Pharmacological properties of medicinal herbs by focus on secondary metabolites

1. Hamed Haddad Kashani, 2. Elahe Seyed Hoseini, 3 Hosein Nikzad*, 4.Mohammadm Hossein Aarabi

Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran[1, 3]; Research Center for Sciences and Biotechnology, Lavizan, Tehran. Iran; [2]Department of Biochemistry and Nutrition, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran[4]. * Nikzad_h@kaums.ac.ir

Abstract: Use of herbs based drugs and chemicals for treating various diseases are as old as human civilization. Herbs have vast ability to synthesize aromatic materials mainly secondary metabolites. Herbs and herbs-based therapies are the source of various modern pharmaceuticals. In many cases, these herbal materials serve as defensive molecules against microorganisms, insects, and herbivores. Further, some of which may involve in plant aroma (terpenoids), pigmentation (tannins and quinines), and flavor (ginger). The aim of this review was to study of secondary metabolites and bioactive chemical constituents of medicinal herbs and their pharmacological activity. Regarding our purpose we searched at Pub Med, MEDLINE, CNKI, EMBASE, Wiley Inter Science, Elselvier databases without language limitation. In this sense we applied different words related to herbal therapy, pharmacology, secondary metabolites and phytochemistry.
[Hamed Haddad Kashani, Elahe Seyed Hoseini, Hosein Nikzad, Mohammad Hossein Aarabi. **Pharmacological properties of medicinal herbs by focus on secondary metabolites.** Life Science Journal 2012; 9(1):509-520]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 77

Keywords: Pharmacology; medicie; herb; metabolite

Introduction:

Since ancient times peoples have applied herbs and its derivatives as therapeutic medicines (Thomson, 1978; Philipeon, 2003; Lesney, 2004; Newman, et al., 2003). It is an evolving practice recorded in both folklore and books of early practitioners. At present, despite the abundance and advancement of synthetic drugs, a significant proportion of the population of developing countries still depend on traditional medicines for their health care needs (Lesney, 2004; Okigbo and Mmeka, 2006). The aim of this literature review was to establish the current level of knowledge regarding the phytochemical specificity of medicinal herbs in the treatment of various diseases. This literature review starts with an overview of the historical and current use of traditional medicinal herbs and their products. This will then be followed by a discussion of most important secondary herbal metabolites with known medicinal and pharmacological activity. Next, will be an overview of general procedures used to bioprospect and to assess herbal materials for their pharmacological properties.

Methods:

We searched for papers published in Pub Med, MEDLINE, CNKI, EMBASE, Wiley Inter Science, Elselvier databases without language limit by retrieving key words “herb/herbal therapy, ancient medicines, human/patients, pharmacology, phytochemistry, secondary metabolites” to identify secondary metabolite of medicinal herbs that used in pharmacology applications. These searches were collected and classified during 2011and conducted by two independent examiners. The last date of searching and revising was January 4, 2012.

Results:

History of herbal medicine

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal uses. Peoples have been utilizing herbal medicine for the treatment, control and management of a variety of diseases since ancient times (Griggs, 1981; Kinghorn and Balandrin, 1993; Kong et al., 2003, Philipeon, 2003). There is plenty archeological evidence to support the fact that prehistoric man used plant and herbs for medicinal purposes. For instance, pollen analysis of numerous plants found in the grave the Neanderthal man buried 60000 years ago in Middle East, indicated that the plants buried with the corpse were all of medical value (Griggs, 1981; Kong, et al., 2003). In another example, medicinal herbs found in the individual belongings of the “Ice man” whose body was frozen in the Swiss Alps for more than 5,300 years, are thought to have been used to treat the parasites found in his intestines (Griggs, 1981; 1990; Kong et al., 2003).In written documentations, evidence that human have been applying plant and herbs for medicinal uses comes from several sources. Firstly, the Sumerian clay tablet (dating about 4000 years ago) recorded and described the medicinal use of plants such as laurel, caraway, and thyme by the ancient Sumerian of Mesopotamia (Kong et al., 2003; Philipeon, 2003). These plants are still employed all

over the world for medicinal uses. The Ebers papyrus, written about 3500 years points to the fact that ancient Egyptians applied plants such as mandrake for pain relief and garlic for the treatment of heart and circulatory disorders (Kong et al., 2003). Ancient China is also a source of information about the early application of medicinal herbs. "The Pun-tsao", a Chinese pharmacopoeia published around 1600 BC contain list several medicinal herbs and their applications including ma–Huang, the shrub that introduced the drug ephedrine to modern medicine (Kinghorn and Balandrin, 1993). In India, herbal medicines date back several thousand years to the Rig-Veda, a collection of Hindu sacred verses (Grover et al., 2002), system of health care known as Ayuverdic medicine, which is still widely practiced in India today. In the ancient Western world, the development of western medicine is believed to have been influenced by the writings of Greek philosophers, in particular, the writings of Hippocrates (460–377 BC) and Aristotle (384–322 BC), and the works of Dioscorides, who collected informations of more than 600 species of plants with medicinal value in his famous book "De Materia Medica". This book, which was written in the first century AD remained the standard medical reference in most of Europe for more than 1500 years (Goldman, 2001; Kong et al., 2001). During the Dark, Middle Ages and the Renaissance (476 -1500 A.D.), herbal medicine continued to play an important role in health care management throughout the world (Barter and Daly, 2000). At about the same time, Persians preserved much expertise, and expanded it to include the use or their own resources, together with those of Chinese and Indian herbs, till then unknown to the Greco-Roman world (Kalhor R. 1997, Cooper EL.2004, Saad B .et al., 2005, Philipeon, 2003). In this sense, Razes (860-930), a Persian physician, who approved treatment based on herbs and foods, and avoided synthesized medicine, except for necessary. Avicenna (980-1037) wrote many book on a wide range of subjects but he is perhaps most famous for his ´Law of Medicine´ which includes divisions on the formulation of medicines in details(Kalhor R 1997, Saad B. et al., 2005, Cooper EL 2004) In the United States, herbal remedies handed down from European settlers and learned from Native Americans were a mainstay of medical care until the early 1900s when expansion of the pharmaceuticals industry, coupled with the advancement of technologies to isolate, purify and characterize natural products as well as increased knowledge of synthetic chemistry, led to a decline of herbal medicine in the United States and other developed countries (Kinghorn and Balandrin, 1993). Prior to the nineteenth century, herbal medicines were administered mostly in their crude forms as infusions (herbal teas), tinctures (alcoholic extracts), decoctions (boiled extract of roots or bark), syrups (extracts of herbs made with syrup or honey) or applied externally as ointments (poultices, balms and essential oils) and herbal washes (Griggs, 1981; Gurib-Fakin, 2006). However, during the late nineteenth and early twentieth centuries, scientists began isolating purifying and identifying active ingredients from medicinal herb extracts. These efforts led to the discovery of some of the most important drugs that are still widely used in modern medicine (Goldman, 2001; Newman et al., 2003; Kong et al., 2003; Gupta et al, 2005). For example, morphine isolated from opium poppy (Papaver somniferum) is a powerful pain reliever and narcotic, quinine isolated from Cinchona plant species is an effective anti-malarial drug; taxol (isolated from Taxus brevifolius) and vincristine (isolated from Catharanthus rosesus) are highly effective against certain types of cancer and serpentine (isolated from the root of the Indian plant Rauwolfia serpentia) is used in the treatment of hypertension (Newman et al., 2003; Lesney, 2004; Gupta et al., 2005; Gurib-Fakin, 2006). In addition to the biologically active plant-derived natural products mentioned above, many other plant derived natural products have served as "lead compounds" for the design, synthesis and development of novel drug compounds (Kinghorn and Balandrin, 1993; Newman et al., 2003; Lesney, 2004). In this context, some herb derived natural products have been modified slightly to render them more effective or less toxic in order to produce the so called "semi-synthetic drugs" (Kinghorn and Balandrin, 1993; Kong et al., 2003). As an example of this type of strategy, aspirin was developed in 1953 through structural modification of salicylic acid which was identified as the active ingredient in a number of plants known for their pain-relieving qualities (Kong et al., 2003; Lesney, 2004). In another example, the development of the current and popular oral hypoglycemic agent, metformin was based on the use of goat's rue (Galega officinalis) to treat diabetes (Kinghorn and Balandrin, 1993). The blood glucose lowering property of Galega officinalis has been attributed to the presence of a guanidine–type of alkaloid, galegine. Because galegine was found to be too toxic for human use, several structural analogs of this compound were synthesized and tested in clinical studies. These efforts culminated in the development and marketing of metformin as an effective antidiabetic drug (Kinghorn and Balandrin, 1993; Gupta et al., 2005).

Herbal medicine today

Although the direct use of herbal extracts in developed countries continued to decrease in the late nineteenth and early twentieth centuries, medicinal herbs still play a key role in health care system of

many parts of the world (Kong et al., 2003; Tapsell, 2006). According to World Health Organization (WHO, 2001) 60% of the world's population depend on traditional medicine, and 80% of the population in developing countries depend almost entirely on traditional medical practices, in particular, herbal medicine for their primary health care needs (Fransworth, 1994; Zhang, 2000). The long tradition of herbal medicine continues to the present day in China, India, and many other countries (Zhang, 2000; Kong et al., 2003; Tapsell, 2006). Medicinal herbs continue to contribute significantly to modern prescription drugs by providing lead compounds upon which the synthesis of new drugs can be made. According to Newman et al., (2003), 60% of the anticancer drugs and 75% of the anti-infectious disease drugs approved from 1981-2002, could be traced to natural origins. In addition, 61% of all new chemical entities introduced worldwide as drugs during the same period could be traced to or were inspired by natural products (Gupta et al., 2005). The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, biochemist, botanists, and natural-products chemists all over the world are currently investigating medicinal herbs for phytochemicals and lead compounds that could be developed for treatment of various diseases (Achaya and Shrivastava, 2008).

Secondary herbal metabolites with reported medicinal properties

The medicinal and pharmacological actions of medicinal herbs are often depended to presence of bioactive compounds called secondary herbal metabolites (Bruneton, 1999; Henrich et al., 2004). Unlike the ubiquitous macromolecules of primary metabolism (e.g. monosaccharides, polysaccharides, amino acids, proteins, nucleic acids, lipids) which are present in all plants, secondary metabolites with medicinal properties are found only in a few species of plants (Henrich et al., 2004). Some of these secondary metabolites serve as defensive compounds against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or reducing the growth of nearby competing plants. (Chynier, 2005; Gurib-Fakim, 2005) Secondary herbal metabolites with reported medicinal properties consist of waxes, fatty acids, alkaloids, terpenoids, phenolics (simple phenolics and flavonoids), glycosides and their derivaties. (Satyajit et al., 2006, Eloff, 2001; Cowan, 1995). Some of these secondary herbal metabolites are briefly discussed as follows.

Carbohydrates and related compounds

Plant-derived carbohydrates and related compounds with medicinal and therapeutic potential include fiber, cellulose and its derivatives, starch and its derivatives, dextrins, fructans, mucillages (uronic acid containing polymers), pectins (polysaccharide complexes formed from partially methoxylated polygalactouronic acid) and gums (Bruneton, 1999). In addition to their use as bulking agents in pharmaceuticals, carbohydrates and related compounds have been shown to have immuno-modulatory, anti-tumor, anticoagulant (e.g. heparin), hypoglycemic or antiviral activities (Gurib-Fakim, 2005).

Alkaloids

Alkaloids often contain one or more rings of carbon atoms, usually with a nitrogen atom in the ring. Many have declared pharmacological activity (Harborne, 1998). Most alkaloids have a strong bitter taste and are very toxic, for these reasons they are used by plant to protect themselves against herbivory, and attacks by microbial pathogens and invertebrate pests (Harborne, 1998). Several alkaloid containing medicinal herbs are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Heinrich et al., 2004; Gurib-Fakin, 2005). Alkaloids are classified into several groups either on the basis of their basic ring system (e.g. atropine, indole, quinoline, isoquinoline, imidazole, piperidine alkaloids), plant sources (e.g. opium, belladonna, vinca, cinchona and ergot alkaloids) or their pharmacological properties (e.g. analgesic, stimulant or anti-malarial alkaloids) (Kinghorn and Balandrin, 1993; Bruneton, 1999, Harborne, 1998; Henrich et al., 2004). Their botanical source(s), as well as their pharmacological properties are summarized in Table (1). Alkaloids normally occur in the herb as salts or free bases. Hence, their extraction from herbal materials is generally based on their differential solubility in aqueous acids and organic solvents (Starmans and Nijhuis, 1996; Jones and Kinghorn, 2005). A regular procedure is to initially extract alkaloids and their salts with 2% sulphuric acid. The resultant acid extract is then made alkaline with an ammonia solution and shaken gently with an organic solvent in a separating funnel. The alkaloids, as the free bases partition in favor of the organic layer leaving behind unwanted free non-basic substances (Jones and Kinghorn, 2005). A general chemical test for alkaloids involves addition of a drop of either Mayer's reagent or Dragendorff's reagent to the acid extract. A pale precipitate or an orange-red precipitate indicates the presence of alkaloids. (Gupta et al. 2005).

Table 1: Botanical source(s), and pharmacological properties of some well known alkaloids.

Alkaloids	Examples	Botanical Sources	Medical Properties	REFERENCES
Opium alkaloids	Morphine , heroin	Papaver Somniferum (Opium poppy)	Analgesics (pain relievers and narcotics)	Kinghorn & Balandrin (1993), Gurib –Fakim (2005) Heinrich et al (2004)
Belladonna alkaloids	Cocaine, atropine, scopolamine, hyoscyamine	Datura species, Atropa belladonna,Hyoscyamus niger(henbane)	Anti cholinergic (local anesthetics) and stimulants	Kinghorn & Balandrin (1993), Newman et al (2003) Heinrich et al (2004)
Cinchona alkaloids	Quinine , quinidine	Cinchona species	Antimalarial, antiarrhythmic activities	Kinghorn & Balandrin (1993), Heinrich et al (2004)
Catharanthus alkaloids (Vinca alkaloids)	Vincristine , vinblastine	Catharanthus roseus (Madagascar rosy periwinkle)	Anti cancer (antileukemic) activity	Kinghorn & Balandrin (1993) Kong et al (2003), Lesney (2004)
Rauwolfia alkaloids	Reserpine	Rauwolfia Species	Anti hypertensive activity	Kinghorn & Balandrin (1993); Brueton (1999); Gupta et al (2005)

Phenolics

Phenolics are a class of herbal secondary metabolites that are characterized by the presence of one or more hydroxyl (-OH) groups attached to a benzene ring or to other complex aromatic ring structures (Bruneton, 1999, Harborne, 1998 and Henrich et al., 2004). Phenolic herb secondary metabolites are widely distributed in herbs and are responsible for color development, pollination and protection against UV radiation and pathogens (Bruneton, 1999; Henrich et al., 2004). They also contribute to the color and astringency of some foods. On the basis of their structure phenolics compounds can be classified into two broad classes: the non-flavonoids and the flavonoid phenolic compounds (Bruneton, 1999; Henrich et al., 2004).

Non-flavonoid phenolic compounds

Non-flavonoid phenolic compounds include simple phenols (eugenol, catechol, hydroquinone, phloroglucinol hydroquinone, and p-anisaldehyde) (Jadhav et al., 2004), the C6-C1 benzoic acids (vanillic acid, gallic acid and protocatechuic acid), the C6-C3 phenyl propanoids and their derivatives (cinnamic acid, caffeic acid, ferulic acid myristicin and sinapyl alcohol), coumarins (scopoletin; warfarin and dicoumarol), hydrozable tannis (gallotannins and ellagitannins) and lignans and related compounds(Kumar R, et al. 2010). Examples of non-flavonoids of pharmacological interest together with their botanical sources and their pharmacological properties are given in Table (2).

Table 2: Botanical source(s) and pharmacolical properties of some non-flavonoid phenolic of pharmacological interest Flavonoid phenolic compounds.

References	Medical Properties	Botanical Sources	Examples	Non-Flavonoid Phenolic
Bruneton,(1999) jadhav et al.,(2004)	Anti-bacterial , anti-inflammatory , local anesthetic activities	Berries,red and green tea, coffee beans	Ellagic acid, tannic acid; vanillin, hydroquinone, eugenol	Simple phenols
Bruneton,(1999) Harborne,(1998)	Choleretic activity, hepatoprotective, anti-oxidant activity	Cynara scolymus (artichoke),rosmarinus officinalis rosemary)	Gallic acid, protocatechuic acid	Benzoic acids
Bruneton,(1999) Harborne,(1998) Gurib-Fakim,(2005)	Antiseptic, component of sunscreen lotions and for treatment of dyspepsia	Fruits and vegetables Cinnamon, Myroxylon, balsamum(Peruvian balsam)	Cinnamic acid,coumaric acid, caffeic acid ,ferulic acid	Phenyl propanoids
Harborne(1998) Heinrich et al. ;(2004)	Antifungal; blood anticoagulants; treatment of capillary fragility	Potato plant(Solanum tuberosum),Citrus	Scopoletin,warfarin, and dicoumarol	Coumarins (benzopyrone derivatives)
Bruneton (1999) Harborne(1998) Heinrich et al., (2004)	Anti-diarrhea; antidote in poisoning by heavy metals	Dicotyledonous herbs	Gallotannins, ellagitannins Stilbenoids	Hydrolyzable tannins
Harborne(1998) Heinrich et al.; (2004)	Antifungal	Heartwood of Pinus species	Resveratrol , Pinosylvin	Stilbenoids
Harborne(1998) Heinrich et al.; (2004)	Anti tumor, antiviral anti allergic, anti-rheumatic activity	Flax seed and other grains	Secoisolariciresinol, pinoresinol	Lignans and related compounds

Flavonoid phenolic compounds

Flavonoids are a large and complex group of compounds containing a three ring structure with two aromatic centers (rings A and B) and a central oxygenated heterocyclic ring (C) (Bohm, 1998; Hollman and Katan, 1999).

The six major classes of flavonoids are flavones, flavonols, flavonones, catechins (flavanols) anthocyanidins and isoflavones (Bohm, 1998; Bruneton, 1999; Pieta, 2000; Scalbert et al., 2005; Goutam and Dilip, 2006). Flavonoids have several proven medicinal properties, such as anti-inflammatory, anti-oxidant, anti cancer, antibacterial and antiviral properties (Valsaraj R, 60, et al 1997, Valsaraj R, 58, et al. 1997, Hollman and Katan, 1999; Harborne and Williams, 2001; Chynier, 2005; Manach et al., 2004). Specific examples of each of the major subclasses of flavonoids, their botanical source s as well as some of their pharmacological properties are summarized in Table (3).

Table 3: Specific examples of each of the major subclasses of flavonoids, their botanical sources as well as some of their pharmacological properties.

References	Medical Properties	Botanical Sources	Examples	Flavonoids
Bruneton, (1999) Harborne et al,(1998) Pietta(2000)	Anti- inflammatory analgesic	Fruits of various citrus trees	Naringenin, hesperetin	Flavonones
Bohm(1998) Bruneton(1999)	Anti-tumor activity	Generally in herbaceous families, e.g. Labiatae, Umbelliferae	Apigenin, luteolin	Flavones
Bohm(1998) Bruneton(1999) Goutam&Delip(2006)	Antioxidant and microbial activities Enzyme inhibitors	Generally in woody angiosperms, anions and green tea leaves	Myricetin kaempferol, quercetin,	Flavonols
Manach et al(2004) Cheynier(2005)	Powerful antioxidants	Found in tea leaves	Catechins, gallocatechins	Flavanols
Hollman & Katan, 1999),Pietta(2000), Scalbert et al(2005)	Anti-hepatotoxic, anti-lipolytic, vasodilatory effects	Fruit and vegetables	Pelargonidin, cyanidin,malvidin	Anthocyanidins
Manach et al(2004) Cheynier(2005)	Powerful anticancer and heart disease properties	Cereals and legumes	Daidzein, genistein, glycitein	Isoflavonoids
Hassan et al(2006) Gurib-Fakim(2005)	Antioxidant, anti-cancer , anti HIV activities	Abundant in grapes, wine and coffee pulp	Procyanidin, prodelphinidins	Condensed tannins (proanthocyanidin)

Less polar flavonoids (e.g. isoflavones, flavones, methylated flavones and flavonols) are generally extracted by solvents of medium polarity and polar solvents (chloroform, dichloromethane, diethyl ether or ethyl acetate). Polar flavonoids (anthocyanidins and flavanols) are generally extracted with alcohol or alcohol-water mixtures in the presence of a small amount (0.1-1%) hydrochloric acid, whereas tannins may be extracted with alcohols and acetone (Elangovan V, et al. 1994, Satyajit et al., 2006). All phenolic compounds (flavonoids and non-flavonoid phenolics) react with ferric chloride to give a characteristic color (Harborne, 1998, Das K. et al., 2010).

Terpenoids

Terpenoids, also known as isoprenoids constitute the largest group of herbal secondary metabolites (Bruneton, 1999). Terpenoids are involved in defense, wound scaling and thermotolerance of plants as well as in the pollination of seed crops (Heirich et al., 2004). They are also responsible for the flavor of fruits, the fragrance of the flowers and the quality of agricultural products. Terpenoids are classified as monoterpenes (C10), sesquiterpenes (C15), diterpene (C20), triterpenes (C30) and tetraterpenes (C40) on the basis of the number of isoprene units. (Banthorpe, 1991; Bruneton, 1999; Heirich et al., 2004; Gurib-Fakim, 2005). Monoterpens and sesquiterpenes are the main components of essential oils and are commonly found in plant families Labiatae, Myrtaceae, Pinaceae, and Rutaceae (Harborne, 1998, Heirich et al., 2004). Diterpenes include resin acids and plant hormones (gibberellins) (Harborne, 1998). Many of the diterpenes are toxic, but some, for example, forskolin (from gymnosperms), taxol (from the Pacific yew) and ginkgolides (from Ginkgo biloba) are used in modern medicine for the treatment of hypertension, cancer and memory loss respectively (Bruneton, 1999; Heirich et al., 2004; Gurib-Fakim, 2005). Triterpenoids are the most abundant plant terpenes, they include plant steroids and are components of saponins and steroidal glycosides (Harborne, 1998; Bruneton, 1999) The most common tetraterpanoids are the carotenoids which are responsible for most of the yellow and orange plant pigments (Heirich et al., 2004). Tetraterpenoids also include the xanthophylls found in many yellow fruits and flowers (Bruneton, 1999). Terpenoids are in general soluble in common organic

solvents. However, low molecular weight terpenoids such as essential oils, are thinly soluble in water. Hence terpenoids are generally extracted with non-polar solvents. However, the volatile essential oils can be steam distilled (Satyajit et al., 2006). Table (4) provides a summary of examples of each class of terpenoids, together with their botanical sources and pharmacological properties.

Table 4: Specific examples of each of the major subclasses of terpenes, their botanical source (s) as well as some of their pharmacological properties.

References	Medical Properties	Botanical Sources	Examples	Terpenes
Harborne, 1998 Heinrich et al., (2004) Gurib Fakim,(2005)	Analgesic and anti-inflammatory activities	Essential oils of some Pinus Spp and coniferous woods	Camphor, limonene	Monoterpenes (C_{10})
Bruneton(1999) Heinrich et al., (2004)	Antibacterial, antifungal,antimalarial, mulluscicidal	Essential oils of many plant species	Bisabolol, Ngaione, Hymenoxin, Santonin	Sesquiterpenes(C_{15})
Bruneton(1999) Heinrich et al., (2004)	Anti hypertensive Anti cancer activities	Gymnosperm woods (Larix spp) Taxus(brevifolia)	Forskolin, Phorbol esters, Taxol (Paclitaxel)	Diterpenes (C_{20})
Bruneton(1999) Heinrich et al., (2004) Gurib-Fakim(2005)	Anti inflammatory Hemolytic properties	Bark of the birch Betulaalba,Larix,Picea,Pinus,Fagus, Quercus spp	Betulin (Pentacyclic triterpene) Phytosterols β-Sitosterol and campesterol	Triterpenes (C_{30})
Heinrich et al., (2004) Gurib-Fakim(2005)	Antioxidant activity	Vegetables such as carrots and pumpkin	β-Carotene	Tetraterpenes (C_{40}) Carotenoids

Glycosides

Glycosides are herbal secondary metabolites made up of two components, a carbohydrate component known as the glycone and a non carbohydrate component known as the aglycone. The glycone component usually consists of one or more glucose units whereas the aglycone may be any one of the secondary herb metabolites discussed above (Bruneton, 1999; Heirich et al., 2004; Gurib-Fakim, 2005). The solubility of glycosides depends on the nature of the aglycone and the number and type of sugar molecules linked to the aglycone (Starmans and Nijhuis, 1996). Aglycones tend to be soluble in organic solvents and sugar part in aqueous solvents. In general, glycosides can be extracted with acetone, ethanol or an aqueous/ethanol mixture (Jones and Kinghorn, 2005). Medicinally important glycosides consist of anthraquinone glycosides, coumarin glycosides and steroidal (cardiac) glycosides.

Anthraquinone glycoside

Herbss such as Cassia senna, rhubarb (Rheum palmentum), cascara (Rhamnus purshiana) and Aloe vera have long been known for their laxative property (Bruneton, 1999; Heinrich et al., 2004; Gurib-Fakim, 2005). This property has been attributed to the presence of anthraquinone and enthrones glycosides present in these plants (Heinrich et al., 2004). When ingested anthraquinone glycosides hydrolyze in the large intestine (colon) to liberate the aglycones which stimulate peristalsis and increase water retention in the colon (Bruneton, 1999).

Coumarin glycosides

Coumarins glycosides are phytoalexins, and are synthesized by the plant in response to bacterial or fungal infection, physical damage, chemical injury, or a pathogenic process (Gurib-Fakim, 2005). For example, scopoletin is synthesized by the potato (Solanum tuberosum) following fungal infection. Coumarin glycosides are very fragrant. They are the source, for instance, of freshly-mown hay scents (Heinrich et al., 2004). Medicinally, coumarin glycosides have been shown to have hemorrhagic, anti fungicidal, and antitumor activities (Bruneton, 1999). The aglycones of a coumarin glycoside dicumarol and its synthetic structural analog, warfarin are used in modern medicine as anticoagulants.

Steroidal glycosides

Steroidal (cardiac) glycosides are naturally occurring drugs whose actions include both beneficial and toxic effects (at higher doses) on the heart (Bruneton, 1999; Gurib-Fakim, 2005; Heinrich et al., 2004). Herbs containing cardiac glycosides contain Digitalis purpurea (foxglove) and Strophanthus. Foxglove is the source of two potent glycosides used as a heart stimulants, digoxin and digitoxin. Both digoxin and digitoxin are widely used in the modern treatment of congestive heart failure, atrial fibrillation and flutter (Heinrich et al., 2004). These glycosides prolong the relaxation phase of the heart (ventricular diastole), thus allowing the left ventricle to fill with more blood. In accordance with Starling's Law of Contraction, the increased blood volume in the left ventricle results in a more forceful contraction (ventricular systole), thereby pumping more blood out into the aorta (Sherwood et al., 2004). Strophanthus, a

genus of a South African shrub produces the cardiac glycoside, ouabain (G-strophanthin). Like digitalis glycosides, ouabain is also used in modern medicine to treat congestive heart failure (Heinrich et al., 2004). The chemical structure of ouabain is similar to that of digitoxin except that it has the sugar rhamnose instead of digitoxose.

Pharmacological investigation of herbal materials

Reviews of literature involving research of medicinal plants suggest that scientists follow more or less the same general strategy to investigate herbal materials for their pharmacological properties (Kinghorn and Balandrin, 1993; Heinrich et al., 2004).

Selection of herbal species

Any plant species and herb parts collected randomly can be investigated using available phytochemical methods. However, a more targeted approach is often preferred to a random selection (Kinghorn and Balandrin, 1993; Harborne, 1998; Heinrich et al., 2004). The herbal material to be investigated can be selected on the basis of some specific traditional uses (ethnobotanical bioprospecting approach). Extract prepared from herb used as traditional remedies to treat certain diseases are more likely to contain biologically active compounds of medicinal interest (Heinrich et al., 2004). Alternatively, the plant can be selected based on chemotaxonomical data. In the chemotaxonomic approach, knowledge that a particular group of plants contain a certain class of natural products may be used to predict that taxonomically related plant may contain structurally similar compounds (Heinrich et al., 2004). Some herbal materials can be selected following a combination of the above mentioned approaches. The use of literature data base early in the selection process can provide some preliminary information on the type of natural products already isolated from the plant and the extraction methods employed to isolate them (Heinrich et al., 2004). Another approach known as the information driven approach, utilizes a combination of ethnobotanical, chemotaxonomic and random approaches together with a data base that contains all relevant information concerning a particular plant species (Kinghorn and Balandrin, 1993; Harborne, 1998; Heinrich et al., 2004). The database is used to prioritize which herbs should be extracted and screened for biological activity. This approach is favored by large organizations (particularly pharmacological companies) interested in screening thousand of samples for bioactivity as it may reduce costs by a process known as dereplication; the process of avoiding the repeated discovery of common or known drugs (Heinrich et al., 2004).

Collection and identification of plant material

The whole plant or a particular part can be collected depending on where the metabolites of interest (if they are known) accumulate. Hence aerial (e.g. leaves stems, flowering tops, fruit, seed, and bark) and underground (e.g. tubers, bulbs, roots) parts can be collected separately. Collection of herb materials can be influenced by factors such as the age of the plant and environmental conditions (e.g. temperature, rainfall, amount of daylight, soil characteristics and altitude) (Williams et al., 1996; Harborne, 1998). Thus, it is important to take this into consideration for the re-collection purpose, in order to ensure reproducible profile (nature and amount) of metabolites (Satyajit et al., 2006). The plant from which the material is collected must also be identified correctly. A plant taxonomist or a botanist should be involved in the detailed authentication of the plant (i.e. classification into its class, order, family, genus and species) (Satyajit et al., 2006). Any feature related to the collection, such as the name of the plant, the identity of the parts collected, the place and date of collection, should be recorded as part of the voucher (a dried specimen pressed between sheets of paper) deposited in a herbarium for future reference (Harborne, 1998; Satyajit et al., 2006).

Extraction of plant materials

Herbal materials are commonly extracted by means of liquid solvents in what is known as the "solid-liquid solvent extraction". Typical solid-liquid solvent extraction processes for herbal materials involve drying and grinding of the herbal material, choosing a suitable extraction solvent and extraction procedure (Starmans and Nijhuis, 1996; Cheng et al., 2001; Jones and Kinghorn, 2005).

Drying and grinding the plant material

Once the herbal material has been collected, it needs to be dried as soon as possible. A common practice is to leave the sample to dry on trays at ambient temperature and in a room with adequate ventilation (Heinrich et al., 2004; Satyajit et al., 2006). Dry conditions are essential to prevent microbial fermentation and subsequent degradation of metabolites. Herbal materials should be sliced into small pieces and distributed evenly to facilitate homogeneous drying. Protection from direct sunlight is advised to minimize chemical reactions (and formation of artifacts) induced by ultraviolet rays (Satyajit et al., 2006). To facilitate the drying process, the material can be dried in an oven. This can also minimize reactions (e.g. hydrolysis of glycosides) that can occur as long as there is some residual moisture present in the herbal material. The dried material should be stored in sealed containers in a dry and cool place. Storage for prolonged periods should be avoided as some constituents may be decomposed (Heinrich et al., 2004; Jones and Kinghorn, 2005).After drying, herbal materials are commonly grounded into a fine powder. Grinding of plant

materials into smaller particles facilitates subsequent extraction procedures by rendering the sample more homogeneous, increasing the surface area, and facilitating the penetration of solvents into cells (Harborne, 1998; Satyajit et al., 2006). Mechanical grinders (e.g. hammer and cutting mills) are employed to shred the herbal material into various particle sizes. Potential problems of grinding include the fact that some material (e.g. seeds and fruits rich in fats and volatile oils) may clog up the sieves and that heat generated may degrade thermolable metabolites (Harborne, 1998).

Choice of a suitable extraction solvent

The choice of the extraction solvent depends mainly on the polarity and hence the solubility of the bioactive compounds of interest. Although water is usually applied as a solvent in many traditional protocols, organic solvents of varying polarities are often used (either alone or in different combinations) in modern methods of extraction to exploit the various solubilities of herbal ingredients (Lapornik B et al., 2005, Handa SS et al., 2008). The polarity and chemical profiles of most of the common extraction solvents have been determined (Ayaffor et al., 1994; Eloff, 2001; Cowan, 1995) and are summarized in Table (5).

Table 5: Polarity and chemical profiles of most of the common extraction solvents.

References	Extracted chemical profile	Solvent	Polarity
Ayaffor et al(1994), Cowan(1999)	Fatty acids, waxes , terpenoids	n- Hexane	Low
Perett et al (1995),Cowan, (1999),Bruneton(1999)	Fatty acids, waxes , terpenoids	Chloroform	
Bruneton(1999), Scalbert et al(2005)	Less polar and polar flavonoids , tannins, terpenoids	Dichloromethane	Medium
Bruneton(1999), Scalbert et al(2005)	Less polar and polar flavonoids , tannins, terpenoids	Ethyl- acetate	
Eloff, (1998), Bruneton(1999), Scalbert et al(2005)	Less polar and polar flavonoids , tannins, terpenoids, glycosides	Acetone	
Cowan, (1999),Bruneton(1999)	Polar flavonoids, tannins , glycosides (saponins)	Ethanol	High
Bruneton(1999),Scalbert et al(2005)	Carbohydrates, lecithin, amino acids, polypeptides, phenolic acids, phenylpropanoids, polar flavonoids, glycosides and alkaloids	Methanol	
Kaul et al(1985), Jones & Kinghorn (2005)	Carbohydrates, lecithin, amino acids, polypeptides, phenolic acids, phenylpropanoids, polar flavonoids, glycosides and alkaloids	Water	
Bruneton(1999)	Alkaloids	Aqueous acid or base	

Thus, if the polarity or the solubility of the compounds of interest is known, information such as the one in the above table can be used to select a proper extractor solvent or a mixture of two or more solvents of different polarity (Kaul et al., 1985). Alternatively, a solvent such as acetone, which has the capacity to extract both polar and non-polar substances, and has been recommended by Eloff (2001) for the extraction of most polar and nonpolar compound. If the polarity of the compounds of interest is not known, the powdered herbal material can be extracted simultaneously with a mixture of different proportions of two or more solvents of different polarity (Bruneton, 1999, Cowan, 1995). Alternatively, the powdered herbal material can be extracted sequentially with solvent of different polarity in what is known as a sequential extraction procedure (Bruneton, 1999).

Choice of the extraction procedure

The choice of the extraction procedure depends on the nature of the source material and the compound to be isolated. Solvent extraction procedures applied to herbal products include but not limited to maceration, percolation, soxhlet extraction, steam distillation and sequential solvent extraction (Starmans and Nijhuis, 1996; Harborne, 1998; Jones and Kinghorn, 2005).

Maceration

This simple, but still widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container at room temperature (Harborne, 1998). Occasional or constant stirring of the preparation (using mechanical shakers or mixers) can increase the speed of the extraction. Maceration involves soaking the herbal material in a suitable solvent, filtering and concentrating the extract (Harborne, 1998; Jones and Kinghorn, 2005). The use of a cold solvent reduces decomposition, but the process takes longer and uses larger amounts of solvent.

Percolation

This is similar to the maceration process, but hot solvent is refluxed through the herbal material. It is quicker and uses less solvent, but decomposition

due heat may occur (Jones and Kinghorn, 2005; Satyajit et al., 2006).

Soxhlet extraction

Soxhlet extraction is a form of continuous percolation with fresh solvent, which uses special glass ware. In this procedure, the herbal material is separated from the extract by encasing it in a paper thimble beneath the dropping condensed solvent. When full, the solvent in the thimble siphons off into the main vessel containing the extractant, and the process continues (Jones and Kinghorn, 2005). The advantage of this procedure is that fresh solvent continually extract the herbal material more effectively with minimum solvent, however, heating and hence decomposition of compounds is again a disadvantage (Nikhal SB et al.,2010).

Steam distillation

There is a special apparatus for distilling volatile oils which are immiscible with water. If compounds being extracted are water soluble, the method is less useful because a large volume of aqueous extract is produced. However, in some cases a partition system may be used to concentrate the extract (Jones and Kinghorn, 2005; Satyajit et al., 2006).

Sequential solvent extraction

If the polarity and solubility of compounds that are isolated is not known, a convenient and frequently used procedure is sequential solvent extraction. In sequential solvent extraction, the herbal material is extracted with a series of solvents of different polarity (Starmans and Nijhuis, 1996). The usual way is to start with a non-polar solvent and exhaustively extract the herbal material followed by a series of more polar solvents until several extracts are obtained of increasing solute polarity. For example, a first step, with dichloromethane, will extract terpenoids, less polar flavonoids (flavones, flavonols, flavonones) and other less polar materials (Jones and Kinghorn, 2005, Okwu DE 2001). A subsequent step with acetone or ethyl acetate will extract flavonoid glycosides and other medium polar constituents. A subsequent extraction with an alcohol or water will extract highly polar constituents (Jones and Kinghorn, 2005).Once the extraction is complete, the extractant is usually concentrated under vacuum, for large volumes or solvents and blown down under nitrogen for small volumes, ensuring at the same time that volatiles are not lost. Aqueous extracts are generally freeze-dried and stored at 20^{o}C as this low temperature reduces the degradation of the bioactive natural product (Starmans and Nijhuis, 1996). Extraction protocols may sometimes be modified depending on the type of molecules being extracted, for example, acids may be added to extract alkaloids as their salts (Jones and Kinghorn, 2005).

Screening the extract for biological activity

Once the extract has been obtained, the biological activity within is usually verified by means of an in vitro bioassay method. In vitro screening methods for biological activity are generally divided into two formats; the low-throughput screening and high-throughput screening methods, depending on the number of extracts to be screened. (Valsaraj R, 58, et al 1997, Okwu DE 2001). In low-throughput screening (LTS), small numbers of extracts (a single extract up to hundred of extracts) are dispensed into a format that is compatible with the bioassay (e.g. microtiter plate or sample tube) (Kinghorn and Balandrin, 1993,). This approach is used widely in academic laboratories where only a relatively low number of extracts are assessed. In high-throughput screening (HTS), thousand of extracts are dispensed into a format (usually a microtiter plate with many wells) and screened in the bioassay (Heinrich et al., 2004). This approach is favored by the pharmaceutical industry. This may have hundreds of thousands of samples (both natural and synthetic) for biological evaluation. (Rawat AK. Et al., 1997). This large scale approach means that decisions can be made rapidly about the status of an extract, which has an effect on the cost of the drug discovery process (Kinghorn and Balandrin, 1993).

Bioassay guided fractionation and isolation of active compounds

Active fractions are fractionated using a bioassay guided fractionation. In bioassay-guided fractionation, a crude mixture is fractionated into its fraction components using chromatographic procedures, followed by biological evaluation (bioassay) of each fraction. (Vaidya and Antarkar 1994). Only fractions which display biological activity in the bioassay are selected for further fractionation. The cycle of fractionation and testing and further fractionation is repeated until a pure compound with the desired activity is isolated (Rimando et al., 2001).

Characterization and structure elucidation of isolated compounds

Once the biological evaluation has been performed and the separation of the natural product has been achieved, the chemist will try to attempt the elucidation of the compound. Structure elucidation depends on classical spectroscopic techniques such as: Nuclear Magnetic Resonance (NMR) 1-D and 2-D Proton NMR as well as C-13 NMR, Infra Red (IR), Mass Spectrometry (MS) and X-Ray analysis (Harborne, 1998).

Preclinical and clinical studies

Once innovation and structure of the bioactive compound has been established, large amounts of the bioactive compound are isolated and the decision is made as to whether the compound can

be synthesized de novo or whether chemical modification needs to be made to enhance the biological activity (Vaidya and Antarkar, 1994). The bioactive compound will undergo extensive in vivo studies to establish activity, toxicity and efficacy. These studies are sometimes known as preclinical studies (Ebadi M, .2002). Only once all these steps have been completed will a drug lead enter clinical studies, which is the most extensive evaluation stage of a drug candidate during which many drug may fail through toxicity or lack of efficacy in humans. (Gupta SS. 1994). Successful achievement of these trials usually results in a product license, which means that the compound is now a drug. (Vaz J, et al. 1998, Dalvi SS. et al. 1994). Given the complexity of the process described above, it is not surprising that many natural product drug leads fail to make their way onto the market. Some estimates state that only 1 in 10,000 of plant-derived drug leads may actually make their way to the market (Kinghorn and Balandrin, 1993). The process is also lengthy and it may take 12-15 years from the collection of the original herbal material to the granting of a license for the new drug.

Conclusion:

Phytochemical monitoring of medicinal herbs is essential to discover new sources of therapeutical and pharmacological compounds. There is an increasing interest in correlating phytochemical ingredients of herbs with its pharmacological activity. Since it is necessary to initiate vital steps for monitoring secondary metabolites of medicinal herbs, scientists also have even started associating the botanical properties of herbs with their pharmacological activity. The phytochemical screening and quantitative and qualitative analysis of chemical constituents of the medicinal herbs showed presence of various secondary metabolites like alkaloids, terpenoids, flavonoids, steroids, coumarins, tannins and saponins. They were known to show medicinal and pharmacological activity. However majority of the drugs are at the experimental stage and have to still undergo clinical trials. There is still a rarity of medical studies which are carried out in randomized, controlled, double blind manner. Today, parallel consumption of medicines from different disciplines is a common finding. Very few studies, however, attend to the problem of drug interactions. Also nonstandardized methods of extraction may lead to the degradation of the secondary metabolites present in the herbs and may fall to the variations of bioactive compounds. So selection of best extraction method to achieve high quality of herbal ingredients is important for producing of herbal medicines.

The herbal studied in this review are potential sources for useful medicines. In future, more co-ordinated multicentral research in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds to detect secondary metabolites properties of medicinal herbs for specific pharmacological activities is expected.

*** Corresponding author:**
Dr. Hosein Nikzad, Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran. P.O.Box:87155111, Email: Nikzad_h@kaums.ac.ir

References:

1. Acharya, D., and Shrivastava, K., (2008). Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices, Aavishkar Publishers Distributor, Jaipur- India. ISBN 9788179102527. pp 440.
2. Ayaffor, J.F., Tehuendem, M.H.K., Nyasse, B., (1994). Novel active diterpenoids from Afromomum aulacocapos. Journal of natural products 57:917-923.
3. Banthorpe, D. V., (1991). Classification of terpenoids and general procedures for their characterization, in Terpenoids (Charlwood, B. V. and Banthorpe, D. V., eds), Methods in Plant Biochemistry (Dey, P. M. and Harborne, J. B., eds.), vol. 7, Academic Press, San Diego, pp. 1–41.
4. Barter, C.D., and Daly, W.J., (2000). "Clinical pharmacology in the Middle Ages: Principles that presage the 21st century", Clinical Pharmacology & Therapeutics 67(5): 447-450.
5. Bohm, B., (1998). Introduction to flavonoids. Amsterdam: Harwood Academic Publishers.
6. Bruneton, J., (1999). Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept. Ltd. England, U.K.
7. Cheynier, V., (2005). Polyphenols in foods are more complex than often thought. Am. J. Clin. Nutr. 81(suppl.): 223S-229S.
8. Cooper EL.,(2004) Drug discovery, CAM and natural products. Evid Based Complement Alternat Med.; 1:215-217.
9. Cowan, M.M., (1999). Plant products as antimicrobial agents. Clinical Microbiology Review 12:564–582.
10. Dalvi SS, Nayak VK, Pohujani SM, (1994). Effect of gugulipid on bioavailability of diltiazem and propranolol. J Assoc Phys India;42:454-5.
11. Das K, Tiwari RKS, Shrivastava DK. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research; 4(2): 104-111.

12. Ebadi M. , (2002). Alternative Therapies, Pharmacodynamic Basis of Herbal Medicines.CRC Press Boca Ratan, London, 10-11.
13. Elangovan V, Ramamoorthy N, Balasubramanian S, (1994). Studies on the antiproliferative effect of some naturally occurring bioflavonoidal compounds against human carcinoma of larynx and sarcoma-180 cell lines. Indian J Pharmacol;26:266-9.
14. Eloff, J.N., (2001). Antibacterial activity of Murula (Sclerocarya birrea (A. rich) Hochst. Subsp. Caffra (Sond) Kokwaro) (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology 76: 305–308.
15. Fransworth, N.R., (1994). Ethnopharmacology and drug development. In Chadwick, D.J., and Marsh, J., (eds). Ethnobotany and the Search for New Drugs, CIBA Foundation Symposium 185, John Wiley and Sons, Chichester, New York, pp 42-51.
16. Goldman, P (2001). "Herbal medicines today and the roots of modern pharmacology". Ann. Intern. Med. 135 (8 Pt 1): 594–600.
17. Goutam, B., and Dilip, G., (2006). Progress in the research on naturally occurring flavones and flavonols: An overview. Current Organic Chemistry 10(8):873-898.
18. Griggs, B., (1981). Green Pharmacy, a history of herbal medicine; J.Norman & Hobhouse Ltd.; London.
19. Grover, J.K., Yadav, S.; and Vats, V.,(2002). Medicinal plants of India with antidiabetic potential. J. Ethnopharmacology 81:81–10.
20. Gupta. R., Gabrielsen, B., and Ferguson1, F.M., (2005). Nature's Medicines: Traditional Knowledge and Intellectual Property Management. Case Studies from the National Institutes of Health (NIH), USA Current Drug Discovery Technologies 2: 203-219.
21. Gupta SS. (1994), Prospects and perspectives of natural plant products in medicine. Indian J Pharmacol;26:1-12.
22. Gurib-Fakim, A., (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27;1–93.
23. Handa SS, Khanuja SPS, Longo G, Rakesh DD, (2008). Extraction Technologies for Medicinal and Aromatic Plants. International centre for science and high technology, Trieste, 21-25.
24. Harbone, J. (1998). Phytochemical methods: a guide to modern techniques of plant analysis. Springer, Nature, 302 pages
25. Harborne, J.B., and Williams, C.A., (2001). Anthocyanins and other flavonoids. Nat. Prod. Rep 18:310-333.
26. Hassan, S.W., F.L. Bilbis, M.J. Ladan, R.A. Umar and S.M. Dangoggo et al., (2006). Evaluation of antifungal activity and phytochemical analysis of leaves, roots and stem barks extracts of Calotropis procera (Asclepiadaceae). Pak. J. Biol. Sci., 9: 2624-2629.
27. Hassan, S.W., R.A. Umar, M. Lawal, L.S. Bilbis and B.Y. Muhammad, (2006). Evaluation of antifungal activity of Ficus sycomorus L. (Moraceae). Biol. Environ. Sci. J. Tropics, 3: 18-25.
28. Heinrich, M., Barnes, J., Gibbons, S., and Williamson, E.M., (2004). Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK.
29. Hollman, P.C., and Katan, M.B., (1999). Health effects and bioavailability of dietary flavonols. Free Radic Res 31(Suppl):S75–S80.
30. Jadhav, B.K., Khandelwal, K.R., Ketkar, A.R., and Pisal, S.S., (2004). "Formulation and evaluation of mucoadhesive tablets containing eugenol for the treatment of periodontal diseases". Drug Dev Ind Pharm. 30 (2):195–203.
31. Jones, W.P., Kinghorn, A.D., (2005). Natural product isolation: Extraction of Plant Secondary Metabolites. Methods in Biotechnology 20:323-351.
32. Kalhor R. (1997). Contribution of Persian physician to development of Islamic medicine. Med Arh;51:9-12.
33. Kaul, T.N., Middletown, Jr. E., Ogra, P.L., (1985). Anitiviral effects of flavonoids on human viruses. Journal of Medical Virology 15: 71-75.
34. Kinghorn, A.D., and Balandrin, M.F., (1993). Human medical agents from plants, American Chemical Society, San Francisco, USA.
35. Kong, J.M., Goh, N.K., Chia, L.S., and Chia, T.F., (2003). Recent advances in traditional plants drugs and orchids. Acta Pharmacologia Scinc 24: 7-21.
36. Kumar R, Sharma RJ, Bairwa K, Roy RK, Kumar A., (2010). Pharmacological review on natural antidiarrhoel agents. Der Pharma Chemica; 2(2): 66-93.
37. Lapornik B, Prosek M, Wondra, A. G., (2005).Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering; 71: 214–222.
38. Lesney, M.S., (2004). Nature's Pharmaceuticals: Natural Products from Plants Remain at the Core of Modern Medicinal Chemistry. TCAW 13(7):26-31.
39. Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jimenez, L., (2004). Polyphenols: food

sources and bioavailability. Am. J. Clin. Nutr. 79:727-747.
40. Newman, D.J., Cragg, G.M., and Snader, K.M., (2003). Natural products as sources of newdrugs over the period 1981-2002. Journal of Natural Products 66: 1022-1032.
41. Nikhal SB, Dambe PA, Ghongade DB, Goupale DC. (2010). Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion. International Journal of Pharmaceutical Sciences; 2 (1): 30-32.
42. Okigbo, R.N., and Mmeka, E.C., (2006) An appraisal of phytomedicine in Africa KMITL Sci. Tech. J. 6(2).
43. Okwu DE (2001). Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl. Sci. 7(3): 455- 459.
44. Philipeon, J.D., (2001). Phytochemistry and medicinal plants. Phytotherapy 56:237-243.
45. Pieta, P.G., (2000). Flavonoids as antioxidants. Journal of Natural Products 63:1035-1042.
46. Rawat AK, Mehrotra S, Tripathi SC, Shome U., (1997). Hepatoprotective activity of Boerhavia diffusa L. roots a popular Indian ethnomedicine. J Ethnopharmacol; 56:61-6.
47. Rimando, A. M., M. Olofsdotter, S.O.D., and Dayan, F.E., (2001). Searching for rice allelochemicals: an example of bioassay-guided isolation. Agron. J 93:16–20.
48. Saad B, Azaizeh H, Said O., (2005). Tradition and perspectives of arab herbal medicine: A Review. Evid Based Complementary Alternat Med; 2:475-479.
49. Satyajit, D., Sarker, Z., Latif, A., and Gray, I., (2006). Natural product isolation. Second edition, Humana Press Inc ISBN 1-59259-955-9.
50. Scalbert, A., Johnson, I.T., and Saltmarsh, M., (2005). Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr 81(suppl):215S-217S.
51. Sherwood, L., (2007). Human physiology: From cells to systems, 6th edition, Thomson Brooks/Cole.UK. p 323.
52. Starmans, D. A. J., and Nijhuis, H. H., (1996). Extraction of secondary metabolites from plant material: a review. Trends Food Sci. Technol. 7:191–197.
53. Tapsell, L.C., (2006). "Health benefits of herbs and spices: the past, the present, the future". Med J Aust 1. PMID 17022438.
54. Thomson, W.A.R., (1978). Medicines from the Earth. Maidenhead, United Kingdom: McGraw-Hill Book Co.
55. Vaidya AB, Antarkar VDS., (1994). New drugs from medicinal plants: opportunities and approaches. J Assoc Phys India; 42:221-8.
56. Valsaraj R, Pushpangadan P, Smitt UW. (1997). Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol;58:75-83.
57. Valsaraj R, Pushpangandan P, Smitt UW. (1997). New anti- HIV-1, antimalarial, and antifungal compounds from Terminalia belerica. J Nat Prod;60:739-42.
58. Vaz J, Kulkarni C, David J, Joseph T., (1998). Influence of caffeine on pharmacokinetic profile of sodium valproate and carbamazepine in normal human volunteers. Indian J Exp Biol;36:112-4.
59. WHO, (2001). Legal status of Traditional Medicines and complementary/Alternative Medicine: A worldwide review. WHO publishing 1.
60. Williams, E.M., Okpato, D.T., Evans, F.J., (1996). Selection, preparation, and pharmacological evaluation of plant material.

2/1/2012