بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Characterization of Flavonoids from *Detarium senegalense* Stem and Their Biological Activity

توصيف الفلافونيدات من ساق نبات أبو ليلة وفعاليتها البيولوجية

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

By

Sakiena Abd Elmageed Fageer Osman

B.Sc. & Ed.

M.Sc. Chemistry

Supervisor: Prof. Mohamed Abdel Karim Mohamed

November, 2016

بسم الله الرحمن الرحيم

قال تعالى:

﴿ وَقُلرَّبِّزِدْنِيعِلْماً ﴾

(صدق الله العظيم)

سورة طه: الآية (114)

Dedication

To the soul of my father, elder brother and my brother Al-Shaheed.

To my mother.

To my brothers.

Acknowledgement

First I would like to thank Allah Almighty for giving me health to complete this study.

Also I thank my supervisor Prof. Mohamed Abdel Karim Mohamed for his scientific guidance and patience.

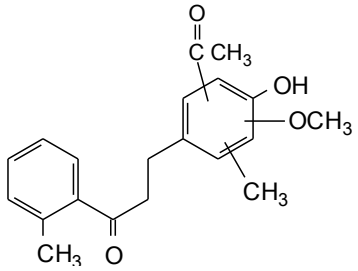
A very special acknowledgement to all my colleagues in Sudan University of Science & Technology for their support.

I am very grateful to the Government of the Sudan through the Administration of Animal Recourses Research Corporation and Central veterinary research laboratories for funding this study.

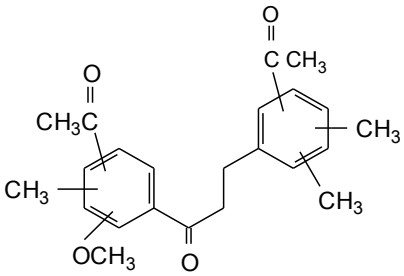
Abstract

Stems of *Detarium senegalense* were screened for secondary metabolites. Phytochemical screening revealed the presence of steroids, triterpenes, flavonoids, tannins, saponins , anthraquinones and cyanogenic glycosides.

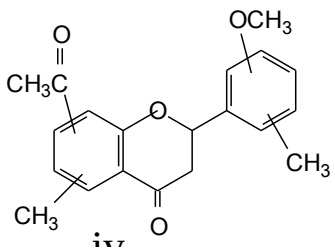
Fractionation by paper chromatography gave three flavonoids : I,II and III. Different fractions (chloroform,ethyl acetate and n-butanol) were evaluated , in vitro, for antimirobial activity against six standard human pathogens : *Bacills subtilis, Staphylococcus auerous , Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* and significant results were obtained.



Compound I



Compound II



Compound III

المستخلص

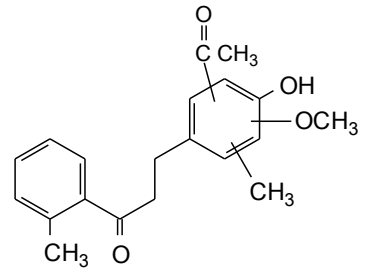
تم مسح فيتوكيميائي لساق نبات أبو ليلة، حيث اتضح وجود استيرولات، تراتربينات، فلافونيدات، تانينات، صابونينات، انتراكيونينات، وجلايكوسيانوجينات.

تم فصل ثلاثة مركبات فلافونيدية (I, II, III) باستخدام كروموتوغرافيا الورق.

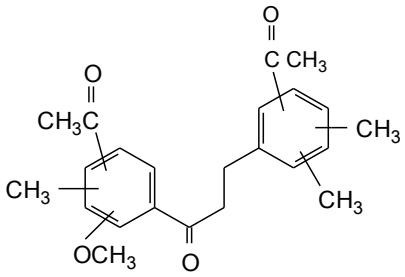
اخضعت المستخلصات المختلفة للنبات (chloroform, ethyl acetate and n-butanol)

لاختبار مضادات الميكروبات حيث استخدمت ستة أنواع من الميكروبات القياسية

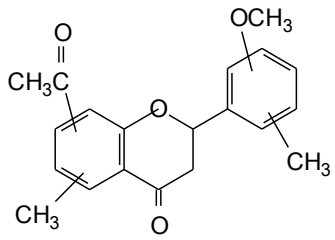
Bacills subtilis, Staphylococcus auerous, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and *Aspergillus niger* وكانت النتائج جيدة جداً.



Compound I



Compound II



Compound III

Table of Contents

List of Tables

List of Figures

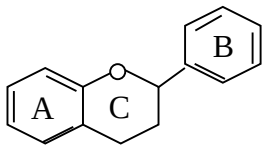
Introduction

1.1-General approach

Flavonoids are important class of natural products. They are generally known to be present in plants and plant-based products. Flavonoids are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, ant-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function[1].

1.2-Structural consideration and classification of Flavonoids

Flavonoids are the largest class of polyphenols, chemically they are defined as a group of polyphenolic compounds consisting of substances that have two substituted benzene rings connected by a chain of three carbon atoms and an oxygen bridge[1,2].



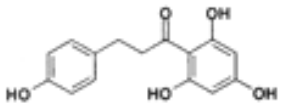
Basic flavonoids skeleton

Flavonoids can be classified into major subgroups : flvanavones, flavonols, chalcones, aurones, flavanones, dihydroflavonols, dihydrochalconnines, isoflavones, catechins and anthocyanins

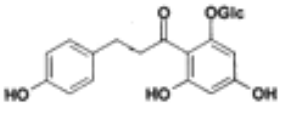
.This classification is mainly based on the degree of oxidation and unsaturation at C-2 and C-3[1,2].

1.3-Chalcones

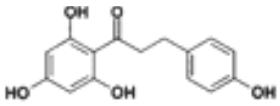
Chalcones are characterized by the absence of "ring C" of the basic flavonoids skeleton structure. Chalcones include phloridzin, Arbutin, phloretin and chalconarigenin[2].



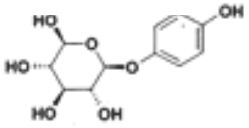
Phloretin



Phloridzin



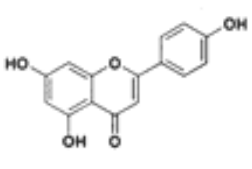
Chalconaringenin



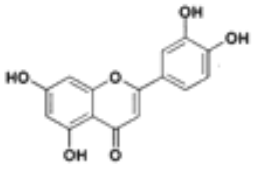
Arbutin

1.4- Flavones

Flavones are widely present in leaves, flowers and fruits as glycosides of celery, parsley, red peppers, chamomile, mint and ginkgo biloba.The following are examples of commonly encountered flavones[1]:



Apigenin



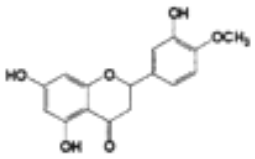
Luteoll

Apigenin Luteolin

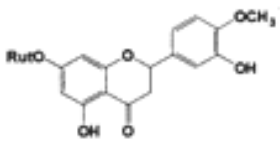
Tangeretin Baicalein

1.5- Flavanones

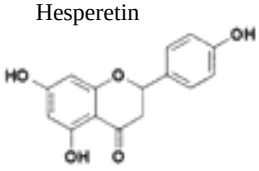
Flavanones are generally present in all the citrus fruits. hesperitin, naringenin, and eriodictyol, are examples of this class of flavonoids. Flavanones are associated with a number of health benefits because of their free radical scavenging properties[1,2].



Hesperetin

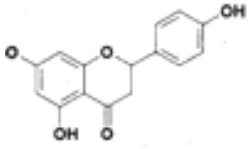


Hesperidin

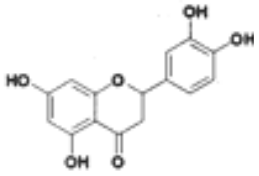


Naringenin

Neohesp



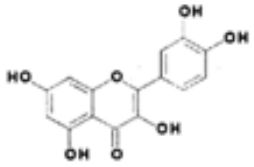
Naringin



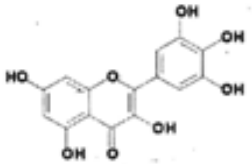
Eriodictyol

1.6-Flavonols

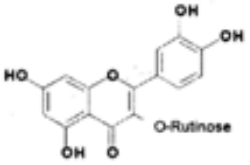
Flavonols are flavonoids containgketonic group at C-4. They occur widely in a variety of fruits and vegetables. Examples of flavonols include : kampferol, Quercetin, myricetin and Fisetin[2].



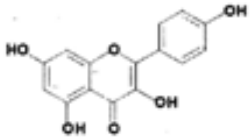
Quercetin



Myricetin



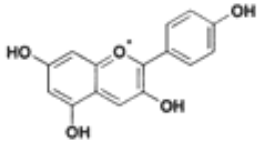
Fisetin



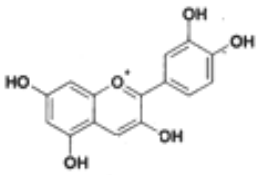
Kampferol

1.7-Anthocyanins

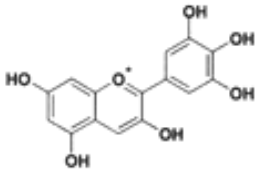
Anthocyanins are pigments responsible for colors in plants, flowers and fruits. Cyanidin, delphinidin, malvidin, pelargonidin and peonidin are the most studied anthocyunins. Anthocyanins display wide rangeofbioligcalactivites including anti-oxidant, anti-inflammatory, anti-microbial and anti-carcinogenic activities, in addition they exhibit significant effects on blood vessels and blood platelets and reduce the risk of coronary heart disease[2].



Pelargonidin



Cyaniding

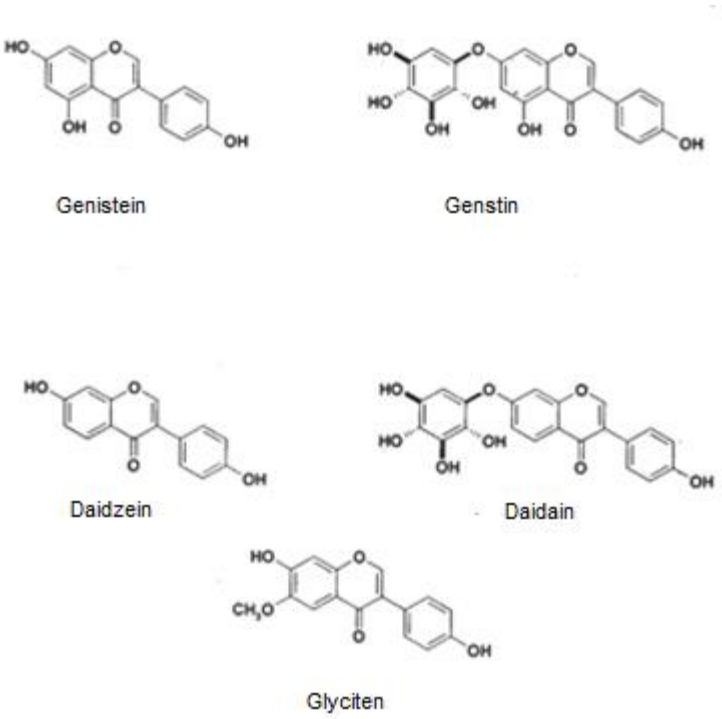


Delphinidin

1.8-Isoflavonoids

Isoflavonoids enjoy a limited distribution in the plant kingdom, and are predominatly found in soyabeans and other leguminous plants. Some isoflavonoidswere reported to occur in microbial organism[1].

Isoflavonoids are a type of phytoestrogen, with chemical structure similar to plant hormone estrogen. Isoflavones such as genistein and daidzein are regarded to be phytoestyrogens.



Studies by Arora et al.[3]and Brivibaet. al.[4]demonstratedthe anti-oxidant properties of genistein and daidzein which inhibited peroxidation of lipid in a liposomal system.

1.9-Biological activity of flavonoids

Flavonoids are well known for their antioxidant activates[5]. Antioxidants are compounds that protect the cell against the oxidative effect of reactive oxygen species, such as singlet oxygen, peroxyl radical, hydroxyl radical, superoxide radical, nitric oxide and peroxynitrite. Oxidative stress leads to cellular damage which is linked to various diseases such as diabetes, cancer, cardiovascular

disorders, neurodegenerative disorders, and ageing. Antioxidants interfere with radical producing systems and increase the function of endogenous antioxidants, protecting the cells from damage by these free radicals. Intake of flavonoids via fruits, vegetable and whole grains helps to increase levels of anti-oxidants in the body[6].The strong antioxidant property of flavonoids is attested by a number of studies [7-12]. Flavonoids are known to exhibit an inhibitory effect on excessive generation of the free radicals.This prevents the damaging effect of reactive oxygen species that includes lipid proxidation, and oxidation of sulfhydril and other susceptible group in proteins[13-15] .Quercetin is well known for its ability to act as antioxidant, it protects the body against reactive oxygen species.Studies have suggested that it helps in suppressing lipid proxidation in model systems[16]. The flavonoids :myricetin, quercetrin and rutin are also known to inhibit the production of superoxide radicals[17,18].

Different studies demonstrated that tea polyphenols- which belong to a sub- class of flavonoids possess potential antioxidant capabilities. A study by Nakagautaet. al., suggested that drinking green tea helps in prevention of cardiovascular disorder by increasing the antioxidant capacity of plasma in humans[19].

Citrus flavonoids, similar to tea polyphenols, are antioxidants that may protect against oxidative stress linked to inflammation and

help reduce the risk of macromolecule damage caused by free radicals. Studies by Zielinska – Przyjemskaet al., [20] indicated the antioxidant effect of citrus flavonoids such as naringin, naringenin and hesperidin by counteraction the effects of reactive oxygen species on apoptosis.

Naturally occurring flavonoids have been recognized for their antimicrobial activity. Many research groups have isolated and identified flavonoids possessing antifungal, antiviral and antibacterial activities. This property of flavonoids enables them to be used extensively in the nutrition, food safety and health. The antiviral effect of flavonoidswas studied[21]. Naturally occurring flavonoids such as quercetin, naringin, hespertin possess a variable spectrum of antiviral activity. They affect the replication and infectivity of certain RNA and DNA viruses[22] .Quercetain and apigenin are known to exhibit antibacterial activies[23,24].

Certain flavonoids exhibit hormone- like activies. They show resemblance to estrogen and other steroid hormones. These compounds exist in fruits, vegetables and tea. Esterogens possess a neuroprotective effect on the brain. Studies have been carried out by various research groups to investigate the estrogenic activity of genistein, daidzein and equalin[25]. The studies determined their treatment of chronic diseases such as hormone-dependent cancer, cardiovascular disorders and osteoporosis.

Genstein is the most promising compound to prevent post-menopausal bone loss in women[26]. Flavonoids are also known to exhibit anti-thyroids effects in animals and humans. Many studies have shown that ingestion of dietary genistein resulted in concentration changes of hormones, such as insulin, thyroids hormones, adrenocorticotropic hormone, cortisone and corticosterone as well as lipid metabolic changes [27].

Flavonoids work as immune modulators. Effects of flavonoids including quercetin on a variety of inflammatory processes and immune functions have been extensively reviewed[28,31]. Studies by Park et.al.[32]demostrated significant anti-inflammatory activity of quercetin.

A large number of studies have highlighted the role of dietary flavonoids in reducing the risk of caner[33-35].Flavonoids have been intensely investigated in the treatment of ovarian, breast, cervical, pancreatic and prostate cancer. A citrus flavonoid-tanageritin - is known to inhibit cancer cell prolleration[36]. Flavonoids such as 3-hydroxy flavones, 3, 4-dihydroxyflavone, 2,3-dihydroxy flavone, fisestin, apgenin and luteolin are potential inhibitors of tumor cell proliferation[37].Daidzein and genistein have been shown to inhibit both hormonal and on-hormonal type of cancer cells.

Neuro-degenerative disease results from the combined effect of oxidative stress, inflammation and transition metal accumulation ; flavonoids have potential interest for their neuroprotective properties. This was attested by studies which suggested that higher consumption of dietary flavonoids is associated with lower population rates of dementia[38]. Similarly a study carried out by Hwang. et, al. suggested that citrus flavones such as hesperidin, and neo-hesperidins could traverse the blood brain barrier and play effective role in the intervention for neurodegenerative diseases[39].

The hepatoprotective activity of flavonoids has been well studied . Flavonoids such as baicalin and quercetin attenuate ion overload -induced mouse liver injury[40]. There are reports which showed the hepatoprotective activity of flavonoids from German Chamomile.They are found to effect sphingolipid metabolism in the aged liver and regulate the levels of their key enzymes [41].Some flavonoids isolated from *Silybummarianum,* have strong antioxidant activity, in addition they exhibit hepartoprotective, and ion chelating properties [42].

Flavonoids have been studied for their anti-diabetic activity. Flavonoids may help to repair beta cell function by reducing free radical-induced tissue damage. They also reduce the hyperglycemic effects by controlling the blood sugar levels.

Studies have shown that intake of specific types of flavonoids, including querecetin and myricetin, is inversely associated with the risk of type 2 diabetes[43]. This is attested by another study which showed that querecetin may relief diabetic symptoms[44]. Quercetinwas found to inhibit the enzyme: aldose reductase. It is the first enzyme of the sorbitol-aldose reductase pathway. It plays an active role in converting glucose to sorbitol (a sugar alcohol) in the body. This result in development of secondary problems, such as neuropathy, retinopathy, diabetic cataracts and nephropathy[45].

Hyperglycaemia leads to the production of free radicals from mitochondria. This free radicals are known to be associated with diabetic micro- and macro-vascular complications and mitochondrial membrane damage. Studies carried out by Waisundaraet et.al. suggested that baicalin, a flavonoid, reduces hyperglycaemia- induced mitochondrial membrane damage and also enhances the effects of metformin which is an ant-diabetic drug. This was observed in the metformin and baicalin treated groups [46].

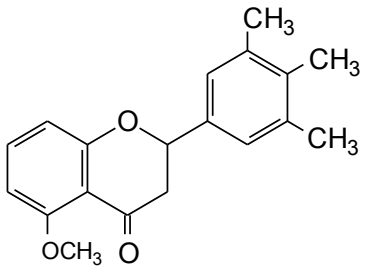
Oxidative stress resulting from the generation of oxygen free radicals is one of the causes responsible for various pathological conditions and ageing. Flavonoids are known as anti-oxidants to fight free radicals, thus reducing the signs of ageing. EGCG, a

compound found in green tea, have gained the interest of various researchers due to its unique range of anti-aging [47,48].

Upcoming research studies have confirmed the effect of flavonoids on skin health. Flavonoids work as anti-oxidizing agents and free radical scavengers, they penetrate deeper into the skin and protect it from UV radiation damage. *Camellia sinensisand Ginkgo bilobae*xtractsandgreen tea are involved in cosmetic formulations and it was suggested that they protect the skin against UV-induced damage and skin aging[49].

Myricetin is considered to be the flavonoids which have the cabability to neutralize the effects of the free radicals which cause photo-aging within the skin. Huanget, al.[50]analyzed the protective effects of myricetin on ultraviolet B- induced damage to keratinocytes. In another study, Fahlmanetet. al. investigated the ability of quercetin to guard the skin against U.V. radiation- induced damage. They suggested that neutralization of UV damage by flavonoids may, in part, be attributed their capacity to scavenge free radicals generated by UV rays [51]

. A flavanone: 5-methoxy-3`, 4`, 5`-trimethylflavanone(1) was isolatedfrom the leaves of Sudanese *Albiziaamara* and its structure was deduced on the basis of its spectral data(IR,UV,^{1}HNMR and MS)[52].

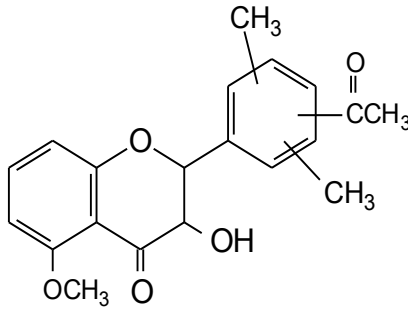


(1)

5-methoxy-3`, 4`, 5`-trimethylflavanone

The isolate was evaluated for its antimicrobial activity against six standard human pathogens: two Gram positive*(Staphylococusaureusand Bacillus subtilis)*, two Gram negative *(Pseudomonas aeruginosaand Escherichia coli)* bacteriaandtwofungalspecies*(Aspergillusniger, Candida albicans)*and promising results suggested that the flavanone is a plausible candidate for further optimization[52].

Alsodihydroflavonol(2) was isolated from the same species by different chromatographic techniques and identified via a combination of spectral tools (IR, UV, ^{1}HNMR and mass spectroscopy)[52].

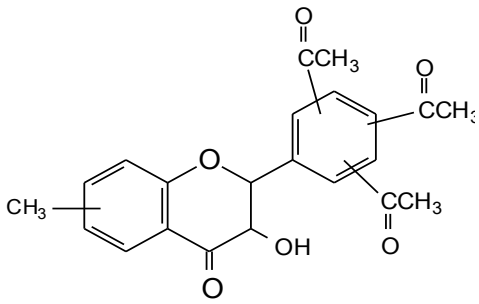


(2)

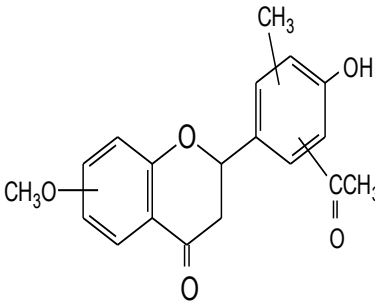
It was screened for its antimicrobial activity against six standard human pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillasniger*, *candida albicans)*, and significant results were obtained[52].

A successive silica gel column chromatography followed by further purification via thin layer chromatography allowed for the isolation[58] of twoflavonoids(compounds 3 and 4) from fruits of *Vangueriainfausta.* Identification of these compounds was based on extensive UV shifting reagents,IR, ^{1}HNMR and mass spectroscopy data.In well diffusion method, the chloroform fraction of *Vangueriainfausta,* compounds I and II were evaluated for their antimicrobial activity[53].

The chloroform fraction of *Vangueriainfausta* did not show antibacterial activity, but it showed significant inhibitory activity against the fungi: Candida albicans and *Aspergantillusniger*. Compounds 3 and 4 also showed antifungal activity. However,they did not reveal antibacterialactivity[53].

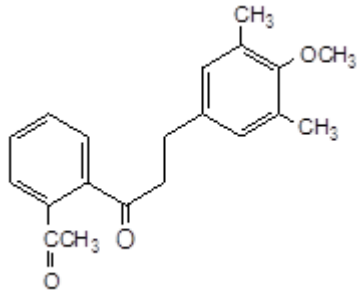


Compond 3

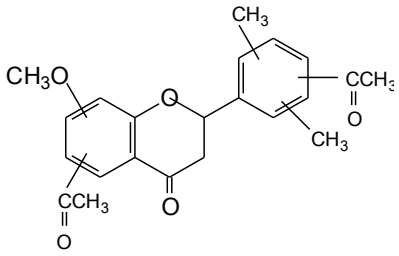


Compound 4

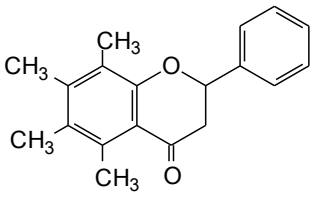
The following compounds were isolated[54] from the leaves of*Catharanthusrose*and *Narissusbrussonetii*. Compounds (5) and (6) were isolated from *Catharanthusroseus*,while compound (7) was isolated as yellow powderfrom *Narissusbrussonetii*leaves by silica gel TLC using BAW(4:1:4) as solvent.



(5)



(6)



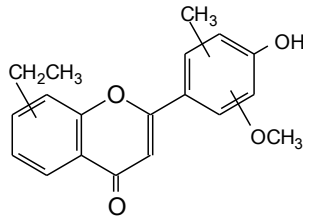
(7)

In cup plate agar diffusion assay,the isolates were screened for antimicrobial activity against five standard human pathogens.

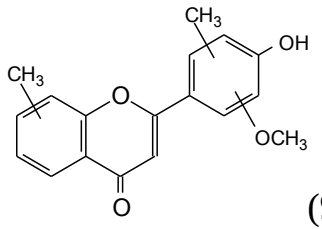
Compound I showed significant activity against the bacterial strain *Bacillus subtilis* and the fungal species *Candida albicans*, while compound II was active against *Escherichia coli,Bacillus subtilis*and the fungus *Candida albicans*. However, compound III gave significant antifungal activity and partial antibacterial activity.

Phytochemical screening of the alcoholic extract of the roots of *Leptadenia heterophylla* revealed[55] the presence of tannins, saponins, terpenes, flavonoids and steroids. Alkaloid and glycosides were not detected. The crude alcoholic extract was fractionated by thin layer chromatography.After the usual workup,

the following compounds(8 and 9) were isolated.The structures of these isolates were elucidated by a combination of spectral tools(UV,IR, ^{1}HNMR and MS).



(8)



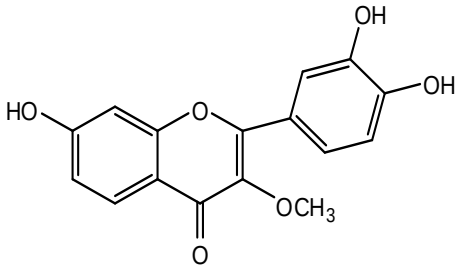
(9)

Different fractions of *Maytenassenegalensis* were evaluated for their antimicrobial activity using the cup plate agar diffusion method. The ethanolic and ethyl acetate fractions of *Maytenassenegalensis* showed activity against all test organisms.The n-butanol fraction was active against all test organisms except *Escherichia coli* andthe fungus *Aspergillusniger*. However,the chloroform extract showed partial activity against the bacterial strains but it did not show any antifungal activity(Table 1).

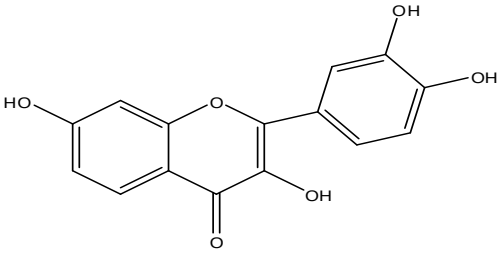
Table (1) : Antibacterial activity of *Maytenassenegalensis*extracts :M.D.I.Z (mm)

Extract	Conc.(mg/ml)	Ec	Pa	Sa	Bs	Ca	An
Ethanolic	100	13	15	16	14	15	14
Ethyl acetate	100	16	14	15	16	13	13
n-butanol	100	12	13	13	16	13	12
Chloroform	100	12	12	12	12	-	-

From the methanolic extractof the heartwood of, *acacia niloticavarnilotica,* two compounds(10 and 11)were isolated (7, 3`, 4`-trihydroxy-3-methoxyflavone and compound II was a 7, 3`, 4`-trihydroxyflavonol respectively). The compounds were purified by different chromatographic techniques and identified via spectroscopic tools: IR, UV, ^{1}HNMR and Mass spectroscopy. The isolated compounds were evaluated for their antimicrobial potential against Gram negative*(Escherichia coli* and*Pseudomonasaeruginos)*and Gram positive *(Bacillus subtilis, Bacillus cereus & Staphylococcus aureus)*bacteria. The two compounds showed varying biological activity. Compounds (10) and (11) were active against both Gram positive and Gram negative bacteria.



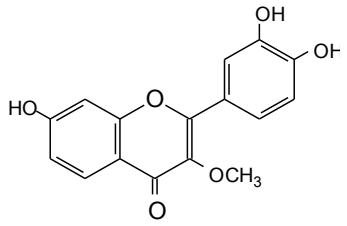
(10)



(11)

Phytochemical investigation[56]of *Acacianiloticavar*

*Adstringens*heart wood led to the isolation of a flavones(12): 7,3`,4`-trihydroxy-3-methoxyflavone from the methanolic extract. The crude extract was purified by a combination of chromatographic techniques(polyamide and Sephadex columns and paper chromatography) . Structure of isolate was elucidated on the basis of extensive spectroscopic procedures including : IR, UV, ^{1}HNMR and MS. The isolated flavonoid was evaluated for its antibacterial potential against Gram negative *(Escherichia coli and Pseudomonasa eruginos)*, Gram positive *(Bacillus subtilis, Bacillus cereus and Staphylococcus aureus)*bacteria. Compound I showed varying antibacterial responses. It showed high potency against Gram positive human pathogens: *Staphylococcus aureus* and *Bacillus subtilis.*



(12)

In well diffusion method[57], all fractions from *Albizia.Amara* roots showed inhibitory activity against *Streptococcusmutans* (Sm) and *Lacto bacillus* (Lb).The activity is expressed as less active, if the zone of inhibition is 9-12 mm, moderate: 15-16 mm and high if greater than 17 mm. The ethyl acetate extract showed high activity on *Streptococcusmutans* (Sm), while the n-butanol extract showed high activity on Lacto bacillus (Lb). The results of antimicrobial activity of the A. amara extracts against the microbial strains are depicted in Table 2.

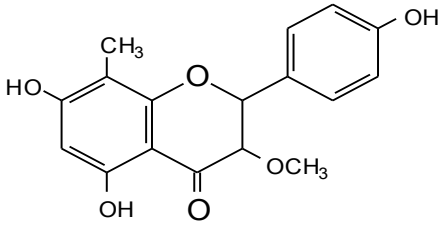
DMSO was used as solvent since it has no effect on the growth of any of the test microorganisms. Standard discs inhibited the growth of all the test microorganisms. There has been an increasing effect on microbial growth inhibition with increasing concentration of the extracts However, the effects observed were less than those produced by the standard chemotherapeutic.

Table 2: Antibacterial activity of different fractions

Microrganism	Fractions	300µl	200 µl	100 µl
Lacto bacillus (LB)	PT	20	19	18
	CH	12	11	12
	EA	14	12	11
	BU	22	22	20
	ET	21	19	18
	AMP	25	25	25
	DMSO	-	-	-
Streptococcus mutans(SM)				
	PT	9	10	11
	CH	15	14	12
	EA	20	23	22
	BU	18	14	15
	ET	20	20	20
	AMP	25	25	25
	DMSO	-	-	-

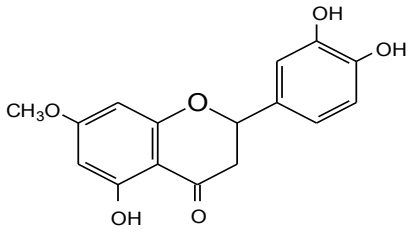
(ET = 0.09 , CH = 0.08, EA = 0.1 , P =0.03 , BU = 0.03) mg/ml , 100 µl of sample + ml of DMSO.

Phytochemical screening[58]of *Anogeissusleiocarpus*fruits indicated the presence of flavonoids,terpenes, tannins and saponins.Compound (13) was isolated from ethanolic extract by column and thin layer chromatography and its structure was established on the basis of its spectral data (IR, UV, NMR and MS). In *in vitro* studies, the isolated flavonoid gave promising antibacterial activity against *Escherichia coli*andmoderate activity against*S. aureus.*



(13)

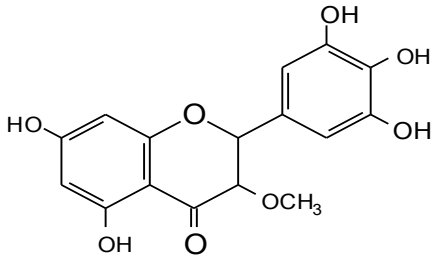
Adil reported[59]the isolation of a flavanone(14) (5,3`,4`-trihydroxy-7-methoxyflavanone) from the Saudi material of *Cassia Italica*. The flavonoid was isolated from the ethyl acetate fraction by column chromatography.The structure was elucidated by sensitive analytical tools (UV,IR,^{1}H NMR,^{13}C NMR,^{1}H-^{1}H-COSY NMR and MS). The flavanone was evaluated,*in vivo*,for anti-inflammatory and anti-ulcer potential and significant results were obtained.



(14)

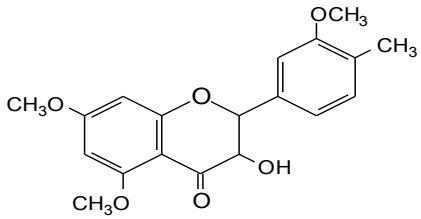
Phytochemical screening[58] of the leaves of *Geigeria alata* revealed the presence of flavonoids, tannins and alkaloids. Compound (15) was isolated from ethanolic extract by column and thin layer chromatography and its structure was established on the basis of its spectral data (IR, UV, NMR and MS). Compound I and different extracts (ethanolic, chloroform, n- butanol and ethyl acetate) of *Geigeria alata* were screened for their antimicrobial activity against six standard human pathogens. The ethyl acetate fraction showed significant antimicrobial activity followed by the n-butanol fraction. However, the chloroform fraction exhibited moderate

activity. Though, compound I showed significant antibacterial activity it did not afford any antifungal properties.



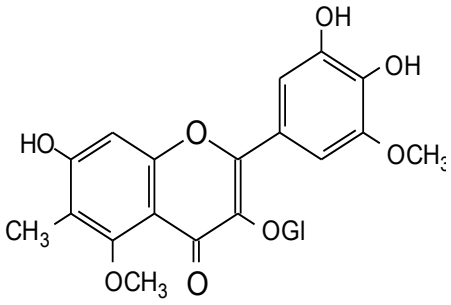
(15)

Amani et.al.[60] reported on the characterization and antimicrobial potency of a dihydroflavonol(16) isolated from the leaves of Sudanese *Tamarix nilotica*. The isolate was purified by different chromatographic techniques and identified via a combination of spectral tools (IR, UV, ^{1}HNMR and Mass spectroscopy). The isolated flavonoid, ethyl acetate and n-butanol fractions were evaluated*(in vitro)* for their antimicrobial potential against Gram negative*(Escherichia coli,Salomonella typhi)*and Gram positive *(Bacillus subtilis, Staphylococcus aureus)* bacteria and the fungi:*Candida albicans* and *Aspergillus niger* and promising results were obtained.The isolated dihydroflavonol seems to be a suitable candidate for future optimization.



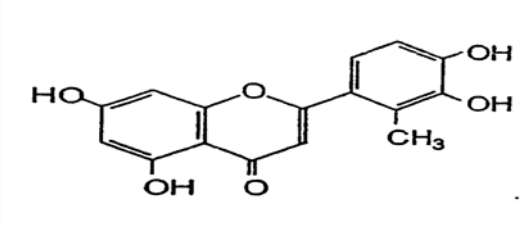
(16)

From the leaves[61] of*Vitexdoniana* (Sweet) a flavone (17) was isolated and characterized. The isolate was purified by different chromatographic techniques and identified via a combination of spectraltools (IR, UV, ^{1}HNMR and Mass spectroscopy). The methanolic fraction of *Vitex doniana* was evaluated*(in vitro)* for its antimicrobial potential against Gram negative*(Escherichia coli, Salomonella typhimurium*and *Pseudomonasa eruginosa)*and Gram positive *(Bacillus subtilis, Bacillus aureus* and *Staphylococcus aureus)*bacteria and the fungus *Candidaalbicans* .Promising results were obtained.*In vitro* antioxidant assay for the methanolic extract was conducted . Evaluation of the antioxidant activity was carried out by measuring the capacity of the extract against stable DPPH radical. The extract showed significant antioxidant activity.



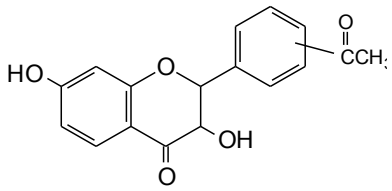
(17)

The crude ethanolic extract of *Croton Zambesicus* gave after paper chromatography a pure flavonoid (18)isolated as brown powder from ethanolic extract of the seeds of *CrotonZambesicus.*



(18)

Compound (19) was isolated as yellow powder from ethanolic extract of the seeds of*Coriandrum Stivum.* In cup plate agar diffusion assay,the crude extracts of *Croton Zambesicus,Coriandrum Sativum* and compounds (19 and 20) showed l different antimicrobial responses against test organisms[62] (Tables 3 and 4).



(19)

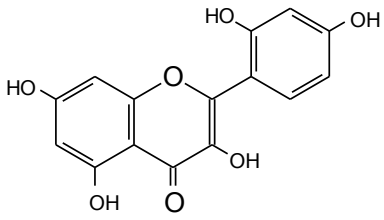
Table 3: The antimicrobial activity crude extractives of *Croton Zambesicus* and compounds (18) ad (19)

Organism	Inhibition growth zone diameter (MIZD) 100 mg \ 100 ml		
	Crude extract	Compound I	Compound II
Bacillus subtitles	22	20	19
Staphylococcusaureus	21	20	17
Escherichia coli	22	19	18
Pseudomonas aeruginosa	22	20	18
Aspergillus niger	19	16	15
Condida albacans	16	15	14

Table 4: The antimicrobial activity of crude extractives of *Coriandrum Sativum*

Organism	Crude extract
Bacillus subtitles	20
Staphylococcusaureus	20
Escherichia coli	19
Pseudomonas aeruginosa	22
Aspergillus niger	18
Condida albacans	20

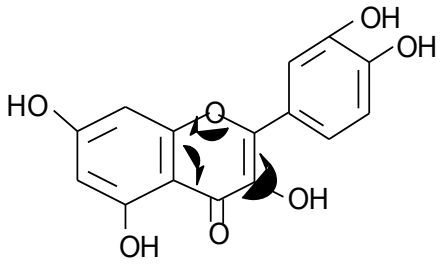
From the ethyl acetate[63]extract of the leaves *Cassia occidentalis* (Leguminosae) a flavonol: 5,7,2',4'-tetrahydroxyflavonol(20) was isolated. The structure was elucidated via a combination of spectral techniques(UV,IR,1H NMR and MS) and its biological potential was evaluated. The flavonol exhibited promising anti-inflammatory and anti-ulcer activity.



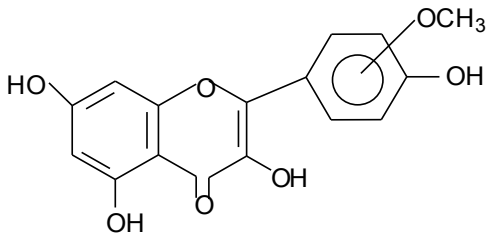
(20)

The antibacterial activity[64] of the crude extract and pure flovonoids of *Withania somnifera* against five human pathogens was carried out. The cup-plate agar diffusion method was adopted with some minor modifications. The test organisms were:*Bacillussubtilis, Escherichia coli, Neisseria gonorrhoeae,*

Pseudomonas aeruginosa and *Staphylococcus aureus.* The methanolic extract of *W. somnifera* showed moderate inhibition against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* andweak inhibition against *Neisseria gonorrhoeae* and *Bacillussubtilis.* A flavonoidof *Withania somnifera*(21) exhibited a moderately inhibition against all five test organisms. Another flavonoid (22) showed weak inhibition against test organisms.



(21)



(22)

1.10-Biosynthesis of flavonoids

Flavonoids differ in their arrangement of hydroxyl, methoxyl and glycosidic side groups and in the connection between A and B rings[65]. A variation in C ring provides division of subclasses[66].

According to their molecular structure they are divided into eight classes[67].

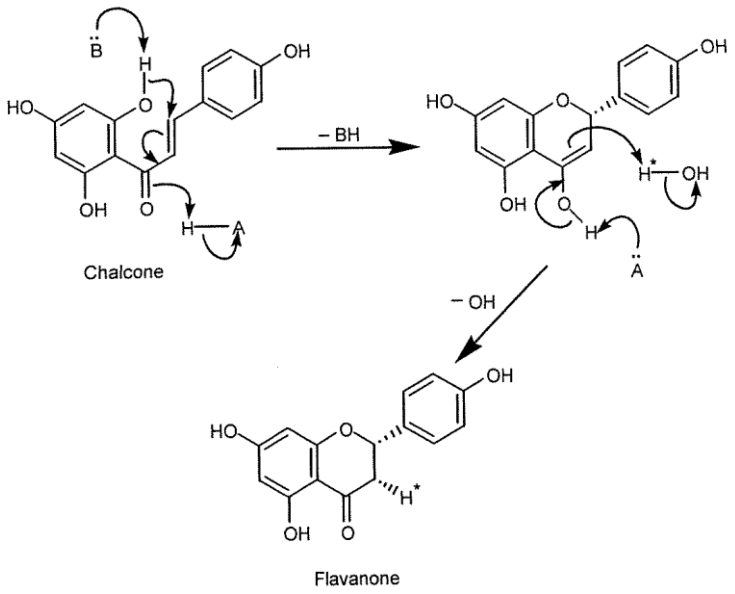
In the plants, flavonoids are often present as O-glycosides or C-glycosides. The O-glycosides possess sugar substituent bond to-OH of aglycone. Usually at position 3 or 7, whereas. C-glycosides possess sugar groups bound to carbon of aglycone usually 6-C or 8-C [68].

1.11- Synthesis of flavonoids

i)Synthesis of flavanone

Flavanones are formed from chalcones by isomerization. There is a good evidence for the *in vitro* and *invivo* existence of equilibrium between flavanones and the corresponding chalcones[73].The interconversion between chalcones and flavanone is catalyzed *in vivo* by an enzyme known as chalcone isomerase. The stereospecificity of this enzymatic reaction is apparent in the (S) chirality of C-2 in flavanone derivative (see scheme I).

Therefore it is not accidental that all the flavanones found in nature have the (S) configuration at C-2 and levorotatory . With chalcones having at least two free hydroxyl groups at C-2 and C-6 the equilibrium in an aqueous solution is shifted completely and rapidly to the flavanone (scheme I). The stabilization energy of the strong hydrogen bond between the carbonyl group and the ortho-phenolic hydroxyl group greatly influences the position



Scheme I

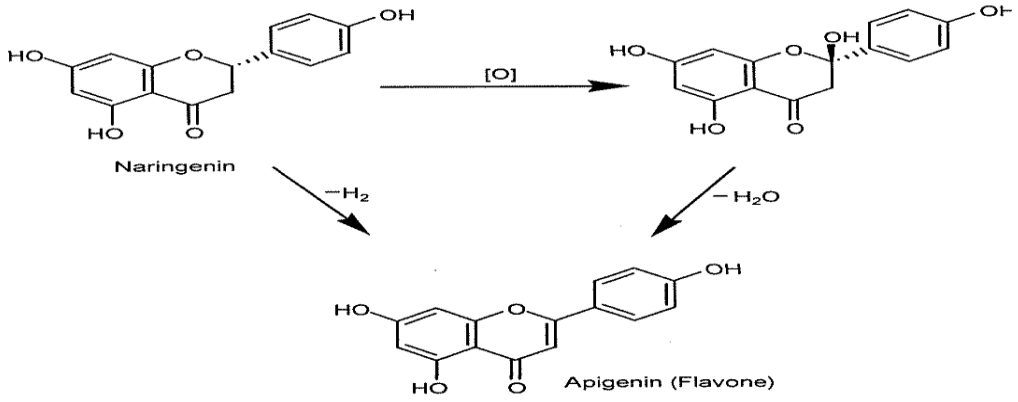
ofEquilibrium and the interconversion rate. When only one hydroxyl is available, either for the cyclization or for hydrogen bonding, the system ends to remain in the open form(chalcone form).

ii)Synthesis of flavones

The *in vitro* conversion of flavanones to flavones was first observed in parsley plant[69]. The reaction has been studied in more detail in parsley cell suspension cultures[70],and in *Antirrhinum* flowers[71].The parsley enzyme requires 2-oxoglutarate, Fe^{2+} and possibly assorbate as co-factors. This co-factor requirement would classify it as belonging to the 2- oxoglutarate- dependent dioxogenase[72].Ascorbate stimulates this and other 2- oxoglutarate-dependent dioxgenases involved in the flavonoid pathway and in

addition exhibits a remarkable stabilizing effect on the enzyme acitvity[73]. In *Antirrhinum*[76] and in a range of other plants including verbena [74]*Dahlid,streptocarpus* and *Zinnia*[80] reduction of flavanones to flavones is catalyzed by a microsomal enzyme requiring NADPH as co-factor. Although mutants recessive with respect to flavones formation are known, yet, evidence for the enzyme being involved in flavone formation is provided by the fact that flowers of plants that naturally lack flavones are also devoid of this NADPH dependent microsomal enzyme activity.

Both the parsley and the flower enzyme catalyzed the reaction from(2S)-naringenin (flavanone) to apigenin (flavone) scheme(II)[75].



Scheme (II)

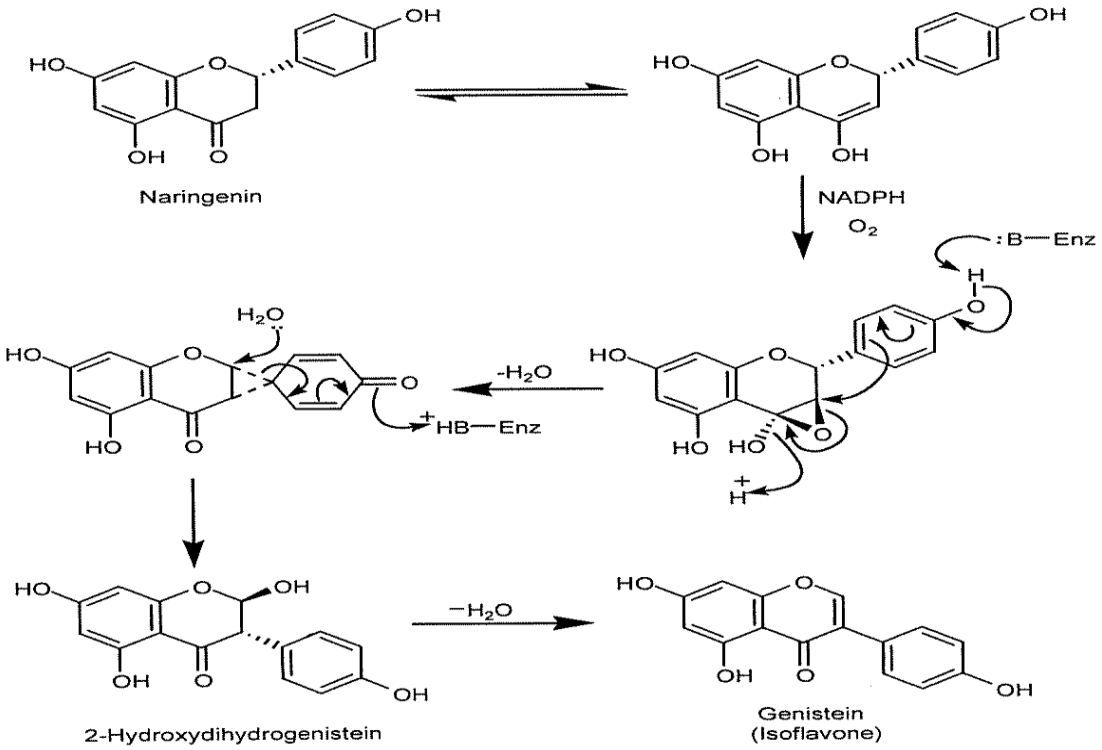
iii)Synthesis of isoflavones

The key step in isoflavone formation is the 2,3-migration of the aryl side chain of a flavanone- chalcone intermediate. An enzyme activity catalyzing this transformation was recently found in microsomal preparation from elicitorchallenged soybean cell suspension cultures. It transforms (2S)-naringenin(flavone) into genistein[76] scheme(III).

It was found that two enzymatic steps are involved in this transformation the first step comprises of oxidation and rearrangement of naringenin to 2-hydroxy-2,3-dihydrogenistein.

This step is strictly dependent on NADPH and molecular oxygen. The second enzyme which catalyses the elimination of water from the 2-hydroxy isoflavanone is identified but has not yet been characterized[77]. For the following reasons,(2S)-flavanones and not the chalcones are very probably the actual substrates: (i) a stereospecific incorporation of (2S)-naringenin into biochanin A(5,7-dihydroxy-4`-ethoxyisoflavone)[78]. (ii) Only the (2S) but only the (2R) enantiomer acts as a substrate *invito*[77].(iii)The equilibrium of 4,2`,4`,6`-tetrahydroxychalcone is at least 1000:1 in favour of the flavanone[79]. The equilibrium of 4,2`,4`,6`- tetrahydroxychalcone is

flavanone to isoflavone (scheme III) is consistent with the participation of NADPH and molecular oxygen.



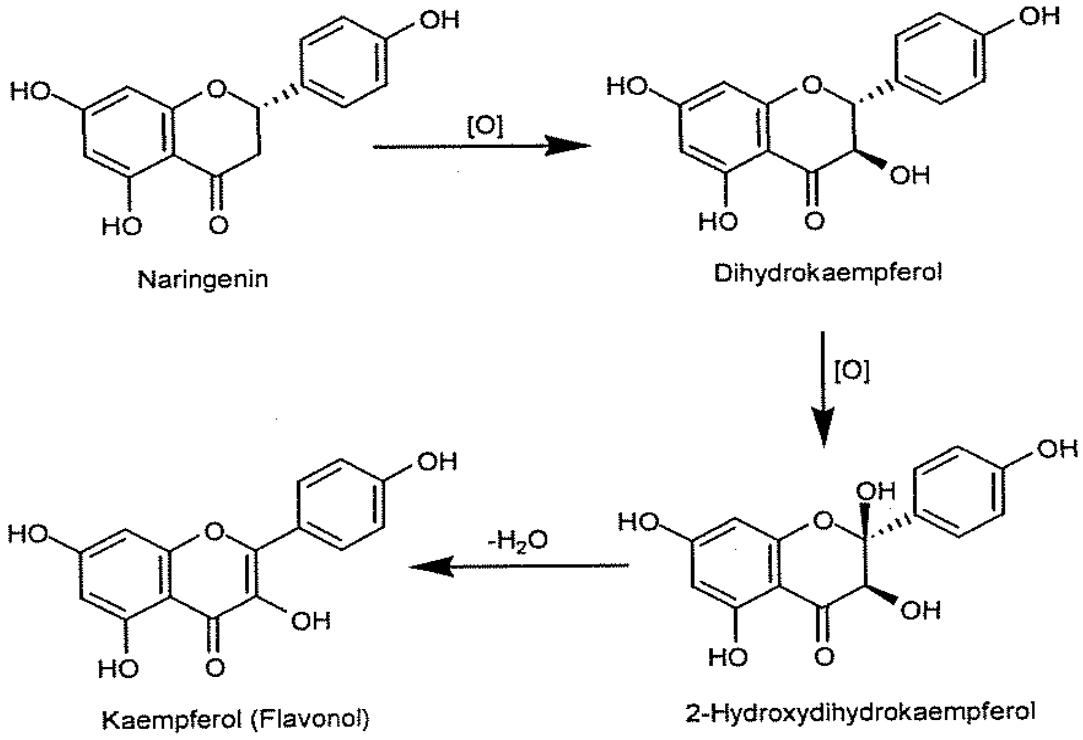
Scheme III

iv)Synthesis of flavonols

Enzymatic conversion of dihydroflavonol to flavonols was first observed with enzyme preparations from parsley cell suspension culture[80]. Synthesis of flavonols was found to be catalyzed by a soluble 2-oxoglutarate dependent oxygenase.

Flavonol synthesis most probably proceeds *via* a 2-hydroxy intermediate such as 2-hydroxydihydrokaempferol with subsequent dehydration giving rise to the respective flavonol[80] (scheme IV). Flavonol synthesis has also been demonstrated in flower extract

from *Matthiola*[81] and *Petunia*[82]. As in parsley flavonol formation in these flowers is catalyzed by a soluble 2-oxoglutarate -dependent dioxygenase.

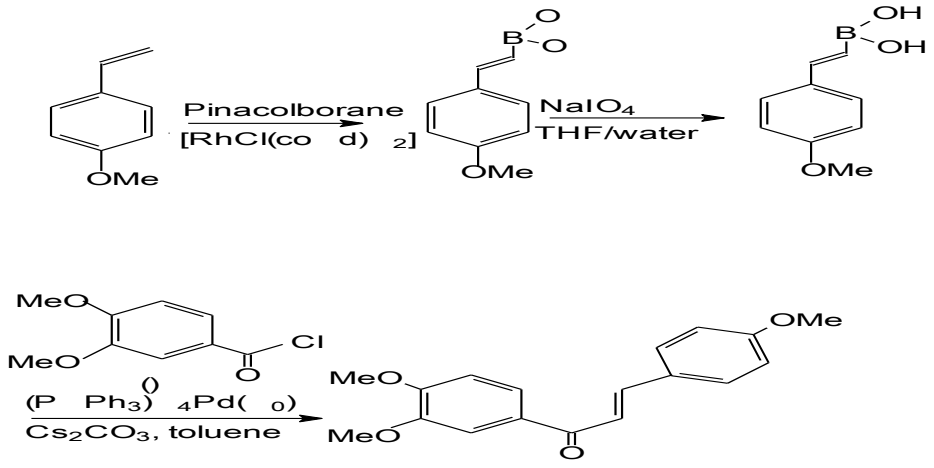


Scheme IV

v)Synthesis of chalcones *via* Suzuki Coupling reaction

Chalcone (**2**) or 1, 3-diphenyl-2-propen-1-one and especially chalcones bearing a hydroxyl function on the aromatic rings are the precursors of all the flavonoids[83]. It was reported that an efficient synthesis of chalcones is based on the Suzuki coupling reaction between benzoyl chloride and phenylvinylboronic acid. Phenylvinylboronic acid was prepared by dehydrogenative borylation of *paramethoxystyrene* by pinacolborane oxidative -

dehydrogenation catalyzed by the rhodium complex, RhCl(cod)2 to give *p*-methoxyphenyl ethenylboronic acid pinacol ester. This was followed by the oxidative cleavage of using sodium periodate in THF/water to form the *p*-methoxyphenylvinyl -boronic acid required for the Suzuki coupling step. The coupling afforded 3',4', 4-trimethoxy -chalcone under the following condition; solvent: anhydrous toluene; catalyst: tetrakis (triphenylphosphine) palladium(O) ; base: cesium carbonate as shown in Scheme(V)[83].



Scheme V

1.12-Optical activity of flavonoids

The favonoids are a class of natural products that gains interest due its great variety and the number of its members. The flavonoids are often hydroxylated in positions 3, 5, 7, 3, 4 and 5 which are frequently methylated, acetylated, or sulphated.

1.13-Distribution of flavonoids

Flavonoids are widely distributed in the plant kingdom [84].They are found in vegetables, fruits, nuts, seeds, stem, flowers, tea etc... Theyare an integral part of our daily diet[85-87].

1.14-Functions of flavonoids in plants

Anthocyanin pigment present in flowers provide colour which is contributing to pollination[88-90]. Flavonoids present in leaves promot physiological survival of plant by protecting it from fungal infections and UV radiations. Flavonoids are involved in energy transfer, respiration and photosynthesis control and morphogenesis sex-determination[91].

1.15-*Detarium senegalenes*

Detariumsenegalenes is a tree up to 25m high ; bark grey, flaking to large scales, exuding resin ; brankes rusty; leaves propionate, gland punctuate, leaflets 2-6 pairs alternate, oblong-elliptic, curvaceous, minutely pubescent below panicles, shorter than leaves; flowers creamy white fragrant, splats 4, pubescent, white petals absent stamens 10 ; fruit round or disc-shaped pods with crustaceous skin and intermediate fibrous larger sometimes small, abortive or galled up to 6cm across; seeds flattened.

Detarium senegalenes is an important medicinal plants. Different parts are extensively used in traditional medicine. The fruit pulp is reported to be useful for kidney pain, spinal tuberculosis, syphilis, cough, rheumatism and used to treat fever [92]. The leaf and shoot decoctions are used for dysentery, anaemia, conjunctivitis inflammation and skin complaints[93]. The bark appears to be the most widely used in traditional medicine. The bark powder is applied to treat wounds, burns and skin problems[94]. Also bark decoctions are claimed to be effective in cases of heavy blood loss, bronchitis pneumonia, stomachache, digestive disorders . The seeds are taken as antidote against arrow poison and snake bite. Roots are used for convolusions[95]. Stem bark is used for measles, hypertension, itch and tiredness[96].

Anthocyanidin alkaloid (2-methoxyamine 3, 4, 5, 7 – tetrahydroxyanthocyanidine) was extracted from stem bark of D. *Senegalenes*[97].

Cyclohexone, β-myrcene, cis-Roseoxide, camphor, citronellol, E-citral, isoledene, palmitic acid, linolic acid, oleic acid were reported from seed of the plant[98].

detarium senegalense

Aims of This study

This study was designed to fulfil the following:

-Extraction of flavonoids from target plant.

-Isolation of chromatographically pure flavonoids.

-Elucidation of structures of isolates.

-Evaluation of antimicrobial activity of different plant fractions.

Materials and Methods

2.1. Materials

2.1.1. Plant Material

The stems of *Detarium senegalense* was collected from western Sudan.The plant was authenticated by direct comparison with a herbarium sample.

2.1.2- Solvents

Analytical grade solvents were used. Methanol HPLC grade is used for spectroscopic purposes (Loba, India).

2.1.3- Chromatographic materials

- **Sheets of Whatman paper** (No.1 and No.3 mm-46x57cm) from Whatman Ltd. Kent, England.

2.1.4- Equipments

The ultraviolet lamp used in visualizing andpaper chromatography was a multiband UV λmax (254 / 365 nm) portable ultaviolet, a product of Hanovia lamps (6 watt S/Y and L/W).

-Ultraviolet spectral analysis

Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV -Visible Spectrophotometer(Shimadzu).

- **Infrared spectral analysis**

Infrared spectra were generally obtained in potassium bromide (KBr) discs using Perkin-Elmer, FTIR, model 1600-Jasco.

- Mass spectrophotometer analysis

The electron impact ionization (EIMS) mass spectra were obtained on a solid probe using Shimadzu QP-class-500.

- Nuclear Magnetic Resonance (NMR)

^{1}H spectra were obtained on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d_6. The chemical shifts values are expressed in δ (ppm) units using (TMS) as an internal standard and the coupling constants (J) are expressed in Hertz (Hz).

2.2- Methods

2.2.1- Preparation of Reagents for phytochemical screening

- Flavonoids test reagents

a- Aluminium chloride solution

(1g) of $AlCl_3$ was dissolved in 100 ml methanol.

b- Potassium hydroxide solution

(1g) of KOH was dissolved in 100 ml water.

c- Ferric chloride solution

(1g) of $FeCl_3$ was dissolved in 100 ml methanol.

-Alkaloids test reagents

-Mayer's reagent

(1.358 g) of $HgCl_2$ was dissolved in 60 ml of water and poured into a solution of (5 g) of KI in (10 ml) of H_2O,then sufficient water was added to 100 ml.

-Wagner's reagent

(2 g) of iodine and (6 g) of KI were dissolved in 100 ml of water.

-glycosides test reagents

-Molisch reagent

(2 g) α-naphthol dissolved in 20 ml EtOH 96%

2.2.2- Shift Reagents

The diagnostic reagents used for the UV spectral measurements of the isolatedflavonoidswereprepared as follows:

-Sodium methoxide (NaOMe)

Freshly cut metallic sodium(2.5 gm.) was dissolved in100 ml spectroscopic methanol.

- Aluminum chloride

Anhydrous $AlCl_3$(5 gm) was cautiously dissolved in100 ml spectroscopic methanol and filtration was carried out after about 24 hours.

-Hydrochloric acid

Fifty ml. concentrated HC1 were mixed with 100 ml. distilled water.

- Sodium acetate

(NaOAc) Anhydrous sodium acetate was melted and allowed to stand

for about 10 minutes. The material was then powdered and stored in a dry bottle.

- Boric acid

Anhydrous powdered reagent grade H_3BO_3 was used

2.2.2.1- Stepwise procedure for use of shift reagents for UV

- The UV spectrum of the compound in methanol was first recorded.
- 3 drops of NaOMereagent 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 shaked and the NaOAC spectrum was recorded. NaOAC/ H_3BO_3 spectrum was then recorded after adding H_3BO_3.

2.2.3.Preparation of plant extract for phytochemical screening

Powdered shade-dried stem (1Kg) of *Detarium senegalense* was macerated with 95% ethanol for 48hr. The solvent was removed in vacuo to give a crude extract used for phytochemical screening.

a)Test for Sterols and Triterpenses

A portion (10ml) of the ethanolic extract was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the colouring matter. The residue was extracted with 7ml chloroform and the chloroform solution was dehydrated over anhydrous sodium sulphite. The chloroform solution (5ml) was mixed with 0.5ml acetic anhydride followed by 2 drops of concn. sulphuric acid. The gradual appearance of green, blue, pink to purple colour was taken as an evidence of presence of sterols (green to blue) and or triterpenes.

b)Test for Alkaloids

A second portion (7.5ml) of ethanolic extract was evaporated to dryness on a water bath and (5ml) of 2N HCI were added and the solution was stirred while heating on a water bath for 10 minutes, cooled, filtered, and divided equally into two test tubes.

To both test tubes few drops of Mayer's reagent were added. A slight turbidity or heavy precipitate, in either of the two test tubes, was taken as presumptive evidence for the presence of alkaloids.

c)Test for Flavonoids

A third portion of (17ml)of the ethanolic extract was evaporated to dryness on a water bath, cooled and the residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in 15ml of 80% ethanol and filtered. The filtrate was used for the following tests:

i)To (3ml) of the filtrate in a test rube, (1ml) of 1% aluminium chloride solution in methanol was added. Formation of a yellow colour indicates the presence of flavonoids.

ii)To (3ml) of the filtrate in a test tube, (1ml) of 1% potassium hydroxide solution was added. Dark-yellow coloration indicates the presence of flavonoid compounds.

d)Test for Tannins

(7ml) of the ethanolic extract were evaporated to dryness on a water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with (10ml) of hot saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to (10ml) with more saline solution and (5ml) of this solution was treated with few drops of gelatin salt reagent. Immediate formation of a precipitate was taken as evidence for the presence of tannins in the plant sample.

To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green colour was taken as an evidence for the presence of tannins (hydrolysable or condensed).

e)Test for Saponins

One gm of the original powdered plant material was placed in a clean test tub and (10ml) of distilled water were added. The tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam. The appearance of honey comb indicates the presence of saponins.

f)Test for cyanogenic glycoside

Three gm of the powdered plant sample were placed in an Erlenmeyer flask and sufficient water was added to moisten the sample followed by addition of (1ml) of chloroform. A piece of freshly prepared sodium picrate paper was carefully inserted between a split corck which was used to stopper the flask. A change in colour of the sodium picrate paper from yellow to various shades of red was taken as an indication for presence of cyanogenic glycosides.

g)Test for anthraquione glycoside

Ten gm of the powdered plant was boiled with (9.5ml) of 5.0N KOH containing (0.5ml) of 3% hydrogen peroxide solution. The mixture was extracted with 10ml of benzene. A portion of (5ml) of the benzene solution was shaken with (3ml) of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones is indicated by the appearance of pink to red coloration in the ammonia layer.

i)Test for coumarins

Three gm of the original powdered plant sample was boiled with (20ml) distilled water in a test tube and a filter paper was immersed in the test tube and left to dry followed by one drop of (0.5N) KOH and examined under UV-light. The appearance of blue fluorescence is taken as an indication for the presence of coumarins.

2.2.4. Extraction of flavonoids

Powdered shade –dried stems of *Detarium senegalense* (2 kg) were macerated with 85% ethanol at ambient temperature for 72 hours. The solvent was removed under reduced pressure to give a crude extract.

2.2.5-Paper chromatography(PC)

The ethyl acetate fraction of*Detarium senegalense*was dissolved in methanol and applied to the Whatman paper (No. 3 mm – 46x57cm), the bands were developed using BAW (n-butanol acetic

acid-water, 5:2:6 and 4:1:6; v:v:v). The developed chromatograms were air-dried and examined under both visible and UV light (ʎ 366,245nm).

The equivalent bands from each PC were then cut out, combined, cut into small strips and slurred with methanol. After several hours of contact occasional shaking, the liquid is filtered and evaporated to dryness. Compound I(R_f 0.97) and II(R_f 0.98) were isolated using BAW(5:2:6) , while compound III(R_f 0.93) was isolated when a ratio of (4:1:6) was used.

2.2.6- Antimicrobial assay

The isolated flavonoids were screened for their antimicrobial activity against four bacterial species, Gram-positive *(Staphylococus aureus* and *Bacillus subtilis)*, gram-negative *(Pseudomonas aeruginosa* and *Escherichia coli)*andtwoFungalspecies*(Aspergillus niger, Candida albicans).*The disc diffusion methodwere used.

2.2.6.1- Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a

suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.2.6.2- Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml

of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.2.6.3- Testing of antibacterial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts.The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.2.6.4-Testing of antifungal susceptibility

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

Results and Discussion

,

3.1-Phytochemical screening

Phytochemical screening of *Detarium senegalense* ethanolic extract revealed the presence of sterols, triterpenes, flavonoids, tannins, saponins, anthraquinones and cyanogenic glycosides(Table 5).

Table 5: Preliminary phytochemical screening

Phytochemical	*Detarium senegalense* extract
Sterols	+ve
Triterpenes	+ve
Alkaloids	-ve
Flavonoids	+ve
Tannins	+ve
Saponins	+ve
Coumarins	-ve
Anthraquinones	+ve
Cyanogenic glycosides	-ve

The ethyl acetate fraction was purified by paper chromatography. After the usual workup, compounds I ,II and III were isolated from *Detarium senegalense*stems in chromatographicallypure form**.**

3.2-Identification of compound I

The IR spectrum of compound I(Fig.1) showed ν(KBr)675 (CH,Ar.bending), 1023(CO), 1450,1531(C=C, Ar.), 1645(C=O), 2923(C-H,aliph.) and 2340cm^{-1} (OH).

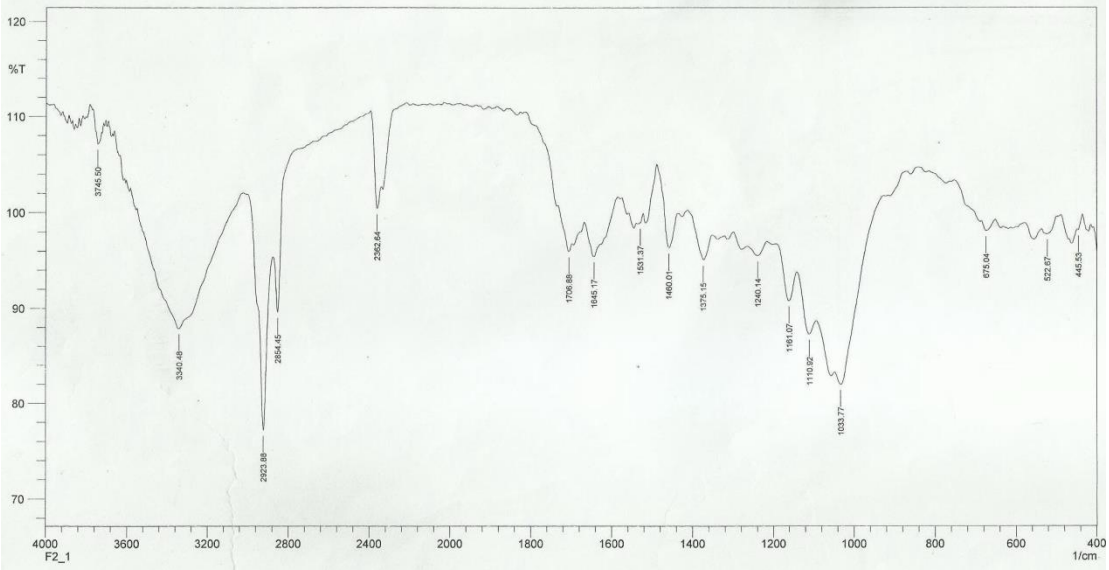


Fig.1: IR spectrum of compound I

Compound I absorbs in the UV(Fig.2) at λ_{max}(MeOH)280nm giving only band II. Such absorption- which originates from a benzoyl chromophore is revealed by : flavanones , isoflavones, dihydrchalcones and dihydroflavonols.

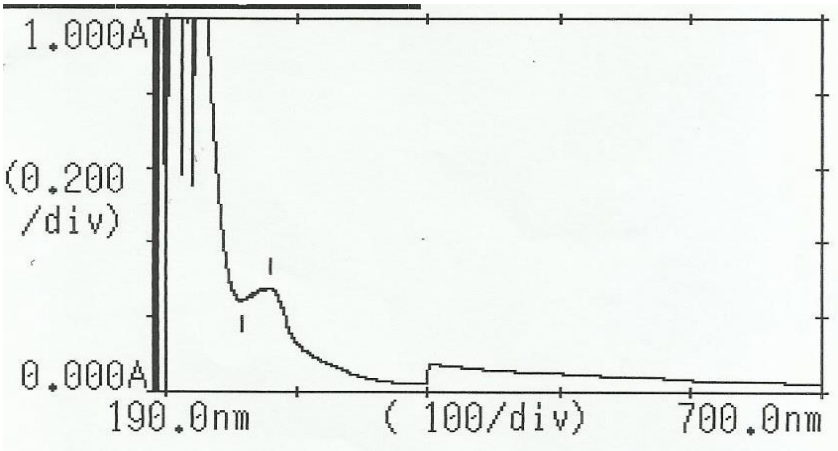
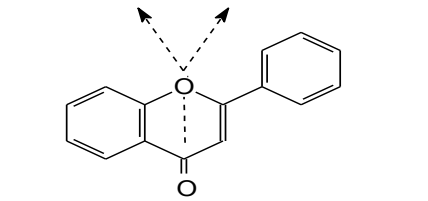
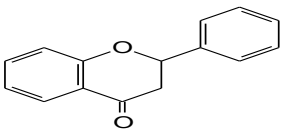


Fig.2: UV spectrum of compound I

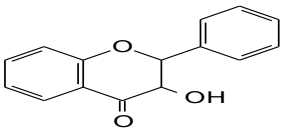
These classes lack conjugation between the carbonyl function and the B ring.Thus only one band- band II is manivested.. Other classes of flavonoids (flavones , flavonols, chalcones and aurones) reveal both bands I and II in their UV spectra due to conjugation between ring B and the carbonyl function at C-4 . Such flavonoids possess two chromophores: the benzoyl(giving band I) and cinnamoyl(giving band II) systems.



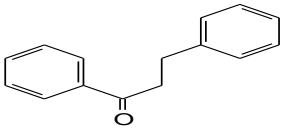
Benzoyl system cinnamoyl system



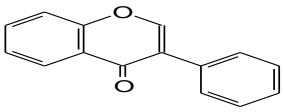
Flavanone



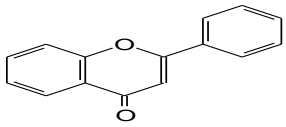
Dihydroflavonol



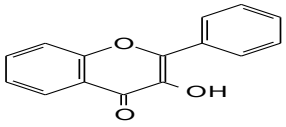
Dihydrochalcone



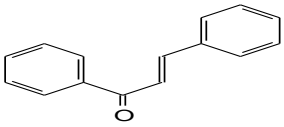
Isoflavone



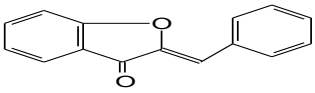
Flavone



Flavonol



Chalcone



Aurone

No shoulder in the range: 300-340nm was observed in the UV spectrum suggesting absence of isoflavones .

Next the hydroxylation pattern of this isolate was investigated using UV shift reagents: sodium methoxide,sodium acetate, aluminium chloride and boric acid.

Sodium methoxide, the strong base, is diagnostic of 3- and 4`-OH functions , in both cases it affords bathochromic shifts, but with decrease intensity in case of a 3-OH group.

The sodium methoxide spectrum of compound I (Fig.3) revealed a bathochromic shift characteristic of a 4`-OH function.This cites evidence for a dihydrochalcone skeleton which gives only band II in the UV spectrum.

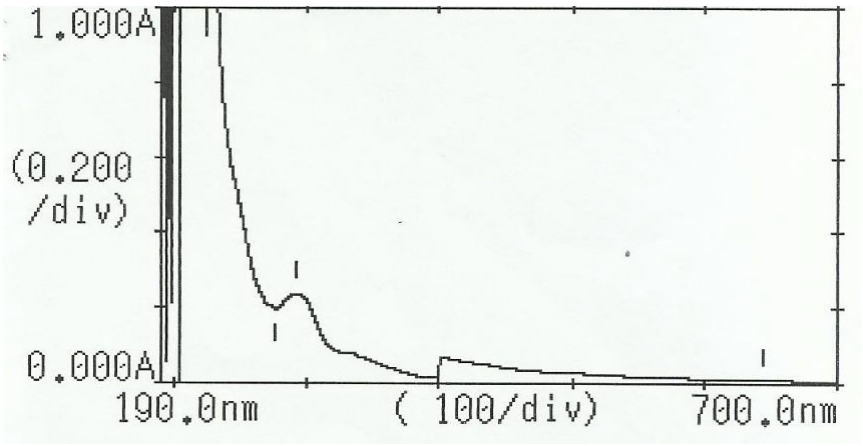


Fig.3:Sodium methoxide spectrum of compound I

.

The shift reagent sodium acetate being a weak base, ionizes only the more acidic hydroxyl groups. It is useful diagnostic reagent for specific detection of a 7-hydroxyl group[1]. When a methanolic solution of compound I was treated with sodium acetate no bathochromic shift was observed(Fig.4). This indicates absence of a 7-OH function.

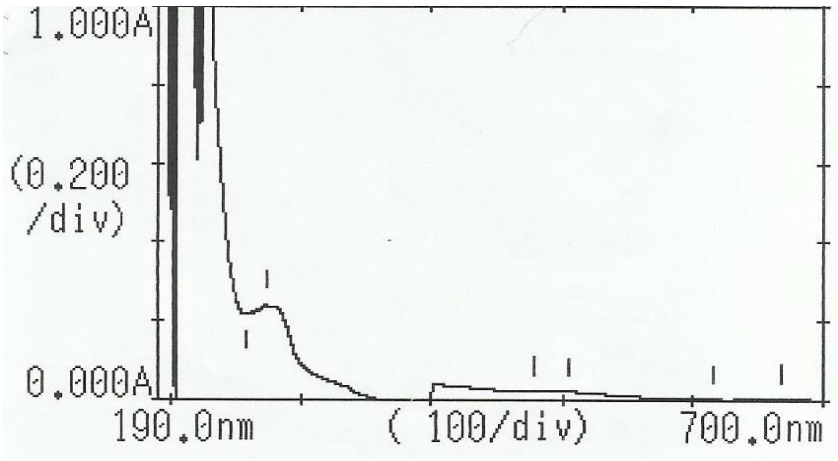


Fig.4: The sodium acetate spectrum of compound I

The shift reagent aluminium chloride forms acid-stable chelates with 3-OH and 4- keto function as well as 5-OH and 4- keto function .It also complexes with catechol systems . However . the ortho- dihydroxy system, unlike the 3-OH and 5-OH complexes afford acid-labile complexes[2]. The aluminium chloride spectrum of compound I (Fig.5)did not reveal any detectable bathochromic shift. This indicates absence of 5- and 3-OH functions as well as catechol moieties.The absence of catechol systems was further furnished by the boric acid spectrum(Fig.6) which did not afford any bathochromic shift.

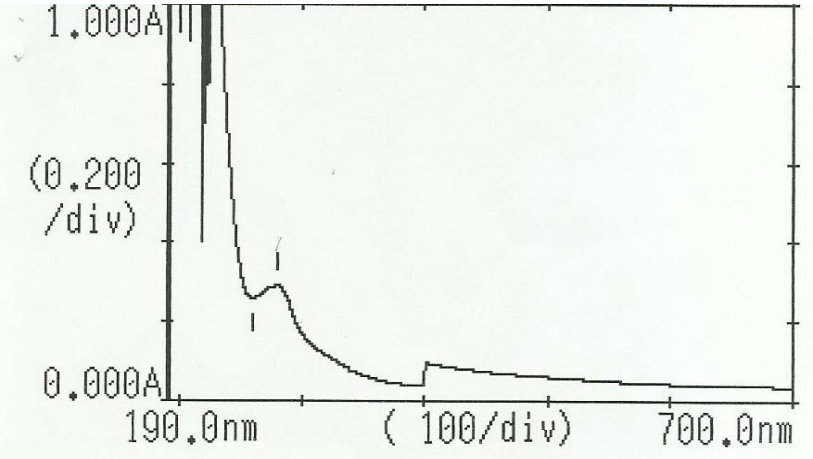


Fig.5: Aluminium chloride spectrum of compound I

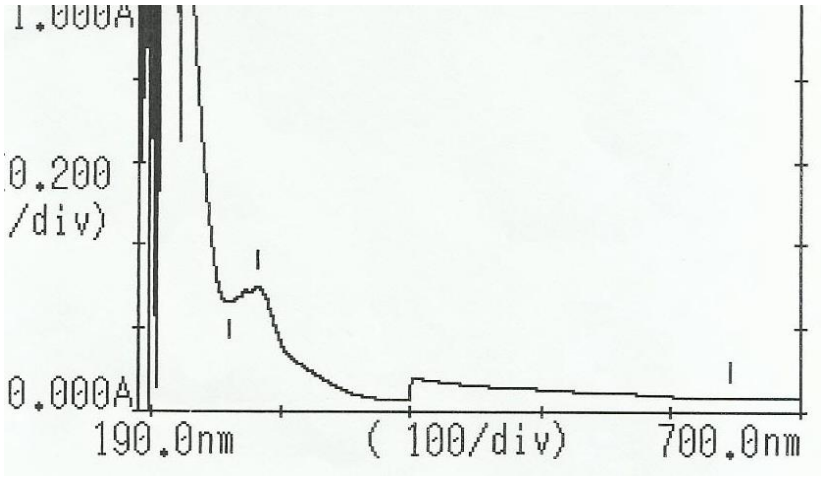


Fig.6: Boric acid spectrum of compound I

The ^{1}HNMR spectrum (Fig.7) showed δ(ppm):1.20(6H) assigned for two methyl functions, while the signal at 1.80(3H) was assigned for an acetyl group.The signal at δ3.73(6H) was attributed to a methoxyl function. The resonance multiplet centered at δ3.50(6H) accounts for a sugar moiety . The signal at δ6.25(1H) accounts for C_6 – proton. Other aromatic protons appeared at δ6.73ppm.

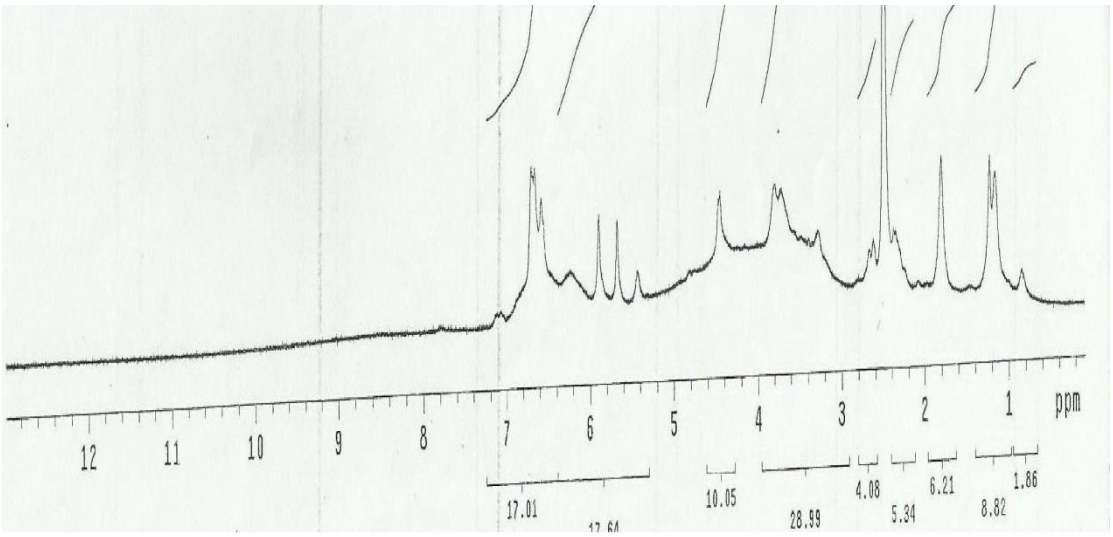


Fig.7: ^{1}HNMR spectrum of compound I

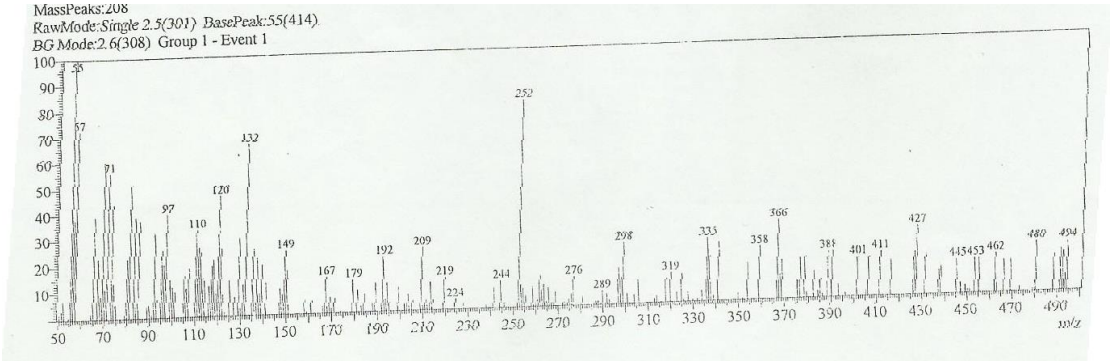
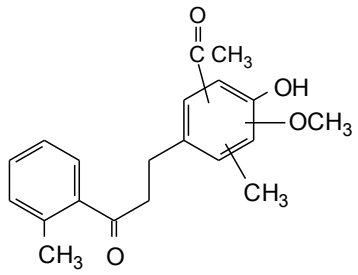


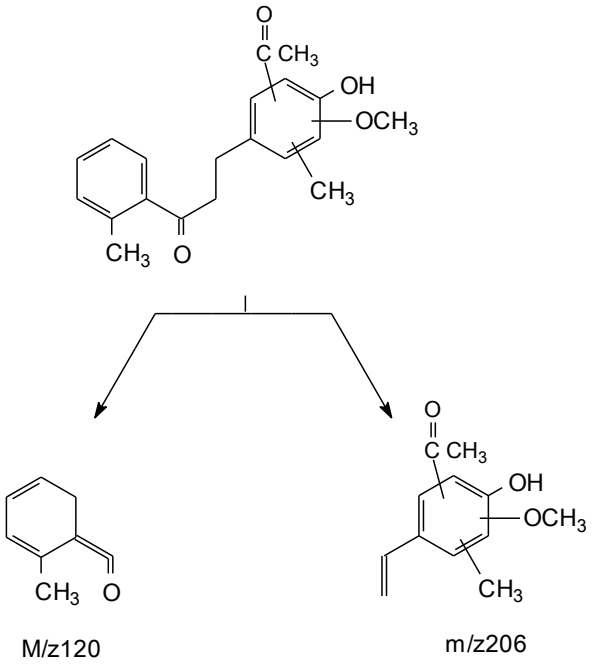
Fig.8: Mass spectrum of compound I

The mass spectrum(Fig.8) gave m/z326 M^{+}. Two important fragments resulting from the fission shown in scheme I, and corresponding to intact A and B rings, appeared at m/z120 and m/z206 respectively. Such fission supports the structure shown below where the assignment of a methyl function at C_5 is testified by the absence of a downfield signal at δ8.00 characteristic of C_5- proton.

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



I



Scheme I:Retro Diels –Alder fission of compound I

3.3-Identification of compound II

The IR spectrum of compound II(Fig.9) showed ν(KBr)611,667,703(C-H,Ar.bending),1064(C-O),1515(C=C, Ar.), 1616(C=O), 2910(C-H,aliph.) and 3350 cm^{-1} (OH).

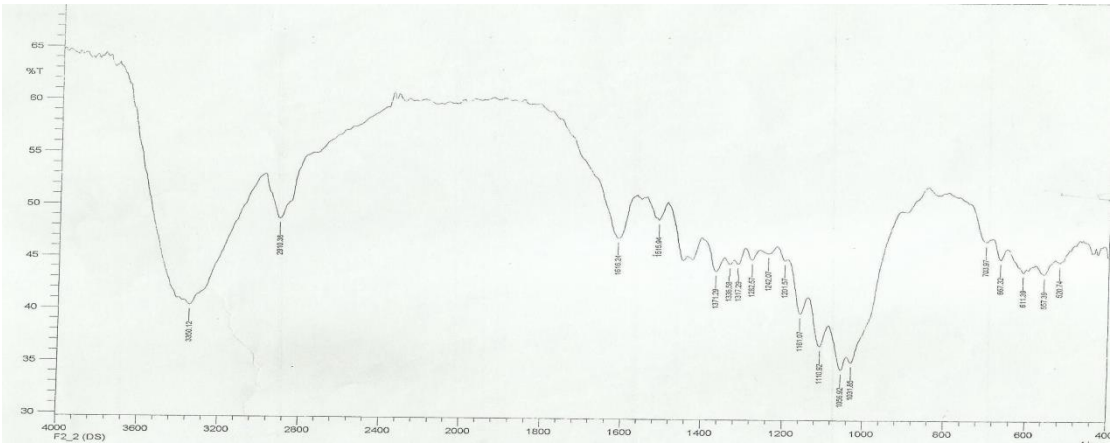


Fig.9: IR spectrum of compound II

Compound II absorbs in the UV(Fig.10) at λ_{max}(MeOH)281nm.Such absorption is characteristicflavano of :isoflavones , flavanones, dihydroflavonol and dihydrochalcones.

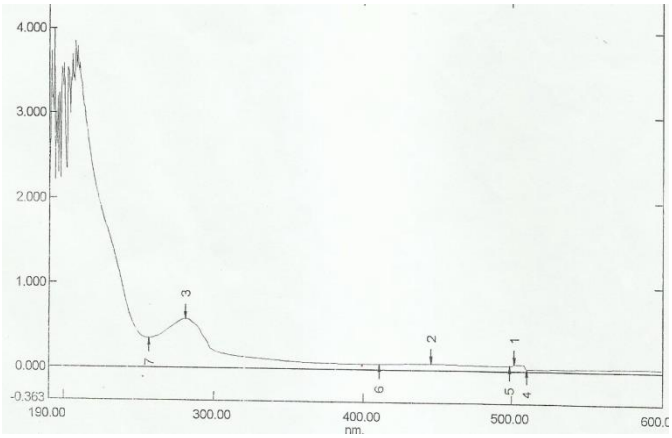


Fig.10: UV spectrum of compound II

However, no shoulder characteristic of isoflavones appeared in the UV spectrum in the range 300-340nm.Furthermore , the shift reagent sodium methoxide spectrum (Fig.11) did not reveal any bathochromic shift diagnostic of a 3-OH function which is a characteristic feature of dihydroflavonols.

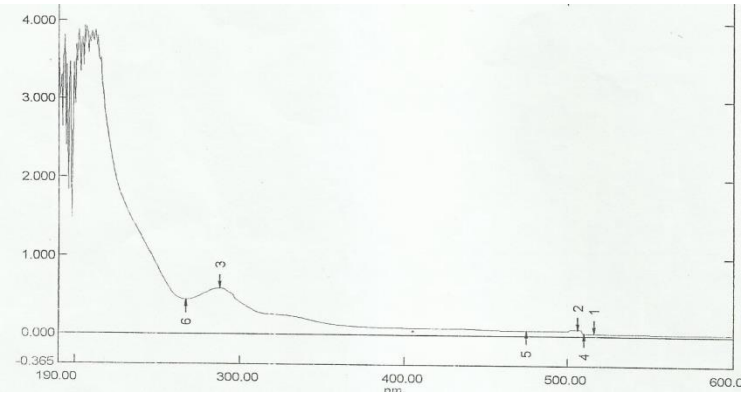


Fig.11: Sodium methoxide spectrum of compound II

Also the NMR spectrum(Fig.14) did not show a double multiplet around δ2.8 and δ5.2 ppm which usually appear in the spectra of flavanones due to mutual spin-spin splitting of the magnetically unequivalent C_2 – protons . The double doublet which appears due to this splitting is further split by C_2 – protons into a pair of multiplets. Such data suggests that compound II is a dihydrochalcone.

When a methanolic solution of compound II was treated with sodium acetate no bathochromic shift was observed in band II (Fig.12). This indicates absence of a 7-OH function.

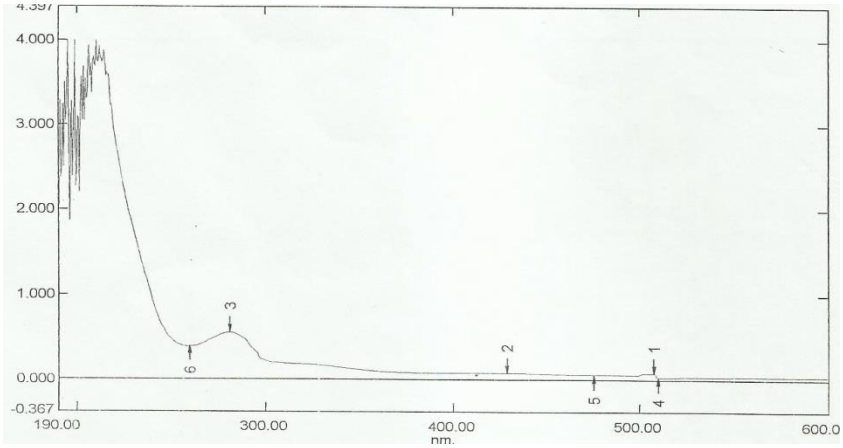


Fig.12: The sodium acetate spectrum of compound II

The aluminium chloride spectrum of compound II (Fig.13)did not reveal any detectable bathochromic shift. This indicates absence of 5- and 3-OH functions as well as catechol moieties.

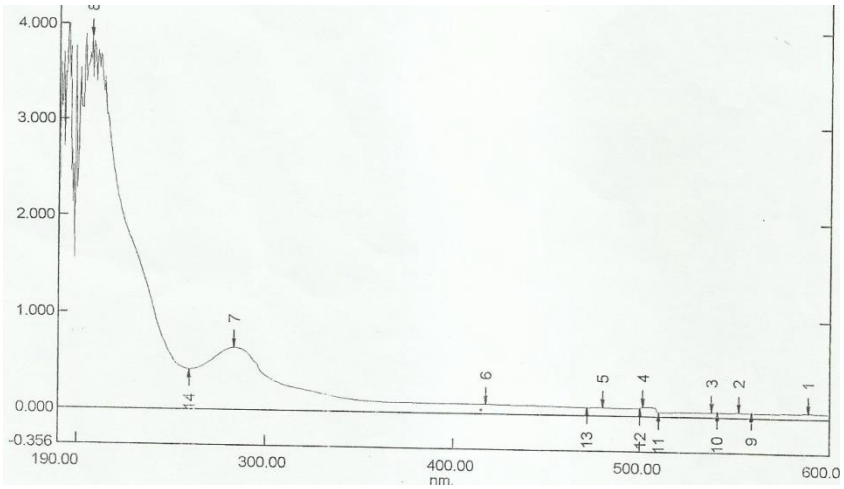


Fig.13: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig.14) showed δ(ppm):0.82(3H),1.12(3H) and 1.20(3H) assigned for three methyl groups.The resonances at δ2.80 and δ 1.88ppm was attributed to two acetyl functions. The signal at δ3.40 accounts for a sugar moiety, while the signals at δ6.67 and 6.80ppm account for aromatic protons.

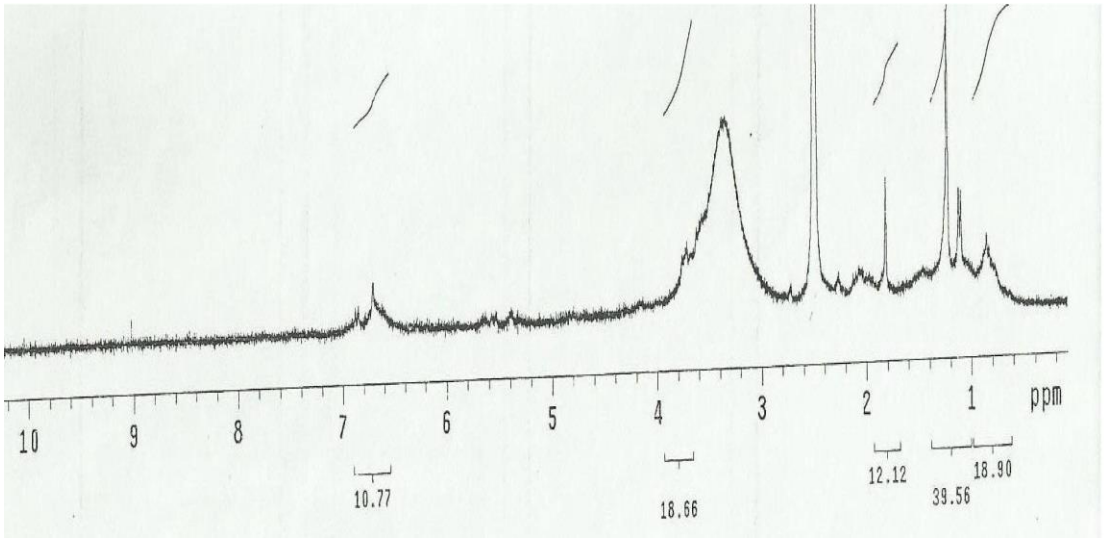


Fig.14:^{1}HNMR spectrum of compound II

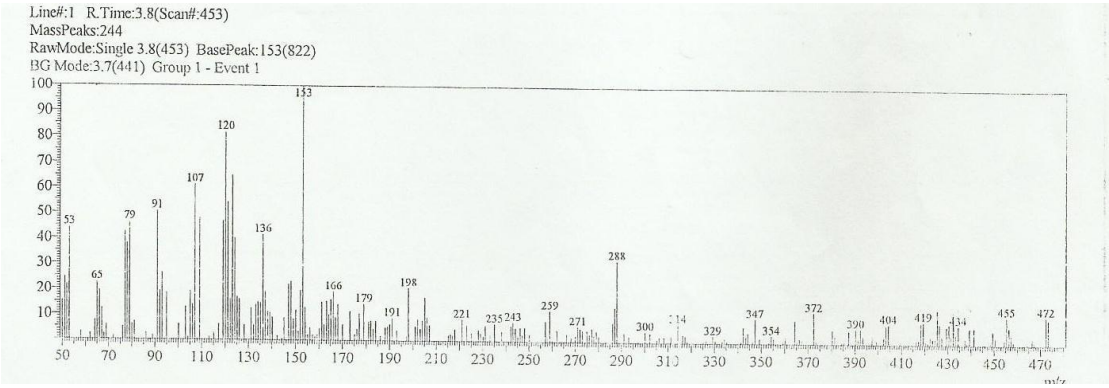
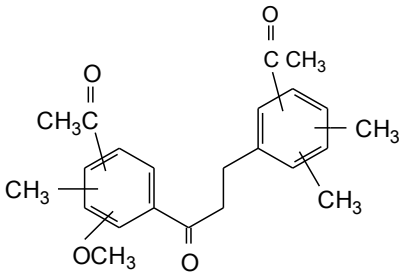
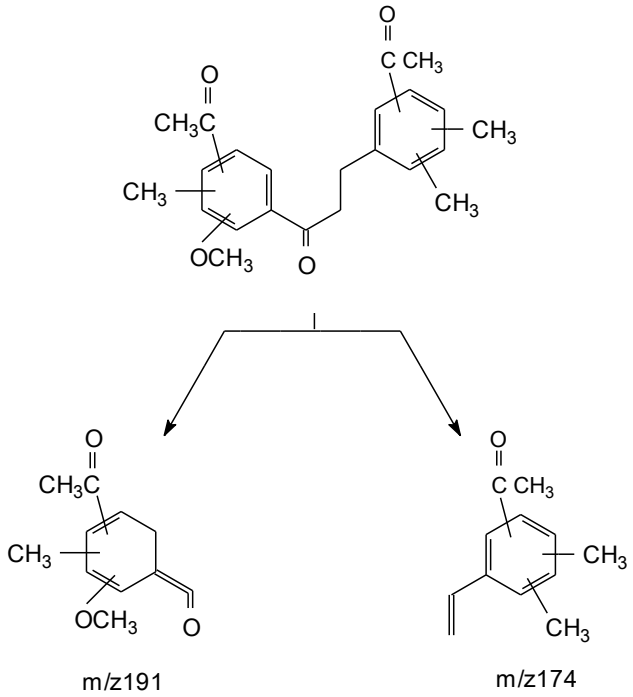


Fig.15: mass spectrum**of compound II**

The mass spectrum (Fig.15) gave m/z365 for M^+ (the aglycone). Other signal exceeding this peak seem to be associated with the fragmentation of the glycoside. Two important fragments corresponding to intact A and B rings, appeared at m/z191 and m/z174 respectively.Such fragments end evidence for the substitution pattern outlined in the following partial structure of compound II:



Compound II



Scheme II: Retro Diels-Alder fission of compound II

3.4-Identification of compound III

The IR spectrum of compound III(Fig.16) showed ν(KBr)770(C-H,Ar.bending),1037(C-O),1454,1510(C=C, Ar.), 1647(C=O), 2920 cm^{-1} (C-H,aliph.) .

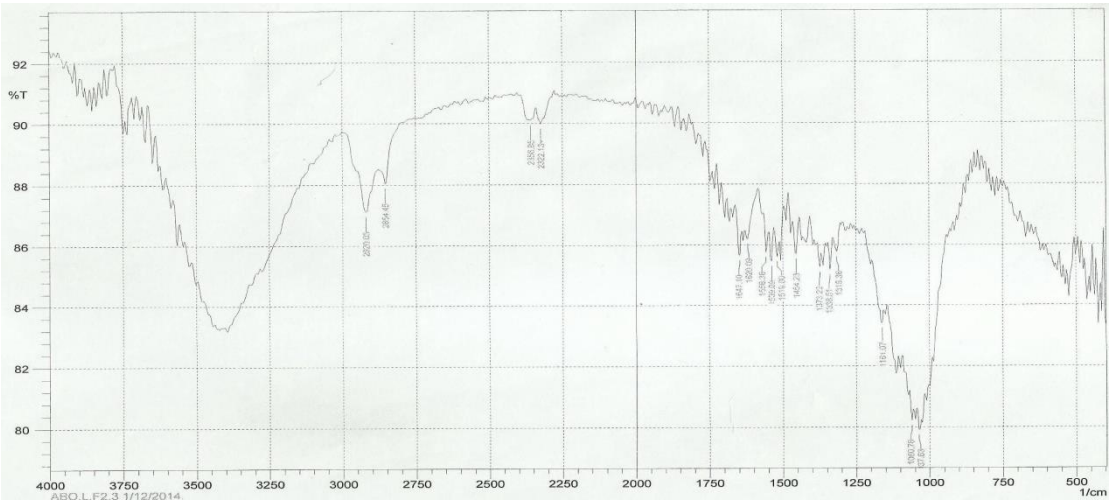


Fig.16: IR spectrum of compound III

Compound III absorbs in the UV(Fig.17) at λ_{max}(MeOH)281nm.Such absorption is characteristicflavano of :isoflavones , flavanones, dihydroflavonol and dihydrochalcones.

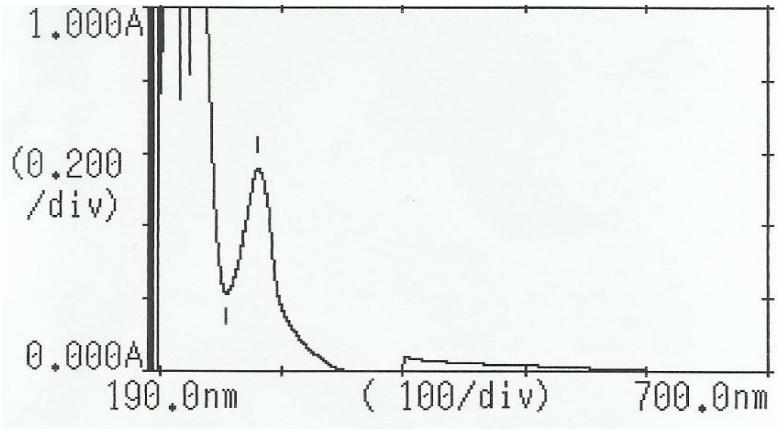


Fig.17: UV spectrum of compound III

No shoulder characteristic of isoflavones appeared in the UV spectrum in the range 300-340nm.Furthermore , the shift reagent sodium methoxide spectrum (Fig.18) did not reveal any bathochromic shift diagnostic of a 3-OH function which is a characteristic feature of dihydroflavonols.

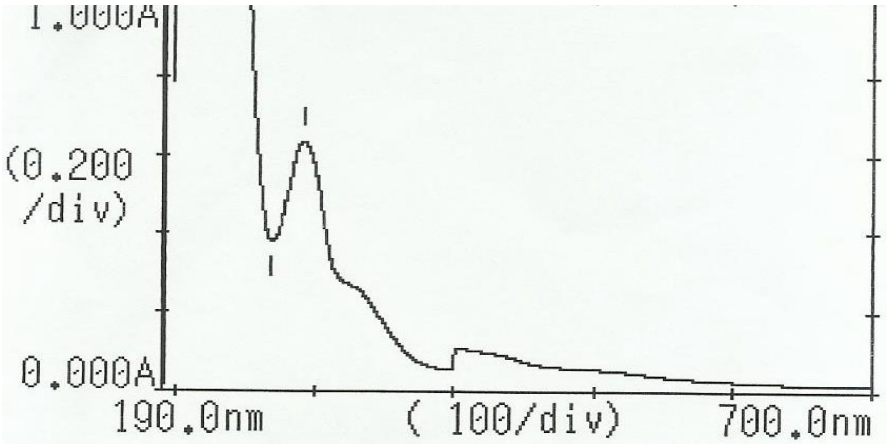


Fig.18: Sodium methoxide spectrum of compound III

When a methanolic solution of compound III was treated with sodium acetate no bathochromic shift was observed in band II (Fig.18). This indicates absence of a 7-OH function.

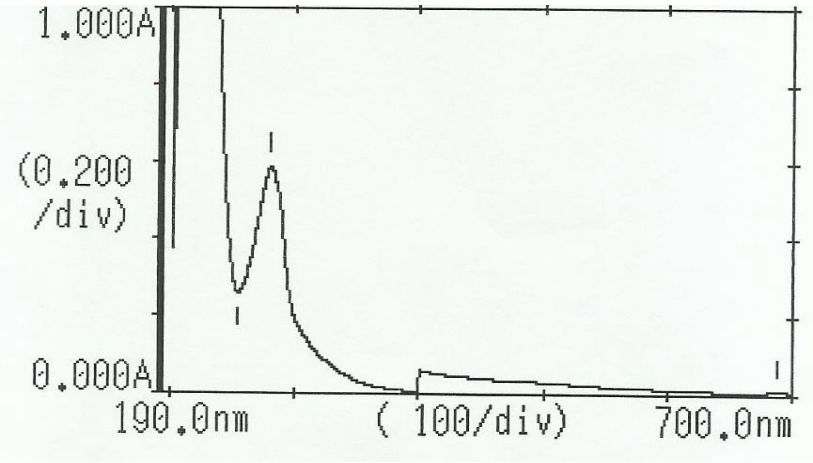


Fig.19: The sodium acetate spectrum of compound III

The aluminium chloride spectrum of compound II (Fig.20)did not reveal any detectable bathochromic shift. This indicates absence of 5- and 3-OH functions as well as catechol moieties.Furthermore, the boric acid spectrum (Fig.21) testified the absence of catechol systems. It was devoid of bathochromic shifts.

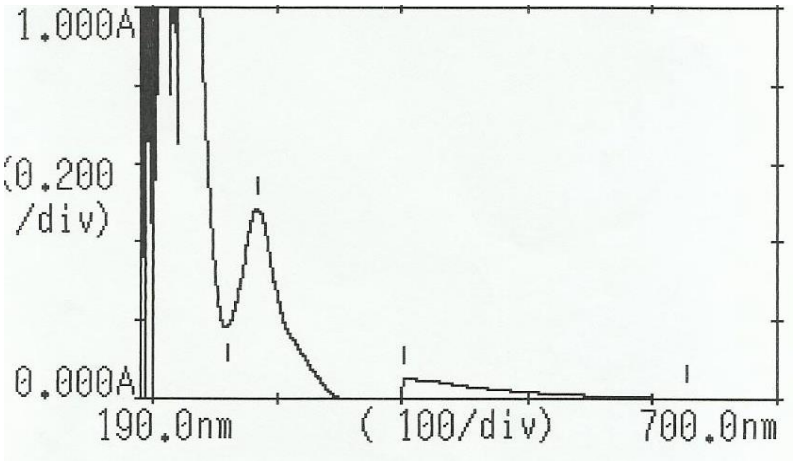


Fig.20: The aluminium chloride spectrum of compound III

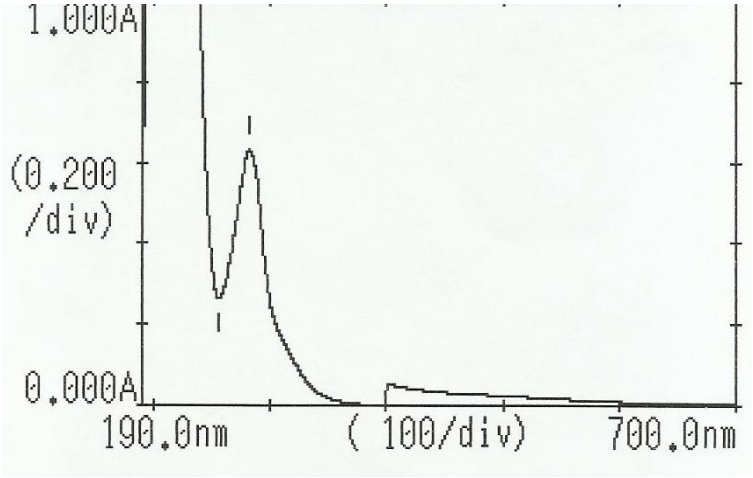


Fig.21:Boric acid spectrum of compound III

The ^{1}HNMR spectrum (Fig.22) showed δ(ppm):1.20(6H) assigned for two methyl groups .The resonances at δ1.80 was attributed to an acetyl function , while the signal at δ4.20 accounts for a methoxyl group. The multiplet centered at δ3.60 accounts for a sugar moiety.Two multiplets characteristic of flavanones appeared at

δ2.20-2.80 and δ5.60-6.60ppm .The resonances at δ5.80, δ5.95, δ6.20 and δ 6.60ppm account for the aromatic protons .

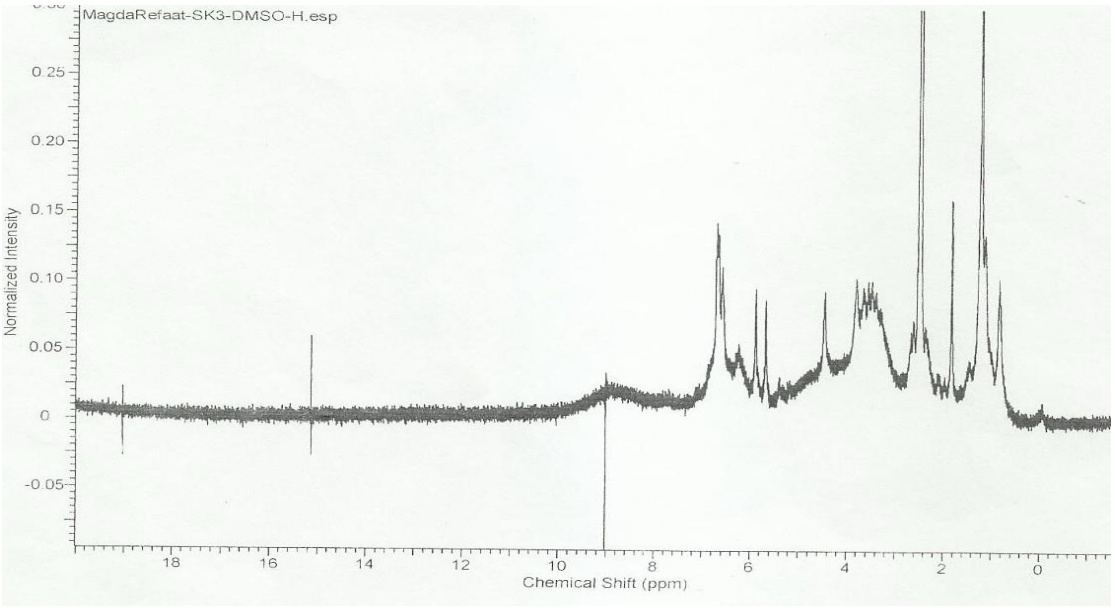
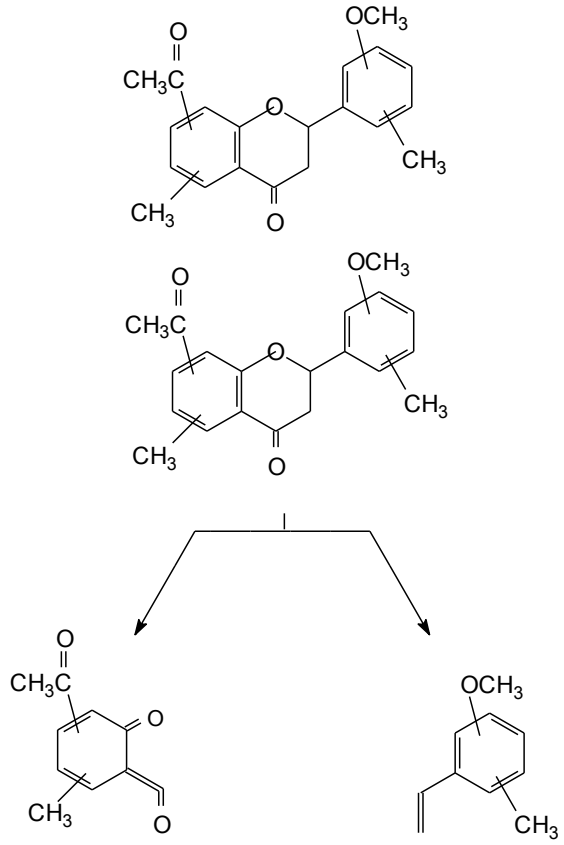


Fig.22:[1]HNMR spectrum of compound III

The mass spectrum (Fig. 23) gave m/z323 for M^+-H^+(aglycone). Other signal exceeding this peak seem to be associated with the fragmentation of the glycoside. Two important fragments corresponding to intact A and B rings, appeared at m/z176 and m/z148 respectively.Such fragments end evidence for the substitution pattern outlined in the following partial structure of compound III:

Compound III



Scheme II: Retro Diels-Alder fission of compound III

3.5-Antibacterial activity

In cup plate agar diffusion assay,the isolates were screened for antimicrobial activity against six standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (6) .The results were interpreted in commonly used terms (<9mm: inative;9-12mm:partially active; 13-18mm: active;>18mm:very active) .Tables (7) and (8) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 6: **Antimicrobial Activity of *Detarium senegalense* fractions**

Concentration		Antibacterial activity				Antifungal Activity	
		Gram-positive		Gram -negative			
100mg/ml	Control Methanol	*B.subtilis*	*St.auerus*	*E. coli*	*Ps. aeruginosa*	*Ca. albicans*	*As. niger*
Ethanolic extract	0.0	23.5	21.5	21.5	25	19.5	19
B	0.0	22.5	16	15	15.5	20.5	20.5
Ethyl acetate fraction	0.0	24.5	26.5	23	22.5	18	22.5
n-Butanol fraction	0.0	20.5	21	23	23.5	19	21

Different fractions of *Detarium senegalense* showed significant antimicrobial activity against test organisms as displayed in Table 6.

Table (7) : Antibacterial activity of standard chemotherapeutic agents against standard bacteria :M.D.I.Z (mm)

Drug	**Conc. mg/ml**	**B.s.**	**S.a.**	**E.c.**	**P.a.**	**S.t**
Ampicillin	40	15	30	-	-	-
	20	14	25	-	-	-
	10	11	15	-	-	-
Gentamycin	40	25	19	22	21	22
	20	22	18	18	15	17
	10	17	14	15	12	14

Table (8) : Antifungal activity of standard chemotherapeutic agents against standard fungi

Drug	Conc. mg/ml	A.n	C.a
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- S.a: *Staphylococcus aureus*
- E.c: *Escherichia coli*
- P.a: *Pseudomonas aeruginosa*
- A.n: *Aspergillus niger*
- C.a: *Candida albicans*
- S.t: *Salmonella typhi*
- B.s: *Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.

Conclusion

Phytochemical screening of the ethanolic extract releaved the presence of steroids, triterpenes, flavonoids, tannins, saponins, anthraquinones and cyanogic.

Three flavonoids (compounds I. II & III) were isolated from the *Detarium senegalense*stem.

The structures of isolates were elucidated via spectral data.

Different fractions were screened for their antimicrobial activity, and significant results were obtained.

Recommendations

1- A future ^{13}C and 2D NMR experiments may fully elucidated the partial structures proposed for some isolates.
2- The crude extracts together with the pure.
3- Flavonoids may be evaluated for their antimalarial, antinflammatary and antidiabetic activities.

References:

1. Math, A., *Appl. Environ Microbial;***74:** 1847-52 (2008).
2. SzkudeIska, K., The *Journal of Steroid Biochemistry and molecular Biology;***105:** (1-5): 37-45 (2007).
3. Arora, A., *Arch. Biochem. Biophys*,**326:** 133-141 (1998).
4. Briviba, K., *In C.A. Rice-Evans and L. Packer (ed.), flavonoids in the health and disease. Marcel Dekker. Inc. New York, N.Y.* 295-302 (1997).
5. Huk, L., *Br. J. Surg*,**85:** 1080-5 (1998).
6. Luczaj, *W., Acta Pol. Pharm.***66** (6): 617 (2009).
7. Kitagawa, S., *Chem. Pharm. Bull;***40:** 304-7 (1992).
8. Lale, A., *J. Wat. Prod.***59:** 273-6 (1996).
9. Hertog, MG.,*Am. J. Clin. Wutr*,**65:** 1489-94 (1997).
10. Haraguchi, H., *Planta. Med.;***62:** 217-21 (1996).
11. Ishikawa, T., *Am. J. Clin. Nutr.***66:** 261-6 (1997).
12. Katan, MB.,*Nutr. Metab. Cardiovascualr Disease,* 1998; **8:** 1-4.
13. Sies, H. Am. *J. Med.;***19:** 315-385 (1991).
14. (a) Halliwell, *B. Drugs;***42:** 569-605 (1991/a).
    (b) Halliwell, B Am. *J. Med.;* 91: 145-225 (1991/b).
15. Halliwell, B., J. Lab. Clin. Med.; **119:** 598-620 (1992).
16. Letan, AJ. *J. Food Sci.;***31:** 395-399 (1966).
17. De Groot, H., *Hepatogastroentrology;***41:** 328-32 (2004).
18. Grace, PA., *Br. J. Surg.;***81:** 637-47. (1994).
19. Nakagawa, K., *J. Agric. Food Chem.;***47** (10): 3967-3973 (1999).

20. Zielinska. Przyjemska, M., *Phytother Res.;* **22**(12): 1557-62 (2008).
21. Wang, HK.,*Adv. Exp. Med. Biol.;***439:** 191-225 (1998).
22. Tej, N. Kaul, *Journal of Medical Virology;***15**(1): 71-79 (2005).
23. Wu. D., *Int. J. Antimicrob. Agents;***32**(5): 421-6 (2008).
24. Li, M., *Arch. Pharm. Res.;***31**(5): 640-4 (2008).
25. Wiseman. H. *Exp.Opin. Invstig.;Drugs***9:**1829-1840 (2000).
26. Metzner, JE.,*Arzneimittelfor – Schug***.; 59**(10): 513-20 (2009).
27. Szkudelska. K., *J.Steroid Biochem. Mol. Biol.;***105:** 37-45 (2007).
28. Comalada; MD., *Eur. J. Immunol;* **35:** 581-591 (2005).
29. Kawada, NS.,*Hepatology;***27:** 1265-1274 (1998).
30. Middleton; E. *Jr. Adv. Exp. Biol.*,**439:** 175-182 (1998).
31. Wang, J., *J. Agric. Food Chem;***50:** 4183-4187 (2002).
32. Park, HJ.,*Int. Immunopharmacol*.**9** (3): 261-7 (2009).
33. Bach; A., *Antioxid. Redox. Signal*.**4** (Dec. 2009).
34. Applet; LC.,*J. Nutr*.**129** (10): 1820-6 (Oct. 1999).
35. Kammaraj, S., *Invest. New Drugs;***27**(3): 214-22 (2009).
36. Arafa, *Cancer Res*.**69**(23): 8910-7 (2009).
37. Fotsis, T., *Cancer Res*.**57**: 2916-2921 (1997).
38. Peking, K., *Public Health Nutri*.**11:** 1-7 (2010).
39. Hwang, SL., *J. Agric. Food Chem*.**13:** 56(3): 859-64 (2008).
40. Zhang, Y., *Eur. J. Pharmacol*.**535:** 263-260 (2006).
41. BabenkosMA.,*Lipids Health Dis*.**28;** 7:1 (Jan. 2008).
42. Gharagozloo; M., *FundamClin. Pharmacol*.**23**(3): 359-65 (2009).
43. Knekt, P., *Am. J. Clin. Nutr*.**76:** 560-568 (2002).

44. Kobori, M., *Mol. Nutri. Food Res.***53**(7): 859-68 (2009).
45. Costantino, L., *J. Med. Chem.***42:** 1881-1893 (1999).
46. Waisundara; VY.,*Diabetes Metab. Res. Rev*.**25**(7): 671-7 (2009).
47. Zhang, L., *Free Radic. Biol. Med*.**46**(3): 414-21 (2009).
48. Meng, Q., *Free Radic. Med.***44**(6): 1032-41 (2008).
49. Dal. Belo, SE.,*Skin Pharmacol. Physiol*.**22**(6): 299-304 (2009).
50. Huag. JH.,*Toxicol. Invitro,***24**(1): 21-28 (2010).
51. Fahlman; BM., *J. Agric. Food Chem.***57**(12): 5301-5 (2009).
52. Monjid Ibrahim, Sudan University of Science and Technology, Ph.D. (2016).
53. Dalia AbdElazizem, Sudan University of Science and Technology, Msc (2015).
54. Fath Elrahman Ahmed. Sudan University of Science and Technology, Ph.D. (2016).
55. Fatima Mohamed Hassan, University of Al-Neelain, MSc. (2016).
56. Al hafezmutafa Alraih, Sudan University of Science and Technology, Ph.D. (2012).
57. AltohamiAlzibairHger, Sudan University of Science and Technology, Ph.D. (2015).
58. Mazen Ismail Mohamed, Sudan Academic Science and Technology, MSc. (2015).
59. Adil Ali Mgwah, Sudan University of Science and Technology, Ph.D. (2012).

60. Amani Hassan Ahmed, Sudan University of Science and Technology, Ph.D. (2012).
61. Slah Homaida, Sudan University of Science and Technology,Ph.D. (2013).
62. Nosiba Alzibair,AlzeemAlazhary University, Ph.D. (2016).
63. Abdel Karim, M. and Adil, A. characterization and some biological activity of flavonol isolated from *Cassia* leave, *Omdurman journal of pharmaceutical* Science, page 35 vol. **2(1)** (2015).
64. Siham Hassan Arbab, Sudan University of Science and Technology, Ph.D.(2012).
65. Fernandez, SP, Wasowski, C., Loscalzo, LM, Granger, RE, Johnston, GAR, Paladini. AC., Marder, M. *Central Bervous system depressant action of flavonoid glycosides European Journal of Pharmacology***539:** 168-176 (2006).
66. Heim. KE. Tagliaferro, AR, Bobliya, DJ. Flavonoids antioxidants: chemistry, metabolism and structure activity relationships. *The Journal of Nutritional Biochemistry;* **13:** 572-584 (2002).
67. Hollman, PCH. Katan, MB. *Dietary Flavonoids:Intake, Health effects and Bioavailability, Food and chemical toxicology***37:**937-942 (1999).
68. Cushnie; TPT. Lamb, AJ. *Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents;***26:** 343-350 (2005).

69. Heim, KE. Tagliaferro; AR. Bobliya; DJ. *Flavonoids antioxidants: Chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry;***13:** 572-584 (2002).
70. Barnes, S., *Br J Nutr***89**, 101 (2003).
71. Sutter, A., Poult, J.E., Grisebach, H., *Arch. biochem. biophys.***170,**547 (1975).
72. Britsh, L., Heller, W., Grisebach H.,Z. *Naturforch* ,**36** ,742 (1981).
73. Sotz, G., Forkmann, *G., Z. Naturforch***36**,737 (1981).
74. Hayaishi, O., Molecular mechanism, Academic Press.new York p.187 (1974).
75. Britsh, L., Grisebach H., " Biochemistry" p 156,569 (1986).
76. Stotz, G., spribill, R., aForkmaan G., *Arch. biochem.biophys.*,**170**,541 (1975).
77. Forkmann, G., Sratz, G., *Planta Medica.*,**161**,261 (1984).
78. Hagman, M., Grisebach, H., *FEBS Lett.*,**175**,199(1984).
79. Kochs, G., Grisebach, H., *Eur.J.Biochem.*,**155**,311 (1986).
80. Patschke, L.,Barz, W., Grisebach, H.,Z. *Naturforch*, **21**,45(1966).
81. Boland, M.J., Wong, *E.,Eur.J.Biochem*,**50**,383 (1975).
82. Hauteville, M., Chadenson, M., Chopin, J., *Bull.soc.Chim.Fr.*,**11**,12 (1979).
83. Spribille, R., Forkmann, G., Z. *Naturforsch*,**39**,714 (1984).

84. Forkmann, G., De Vlaming, P., Spribille, R., Wiering, H., Schram, A.W., *Naturforsch*, **41**,179 (1986).

85. Edrarir, S., Cotelle, N., Bakkour, Y. and Rolando,*Tetrahedron Letters*,(2003).

86. Peterson, J. Dwyer, MSJ. RD. DSC. *Flavonoids: Dietary occurrence and biochemical activity. Nutrition Research***18:**1995-2018 (1998).

87. Tsuchiya, H. *Structure-dependent membrane and interaction of flavonoids associated with their bioactivity.Food chemistry*,**120:** 1089-1096 (2010).

88. Heim. KE. Tagliaferro, AR, Bobliya, DJ. Flavonoids antioxidants: chemistry, metabolism and structure activity relationships. *The Journal of Nutritional Biochemistry;* **13:** 572-584 (2002).

89. Tsuchiya, H. *Structure-dependent membrane and interaction of flavonoids associated with their bioactivity. Food chemistry,*;**120:** 1089-1096 (2010).

90. Tsuchiya, H. *Structure-dependent membrane and interaction of flavonoids associated with their bioactivity. Food chemistry,;***120:** 1089-1096 (2010).

91. Rijke, ED. OUT, P. Niessen, WMA, Ariese, F. Goojer, C., Brinkman, UAT, 2006. *Analytical separation and detection method for flavonoids Journal of chromatography A*.**1112:** 31-63 (2006).

92. Cushnie, TPT. Lamb. AJ. *Antimicrobial and activity of flavonoids. International Journal of Antimicrobial Agents,***26:** 343-356 (2005).
93. Cook, NC, Samman, S. *Flavanoids: Chemistry, Metabolism. Cardiopotective effects and dietary sources. Nutritional Biochemistry;* 7: 66-76 (1996).
94. Sahu, SC, Gray, GC. *Pro-oxidant activity of flavonoids; effect on glutathione and glutathione S transferees in isolated rat liver nuclei letters;***104:** 193-196 (1996) (2003).
95. Prey, JO. Brown, J. Fleming, J. Harrison, PR. *Effect of dilatory flavonoids on major signal transduction pathways in human epithelial cells. Biochemical Pharmacology;***66:** 2075-2088 (2003).
96. Heim, KE. Tagliaferro, AR. Bobliya, DJ. *Flavonoids antioxidants: chemistry, metabolism and structure activity relationships. The Journal of Nutritional Biochemistry* **13:**572-584 (2002).
97. Cushnie, TPT. Lamb. AJ. *Antimicrobial and activity of flavonoids. International Journal of Antimicrobial Agents,***26:** 343-356 (2005).
98. Aderogba; MA. Ogundaini; AO. Eloff, *JN. Isolation of two flavonoids from Bauhinamonandra leaves and their ant- oxidative effects. African Journal of Traditional Complementary and Alternative Medicines;***3:** 59-65 (2006).
99. Cushnie; TPT. Lamb, AJ. *Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents;***26:** 343-350 (2005).
100. Cushnie, TPT. Lamb. AJ. *Antimicrobial and activity of flavonoids. International Journal of Antimicrobial Agents,***26:** 343-356 (2005).

101. Murray, MT. *Quercetin: Nature's antihistamine. Better Nutrition* (1998).
102. Cook, NC, Samman, S. *Flavonoids: chemistry, metabolism, cardio-protective effects and dietary sources. Nutritional Biochemistry;* **7**:66-76 (1996).
103. Williams, RJ, Spencer, JPE. Rice-Evans, C. *Serial review: flavonoids and isoflavonones (Phytoestrongens) Absorption, Metabolism and Bioactivity. Free Radical Biology and Medicine;***36:** 838-849 (2004).
104. Murray, MT. *Qercetin: Nature's antihistamine. Better Nutrition* (1998).
105. Tsuchiya, H. *Structure-dependent membrane and interaction of flavonoids associated with their bioactivity.Food chemistry,***120:** 1089-1096 (2010).
106. Chebil, L. Humeau, C. Falcimaigne, A. Engasser, J. Ghoul, M. *Enzymatic acylation of flavonoids. Process Biochemistry;***41:** 2237-2251 (2006).
107. Cook, NC, Samman, S. Flavanoids: *Chemistry, Metabolism. Cardiopotective effects and dietary sources. Nutritional Biochemistry;* **7:** 66-76 (1996).

108. Chebil, L. Humeau, C. Falcimaigne, A. Engasser, J. Ghoul, M. *Enzymatic acylation of flavonoids. Process Biochemistry;* **41:** 2237-2251 (2006).
109. Middleton, EJR. Kandaswami, C. Teoharides, TC. *The effects of Plant Favonoids on Mammalian Cells: Implications for inflammation, heart disease and cancer. Pharmacological Reviews***52**:673-751 (2000).
110. Narayana, KR. Reddy, SR. Chaluvadi, MR. Krishna, DR. *Bioflavonoid classification, pharmacological, biochemical effects and therapeutic potential. Indian Journal of pharmacology;***33:** 2-16 (2001).
111. Hamza, M. El Amin, *Trees and Shrubs of Sudan*FAO p. 209 (1990).
112. Kaey, RWJ. Phil. D. Biol. TT. *trees of Nigeria, Oxford University Press London,* pp. 204-207 (1998).
113. Burkill, HM. *The useful plants and of west tropical Africa. Royal Botanical Gardens Kew London,* p. 101 (1995).
114. Burkill, HM. *The useful plants and of west tropical Africa. Royal Botanical Gardens Kew London,;* p. 101 (1995).
115. Kaey, RWJ. Phil. D. Biol. TT. *Trees of Nigeria. Oxford University Press London.* pp. 204-207 (1998).
116. Kaey, RWJ. Phil. D. Biol. TT. *Trees of Nigeria. Oxford University Press London.* pp. 204-207 (1998).
117. Gamal, E.B. El Ghazali, *Poisonous plants of the Sudan;* p. 20 (Dec. 2008)Sudan.

118. El Kamali. H.H. and Khalid, SA. *The most common Herbal Remedies in Central Sudan. Fitaterapia;* **4:**301-306 (1996).

119. Boulos, L. Medicinal *Plants of North Africa. Reference Publications. Inc. Michigan***4,** 301. London. pp. 398-403 (1983).

120. Okwu, DE. Uchegbu, R. *Isolation, characterization and antibacterial activity screening of ethoxyaminetetrahydroxyl – anthocyanidines from Detariumsenegalense. Gmelin stem bark. African Journal of Pure and Applied Chemistry.;***3**(1), 1-5 (2009).

121. Sowemino, AA. Pendota, C. Okoh, B. Omotosho, T. Idika, N. Adekunle, AA. Afolayan, AJ. *Chemical composition, antimicrobial activity, proximate analysis and mineral contents of the seed of Detariumsenegalense JF. Gmelin. African Journal of Biotechnology;* **10** (48); 9875-9879 (2011).

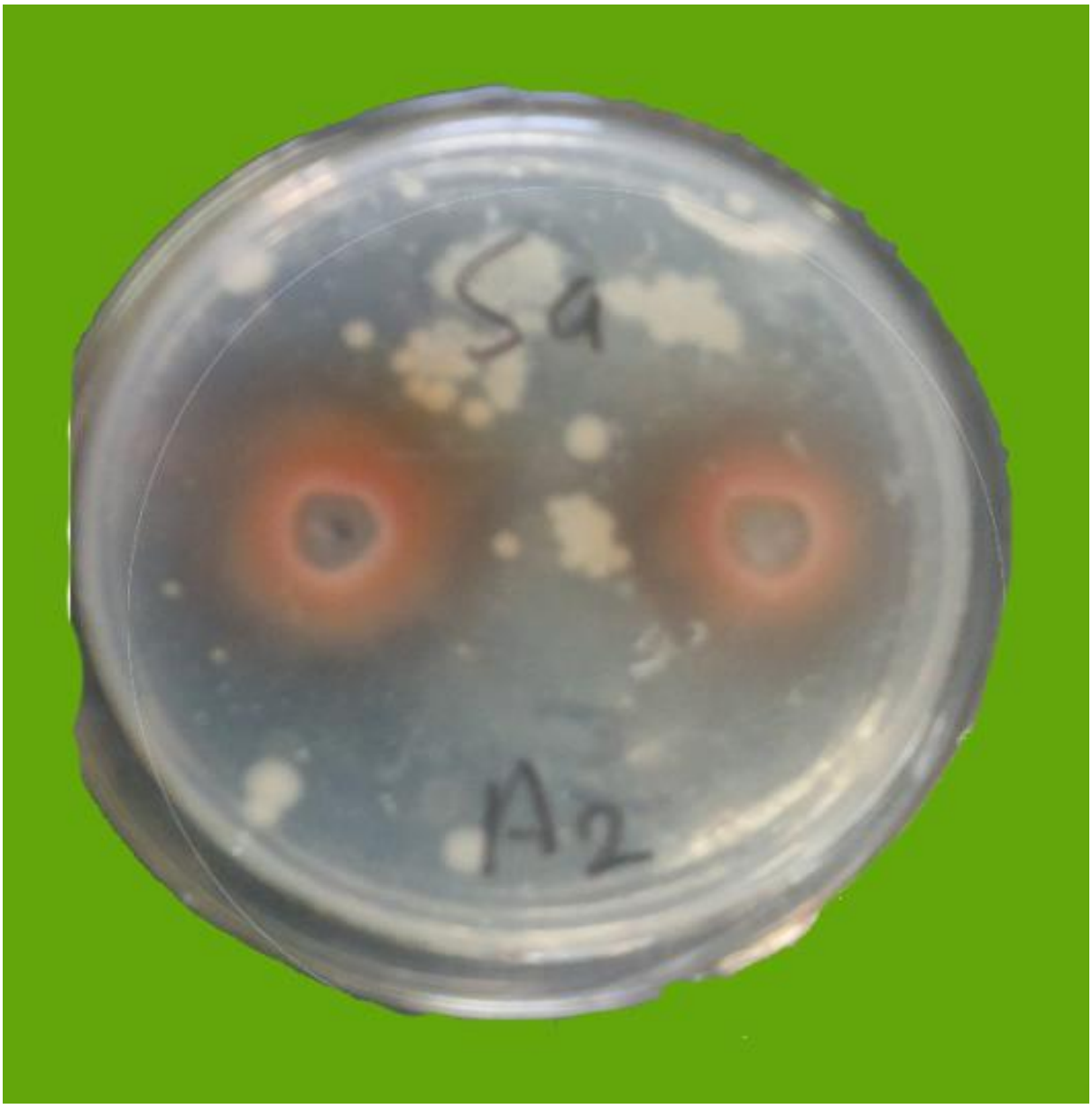


Plate 1: Inhibition zone of fraction 2 against *Staphylococus aureus*

Plate 2: Inhibition zone of fraction 2 against: *Escherichia coli*

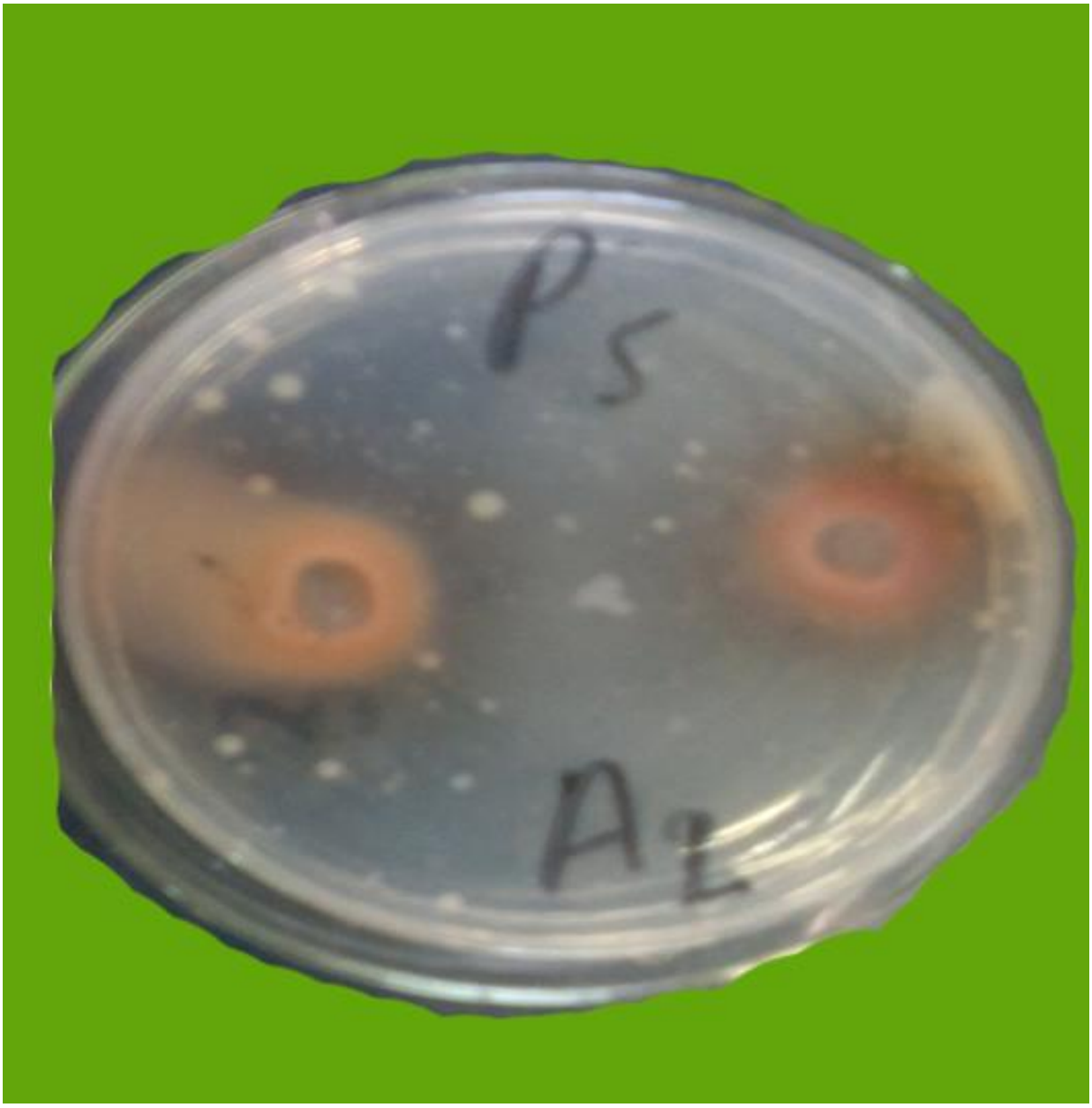


Plate 3: Inhibition zone of fraction 2 against *Pseudomonas aeruginosa*

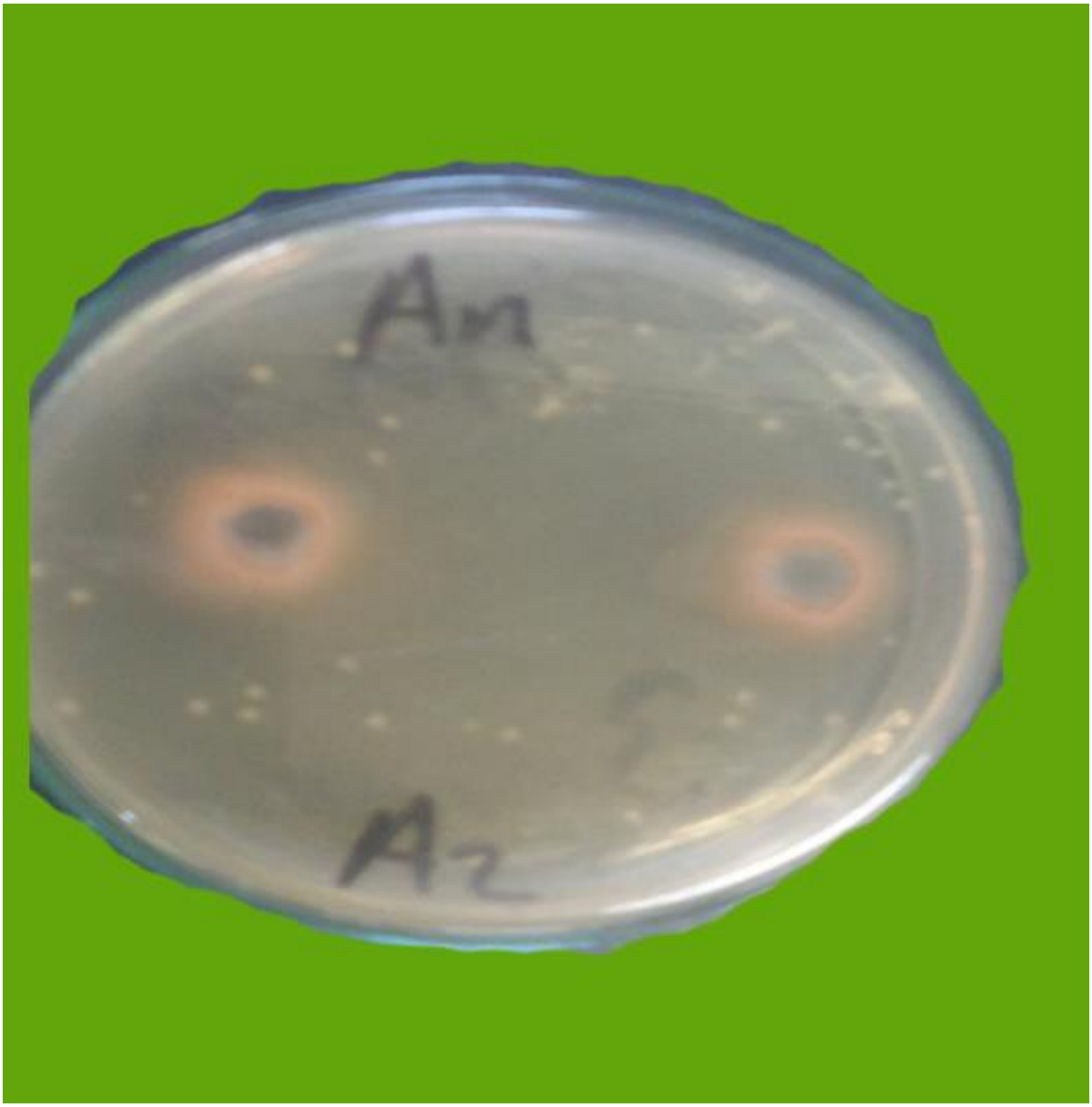


Plate 4: Inhibition zone of fraction 2 against *Aspergillus niger*

Plate 5: Inhibition zone of fraction 2 against *canadida albicans*

Plate 6: Inhibition zone of fraction 3 against *Staphylococus aureus*

Plate 7: Inhibition zone of fraction 3against *Bacillus subtilis*

Plate 8: Inhibition zone of fraction 3 against *canadida albicans*