بسم الله الرحمن الرحيم

Sudan University of Science and Technology

The Graduate College

Phytochemical Study of the Major *Flavonoid fromWaltheriaindica*

دراسة كيميائية نباتية للفلافونيد الرئيسي في نبات عرق النار

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

By:

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(B.Sc., Chemistry)

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بسم الله الرحمن الرحيم

الآية

قال تعالى:

﴿ وَقُلِ اعْمَلُواْ فَسَيَرَى اللّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ﴾

صدق الله العظيم

سورة التوبة الآية (105)

Dedication

Dedication to:

My parents, sisters and brothers.

ACKNOWLEDGEMENT

I would like to express my kind regards to my family and any friends for their help and encouragement.

Thanks are ext After thanking Allah Aimighty I would like to express my gratitude to my supervisor Prove .Mohammed Abdelkarim for his intmate supervisions, variable activate kind and found done the course of this work.

ended to chemistry department , Faculty of seience,Sudan University of Science and technology for all facilities.

Also I would like to thank miss Amira, Elneileen University for the spectral measurements.

ABSTRACT

In this study phenolic compounds were extracted from Waltheria indiea using 95% ethanol.

The crude extracts were subjected to paper chromatography using (7,3) methanol: water for irrigation. In this way compound (A) was isolated from Waltheria indicia.

The IR spectrum gave the expected functional groups for compound (A) . Uv studies illustrated the hydroxylation pattern of the isolated phytochemicals.

مستخلص الدراسة

استخلصت المركبات الفينولية في عرق النار بواسطة 95% ايثانول وعن طريق كروماتوغرافيا الورق تم فصل المركب(A)مننبات عرق النار بأستخدام الميثانول والماءبنسبة (7:3) كمذيب.

اوضح طيف الاشعة تحت الحمراء وجود الزمر الوظيفية المتوقعة . اما طيف الاشعة فوق البنفسجية فقد اوضحت نمط الهيدروكسيل في المركب (A).

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1-Introduction

1.1-General approach

Flavonoids are plant secondary metabolites with a C_6- C_3- C_6 skeleton and can be divided into three main classes, *i.e.*, flavonoids, isoflavonoids and neoflavonoids (4-arylcoumarins). Chalcones, the biogenetic precursor to flavonoids, are often classified as flavonoids. Different oxidation states and different substituents contribute to the diversity of flavonoid structures. Flavonoids play an important role in plant physiology and are of interest to humans as a result of biological activities such as antioxidant, anticancer and estrogenic activity of individual flavonoid derivatives.

As a result of the biological activity of flavonoids, there is an interest in the development of synthetic procedures that can conveniently give access to these molecules and their derivatives. One of the methods that has recently been employed successfully in the synthesis of flavonoids is the Suzuki-Miyaura reaction. The Suzuki-Miyaura reaction normally involves insertion of palladium into a sp2-hybridized C-X bond and consequently the major application of this reaction is in the construction of the flavonoid nucleus of chalcones, flavones, isoflavones and neoflavones rather than in the synthesis of their reduced derivatives. Unlike other methods that have been employed in the synthesis of flavonoids, the Suzuki-

Miyaura reaction often employs mild conditions that are compatible with a variety of functional groups. This enables the synthesis of flavonoids of natural origin and derivatives from precursors bearing sensitive substituents[1-4]. Moreover, the method readily offers access to a variety of flavonoids for biological activity studies by using different organoboron starting materials in the final stages of the synthesis[5-10]. The method is amenable to large scale synthesis due to the stability and commercial availability of a wide range of boronic acids/esters, and the ease of working up the reaction mixture[6,11].

Flavonoids, or bioflavonoids, are a ubiquitous group of polyphenolic substances whichare present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers. A great number of plant medicines contain flavonoids, which have been reported by many authors ashaving antibacterial, anti-inflammatory,antiallergic,antimutagenic,antiviral,antineoplastic, anti-thrombotic, and vasodilatory actions. The structural components common to these moleculesinclude two benzene rings on either side of a 3-carbon ring .Multiplecombinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids: flavanols,flavanones,flavones,flavan-3ols(catiechins),anthocya-nines and isoflavones. Flavonoids have been shown in a number of studies tobe potent antioxidants, capable of scavenging

hydroxyl radicals, superoxide anions, and lipidperoxy radicals.[12]

1.2- Classification of flavonoids

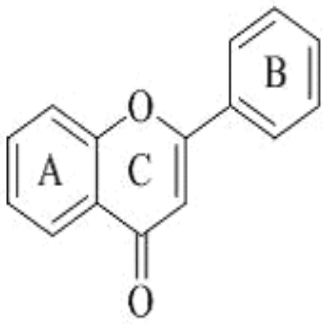
Flavonoids are polyphenols of plant origin that are among the most important compounds in human diet due to their widespread distribution in foods and beverages. They can occur both in the free form (aglycones) and as glycosides, and differ in their substituents (type, number and position) and in their unsaturation. The most common classes are the flavones, flavonols,flavanones, catechins, isoflavones and anthocyanidins, which account for around 80 % of flavonoids.All flavonoids share a basic C_6-C_3-C_6 phenyl-benzopyran backbone. The position of the phenyl ring relative to the benzopyran moiety allows a broad separation of these compounds into flavonoids (2-phenyl-benzopyrans), isoflavonoids (3-phenyl-benzopyrans) and neoflavonoids (4-phenyl-benzopyrans) . Division into further groups is made on the basis of the central ring oxidation and on the presence of specific hydroxyl groups.Most common flavonoids are flavones (with a C_2-C_3 double bond and a C_4-oxo function), flavonols (flavones with a 3-OH group) and flavanones (flavone analogues but with a C_2-C_3 single bond), and abundant isoflavonoids include isoflavones (the analogue of flavones), 4-arylcoumarins (a neoflavonoid with a C_3-C_4 double bond) and its reduced form, 3,4-dihydro-4-arylcoumarin, are the major neoflavonoids. Other natural compounds, such as

chalcones and aurones also possess the C_6-C_3-C_6 backbone, and are henceforth included in the general group of flavonoids.

Flavonoids except chalcones, aurones and isoflavones share the same basic skeleton. The flavanone nucleus contains two hexacarbonic aromatic rings (A and B) interconnected with a heterocylic ring composed of three carbon atoms and one oxygen atom.This nucleus can undergo many modifications such as hydroxylation, alkylation or glycosylation. Depending on these modifications, the flavonoids are classified into 9 groups (chalcones, aurones, flavanones, dihydroflavanols, flavones, isoflavones, anthocyans, flavonols and flavanols). Compounds belonging to the same group differ in the degree and the position of hydroxylation, the presence of substituents on the nucleus and the state of their polymerization[12] .

1.2.1-Flavones

Flavones and flavonols are characterized by fully unsaturated C-rings that connect the A and B rings in a single conjugated system. They are generally photochemically inert as indicated by their reported use as photosensiting, photoquenchers and ultraviolet absorption filters. Their inertness prompted and allowed investigation into the potential of photochemically generated singlet oxygen to afford chemical transformations[13].

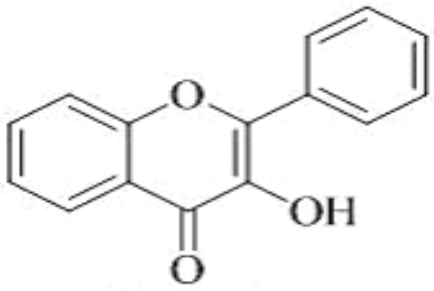


flavone

Chen and co-workers[14] investigated the photo-induced electron transfer reactions of flavones with amines. Irradiation of flavone swith 0.1 M triethylamine in acetonitrile under argon at >300 nm yielded *meso*-2,2'-biflavanone, (±)-*meso*-2,2'-biflavanone and flavones- [4,2]-flavanol . These products resulted from radical addition of 4-ketyl and/or its isomeric 1,2-ketyl anion to flavones, respectively . Single electron transfer (SET)[15] is a well-known photo-reaction between amines and α,β-unsaturated carbonyl compounds. The amine donates an electron to form a contact ion radical pair[16] (CIP) that undergoes hydrogen transfer to yield the radical responsible for dimerisation Bhatacharya and co-workers[17] studied the photo-physics of flavones. They concluded that flavone almost instantaneously forms a triplet state with a 90% inter-system crossing (ISC) formed after absorption of UV light. Polar solvents enhanced yields indicating a π,π*-character for the lowest triplet excited state. The flavone's triplet is quenched by several typical triplet quenchers like hydrogen donors[18], including amines[19].

1.2.2- Flavonol

Flavonols (examples : kaempferol, quercetin and myricetin) are pale yellow, poorly soluble substances present in flowers and leaves of at least 80% of higher plants and also in fruits and berries[19] . Flavonols occur in foods usually as *O*-glycosides, D-glucose being the most common sugar residue. Other sugar residues are D-galactose, L-rhamnose, L-arabinose, D-xylose and D-glucuronic acid. The preferred binding site for the sugar residue is C_3 and less frequently the C_7 position[17,18].



flavonol

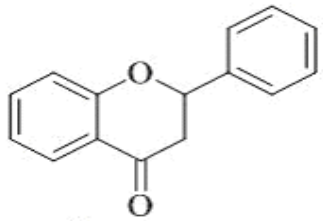
Flavonoids are generally stable compounds and may be extracted from fresh or dried, ground plant material with cold or hot solvents. Suitable solvents are aqueous mixtures containing ethanol, methanol, acetone or dimethylformamide[20]. Extraction of flavonols has been performed by maceration of the fresh, undried fruit or plant sample in the extracting solvent[21], by extracting an aliquot of homogenised fresh fruit sample or by extracting a freeze-dried (lyophilised) sample. Photolysis of quercetin 5,7,3',4'-tetra-O-methyl ether in pyridine in the presence of rose bengal (300 W tungsten lamp) followed by

diazomethane methylation of the reaction mixture afforded methyl 2-hydroxy-4,6-di-O-methylbenzoate(depside) and methyl 3,4-di-O-methylveratrate . Enzymatic oxygenation also yielded depside and there seemed to be a resemblance between enzymatic and photosensitised oxygenation. Liberated carbon monoxide (31%) and carbon dioxide (17%) were determined. Similar results were obtained with 3-hydroxyflavone . In a later publication Matsuura and co-workers[22] irradiated (100 W high-pressure lamp) free phenolic quercetin in methanol in the presence of rose bengal to get similar products . In the absence of an oxygen sensitiser no reaction took place. No reaction was observed with quercetin 3,7,3',4'-tetra-O-methyl ether. It was suggested that the 3-hydroxy group was essential for photo-oxygenation[23].

1.2.3- Flavanone

Flavanones (2,3-dihydro-2-phenyl-4H-1-benzo-pyran-4- one derivatives) are the main biosynthetic precursors for major flavonoids such as flavones or isoflavones and for two important flavonoid intermediates the flavan-4-ols(biosynthetic precursors for the formation of 3-deoxyanthocyanins) and the dihydroflavonols (biosynthetic intermediates in the formation of catechins,flavonols, anthocyanins and proanthocyanidins)[24]. The flavanone skeleton is present in a wide range of synthetic or naturally occurring products exhibiting various interesting pharmacological activities[25]. Flavanones which are widely

distributed in nature, continue to attract attention due to their ample range of biological activities (like hypotensive, antifungal antibacterial, antitumoral)[26].The cyclization of chalcones to flavanones has been reported using acids , heat , light, electrolysis, cobalt (II) Schiff-base complexes , zeolites ,L-proline and microwave conditions but the obtained yields are often moderate and sometimes poor.



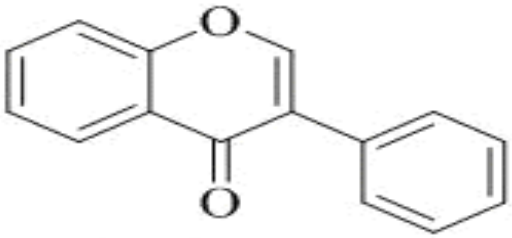
flavanone

The acid catalyzed cyclization can be carried out by refluxing the chalcone in acetic acid or also in ethanol or other suitable solvent in the presence of an acid catalyst such as H_3PO_4 . Flavanones can also be obtained from precursors other than chalcones, namely by reacting 3-chloro-2,3-dihydro-3-nitro-2-phenyl-4H- 1-benzopyran-4-ones with tributyl tin hydride and 2,2'-azobisisobutyronitrile or by treating 3-bromo-1-phenyl prop-2-ynil aryl ethers with mercury (II) trifluroroacetate[27].

1.2.4- Isoflavones

Isoflavone comprise a class of organic compound srelated to the isoflavonoids. Many act as phytoestrogens in mammals[27]. Some are termed antioxidants because of their ability to trap singlet oxygen[28].Some isoflavones, in particular soy isoflavones,

when studied in populations eating soy protein, have indicated that there is a lower incidence of breast cancer and other common cancers because of its role in influencing sex hormone metabolism and biological activity through intracellular enzymes, protein synthesis, growth factor actions, malignant cell proliferations, differentation and angiogenesis[29]. However, the risk reduction in breast cancer from soy isoflavones was only shown in Asian populations (Shanghai Breast Cancer Survival Study). Isoflavones are produced almost exclusively by the members of the *Fabaceae* (i.e., *Leguminosae*, or bean) family.



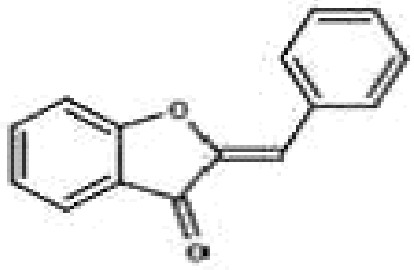
isoflavone

The first representatives of flavone epoxides are prepared either by alkaline hydrogen peroxide epoxidation of isoflavones or by an intramolecular Darzens reaction of α-bromo -*O*-acyoxyaceto-phenones[30].

1.2.5- Aurones

Aurones (2-benzylidene benzofuran-3-ones) are naturally occurring molecules belonging to the flavonoid family, the

pharmacological potential of which we reported for the first time several years ago[31]. Some aurone derivatives were synthesized by means of aldolcondensation of a substituted benzofuran-3(2*H*)-one with a benzaldehyde derivative, in basic KOH/MeOH conditions under reflux. Aurones bearing fluoro groups were obtained with a softer method using neutral alumina, as described by Varma*et* in order to avoid nucleophilic substitution by in situ- formed potassium methoxide[32].4,6-Dihydroxy aurones were synthesized by preparing the corresponding 4,6-dimethoxy analogues, followed by protection with boron tribromide. In some cases it was possible to obtain these derivatives directly by simple basic condensation in KOH/EtOH.



aurone

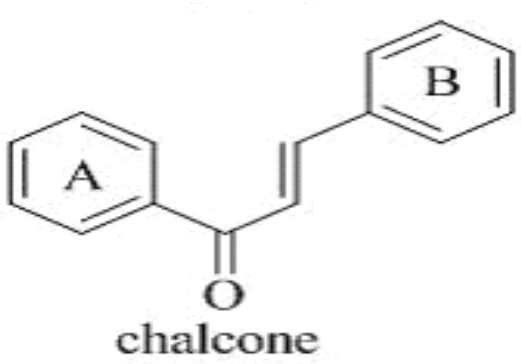
The position and electronic nature of the substituent on ring B seem to significantly affect the activity of these compounds against *L.infantum* parasite as well as their cytotoxicity the most active one of the less toxic compounds of the series, 2′,4,6-

trimethoxyaurone bears a methoxy group on position 2′ of ring B and exhibits potentantileishmanial activity by changing the electron-donating methoxy group to the nonoxygenated electron-Donating methyl group on the same position but slightly higher toxicity was observed. Aurone in which ring B is unsubstituted possesses significant anti-leishmanial activity although lower than the 2′-substituted.

1.2.6-Chalcones

The chemistry of chalcones has generated intensive scientific studies throughout the world. Specially interest has been focused on the synthesis and biodynamic activities of chalcones. The name "Chalcones" was given by Kostanecki and Tambor.[30] These compounds are also known as benzalacetophenone or benzylideneacetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcone bears a very good synthon so that variety of novel heterocycles with good pharmaceutical profile can be designed. Chalcones are unsaturated ketones containing the reactive keto-ethylenic group –CO-CH=CH-. These are coloured compounds because of the presence of the chromophore -CO-CH=CH-, which depends in the presence of other auxochromes **.**Different methods are available for the preparation of chalcones[33-35] .The most convenient method is the Claisen-Schimdt condensation of equimolarquantities of arylmethylketone with aryl aldehyde in

the presence of alcoholic alkali.Chalcones are used to synthesize several derivatives like cyanopyridines, pyrazolinesisoxazoles and pyrimidines having different heterocyclic ring systems[36-39] .

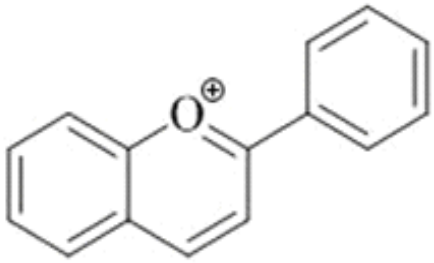


chalcone

Chalcones exist in nature with a variety of substituents. The MS spectra of these compounds are characterized by substituent loss, fragmentations of the substituents and chalcone fragmentations. Chalcone fragmentations are dominated by cleavage of a single bond, yielding a B ring derived ion with the attached C=O group in the charged form (C≡O+), from which a CO loss yield the free B ring[40] besides that, 2'-OH-chalcones, the most common ones, with an OH group in ring B adjacent to the propenone chain, are known to be converted to the corresponding flavanones by various processes, and that has also been observed to occur in ESI MS; the pattern of chalcone fragmentation will then be the same of flavanone fragmentation[41] .

1.2.7- Anthocyanins

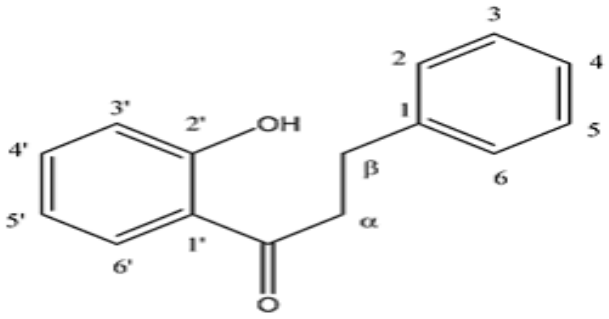
Anthocyanins are the most important group of pigments, after chlorophyll, that are visible to the human eye[41] .Chemically, anthocyanins(fromthe Greek *anthos*, a flower, and *kyanos*, dark blue) are flavonoids (flavan like), and consequently based on a C_{15} skeleton with a chromane ring bearing a second aromatic ring B in position 2 (C_6-C_3-C_6) and with one or more sugar molecules bonded at different hydroxylated positions of the basic structure .Anthocyanins are substituted glycosides of salts of phenyl-2- benzopyrilium (anthocyanidins)[42,43] .The basic C_6-C_3-C_6 anthocyanin structure is the source of an infinity of colors produced by its chemical combination with glycosides and/or acyl groups and by its interaction with other molecules and/or media conditions. Harborne and Gryer mentioned the existence of 17 anthocyanidins, with differences in the number and position of hydroxyl groups and/or methyl ether groups, but six of them are the most common anthocyanidin constituents of this kindofpigments . From these 17 structures many structures have arisen with at least one sugar molecule to obtain anthocyanin compounds[41].



anthocyanin

1.2.8-Dihydrochalcones

To establish the synthetic method for dihydrochalconesas an important bioactive compounds, 2'- hydroxydihydrochalcone has been synthesized from the reduction of flavone in 20% yield. Flavone with five equivalents of ammonium formate in the presence of Pd/C in methanol under nitrogen atmosphere produced the C-ring opened product. The product was characterized by UV-VIS, ESI-MS and 1H-NMR spectroscopy, and identified as 2'-hydroxydihydrochalcone .Dihydrochalcones are minor flavonoids found in some plants[44,45] and show various biological activities. Derivatives of phlozirin are known to be potent inhibitors of *Plasmodium falciparum*-induced erythrocyte permeation[46] and C-benzylated dihydrochalcones show cytotoxicity towards human promyelocytic leukemia HL-60 cells. The reduction of flavones with excess amount of ammonium formate in the presence of Pd/C could provide general procedure for the synthesis ofdihydrochalcones from flavones[47].

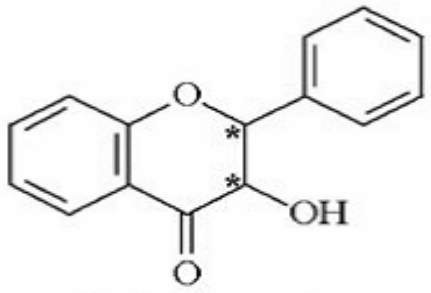


2'-hydroxydihydrochalcone

1.2.9-Dihydroflavonols

Although the Algar-Flynn-Oyamada (AFO) protocol and the Weeler reaction were mainly used for the synthesis of aurones, it was demonstrated that these reactions can be adapted for the formation of racemic dihydroflavonols in moderate to good yields. Cyclization of 2′-hydroxy-α,3,4,4′-tetramethoxy chalcone with sodium acetate in ethanol furnished both 3,3′,4′,7-*O*-tetramethyl-2,3-trans- and 3,3',4',7-*O*-tetramethyl-2,3-*cis*-dihydroflavonols in 22% and 11% yields, respectively. However, this method was not applicable to cyclization of α-OH-chalcones. Initial attempts toward acid catalyzed cyclization of the chalcone epoxide to the corresponding (2*R*,3*R*)-2,3-*trans*- and (2*S*,3*R*)-2,3-*cis*-dihydroflavonols were hampered by two difficulties, i.e., aryl migration with formation of 4′,7-dimethoxy isoflavone and the epimerization / racemization of the thermodynamically less stable (2*S*,3*R*)-2,3-*cis*-4′,7-dimethoxy- dihydroflavonol to yield (2S,3S)-2,3-trans-dihydro-flavonol. The “loss” of optical purity in the conversion indicates

competition between protonation of the heterocyclic oxygen and hydrolysis of the 2′-*O*-acetal functionality, hence leading to a considerable degree of S_N1 character for the cyclization step with concomitant racemization at C-β of apresumed carbocationic intermediate, yielding dihydroflavonols. The thermodynamically less stable (2*S*,3*R*)-2,3-*cis*-dihydroflavonol is rapidly racemized at C-3 to give a mixture of productsunder the prevailing acidic conditions. Formation of the isoflavone is attributed to acid-catalyzed cleavage of the highly reactive oxirane functionality prior to deprotection[50,51].



dihydroflavonol

1.3 Synthsis of flavones

The main synthetic methods known for the flavones are the cyclodehydration of 1-(2-hydroxyphenyl)-3-phenyl-1,3- propane diones, the oxidative cyclization of 2'-hydroxy chalcones, and synthesis via an intramolecular Wittig reaction[52]. The rearrangement of benzoyl esters of 2'-hydroxy acetophenones (Baker-Venkataraman process)[53] and the direct benzoylation of 2'-hydroxyacetophenones with benzoyl chlorides[54] or methyl

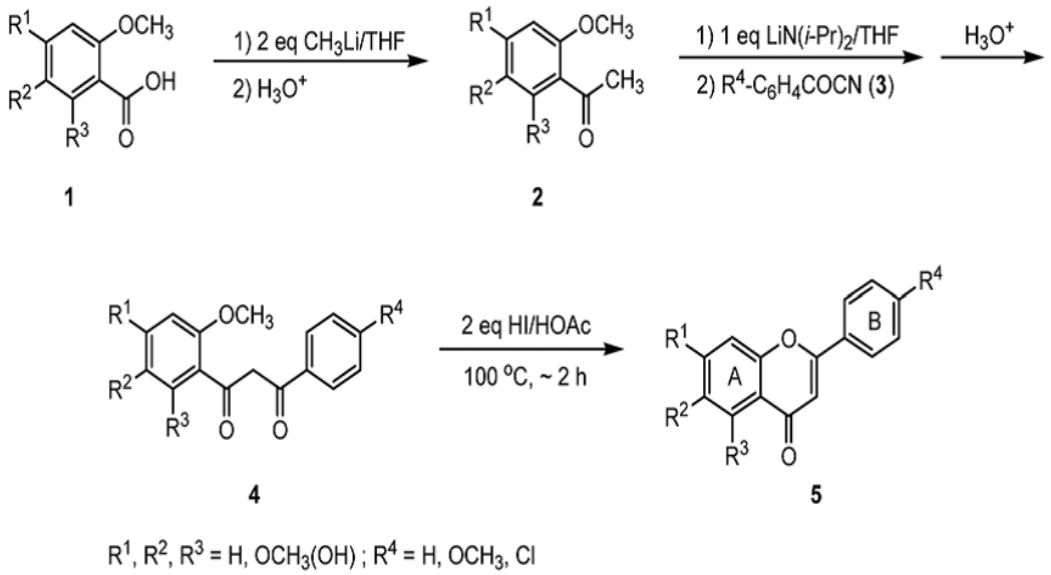
benzoates[50] affords 1-(2- hydroxyphenyl)-3-phenyl-1,3-propanediones, which are cyclodehydrated to give flavones in acidic conditions. The treatment of 2'-hydroxychalcones which are prepared from 2'-hydroxyacetophenones and benzaldehydes in the presence of 2 equivalents of lithium diisopropylamide with oxidizing agents also affords flavones at high temperature[51]. Alternatively ,Wittig reaction[52] involves the intramolecular olefination of phosphoranes obtained from triphenylphosphine and 2-acetoxyphenacyl bromides. A common feature in all these methods is that they invariably use 2'- hydroxyacetophenones as the starting material. However, there are no reports on the synthesis of flavones from 2' methoxyacetophenones.Although1-(2-methoxyphenyl)-3-methyl-1,3-propanedione is cyclized with boiling HI to give 2-methyl chromone, the scope of the reaction is not fully investigated and there are no reports on the synthesis of flavones with 2-substituted phenyl group[53]. Furthermore, it has been reported that the condensation of 2'-methoxyacetophenone with methyl 2-methoxybenzoate using sodium or sodium hydride failed to produce the corresponding 1,3-diketone[54]. It was reported that flavones can be synthesized in two steps via 1-(2-methoxyphenyl)-3-phenyl-1,3-propane diones from2'-methoxy acetophenones which is cheaper than2'-hydroxyacetophenones in general.2'-Methoxyacetophenones were readily prepared by the treatment of 2-methoxybenzoic acids with 2 equivalents of

methyl lithium in THF for 2 hr. at −78^{o}C . However, the reaction of2,6-dimethoxybenzoic acid with methyl lithium proceeded slowly due to the steric effect and thus 2',6' – dimethoxy acetophenone was prepared by the treatment of N-methoxy-N-methyl 2,6-dimethoxy benzamide with methyl magnesium bromide at room temperature giving a 75% yield.

To find out the optimum reagent for the benzoylation of 2, benzoyl chloride, 2-pyridyl benzoate, and benzoyl cyanide were added to the lithium enolate solution of 2 in THF at −78^{o}C, which was generated from 2'-methoxy acetophenone and equivalent of lithium diisopropylamide for 2 hr. at −20^{o}C. The resulting yellow solution was allowed to warm to room temperature and 1-(2 methoxy phenyl)-3-phenyl-1,3-propane dione was obtained after chromatographic separation. The condensation of the lithium enolate of 2 with 3 worked well regardless of the kind of substituents on both 2'-methoxy acetophenones and benzoyl cyanides under the present reaction conditions and 4 was obtained in high yields (80-95%). The cyclization of 4 was successfully accomplished by heating with hydroiodic acid in glacial acetic acid. The initial cyclization of 1-(2-methoxyphenyl)-3-phenyl-1,3-propanedione with sulfuric acid, hydrobromic acid, and hydoriodic acid in acetonitrile didn't proceed at room temperature.

However, the cyclization accompanied by the cleavage of the 2-methoxy group of 1-(2-methoxy phenyl)-3-phenyl-1,3- propane

dione with 47% HI proceeded well in glacial acetic acid for 1.5 h at 100^{o}C to afford flavone in 78% yield. The use of 48% HBr was also effective, but the yield of flavones was decreased to 55%. Various flavones were synthesized in overall high yields (47-67%) from the starting 2 methoxybenzoic acids. The present method was generally applicable for the synthesis of 5 having methoxy- and chloro- substituents on the A- and/or B-ring. However, the treatment of 1-(2,6-dimethoxyphenyl)-3-(4'-chlorophenyl)-1,3-propanedione with 47% HI in glacial acetic acid at reflux resulted in the cleavage of the two methoxy groups and the successive cyclization to produce 5-hydroxy-4'-chloroflavone (5) in 85% yield[55].



R^1, R^2, R^3 = H, OCH_3(OH) ; R^4 = H, OCH_3, Cl

1.3.1 -Synthsis of flavanones

The flavanones (2-phenyl chroman-4-ones) are mainly found in plants of the family Leguminosae, Compositae, and Moraceae[56] and have attracted a good deal of attention because they possess pharmacological activities, such as antioxidant effect, toxicity to several bacteria, and inhibition of hormone-dependent proliferation of cancer cells[57]. The most widely used route to flavanones is the oxidative cyclization of 2'-hydroxy chalcones which are prepared from 2'-hydroxy acetophenones and benzaldehydes with 2 equivalents of base using various reagents[58]. The reaction of 2'-hydroxy chalcones with palladium(II) acetate, Co under oxygen atmosphere, and potassium ferricyanide using phase transfer catalysis leads to the formation of flavanones, but the yields are low to moderate. Alternatively flavanones are prepared from the oxidative cyclization of cinnamic acids and phenols with polyphosphoric acid[59]. However, the preparation of 2'-hydroxychalcones from 2'-hydroxyacetophenones and benzaldehydes has underwent trouble someness because they are always cyclized to flavanones partially during their synthesis. The synthetic strategy to avoid this undesirable reaction is to prepare β-hydroxy ketones as precursors of flavanones from 2'-hydroxy acetophenones and benzaldehydes. The use of 2' methoxya cetophenones can also avoid the undesirable cyclization of chalcones to flavanones and furthermore they are generally

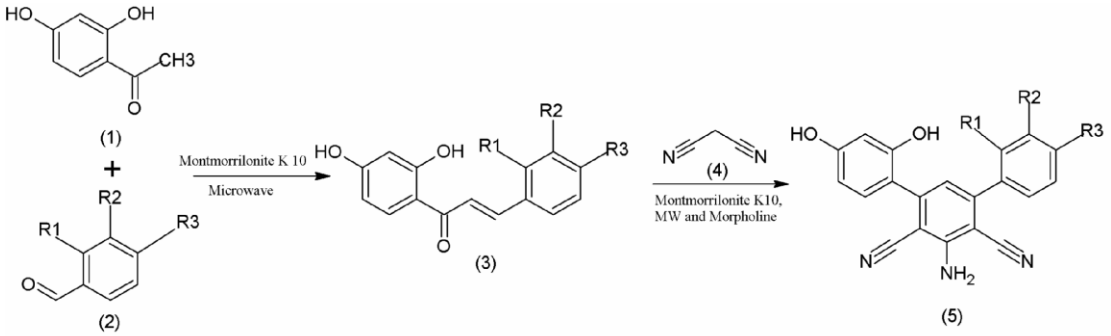
cheaper than 2'-hydroxyacetophenones. Flavanones can also be synthesized via 1-(2'-methoxyphenyl)-1-oxo-propan-3-phenyl-3-ols from 2'-methoxyacetophenones as a new synthetic route. 2'-Methoxyacetophenones were readily prepared by the treatment of 2-methoxybenzoic acids with 2 equivalents of methyllithium in THF for 0.5-2 hr. at −78°C in 88-93% yields[60].

1.3.2 - synthsis of chalcones

The chalcones (1, 3-diaryl-2-propenones) and their derivatives are important intermediates in organic synthesis[61-63]. They serve as starting material for the synthesis of variety of heterocyclic compounds which are of physiological importance. The presence of enone functionality in chalcone moiety confers biological activity upon it, like antiinflammatory[64], antifungal[65], antioxidant[66],antimalarial[67], antituberculosis[68],analgesic[69], anti HIV[70] and antitumor[71] activities. In recent years, microwave assisted solid support solvent- free organic synthesis have attracted attention as they offer several advantages such as simple procedure, fast reaction rate, mild reaction condition, eco-friendly and improved yields as compared to conventional methods. Further the reaction in dry media conditions is especially appealing as they provide an opportunity to work with open vessels, thus avoiding the risk of high pressure development and offer the possibility of carrying out reactions that can be scaled up by the industries. The m-terphenyl moiety is an important structural unit, useful intermediate and act as

building blocks for constructing optically active cyclophans[72,73], cyclic ketones[74-75] , and liquid crystals[76,77]. Though several approaches have been developed for the synthesis of 2-aminobenzene-1, 3-dicarbonitriles , the majority of them are multistep with poor yield. In continuation of our earlier endeavor [86-88] on MORE (Microwave induced organic reaction enhancement) chemistry for the synthesis of bioactive compounds using solid phase conditions, we herein describes the synthesis of some novel chalcones and their 2-aminobenzene-1,3-dicarbonitrile derivatives by conventional and microwave irradiation coupled with solid support and under neat conditions. Prompted by the varied biological activities of chalcones and their derivatives and increasing applications of microwave irradiation in organic synthesis , it was thought of interest to synthesis variously substituted chalcones and their transformation products 2-aminobenzene-1,3-dicarbonitrile derivative mutimode commercial microwave oven[78].

Condensation of variously substituted aromatic aldehydes and 2,4-dihydroxy acetophenone furnished the corresponding chalcones which on treatment with malononitrile in presence of catalytic amount of morpholine afforded the desired chalcones[79].



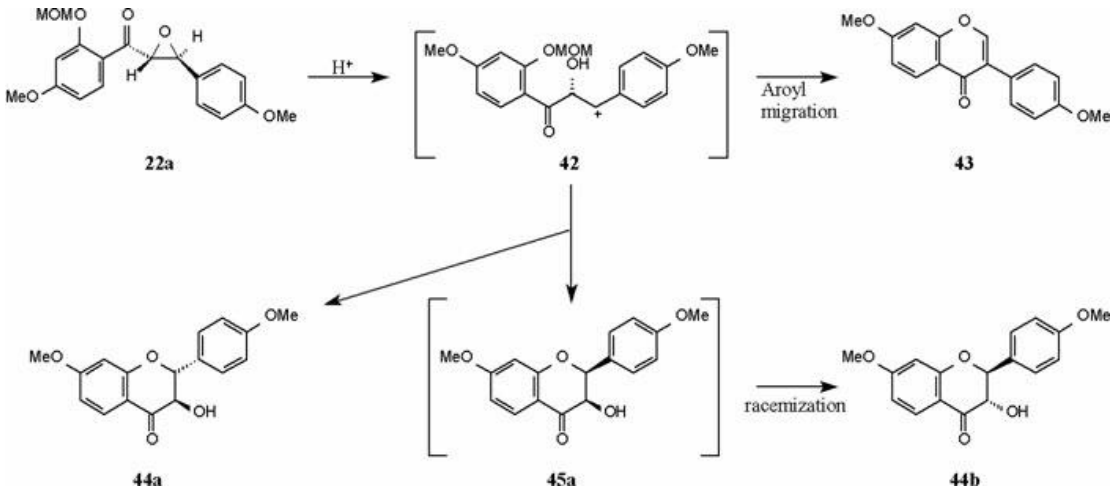
Scheme 1: *Synthesis of chalcones Chalcones 3_{a-k} and their 2-aminobenzene-1,3-dicarbonitriles 5_{a-k}*

1.3.3 -Synyhsis of dihydroflavonol

Initial attempts towards acid catalyzed cyclization of the chalcone epoxide to the corresponding (2*R*,3*R*)-2,3-*trans*-44aand (2*S*,3*R*)-2,3-*cis* dihydroflavonols were hampered by two difficulties, i.e., aryl migration with formation of 4′,7-dimethoxy isoflavone and the epimerization / racemization of the thermodynamically less stable (2*S*,3*R*)-2,3-*cis*-4′,7-dimethoxy dihydroflavonol to yield (2*S*,3*S*)-2,3-*trans*-dihydroflavonol[80] (Scheme1.4). The “loss” of optical purity in the conversion indicates competition between protonation of the heterocyclic oxygen and hydrolysis of the 2′-*O*-acetal functionality, hence leading to a considerable degree of S_N1 character for the cyclization step with concomitant racemization at C-β of a presumed carbocationic intermediate, yielding dihydro flavonols.The thermodynamically less stable(2*S*,3*R*)-2,3-*cis*-dihydroflavonol is rapidly racemized at C-3 to give a mixture under the prevailing acidic conditions. Formation of the

isoflavone is attributed to acid-catalyzed cleavage of the highly reactive oxirane functionality prior to deprotection.

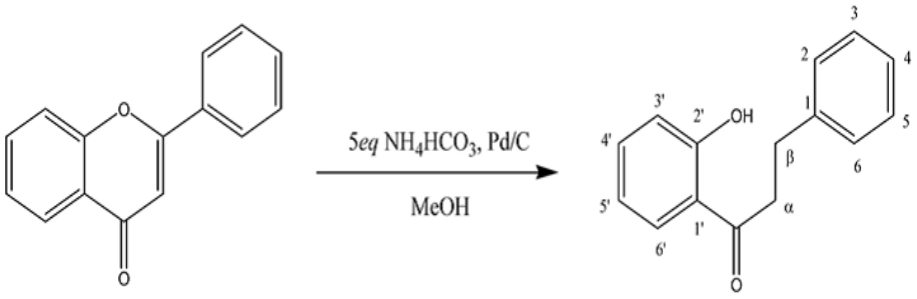
In order to enhance the S_N2 nature of the ring closure step, methods aimed at the selective removal of the 2'-*O*-methoxymethyl group under mild conditions were explored. It was anticipated that deprotection of the 2′-*O*-methoxymethyl group with concomitant cyclization would enhance the preservation of optical integrity. In order to circumvent the problem of isoflavone formation,[81,82] methods aimed at the initial nucleophilic opening of the oxirane functionality, followed by deprotection and cyclization were investigatged. The excellent nucleophilic and nucleofugic properties of Mercaptans[83]prompted evaluation of thiols in the presence of Lewis acids and resulted in the selection of the phenylmethanethiol–tin(IV) chloride (BnSH/SnCl4) system as the reagent of choice for the oxirane cleavage [84].



1.3.4-ynthsis of dihSydrochalcone

Dihydrochalcones are minor flavonoids found from some plants[84,85,]and show various biological activities. Derivatives of phlozirin are known to be potent inhibitors of *Plasmodium falciparum*-induced erythrocyte permeation[86] and C-benzylated dihydrochalcones show cytotoxicity towards human promyelocytic leukemia HL-60 cells [87]. Hence, simple preparation of dihydrochalcones can provide many useful bioactive compounds. Flavones are structurally robust and more than hundreds derivatives are available. If flavones can be used for the synthesis of dihydrochalcones through C-ring cleavage, numerous dihydrochalcones can be obtained for the relevant study. Simple and convenient synthetic method of 2' hydroxy dihydrochalcone from flavones were describwd, as a possible expansion to the general synthesis of dihydrochalcones[88]. The preparation and identification of 2'-hydroxy dihydrochalcone were done as follows: The reduction of flavone was carried out in the presence of ammonium formate and Pd/C in an inert atmosphere glove box . Flavone (600 mg, 2.70 mmol), Pd/C (550mg), NH4HCO3 (1050 mg, 13.3 mmol) were dissolved in anhydrous MeOH (50 ml). The reaction mixture was stirred for a day and Pd/C was filtered off through cotton plugged Pasteur pipette. More than 8 compounds were identified on silica gel TLC plate after developing in chloroform and the product with Rf= 0.6 (2'-dihydroxy dihydrochalcone) was isolated in 20%

yield (120 mg, 0.53 mmol), by using prepTLC with concentrating zone UV-Vis spectrum of the 2'-hydroxy dihydro chalcone showed(λmax at 323 nm, 251 nm and 218 nm in MeCN). ESI-MS measurement identified the molecular ion peak at 226.7 m/z .

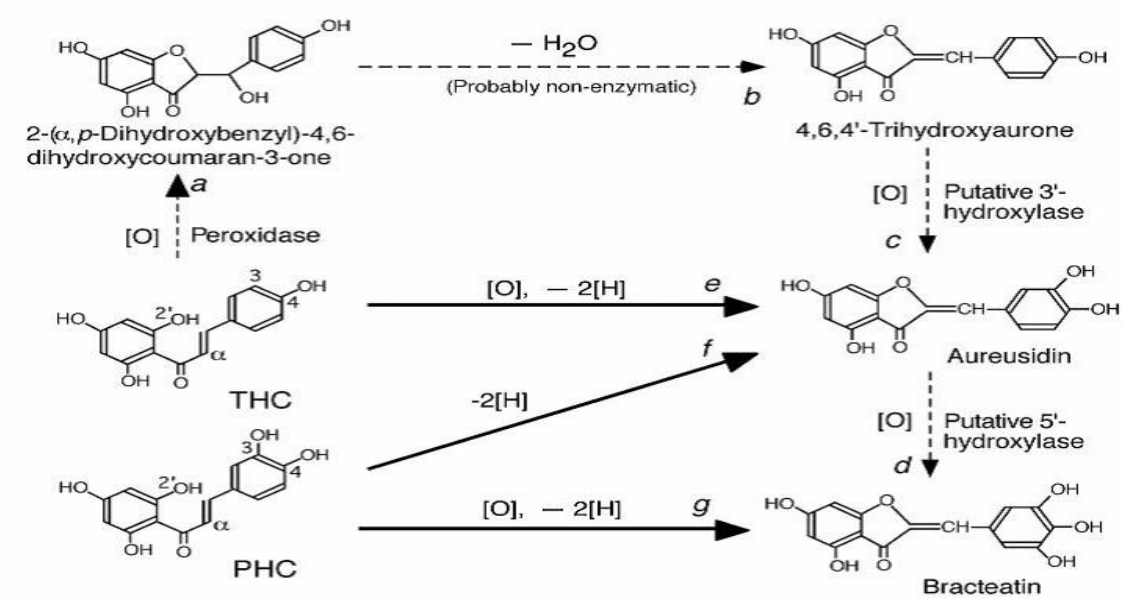


flavone 2'-hydroxydihydrochalcone

1.3.5-Synthsis of aurones

Most floral colors present in nature arise from flavonoids [89]. Genetic and biochemical knowledge of flavonoid biosynthesis in plants has provided a basis for controlling floral color through genetic engineering approaches[90]. Aurones[91], a class of plant flavonoids, confer bright yellow color to flowers such as cosmos, coreopsis, and snapdragon (*Antirrhinum majus*) . Although the aurone biosynthetic gene(s) is an attractive tool to

engineer yellow flowers, the biochemical and genetic details of aurone biosynthesis have remained unclear [92]. One mechanism proposed for aurone biosynthesis in plants (soy seedling) is a two-step pathway, in which an H_2O_2-dependent peroxidase is responsible for aurone biosynthesis (called aureusidin synthase) [93,94].A Synthetic protocol for aurones is shown below:

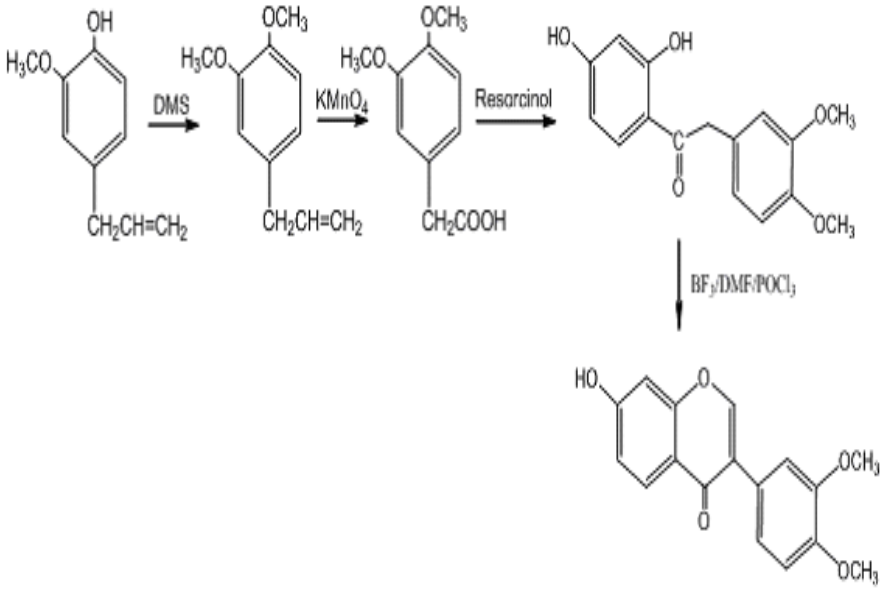


1.3.6-Synthsis of isoflavones

Isoflavones are secondary metabolites which can be classified as flavonoids [95]. Isoflavone displays some important biological activity, such as the growth inhibitor of breast [96], servical[97]and liver cancer cells [98]. Despite isoflavone has a lot of benefits, isolation from natural products only gives very limited amount. Thus, convenient synthesis of isoflavone with various structures is really needed to produce this compound in the effort to design and develop new drugs with more interesting as well as potential therapy effects. Some synthetic anticancer of isoflavone are genistein, daidzein, biochanin A and formonometin. Isoflavones could be obtained via intermediates of chalcone[99-101]chromone [102] or deoxybenzoin[103]. The synthetic route from deoxybenzoin intermediate is mostly conducted, i.e. based on the Friedel-Craft acylation between phenolic compound and benzyl carboxylic acid[104]. Therefore, benzyl carboxylic acid derivative is a key compound in the isoflavone synthesis through deoxybenzoin. An effort to provide the key compound might be done by applying abundance of the natural product eugenol. Eugenol is a major component of clove leaves oil (80%). Chemical property of eugenol, i.e. the presence of allylic group, gives possibility to convert eugenol into 3,4- dimethoxybenzyl carboxylic acid.

Friedel-Craft acylation with recorcinol in the presence of BF_3 as Lewis acid catalyst yields deoxybenzoin intermediate. Further

reaction of the intermediate with reagents of $BF_3.OEt_2$/DMF/ $POCl_3$ produces 7-hydroxy-3',4'-Dimethoxy isoflavone[105].



1.4-*Waltheria indica*

Description

Waltheria indica is a small shrub 2 to 6 feet tall with velvety hairs covering all parts of the plant. The oblong to oval leaves are up to 6 inches long and 2 inches wide with toothed edges and conspicuous veins. The fragrant yellow flowers grow in small, dense clusters in the leaf axils[106]

Waltheria indica is found throughout the tropics and is considered to be indigenous to Hawai'i. It grows on dry, often disturbed sites from sea level to almost 4,000 feet on all of the main islands and on Midway Atoll[107].

The fruit of *Waltheria indica* is a small, round, dry capsule containing one seed. *Waltheria indica* can be grown from seed. Germination takes 1 to 3 months. *Waltheria indica* (Waltheria americana) is commonly used in traditional medicine in Africa, South America and Hawaii, mainly against pain, inflammation, conditions of inflammation, diarrhea, dysentery, conjunctivitis, wounds, abscess, epilepsy, convulsions, anemia, erectile dysfunctions, bladder ailments and asthma.. *Waltheria indica* was investigated and showed analgesic, anti-inflammatory, antibacterial, antifungal, antimalarial, anti-anemic, anti-oxidant, sedative and anticonvulsant activities. The phytochemical investigations showed the presence of cyclopeptid , flavonoids, tannins, sterols, terpenes, saponins, anthraquinones. Studies of acute toxicity in animal indicated that *Waltheria indica* can be toxic.*Waltheria indica* possess therapeutic potential in the treatment of infectious diseases (e.g., lungs infection due to *Klebsiella pneumoniae*, diarrhea due to *Candida albicans* or *Escherichia coli*) and prevention of oxidative stress. Further studies are necessary to explore pure compounds responsible for the pharmacological effects and the mechanisms of action. Further investigations are also needed to provide an evidence base for traditional uses of this species against pain, anemia, convulsions and epilepsy. In addition, there is a pressing need to investigate the other traditional uses such as dysentery, syphilis, erectile dysfunctions and asthma[108].

Photo of *Waltheria indica*

1.5-Aim of this study

This study was aimed to :

- Extracted of phenolics from *Waltheria indica*
- Isolated of the major flavonoid from *Waltheria indica*
- Conducted some spectral studies (UV and IR) on the isolated flavonoid.

2-Materials and Methods

2.1-Materials

2.1.1-Apparatus.

Analytical grade solvents were used. The UV spectra were recorded on a Shimadzo 1601 spectro-photometer and UV lamp used for localization of fluorescent spots on PC. The IR spectra were recorded as KBr disks, using Shimadzu IR-8400 spectrophotometer.

2.1.2-Collection of plant material.

The stems of *Waltheria indica* were collected from Kordofan state during August 2013 . The plant was authenticated by National Research Center, Khartoum, Sudan.

2.2- Methods.

2.2.1 Preparation of test reagents for phytochemical screening .

a) Flavonoids test reagents:

(i)Aluminum chloride solution: (1g) of aluminum chloride was dissolved in (100ml) methanol.

(ii)Potassium hydroxide solution: (1g) of potassium hydroxide was dissolved in (100ml) water.

(iii)Ferric chloride solution : (0.5g) of ferric chloride was dissolved in (100ml) 95% ethanol.

b) Alkaloids test reagents:

Dragendroffs Reagents

(i) Stock solutions

Solution **A** and **B** were prepared as follows. Solution **A** was

obtained by dissolving 20 gram of tartaric acid and 1.7 grams of bismuth subnitrate in 80ml of water, shaken well for one hour. Solution **B** was prepared by dissolving 16 grams of potassium iodide (KI) in 40 ml of water, the above solution were mixed by 1:1 (v/v) and store in a cooled place .

(ii) Spray Reagents

10 ml of stock solution with 100 ml of water containing 20 grams of tartaric acid.

(iii) Mayer's Reagent

$HgCl_2$ 1.35 grams was dissolved in 60 ml of water, 5 grams of KI was dissolved in 10 ml of water. The two solutions were mixed and the total volume adjusted to 100ml with water.

(iv) Wagner's Reagent

2grams of KI and 1.27 grams of I_2 were dissolved in 75 ml of water and the total volume adjusted to 100 ml with water.

(v) Ammonium Reineckates Reagent

One gram of amm. Reineckat($NH_4\{Cr(NH_3)_2(SCN)_4\}.H_2O$)and 0.3 grams of hydroxylamine hydrochloride, were dissolved in 100 ml of ethanol and stored in a refrigerator.

c)Glycoside test reagent

(i) Molish reagent.(10%solution).

10 grams of 2-naphthol dissolved in 5ml of ethanol, then complete the volume to 100 ml.

2.2.2-Preparation of plant extract for phytochemical screening.

The pods of *Waltheria indica* were air dried and powdered. Part of the powdered material (30 – 50 g) was extracted with (2×50 ml) 95% ethanol(soxhlet) for 5 hours . The alcoholic extract was filtered, and concentrated under reduced pressure. This prepared extract (PE) was used for phytochemical screening.

2.2.3- Test for steroids and/or terpenoids .

(40ml) aliquot of the prepared extract was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether(40 – 60 ^{0}C) to remove most of the colouring matter. The residue was extracted with (20ml) chloroform. The chloroform solution was dehydrated over anhydrous sodium sulphate. (5ml) portion of the solution was mixed with (0.5ml) acetic anhydride, followed by two drops of concentrated sulphuric acid. A green colour was observed.

Two specific tests are the Salkowsks and Libermann-Burchards (L-B) reactions. The alcoholic extract was evaporated, the residue was dissolved in anhydrous $CHCl_3$ and filtered (this essential step since these are dehydration reaction). The latter filtrate was divided into two portion, and each was tested with two reagents. Blue-green colour with steroids and red –pink or purple colour with triterpenoids are formed in (L-B) test.

In **Salkowsk is**, the yellow colored ring noted after 1-2 minutes, which changed to cherry red colour see table (3.1).

2.2.4-Test for alkaloids.

(i)30 ml aliquot of the prepared extract was evaporated to dryness on a water bath. (5ml) of 2N hydro chloric acid was added and the solution was heated with stirring in a water bath

for 10 minutes. To the cooled solution, few drops of dragen-droffs reagent were added. precipitate was formed.

(ii) 5 grams of the plant material defatted using petroleum ether (40-60^0 C), were filtered and dried (in an oven at 50^{0}C) . A few ml of 28% ammonium hydroxide solution was added. This alkaline solution was extract three times with $CHCl_3$ and filtered .To the filtrate, 4N hydrochloric acid was added until the aqueous solution becomes acidic to litmus paper. The layers were then separated and part of the acidic layer was treated with 35% amm.hydroxide solution until it becomes alkaline to litmus paper. Finally, it was extracted with $CHCl_3$ several times and the organic layer was then tested with alkaloid detecting reagents, such as Mayer's, Wanners and Dragenendorffs reagents. The formation of precipitate was indicative of the presence of 1^0,2^0,3^0alkaloids.

(iii) For quaternary alkaloids, 2ml of the rest the acidic layer was tested with ammonium rieneckate reagent . The formation of precipitate with this reagent was indicative of presence of 4^0alkaloids. The results are given in table (3.1).

2.2.5- Test for flavonoids

(85ml) aliquot of the prepared extract (PE) was evaporated to dryness on a water bath. The cooled residue was defatted and the residue was dissolved in (30ml) 95% ethanol and filtered. The filtrate was used for the following tests:-

(i) To (3ml) of the filtrate few drops of methanolic aluminum chloride were added. Formation of a dark yellow colour was taken as a positive test of flavonoids.

(ii) To (3ml) of the filtrate few drops of potassium hydroxide solution were added. A dark yellow colour indicated the presence of flavonoids.

(iii) To (3ml) of the filtrate (0.5 ml) of concentrated hydrochloric acid was added followed by few magnesium turnings. No red was colouration formed and this indicates absence of flavanones.

(iv) To (3ml) of filtrate few drops of ferric chloride solution were added. Development of a blue colouration was taken as a positive test for flavonoids. The results are given in table (3.1).

2.2.6- Test for glycosides

(20ml) of the prepared extract (PE) was vigorously shaken in a test tube. The presence of a froth that persisted for one hour was taken as a positive test for glycosides.

Molish reagent was used to test the presence of glycosides and carbohydrates. By using 2 ml of the alcoholic extract, and intense violet ring appeared at the junction of the two layers confirming the presence of glycosides and/or carbohydrates. The result of these tests are presented in table (3. 1) .

2.2.7- Extraction of flavonoids

Powdered air-dried stems (1.0kg) of *Waltheria indica* were extracted with filtered and the residue was re-extracted over night in 50% aqueous methanol (500 ml) at room temperature . Paper chromatography was then employed for fractionation.

2.2.8-Paper Chromatography

Part of the crude product (ca.0.5g) was dissolved in the minimum amount of ethanol and applied on Whatman No. 2 sheets. Different solvent systems were attempted. However , the solvent that gave the best separation was : 70% methanol in water .

The crude product was then applied as a narrow zone on the papers and irrigated with 70% methanol in water . The zones were located under UV

light . Only one major component was detected. This major phenolic was eluted from paper by ethanol. Removal of the solvent gave compound A.The IR and UV spectra of compound A was then recorded.

2.2.9- Spectral data of compound

2.2.9.1-UV shift reagents

Stock solutions of sodium methoxide, aluminum chloride, boric acid and hydrochloric acid were prepared as follows:-

(i) **Sodium methoxide stock solution**: freshly cut metallic sodium (2.5g) was added cautiously in small portions to dry spectroscopic methanol (100 ml). the solution was stored in a glass container with a tightly fitting plastic stopper.

(ii) **Aluminum chloride stock solution** :(5g) of fresh anhydrous aluminum chloride were added cautiously to spectroscopic methanol (100 ml).

(iii) **Hydrochloric acid stock solution** :concentrated hydrochloric acid (50 ml) was mixed with water (100ml) and stored in a glass Stoppard bottle.

(iv) **Boric acid stock stion** : spectroscopic methanol (100 ml) was saturated with anhydrous boric acid.

2.2.9.2- The UV spectrum of compound A in presence NaOMe.

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

2.2.9.3- The UV spectrum of compound A in presence of aluminum trichloride.

Six drops of aluminum chloride were added to a solution of **compound A** in methanol (2ml) and the UV spectrum was immediately recorded.

2.2.9.4- The UV spectrum of compound A in presence of $AlCl_3$/HCl

Three drops of the stock solution of hydrochloric acid were added to a solution of **compound A** in methanol / aluminum trichloride (2.2.10.3) (5 ml) and the spectrum was immediately recorded.

2.2.9.5- The UV spectrum of compound A in presence of NaOAc.

Excess of coarsely powdered anhydrous NaOAc was added with shaking to a cuvette containing (2-3 ml) of solution of **compound A** in methanol and the UV spectrum was recorded after two minutes.

2.2.9.6- The UV spectrum of compound A in presence of NaOAc /H_3BO_3.

Sufficient powdered anhydrous H_3BO_3 was added with shaking to a cuvette containing a solution of **compound A** in methanol/sodium acetate (5 ml)(2.2.10.5) and the UV spectrum was recorded after two minutes.

3- Results and Discussion

3.1- Extraction of flavonoids from plant material

Powdered air-dried bark of *Waltheria indica* was extracted with 95% ethanol at room temperature for 48hrs. Evaporation of the solvent *in vacuo* gave a crude product which was fractionated over Whatman No.3 paper sheets using 70% methano as eluant **.** After the usual workup a pure compound – compound A was isolated. The purity was monitored by TLC experiments where only a single spot was observed when three different solvent systems were employed.

3.2- Phytochemical screening

Analytical tests on the crude ethanolic extract were positive for flavonoids and steroids, but negative for alkaloids and glycosides(Table 3.1).

Table 3.1: Phytochemical screening of

Species	Flavonoids	Steroids	Alkaloids	Glycosides
	+	+	-	-

3.3- spectral dapta of compound A

The IR spectrum of compound I (Fig.1) showed ν(KBr) 3429 (O-H) , 2925 (C-H) , 1733 (C=O), 1458 (St.C-O) , 1558 (C=C) , 590 (C-H , Ar. Bending)

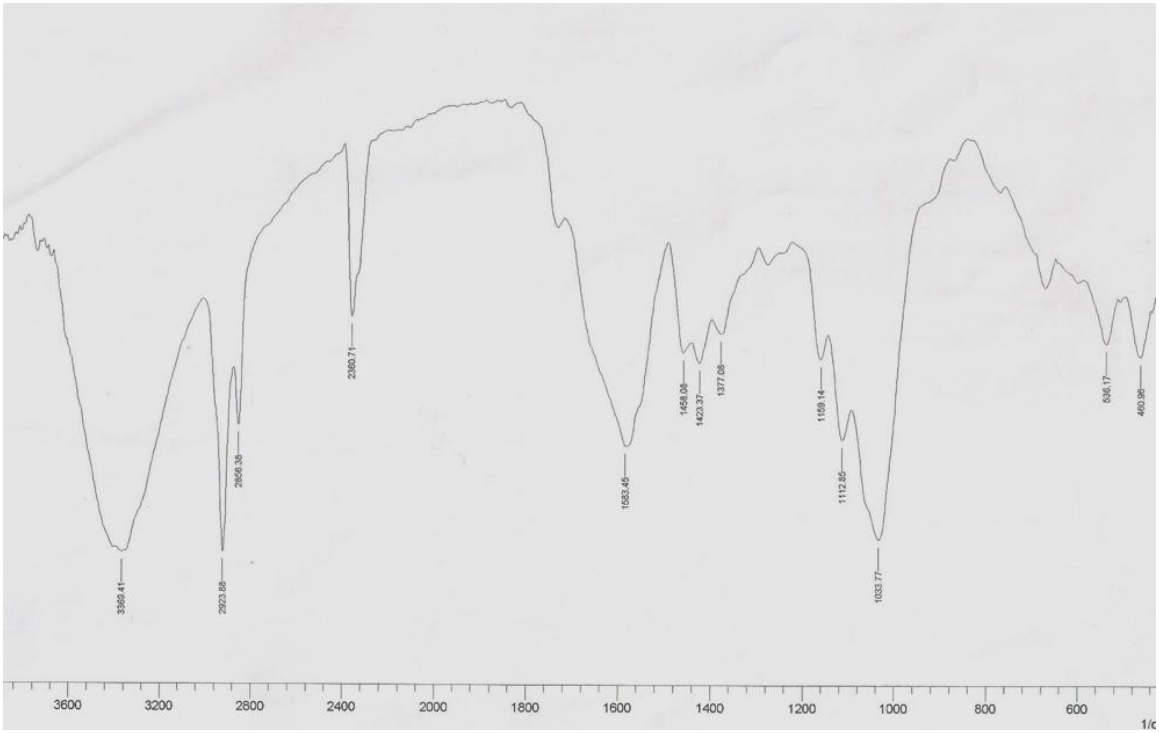
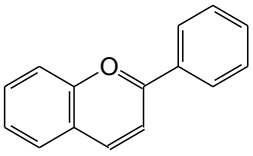
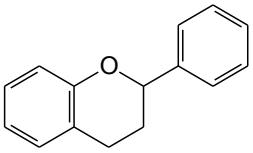


Fig. 1: IR spectrum of compound A

A carbonyl function was observed in the IR spectrum indicating that compound A is neither an anthcyanin nor a flavan. These classes of flavonoids are devoid of carbonyl functions.

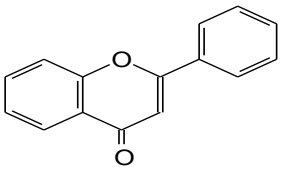


Anthocyanin

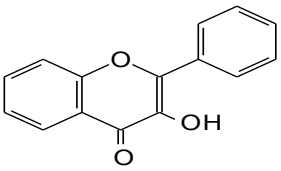


Flavan

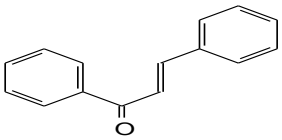
Compound A could be:a flavone , a flavonol, chalcone ,aurone, flavanone, isoflavone, dihydrochalcone or dihydroflavonol.



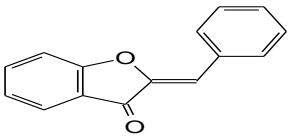
Flavone



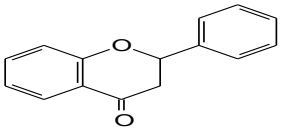
Flavonol



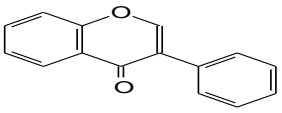
Chalcone



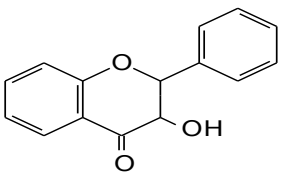
Aurone

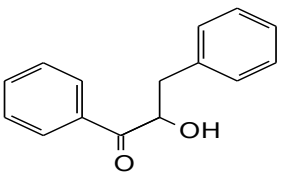


Flavanone



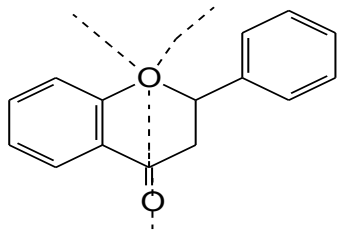
Isoflavone





Dihydroflavonol Dihydrochalcone

The UV spectrum of compound A revealed λ_{max}(MeOH) 298,364nm . The UV spectra of most flavonoids consists of two major absorption maxima , one of which occurs in the range 230-290nm (band II). Such absorption is due to A- ring benzoyl system .The other band appear in the range 300-400nm (band I) which is due to B-ring cinnamoyl system[200].



Benzoyl system Cinnamoyl system

Flavones, flavonols , chalcones and aurones give two UV peaks, band I and band II, due to conjugation between the C=O function and the B ring[201] , while flavanones, isoflavone, dihydroflavonols and dihydrochalcone afford only band II . These classes are characterized by loss of conjugation between the carbonyl function and the B ring.

Since the UV spectrum of compound A showed (Fig.2) both bands , then this compound is either : a flavone , flavonol, chalcone or aurone.

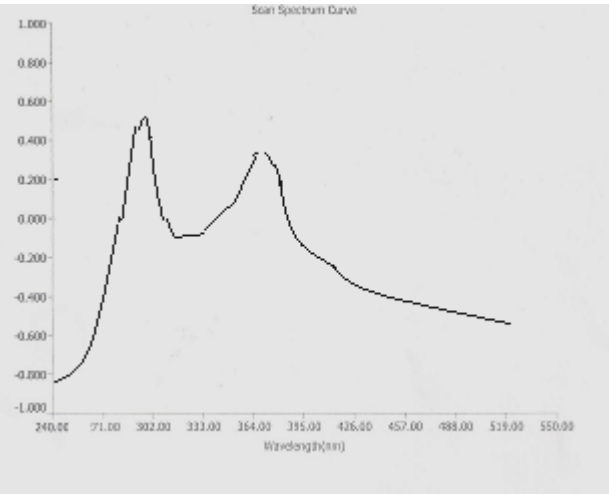


Fig.2: UV spectrum of compound A

However, the UV spectrum did not reveal a dominant absorption for the cinnamoyl chromophore which is diagnostic of chalcones.

Also the absorption of the cinnamoyl chromophore is not at all consistent with that of flavones . This suggests that this compound is either a flavonol or an aurone.

Flavonols are characterized by a 3-OH function which was not detected by the shift reagent sodium methoxide. This shift reagent is diagnostic of 3- and 4`-OH functions where it gives bathochromic shifts in presence of such functions. The sodium methoxide spectrum(Fig.3) of compound I did not show any bathochromic shift indicating absence of a 3-OH function and consecuently absence of a flavonol.

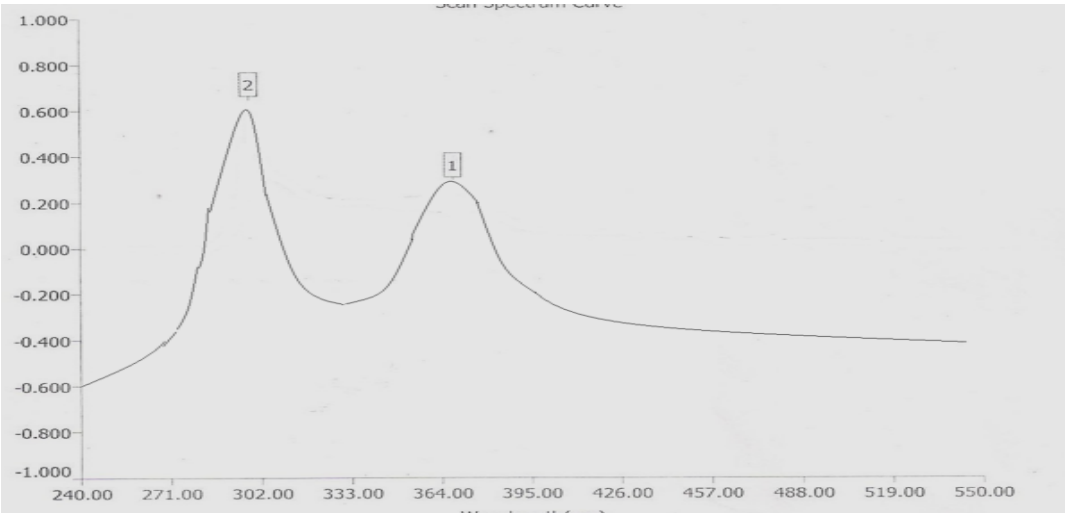
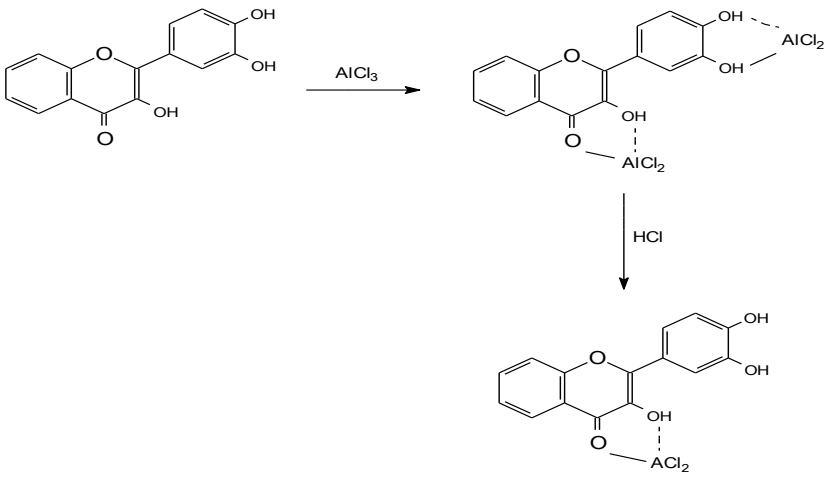


Fig. 3 : Sodium methoxide spectrum of compound A

The above argument suggests that compound A is probably an aurone.

Next , the hydroxylation pattern of the isolated flavonoid was investigated by further UV studies involving other useful shift reagents, namely , aluminium chloride, sodium acetate and boric acid .

The shift reagent aluminium chloride is diagnostic for 3-OH, 5-OH groups as well as catechol systems, where it gives bathochromic shifts when added to a methanolic solution of the flavonoid . Catechols form acid – labile complexes with aluminium chloride , while the 3-OH (or 5-OH) give acid – stable chelates as shown below[200].



When this shift reagent was employed for compound A (Fig.4) no bathochromic shift was detected indicating absence of 3- and 5- OH as well as catechol systems.

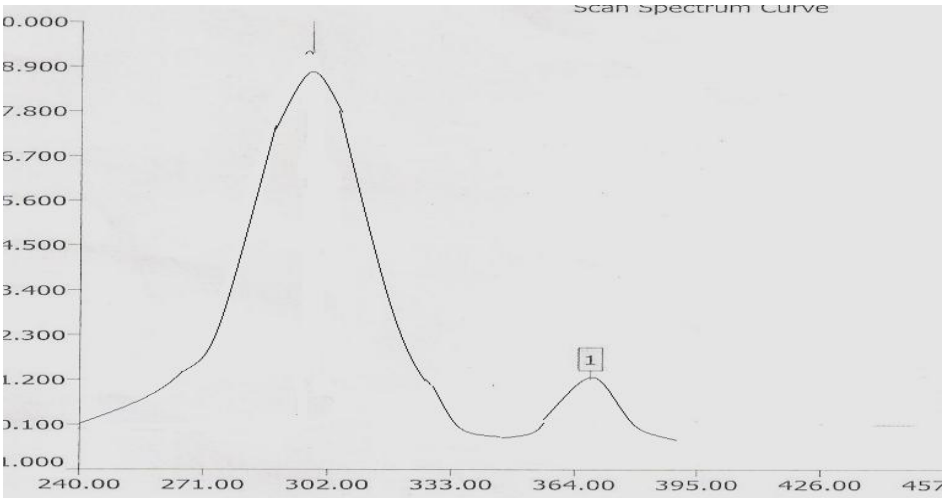


Fig.4: Aluminium chloride spectrum of compound A

The weak base, sodium acetate, only ionizes the more acidic 7-OH group . When sodium acetate was added to a methanolic

solution of compound A no bathochromic was observed (Fig.6)) indicating absence of a 7-OH group[200,201] .

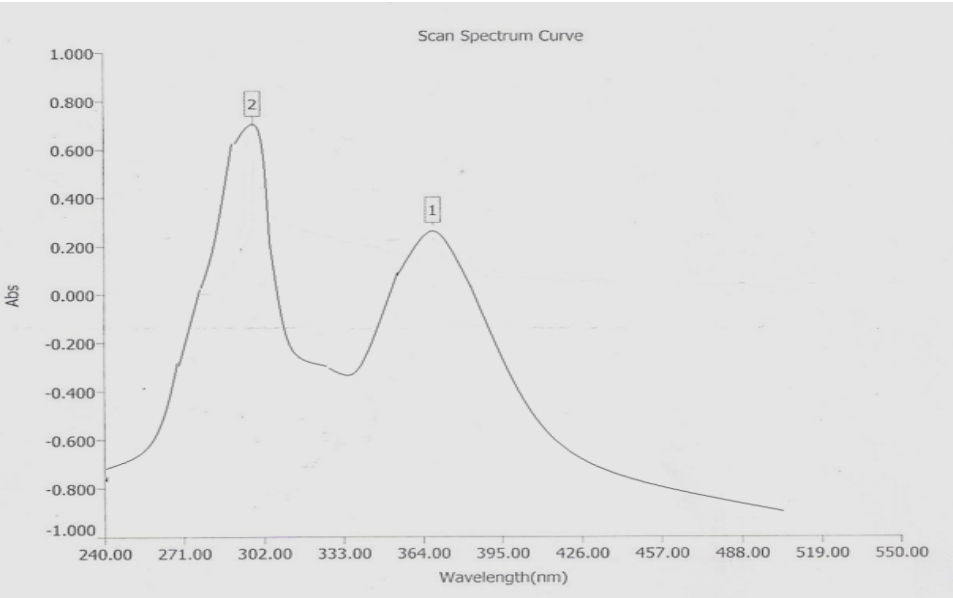


Fig. Sodium acetate spectrum of compound A

The shift reagent , boric acid is diagnostic of catechol systems. It induces bathochromic shifts , where it chelate with ortho – dihydroxy systems at all locations on the flavonoid nucleus[200] , except at $C_{5,6}$. When boric acid was added to a methanolic solution of compound A in presence of sodium acetate (Fig.7) no

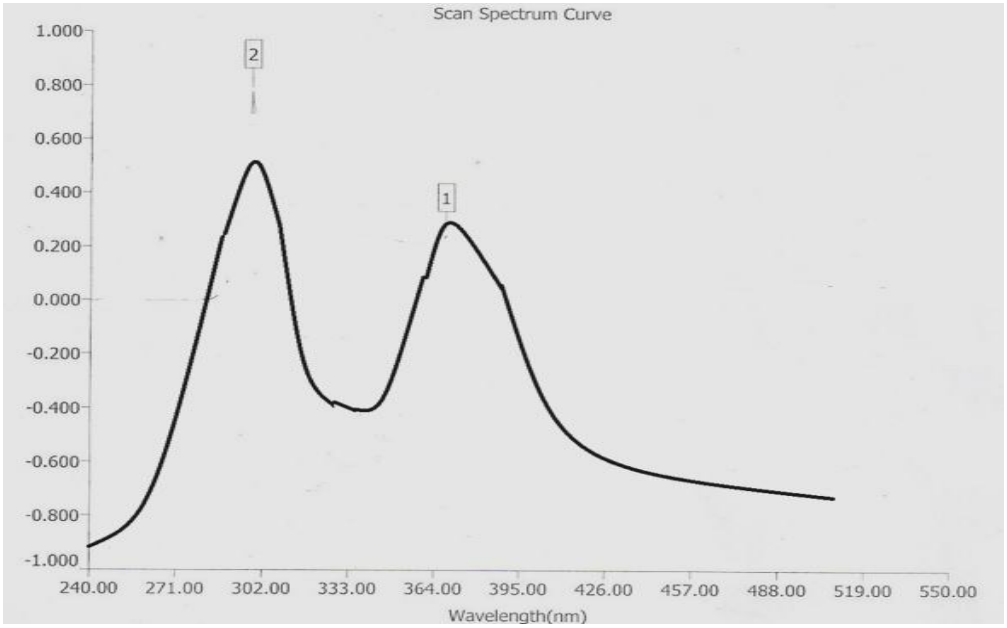


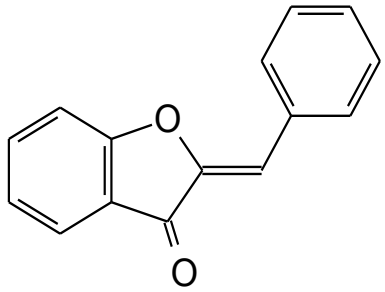
Fig. Boric acid spectrum of compound A

bathochromic shift was observed indicating absence of catechols and supporting the findings previously obtained by the shift reagent aluminum chloride .

The above argument suggests the following:

- Compound A is an aurone.
- Compound A is not hydroxylated at position 3 or 4` (shift reagent -sodium methoxide)
- Compound A is not hydroxylated at position -5 and it is devoid of catechol systems (shift reagent- aluminium chloride).
- Compound A is not hydroxylated at position -7 (shift reagent – sodium acetate).

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



Compound A

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