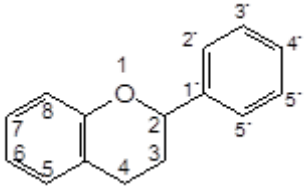
Introduction

1.1-General overview

Flavonoids are a large group of phenolic plant constituents. To date, more than 8000 flavonoids have been identified[1] , although a much smaller number is important from a dietary point of view. That flavonoids possess bioactive potential has been recognized for long, but until recently, data about their bioavailability, metabolic fate, and health effects were limited. In the 1990s, interest in these compounds truly commenced and has been growing ever since. Flavonoids are potent antioxidants in vitro, and therefore one of the main interests in thecompounds has involved protection against cardiovascular disease. Antioxidation is, however,only one of the many mechanisms through which flavonoids could exert their actions. Flavonoids are divided to several subgroups, and it is important to keep in mind that thebiological and chemical properties of flavonoids belonging to different subgroups can be quite different.

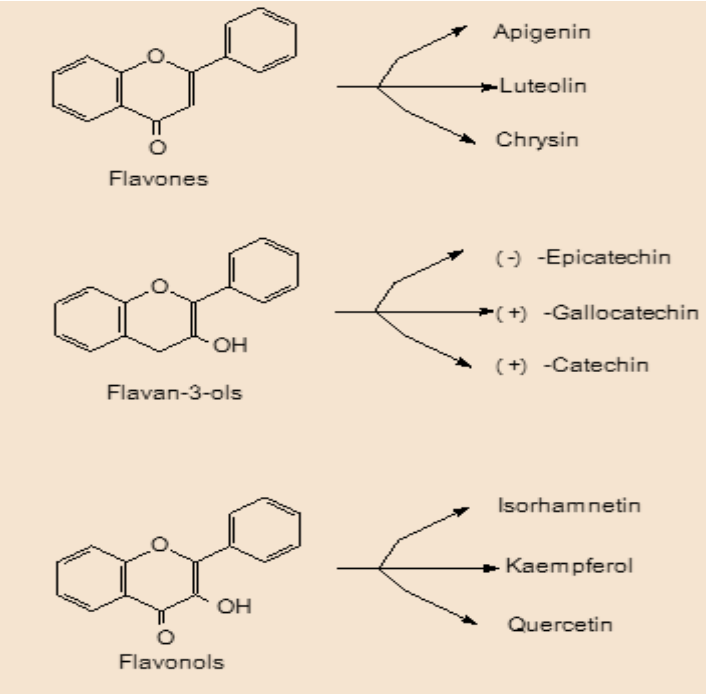
Flavonoids consist of 2 benzene rings (A and B), which are connected by an oxygen-containing pyrene ring (C) as shown below:



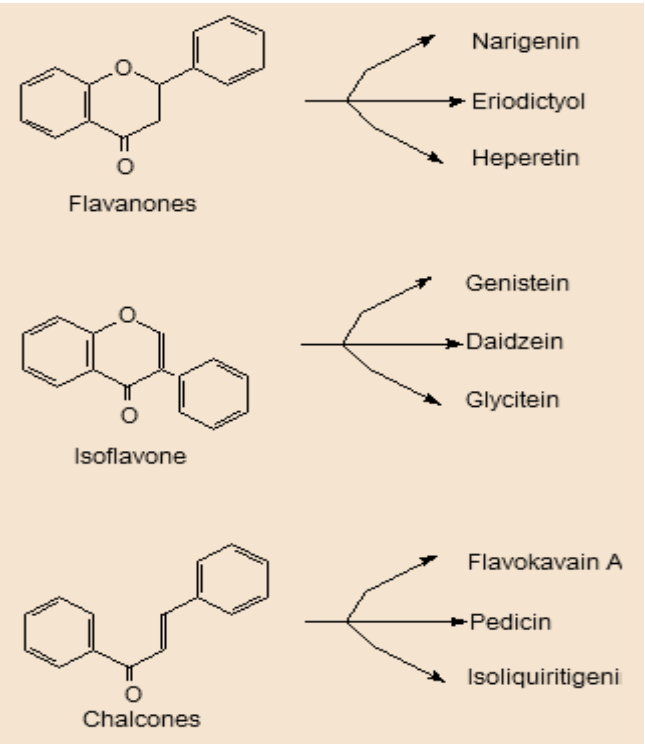
Flavonoids containing a hydroxyl group in position C-3of the C ring are classified as flavonols . Beside this class flavonoids are generally classified into : flavones, chlacones, aurones, flavanones, isoflavones, dihydroflavonols, dihydrochalcones, catechins(flavans) and anthocyanins. The general structures of such classes are outlined in scheme I. Further distinction within these families is based on whether and how additional substituents (hydroxyls or methyls, methoxyls …etc) have been introduced to the different positions of the molecule.In isoflavonoids, the B ring is bound to C-3 of ring C (instead of C-2 as in flavones and flavonols). Anthocyanins and catechins, on the other hand, lack a carbonyl function on C-4[2]. Flavonoids are mainly present in plants as glycosides. Aglycones (the forms lacking sugarmoieties) occur less frequently. At least 8 different monosaccharides or combinations of these (di- or trisaccharides) can bind to the different hydroxyl groups of the flavonoid aglycone[3] .

The large number of flavonoids is a result of the many different combinations of flavonoid aglycones and these sugars. The most common sugar moieties include d-glucose and l-rhamnose. The glycosides are usually O-glycosides, with the sugar moiety bound to the hydroxyl group at the C-3 or C-7 position.

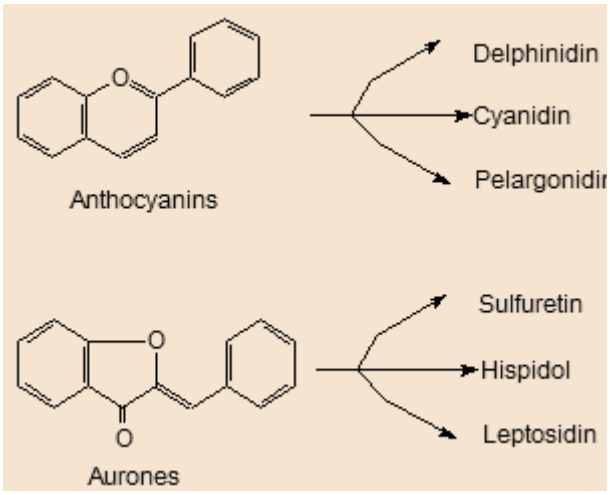
Examples of the major classes of flavonoids are presented below:



Examples of flavones , flavan-3-ols and flavonols



Examples of flavanones, isoflavones and chalcones



Examples of anthocyanins and aurones

Natural phytochemicals available have a wide range of therapeutic efficacy as they exhibit numerous biological activities in the treatment of chronic diseases. These phytochemicals attract the attention of public as they are free/minimal side effects on consumption for long term treatment of diseases compared to any other modern medication[4] . Flavonoids are an important class of plant based compounds found in many fruits, vegetables, legumes, grains, nuts and have a wide range of actions and medicinal uses too. They are hydroxylated phenolic substances and the hydroxyl group mediate their antioxidant effects by scavenging free radicals by chelating metal ions[5]. They form the biggest group of poly-phenols in plants with health beneficiary like preventing degenerative diseases like cancer, cardiovascular, metabolic and neurodegenerative diseases. As “Redox Regulators”, they are even more powerful than the common antioxidants like

Vitamins E and C in preventing free radical mediated cellular damage. They are found to interfere with the digestion, absorption, and metabolism of carbohydrates and also evident to have anti-inflammatory, antiallergic, antiviral and anticancer properties[6] .

1.2-Occurrence of flavonoids in diet

Flavonoids are present in most edible fruits and vegetables, but the type of flavonoids obtained from different dietary sources varies. The main dietary flavonoids and their sources are shown[4,6] in Table 1. Intake estimates for flavonoids on a population level are only available for a few flavonoid subclasses, such as flavonols, flavanones, and isoflavones.

Table 1 : Dietary source of flavonoids

Type	Source
Flavonol	
Quercetin-3,4V-glucoside	Onion
Quercetin-3-glucoside	
Quercetin-3-rhamnoglucoside (rutin)	Black tea
Quercetin-3-galactoside	Apple
Quercetin-3-rhamnoside	
Quercetin-3-arabinoside	
Quercetin-3-glucoside	
Quercetin-3-rhamnoglucoside	Black currant
Quercetin-3-rhamnoside	
Quercetin-3-galactoside	
Myrisetin-3-glucoside	
Flavone	
Luteolin-7-apiosylglucoside	Red pepper
Flavanone	
Hesperetin-7-rhamnoglucoside	Orange juice
Naringenin-7-rhamnoglucoside	
Naringenin-7-rhamnoglucoside	Grapefruit juice
Naringenin-7-rhamnoglucoside	
Flavonols	
(+)-Catechin	Apple
(_)-Epicatechin	
(+)-Catechin	Red wine
(_)-Epicatechin	
9-42 [14]	
(Epi)catechin and their gallates	Black tea
Anthocyanins	
Cyanidin-3-glucoside	Black currant
Cyanidin-3-rutinoside	
Delphinidin-3-glucoside	
Delphinidin-3-rutinoside	
Isoflavones	
Genistein-7-glycoside	Soybeans
Daidzein-7-glycoside	

1.3- Biological potential of flavonoids

In vitro studies indicate a wide range of biologic activities for different flavonoids. These studies have mainly been performed with flavonoid aglycones or glycosides. Until very recently, flavonoid metabolites were rarely used, mainly because data about their identity were scarce; moreover, chemical standards for only a few potential metabolites are commercially available. A few general reviews on flavonoids[7-9] have been published during the past few years. These reviews mainly concern quercetin, which is the most studied flavonoid. Because of the large number of studies on quercetin (over 3000 citations listed in PubMed), only representative examples of its bioactivities can be given here. Quercetin has been reported to exhibit antioxidative[10,11], anticarcinogenic[12-14], anti-inflammatory[15], anti-aggregatory[16], and vasodilating[17] effects. The mechanisms behind the effects are largely unknown, but it is possible that several different types of biochemical events precede them. Antioxidation, for instance, could be a result of metal chelation[18,19], scavenging of radicals[21,22], enzyme inhibition[22,23] , and/or induction of the expression of protective enzymes[24]. Anticarcinogenesis, on the other hand, could result from enzyme inhibition[25,26], antioxidation, or effects on gene expression[27-29]. Altered gene expression could lie behind the anti-inflammatory effect as well[30]. Regarding anticarcinogenesis, it should be noted that in the 1970s, quercetin was actually considered a

carcinogen because the compound showed mutagenicity in the Ames test[31].However, a number of long-term animal studies subsequently performed with different species have indicated that this is not the case. On the contrary, quercetin has been shown to inhibit carcinogenesis in laboratory animals[32]. During the past few years, some reports on the biological activities of potential quercetin metabolites have been published. In these studies, it has been shown that the site and/or type of conjugation are important determinants of its antioxidant activity[33-35]. The flavanones have gained less interest than quercetin and have been covered less extensively in previous reviews. A lot of attention has been paid to their anticarcinogenic properties. Hesperetin (and orange juice) has been shown to inhibit chemically induced mammary[36], urinary bladder[37], and colon[38,39] carcinogenesis in laboratory animals. Hesperetin[40], as well as the other major citrus flavanone naringenin[41,42], also possess some antioxidant activities, although this activity is poorer compared with many other polyphenols. Other possible effects of hesperetin and naringenin are on lipid metabolism. They have been reported to regulate apolipoprotein B secretion by HepG2 cells, possibly through inhibition of cholesterol ester synthesis[43], and to inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase and acyl coenzyme A:cholesterol O-acyltransferase in rats[44,45]. Furthermore, a decrease in plasma low-density lipoprotein levels

and hepatic cholesterol levels in rabbits fed a high-cholesterol diet has been observed[46]. An increase of high-density lipoprotein levels in hypercholesterolemic human subjects after consumption of orange juice was also reported[47]. According to a literature search in PubMed, these results had not been confirmed or refuted in other human studies. Other biological activities attributed to naringenin include antiinflammatory[48] actions and different types of effects on sex hormone metabolism[49-52]. The compound has, for instance, been shown to bind to estrogen receptors[53].

Most publications on naringenin concern its possible role in grapefruit juice–drug interactions[54,55]. The considerable increase in plasma concentrations of many drugs metabolized by intestinal cytochrome P-450 IIIA (CYP3A4) when administered concominantly with grapefruit juice is well documented and is of clinical relevance[56,57]. Naringenin is an inhibitor of the enzyme[58] and could be one of the compounds causing the interaction. However, other grapefruit constituents seem to be more important in this phenomenon. In any case, the grapefruit juice–drug interaction is a very interesting indication of how dietary components may influence our health (ie, by modulation of the biotransformation system).

1.3.1-Flavonoid intake and risk of chronic diseases

The association between flavonoid intake and the risk of cardiovascular disease and cancer has been investigated in

several epidemiological studies. In most studies, the term flavonoid refers to flavonols and flavones, with quercetin being quantitatively the most important flavonoid.

Most, but not all, prospective cohort studies have indicated some degree of inverse association (from borderline to modest) between flavonoid intake and coronary heart disease. An inverse association was found in the Zutphen Elderly Study[59], the Finnish Mobile Clinic Study[60,61], the Iowa Women's Health Study[62], the Alpha-Tocopherol, Beta- Carotene Cancer Prevention (ATBC) Study[63], and the Rotterdam Study[64]. No association was found between flavonoid intake and risk of coronary heart disease in subjects free of disease at baseline in the Health Professionals Follow-up Study[65] and in the Women's Health Study[66]. Interestingly, in the Caerphilly Study[67], flavonol intake in 1900 men was directly associated with the risk of ischemic heart disease and all-cause mortality. The result may be explained by tea being a very important source of flavonoids in Scotland; its consumption was associated with lower social class, smoking, and higher fat intake[67] . In the above-mentioned ATBC Study, no association was found between flavonoid intake and risk of stroke[68]. The epidemiological evidence regarding the cancer-protecting effects of flavonoids is conflicting. Some of the case-control studies have indicated an inverse association between intake of flavonoids and cancer risk (lung cancer[69,70], upper digestive tract cancer[71], and gastric

cancer[72]. Other case-control studies found no associations (lung cancer[73] and bladder cancer[74]. In 2 cohort studies, no association between intake of flavonoids and risk of several cancer types was present[75], but in 2 other cohort studies, an inverse association was shown for lung cancer[76,77]. Knekt et al[61] studied the association between the intake of flavonoids and the risk of several chronic diseases in 10054 participants of the Finnish Mobile Clinic Health Examination Survey. Higher quercetin intakes were associated with lower risk of asthma and lung cancer, and there was a trend toward a decrease in the risk of type 2 diabetes. Persons with higher quercetin intakes had lower mortality from ischemic heart disease. The incidence of cerebrovascular disease and asthma was lower with higher intakes of hesperetin and naringenin. Overall, the epidemiological evidence concerning flavonoids and chronic diseases is difficult to interpret. Often, the reported associations have not been very strong. Furthermore, confounding factors (a common problem of epidemiological studies) probably affect the outcome of the studies. In many countries, drinking tea and consuming high quantities of vegetables and fruit are merely indicators of a generally healthy lifestyle or a high level of education. This is not the case for onion, which is obtained from so-called unhealthy food as well, but still, this source of flavonoids may be problematic in epidemiological studies. Onion is qualitatively, and in many countries, quantitatively, the

most important source of quercetin[6578,79]. The accurate assessment of an individual's onion consumption is quite difficult with dietary survey methods because it is a commonly used hidden ingredient of many homemade and processed food, such as soups, salads, sausages, hamburger meat, and others. Thus, it is likely that intake estimates for onions contain a large margin of error.

Assessment of tea intake, on the other hand, another important source of quercetin in many countries, is probably accurate. However, the bioavailability of quercetin from tea is poorer than from onion. Considering the possible impact on the results of epidemiological studies, it is rather surprising that these problems have not been discussed in the reports. In summary, the epidemiological evidence concerning the association between flavonoid intake and the risk of chronic diseases is conflicting. Whether flavonoids protect against chronic diseases may be difficult to show using traditional epidemiological methods. An alternative, which may help to overcome some of the problems associated with intake assessment, is to use serum concentrations as biomarkers of intake. This approach is not faultless, but the sources of error are different.

1.3.2-Flavonoids as antiinflammatory agents

Inflammation[80] is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the

injurious stimuli as well as initiate the healing process for the tissue. It is regulated to prevent over activation of the immune system and unwanted immune response. Many types of cells involved with the immune - including T cells, B cells, NK cells, mast cells, and neutrophils - have been shown to alter their behavior in the presence of flavonoids.

The activities of a number of regulatory enzymes (e.g., protein tyrosine kinases, protein kinase C, phosphodiesterase, phospholipase A2, lipoxygenases, and cyclooxygenase) are essential to inflammation and the immune response. These enzymes are central to the activation of endothelial cells and numerous other specialized cells involved in inflammation, and it is significant that in many cases the anti-inflammatory actions of flavonoids can be associated with their inhibition of these enzymes. Cyclooxygenase (COX) is an enzyme that plays an important role as inflammatory mediator and is involved in the release of arachidonic acid, which is a precursor for biosynthesis of eicosanoids like prostaglandins and prostacyclin. The release of arachidonic acid can be considered starting point for a general inflammatory response. Prostaglandins and nitric oxide biosynthesis is involved in inflammation, and isoforms of inducible nitric oxide synthase (iNOS) and of cyclooxygenase (COX-2) are responsible for the production of a great amount of these mediators. Phosphodiesterases and phosphorylating kinases[81] also play an important role in inflammation.

Phospodiesterases are involved in cell activation through their influence on intracellular 3', 5'-cyclic monophosphate (cAMP) levels, and play important roles in controlling platelet function and the expression of pro-inflammatory cytokines. Cell activation during inflammation additionally involves a variety of kinases (e.g., protein tyrosine kinase, protein kinase C, and phosphatidylinositol kinase) responsible for signal transduction via protein and lipid phosphorylation.

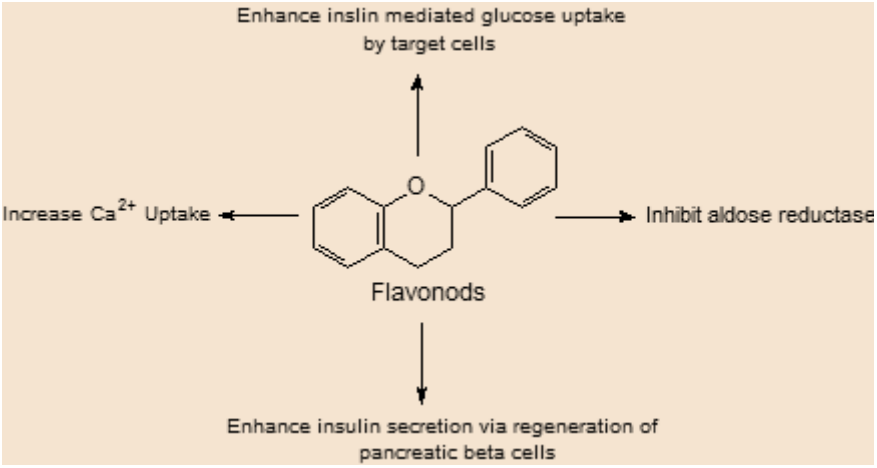
1.3.3-Inhibition of histamine release

Studies have shown flavonoids attenuation of histamine release during the late phases of the allergic reactions. The release of histamine during this stage of the allergic reaction is tightly regulated by leukotrienes generated by the lipoxygenase catalyzed reactions. The inhibition of this process occurs with a number of hydroxylated flavones, aglycones, whereas much lower inhibition occurs with methoxylated flavones[82] . In these studies, the relative inhibition of basophil histamine release by flavonoids correlated with the structure-activity requirements previously noted for lipoxygenase.

1.3.4-Antidiabetic effect

Mechanism of Anti hyperglycaemic activity of flavonoids Flavonoids of all sub-class have proven to have antidiabetic properties by (1) enhancing insulin secretion via regeneration of pancreatic β-cells, (2) enhancing insulin mediated glucose

uptake by target cells, (3) inhibiting of aldose reductase (4) increasing Ca2+ uptake. Flavonoids with potent antioxidant activity were shown to be effective in management of diabetes[83] . Antidiabetic activity of flavonoids depends on the chemical criterion (C-2-C-3 double bond and ketonic group at C-4 position on ring B) which is fundamental for the bioactivity of poly-phenol compounds[84]. These phytomedicines play significant role in maintaining blood glucose levels, glucose uptake, insulin secretory and immuno modulatory functions to prevent specific DM[85].The hypoglycemic effect of flavonoids is summarized schematically below:

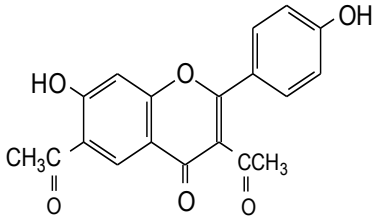


Hypoglycemic activity of flavonoids

1.4-Flavonoids isolated from Sudanese plants

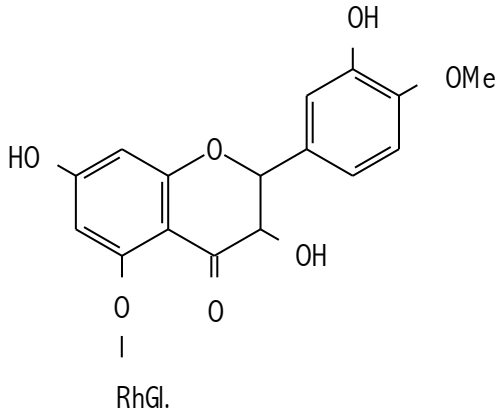
The roots of the medicinally important species *Balanites aegyptiaca* was investigated[86] for secondary constituents.The ethanolic extract was partitioned successively by hexane, chloroform, ethyl acetate and n-butanol. The ethyl acetate

fraction was fractionated by paper chromatography and an acylated flavone was isolated. The structure of the isolate was partly elucidated on basis of its spectral data (IR, UV, NMR and MS).The isolated flavonoid was assigned structure (1):



(1)

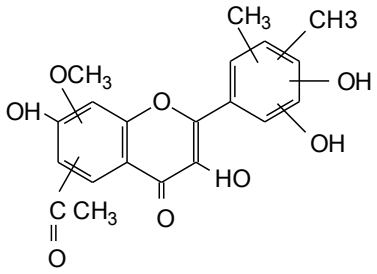
A Flavonol : 7, 3` -dihydroxy – 4` - methoxy - 5-rhamnoglucosylflavonol(2) was isolated[87] and characterized for the first time from the Sudanese material of *Guiera senegalensis* . Its structure was deduced on the basis of its IR , UV, NMR and mass spectra .



(2)

The flavonoids of the medicinally important *Terminila browni* have been studied[88]. From the roots of *Terminila browni* two flavonoids (compounds 3 and 4) were isolated in a

chromatographically pure form via a combination of chromatographic techniques. The structures of these flavonoids have been elucidated using spectral tools(UV and ^{1}HNMR).



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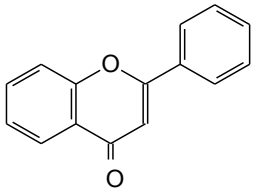
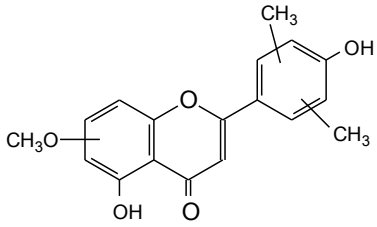


(4)

Compounds (5) and (6) have been isolated[89] from the seeds of *Hagenia abssynica* by paper chromatography.



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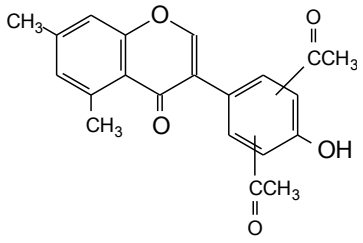
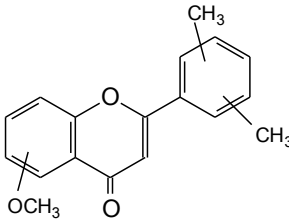


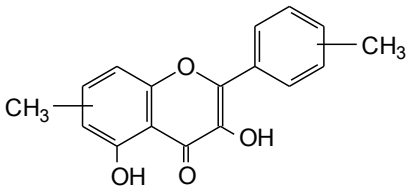
(6)

A Flavonoid (7) has been isolated from the roots of *Cisuss petiolata*. On the basis of some spectral data the following structure was proposed :



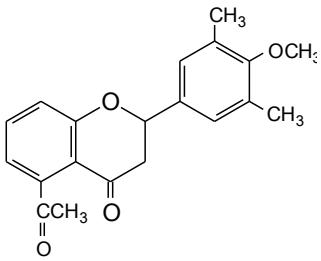
(7)

The barks of *Acacia mellifera* were macerated with 95% ethanol at room temperature for 48hr. Removal of the solvent under reduced pressure gave[90] a crude product. Paper chromatography of the crude extract gave a pure flavonoid – compound 8.



(8)

Compounds (9) and (10) have been isolated[91] from *Catharanthus roseus* leaves by silica gel TLC using BAW(4:1:4) as solvent.



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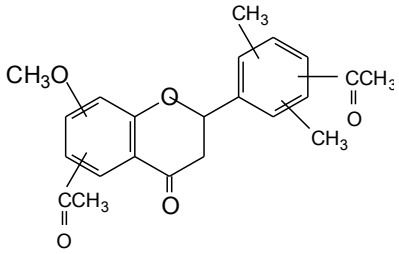
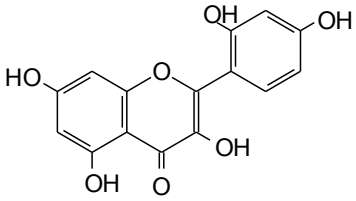


(10)

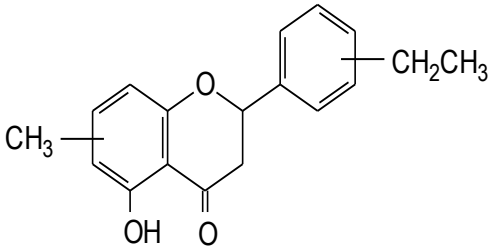
From the ethyl acetate extract of the leaves *Cassia occidentalis* (Leguminoseae) a flavonol: 5,7,2',4'-tetrahydroxyflavonol(I1) has been isolated[92]. The structure was elucidated via a combination of spectral techniques(UV,IR,1H NMR and MS). The flavonol was evaluated *in vivo* for anti-inflammatory and anti-ulcer activity.Treatment of the animal models with compound (11) inhibited formaldehyde-induced oedema up to 93.99%.The flavonol also showed significant anti-ulcer activity.



(11)

The leaves of *Cajanus cajan* were extracted with 95% ethanol at room temperature for 72hr. Removal of the solvent *in vacuo* gave a crude product. A Silica gel column followed by a Sephadex column gave[93] two chromatographically pure flavonoids – compounds (12) and (13). The structures of these

flavonoids have been elucidated via a combination of spectral techniques(UV and NMR).



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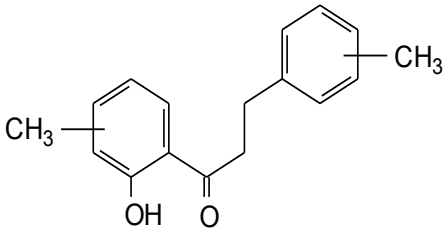
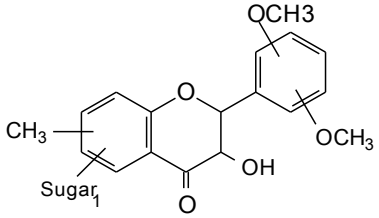


(13)

Phytochemical screening of the roots of *Acacia nilotica* revealed the presence of flavonoids, tannins, saponins, alkaloids and glycosides . A silica gel column followed by thin layer chromatography allowed isolation[94] of three compounds –(14) , (15) and (16) .The structures of the isolates were deduced on the basis of their spectral data(UV,NMR and MS).



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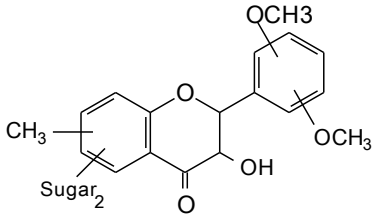

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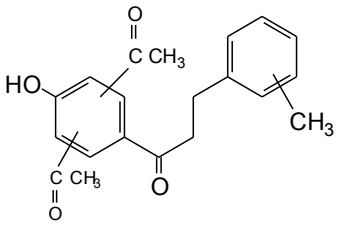
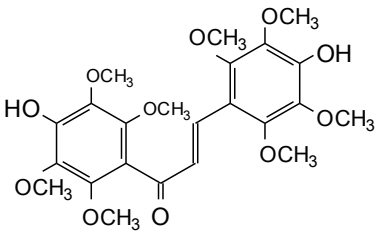


(16)

The isolated compounds were evaluated ,*in vitro*, for their potential antimicrobial activity against five standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative : *Escherichia coli* and *Pseud*om*onasa aeruginos*a and the fungus *Candida albicans*) using the cup plate agar diffusion bioassay . Different antimicrobial responses were observed. The activity ranged from high to moderate .

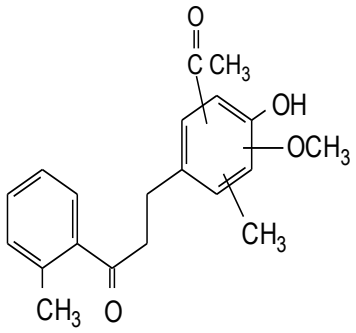
Powdered air- dried Twigs of *Eucalyptus Camaldulensis* Dehnh were extracted[95] with 95%. The crude product was fractionated by thin layer chromatography . A pure flavonoid – compound (17) has been isolated and its structure was elucidated.



(17)

In cup plate agar diffusion bioassay, compound (17) showed good activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It also exhibited excellent antifungal activity against the yeast *Candida albicans*. However, it was inactive against *Bacillus subtilis* and *Escherichia coli*.

The flavonoids of the medicinally important *Detarium senegalense* has been stdied. Three compounds (18), (19) and (20) haveeen b isolated[96] roots in chromatographically pure form**.**



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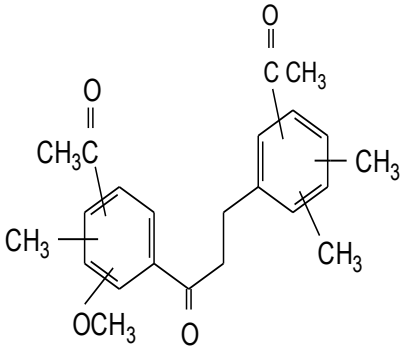

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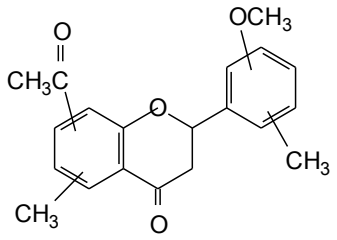
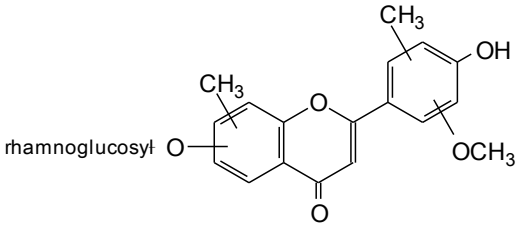


(20)

A Study was designed to investigate the major flavonoid of *Lepidium sativum*. The flavonoids were extracted with ethanol and the crude extract was purified by thin layer chromatography where a pure flavones(21) has been isolated[97]. The structure of this flavone has been partially characterized by some spectral tools(UV and ^{1}HNMR).

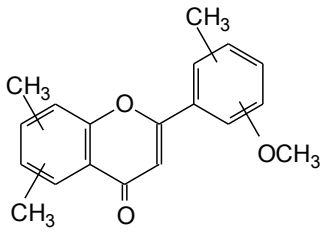


(21)

Ammi visnaga is widely used in Sudanese herbal medicine where it is used against a wide range of diseases including asthma , urinary calculi and mild anginal symptoms.The plant is diuretic and is claimed to relieve menstruation pain..

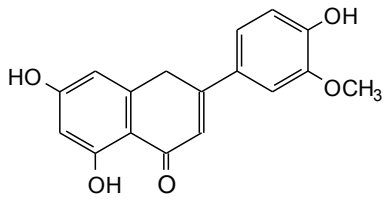
A Study was undertaken to investigate the major flavonoid of *Ammi visnaga* . The flavonoids were extracted with ethanol and the crude extract was purified by thin layer chromatography

where a pure flavones(22) has been isolated. The structure of the isolated flavone has been partially characterized by : (UV and ^{1}HNMR)[97].



(22)

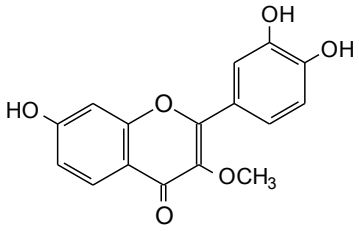
Amani reported on the isolation[98] of a flavone :5,7,4`-trihydroxy-5`-methoxylflavone(23)).This has been a first isolation for such flavone from the Sudanese material of *Tamarix nilotica.* The flavonoid was isolated from the n-butanol fraction by column chromatography.The structure was elucidated by a combination of analytical tools (UV,IR,1H NMR,13C NMR,HMBC and MS).



(23)

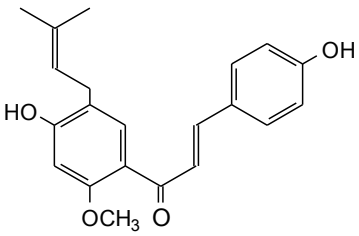
From the methanolic extract of the heartwood of *Acacia nilotica* var *nilotica,* a flavonoid (24) has been isolated[99].The

isolate has been purified by different chromatographic techniques and identified via spectroscopic tools: IR, UV, 1HNMR and Mass spectroscopy.The isolated compound has been evaluated for its antimicrobial potential against a panel of human pathogens. The compound showed varying antimicrobial responses.



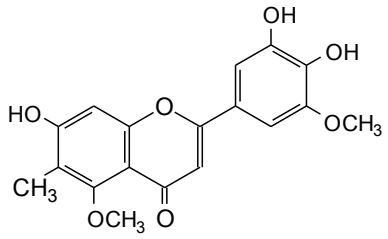
(24)

A prenylated chalcone(25) has been isolated[100] from the ethanolic extract of *Pistacia lentiscus* leaves by preparative thin-layer chromatography technique. The structure of the isolate was elucidated via a combination of sensitive spectral tools including : UV, IR, 1D and 2D NMR and high-resolution mass spectrometry.



(25)

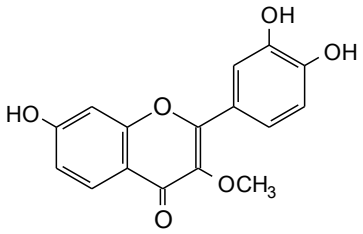
From the leaves of *Vitex doniana* (Sweet) , a flavone (26) was isolated[101] and characterized. The isolate was purified by different chromatographic techniques and identified via spectral tools(IR, UV, 1HNMR and mass spectroscopy). The methanolic fraction of *Vitex doniana* was evaluated(*in vitro*) for its antimicrobial potential against Gram negative (*Escherichia coli, Salomonella typhi* and *Pseudomonasa eruginosa*) and Gram positive (*Bacillus subtilis, Bacillus aureus and Staphylococcus aureus*) bacteria and the fungus *Candida albicans* .Promising results were obtained. *In vitro* antioxidant assay for the methanolic extract was conducted .Evaluation of the antioxidant activity was carried out by measuring the capacity of the extract against stable DPPH radical. The extract showed significant antioxidant activity[101].



(26)

Phytochemical investigation of *Acacia nilotica* var *adstringens* heartwood led to the isolation[102] of a flavone : 7,3`,4`-trihydroxy-3-methoxyflavone(27) from the methanolic extract. The crude extract was purified by a combination of chromatographic techniques(polyamide and Sephadex columns and paper chromatography) . Structure of isolate was elucidated

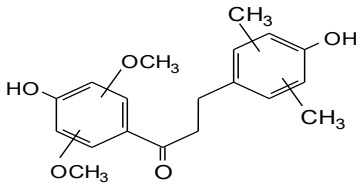
on the basis of extensive spectroscopic procedures including : IR, UV, ^{1}HNMR and MS. The isolated flavonoid was evaluated for its antibacterial potential against Gram negative (Escherichia coli and Pseudomonasa eruginos)Gram positive (Bacillus subtilis, Bacillus cereus and Staphylococcus aureus) bacteria. Compound I showed varying antibacterial responses. It showed high potency against Gram positive human pathogens : Staphylococcus aureus and Bacillus subtilis.



(27)

A study was set to investigate[103] the phenolics of the medicinally important species *Borassus aethiopium* which is widely used in ethnomedicine to treat an array of human diseases. Phytochemical screening of *Borassus aethiopium* bark ethanolic extract revealed the presence of sterols, triterpenes, flavonoids, tannins, saponins and glycosides. A dihydrochalcone(28) was isolated[103] from stem bark and its structure was partially elucidated on the basis of its spectral data(IR,UV,1HNMR and MS). The isolate was evaluated, *in vitro*, for its antimicrobial activity against six standard human

pathogens: two Gram positive (*Staphylococus aureus* and *Bacillus subtilis*), two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria and two fungal species (*Aspergillus niger*, *Candida albicans*) and significant results were obtained.



(28)

1.5- Structural elucidation of flavonoids

In identifying a plant constituent, once it has been isolated and purified, it is necessary first to determine the class of compound and then to find out which particular substance it is within that class. It should travel as a single spot in several TLC and or PC system. The class of compound is usually clear from its response to colour test, its solubility and R_f properties. Complete identification within that class depend on measuring other properties and then comparing these data with those in the literature. These properties include melting point, boiling point, R_f, and optical reaction. However, equally informative data on plant substance are its spectrala characteristics. These include ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectra (MS). A known plant compound can

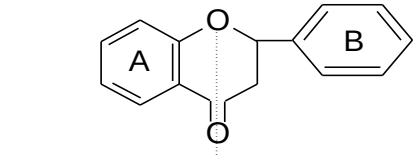
usually be identified on the above basis. If a new compound is present all the above data should be sufficient to characterize it.

1.5.1- The Infrared spectroscopy

Infrared spectroscopy has been a major source of information for natural product chemists for many years. The characteristic absorption of energy in the infrared region of the electromagnetic spectrum by different functional groups (causing, stretching, rocking of bonds), gives the chemist a powerful mean of determine what the essential features of any given molecule are[104]. The method is an extremely useful one that has the capacity to determine the environment in which a given functional group is located, example whether a carbonyl group is free, hydrogen bonded, or conjugated with an β- double bond[105]. If no other spectroscopic information is available, the IR spectrum will at least tell the chemist the class of compounds to which the unknown belongs. This information is vital and serves as a key in deciding the direction of subsequent steps[106]. Since flavonoids manifest themselves readily by means of their informative UV spectra, IR rarely used to be sure, there are structural features of flavonoids that are revealed by IR analysis, but for the most part, there are more of theoretical than practical interest[107].

1.5.2- The Ultraviolet / Visible spectroscopy

The UV spectra of most flavonoids consist of two major absorption maxima,one of which occurs in the range of 240-285nm (band II) and other in the range 300-400(band I). Band II occur from A-ring benzoyl system[108], while band I originates from B-ring cinnamoyl system .



Benzoyl System Cinnamoyl System

Flavonoids containing conjugated aromatic systems show intense absorption bands in UV region of spectrum (Table 2)

Table (2) Spectral characteristics of main flavonoid classes

Class Of Flavonoid	Band I λ nm	Band II λ /nm
Flavones	300-350	230-285
Flavonol	350-390	250-280
Flavonone	300-330 (sh)	275-295
Isoflavones	310-330 (sh)	270-280
	320(one peak)	
Anthocyanin	465-550	270-280
Chalcone	365-390	220-270 (low intensity)
Aurone	390-430	230-270 (low intensity)

UV spectrum has become a major technique for the structure analysis of flavonoids for two reasons: the first is that only a small amount of pure materials is required, often a single flavonoid spot on paper chromatogram will yield sufficient compound to several UV studies .The second reason is that the amount of structural information gained from a UV spectrum is considerably enhanced by the use of specific reagents (shift reagents), which react with one or more functional group on the flavonoids nucleus. The addition of each of these reagents separately to an alcoholic solution of the flavonoid induces structurally significant shifts in the UV spectrum. Shifts are commonly induced by the addition of sodium methoxide ,sodium acetate, sodium acetate\boric acid , aluminum chloride and aluminum chloride \ hydrochloric acid [109].

Sodium methoxide is a strong base and ionizes to some extent all hydroxyl groups on flavonoid nucleus .However, use has been made of the effect of (NaOme) on the UV spectra of flavones and flavonols for detection of 3 and for 4`-hydroxyl groups , in both case it give a bathochromic shift in band I but with decrease in intensity in case 3-OH [110].

The absence of shift in the major band indicates the absence of isoflavones of A-ring hydroxylation, while the presence of 5,6,7 and 5,7,8- hydroxyl system is evidenced by degeneration of sodium methoxide spectra with time[110-112].

Flavanones and dihydroflavonols with 5,7- dihydroxyl system exhibit a consistent 35-40 nm bathochromic shift of band (II). Flavanones in particular,those laking a free 5-hydroxyl group, isomerze to chalcones and have band (I) peak in 400 nm region[110].

4^{-}-hydroxyl groups in aurones and 4^{-}- hydroxyl groups in chalcones are evidenced,by band (I) bathochromic shift of 80-96 nm and 60-100 nm respectively each with an increase in intensity , while 6- hydroxy- aurones give a smaller shift (60-70 nm) than 4^{-}- hydroxyaurones. Chalcones give a 60-100 nm bathochromic shift[113].

The only anthocyanidine that give stable spectra in sodium methoxide are the 3-deoxyanthocyanidins[114], the bathochromic shift in band (I) being 50-60 nm.

Sodium acetate is weaker base than sodium methoxide, and as such, ionizes only the more acidic hydroxyl groups in flavonoids ie :3, 7- and 4'-hydroxyl groups. Since Ionization of the 7- hydroxyl groups mainly effect (band II), where as ionization of 3- and/or 4^{-}-hydroxyl groups mainly effect (band I). In the presence of sodium acetate the UV spectra of flavones and flavonols containing free 7- hydroxyl groups, with few exception exhibit a diagnostic 5-20 nm bathochromic shift [115] .

The presence of 7-hydroxyl group in isoflavones is evidenced by a band (II) bathochromic shift of 6-20 nm, in 5,7- hydroxyl

flavanones and dihydroflavonols by a shift of 35nm. Alkali-sensetive grouping in the A-ring cause the spectrum to degenerate with time.

Hydroxyl groups at position 4`- and\or 4 in chalcones and at 4`- and\or 6 in aurones are evidenced by a bathochromic shift of band (I) or by the appearance of a long wavelength shoulder.

In the presence of sodium acetate, boric acid will chelate with ortho-dihydroxyl groups at all location on the flavonoid nucleus, except at C-5,C-6 .Flavones and flavonols containing ortho-dihydroxyl group show a cosistent 12-30 nm bathochromic shift of band (I) in the presence of ($NaOAc\backslash H_3BO_3$) . A-ring catecholes at C-6, 7 and C-7, 8 in flavonoids are also detectable by the effect of ($NaOAc\backslash H_3BO_3$) on the UV spectra. A band (I) bathochromic shift of 5-10 nm is observed[116].

Isoflavones, flavanones and dihydroflavonols exhibits a bathochromic shift of 10-15 nm for band (II).Aurones and chalcones give bathochromic shift of 28-36 nm band (I), ortho-dihydroxyl groups in the A-ring give rise to a smaller shift.

With aluminum chloride ,Flavones and flavonols which contain hydroxyl groups at C-3 or C-5. form acid-stable complexes [156,157] ;in addition $ALCl_3$ froms acid-labile complexes with flavonoids which contain ortho-dihydroxyl system.The complex formed between $ALCl_3$ and the A-and B-ring ortho-dihydroxyl group; decompose in the presence of acid media [116]. In contrast,

the $ALCl_3$ complex between the C-4 keto function and either 3- or 5-hydroxyl group is stable in presence of acid. The presence of ortho-dihydroxyl group in the B-ring of flavones and flavonols can be detected by a comparsion of the spectrum of the flavonoids in the presence of ($AlCl_3$) with that obtained in ($AlCl_3$/HCl).

The presence of 5-hydroxyl group is revealed,by a10-14nm,band (II)batho chromic shift in the spectra of flavones and a20-26nm in the spectra of flavonones and dihydro flavonois. Ortho-dihydroxyl group are detectable only whore present in the A-ring ,and the spectrum shows aband (II)bathochrcmic shift of 11-30 nm .

The presence of 2'- hydroxyl group in chalcones and a 4--hydroxyl group in aurones is evidenced[112] by band (II) bathochromic shift of 48-64 nm.

B-ring ortho-dihydroxyl groups give rise to a 40-70 nm bathochromic shift of band (I) with Alcl . A-ring ortho-dihydroxyl groups give rise to a smoller shift[114] .

Anthocyanidines and anthocyanins containing ortho-dihydroxyl groups give band (I) bathochromic shift of 25-35 nm, longer shifts are observed with 3-deoxyanthocyanidins [109].

1.5.3- Mass spectrometry

The value of the technique is that requires only microgram amounts of material. It can provide an accurate molecule weight and may yield a complex fragmentation pattern, which is often characteristic of that particular compound[117,118].

Mass spectrum has been applied successfully to all classes of flavonoid aglycones, and more recently to a number of different type of glucosides including mono and di-c glycosyl flavones and mono- to tetra o-glycosides[119]. Electron impact spectroscopy of both flavonoid aglycones and glycoside serve as available aid in determing other structures specially when only small quantities (i.e less than I mg) of the flavonoid are available[120].Most flavonoid glycone yield intense peaks for the molecule ion [M]+ and indeed this is often the base peak[121]. In addition to the molecule ion, flavonoid glycones usually afford major peak for $[M-H]^+$ and when methoxylated $[M-CH_3]^+$. Perhaps the most useful fragmention in term of flavonoid indentification is those which involve cleavage of intact A- ring and B- ring fragments[122].

1.5.4- Nuclear magnetic resonance spectroscopy

The major use of proton NMR is for structural determination, in combination with other spectral technique. It used for determing the class of compound is quite considerable.

The application of NMR spectroscopy to the structure analysis of flavonoid is now well established. Most naturally occurring flavonoids, including all of the flavonoid glycosides, have low solubility in deuterio chloroform (CD CL_3). The dimethyl sulfoxide (DMSO- d6) has been used as solvent for number of extensive investigations of flavonoid structure by NMR spectroscopy[123,124]. Some of the advantage of this method, most flavonoid glycones and glycosides are sufficiently soluble in DMSO- d6. Its signal occurs in narrow band between δ 2.4 -2.6 ppm, outside the region where most flavonoid protons absorb. DMSO- D6 can be used for observing protons on phenolic hydroxyl group. It is anhydrous the hydroxyl proton signals are readily distinguishable. Water in the solvent, however, cause the flavonoid hydroxyl proton signals to broaden, thus making their detection difficult[125].

Proton of B-ring usually appear in the rang δ 6.7- 7.6 ppm, which is downfield from the region where the A- ring protons absorb. Considerable variation is found in the chemical shift of C-ring protons among the different flavonoid classes depending upon the oxidation level of the C-ring.

The chemical shift of the proton of sugar directly attached to the flavonoid hydroxyl group depends both on the nature of the flavonoid and on the position and stereochemistry of attachment. Methoxyl proton signals with few exceptions appear

in the region δ 3.5-4.1 ppm. While most aromatic acetoxyl proton signals occur in the range δ 2, 25 – 2,50 ppm[125,126].

1.6- The target species

1.6.1- *Bauhinia Rufescens*

About 600 species of bauhinia grow in the tropical regions of the world [127]. The genus includes tree, vines, and shrubs that are frequently planted for their showy flowers and ornamental foliage[128]. A native of southeastern Asia, B. monandra is found in the tropical regions of the world. Cultivated in the West Indies, it has naturalized throughout the islands.

Bauhinia Rufescens is deciduous in drier areas and is an evergreen in wetter areas. It is often found in dry savannah, especially near stream banks. It is found in the other Sahel and adjacent Sudan zone, from Senegal and Mauritania across North Ghana and Niger to central Sudan and Ethiopia[129]. This is a beautiful little tree shrub. It stays green all year round and has small leaves like lima beans and little white flowers like confederate jasmine. Bauhinia used externally to treat ulcers and rheumatism; these and several other medicinal uses. Today, the plant is still sometimes used for these purposes in folk medicine[130].

Bauhinia Rufescens

1.6.2-*Coriandrum sativum*

Coriandrum sativum, also known as cilantro, Chinese parsley or coriander is an herb in the family Apiaceae. Coriander is native to regions spanning from Southern Europe and North Africa to South western Asian[131]. It is soft plant growing to 50cm (20) in tall. The leaves are variable in shape, broadly lobed at the base of the plant, and slender and feathery higher on the flowering stems. The flowers are borne umbels in small, white or very pale pink, asymmetrical, with the petals pointing away from the centre of the umbel longer (5-6 mm) than those pointing toward it (only 1-3 mm long) [191]. The fruit is a globular, dry schizocarp 3-5 mm (0.12-0.20 in) in diameter. Although sometimes eaten alone, the seeds often are use as spice or an added ingredient in other foods. Coriander like many spices, contain antioxidants, which can delay or prevent the spoilage of food seasoned with the spice. A study found both the leaves and seed contain antioxidants, but the leaves were found to have a stronger effect[132]. Chemicals derive from coriander leaves were found to have

antibacterial activity against Salmonella choleraesuis, and activity this was found to be caused in part by these chemicals acting as nonionic surfactants[133].

Coriander has been used as a folk medicine for the relief of anxiety and insomnia in Iran. Coriander seeds are used in traditional Indian medicine as diuretica by boiling amounts of coriander seeds then cooling and consuming the resulting liquid. In holistic and traditional medicine it used as a carminative medicine and as a digestive aid[134]. Coriander has been documented as a traditional treatment for type 2 diabetes. Study on mice found coriander extract had both insulin-releasing and insulin- like activity[135]. Coriander seeds were found in a study on rats to have a significant hypolipidaemic effect, resulting in lowering of levels of cholesterol and triglycerides, and increasing levels of high-density lipoprotein. This effect appeared too caused by increasing synthesis of bile by the liver and increasing the breakdown of cholesterol into other compounds[136]. The essential oil produced from *Coriandrum sativum* has been shown to exhibit antimicrobial effects[136].

1.6.3*Albizza 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 , Madagascar); North America and

Albizia amara

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[137].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[138]. 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 are used as ati-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[138].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[139].

Aim of this study

This study was carried out to:

-Investigate the flavonoids of three key species widely used in Sudanese phytotherapy : *Albizza amara*, *Coriandrum sativum* and *Bauhinia Rufescens*.

-Elucidation of structures of the isolated flavonoids via sensitive spectroscopic tools (IR, UV and NMR).

-Evaluation of the antimicrobial potential of the isolated flavonoids and some of their crude extracts.

Materials and methods

2.1.Materials

2.1.1.Plant material

The roots of *Bauhinia rufescens* , leaves of *Coriandrum sativum,* and stem bark of *Albizza amara* were collected from Nyala, western Sudan. The plant was identified and authenticated by direct comparison with a herbarium sample.

2.1.2.Materials for paper chromatography

i. Whatman paper No (3mm) for preparative paper chromatography (Whatman Ltd., England).

ii- Glass jars (rectangular glass tanks 100 x80 x 40cm) for developing PC chromatograms.

2.1.3.Test organisms

- Gram +ve

Bacillus subtilis and Staphylococcus aureus.

- Gram -ve

Escherichia coli and *Pseudomonas aeruginosa* .

- Fungal strains

Candida albicans.

- Positive controls

i. Pencilin: for G+ve bacteria.

ii. Gentamycin: for G-ve bacteria.

iii. Clotrimazole: antifungal standard.

-Media for G+ve bacteria

Macconkey agar is used as media for G+ ve bacterial growth.

-Media for G-ve bacteria

Muller –Hinton agar

- Media for fungi

Sabouraud Agar (oxoid, England) is used as media for fungal growth:

2.1.4. Equipments

1- Ultra - Violet - Visible spectrophotometer (Shimadzu model UV240 and 240PC) .

2- Joel- Nuclear Magnetic Resonance (NMR) spectrophotometer, (Brucker AC-250) operating at 500 MHz.

2.1.5. Solvents

Analytical grade solvents were used.

Methanol(Merck,Germany) was used for spectrophotometric analysis . DMSO-d_6 was used as solvent and TMS as internal standard.

2.2. Methods

2.2.1. Extraction of flavonoids

The target plant material(1Kg) was macerated with 95% ethanol for 48h. at room temperature.The extract was filtered and the solvent was removed *in vacuo*.

2.2.2.Phytochemical screening

The target plants were screening for the presence of flavonoids ,phenolics, tannins, terpenoids, saponins and alkaloids as follows.

a)Test for alkaloids

To the extract, dilute hydrochloric acid was added,then it was shaken well and filtered.For the filtrate, the following tests were performed.

-Mayer's reagent test

To 3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates presence of alkaloids.

-Wagner test

To 2 ml of filtrate, few drops of Wagner's reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids.

b) Test for steroids

The ethanolic extract(20ml) of each sample was evaporated to dryness. (2ml) of acetic anhydride were added followed by (2ml) concentrated sulphuric acid . The presence of steroids is indicated by a change in color in the test sample.

c)Test for flavonoids

i.Alkaline reagent test :The extract was treated with few drops of sodium hydroxide solution separately in a test tube.
Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

ii. Lead Acetate ;The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

d)Test for tannins

i)Ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of tannins.

ii)Lead acetate test

A small amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution were added. Formation of white precipitate indicates the presence of phenolic compounds.

e)Test for Saponins

Froth test

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of a persistent

slayer of foam indicates the presence of saponins.

2.2.3. Antimicrobial assay

An inoculum suspension was swabbed uniformly to solidify 20 mL Mueller-Hinton Agar (MHA) for bacteria, and the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using glass Pasteur pipettes. Aliquot of 20 µl from each plant crude extract (200 mg/ml) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37^{o}C for 24 h. The resulting inhibition zones were measured in millimeters (mm)The assays were repeated in triplicate and the concurrent values were taken.The activity is expressed as less active, if the zone of inhibition is 9-12 mm, moderate 13-16 mm and high greater than 17 mm.

2.2.4.Isolation of flavonoids

Concentrated plant extract was applied on Whatman 3mm paper (46×57 cm) and run in BAW(4:1:5;v:v:v) for *Bauhinia rufescens*, BAW(6:1:5;v:v:v) for *Coriandrum sativum* and BAW(6:1:4;v:v:v) for *Albizza amara*. The dried paper were viewed and examined under visible and ultraviolet light. The chromatograms were then located under UV light , cut off and similar bands were joined and cut into small pieces and slurred with methanol. After several hours of contact the solvent was removed.Compound I was isolated from *Coriandrum sativum,*

compound II from *Bauhinia rufescens* and compound III from *Albizza amara* .

2.2.5.Structural elucidation of flavonoids

The structure of the isolated compound was elucidated via a combination of spectral techniques as illustrated below.

2.2.5.1.UV-Visible Spectroscopy

UV-Visible spectra of the isolated flavonoids were recorded on a Shimadzu spectrophotometer. Spectra were recorded in a quartz cuvette (1cmx1cmx4.5cm) which did not absorb over the spectral region of interest. One (mg) of the isolated flavonoid was dissolved in HPLC grade methanol (10mL) to create a stock solution. The UV-visible spectrum was taken for this solution at a rapid scan rate (600nm/min) to check the optical density. The concentration of the flavonoid solution was then adjusted so that the optical density of the major absorption peak (between 250-400nm) was within 0.6 to 0.8AU. The spectrum of the flavonoid stock solution (2-3mL) was measured at a normal scan speed (50nm/min) over the 200-500nm region. Additional spectra were measured over the regions of peak maxima at a reduced scan rate (10nm/min) to determine the wavelength of maxima accurately. Sodium methoxide solution (3 drops) was added to the cuvette of flavonoid in methanol. The spectrum was then recorded immediately at a normal scan rate. The solution in the cuvette was then discarded. Aluminium chloride solution (6 drops) was then added to fresh flavonoid stock solution (2-3mL)

in the cuvette. The spectrum was then recorded at a normal scan rate. Hydrochloric acid (3 drops) was then added to the cuvette containing aluminium chloride and flavonoid in methanol. The spectrum was recorded again and the solution discarded. An excess of powdered anhydrous sodium acetate was added to fresh flavonoid stock solution (2-3mL) in the cuvette and shaken. The powdered sodium acetate formed a layer (1-2mm) at the bottom of the cuvette. The spectrum was recorded immediately and again after ten minutes to check for decomposition. Finally, an excess of powdered anhydrous boric acid was added with shaking to the saturated sodium acetate solution. The spectrum was then recorded . In cases where the flavonoid decomposed in the presence of sodium methoxide, a different method was used to obtain a boric acid/sodium acetate spectrum. Boric acid solution (5 drops) was added to fresh flavonoid stock solution (2-3mL) which was quickly saturated with powdered sodium acetate and the recorded immediately.

2.2.5.1.1. UV - Shift Reagents

-Sodium methoxide solution

Freshly cut 2.5g metallic sodium was dissolved, cautiously, in 100 ml spectroscopic methanol (dry methanol).

-Aluminum chloride solution

(5g) anhydrous aluminium chloride was cautiously dissolved in 100 ml spectroscopic methanol and filtration was carried out after about 24 hours.

- Hydrochloric acid

50 ml concentrated hydrochloric acid was mixed with 100 ml distilled water, then keep in glass bottle.

- Sodium acetate

Anhydrous reagent grade sodium acetate was melted and allowed to stand for about 10 minutes. The material was then powdered and stored in a dry bottle.

- Boric acid

Anhydrous powdered reagent grade boric acid was used.

2.2.5.2.Infrared (IR) Analysis

An IR transmittance spectrum was recorded on a Perkin Elmer FTIR spectrophotometer at room temperature (25°C) in KBr disks.

2.2.5.3.Nuclear Magnetic Resonance Spectroscopy of (NMR)

NMR spectrum was obtained on a Bruker Advance DRX instrument. Sample was dried by freeze drying over several days and dissolved in DMSO-*d6* and then analyzed.

Results and Discussion

Flavonoids are plant polyphenolics reputed for their health promoting properties , where they primarily act as antioxidants. The accumulation free radicals in hman body results in many harmful processes including development of cancer.

In our search for biologically interesting molecules from plants grown in Sudan, it was aimed to investigate the flavonoids of *Coriandrum sativum*, *Bauhinia rufescens* and *Albizza amara* which are key species extensively used in Sudanese system of medicine.Herewith, we report isolation and structural elucidation of three flavonoids : (i) compound A from the leaves of *Coriandrum sativum* (ii) compound B from the roots of *Bauhinia rufescens* and (iii) compound C from the stem bark of *Albizza amara* .Two of these compounds have also been tested for their antimicrobial inhibitory effect against some clinically isolated microbes (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Condida albacans*).

The phytochemical screening of *Coriandrum sativum* leaves and *Albizza amara* stem bark is presented in Table 1.

Table 1: Phytochemical of *Coriandrum Stivum* and *Albizza amara*

Plant	Tannins	Alkaloids	Flavonoids	Steroids	Saponins
Albizza amara	+ve	+ve	+ve	+ve	+ve
Coriandrum Stivum	+ve	+ve	+ve	+ve	+ve

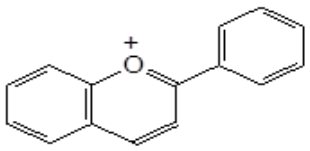
3.1 Compound A

Using paper chromatography,the ethanolic extract of *Coriandrum sativum* gave a flavonoid - compound A .

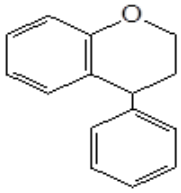
3.3.1IR spectrum of Compound A

The IR spectrum (Fig.1) showed ν (KBr) 3330.84(OH) , 2927.7 (C-H ,alkane) , 1610.45 (C = O), 1525.5, 1448.4 (C = C , aromatic) , 1205.4 (C-O , ether), and 1035.7 (C – O , phenolic)

Thus compound A is evidently not a flavan or anthocanin due to the presence of a carbonyl stretching at 1610cm^{-1} . flavans and anthocanins do not possess a carbonyl group.



Anthocanin



Catechin

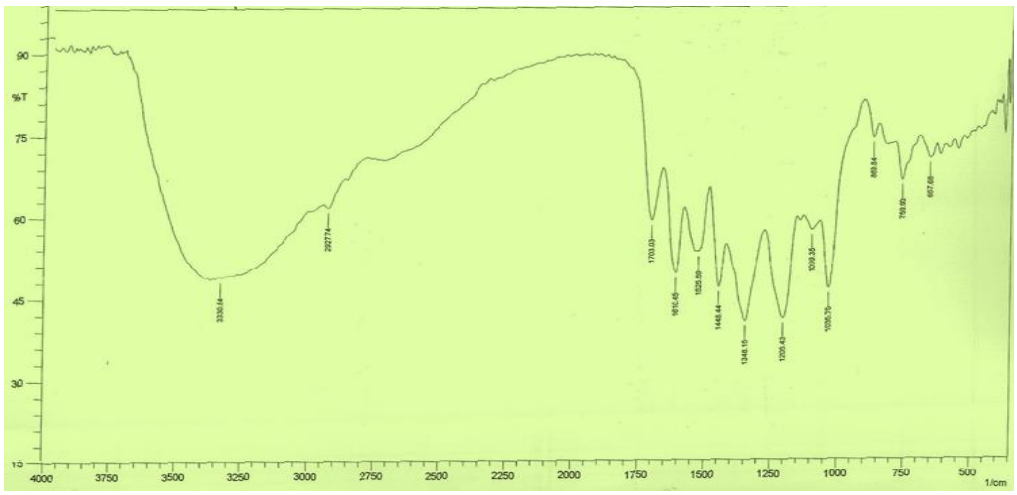


Fig. 1: The IR spectrum of compound A

3.1.2 UV of Compound A:

The UV spectra of most flavonoids consist of two major absorption bands appearing at : 230-290 nm (called band II) and the other in the range 300-400nm (called band I). The appearance of both bands is a characteristic feature of: flavones,flavonols,chalcones and aurones.However, the appearance of a single absorption band (band II) is a distinctive feature of : flavanones, isoflavones, dihydroflavonols and dihydrochalcones.The latter are characterized by saturation at C_2-C_3 linkage. The UV spectrum of compound I (Fig.2) showed λmax(MeOH) 234, 354nm .

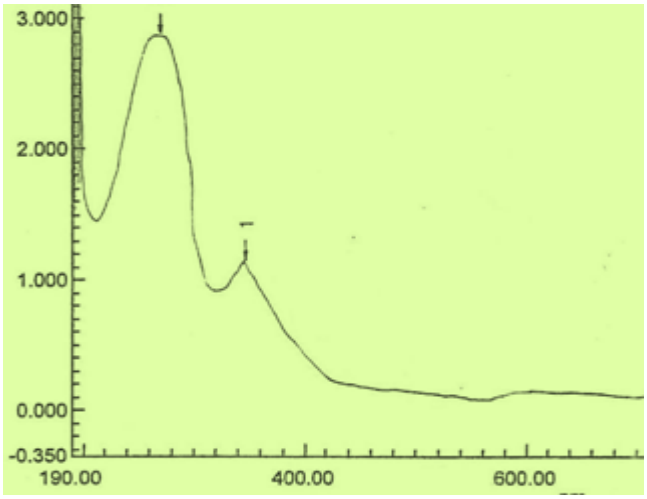
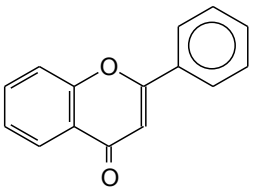
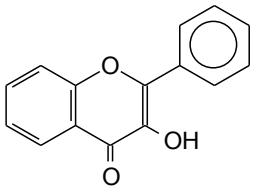


Fig.2 : UV spectrum of compound A

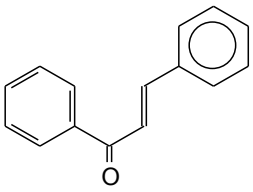
The appearance of both of band I and II in the spectrum suggests effective conjugation between the two aromatic rings(A and B) and compound I is probably (i)a flavone, (ii)a flavonol,(iii)a chalcone or(iv)an aurone.



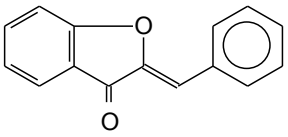
Flavone



Flavonol



Chalcone



Aurone

Chalcones have a dominant band I absorption and this is not the case with the spectrum of compound A .Also it is known that aurones absorb at λmax 400- 500 nm. Flavonols absorb at λmax 358 -390 (bandI), while flavones absorb in the range : 304 – 356 nm. Hence compound I which gave λ_{max}(MeOH) 234, 354nm is a flavone.

The UV shift reagent – sodium methoxide – can detect the presence of 3- and 4` - OH functions in the nucleus of flavonoids by inducing a blue shift. However, the sodium methoxide spectrum of compound A(Fig.3) did not exhibit a bathochromic shift indicating absence of 3- and 4`-OH groups.

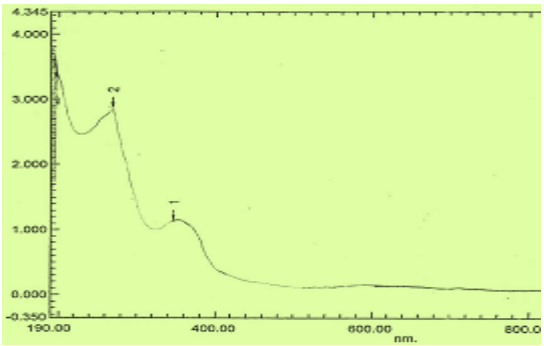


Fig. 3: Sodium methoxide spectrum of compound A

Very useful structural features are also gained by employing sodium acetate, boric acid and aluminum chloride.

Sodium acetate is diagnostic of a 7-OH where a bathochromic is observed when the sodium acetate spectrum is recorded. No bathochromic shift was observed in the sodium acetate spectrum of compound A (Fig.4) indicating absence of a 7 – OH function.

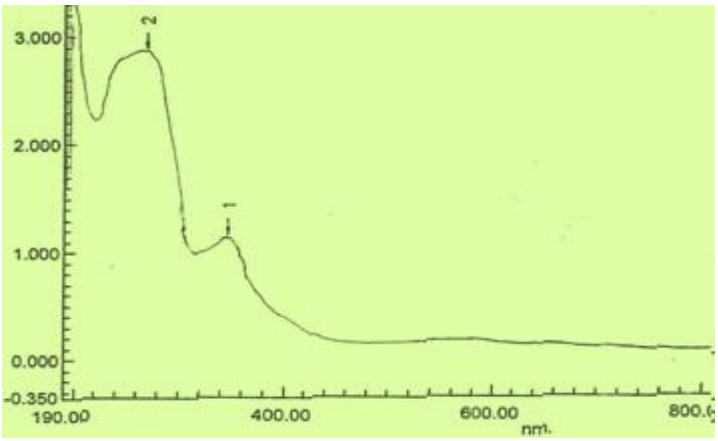
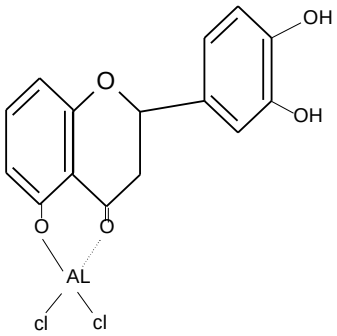
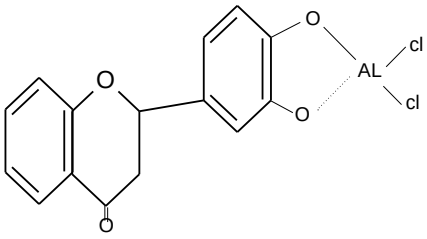


Fig. 4: Sodium acetate spectrum of compound A

Aluminum chloride is an extremely useful complexing agent which can detect 3- , 5-OH as well as *ortho*- dihydroxy systems. The complexes formed by $ALCl_3$ with the 4-keto group and 3- OH (or 5-OH) functions or catechol moieties are presented below. Catechol complexes are known to decompose in acidic media and can easily be distinguished from the 3- OH (or 5-OH) complexes.



5.4 complex



3-,4-complex

The aluminum chloride spectrum (Fig.5) failed to reveal a bathochromic shift indicating absence of 3-, 5- OH groups and catechol systems.

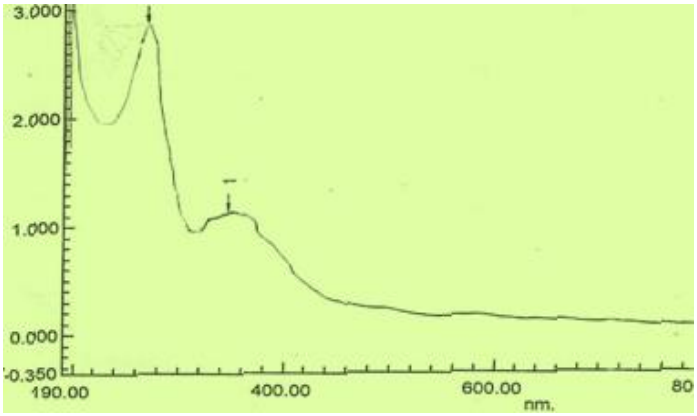


Fig. 5: Aluminium chloride spectrum of compound A

The above spectral data of compound A suggests the following:

-The absence of a 4`- OH group (Sodium methoxide spectrum).

-The absence of a 7-OH group (Sodium acetate spectrum).

-The absence of 3- and 5-OH groups and catechol systems ($ALCl_3$ spectrum).

3.1.3 ^{1}HNMR spectrum of compound A:

The ^{1}HNMR spectrum of compound A (Fig.6) gave δ(ppm): 0.89,1.35(assigned for two methyl groups); 1.78(acetyl group); 3.40(methoxyl);5.95,7.75 and 8.45(aromatic protons).

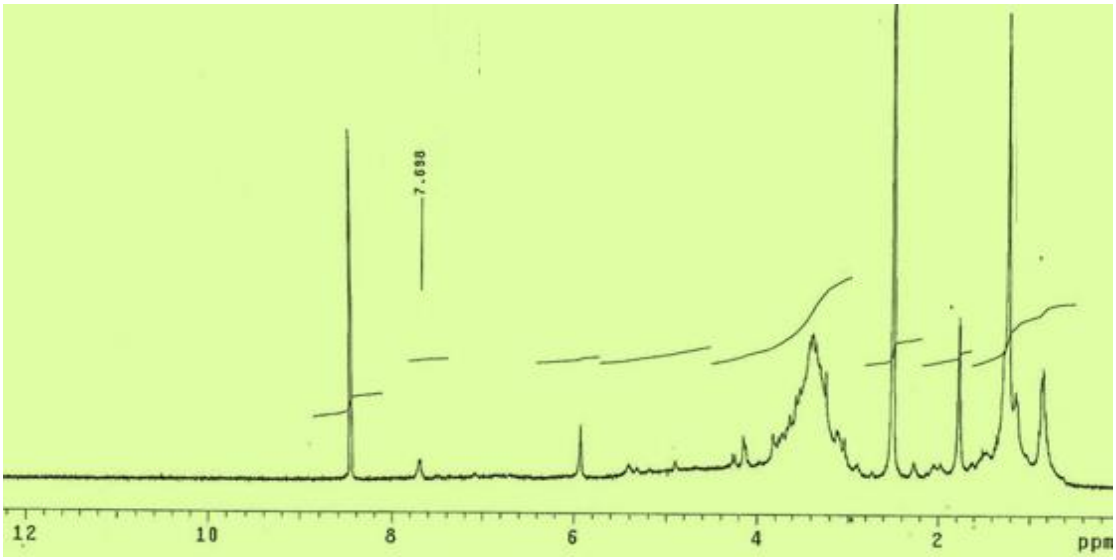
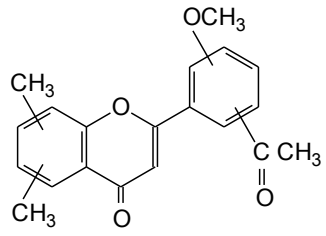


Fig.6 : ^{1}HNMR spectrum of compound A

On the basis of the above spectral data the following partial structure was proposed for compound A:



Compound A

3.2-Compound B

Compound B was isolated from the roots of *Bauhinia rufescens* .

3.2.1UV spectrum of compound B:

The UV spectrum of compound B(Fig.7) gave λ_{max}(MeOH) 261 nm .

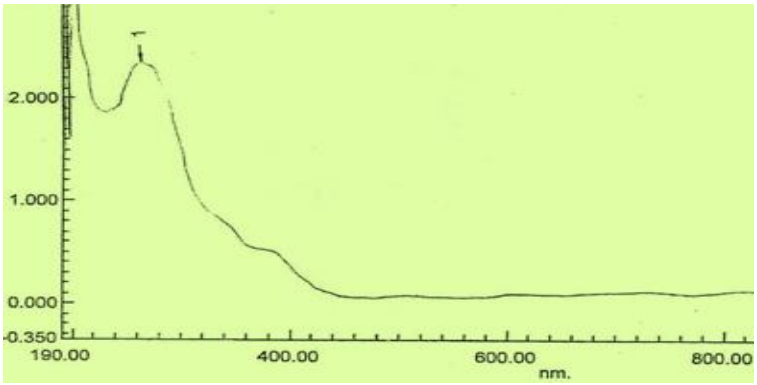


Fig. 7 : UV spectrum of compound B

The appearance of only one band-band II suggests: (i)a flavanone, (ii) an isoflavone, (ii)a dihydoflavanol or (iv) a dihydrochalcone.

The sodium methoxide spectrum revealed a bathochromic shift with decrease in intensity indicating a 3-hydroxyl group in compound B.This suggests that the isolated flavonoid is a dihydroflavonol.

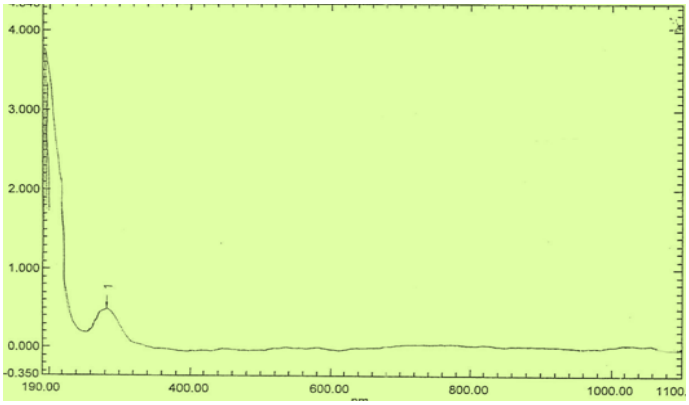


Fig. 7 : Sodium methoxide spectrum of compound B

The sodium acetate spectrum(Fig.8) failed to give a bathochromic shift thus suggesting the absence of a 7 – OH group. Also the aluminium chloride spectrum did not give a bathochromic shift suggesting absence of 3-,5-OH groups as well as catechol systems(Fig.9)

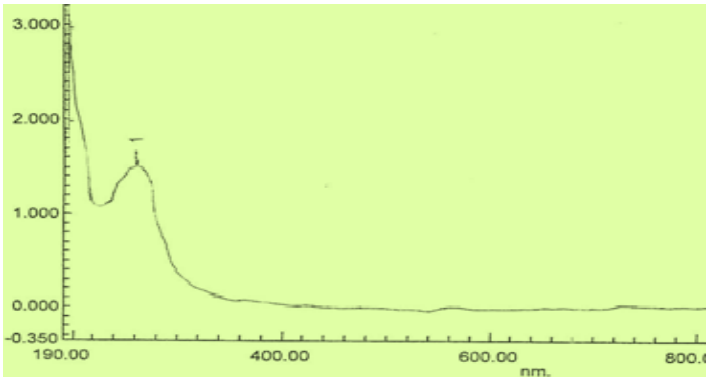


Fig. 8 : Sodium acetate spectrum of compound B

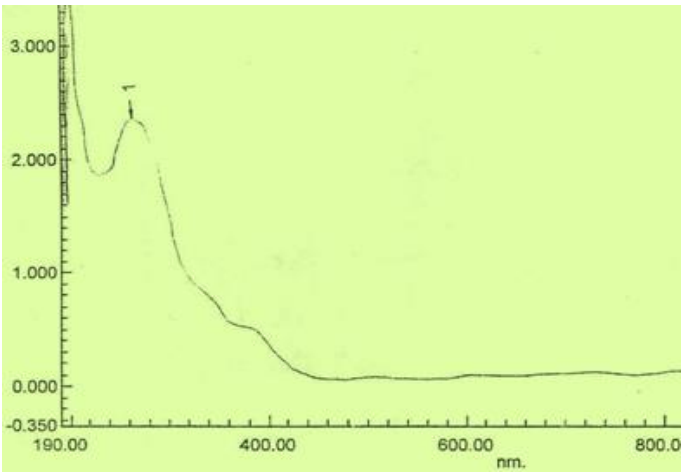


Fig. 9 : Aluminium chloride spectrum of compound B

The above argument suggests:

- The presence of a 3- OH group (the sodium methoxide spectrum).
-Absence of a 7-OH group (Sodium acetate spectrum).
-Absence of 3- and 5-OH and catechols (aluminium chloride spectrum).

3.2.2 ^{1}HNMR spectrum of compound B

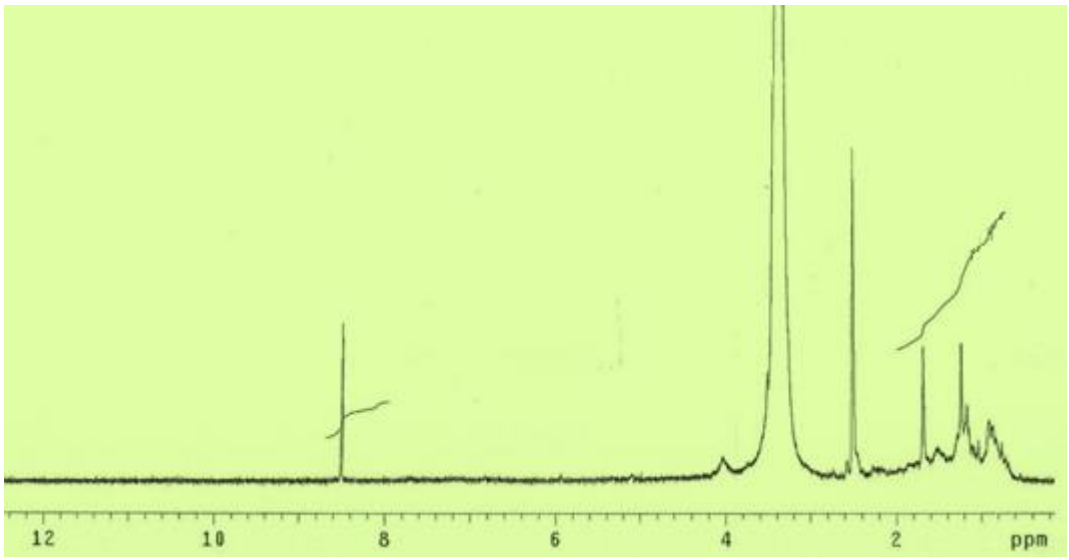
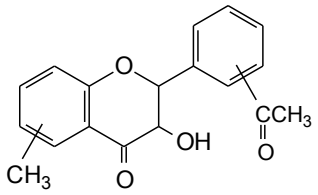


Fig.10 : ^{1}HNMR spectrum of compound B

The ^{1}HNMR spectrum of compound B (Fig.10) gave δ(ppm): 1.35(assigned for a methyl group); 1.78(acetyl group) : 8.45(aromatic protons).

On the basis of its spectral data, the following partial structure was suggested for compound B:



Compound B

3.3-Compound C

Compound C was isolated from stem bark of *Albizza amara* by paper chromatograph.

3.3.1 UV spectrum of compound C:

In the UV it absorbs(Fig.11) at λ_{max}(MeOH)279nm, hence it belongs to those flavonoids which are characterized by saturation at $C_2 - C_3$ linkage i,e. it could be : a flavanone, dihydroflavonol, dihydrochlcone or an isoflavone.The latter class is ruled out since it is characterized in the UV by a shoulder in the UV range : 300-340nm and such feature was not detected in the UV spectrum of this compound.On the other hand dihydroflavonols are known to possess a 3-OH function. This group was not detected by the sodium methoxide spectrum – no bathochromic shift(Fig.12)

However, flavanones and dihydrochalcones are distinguishable by their ^{1}HNMR. Flavanones, unlike dihydrochalcones give double multiplets around δ2.80 and δ5.20ppm . One of these multiplets is due to mutual splitting of the magnetically unequivalent protons at C_3 . The double doublet arising from such splitting suffers further splitting by the neighboring C_2 proton yielding a multiplet. The other mulriplet is due to the splitting of C_2 resonace by the neighboring unequivalent C_3 protons.However , such multiplets were not detected in the ^{1}HNMR spectrum of compound C(Fig.16). Hence this compound is a dihydrochalcone.

The sodium acetate spectrum(Fig.13) did not afford any bathochromic shift confirming absence of a 7-OH function. The aluminium chloride spectrum and the boric acid spectra(Fig.14 and 15) were also devoid of bathochromic shifts suggesting absence of catechol systems as well as 3- and 5-OH groups.

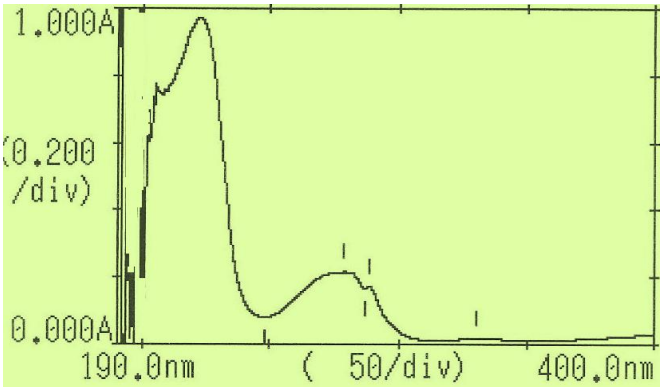


Fig.11 : UV spectrum of compound C

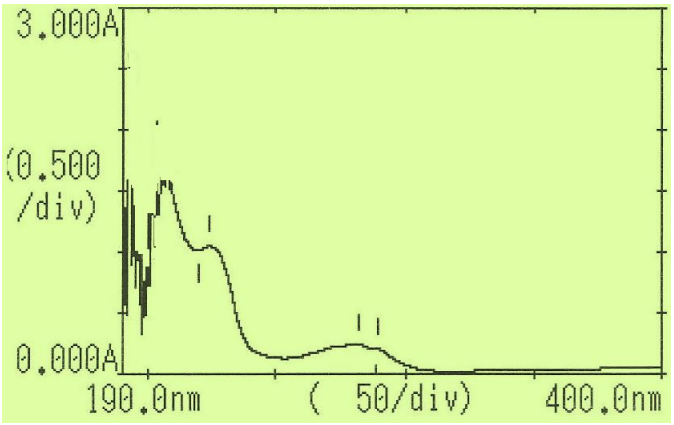


Fig. 12: Sodium methoxide spectrum of compound C

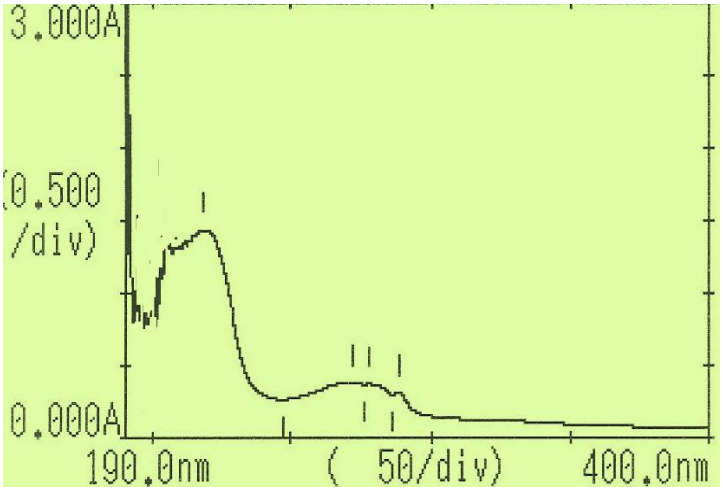


Fig13. : Sodium acetate spectrum of compound C

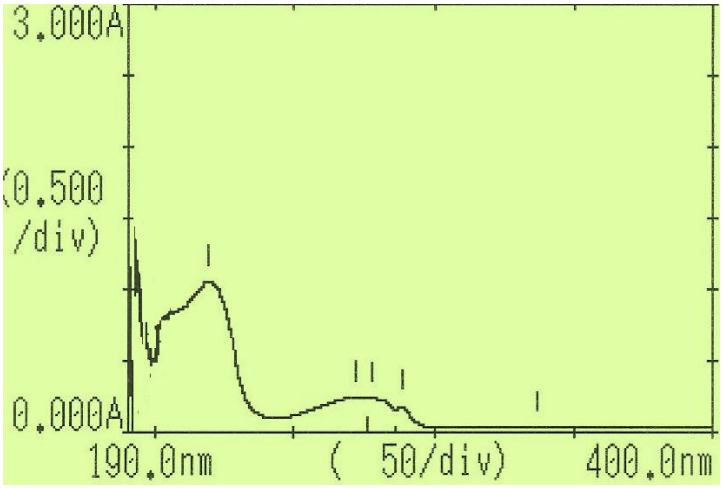


Fig.14 : Alminium chloride spectrum of compound C

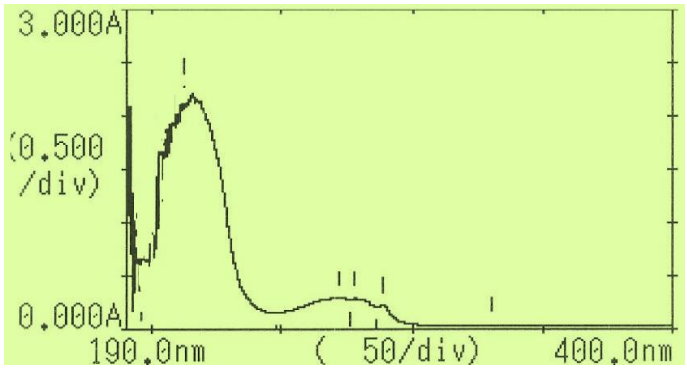


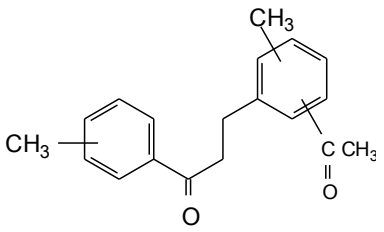
Fig.15 : Boric acid spectrum of compound C

3.3.2 ^{1}HNMR spectrum of compound C:

The ^{1}HNMR spectrum of compound C (Fig.16) gave δ(ppm): 1.35(assigned for two methyl groups); 1.82(acetyl group) : m(3.30-3.82)

attributed to a sugar moiety(not identified in this study); m(6.69-7.60) assigned for aromatic protons.

On the basis of its spectral data, the following partial structure was suggested for compound C:



Compound C

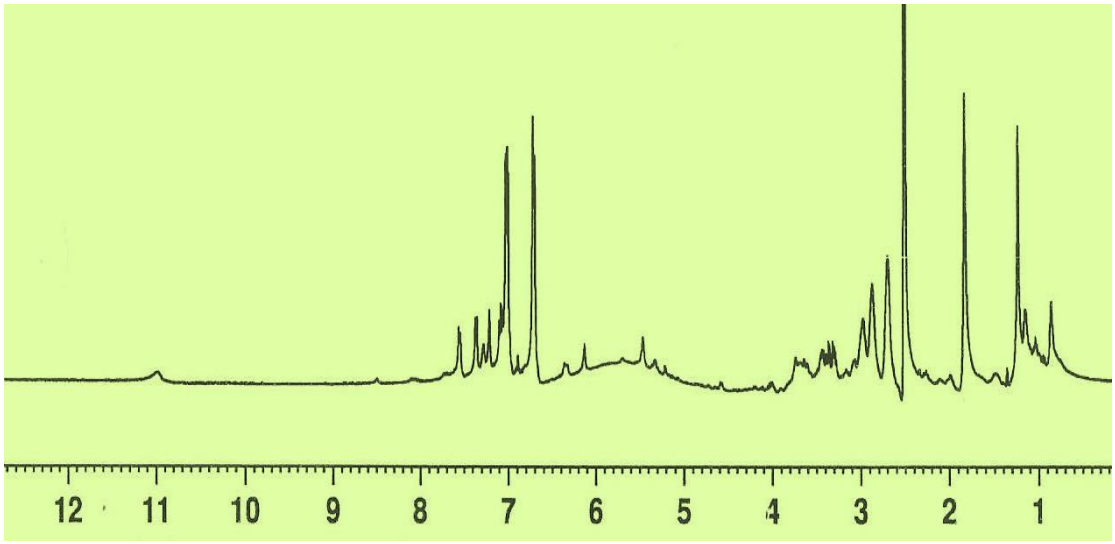


Fig.16 : ^{1}HNMR spectrum of compound C

3.4-Antimicrobial assay

Compound A which was isolated from *Coriandrum sativum* was evaluated for antimicrobial activity against six standard pathogenic bacteria.The results of Table (2) showed significant anticandidal activity exhibited by compound A. The compound also showed significant antibacterial activity against *Pseudomonas aeruginosa*

and *Bacillus subtitles.* Good activity has been detected against other test organisms

Table 2: Antimicrobial activity of compound I

Organism	Inhibition growth zone diameter (MIZD) 100 mg /100 ml
	Compound A
Bacillus subtitles	17
Staphylococcus aureus	16
Escherichia coli	15
Pseudomonas aeruginosa	18
Aspergillus niger	15
Condida albacans	18

Both of the crude ethanolic extract of *Bauhinia rufescens* and chromatographically pure flavonoid isolated from this species were assessed for their antimicrobial activity against six standard human pathogens. Ampicilin, gentamycin and clotrimazole were used as positive controls.

The diameters of inhibition zones are listed in Table 3 . The crude extract showed better inhibitory effect compared to compound B. While the crude extract showed significant antibacterial and antifungal properties, compound A exhibited moderate antibacterial activity and weak antifungal properties.

Table 3: Diameters of inhibition zones(mm)

Organism	Inhibition growth zone diameter at 100 mg/ml	
	Crude extract	Comp. B
Bacillus subtitles	19	15
Staphylococcus aureus	20	16
Escherichia coli	22	15
Pseudomonas aeruginosa	20	15
Aspergillus niger	18	12
Condida albacans	18	14

***** *Activity:*
10 mm – 13 mm → weak.
14 mm – 18 mm → medium.
18 mm - over → high.

Table 4 : Antibacterial activity of standard drugs

Drug	Conc. (mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5 : Antifungal activity of standard drug

Drug	Conc. (mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

Conclusion

The flavonoids of *Coriandrum sativum*, *Bauhinia rufescens* and *Albizza amara* have been investigated. Three flavonoids : (i) compound A from the leaves of *Coriandrum sativum* (ii) compound B from the roots of *Bauhinia rufescens* and (iii) compound C from the stem bark of *Albizza amara* have been isolated and partial structures have been proposed .Two of these compounds have also been tested for their antimicrobial inhibitory effect against some clinically isolated microbes (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Condida albacans*) and significant to moderate responses have been detected.

Recommendations

The following is recommended:

-Other phytochemicals included in the studied species(steroids, essential oils, alkaloids…etc may be isolated and their structures elucidated.

-The isolated flavonoids (compounds A, B and C) may be screened for their antiinflammatory, antimalarial antileshmenial properties.

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