بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Study of the Major Flavonoids from *zingeber officinle bark* by UV-Visible Spectroscopy

دراسة الفلافونيد الرئيس فى لحاء نبات الزنجبيل 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 2008)

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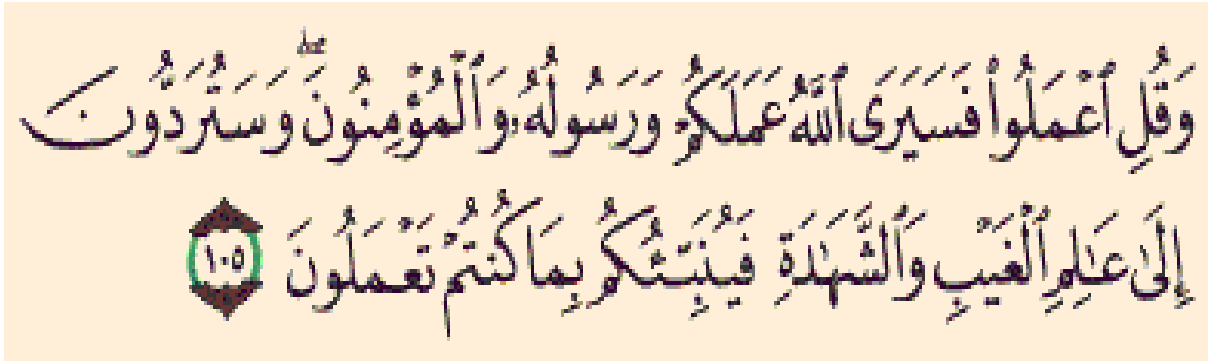
August, 2016



(التوبة-105)

Dedicaion

Dedicated to:

My parents,

My husband,

My brothers and sister.

Acknowledgement

First of all I would like to thanks Almighty Allah whose benevolent blessing enabled me to complete this work.

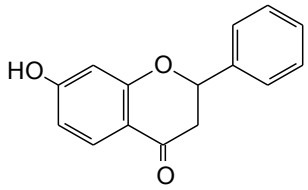
I wish to express my heartiest gratitude to my supervisor, Professor Mohamed Abd Elkarim Mohamed for his keen interest, invaluable help, expert guidance and continuous encouragement during the period of this research work.

My thanks are also extended to my family and friends for their patience and support.

Abstract

Phytochemical screening of the stem bark of Zingiber officiale revealed the presence of tannins,flavonoids and alkaloids.

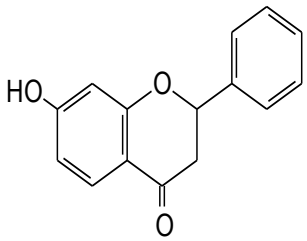
The bark of this species was macerated with 95% ethanol. A silica gel column developed with chloroform:methanol(3:2;v:v) gave apure flavonoid compound I. The structure of this isolate was elucidated using UV and IR data and The following partial structure was proposed:



المستخلص

في هذه الدراسة استخلص لحاء نبات الزنجبيل بالإيثانول واخضع المستخلص الإيثانولي للمسح الفيتوكيميائي والذي أوضح وجود الفلافنويدات،التنينات والقلويدات .

وعن طريق كروماتوغرافيا العمود تم فصل المركب الفلافنويدي- مركب 1 وتم تحديد التركيب الجزئي بالطرق المطيافية (طيف الاشعة فوق البنفسجية،طيف الأشعة تحت الحمراء) وقد اقترح التركيب الجزئي الأتي:



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I-Introduction

1.1 General approach

Flavonoids and their conjugates form a very large group of natural products. They are found In many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The chemical structures of this class of compounds are based on a C_6-C_3-C_6 skeleton. They differ in the saturation of the hetero atomic ring C,in the placement of the aromatic ring B at the positions C_2 or C_3 of ring C , and in the overall hydroxylation patterns (Figure1.1). The flavonoids may be modified by hydroxylation, methoxylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton[1].

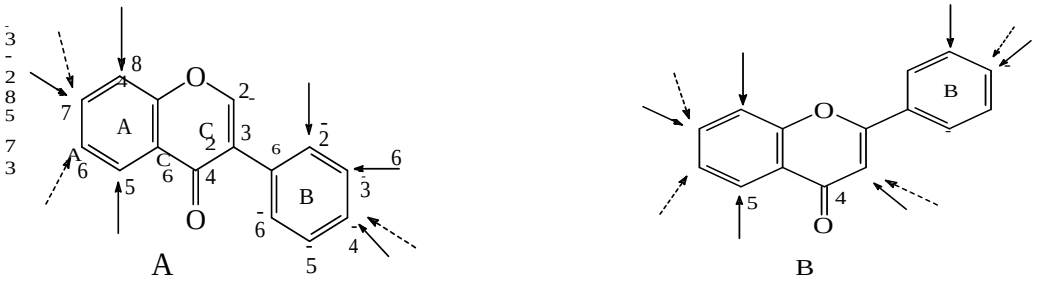


Figure1.1 Flavonoid structures, ring labeling, and carbon atom numbering. (A) Isoflavones.(B) Flavones and flavonols. Full arrows indicate most frequent hydroxylation sites and dashed arrows indicate most frequent C- and/or O- glycosylation sites.

In addition, alkyl groups (often prenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core. The last

modification takes place most often in the case of isoflavonoids, where the B ring is condensed to the C-3 carbon atom of the skeleton[1].

Flavonoid glycosides are frequently acylated with aliphatic or aromatic acid molecules. These derivatives are thermally labile and their isolation and further purification without partial degradation is difficult. The multiplicity of possible modifications of flavonoids result in more than 6,000 different compounds known in the end of The last century and this number continues to increase[2]. In the plant Kingdom, different plant families have characteristic patterns of flavonoids and their conjugates. All these compounds play important biochemical and physiological roles in the various cell types or organs (seed, root, greenpart, andfruit) where they accumulate.[1]

Different classes of flavonoids and their conjugates have numerous functions during the interactions of plant with the environment, both in biotic and abiotic stress conditions.[3,4] Additionally, flavonoid conjugates, because of their common presence in plants, are important components of human and animal diet. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative[5-9].

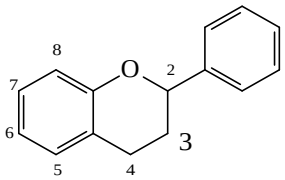
For the mentioned reasons, methods for the efficient and reproducible analysis of flavonoids play acrucial role in research conducted in different fields of the biological and medical sciences. The

Identification and structural characterization of flavonoids and their conjugates isolated from plant material, as single compounds or as part of mixtures of structurally similar natural products, create some problems due to the presence of isomeric forms of flavonoid aglycones and their patterns of glycosylation. A number of analytical methods are used for the characterization of flavonoids. In many cases, nuclear magnetic resonance (NMR) analyses (^{1}Hand^{13}C) are necessary for the unambiguous identification of unknown compounds; other instrumental methods (massspectrometry, UV and IR spectrophotometry) applied for the identification of organic compounds fail to provide the information necessary to answer all the structural questions. Utilization of standards during analyses and comparison of retention timesas wellas spectral properties, especially when compounds are present in a mixture, is critical. An important area of research on flavonoids is the identification of their metabolites in animal tissues and body fluids (urine, blood, spinalfluid). For this, investigators have to deal with different modifications of the flavonoid moieties, modifications often not found in plant tissues [10]. The metabolism of flavonoids in human and animal organisms, amongothers, is based on glucuronidation, sulfation, ormethylation[11,12]. The first two above-mentioned types of flavonoid aglycone modifications, occurring after their consumption by humans or animals, are less often found in the samples of plant origin. One of the goals in functional genomics and systems biology studies is metabolite profiling. Qualitative and quantitative monitoring of

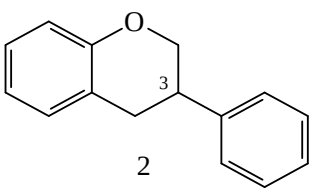
flavonoid derivatives, together with information about the level of transcription and protein expression, enables the elucidation of gene functions[13-16]. Another challenge in the field is to establish the flavonoid conjugate profiles ingenetically modified plant lines, e.g. engineered for higher resistance against environmental conditions (pathogenic microorganisms, insects, and physical stress factors such as temperature, drought, or UV light). The plant performance in different environmental conditions and the resulting effect on crop yield may be accompanied by increased synthesis of desired and/or undesired natural products in particular plant tissues, among the biologically active flavonoids or isoflavonoids[17-21].

1.2 Classification

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 aphenyl benzopyran 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.[1]



1



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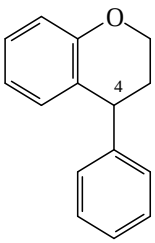
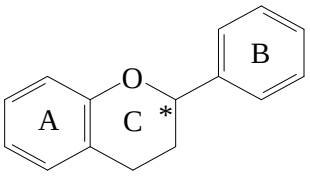


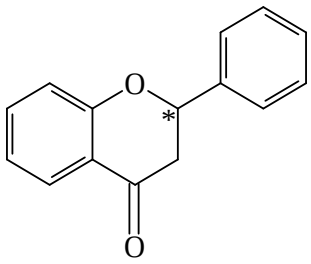
3

1.2.1 Phenylbenzopyrans

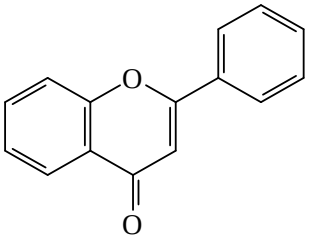
Based on the degree of oxidation and saturation Present in the heterocyclic C-ring, the flavonoids may be divided into the following groups:[1]



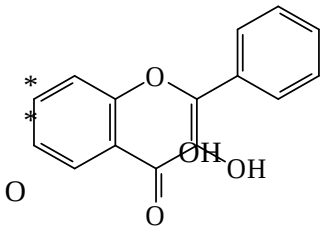
Flavan



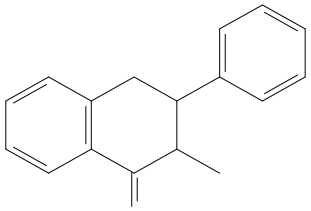
flavanone



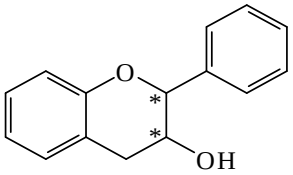
flavone



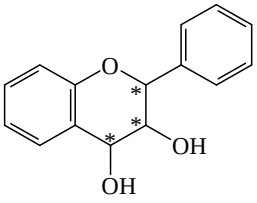
Flavonol



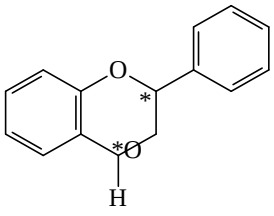
dihydroflavonol



flavan-3-ol



Flavan-3,4-diol



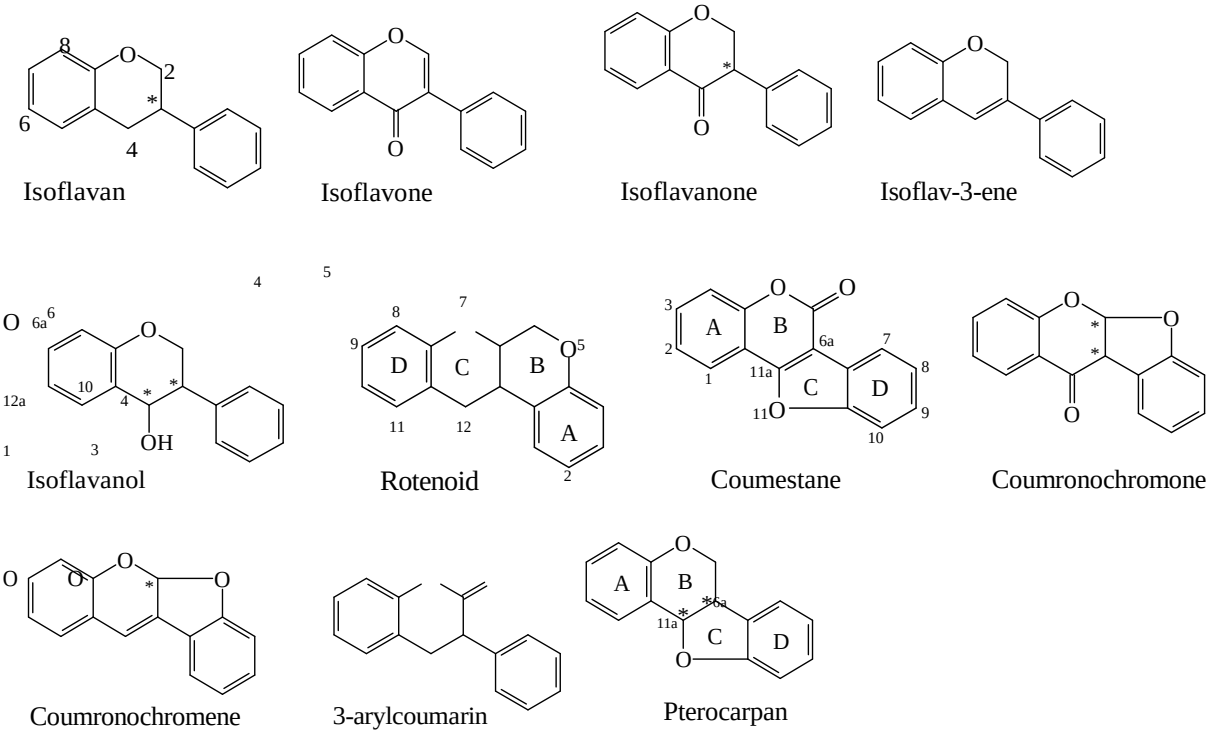
flavan-4-ol

streocenters

1.2.2 Isoflavonoids

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.

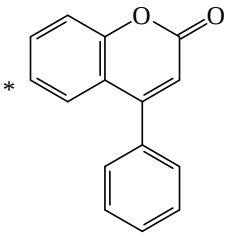
Isoflavonoids are subdivided into the following groups:[1]



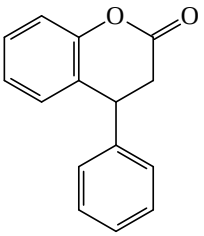
*stereocenters

1.2.3 Neoflavonoids

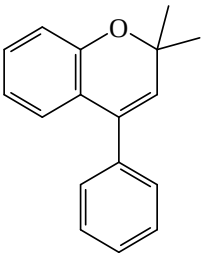
The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2*H*-1-benzopyran-2-ones), 3,4-dihydro-4-arylcoumarins, and neoflavenes.[1]



4-arylcoumarin



3, 4-dihydro-4-arylcoumarin

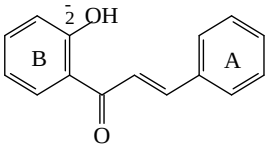


neoflavene

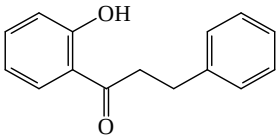
*stereocenters

1.2.4 MinorFlavonoids

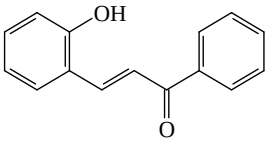
Natural products such as chalcones and aurones also contain a C_6-C_3-C_6 backbone and are considered to be minor flavonoids. These groups of compounds include the 2′-hydroxychalcones, 2′-OH-dihydrochalcones, 2′-OH-*retro*-chalcone, aurones(2-benzylidenecoumaranone), andauronols.[1]



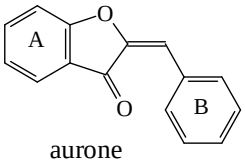
2-OH-chalcone



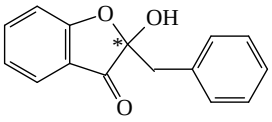
2 -OH-dihydrochalcone



2 -OH-retro-chalcone



aurone



auronols

1.3 Synthesis of flavonoids

1.3.1 Chalcones, Dihydrochalcones, and Racemic Flavonoids

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 abase-catalyzed aldol condensation or acid-mediated aldolization of 2-hydroxyacetophenones **4** and benzaldehydes**5**[22-24] (Figure1.2). The base-catalyzed aldol condensation is usually the preferred route towards chalcone **6** formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of corresponding racemic flavanones**7**.[25] Dihydrochalcones **8** are generally obtained via reduction (H_2/Pd) of the preceding chalcones (Figure1.2).

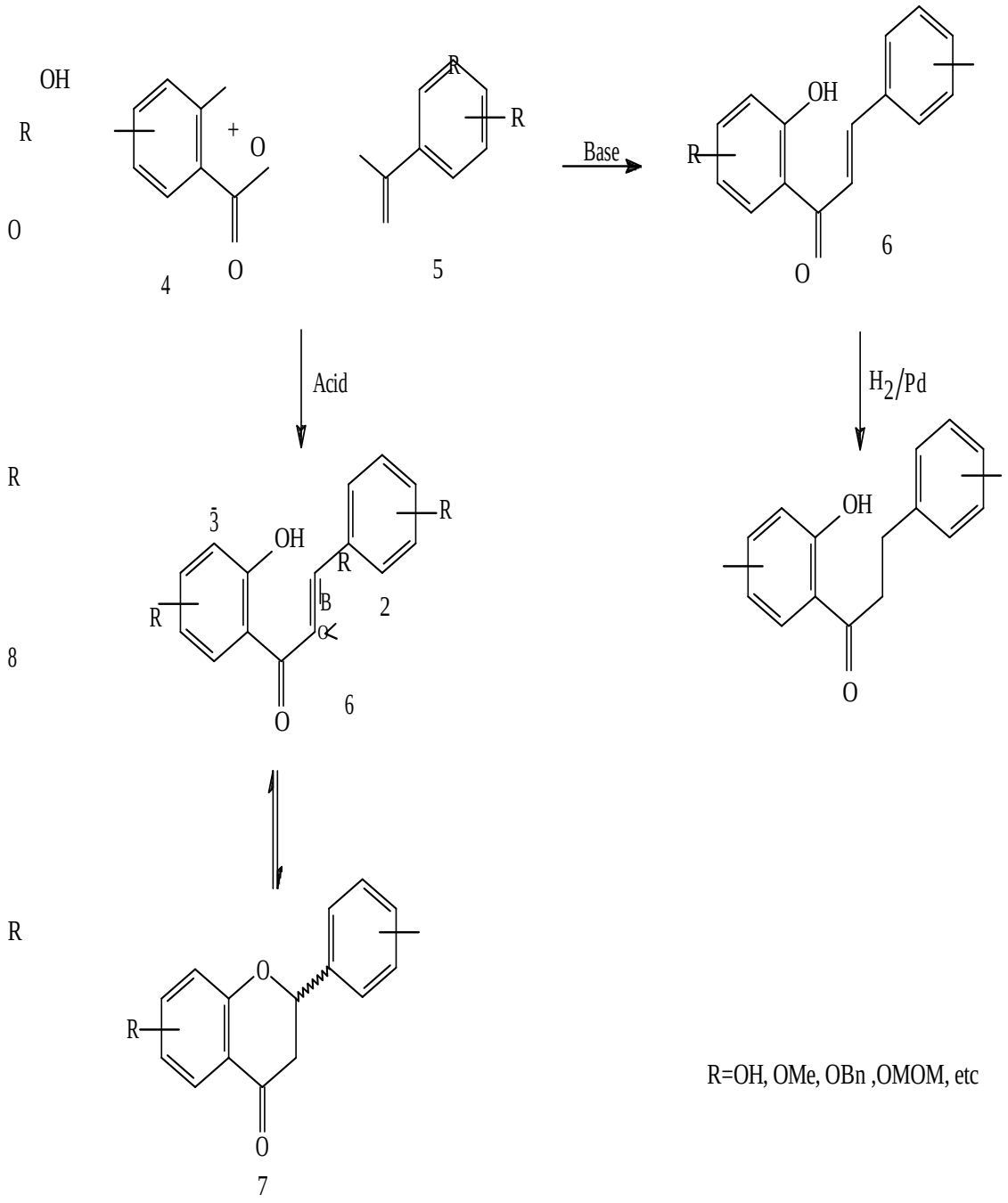


Figure1.2 Acid- and base-catalyzed synthesis of chalcones, racemic flavanones, and dihydrochalcones.

1.3.2 Individual Pathways of Flavonoid Biosynthesis

All classes of flavonoids are biosynthetically closely related, with achalcone being the first common intermediate. Earlier feeding

Experiments with radioactively labeled precursors have established that the carbon skeleton is derived from acetate and phenylalanine; ring A is formed by ahead-to-tail condensation of three acetate units and ring B as well as carbon atoms 2,3 and 4 of the heterocyclic ring C arise from phenylalanine. The biosynthetic relationships of the flavonoids asconcluded mainly from labeling experiments *in vivo* are illustrated in Fig.1.3. More recent investigations at the enzymic level have largely confirmed the hypothetic steps which had been deduced from incorporation experiments. In particular, detailed knowledge of the central reaction of flavonoid biosynthesis, the condensation of the acyl residues from one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA (Fig.1.3), was obtained by several authors. Extensive studies of the enzymology and regulation of flavones and flavonol glycoside biosynthesis revealed many details of the reactions involved in the formation of these two important classes of flavonoid.[26]

On the other hand, relatively little beyond the results of tracer studies is known about the synthesis of anthocyanins, isoflavonoids and other flavonoids.[26]

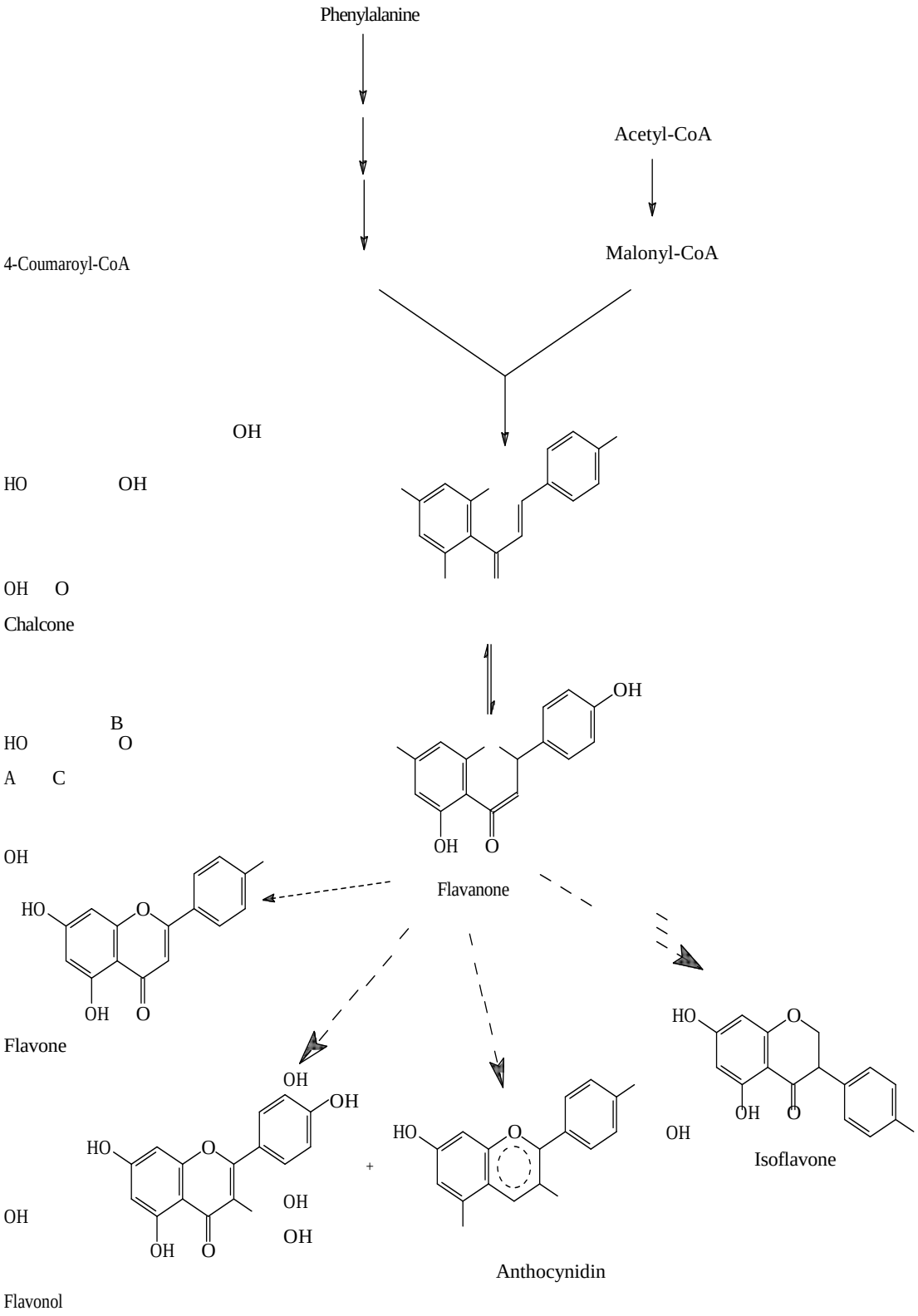


Fig.**1.3:** Schem eillustrating the position of the chalcone as the first common in termediate in the biosynthesis of all classes of flavonoid.

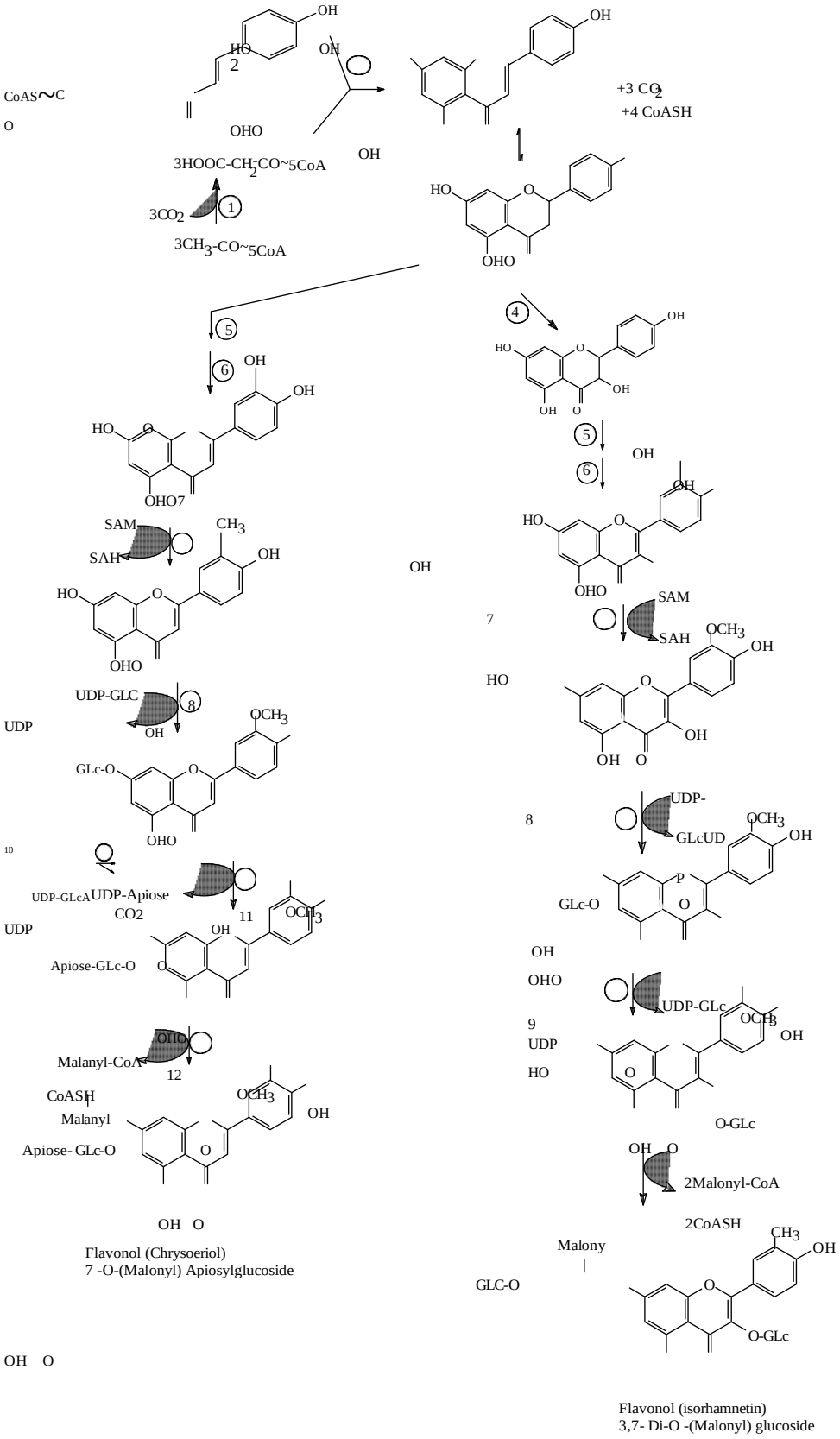


Fig.1.4: Scheme ilIustrating the sequence of reactions of the flavones and flavonol g1ycoside pathways.

The chalcone/flavanone isomers are the central intermediates in the synthesis of all flavonoids.[27,28] Compelling evidence has now been presented that the chalcone is the immediate product of the synthase reaction that catalyses the stereospecific formation of the (-)(2S)-flavanone. In a second step, chalcone isomerase form the corresponding chalcone. The frequent co-occurrence of chalcones, flavanones, flavones and flavonols suggests that their biosynthetic Pathways are closely related. *In vivo,* chalcones with a phloroglucinol-type substitution in ring A are exclusive intermediates in the formation of 5, 7-dihydroxyflavonoids, while chalcones with a resorcinol-type substitution in ring A are selectively converted into 7-hydroxyftavonoids.[27] However, an enzyme catalyzing the synthesis of a chalcone with a resorcinol structure in ring A has not been reported. Further , the actual mechanism sthrough which flavanones are Converted into flavones, flavonols and other ftavonoids are in most cases not well understood. Only a few studies with cell-free extracts have demonstrated the oxidation of flavanones to the corresponding flavones and the hydroxylation of flavanones in the 3 position to yield the corresponding dihydroflavonols. Several properties of the enzyme scatalysing flavanone oxidation and 3-hydroxylation indicate that they are not peroxidases. This is in contrast to earlier reports which demonstrated the conversion of 4,2',4'- trihydroxychalcone to the corresponding dihydroflavonol, flavonol and aurone either by Plant extracts or by peroxidase.[29-31]

The ftavonoid glycoside pathway in parsley consists of about 13 enzymes which catalyse several consecutive steps of flavones and flavonol biosynthesis. The first step is the conversion of acetyl-CoA to malonyl-CoA, which serves as substrate for three enzymes, chalcone synthase and two malonyltransferases. In the second step, the chalcone is formed from 4-coumaroyl-CoA (and possibly from other substituted cinnamoyl-CoA esters) and malonyl-CoA. The chalcone is isomerized to the corresponding flavanone, which is further converted into the basic flavones and flavonol structures. The subsequent steps are substitutions of ring B of the aglycones by hydroxylation and O methylation. The final steps are glycosylation of the aglycones and acylation of the resulting glycosides. The enzymes Involved in the individual steps, as elucidated with parsley cells.[26]

1.4 Isolation Techniques for Flavonoids

1.4.1 Plant preparation and extraction

Flavonoids occur in virtually all parts of the plant, the root, heartwood, sapwood, bark, leaf, fruit and flower, and the method of isolation depends to some extent both on the source material and the type of flavonoid being isolated. In cases when flavonoids occur in the surface oils or waxes, they may be obtained simply by scraping or washing the surface with an appropriate solvent.[32-34]

Ingeneral, however, the plant material is ground up or macerated before extraction. The possibility of enzyme action occurring during this early period of isolation, leading inparticular to hydrolysis of

glycosides,[35,36] may be avoided by plunging the plant material into boiling solvent or by rapid drying prior to extraction,[37] Pre-drying of plant material generally appears to increase the yield of extractives, possibly due to rupture of the cell structure and to the better solvent access provided as a consequence.

Solvents used for extraction are chosen according to the polarity of the flavonoids being studied. The less polar solvents are particularly useful for the extraction of flavonoid aglycones, whilst the more polar solvents are used if flavonoid glycosides or anthocyanins are sought. The less polar aglycones, such as isoflavones, flavanones and dihydroflavonols, or flavones and flavonols which are highly methylated, are usually extracted with solvents such as benzene, chloroform, ether or ethylacetate.[38-45]

Apre-extraction with light petroleum or hexane is frequently carried out to rid plant material of sterols, carotenoids, chlorophylls, etc.[46-48] However, it should be noted that even this treatmen tmay cause the loss (byextraction) of certain flavonoid ag1ycones. For example, the isoflavones, ichthynone and rotenone, have been isolated by extraction of *Piscidia erythrina* root wood with hexane[49] and methylated flavones such as hispidulin are known[40] to be significantly soluble in light petroleum.

Flavonoid glycosides and the more polar aglycones such as hydroxylated flavones, flavonols, biflavonyls, aurones and chalcones are generally isolated from plant material by extraction with acetone,

a1cohol, water or a combination of these,[46,50-54] Perhaps the most useful solvent for the extraction of this group of compounds is a 1:1 mixture ofwater and methanol. Boiling water has been found suitable for the extraction of polyglycosides such as a cacetin 7-0-diglucuronide[55] and the flavones polysaccharide compound from *Monoclea forsteri*[56] and also for the isolation of compounds such as flavandiols, catechins and procyanidins. Traces of acid are occasionally incorporated in the solvent for the extraction of flavonoid glycosides.[57] AIthough this practice is normally reserved for the extraction of anthocyanins and anthocyanidins.[37,58] The use of acid, however, can lead to hydrolysis of glycosidic materials.

1.4.2- Preliminary purification

When flavonoids of varying types are to be extracted from asingle batch of plant material, a worthwhile method for preliminary separation is sequential solvent extraction with a number of solvents of varying polarity. This can lead to separation of glycosides from Aglycones and to the separation of polar from non-polar aglycones.[47,44,42] Alternatively, sequential solvent extraction of a crude extract may be used to produce the same type of separation.[52,46,60,69] Counter current separation techniques may also be of value, particularly for the separation of flavonoids.[61-65] Distributions between water and an organic phase such as ethy lacetate,[48,66-68] or butanol: light petroleum,[69,48] have been found effective for this purpose.

Precipitation with lead acetate has been widely used in the past as a method of isolating phenolics (especially those with o-dihydroxyl groups) from other extractives in crude extracts,[37,70] It has the disadvantage, however, that it doesnot precipitate some phenols and may co-precipitate other compounds.

Decomposition of the lead salts formed is best carried out with sulphate or phosphate rather than with sulphide, in view of the highly adsorptive nature of the precipitated lead sulphide. Recently, a method using polyvinylpyrrolidone (PVP) as precipitant has been suggested for use in cases where lead acetate is unsatisfactory.[71] The optimum conditions for PVP-phenol bonding were established as pH 3.5 in 1-10% methanol in water. Treatment of crude plant extracts with charcoal powder is also a useful method for the preliminary purification of flavonoids, particularly glycosides.[59]

In one example of this procedure[72] flavonoids from *Baptisia lecontei* were eluted from activated charcoal by washing successively with methanol, boiling water, 7% aqueous phenol and 15% methanolic phenol. The bulk of the flavonoid material appeared in the 7% phenol fraction, which was subsequently ether-extracted to give aphenol-free aqueous solution rich in flavonoid glycosides.[59]

In certain circumstances dialysis has been found helpful as a preliminary clean-up procedure. It is of particular use in the isolation of high molecular weight flavonoids (and tannins) which are water soluble and are mixed with sugars or soluble in organic material in the

Crude extract. Markharn (1972) found dialysis useful in the separation of a flavone-polysaccharide compound (MW about 3000) from an aqueous plant extract of *Monoclea forsteri,* and considerable use has been made of this technique in the isolation of high molecular weight blue flavonoid 'complexes' from cornflower (MW 6200).[73] Commelina communis74 and Professor Blaauw iris.75 all of which were non-dialysable through a cellulose membrane. Flavonoids produced on hydrolysis of the non-dialysable commelinin.74 were dialyzable under the same conditions.59

1.4.3- Column chromatography

Column chromatography remains the single most useful technique for the isolation of large quantities of flavonoids from crude plant extracts.[65,72,76,77] The advent of the relatively new chromatographic media, polyamide and Sephadex, has had a dramatic effect on the type and efficiency of separations achieved, and 'dry column' techniques also seem to offer prospects of markedly improved chromatographic separations. These changes appear to at least partly fulfil the Requirement expressed by Harborne (1959b) for new adsorbents for column chromatography.[59]

Adsorbents commonly used for the separation of flavonoids include silica gel, kieselguhr, magnesol, cellulose, alumina, polyamide, sephadex and ion exchange resins. The adsorbents of choice have generally been silicagel, cellulose and polyamide.[59]

1.4.4- Thin-Layer chromatography

TLC is a technique which has developed rapidly during the last decade and to a limited extent It has replaced paper chromatography in analytical and small scale separations of flavonoid. However, it is also complementary to paper chromatography in that it provides new media for the separation of flavonoids on a small scale, and permits the use of awider variety of detecting reagent. As in column chromatography, the adsorbents of choice for the separation of flavonoids are silica, polyamide and cellulose. The mechanism of separation and the eluotropic series for each chromatographic medium as in column chromatography, but the solvent systems used often vary widely from those used for columns. Ingeneral, flavonoids are much more strongly held on thin-layers and as a result solvents of higher Polarity are required for their elution.[59]

1.4.4.1 Preparative scale TLC

TLC is essentially a technique for the separation of milligram quantities of material. However, it can be upgraded to handle up to a gram when layers of from 1-5 mm thick are used in conjunction with plates of up to 20 x100 cm in size.[76-78] A more promising method is that of 'dry column' chromatography,[79-81] which involves the use of columns packed with fine-powdered, dry adsorbent. Although this has Not as yet been used for the separation of flavonoids, resolution with other compounds is claimed to be as good as TLC when low sample loadings (e.g. 1 :500) are used.[82]

1.4.4.2 – Spray reagents and detecting methods

A part from the anthocyanins and some of the more intensely coloured chalcones and aurones, flavonoids are not sufficiently coloured to be visible to the naked eye on a thin-layer plate; thus, some form of visualization I snecessary for spot detection. In many cases this is achieved by viewing the plate in UV light (366 nm) either in the presence or absence of ammonia vapour.[72] Detection under UV is often assisted by the use of layers which contain a UV-fluorescent indicator (e.g. silica gel GF 254).[59]

Flavonoids appear as dark spots against a fluorescent green background. Another useful method of detection is brief exposure of the plate to iodine vapour which produces yellow-brown spots against a white background with most flavonoids. These techniques all have the advantage that they are non-destructive.[59]

1.4.4.3 – TLC Separations on silica gel

Flavones, Flavonols and Biflavonoids. 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-diOH, 6,8,3',4'-tetraOMe), scaposin(5,7,3'-triOH, 6,8,4',5'tetraOMe), and demethoxysudachitin (5,7,4'-triOH, 6,8,-diOMe) have been chromatographed using chloroform-methanol (15 :1),[44] and digicitrin (5,3'-diOH, 3,6,7,4',5'pentaOMe)and a number of related compounds were separated using benzene-ethyl acetate (3: 1).[83] Flavonol polyacetates and polymethyl ethers have been successfully

Chromatographed by Egger (1969) 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),[84] *hexane-acetone-n-butano1* (8:1:1 and 17:2:1),[85] benzene-acetone (3:1), (9:1), (49:1), (92.8:7.2),[85-87] and chloroform-ethyl acetate (1 : 1),[88] the degree of solvent polarity depending largely upon the extent of methylation in the flavonoids.[59]

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'tetraOH) and morin (3,5,7,2',4' -pentaOH) separate well in toluenechloroform-acetone (8 : 5 : 7), [87] *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 by Hörhammer *et al.* (1964) 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 Isoi (1965) have had success with chloroform-acetic acid (9:1) and toluene-ethyl formate-formic acid (5:4:1) for the chromatography of C-methyl flavones such as sideroxylin and eucalyptin (the 7-and 7,4'-methyl ethers of 6,8-d C methylapigenin respectively). Other solvents, such as benzene-dioxan acetic acid (90: 25 :4),[89] benzenepyridine-ammonia (80 :20:1),[89] and acetone-benzene (1:3),[31] have also been used with Flavones and flavonols.

Biflavonoids are chromatographed in solvents similar to those above; for example, toluene-ethyl formate-formic acid (5:4:1),[53,90] and benzenepyridine- formic acid (36: 9: 5),[91] Fully methylated biflavonoids have been separated by Chexal *et al.* (1970) in benzene-pyridine-ethyl formate-dioxan (5: 1: 2 :2). This solvent even permitted separation of the isomeric pairs of hinokiflavones (i.e. those containing the *4-O-8'* and *4-O-6'* interflavonoid linkages) and of amentoflavones (i.e. with the 3-8' and 3-6' linkages). Flavone and flavonol glycosides are not commonly chromatographed on SiO_2. However, where this has been done, polar solvents such as ethyl acetate-butanone-formicacid-water (5: 3:1:1), n-butanol-2NHCI (1:1, upper phase) and isoamyl a1cohol-acetic acid-water (2:1:1)have been used.[92,90,87,93] Mono-, di-and triglycosides of avariety of flavones and flavonols have been chromatographed in the first of these solvents,[87] but distinction of flavonoids within each glycoside group was not good. However, monoglycosides (with *Rf* values of 0.46-0.65) were clearly separated from diglycosides (with *Rf* values of 0.21-0.36) and triglycosides (with *Rf* values of 0.06-0.16). Chopin (1971) found $Si0_2$ more sensitive than paper for distinguishing Flavones C-glycosides.[59]

In particular, the solvent system, ethyl acetate-pyridine-water methanol (80: 12: 10: 5) separated the paper chromatographically indistinguishable C-glucosides and C-galactosides. *Rf* values obtained for acacetin glycosides were as folIows: 6- and 8-C-glucosides 0.44,0.63: 6- and 8-C-galactosides 0.32, 0.46: 6- and 8-C-xylosides 0.6

0.70: 6,8-di-C-glucoside 0.11; 6,8-di-C-xyloside 0.30; 6-C-xyloside-8-C-glucoside 0.19. Magnesol has been considered superior to silica for flavonolglycosides,[94] and quercitrin was separated from isoquercitrin using toluene-ethyl formate-formic acid (5: 4: 1). Silica gel G impregnated with complexing or buffering anion ssuch as borate, molybdate, tungstate and acetate has been used successfully for the isolation of kaempferol and quercetin triglycosides and their *p-coumaryl* derivatives from *Pisum sativum.*[95] Silica gel-cellulose(1:1),[96] and silica gel-starch,[97] mixtures have also been used with some Success and it is claimed that they combine the advantages of $Si0_2$ and cellulose.[59]

Isofavones, flavanones, and dihydroflavonols. These flavonoids are generally chromatographed using less polar solvents than those required for the common flavones and flavonols. For example, the isoflavones daidzein, formononetin, genistein and biochanin A have been separated using chloroform-methanol (92: 8,3: 1 and 1: 1),[98,99] and ethyl acetate-light petroleum (3:1 and 1:1).[98] Isoflavones from the heartwood extracts of *Cladrastis lutea* have been chromatographed with chloroform-methanol (4:1) and ether.[41] With the latter solvent, the order of elution was related to the extent of substitution in that the *Rf* value of formononetin (7-0H, 4'-OMe) > c1adrin and afrormosin (7-0H, 3'4'-diOMe and 7-0H,6,4'-diOMe) > cladrastin (7-0H,6,3',4'-triOMe). The highly methylated isoflavonoids of *Cordyla ajricana* heartwood were isolated by preparative TLC with chloroform-benzene-acetone(10: 10: 1).[100]

F1avanones such as naringenin, hesperetin and isosakuranetin have been distinguished and identified by Mizelle *et al.* (1965), using benzene-acetic acid-water(125: 72: 3), benzene-nitromethane-water (3: 2: 5, upper layer), chloroform-acetic acid-H_2O (2: 1: 1) and by Hörhammer *et al.* (1964), using benzene-pyridine-formic acid(36: 9: 5). Dihydroflavonolssuch as taxifolin and aromadendrin are conveniently separated[101] with chloroform-methanol-acetic acid (7: 1: 1). Glycosides of isoflavones and flavanones have also been chromatographed successfully on $Si0_2$.The *8-C-* and 7-O-glycosides of daidzein were separated using n-butanol-acetic acid-water (4: 1: 5, lower layer)[46] as also were the 7-O rhamnoglucosides of the flavanones hesperetin, eriodictyol and naringen in.[102] However for the separation of these latter glycosides Mizelle *et al.* (1965) preferred to use cellulose and polyamide layers. Mixtures of glycosides and acety1ated glycosides of formononetin, genistein and biochanin A (ex. *Trifolium* species) have been successfully separated[99] using chloroform- acetone-methanol (20: 6: 5) and ethyl acetate-methano1-

water (100: 16.5: 13.5).[59]

Chalcones and aurones. A number of synthetic aurones have been chromatographed by Hanse1 *et al.* (1963), on $Si0_2$ G plates buffered with sodium acetate, in benzene-ethyl acetate-formic acid (9:7:4), chloroform-ethyl acetate-formic acid (6: 3: 1) and toluene-ethyl formate-formic acid (5: 4: 1). The same workers separated chalcones on Si02 –kieselguhr plates with cyc1ohexane-ethyl acetate (7:1)saturated with formamide-water (2:1). Harbome (1966) used benzene-

Ethyl acetate-formic acid (9: 7: 4) in the identification of isosalipurposide (chalcononaringenin2'-glucoside), and Dhar (1972) separated isomeric chalcones and flavanones with ligroin-ethyl acetate (1:1).[59]

Anthocyanidins and anthocyanins**.** Although chromatography on paper or TLC-cellulose is the method of choice for the separation of anthocyanins and anthocyanidins,[76,58,103] a number of separations have been achieved on silica. Harbome (1967b) recommends ethyl acetate-formic acid-2N HCI (85 : 6 : 9) for the separation of anthocyanidins generally, and in particular for the separation of malvidin (3,5,7,4'-tetraOH, 3'5'diOMe) and peonidin (3,5,7,4'-tetraOH, 3'-OMe) which are difficult to distinguish by paper chromatography. Solvents such as n-butanolacetic acid-water(4:1: 2) and ethyl formate-methyl ethyl ketone-formic acid-water (3: 4: 1: 2) are satisfactory for the separation of both anthocyanins and anthocyanidins.[76,104] Effective separation of anthocyanidin derivatives is thought to be dependent on the presence of trace metals in the $Si0_2$ which retard the movement of catechol derived pigments, such as cyaniding and delphinidin, by complex formation. [58] The anthocyanins of species of *Medicago,* e.g. the 3,5-diglucosides of delphinidin, petunidin and malvidin separate in ethyl acetate-butanone-formic acid-water (5: 3: 3: 1),[103] but some hydrolysis of the anthocyanins occurred with solvents containing higher levels of formic acid. The anthocyanins from *Vitis vinifera* skins (peonidin, malvidin, petunidin and delphinidin 3-glucosides and their acyl

derivatives) have been effectively separated using n-butanol-ethyl acetate-benzene-formic acid (l : 1 : 1 : 1) saturated with paraformaldehyde on p1ates buffered with sodium acetate.[105] Acid-washed $Si0_2$ was used for the preparative separation of the 3-O-glucoside and 3-O-rhamnoglucoside of cyaniding from blackcurrant juice with ethyl acetate-butanone-formic acid-water (6: 3: 1: 1).[106] Asen (1965) used 1 mm thick layers of silica gel-cellulose (2: 1) and n-butanol-2N HCl (l: 1) to separate cyaniding (3,5,7,3' ,4'-penta OH) glycosides from their pelargonidin (3,5,7,4'-tetraOH) equivalents, and claimed that the separations achieved were better than with $Si0_2$ alone. Individual glycosides were further purified using a water-2N HC1-formic acid (8: 4: 1).[59]

1.5- Spectroscopic techniques applied to flavonoids

Atypical analysis involving spectroscopic techniques embraces structural elucidation including determination of stereochemical attributes. However, it may also be aimed at tracing specific compounds and presenting quantitative aspects, or revealing color depiction.[107]

1.5.1- NMR Spectroscopy

NMR spectroscopy is an extremely powerful analytical technique for the determination of flavonoid structures,[108-111] but it is limited by poor sensitivity, slow throughput, and difficulties in analysis of mixtures .Recent developments have, however, made NMR arguably the most important tool for complete structure elucidation of

flavonoids. Today, it is possible to make complete assignments of all proton and carbon signals in NMR spectra of most flavonoids isolated in the low milligram range. These assignments are based on chemical shifts (δ) and coupling constants (J) observed in 1D ^{1}H and ^{13}C NMR spectra combined with correlations observed as crosspeaks in homo- and hetero nuclear 2D NMR experiments. Other nuclei like ^{17}O NMR spectroscopy has been used to study flavonoids only in a few cases. Natural abundance ^{17}O NMR spectra have been recorded for 11 methoxyflavones,[112] and ^{17}O NMR data for some 3-arylidenechromanones and flavanones have recently been discussed interms of mesomeric and steric substituent interactions.[113] ^{17}O NMR spectroscopy has also been used to study the effect of sugar on anthocyanin degradation and water mobility in aroselle anthocyanin model system.[114]

1.5.1.1 NMR solvents

The most frequently used NMR solvents for flavonoid analyses are hexadeuterodimethylsulfoxide (DMSO-d6) and tetradeuteromethanol (CD_3OD). Anthocyanins require the addition of an acid to ensure conversion to the flavylium form. For the analysis of relatively nonpolar flavonoids, solvents such as hexadeuteroacetone (acetone-d6), deuterochloroform ($CDCl_3$), carbontetrachloride (CCl_4), and pentadeuteropyridine have found some application. The choice of NMR solvent may depend on the solubility of the analyte, the temperature of the NMR experiments, solvent viscosity, and how

Easily the flavonoid can be recovered from the solvent after analysis. In recent years, the problem of overlap of solvent signals with key portions of the NMR spectrum has been reduced by solvent suppression and the application of improved 2D and 3D NMR techniques.[107]

1.5.1.2- NMR experiments

The purpose of a standard ^{1}H NMR experiment is to record chemical shifts, spin–spin couplings, and integration data, thus providing information about the relative number of hydrogen atoms. Applied to a flavonoid, this information may help in identifying the aglycone and acyl groups, the number of monosaccharides, and the anomeric configuration of the monosaccharides. However, for most flavonoids the information provided by a standard ^{1}H NMR experimentis insufficient for complete structural elucidation. Thus, ^{13}C NMR experiments (spin–echo Fourier transform, SEFT, compensated attached protontest, CAPT, etc.) combined with various 2D NMR experiments, especially those using gradient techniques that imply increased sensitivity, have to be used for assignments of all ^{1}H and ^{13}C NMR signals.[107]

1.5.1.3 COSY and TOCSY

Two-dimensional ^{1}H–^{1}H COSY (correlation spectroscopy) experiments allow determination of the protons that are spin–spin coupled, and the spectrum shows couplings between neighboring protons ($^{2}J_{HH}$, $^{3}J_{HH}$, and $^{4}J_{HH}$) revealed as crosspeaks in the spectrum.

The $^{1}H–^{1}H$ DQF-COSY (double-quantum filtered) experiment is a modification of the standard $^{1}H–^{1}H$ COSY experiment.[107]

The main advantage of the DQF techniqueis that noncoupled proton signals are eliminated. The DQF technique eliminates the strong solvent signal and the often very strong H_2O signal as sociated therewith, which may overlap with flavonoid sugar signals. The DQF-COSY experiment is routinely used in flavonoid analysis to assign all the sugar protons. The use of a ‘‘sequentialwalk’’ approach may provide information on the relative positions of individual proton signals along aspin system.[107]

The 2D homonuclear $^{1}H–^{1}H$ TOCSY (Total Correlation Spectroscopy) experiment identifies protons belonging to the same spin system. As long as successive protons are coupled with coupling constants larger than 5 Hz, magnetization is transferred successively over up to five or six bonds. The presence of heteroatoms, such as oxygen, usually disrupts TOCSY transfer. Since each sugar ring contains a discrete spin system separated by oxygen, this experiment is especially useful for assignments of overlapped flavonoid sugar Protons in the 1D ^{1}H NMR spectrum. It must be understood that the Crosspeak intensity is not an indicator of the distance between the protons involved, and that all expected correlations may not appear in a TOCSY spectrum. To avoid this latter problem It may be helpful to record a second spectrum with an other mixing time.[107]

Most of the sugar proton signals found in flavonoids occurs in the narrow spectral region of 4.5 to 3.0 ppm. Thus, for complex flavonoids containing several sugar units, extensive overlap occurs in this part of the spectrum. $^{1}H–^{1}H$ sugar coupling constants for such compound scan, however, be accessible by using the selective 1D TOCSY experiment, also known as the HOHAHA (homonuclear Hartman–Hahn) experiment. In the 1D TOCSY experiment, the Resonances of one proton are selected and the signal formed is transferred in a stepwise process to all protons that are J-coupled to this proton. Instead of crosspeaks, magnetization transfer is seen as increased multiple intensity. Thus, this 1D TOCSY spectrum looks like a normal ^{1}H NMR spectrum including only the protons that belong to the same spin system as the chosen proton. TOCSY experiments have together with other NMR experiments been used for the structural elucidation of flavonols from.[107]

1.5.2 Mass spectrometry

Modern mass spectrometric techniques are very well suited for the analysis of flavonoids isolated from plants and foodstuffs and in their in vivo metabolite forms. Progress during the last two decades has made MS the most sensitive method for molecular analysis of flavonoids. MS has the potential to yield information on the exact molecular mass, as well as on the structure and quantity of compounds with the nature and within the mass range of flavonoids. Further more, due to the high power of mass separation, very good selectivities can also be obtained. The purpose of the MS techniques

Is to detect charged molecular ions and fragments separated according to their molecular masses. Most flavonoid glycosides are polar, nonvolatile, and often thermally labile. Conventional MS ionization methods like electron impact (EI) and chemical ionization (CI) have not been suitable for MS analyses of these compounds because they require the flavonoid to be in the gas phase for ionization. To increase volatility, derivatization of the flavonoids may be performed. However, derivatization often leads to difficulties with respect to interpretation of the fragmentation patterns. Analysis of flavonoid glycosides without derivatizationbecame possible with the introduction of desorption ionization techniques. Field desorption, which was the first technique employed for the direct analysis of polar

Flavonoid glycosides, has provided molecular mass data and little structural information.[115] The technique has, however, been described as ‘‘notorious for the transient nature of the spectra,’’[240] and drawbacks related to the preparation of the MS samples have restricted application of this technique. Another method, desorption chemical ionization (DCI), provides rapid heating of the analyte and Overcomes the problem of the thermal decomposition inherent in conventional CI. The combined use of positive-and negative-ion DCIMS has been shown to be an alternative approach for the structural characterization of flavonoid glycosides;[117] however, this method has been applied in frequently to flavonoid analysis inrecent years.[118,119] Plasma desorption mass spectrometry (PD-MS) is another MS method used for flavonoid analysis; however, its application has

In recent years been limited to some papers on anthocyanins including deoxyanthocyanidins.[120-122] Fast atom bombardment (FAB) MS is still popular for flavonoid analysis. In this technique, the flavonoid is solubilized in a non volatile polar matrix and deposited on acopper target, which is bombarded with fast neutral energized particles such as xenon or argon and thereby inducing the desorption and ionization.[107]

In addition to giving accurate molecula rmasses of molecular ions, fragmentation patterns revealed by some MS methods may provide (a) structural information about the nature of the aglycone and substituents (sugars, acylgroups, etc.), (b) interglycosidic linkages and aglycone substitution positions, and (c) even some stereochemical information. The amount of structural information obtained for flavonoids from a masss pectrum depends on the ionization method used.[123]

1.5.3 Vibrational spectroscopy (IR and Raman)

Two different types of spectroscopic techniques are most frequently used to view the fundamental modes of molecular vibrations, namely mid-IR spectroscopy and Raman spectroscopy[124] The first method measures the absorption, transmission, or reflection of IR radiation with wavelengths in the range of 2.5 to 25 µm. The Raman method irradiates the sample with radiation of much shorter wavelengths and Measures the fraction of scattered radiation for which the energy of the photon has changed. The vibrational spectra may serve as fingerprints of structure, composition, interactions, and dynamics. The

Reciprocal of wavelength, wave number (cm^{-1}), is commonly used to characterize the energy in the field of vibrational spectroscopy.[107]

These investigations have usually been accompanied by UV–Vis spectroscopic and x-ray crystallographic analysis, as well as quantum chemical calculations. The main focus has been on the effects of position and nature of substituent (hydroxyl or methoxyl groups) on the molecular structure, including investigations of the dihedral angle between the phenyl ring and the chromone part of the molecule. The vibrational spectra of various simple flavonoids in solid state have been compared with those obtained in solutions, and differences between the solid state spectra and solution-state spectra were explained by the possibility of the formation of intramolecular hydrogen bonds present in the solid state and under specific solution conditions, or formation of intermolecular hydrogen bonds with the Solvent (CH_3OH).[107]

The raman spectra were preferred in most of these studies because they were considerably less complex than the corresponding IR spectra. The structures of a variety of flavonoid–aluminum ion complexes, including the complexes of aluminum(III) with 3-hydroxyflavone,[125] 5-hydroxyflavone,[126] 3',4' dihydroxyflavone,[127] quercetin,[128]and quercetin 3-glucoside,[129] have also been examined by this research group. The influence of pH and Al^{3+} concentration on the complex formation was considered, and molecular conformations of both the free and complexed flavonoids were proposed. Recently,

these flavonoids have been used as model compounds for the study of the behavior of humic substances toward Al(III) complexation.[130] Other complexes between flavonoids and metal ions investigated by IR spectroscopy include an alumina–(+)-catechin solution system,[131] and some prepared organotin(IV) complexes with the flavonoid glycosides, rutin, hesperidin, and 2',4',3-trihydroxy-5',4-dimethoxychalcone4-rutinoside, and with the aglycones, quercetin, morin, hesperitin, and some flavones.[132] The FTIR spectra of these latter complexes were consistent with the presence of Sn–O (phenol or carbohydrate) vibrations in the compounds, and the structures of the complexes were measured by Mössbauer spectroscopy.

FTIR spectroscopy has been used for the reexamination of the carbonyl stretching frequency of some simple hydroxylflavones in argon and methanol–argon matrices,[133] and IR spectra have been recorded for some simple flavonoids including sulfonic acid derivatives.[134]

In study of the Raman spectrum of Artocarpus heterophyllus heartwood has shown to exhibit two characteristic bands at 1247 and 745 cm^{-1}.[135] Based on Raman measurements of pure flavones and related compounds, it was predicted that the Raman band at 1247 cm^{-1} was attributed to flavonoid-type compounds. In this case, no vibrational band corresponding to the characteristic Raman bands was observed by diffuse reflectance IR spectroscopy.[135]

By using solid-state FTIR and Raman spectroscopies an inclusion complex between 2',6'-dimethoxyflavone and formic acid has been identified.[136] The broad and intense IR absorption observed in the Range 3400 to 1900cm^{-1}, assigned to the hydrogen-bonded OH-group stretching vibration, exhibited the characteristic ABC structure of strong hydrogen-bonded complexes in good agreement with previous x-ray data showing that cis-formic acid was strongly hydrogen bonded to 2',6'-dimethoxyflavone. The inclusion complex was quite unstable, and the IR spectrum clearly showed that formic acid disappeared after a period of a few months.[107]

1.5.4 - Ultraviolet–visible absorption spectroscopy

The application of standardized UV (or UV–Vis) spectroscopy has for years been used in analyses of flavonoids. These polyphenolic compounds reveal two characteristic UV absorption bands with maxima in the 240 to 285 and 300 to 550 nm ranges. The various flavonoid classes can be recognized by their UV spectra.[137] the combination of HPLC equipped with a UV–Vis DAD (diode array detection) has for the two last decades been the standard method for the detection of flavonoids in mixtures.[107]

The use of UV shift reagents such as $AlCl_3$ (5% in methanol)–HCl (20% aqueous), NaOMe (2.5% in methanol), and NaOAc (3mg)–H_3BO_3 has provent obevery useful as guidelines for substitution patterns of many flavonoids; however, the use of these reagents has mainly been applicable for purified flavonoids.[137,138] By adding suitably modified shift reagents to the eluate leaving a HPLC column,

Similar shifts of the UV absorption maxima of flavonoids in the eluate have been induced.[139]

Detection can be performed at the wavelength maximum of the compound in question. These are typically to be found[140] at 270 and 330 to 365 nm for flavones and flavonols, at 290 nm for flavanones, at 236 or 260 nm for isoflavones, at 340 to 360 nm for chalcones, at 280 nm for dihydro chalcones, at 502 or 520 nm for anthocyanins, and at 210 or 280 nm for catechins.[107]

For example Structure elucidation of flavonoid E, in combination with shift Reagents, The shift of 11 nm of the band with weak base, 0.1 M Na2HPO4, was characteristic for a nonsubstituted 7-hydroxyl group. A 15 nm shift with boric acid reagent was typical for ortho-dihydroxyl groups on the B-ring[140]. The shift of 42 nm of band I obtained for aluminum chloride without neutralization of the eluate was specific for a 5-hydroxyl substituent. Addition of aluminium chloride after neutralization gave a 56 nm shift of the band.[141]

1.6- *Zingiber officinale* (Ginger)

Zingiber officinale (Ginger) **Roscoe** is a member of the family Zingiberaceae and it is well known in Asia. The plant is widely cultivated in village gardens in the tropics for its medicinal properties and as a marketable spice[142]. In Asia and especially in Malaysia rhizomes of young ginger have been widely used as spices or condiments. Ginger is the third most important spice originated in south Asia. The components in ginger include: extractable oleo-

resins, many fats, carbohydrates, vitamins, minerals bioconstituents such as: antioxidants, flavonoids and anticancers.[143]

Zingiber officinale

Dried Ginger

The pungency of ginger is due to gingerol, an oily liquid consisting of homologous phenols. It is formed in the plant from phenylalanine, malonate and hexonate. In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil; 9% protein; 60-70% carbohydrates; 3-8% crude fiber; about 8% ash; 9-12% water; and 2-3% volatile oil. The Volatile oil consists of mainly monoterpenes[144].

Some constituents of ginger inhibit the growth of some colon bacteria like *Escherichia coli*, *Proteus* species, *Staphylococci, Streptococci* and *Salmonella*. It has been found that out of 29 plant extracts, ginger extract had the broadest range of antifungal activity measured either by the fungi inhibited or as the average diameter of the zones of inhibition[145-146].

The antioxidant properties of gingerol which is a very effective agent for Ultra Violet B (UVB)-induced reactive oxygen species production and COX-2 idiom, and apromising the rapeutic agent against UVB-induced skin disorders, has been studied both *in vitro* and *in-vivo.* It Also has a protective role to toxicity and lethality against some agent like carbontetra chloride, cisplatin etc[147,148] .

Aim of this work

This study was designed to:

-Extract flavonoids from rhizomes of medicinally important Ginger.

-Separation of the major flavonoid by preparative TLC.

-Conducting UV studies on the isolate.

-Proposing partial structure for the isolate.

2-Materials and Methods

2.1. Materials

2.1.1 Plant material

The stem bark of *Zingiber officinale* were purchased from the local market- Khartoum.The plant was kindly authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum.

2.1.2- Instruments

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

(100 g) of powdered air- 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 prepared extract of each plant was screened for major secondary constituents.

2.2.2.1.1- Test for unsaturated sterols and for triterpenes

(10 ml)of the (PE) of each plant were 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.

2.2.2.1.2- Test for flavonoids

(20 ml) of the (PE) were 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.

2.2.2.1.3- Test for alkaloids

(10 ml) of the (PE) of each plant were separately 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 Maeyer reagent were added., to the other portion few drops of Wagner reagent were added.

2.2.21.4- 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.

2.2.2.1.5 -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 successively with: chloroform:methanol(4:1;3:2 and 1:4;v:v). (5ml) fractions were collected.The ratio (3:2) was rich in flavonoids. Fractions (5-32) 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 flavonoids 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

Phytochemical screening of the stem bark of *Zingiber Officinale* revealed the presence of tannins, flavonoids and alkaloids. The bark of this species were macerated with 95% ethanol. Column chromatography gave a pure flavonoid–compound I. The structures of this isolate was elucidated partially using UV and IR data.

3.1- Compound I

The IR (KBr disc) spectrum of compound I (Fig. 3.1) showed ν 667, 781, 885 (C-H bending, Ar), 1053 (C-O, ether), 1446, 1521 (C=C, Ar), 1612 (C=O) and 3384 cm^{-1} (OH).

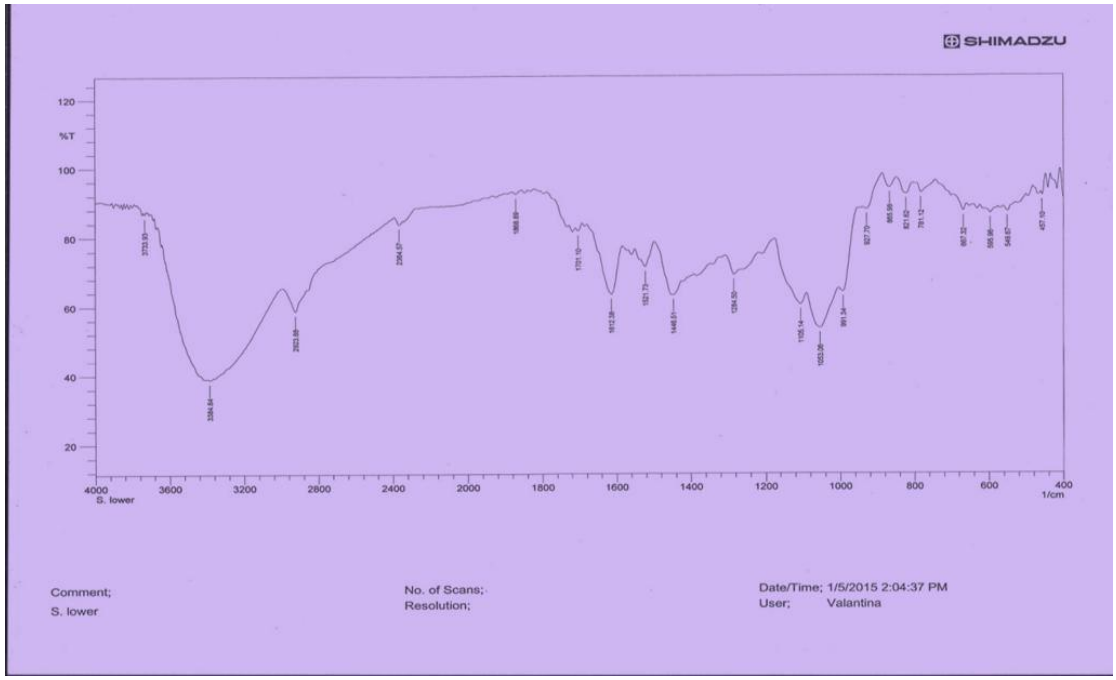


Fig. 3.1: The IR spectrum of compound I

The presence of a carbonyl stretching suggests: aflavone, flavonol, isoflavone, flavonone, chalcone or aurone. In the UV nm. Such absorption is characteristic of flavonols.

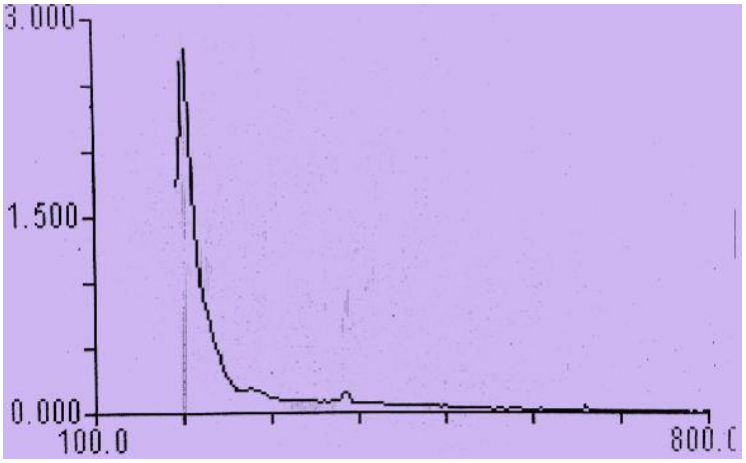
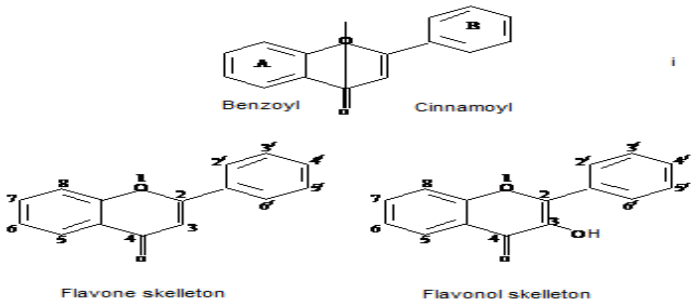


Fig. 3.2: The UV spectrum of compound I

The majority of flavonoids show two absorption bands in the region 240-400 nm (referred to as band I-usually 300-380 nm- and band II –usually 240-280 nm-[11]). Band I is considered to be associated with absorption due to the B-ring cinnamoyl system, while band II with the A ring benzoyl system. Flavones, flavonols, chalcones and aurones exhibit both bands.



As a result of little, or no conjugation, between the A and B rings, isoflavones, flavanones and dihydroflavonols all give similar UV spectra; they exhibit only one band-band II.

Very significant structural feature may also be obtained by utilizing other UV shift reagents: sodium acetate, aluminum chloride and boric acid. These reagents produced shifts in the UV absorption maxima in accordance with the location of the various functional groups in the flavonoid nucleus**.**

Sodium acetate ionizes only the more acidic hydroxyl groups in flavonoid nucleus, 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-hydroxy lgroup. Certain 3',4'–deoxygenated derivatives without the 7-OH group showed Bathochromic shifts of 20 – 25 nm[11]. The sodium acetate Spectrum of compound I revealed a bathochromic shift suggesting a free 7-OH function (Fig. 3.3).

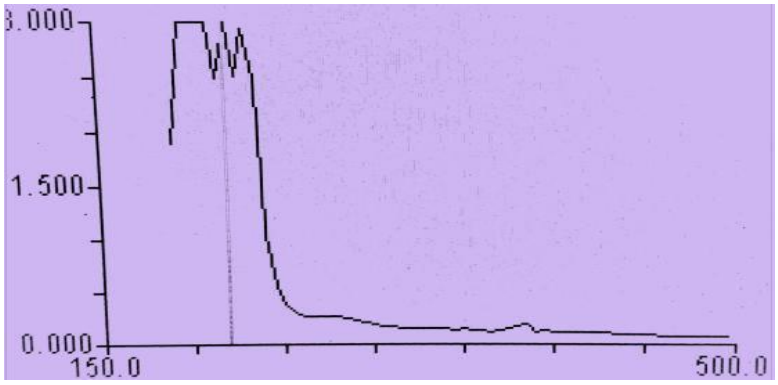
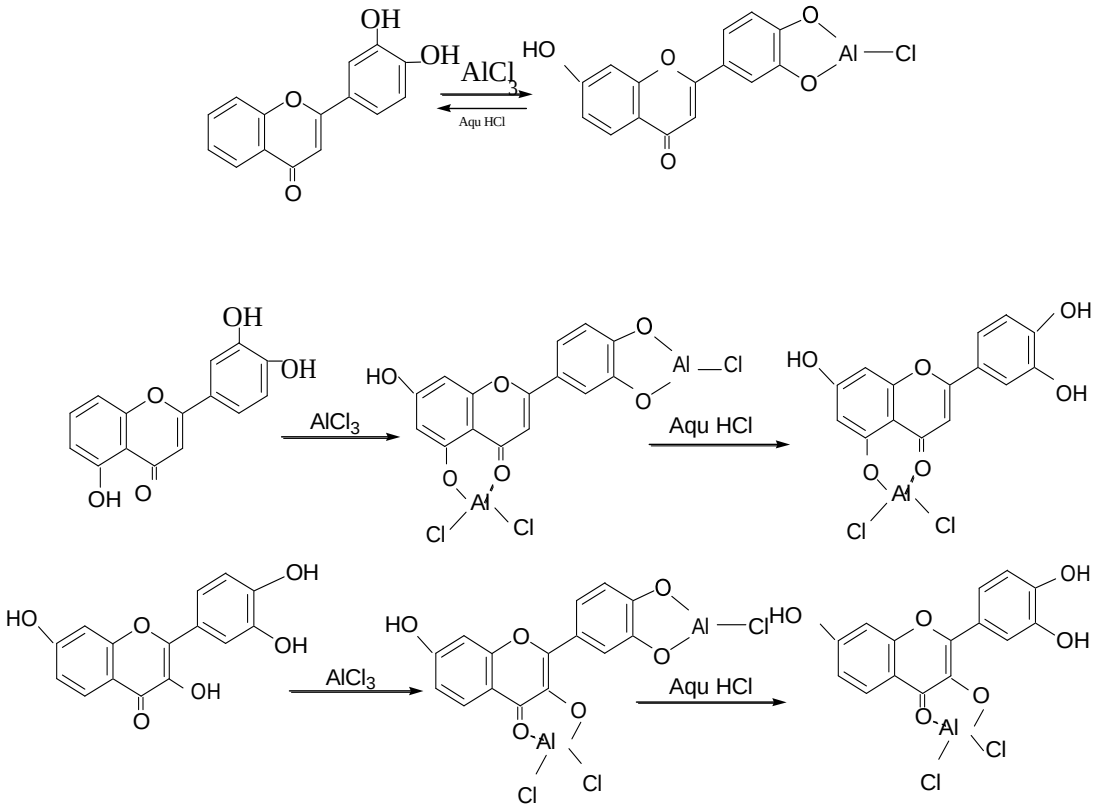


Fig. 3.3: Sodium acetate spectrum of compound I

Flavonoids with 3-, 5-OH and catechol systems can form complexes with the shift reagent: aluminum chloride. The aluminum chloride complexes with catechols are not stable in acidic media, but hydroxyl groups at C-3 or C-5 can form acid stable complexes. Such acid-stable and acid–labile complexes are shown below:



The aluminium chloride spectrum of compound I is depicted in Fig. 3.4. No bathochromic shift which is diagnostic of 5-, 3-OH or catechol systems was observed.

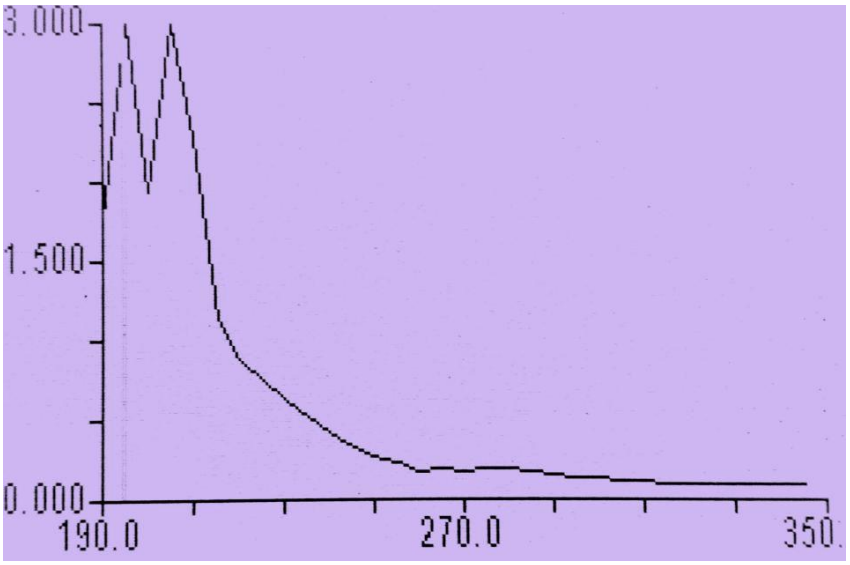
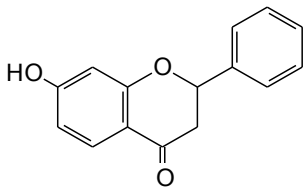


Fig. 3.4: Aluminium chloride spectrum of compound I

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



Compound I

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