**Sudan University of Science and Technology**

**College of Post Graduate Studies**

# Isolation and Characterization of Flavonoids From *Weltheria indica* , *Cistanch phelypea* and *Fagonia critica* and Their Biological Activity

# فصل وتوصيف فلافونيدات الفكهت ، عرق النار وشوكة الجمل وفعاليتها البيولوجية

**A Thesis Submitted in Fulfillment of the Requirements of the Ph.D. Degree in Chemistry**

**By**

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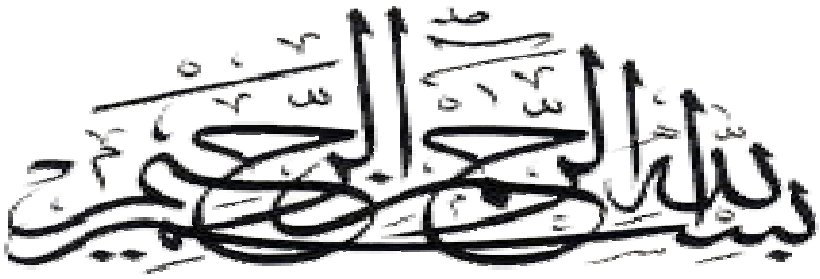
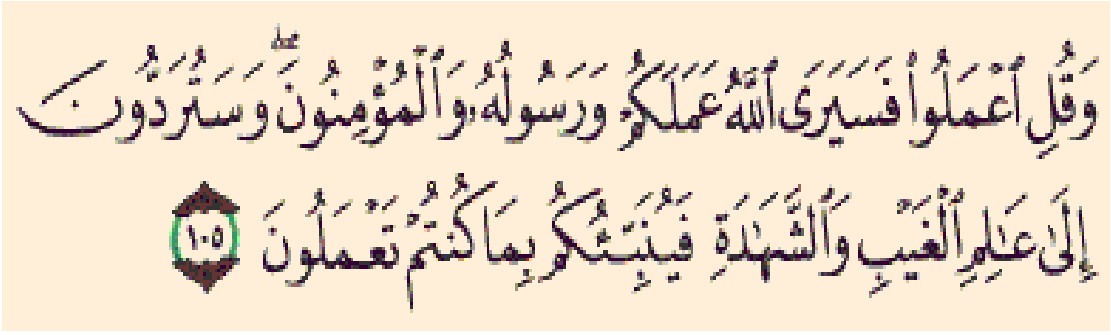
**September,2019**





وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ
إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ﴿١٠٥﴾

**(التوبة-١٠٥ )**

# Dedication

To,

My parents : Mohamed and Ghalia

My dear brother : Khalid

Brothers and sisters

# Acknowledgment

First I would like to thank **Almighty Allah** for giving me the will and strength to finish this work.

I would like to express my deepest gratitude to my supervisor Prof. Mohammed Abdel Karim for his suggestions , guidance , encouragement and useful criticism throughout the course of the study .

Thanks to the technical staff of dept. of Chemistry-Faculty of Science, Sudan University of Science and Technology for their kind help .

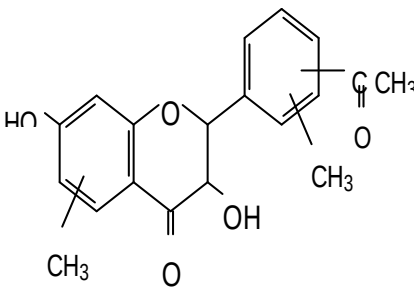
I would like to thank the staff of the Medicinal and Aromatic Plants Research Institute for all facilities. Also thanks are extended for the National Research Center, Cairo for the spectral measurements.

Thanks for my family for the infinite support and assistance.

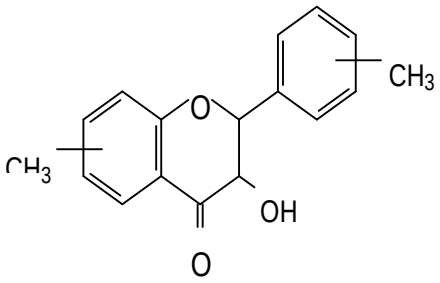
# Abstract

This study was carried out to investigate the flavonoids three key species in Sudanese traditional medicine: *Wetheria indica* , *Cistanch phelypea* and *Fagonia critica*. Three flavonoids ,one flavonoid from each plant, have been isolated and their tentative structures have been deduced on the basis of their spectral data. Furthermore the antimicrobial activity of these plants have been screened *in vitro*.

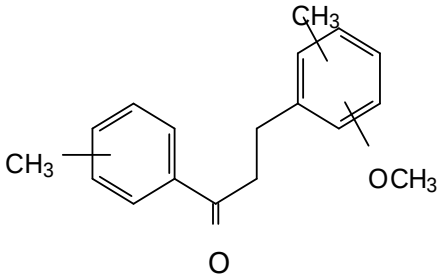
The flavonoids were extracted with 95% ethanol and the crude extracts were purified by thin layer chromatography . The structures of the isolated flavonoids have been partially characterized by some spectral data (UV and $^{1}$HNMR). One flavonoid-compound I- was isolated from the leaves of *Waltheria indica* another one-compound II- was isolated from the leave of *Cistanch phelypea* , while a third flavonoid-compound III- was isolated from *Fagonia critica*.




Compound I




Compound II

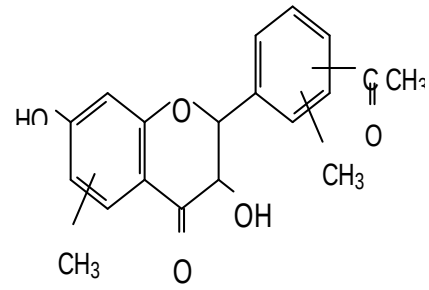



Aglycone of compound III

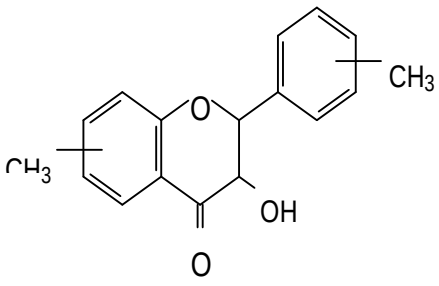
In the cup plate agar diffusion bioassay, The ethanolic extract of *Wetheria indica* showed moderate antibacterial and antifungal activity against test organisms. The extract **also** exhibited significant free radical scavenging capacity. The ethanolic extract of *cistanch phelypea* was inactive against the test bacteria ,but it gave good antifungal acivity against the yeast : *Candida albicans*.The extract exhibited exhibited significant antioxidant activity close to that of the positive standard propyl gallate. The ethanolic extract of *Fagonia critica* showed moderate activity against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*.It also exhibited partial activity against *Pseudomonas aeruginosa* , but it failed to show any anticandidal potential.

# المستخلص

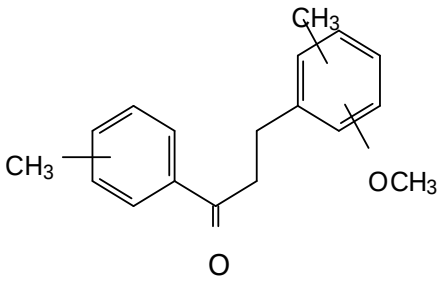
في هذا البحث تمت دراسة الفلافونيدات الرئيسة لنباتات عرق النار , شوكة الجمل والفكهت , كما واختبرت مستخلصات هذه النباتات كمضادات ميكروبات وكمضادات اكسدة. استخلصت الفلافونيدات بالاثانول وتمت التنقية بتقنية كروموتوغرافيا الطبقات الرقيقة وتم التوصيف الجزئى للفلافونيدات عن طريق طيف الاشعة فوق البنفسجية –المرئية وطيف الرنين النووي المغنطيسى للبروتون. تم فصل المركب I من نبات الفكهت والمركب II من عرق النار اما المركب III فقد تم فصلة من نبات شوكة الجمل .




Compound I




Compound II




Aglycone of compound III

في اختبار مضاد الميكروبات اعطى مستخلص الاثانول لنبات الفكهت فعالية معتدلة ضد جميع الميكروبات قيد الاختبار.ايضا اعطى المستخلص فعالية عالية كمضاد اكسدة.

اما مستخلص عرق النار فقد ابدى فعالية جيدة ضد فطر كانديدا, الا انة لم يكن فعالا ضد بقية الميكروبات قيد الاختبار. وقد اعطى مستخلص نبات شوكة الجمل فعالية معتدلة ضد :

*Staphylococcus aureus, Escherichia coli , Bacillus subtilis*

أيضا تم اختبار مستخلص نبات شوكة الجمل كمضادا اكسدة حيث أعطى هذا المستخلص فعالية عاليه.

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## Chapter Three

# 1-Introduction

## 1.1- **General approach**

Natural products have long been sources of drugs, and a large proportion (30 -40%) of the pharmaceuticals available in modern medicine is directly or indirectly derived from natural sources [1].

Furthermore , natural products derived from plants are also of great interest in the process of drug discovery. Due to their large diversity in nature, they permit the identification of leading molecules of interest for the development of new therapeutic agents. Furthermore they provide biochemical and molecular tools needed to clarify complex cellular and molecular mechanisms of action involved in most physiological and pathological processes. Ultimately, a growing world – wide interest in the use of phytopharmaceuticals as complementary alternative medicine either for the prevention or treatment of many diseases, has been noted in recent years. It is believed that about 80% of world`s population uses plants as their primary sources of medicinal agents[2].

## 1.2-The flavonoids

Plant phenolics - flavonoids - are considered to be one of the most important natural products and one of the most commonly used compounds around the world[1]. Flavnoids belong to the recently popular class of phytochemicals, which are plant product with potential benefit for human health. Since these compounds exist as secondary metabolites. They are an

important part of human diet. They are also considered to be the active principles in many medicinal plants[3].

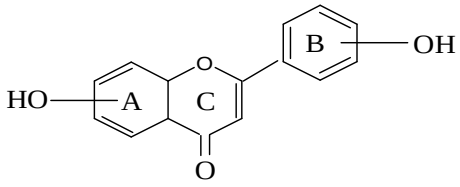
Flavonoids have been isolated from a wide range of vascular plants[4], and more than 8000 different flavonoids have been reported in literature[1,5].

Flavonoids have various functions in plants [6]. They act in plants as antioxidants , antimicrobial , photoreceptors , visual attractors , feeding repellents , and for light screening [7] . Many studies showed that the flavonoids exhibit biological and pharmacological antibacterial, cardio protective, hepatoprotective, neuroprotective, antimalarial, antileishmanial, antitrypanosomal, and antiamebial properties[8-14] . These biological and pharmacological properties are usually attributed to their free radical scavenging efficacies, metal complexion capabilities, and their ability to bind to proteins with a high degree of specificity [15].

The basic skeleton of flavonoids is the flavan nucleus which consists of 15 carbon atoms derived from a C6 - C3 - C6 skeleton. A flavonoid skeleton is composed of two aromatic rings (commonly designated as A and B ), which are linked by a three –carbon chain[16].The connecting carbon chain combines with an oxygen to form a heterocyclic central or C- ring for most flavonoids with the exception of chalcones in which the carbon chain between the A and B ring is linear[17] .

Twelve different classes of Flavonoids are known, differing from each other by the degree of oxidation of the C – ring.These are : flavones, flavonols, flavans, flavanones, flavanols, chalcones, dihydrochalcones, iso flavones,aurons, anthocyanins, anthocyanidins,and catechins [18-20].

Polyphenols are defined as compounds consisting of more than one aromatic ring with each containing at least one hydroxyl group(see Fig. 1) [21].

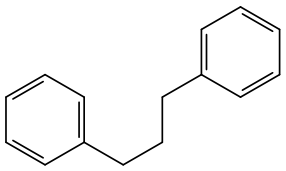



**(1)**

The structure of flavonoids contains two aromatic rings(designated as A and B)[23]. These plant phenolics have a characteristic backbone ring structure $C_6 - C_3 - C_6$ , namely diphenyl propane. The two aromatic ring generally contain a number of phenolic hydroxyl groups[24].

The "A" ring of flavonoids arises by condensation of acetate units , while the "B" ring is constructed of phenyl propane units via shikimic acid pathway[25].

Both aromatic rings of flavonoids (A and B rings) are joined by a linear carbon chain, which may be open or closed as shown in figures below. In structure ( 3), the benzene ring "A" is linked with a six – membered heterocyclic ring "C" , which in tern carries a benzene ring "B" at position 2 as shown in Fig. 3. Ring "C" may be a heterocyclic pyrane, or pyrone ring [26].



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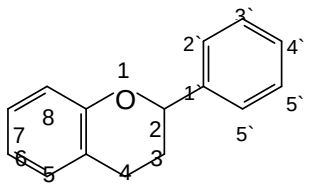
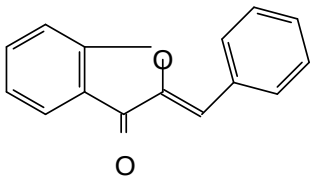



⌣ 3

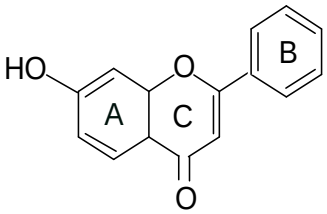
However, sometimes the six- membered heterocyclic ring "C" exist in an isomeric open form or is replaced by five-membered ring, giving aurones (2-benzeylidenecoumaranone)[27] as depicted in Fig. 4.




(4)

In the majority of cases, the flavonoid nucleus is linked to sugar. Flavonoids usually occur naturally as water – soluble glycosylated and methylated derivatives[28]. The glycosidic linkages are normally located in the 3- or 7-position, and are frequently hydroxylated at the 4` position[29] . Generally the presence of sugars bound as glycoside in flavonoids may occur in a single plant in several glycosidic combinations[30]. For this reason when analyzing flavonoids it is usually better to examine the aglycones present in hydrolyzed plant extracts before considering the complexity of glycosides that may be present in the original extract.

Flavonoids are considered to be derived from the parent compound flavone (5). These plant phenolics bear phenolic hydroxyls and hence change in colour when treated with base or ammonia. Thus flavonoids are easily detected on chromatograms or in solution[23].




**(5)**

Flavonoids usually show intense absorption band in UV light and visible regions of spectrum[23,31], consequently some flavonoids are intensely coloured, providing a spectrum of colours from red to blue in most plant parts. Essentially colourless flavonoids produce, "whiteness" of white flower. Beside their contribution to plant colour, flavonoids have a variety of other roles in the growth, development, texture and taste of plant food[32].

Flavonoids are categorized into : flavones, flavonols, flavanones, isoflavones, anthocyanins, chalcones[33, 34], and aurones[35] (table 1). All subclasses of flavonoid possess additional phenolic hydroxyl group at different possition in rings A and B[36].

Dimers flavonoids, in which two classes of flavonoids, mostly flavones and flavanones are joined together are known. The dmer may be composed of the same or different types of flavonoids, such as flavones–flavones or flavones – flavanones complex. They may be bounded together directly through their carbons and most often by C- 8 and C –6 or by C – O – C inter flavonyl link[37].

The occurrence of flavonoids in plants is not restricted to a certain organ but include all parts of the plant[38], root, heartwood, sap wood, bark, stem, leaf, fruit and seed. Some kinds of flavonoids are more characteristic of certain tissues[39] (Table 2) which are present in all vascular plant. Some classes are more widely distributed than others, while flavones and flavonols are almost universal. isoflavones and biflavonyls are found in few plant families[40]. However,the presence of flavonoids in plants is largely influenced by genetic factors and environmental conditions. Other factors such as germination, degree of ripeness, processing and storage also influence the content of plant phenolics[41,42].

One of the roles of flavonoids in plants iw to induce coloration of the flowers, fruits and sometimes the leaves . Colourless flavonoids are also abundant and

many-such as flavones and flavonols act as co- pigments[30]. The yellow colour of flowers and fruits are derived from chalcones and aurones, whilst the anothocyanins give rise to other shades : red, blue and violet colours.

One of the biological functions of flavonoids is the protection of plants against the harmful and damaging effect of UV- radiation[43-44]. These phenolics also play an important metabolic role[45, 46]. They are also considered as important nutrients[47-48]. The function of the flower pigment in pollination is fairly clear, insects and birds pollinators are attracted by colored petals[49].

Flavonoids have significant impact on various species of plant biology. They exhibit a wide range of functions in physiology, biochemistry, and ecology. Moreover, for long time flavonoids constitute useful tool in phytogenetic studies[50]. Flavonoids are believed to protect humans by providing protection against certain form of cancer and reduction of cardiovascular disease[51].

Flavonoids one of the major secondary metabolites found in food plants possessing a wide range of physiological activity[52]. The presence of flovonoids in foods and other compounds is thought to be one of the reasons for the beneficial influence on human health[53]. They have long been recognized in folkmedicine[54] Finally, flavonoids possess strong anti-oxidative activity[55] as well as other potential beneficial effects including anti-inflammatory[56], anti-viral[57], anti-atherosclerotic[58], anti-cancer[59], and anti-osteoptic effects[60-62].

Flavonoids are present in a diversed classes of secondary plant metabolities with about 9000 structures[63]. Some characteristic properties of the different flavonoid classes are shown in Table 1 ,while colour properties and occurence of the different classes are depicted in Table 2.

**Table 1: Characteristic properties of the different flavonoid classes.**

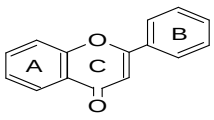
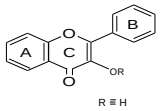
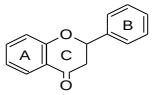
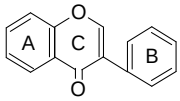
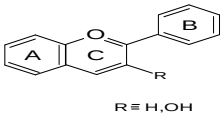
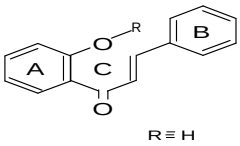
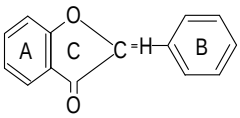
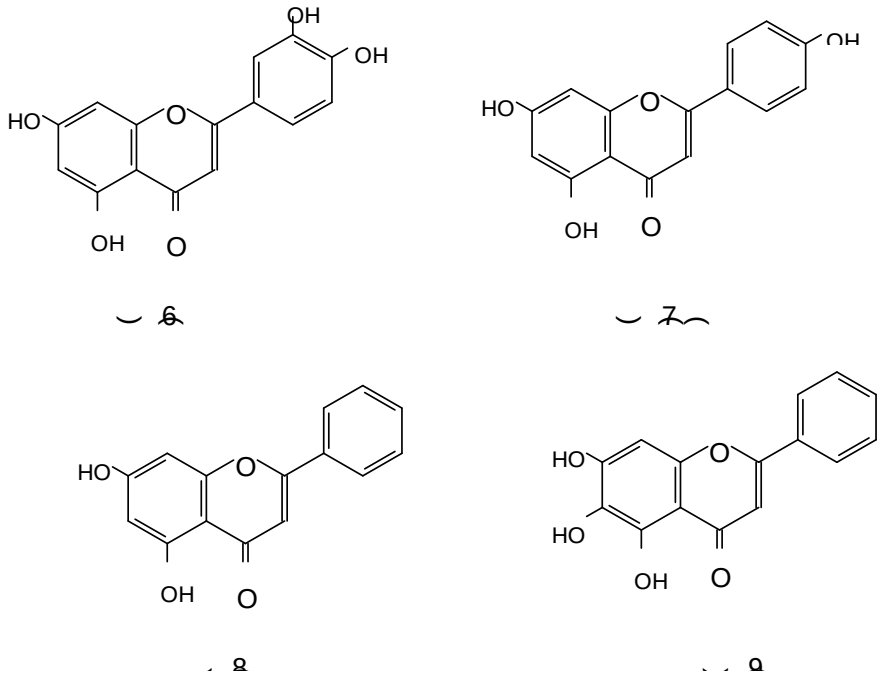
| Sub-Class of Flavonoid | Basic Structure of Flavonoid | Characteristic properties |
|---|---|---|
| Flavone |  | differ from flavonol in lacking a 3-O substituention |
| Flavonol |  | differ from flavones in having a 3-O substitution |
| Flavanone |  | Differ from all classes of flavonoid in lacki the double bond in 2,3 position |
| Isoflavon |  | Isomeric with flavones, having the B-ri attached at the 3-position in flavones instead 2-position in flavones |
| Anthocyanin |  | Differ from all classes of flavonoid in lacki the carbonyl group at 4-position |
| Chalcone |  | Isomeric with flavanone having the open cha instead of close chain in flavanone (ring c). |
| Aurone |  | Differ from all classes of flavonoids having membred ring-c instead of six-membred ring certain classes |

Table (2): Colour Properties and Occurence of The Different Flavonoid Classes.

| Flavonoid | Occurrence | Colour in nature |
|---|---|---|
| Flavone | Found in all parts of plant, widespreading flowers and leaves | Yellow Colour |
| Flavonol | The same as flavones | Yellow Colour |
| Flavanone | The same as flavones | Colourless |
| Isoflavone | Found in root; only common in one family; the Leguminacea | Colourless substance |
| Anthocyanin | Flower pigments; also in leaves, fruits petals and other tissues. | Scarlet,mauve, blue, pink and violet |
| Chalcone | Flower pigments, occasianally present in other tissues | Bright yellow colour |
| Aurone | Flower pigments, widespead in leaves, fruits and bark wood | Bright yellow colour |

As far as the occurrence of flavonoids in plants is concerned, flavonoids occur either in the form of glycosides or aglycones . Whle aglycones are absorbed easily from gut by passive diffusion, while the glycosides are usually hydrolyzed to the corresponding molecules prior to its gastrointestinal absorption[68].Flavonoids are present in plant as mixture and it is very rare to find only a single flavonoid component in plant tissue. In addition ,there are often mixture of different classes and they are generally present in all vascular plants, but some classes are more widely distributed than others[23].

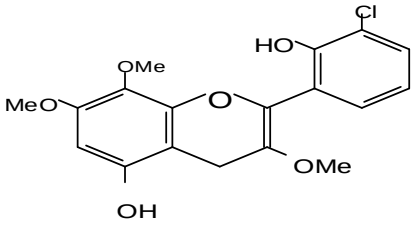
Flavones are yellow pigments and are one of the most significant classes of flavonoids . They are widely distributed in leaves and flowers of angiosperm[69]. Common examples are luteoline( 6)[70], apigenin (7)[71], chrysin( 8), and baicalin(9)[72] .




Generally, flavones are found in all parts of plantincluding vegetable and generative organs. They exist in barks, heartwood, thorns , stems, leaves, buds, rhizomes, flowers and also in root and leaf exudates. Flavones were detected in more than 70 different families within the plant kingdom[39] representing an abundant class of phytochemicals in our daily diet. Lastly, flavones attracted considerable scentific and therapeutic interest, because of the assumed beneficial health effects of flavones in the prevention of some human diseases. Besides their physiological importance they have important function in the biochemistry, physiology and ecology of plant[73-74]. Flavones occur naturally in the plant in free state ( aglycone), or as glycosides, or associated with tannins[29].

Flavonols are flavones in which the 3- position is substituted by a hydroxyl function. Flavonols are practically ubiquitous in woody angiosperms and appear less frequently in herbaceous angiosperm. There are no records of

flavonoids occurring in bacteria and algae, and chlroflavonin (10) is ,so far, the only fully characterized flavonol isolated from a fungus. It was isolated from a strain of *Aspergillus candidus* . The hydroxylated pattern of chloroflavonin, although not a common one, incorporates features usually associated with flavonols from higher plant. Kaempferol (11), quercetin(12) and less frequently myrcetin (13) occur singly or jointly in a vast proporation of analysed species[40].

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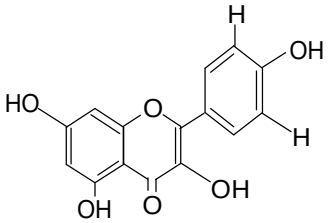


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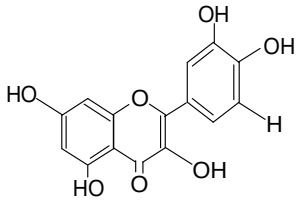


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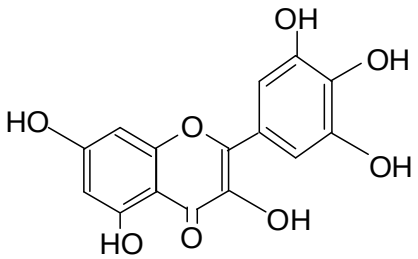
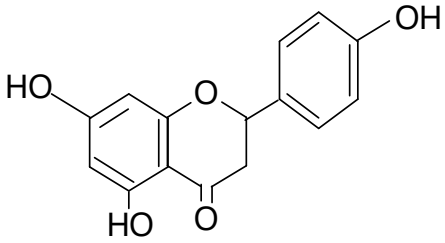



(13)

## 1.3-The Flavanones

Unlike the yellowish flavones, flavanones are colourless[23]. Hydroxylated flavanones may exist either as aglycones or in the form of glycosides. They are distributed in different parts of the plant ; in flowers, fruits, leaves, barks and roots and appear to be of fairly general distribution, especially in higher plants[75].

Flavanones, unlike the flavones , are saturated between 2- and 3 position and thus lacking the conjugation of the 2- phenyl group (B- ring)[28,75]. Common examples are: naringenin (14), eriodictyol (15)and hesperetin(16).

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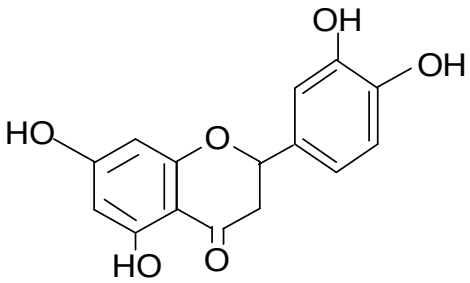


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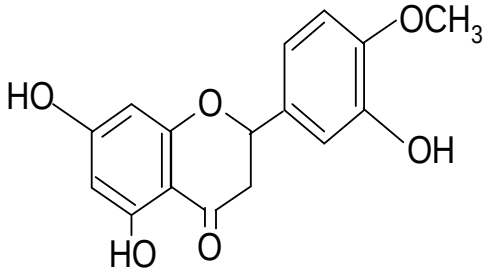



(16)

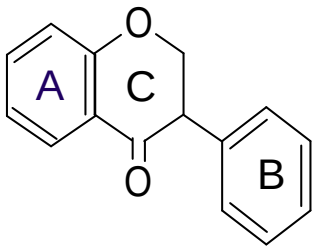
The dihydropyrone ring of the flavanones may be opened between O(1) and CH(2), giving rise to chalcones .Such ring opening occur when flavanones are reacted with acetic anhydride[76].

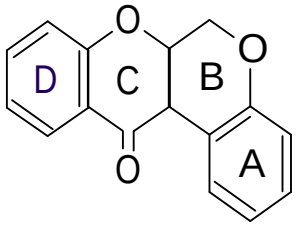
Flavanones isomerizes to the corresponding chalcones by ring- fission and this transformation is mediated by alkalis. Flavanones decompose to benzaldehyde, acetic acid and phenol when a strongly alkaline reaction mixture is heated drastically.Some flavanones give bright yellow-green or light-blue colours on paper when viewed in UV-light with the help of ammonia vapour, but this is not reliable enough to be used as diagnostic test[77].

Flavanones are characterized by unique spectral properties from other flavonoids, with one intense peak at about 225nm, and another one at either 278nm or 288nm, and a weak peak or inflection above 300nm. In some cases, flavanones undergo ring opening or ring fission in alkaline solution and are readily converted to the corresponding chalcone[23].

**1.4- The isoflavones**

The isoflavones (17) are comprising a large class of phytochemicals. Structurally they are closely related to the rotenoids (18)[75] .



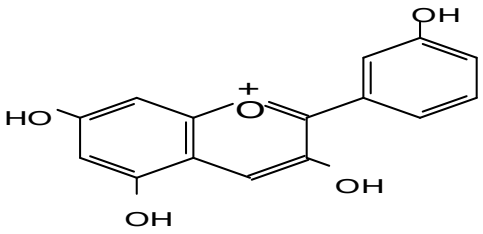

(17) (18)

Isoflavones are not as widespread as the flavonols and flavones. They occur either as aglycones or as glycosides[78] and the glycosides of isoflavones have been known since a very early date. Natural glycosides, however, have been reported, mainly as a result of more systematic analysis of plant extractives . The majority of glycosides are (7-glucosides) or (7- rhamnosylglucoside) and (4- glucosides) or (4- rhamnosylglucosides)[39.]

The chemical characterization of isoflavones is difficult since they do not respond specifically to any specific color reaction. Some isoflavones give a light blue colour in UV- light in the presence of ammonia, but most other appear as dull- purple absorbing spot, changing to dull- brown with ammonia[40]. In fact in some early investigations, isoflavones have been mistaken for flavones owing to their similar behavior in certain colour tests. Various color reaction may be used to test for benzopyrone structure, but they do not apparently differentiate between flavones and isoflavones[75.]

Another important class of flavonoids is known as anthocyanins, which are the most important and widespread group of colouring matter in plant. These intensely-coloured, water-soluble pigments are responsible for nearly all the pink , scarlet, red, mauve, violet and blue shades in petals, leaves and fruits of higher plant.

The magenta coloured - cyanidin (19) - is the most common anthocyanin. This class of flavonoids is based chemically on the structure of the cyandin. All anthocyanins are derived from cyandin by addition or subtraction of hydroxyl groups or by methylation or glycosylation. Orange-red are due to pelargonidin (20), with one less hydroxyl group than cyandin, while mauve, purple and blue colour are generally due to delphinidin (21), which has one more hydroxyl group than cyaniding.

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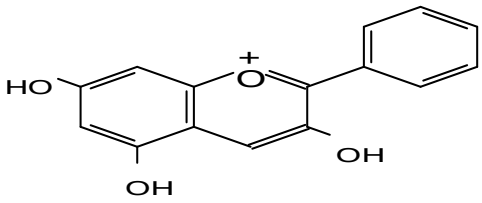


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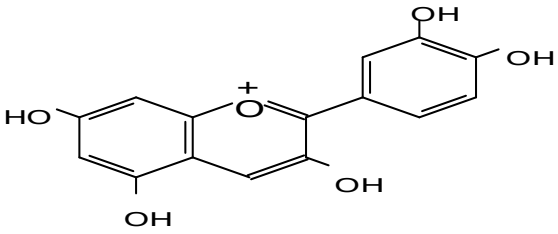
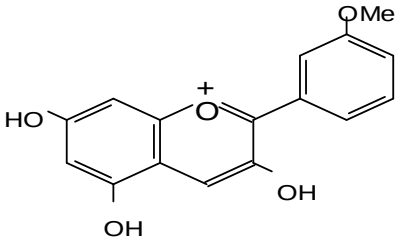



(21)

Also among common anthocyanins are; peonidin(22), petunidin(23) and malvidin(24):

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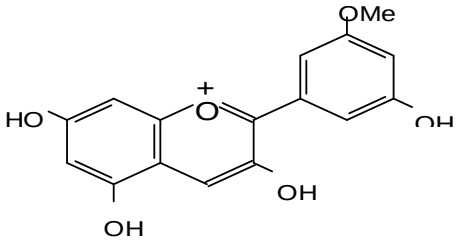


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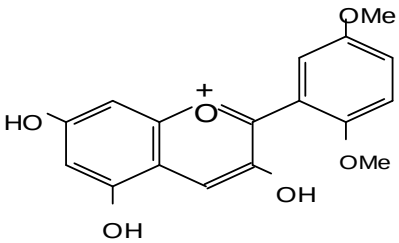



(24)

These anthocyanidin may occur as glycosides, the main varying in nature of sugar , the number of sugar units (mono, di, tri, glycoside) and the position of attachment of the sugar [67].

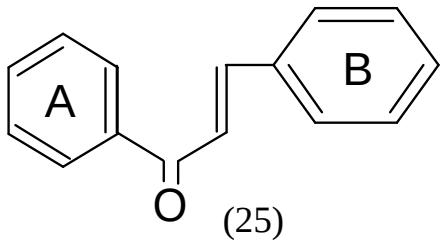
Anthocyanins impart colors to fruits, flowers and leaves, when they do occur in other parts of plant they are often confined to, or occur in highest concentration in one kind of tissue. Deeply coloured flowers may be born on plant with essentially anthocyanin in tree stems and leaves. In general, the capacity of plant to synthesize anthocyanin result in the formation of at least traces of pigment in the green parts of plant. Occasionally, heavy anthocyanin pigmentation cause plant leaves and stems acquire red or brown colour, examples are found in the conspicuous coloration of many autumn leaves, and in the colour of young leaves of some plants[75].

In the UV anthocyanins give two absorption bands. band **I**, 475-560 nm , and band **II**, 275 -280 nm. Band **I** depends on the number and position of hydroxyl and methoxyl groups[78].

### 1.5-The Chalcones

Chalcones lack the heterocyclic (C) ring .They possess two aromatic rings joined by a three carbon , ά,β- unsaturated carbonyl system[79].In chalcones the two aromatic rings ( A and B) are linked by an aliphatic three carbon chain which does not participate in forming a hetero ring as is usually found in other types of flavonoid compounds.

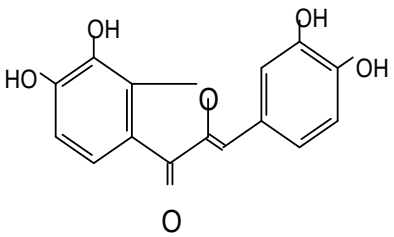
Natural chalcones are all hydroxylated to greater or lesser extent[79].



(25)

Chalcones are known to play an ecological role in nature, in relation to plant colour. These coloured phytochemicals are found in many plant organs, but most conspicuously in flower. Most yellow flower colour is due to the presence of caroteniods, but in the case of certain member of Compositeae, Couthaceae, Oxalidaceae, etc, the chalcones contribute significantly to the corolla pigmentation.

Chalcones, in paper chromatography give intense deep brown UV colour . On fuming the paper with ammonia, the color may change to rich deep red, although a few chalcones fail to respond in this way. Chalcones are easily separated by paper charomotography in the usual solvents. In the UV chalcones absorb at $\lambda_{max}$ 365-390 nm, which distinguishes them from aurones $\lambda_{max}$ 390-430nm[80]. The aurones are colored flavonoids found in many flowered species[39]. Aurones are secondary metabolites and structurally isomeric with flavones, which are widely distributed in fruits and floweres, and play significant role in the pigmentation of the part of plant in which they occur. They are not restricted to floral tissues, but have been obtained from bark, wood and leave as well. First aurones were discovered only in 1943. Aurones however, have a limited occurrence and limited method of synthesis.[30.41] Chemically aurones are on the (2- benzylidene- coumaranone) or (2- benzylidene -3 (2H)- benzofuranone) system[39]. In aurones there is a 5-membered C- ring. Examples of aurones are : maritimetin(26) and resorcinol (27)[67].

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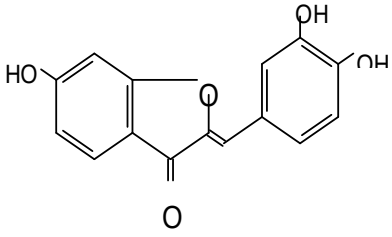



27

Chalcones and aurones show UV absorption in the range : 260-420nm and the absorption spectra can differentiate between the two types of pigments since chalcones and aurones show quite different absorption spectra. The study of the spectra of acetates of chalcones and aurones is particularily instructive. The absorption spectra of two series of aurone pigments and their derivatives have been determined and compared[81]. Aurones appear on paper chromatograms as yellow spots in day light, however, in the uv- light, they are very different, the colour of aurones is an intense bright yellow, changing with ammonia to bright orange- red.

Analogy have interesting biological properties[82]. Some aurones were synthesized and tested for the ability to inhibit erythrocyte stages of *Plasmodium flaciparun* strains. Some of these compounds exhibit antiplasmodial activity in the micro range[83,84].

**1.6- Medicinal uses of flavonoids**

Pharmacological and chemical screening of plant used in traditional medicine have provided important advances in the therapeutic approach to several pathologies. A number of medicinal plant containing flavonoids and alkaloids are used in traditional medicine and are known to contain important therapeutic agents[85].

Some plants used in ethnomedicine contain flavonoids with anti-inflammatory, anti-allergic, anti-thrombotic and vasodilatory activities[86], also some have anti-viral and anti- bacterial properties[87] .Flavonoids exist in many formulations of ethnomedicine including treatment for cardiovascular disease , peripheral vascular disease, stroke and cancer[88]. Several epidemiological studies provided support for a protective effect of the consumption of fresh fruit and vegetable against cancer[89], heart disease and stroke[68].

Ingested flavonoids enter the plasma, to elevate the redox and anti- oxidant levels. The physiological benfits of flavonoid are generally thought to be due to their anti-oxidant and free radical scavenging properties[90].

Among the powerful antioxidants is quercetin (12). It also exhibits anti-flammatory and was found to inhibit both tumour promoter and human cancer cell[91].

A Major function of flavonoids is their accumulation as phytoalexins, which protect plant from microbial invasion[92]. Phytoalexins are compounds that are formed in response to microbial or other invasions. Naringenin (14), found in the heartwood of trees from the Rosaceae is an anti-fungal agent[93]. It can also function as stress protectants in plant cell by scavenging reactive oxygen species (ROS) produced by the photosynthetic electron transport system[94]. Furthermore, because of their UV absorbing properties, flavonoid protect plants from the UV radiation[95]. The fungicidal properties of flavonoids are effected by phenolic substituation and in many cases it has been shown to decrease with increasing substitution[43]. Isoflavonoids, flavanones and flavones are the most effective anti- microbial agents[87].

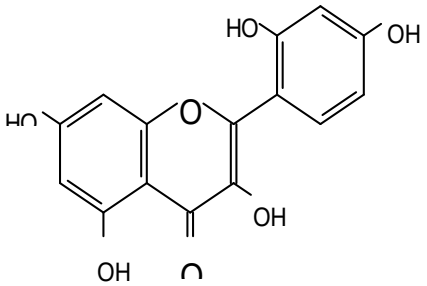
Anthocyanins are also used to treat skin diseases specifically dermatological hypersensitivity. Flavonoids may inhibit the enzyme involved in the glycosylation process, which gives rise to sorbitol which causes swelling complication in diabetes[96].

The biological activities of chalcone is including anti- bacterial, anti– cancer, anti-ulcer, anti-protozoal, amoebicidal , cytotoxic, and immune suppressive activates[97].

Flavonoids are usually found alongside vitamin C in nature. Studies have shown that vitamin C alone may not be effective as being supplemented with flavonoids. Flavonoids may correspondingly increase the amount of vitamin C in tissues, by preventing the break- down of this vitamin[98] .

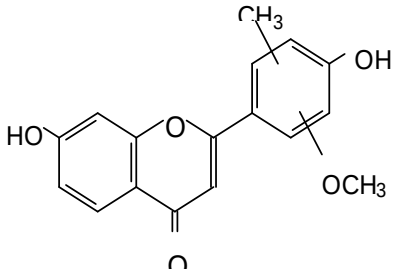
## 1.7-Some flavonoids from Sudanese plants

From the ethyl acetate extract of the leaves *Cassia occidentalis* (Leguminoseae) a flavonol: 5,7,2',4'-tetrahydroxyflavonol(28) was isolated[99] . The structure was elucidated via a combination of spectral techniques(UV,IR,$^{1}$H NMR and MS ). The flavonol was evaluated *in vivo* for anti-inflammatory and anti-ulcer activity.Treatment of the animal models with compound I inhibited formaldehyde-induced oedema up to 93.99%.The flavonol also showed significant anti-ulcer activity.Treatment with compound 28 (30)min. before alcohol-induced ulcer completely suppressed ulcer.



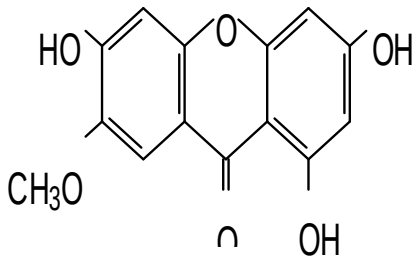

(28)

From ethyl acetate extract of the leaves of *Combretum aculeatum* a flavones(29) was isolated[100] via column and paper chromatography. The structure of the flavone was partially elucidated by a combination of spectral techniques (UV, IR, 1HNMR and MS). The methanolic and ethyl acetate fractions were evaluated for their antimicrobial activity. The methanolic extract showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. It also exhibited moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. This extract also exhibited significant antifungal activity against the fungi: *Candida albicans and Aspergillus niger*. However, the ethyl acetate extract was devoid of activity.



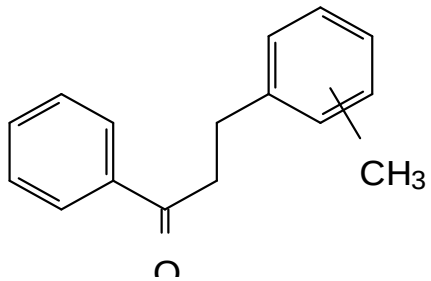

( 29)

**A** xanthone (30) was isolated[101] from the chloroform extract. The structure of this compound was established by spectral methods (IR, UV,$^{1}$H-NMR,$^{13}$C-NMR, HMQC,HMBC and MS). The crude extractives as well as the isolated compound were evaluated for their antibacterial and larvicidal potential. The antibacterial activity test against four standard microbes, *Staphylococcus aureus* (ATCC25923- G +ve), *Escherichia coli* (ATCC25922- G –ve), *Pseudomonas aeruginosa* (ATCC27853- G-ve) and *Proteus vulgaris* (NCTC8196). The crude chloroform and ethyl acetate extracts of *Garcinia mangostana* gave moderate inhibition against *S. aureus* only. The pure compound (30) showed moderate activity against *S. aureus*. The hexane, chloroform and ethylacetate crude extracts of *Garcinia mangostana* were screened for larvicidal activity against *Aedes aegypti*. The crude chloroform was very active, the crude hexane was moderately. The crude ethyl acetate and compound (30) devoid of activity.



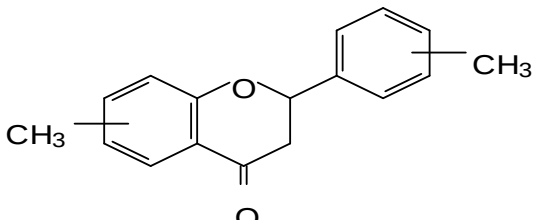

( 30 )

*Acacia melifera* – Known locally as "Kitir" – is a commonly occurring plant throughout the savannah in southern, eastern and western Africa. The plant has many potential medical benefits and is widely used in African system of medicine.A methylated dihydrochalcone(31) was isolated[102] from bark and its structure was partially elucidated via a combination of spectral techniques (UV-Vis. and 1HNMR and MS).



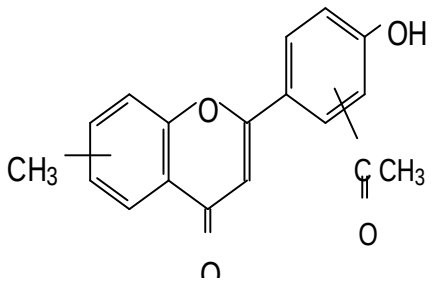

(31)

Genus *Mitragyna* is distributed in tropical and subtropical regions of Africa and Asia. Different species of *Mitragyna* are widely used in ethnomedicine against malaria, fever, worms, diarrhea and cough. *Mitragyna speciosa* is a natural remedy for fatigue. *Mitragyna ciliate, Mitragyna inermis and Mitragyna stipulosa* have been used traditionally against hypertension, inflammation, rheumatism, gonorrhea and bronchpulmonary diseases. *Mitragyna africanus* is used traditionally against mental diseases. In this study stem barks of *Mitragyna inermis* were extracted with 95% ethanol and the crude extract was fractionated over silica gel plates to yield a chromatographically pure flavonoid – compound (32). The structure of the isolated[103] compound has been partially characterized by its spectral data (UV and $^{1}$HNMR).



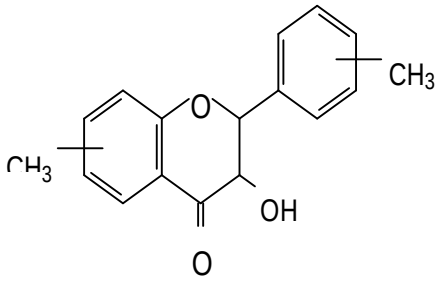

(32 )

*Pulicaria crispa* is used in folk medicine for the treatment of colds, coughs, colic pain, excessive sweating and as carminative. Phytochemical screening of *pulcaria crispa* stems rev*ea*led the presence of flavonoids. A flavonoid (33) was isolated[104] from the ethanol extract by column and paper chromatography and its structure was partially characterized on the basis of its spectral data (IR, UV and NMR). The crude extract and different fractions (chloroform, n- butanol and ethyl acetate) of *pulcari crispa* were screened for their antimicrobial activity against five standard human pathogens. The ethyl acetate fraction of *pulcaria crispa* showed moderate activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans.* However, the ethanol extract and chloroform fraction were inactive against the tested bacteria, but they gave partial antifungal activity against the fungus *Candida albicans.* The n-butanol fraction exhibited partial activity against *Staphylococcus aureus* and *Candida albicans.*




( 33 )

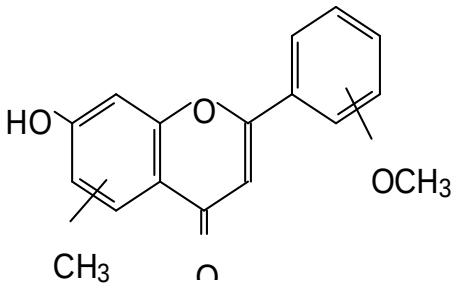
Phytochemical screening of *Cistanch phelypea* stems revealed the presence of flavonoids, tannins steroids , saponins and alkaloids. A flavonoid - compound (34)- was isolated[105] from ethanol extract by thin layer chromatography and its structure was partially characteized on the basis of its spectral data (IR, UV, NMR and MS). The ethanol extract of *Cistanch phelypea* was screened for antimicrobial activity against five standard human pathogens : *Bacillus subtilis* (Gram((+ve) , *Staphylococcus aureus* (Gram (+ve), *Pseudomonas aeroginosa* (Gram(–ve) , *Escherichia coli*(Gram (–ve) and the fungal species *Candida albicans* .The ethanolic extract of *cistanch phelypea* was inactive against the test bacteria ,but it gave good antifungal activity against the yeast : *Candida albicans* .In the DPPH assay , the ethanol extract of *Cistanch phelypea* stems exhibited significant antioxidant activity close to that of the positive standard propyl gallate




(34)

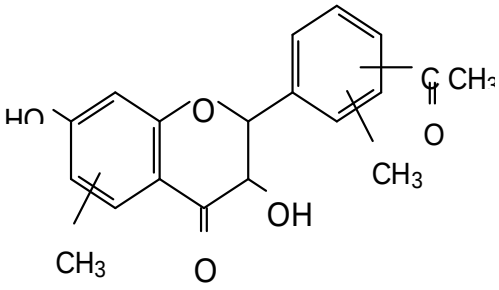
*Terminalia brownii* is widely used in African system of medicine as a natural remedy for cough, tonsillitis, typhoid, tooth-ache, snake bite and rheumatic pain . Phytochemical screening of *Terminalia brownii* root rev*ea*led the presence of flavonoids , alkaloids,steroids, tannins, terpenes, saponins and coumarins. A flavonoid - compound (35)- was isolated[106] from the ethanol extract by paper chromatography and its structure was partially characterized via some spectral data ( UV and 1HNMR).In the agar diffusion bioassay,the ethanol extract showed significant activity

against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* It exhibited moderate activity against *Escherichia coli* and the yeast *Candida albicans* . It also showed moderate free radical scavenging capacity against stable DPPH radicals.




(35 )

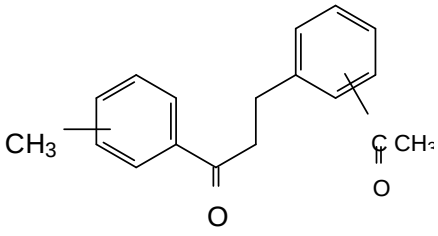
Phytochemical screening of *Wetheria indica* stems revealed the presence of flavonoids, tannins steroids , saponins and alkaloids. A flavonoid - compound (36)- was isolated[107] from ethanol extract by thin layer chromatography and its structure was partially characteized on the basis of its spectral data (IR, UV, NMR and MS). The ethanol extract was screened for antimicrobial activity against five standard human pathogens : *Bacillus subtilis* (Gram((+ve) , *Staphylococcus aureus* (Gram (+ve), *Pseudomonas aeroginosa* (Gram(–ve) , *Escherichia coli*(Gram (–ve) and the fungal species *Candida albicans* .The ethanolic extract of *cistanch phelypea* was inactive against the test bacteria ,but it gave good antifungal activity against the yeast : *Candida albicans* .In the DPPH assay , the ethanol extract of stems exhibited significant antioxidant activity close to that of the positive standard propyl gallate



(36)

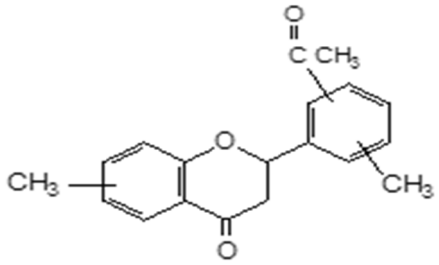
*Bauhinia* species are trees up to 6-12m in height. Traditionally, leaves and stem barks have been used against pain, inflammation, dysentery, mycosis, diarrhea and diabetes. *Bauhinia rufescens* Lam. is a tropical forage up to 8 m in height. In African system of medicine, the plant has been used against gout, leprosy, and malaria.

The major flavonoid of *Bauhinia rufescens* roots was isolated[108] the antimicrobial activity of the crude ethanolic extract and the isolated flavonoid were assessed. The flavonoids were extracted with ethanol and the crude extract was purified by paper chromatography where a pure flavonoid (37) was isolated. The structure of this compound has been partially characterized by some spectral tools (UV, IR and $^{1}$HNMR). In the antimicrobial assay, the crude ethanolic extract showed better inhibitory effect against a panel of human pathogens compared to compound 37. While the crude extract showed significant antibacterial and antifungal properties, compound 37 exhibited moderate antibacterial activity and weak antifungal properties.



(37)

The flavonoids of *Albizia amara* stem bark was investigated and the antibacterial activity of root ethyl acetate and n-butanol fractions was screened. The flavonoids were extracted with ethanol and the crude extract was purified by paper chromatography where a flavanone(38) was isolated[102]. The structure of this compound has been partially characterized by UV and $^{1}$HNMR data. In the antimicrobial assay , the ethyl acetate and n-butanol fractions from *Albizia.Amara* roots were assessed for antibacterial activity.These extracts showed responses against the bacterial strains : *Streptococcus mutans* and *Lacto bacillus* . Both of the ethyl acetate and the n-butanol fractions showed significant activity against *Lacto bacillus* within the test concentracions(100,200mg/ml). However, within the test concentrations, the ethyl acetate fraction was more effective against *Streptococcus mutans* than the n-butanol fraction.



(38)

## 1.8-The target plant species

### 1.8.1- *Cistanche phelypea* L.

*Cistanche* is a genus of about twenty species in the family Orobanchaceae[109]. The genus is widely distributed in Arica, Mediterranean region and Asia where it grows in arid and semi-arid areas[110]. Some of these species are widely used in ethnomedicne against various ailments including kidney disorders, infecions, infertility, constipation, profuse metrorrhagia[111]. Inflammation[112]. They are also used as smooth muscle

relaxant[113] .Some phytochemicals like iridoids , lignans,phenylethanoid glycosides and alkaloids have been reported from Cistanche genus[114-118].

*Cistanche phelypea* L. is a perennial plant. The plant is used traditionally against diabetes, diarrhea, infections, intestinal disorders and as diuretic[119-120].

*Cistanche phelypea*

### 1.8.2- *Wetheria indica* L.

*Wetheria indica*

*Wetheria indica* L. (also called monkey bush, buff coat, marsh-mallow) is a plant in the family Sterculiaceae. The plant is native to Florida and Texas and widely distributed along tropics and worm tropics. In ethnomedicine, *Wetheria indica* is a plant of many attributes. It has been used in folklore medicine for the remedy of many pathologies in Hawaii[122] ,India[123] ,South[124] America[125] ,South Africa[126] ,East Africa[127] and West Africa[128] . *Wetheria indica* is used as tonic, analgesic, astringent, purgative and febrifuge[129].Various extracts of *Wetheria indica* have been used traditionally against cough, skin diseases and infertility[130]. The plant is also used as immunomodalatory[131] and as a natural remedy for internal hemorrhage, syphilis[132] , inflammation and circulatory problems[133,133]

### 1.8.3- *Fagonia critica*

*Fagonia critica* is a plant in the famile Zygophyllaea. *Fagonia* is a genus of 34 species disributed in worm and dry areas of all continents [134] .The anti-inflammatory, antiemetic, astringents and febrifuge properties of

*Fagonia critica* has been reported[135] . The plant is useful for the treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharge, liver trouble, typhoid, toothache, stomach trouble and skin diseases[136]. Aqueous extract of *Fagonia critica* is used traditionally to induce abortion. The plant it is applied externally as poultice for tumors and other swelling of the neck and an aqueous decoction of the herb is popular treatment for cancer in the indigenous system of medicine[137].

It has been reported that *Fagonia critica* possesses antidiabetic action when used in blending with stem of *Aloe vera* and fresh branches of *Tylophora herustal* [138]. The plant showed *in vitro* activity of dipentidyl ppeptidase-4 (DPP-4P) prevention, due to presence of quinonic acid which can be helpful in diabetes control[139]. *Fagonia critica* is used in some Asian countries as a medicine for snake bite[139].

*Fagonia critica*

**Aim of this study**

This study was carried out to:

-Extract flavonoids from three Sudanese medicinal plants : *Fagonia critica, Wetheria indica* and *Cistanche phelypea.*

-Elucidate the partial structures of the isolated flavonoids.

-Ev*aluate* the studied plants for their antimicrobial potential.

-Assess the antioxidant activity of the target plants.

# 2-Materials and Methods

## 2.1-Materials

### 2.1.1-Plant material

*Cistanche phelypea* stems,*Waltheria indica* stems and *Fagonia critica* leaves were collected from Omdorman , Sudan. The plant was authenticated by the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum.

### 2.1.2-Instruments

IR spectra were run on a Shimadzu IR spectrophotometer, UV spectra were run on a Shimadzu 2401PC UV- Visible Spectrophotometer. NMR spectra were measured on a Joel ECA 500MHZ NMR Spectrophotometer.

### 2.1.3-Test organisms

The following standard microorganisms were used to assess the antimicrobial potential of the target plant extracts *: Bacillus subtilis* (Gram (+ve), *Staphylococcus aureus* (Gram(+ve), *Pseudomonas aeroginosa* (Gram –ve) , *Escherichia coli* (Gram – ve) and the fungal species *Candida albicans.*

## 2.2-Methods

### 2.2.1-Preparation of plant extract for phytochemical screening

(200 g) Of powdered air- dried plant material were extracted with 95% aqueous ethanol by maceration . This prepared extract (PE) was used for phytochemical screening . Phytochemical screening was accomplished according to the method described by Harborne[19].

### 2.2.2-Extraction and isolation of flavonoids

(1 kg) of powdered air-dried plant material was macerated with 95% ethanol (5L) for 48hr at room temperature . The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated under reduced pressure at $40^{o}$ C yielding a crude product.This crude product was applied on silica gel plates as narrow zones.The plates were developed with 40% acetic acid. After the usual workup a chromatographically pure flavonoid-compound I- was isolated.

### 2.2.3-Biological activity

### 2.2.3.1-Antimicrobial assay

By using the agar diffusion bioassay, plant extracts were assessed for antimicrobial activity against four standard pathogenic bacteria and one pathogenic fungus : (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans* ) .

### (i)-Preparation of bacterial suspensions

Broth cultures of the test organisms were distributed into agar slopes and incubated at $37^{o}$C for 24 hours. Bacterial growth was harvested and suspended in 100 ml of normal saline to give about $10^{8}$-$10^{9}$ colony forming units(CFU) per ml. The Average number of viable organism per ml was determined using the surface viable counting technique.

Serial dilutions of the stock suspension were prepared in sterile normal saline. (0.02 ml) of the appropriate dilution was transferred into the surface of dried nutrient agar plates. After drying, the plates were incubated at 37$^{o}$ C for 24 hours. Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25$^{o}$C for four days.

## (ii)-Testing for antimicrobial activity

(2 ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45$^{o}$ C in a water bath. (20 ml) aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and each plate was divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 4).Agar discs were removed and cups were filled with (0.1 ml) of each test sample and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37$^{o}$ C for one hour. Tests were performed in duplicates. After incubation the diameters of the resultant growth inhibition zones were measures and averaged.

For antifungal activity, instead of nutrient agar, Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

## 3.3.3.2-Antioxidant activity

The test sample was dissolved in DMSO while DPPH was prepared in ethanol[22]. After incubation, decrease in absorbance was measured at 517nm. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests were run in triplicate.

# 3-Results and Discussion

This study was designed to investigate the flavonoids of three medicinal plants of many attributes to Sudanese system of traditional medicine. These plants are: *Wetheria indica* L. , *Cistanch phelypea* and *Fagonia critica*. Three flavonoids ,one flavonoid from each plant, have been isolated and their tentative structures have been deduced on the basis of their spectral data. Furthermore the antimicrobial activity of these plants have been screened *in vitro*.

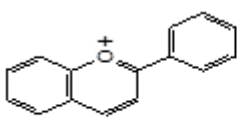
## 3.1-*Waltheria indica*

### 3.1.1-Characterization of compound I

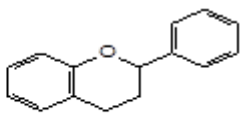
Phytochemical screening of the leaves of *Waltheria indica* showed the presence of tannins, flavonoids , saponins, steroids , and alkaloids. From the ethanol extract of *Waltheria indica* , a flavonoid- compound I- was isolated by thin layer chromatography and its structure was elucidated partially via a combination of spectral techniques (UV, IR, $^{1}$HNMR ).

The IR spectrum of compound I showed a pattern characteristic of flavonoids. It revealed ν (KBr): 829 (C-H, Ar. ,bending),1174(C-O) ,1560,1598 (C=C, Ar.),1699 (C=O) , 2804 (C-H,aliph, stretching) and 3733cm$^{-1}$(OH).

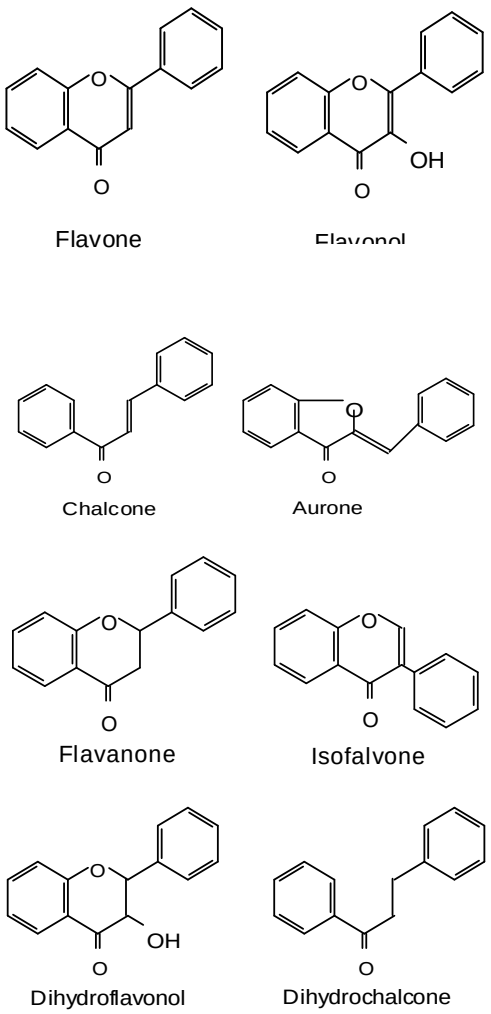
Flavonoids are classified into : flavones, flavonols, chalcones , aurones, flavanones, dihydroflavonols, dihydrochalcones ,isoflavones, anthocyanins and flavans.



Anthocyanin



Flavan




Among these classes, anthocyanis and flavans are easily detected via their IR spectra. These classes lack the 4- keto function which distinguishes the 8 other classes.

Isoflavones, flavanones, dihydrochalcones and dihydroflavonols all give similar UV spectra as a result of having little or no conjugation between the A- and B-rings. In their UV spectra, they typically exhibit an intense Band II absorption with only a shoulder or low intensity peak representing Band I. Due to effective conjugation between the two aromatic rings of flavonoids(rings A and ring B), the flavones, flavonols,chalcones and aurones show both band I and II. In flavonoids band I(in the range:300-400nm) is associated with the absorption of the cinnamoyl chromophore , while band II( the range:235-290nm) originates from absorption of the benzoyl chromophore.

The UV spectrum(Fig.1) of compound I showed $\lambda_{max}$ 276nm, i.e. it gave only one band- band II. Such absorption is characteristic of flavanones, dihydroflavonols, dihydrochalcones and isoflavones.

The band II absorption of isoflavones usually occurs in the region 245 - 270 nm with a characteristic shoulder in the UV range : 300-340nm. However, such shoulder was not detected in the UV spectrum of compound I (Fig.1).

The UV shift reagent : sodium methoxide is used in the chemistry of flavonoids for the specific detection of 3- and 4`-OH functions. In the presence of both groups it exhibits a characteristic bathochromic shift but with decrease in intensity in case of a 3-OH substituent.However , the sodium methoxide spectrum (Fig. 2) revealed a bathochromic shift with decrease in intensity.Such shift is diagnostic of a 3-OH function.Hence the isolated flavonoid is a dihydroflavonol.

Next the hydroxylation pattern on the nucleus of the flavonoid was investigated using different UV shift reagents : sodium acetate(diagnostic of a 7-OH) and boric acid (diagnostic of catechols).

The sodium acetate spectrum (Fig.3) showed a bathochromic shift characteristic of a 7-OH function. However, the boric acid spectrum (Fig.4) failed to afford any bathochromic shift indicating absence of catechol systems.

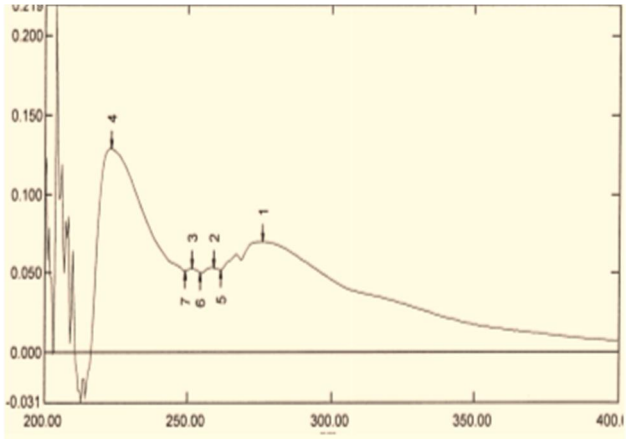



Fig. 1: UV spectrum of compound I

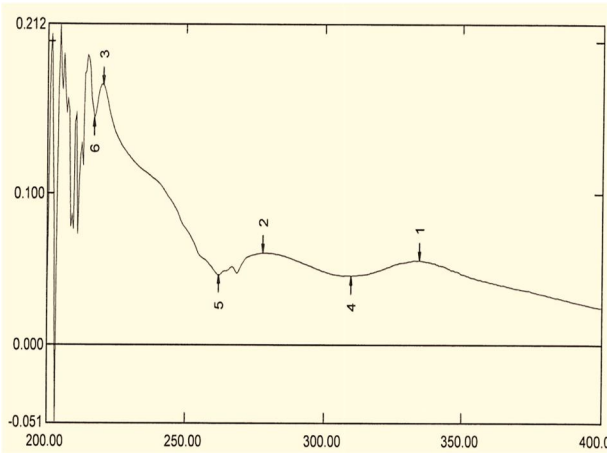



Fig.2: Sodium methoxide spectrum of compound I

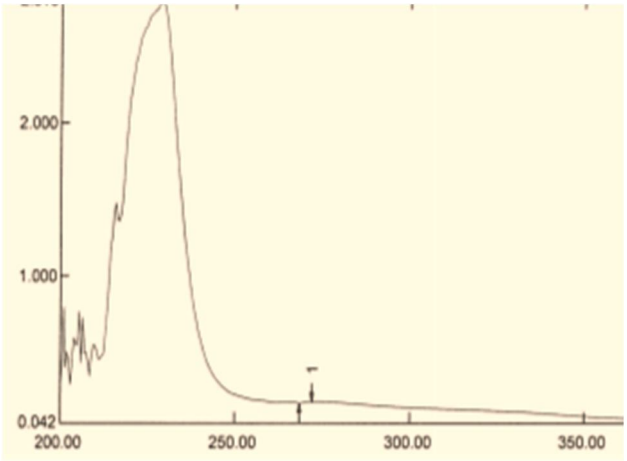



Fig.3: Sodium acetate spectrum of compound I

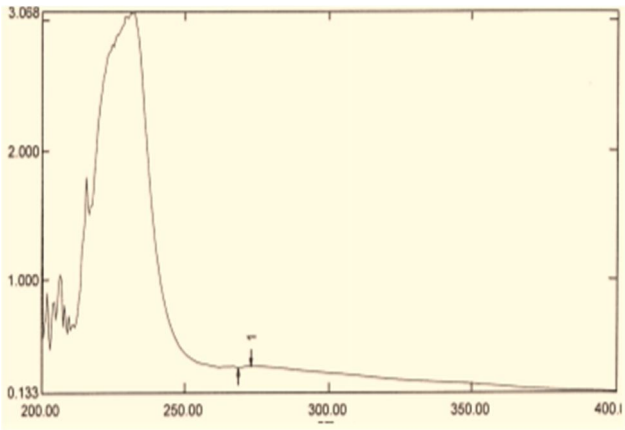
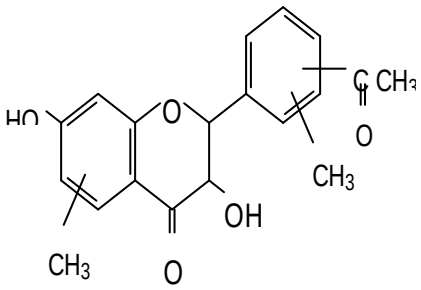



Fig. 4: Boric acid spectrum of compound I

The $^{1}$HNMR spectrum(Fig.5) showed , δ(ppm : 1.23,1.62 - assigned for two methyl groups. The signal at δ2.00ppm was assigned for an acetyl group. The resonance at δ6.61 , 7.20 and 8.50 ppm accounts for the aromatic protons. The signals at δ2.50 and δ3.40 ppm are due to the solvent(DMSO) residual protons and residual water respectively. On the basis of the its spectral data , the following partial structure was propososed for compound I:




Compound I

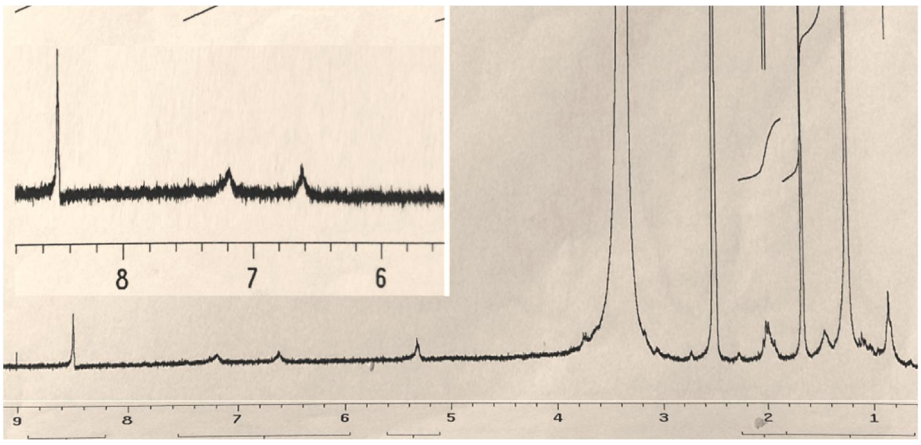



**Fig. 5 :** $^{1}$HNMR spectrum of compound I

## 3.1.2-Antimicrobial activity

The ethanolic extract of *Wetheria indica* stems was screened for the antimicrobial activity against five standard microorganisms(Table 1).The inhibition zones are shown in Table (2) .Results were interpreted as follows : (>9mm: inative;9-12mm:partially active;13-18mm:

active;<18mm:very active) . The ethanolic extract showed moderate antibacterial and antifungal activity against test organisms(Table 2). Ampicilin , gentamycin and clotrimazole were used as positive controls(Tables 3 and 4).

**Table 1:** Test organisms

| Ser. No. | Moicroorganism | Type | Source |
|---|---|---|---|
| 1 | *Bacillus subtillus* | G+ve | ATCC 2836 |
| 2 | *Staphylococcus aureus* | G+ve | ATCC 29213 |
| **3** | *Pseudomonas aeroginosa* | G-ve | NCTC 27853 |
| 4 | *Escherichia coli* | G-ve | ATCC 25922 |
| 5 | *Candida albicans* | fungi | ATCC 7596 |

* NCTC. National collection type culture, Colindale. England

*ATCC. American type culture collection, Maryland, USA

**Table 2 :** Antibacterial activity of ethanolic extract

| Extract | Conc.(mg/ml) | **Sa** | Bs | Ec | Ps | Ca |
|---|---|---|---|---|---|---|
| Ethanolic extract | 100 | 15 | 14 | 14 | 14 | 13 |

**Table 3** : Antibacterial activity of standard chemotherapeutic agents

| 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 4 :** Antifungal activity of standard chemotherapeutic agent

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

## 3.1.3-Antioxidant activity

The antioxidant capacity of the ethanol extract of *Wetheria indica* stems has been measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test extract against stable DPPH radical. The change in color was measured spectrophotometrically at 517 nm. As displayed in (Table 5) the extract exhibited significant free radical scavenging capacity.

**Table 5:** Radical scavenging activity of ethanol extract

| Sample | Antioxidant activity |
|---|---|
| Propyl gallate | 92.00% |
| Ethanol extract | 89.02% |

## 3.2- *Cistanch phelypea*

## 3.2.1-Characterization of compound II

Phytochemical screening of the leave of *Cistanch phelypea* unmask the presence of tannins, flavonoids , saponin, steroids ,triterpen, and alkaloids.

From the ethanol extract compound I was isolated by thin layer chromatography and its structure was elucidated via a combination of spectral techniques (UV, IR, $^{1}$HNMR).

The IR spectrum of compound I (Fig.6) revealed ν (KBr): 675, 823 (C-H, Ar..,bending),1099 (C-O), 1454,1514 (C=C, Ar.),1704 (C=O) , 2808 (C-H,aliph, stretching) , 3303 and 3733cm$^{-1}$ (OH).

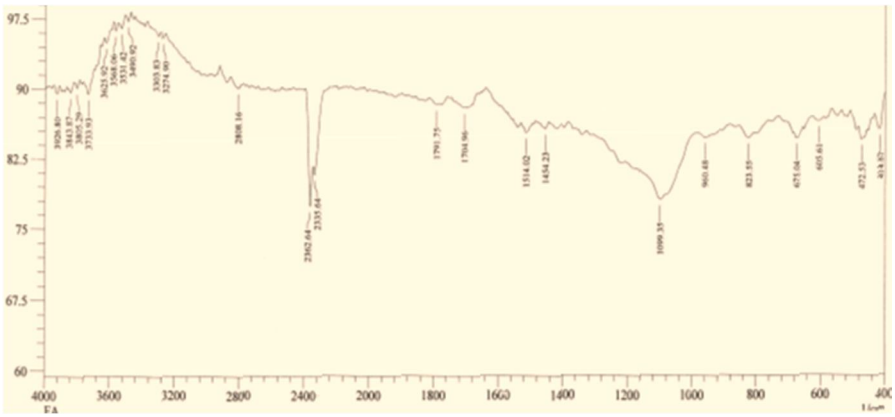



Fig.6: The IR spectrum of compound I

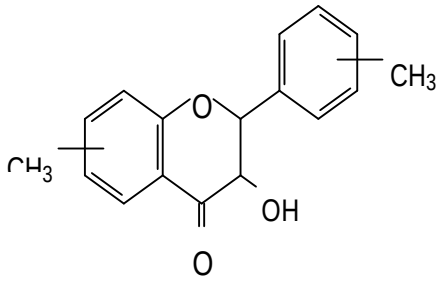
In the UV, compound II absorbs at $\lambda_{max}$274 (Fig.7). Such absorption is characteristic of : flavanones, isoflavones, dihydrochalcones and dihydroflavonols . The characteristic shoulder of isoflavones-which appears in the range 300-340nm- was not detected in the UV spectrum of compound I. However, the sodium methoxide spectrum(Fig.8) showed a bathochromic shift with decrease in intensity suggesting the presence of a 3-OH which is a characteristic feature of dihydroflavonols. Hence compound II is a dihydroflavonol.

Different UV shift reagents were used to illustrate the hydroxylation pattern on the nucleus of the isolated dihydroflavonol; these are (i) sodium acetate(detects 7-OH group) and (ii) boric acid (detects catechols).

The sodium acetate spectrum did not reveal any bathochromic shift suggesting absence of a 7-OH group(Fig.9).Also the boric acid spectrum(Fig.10) indicated absence of catechol moieties, it failed to show a bathochromic shift.

The $^{1}$HNMR spectrum of compound II (Fig.11) showed δ(ppm) : 1.23, 1.61 assigned for two methyl groups. The aromatic protons resonated at δ6.65,6.80,7.38 and 7.95ppm. The signals at δ2.50 and δ3.40 ppm are due the solvent (DMSO) residual protons and residual water respectively.

On the basis of the above argument the following partial structure was proposed for compound II:




Compound II

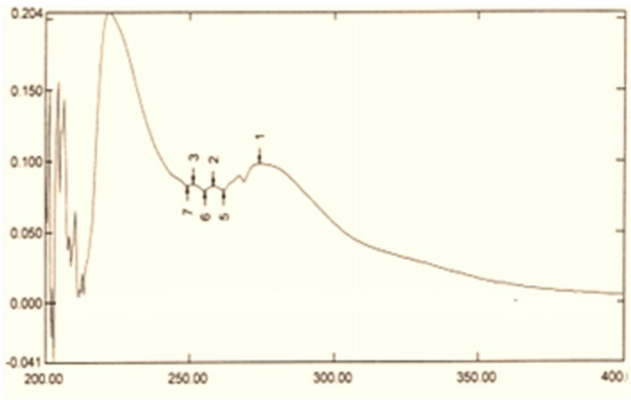



Fig.7: UV spectrum of compound II

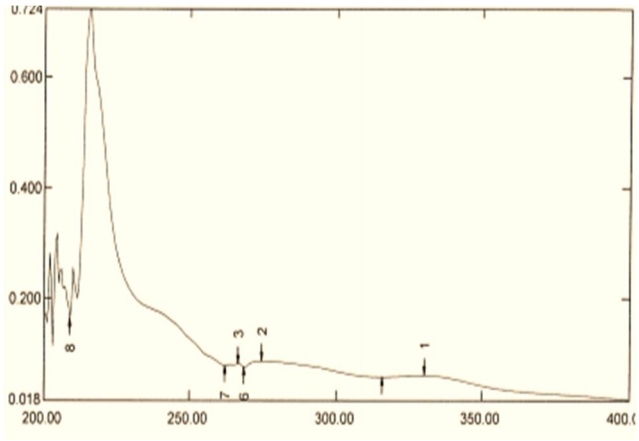



Fig.8: Sodium methoxide spectrum of compound II

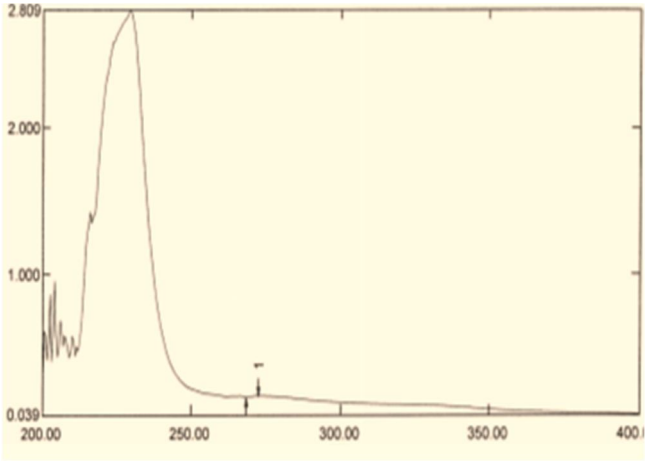



Fig. 9: Sodium acetate spectrum of compound II

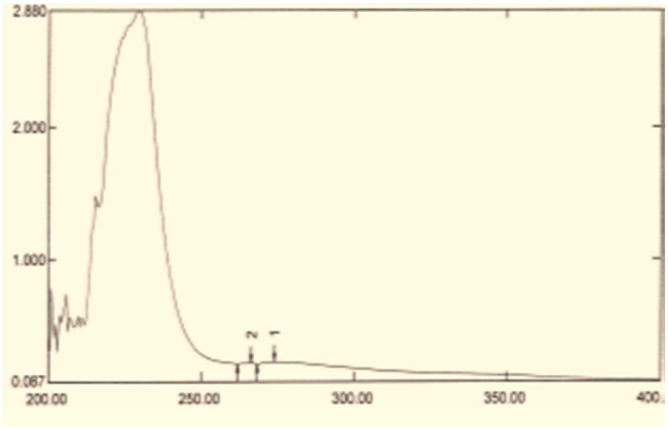



Fig. 10: Boric acid spectrum of compound II

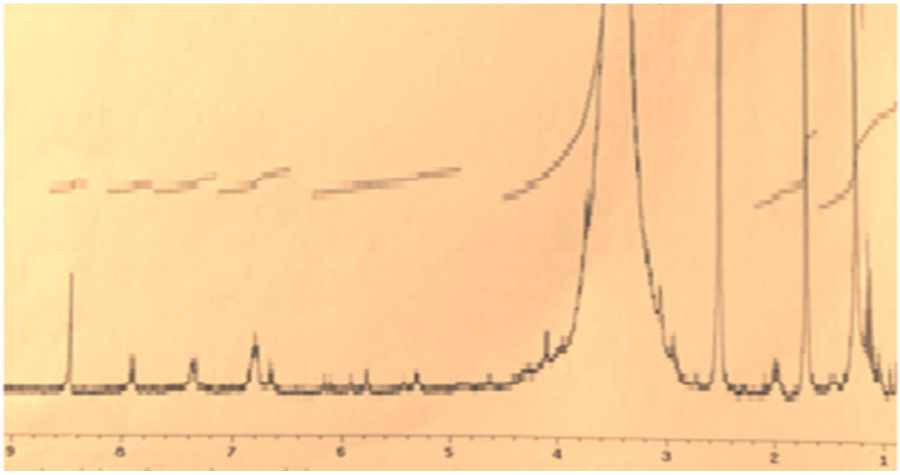


Fig. 11 : $^{1}$HNMR spectrum of compound II

## 3.2.2-Antimicrobial activity

The ethanolic extract of *Cistanch phelypea* was evaluated for antimicrobial activity against five standard microorganisms (Table 6).The results are depicted in Table (7) .Results were interpreted in the following terms : (>9mm: inative; 9-12mm:partially active; 13-18mm: active; <18mm:very active) .Ampicilin,gentamycin and clotrimazole were used as positive controls.

The ethanolic extract of *cistanch phelypea* was inactive against the test bacteria ,but it gave good antifungal acivity against the yeast : *Candida albicans* (Table 6).

**Table 6:** Test organisms

| No | **Micro organism** | Type | Source |
|---|---|---|---|
| 1 | *Bacillus subtillus* | G+ve | ATCC 2836 |
| 2 | *Staphylococcus aureus* | G+ve | ATCC 29213 |
| **3** | *Pseudomonas aeroginosa* | G-ve | NCTC 27853 |
| 4 | *Escherichia coli* | G-ve | ATCC 25922 |
| 5 | *Candida albicans* | fungi | ATCC 7596 |

* NCTC. National collection of type culture, Colindale. England

*ATCC. American type culture collection, Maryland, USA

**Table 7 :** Inhibition zones of ethanolic extract

| Fraction/ Comp | Conc.(mg/ml) | **Sa** | Bs | Ec | Ps | Ca |
|---|---|---|---|---|---|---|
| Ethanolic extract | 100 | --- | --- | --- | --- | 15 |

Sa.: *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Ca.: *Candida albicans*

Bs.: *Bacillus subtilis*

## 3.2.3-Antioxidant activity

The antioxidant capacity of the ethanol extract of *Cistanch phelypea* stem has been measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test extract against stable DPPH radical. The change in color was measured spectrophotometrically at 517 nm. As displayed in (Table 8) exhibited significant antioxidant activity close to that of the positive standard propyl gallate.

**Table 8:** Radical scavenging activity of ethanol extract

| Sample | Antioxidant activity |
|---|---|
| Propyl gallate | 92.00% |
| Ethanol extract | 91.03% |

## 3.3-*Fagonia critica*

## 3.3.1-Characterization of compound III

The IR spectrum of compound III (Fig.12) revealed ν (KBr): 671, 831(C-H,aliph.,bending),1072(C-O),1261(C-H,Ar, bending) ,1458,1564(C=C, Ar.),1741(C=O) , 2931(C-H,aliph, stretching) , 3488(C-H,Ar,stretching) and 3755cm-1(OH).

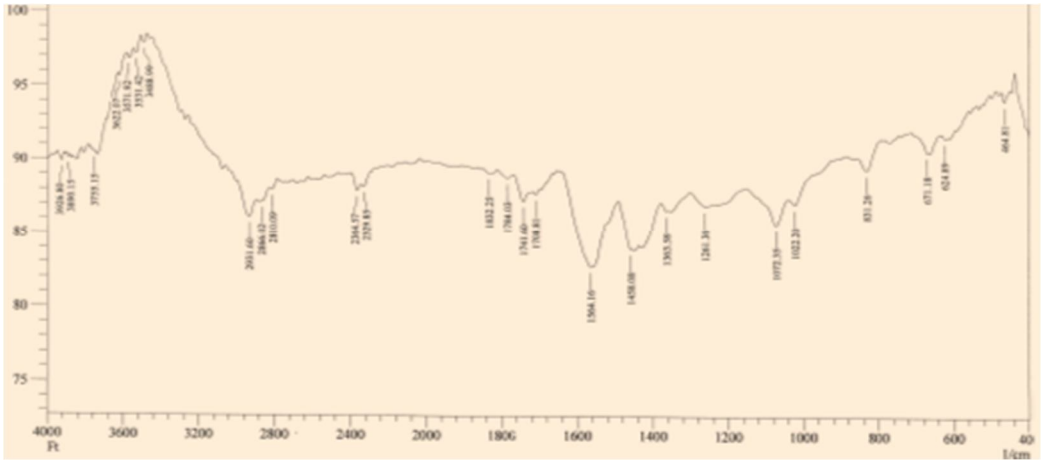


Fig.12 : IR spectrum of compound III

In the UV, compound I absorbs at $\lambda_{max}$279 (Fig.13). Such absorption is characteristic of : flavanones , dihydroflavonols , dihydrochalcones and isoflavones. No shoulder was observed in the 300-340nm region- such shoulders are characteristic of isoflavones. On the other hand dihydroflavonols are distinguished by a 3-OH function which is detected by the UV shift reagent – sodium methoxide.The sodium methoxide spectrum(Fig.14) showed absence of a 3-OH group(no bathochromic shift being detected) and consequently absence of dihydroflavonols. Thus the isolated flavonoid is either a flavanone or a dihydrchalcone. These classes are distinguished by their $^1$HNMR spectra. Flavanones exhibit a double doublet(usually merging into a multiplet) around δ2.80ppm due to the mutual splitting of $C_3$ protons , which suffers further splitting by the neighboring $C_2$ proton. Furthermore a second multiplet appears around δ5.20ppm due to the $C_2$ resonance which is split by the magnetically unequivalent $C_3$ protons. However, the $^1$HNMR spectrum (Fig.18) did not reveal those multiplets which are characteristic of flavanones , but a multiplet for the two methylene moieties of a dihydrochalcone appeared at δ 1.85-2.24 ppm indicating that the isolated flavonoid is a dihydrochalcone.

Next the hydroxylation pattern of the isolated chalcone has been investigated by some UV shift reagents . These are :sodium acetate(gives bathochromic shift diagnostic of a 7-OH group); aluminium chloride(shows bathochromic shifts diagnostic of 3- , 5-OH groups and catechols); boric acid(diagnostic of catechol systems).

The sodium acetate spectrum (Fig.15 ) revealed a bathochromic shift indicative o a 7-OH function. However, the shift reagent-aluminium chloride failed to show any bathochromic shift(Fig.16 ) suggesting absence of 3-, 5-OH groups and catechol moieties.The same behavior was shown by the shift reagent –boric acid- which revealed absence of catechols(no bathochromic shift has been detected in the spectrum(Fig.17).

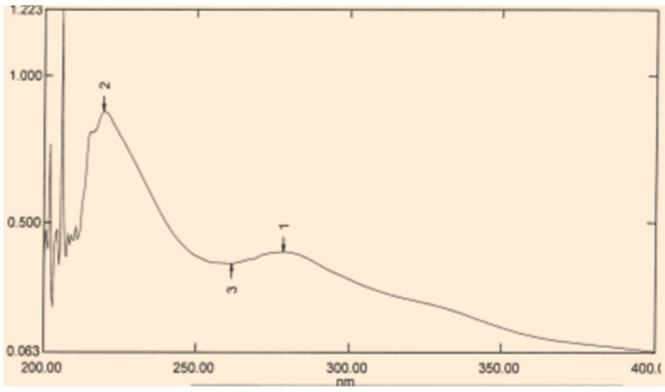



Fig.13 : UV spectrum of compound

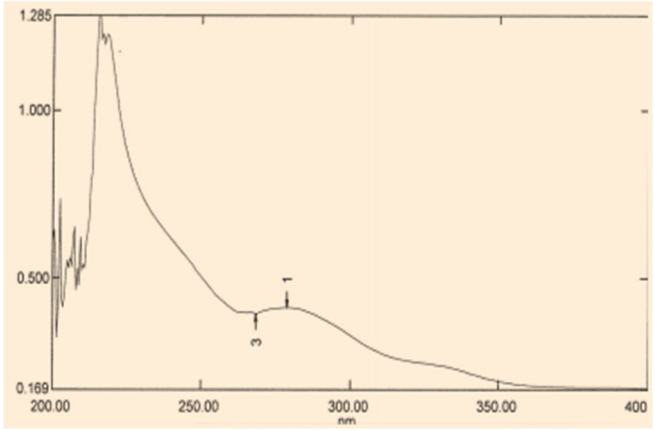



Fig.14 : Sodium methoxide spectrum

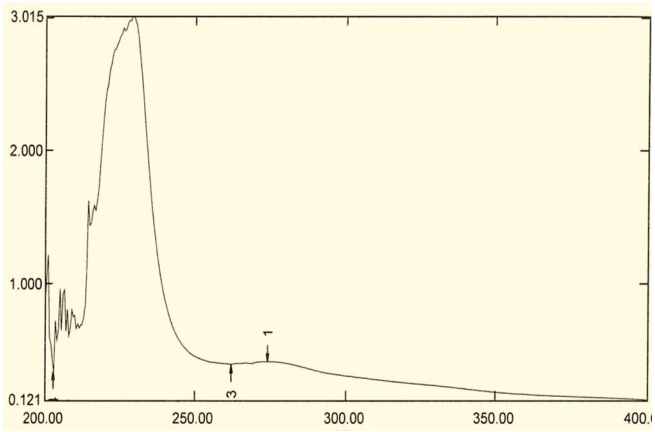



Fig.15 : Sodium acetate spectrum of compound

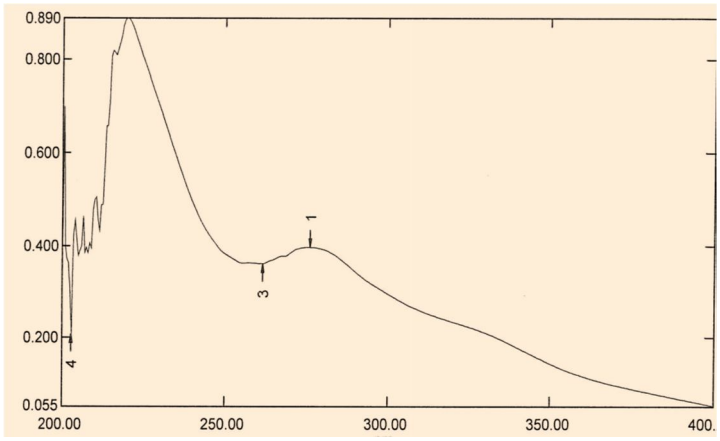



Fig.16 : Aluminium chloride spectrum of compound

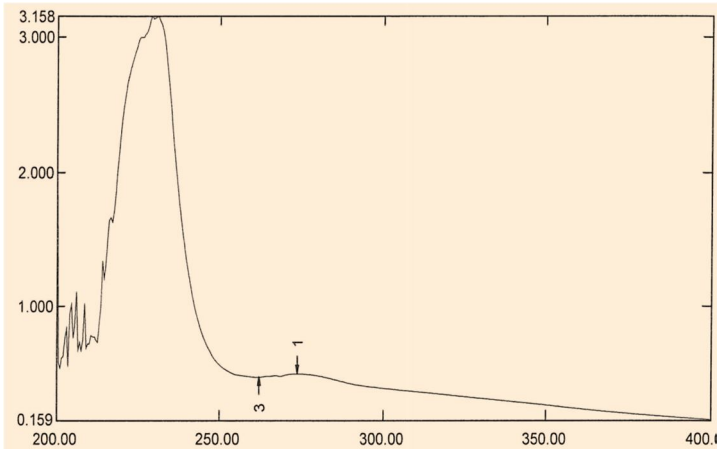
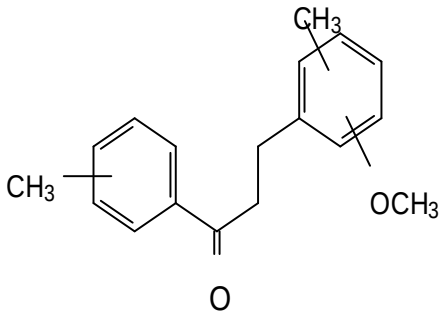



Fig.17 : Boric acid  spectrum of compound

The $^{1}$HNMR spectrum(Fig.18) showed δ(ppm) : 1.13 , 1.24 (assigned for two methyl groups), multiplet(1.85-2.24) assigned for two methylene moieties in a dihydrochalcone ; 3.82 accounting for a methoxyl function ;the multiplet (4.10-5.00) was attributed to a sugar residue(this sugar was not identified in this study) ; The aromatic protons resonated at δ6.63,7.00,7.22 and 7.83ppm. The signals at δ2.50 and 3.36ppm are due to the solvent (DMSO) residual protons and residual water respectively.

On the basis of the above spectral data , the following partial structure was proposed for the aglycone of compound III:




Aglycone of compound III

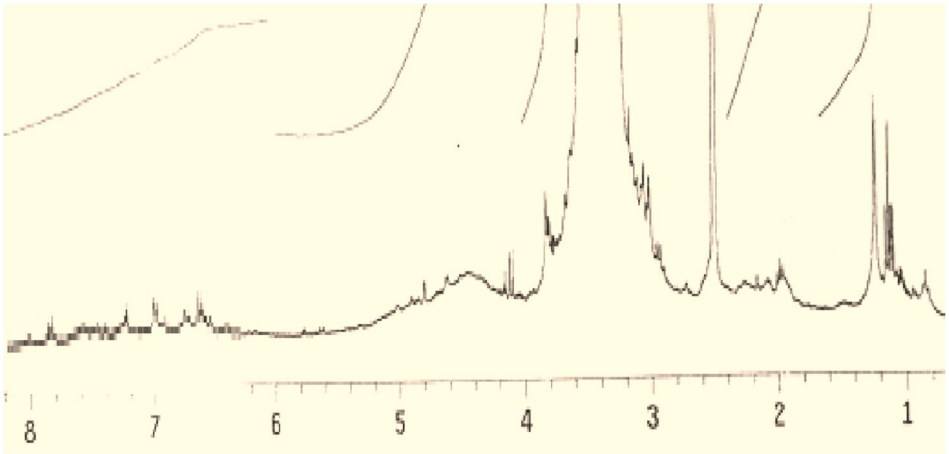



Fig.18 :$^{1}$HNMR spectrum of compound III

## 3.3.2-Antimicrobial activity

The ethanolic extract of *Fagonia critica* was screened for its antimicrobial activity against five standard microorganisms.The results are depicted in Table (19) .Results were interpreted in the following conventional terms : (>9mm: inative;9-12mm:partially active;13-18mm: active;<18mm:very active) .

**Table 19 : Antibacterial activity of ethanolic extract**

| Sample | Conc.(mg/ml) | **Sa** | Bs | Es | Ps | Ca |
|---|---|---|---|---|---|---|
| Ethanolic extrac | 100 | 13 | 12 | 13 | 10 | --- |

Sa.: *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Ca.: *Candida albicans*

Bs.: *Bacillus subtilis*

The ethanolic extract of *Fagonia critica* showed moderate activity against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*.It also exhibited partial activity against *Pseudomonas aeruginosa* , but it failed to show any anticandidal potential.

# Conclusion

In this research was carried out to study the flavonoids of *fagonia critica*, *Cistanch pheleapea* and *waltheria indica*.

Two components 1 and 2 were isolated form the roots of *waltheria indica*

One flavonoid was isolated from stem of *cistanch pheleapa*

One flavonoid was isolated from leaves of *fagonia critica*.

The partial structures of both compounds were deducted on the basic of their: IR, UV and NMR data and partial structures.

Evaluations of the antimicrobial activity of the ethanol extract of the three plant.

The result exhibited variables susceptibilities of microorganism for crude extract.

The antioxidant capacity of the alcoholic extracts of *fagonia critica*, *cistanch phelea* and *waltheria indica* were screened.

The extract showed significant antioxidant activity using the DPPH bioassay.

## Recommendations

The following is highly recommended:

1- The isolated flavonoids may be subjected to 2d NMR experiment for full characterization.

2- Other phytochemicals of the studied species may be isolated and their structure may be elucidated and biological activity could be screened.

3- The isolated flavonoid may be screened for both biological effects such as anti-inflammatory, antiviral …etc.

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