1-Introduction

1.1-General approach

Nowadays, diseases transmitted by microbes such as fungi and other bacteria are one of the health problems in many countries worldwide. *Gram*-positive bacteria like *Staphylococcus aureus* causes several serious problems such as food poisoning, post-operativ- endocarditis, osteomyelitis, toxic shock syndrome, and wound infections[1]. *Gram*- negative bacteria such as *Pseudomonas aeruginosa* is mainly responsible for urinary tract infections, ventilator-associated pneumonia, surgical site infection, respiratory infections, ocular infections, burn sepsis[2].In the past 60 years, effective antibiotic agents to treat most of infections and diseases caused by bacteria and fungi are commercially available and known as antimicrobials agents[2]. However, the indiscriminate use of avalibale antimicrobials drugs, the wide and long use of antibiotics agents, the specific nature of the relationship of bacteria and fungi to antimicrobials agents and environmental factors lead some bacteria and fungi to develop resistance to commercially available antibiotics. The prevalence of resistance to antimicrobial agents in the recent years actually became the main reasons for increased failure of treatment by antimicrobials. People infected with antimicrobial-

resistant strains are more likely to have longer, more expensive hospital stays, and may be more likely to die as a result of the infections; this problem has forced researchers to search for new antimicrobial substances from various sources as novel antimicrobial therapeutic agents .One of these novel antimicrobial sources are the plants which are very rich source for a wide variety of phytochemicals such as alkaloids, terpenoids, saponins, flavonoids, phenols and tannins[3] which have been reported to have biological activities. By this way safer, environment friendly, cheaper in price natural drugs can be originated. However, several medicinal plants for different reasons have not received sufficient scientific studies and sometimes are classified as 'forgotten plants'. The growing interest in medicinal plants or herbs as a source of new pharmaceuticals and the increasing demand for herbal products in the world, encourage us to revise the 'forgotten plants' by evaluating their applicability and benefits using advanced scientific approaches to increase our information about their pharmacological effects and the phytochemicals responsible for their biological activity[4].Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that

damage the components of the cell, including proteins, lipids, and DNA. The ROS overproduction is associated to degenerative human diseases such as cancer or heart disease[5]. Phytochemicals exerting antioxidant actions are largely recognized as beneficial to human health and disease prevention[6], possibly because they interfere with the processes involved in reactive oxygen and nitrogen species mediated pathologies including coronary diseases and cancer, among others. Epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits, which are known sources of antioxidants, and the risk of cardiovascular diseases[7]. And this antioxidant effect is mainly attributed to the presence of the flavonids in these vegetables and fruits. In addition, flavanoids have a wide array of other pharmacological and medicinal properties[8].Reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxide are known to play important roles in oxidative damage. These ROS are considered to be important causative factors in the development of diseases such as cardiovascular diseases, cancer, liver disease, inflammatory diseases and the aging process[9]. Several studies have demonstrated that natural antioxidants, such as phenolics, flavonoids, carotenoids and tocopherols, can effectively prevent and cure oxidative stress-related diseases[10]. Plants are rich

source of natural antioxidants and have been found to possess a variety of biological activities including antioxidant potential.

1.2- Natural products as medicines

Since the ancient time, medicinal plants are important part of healthcare system. The heavy reliance on plant medicine is attributed to their relative accessibility, low prices, local availability and acceptance by local communities. Due to shortage of dispensaries and professional standard doctors for western healthcare needs, especially in rural areas, medicinal plants have found applications in pharmaceutical, osmotic, agricultural and food industry. The use of medicinal herbs for curing diseases has been documented in the history of all civilizations[11].Medicinal plants and derived products are used for the treatment of major disease such as typhoid fever, cardiac edema, diabetes, malaria, obesity and high blood pressure[12]. Likewise, herbal drugs have been in use by different civilizations in different parts of the world for centuries to fight a large number of diseases. Many of these are in common use even today. According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine. About 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources. Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics. Between the

years 1981-2006, about a hundred anticancer agents have been developed, of which, twenty five are natural product derivatives, eighteen are natural product mimics, eleven candidates are derived from a natural product pharmacophore, and nine are pure natural products. Thus natural sources make a very significant contribution to the health care system[13].

1.2.1 -Types of natural products

As noted above, several drug candidates are derived from various naturally occurring medicinal sources. These can be broadly divided into four categories:

1.2.1.1-Natural products from microorganisms

Microorganisms as a source of potential drug candidates were not explored until the discovery of penicillin in 1929. Since then, a large number of terrestrial and marine microorganisms have been screened for drug discovery. Microorganisms have a wide variety of potentially active substances and have led to the discovery of antibacterial agents like cephalosporins , antidiabetic agents like acarbose , and anticancer agents like epirubicin.

1.2.1.2-Natural products from marine organisms

The first active compounds to be isolated from marine species were spongouridine and spongothymidine from the Carribean sponge *Cryptotheca crypta* in the 1950s. These compounds are nucleotides and show great potential as anticancer and antiviral agents. Their discovery led to an extensive research to identify

novel drug candidates from marine sources. About 70% of the earth's surface is covered by the oceans, providing significant biodiversity for exploration for drug sources. Many marine organisms have a sedentary lifestyle, and thereby synthesize many complex and extremely potent chemicals as their means of defense from prediators[13].These chemicals can serve as possible remedies for various ailments, especially cancer. One such example is discodermolide , isolated from the marine sponge, *Discodermia dissoluta*, which has a similar mode of action to that of paclitaxol and possesses a strong antitumor activity. It also exhibits better water solubility as compared to paclitaxol. A combination therapy of the two drugs has led to reduced tumor growth in certain cancers[13].

1.2.1.3-Natural products from animal sources

Animals have also been a source of some interesting compounds that can be used as drugs. Epibatidine , obtained from the skin of an Ecuadorian poison frog, is ten times more potent than morphine[14].Venoms and toxins from animals have played a significant role in designing a multitude of cures for several diseases.Teprotide, for example, extracted from a Brazilian viper, has led to the development of Cilazapril and Captopril, which are effective against hypertension[15] .

1.2.1.4-Natural products from plant sources

The use of plants as medicines has a long history in the treatment of various diseases. The earliest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies[16].To date, 35,000-70,000 plant species have been screened for their medicinal use[17]. Several important drugs such as Taxol, Camptothecin, Morphine and Quinine have been isolated from plant sources. The first two are widely used as anticancer drugs, while the remaining are analgesic and antimalarial agents, respectively. The geographical location and multi-climatic nature of Sudan lead to the availability of several types of the plants which include numerous medicinal plants grouped into different plant families.Low level of research is done in these medicinal plants and their phytochemicals, in comparison to the number of different types available.

1.3-*Albizia amara*

A.Amara (Fabaceae)is one of the 150 species available from the genus Albizia. The genus is pan tropical, it has a wide distribution in Africa, occurring in Sudan, growing as a savanna tree .It is also found in: many other parts of Africa including Kenya, Zambia, and Madagascar); North America and Australia, but mostly in the old world tropics and has been used for various ailments in the traditional system of medicine. *A.Amara* is a tree of moderate size, much branched with smooth, dark green, scaly

bark. Leaves; pinnately compound,with 15-24 pairs of small, linear leaflets, on 6-15 pairs of pinnate. The flowers are globose and in clusters with 12-20 globose heads.Fruits are oblong pods, about 10-28 X 2-5 cm, light brown, puberulous, thin, and 6-8 seeded[18].The seeds are used as astringent and in treating piles, diarrhea, gonorrhea, leprosy, leucoderma, erysipelas and abscesses. The leaves of the flowers have been applied to boils, eruptions, swellings, emesis, coughs, ulcers, dandruff and malaria[19]. In traditional medicinal the roots are chewed and applied to an eye infection of cattle. It is also used in making soap for washing .Fruits is used as anti-emetic and for treating coughs and malaria. Tannins and gums are constituents of bark. The gum is used against ulcers. Phytochemical investigation revealed the presence of triterpenes, flavonoids, rare amino acids, lipids, steroids and macrocyclic alkaloids[20].The previous studies indicated that the compounds isolated from bark of *A.Amara* showed anti-oxidant, and anti hyperlipidemic activities.Whereby compounds isolated from its leaves showed anti-oxidant,anti-microbial and anti–cancer properties.In addition, the root of *A. Amara* also showed anti-inflammatory and anti-analgesic activities. *Albizia amara* belongs to a family rich in alkaloids, and the extracts have been reported to possess various bioactivities[21, 22].

Fig.1.1: *Albizia amara*

1.4-*Acacia mellifera*

Acacia mellifera (Vahl)Benth(Fabaceae-Mimosoideae) is a low branched tree with a more or less spherical crown.Black bark on stem become ash-grey to light brown on the branches, bearing small, short, sharply hooked spines in pairs. It has a shallow but extensive root system radiating from crown,allowing the plant to exploit soil moisture and nutrients from large volume of soil. The root rarely penetrate than 1m[23].

Leaves characterized by 2 pairs of pinnulae,each with a single pair of leaflets.Leaflet elliptic,0.6-2cm long and highly coloured beneath.Flowers sweetly scented,in elongated spikes,cream to white in spiciform racemes,up to 3.5m long[23].

Flowering and fruiting start three years after planting and generally occur twice a year.Flowers are borne on shoots produced the previous year.

Acacia mellifera is a commonly occurring shrub on rangelands throughout savanna in western, eastern and southern Africa. The preference is rocky hillsides with rainfall along seasonal Nat.

The plant is native to Sudan, Angola, Chad and other countries. Gum from injured stems is edible and relished by children,animals and birds. Camels and goats browse the leaves which are rich in protein. The wood is used for fuel. The bark decoction is used for stomach-ache,sterility,pneumonia,malaria and syphilis[23].

The stem bark extract of Acacia mellifera was examined for antimicrobial activity using the disc diffusion method.The methanolic and methanol:dichlormethane(1:1)showed significant activity against some bacterial strains.Activity-guided fractionation led to the isolation of some metabolites[24].

One new and eight previously described lupine –type metabolites were isolated and their structures elucidated.Some of these isolates exhibited significant levels of cytotoxic activity[25].

Fig. 1.2: Acacia mellifera

Acacia mellifera

1.5- Flavonoids

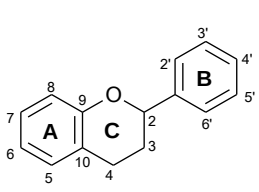
The term of flavonoids is drived from Greek word *flatus* (yellow). Flavonoids are phenolic substances isolated from a wide array of vascular plants, and more than 8150 different flavonoids have been reported[26].The flavonoids are large and important group of polyphenolic natural products which occurs in a variety of structural forms. Flavonoids, in general, are universally distributed in higher plants being located inside the cells or on the surface of various plant organs they also occur in fungi, but there are no records so far from the bacteria. Flavonoids occur primarily conjugated with either one or more sugar residues that are linked to hydroxyl groups, but association with other compounds including carboxylic and organic acids, amines and lipids also takes place. Flavonoids with sugar moieties are referred as the flavonoids glycosides whereas aglycones are flavonoids without a sugar moiety.

Flavonoids occur predominantly in plants as glycosides. Glycosylation increases their solubility in water facilitating their storage in vacuoles of flowers, leaves, stems and roots[27]. Flavonoids occur as O- or C-linked glycosidic conjugates. Although any hydroxyl group can be glycosylated, certain positions are favoured: e.g. the 7-hydroxyl group in flavones, flavonones and isoflavones, the 3- and 7- hydroxyls in flavonols and flavan-3-ols, and the 3- and 5-hydroxyls in anthocyanidins[28]. Flavonoids are also known as plant secondary metabolites which does not affect the normal growth, development or reproduction of organisms like the primary metabolites neither their absence result in immediate death[29]. But, these compounds are used for defense against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc) and hence they are called as the "First Line of Defense"[30]. Flavonoids have various functions in plants[31]. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors,feeding repellents, and light screening substances[32].Many studies have shown that flavonoids exhibit biological and pharmacological activities, including antioxidant, cytotoxic,anticancer,antiviral,antibacterial,cardioprotective,hepatoprotective,neuroprotective,antimalarial,ant-ileishmanial,anti trypanosomal and anti-amebial properties[32-37].These biological and pharmacological properties are usually attributed to their

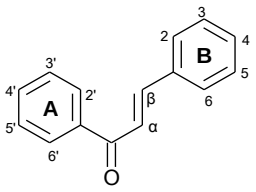
free radical scavenging efficacies, metal complexing capabilities and their ability to bind to proteins with a high degree of specificity[38]. It has been estimated 109 tons of flavonoids and related compounds are produced annually by plants, this is equivalent to about 2% of all photosynthesized carbon .Some are coloured(e.g.anthocyanins) and provide a wide range of red to blue colors in flowers, fruits and leaves .Others like the flavones ,essentially colorless and yet they provide the whiteness of white flowers.

1.6- Flavonoids structure

The basic flavonoid structure contains the flavan nucleus (**1**), which consists of 15 carbon atoms derived from a C_6-C_3-C_6 skeleton. A flavonoid skeleton is composed of two aromatic rings (commonly designated as A and B), which are linked by a three-carbon chain. The connecting carbon chain combines with an oxygen to form a heterocyclic central C-ring for most flavonoids with the exception of chalcones (**2**) in which the carbon chain between the A and B rings is linear[39]. The numbering scheme for chalcones differs from three-ring flavonoids in that the A ring, rather than the B ring carbons are labeled as prime.

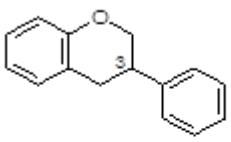


(1)



(2)

Depending on the position of the linkage of the aromatic B-ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans)(1), isoflavonoids (3-phynylbenzopyrans) (3), and the neoflavonoids(4-phynylbenzopyrans)(4). These groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related[31].



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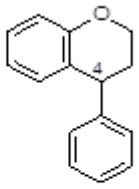


(4)

1.7- Properties of flavonoids

Most flavonoids are crystalline solids, and only a few are amorphous powders .The colors of flavonoids are dependent on the conjugated system and the number and position of substituents of auxochromes. For example, the hydroxyl and methoxy at 7- or 4'- position will deepen the color of

compounds due to their acceleration to the electron rearrangement. In general, flavones, flavanols, and their glycosides are grayish yellow or yellow solids, while flavanones and flavanonols have no color due to lack of conjugated system. But with the treatment of aqueous ammonia, they feature characteristic color or fluorescence and change color. The color of isoflavones is pale yellow due to short conjugated structure. The color of chalcone ranges from yellow to orange. There is a strong relationship between the color of anthocyanins and pH value. The color is red, purple, and blue at pH <7, pH = 8.5 and pH > 8.5, respectively. The solubility of flavonoids in solvents depend on their existing forms; aglycones of flavonoids are less soluble in water, but easily soluble in methanol, ethanol, dichloromethane, and other organic solvents. Flavonoids glycosides are easily dissolved in hot water, methanol, and other polar solvents, but they are solid in benzol,dichloromethane, and other organic solvents. The more sugars connected to the aglycone, the more soluble the glycoside is in water. Most flavonoids containing phenolic hydroxyl groups are soluble in alkaline aqueous solution (such as sodium carbonate solution) and alkaline organic solvents (such asdimethylformamide). Favonoids are usually weakly acidic due to the presence of phenolic hydroxyl groups, and can dissolve in basic solutions. Because of the presence of phenolic hydroxyl groups and gamma- pyrone, flavonoids compounds have the capacity to

produce various colors when reacting with some reagents, as listed in Table 3.1[40].

Table 1 .1. Properties of flavonoids with some reagents

Reagent	flavone	Flavonol	Flavanone	Isoflavone	Chalcone
HCl-Mg	Yellow red	Red mauve	Red. blue	—	—
HC1-Zn	Red	Mauve	Mauve	—	—
$NaBH_4$	—	—	Blue-mauve	—	—
$AICl_3$	Yellow	Olivine	Blue	Yellow	Yellow
$MgAcO_3$	Yellow	Yellow	Blue	Yellow	Yellow
NaOH aq.	Yello	Deep yellow	Yellow orange	Yellow	Orange red
H_2SO_4	Yellow orange	Yellow orange	Orange purple	Yellow	Orange

1.8-Human Uses of flavonoids

Flavonoids have applications in food industry as well as cut flower industry.Flavonoids are generally responsible for color, taste, prevention of fat oxidation and protection of vitamins and enzymes in foods[41].The flavonoids are considered as bioactive compounds containing the extra-nutritional constituents that are typically naturally occurring in small quantities in plant products and lipid rich foods.The bioactive compounds of plant origin include phenolic compounds (flavonoids, resveratrol and

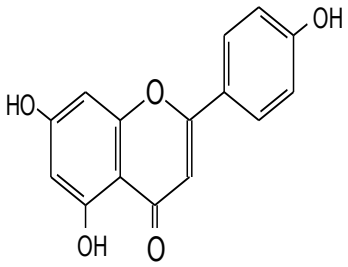
phytoestrogens) and are present in tea,fruits,vegatables; organosulfur compounds; plant sterols; dietary fibers; isothiocyanates; and monoterpenes.The bioactive compounds have beneficial health effects[42] ,and contribute to the antioxidant properties of green vegetables, fruits, olive and soybean oils, chocolate and tea. Some flavonoids are antiallergic, anti-inflammatory, antiviral, antidiabetic, antiproliferative, anticarcinogenic and have effects on mammalian metabolism. Flavanoids act as antioxidants in the prevention of cancer and cardiovascular diseases. They also protect from ulcers, allergies, vascular fragility and viral and bacterial infections[41]. Several epidemiological studies have reported an inverse association between flavonoid intake and the risk of coronary disease and cancer.

1.9-Classification of flavonoids

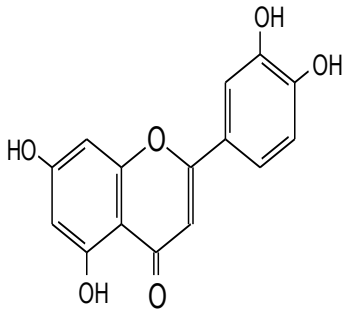
Flavonoids can be subdivided in the following subclasses according to the differences in functional groups and their relative positions on the 15-carbon skeleton: flavones, flavanones, flavonols, isoflavonoids, anthocyanidins, and chalcones[43]. The great variety of flavonoids within a class is the result of further modification such as hydroxylation, methylation, acylation, and glycosylation(Scheme 1.1)[44].

1.10-Flavones

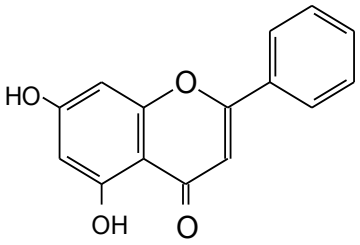
Flavones refer to flavonoids that share structural features, which include the B ring substituted at C2, a double bond at C2-C3, and carbonyl group at C,Flavones are one of the important subgroups of Flavonoids. Flavones are widely present in leaves, flowers and fruits as glucosides. Celery, parsley, red peppers, chamomile, mint and ginkgo biloba are among the major sources of Flavones. Flavones are structurally very similar to flavonols and differ only in the absence of hydroxylation at the 3-position on the C-ring. Examples of flavones are:apigenin, luteolin and chrisin[45].



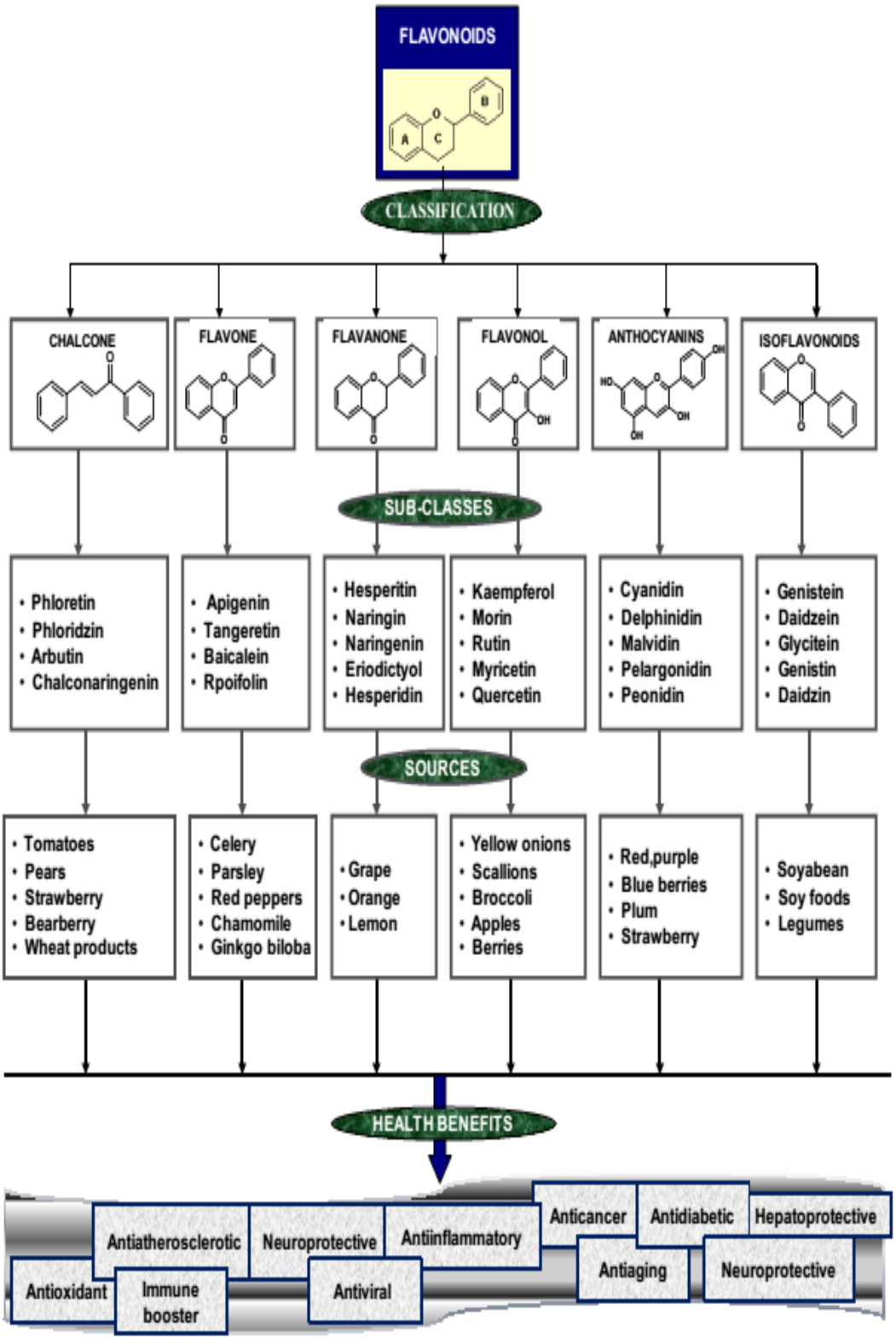
Apigenin



Luteolin



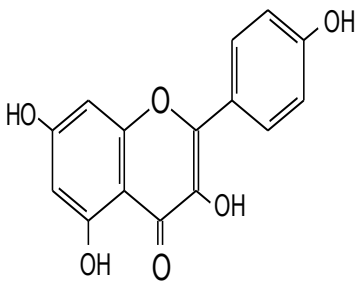
Chrysin



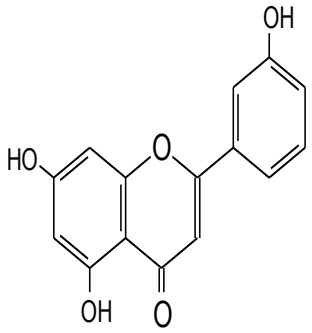
Scheme 1.1: Classification of flavonoids

1.11-Flavonols

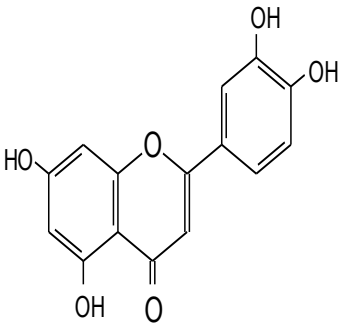
Flavonols refer to flavonoids that share structural features with flavones including the B ring substituted at C_2, a double bond at C_2-C_3, and a carbonyl function at C_4, but with an addition of a hydroxyl group substituted at C_3. Flavonols are flavonoids containing a keto function. They are building blocks of proanthocyanins. Flavonols occur widely in a variety of fruits and vegetables including: onions, kale, lettuce, tomato, apple, grape, and berries. Apart from fruits and vegetables, beverages such as tea are also important source of flavonols. Flavonols are widespread in the plant kingdom but the amounts detected in fruits and vegetables vary greatly due to seasonal changes and varietal differences. Intake of flavonols is found to be associated with wide range of health benefits which including: antioxidant potential and reduced risk of vascular disease.Flavonols are the most widespread among the flavonoids in plant food. They vary in color from white to yellow and are closely related in structure to the flavones. They are represented mainly by quercetin, kaempferol, and myricetin while the methylated derivative isorhamnetin is also quite common. Of the various flavonols found in the diet, quercetin is the most ubiquitous. It is present in various fruits and vegetables, with especially high concentrations, occurring in onions (*Allium cepa*)[46-48].



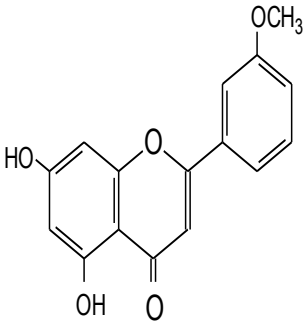
Kaempferol



Quercetin



Myricetin

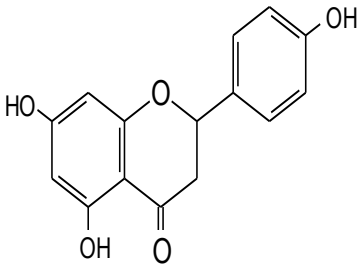


Isorhamnetin

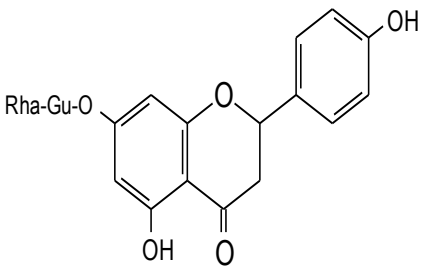
1.12-Flavanones

Flavanones refer to flavonoids that share structural features with flavones including the B ring substituted at C_5, and a carbonyl group at C_4, but without a double bond at C_2-C_3.Flavonones cnstitute another important class of flavonoids which is generally present in all the citrus fruits in their glycosidic forms. Hesperitin, Naringenin, are examples of this class of flavonoids. Flavonones are associated with a number of health benefits because of their free radical scavenging properties. The flavanone structure is highly reactive and has been reported to undergo hydroxylation, glycosylation, and O-methylation

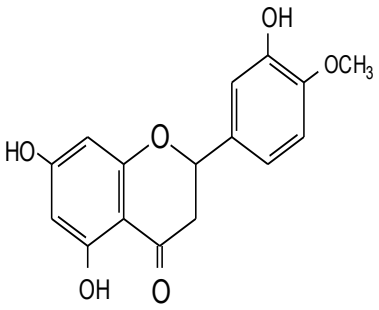
reaction. Grapefruit (*Citrus paradisi*) juice contains naringin (naringenin-7-O-neohesperidoside) and orange juice narirutin (naringenin-7-O-rutinoside)[49,50]. The peel is by far the richest part of citrus fruit in terms of its flavanone content. Substantial quantities of eriodictyol-7-O-rutinoside have been reported in lemon (*Citrus limon*)and lime(*Citrus aurantifolia*)[51].Flavanone rutinoside : hesperetin-7-O-neohesperidoside (neohesperidin) is found in bitter orange (*Citrus aurantium*) while naringenin-7-O-neohesperidoside (naringin) is present in grapefruit peel (*Citrus paradisi*).Both have an intensely bitter taste. Naringenin is also found in tomatoes and tomato-based products. Fresh tomatoes, especially the skin, also contain naringenin chalcone, which is converted to naringenin during the manufacture of tomato ketchup[52]. Hesperetin-7-O-rutinoside has also been detected in kiwi fruit, while hesperetin-7- O- neohesperidoside was reported in bananas (*Musa cavendishii*)[53,54] .The structures of some key flavonones are shown below:



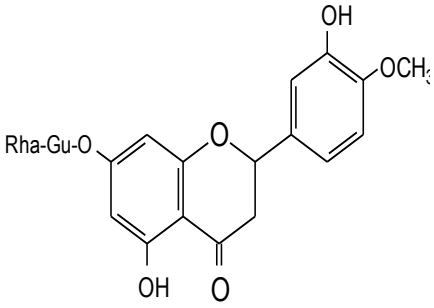
Naringenin



Naringin



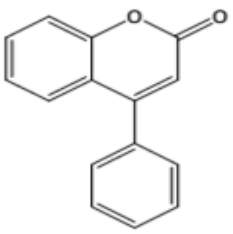
Hesperetin



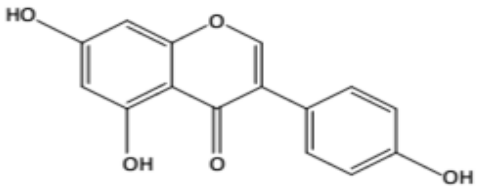
Hesperidin

1.13- Isoflavonoids

Isoflavonoids contain a benzo-γ-pyrone ring with phenyl substitution at position 3 (instead of position 2 in other flavonoids). There is also a group of chromane derivatives with B ring at position 4 (4-phenyl-coumarins = neoflavonoids),Isoflavones have been found in species of Leguminosae like *Trifolium pratense* L. (Red Clovers)[55] and in soybeans[56]. The isoflavonoids have several applications in the prevention of cardiovascular diseases. Due to the chemical structures of their aglycones similar to the 17-β-estradiol, they are often used to treat the menopause symptoms, post-menopause, osteoporosis, and other estrogen-related disorders[57]. Neoflavonoid and the isoflavone-genistein are shown below:



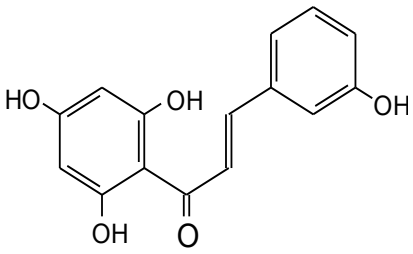
Neoflavonoid



Genistin

1.14-Chalcones

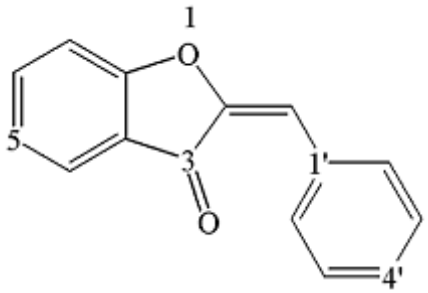
Chalcones: refer to flavonoids that share structural features of flavones including a double bond at C_2-C_3 and a carbonyl group at C_4, but the C ring is opening at position 1. Chalcones are a subclass of flavonoids. They are characterized by the absence of "ring C" of the basic flavonoid skeleton structure. Hence, they can also be referred to as open chain flavonoids.Major examples of chalcones include: phloridzin, arbutin, phloretin and chalconaringenin. Chalcones occur in significant amounts in tomatoes, pears, strawberries, bearberries and certain wheat products. The dihydro derivatives are called dihydrochalcones[58]. The structure of a hydroxylated chalcone is displayed below:



Hydroxylated chalcone

1.15-Aurones

Aurones refer to flavonoids that have the 2-benzylidene coumaranone skeleton; its C ring is a five-member ring. They possess the skeleton of 2-benzylidene-coumaranone or 2-benzylindene-3-(2H)-benzofuranone.They are formed by cyclization of chalcones,whereby the meta-hydroxyl groups reacts with the α-carbon to form a five member heterocycle[59] (furan type) e.g sulfuretin or 3',4',6-trihydroxyaurone .Aurones are also yellow pigments present in flowers.The basic structure of aurones is displayed below:

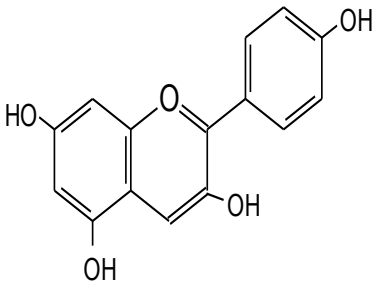


Aurone

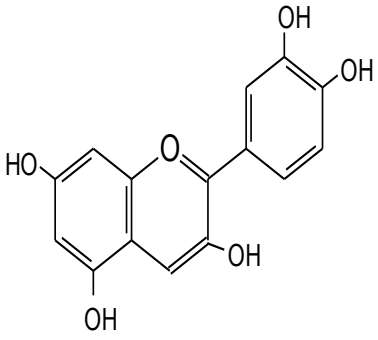
1.16-Anthocyanins

Anthocyanidins: refer to flavonoids that have 2-phenylbenzopyrylium salts structures. Anthocyanins are pigments responsible for colors of flowers and fruits. cyanidin, delphinidin, almvidin, pelargonidin and peonidin are the most commonly studied anthocyanins.They occur predominantly in the outer cell layers of various fruits such as cranberry, black currant, red grape, merlot, raspberry, strawberry, blueberry,

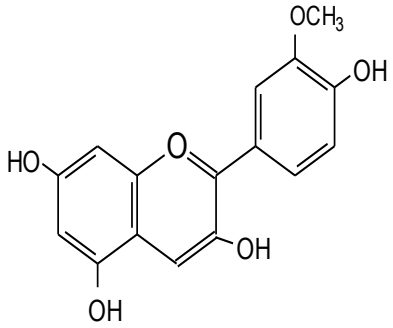
bilberry and blackberry. Stability coupled with health benefits of these compounds enable them to be used in the food industry in a variety of applications[60]. Anthocyanins display wide range of biological activities including anti-oxidant, anti-inflammatory, anti-microbial and anti- carcinogenic activities. In addition, they exhibit significant effects on blood vessels and blood platelets,and reduce the risk of coronary heart disease. There are about 17 anthocyanidins found in nature, but only six:cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin are ubiquitously distributed and of dietary importance.The variation of anthocyanins is due to the number and position of hydroxyl and methoxy groups on the basic anthocyanidin skeleton, the identity, number, and positions at which sugars are attached and the extent of sugar acylation and the identity of the acyl function[61].The structure of some key anthocyanins is displayed below:



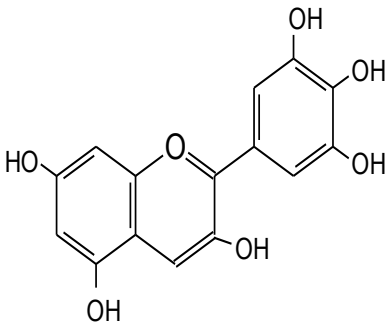
Pelargonidin



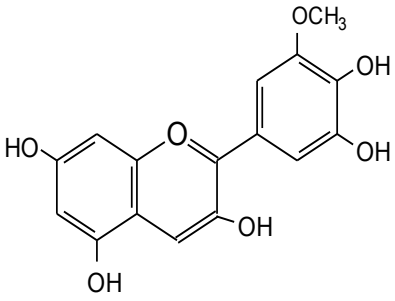
Cyanidin



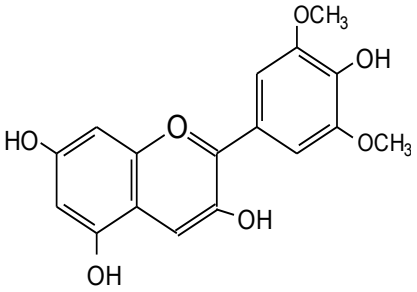
Peonidin



Delphinidin



Petunidin



Malvidin

1.17-Biosynthesis of Flavonoids

Biosynthesis of all flavonoid 15-carbon skeletons is achieved by two basic metabolites: malonyl-CoA and p-coumaroyl-CoA[61] .Basically,flavonoids are derivatives of 1,3- diphenylpropan -1-one (C_6–C_3–C_6).The crucial biosynthetic reaction is the condensation of three molecules of malonyl-CoA with one molecule of p-coumaroyl-CoA to a chalcone intermediate. Chalcones and dihydrochalcones are classes of flavonoids that consist of two phenolic groups which are connected by an open three carbon bridge. Derived from the chalcone structure, a flavonoid-class containing three rings, the flavanones, can be formed. Here, the three-carbon bridge is part of an additional

heterocyclic six-membered ring that involves one of the phenolic groups on the adjacent ring. Based on these flavanones all other flavonoid-classes are generated, including isoflavones, flavanols, anthocyanidines, flavonols and flavones.

1.18-Flavanone formation

Flavanone are formed from chalcone by intra- molecular cyclization (isomerization).Literature has revelaed the existence of equilibrium between flavavnones and corrsponding chalcones[62].The interaconversation between chalcone and flavanones is catalyzed *in vivo* by enzyme known as chalcone isomerase.The important feature of this enzymatic reaction is the sterospecificily apparent in the (S) chirality of C-2 in flavanone derivative .Therefore it is not accident that all the flavones found in nature have the (S) configuration at C-2 and are levorotatory.With chalcones having at least two free hydroxyl group at C-2 and C-6 ,the equilibrium in an aqueous solution is completely and rapidly shifted to the flavanone[63]. The stabilization of strong hydrogen bond between the carbonyl group and the *ortho* phenolic hydroxyl group greatly influence the position of equilibrium and the interconversion rate. When only one hydroxyl is available ,either for the cyclization or for hydrogen bonding the system tends to remain in the open form(chalcone form) .

1.19-Flavone Formation

In vitro conversion of flavanones to flavones was first observed in parslehy plants.The reaction has been further studied in parsley cell suspension cultures and in Antirrhinum flowers. Both the parsley and the Antirrhinum flower enzyme catalyzed the conversion of (2S)- narinenin[63](flavanone) to apigenin[64] (flavone). The mechanism of double bond formation is however,still unclear.It has been suggested that 2-hydroxyflavanone is formed in the first step , and water is then eliminated via a dehydratase[65-67].

1.20-Isoflavone formation

The aryl side chains of flavanone-chalcone intermediate undergo 2,3 migration in the key step in isoflavone biosynthesis.An enzyme activity catalyzing this transformation was recently found in microsomal from elicitor – challenged soyabean cell suspension cultures.It transforms (2S)- naringenin (flavanone) into genisten[68].It was found that two enzyme-catalyzed steps are involved in this transformation.The first step is the oxidation and reaarangment of naringenin to 2-hydrox 2,3 dihydrogenstein.This step is strictly dependant on the availability of NADPH and molecular oxygen .The second enzyme-catalyzed step involved the elimination of water from 2-hydroxyisoflavone in the soluble fraction

1.21-Flavonol formation

Biosynthesis of flavonols was found to be catalyzed by a soluble enzyme: 2-oxoglutarate-dependent oxygenase .Flavonol synthesis most probably proceeds via a 2-hydroxy intermediates such as 2-hydroxydihydrokamferol[69]with subsequent dehydration, giving rise to the respective flavonols .

1.22-Antioxidant potential of flavonoids

Flavonoids are well known for their antioxidant activities[70]. Antioxidants are compounds that protect the cells against the oxidative effect of reactive oxygen species, such as singlet oxygen, peroxyl radical, hydroxyl radical, superoxide radical, nitric oxide and peroxynitrite The impaired balance between these reactive oxygen species and antioxidants results in a condition commonly referred to as oxidative stress. This oxidative stress may lead to cellular damage which is linked to various diseases such as diabetes, cancer, cardiovascular disorders, neurodegenerative disorders and aging. Furthermore, oxidative stress can damage many biological molecules. Proteins and DNA are significant targets of cellular injury. Antioxidants interfere with free radical producing systems and increase the function of endogenous antioxidants, protecting the cells from damage by these free radicals. Intake of flavonoids via fruits, vegetables and whole grains helps to increase levels of anti-oxidants in the body[71].The strong antioxidant property of flavonoids is attested by a number of studies[72-77].The structural

consideration of flavonoids is very important for their antioxidant activity. The structural requirements include the presence of a hydroxyl group at the third position of carbon, a double bond between the second and third position of carbon atoms, a carbonyl group at the fourth position of carbon and polyhydroxylation of both aromatic rings A and B of the basic flavonoid structure[78].Flavonoids exhibit an inhibitory effect on excessive generation of free radicals. This prevents the damaging effect of reactive oxygen species that includes lipid peroxidation, and oxidation of sulfhydril and other susceptible group in proteins[79-81].In general, lipid peroxidation is found to be responsible for various diseases such as atherosclerosis, diabetes,hepatotoxicity and inflammation as well as aging. Quercetin, a flavonoid compound, is well known for its ability to act as an antioxidant. Quercetin seems to be one of the most powerful flavonoids used for protecting the body against reactive oxygen species. Studies have suggested that it helps suppress lipid peroxidation in model systems[82].In addition to quercetin, other flavonoids such as myricetin and rutin help inhibit the production of superoxide radicals[83,84].Tea polyphenols also belong to a subclass of flavonoid. A large number of studies have reported their antioxidant capabilities. One of the tea catechins, named epigallocatechin gallate (EGCG),is among the most potent antioxidants. Antioxidant capabilities of tea catechins explain their capabilities to protect

the cell components. A study by Nakagawa et al. suggested that drinking green tea helps in prevention of cardiovascular disorders by increasing the antioxidant capacity of plasma in humans[85]. Similarly, another study by Rene *et. al*.[86] showed that one or two cups of tea have the same 'radical scavenging capacity' as five portions of fruit or 400 mg vitamin C equivalents[87].

1.23-Antimicrobial and antiviral effect of flavonoids

Naturally occurring flavonoids have been recognized for their antimicrobial activity. This makes them significantly important in the field of medical microbiology. Many research groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity. This property of flavonoids enables them to be used extensively in the area of nutrition, food safety, and health. The antiviral effect of flavonoids was shown in a study carried out by Wang et.al.[88]. All the flavonoids, with some exceptions, are used in the therapy for viral disease and are effective against a number of viral infections. Naturally occurring flavonoids such as quercetin, naringin, hesperetin, and catechin possess a variable spectrum of antiviral activity. They affect the replication and infectivity of certain RNA and DNA viruses[89]. Quercetin and apigenin are among the most studied flavonoids which have been known to exhibit antibacterial activities[90]. The mechanism of action includes multiple targets. Li et al. has reported that the quercetin

extracted from lotus leaves may be a promising antibacterial agent for periodontitis[91].

Antibacterial activity has been displayed by a number of flavonoids. Quercetin has been reported to completely inhibit the growth of *Staphylococcus aureus*. Most of the flavonones having no sugar moiety showed antimicrobial activities[92]. A number of flavonoids isolated from the peelings of tangerine orange, when tested for fungistatic activity towards *Deuterophoma tracheiphila* were found to be active; while hesperidin could stimulate fungal growth slightly. Chlorflavonin was the first chlorine-containing flavonoid-type with antifungal properties[93] .Naturally occurring flavonoids with antiviral activity have been recognized since the 1940s but only recently have attempts been made to make synthetic modifications of natural compounds to improve antiviral activity. Quercetin,morin, rutin ,dihydroquercetin (taxifolin),apigenin, catechin, and hesperidine have been reported to possess antiviral activity against some of the 11 types of viruses[94].The antiviral activity appears to be associated with the nonglycosidic compounds, and hydroxylation at the 3-position is apparently a prerequisite for antiviral activity. It has been found that flavonols are more active than flavones against *Herpes simplex* virus type 1 and the order of importance was: galangin>kaempferol>quercetin[95]. Recently, a natural plant flavonoid polymer of molecular weight 2100 daltons was found

to have antiviral activity against two strains of type 1 *Herpes simplex* virus and type 2 *Herpes simplex* viruses[96]. There have appeared several recent reports on the anti-AIDS activity of flavonoids. Out of twenty eight flavonoids tested, the flavans were generally more effective than flavones and flavonones in the selective inhibition of HIV-1 and HIV-2 or similar immunodeficiency virus infections97.

1.24-Anticancer effect of Flavonoids

A large number of studies have highlighted the role of dietary flavonoids in reducing the risk of cancer[98-100]. The anticancer effect of these flavonoids may be attributed to their effect on several mechanisms that lead to cancer. Flavonoids are dietary antioxidants which may play a role as nutritional supplements during treatment of cancer or during inflammatory disorders. In recent years, flavonoids and their synthetic analogues have been intensely investigated in the treatment of ovarian, breast, cervical, pancreatic, and prostate cancer. For example, tangeritin, a citrus flavonoid is known to inhibit cancer cell proliferation[101]. Similarly, flavonoids such as 3-hydroxyflavone, 3',4'- dihydroxyflavone, 2',3'-dihydroxyflavone,fisetin, apigenin, and luteolin are potential inhibitors of tumor cell proliferation[102]. Furthermore, daidzein and genistein have been shown to inhibit both hormonal and non-hormonal types of cancer development[103]. All these flavonoids may help to develop new flavonoid-based herbal medicine to combat the risk of cancer.

1.25-Anti-diabetic activity of flavonoids

Flavonoids have been studied for their antidiabetic activity, although there are only a few studies in relation to this. It may be thought that flavonoids may help to repair beta cell function by reducing free radical induced tissue damage. It also reduces the hyperglycemic effects controlling the blood sugar levels. Studies have shown that intake of specific types of flavonoids, including quercetin and myricetin, is inversely associated with the risk of type 2 diabetes[104]. This is attested by another study which showed that quercetin may relieve diabetic symptoms[105].The study illustrated the mechanism for protective effects of quercetin on diabetes-induced hepatic injury. Quercetin is found to inhibit enzyme aldose reductase. It is the first enzyme of the sorbitol-aldose reductase pathway.It plays an active role in converting glucose (sugar) to sorbitol (a sugar alcohol) in the body. Hyperglycemia leads to increased generation of sorbitol in the body.This result in development of secondary problems, such as neuropathy, retinopathy, diabetic cataracts, and nephropathy[106].Quercetin may therefore, be beneficial in the nutritional management of diabetes and its complications. Hyperglycemia leads to the production of free radicals from mitochondria. These free radicals are known to be associated with diabetic micro- and macro-vascular complications and mitochondrial membrane damage. Studies carried out by Waisundara et.al. Suggested that baicalin,a

flavonoid, reduces hyperglycemia-induced mitochondrial membrane damage and also enhances the effects of metformin which is an antidiabetic drug. This was observed in the metformin and baicalin treated groups[107].

1.26-Anti-inflammatory activity

Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators.They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines. Selected phenolic compounds were shown to inhibit both the spatula DD cyclooxygenase and 5-lipoxygenase pathways[108].This inhibition reduces the release of arachidonic acid[109].Flavone/flavonol glycosides as well as flavonoid aglycons have been reported to exert significant anti-inflammatory activity in the animal model of both acute and chronic inflammation when given orally or topically[110,111].Hesperidin, a citrus flavonoid, possesses significant anti-inflammatory and analgesic effects[112].Recently apigenin, luteolin and quercetin have been reported to exhibit anti-inflammatory activity[113].

1.27-Extraction techniques

Flavonoids occur in virtually all the plant organs. Their for the method of isolation depend to some extent on the source material .Solvents for extraction choice according to the polarity of flavonoids .The less polar solvent are particularly used to extracted flavonoids aglycones, while more polar solvens are employed for the extraction of flavonoids glycoside especially anthocyanins. So less polar aglycones such as isoflavones,flavaonons,flavones or highly methylated flavonols are usually extracted with solvents like benzene chloroform,ether, or thylacetate .Flavonoids glycoside and more polar aglycones such as hydroxylated flavones,flavonols,and chalcones are generally isolated from plant material extraction with acetone ,alcohol,water or a combination of these.The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet),aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants,hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfl eurage (cold fat extraction) may be employed. Some of the latest extraction

methods for aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, microdistillation, thermomicro distillation and molecular distillation[114].

1.28-Phytochemical screening assay

Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analysis by the standard phytochemical screening assays[115].

1.29-Chromatographic techniques

A number of different techniques have been employed over the years for the isolation and separation of the flavonoids including paper chromatography(PC),thin layer chromatography (TLC) and high performance liquid chromatography HPLC[115].

1.29.1-Thin-layer Chromatography (TLC)

TLC is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. Additional tests involve the spraying of phytochemical screening reagents, which cause color changes according to the phytochemical existing in a plants extract; or by viewing the plate under the UV light. This has also been used for confirmation of purity and identity of isolated compounds[115].

1.29.2-Paper Chromatography (PC)

paper chromatography (PC) is the oldest of the chromatographic methods it is still used because of its easiness and low cost. Indeed two-dimensional paper chromatography (2D-PC) is still often used for the preliminary analysis of crude phenolic extracts or as a preparative tool. Information on the structures of flavonoids can be elucidated on the basis of their R_f values (mobility)or their response to UV exposure and various spray reagents[116].

One of the main advantages of PC is the great convenience of carrying out separations simply on sheets of filter paper, which serve both as the medium for separation and as the support. Another advantage is the considerable reproducibility of R_f values determined on paper, so that such measurements are valuable parameters for use in describing new plant compounds[117].

1.29.3-Column Chromatography

The major applications of column chromatography are in the purification of crude plant extracts or the preparative isolation of large quantities of compounds where it has the advantages of convenience and low cost. The column packing used include cellulose (microcrystalline), silica (0.06-0.3 mm partical size), polyamide, Sephadex LH-20, Amberlite XAD-7 and reversed phase Cs and CIS supports[117].

1.29.4-High performance liquid chromatography

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products[118]. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants[119].

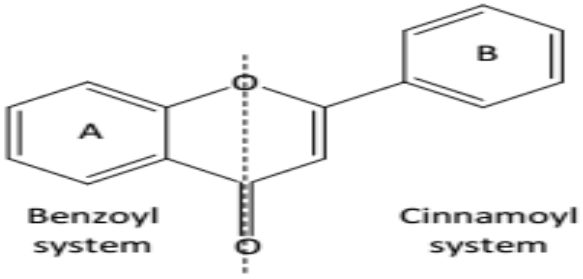
1.30-Identification of Flavonoids

Traditional methods for the identification of flavonoids made use of color reactions with reagents such as aqueous sodium hydroxide, concentrated sulphuric acid, magnesium-hydrochloric acid and sodium amalgam with acid. Color changes observed when the flavonoids undergoes reaction with these reagents gives information on the oxidation state of the C_3 bridging unit and hence the class of the flavonoids. Variations in the colour changes can also be used to identify substitution patterns of flavonoids to a limited extent. The information which can be gained through ultraviolet and visible absorption (UV-Vis) and nuclear magnetic resonance (NMR) spectroscopy is enough to determine the structure of most flavonoids[120-122].

1.31-Ultraviolet-Visible spectroscopy

The UV-Visible spectroscopy is a single most useful technique to analyze both flavonoids structure and to recognize the classes of flavonoids[123].The UV spectra of flavonoids shows in general two absorption bands (Band I and II).The band I is situated between 300-550 nm and it appears to be due to the electronic

transitions in the B ring system (Cinnamoyl system). Band II is observed in the region of 240-285 nm and corresponds to the A ring (Benzoyl system).



The UV absorption of flavonoids is depicted in Table (1.2).

Table 1.2: Absorption bands for flavonoids

Band I (nm)	Band II (nm)	Class of flavonoids
310-350	250-280	Flavones
330-360	250-280	Flavonols (3-OH substituted
350-385	250-280	Flavonols (3-OH free)
310-320 (sh)	245-275	Isoflavones
320 peak		Isoflavones (5-deoxy-6,7-dioxygenated)
300—330 (sh)	275-295	Flavanones and dihydroflavonols
340-390	230-270 (l i)	Chalcones
380-430	230-270 (l i)	Aurones
465-560	270-280	Anthocyanidin and anthocyanins

In addition, modifications in the substitution of the A-ring are generally reflected in the band II ,while alterations in B and C rings tend to be revealed in band I. Additional hydroxylation usually provokes bathochromic shifts (to longer wavelengths) of the appropriate band. In contrast, methylation or glycosylation produce hypsochromic band shifts. In flavones and flavonols, the presence of a 3', 4'-diOH system usually shows a second peak in band II, sometimes a shoulder.The oxygenation pattern and even the citing of the phenolic hydroxyl groups on the flavonoids nucleus may be identified by adding some shift reagents in the solutions of the pure flavonoids e.g NaOMe, NaOAc, NaOAc/HCl, H_3BO_3, $AlCl_3$, $AlCl_3$/HCl. Thus, indirectly, the resulting shifts in the spectrum are useful to determine the location of groups other than hydroxyls[124] .

1.32-Fourier-transform infrared spectroscopy (FTIR*)*

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups(chemical bonds) present in an unknown mixture of plants extract[**125**]. In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride.

The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet which can be analyzed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the solution then placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate.

1.33-Proton Nuclear Magnetic Resonance spectroscopy

The ^{1}HNMRspectrum appears predominantly in the range of 0-10ppm (Table2.2).Through the interpretation of the ^{1} HNMR spectra important information such as definition of the oxygenation pattern of all the rings, including number and position of the methoxyl groups, determination of the number and position of the sugars present and other groups, and also the distinction among isoflavones, flavanones and dihydroflavonols may be carried out[124].The proton chemical shifts for some flavonoids is depicted in Table(1.3).

Table 1.3: Proton chemical shift ranges of some classes of flavonoids

Chemical shift range (ppm)	Proton type
1.0	Rhamnose C-CH 3 (broad doublet)
2.0	-OCOCH $_3$ and aromatic C-CH $_3$
2.0-3.0	H-3 of flavanones (two proton multiplet)
3.5-4.1	Most sugars C-H and methoxyl groups
4.2-6.0	H-1 of sugars (also H-2 of

	dihydroflavonols at 5.0 ppm and H-2 of flavanones at 5.0-5.5 ppm)
-6.0	Methylenedioxy (O-CH 2-O), singlet
6.0-8.0	Protons of the A and B rings
7.5-8.0	H-2 of isoflavones (singlet)
9.5-12.5	5-OH (observed when DMSO-d6 is used)

The proton chemical shifts of the flavonoids have two signal regions, typical of the A and B benzene rings. Signals of the A-ring protons occur to upper field (in the range of 5.7- 7.0 ppm) and those of the B-ring occur in the range of 6.5-8.0 ppm . The most frequent substitutions in A-ring occur at C-5 and C-7. Therefore, protons at C-6 and C-8 show two doublets of J≈ 2.5 Hz, the H-6 appears at higher field than the H-8. The A-ring may also be trisubstituted, hence a singlet appears. To determine the positions of substituents on A-ring, the use of ^{13}C-NMR or bidimensional techniques is necessary .The most common oxygenation pattern of the B-ring occurs in 4` (B-ring type kaempferol), 3`,4` (quercetin) and 3`,4`,5` (myricetin). Due to the free rotation of the B ring and the similar chemical environment of the H-2`,H-6` and H-3`, H-5` in the case of B-ring type kaempferol two pairs of ortho coupled doublets with J≈ 8.5 Hz are observed. The exact position of the H-2`-6` doublet depends of the class of flavonoids (C-ring).

In addition, the H-3`,5` doublet always appears at higher field than H-2`,6` as a result of the shielding effect of the oxygen substituent and the deshielding influence of C-ring on H-2` and H-6`. The 3`,4` substitution patterns for flavones and flavonols exhibit a doublet of J≈ 8.5 Hz due to the H-5`. The H-2` also reveals a doublet of J≈ 2.5Hz at higher field than H-6` which appears as a doublet of doublet J≈ 2.5 Hz and J≈ 8.5Hz. The 3`-OH and 4`-$OCH_3$ show the same effect in the position of the signals whereas3`-OCH_3 and 4`-OH reveal the opposite consequence. The 3`,4`,5` trisubstituted flavonoids may show two doublets if the oxygenated groups linked to the C-3` and C-5`are not equivalent if not the signals appear as a doublet with J≈ 2.0 Hz .As it has been discussed above, the classification of the flavonoids is based on the C ring, hence the different signals provided for the protons of the C-ring are essential.In flavones the C-3 proton appears as a sharp singlet near the region of the A-ring protons (ca 6.3 ppm). This signal could be confused with a singlet of a proton of the trisubstituted A-ring. The C-2 proton in isoflavones also appears as a singlet usually to downfield from most aromatic protons and the chemical shift of this signal vary strongly in different solvents. In flavanones, the C-2 proton occurs as a quartet with centered at 5.2 ppm (J_{trans}≈ 11.0 Hz, J_{cis}≈ 5.0 Hz). The C-3 protons also appear to quartets near 2.8 ppm, attributable to the spin-spin interaction with each other (J= 17.0 Hz) and also with the H-2. The C-2 and C-3 protons of the

dihydroflavonols are in a *trans* situation and appear as two doublets well separated at about 4.9 ppm and 4.3 ppm respectively (J≈ 11.0 Hz). In chalcones, the H-α and H-β appear as doublets (J≈ 17.0 Hz) in the ranges of 6.7-7.4 and 7.3-7.7 ppm respectively. In aurones, the benzyl proton occurs as a singlet in the range of 6.5-6.7. The exact position depends on the kind of solvent used and also of the oxygenation pattern. Different groups may be linked to the nucleus of flavonoids e.g -OH, -OCH_3, -$OCOCH_3$,prenyl, sugars, among others. The most common substituents in flavonoids are –OH, -OCH_3 and sugars. In fact, in DMSO-d 6, generally the hydroxyl groups may be assigned. For example in 3,5,7 trihydroxyflavones the 3-OH appears at 9.70 ppm, 5-OH at 12.40and the 7-OH at 10.93 . The -OCH_3 groups occur in the range of 3.5 - 4.1 ppm. The anomeric proton of the sugars H-1`` allows to distinguish the position of the sugars.In flavonol 3-O-glucosides, the H-1`` signal appears at 5.7-6.0 ppm. In contrast, the H-1`` of the flavonoid 5, 7, 4'-O-glucosides occurs in the range of 4.8-5.2ppm as well as for signals of H-1`` in 6 and 8-C-glycosides. Furthermore, the 3-O-glucosides of flavonols are distinguishable from the 3-O-rhamnosides.

1.34-Carbon -13 Nuclear Magnetic Resonance spectroscopy

The ^{13}C-NMR is useful for detecting the total number of carbon atoms in a molecule, the number of oxygenated carbons on the flavonoids skeleton and the sugar moiety. Through the ^{13}C-

NMR spectrum, the identification of C- and O- linked sugars and even the site of the C-linkages can be established (e.g. in C-glycosides and biflavonoids)[124].The C-13 chemical shifts of flavonoids is depicted in Table (1.4).

Table 1.4:[13]C chemical shifts for flavonoids

Chemical shift range (ppm)	Carbon type
210-170	Carbonyl (4-keto, acyl) Aromatic and olefinic:
165-155 (no o/p oxygenation) 150-130 (with o/p oxygenation	a) oxygenated substituent
135-125 (no o/p oxygenation) 125-90 (with o/p oxygenation)	b) non-oxygenated substituent
	Aliphatic
83-69 (C-1 of O-glycosides c a 100 ppm)	a) oxygenated (sugars)
80-40	b) non-oxygenated (C-2, C-3 flavanones)
c a 100	Methylenedioxy
55-63	-OCH 3
c a 17-20	-C-CH 3, -COCH 3
21 (CH 2), 122 (CH), 131 (C), 18 (CH 3)	Isopropenyl (-CH 2CH=C(CH 3)2)

Chemical shifts measured in DMSO-d 6 or DMSO-d 6/CDCl 3 solutions.

Generally, UV, 1H-NMR, and mass spectra for isomeric polyhydroxyflavones are similar, thus the position of the substituent in the A-ring must be determined with care. The ^{13}C-NMR is the most suitable method for the structural elucidation of polyhydroxyflavonoids. The chemical shifts of several polyhydroxylated flavones and flavonols have been studied[126] .On the other hand, the ^{13}C-NMR may be used for distinguishing the class of flavonoids in some situations[127] as shown in Table (1.5).

Table 1.5: Carbon-13 chemical shift ranges of some classes of flavonoids

Type of flavonoid	C-2	C-3	C=O
Chalcones	136.9-145.4	116.-128.1	188.6-194.6
Flavanones	75.0-80.3	42.8-44.6	189.5-195.5
Flavones	160.5-165	103-111.8	176.3-184
Flavonols	145-150	136-139	172-177
Isoflavones	149.8-155.4	122.3-125.9	174.5-181
Aurones	146.1-147.7	111.6-111.9 (=CH-)	182.5-182.7

1.35-Mass spectrometry

The mass spectrometry is a useful technique to obtain structural elucidation concerning the molecular weights, the distribution of the substituent between the A and B-rings and also the nature and site of linkage of the sugars in flavonoids glycosides especially with a very small quantity (i.e. less than 1mg).

Most flavonoids aglycones produce intense molecular ion (M^+) and generally it is the base peak. With the molecular ion, flavonoids aglycones generally give major peaks $[MH]^+$.The fission of the M^+ may produce two common different fragmentation pathways. For structural identification of the flavonoids, the most useful fragmentations are those which involve the retro-Diels-Alder A and B-ring fragmentations.

Problem statement

Literature survey revealed that there was no study concerning the flavonoids of the Sudanese material of *Albiza Amara* and *Acacia mellifera* providing solid scientific evidence for the use of these species in Sudanese traditional medicine.

Significance of the study

A.Amara species belong to the family Fabaceae that provide a host of bioactive compounds (Alkoloides, Steroides, phenols and Flavonoids) of important biological activities, like prevention and treatment of heart and vascular disease and some types of cancer. Researchers reported that the leaves and flowers of *A. amara* possess strong antioxidant and free radical scavenging properties. Based on the above knowledge, the present study was designed to investigate the antioxidant properties and antimicrobial activities of *A. amara* roots flavonoids.The same is true for the medicinally important species- Acacia mellifera

1.6. Objectives of this study

Objectives of this research include:

-Extract of flavonoids from roots of *Albiza amara* and barks of *Acacia mellifera*.

-Evaluate of the anti-oxidant and antimicrobial potential of *A. Amara* extracts .

-Phytochemical screening for different secondary metabolites.

-Isolation of flavonoids from the targeted species.

-Elucidation of structures of the targeted molecules.

Scope of this study:

There are some important stages to be carried out to accomplish all of the objectives in this research. Important scopes are:

-The extraction of phenolics from targeted species is accomplished by soxhlet apparatus.

-The fractionation loof the ethanolic extract is feasible by solvent-solvent extraction using: n-hexane, chloroform, ethyl acetate and n-butanol.

-Antioxidant and antibacterial activity may be evaluated via disc diffusion method and DPPH scavenging respectively.

-For phytochemical screening for flavonoids. Alkaloids, terpenoids and phenolics standard methods may be employed.

-For the isolation of the flavonoid from plant species a combination of chromatographic techniques (TLC, CC and PC) may be used.

-For elucidation of structures of the targeted molecules sensitive spectral tools including UV, FTIR, MS and NMR spectroscopy may be employed.