بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Study of the Major Flavonoids from *Guiera senegalensis* by UV-Visible Spectroscopy

دراسة الفلافونيد الرئيس فى نبات الغبيش بمطيافية الاشعة فوق البنفسجية ـ المرئية

A Thesis Submitted in Partial Fulfillment of the Requirements of the M.Sc. Degree in Chemistry

By:

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(B.Sc. (Hons) Chemistry 2007)

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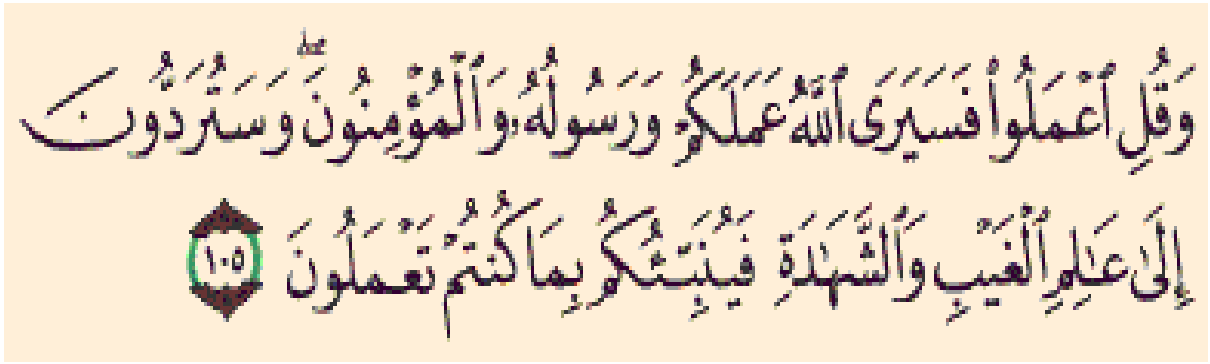
August, 2016



(التوبة-105)

Dedication

To

My parents

My husband

My brothers

And sisters.

Acknowledgement

I would like to Thanks Allah Almighty for giving me strength and health to complete this work.

I wish to extend my utmost gratitude to my supervisor, Professor Mohamed Abd Elkarim Mohamed for his tremendous help, advice and support during the period of this study.

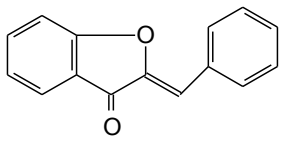
I would like to thank the laboratory staffs Sudan university of sciences and technology for their kind help and infinite support.

I would also like to thank my family and friends for their patience and support.

Abstract

phytochemical screening of the ethanolic extract of Guiera *senegalensis* leaves revealed the the presence of flavonoids, alkaloids, glycosides, tannins, saponnins and terpines.

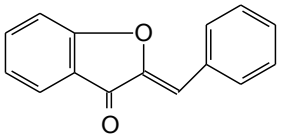
the ethanolic extract of Guiera *senegalensis* leaves was purified by a silica gel column eluted with chloroform:methanol(3:2). .The characterization of isolate was accomplished by UV studies; the UV data suggested a pattern characteristic of aurones. The following tentative structure was proposed:



المستخلص

في هذه الدراسة اخضع مستخلص اوراق نبات الغبيش لمسح فيتوكيميائى اوضح وجود الفلافونيدات، التنينات،القلويدات،الصابونينات،التربينات والجلايكوسيدات.وقد تمت تنقية المستخلص بكروماتوغرافيا العمود.

وتم تحديد التركيب المبدئى عن طريق طيف الاشعه فوق البنفسجية والمرئية واظهرت هذة المطيافيات ان هذا الفولافنويد ينتمى لطائفة الاوروانات وقد اقترح التركيب المبدئى التالى :



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1-Introduction

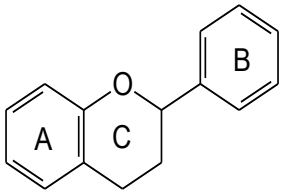
1.1-General approach

Flavonoids are an important class of natural products. They are generally known to be present in plants and plant based products. These include various fruits, vegetables, herbs and beverages such as tea and red wine. Flavonoids are associated with a broad spectrum of health promoting effects. They are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function[1].

Flavonoids are a group of natural compounds with variable phenolic structures and are found in plants. In 1930 a new substance was isolated from oranges. At that time it was believed to be a member of a new class of vitamins and was designated as vitamin P. Later on it became clear that this substance was a flavonoid (rutin) and till now more than4000 varieties of flavonoids have been identified. Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and B as shown in Figure 1) linked via a heterocyclic pyrane ring (C). They can be divided into a variety of classes such as flavones (e.g., apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin),

flavanones (e.g., flavanone, hesperetin, and naringenin), and others.. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings [2] .

Flavonoids occur as aglycones, glycosides, and methylated derivatives. The basic flavonoid structure is aglycone (1). Six-member ring condensed with the benzene ring is either a α-pyrone (flavonols and flavanones) or its dihydroderivative (flavonols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position).



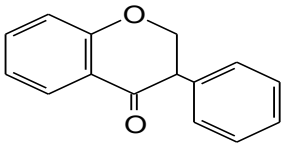
(1)

Flavonols differ from flavanones by hydroxyl group at the 3-position and a C_2–C_3 double bond [3] .Flavonoids are often hydroxylated in positions 3, 5, 7, 2`, 3`, 4`, and 5 methyl ethers and acetyl esters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose [4] .

Flavonoids are the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesising plant cells. They are a major coloring component of flowering plants.

Flavonoids are an integral part of human and animal diet. Some food sources containing different classes of flavonoids. Being phytochemicals, flavonoids cannot be synthesized by humans and animals [5] .Thus flavonoids found in animals are of plant origin rather than being biosynthesized *in situ*. Flavonols are the most abundant flavonoids in foods. Flavonoids in food are generally responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes [6].Flavonoids found in the highest amounts in the human diet include the soy isoflavones, flavonols, and the flavones. Although most fruits and some legumes contain catechins, the levels vary from 4.5 to 610mg/kg[7] .Preparation and processing of food may decrease flavonoid levels depending on the methods used. For example, in a recent study, orange juices were found to contain 81–200mg/L soluble flavanones, while the content in the cloud was 206–644mg/L which suggest that the flavanones are concentrated in the cloud during processing and storage[8] . Accurate estimation of the average dietary intake of flavonoids is difficult, because of the wide varieties of available flavonoids and the extensive distribution in various plants and also the diverse consumption in humans [9] .

The term “flavonoid” is generally used to describe a broad collection of natural products that include a C_6-C_3-C_6 carbon framework, or more specifically a phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans) **1**, isoflavonoids (3-benzopyrans) **2**, and the neoflavonoids (4-benzopyrans) **3**. These groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related.



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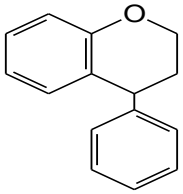
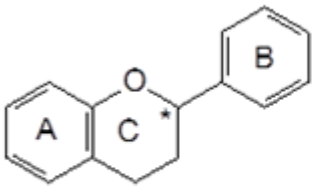


(3)

1.2- 2-Phenylbenzopyrans

Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into the following groups: (flavans)4,(flavanones)5,(flavones)6,(flavonols)7,(dihydroflavonols)8, (flavan-3-ols)9,(flavan-4-ols)10,(flavan-3,4-diols)11[10-17].



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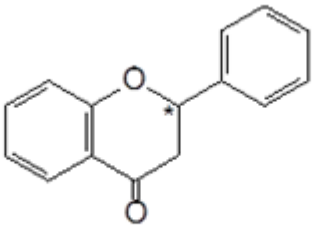

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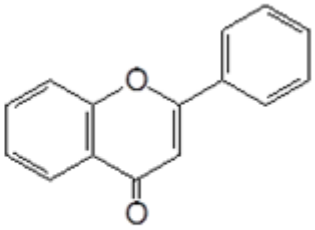

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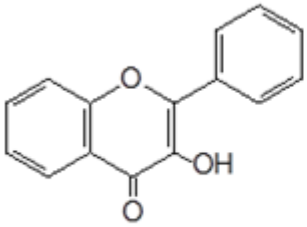

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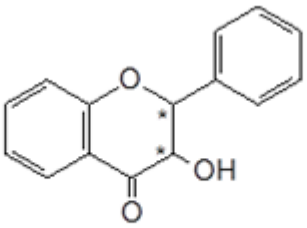

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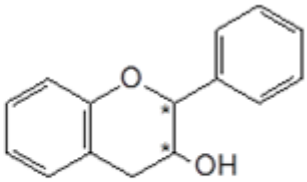

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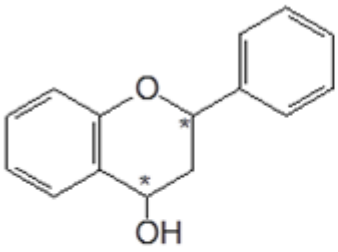

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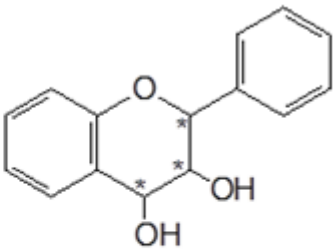

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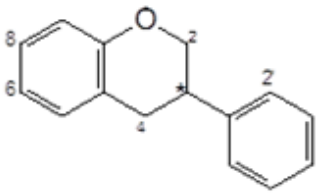
* stereocenters

The isoflavonoids are a distinctive subclass of the flavonoids. These compounds possess a 3-phenylchroman skeleton that is biogenetically derived by 1,2-aryl migration in a 2-phenylchroman precursor. Despite their limited distribution in

the plant kingdom, isoflavonoids are remarkably diverse as far as structural variations are concerned. This arises not only from the number and complexity of substituents on the basic 3 phenylchroman system, but also from the different oxidation levels and presence of additional heterocyclic rings. Isoflavonoids are subdivided into the following groups: (isoflavans)12,(isoflavones)13,(isoflavanones)14,(isoflanenes)15,(isoflavanols)16,(rotenoids)17,(coumestanes)18,(3arylcoumarins)19,(coumaronochromenes)20,(coumaronochromones)21,(pterocarpans)22[10-17].



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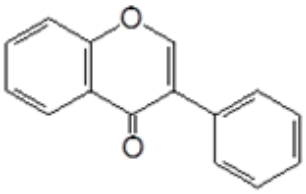

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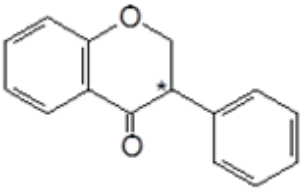

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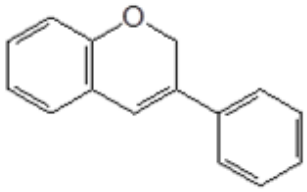

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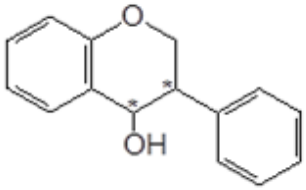

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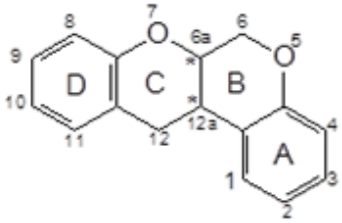

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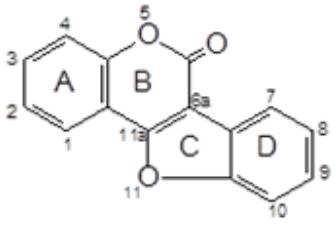

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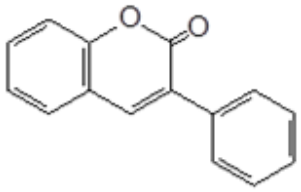

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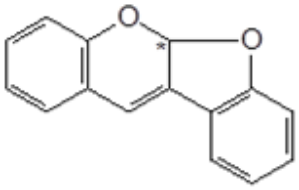

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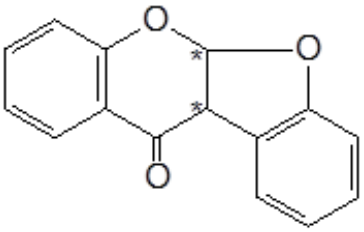

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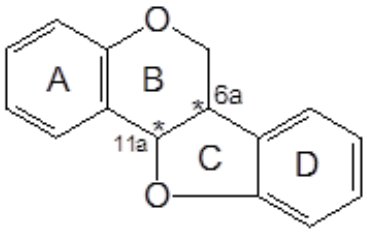

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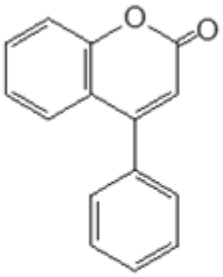
*stereocenters

Isoflavones constitute the largest group of natural isoflavonoids, with some 364 known aglycones reported.This number of new additions over only five years is remarkable,it illustrates the structural diversity existing in nature, and leads to speculation on how long it will be before every natural structural permutation has been demonstrated[18].

1.3- Neoflavonoids

The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2*H*-1- benzopyran-2-ones)23,(3,4-dihydro-4-arylcoumarins)24, and(neoflavenes)25[10-17].



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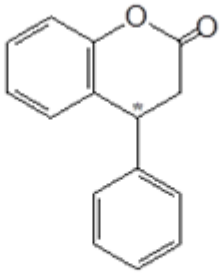

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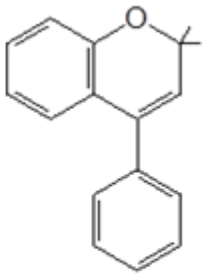

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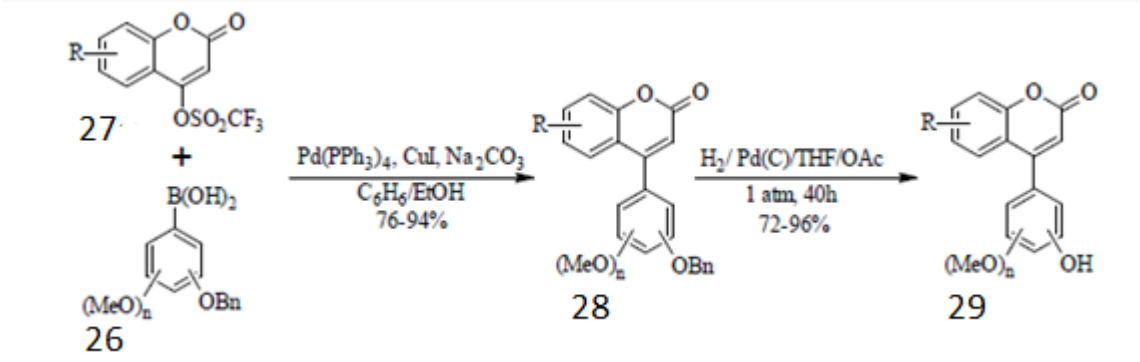
*stereocenters

The neoflavones (4-arylcoumarins) constitute the largest subclass of neoflavonoids. Traditional procedures for the preparation of neoflavones include the Pechmann or Perkin reactions and the Kostanecki acylation of 2-hydroxybenzophenones followed by base-catalyzed ring closure . Other methods that have been developed include the Wittig reaction of 2-benzophenones .and metal-catalyzed cross-coupling reactions such as the Negishi-type, Stille-type and Suzuki-type reactions [19-24]. More recently, 4-arylcoumarins have been synthesized by direct arylation by the palladium-catalyzed oxidative Heck coupling of arylboronic acids to coumarins [25,26].

The synthesis of neoflavones by the Suzuki reaction was first reported by Donnelly and co-workers in 1996 [27]. Their approach involved coupling of 4-trifluoromethanesulfonyloxycoumarins with arylboronic acids in the presence of $Pd(PPh_3)_4$ and copper(1) iodide as a co-catalyst. The same research group later extended the procedure to the synthesis of hydroxylated

neoflavones from benzyloxyboronic acids **26** and 4-trifluoromethanesulfonyloxycoumarins **27** as shown below[28]. The benzyl protecting group was removed in the late stage by hydrogenation of **28** in the presence of Pd(C) in THF and AcOH to give **29**.

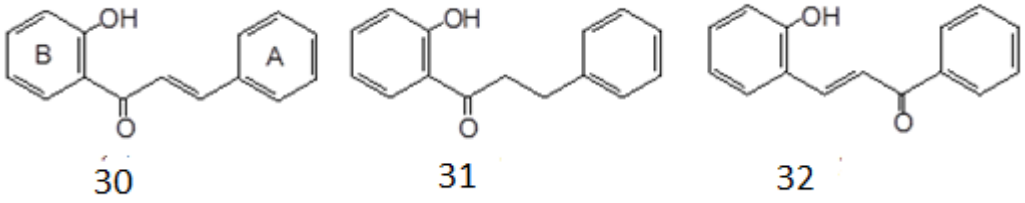


Subsequently, similar conditions have been applied in several instances in the syntheses of a series of neoflavones for the investigation of their biological activities [19,21]. For instance, neoflavones with a substitution

pattern similar to the combretastatins have been prepared and evaluated for pharmacological activities that include, amongst others, cytotoxicity against CEM leukemia and HBL 100 epithelium cell lines [19,21]and antiprotozoal activity against *Plasmodium falciparum* and *Leishmania donovani* .

Natural products such as chalcones and aurones also contain a C_6-C_3-C_6 backbone and are considered to be minor flavonoids. These group of compounds include the 2′-hydroxychalcones(30), 2′-OH-dihydro- chalcones(31), 2′-OH-

retro-chalcone(32), aurones (2- benzylidenecoumaranone)(33), and auronols(34) [10-17].

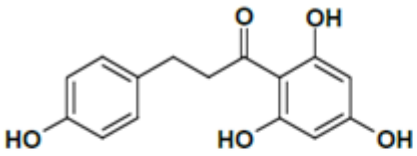


30 31 32

33 34

*stereocenters

Chalcones are a subclass of flavonoids. They are characterized by the absence of "ring C" of the basic flavonoid skeleton structure. Hence, they can also be referred to as open chain flavonoids. Major examples of chalcones include phloridzin(36), arbutin(38), phloretin(35) and chalconaringenin(37). Chalcones occur in significant amounts in tomatoes, pears, strawberries, bearberries and certain wheat products. Chalcones and their derivatives have gained considerable attention because of numerous nutritional and biological benefits. The structures of some key chalcones are displayed below[1]:



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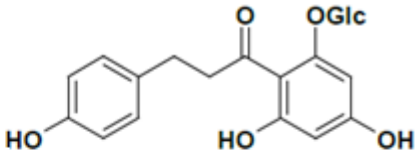

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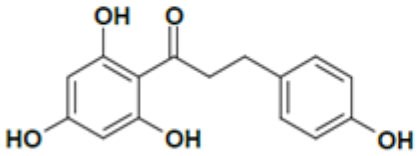

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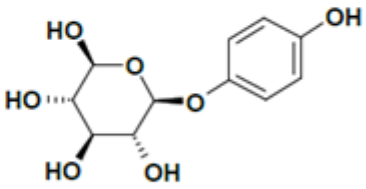
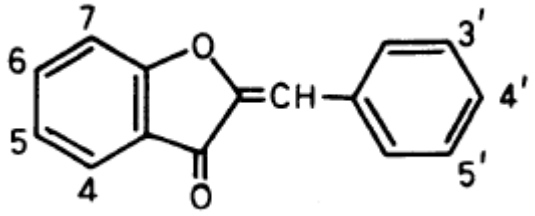


38

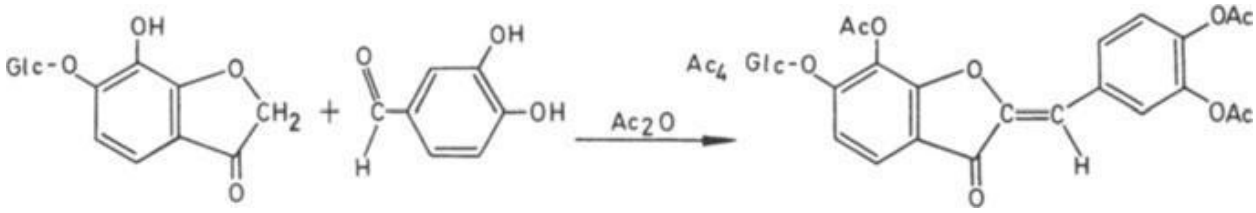
Aurones are hydroxylated 2-benzylidenecoumaranones. The 'normal' numbering system app1ies to this group of compounds: positions on the A ring are identified by unprimed numbers and the B ring positions by primed numbers. Note that in aurones position 4 corresponds bio- synthetically to position 5 of other heterocyc1ic ftavonoids .



Aurone

All practical methods for the preparation of aurones are based on the condensation of coumaran-3-ones with aromatic aldehydes . The use of alkali or hydrochloric acid as condensing agent fails with 4,6-dihydroxycoumaran- 3-ones

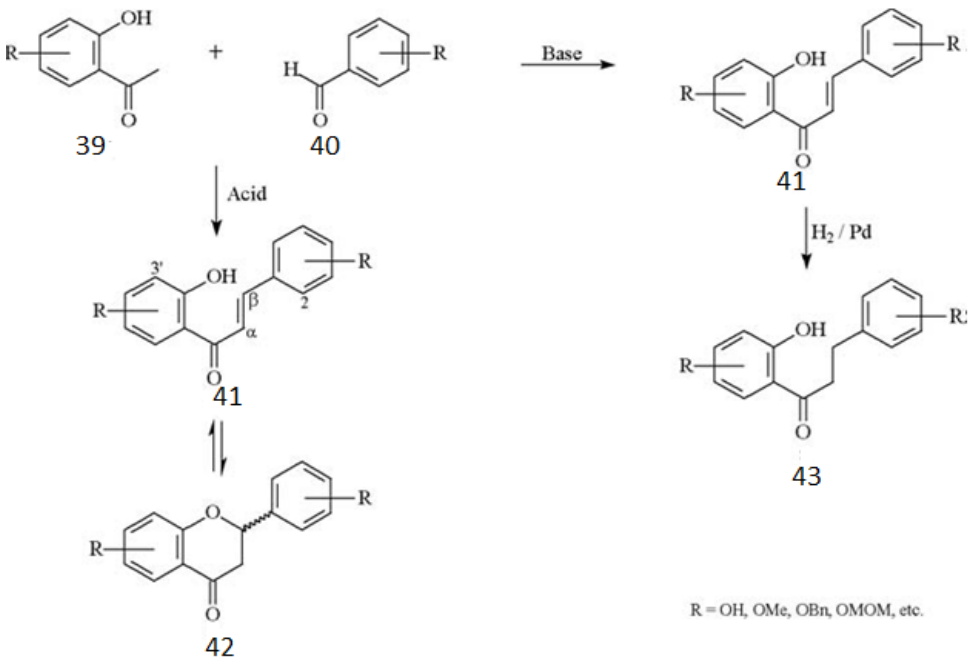
(phloroglucinol type) and acids can of course not be used when the components have sugars attached to them. A method of general applicability is the condensation in boiling acetic anhydride without the use of a catalyst . This also offers the advantage of giving the acetates directly which can be easily characterized and purified. Except for leptosin which was prepared by coupling the 6-hydroxy-3' ,4'-dibenzoyloxyaurone with acetobromoglucose, all the natural aurone glycosides have been synthesized by the condensation of the appropriately glucosylated components in acetic anhydride , as shown in the example of maritimein below. Rengasin, leptosin bractein and hispidol have also been synthesized by the acetic anhydride method[29-32].



Synthesis of maritimein heptaacetate

Chalcones and dihydrochalcones are considered to be the primary C_6-C_3-C_6 precursors and constitute important intermediates in the synthesis of flavonoids. Chalcones are readily accessible via two well-established routes comprising a base-catalyzed aldol condensation or acid-mediated aldolization of 2-hydroxyacetophenones **39** and benzaldehydes **40**. The base-

catalyzed aldol condensation is usually the preferred route toward chalcone **41** formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of corresponding racemic flavanones **42** . Dihydrochalcones **43** are generally obtained via reduction (H2/Pd) of the preceding chalcones .

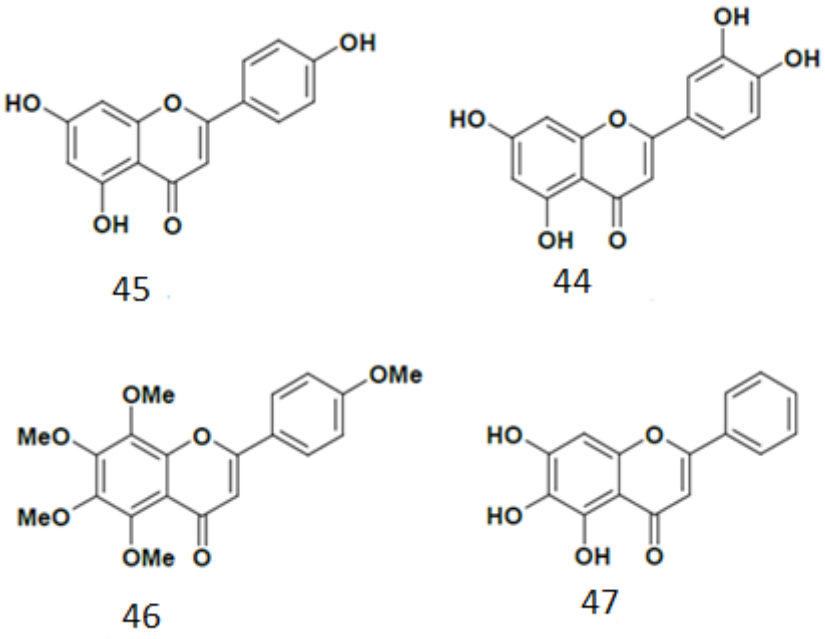


Synthesis of chalcones and dihydrochalcones

1.4. Flavone

Flavones are one of the important subgroups of flavonoids. Flavones are widely present in leaves, flowers and fruits as glucosides. Celery, parsley, red peppers, chamomile, mint and ginkgo biloba are among the major sources of flavones. Luteolin(44), apigenin(45) and tangeritin(46) belongs to this sub-class of flavonoids. The peels of citrus fruits are rich in the

polymethoxylated flavones tageretin, nobiletin and sinensetin. Flavones display various biological functions. The structures of some key flavones:apigenin(45),luteolin(44),tangetrtin(46),baicalein(47) are displayed below:



Structures of some commonly studied flavones

1. 5-Methods of isolation of flavonoids

i)Column chromatography

As in paper chromatography, the solvents most favored for use with cellulose columns are of the aqueous alcohol and acid types. Some typical examples include the separation of flavones from *Spartium junceum* flower extracts by elution with water-saturated butanol[33] .The isolation of flavonols and their glycosides was achieved by using the solvents : ethyl

acetate:methanol:water (50: 3 : 10) and ethyl acetate-methanol-acetone-water (50 : 3 : I : 10) [34], and the partial separation of complex mixtures of flavones C- glycosides from *Vitex lucens* extracts was accomplished using 5% acetic acid and BAW (n-butanol-acetic acid-water, 4 : I : 5). In this latter example, rolled paper columns were also used and it was claimed that in general, cellulose columns were preferable to polyamide for the separation of these compounds [35]. C-Glycosides of the lucenin and vicenin types have recently been isolated from extracts of the green alga *Nitella hookeri,* using cellulose column chromatography with water as eluent. Particularly effective use was made of cellulose chromatography in the separation of heartwood flavonoids of *Acacia* species. In this application the ether extractives of *A. kempeana* (later corrected to *A. cuthbertsoni)* heartwood were applied to a cellulose column in 2% aqueous acetic acid. Elution with this solvent yielded constituent flavonoids in the following order: flavan-3,4-diols, dihydroflavonols, flavanones, 3-methoxyflavones, and flavonols and chalcones. These flavonoids consisted of three basic substitution types: 7,8,4' -trihydroxy-, 7,8,3' ,4' -tetrahydroxy-, and 7 ,8-dimethoxy-3' ,4' -dihydroxy-, and some separation of these types, within each flavonoid group, was observed. For example 7,3',4'-trihydroxy-3,8-dimethoxyflavone (eluted with 2% acetic acid) preceded 3,7,3' ,4'-tetrahydroxy-8-

methoxyflavone which in turn preceded 3,7,8,3'4'-pentahydroxyflavone (both eluted with aqueous methanol)[36].

ii) Separations on silica gel

Highly methylated or acetylated flavones and flavonols require relatively non-polar solvents for TLC on silica gel (SiO_2). Thus, flavones such as hymenoxin (5,7-di-OH-6,8,3',4'-tetra-OMe), scaposin (5,7,3'-tri-OH-6,8,4',5'-tetra-OMe), and demethoxysudachitin (5,7,4'-tri-OH-6,8,-di-OMe) have been chromatographed using chlorofonn-methanol (15 : 1) [37], and digicitrin (5,3'-di-OH-3,6,7,4',5'-penta-OMe) and a number of related compounds were separated using benzene-ethyl acetate (3: 1) [53] . Flavonol polyacetates and polymethyl ethers have been successfully chromatographed by using benzene-acetone (9 : 1) and toluene: acetone (19: 1). Other highly methoxylated flavones and flavonols have been chromatographed with benzene-methanol-n-butyl acetate (20 : 4 : 1) [38], hexane-acetone-n-butano1 (8 : 1 : 1 and 17 : 2 : 1) [54], benzene-acetone (3 : 1), (9 : 1), (49 : 1), (92.8 : 7.2) ,and chloroform-ethyl acetate (1 : 1) . It seems that the degree of solvent polarity depends 1argely upon the extent of methylation in the flavonoids. More polar flavones and flavonols require more polar solvents. Thus apigenin, luteolin, galangin, kaempferol, quercetin, myricetin, isorhamnetin (3,5,7,4'-tetra-OH- 3'-OMe), datiscetin (3,5,7,2'-tetrOH) and morin (3,5,7,2' ,4' –penta-OH) separate weIl in toluene : chlorofonn- acetone (8 : 5 : 7) [57], *Rf* values being 0.43,

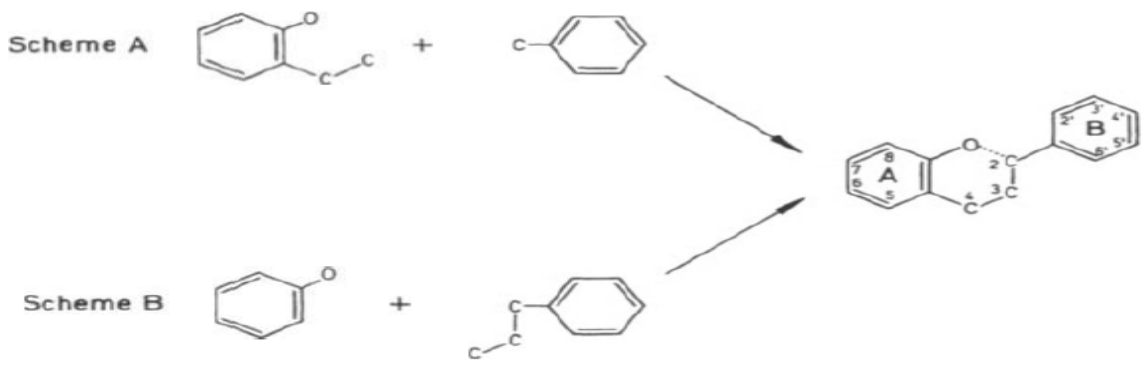
0.28,0.62,0.39,0.27,0.13,0.26,0.36 and 0.06 respectively. Similar flavonoid mixtures were separated using benzene-pyridine-formic acid (36: 9 : 5). We have found chloroform-methanol (96 : 4) useful for distinguishing flavones such as apigenin, chrysoeriol and luteolin, and Hillis and have had success with chloroform-acetie acid (9: 1) and toluene-ethyl formate-formic acid (5 : 4 : 1) for the chromatography of C-methylflavones such as sideroxylin and eucalyptin (the 7- and 7,4'-methyl ethers of 6,8-di-C-methylapigenin respectively). Other solvents, such as benzene-dioxan-acetic acid (90: 25 : 4) benzene-pyridine- ammonia (80 : 20 : 1) and acetone-benzene (1 : 3) have also been used with flavones and flavonols[39,40].

TLC on cellulose layers has to some extent replaced paper chromatography in analytical work, since the high surface area, fine-grained cellulose thin-layers offer the advantages of greater speed [41] and better resolution . Flavone and flavonol aglycones were found [42]to be conveniently separated on cellulose with chloroform-acetic acid-water (10 : 9 : 1) , whereas the glycosides were better resolved with butano1-acetic acid-water (4: 1 : 5). It was found that modest separations could be achieved with 2% formic acid, isopropyl alcohol-ammonium hydroxide-water (8 : 1 : 1), and 10% acetic acid. Two-dimensional cellulose TLC has been carried out with solvent pairs of the type commonly used in paper chromatography (e.g. water, or 5% methanol in water, and n-butanol-acetic acid-

water, 4 : 1 : 5). Similar solvents were also used for the separation of flavone C-glycosides on microcrystalline cellulose . However, twelve solvents developed for use in paper chromatography were tested for the separation of *Medicago* flower petal flavonoids, and only three were found useful for TLC, namely, n-butanol-2N HCl (l : 1), n-amyl alcohol-acetic acid-water (2 : 1 : 1) and ethyl acetate-formic acid-water (8 : 2 : 3)[43].

1.6-Synthesis of flavones

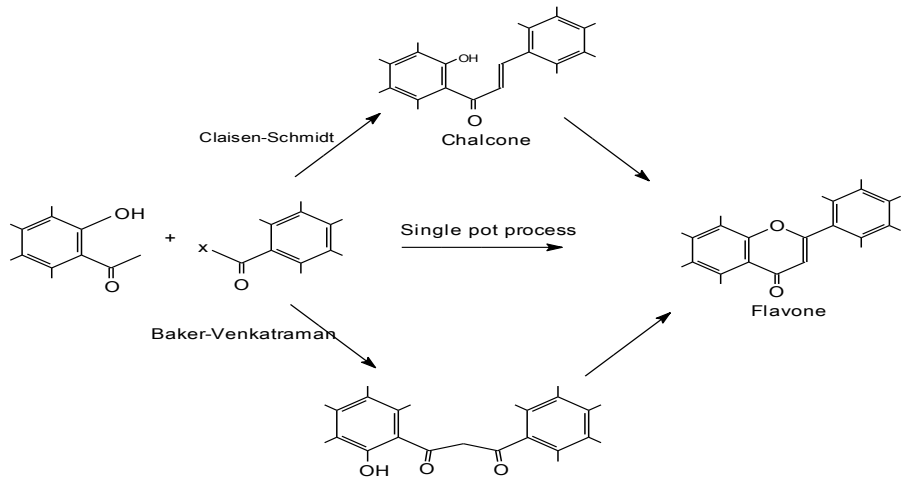
Theoretically, there are at least four ways of forming the C_6 -C_3 -C_6 flavonoid skeleton from simple starting materials, but only two have achieved importance for the laboratory synthesis: (i) condensation of a C_6-C_2 unit (2-hydroxyacetophenone) with a C_6-C_l unit (aromatic aldehyde) according to scheme A; and (ü) Acylation of phenols (C_6 unit) with a cinnamic acid derivative or its equivalent (C_6-C_3 unit) according to scheme B , which also corresponds to the biosynthetic pathway .



Synthesis of flavones

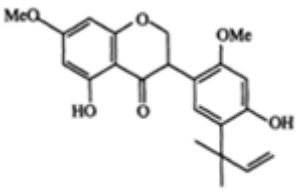
In addition, many flavonoids can be prepared by modifying existing C_{I5} structures by oxidation, reduction, isomerization, partial O- and C-alkylation, dealkylation, selective glycosylation or partial hydrolysis.

Traditionally,flavones have been prepared by Baker-Venkatraman rearrangement and Claisen-Schmidt condensation.which involves the conversion of 2-hydroxyacetophenones into benzoyl esters, followed by rearrangement in base to 1,3-diphenylpropane-1,3-diones which upon cyclization under acidic conditions furnishes flavones.On the other hand hydroxychalcone synthesized from 2-hydroxyacetophenone and benzaldehyde under Claisen-Schmidt conditions can undergo oxidative cyclization to furnish flavone ring.

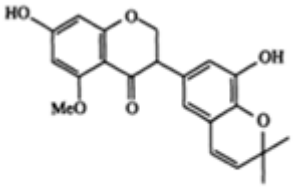


Synthesis of flavones via two routes

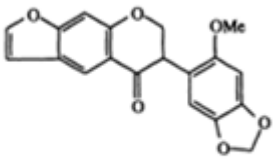
Isoflavanones are considerably rarer than isoflavones, but several new structures and isolations have been reported . Since isoflavanones are biosynthetic intermediates between isoflavones and pterocarpans or isoflavan phytoalexins, they often accumulate when leguminous plants are challenged by fungi or abiotic agents. Treatment of *Phaseolus lunatus* seedlings with aqueous copper(II) chloride resulted in the isolation of 25 isoflavonoids, including some isoflavanones[43].



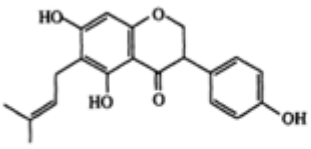
Echinoisosophoranone



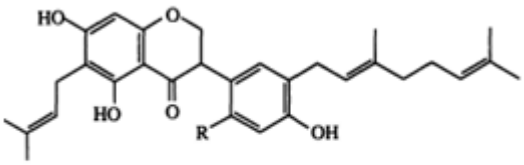
Glycyrrhisoflavanone



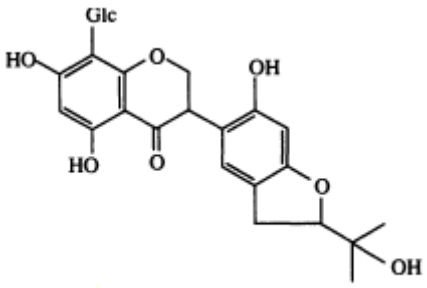
Neotenone



Dihydrowighteone



R = H, Sophoraisoflavanone C
R = OH, Sophoraisoflavanone D



Dalpanin

Structurally interesting isoflavanones reported for the first time include echinoisosophoranone from the root of *Echinosophora koreensis,* which contains a 1,1-dimethylallyl substituent, and the two compounds, sophoraisoflavanone C and sophoraisoflavanone D from roots of *Sophora chrysophylla*. These latter two isoflavanones contain a C- geranyl substituent as weIl as a C-3,3-dimethylallyl group. *Echinosophora koreensis* also yielded a further example of a 3-hydroxyisoflavanone, echinoisoflavanone , which, with the previously known examples bolusanthin (from *Bolusanthus speciosus)* and secondifloran (from *Sophora secondiflora),* brings the total in this group to three compounds. However, spectral similarities between echinoisoflavanone and sophoronol, previously isolated from *Sophora tomentosa* and formulated as a 3 hydroxyflavanone, have resulted in sophoronol now being reformulated as a fourth 3-hydroxyisoflavanone. In the coupled ^{13}C NMR spectrum for sophoronol, C-2 and C-3 appeared as a triplet and singlet

respectively, clearly inconsistent with the 3-hydroxyflavanone formulation.

Most isoflavanones are isolated in racemic form, with optically active examples being the exception, since racemization can easily be brought about during extraction and purification procedures. The use of milder techniques is thus probably a feature that has resulted in the characterization of some more optically active examples. Echinoisosophoranone[44,45], glycyrrhisoflavanone from *Glycyrrhiza* species [46], dihydrowighteone and the known dalbergiodin (5,7,2' ,4'-tetrahydroxyisoflavanone) from *Vigna angularis* [47], 7,2' ,3'-trihydroxy-4'-methoxyisoflavanone from *Dalbergia odorifera* and neotenone from *Neorautanenia mitis* have all been isolated in optically active form. Since the sign of rotation is not indicative of the chirality at C-3, CD spectra are required to assign absolute configurations. This has now been recorded in the cases of 7,2',3' -trihydroxy-4'-methoxyisoflavanone and glycyrrhisoflavanone, which have both been assigned the 3S configuration (negative Cotton effect at about 330-340 nm). An X-ray crystallographic examination of the optically active neotenone has confirmed the structure, and shown the B-ring to be twisted some 490^{o} from the mean plane of the rest of the molecule . Isoflavanone glycosides are extremely uncommon, and only one O-glycoside (2,3-dihydrononin or dihydroformononetin- 7-O-glucoside) and one C-glycoside

(dalpanin) were known. The heartwood of *Pterocarpus macrocarpus* has been shown to contain a new isoflavanone C-glycoside, dalbergioidin-6-C-glucoside [48].

Isoflavones may be smoothly reduced to isoflavanones using di-isobutylaluminium hydride , but, prior to the availability of this method, the catalytic hydrogenation of isoflavones featured as the normal synthetic approach to isoflavanones for many workers. However, since the required product may be reduced further , eventually to an isoflavan, such reductions have to be monitored closely. Catalytic transfer hydrogenation has been investigated as an alternative, more reliable, method . Several isoflavones were converted into isoflavanones in 50-60% yield if heated under reflux in methanol with palladium charcoal and ammonium formate as hydrogen donor [49] . These workers found that up to half of the starting material remained after 3-4 h, and no conditions seemed to drive the reaction further to completion. In contrast, up to 90% yields were obtained if the reaction was carried out at room temperature, over shorter reaction times. Small amounts of *trans* -isoflavan- 4-oIs were produced as by-products, but the isoflavanones themselves were stable to further reduction under the hydrogen transfer conditions [50].

1.7-Guiera *senegalensis*

Guiera senegalensis (family Combretaceae) has been used traditionally in Burkina Faso for therapeutic purposes [51]. The galls of *G. senegalensis* possess effective

antiacetylcholinesterase, antilipid peroxidation in rat brain homogenates and erythrocytes hemolysis inhibitory activities . The galls of *Guiera senegalensis* demonstrated pronounced antioxidant potential, showed high polyphenols, totals tannins and total flavonoids contents [52]. The flavonoids are not only present in plants as constitutive agents but have also accumulated in plant tissues in response to microbial attack [53]. Opportunistic fungal infections have increased over the past several years. Leaves, young shoots and galls of *G senegalensis* are used in Burkinabe folk medicine for their antibacterial and antifungal properties[51]. It has also been reported that crude methanolic extracts of *G senegalensis* exhibit antimicrobial properties on bacteria and fungi [54]. Plant-derived antimicrobial are always a source of novel therapeutics.

G. senegalensis

Aim of this study

This study was designed to conduct a preliminary phytochemical screening for the secondary metabolites of *G senegalensis* ; isolation of the major flavonoids via chromatographic techniques and then conducting UV studies on the isolate to propose a partial structure.

2-Materials and Methods

2.1. Materials

2.1.1 Plant material

Leaves of *Gueira senegalensis* were purchased from the local market.The plant was authenticated by direct comparison with a herbarium sample.

2.1.2- Instruments

The UV spectra were run on a Shimadzu UV – 2401PC UV-Visible Spectrophotometer .

2.2- Methods

2.2.1- Preperations of reagents for phytochemical screening.

Flavonoid test reagents

- Aluminium chloride solution

(1 g) of aluminum chloride was dissolved in 100 ml methanol

- Potassium hydroxide solution

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

-Ferric chloride solution

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

Alkaloid test reagents

Maeyer reagent

- **Mercuric chloride solution**: 1.36 g in 60 ml. distilled water.

- **Potassium iodide solution** : 5 g in 10 ml. distilled water

The two solutions were combined and then diluted with distilled water up to 100 ml.

Wagner reagent

(1.27 g) iodine and(2 g) of potassium iodide in (100 ml) distilled water.

2.2.2-Preparation of plant extract for phytochemical screening

(200 g) of powdered shade- dried plant material were macerated with 95% aqueous ethanol until exhaustion. This prepared extract(PE) was used for phytochemical screening.

2.2.2.1-- Phytochemical screening

The leaves of *Gueira senegalensis* were screened for major secondary constituents.

i)- Test for unsaturated sterols and for triterpenes

(10 ml)of the (PE) of was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chlorform solution was dehydrated over anhydrous sodium sulphite . (5 ml) portion of the solution was mixed with(0.5 ml) of acetic anhydride, followed by two drops of concentrated sulphuric acid.

ii)- Test for flavonoids

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

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaked and then few drops of concentrated hydrochloric acid were added.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

iii)- Test for alkaloids

(10 ml) of the (PE) were evaporated to dryness on water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into two portions:

To one portion a few drops of Mayer reagent were added., to the other portion few drops of Wagner reagent were added.

iv)- Test for tannins

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtered. The insoluble residue was stirred with n-hexane and (10 ml) of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled , filtered and the volume adjusted to 10 ml. with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution.

v) -Test for Saponins

(1 g) of dried powdered plant material was placed in a clean test tube. (10 ml) of distilled water were added and the tube was stoppered and vigorously shaked for about 30 seconds, and allowed to stand.

2.2.3- Extraction of flavonoids

(1 kg) of powdered shade-dried plant material was macerated with 95% ethanol (5L) for 48hr. at room temperature with occasional shaking and then filtered off . The extraction process

was repeated till exhaustion. Combined filtrates were concentrated under reduced pressure until all ethanol was removed yielding a crude product.

2.2.4-Isolation of flavonoids

The ethanolic extract was fractionated by a silica gel column eluted with: chloroform:methanol in increasing order of polarity i.e.(4:1;3:2 and 1:4;v:v). (10ml) fractions were collected.The ratio (3:2) was rich in flavonoids. Fractions (6-20) gave a pure component-compound I.

2.2.5- The UV spectrum of compound I in presence of NaOMe

Three drops of NaOMe were added to a solution of the flavonoid in methanol (2 ml) and the UV spectrum was immediately recorded.

2.2.6- The UV spectrum of compound I in presence of aluminium chloride

Six drops of the stock solution of aluminium chloride were added to a solution the flavonoid in methanol (2 ml) and UV spectrum was immediately recoreded.

2.2.7- The UV spectrum of compound I in presence of NaOAc

Exess coarsely powdered anhydrous NaOAc was added with shaking to a cuvette contaning (2-3 ml) of the solution of flavonoid in methanol and the UV spectrum was recorded after 2 minutes.

2.2.8- The UV spectrum of compound I in presence of NaOAc/H_3BO_3

Sufficient powdered anhydrous H_3BO_3 was added with shaking to a cuvette containing the solution (2.2.5.4) to give a saturated solution. The UV spectrum was recorded after 2 minutes

3-RESULTS AND DISCUSSION

Recently, flavonoids aroused considerable interest because of their potential beneficial effects on human health. They are claimed to possess antiviral, anti-allergic, anti-inflammatory, anti-tumer , anti-malarial and antioxidant activities.The biological properties of these interesting compounds promoted the present investigation and this study was designed to investigate the flavonoids of the medicinally important species *Gueira senegalensis*..

3.1-Phytochemical screening

Qualitative tests on the leaves of *Gueira senegalensis* revealed the presence of : alkaloids, glycosides, tannins, saponins and terpines.

3.2-Characterization of compound I

Compound I was isolated as yellow powder from the leaves of *G.snegalensis* by column chromatography. . In the UV (Fig.1) , compound I absorbs at λ_{max} (MeOH) 275, 380 nm.Such absorption is characteristic of aurones.

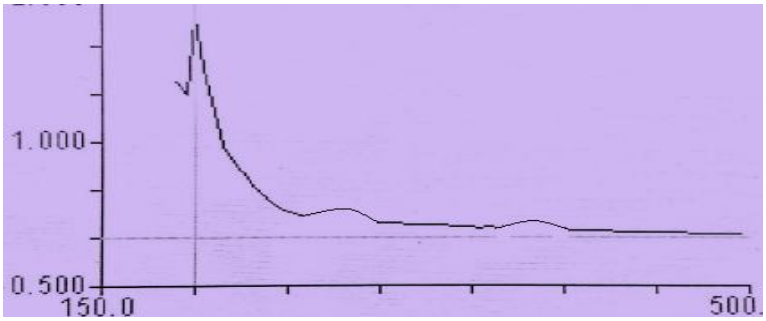


Fig.1 : UV spectrum of compound I

Further structural features accumulated by using UV shift reagents (sodium methoxide, sodium acetate, aluminum chloride and sodium acetate/boric acid).These UV shift reagents induce characteristic bathochromic shifts in accordance with specific location of hydroxyl groups in the flavonoid skeleton.Sodium methoxide (NaOMe) is strong base and ionizes to some extent all hydroxyl groups on the flavonoid nucleus. However, use has been made of the effect of sodium methoxide on the UV spectra of flavonoids for detection of free 3- and /or 4` - hydroxyl groups.

When the shift reagent NaOMe was added to a methanolic solution of compound I(Fig.2) no bathochromic shift was observed .This suggests absence of 3-,4`- OH functions as well as catechol systems .

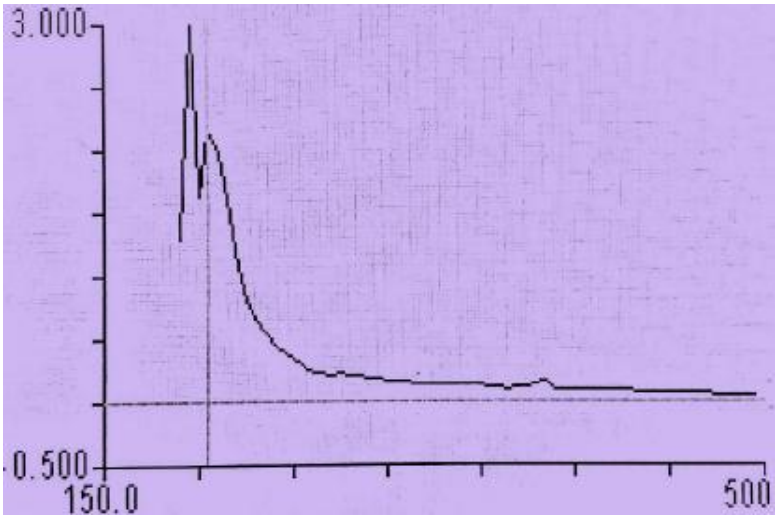


Fig.2: Sodium methoxide spectrum of compound I

The UV shift reagent sodium acetate is considered as a weak base. It ionizes only the more acidic hydroxyl groups in flavones and flavonols, i.e., the 3-,7- and 4'-hydroxy groups. The ionization of the 7-hydroxy group mainly affects band II. Particularly NaOAc is a useful diagnostic reagent for the specific detection of 7- hydroxyl groups . In presence of sodium acetate, flavones and flavonols containing free 7-hydroxy groups exhibit a diagnostic 5-20 nm bathochromic shift of band II. In presence of 6- and 8-oxygenation , the bathochromic shift is often smaller due to the reduced acidity of the 7- hydroxyl groups. Certain 3' , 4' –dioxygenated derivatives without the 7-OH group showed bathochromic shifts of 20 – 25 nm[11]. The sodium acetate spectrum of compound I did not reveal any bathochromic shift(Fig.3) indicating absence of free 7-OH function.

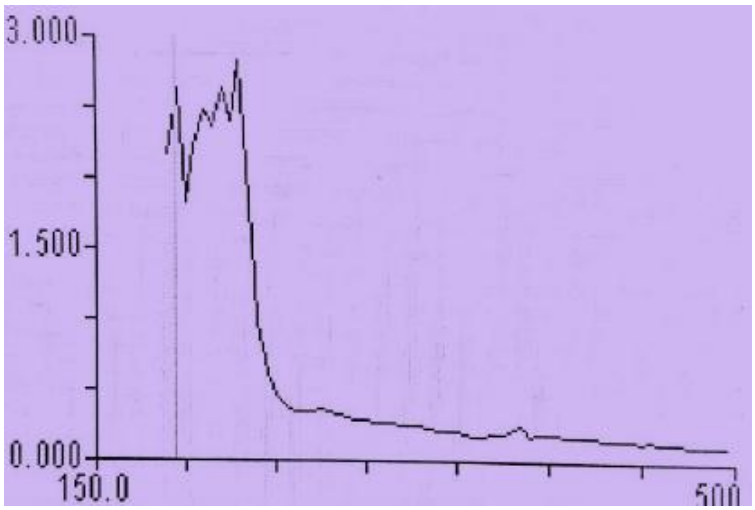
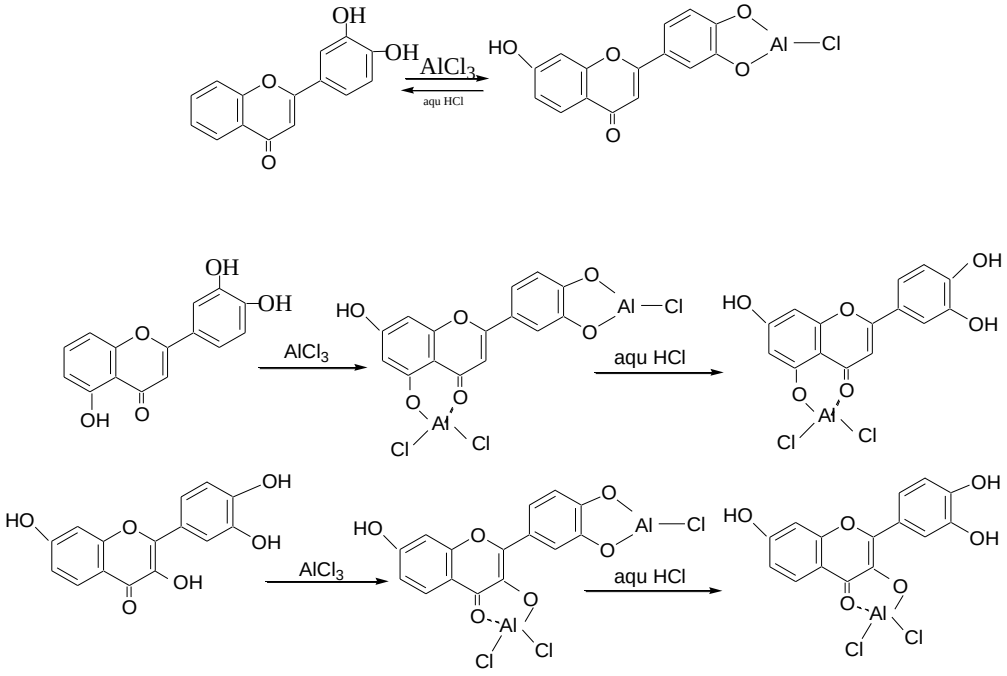


Fig.3 : Sodium acetate spectrum of compound I

Flavonoids with hydroxyl functions at C-3 or C-5 form acid stable complexes with aluminum chloride, whereas the aluminum chloride complexes with catechol systems are not stable in acidic media. Such acid- stable and acid – labile complexes are shown below:



Catechol systems in the B-ring of flavonoids can be detected by comparison the spectrum of the flavonoid in the presence of $AlCl_3$ with that obtained in $AlCl_3$/HCl. The presence of catechols the B –ring gives about a 20 nm hyposchromic shift on the addition of acid to $AlCl_3$ solution .The aluminium chloride spectrum of compound I(Fig.4)did not reveal a bathochromic .This indicates absence of a 3-,5-OH functions as well as catechol systems .No bathochromic shift was observed in the boric acid spectrum (Fig.5)indicating absence of catechol systems.

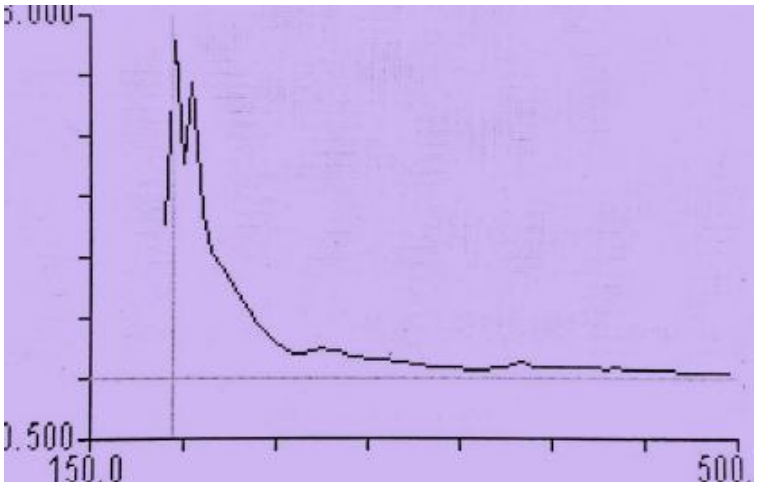


Fig. 4 : Aluminium chloride spectrum of compound I

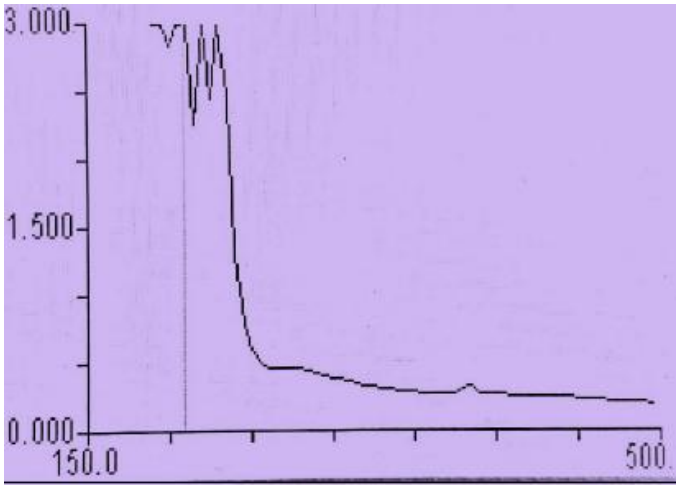
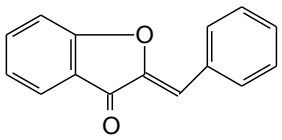


Fig. 5 : Boric acid spectrum of compound I

On the basis of the above spectral data it seems that compound I is an aurone which lacks hydroxylation at : 3-, 5-,7- and 4`-positions . Also the shift reagents aluminium chloride and boric acid indicated absence of catechol systems. Hence the following partial structure was proposed for compound I :



Aurone

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