacol Sin* 25 (8), 1070-1076.

118. Bafna, P.A. and Balaraman, R. (2005). Antiulcer and antioxidant activity of pepticare, a herbomineral formulation. *Phytomed* 12, 264-270.

119. Kumar, R.S., Sivkumar, T., Sunderm, R.S., Gupta, M., Mazumdar, U.K., Gomothi, P., Rajeswar, Y., Sarvanan, S., Kumar, M.S., Murgesh, K. and Kumar, K.A. (2005). Antioxidant and antimicrobial activities of *Bahunia racemosa* L. stem bark. *Braz J Med Biol Res* 38 (7), 1015-1024.

120. Katzung, B.G. (1998). Basic and clinical pharmacology. 7th ed, Appleton & Lange, Stamford, Connecticut, U.S.A. pp. 62-67, 242-244.

121. Abraham, E.P. (1981). Antimicrobial activity of herbal formulations. *Scientic America* 76, 244.

122. Goodman and Gilman. (1996). The pharmacological basis of Therapeutics. Vol-8, Pergamon Press, New York, pp. 935.

123. Abraham, E.P. Newton, G.G.F. (1960). Chemotherapeutic agent from natural origin. *Brit Med Bull* 19, 1.

124. Davis, J. (1984). Antimicrobial Therapy, 5th ed., A.M. Ristuccia and B. A. Cunha, New York, Raveen Press, pp.2

125. Davis, S.D. (1980). Antimicrobial Therapy, 3rd ed., B.M. Kogan and B. Saunders, New York, Raveen Press, pp. 4.

126. Hugo, B. and Russel, A.D. (1985). Pharmaceutical Microbiology, 3rd ed., Blackwell scientific publications, London, pp. 3.

127. Egorov, N.S.C. (1985). Antibiotics, a scientific approach, Mir Publications, Moscow, pp. 1.

128. Howard, B.J., Klass, J.I., Rubin, S.J., Weissfeld, A.S. and Titton, R.C. (1987). Clinical and Pathogenic Microbiology, St, Louis, Missouri; C.V. Mosby Company, pp.123-130.

129. Pelcazer, M.J.Jr., Chan, E.C.S. and Krieg, N.R. (1993). Microbiology: Concept and Applications, International Edition, McGraw-Hill, Inc., U.S.A., pp. 161, 557.

130. Skinner, F.A. (1995). Modern Methods of Plant Analysis, Springer-verlag, Berlin, pp. 626.

131. Verpoorte, R., Tjenatsoi, A., Van-Doorne, J. and Svedsen, A.B. (1982). Medicinal plants of Surinaïme: Antimicrobial activity of some medicinal plants. *J Ethnopharmacol* 5, 221-226.

132. Ratnayake, C.K., Arambewela, L.S.R., De Silva, K.T.D., Atta-ur-Rahman. And Alvi, K.A. (1987). Alkaloids of *Alstonia macrophylla*. *Phytochem* 26 (3), 868-870.

133. Roychowdhury, R. (1991). Herbal Medicine for human Health, CBS Publishers and Distribures Ltd., New Delhi, pp. 85-86.

134. Newman, D.J., Cragg, G.M. and Snader, K.M. (2000). The influence of natural products upon drug discovery. *Nat Prod Rep* 17, 175-285.

135. Kirby, G.C. (1996). Medicinal plants and the control of parasites. *Trans. Roy Soc Trop Med Hyg* 90, 605-609.

136. Cowan, M.M. (1999). Plants products as antimicrobial agents. *Clin Microbio. Rev* 12, 564-582.

137. Nair, A. and Bhide, S.V. (1996). Antimicrobial properties of different parts of *Semecarpus anacardium*. *Ind Drugs* 33, 323-328.

138. Sakharkar, P.R. and Patil A.T. (1998). Antimicrobial activity of *Cassia alata*. *Ind J Pharma Sci* 60, 311-312.

139. Kazmi, M. H., Malik, A., Hameed, S., Akhtar, N. and Noor Ali, S. (1994). An anthraquinone derivative from *Cassia italica*. *Phytochem* 36, 761–763.

140. Duke, J. A. (1985). Handbook of Medicinal Herbs. CRC Press, Inc., Boca Raton, Fla. pp. 14.

141. Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochem* 30, 3875-3883.

142. Jones, G. A., McAllister, T. A., Muir, A. D. and Cheng. K. J. (1994). Effects of sainfoin (*Onobrychis viciifolia* scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Appl Environ Microbiol* 60, 1374-1378.

143. Amaral, J. A., Ekins, A., Richards, S.R. and Knowles, R. (1998). Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. *Appl Environ Microbiol* 64, 520-525.

144. Tassou, C. C., Drosinos, E. H. and Nychas, G. J. E. (1995). Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4° and 10°C. *J Appl Bacteriol* 78, 593-600.

145. Mendoza, L., Wilkens, M. and Urzua, A. (1997). Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean *Pseudognaphalium* (Asteraceae). *J Ethnopharmacol* 58, 85-88.

146. Aureli, P., Costantini, A. and Zolea, S. (1992). Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. *J Food Prot* 55, 344-348.

147. Agnihotri, S. and Vaidya, A.D. (1996). A novel approach to study antibacterial properties of volatile components of selected Indian medicinal herbs. *Ind J Exp Biol* 34, 712-715.

148. Shirataki, Y., Endo, M., Yokoe, I., Noguchi, M., Tomimmori, T. And Komatsu, M. (1988). Studies on the constituents of *Sophora* species. XXII. Constituents of the root of *Sophora moorcroftiana* Benth. Ex Baker. (I). *Chem Pharm Bull* 36, 2220-2225.

149. Dastidar, S.G., Mahapatra, S.K., Ganguly, K., Chakrabarty, A.N., Shirataki, Y. and Motoshashi, N. (2001). Antimicrobial activity of prenylflavanones. *In vivo*, 15, 519-524.

150. Owasis, M., Sharad, K.S., Shehbaz, A. and saleemuddin, M. (2005). Antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomed* 12, 229-235.