Sudan University of Science and Technology

Collage of Graduate Studies

Phytochemical Study of The Leaves of Adansonia digitata

دراسه فيتوكيميائية لاوراق شجرة التبلدي

A Thesis Submitted In Partial Fulfillment of The Requirements For The M.Sc Degree In Chemistry

By

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(B.Sc. (honors) Chemistry)

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بسم الله الرحمن الرحيم

((أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ ثَمَرَاتٍ مُخْتَلِفًا أَلْوَانُهَا وَمِنَ الْجِبَالِ جُدَدٌ بِيضٌ وَحُمْرٌ مُخْتَلِفٌ أَلْوَانُهَا وَغَرَابِيبُ سُودٌ))

سورة فاطر الاية(27)

صدق الله العظيم

Dedication

To my dear parents,

brother, and sisters.

Acknowledgements

After thanking Allamity Alla.

I send my full thanks to my Supervisor Dr. Elfatih Ahmed Hassan for giving me strength to complete this work and for his prefect supervision during the course of the work.

My best regards and thanks also go to Ahmed Abd Elazeem for providing materials.

Special thanks are also extended to my colleagues for their interest and encouragement.

Finally, my deepest thanks to every one who supported me in this research until it reached it is final stage.

Abstract

This study was carried out on the leaves of *Adansonia digitata*.

In this work, powdered sample of *Adansonia digitata* leaves were sequentially extracted using water, methanol, ethanol, acetone, ethyl acetate, and hexane solvents. The resultant extracts were subjected to phytochemical tests.

The results obtained showed that terpenoids were present over all extracts except methanol and ethanol.

Alkaloids were present in water, methanol, ethanol, and acetone extracts; flavonoids in water, methanol, ethanol, and ethyl acetate extracts.

Phenolics and tannins were present in water, methanol, ethanol, and acetone; saponins; in water, methanol, and ethanol extracts.

Steroids were present only in ethyl acetate and hexane extracts.

The yield percentage of flavonoid in the sample was 12.41%.

مستخلص الدراسة

اجريت هذه الدراسه علي عينه من اوراق شجرة التبلدي

اجريت دراسه فيتوكيميائيه لبدرة العينه المأخوذة من التلبدي باستخدام الماء, الميثانول, الايثانول, الاستون, ايثانوات الايثيل, والهكسان كمذيبات للاستخلاص.

اوضحت الدراسه ظهور التربينات في مستخلصات كل المذيبات عدا الميثانول والايثانول.

القلويدات ظهرت في مستخلصات الماء, الميثانول, الايثانول, والاستون; الفلافونيدات في مستخلصات الماء, الميثانول, الايثانول, ايثانوات الايثيل.

الفينولات والتانين ظهرت في مستخلصات الماء, الميثانول, الايثانول, والاستون; الصابونيدات في الماء, الميثانول, الايثانول.

الاسترويدات ظهرت في مستخلصات ايثانوات الايثيل والهكسان فقط.

كما وجد ان النسبه المئويه للفلافونيدات في العينه كانت 12.41%.

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Introduction

1.1 Adansonia digitata trees:

Adansonia digitata (Bombaceae family) is a native deciduous tree from the African savanna. The English common name is baobab, probably derived from the Arabic buhibab, which means “fruit with several seeds”. There are many different local names used in southern Africa :Umkhomo and Muuju in Zimbabwe; Moana, Dovuyu, Ibozu, Mbuyu and Mobuyu in Botswana; Mnambe and Mbuye in Malawi; Muuyu, Mbuyu, Mkulukumba, Mlambe in Zambia[1], also it is known as cream of tartar, lemonade tree and monkey bread tree. In Sudan it’s locally known as Homeria or Tabeldi; the fruit are named “gunguleiz”. The Tabeldi tree has many ranges of uses extended from food and beverages to medicinal uses and also it is a good fodder for thedomestic animals[2].

1.1.1 Scientific classification:

Kingdom : Plantae
Division : Tacheophyta
Class : Magnoliopsida
Order : Malvaless
Family : Malvaceae
Genus : Adansonia
Species : Adansonia digitata – baobab
Botanical name :Adansonia digitata[3].

1.1.2 Description:

All Baobab are deciduous trees ranging in height from 5 to 20 meters. The Baobab tree is a strange looking tree that grows in low-lying areas in Africa and Australia. It can grows to enormous sizes and carbon dating indicates that they may live to be 3,000 years old. One ancient hollow Baobab tree in Zimbabwe is so large that up to 40 people can shelter inside its trunk.Various Baobabs have been used as a shop, a prison, a house, a storage barn and a bus shelter. The tree is certainly very different from any other. The trunk is smooth and shiny, not at all like the bark of other trees, and it is pinkish grey or sometimes copper coloured.

When bare of leaves, the spreading branches of the Baobab look like roots sticking up into the air, rather as if it had been planted upside-down. Baobab are very difficult to kill, they can be burnt, or stripped of their bark, carry on growing[4]. The thick, fibrous bark is remarkably fire resistant, and even if the interior is completely burnt out, the tree continues to live; Re growth after fire results in a thickened, uneven integument that gives the tree its gnarled appearance resembling an elephant's skin but that serves as added protection against fire[5]. When they do die, they simply rot from the inside and suddenly collapse, leaving a heap of fibres, which makes many people think that they don't die at all, but simply disappear.

An old Baobab tree can create its own ecosystem, as it supports the life of countless creatures, from the largest of mammals to the thousands of tiny creatures scurrying in and out of its crevices, also birds nest in its branches[4].

The stem is covered with a bark layer , which may be 50-100mm thick. The leaves are hand-sized and divided into 5-7 finger like leaflets attached to a central point, The leaves are simple or digitally compound, dark-green on top, and borne at the end of a 16 cm-long petiole. The leaflets are between 5-15 cm long and 1.5-7 cm broad. The baobab shed its leaves during the early dry season and new leaves appear after flowering. The pentamerous flowers are white, large (20 cm in diameter and 25 cm long), and hang from stalks on pedicels up to 90 cm long. The fruit is a voluminous (35 cm long and 17 cm in diameter) ovoid capsule with a hard woody envelope containing a pulp and black seeds. Once ripe, the fruit envelope becomes brittle and the pulp takes on a chalky consistency. The tree starts producing fruits 8-10 years after planting but consistent production only occurs after 30 years[6].

1.1.3 Distribution:

Although the baobab is the most easily recognised tree in the savannas of tropical and southern Africa, its precise distribution between the margins of the forest fringes has not been completely mapped. The map (Figure 1) shows an almost total encirclement of the Congo basin apart from the gap between Lake Chad and the Sudan Republic[7].

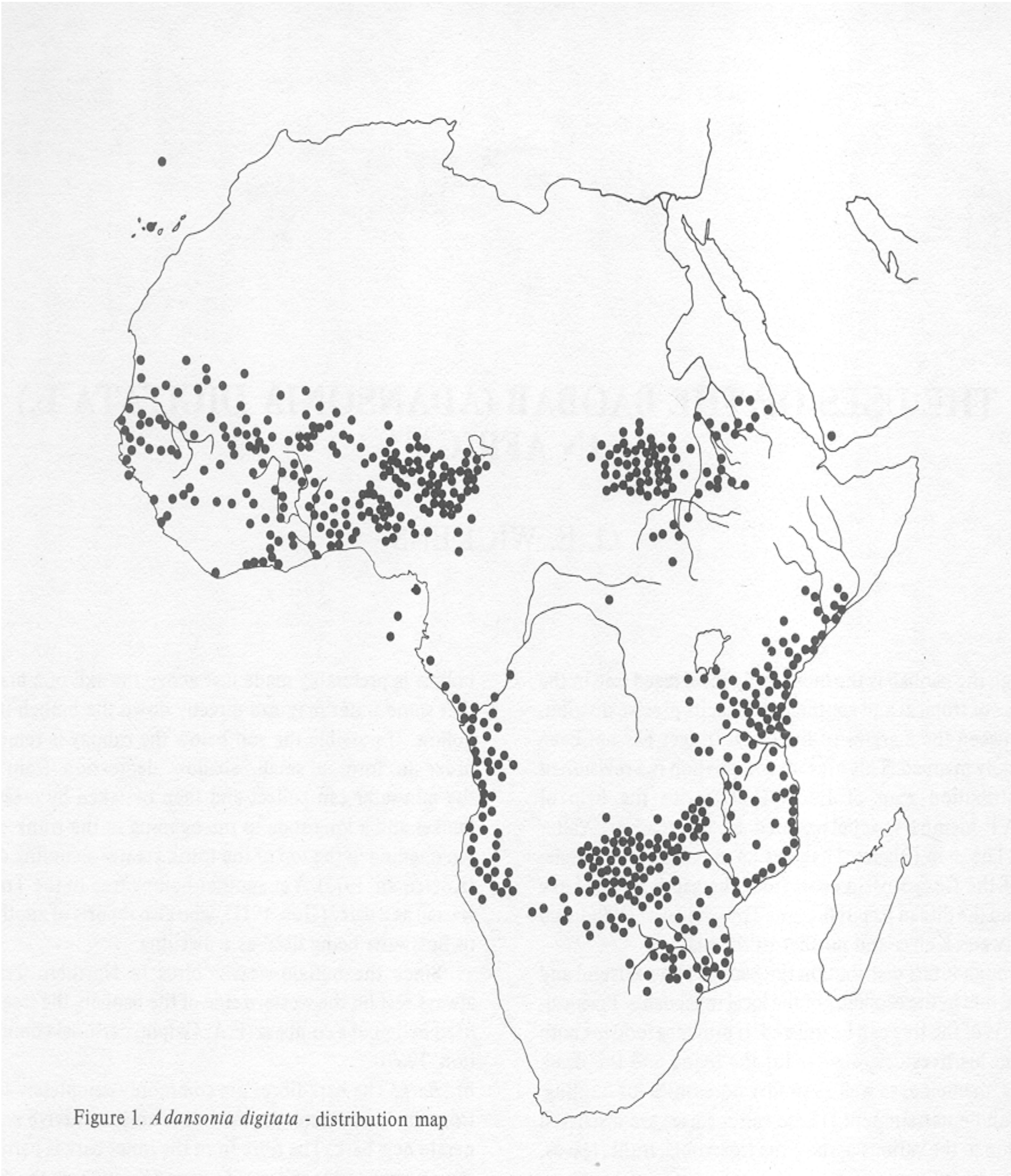


Figure 1. *Adansonia digitata* - distribution map

Although the baobab grows satisfactorily outside, it seems unlikely to become a significant vegetable resource in location where it is not now employed in that manner[8].

1.1.4Uses:

1.1.4.1 Main uses:

Adansonia digitata has numerous medicinal and non-medicinal uses in Africa. Every part of the baobab tree is reported to be useful[9]. In overall utility, perhaps no tree on earth surpasses baobab[8].

The leaves of Adansonia digitata are eaten as a leaf vegetable, they are said to be rich in vitamin C contain uronic acids, sugars, potassium tartrate and calcium. In ferlo, North Senegnal, an extract of the leaves, called “lalo” is used to give couscous (millet porridge) a smooth consistency, also they form an excellent condiment and seasoning. Research has shown it to be of such nutritional quality that it may be therapeutically useful in the management of protein calorie malnutrition[8].Caterpillars, which feed on the leaves, are collected and eaten by African people as an important source of protein[6]. Typically, the young leaves are used as a soup ingredient. The fruit pulp is used in preparing cool and hot drinks in rural areas and has recently become a popular ingredient in ice products in urban areas. In some areas the use of the ‘baobab milk’ is very common. The dried pulp is scraped from baobab fruits and made into a solution. The milk is a highly nutritious drink. The pulp is also eaten fresh in Sudan. The seeds are characterized as a potential protein source. In Sudan they are pounded whole into a coarse meal and added to soups and other dishes like “Burma”. In some areas roasted seeds are used as a coffee substitute. The bark, which produces strong fibre, is used in making ropes, mats, bags, and hats. The smooth fibres of the inner side of the bark are more important than the outer bark for weaving. The wood is whitish, spongy and light (air-dried 320kg m^{-3}) and is used mainly for fuel. Hollow trees provide reservoirs of fresh water which are used by nomads, particularly in the western part of the Sudan. Water storage capacities range from 1000 to 9000 liters per tree[9], (may keep for months or even years without fouling) . As a source of nectar baobab flowers are excellent. All in all, these trees contribute greatly to African’s honey supply. The tender root of the very young baobab is edible. Older roots are not, but they provide a strong red dye[8].

1.1.4.2 Medicinal Uses:

Baobab leaf powder is credited with various medicinal powers and is commonly taken as a general tonic as well as a treatment for anemia and dysentery. The leaves are also used in treating other affictions : asthma, kidney and bladder disease, insect bites, fevers, malaria, and even copious perspiration[8]. Pulp extract is applied as eye-drops in cases of measles. Asemifluid gum, obtained from baobab bark , is used to treat sores. The seed paste is used for curing tooth and gum diseases. The bark is boiled and taken as a cure for body pains[9].

1.1.5 Cultivation:

Adansonia digitata is widely spread over the African savanna through natural re- production. The species has not commonly been cultivated, partly because of the reputation for growing slowly. Near villages, transplanting naturally germinated seedling has traditionally propagated baobab. Cultivation requires that the seeds be treated before sowing, in order to break dormancy. To do this,the Forest Research Institute in Mali uses sulfuric acid for 6 to 12 hours, followed by rinsing in water for 24 hours. With this method, they achieve a germination rate of over 90%. Direct sowing has not been very successful. The seedlings should be at least 3 to 4 months old before they are transplanted with 10 x 10 meter spacing.

Genetic selection makes it possible to select plants with particularly good characteristics. It has been shown that the Vitamin C content is variable depending on the origin. Agronomical studies by the Forest Research Institute in Mali have shown that baobab can be easily grafted. The advantage is that the plants will be smaller, which facilitates collection of the fruits. Furthermore, grafting shorts the time until flowering. While plants grown from seed start flowering after 8-23 years, grafted plants will start flowering in only 3 years. However, grafted plants give 30% less fruit[1].

1.1.6. Nutrition:

Baobab leaf provides at least four nutritious ingredients: protein, vitamins, minerals, and dietary fiber.

As noted, fresh leaf samples are protein rich. Leaves analyzed in the above-mentioned report contained 10.6 percent protein. The amino-acid composition—the one comparing favorably to the “ideal”—was valine (5.9 percent), phenylalanine/tyrosine (9.6 percent), isoleucine (6.3 percent), lysine (5.7 percent), arginine (8.5 percent), threonine (3.9 percent), cysteine/methionine (4.8 percent), and tryptophan (1.5 percent). In sum, there were adequate amounts of all the essential amino acids excepting the two, cysteine and methionine, containing sulfur.

Baobab leaves contain a very high level of the carotenoids that give rise to vitamin A. The actual amounts (9-27 mg per kg) depend on the tree and on the method of drying. The carotenoids are not unlike those found in carrots (and mangoes), but are less concentrated and less available than their carrot counterparts.

Other researchers have reported up to 15 percent protein. Measurements are on a dry-weight basis. Recent research determined the levels of provitamin A for various leaf types, drying methods, and processing systems. It was found that drying the leaves in shade rather than sun doubled the leaf powder’s provitamin A content a very important discovery for those using baobab in health campaigns. The age of the tree had no effect on provitamin A levels but leaves from small-leafed trees contained more than from large-leafed trees.

The leaf samples are also high in ash (9-13 percent), which includes minerals such as calcium, magnesium, manganese, potassium, phosphorus, iron, sodium, and zinc. In certain samples, however, some of these elements occurred at lesser levels, probably reflecting deficiencies in the soil where the particular tree grew. One test indicated that 100 g of baobab leaves provide about three times the daily calcium requirements, twice the daily magnesium and copper requirements, and four times the daily manganese requirement. It is unclear how available these minerals are in fresh or processed leaves.

The leaf is also high in crude fiber, with levels of 15 to 18 percent measured. They also have an important amount of mucilage[8].

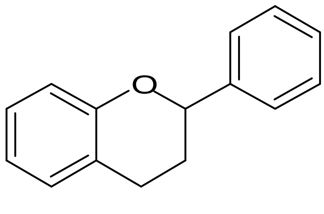
1.2. phytochemical:

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. The therapeutic use of plants certainly goes back to the Sumerian and the Akkadian civilizations in about the third millenium BC. Hippocrates (ca. 460–377 BC), one of the ancient authors who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian. Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto-' from Greek - *phyto*meaning 'plant') or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests. The study of natural products on the other hand is called phytochemistry. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources. The science of application of these indigenous or local medicinal remedies including plants for treatment of diseases is currently called ethno pharmacology but the practice dates back since antiquity. Ethno pharmacology has been the mainstay of traditional medicines the entire world and currently is being integrated into mainstream medicine. Different catalogues including *De MateriaMedica, HistoriaPlantarum, Species Plantarum*have beenvariously published in attempt to provide scientific information on the medicinal uses of plants. The types of plants and methods of application vary from locality to locality with80% of rural dwellers relying on them as means of treating various diseases. For example,the use of bearberry (*Arctostaphylosuva-ursi*) and cranberry juice (*Vacciniummacrocarpon*) totreat urinary tract infections is reported in different manuals of phytotherapy, while speciessuch as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleucaalternifolia*) are described as broad-spectrum antimicrobial agents. A single plant may be used for the treatment of various disease conditions depending on the community. Several ailments including fever, asthma, constipation, esophageal cancer and hypertension have been treated with traditional medicinal plants. The plants are applied in different forms such as poultices, concoctions of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral, nasal (smoking, snoffing or steaming), topical (lotions, oils or creams), bathing or rectal (enemas). Different plant parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin. Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhoea, and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal health. Unfortunately, rapid explosion in human population has made it almost impossible for modern health facilities to meet health demands all over the world, thus putting more demands on the use of natural herbal health remedies. Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus*,*Helicobacter pylori,* and MDR *Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties. In addition, the increase in cases of opportunistic infections and the advent of Acquired Immune Deficiency Syndrome (AIDS) patients and individuals on immuno suppressive chemotherapy, toxicity of many antifungal and antiviral drugs has imposed pressure on the scientific community and pharmaceutical companies to search alternative and novel drug sources[10].

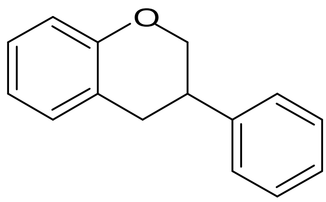
1.2.1. Classes of Phytochemicals:

1.2.1.1. Flavonoids:

The study of flavonoids chemistry have emerged, like that of most natural products, they areimportant group of polyphenols widely distributed among the plant baobabs. The term "flavonoid" is generally used to describe abroad collection of natural products that include a C6-C3-C6 carbon framework, or more specifically a phenylbenzopyran functionality, depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans)(1), isoflavonoids (3- phenylbenzopyrans)(2), and the neoflavonoids (4-phenylbenzopyrans)(3).



(1)



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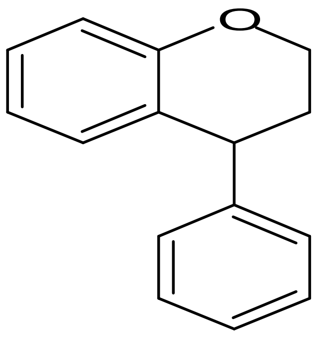

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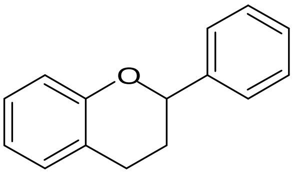
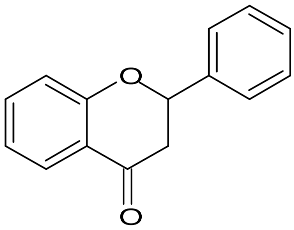
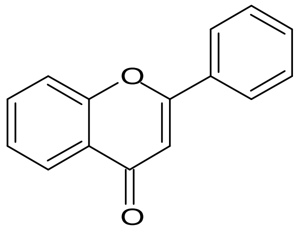


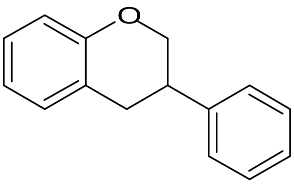
Flavan



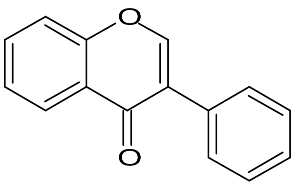
Flavanone



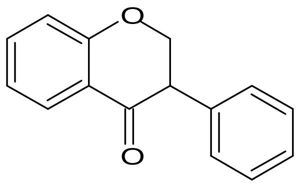
Flavone



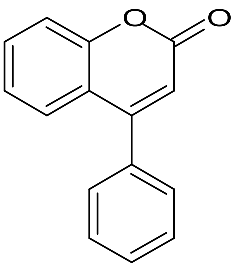
Isoflavan



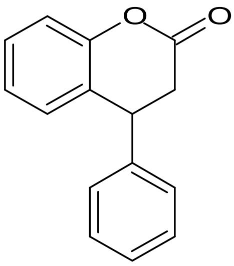
Isoflavone



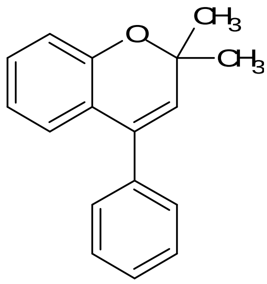
Isoflavanone



4-aryl coumarin

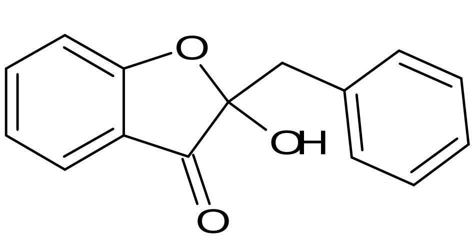


3,4-dihydro-4-aryl coumarin



neoflavene

Minor flavonoids



Auronol

Fig(2). Basic structures of some flavonoids

1.2.1.1.1 Analytical Methods:

Agood separation and analytical method is essential to the characterization Of flavonoids for both chemical and biological properties. The heightened interest in flavonoids from fruits and vegetables and their potential health benefits has resulted in several good reviews on the separation and analysis of phytochemicals including flavonoids, we briefly summarize the most frequently used methods for extraction, separation, and analysis of flavonoids found in commonly consumed fruits and vegetable.

Plant including fruits and vegetable are vast reservoir of different phytochemicals. As state previously, flavonoids are adiverse group of polyphenolic compounds, some of which are relatively stable, whereas other such as anthocyaninsare labile under ambient conditions. Sample preparation is of paramount importance in studing flavonoids because agood method prevents compound of interest from being degraded during sample collection, storage, and any drying processes. Degradation can also happen under elevated temperature or exposure to air and light. It is always advisable to collect fresh samples, extract, and analyze immediately; however, when they must be kept for long time, freezing and drying the samples is preferred.

The purpose of extraction is to maximize recovery of the compound of interest, that is, flavonoids, from the samples. It generally carried out by using water, organic solvents, or liquefied gas, or combination of these under various temperature and pressure condition. The efficiency of solvent depends on several factors, including physicochemical properties of the solvent(e.g., polarity, temperature, PH sample/solvent ratio, extraction steps (repeat), in addition, the sample particle characteristics (size and shape), also the physicochemical properties of compound under investigation.

Flavonoids are normally stored in the vacuoles of the cell; therefore, solvent molecules must be able to penetrate the cell walls to reach the compounds of interest.

Different extraction technologies exist that can enhance the efficiency of flavonoids extraction, such as hydrodistillation, maceration, soxhlet extraction, ultrasonic extraction, etc…

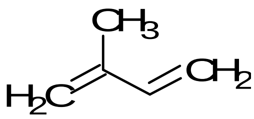
Purification procedure is critical for identification of unknown compound. Concentrations of individual flavonoids in fruits and vegetables have been determined using various methods, particularly chromatographic techniques. Conventional chromatographic separations of flavonoids include the use of thin- layer chromatography (TLC), open column chromatography (CC),(these are simple and cost less), gas chromatography (GC), capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC), provide high sensitivity separation efficiency. HPLC coupled with different detection techniques such as UV-Vis and mass spectrometry (LC-MC) has been pivotal in the characterization of different classes of flavonoids.

When standard flavonoids are not available, or when unknown compounds are encountered in particular fruit or vegetable, use of MS detector is essential.

Identification using technologies such as MS, nuclear magnetic resonance (NMR) spectroscopy, and infrared (IR) spectroscopy, the UV-Vis absorption spectra can be used to distinguish and identify the subgroup they belong to[11].

1.2.1.2 Terpenes:

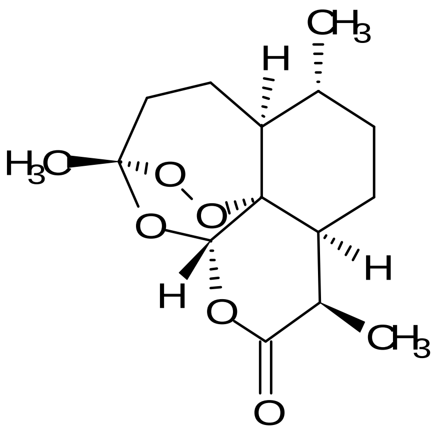
Terpenes are among the most widespread and chemically diverse groups of natural products.They can be hydrocarbons,or they can contain oxygen and be alcohols, ketones or aldehydes. Terpenoids includes hydrocarbons of plant origin of general formula $(C_5H_8)n$, they are commonly classified as monoterpenes (C_{10})[12], include terpinen-4-ol, thujone, camphor, eugenol and menthol,sesquiterpenes (C_{15}),diterpenes (C_{20}), are classically considered to be resins and taxol, sesterterpenes (C_{25}), triterpense (C30), include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity, common triterpenes: amyrins, ursolic acid and oleanic acid. These terpenoids display a wide range of biological activities against cancer (Taxol), malaria (Artimesinin), inflammation, and a variety of infectious diseases (viral and bacterial). Terpenoids are classified according to the number of isoprene units involved in the formation of these compounds. The difference between terpenes and terpenoids is that terpenes are simple hydrocarbons, whereas terpenoids contain additional functional group[13].



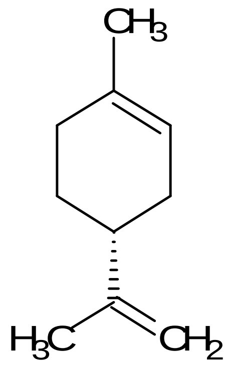
Isoprene unit

Type of terpenoids	**Number of carbon atoms**	**Number of isoprene units**	**Example**
Monoterpene	10	2	Limonene
Sesquiterpene	15	3	Artemisinin
Diterpene	20	4	Forskolin
Triterpene	30	6	a-amyrin
Tetraterpene	40	8	b-carotene
Polymeric terpenoid	several	several	Rubber

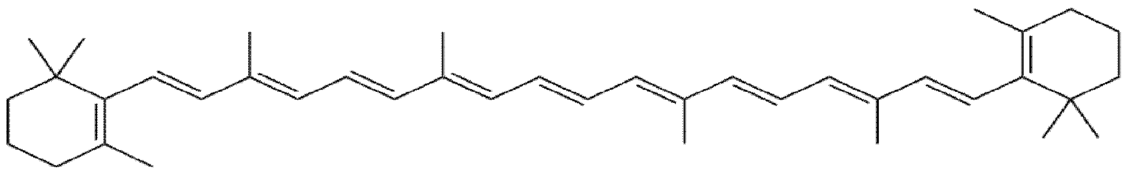
Table(1). Types of terpenoids according to the number of isoprene units.



Artemisinin



Limonene



B-carotene

Fig(3). Basic structures of some terpenoids

1.2.1.3 Alkaloids:

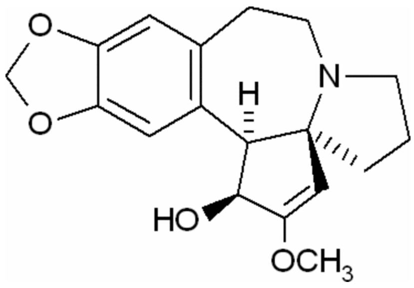
Alkaloid, a chemical substance of plant origin composed of carbon, hydrogen, nitrogen, and (usually) oxygen. The alkaloids are organic bases similar to the alkalies (inorganic bases); the name means alkali-like. There are many types of alkaloids which are derived from tryptophan (e.g. indolylalkylamines, physostigmines, and B-carbolines), also derived from anthranilic such as skimmianine, monoterpene such as gentianine, and sesquiterpene alkaloids such as nymphaeaceue.

Several alkaloids are used as valuable drugs in medicine, e.g. morphine as apain reliever, reserpine in psychlarry as atranquillizer, curare alkaloids in general anaesthesia, (e.g. cocaine,but is nowadays replaced by other drugs since it cause addiction), also utlized by south american indians as an arrow-poison, atropine in eye surgery, ergonovine to induse or make childbirth easier, quinine anti malarial, sanguinafin as antibiotics, and vinblastine as anti cancer agent.

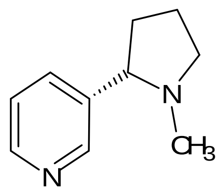
Most alkaloids are readily soluble in alcohol and though they are sparingly soluble in water, their salts are usually soluble[14].

Reagent/test	**Composition of the reagent**	**Result**
Meyer's reagent.	Potassiomercuric iodide solution.	Cream precipitate
Wagner's reagent.	Iodine in potassium iodide.	Reddish-brown precipitate
Tannic acid.	Tannic acid.	Precipitation
Hager's reagent.	A saturated solution of picric acid.	Yellow precipitate
Dragendorff's reagent.	Solution of potassium bismuth iodide potassium chlorate, a drop of hydrochloric acid, evaporated to dryness.	Orange or reddish-brown precipitate (except with caffeine and a few other alkaloids)
Murexide test for caffeine.	residue is exposed to ammonia vapour.	Purine alkaloids produce pink colour

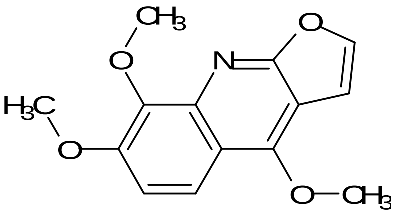
Table(2). Methods for detection of alkaloids



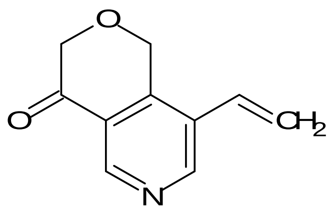
Cephalotaxine



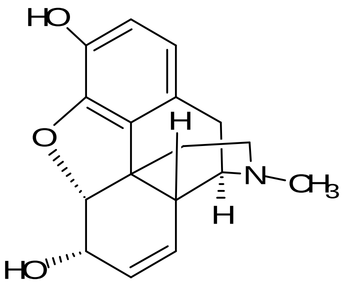
Nicotine



Skimmianine



Gentianine



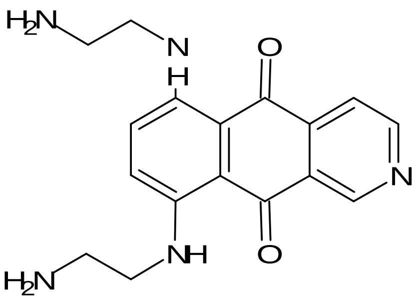
Morphine

Fig (4). Basic structures of some alkaloids

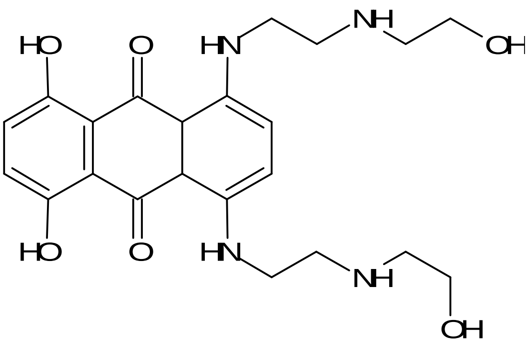
1.2.1.4 Anthraquinones:

Anthraquinones are the largest group of quinones, they are derivatives of phenolic and glycosidic compounds. They have been used as mordant dyes e.g. alizarin from Rubiatinctorum and purgatives, e.g. emodin from Rheum, Rumex. They lost their importance, like so many other natural dyes, with the development of the synthetic dye industry. They are widely speard in lower and higher plants and occur also in the animal kingdom. In addition they are present as glycosides in young plants.

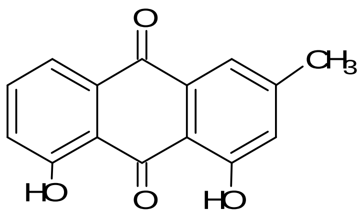
As ageneral rule fungal anthraquinones and plant anthraquinones with hydroxy groups are derived from polyketides, whereas plant anthraquinones devoid of hydroxy groups in one ring, e.g. alizarin, come from mixed pathways[14].



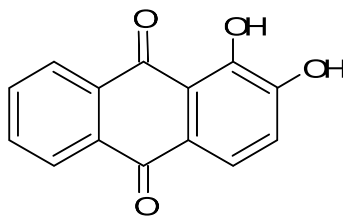
Pixantrone



Mitoxantrone



Emodin

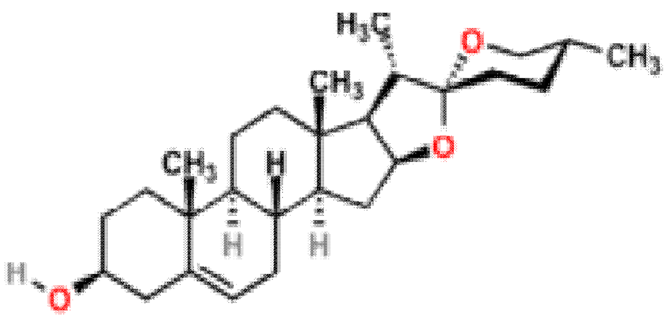


Alizarin

Fig(5). Basic structures of some anthraquinones

1.2.1.5 Steroid:

Plant steroids (or steroid glycosides) also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illnes. Caution should be taken when using steroidal glycosides as small amounts would exhibit the much needed stimulation on a diseased heart, whereas excessive dose may cause problems, even death. Diosgenin and cevadine (from *Veratrumveride*) are examples of plant steroids[10].

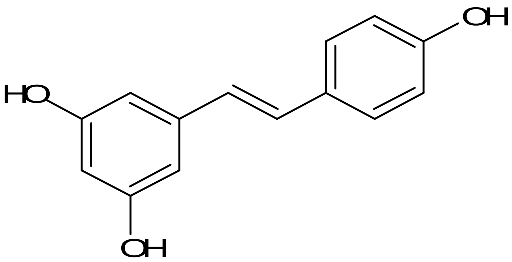


Fig(6). Structure of Diosgenin

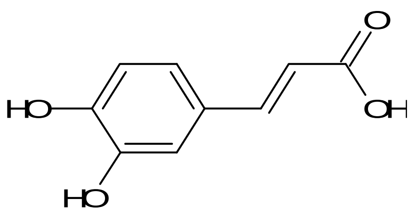
1.2.1.6. Phenolics:

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 plant, with more than 8,000 phenolic structures, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. They are ubiquitous in all plant organs and therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetable, cereals, legumes, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods. For example, phenolics contribute to the bitterness and astringency of fruits and fruit juices[15]. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogen, parasites, and predators, as well as contributing to plants' colors[15].

Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant, other examples include flavones, rutin, naringin , hesperidin and chlorogenic.



Resveratrol



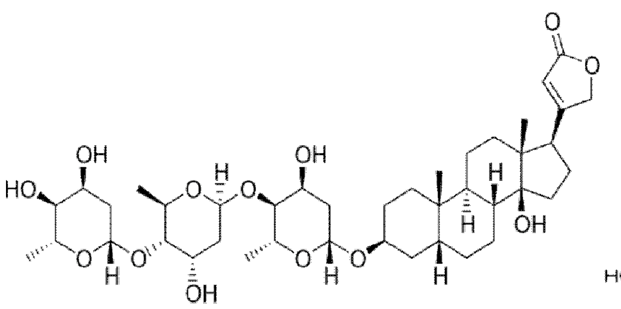
Caffeic acid

Fig(7). Basic structure of some phenolics.

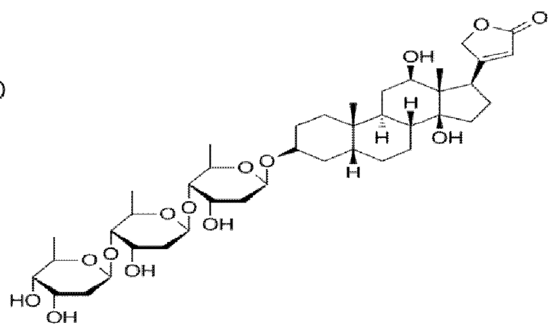
1.2.1.7.Cardiac glycosides:

The cardiac glycosides are an important class of naturally occurring drugs which actions include both beneficial and toxic effects on heart, and have played an outstanding role in the therapy of congestive heart failures, also cardiac-glycosides-based drug have now entered clinical trials for treating cancer. In addition, the inclusion of several cardiac glycosides in large compound libraries for increasing needs of hypothesis-neutral, high-throughput screening assays has uncovered further candidate therapeutic aspects of these drugs for a number of non-cancer pathologies. The term ‘cardiac glycosides’ or ‘digitalis’ are used throughout to refer to any of steroid or steroid glycoside compounds that exert characteristic positively inotropic effect on the heart.

The cardiac glycosides are composed of two structural features; the suger (glycoside) and the non-sugar (aglycon) moieties, and the two class have been observed in nature, cardenolides and bufadienolides according to their chemical structure. Digitalis Purpurea, Digitalis Lanata, Strrophanthiusgratus and Strophanthuskombe are the major source of cardiac glycosides and digoxin, digitoxin, and ouabain(G-strophanthin) are well known cardiac glycosides[16].



Digitoxin



Digoxin

Fig(8). Basic Structure Of Some Cardiac Glycosides

1.2.1.8 Tannins:

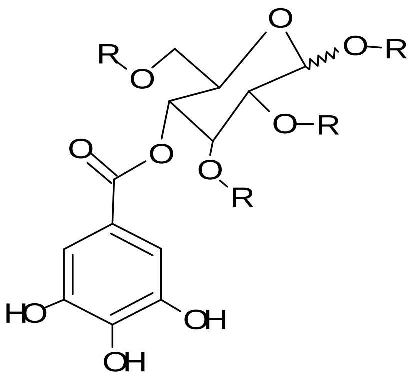
Tannins are polyphenols sometimes called plant polyphenols, although originally the name tannins was given to plant extract exhibiting astringency, without knowing their chemical structure. The features distinguishing tannins from plant polyphenols of other types are basically the properties of the former: binding to proteins, basic compound, pigments, large-molecular compounds and metallic ions, and also anti-oxidant activities, etc. These features of tannins lead to qualitative and quantitative analytical differences between tannins and other polyphenols.

The properties of tannins are based on their chemical structures having two or three phenolic hydroxyl groups on phenyl ring in amolecules of moderately large size[17].

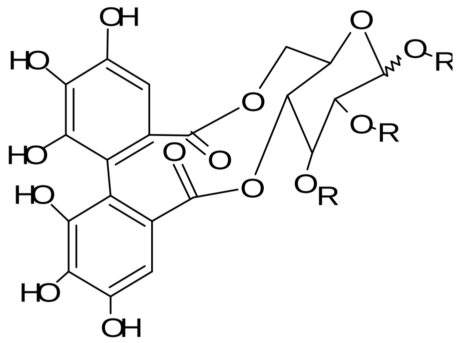
The tannins are applied widely, with uses ranging from medicinal uses to uses in food industry. In medicine, especially in asian(Japanese and Chinese) natural healing, the tannins-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, anti-septic, and haemostatic pharmaceuticals. As tannins can precipitate heavy metals and alkaloids (except morphine), they can be used in poisonings with these substances.

Tannins are used in dyestuff industry as caustics for cationic dyes (tannins dyes), and also in the production of inks (iron gallate ink). In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, as anti-oxidants in fruit juice, beer, and wine industries, and as coagulants in rubber production.

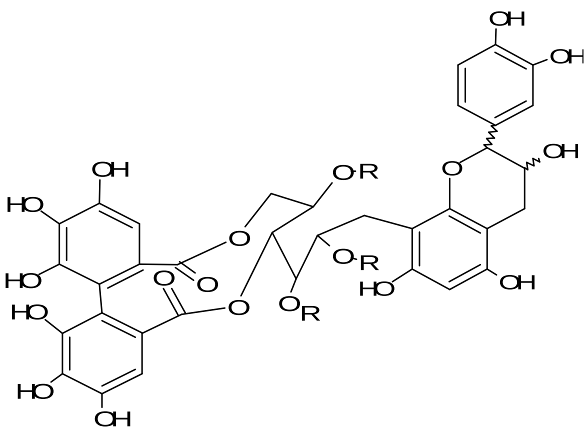
Tannins were classified as ‘hydrolysable tannins’, non hydrolysableoligomeric and polymeric proanthocyanidins were classified as condensed tannins. Therefore,the term ‘hydrolysable tannins’ includes both the gallotannins and ellagitannins[18].



Gallotannins

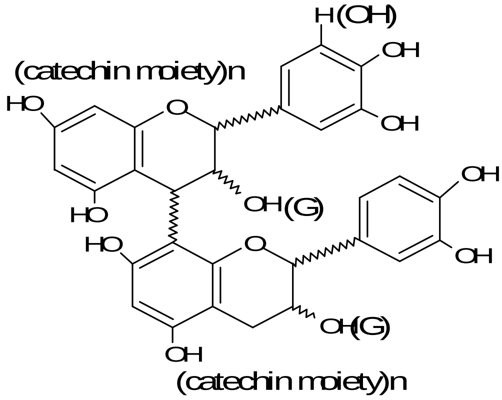


Ellagitannins



R = Galloyl moiety (G)
or other substituents

Complex Tannins



Condensed Tannins

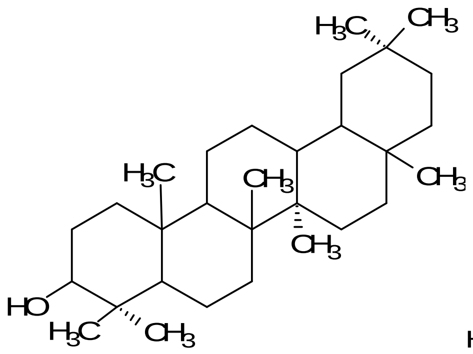
Fig(9). Basic structure of Tannins

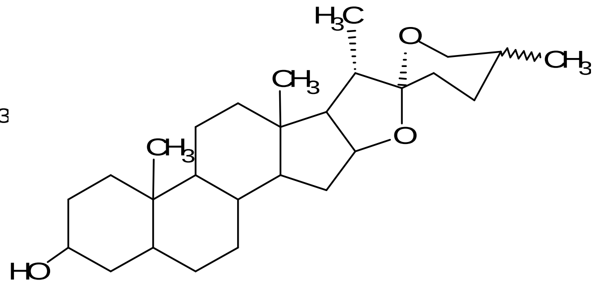
1.2.1.9 Saponins:

Saponins are natural high-molecular weight glycosides of triterpene or steroids with a very wide distribution in the plant kingdom, as well in lower marine animals, such as starfish. Many saponins have detergent properties. They lower the surface tension of aqueous solutions and therfore give stable foams when in contact with water. In fact, the name’’saponins’’ stems from latinword(soap). Many plant drugs and flok medicine, especially those that have origins in asia, contain saponins. For this reason, there is a great interest in characterization and in the investigation of their pharmacolgical and biological properties. 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 anti-virus[19].

The non-sugar or the aglycone unit of the saponins molecule is called the sapogenin or just the genin. The saponins can be divided into three major classes according to the structure of genin: Triterpene glycosides, steroid glycosides, and steroid alkaloid glycosides[20].

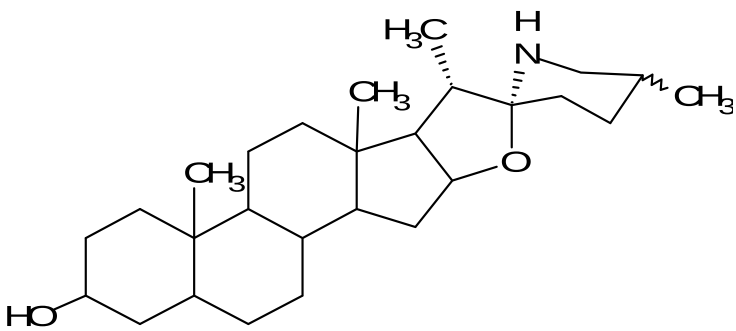
The saponin areSoluble in water, and they are stable under ordinary condition of use and storage.





Triterpene class

Steroid class



Steroid alkaloid class

Fig(10). Basic structure of saponins

Objective:

The phytochemical analysis of the plant is very important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing various diseases.

The objective of this study was:

1- To study presence of phytochemical in adansonia digitata by using different solvents

2- To extract flavonoid

3- To determine the yield percentage of flavonoid in the sample.

Chapter Two
Experimental

Chapter Two

Experimental

2.1 Materials

2.1.1 Collection and preparation of plant sample:

The plant sample for the study were collected from Alamab Nasir, in Khartoum. The leaves were washed, cleaned, dried at room temperature, and reduced to fine powder using a blender. Twenty grammes (20g) of sample was accurately weighed into five different 500ml beaker and 300ml each methanol, ethanol, distilled water, ethyl acetate, acetone and hexane were added to the beakers respectively. These were left for 72 hours. The crude extracts were then decanted and kept for the various analyses.

2.2 Chemicals

1- Methanol

2- Ethanol

3- Distilled water

4- Ethyl acetate

5- Hexane

6- Acetone

7- Ferric chloride 0.1%

8- Chloroform

9- Concentrated tetraoxosulphate (VI) acid

10- Glacial acetic acid

11- Ammonia solution

12- Benzene

13- Hydrochloric acid 1%

14-Iodine

15-Potassium iodide

2.2 Methods:

2.2.1 Phytochemical Screening:

2.2.1.1 Tannins:

Five ml of the extracts was measured and boiled with 10ml of water in a test tube, followed by filtration. Few drops of 0.1% ferric chloride were added. Brownish green precipitate indicates the presence of tannins.

2.2.1.2 Terpeniods:

Five ml of the extracts was measured and was added to 2ml of chloroform. 3ml of concentrated tetraoxosulphate (VI) acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2.2.1.3 Flavonoid:

5 ml of dilute ammonia solution were added to a portion of the extracts followed by addition of concentrated H_2SO_4. Ayellowcolouration that is observed indicates the presences of flavonoids. The yellow colouration disappeared on standing.

2.2.1.4 Alkaloids:

Five ml of the extracts was diluted with 10ml alcohol, boiled and filtered. 5ml of filtrate was added to 2ml of ammonia. 5ml of chloroform was also added and shaken gently, 10ml of acetic acid was added. Then Wagner's reagent was also added. Reddish brown precipitatewas positive for the presence of alkaloids.

2.2.1.5 Anthraquinones:

Five ml of the extracts was shaken with 4ml benzene, it was filtered when hot, the filtrate was shaken with 2ml of 10% ammonia solution The absence of violet colour in the ammoniacal (lower phase) indicates the presence of free anthraquinones.

2.2.1.6 Steroids:

Two ml of the extracts was mixed with 2ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. The formation of a reddish-brown colour at the interface indicates the presence of steroids

2.2.1.7 Cardiac glycoside:

Two ml of the extracts was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was followed by the addition of 1ml of concentrated sulphuric acid. A brown ring which was formed at the interface confirmed the presence of cardiac glycosides.

2.2.1.8 Phenolics:

Two ml of the extracts was mixed with ferric chloride solution. A green or dirty green precipitate indicates the presence of phenolic compound.

2.2.1.9 Saponins

5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

2.2.2 Extraction of flavonoid:

10g of plant was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The whole solution was then filtered through filter paper, and the filtrate was later ontransferred into a water bath and solution was evaporated into dryness. The sample was then weighed .

2.2.3 Thin Layer Chromatography

(0.2g) of the sample was dissolved in minimum amount of 95% ethanol and applied as concentrated spot on TLC plates. The plates were developed with butanol: acetic acid: water (5:2:6). Only a single spot was detected in TLC plate.

Chapter Three

Results and Discussion

Chapter Three

Results and Discussion

The result of the analysis revealed that baobab leaves contain significant nutritional componants that are good for human health.

The leaves are staple for many population in Africa, young leaves are widely used, cooked like spinach, and frequently dried, often powder and used for sauces over porridges, thick gruels of grains, or boild rice. Baobab leaves are superior to fruit pulp in nutritional quality, and contain interesting levels of vitamin A. They appear to be a good source of protein, and contain particularly significant amounts of the amino acids tryptophan. Baobab leaves are significant source of Fe, Ca, K, Mg, Mn, P, and Zn.

In this study different solvent polar and non polar were used to test presence of different phytochemical. The solvents used were, water, methanol, ethanol, ethyl acetate, acetone, and hexane, and the results are depicted in (Table1):

Table (1):phytochemical test of leave extract of Adansonia digitata

Phytochemical	Water	Methanol,and Ethanol	Ethyl acetate	Acetone	Hexane
Flavonoids	+	+	+	-	-
Terpenoids	+	-	+	+	+
Alkaloids	+	+	-	+	-
Anthraquinones	-	-	-	-	-
Steroids	-	-	+	-	+
Phenolics	+	+	-	+	-
Tannins	+	+	-	+	-
Cardiac-glycosides	-	-	-	-	-
Saponins	+	+	-	-	-

(-): Negative test

(+): Positive test

From this result the polar solvents were superior than non polar solvents as most of phytochemicals were efficiently extracted by them.

The yield percentage of flavonoid in the sample was 12.41%.

The IR spectrum of sample of ethanol extract showed absorption at 1614.73cm^{-1} (C=O),

3400.40 cm^{-1} (OH), 1052.23 cm^{-1} (C-O), 1397.22 cm^{-1}(C=C, Ar), and 2925.74 cm^{-1} (C-H, alkane), 624.50cm^{-1} (C-H , Ar, bending).

Since the IR spectrum gave carbonyl stretching at 1614.73 cm^{-1}, it indicates presence of: aflavone, flavonol, flavanone, achalcone, an aurone, an isoflavone, adihydroflavonol or adihydrochalcone.

The UV spectrum of sample is depicted in fig(12).

The flavonoids show two band; band I and band II. Band I is considered to be associated with the absorption of the cinnamoyl system, while band II originates from the benzoyl system.

aflavone, flavonol, achalcone, and aurone, give band I and band II, due to conjugation betweenbenzoyl and cinnamoyl chromophores.

Other flavonoids; isoflavone, adihydroflavonol, adihydrochalcone and flavanones give only band II due to loss conjugation between the carbonyl function and ring (B).

The UV spectrum showed λ_{max} MeOH 275nm.

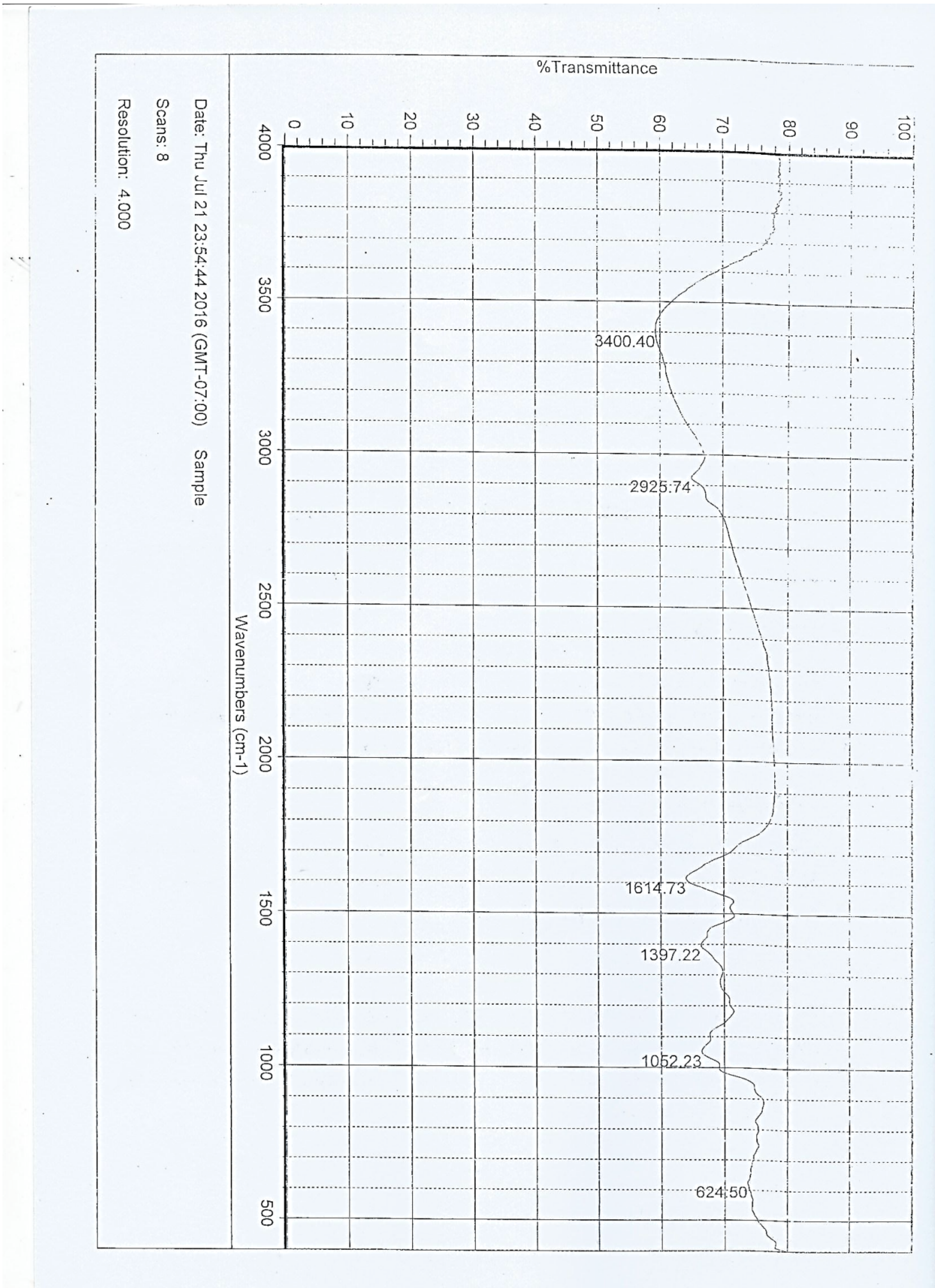
Isoflavone are characterized by band II beside a shoulder in the 300-340nm. Thus the compound cannot be an isoflavone.

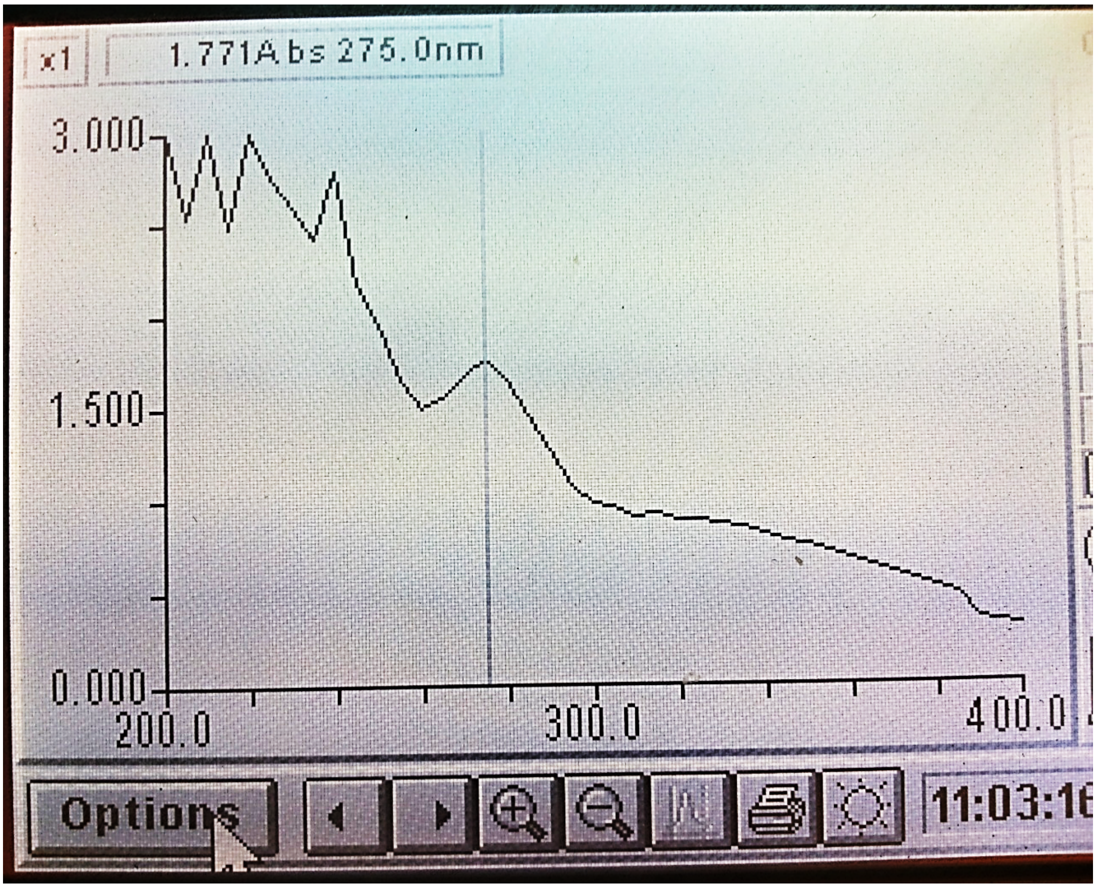
The presence of one band indicates that the sample is probably:

(i)aflavanones or (ii) adihydroflavonol.

Conclusion:

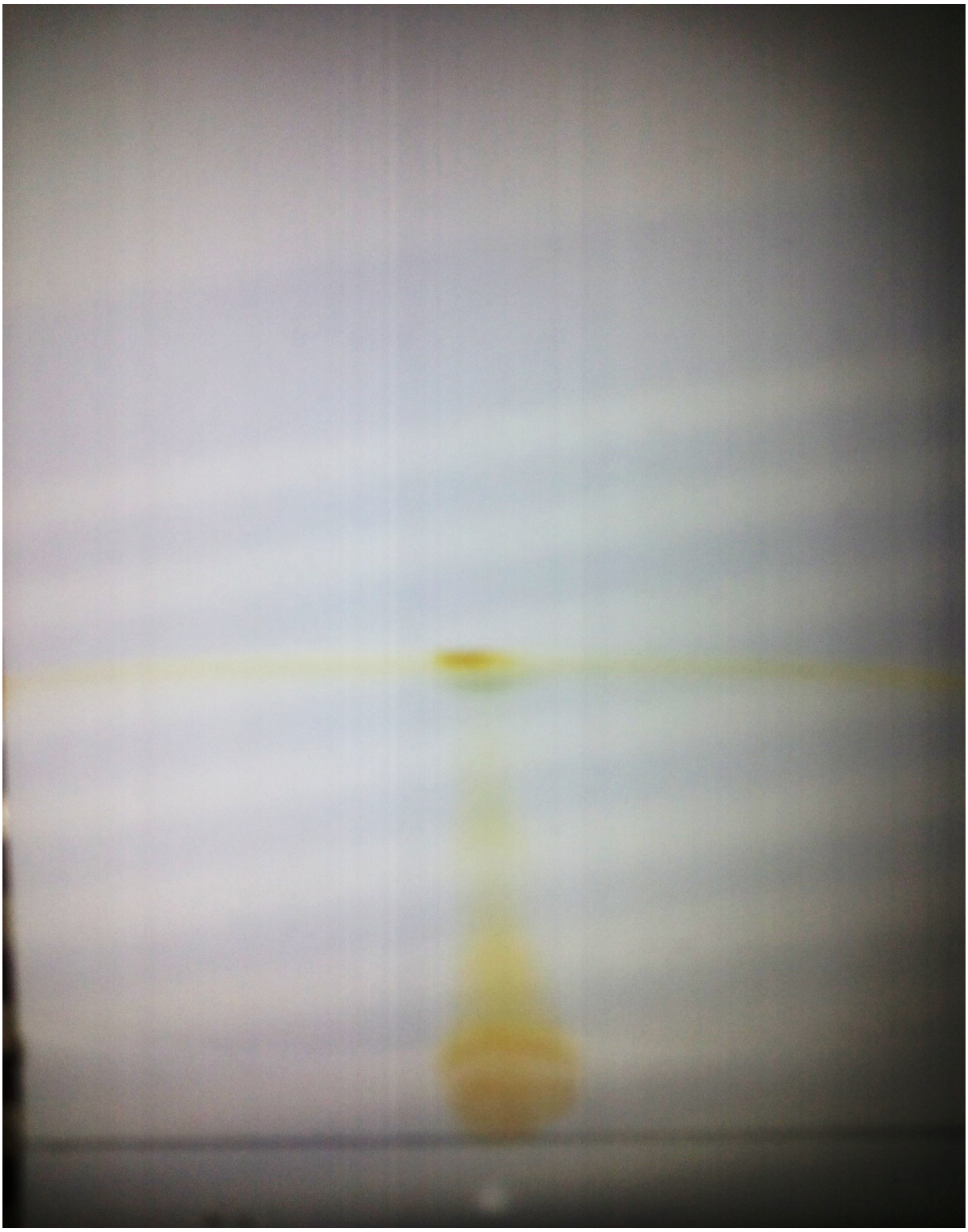
The results of the analysis revealed that baobab leaves contain significant nutritional components that are good for human health and maintenance, such as tannins, terpenoids, alkaloids, steroids, phenolics, flavonoids, and saponins.


%Transmittance
Date: Thu Jul 21 23:54:44 2016 (GMT-07:00) Sample
Scans: 8
Resolution: 4.000
Wavenumbers (cm-1)
3400.40
2925.74
1614.73
1397.22
1052.23
624.50



Fig(12): The UV spectrum of sample (flavonoid).

Fig(13): TLC of sample (flavonoid).



Fig(13): TLC of sample (flavonoid).

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