Sudan University of Science and Technology

College of Postgraduate Studies

Characterization of Flavonoids From *Combretum aculeatum* Root, *Dichrostachys cinera* Leaves and Antimicrobial Activity of *Combretum aculeatum*

توصيف التركيب لفلافونويدات جذور الشحيط وأوراق الكداد والفعالية المضادة للميكروبات لنبات الشحيط

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

By:

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الآستهلال

﴿ بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ ﴾

فَلْيَنظُرِ الْإِنسَانُ إِلَى طَعَامِهِ ﴿٢٤﴾ أَنَّا صَبَبْنَا الْمَاءَ صَبًّا

﴿٢٥﴾ ثُمَّ شَقَقْنَا الْأَرْضَ شَقًّا ﴿٢٦﴾ فَأَنبَتْنَا فِيهَا حَبًّا

﴿٢٧﴾ وَعِنَبًا وَقَضْبًا ﴿٢٨﴾ وَزَيْتُونًا وَنَخْلًا ﴿٢٩﴾

وَحَدَائِقَ غُلْبًا ﴿٣٠﴾ وَفَاكِهَةً وَأَبًّا ﴿٣١﴾ مَّتَاعًا لَّكُمْ

وَلِأَنْعَامِكُمْ ﴿٣٢﴾ سورة عبس

DEDICATION

Dedicated to:

My loving parents, Malak and Eltayeb

.

my husband, Haythem

my father in-law Hassona

my sons, wael, Mohanned, Hala and Hadeel

Acknowledgement

First and foremost, I must acknowledge my limitless thanks to **Allah,** the Ever-Magnificent; the Ever-Thankful, for His helps and bless.

I am grateful to some people, who worked hard with me from the beginning till the completion of the present research particularly my supervisor Dr. Mohamed Abdel Karim, who has been always generous during all phases of the research, and I highly appreciate the efforts expended by my co-supervisor, Dr. Amira Abdel Aziz.

I owe a deep debt of gratitude to the Sudan University of Science and Technology for giving us an opportunity to accomplish this work.

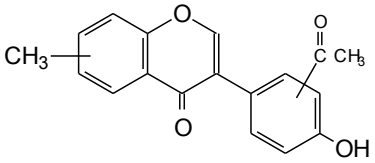
I would like to take this opportunity to say warm thanks to all my beloved friends, who have been so supportive along the way of doing my thesis.

I also would like to express my whole hearted thanks to my family, specially my father and my husband for their generous support. They provided me throughout my entire life and particularly through the process of pursuing the PhD degree.

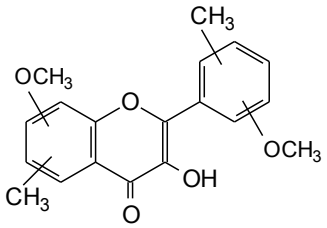
Abstract

The flavonoids of two key species in Sudanese ethnomedicine have been investigated and three compounds (I-III) have been isolated.

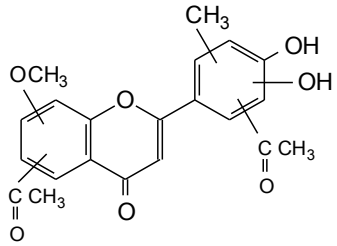
Compound I was isolated from *Combretum aculatum* roots via paper chromatography, while compounds II and III were isolated from the leaves of *Dichrostachys cinerea*. The structures of these flavonoids have been partially characterized by their spectral data (UV and ^{1}HNMR).



Compound I



Compound II



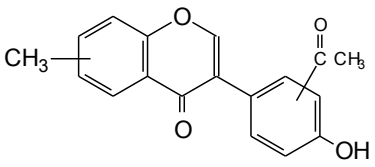
Compound III

The methanolic extract of *Combretum aculeatum* was assessed for its antimicrobial activity. It showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. It also showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. The extract also exhibited significant antifungal activity against the fungi: *Candida albicans and Aspergillus niger* (Table 1).

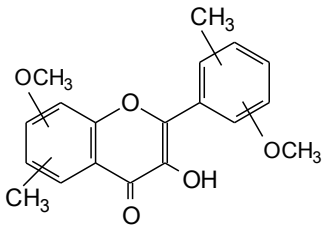
المستخلص

درست الفلافونيدات لاثنان من النباتات المستخدمة في الطب الشعبى السودانى هما نباتى الشحيط والكداد , حيث تم فصل ثلاثة مركبات.

تم فصل المركب I من جذور نبات الشحيط , اما المركبان II,III فقد فصلا من نبات الكداد. وقد اقترحت تراكيب مبدئية لهذة المركبات بناء على البيانات الطيفية(طيف الاشعة فوق البنفسجية وطيف الرنين النووي المغنطيسى).



Compound I



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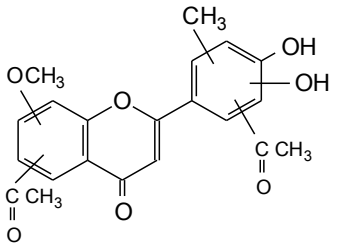

Compound III

في اختبار مضاد الميكروبات اعطى المستخلص الميثانولى لنبات الشحيط فعالية عالية ضد : *Escherichia coli* and *Pseudomonas aeruginosa*

كما وابدى هذا المستخلص فعالية معتدلة ضد : *Bacillus subtilis* and *Staphylococcus aureus*. كذلك ابدى هذا المستخلص فعالية عالية ضد فطرى : *Candida albicans and Aspergillus niger*

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Chapter One

Introduction

1. Introduction

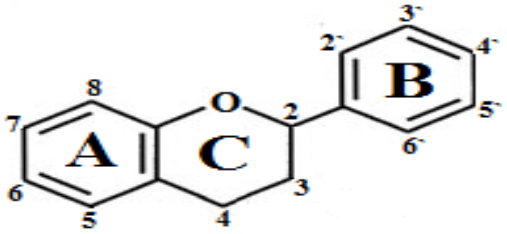
1-1: General overview

Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in the plant kingdom[1,2,3,4,5,6] and prokaryotes[7,8]. Thus far, approximately 9000 different flavonoids have been identified and they form the largest group of naturally occurring polyphenols[9]. This list is constantly growing owing to the enormous structural diversity associated with these compounds.

The original "flavonoid" research apparently began in 1936, when Hungarian scientist Albert Szent- Gyorgi was uncovering a synergy between pure vitamin C and as yet unidentified cofactors from the peels of lemons, which he first called "citrine," and, later, "vitamin P"[10].

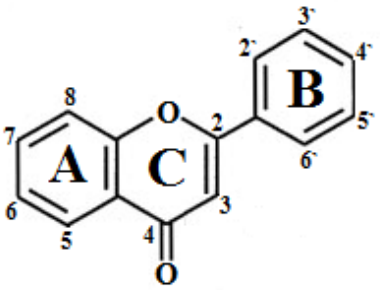
Flavonoids are low molecular weight[11,12] bioactive polyphenols[13]which play a vital role in photo synthesizing cells[3]. The biosynthesis of flavonoids occurs in higher plants through the shikimic acid and malonic acid pathways[14,15,16]. Flavonoids are found in vegetables, fruits, nuts, grains, seeds flowers,[17], bark, roots, stems, leaves[7,18], black tea[19], green tea[20], herbs, spices, cocoa[21,22] and soybean [23,24,25]. These are an integral part of our daily diet[26, 27, 28].

Flavonoids are secondary metabolites[29,30] characterized by flavan nucleus[12] and C_6-C_3-C_6 carbon-skeleton[31,32]. These are group of structurally related compounds with a chromane-type skelton having phenyl substituent in C_2-, C_3- or C_4-position[33]. The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C) as shown below[3,34,35].



Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge[1]. They are differing in their arrangement of hydroxyl, methoxy and glycosidic side groups and in the conjunction between A and B rings[12].

Flavonoids have been divided into several sub-classes based on their C-ring structure [36,37,38]. According to their molecular structure, they are divided into eight classes; flavones (1), flavonols (2), flavanones (3), dihydroflavonols (4), chalcones (5), anthocyanidins (6), isoflavones (7) and catechins (8)[32]:



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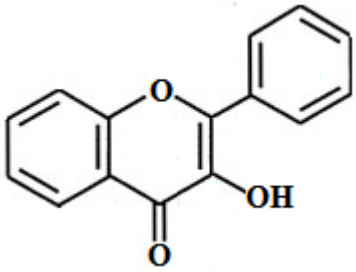

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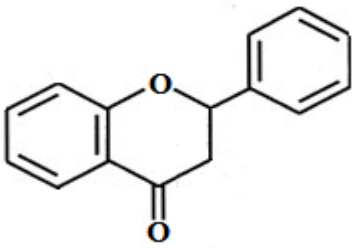

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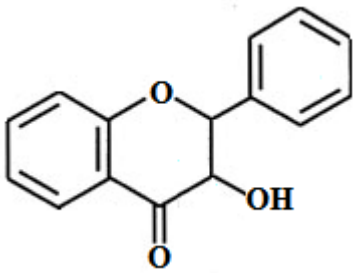

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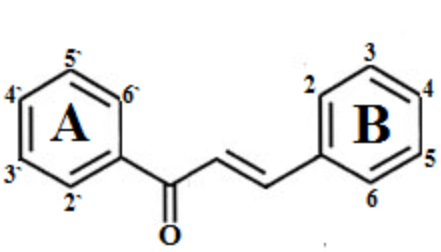

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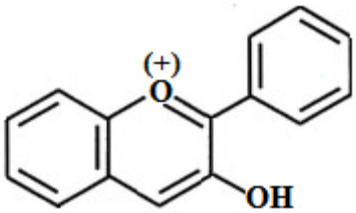

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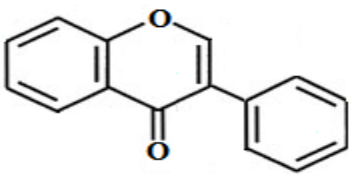

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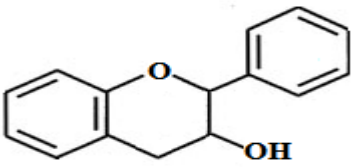
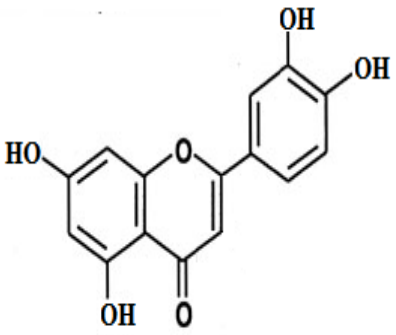


(8)

Flavones (1) have a very close structural relationship to flavonols. Although flavones, such as luteolin (3',4',5, 7-tetrahydroxyflavone)(9) and apigenin (4',5,7-trihydroxyflavone) (10), have A- and C-ring substitutions, they lack oxygenation at C_3. A wide range of substitutions is also possible with flavones, including hydroxylation, methylation, O- and C-alkylation, and glycosylation. Most flavones occur as 7-O-glycosides. Unlike flavonols, flavones are not distributed widely with significant occurrences being reported in only celery, parsley and some herbs. In addition, poly methoxylated flavones, such as tangeretin (4',5,6,7, 8- penta methoxy flavone) (11), have been found in citrus species. The animal research shows the potential of tangeretin as a cholesterol lowering agent[39]. A hamster study showed potential protective effects against Parkinson's disease[40]. Flavones in millet have been associated with goiter in West Africa [38].

Flavones and their 3-hydroxy derivatives flavonols, including their glycosides, methoxides and other acylated products on all three rings, make this the largest subgroup among all polyphenols.



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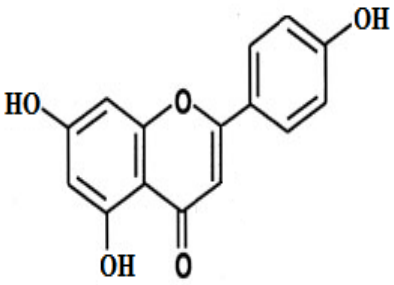

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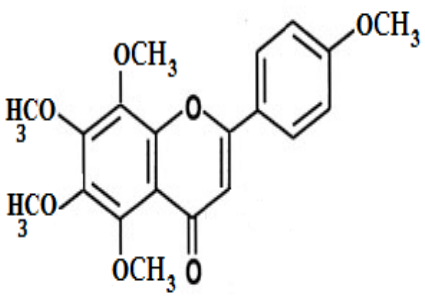
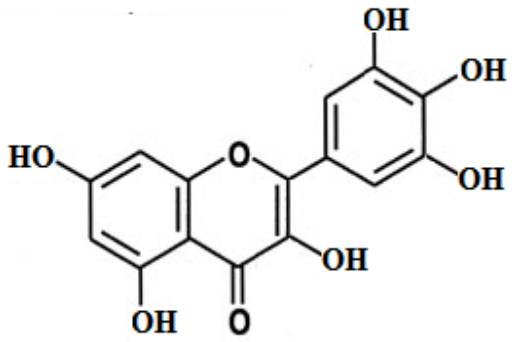


(11)

Flavonols (2) are arguably the most widespread of the flavonoids, being dispersed throughout the plant kingdom with the exception of fungi and algae. The distribution and structural variations of flavonols are extensive and have been well documented. Flavonols such as myricetin (12), quercetin (13),

isorhamnetin (14) and kaempferol (15) are most commonly found as O-glycosides. Conjugation occurs most frequently at the 3 position of the C-ring but substitutions can occur at the 5,7,4, 3 and 5 positions of the carbon ring.

Although the number of aglycones is limited there are numerous flavonol conjugates with more than 200 different sugar conjugates of kaempferol alone[41]. There is information on the levels of flavonols found in commonly consumed fruits, vegetables and beverages[42,43].



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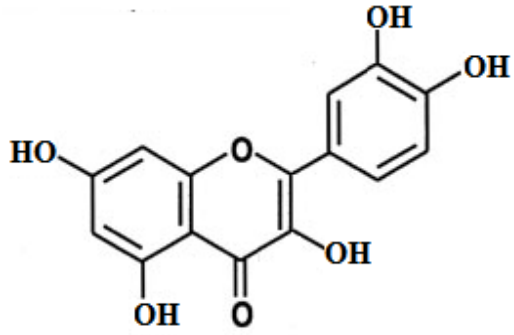

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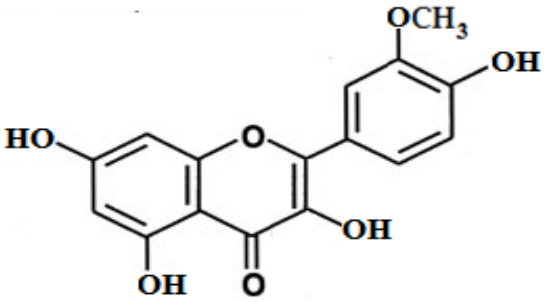

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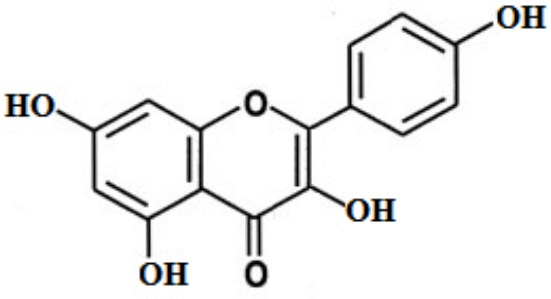
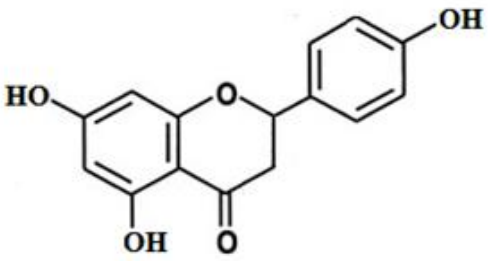


(15)

Another class of flavonoids – the flavanones- are based upon structure (3) – 2-phenyl-benzopyran-4-one – which is the flavanone itself. They are interesting compounds, since they are obligate intermediates in flavonoid biosynthesis. They are dietary components that are present in especially high concentrations in citrus fruits[44,45].

The naturally occurring flavanones will be treated according to their B-ring hydroxylation pattern, e.g. naringenin (16) and hesperetin (17). Hesperetin is a bioflavonoid and, to be more specific, a flavanone. Hesperidin (a flavanone glycoside) is water-soluble due to the presence of the sugar part in its structure, so on ingestion it releases its aglycone, i.e, hesperetin. This flavonoid is found in Citrus fruits.

Naringenin is a flavanone, a type of flavonoid, that is considered to have a bioactive effect on human health as antioxidant, anti-inflammatory, carbohydrate metabolism promoter, and immune system modulator. It is the predominant flavanone in grapefruit[46]. The sources of naringenin are; grapefruit, oranges, and tomato. Grapefruit juice can provide much higher plasma concentrations of naringenin than orange juice[47], and can be absorbed from cooked tomato paste[48].



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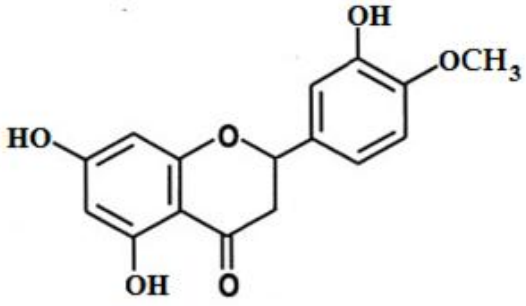
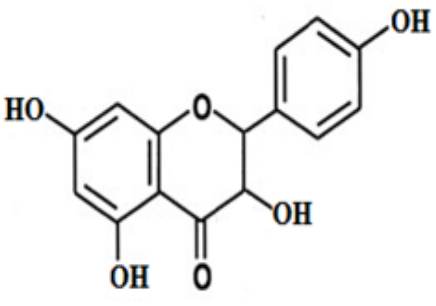


(17)

The dihydroflavonols -another class of flavonoids - are constructed upon the same fundamental ring system (4) as the flavonones and are 2-phenyl-3-hydroxy-benzopyran-4-ones. The numbering system is the same as that for the flavonones. 7-Hydroxydihydroflavonol is the simplest known naturally occurring member of the series[49]. Dihydroflavonols having one B-ring hydroxyl, dihydrokaempferol (18). Aromadendrin occurs as the free phenol in a wide variety of plants. Dihydroflavonols having two B-ring hydroxyls e.g. fustin (19) which possesses stereoisomers: (-)-Fustin and (+)-Fustin.



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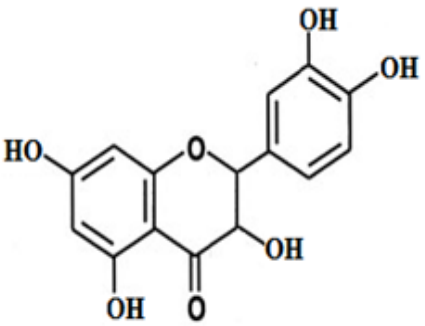
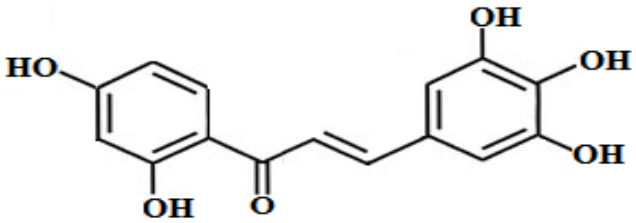


(19)

Chalcone (5) belong to the flavonoids. They are aromatic ketone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones. They show antibacterial, antifungal and anti-inflammatory properties. Some chalcones demonstrated the ability to block voltage-dependent potassium channels[50]. The open-ring chalcones are found in fruits such as apples[51] and hops or beers[52]. An example of chalcones having three B-ring hydroxyls is robtein (20) – 2`, 4`,3,4, 5- penta hydroxychalcone known from the heartwood of Acacia.



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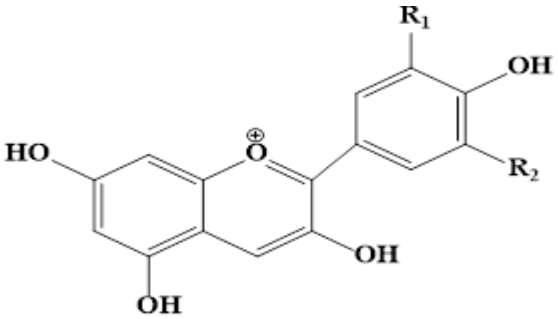
(20)

The anthocyanidins (6) constitute an important class of flavonoids[44]. About 90% of anthocyanins are based on the structure of cyanidin, delphinidin and pelargonidin and their methylated derivatives[44] (Fig.1). A total of more than 500 anthocyanins are known depending on the hydroxylation,

methoxylation patterns on the B ring, and glycosylation with different sugar units [53,54].

Anthocyanidins, principally as their conjugated derivatives, anthocyanins, are widely dispersed throughout the plant kingdom, being particularly evident in fruit and flower tissue where they are responsible for red, blue and purple colours. In addition they are also found in leaves, stems, seeds and root tissue. They are involved in the protection of plants against excessive light by shading leaf mesophyll cells and also have an important role to play in attracting pollinating insects.



Anthocyanidin	**R_1**	**R_2**
Cyanidin	-OH	-H
Delphinidin	-OH	-OH
Pelargonidin	-H	-H
Malvidin	$-OCH_3$	$-OCH_3$
Peonidin	$-OCH_3$	-H

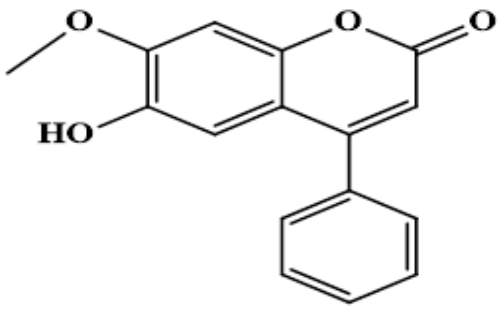
Fig. 1: The most common anthocyanidins

Another category of flavonoids is known as Isoflavones (7) having their B ring attached to the C_3 position of ring C. They are mostly found in the leguminous family of plants[55]. Since beans, particularly soybean, are a major part of the diet in many cultures, the role of isoflavones have, thus, great impact on human health.

Genistein and daidzein are the two main isoflavones found in soy along with glycetein, biochanin A and formononetin[56,57] . They are also found in red clovers[58].

Flavanols or flavan-3-ols-an important class of flavonoids- is often commonly called catechins. Different from most flavonoids, there is no double bond between C_2 and C_3, and no C_4 carbonyl in ring C of flavanols. This and the hydroxylation at C_3 allows flavanols to have two chiral centers on the molecule (on C_2 and C_3), thus four possible diastereoisomers exist. Catechin is the isomer with *trans* configuration and epicatechin is the one with *cis* configuration. Each of these two configurations has two steroisomers, i.e., (+)-catechin, (−)-catechin, (+)-epicatechin and (−)-epicatechin. (+)-catechin and (−)-epicatechin are the two isomers often found in food plants. Flavanols are found in many fruits, particularly in the skins of grapes, apple and blueberries[51]. Monomeric flavanols (catechin and epicatechin) and their derivatives (e.g., gallocatechins) are the major flavonoids in tea leaves and cacao bean (chocolate)[59,60].

Neoflavonoids are a class of polyphenolic compounds. They are not often found in food plants, but dalbergin (21) is the most common and relatively widely distributed neoflavone in the plant kingdom[61].



(21)

1- 2: Biosynthesis of flavonoids

The B-ring and part of the heterocyclic ring of the flavonoid skeleton are provided by a suitable hydroxy-cinnamic acid-CoA ester, usually 4-coumaroyl-CoA, whereas the A-ring originates from three acetate units via malonyl-CoA (Fig.2). Both precursors are derived from carbohydrates. Malonyl-CoA is formed from acetyl-CoA and CO_2 catalyzed by acetyl CoA carboxylase. 4-Coumaroyl-CoA and related hydroxy cinnamic acid esters are supplied by the first steps of the general phenylpropanoid pathway. This pathway starts from the aromatic amino acid phenylalanine, which is synthesized via the shikimate arogenate pathway. The key reaction is the deamination of phenylalanine catalyzed by phenylalanine ammonialyase (PAL). This enzyme links the primary metabolism with the phenylpropanoid pathway. The product of the reaction, trans- cinnamate, is hydroxylated to 4-coumarate by cinnamate 4-hydroxylase, a cytochrome P450 mixed-

function mono oxygenase. Activation of 4-coumarate by formation of the CoA ester is catalyzed by 4-coumarate-CoA ligase. 4-Coumaroyl-CoA can be hydroxylated in position 3 to caffeoyl- CoA, which may serve as a substrate for chalcone formation besides 4-coumaroyl-CoA in some plant species. Three different enzyme activities have been demonstrated for caffeoyl- CoA formation from 4-coumaroyl-CoA.

The key enzyme for the formation of the flavonoid skeleton is chalcone synthase (CHS), which catalyses the stepwise condensation of three acetate units from malonyl- CoA with 4-coumaroyl-CoA to the fifteen carbon intermediate 2´,4´,6´,4-tetrahydroxychalcone (Fig. 2). The respective 6'-deoxychalcone, isoliquiritigenin, is likewise synthesized from malonyl- CoA and 4-coumaroyl-CoA by chalcone synthase but in coaction with a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductase. Both chalcone types may be the direct precursors for aurones and other diphenyl propanoids. The enzymes involved in these reactions are still unknown. But, in particular, the 6´-hydroxy- and 6´deoxychalcones are the immediate precursors for all flavonoid compounds. The stereo specific cyclization of the chalcone, catalyzed by chalcone isomerase, provides 25-flavanones with the typical flavonoid skeleton (Fig 2). Two types of chalcone isomerases are known: one catalyzing cyclization of 6´-hydroxy-chalcone to 5-hydroxy flavanone and another isomerizing both 6´-hydroxy- and 6´-

deoxychalcone to 5-hydroxy-and 5-deoxyflavan-one, respectively.

Flavanones are the direct precursors for other natural products, such as the large class of flavones, isoflavones that are involved in phytoalexin synthesis, and the two flavonoid intermediates, the flavan-4-ols and the dihydroflavonols. Flavones are synthesized from flavanones by introduction of a double bond between C-2 and C-3. Two types of enzymes, flavone synthase I, (a 2-oxoglutarate-dependent di oxygenase), and flavone synthase II, (a cytochrome P450 mixed-function mono oxygenase), were found to catalyze this reaction. Formation of isoflavones from flavanones is catalyzed by 2-hydroxy-isoflavanone synthase, another cytochrome P450 mixed-function mono oxygenase, coating with a dehydrates protein. The enzyme accepts both 5-hydroxy- and 5-deoxyflavanones as substrates. The reaction involves an oxidative rearrangement of the flavanone, including a shift of the aryl ring from position 2 to 3.

The reduction of the carbonyl group of flavanones gives rise to flavan-4-ols. The reaction is catalyzed by flavanone-4-reductase and provides the immediate precursors for the formation of 3-deoxy-anthocyanins (Fig. 2).

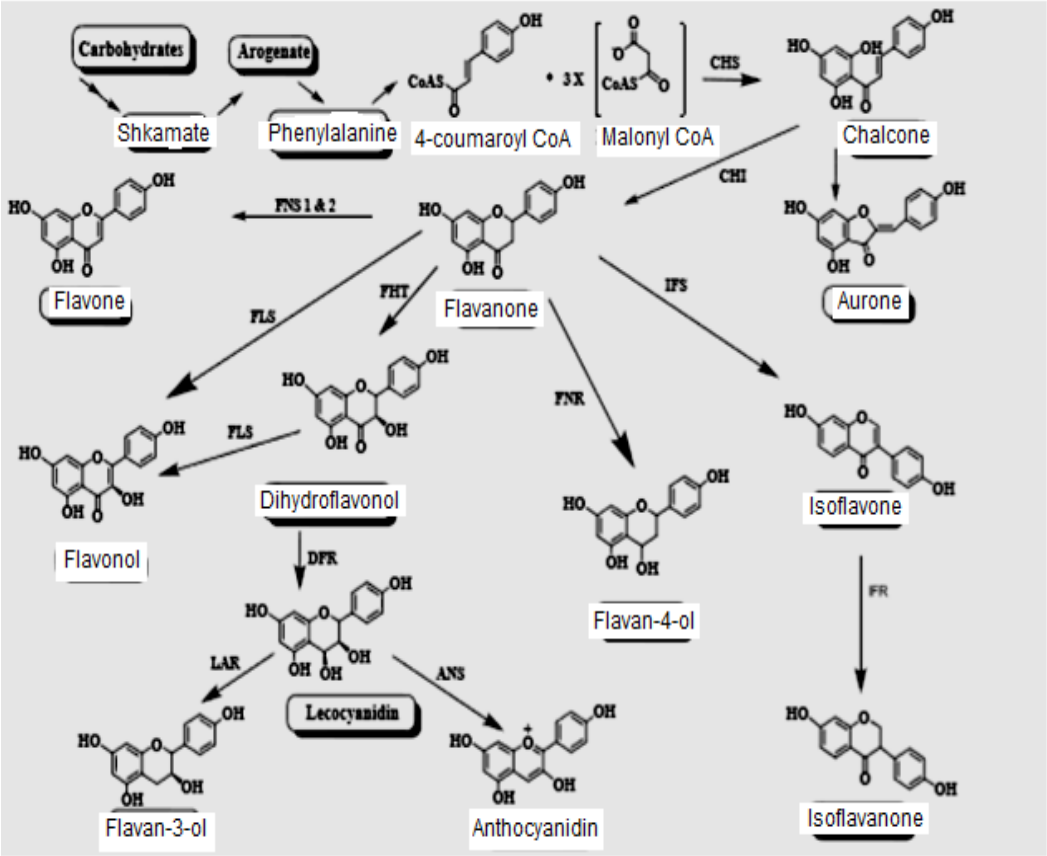


Fig. 2: Biosynthesis of flavonoids

Finally, flavanones can be hydroxylated in position 3 to dihydroflavonols, which are biosynthetic intermediates in the formation of flavonols, catechins, proanthocyanidins and anthocyanidins. This reaction is catalyzed by flavanone 3-hydroxylase, a 2-oxoglutarate-dependent di oxygenase.

Dihydroflavonols are the direct substrates for most of the flavonols and flavan-3,4- diols, which are also known as leucoanthocyanidins[62,63].

1-3: Role of flavonoids in plant

Flavonoids have long been reported as serving multiple functions in plants[64]. They are important for the survival of a

plant in its environment; they regulate plant growth[4,65], inhibit or kill many bacterial strains, inhibit major viral enzymes, destroy some pathogenic protozoans[66], stimulants for germination of spores[67,68] and protect the plants from insects[69,70,71,72]. Also, they act in plants as visual attractors[73], feeding repellents, photoreceptors[73,74,75].

Flavonoids act as signal molecules [75,76], phytoalexins [77,78], detoxifying agents[79,80,81], pigmentation, stimulation of nitrogen-fixing nodules [82,83]. They play significant activities in seed germination [84,85], act as UV filters [3,75,82,83,86,87,88]. Flavonoids are also involved in temperature acclimation [89] and in drought resistance [90] and they act as allelochemical agents[91,92].

Anthocyanin pigment present in flowers provide colour to it contributing to pollination[3,12,93]. Flavonoids present in leaves promote physiological survival of plant by protecting it from fungal infections. In addition, flavonoids are involved in photosensitisation, energy transfer, respiration and photosynthesis control, morphogenesis, sex determination, energy transfer[3].

Flavonoids protect plants against various biotic and abiotic stresses[94] and exhibit a diverse spectrum of biological functions and play an important role in the interaction between the plant and their environment[95]. Recent evidence of a nuclear location of flavonoids (as well as of enzymes of flavonoid biosynthesis) supports that flavonoids are capable of modulating the activity

of proteins involved in cell growth. Flavonoids may therefore act as transcriptional regulators[96,97].

1- 4: The Medicinal uses of flavonoids

Up to date, flavonoids have been found only in plants. Being not only colored pigments, but also enzyme inhibitors and stimulants, metal chelators and reducing agents[4,65]. In addition, several papers including epidemiological studies and meta-analyses report on their beneficial effects on human health.

Flavonoids possess a wide range of biological activities, medicinal and pharmacological effects[98,99,100]. Their medicinal and pharmacological effects and their contributions to human health have made them prominent in the past 10 years[98]. Many flavonoids are active principles of medicinal plants and exhibit pharmacological effects[66,101].

The most important medicinal properties of the flavonoids are briefly discussed below:

1- 4- 1: Anti-cancer activity

Recently, epidemiological studies have strongly suggested that consumption of plant flavonoids contributes to reducing the risk or incidence of some cancers[102,103], slow the division of tumor cells[104,105], can also be preventive agents against cancer[3,37,106,107,108,109,110] , because they have ability to modulate enzyme activities resulting in decreased carcinogenicity of xenobiotics that are responsible for oxidative stress- induced

cancer[111]. Fruits and vegetables having flavonoids have been reported as cancer chemo preventive agents[112,113].

Flavonoids can slow or protect against certain kinds of cancerous processes[114,115,116,117,118]. Flavanols, flavonols and anthocyanins are relatively abundant in human diet and possibly are involved in prevention of cancers[110,119,120,121,122].

Flavonoids for a long time have been part of the herbal treatment by lay practitioners, but they were recognized only recently as effector substances. Examples of herbal preparations owing their growing recognition as effective anticancer drugs to flavonoids are propolis and Essiac[66].

1- 4- 2: Anti-microbial activity

Flavonoids have been used extensively since centuries for the treatment of various diseases. Propolis has been referred even in old testament for its healing properties. The antimicrobial activity[3] of propolis has been attributed to its high flavonoid content. Galangin is a flavonol commonly found in propolis. It has been reported to possess inhibitory actions against *Aspergillus tamarii, Aspergillus flavus, Cladosporium sphaerospermum, Pencillium digitatum, Penicillium italicum* [3].

1- 4- 3: Anti-inflammatory activity

Flavonoids are known to exhibit anti-inflammatory activity[123,124,125,126,127]. A number of flavonoids such as hesperidin, apigenin, luteolin, and quercetin are reported to possess anti-inflammatory and analgesic effects. Flavonoids

may affect specifically the function of enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases[128-132].

1- 4- 4: Antiviral activity

Naturally occurring flavonoids with antiviral activity[133] have been recognized since the 1940s and many reports on the antiviral activity of various flavonoids are available[134].

1- 4- 5: Anti-oxidant activity

The polyphenolic nature of flavonoids equates with ready oxidation and the formation of stable radicals and it is widely believed that flavonoids protect against free radical damage (caused by photolytically generated singlet oxygen and metabolic processes in living organisms)[135]. Also, more and more attention has been focused on the antioxidant activity of medicinal plants, fruits and vegetables that contain plentiful flavonoid compounds. However, the content of flavonoid compounds is influenced by many factors, including the genus, the place of the plant growth, the extraction conditions and technology[136].

Flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants[2,3,12,85,108,135,137,138,139,140,141,142,143,144,145], free radical scavengers[135,137,139,140,141,142,143,144,145,146], chelators of divalent cation[26, 37,147].

1- 4- 6: Anti-diabetes mellitus agents

Diabetes mellitus is a serious chronic disease. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients[148]. Flavonoids showed prospective benefits in the treatment and/or ease of symptoms of several serious illnesses such as diabetes[149,150,151].

1- 4- 7: Hepatoprotective agents

Flavonoid compounds, which are a large group of secondary metabolites in higher plants[152], are known to be responsible for hepatoprotective potential[3,123,153,154].

Several flavonoids such as catechin, apigenin, quercetin, naringenin, rutin, andvenoruton are reported for their hepato protective activities[108].

1- 4- 8: Other biological activities

Flavonoids have been reported to exert wide range of biological activities. These includes: antiallergic[3,10,26,123,155], cytotoxic antitumour, treatment of neurodegenerative diseases[156], vasodilatory action[10,32,37,147,157], antithrombotic[37], antimutagenic activities, reducing the risk of cardiovascular disease[26,71,119,156,158,159,160,161,162,163], stroke[71], reduced risk for certain chronic diseases[164,165], antiosteoporotic, and antihepatotoxic actions[3,4], reduced risk of coronary heart disease , premature aging[166] and immunomodulator activities[3].

Flavonoids exhibit also beneficial effects on capillary fragility[167] and an ability to inhibit human platelet aggregation[163], antiulcer[168,169] properties. In addition, reduce hypertension[170,171], as well as protect and strengthen vascular walls[172,173], asthma bronchiale[66], gastro-intestinal ulcers[168,174] due to their immunmodulatory, pain-killing, and smooth muscle relaxant effects[3,66,135,155,175]. Flavonoids also render valuable help in minor problems e.g. wounds, bites, burns, or common cold[66,176]. Their modulatory role in several biological processes has also been identified like detoxification of enzymes, apoptosis, host immune system and several others. Their potential to interfere with numerous cellular processes such as protection of genomic vitality suggest that flavonoids may used as dietary compounds for chronic degenerative diseases[108].

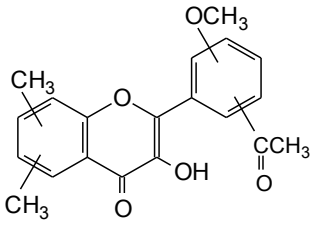
Animal studies have declared that, flavonoids cause the inhibition of degranulation of mast cells, basophils and neutrophils. These could protect the rat brain from LPS (lipo poly saccharide) induced shock through attenuation of lipid peroxidation and nitric oxide generation[177]. Furthermore, they have shown improved biological actions upon combination with each other that indicates their potential to form synergisms[178].

1- 5: Flavonoids from some Sudanese medicinal plants

Abdel Karim and Haga[179] reported the isolation and structural elucidation of three flavonoids: (i) compound A from the leaves of *Coriandrum sativum* (ii) compound B from the roots of *Bauhinia*

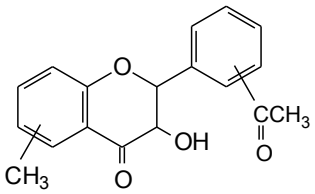
rufescens and (iii) compound C from the stem bark of *Albizza amara* .Two of these compounds have also been tested for their antimicrobial inhibitory effect against some clinically isolated microbes (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Condida albacans*).

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



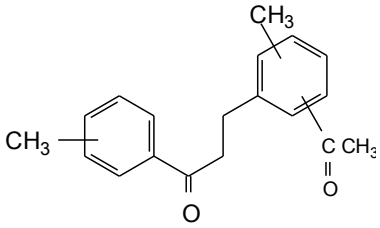
Compound A

The following partial structure was suggested for compound B:



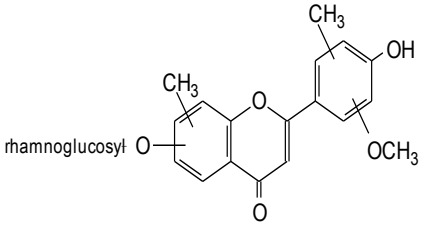
Compound B

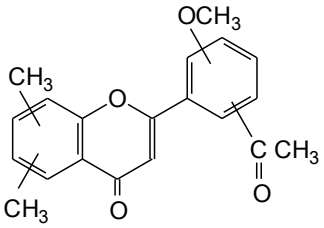
Compound C was assigned the following tentative structure:



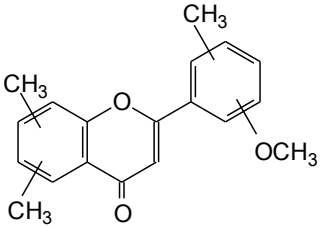
Compound C

Two Flavones have been isolated[180] from the seeds of *Lepidium sativum* and the following partial structures have been proposed on the basis of some spectral data:

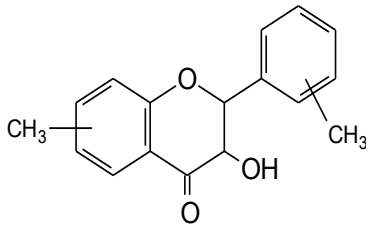




Another flavones was reported[180] from *Ammi visnaga* and the following tentative structure has been suggested:

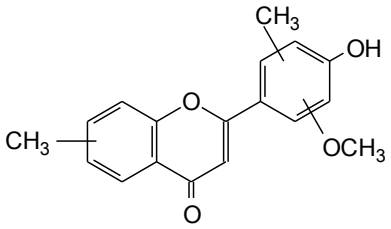


The ethanol extract of *Smilax regelii* was purified by thin layer chromatography where flavonoids - compound I - was isolated[181] and partially characterized by spectral data (UV, 1HNMR).

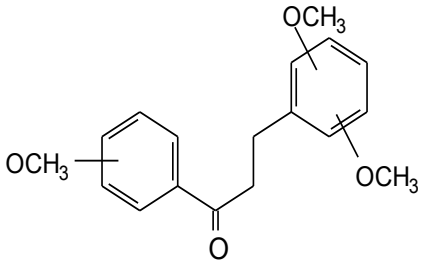


vi

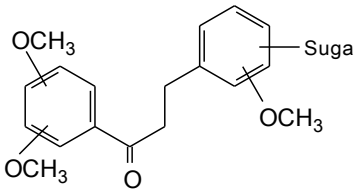
From the ethanolic extract of *Acacia nilotica* var. *adstringens* a flavonoid has been isolated[181] and characterized via some spectral data and it was assigned the following partial structure:



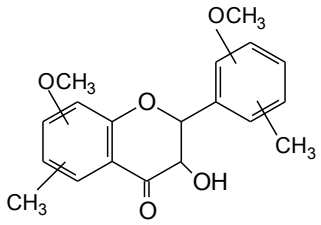
From *Acacia seiberiana* stems a dihydrochalcone has been isolated[181] and its structure partially characterized:



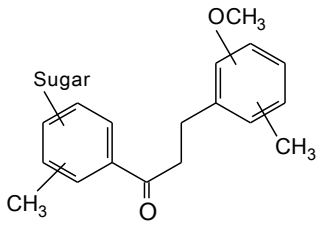
A flavonoid has been isolated[181] from the leaves of *Acacia tortilis* and it following structure was proposed:



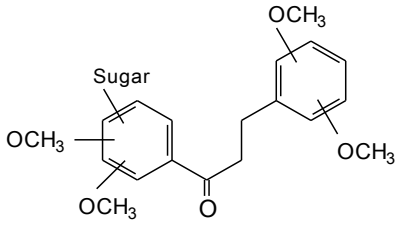
Shaza reported[182] the isolation of a flavonoid from the pods of *Acacia nilotica* subsp. *nilotica* and the following structure has been suggested on the basis of its spectral data:



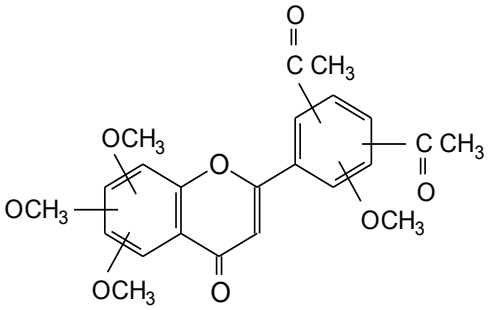
From the leaves of *Leptadenia pyrotechnica* a dihydrochalcone has been isolated[182] and a partial structure has been proposed:



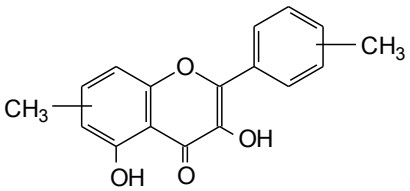
Another dihydrochalcone has been reported[182] from the stems of *Acacia polacantha* and the following tentative structure has been suggested:



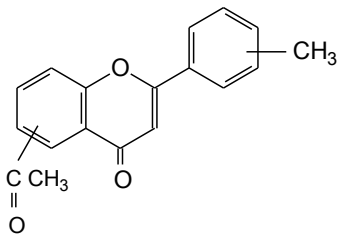
Acetylated flavones have been isolated[182] from *Acacia polyacantha*. The following tentative structure was deduced on the basis of some spectral data:



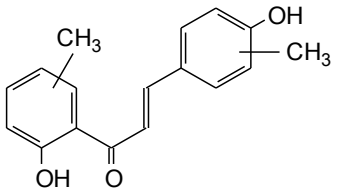
A Methylated flavonol was isolated[183] from the barks of *Acacia nilotica* var. *tomentoza* and it was assigned the following partial structure on the basis of some spectral data:



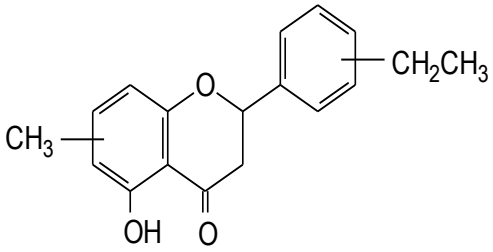
Indigofera oblongifolia was screened for major secondary metabolites and then the flavonoids of this species were investigated. An acetylated flavonol was isolated[183] by paper chromatography and its structure has been partially characterized:



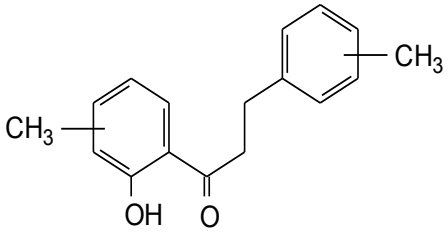
A Methylated chalcone has been isolated[183] from *Acacia ehrenbergiana* growing in Sudan and following tentative structure was proposed:



Two flavonoids –compounds I and II were isolated[184] from the leaves of *Cajanus cajan* by thin layer chromatography. Depending on their spectral data they were assigned the following tentative structures:



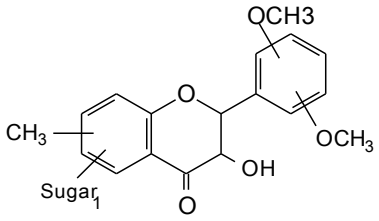
Compound I



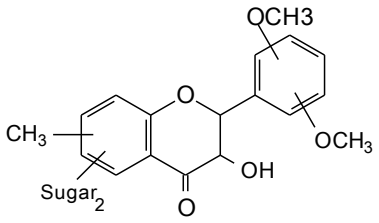
Compound II

Phytochemical screening of the roots of *Acacia nilotica* revealed the presence of flavonoids, tannins, saponins and glycosides in both species. A silica gel column and thin layer chromatography eluted with methanol: chloroform allowed isolation[185] of two

flavonoids (I, II). The structures of the isolated flavonoids were deduced on the basis of their spectral data (UV, NMR and MS).



I



II

1- 6: *Dichrostachys cinerea*

Dichrostachys cinerea (L.) **Wight and Arn**. belongs to the family- Mimosaceae . It is leguminous shrub commonly known in Sudan as Kadad [186].

Dichrostachys cinerea is more common at low altitudes where it grows in a wide variety of soils in wooded grasslands. *Dichrostachys cinerea* is a spiny Acacia-like tree-let, common in Africa, from the sub-Saharan part to the south [187]. It grows in disturbed areas and impoverished soils and is sometimes planted as a defensive thorny fence. In some places, it forms invasive and impenetrable thickets and represents a nuisance. The tree often forms secondary bush on impoverished ground. The bark is dark grey-brown, the stems often twisted and

seamed and the branches intertwined giving thick matted appearance. Dwarf lateral shoots are modified to form short compact spines. Leaves are often compound with 4-13 pairs of pinnae, each carrying up to 27 pairs of leaflets. Leaflets are narrowly obovate to lancelote, up to 10 x 3 mm, dark green, rather glossy above but dull below. Glands are conspicuous on the petiole and on the rachis [188].

Flowers are in auxiliary spikes, all floral parts in fives, stamens, pink, sterile staminodes and the other half formed by yellow fertile flowers. The fruits are in the form of a cluster of pods, each up to 10 x 1 cm twisted, contorted and indehiscent. They fall from the tree rot on the ground [188]. It is found in Sudan and the north, west, central, east and southern regions of Zimbabwe.

Traditionally, the roots are chewed and placed on the sites of snake bites and scorpion stings. The leaves, which are believed to produce a local anesthetic effect, are used as a remedy for sore eyes and tooth ache. *Dichrostachys cinerea* is also used to treat influenza, cough, scabies, leprosy and oedema.

Dichrostachys cinerea plant extracts have been examined and found to possess appreciable antibiotic activity[189,190]. Earlier claims reported that the roots are bitter, astringent, acrid, anti-inflammatory, anodyne, lithnotriptic and diuretic [191]. It is also reported that, roots are also used in urinary calculi and renal troubles, disease of vagina, uterus, and pain in joint [192]. Tender shoots of the plant are bruised and applied to eyes in case of

opthalmia [193].

The antibacterial activity of leaves and fruits of plant were reported [194]. Methanolic extract of root of *Dichrostachys cinerea* was investigated as a natural remedy for sexually transmitted diseases (STD) [195].

Numerous uses have been found for *Dichrostachys cinerea* in traditional medicine [11]. The roots are used as a diuretic, febrifuge, antivenom, and antirheumatic and against leprosy. The trunk bark is believed to be antivenom and antidysenteric and to be active against tooth decay and leprosy[196].

The leaves are used to treat eczema, abscesses, measles, and rheumatism. Fresh twigs with leaves are recommended to prevent miscarriage[197]. The fruit is utilized for the prevention of otitis, umbilical hernia, and malaria in children. Despite all these interesting properties, chemical investigations on the plant are scant and mostly limited to tannins, with the noticeable exception of a very recent preliminary report on the antitumor activity of some still unindenfied constituents[197].

Dichrostachys cinerea is endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity[198,199]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti- atherosclerotic, antitumor, antimutagenic,

anticarcinogeni, antibacterial, and antiviral activities[200,201].

1- 7: *Combretum aculeatum*

Classification:

Phylum Magnolioph

Class Magnoliopside

Order Matales

Family Combretaceae

Genus Combretum

Synonyms: *Combretum leuconili* Schweinf., *C. holstii*

Vernacular/common names: Bularal, laonadi, laongi, (Peulh), agersigil (Tamachek), kodentabga (Mooré), shihheit (Sudan), gedajedo, mardaf (Somali).

1- 7- 1: Distribution and habitat

Combretum aculeatum is sub-Sahelian dry zone species with a distribution range stretching across Africa from Senegal and Mauritania, to Somalia and Tanzania. It is widespread in dry areas, in bushland, woodland, savannah, and wooded grassland. It is often found along rivers, riverine forest and ground water forests, as well as on rocky slopes. It grows in bushland on fixed dunes, on sandy alluvium or in rocky places. It has a wide edaphic adaptation growing on alluvial soils and sandy, stony or clay soils. It can grow at altitudes of up to 1800 miles. It is reported to withstand flooding; however in the seasonally flooded areas of Sudan, it is restricted to termite mounds, which are generally above the flood level. Its distribution is irregular and is locally common. There is no recorded threat to *C.*

aculeatum; however, the species is strongly browsed and regeneration suffers in heavily overgrazed areas[202].

1- 7- 2: Uses

C. aculeatum is important source of nutrients for animals, which consume the leaves, flower and young shoots. its appreciated for its nutritive value and also for its palatability to stall fed and browsing sheep. The green leaves and young branches are much sought after as browse by both wild and domestic animals, and even the fallen leaves are eaten. Seeds of *C. aculeatum* are edible and in some places used for consumption, they are also eaten by wild and domestic animals[203].

1- 7- 3: Medicinal uses

The plant is used for its purgative and diuretic properties. It is used to treat blennorrhoea, colic, diarrhea, intestinal worms, wounds, fever, gastritis, and loss of appetite. Some more speculative traditional uses include treatment against female sterility and mental disorders. Water in which the leaves have been boiled is drunk in northwest Senegal to promote micturition in cases when venereal disease obstructs the urethra. It is also used in Burkina Faso and Senegal for leprosy. In Senegal, the Soce tribe claims that a root decoction has a well-established reputation in the treatment of catarrha; the Serer tribe uses sap from the center of the stem for eye troubles. The boiled roots are taken in Kenya for stomach upsets. Also the macerations of the roots of *C. aculeatum* are used to enhance

wounds healing and the water extract of its roots is used as a purgative and as a poultice for skin tuberculosis in Sudan [202-204].

1- 7- 4: Botanical description

Combretum aculeatum is a climbing shrub that subsists often on its annual shoots after been eaten. It can grow up to 4 m, even taller if support is available. The bark is fibrous grey-beige or dark red, with brown rhytidome, greenish or pale yellow slash. It often has long sarmentose branches. The leaves are alternate to sub-opposite. They can vary in size on the same branch. The blades can grow up to 7 cm long and 5 cm wide, but are usually smaller. They are elliptic, obovate or orbicular with acute to emarginated apex; both surfaces are pubescent. The nerves are pinnate, more or less prominent, with 4-6 pairs of mostly fused Lateral nerves. Petioles are 1-10 mm long, and persist after the rest of the leaf has fallen, forming a recurved spine that is up to 30 mm long. Its hairy branches with curved thorns allow the plant to hook onto surrounding trees and shrubs. The yellowish-white fragrant flowers are bisexual, with greenish to dark red sepals. The petals are 4-8 mm long by 1-2 mm wide, oblanceolate to obovate to spatulate, and pubescent on the back. The inflorescence is spike like, from the axils of the leaves. The amenfilaments are longer than the petals[204].

Flowering occurs at the end of the dry season and during rainly season. In Sudan flowering occurs from March to June and

fruits from July to November. A tree cans beer flowers and ripe fruit simultaneously.

The pale yellow or pale reddish fruit is an ovoid samara. It is 5-winged, 1-2 by 1-3 cm, and with a stalk 0.6-1 cm long. The papery, yellow-brown wings are 0.4-0.6 cm wide[202].

Combretum aculeatum

Preliminary phytochemical screening of *Combretum aculeatum* leaves revealed that it is rich of tannins, unsaturated sterols and or triterpenes and flavonoids .Also there is presence of coumarins in low concentration and trace of alkaloids[205].

Extracts of leave and root show anti-cercarial activity against cercariae of *Schistosoma mansonia* [205]. Extracts of *C. aculeatum* showed anti- microbial activity against Gram-positive bacteria (*Bacillus subtilis* and *staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*)[206].

In Sudan, the genus *Combretum* represents a common constituent of forests on high drained rainfall savanna on wells or alluvial soils along streams, rivers and valleys in south Kassala, Kordofan and south Darfor[207].

Phytochemical studies carried out in the genus *Combretum* have demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans and amino acids. Also phenanthrene, dihydrophenanthrene, methoxylated flavonoids, stibenes were reported from the different species[208].

About 24 different species of *Combretum* are well known in traditional medicine and used for treatment of an array of human disorders, such as abdominal pain, back-pain, cough, cold, conjunctivitis, diarrhea, dysmenorrhea, earache, fever, headache, fighting worms, infertility in women, fattening babies, leprosy, eumonia, swelling caused by mumps, scorpion stings and snake bites [209-211].

Since the 1970s, several unusual compounds have also been isolated from *Combretum* species, for example, 9, 10-dihydrophenanthrenes and a substituted bibenzyl from *C.*

molle[212] was isolated. Eleven triterpenes and theirglycosides were isolated from *C. laxum* among them, oleanane -, ursane-glycosides, arjunolic acid, arjunglucoside II, bellericoside, chebuloside II, quadranoside IV, asiatic acid and betulinic acid [135] .Alkaloids (combretine and betonicine) were isolated from the leaves of *C. micranthum* [213]. Some flavonoids, rhamnoctrin, quercetin-5, 3'-dimetylether, ramnazi and kaempferol were isolated from *C. erythrophyllum*[213], as well as quercetrin, kaempferol and pinocembrin (flavanone) from *C. apiculatum C rdamonin*. A Chalcone was also isolated from *C.apiculatum* [214] and ellagic acid derivatives from *C. kraussii* [215].Combretastatins, a group of stilbenes, have been isolated from several species of *Combretum* [216].

Several phytochemical investigations on this genus focus mainly on pentacyclic triterpenoids, various polyphenols like flavonoids and stilbenoids[217]. GC/ MS showed presence of triterpenoids and stilbenoids in dichloromethane fractions of leaf and stem bark of *C. aculeatum, C. glutinosum and C. micranthum* . Ursolic acid was identified in the leaf extracts of all the three species whereas combretastatin A4 was found in small amounts only in the bark extract of *C glutinosum* species[218].

Oleanene-type of pentacyclic terpenoids containing 29-carboxyl-1α-hydroxyl groups were isolated from various species of *Combretum* e.g. *C.molle* and *C. imberbe* confirming

chemotaxonomically significant bifurcation in triterpenoids synthesis in *Combretum* species[218].

Aim of this study

This study was designed to fulfill the following:

-Extraction of flavonoids from two medicinal plants- *Combretum aculeatum* and *Dichrostachys cinerea*.

-Isolation of pure flavonoids via some analytical tools.

-Elucidation of structures of the isolated compounds via some spectral tools.

-Evaluation of the antimicrobial potential of the target plants.

Chapter Two

Materials and Methods

2. Materials and Methods

2- 1: Materials

2- 1- 1: Plant Material

The roots of *Combretum aculatum* were collected from a forest reserve around Hawata western Sudan. *Dichrostachys cinerea* leaves were collected from Alnohud - western Kordufan -Sudan. The plants were identified and authenticated by the Institute of Aromatic and Medicinal Plants-Sudan.

2- 1- 2: Equipments

Ultraviolet spectra were recorded in spectroscopic methanol on a Shimadzu UV -Visible Spectrophotometer. ^{1}HNMR spectra were measured on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d_6.

2- 2: Methods

2- 2- 1: Flavonoid and phenolic test reagents

- Aluminum chloride solution

1 g of aluminum chloride was dissolved in 100 ml methanol

- Potassium hydroxide solution

1 g of potassium hydroxide was dissolved in 100 ml distilled water.

- Ferric chloride solution

1 g of ferric chloride was dissolved in 100 ml methanol.

2- 2- 2: Test for flavonoids

20 ml of the (PE) was evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added. Red colour was observed.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added. A dark yellow colour was formed.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added. A dark yellow colour was observed.

2- 2- 3: Isolation of flavonoids from *Combretum aculeatum*

Powdered roots of *Combretum aculeatum* (950g) were macerated with 95% ethanol for 72 hours. The solvent was evaporated under reduced pressure. The resulting extract was applied on Whatman No. 3mm papers as concentrated narrow

zones. The bands were developed with BAW (4:1:5;V:V:V). The developed papers were air-dried and examined under long wavelength UV light (λ_{max} 366nm). The equivalent bands from each paper were combined, cut into small pieces and the product was eluted with methanol. The solvent was evaporated to give a powder -compound I.

Different UV shift reagents (sodium methoxide, sodium acetate and aluminium chloride) were used to elucidate the hydroxylation pattern on the nucleus of the isolated flavonoid.

2- 2- 4: Extraction and isolation of flavonoids from *Dichrostachys cinerea*

Powdered shade-dried leaves of *Dichrostachys cinerea* (450g) were defatted with petroleum ether and macerated with70% methanol for 48h. The solvent was removed under reduced pressure and the residue was dissolved in water (150ml) and filtered. The filtrate was partitioned with successive portions of n-hexane, chloroform, ethyl acetate and n-butanol.

The n-butanol extract which was rich in flavonoids, was concentrated to dryness to yield a dark amorphous material. This extract was chromatographed on polyamide column eluted by H_2O/ EtOH (3:4;v:v) where three sub fractions (i), (ii) and (iii) were obtained. Fraction (iii) was chromatographed on a Sephadex LH-20 column using 50% methanol as an eluant. Thus two flavonoids (compounds II and III were isolated. The

isolated flavonoids were further purified before spectral analysis by paper chromatography eluted with BAW (n-BuOH-HOAc-H_2O 4:1:5, upper layer).

2- 2- 5: Stepwise procedure for use of shift reagents for UV

- The UV spectrum of the compound in methanol was first recorded.
- 3 drops of NaOMe reagent were added to the sample and the NaOMe spectrum was recorded, and after 8 minutes the NaOMe spectrum was re-recorded.
- 6 drops of $AlCl_3$ reagent were added to the fresh sample and the $AlCl_3$ spectrum was recorded, 3 drops of HCl were added and after mixing, the $AlCl_3$/ HCl spectrum was recorded.
- Powdered NaOAC was then added to the fresh sample, the mixture was shacked and the NaOAC spectrum was recorded. NaOAC/ H_3BO_3 spectrum was then recorded after adding H_3BO_3.

2- 2- 6: Preparation of plant extract for phytochemical screening

(100g) of powdered shade-dried plant material were extracted with 80% methanol (Soxhlet) for 6 hours. The cooled solution was filtered and evaporated to dryness. This prepared extract (PE) was subjected to preliminary phytochemical screening for the presence of flavonoids.

2- 2- 7: Antimicrobial assay

The methanolic extract and ethyl acetate fraction were screened for their antimicrobial activity against six standard human pathogens (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*) using the cup plate agar method with some minor modifications.

2- 2- 7- 1: Preparation of bacterial suspensions

One ml. aliquots of 24 hours broth culture of the test organisms were distributed onto agar slopes and incubated at 37^{o} C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce suspension containing about 10^{8}- 10^{4} colony forming units per ml. The suspension was stored in refrigerator at 4^{o}C until used. The average number of viable organism per ml of the saline suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volume (0-02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature to dry, and then incubated at 37^{o} C for 24 hours.

2- 2- 7- 2: Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25^{o}C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

2- 2- 7- 3: Testing for antibacterial activity

The cup plate agar diffusion method was adopted with some minor modification, to assess the antibacterial activity of the methanolic extract and ethyl acetate fraction of *Combretum aculeatum* .Two ml of the standardized bacterial stock suspention were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45^{o} C in water bath.

(20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes and the agar was left to settle in each of these plates which were divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 4). Each of the halves was designed for one of the extracts.

The agar discs were removed and cups were filled with (0.1) ml of each extract using adjustable volume micro titer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ^{o} C for 24 hours.

The above procedure was repeated for different concentrations of the extracts and the standard antimicrobial chemotherapeutics. After incubation the diameters of the resultant growth inhibition zones were measures.

2- 2- 7- 4: Testing for antifungal activity

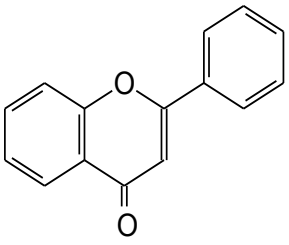
The above mentioned method was adopted for antifungal activity, but instead of nutrient agar dextrose agar was used. Samples were used here by the same concentrations used above.

Chapter three

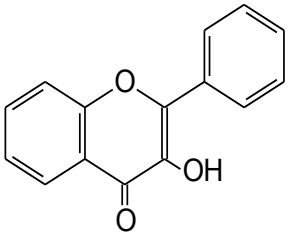
Results and Discussion

3. Results and Discussion

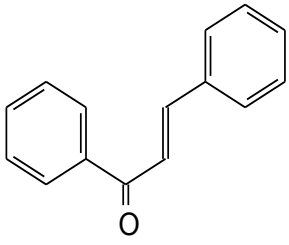
In most cases, the UV spectra of flavonoids are a valuable tool that can distinguish the class of a specific flavonoid. Some flavonoids exhibit two UV absorption bands: band II – due to benzoyl chromophore – and band I – due to cinnamoyl chromophore. Band II appear in the range 230-290 nm, while band I occur usually in the range 300-400nm.Those flavonoids which are characterized by conjugation between the carbonyl function and the aromatic B ring of flavonoids exhibit both bands. Thus flavones, flavonols, chalcones and aurones show both bands. Other classes of flavonoids – flavanones, isoflavones, dihydrochalcones and dihydroflavonols – exhibit only one band (band II) due to loss of conjugation between the carbonyl group and ring B.



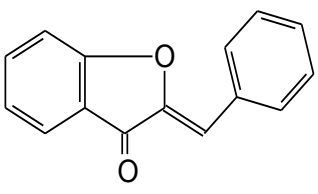
Flavone



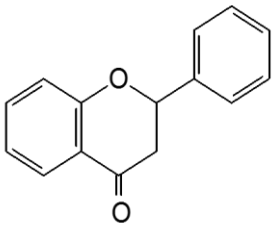
Flavonol



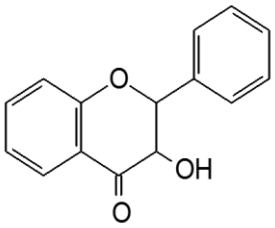
Chalcone



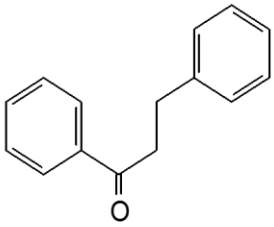
Aurone



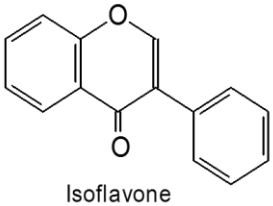
Flavanone



Dihydroflavonol



Dihydrochalcone



Isoflavone

3- 1: *Combretum aculatum*

3- 1- 1: Characterization of compound I

Compound I, which was isolated from *Combretum aculatum* roots via paper chromatography, was partially characterized by its spectral data. The UV spectrum of compound I showed (Fig. 3) λ_{max} (MeOH) 281, 340 (sh.) nm. The appearance of only one band- band II – together with a shoulder at 340nm suggests an isoflavone.

The hydroxylation pattern of this compound was studied using the UV shift reagents; sodium methoxide (diagnostic of 3- and 4`-OH); sodium acetate (diagnostic of 7-OH); aluminium chloride (diagnostic of 3- and 5-OH and catechol systems) and boric acid (diagnostic of catechol moieties). The sodium methoxide spectrum (Fig. 4) showed a bathochromic shift without decrease in intensity indicating a 4`-OH function. The sodium acetate spectrum (Fig. 5) did not reveal any

bathochromic shift indicating absence of a 7-OH. Also no bathochromic shifts were observed in the aluminium chloride (Fig. 6) and boric acid (Fig. 7) spectra. Such findings suggest absence of 3- , 5-OH groups as well as catechol systems.

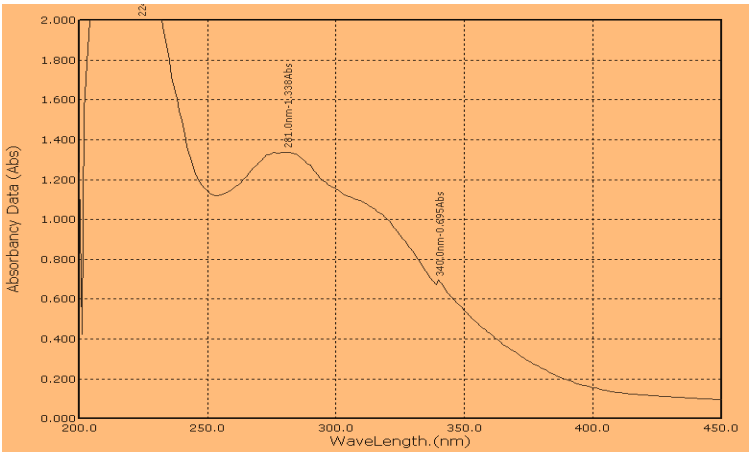


Fig. 3: UV spectrum of compound I

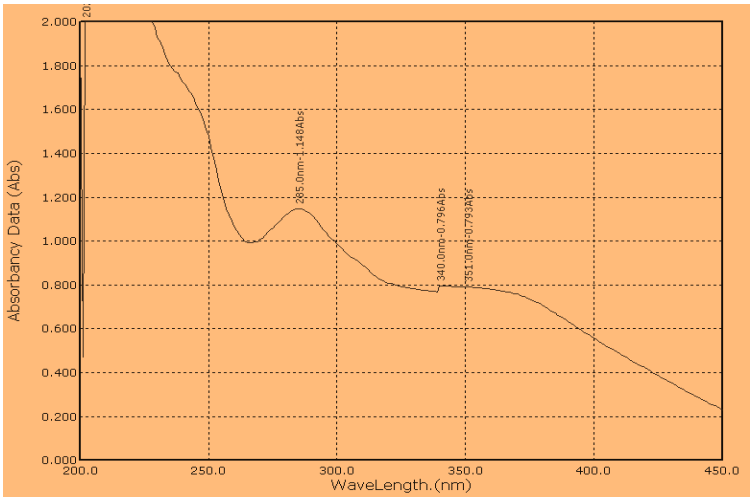


Fig. 4: Sodium methoxide spectrum of compound I

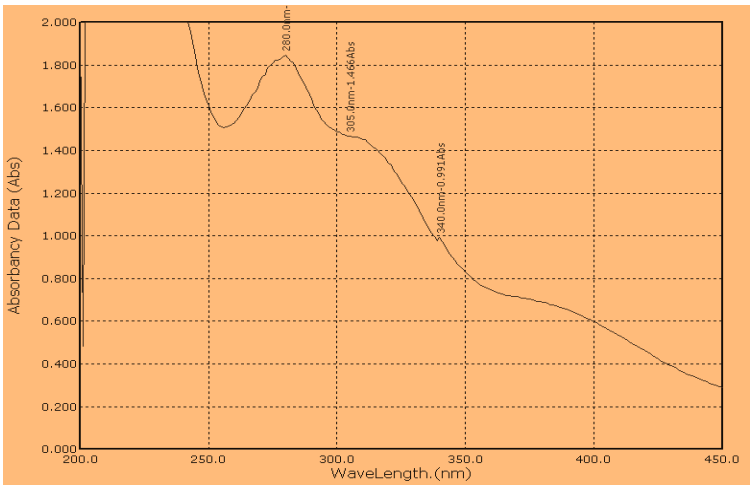


Fig. 5: Sodium acetate spectrum of compound I

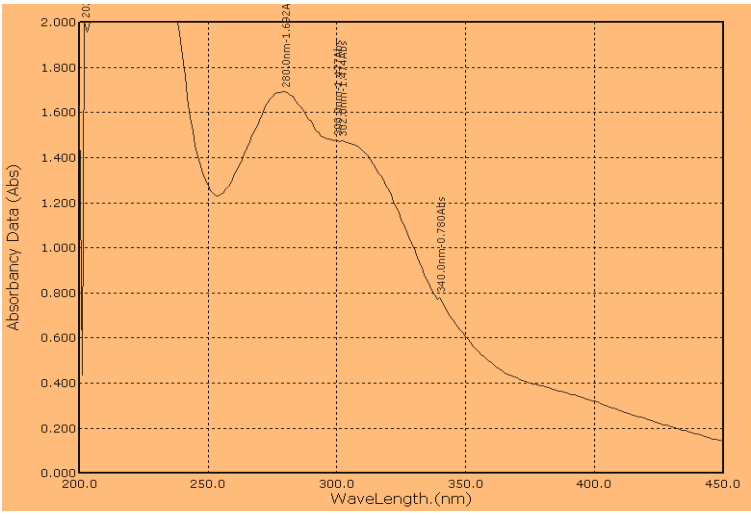


Fig. 6: Aluminium chloride spectrum of compound I

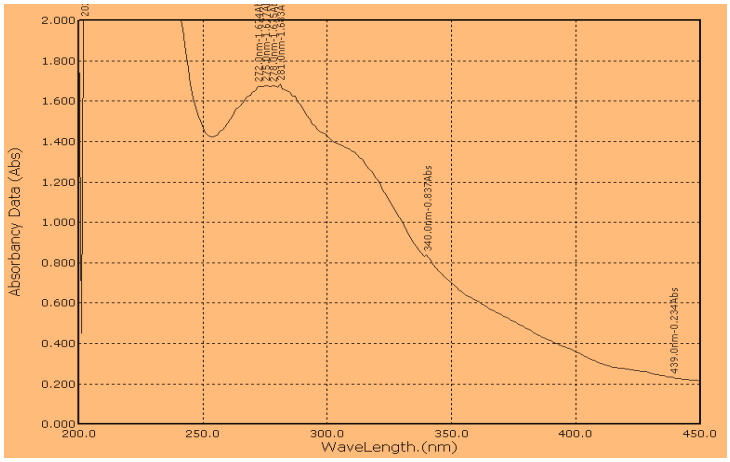


Fig. 7: Boric acid spectrum of compound I

The 1HNMR spectrum (Fig. 8) showed δ (ppm): 1.25 (assigned for a methyl); 2.90 (Acetyl); 4.00-5.50 (assigned for a sugar residue-not identified in this study); 6.60 - 7.40 (multiplet, Ar. protons), 7.50 and 7.70 (Ar. protons). The signal at δ2.50 and δ3.50 is due to solvent (DMSO) residual protons and residual water respectively.

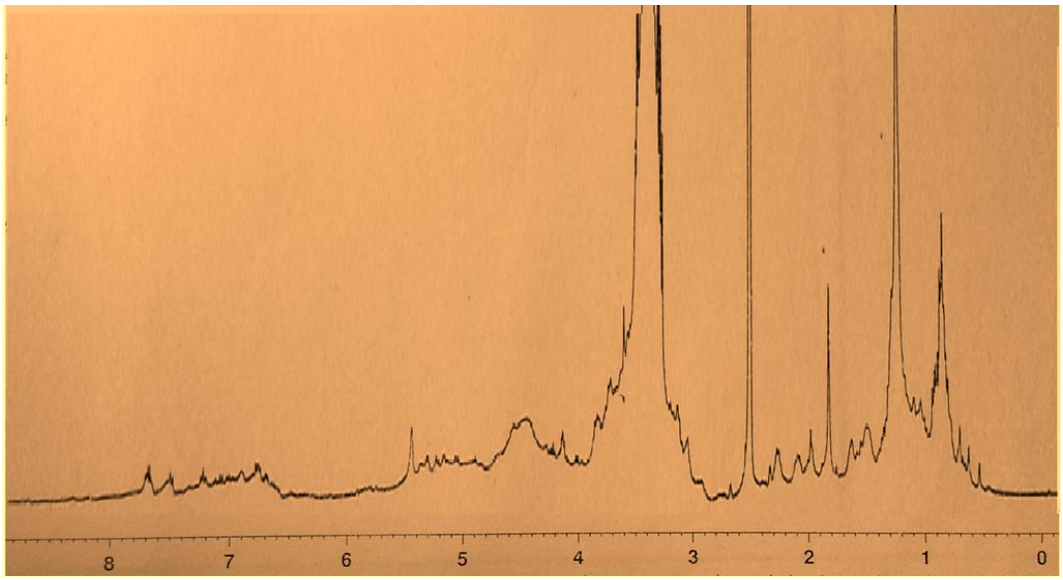
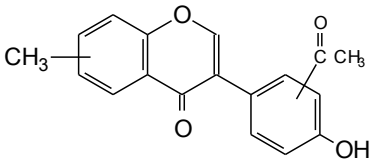


Fig. 8: [1]HNMR spectrum of compound I

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



Compound I

3- 1- 2: Antimicrobial assay of *Combretum aculeatum*

The methanol extract and of *Combretum aculeatum* was screened for antimicrobial potential against six standard human pathogenic

(*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*).

The methanolic extract showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*. It also showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus*. The extract also exhibited significant antifungal activity against the fungi: *Candida albicans and Aspergillus niger* (Table 1).

Table 1: Inhibition zones for methanol extract

	Inhibition zone diameter(mm) ; (100mg/ml sample)					
	*Ec	Pa	Bs	Sa	Ca	An
Methanolic Extract	22	30	17	17	19	22

*Ec= *Escherichia coli*

Bs= *Bacillus subtilis*

Sa= *Staphylococcus aureus*

Pa= *Pseudomonas aeruginosa*

Ca= *Candida albicans*

An= *Aspergillus niger*

3- 2: *Dichrostachys cinerea*

3- 2- 1: Identification of compound II

The UV spectrum (Fig. 9) of compound II showed λ_{max} 253,347nm.

Such absorption is characteristic of (i) flavones (ii) flavonols and (iii) chalcones. However, the UV shift reagent – sodium methoxide – gave a bathochromic shift (Fig. 10) with decrease in intensity indicating a 3-OH function which is a characteristic feature of flavonols.

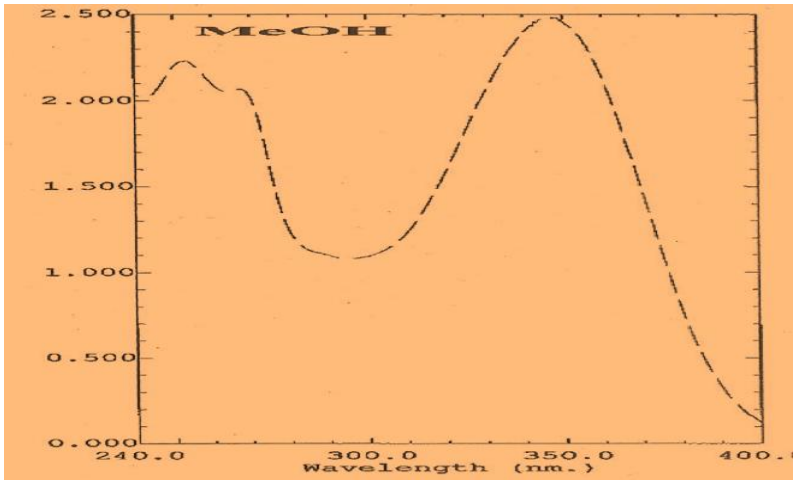


Fig. 9: UV spectrum of compound II

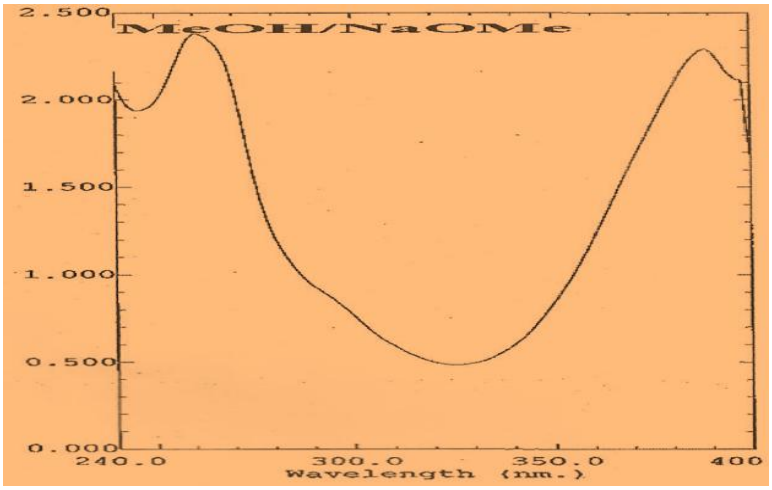


Fig. 10: Sodium methoxide spectrum of compound II

Next, the hydroxylation pattern of the isolated flavonol was investigated by using the shift reagent – aluminium chloride which raveled a bathochromic shift indicative of 3- , 5-OH functions and

catechol systems.

The shift reagent aluminium chloride gave a bathochromic shift diagnostic of a 3-OH group (Fig. 11) - the spectrum was quite stable in acidic media (Fig. 12). It is known that catechol form acid labile complexes with aluminium chloride.

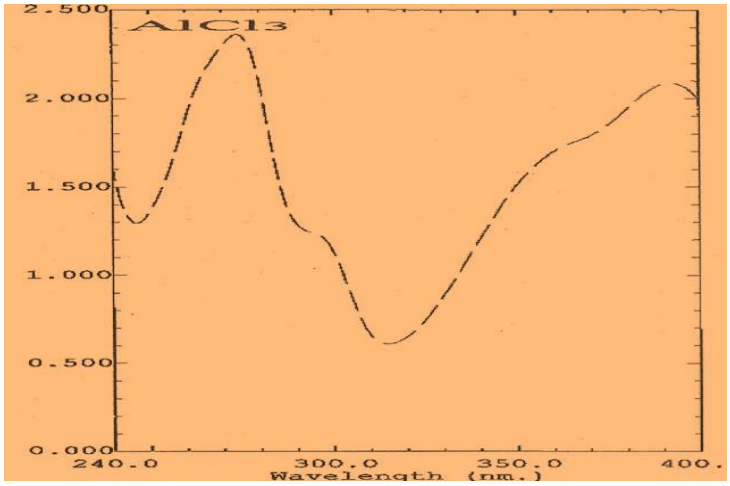


Fig. 11: Aluminium chloride spectrum of compound II

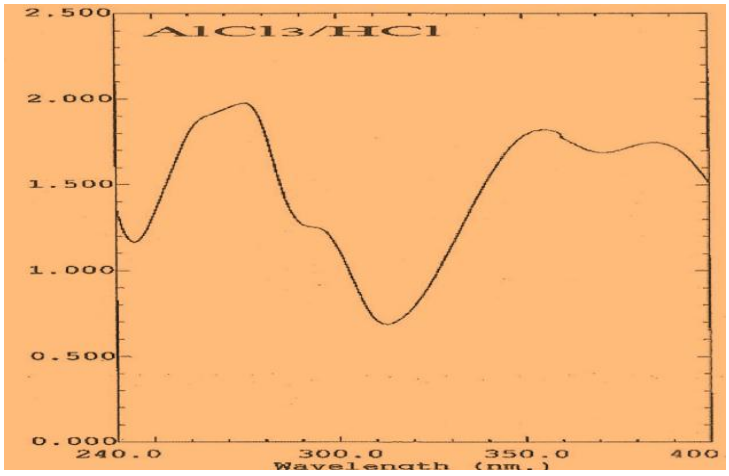


Fig. 12: $AlCl_3$ /HCl spectrum of compound II

The boric acid spectrum failed to reveal any bathochromic shift indicating absence of catechol moieties (Fig. 13).

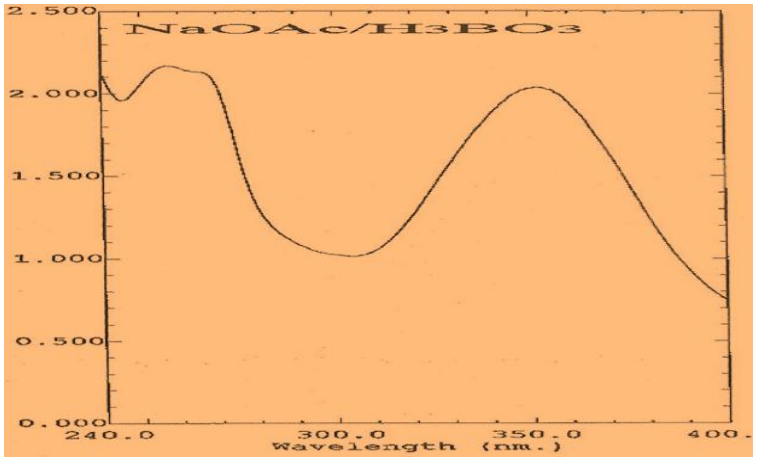


Fig. 13: Boric acid spectrum of compound II

The ^{1}HNMR spectrum (Fig. 14) showed δ (ppm): 1.14 (Integrating for six protons and assigned for two methyl groups); 3.85 (6H, assigned for two methoxyl functions); 5.12, 5.31 (accounting for a sugar moiety which was not identified in this study); 6.38 (1H, attributed to C_6-H); 6.82 (1H, assigned for C_8 proton). Other aromatic protons appeared at 6.91, 7.39 and 7.56ppm.

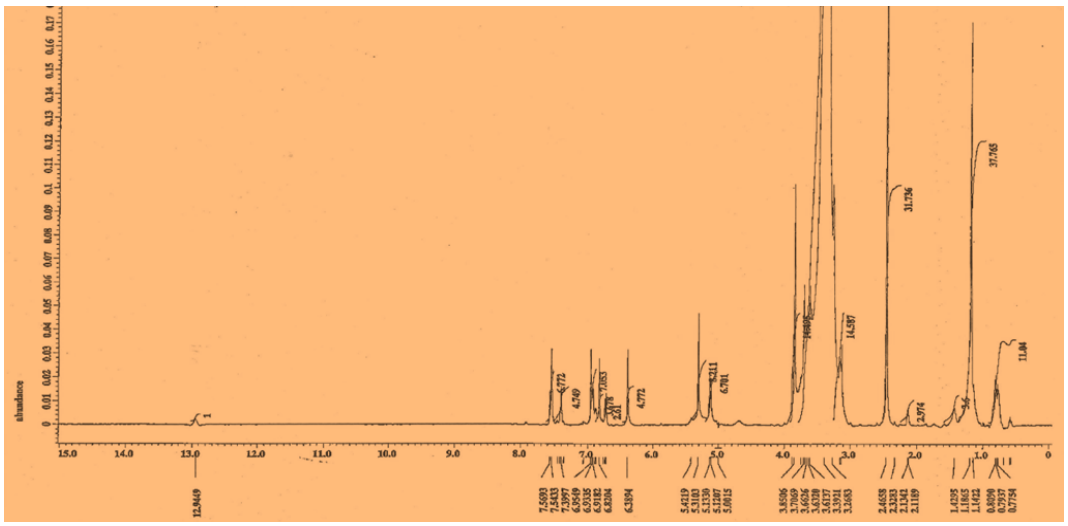
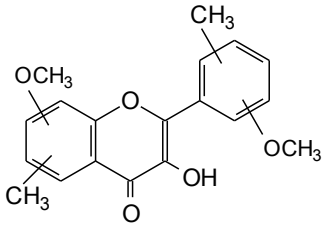


Fig. 14: ^{1}HNMR spectrum of compound II

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



Compound II

3- 2- 2: Identification of compound III

The UV spectrum of compound III showed (Fig. 15) λ_{max} (MeOH) 289,323nm. Such absorption suggests a flavone. The hydroxylation pattern of this compound was studied using different UV shift reagents; sodium methoxide (diagnostic of 3- and 4`-OH); sodium acetate (diagnostic of 7-OH); aluminium chloride (diagnostic of 3- and 5-OH and catechol systems) and boric acid (diagnostic of catechol moieties).

The sodium methoxide spectrum (Fig. 16) showed a bathochromic shift in band I with increase in intensity indicating a 4`-OH function. The sodium acetate spectrum (Fig. 17) failed to afford a bathochromic shift suggesting absence of a 7-OH group. The aluminium chloride spectrum (Fig. 18) gave a 6nm bathochromic shift in band I. This shift is diagnostic of a catechol system (the aluminium complex decomposed on addition of HCl as suggested by (Fig. 19). Further evidence in favor of a catechol moiety comes from the boric acid

spectrum (Fig 20) which revealed a bathochromic shift(see also figures 21-23).

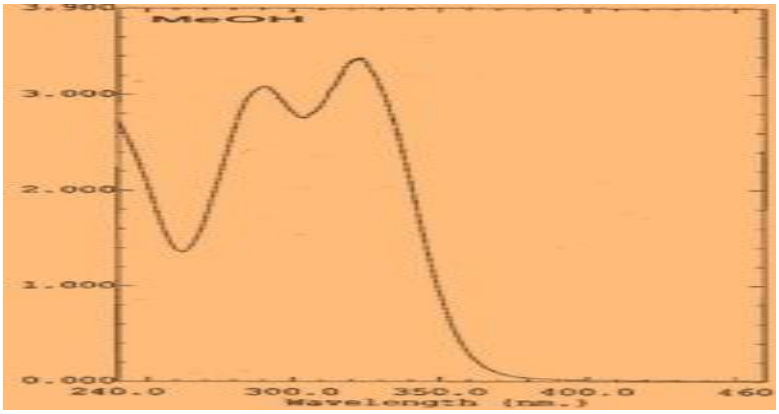


Fig. 15: UV spectrum of compound III

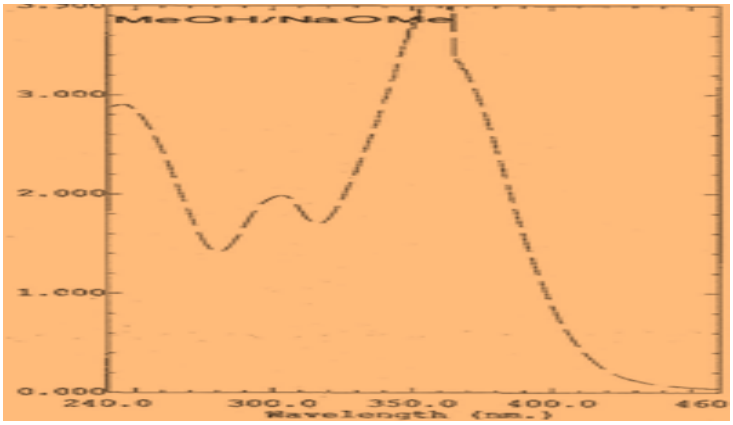


Fig. 16: Sodium methoxide spectrum of compound III

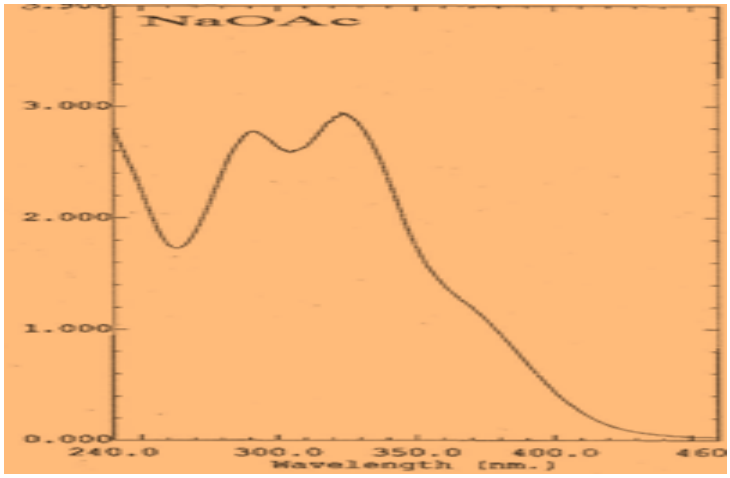


Fig. 17: Sodium acetate spectrum of compound III

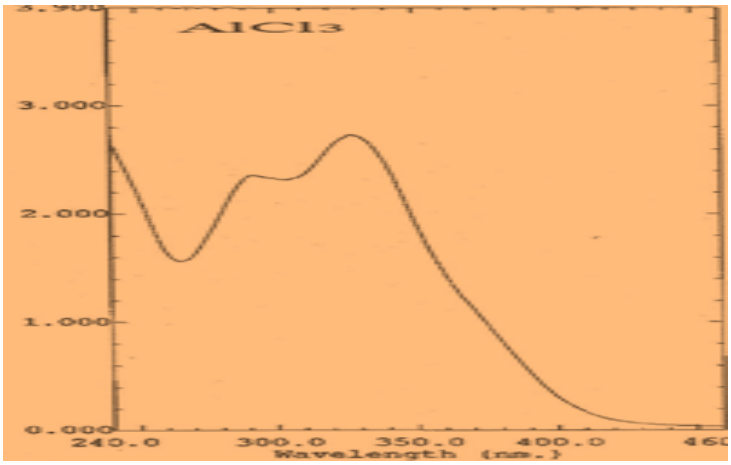


Fig. 18: Aluminium chloride spectrum of compound III

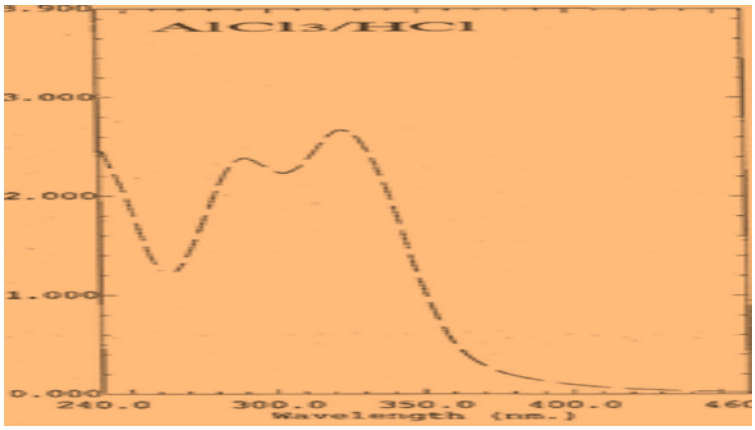


Fig. 19: Aluminium chloride / HCl spectrum of compound III

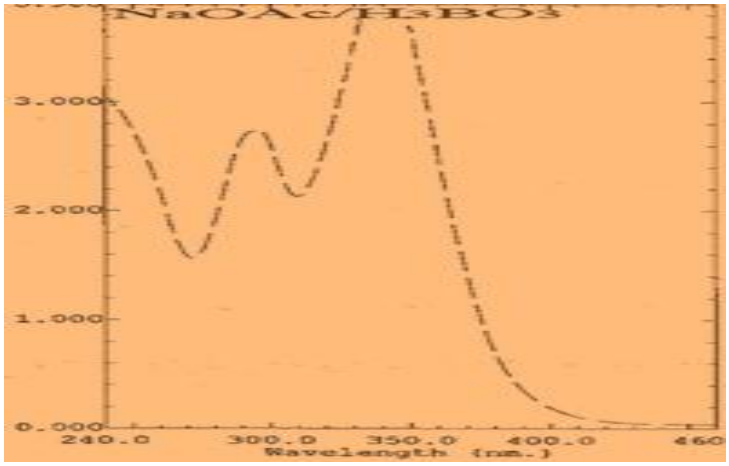


Fig. 20: Boric acid spectrum of compound III

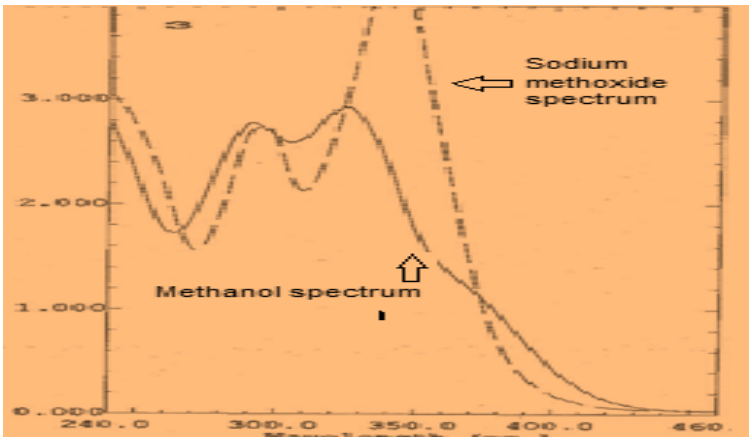


Fig. 21: Methanol/sodium methoxide spectrum

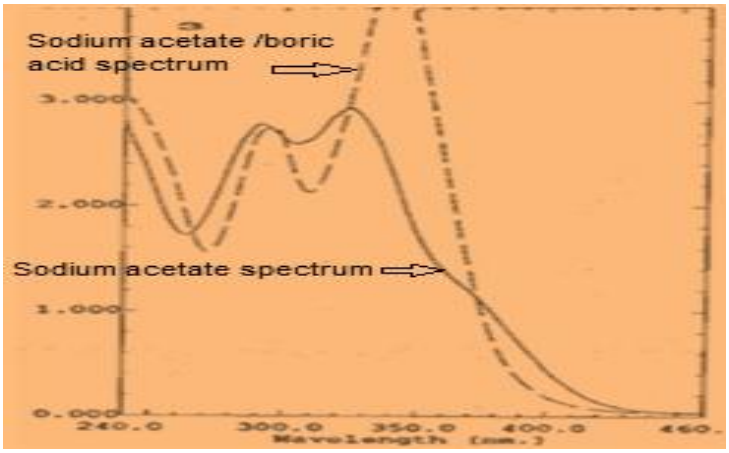


Fig. 22: Sodium acetate / boric acid in sodium acetate spectra

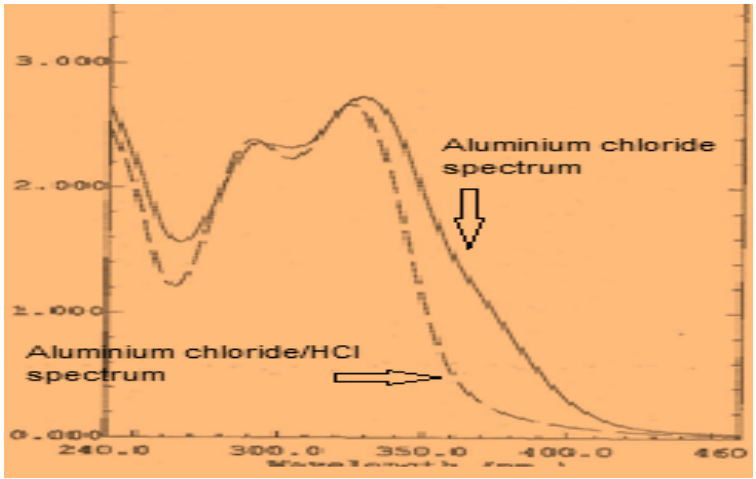


Fig. 23: Aluminium chloride /Aluminium chloride/HCl spectra

The ^{1}HNMR spectrum (Fig. 24) showed δ (ppm): 1.18 (assigned for a methyl group); 2.66, 2.81(2 acetyl groups); 4.31(methoxyl); 6.18-7.21-multiplet (Ar. protons); 7.91(Ar. proton).

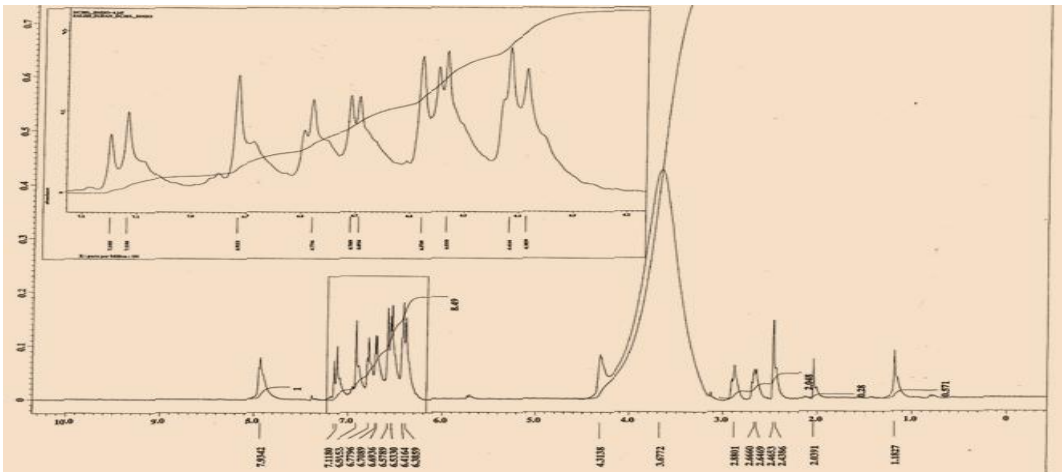
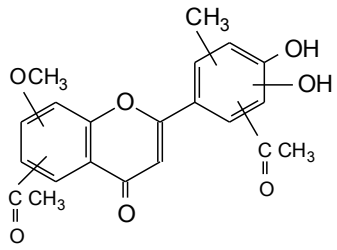


Fig. 24: ^{1}HNMR spectrum of compound III

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



Compound III

Conclusion

The flavonoids of two key species in Sudanese ethnomedicine have been investigated and three flavonoids (I-III) have been isolated. Compound I was isolated from *Combretum aculatum* roots via paper chromatography , while compounds II and III were isolated from the leaves of *Dichrostachys cinerea* . The structures of these flavonoids have been partially characterized by their spectral data (UV and ^{1}HNMR). The methanolic extract of *Combretum aculeatum* was assessed for its antimicrobial activity. It showed significant activity against *Escherichia coli* and *Pseudomonas aeruginosa*

Recommendations

The following is recommended:

1-The structures of the isolated flavonoids may fully be characterized by a future 2D NMR measurement.

2-The isolated flavonoids could be evaluated for their biological activity.

3-Other phytochemicals of the target species may be isolated, characterized and then screened for their biological activity.

References

References

1. Harborne, J.B., The Flavonoids: Advances in Research Since 1986, Chapman & Hall, London. xd,(1993).
2. Croft KD., The chemistry and biological effects of flavonoids and phenolic acids. *Ann N Y Acad. Sci*., **854**, 435-42(1998).
3. Cushnie, TPT, Lamb, AJ. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, **26**, 343-356(2005).
4. G. Di Carlo, N. Mascolo, A.A. Izzo, F. Capasso, Flavonoids: Old and new aspects of a class of natural therapeutic drugs, *Life Sci.*, **65**, 337–353(1999).
5. Andersen, Ø.M.; Markham, K.R. Eds. Flavonoids: Chemistry, Biochemistry and Applications, CRC Press: Boca Raton, (2005).
6. Grotewold, E. Ed., The Science of Flavonoids, Springer: New York, (2006).
7. Middleton EJ., Effect of plant flavonoids on immune and inflammatory cell function, *Adv. Exp. Med. Biol.*, **439**:175–182(1998).
8. Woo HH, Kuleck G, Hirsch AM, Hawes MC., Flavonoids: signal molecules in plant development, In Bela SB, Michael EB. (eds.), Flavonoids in cell function, New York, USA, *Kluwer Academic/ Plenum Publishers*, 51-60(2002).
9. Williams, C.A.; Grayer, *R.J. Nat. Prod. Rep.*, **21**, 539(2004).
10. Murray, MT., Quercetin: Nature's antihistamine, *Better Nutrition*, (1998).
11. Fernandez, SP, Wasowski, C, Loscalzo, LM, Granger, RE, Johnston, GAR, Paladini, AC, Marder, M. Central

nervous system depressant action of flavonoid glycosides, *European Journal of Pharmacology* , **539**, 168-176(2006).

12. Heim, KE, Tagliaferro, AR, Bobliya, DJ. Flavonoids antioxidants: Chemistry, metabolism and structure activity relationships, *The Journal of Nutritional Biochemistry*, **13**, 572-584(2002).
13. Hollman, PCH, Katan, MB. Dietary Flavonoids: Intake, Health Effects and Bioavailability. *Food and Chemical Toxicology*, **37**, 937-942(1999).
14. Rupasinghe HPV. The role of polyphenols in quality, postharvest handling and processing of fruits, Ed: Paliyath G, Lurie S, Murr D. Handa A. Postharvest Biology and Technology of Fruits, Vegetables, and Flowers, *Wiley-Blackwell Publishers*, 260-81(2008).
15. Waterman, P. G., Mole, S. , Structure and biosynthesis of phenolic compounds, in Analysis of phenolic plant metabolites, *Blackwell Scientific Publications*, London, UK,1-35(1994).
16. Quideau, S., Deffieux, D., Douat-Casassus, C., Pouységu, L., Plant polyphenols: Chemical properties, biological activities, and synthesis, *Angew. Chem. Int. Ed*. **50**,586–621(2011).
17. USDA Database for the Flavonoid Content of Selected Foods, (2003). http://www.nal.usda.gov /fnic/foodcomp/Data /Flav/flav.html
18. Robles, C., Greff, S., Pasqualini, V., Garzino, S., Bousquet-Melou, A., Fernandez, C., Korboulewsky, N. & Bonin, G., Phenols and flavonoids in Aleppo pine needles as bioindicators of air pollution, *J. Environ. Qual.*, **32**, 2265-2271(2003).

19. Robertson, A. The Chemistry and Biochemistry of Black Tea Production – the non-volatiles, In Tea: Cultivation to Consumption; Wilson, K.C., Clifford, M.N., Eds.; Kluwer Acad. Publ.: Dordrecht, *The Netherlands*, 574-580(1991).
20. Jain P.K., Kharya M.D., Gajbhyie A., Sara U.V.S., Sharma V.K., *Herba Pol*. **56**, 105 (2010).
21. He, J.; Santos-Buelga, C.; Mateus, N.; de Freitas, V. Isolation and Quantification of Oligomeric Pyranoanthocyanin-flavanol Pigments from Red Wines by Combination of Column Chromatographic Techniques. *J. Chromatogr. A*, **1134**, 215-225(2006).
22. Hergert, H.L. Condensed Tannins in Adhesives: Introduction and Historical Perspectives; Repap Technologies Inc., *Valley Forge, PA, USA*, **385**, 155–171(1989).
23. Agricultural Research Service. US Department of Agriculture (USDA) Database for the flavonoid content of selected foods, Beltsville (2014). http://www.ars.usda.gov/SP2UserFiles/Place/80400525/Data/Flav/Flav_R03-1.pdf. Accessed (5 Jun 2015)
24. Agricultural Research Service. US Department of Agriculture (USDA) Database for the isoflavone content of selected foods, Beltsville (2008). http://www.ars.usda.gov/SP2UserFiles/Place /80400525/Data/isoflav/ Isoflav_R2.pdf. Accessed (9 Jun 2015)
25. Agricultural Research Service. US Department of Agriculture (USDA) Database for the proanthocyanidin content of selected foods, Beltsville (2004). http://www.ars.usda.gov/SP2UserFiles/Place/80400525/Data/ PA/PA.pdf. Accessed (7 Jun 2015)

26. Cook, NC, Samman, S., Flavonoids: Chemistry, metabolism, cardioprotective effects and dietary sources, *Nutritional Biochemistry*, **7**, 66-76(1996).
27. Sahu, SC, Gray, GC. Pro-oxidant activity of flavonoids: effect on glutathione and glutathione-Stransferase in isolated rat liver nuclei, *Cancer letters*, **104**, 193-196(1996).
28. Prey, JO, Brown, J, Fleming, J, Harrison, PR. Effect of dietary flavonoids on major signal transduction pathways in human epithelial cells. Biochemical Pharmacology, **66**, 2075-2088(2003).
29. S. Hamamatsu, K. Yabe and Y. Nawa, "Compositions of Anthocyanin and Other Flavonoids in Cultured Rabbiteye Blueberry (Va ccinium ashei Reade cv. Tiiblue)," *Food Science and Technology Research*, **10**(3), 239-246(2004). doi:10.3136/fstr.10.239
30. A. H. Laghari, S. Memon, A. Nelofar, K. M. Khan, A. Yasmin, M. N. Syed and A. Aman, "A New Flavanenol with Urease-Inhibition Activity Isolated from Roots of Manna Plant Camelthorn (Alhagi maurorum)," *Journal of Molecular Structure*, **965**(1-3), 65-67(2010). doi: 10.1016 /j.molstruc. 2009.11.039
31. Peterson, J., Dwyer, M., *Nutrition Research*, **18**, 1995(1998).
32. Tsuchiya, H., Structure-dependent membrane interaction of flavonoids associated with their bioactivity. Food Chemistry, **120**, 1089-1096(2010).
33. Rijke, E. D., Out, P., Niessen, W. M. A., Ariese, F., Goojer, C., Brinkman, U. A. T., *Journal of Chromatography* ,**1112**, 31(2006).

34. D'Archivio M, Filesi C, Benedetto RD, Gargiulo R, Giovannini C, Masella R., Polyphenols, dietary sources and bioavailability. Ann 1st Super Sanita, **43**(4), 348-61(2007).
35. Aherne, S.A.; O'Brien, N.M. Nutrition, **18**, 75(2002).
36. Iwashina, T., The structure and distribution of the flavonoids in plants. *J. Plant Res.*, **113**, 287– 299(2000).
37. Middleton E, Kandaswami C, Theoharides T, The effect of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol. Rev.* **52**(4), 673–751(2000).
38. Kurowska E. M., Manthey J. A., *J. Agric. Food Chem.*, **52**(10), 2879(May 2004).
39. Datla K. P., Christidou M., Widmer W. W., Rooprai H. K., Dexter D. T., *Neuroreport*, **12** (17), 3871(December 2001).
40. Gaitan, E.,Lindsay, R.H., Reichert, R.D.etal. Antithyriodic and goiterogenic effects of millet: role of C-glycosyl flavones. *J. Clin. Endocrinol. Metab.,* **68**, 707–714(1989).
41. Strack, D. and Wray V. Anthocyanins. In J.B. Harborne (ed.), The Flavonoids: Advances in Research Since 1986. Chapman & Hall, London, 1–22(1992).
42. Hertog, M.G.L., Hollman, P.C.H. and Katan, M.B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed, The Netherlands. *J. Agric. Food Chem.*, **40**, 2379–2383(1992).
43. Hertog, M.G.L., Hollman, P.C.H. and vande Putte, B., Content of potentially anticarcinogenic flavonoids of tea, infusions, wines and fruit juices. *J. Agric. Food Chem.*, **41**, 1242–1246 (1993).

44. Grayer, R.J.; Veitch, N.C. Flavanones and dihydroflavonols, In Flavonoids: Chemistry, Biochemistry and Applications; Anderson, O.M., Markham, K.R., Eds.; CRC Press/Taylor & Francis Group: Boca Raton, FL, USA, 472-1002(2006).
45. Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. Quantitation of flavonoid constituents in citrus fruits. *J. Agric. Food Chem.*, **47**, 3565-3571(1999).
46. http://ajpgi.physiology.org/content/279/6/G1148.short
47. http://jn.nutrition.org/content/131/2/235.abstract?ijkey=6cd7e72885072c7f69948835b6c36cbef4ab0e35&keytype2=tf_ipsecsha
48. http://jn.nutrition.org/content/132/11/3349.full
49. Harborne, J. B., Mabry, T., J., "The Flavonoids" Chapman and Hall Ltd., London (1982).
50. Yarishkin, O. V., Bioorg. Med. Chem. Lett., **18**, 137(2008).
51. Tsao, R.; Yang, R.; Young, J.C.; Zhu, H. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *J. Agric. Food Chem.*, **51**, 6347-6353(2003).
52. Zhao, F.; Watanabe, Y.; Nozawa, H.; Daikonnya, A.; Kondo, K.; Kitanaka, S. Prenylflavonoids and phloroglucinol derivatives from Hops (Humulus lupulus). *J. Nat. Prod.*, **68**, 43-49(2005).
53. Tsao, R.; McCallum, *J. Chemistry of Flavonoids*. In Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value and Stability; de la Rosa, L.A., Alvarez-Parrilla, E., Gonzalez-Aguilar, G., Eds.; Blackwell Publishing: Ames, IA, USA, Chapter 5, 131-153(2009).

54. McCallum, J.L.; Yang, R.; Young, J.C.; Strommer, J.N.; Tsao, R. Improved high performance liquid chromatographic separation of anthocyanin compounds from grapes using a novel mixed-mode ion-exchange reversed-phase column. *J. Chromatogr*., **1148**, 38-45(2007).

55. US Department of Agriculture, Agricultural Research Service USDA-Iowa State University Database on the Isoflavone Content of Foods. Nutrient Data Laboratory Web Site (2002) http://www.nal. usda.gov/fnic/foodcomp /Data /isoflav/isoflav.html.

56. Wang, H.; Murphy, P.A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.*, **42**, 1666-1673(1994).

57. Mazur, W.M.; Duke, J.A.; Wahala, K.; Rasku, S.; Adlercreutz, H. Isoflavonoids and lignans in legumes: Nutritional and health aspects in Humans. *J. Nutr. Biochem.* **9**, 193-200(1998).

58. Tsao, R.; Papadopoulos, Y.; Yang, R.; Young, J.C.; McRae, K. Isoflavone profiles of red clovers and their distribution in different parts harvested at different growing stages. *J. Agric. Food Chem.*, **54**, 5797-5805(2006).

59. Si, W.; Gong, J.; Tsao, R.; Kalab, M.; Yang, R.; Yin, Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *J. Chromatogr.*, **1125**, 204-210(2006).

60. Prior, R.L.; Lazarus, S.A.; Cao, G.; Muccitelli, H.; Hammerstone, J.F., Identification of procyanidins and anthocyanins in blueberries and cranberries (Vaccinium spp.) using high-performance liquid chromatography/mass spectrometry, *J. Agric. Food Chem.*, **49**, 1270-1276(2001).

61. Garazd, M.M.; Garazd, Y.L.; Khilya, V.P. Neoflavones. Natural distribution and spectral and biological properties, *Chem. Nat. Comp.*, **39**, 54-121(2003).
62. J.B. Harborne, The flavonoids advanced in research since 1986, Chapman &Hall, London, New York, Tokyo, 499-514(1994).
63. J. Bruneton, Pharmacognosy, phytochemistry of medicinal plant, 2nd edition, TEC & DOC., London, Paris, NewYork, 316-319(1999).
64. B. W. Shirley, "Flavonoid biosynthesis: "new" functions for an "old" pathway," Trends in Plant Science, **1**(11), 377– 382(1996).
65. J. B. Harborne, The Flavonoids. London: Chapman & Hall, (1988).
66. B. H. Havsteen, "The biochemistry and medicinal significance of the flavonoids," *Pharmacol. and Ther.*, **96**, 67–202(2002).

67. Bagga S, Straney D. Modulation of cAMP and phosphodiesterase activity by flavonoids which induce spore germination of Nectria haematococca MP VI (Fusarium solani). Physiol. Mol. Plant Path., **56**(2), 5161(2000).
68. Morandi D, Branzanti B, Gianinazzi-Pearson V. Effect of some plant flavonoids on in vitro behaviour of an arbuscular mycorrhizal fungus. Agronomie. **12**(10), 811-816(1992).
69. Griesbach RJ., Biochemistry and genetics of flower color, *Plant Breed. Rev.*, **25**, 89-114(2005).

70. Bohm BA., Introduction to flavonoids, Amsterdam, The Netherlands, *Hardwood academic publishers,* 365-394(1998).
71. Yao LH, Jiang YM, Shi J. et al. Flavonoids in Food and Their Health Benefits, *Plant Foods Hum. Nutr.* (Formerly Qualitas Plantarum)., **59**, 113-122(2004).
72. Iwashina T. Flavonoid function and activity to plants and other organisms, *Biol. Sci. Space*., **17**(1), 24-44(2003).
73. Harborne, J.B. In Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships; Cody, V.; Middleton, Jr. E.; Harborne, J.B., Eds.; Alan R. Liss: New York, 15-24(1986).
74. Harborne, J.B.; Williams, C.A., Phytochemistry, **55**, 481(2000).
75. Treutter, D., Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* **4**,147–157 (2006).
76. Peer WA, Murphy AS. Flavonoids as signal molecules: Targets of flavonoid action. In Peer page-29 WA, and Murphy AS. (eds.), The Science of Flavonoids. Springer, New York, 239-268(2006).
77. Fawe A, Abou-Zaid M, Menzies JG, Belanger RR. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathology*, **88**(5), 396-401(1998).
78. McNally DJ, Wurms KV, Labbe C, Belanger RR. Synthesis of C-glycosyl flavonoid phytoalexins as a site-specific response to fungal penetration in cucumber. *Physiol. Mol. plant path.* **63**(6), 293-303(2003).
79. Yamasaki H, Sakihama Y, lkehara N. Flavonoid-Peroxidase Reaction as a Detoxification Mechanism of Plant Cells against H202. *Plant Physiol*., **115**, 1405-1412(1997).

80. Jansen MAK, Van den Noort RE, Tan MY. *et al.*, Phenol-oxidizing peroxidases contribute to the protection of plants from ultraviolet radiation stress, *Plant Physiol*. **126**, 1012-1023(2001).
81. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress, *Polish J. of Environ. Stud.* **15**(4), 523-530(2006).
82. Koes, R.E., Quattrocchio, F. and Mol, J.N.M. The flavonoid biosynthetic pathway in plants: function and evolution. *BioEssays*, **16**, 123–132(1994).
83. Pierpoint, W.S. Why do plants make medicines, *Biochemist*, **22**, 37–40(2000).
84. Shirley BW, Flavonoids in seeds and grains: physiological function, agronomic importance and the genetics of biosynthesis, *Seed Sci. Res.* **8**, 415-422(1998).
85. Gould KS, Lister C. Flavonoid Functions in plants. In Andersen ØM, Markham KR. (eds.), Flavonoids. CRC Press, Boca Raton , 397-442(2006).
86. Lanot A, Morris P. Elicitation of isoflavan phytoalexins. In Marquez AJ (ed.), Lotus japonicus handbook. Springer, *The Netherlands*, 355-361(2005).
87. Vergas FD, Lopez OP. Natural colourants for food and nutraceutical uses. CRC press, USA, 93-109(2003).
88. Takahashi A, Ohnishi T. The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the internal space station, *Biol. Sci. Space* **18**(4), 255-260(2004).
89. Kaplan E, Kopka J, Haskell DW. *et al.* Exploring the Temperature-Stress Metabolome of Arabidopsis, *Plant Physiol.* **136**, 4159-4168(2004).

90. Hernandez I, Alegre L, Munne-Bosch S. Drought-induced changes in flavonoids and other low molecular weight antioxidants in Cistus clusii grown under Mediterranean field conditions, *Tree Physiol.*, **24**, 1303-1311(2004).
91. Mamolos AP, Kalburtji KL. Significance of Allelopathy in crop rotation. In Kohli RK, Singh HP, Batish DR. (eds.), Allelopathy in Agroecosystems. Published by *Food Products Press, Binghamton, USA,* 197-218(2001).
92. Duke SO. The emergence of grass root chemical ecology. *Proc. Natl. Acad. Sci.***, 104**(43), 16729-16730(2007).
93. Aderogba, MA, Ogundaini, AO, Eloff, JN. Isolation of two flavonoids from Bauhinia monandra leaves and their antioxidative effects, *African Journal of Traditional Complementary and Alternative Medicines***, 3**, 59-65(2006).
94. G. Agati, E. Azzarello, S. Pollastri, and M. Tattini, "Flavonoids as antioxidant sinplants: location and functional significance," *Plant Science,* **196**, 67–76(2012).
95. Pourcel L, Routaboul JM, Cheynier V. et al. Flavonoid oxidation in plants, from biochemical properties to physiological functions. *Trends Plant Sci.* **12**(1): 29-36(2007).
96. D. E. Saslowsky, U. Warek, and B. S. J. Winkel, "Nuclear localization of flavonoid enzymes in Arabidopsis," Journal of Biological Chemistry, **280**(25), 23735–23740(2005).
97. M. Naoumkina and R. A. Dixon, "Subcellular localization of flavonoid natural products," *Plant Signaling and Behavior*, **3**(8), 573–575(2008).

98. Parr, A. J. & Bolwell, G. P. Phenols in the plant and in man. The potential for possible nutritional enhancment of the diet by modifying the phenols content or profile, *Journal of the Science of Food and Agriculture,* **80**, 985–1012(2000).
99. Noori, M. Characterization of the Iranian species of Sophora and Ammodendron (Leguminosae; Sophoreae), PhD Thesis, University of London and Royal Botanic Gardens, Kew, UK(2002).
100. Noori, M., Chehreghani, A. & Kaveh, M. Flavonoids of 17 species of Euphorbia, (Euphorbiaceae) in Iran, Toxicological and Environmental Chemistry, **91** (3), 409-418(2009).
101. Yilmaz, Y. & Toledo, R. T. Health aspects of functional grape seed constituents, *Trends Food Sci. Technol.*, 15422-15433(2004).
102. Bobe, G.; Peterson, J.J.; Gridley, G.; Hyer, M.; Dwyer, J.T.; Brown, L.M. Flavonoid consumption and esophageal cancer among black and white men in the United States, *Int. J. Cancer,* **125**, 1147–1154(2009).
103. Wang, L.; Lee, I.M.; Zhang, S.M.; Blumberg, J.B.; Buring, J.E.; Sesso, H.D. Dietary intake of selected flavonols, flavones, and flavonoid-rich foods and risk of cancer in middle-aged and older women, *Am. J. Clin. Nutr.* **89**, 905–912(2009).
104. S. Caltagirone, C. Rossi, A. Poggi, F. O. Ranelletti, P. G. Natali, M. Brunetti, F. B. Aiello, and M. Piantelli, “Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential,” *Int. J. Canc.*, **87**(4), 595–600(2000).

105. T.Fotsis, M. S. Pepper, E. Aktas, S. Breit, S.Rasku, H. Adlercreutz, K. Wahala, R. Montesano, and L. Schweigerer, "Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis," *Canc. Res.*, **57**, 2916–2921(1997).
106. H. Wei, L. Tye, E. Bresnick and D. F. Birt, "Inhibitory Effect of Epigenin, a Plant Flavonoid, on Epidermal Ornithine Decarboxylase and Skin Tumor Promotion in Mice," *Cancer Research*, **50**(3), 499- 502(1990).
107. Le Marchand, L. Biomed., *Pharmacother.*, **56**, 296(2002).
108. Tapas AR, sakarkar DM, Kakde RB Flavonoids as nutraceuticals: A review. Trop. *J. Pharm. Res.*, **7** (3), 1089-1099(2008).
109. Cibin, T.R.; Devi, D.G.; Abraham, A. Chemoprevention of skin cancer by the flavonoid fraction of Saraca asoka. Phytother. Res. **24**, 666–672(2010).
110. Fang, S.-C.; Hsu, C.-L.; Lin, H.-T.; Yen, G.-C. Anticancer Effects of Flavonoid Derivatives Isolated from Millettia reticulata Benth in SK-Hep-1 Human Hepatocellular Carcinoma Cells. *J. Agric. Food Chem.*, **58**, 814–820(2010).
111. Barros L., DueÚas M., Carvalho A.M., Ferreira I.C.F.R.: Food Chem. Toxicol. **50**, 1576 (2012).
112. A. Mishra, A. K. Sharma, S. Kumar, A. K. Saxena, and A. K. Pandey, "Bauhinia variegata leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities," BioMed Research International, **2013**, Article ID 915436, 10 pages(2013).
113. C. T. Ho, T. Osawa, M. T. Huang, and R. T. Rosen, Food Phytochemicals for Cancer Prevention II. Teas, Spices,

and Herbs, American Chemical Society, Oxford University Press (1994).

114. Nichenametla, S. N.; Taruscio, T. G.; Barney, D. L.; Exon, J. H. A review of the effects and mechanism of polyphenolics in cancer. Crit. Rev. *Food Sci.*, **46**, 161–183(2006).

115. Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer prevention: a review. *J. Am. Diet. Assoc.*, **96**, 1027–1039(1996).

116. Moon, Y. J.; Wang, X. D.; Morris, M. E. Effects on xenobiotic and carcinogen metabolism. Toxicol. in Vitro **20**, 187–210(2006).

117. Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Etherton, T. D. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. *J. Med.* **113**, 71–88(2002).

118. Le Marchand, L.; Murphy, S. P.; Hankin, J. H.; Wilkens, L. R.; Kolonel, L. N. Intake of flavonoids and lung cancer, *J. Natl. Cancer Inst.*, **92**, 154–160(2000).

119. Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L, Whelton PK Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *A J Clin Nutr* **76**(1), 93–99(2002).

120. Clifford MN Diet-derived phenols in plasma and tissues and their implications for health. *Planta Med***., 12**, 1103–1114(2004).

121. Atmani D, Chaher N, Atmani D, Berboucha M, Debbache N, Boudaoud H., Flavonoids in human health:

from structure to biological activity, *Curr Nutr Food Sci* **5**, 225–237(2009).

122. Xiao ZP, Peng ZY, Peng MJ, Yan WB, Ouyang YZ, Zhu HL , Flavonoids health benefits and their molecular mechanism, *Med Chem* **11**(2),169–177(2011).

123. Malešev D, Kunti V Investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions, *J. Serb. Chem. Soc.* **72**(10), 921-939(2007).

124. Kim, H. P.; Son, K. H.; Chang, H. W.; Kang, S. S. Anti-inflammatory plant flavonoids and cellular action mechanisms, *J. Pharmacol. Sci.* **96**, 229–245(2004).

125. Y. Yamamoto and R. B. Gaynor, "Therapeutic Potential of Inhibition of the NFkB Pathway in the Treatment of Inflammation and Cancer," *The Journal of Clinical Investigation,* **107(**2), 135-142(2001). doi:10.1007/s10457-010-9316-9

126. Jin, J.H.; Lim, H.; Kwon, S.Y.; Son, K.H.; Kim, H.P. Anti-inflammatory activity of the total flavonoid fraction from Broussonetia papyrifera in combination with Lonicera japonica., *Biomol. Ther.* **18**, 197–204(2010).

127. Yam, M.F.; Lim, V.; Salman, I.M.; Ameer, O.Z.; Ang, L.F.; Rosidah, N.; Abdulkarim, M.F.; Abdullah, G.Z.; Basir, R.; Sadikun, A.; Asmawi, M.Z. HPLC and anti-inflammatory studies of the flavonoid rich chloroform extract fraction of Orthosiphon stamineus leaves. *Molecules,* **15**, 4452–4466(2010).

128. Y.Nishizuka, "The molecular hetero geneity of protein kinase C and it simplications for cellular regulation," Nature, **334**(6184), 661–665(1988).

129. T.Hunter, “Protein kinases and phosphatases: they inandyang of protein phosphorylation and signaling,” Cell, **80**(2), 225–236(1995).
130. A. Mishra, S. Kumar, and A. K. Pandey, “Scientific validation of the medicinal efficacy of Tinos poracordi folia,” *The Scientific World Journal*, **2013**, (2013). Article ID 292934.
131. A.Mishra, S.Kumar, A.Bhargava, B.Sharma, and A.K.Pandey, “Studies on in vitro antioxidant and antistaphylococcal activities of some important medicinal plants,” *Cellular and Molecular Biology*, **57**(1), 16–25(2011).
132. A. K. Pandey, A. K. Mishra, A. Mishra, S. Kumar, and A. Chandra, “Therapeutic potential of C. zeylanicum extracts: an antifungal and antioxidant perspective,” *International Journal of Biological and Medical Research*, **1**, 228–233(2010).
133. Asres, K.; Seyoum, A.; Veeresham, C.; Bucar, F.; Gibbons, S. Naturally derived anti-HIV agents, *Phytother. Res.* **19**, 557–581(2005).
134. B. Gerdin and E. Srensso, “Inhibitory effect of the flavonoid on increased microvascular permeability induced by various agents in rat skin,” *International Journal of Microcirculation, Clinical and Experimental*, **2**(1), 39–46(1983).
135. Pietta, P.G. Flavonoids as Antioxidants, *J. Nat. Prod.* **63**, 1035-1042(2000).
136. Mao, W.H.; Han, L.J.; Shi, B. Optimization of microwave-assisted extraction of flavonoid from Radix

Astragali using response surface methodology. *Sep. Sci. Technol*, **43**, 671–681(2008).

137. Liu RH, Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals, *Am. J. Clin. Nutr.* **78**, 517-520 (2003).

138. F. Shahidi and P. K. Wanasundara, "Phenolic Antioxidants," Critical Reviews in Food Science and Nutri, **32**, 1, 67-103(1992). doi:10.1080/10408399209527581

139. Zhang, H. Y.; Yang, D. P.; Tang, G. Y. Multipotent antioxidants: from screening to design. *Drug Discov. Today* **11**, 749–754(2006).

140. Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits, *J. Agric. Food Chem.* **44**, 701–705(1996).

141. Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radic. Biol. Med.,* **22**, 749–760(1997).

142. Rice-Evans, C. Flavonoid antioxidants, *Curr. Med. Chem.* **8**, 797–807(2001).

143. Kaur, C.; Kapoor, H. C. Antioxidants in fruits and vegetables – the millennium's health, *Int. J. Food Sci. Tech.* **36**, 703–725(200).

144. Sánchez Moreno, C.; Plaza, L.; De Ancos, B.; Cano, M. P. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juices, *J. Sci. Food Agric.* **83**, 430–439(2003).

145. Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids, *J. Agric. Food Chem.* **49**, 2774–2779(2001).

146. Jia Z., Tang M., Wu, *J. Food Chem.* **64**, 555 (1999).

147. Chebil, L, Humeau, C, Falcimaigne, A, Engasser, J, Ghoul, M. Enzymatic acylation of flavonoids, Process Biochemistry, **41**, 2237-2251(2006).
148. Zhou, T, Luo, D, Li, X, Luo, Y. Hypoglycaemic and hypolipidemic effects of flavonoids from lotus (Nelumbo nuficera) leaf in diabetic mice. *Journal of medicinal Plants Research,* **3**, 290-293(2009).
149. Y. Miyake, K. Yamamoto, N. Tsujihara, and T. Osawa, "Protective effects of lemon flavonoids on oxidative stress in diabetic rats," *Lipids*, **33**, 689–695(1998).
150. Y. Song, J. E. Manson, J. E. Buring, H. D. Sesso, and S. Liu, "Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: A prospective study andcross-sectional analysis," *J. Am. Coll. Nutr.*, **24**, 376–384(2005).
151. S. D. Varma, A. Mizuno, and J. H. Kinoshita, "Diabetic cataracts and flavonoids," *Science*, **195**, 205–206(1977).
152. Gattuso, G.; Barreca, D.; Gargiulli, C.; Leuzzi, U.; Caristi, C. Flavonoid composition of Citrus juices. Molecules **12**, 1641–1673(2007).
153. Jain, A.; Singhai, A.K.; Dixit, V.K. A comparative study of ethanol extract of leaves of Tephrosia purpurea pers and the flavonoid isolated for hepatoprotective activity. *Indian J. Pharm. Sci.,* **68**, 740–743(2006).
154. Sannigrahi, S.; Mazumder, U.K.; Pal, D.K.; Mondal, A.; Roy, S. Hepatoprotective potential of flavonoid rich fraction of Enhydra fluctuans against CCl4-induced oxidative damage in rats. Pharmacology online, **2**, 575–586(2009).

155. Middleton, E.; Kandaswami, C. Effects of flavonoids on immune and inflammatory cell functions, *Biochem. Pharmacol*, **43**, 1167–1179(1992).
156. Stevenson DE, Hurst RD. Polyphenolic phytochemicals just antioxidants or much more, *Cell Mol Life Sci.*, **64**(22), 2900-16(2007).
157. Williams, RJ, Spencer, JPE, Rice-Evans, C. Serial review: Flavonoids and isoflavonones (Phytoestrogens): Absorption, Metabolism and Bioactivity. *Free Radical Biology and Medicine*, **36**, 838-849(2004).
158. Yochum, L; Kushi, L. H.; Meyer, K.; Folsom, A. R. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women, *Am. J. Epidemiol.*, **149**, 943–949(1999).
159. Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. Brit. Med. J. **312**, 478–481(1996).
160. Hertog, M. G. L. Flavonoid intake and long-term risk of coronary heart-disease and cancer in the 7 countries study. *Arch. Intern. Med.*, **155**, 381–386(1995).
161. Gross, M. Flavonoids and cardiovascular disease. Pharm. Biol., **42**, 21–35(2004).
162. M.G .Hertog, E.J.Feskens, P.C.Hollman, M.B.Katan, and D.Kromhout, "Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study," *Lancet*, **342**, 1007–1011(1993).
163. L. Tijburg, T. Mattern, J. Folts, U. Weisgerber, and M. Katan, "Tea flavonoids and cardiovascular disease: a review." *Crit. Rev. Food Sci. Nutr.*, **37**, 771–785(1997).

164. Kris-Etherton, P. M.; Lefevre, M.; Beecher, G. R.; Gross, M. D.; Keen, C. L.; Etherton, T. D. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis, *Ann. Rev. Nutr.*, **24**, 511-538(2004).

165. Drewnowski, A.; Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: *a review. Am. J. Clin. Nutr.*, **72**, 1424–1435(2000).

166. Mojzisova, G.; Kuchta, *M. Physiol. Res.*, **50**, 529(2001).

167. Benavente-García, O.; Castillo, J.; Marín, F. R.; Ortuño, A.; Del Río, J. A. Uses and properties of Citrus flavonoids. *J. Agric. Food Chem.*, **45**, 4505–4515(1997).

168. Borrelli, F.; Izzo, A. A. The plant kingdom as a source of anti-ulcer remedies. *Phytother. Res.*, **14**, 581–591(2000).

169. Wightman, J. D. Red berries and their health benefits. In Nutraceutical Beverages: Chemistry, Nutrition, and Health Effects (ACS Symp. Ser. 871), 123–132(2004).

170. M. F. Garcia-Saura, M. Galisteo, I. C. Villar, A. Bermejo, A. Zarzuelo, F.Vargas, andJ.Duarte, "Effects of chronic quercetin treatment in experimental renovascular hypertension," *Mol. Cell. Biochem.*, **270**, 147– 155(2005).

171. J. Moline, I. F. Bukharovich, M. S. Wolff, and R. Phillips, "Dietary flavonoids and hypertension: is there a link," *Med. Hypoth.*, **55**, 306–309(2000).

172. J. M. Geleijnse, L. J. Launer, A. Hofman, H. A. P. Pols, and J. C. M. Witteman, "Teaflavonoids mayprotect againstatherosclerosis," *Arch. Intern. Med.*, **159**, 2170-2174(**1999)**.

173. J. Reed, “Cranberry flavonoids, atherosclerosis and cardiovascular health,” *Crit. Rev. Food Sci. Nutr.*, **42**, 301–316(2002).
174. D. A. Lewis and P. J. Hanson, “Anti-ulcer drugs of plant origin,” *Prog. Med. Chem.*, **28**, 201–231(1991).
175. R. J. Nijveldt, E. van Nood, D. E. van Hoorn, P. G. Boelens, K. van Norren, and P. A. M. Van Leeuwen, “Flavonoids: a review of probable mechanisms of action and potential applications,” *Clin. Nutr.*, **74**, 418– 425(2001).
176. W. B. Mors, M. C. do Nascimento, B. M. R. Pereira, and N. A. Pereira, “Plant natural products active against snake bite: The molecular approach,” *Phytochem.*, **55**, 627–642(2000).
177. Abd El-Gawad HM, Khalifa AE Quercetin, coenzyme Q10, and L- canavanine as protective agents against lipid peroxidation and nitric oxide generation in endotoxin-induced shock in rat brain, *Pharmacol. Res.*, **43**(3), 257-63(2001). http://dx.doi.org /10.1006/phrs. 2000. 0781
178. Alvarez MA, Debattista NB, Pappano NB Antimicrobial activity and synergism of some substituted flavonoids, *Folia. Microbiol.*, **53**(1), 2328(2008). http://dx.doi.org/10.1007/s12223-008-0003-4
179. Haga, S., Ph.D. Thesis, Sudan University of Science and Technology (2019).
180. Rogia, A. Ph.D. Thesis, Sudan University of Science and Technology (2019).
181. Mayada, A., Ph.D. Thesis, Sudan University of Science and Technology (2019).

182. Shaza, A. Ph.D. Thesis, Sudan University of Science and Technology (2019).
183. Ibrahim, H. Ph.D. Thesis, Sudan University of Science and Technology (2018).
184. Weam, A. Ph.D. Thesis, Omdurman Islamic University (2018).
185. Tamador, A. Ph.D. Thesis, Omdurman Islamic University (2016).
186. Nadkarni KM. Indian Materia Medica.Vol-I, Popular Book Depot, Bombay, India; PP.798 (1982).
187. Palgrave K. C. Trees of Southern Africa, 4th ed.; C. Struik Publishers: Cape Town; p 254(1984).
188. Palgrave KC, Moll EJ, Drummond RB: Trees of Southern Africa 4th Ed. C. Struik Publishers; Cape Town. pp. 254(1984).
189. Gelfand M, Mari S, Drummond RB, Ndemera B: The Traditional Medical Practitional in Zimbabwe. 1st Ed. Mambo Press: Harare: pp.141-142(1985).
190. Eisa MM, Amaghoul Az, Omer MEA, Elegami AA, *Fitoterapia*, **71**(3): 324 – 347(2000).
191. Vaidyaratnam PS. Varier. Indian Medicinal Plants. A compendium of 500 species. vol-I, Orient Longman Ltd, Chennai, India; PP. 330-33(1998).
192. Kirthikar KR and Basu B.D. Indian Medicinal Plants. Publications and Information Division, vol II, CSIR,

New Delhi, India; pp. 912-913(1975).

193. Anonymous.The wealth of India. Publications and information Directorate. CSIR, pp. 55-56(1952).

194. Eisa MM, Alamagboul AZ, Omer MEA Elegami AA. Anti-bacterial activity of Dichrostachys cinerea. *Fitotherapia,* **71**(3), 324-327(2000).

195. Kambizil, Afolayan AJ. (2001) An Ethno botanical study of plants used for the treatment of sexu- ally transmitted diseases. *Journal of Ethno pharmacology*, **77**(1), 5-9 (2001).

196. Kerharo, J.; Adam, J. G.: La Pharmacope´e se´ne´galaise traditionnelle, Plantes me´dicinales et toxiques; Vigot Fre`res: Paris; pp 573-575(1974).

197. Abou Zeid, A. H. S.; Hifnawy, M. S.; Mohamed, R. S., *Planta Med.* , **74**, 1020–1021(2008).

198. Zheng W, Wang SY (2001).Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem 49: (11):5165–5170.

199**.** Cai YZ, Sun M, Corke H, Antioxidant activity of betalains from plants of the Amaranthaceae. *J. Agric Food Chem.,* **51**(8):2288–2294 (2003).

200- Sala A, Recio MD, Giner RM, Manez S, Tournier H, Schinella G, Rios JL , Anti-inflammatory and

antioxidant properties of Helichrysumitalicum. *J. Pharm. Pharmacol*., **54**(3):365–371(2002).

201- Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM,Pridham JB , The relative activities of Plant- derived polyphenolic flavonoid. *Free radical Resource*, **22**, 375–383 (1995).

202- Harborne, J.B. Phytochemical Methods; A guide to Modern Techniques of Plant Analysis, Chapman and Hall Ltd., London, Great Britain *Column* 1998 127.Anderson O.M., Markham K.R.,. Flavonoids: Chemistry, Biochemistry and Applications. CRC Press/Taylor & Francis Group; Boca Raton, FL, USA: 2006.pp1-86 (1998).

203- Mabry, T.J., K.R. Markham, and M.B Thomas, The Systematic identification of flavonoids, Spring-Verlag New York (1970).

204- Sanon, Mathurin, Sacande, Moctar, Schmidt Combretum aculeatum vent *Seed leaf let*, 127 (2007).

205- Le Houérou H. N. (ed). *Browse in Africa: the current state of knowledge*. International. Livestock Center for Africa, Addis Ababa, Ethiopia, 491 (1980).

206- Orwa C, A Mutua, Kindt R, Jamnadass R, S Anthony. Agrofores tree Database:a tree reference and selection

guideversion,(2009)(http://www.worldagroforestry.org/sites /treedbs/treedatabases.asp).

207- Ahmed H. Albagouri, Awatif A. Elegami1, Waleed S. Koko1*,Elbadri E. Osman2, Mahmoud M. Dahab2. *In Vitro* Anticercarial Activities of some Sudanese Medicinal Plants of the Family *Combretaceae JOURNAL OF FOREST PRODUCTS and INDUSTRIES*, 2014, **3**(2), 93-99(2014).

208- Awatif a., Sara M. Osman ,Mohammed E. Omer in vitro anti bacterial activity of some Sudanese Combretum species *International journal of tropical medicine* 2(2):45-51(2007).

209- El Amin H. E. Trees and Shrubs of the Sudan. *Khartoum University Press* , Pietrovski, E.F.; Rosa, K.A.; Facundo, V.A.; Rios, K.; Marques, M.C.A.; Santos, A.R.S.(1990).

210- Antinociceptive properties of the ethanolic extract and of the triterpene 3β, 6β, 16β-trihidroxilup-20 (29)-ene obtained from flowers of *Combretum leprosum* in mice. *Pharmacol. Biochem. Behav.*, **83**, 90–99(2006).

211- Fyhrquist, P. Traditional medicinal uses and biological activities of some plant extracts of African *Combretum*

Loefl., *Terminalia* L. and *Pteleopsis* Engl. species (Combretaceae). Academic dissertation (2007).

212- Angeh J.E. Isolation and characterization of antibacterial compound present in members of Combretum section, Hypocrateropsis. A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in the Phyto medicine programme, Department of paraclinical sciences, faculty of veterinary sciences, University of Peritori. (2006).

213- Rogers, C.B.; Verotta, L. Chemistry and Biological Properties of the African Combretaceae, CR Press, London (2000).

214- In Chemistry, Biological and Pharmacological Properties of African Medicinal Plants; K., Hostettman, Chinyanganga, F., Maillard, M., Wolfender, J.L., Eds.; University of Zimbabwe Publications: Harare, Zimbabwe, (1996).

215- Banskota, A.H.; Tezuka, Y.; Kim, Q.T.; Tanaka, K.; Saiki, L.; Kadota, S. Thirteen novel cycloartane-type triterpenes from *Combretum quadrangulare*. *J. Nat. Prod.* , **63**, 57–64(2000).

216- Martini, N.D.; Katerere, D.R.P.; Eloff, J.N. Biological activity of five antibacterial from *Combretum*

erythrophyllum (Combretaceae). *J. Ethnopharmacol.*, **93**, 207–212(2004).

217- Aderogba, M.A.; Kgatle, D.T.; McGaw, L.J.; Eloff, J.N. Isolation of antioxidant constituents from *Combretum apiculatum* subsp. *apiculatum.*, *South Afr. J. Bot.* ,**79**,125-31(2012).

218- Ch1aabi, M.; Benayache, S.; Benayache, F.; N'Gom, S.; Koné, M.; Anton, R.; Weniger, B.; Lobstein, A. Triterpenes and polyphenols from *Anogeissus leiocarpus* (Combretaceae). *Biochem. Systemat. Ecol*., **36**, 59–62(2008).