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Journal of Chemical and Pharmaceutical Research, 2016, 8(4):1423-1443



Review Article

ISSN: 0975-7384 CODEN(USA): JCPRC5

The active constituents of herbs and their plant chemistry, extraction and identification methods

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ABSTRACT

The active constituents of plants have only relatively recently been isolated. The active constituents in plants are the chemicals that have a medicinal effect on the body. These are the active ingredients of the plant, the chemicals that have a marked, definable physiological and therefore, possibly medical activity upon the body. These constituents and their actions within the body are also referred to as their pharmacology. They have been divided into 16 main groups: Alkaloids, Anthocyanins, Anthraquinones, Cardiac Glycosides, Coumarins, Cyanogenic Glycosides, Flavonoids, Glucosilinates, Phenols, Saponins, and Tannins.

Keywords Phytochemicals, extraction, plant constituents, plant chemistry.

INTRODUCTION

Phytochemicals are chemical compounds that occur naturally in the plant kingdom. Some are responsible for the organoleptic properties of the natural sources in which they are present. The term is generally used to refer to those chemicals that may have biological significance, for example carotenoids, flavonoids, coumarins or chromones, but not all are established as essential nutrients. There may be as many as 4,000 different phytochemicals having potential activity against several diseases such as cancer and metabolic or degenerative diseases. Before the sixteenth century, most of the mainstream medical systems were based on the idea that one should work with nature and that the body's own healing capacity could be strengthened and complimented by the right herbs [1,2].

All the old medical systems had, at their center, a belief in a primal energy that sustained life and health. The Chinese called it "qi," while the Indians referred to it as "prana".

Western herbalists called it the "vital force". When modern medicine took over in the nineteenth century, these concepts were dismissed as remnants of the superstition and ignorance of earlier healing practices. The age of western medicine had dawned and had overshadowed traditional practices in China and India [2].

Plant chemistry includes the miracle of photosynthesis, plant respiration, structure, growth, development, and reproduction. Much of the chemical basis of life is common to both plants and animals. From a holistic perspective the whole of the plant must be respected as an integrated biologically evolved unit that is beyond the analytical comprehension of science [3].

Plant Constituents

Physiologically active plant constituents are usually classified by their chemical structure rather than specific actions. The list here will assumes a certain degree of chemical knowledge: [1,3]

- Alkaloids
- Anthocyanins
- Anthraquinones
- Cardiac Glycosides
- Coumarins
- Cyanogenic Glycosides
- Flavonoids
- Glucosilinates
- Phenols
- Saponins
- Tannins

Alkaloids:

Alkaloids are basic (alkali-like), nitrogen-containing organic constituents found in some plants. Alkaloids are organic bases. Many alkaloids are poisonous, others are addictive (e.g. cocaine), and some are used clinically (e.g. morphine). More than 10 000 alkaloids are now known, the first discovered being narcotine, isolated from opium by Derosne in 1803. Alkaloids exist as salts in the cell sap. They may be extracted from the cell with acidified water or alcohol, or alternatively they are soluble in organic solvents (e.g. chloroform) when the plant is rendered alkaline [4].

1.1 Chemistry: Alkaloids are normally classified according to the heterocyclic ring system they possess, but some authors prefer a classification based on their biosynthetic origins from amino acids, e.g. phenylalanine, tyrosine or tryptophan [4].

1.2 Occurrence in the Plant Kingdom Alkaloids are common in the Angiosperms (Mono- and Dicotyledons), but rare in lower plants, although there are exceptions, for example pacletaxel from yew(a Gymnosperm), lycopodine from Lycopodium and palustrine from Equisetum (both Pteridophytes), and even fungi, e.g. ergometrine (Claviceps). These structures are shown in Figure 1.0. [4].



Figure 1.0 : structure of Lycopodine and Palustrine

1.3 The distribution of alkaloids in the plant kingdom with pharmacological usage is listed in Table 1. [4].

Family	Alkaloid	Plant genus ^a	Biological activity ^b
Agaricaceae	Bufotenine	Amanita (m)	Hallucinogen
	Muscarine	Amanita (m)	Acetylcholine-like
	Psilocybine	Psilocybe (m)	Hallucinogen
Amaryllidaceae	Lycorine	Amaryllis (b)	
	Galanthamine	Galanthus (b)	Alzheimer disease
		Narcissus (b)	
Apocynaceae	Alstonine	Alstonia (bk)	Antimalarial
	Aspidospermine	Aspidosperma (bk)	Respiratory stimulant
	Yohimbine	Yohimbe (bk)	Aphrodisiac
	Conessine	Holarrhena (bk)	Antidysenteric
	Ellipticine	Ochrosia (bk)	Anticancer
	Akuammigine	Picralima (s)	Antimalarial
	Reserpine	Rauwolfia (rh)	Tranquillizer
	Serpentine		
	Vinblastine	Catharanthus (1)	Anticancer
	Vincristine		
Aristolochiaceae	Aristolochic acid	Aristolochia (rh)	Tumour-inducing
Berberidaceae	Berberine	Berberis (bk)	Antibacterial
		Mahonia (bk)	Antimalarial
Boraginaceae	Indicine N-oxide	Heliotropium (1)	Anticancer
Cactaceae	Mescaline	Lophophora (1)	Hallucinogen
Campanulaceae	Cathine	Catha (1)	CNS stimulant
07.0	Ephedrine		
Celastraceae	Maytansine	Maytenus	Anticancer
Chenopodiaceae	Anabasine	Anabasis (1)	Insecticidal
Clavicepitaceae	Ergometrine	Claviceps (fb)	Postpartum haemorrhage
	Ergotamine		Migraine
Convolvulaceae	Calystegines	Calystegia (r)	Antiviral
	Agroclavine	Ipomoea (1)	Hallucinogen
Ephedraceae	Ephedrine	Ephedra (hb)	CNS stimulant
Equisitaceae	Palustrine	Equisetum (1)	
Erythroxylaceae	Cocaine	Coca (1)	Local anaesthetic
Graminae	Loliine	Lolium (1)	
Leguminosae	Castanospermine	Castanosperma (s)	Antiviral, 'locoism' (stock)
	Cytisine	Cytisus (hb)	Very toxic
		Anagyris (hb)	Teratogenic
	Sparteine		Diuretic
	Swainsonine	Swainsona	Glysosidase inhibitor 'locoism' (stock)
	Monocrotaline	Crotalaria (1)	Hepatotoxic, tumour-inducing
	Physostigmine	Physostigma (s)	Ophthalmology
	 Statistics and a statistical statistics and a statistical statistics. 	na mining ng pang kalan na kanan katala Sabata (Katala Sabata) (Katala Sabata) (Katala Sabata) (Katala Sabata)	Cholinesterase inhibitor
Liliaceae	Colchicine	Colchicum (c)	Induces polyploidy
	Cevadine	Schoenocaulon (s)	Insecticidal
	Rubijervine	Veratrum (r)	Antihypertension

Table 1 Distribution of alkaloids in the plant kingdom

Family	Alkaloid	Plant genus ^a	Biological activity ^b
Loganiaceae	Strychnine	Strychnos (s)	Very poisonous
Lycopodiaceae	Lycopodine	Lycopodium (1)	
Menispermaceae	Tubocurarine	Chondrodendron (bk)	Neuromuscular blocking agent, muscle relaxant
Moraceae	Calystegines	Morus (1)	Antiviral, oral hyperglycaemic agent
Orchidaceae	Dendrobine	Dendrobium (hb)	
Palmae	Arecoline	Areca (s)	Anthelmintic
Papaveraceae	Chelerythrine	Dicentra	Diuretic
	Codeine	Papaver (lt)	Analgesic
	Morphine		Analgesic, narcotic
	Narcotine		Cough suppressant
	Papaverine		Anti-impotence vasodilator
	Thebaine		
Ranunculaceae	Aconitine	Aconitum (hb)	Diaphoretic, rheumatism, neuralgia (topical)
	Ajaconine	Delphinium (hb)	
Rubiaceae	Emetine	Cephaelis (rh)	Amoebic dysentery
		Psychotria (r)	
	Quinine	Cinchona (bk)	Antimalarial
	Quinidine		Antiarrhythmia (heart)
Rutaceae	Harmaline	Peganum (sd)	Anthelmintic
	Pilocarpine	Pilocarpus (1)	Glaucoma, ophthalmology
	Canthin-6-one	Pentacerus	Antibacterial
Solanaceae	Capsaicin	Capsicum (fr)	Hot taste
	Hyoscine	Atropa (hb)	Travel sickness, amnesia
	Hyoscyamine	Datura (hb)	Antagonist of acetylcholine
		Hyoscyamus (hb)	
		Duboisia (hb)	Preoperative treatment
		Mandragora (r)	
	Tigloidine	Duboisia (hb)	Parkinson disease
	Solanidine	Solanum (tb)	Toxic
	Nicotine	Nicotiana (1)	Insecticidal, smoking
Taxaceae	Pacletaxel	Taxus (1)	Anticancer drugs
	Baccatin		Anticancer drugs

Table 1 - continued

^aPart of plant used: (l), leaves; (s), seeds; (hb), herbs; (bk), bark; (rh), rhizome; (tb), tuber (green); (fr), fruits; (lt), latex; (r), roots; (c), corm; (fb), fruiting body; (b), bulb; (m), mushroom.

^bBiological activity relates to the plant and not necessarily the alkaloids that are listed as representative examples of the families.

1.3Phytochemical analysis test for alkaloids

100 mg of powdered sample was dissolved in 5 ml of methanol and then filtered. Then 2 ml of filtrate was mixed with 5 ml of 1% aqueous HCl. One milliliter of mixture was taken separately in two test tubes [5].

1.3.1 Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive [5].

1.3.2 To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids[5].

1.3.3 Wagner's test: Alkaloids give a reddish brown precipitate with wagner's reagent (solution of iodine in potassium iodide)[6].

1.3.4 Hager's test: Alkaloids gives yellow color precipitate with hager's reagent (saturated solution of picric acid)[6].

1.3.5 Tannic acid test: Alkaloid gives buff color precipitate with 10% tannic acid solution[6].

2. Anthocyanins:

Anthocyanins are the most abundant and widespread of the flavonoid pigments. They absorb light at the longest wavelengths, and are the basis for most orange, pink, red, magenta, purple, blue and blue-black floral colors. Key to providing such color diversity is the degree of oxygenation of the anthocyanidins (the central chromophores of the anthocyanins) and the nature and number of substituents (e.g. sugar moieties) added to these chromophores[7]. 2.1 Chemistry: At a primary level, the degree of oxygenation of the B-ring has the greatest impact on the colour of anthocyanin pigments. Most anthocyanins are derived from just three basic anthocyanidin types: Pelargonidin, Cyanidin and Delphinidin. The difference between them is in the number of hydroxyl groups on the B-ring[7].



Figure: 2.1 Basic structure of a main anthocyanidins. R1, R2 and R3 substitutions determine the various common anthocyanidins. The common 3-hydroxyanthocyanidins (R_3 = OH) are Pelargonidin (R_1 =H and R_2 =H), Cyanidin (R_1 =OH and R_2 =H), Delphinidin (R_1 =OH and R_2 =OH)[7]



Fig. 2.2: Example of a complex anthocyanin structure; delphinidin3,7,30, 50 -tetra-(6-O-p-coumaroyl-b-glucoside)[7]

An increased number of hydroxyl groups on this ring have a bluing effect on the colour manifested by the anthocyanin. In general, there is a strong correlation between the flower colour and the predominant type of anthocyanin that accumulates. Orange and pink colours tend to be based on pelargonidin derivatives, magenta

colours on cyanidin derivatives and purple and blue colours on delphinidin derivatives. Flowers can also accumulate mixtures of anthocyanin types, providing further variation in colour[7].

Willstatter and Everest identified the first anthocyanin in 1913, from the blue cornflower Centaureacyanus. Since then approximately 630 different anthocyanins have been structurally defined. Secondary modifications to the core anthocyanidins are the basis for these diverse structures. In some cases, very complex anthocyanins may be formed, with multiple glycosyl and acyl groups, and an example of one of these complex structures is shown in Fig. 2.2[7]. Anthocyanin modification typically involves O-glycosylation, O-acylation and O-methylation.

2.2 Medical Uses:

Anthocyanin pigments, has been linked to an incredibly diverse range of biological functions in human metabolism. Recently the USDA ARS constructed an extensive database for flavonoid content of selected foods www.nal.usda.gov/fnic/foodcomp/index.html. Folk medicine has relied on anthocyanins taken from *Hibiscus sabdariffa* L. as a remedy for liver dysfunction and hypertension; the antioxidant capacity of the pigments is considered integral to efficacy in these cases. Particularly complex anthocyanin profiles in members of the genus Vaccinium including bilberry (in Europe) and blueberry (in North America) have been credited with superb antioxidant capacities, especially inacylated anthocyanin mixtures[7].

Anthocyanin pigments have been administered to remedy vision disorders, enhance visual acuity, and increase capillary resistance and improving eyesight, including night vision[7].

Retinopathy and cataracts, serious consequences of diabetes mellitus, can be combated using plant-derived anthocyanin pigments [7].

Anthocyanins were found to confer significant protection from oxidative stress and to be highly bioavailable in endothelial cells, which has direct relevance to atherosclerosis and neurodegenerative disorders[7].

A typical daily dietary intake (25–215 mg) without supplementation is sufficient to provide pharmacological benefits. The rich anthocyanin pigment content of red wines, as well as associated flavonoids, are purported to be responsible for the well-publicized correlation between red wine consumption and reduced cardiovascular mortality[7].

Anthocyanins (and the aglyconecyanidin) were noted to inhibit cycloxygenase enzymes, which can be one marker for the initiation stage of carcinogenesis. Recently, both the anthocyanins and cyanidinaglycone from tart cherries reduced cell growth of human colon cancer cell lines[7].

2.3Phytochemical screening of anthocyanin:

The presence of anthocyanins has been demonstrated by adding 2 mL of the plant extract with 2 mL of 2 N HCl. The appearance of a pink-red color that turns purplish blue after addition of ammonia indicates the presence anthocyanins[8].

fruits	vegetables	nuts and dried fruits	spices	other foods
fruits apples ^b apricot avocado bananas blackberry ^b clarbeberry ^b cantaloupe cherries ^b chokeberry ^b cranberry ^b currant, black ^b currant, black ^b currant, red ^b elderberry ^b grapefruit grapes ^b honeydew kiwifruit mango nectarines ^b orange, navel peaches ^b pears pineapples plums ^b	vegetables artichoke asparagus beans, black ^b beans, navy beans, net beats, red kidney beats, red kidney beats, small red ^b beets broccoli cabbages ^b carrots cauliflower celery com cucumber eggplant ^b lettuces ^b onions ^b peas peas peppers potatoes pumpkin red radishes ^b spinach sweet potatoes	nuts and dried fruits almonds Brazil nuts cashews hazelnuts pecans pine nuts pistachios ^b walnuts dates figs prunes raisins	spices basil leaf chili powder cinnamon cloves, ground curry powder garlic powder garlic powder garlic powder oregano leaf paprika parsley pepper poppy seed turmeric	other foods bread breakfast cereals snack apple sauce baby foods chips chocolate native American food juice, grapefruit juice, Iemon juice, Iemon juice, Iemon juice, Iemon juice, Iomato juice, tomato juice, V8 vegetable ketchup milk peanut butter rice salsa tomato sauce

Table 2.0 : Foods Screened for the Presence of Anthocyanins[9]

^a Foods listed as only major categories. ^b These foods were found to contain anthocyanins.

3.Anthraquinones

Anthraquinones are commonly found as glycosides in the living plant and several groups are distinguished based on the degree of oxidation of the nucleus and whether one or two unites make up the core of the molecule. These are derivatives of phenolic and glycosidic compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols. The anthrones and less oxygenated than the anthraquinones and the dianthrones are formed from two anthrone units[10].

Anthraquinone occurs naturally in certain plants, fungi and insects and it contributes to the coloring pigment of such organisms. Due to this property, the compound is used commercially to manufacture dyes. In powdered form, anthraquinone exhibits a color that ranges from gray to yellow and green. However, it produces a variety of different colored dyes, including alizarin (red), oil blue A and oil blue 35, quinizarine green SS and solvent violet 13. Anthraquinone is a derivitive of anthracene, a coal-tar byproduct characterized by a chemical structure consisting of a polycyclic aromatic hydrocarbon and three fused rings of benzene[11].

3.1 Chemistry:

Anthraquinone, also called anthracenedione or dioxoanthracene is an aromatic (a hydrocarbon characterized by general alternating double and single bonds between carbons) organic compound. This compound is an important member of the quinone family. Quinone is a class of organic compounds that are formally derived from aromatic compounds. The term is also used in the more general sense of any compound that can be viewed as an anthraquinone with some hydrogen atoms replaced by other atoms or functional groups. These derivatives include many substances that are technically useful or play important roles in living beings. Anthraquinone is identified by many other names, such as anthrachinon, dioxoanthracene, and several different trade names, including Hoelite and Corbit[11].



Figure 3.1:Structure of9,10–Anthraquinone[11]



3.2 Medicine use of Anthraquinone:

Derivatives of 9,10-anthraquinone include many important drugs (collectively called anthracenediones). They include

- Laxatives such as dantron, emodin, and aloe emodin, and some of the senna glycosides
- Antimalarials such as rufigallol[12].
- Antineoplastics used in the treatment of cancer, such as mitoxantrone, pixantrone, and the anthracyclines[12].

3.3 Phytochemical analysis test for Anthraquinone:

3.3.1 Borntragor's Test: To 1 gm of drug add 5-10 ml of dilute HCl or dilute H_2SO_4 boil on water bath for 10 minutes and filter. Filtrate was extracted with CCl4/benzene and add equal amount of ammonia solution to filtrate and shake. Formation of pink or red colour in ammonical layer due to presence of anthraquinone moiety [5,13].

3.3.2 Modified Borntragor's Test: To 1 gm of drug add 5 ml dilute HCl followed by 5 ml ferric Chloride (5% w/v). Boil for 10 minutes on water bath, cool and filter, filtrate was extracted with carbon tetrachloride or benzene and add equal volume of ammonia solution, formation of pink to red colour due to presence of anthraquinone moiety. This is used C-type of anthraquinone glycosides [5,13].

3.4 Source: Plants like Chinese Rhubarb that have this active ingredient stimulate the large intestine, causing contractions and bowel movement. This is used C-type of anthraquinone glycosides [12].

Herbal examples:

- Aloe barbadensis (Aloe)
- Cassia sp. (Senna)
- Rheum palmatum (Turkey Rhubarb)
- Rhamnusfrangula/purshiana (Cascara sagrada)
- Rumexcrispus (Yellow Dock)

4. Cardiac Glycosides:

Cardiac glycosides are a diverse family of naturally derived compounds that bind to and inhibit Na⁺/K⁺-ATPase. Members of this family have been in clinical use for many years for the treatment of heart failure and atrial arrhythmia, and the mechanism of their positive inotropic effect is well characterized. Exciting recent findings have suggested additional signaling modes of action of Na⁺/K⁺-ATPase, implicating cardiac glycosides in the regulation of several important cellular processes and highlighting potential new therapeutic roles for these compounds in various diseases. Perhaps most notably, the increased susceptibility of cancer cells to these compounds supports their potential use as cancer therapies, and the first generation of glycoside-based anticancer drugs are currently in clinical trials [14].

4.1 Chemistry:

Cardiac glycosides are divided into two main types:

4.1.1 Bufadienolides are C24 steroids

The bufadienolides are C_{24} homologues of the Cardenolides and carry a doubly unsaturated 6-membered lactone ring at the 17-position. The primary cardiac glycoside present in *Helleborus* is the bufadienole, hellebrin. Hellebrigenin, the aglycone of hellebrin is more potent than the glycoside itself[14].



Bufadienolides

4.1.2 Cardenolides are C23 steroids

They are C_{23} steroids that have α 17- β side chain and α , β unsaturated 5-membered lactone ring. Cardenolides are classified according to the chemical composition of their aglyconesaslanataglucosides A, B, C, D and E. Only *Digitalis lanata*, the woolly foxglove contains all five forms.e. The entire foxglove plant is toxic. Symptoms of poisoning include dizziness, vomiting, irregular heartbeat, and delerium or halucinations.



This powerful constituent is found in plants like Foxgloves. They have a strong direct action on the heart and support and strengthen the rate of contraction. Significantly diuretic, these plants help lower blood pressure[14]. 4.2 Phytochemical analysis test

Keller-Killani test:

To 2 ml of extract, 1 ml of glacial acetic acid, one drop 5 % ferric chloride and concentrated sulphuric acid were added. Appearance of reddish brown color at the junction of the two liquid layers indicates the presence of cardiac glycosides[15]. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might form just gradually throughout thin Layer[16].

4.3 Medical Uses:

From ancient times, humans have used cardiac-glycoside-containing plants and their crude extracts as arrow coatings, homicidal or suicidal aids, rat poisons, heart tonics, diuretics and emetics. In modern times, purified extracts or synthetic analogues of a few have been adapted for the treatment of congestive heart failure and cardiac arrhythmia[17].

Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure. Their utility results from an increased cardiac output by increasing the force of contraction. By increasing intracellular calcium as described below, cardiac glycosides increase calcium-induced calcium release and thus contraction. They also delay depolarization thus decreasing heart rate.Bufalin, Ouabain and Digoxin are a few toxic cardiac glycosides. Digoxin from the foxglove plant is used clinically, whereas Bufalin and Ouabain are used only experimentally due to their extremely high potency[17].

5.Coumarins

Coumarin was isolated from the seed of D. odorata. Coumarins are secondary metabolites of higher plants, few microorganisms (bacteria and fungi), and sponges. Coumarins are found free or as heterosides in many

dicotyledonous families, including the Apiaceae, Asteraceae, Fabiaceae, Moraceae, Rosaceae, Rubiaceae, Rutaceae and Solanaceae. Many monocotyledonous plants, especially the Gramineae and orchids, also contain large amounts of coumarins. Although mainly synthesised in the leaves, coumarins occur at the highest levels in the fruits, followed by the roots and stems. In addition, seasonal changes and environmental conditions may affect the occurrence in various parts of the plant. The distribution of biologically active coumarins in a wide range of plants seems to correlate with their ability to act as phytoalexins, i.e. they are formed as a response to traumatic injury, during the wilting process, by plant diseases or through drying, they accumulate on the surface of the leaves, fruits and seeds, and they inhibit the growth and sporulation of fungal plant pathogens and act as repellents against beetles and other terrestrial invertebrates. Coumarins are leached from the roots of some plants, such as wild Avena, into the soil, where they provide a defense tool against hostile micro-organisms. Coumarins are also active in plant metabolism, taking part in growth regulation . In particular furanocoumarins, are known to inhibit root tip growth and seem to induce membrane disturbances, and their excretion on seed surfaces might be a means to delay germination[18].

5.1 Phytochemistry of coumarins:

Coumarins owe their class name to 'coumarou', the vernacular name of the tonka bean (Dipteryxodorata, Fabaceae), from which coumarin itself was isolated. Coumarins belong to a group compounds known as the benzopyrones, all of which consist of a benzene ring joined to a pyrone. Coumarin and the other members of the coumarin family are benzo- α - pyrones, while the other main members of the benzopyrone group-the flavonoids-contain the γ -pyrone group. Coumarins may also be found in nature in combination with sugars, as glycosides. The coumarins can be roughly categorised as follows. Simplethese are the hydroxylated, alkoxylated and alkylated derivatives of the parent compound, coumarin, along with their glycosides[18].

Furanocoumarins: These compounds consist of a five-membered furan ring attached to the coumarin nucleus, divided to linear and angular types with substituents at one or both of the remaining benzenoid positions[18]. Pyranocoumarins: Members of this group are analogous to the furanocoumarins, but contain a six-membered ring coumarins substituted in the pyrone ring. Like other phenylpropanoids, coumarins arise from the metabolism of

phenylalanine via a cinnamic acid, p-coumaric acid[18].



Figure 5.0: Structure of Coumarin[2].



Figure 5.1: Principal types of coumarins isolated from plants[2]

Coumarins are multi-tasking constituents that thin the blood, relax smooth muscle and can act as a sunscreen all at once. You can find this active constituent in plants like Celery.

5.2 Phytochemical analysis test

a. FeCl₃ test: To the concentrated alcoholic extract of drug few drops of alcoholic FeCl₃ solution was added. Formation of deep green colour, which turned yellow on addition of conc. HNO₃, indicates presence of coumarins[13].

b. Fluorescence test: The alcoholic extract of drug was mixed with 1N NaOH solution (one ml each). Development of blue-green fluorescence indicates presence of coumarins[13].

5.3 Medical Uses:

Coumarin has blood-thinning, anti-fungicidal and anti-tumor activities. Coumarin should not be taken while using anticoagulants. Coumarin increases the blood flow in the veins and decreases capillary permeability. Coumarin can be toxic when used at high doses for a long period[2].

6. Cyanogenic Glycosides

Cyanogenic glycosides are widely distributed among 100 families of flowering plants. They are also found in some species of ferns, fungi and bacteria. There are manyeconomical important plants highly cyanogenic, including white clover, linum, almond, sorghum, the rubber tree and cassava[19].

6.1 Chemistry:

Cyanogenic glycosides have a chemical structure that contains one carbon with a cyanide group linked to a sugar ("glyco" means sugar). During digestion, the cyanide group is released and forms hydrocyanic acid (HCN) known as prussic acid.Cyanogenesis is the ability of some plants to synthesize cyanogenic glycosides, which when enzymically hydrolyzed, release cyanohydric acid (HCN), known as prussic acid. There is strong evidence that cyanogenesis is one of the mechanisms that can serve to the plant as a protective device against predators such as the herbivores. The level of cyanogenic glycosides produced is dependent upon the age and variety of the plant, as well as environmental factors. It is usual to find cyanogenic and acyanogenic plants within the same species, where the function of cyanogenesis is revealed through their phenotypic characteristics. Cyanogenesis may not necessarily be

used for plant survival; it may take part in metabolic and excretory processes but there certainly is a characteristic of value for these species[19].

In this case, the aglycone contains a cyanide group. All of these plants have these glycosides stored in the vacuole, but, if the plant is attacked, they are released and become activated by enzymes in the cytoplasm. These remove the sugar part of the molecule and release toxichydrogen cyanide. Storing them in inactive forms in the vacuole prevents them from damaging the plant under normal conditions[20].

An example of these is amygdalin from bitter almonds (but not sweet almonds). Cyanogenic glycosides can also be found in the fruit seeds (and wilting leaves) of many members of the rose family (including cherries, apples, plums, bitter almonds, peaches, apricots, raspberries, and crabapples). Bamboo shoots a staple food inSouth East Asia, must be thoroughly cooked in order to inactivate the present toxin in its raw form[20].

e.g. Amygdalin:-obtained from bitter almond (Prunusamygdalus), Prunasin: obtained from wild cherry bark.



Figure 6.0 : Amygdalin structure [20]

These active constituents are found in Elder plants, amongst others. They have a sedative and relaxing effect on the heart and muscles.

6.2 Phytochemical analysis test

6.2.1 Ferriferrocyanide test:

Macerate 1 g of the powdered drug with 5 ml of alcoholic KOH for 5 min. transfer it to an aqueous solution containing $FeSO_4$ and $FeCl_3$, and maintain at 60-70°C for 10 minutes. Now transfer the contents to HC1 (20%) when the appearance of a distinct Prussian blue color confirms the presence of HCN[19,20].

6.2.2 Precipitation of Hg from HgNO₃:

The reduction of aqueous mercurous nitrite solution to metallic Hg by HCN being observed by an instant formation of black metallic Hg in the cells[19,20].

6.2.3 Cuprocyanate test:

To saturate the pieces of filter paper in a freshly prepared solution of guaic resin dissolved in absolute ethanol and allows them to dry completely in air. Now, carefully moisten a piece of the above paper with a very dilute solution of CUSO4 and place it into contact with a freshly exposed surface of the drug. In case, HCN is generated, it will give rise to a distinct strain on the paper[19,20].

Take few g of sample powder in a conical flask and moisten with a few drop of water. Moisten a piece of picric acid paper with sodium carbonate solution. Suspend the strip of sodium picrate (sodium 2, 4, 6 trinitrophenate) paper by means of cork in the neck of the flask. Warm gently to about 37°C by placing on lamp or thermostat- controlled water bath and allow standing.

Observe the change of colour of the test paper. Hydrogen cyanide is liberated from cyanogenic glycoside by enzyme activity. HCN react with sodium pirated to form the reddish-purple sodium isopurpurate[19,20].

6.3 Medical Uses:

The most controversial evidence involves the cyanogenic glycosides from *Prunus* spp (cherry), particularly amygdalin (sold under the trade name Laetrile) and prunasin. These compounds do have anticarcinogenic activity in

vitro according to recent research. Previous animal and human studies have, however, failed to show convincing clinical effectiveness of isolated injections of amygdalin. Prunasin has also been shown to inhibit DNA polymerase in vitro[20].

6.4 Source:

Apricots, bamboo shoots, cassava, corn, wild cherry, elderberries, flaxseed, and lima beans [20].

7. Glucosilinates

Glucosinolates, a class of secondary metabolites, are nitrogen- and sulfur-containing compounds mainly found in *Capparales* and almost exclusively in *Brassicaceae*, which include Brassica crops of economic and nutritional importance, as well as the model plant, *Arabidopsis thaliana*[21].

7.1 Chemistry:

Glucosinolates have a common core structure containing a β -D-thioglucose group linked to a sulfonatedaldoxime moiety and a variable side chain derived from amino acids. Glucosinolates can be divided into three classes based on the structure of different amino acid precursors: 1. aliphatic glucosinolates derived from methionine, isoleucine, leucine or valine, 2. aromatic glucosinolates derived from phenylalanine or tyrosine, and 3. indoleglucosinolates derived from tryptophan. The biosynthesis of glucosinolates comprises three phases: (i) amino acid chain elongation in which additional methylene groups are inserted into the side chain, (ii) conversion of the amino acid moiety to the glucosinolate core structure, (iii) and subsequent side chain modifications. More than 130 glucosinolates have been identified. Their structural diversity arises from side chain elongation of the amino acid precursors prior to the formation of the glucosinolate core structure and from a wide range of secondary modifications including oxidation, desaturation, hydroxylation, methoxylation, sulfation and glucosylation[21].



Figure 7.0: General structure of Glucosilinates. R denotes the variable side chain from amino acids

7.2 Source:

For example, Glucosinolatesoccer in cabbages (white cabbage, Chinese cabbage, Broccoli), Watercress, Horseradish, Capers and Radishes[21].

When plants like Radish, which contain Glucosilinates, are applied as a soft, moist mass onto painful joints, they increase blood flow to the area. This aids in healing as it helps remove the build-up of waste products[21].

8. Natural phenolics: Phenolics acids and flavonoids

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors[22].

Phenolic compounds are a large class of plant secondary metabolites, showing a diversity of structures, from rather simple structures, e.g. phenolic acids, through polyphenols such as flavonoids, that comprise several groups, to polymeric compounds based on these different classes. Phenolic compounds are important for the quality of plant based foods: they are responsible for the color of red fruits, juices and wines and substrates for enzymatic browning, and are also involved in flavor properties. In particular, astringency is ascribed to precipitation of salivary proteins by polyphenols, a mechanism possibly involved in defense against their anti-nutritional effects[22].

8.1 Chemistry: Plant phenolics include phenolics acids, flavonoids, tannins.

Phenolic acids can be divided into two classes: Derivatives of benzoic acid such as gallic acid, and Derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall[22].

Flavonoids are the most abundant polyphenols in our diets. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), which are labeled as A, B and C. Flavonoid are themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. Some of the most common flavonoids include quercetin, a flavonol abundant in onion, broccoli, and apple; catechin, a flavanol found in tea and several fruits; naringenin, the main flavanone in grapefruit; cyanidin-glycoside, an anthocyanin abundant in berry fruits (black currant, raspberry, blackberry, etc.); and daidzein, genistein and glycitein, the main isoflavones in soybean[22].



Gallic acid

Ferulic acid

Caffeic acid

Figure 8.0: Structure of phenolics acids, flavonoids[22]

8.2 Phytochemical analysis test:

8.2.1 Shinoda's test for flavonoids

Five hundred milligram of sample was dissolved in 5 ml of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of conc. HCl. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids[5].

9.Saponins

Saponins are natural high-molecular-weight glycosides of triterpene or steroids with a very wide distribution in the plant kingdom , as well as in lower marine animals, such as starfish. In the past, saponins were characterized according to their surface-active properties and ability to form persistent foams[23].

There are two types of this constituent, namely steroidal saponins and triterpenoidsaponins. The latter are strong expectorants. Expectorants are agents that increase bronchial secretions and facilitate their expulsion through coughing, spitting or sneezing. These agents can also aid in nutrient absorption. Steroidal saponins have a marked effect on hormonal activity. Plants like Liquorice contain saponins[23].

9.1 Chemistry:

Saponins are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin[23].



Figure 9.0 : Chemical structure of the saponin- Solanine

9.2 Medical Uses:

Saponins exhibited a range of biological activities. On the other hand, saponins also have beneficial pharmacological effects. They are anticholesterolemic due to the formation of a complex with cholesterol in gastrointestinal tract thus preventing absorption. Other activities include anti-inflammation, anti-parasite and antivirus. Numerous lines of evidence now indicate that saponins can kill tumor cells by triggering tumor cell death via different signaling pathways, by activating death receptors, targeting mitochondria, and inducing oxidative stress. Saponins, by virtue of their multiple apoptotic actions on cancer cells, may provide a new line of anticancer agents. They are also effective against drug-resistant cancer cells[23].

9.3 Phytochemical analysis test:

One gram of powdered sample was boiled in 10 ml of distilled water and then filtered. 3 ml of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins[5].

10.Tannins

Tannins are defined as phenolic compounds of high molecular weight ranging from 500 Da to more than 3000 Da which they found in plants leaves, bark, fruit, wood and roots located basically in the tissues in the vacuoles. They have been closely associated with plant defense mechanisms against mammalian herbivores, birds and insects. Except of some higher molecular weight structures tannins are soluble in water (20- 35°C). Oligomeric compounds with multiple structure units with free phenolic groups can complex with

proteins, starch, cellulose and minerals. In the plant kingdom tannins are found in both flowering plants and non-flowering plants[24].

Tanniniferous plants are widespread in nature and although a lot of attention has been given to their study in recent years, the term "tannin" continues to be difficult to define accurately. Indeed, whereas related phenolic compounds such as simple phenolics, neolignans and flavonoids are characterized and classified according to their chemical structure, tannins are a diverse group of compounds that are related primarily in their ability to complex with proteins[24].

10.1 Chemistry:

According to their chemical structure and properties, tannins are divided into two main groups: Hydrolysable (HT) and Condensed tannins (CT).

The characteristics of the two groups are different in molecular weight, structure and produce a different effect on the herbivorous animals especially on ruminant when ingested. According to the chemical structure of HTs (gallotannins and ellagitannins) are molecules which contain a carbohydrate, generally D-glucose, as a central core. The hydrolysable groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid. Hydrolysable tannins are usually found in lower concentrations in plants than CTs. Hydrolysable tannins are subdivided into taragallotannins (gallic and quinic acid) and caffetannins (caffeic and quinic acid). They are hydrolyzed by tanninase enzymes which engage in ester bond hydrolysis. HTs can form compounds such as pyrogallol which is toxic to ruminants. Toxic compounds from more than 20% HT in the diet can cause liver necrosis, kidney damage with proximal tuberal necrosis, lesions associated with hemorrhagic gastroenteritis and high mortality, which were observed in sheep and cattle. Hydrolysable tannins can also affect monogastrics by reducing growth rates, protein utilization and causing damage to the mucosa of the digestive tract and increasing the excretion of protein and amino acids[24].

Condensed tannins (CT or proanthocyanidins), are the most common type of tannins found in forage legumes, trees and stems. These types of tannins are widely distributed in legume pasture species such as Lotus corniculatus and in several kinds of acacia and other plant species. Condensed tannins have a variety of chemical structures affecting their physical and biological properties. They are consist of flavanoid units (flavan-3-ol) linked by carbon-carbon bonds. The complexity of CT depends on the flavanoid units which vary among constituents and within sites for interflavan bond formation. The term proanthocyanidins (PAs) is derived from the acid-catalyzed oxidation reaction producing red anthocyanidins upon heating PAs in acidic alcoholsolutions. Anthocyanidin pigment is responsible for the colors observed in flowers, leaves, fruits juices and wines. The astringent taste of some leaves, fruits and wines is due to the presence of tannin[24].

Base Unit:	HO OH OH Gallic Acid	Flavone	
Class/Polymer:	Hydrolyzable Tannins	Condensed Tannins	
Sources	Plants	Plants	

Figure 10.0: Structure of different tannins[25]

Tannin-rich plants like the Oak tree can contract the skin's tissue, thereby improving the skin's resistance to infection.

10.2 Phytochemical analysis test:

10.2.1 Gelatin test:

To a solution of tannin, aqueous solution of gelatin and sodium chloride are added. A white buff colored precipitate is formed [26].

10.2.2 Goldbeater's skin test:

A small piece of goldbeater skin (membrane prepared from the intestine of an ox) is soaked in 20% hydrochloric acid, ringed with distilled water and placed in a solution of tannin for 5 minutes. The skin piece is washed with distilled water and kept in a solution of ferrous sulphate. A brown or black colour is produced on the skin due presence of tannins [26].

10.2.3. Phenazone test:

A mixture of aqueous extract of a drug and sodium acid phosphate is heated and cooled and filtered. A solution of phenazone is added to the filtrate. A bulkycoloured precipitate is formed [26].

10.2.4 Match stick test (Catechin test):

A match stick is dipped in aqueous plant extract, dried near burner and moistened with concentrated hydrochloric acid. On warming near flame, the matchstick wood turns pink or red due to formation of phloroglucinol [26].

10.2.5 Chlorogenic acid test:

An extract of chlorogenic acid containing drug is treated with aqueous ammonia. A green colour is formed on exposure to air [26].

10.2.6 Vanillin-hydrochloric acid test:

Sample solution and added vanillin- hydrochloric acid reagent (Vanillin 1 gm, alcohol 10 ml, concentrated hydrochloric acid 10 ml). A pink or red colour is formed due to formation of phloroglucinol [26].

11. Extraction:

11.0 Methods of extraction of medicinal plants Maceration:

In this process, the whole or coarsely powdered crude drug is placed in a stopper container with the solvent. And allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained; the Marc (the damp solid material) is pressed. And the combined liquids are clarified by filtration or decantation after standing [27].

Infusion :

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs [27].

Digestion :

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased [27].

Decoction :

In this process, the crude drug is boiled in a specified volume of water for a defined time. It is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents. This process is typically used in preparation of Ayurvedic extracts called "quath" or "kawath". The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16. The volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then the concentrated extract is filtered and used as such or processed further [27].

Percolation :

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting[27].

Hot continuous extraction (Soxhlet) :

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber Esiphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale[27].

Ultrasound Extraction (Sonication):

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfi a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules [28].

Counter-current Extraction:

In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce a fi ne slurry. In this process, the material to be extracted is moved in one direction (generally in the form of a fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. The further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end [28].

Extraction by Fermentation:

Some medicinal preparations of Ayurveda (like *asava* and *arista*) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (*kasaya*), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel. In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are *karpurasava, kanakasava, dasmularista*. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts [28].

Supercritical Fluid Extraction:

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample

volume, analyze collection, modifier (co-solvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt. The collection of the extracted analyze following SFE is another important step: significant analyze loss can occur during this step, leading the analyst to believe that the actual efficiency was poor [28].

There are many advantages to the use of CO2 as the extracting fluid. In addition to its favorable physical properties, carbon dioxide is inexpensive, safe and abundant. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present. Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations. Of late, instead of carbon dioxide, argon is being used because it is inexpensive and more inert. The component recovery rates generally increase with increasing pressure or temperature: the highest recovery rates in case of argon are obtained at 500 atm and 150° C [28].

Phytonics Process:

A new solvent based on hydrofluorocarbon-134a and a new technology to optimize its remarkable properties in the extraction of plant materials offer significant environmental advantages and health and safety benefits over traditional processes for the production of high quality natural fragrant oils, flavors and biological extracts [28].

Advanced Phytonics Limited (Manchester, UK) has developed this patented technology termed "phytonics process". The products mostly extracted by this process are fragrant components of essential oils and biological or phytopharmacological extracts which can be used directly without further physical or chemical treatment [28].

The properties of the new generation of fluorocarbon solvents have been applied to the extraction of plant materials. The core of the solvent is 1,1,2,2-tetrafluoroethane, better known as hydrofluorocarbon-134a (HFC-134a). This product was developed as a replacement for chlorofluorocarbons. The boiling point of this solvent is -25° C. It is not flammable or toxic. Unlike chlorofluorocarbons, it does not deplete the ozone layer. It has a vapor pressure of 5.6 bar at ambient temperature. By most standards this is a poor solvent. For example, it does not mix with mineral oils or triglycerides and it does not dissolve plant wastes [28].

11.1 Aqueous extraction

10 g of powdered sample was dissolved in 100 ml of distilled water and boiled for 2 h on slow heat. The residue was removed by filtering through 8 layers of muslin cloth; the filtrate was then centrifuged at 500rpm for 10 min. The supernatant was collected and further boiled till the volume was reduced to one fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml. It was then autoclaved at 121 $^{\circ}$ C and at 15 lbs pressure and stored at 4 $^{\circ}$ C[5].

11.2 Methanol extraction

10 grams of powdered sample was dissolved in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190–220 rpm for 24 h. The supernatant was collected slowly and evaporated in wide mouthed evaporating bowls at room temperature for 2–3 days till the final volume was reduced to one fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml. and stored at 4 $^{\circ}$ C in airtight bottles [5].

12.0 Solvent selection for extraction:

Pharmacologically test a traditionally used phytomedicine that is usually used as a water decoction (hot) or extraction (hot or cold), you should use water in the same manner as the traditional medicine is prepared. When testing this in an animal model, take care to apply it orally (as in human treatment).

For identification and isolation of secondary plant compounds, use methanol (lower boiling point) or ethanol (somewhat higher boiling point), pure acetone or acetone/water mixtures. This is for most of the polar and semipolar constituents. Alcohol is a good solvent for the herbal active components it is also an excellent preservative. Alcohol has been used to make herbal preparations for hundreds, perhaps thousands, of years. Old texts describe steeping herbs in wine for long periods and then using the resultant liquid. With our increased knowledge of plant constituents herbalists now choose the appropriate Alcohol :Water mix to optimize the effectiveness of the extract. However, for lipophilic compounds you should use lipophilic solvents such as petrol or (bit more polar) chloroform. For some of these solvents you should take care; acetone is highly fire sensitive and liver toxic; chloroform shows

liver toxicity; thus take care for a well working hood. Don't use diethylether because of its high tendency for explosion.

REFERENCES

[1] The active constituents of herbs, **2013**. http://www.health24.com/Natural/Herbs/The-active-constituents-of-herbs-20120721.

[2] Dr. VenketeshwerRao (Ed.), Maria João Matos, Lourdes Santana, Eugenio Uriarte, Orlando A. Abreu, Enrique Molina and Estela GuardadoYordi (authors)Chapter 5 Coumarins - An Important Class of Phytochemicals; Phytochemicals - Isolation, Characterisation and Role in Human Health, **2015**. ISBN: 978-953-51-2170-1, InTech, DOI: 10.5772/59982.http://dx.doi.org/10.5772/59982

[3] Dr. Marisa Marciano. Plant constituents. The Naturopathic Herbalist. http://thenaturopathicherbalist.com/plant-constituents/

[4] Plant alkaloids.Jack G woolly. Encyclopedia of life sciences. Nature publishing group, 2001. www.els.net

[5] Sooad Al-Daihan; Manar Al-Faham; Nora Al-shawi; RawanAlmayman; AmalBrnawi;Seemazargar et al. Antibacterial: Activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. Journal of King Saud University –Science, **2013**, 25, 115-120.

[6] Kevin m. Davies, editor; Plant pigments and their manipulation. Annual Plant Reviews, Blackwell Publishing Ltd., **2004**, (14) 95-97, 251-256.

[7] JI Yubin; Yu Miao; Wang Bing and Zhang Yao. *Journal of Chemical and Pharmaceutical Research*, **2014**, 6(1), 338-345.

[8] Abba PacômeObouayeba; MoussaDiarrassouba; Eric Francis Soumahin; TanohHilaireKouakou. Phytochemical analysis, purification and identification of *Hibiscus Anthocyanins*. *Journal of Pharmaceutical, Chemical, and Biolological Sciences*, **2015**, 3(2), 156-168.

[9] Khalil I. Ereifej;HaoFeng;TahaRababah et al. Chemical Composition, Phenolics, Anthocyanins Concentration and Antioxidant Activity of Ten Wild Edible Plants. *Food and Nutrition Sciences*, **2015**, 6, 581-590.(http://www.scirp.org/journal/fns and http://dx.doi.org/10.4236/fns.2015.67061)

[10] What is Anthraquinone.Innovate Us. http://www.innovateus.net/science/what-anthraquinone

[11]Xianliwu; Gary r. Beecher; Joanne M. Holden et al.Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. Journal of Agricultural and Food Chem, **2006**,54, 4069-4075. [12]DrVenketeshwerRao (Ed.). James HamuelDoughari. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, Phytochemicals - A Global Perspective of their Role in Nutrition and Health, **2012**. ISBN: 978-953-51-0296-0.

[13] Ch.s.d. Phanideepthiyadav; N.s.p. Bharadwaj; M. Yedukondalu; Ch. Methushala; A. Ravi kumar. Phytochemical evaluation of *nyctanthesarbortristis*, *nerium oleander* and *catharathnusroseus*. *Indian Journal of Research in Pharmacy and Biotechnology*, **2013**, 1(3), 333-338.

[14] IoannisPrassas; Eleftherios P; Diamandis. Novel therapeutic applications of cardiac glycosides. *Nature Reviews Drug Discovery*, **2008**, 7, 926-935.

[15] Mir Ashfaq; K.W. Shah; Showket Ahmad; Dharmendra Singh. Preliminary phytochemical screening of alcoholic & aqueous extracts of *menthalongifolia*linn. Leaves. *International Journal of Biological & Pharmaceutical Research*, **2012**, 3(3), 384-386.

[16] Nilofer Sheikh; Yogendra Kumar; A.K. Misra; LokhoPfoze. Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of Meghalaya, North East India. Journal of Medicinal Plants Studies, **2013**, 1(6), 62-69.

[17] Cardiac glycoside. Wikipedia. https://en.wikipedia.org/wiki/Cardiac_glycoside

[18] Mohammad Asif. Pharmacological activities and phytochemistry of various plant containing coumarin derivatives. *Current Science Perspectives*, **2015**, 1(3), 77-90.

[19] Ilza A. Francisco and Maria Helena PimentaPinotti. Cyanogenic Glycosides in Plants. Brazilian Archives of Biology and Technology, **2000**, 43(5), 487-492.

[20] https://en.wikipedia.org/wiki/Glycoside#Cyanogenic_glycosides

[21] IvanaRadojcicRedovnikovic et al. Glucosinolates and their potential role in plant. PeriodicumBiologorum, **2008**,110 (4), 297–309.

[22] Jin Dai and Russell J. Mumper. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*, **2010**,15,7313-7352. doi:10.3390/molecules15107313.

[23] Veena Sharma and RituPaliwal. Isolation and Characterization of Saponins from MoringaOleifera (Moringaeceae) Pods. *International Journal of Pharmacy and Pharmaceutical Sciences*, **2013**, 5(1), 179-183.

[24] ShahinHassanpour; NaserMaheri-Sis; BehradEshratkhah and FarhadBaghbaniMehmandar. Plants and secondary metabolites (Tannins): A Review. *International Journal of Forest, Soil and Erosion*, **2011**, 1 (1), 47-53. [25] Praveen Kumar Ashok;KumudUpadhyaya. Tannins are Astringent. *Journal of Pharmacognosy and Phytochemistry*,**2012**, 1(3), 45-50.

[26] ManpreetKaur. Tannins: Classification, Properties and Chemical tests. http://www.yourarticlelibrary.com/pharmacognosy/tannins/classification-properties-and-chemical-tests/49892/

[27] Tasneef Ahmad; Swatantra Bahadur Singh; Shivshankar Pandey. Phytochemical Screening and Physicochemical Parameters of Crude Drugs: A Brief Review. *International Journal of Pharma Research & Review*, **2013**, 2(12), 53-60.

[28] S. S. Handa, S.P.S Khanuja, G. Longo, D.D.Rakesh(Ed.). Extraction Technologies for Medicinal and Aromatic Plants. *International Centre For Science And High Technology Trieste*, **2008**, 21-52.