



**Sudan University of Science  
and  
Technology**

**College of Graduate Studies**

***Extraction and Identification of Quercetin in  
Allium Cape Skin (Onion Skin)***

**إستخلاص الكيرسيتين من قشر البصل وتشخيصه**

**A thesis submitted in partial fulfilment of  
the requirements for M.Sc. degree in  
chemistry**

**BY**

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## الآية

قال تعالى :

(وَأَلْقِ مَا فِي يَمِينِكَ تَلْقَفْ مَا صَنَعُوا إِنَّمَا صَنَعُوا كَيْدٌ سَاحِرٌ وَلَا يُفْلِحُ

السَّاحِرُ حَيْثُ أَتَى)

صدق الله العظيم

سورة طه الآية (٦٩)

## **Dedication**

To my dear mother

My dear husband

My dear father

I dedicate this research

## **Acknowledgments**

I would like to convey my thanks to all who contributed to carry out this research work. In particular, I would like to thank my supervisor Dr: Mohamed El Mukhtar Abdel Aziz for his encouragement and assistance.

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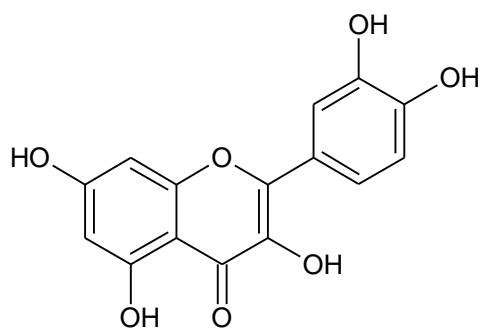
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## Abstract

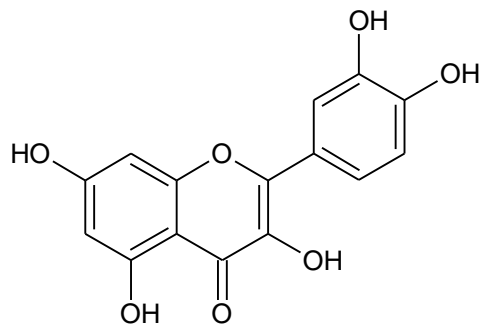
In the present work powdered air-dried allium cepa was extracted with absolute methanol. The chlorophyll and waxy materials were removed by adding activated carbon. The crude extract obtained after removing the methanol was hydrolyzed by using hydrochloric acid and ethanol to produce aglycones. The resulting aglycones were extracted three times with ethyl acetate. The brown solid, compound obtained after removing ethyl acetate, was isolated by thin layer chromatography using: benzene: acetic acid: Water (125:72:3) solvent system giving three compounds. The structure of the extracted compound, at  $R_F$  value 0.78, was identified as 5, 7, 3', 4'-tetrahydroxy flavonol using UV and IR spectral data.



5, 7, 3', 4'-tetrahydroxy flavonol

## المُستخلص

في هذا البحث تم استخلاص بودرة قشر البصل المجففة بالهواء باستخدام الميثانول المطلق. تم ازالة الكلوروفيل والمواد الشمعية باضافة الفحم المنشط. تم تحليل المسخلص الذي تم الحصول عليه بعد التخلص من الميثانول باستخدام حمض الهيدروكلوريك والإيثانول لانتاج الاجلكونات. وتم استخلاص الاجلكونات ثلاث مرات باستخدام الايثايل استيت، وتم عزل المركب الصلب البني الذي تم الحصول عليه بعد التخلص من الإيثايل استيت باستخدام كروماتوغرافيا الطبقة الرقيقة باستخدام نظام المذيب:بنزين:حمض الخل:الماء (١٢٥:٧٢:٣). أعطت عملية الفصل ثلاث مركبات. تم تحديد بنية المركب الذي معامل عرقلته يساوي ٧٩ و. بالصيغة ٧,٥، ٣، ٤- رباعي فلافونول باستخدام طيف الاشعة فوق البنفسجية و طيف الاشعة تحت الحمراء



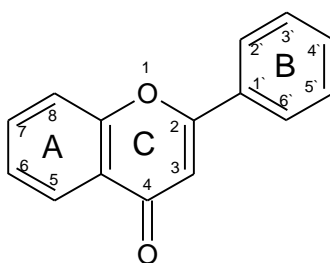
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# Chapter 1

# 1-Introduction

## 1.1 General approach

Flavonoids (2-phenylbenzopyrone)-(1) are a large group of polyphenolic compounds that occur commonly in plant (Catherine and Laster 2003). The name “flavonoid” is derived from Greek word “flavus” it means yellow (Zechemeister 1957).

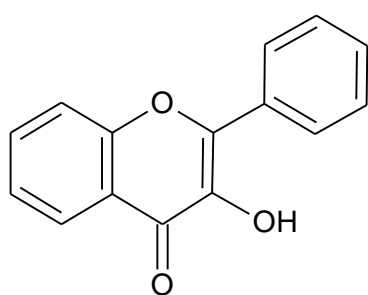


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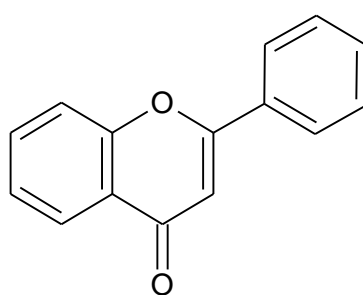
These phytochemicals are distributed widely in higher plant parts (barks, roots, stems, seeds, flowers) but also found in some lower plants including algae (Satyajit, Zahid and Lexandar 2006). Many of these compounds are responsible for the attractive colors of flowers and fruits and leaves. Flavonoids may occur as aglycones (consisting of a benzene ring condensed with a six membered ring which possesses a phenyl ring at the 2, 3 or 4 position). Glycosides which carry one or more sugar residues and methylated derivatives are very common. The chemical structures of these compounds are based on a ( $C_6 - C_3$ ) skeleton. They differ in saturation of heteroatomic ring C, in the placement of the aromatic ring B at the positions 2, 3 or 4. According to the modification of the central C-ring,

they can be divided into different structural classes including flavonols (2), flavones (3), flavan-3-ols (4), flavanones (5), isoflavones (6), and anthocyanins (7). In a few cases, the 6- membered heterocyclic ring C occurs in an isomeric open form or is replaced by a 5-membered ring as in the case chalcones (8) and aurones (9) (Grote 2006) (Ceser 2010) (Wilfred and Ralph 2006). In plants, flavonoid aglycones (flavonoids without attached sugars) occur in a variety of structures. Most frequently encountered groups of flavonoid aglycones include flavones, flavonols, anthocyanidins, isoflavones, flavanones, dihydroflavonols, biflavonoids, chalcones, and aurones.

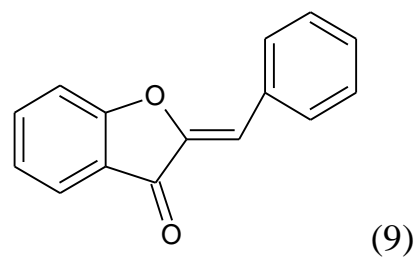
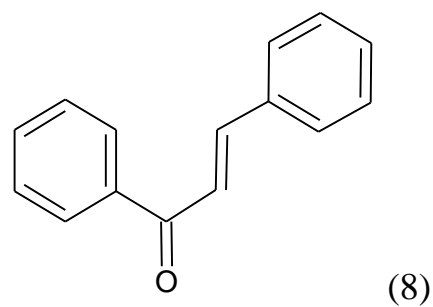
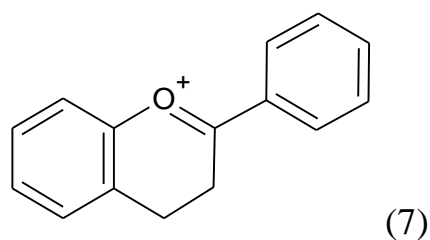
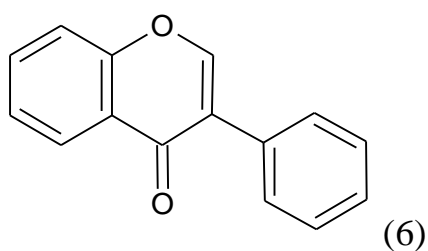
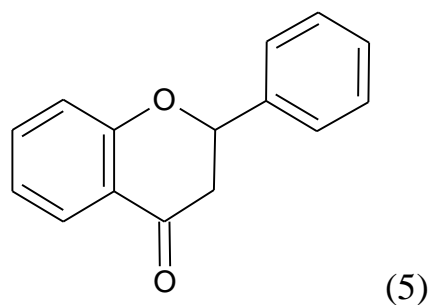
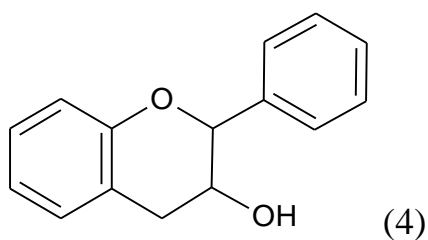
Flavonoid aglycones possess the chemical properties of phenolics, and thus they are slightly acidic. Those possessing a number of unsubstituted hydroxyl groups, or sugar moieties, are polar substance and soluble in polar organic solvents. The presence of sugar makes flavonoid more water soluble, while less polar aglycones like isoflavones, flavanones and highly methoxylated flavones and flavonols tend to be more soluble in ether or chloroform (Umezawa 2003) (Monica 2008).



(2)



(3)

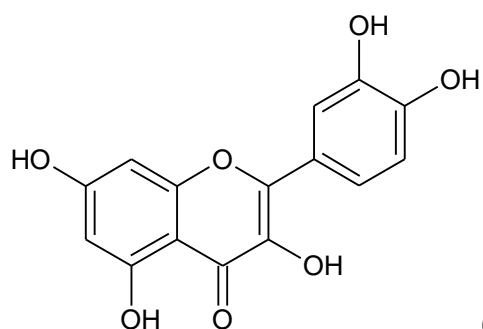


### 1.1.2 Flavonols

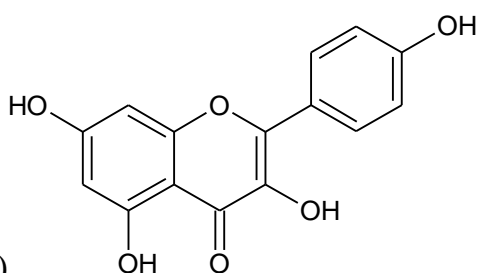
The flavonols (3-Hydroxy-2-phenyl-chromen-4-one) (2) are the most widespread of the flavonoids in plant food. They vary in colour from white to yellow. Flavonols have a double bond between C<sub>2</sub> and C<sub>3</sub> and an oxygen atom at the C<sub>4</sub> position. Furthermore flavonols also have a hydroxyl group at the C<sub>3</sub> position, and are represented mainly by quercetin (10), kaempferol (11) and



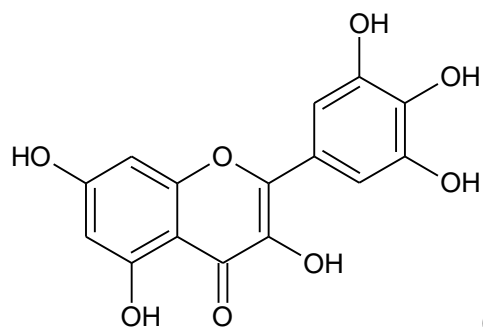
myricetin (12) and methylated derivative isorhamnetin (13). They are frequently found as O-glycosides, in which glycosidation occurs mainly at the 3-position of the C-ring, but substitution can also occur at the 5', 7', 4' or 3' positions. Many types of glycosides are derived from flavonol aglycones because various sugar groups can conjugate to the hydroxyl groups of flavonols at different positions (Crozier, Jaganth and Clifford 2006) (Hertog, Hollman and Katan 1992). Flavonols have a saturated three-carbon chain with a hydroxyl group in the C<sub>3</sub> position. In foods they are present as monomers or as proanthocyanidins, which are polymeric flavonols (4 to 11 units) known also as condensed tannins. In foods they are never glycosylated (Laura 2010)



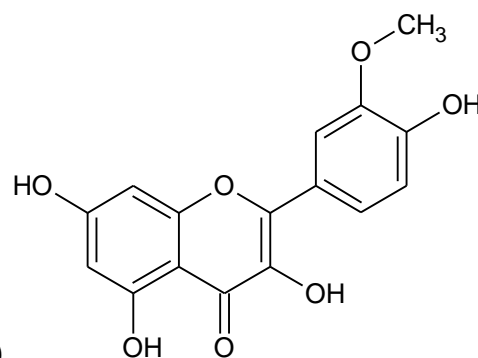
(10)



(11)



(12)



(13)

### **1.1.3 Flavones**

The ring C of the flavones (2-phenyl-chromen-4-one) (3) contains a double bond between positions 2 and 3, and a keto function in a position 4. Most flavones of fruits and vegetables hold a hydroxyl group on position 5 of ring A, whereas hydroxylation on other positions, most often position 7 of ring A or positions 3' and 4' of ring B, can vary depending on the taxonomic classification of a particular fruit or vegetable.

### **1.1.4 flavanones**

Flavanones (2-phenyl-chroman-4-one) (5) (also called dihydroflavones) lack the double bond between carbon 2 and 3 in the C-ring of the flavone skeleton, which is present in flavones and flavonols. Thus, in flavanones, C-2 bears one hydrogen atom in addition to the phenolic B-ring, and C-3 two hydrogen atoms. Two stereoisomeric forms of each flavanone structure are possible, since C-2 is a center of asymmetry (epimeric center).

Consequently, the B-ring can be either in the (2S) or (2R) configuration. The great majority of the flavanones isolated from plants are levorotatory (2R) or (2S)-flavanones, because the enzymatic reaction catalyzing the conversion of chalcones to flavanones is stereospecific. The C-3 atom of dihydroflavonols bears both a hydrogen atom and a hydroxyl group, and is therefore an additional center of asymmetry. Thus, four stereoisomer's are possible for each di hydro

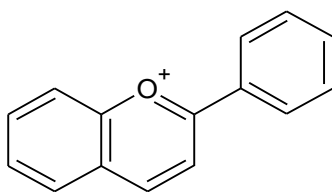
flavonol structure, (2R, 3R), (2R,3S) , (2S,3R) and (2S,3S). All four configurations have been found in naturally occurring dihydroflavonols, but the (2R, 3R)-configuration is by far the most common (Oyvind and Kenneth 2006).

### 1.1.5 Isoflavones

Isoflavones (3-phenyl-chromen-4-one) (6) also have a diphenylpropane structure in which the B ring is located in the 3 position.

### 1.1.6 Flavanols

Flavanols (4) are often referred to as flavan-3-ols, as the hydroxyl group is almost always attached to the position 3 carbon of ring C , but it may occupy position 4 or the 3,4-positions as in flavan-3,4-diols. Flavanols are based on the flavylium salt (14) structure and are water-soluble pigments in plants.



(14)

### 1.1.7 Aurones

Aurones (9) were first described from flower of *Coreopsis Grandiflora* by Geissman and Heaton in 1934. These conspicuously colored compounds have been found in a variety of yellow flowered species since that time. However, the

aurones are not restricted to floral tissue but have been obtained from bark, wood and leaves as well.

Chemically aurones are based on the 2-benzylidene-coumaranone or 2-benzylidene-3(2H)-benzofuranone system (9) the term ‘aurones’ recognized both yellow golden colour and their isomeric relationship with the flavones. Structures of natural aurones are most easily discussed by grouping them according to the number of hydroxyl groups present in the ring B-ring (Harborne and Mabry 1982).

- Aurones lacking B-ring hydroxyls
- Aurones having one B-ring hydroxyl
- Aurones having two B-ring hydroxyl
- Aurones having three B-ring hydroxyl

#### **1.1.8 Medicinal uses of flavonoid compounds**

Pharmacological and chemical investigations of medicinal plant have provided important advances in the therapeutic approach to several pathologies. A number of medicinal plant containing flavonoids and alkaloids are used in traditional medicine and are known to contain important therapeutic agents (Brown, Kelly, and Husband 2005).

A great number of plant medicines contain flavonoids having anti-inflammatory, anti-allergic, anti-thrombotic and vasodilatory activities

(Koganov, Duev and Trorin 1999) also some have anti-viral and anti-bacterial properties (Shamma and Stiver 1969).

Flavonoids could be found in many formulations of alternative medicine. Ranging from treatment for cardiovascular disease to peripheral vascular disease, stroke and cancer (Kumiko 2001). Several epidemiological studies provided support for a protective effect of the consumption of fresh fruit and vegetable against cancer (Kuos 1996), heart disease and stroke (Shamma and Stiver 1969).

Flavonoids are also considered to be the active ingredient in some medicinal plants, because ingested flavonoids enter the plasma, to elevate the redox and anti-oxidant levels. The physiological benefits of flavonoid are generally thought to be due to their anti-oxidant and free radical scavenging properties (Grayer and Harbone 1994).

Quercetin (10) is considered a good antioxidant. It also exhibits anti-inflammatory activity and was found to inhibit both tumor promoter and human cancer cell (Parmar, Vardham, Nagarjan and Jain 1995).

One of the main functions of flavonoids is their accumulation as phytoalexins, which protect plant from microbial invasion (Marquart 1935). Phytoalexins are compounds that are formed in response to microbial or other invasions. Nringenin (25) found in the heartwood of trees from the Rosaceas is an anti-fungal agent (Harbon 1994). It can also function as stress protectants in plant

cell by scavenging reactive oxygen species (ROS) produced by the photosynthetic electron transport system (Shirely 1996). Furthermore, because of their UV absorbing properties, flavonoids protect plants from the UV radiation (Chaudhry 1995). The fungicidal properties of flavonoids are affected by phenolic substitution and in many cases it has been shown to decrease with increasing substitution (Davies and Kost 1988). Isoflavonoids, flavonones and flavones are the most effective anti-microbial agents (Shamma and Stiver 1969). Anthocyanins are also used to treat skin diseases especially dermatological hypersensitivity. Flavonoids may inhibit the enzyme involved in the glycosylation process, which gives rise to sorbitol which causes swelling complication in diabetes.

The biological activities of chalcone is including anti-bacterial, anti-cancer, anti-ulcer, anti-protozoa, amoebicidal, cytotoxic, and immune suppressive activates (Bertuglia 1995) .

Flavonoids are usually found alongside vitamin C in nature. Studies have shown that the vitamin C alone may not be effective as being supplemented with flavonoids. Flavonoids may correspondingly increase the amount of vitamin C in tissues, by preventing the break-down of this vitamin (Harbone, 1958).

## **1.2 Quercetin**

Quercetin is a bioflavonoid (or flavonoid), which is a type pigment found in almost all herbs, fruits, and vegetables. Quercetin is the aglycone form of other

flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. Quercetin forms the glycosides, quercitrin and rutin, together with rhamnose and rutinose, respectively.

### **1. 2.1 Synonyms**

Quercitroside; Quercimelin; Quercitin – 3-L-rhamnoside; Thujin; Quercitin; Quercetin, Sophoretin, Meletin, Quercetine, Xanthaurine, Quercetol, Quertine, Flavin meletin.

### **1.2.2 Biological sources**

Quercetin occurs in the bark of *Quercus tinctoria* and some other species of *Quercus*. It is also obtained from *Aesculus hippocastarum* L, horse chestnut, belonging to family Hippocastanaceae.

It is also found in *Thuja occidentalis*, *Morus Alba* L, *Humulus lupulus* L, *Fraxinus excelsior* L, *Vitis vinifera* and the other plants.

### **1.2.3 Description**

The crystals are yellow in colour when obtained from ethanol or methanol. It is practically insoluble in cold water and ether. Flavine yellow shade obtained from the quercitron bark by extraction under high pressure steam which is used exclusively in dyeing fabrics.

### 1. 2.4 IUPAC name

2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one

Quercetin on hydrolysis in an acidic medium gives rise to rhamnose and quercetin (i.e., 5, 7, 3', 4'-tetrahydroxy flavonol) (Cruz, Shoskes and Sanchez 2006).

**Table 1: Properties of quercetin**

Molecular formula	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>
Molecular weight	302.236 g/mole
Exact mass	302.042653
Density	1.799g/ml
Melting point	316 °C

### 1. 2.5 Health benefits

1. Cancer
2. Diarrhea
3. Allergies, Asthma, Hay Fever
4. Heart Disease
5. Hypertension
6. Interstitial Cystitis
7. Prostatitis



8. Diabetes
9. Rheumatoid Arthritis (RA)
10. Athletic Endurance

### **1.2.6 Quercetin side effects**

- **Quercetin cause cancer**

On the other hand, a handful of studies from reputable sources claim high doses of quercetin cause cancer. Researchers found that the high concentrations of the element would actually bind with and damage chromosomes and DNA structures resulting in a cancerous mutation. High concentrations of the flavonoid also disrupted the activity of enzymes and particularly interfered with oestrogen and thyroid hormones. These studies claim that there is a documented risk to young children consuming high doses of it and getting sick with a rare form of leukaemia.

- **Birth defects**

Scientists theorize that birth defects could occur in the unborn children of women who consumed high doses of quercetin at the time of conception and throughout pregnancy (Schabath, Hernandez, Pillow and Spitz 2005).

### **1.3 Techniques used in flavonoids analysis**

The separation and purification of plant constituents is mainly carried out using one or other or combination of five chromatographic techniques. Paper chromatography (PC), thin-layer chromatographic (TLC), gas-liquid chromatography (GLC), high performance liquid chromatography (HPLC) and high speed counter current chromatography (HSCCC). The choice of the technique depends largely on the solubility properties and volatility of compound to be separated (Harbone and Williams 2000).

#### **1. 3.1 Paper chromatography (PC)**

The technique of paper chromatography (PC) is a common one in the field of flavonoid analysis and separation (Roux, maihs and paulus, E.1961).PC is suitable for the separation of complex mixture of all type of flavonoids and their glycosides. It is convenient for isolating of both small and relatively large amounts and is associated with the low cost of the necessary equipment and material (Ribrean 1972).

One of the main advantages of PC is the great convenience of carrying out separation simply on sheets of filter paper, which serve both as the medium for separation and as the support. Another advantage is the considerable reproducibility of  $R_f$  determined on paper, so that such measurements are valuable parameters for use in describing new plant compounds. Indeed for substance such as the anthocyanin, which do not have other clearly defined

physical properties, the R<sub>f</sub> is the most important means of describing and distinguishing the different pigments (Done, Kennedy and Konx, 1972).

Most flavonoids appear as colored spot on paper chromatogram when viewed in UV-light, and fuming with ammonia often produced significant changes in these colors. Often reaction with a chromogenic reagent which is used as spray is extremely useful (Young and Britton 1993).

### **1. 3.2 Thin layer chromatography (TLC)**

TLC is a technique which has developed rapidly. However, it is complementary to PC in that it provided new media for separation of flavonoids on small scale and permits the use of a wider variety of detecting reagent. The special advantages of TLC compared to PC include speed, versatility and sensitivity. The greater speed of TLC is due to the more compact nature of adsorbent when spread on plates and is save when working with labile compounds. The sensitivity of TLC is such that separation on less a milligram amount of material can be achieved and if necessary detection of compounds on TLC plates is normally carried out by spraying procedure. One advantage over PC is that glass plates may be sprayed with Conc.H<sub>2</sub>SO<sub>4</sub>, an extremely useful detecting reagent for steroids and lipids (Harbone and Williams 2000). Thin layer chromatography is a technique, which has replaced paper chromatography in analytical and small scale separations of flavonoids (Douglas 2001) (Harbone 1984).

As in column chromatography, the adsorbents of choice for the separation of flavonoids are silica, polyamide and cellulose (Gamache and Ryan 1993). Thin layer chromatography 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 1-5 mm thick are used in conjunction with plates of up to 20x100 cm in size.

Apart from the Anthocyanins and some of the more intensity colored chalcones and aurones, flavonoids are not sufficiently colored to be visible to naked eye on a thin-layer plate, thus some form of visualization is necessary for spot detection (Thomass and Mabry 1968). UV light is often used as detection agent. Another useful method of detection is brief exposure of the plate to iodine vapor where flavonoids appear as yellow-brown spots against a white background. These techniques all have the advantages that they are non – destructive (Gamache and Ryan 1993). Most flavonoids are detectable by one or other of flavonoid spray agents, and some of the most frequently used are listed in table (2).

**Table 2: spray reagent for the detection of flavonoids in TLC**

No	Reagent	Flavonoid type detectio
1	Boric acid-ethanol-arin complex (1% in ethanol)	Most flavonoids
2	Ferric chloride(methanolic)	Most flavonoids
3	Ferric chloride-potassium ferric cyanide(1%aq-slons mixed”1:1”)	Most flavonoids
4	Antimony chloride(in CHCl <sub>3</sub> ),UV	Most flavonoids
5	Lead acetate-basic, UV	Most flavonoids
6	Aluminum chloride (2%in methanol)	Most flavonoids
7	Zirconium oxy chloride(in methanol), UV	Most flavonoids
8	Ceric sulphate	Isoflavones
9	Conc. sulphuric acid	Isoflavones
10	Hydrogen chloride(Gas)	Fully methylated flavones
11	Zinc-hydrochloric acid	Dihydroflavonols
12	Oxalic acid(10% in Acetone:H <sub>2</sub> O,1:1)	Anthocyanins and Anthocyanidins
13	Diazotized p-nitro aniline	Most flavonoids
14	Diazotized sulphuric acid	Most flavonoids
15	Fast red salt B(Diazotized-5-nitro-2-amino anisol)0.5%, then 0.1N NaOH	Most flavonoids
16	Fast blue salt B(Tetrazotized di-o-anisidine) 0.5%, then 0.1N NaOH	Most flavonoids
17	Bis diazotized benzidine	Flavones and flavonols
18	Sodium borohydried(1%in isopropanol and HCL) or AlCl <sub>3</sub>	Flavanols and isoflavanones
19	Ammoniacal silver nitrate	Most flavonoids
20	Sodium hydroxide solution (1% in methanol)	Most flavonoids

Highly methylated or acetylated flavones or flavonols require relatively non-polar solvents for thin-layer chromatography on silica gel. Flavonol poly acetate and poly methyl ethers have been successfully chromatographed by using benzene: acetone (9:1) and toluene: acetone (19:1). More polar flavones and flavonols require more polar solvents.

Flavone and flavonol glycosides are not commonly chromatographed on  $\text{SiO}_2$  (Gamache and Ryan 1993). However, when this has been done, polar solvents such as ethyl acetate: butanone: formic acid: water (5:3:1:1), have been used (Bhutani, Chibber and Seshadri 1969). Isoflavones, flavonones and dihydroflavonols are generally chromatographed using less polar solvents than those required for the common flavones and flavonols.

A number of synthetic aurones have been chromatographed on  $\text{SiO}_2$  (Endres and Hormann 1963). Anthocyanins and anthocyanidins which are usually fractionated on paper or cellulose were successfully fractionated on silica (Horhammey 1964).

TLC on cellulose layer has to some extent replaced paper chromatography in analytical work, since the high surface area, fine-grained cellulose thin layers offer the advantage of greater speed. Cellulose, when admixed with 3% by weight of polyamide, was found useful for the chromatography of a wide range of flavones and flavonols and their mono – and di glycosides using 15, 40 or 60% acetic acid as solvents (Ice and Wender 1952).

Good separations of anthocyanidins and Anthocyanins have been achieved using cellulose TLC (Siekel 1962). Commercially available polyamides have various physical and chromatographic properties. An excellent polyamide powder, however, may be prepared from polyamide pellets.

Polyamide separated flavonoids either by partition or adsorption processes depending upon the solvent used (Kirchner 1967). The adsorption process is favored with water – alcohol mixtures. Polyamides are generally considered unsatisfactory for the chromatographic separation of Anthocyanins and anthocyanidins (Kirchner 1967)(Stahl 1969).

TLC is an ideal technique for the screening of drugs because of its low cost, easy maintenance and selectivity of detection reagent. TLC on silica gel is very favorable for the analysis of flavonoids (Harbone 1984).

### **1. 3.3 Gas chromatography (GC)**

Two types of gas chromatography are encountered: gas-solid chromatography (GSC) and gas-liquid chromatography (GLC). GLC finds widespread use in all fields of science, where its name is usually shortened to GC (Egeer 1969). GC is no more complicated than other chromatographic procedures. The main variable is the nature of stationary phase of column and the temperature of operation; these are varied according to the polarity and volatility of compound being separated.

Most frequently, GC is automatically linked to mass spectroscopy (MS) and the combined GC-MS apparatus has emerged as one of the most important techniques for phytochemical analysis (Harbone and Williams 2000).

### **1. 3.4 High performance liquid chromatography (HPLC)**

HPLC is analogous to GLC in its sensitivity and ability to provide both quantitative and qualitative data in a single operation. HPLC is mainly used for those classes of compounds which are non-volatile, e.g. higher terpenoids, phenolic of all types, alkaloids, lipids and sugars (Harbone and Williams 2000). The qualitative analyses which produce a “finger print” chromatogram obtained under slandered concentration can be very useful for quality control of phytochemicals. Although TLC is a powerful and simple technique used for this purpose, there are situations in which it can produce doubtful results. HPLC can also be a useful tool on chemosystematics helping, for example, to characterize species on the basis of their secondary metabolite contents. HPLC has been used in a number of occasions for the analysis of flavonoids in plant. In one study (Harbone, Boardly and Linder 1985) HPLC was used to distinguish species based on the quantitative variation of flavonoids among them. In other study (Grozier, Jensen and lean 1997), it was used for the quantitative analysis of flavonoid aglycones. The literature is replete with chromatographic systems which are capable of measuring flavonoid content across one, two and three of the five common sub-classes of flavonoids found in foods. However, many



foods and mixed diets, in particular, contain member of all five sub-classes of flavonoids (Merken and Beecher 2000).

Advantages for HPLC analysis include. (i) short analysis time, (ii) high resolution, (iii) no derivatization is required, (iv) no risk for thermal decomposition and (v) easy quantification.

Where twenty years ago the principle chromatographic methods for analysis of flavonoids were TLC and PC, in last decade HPLC has taken over almost completely separation of widely differing compounds (for example, aglycones and their conjugates) which is a common problem in both phytochemical and clinical studies. This is now routinely resolved by use of HPLC on reversal-phase columns with gradient elution (Willkinson, A.P, Wahala and WilliamSon, G. J 2002). Natural products are often obtained by some conventional protocols of extraction and separation technique, such as using organic solvent to extract and column chromatography, including silica gel and HPLC, to isolate. However, some organic solvents are unfriendly to our environment and the conventional separation methods are usually tedious, time consuming and needing multiple steps and the samples are adsorbed on the stationary phase irreversibly (Harbone, Boardly, and Linder 1985).

### **1. 3.5 High speed counter current chromatography (HSCCC)**

Recently, two techniques, supercritical fluid extraction (SFE) and high-speed counter current chromatography (HSCCC) are widely used to extract and separate natural products from medical plants (Lin, Lee and Kim 2002).

(HSCCC) is a major tool for the fast separation of natural products from plants. It was used for the preparative isolation of the flavonoid mono glycosides present in aerial parts of *Davila Elliptical* (Lin, Lee and Kim 2002). Several classes of natural products were already isolated using (HSCCC), including flavonoids. Separation using (HSCCC) provide natural products with a very efficient method for the separation of compounds derived from plant origin in short separation time and with the possibility of large range of aromatic solvents (Marston and Hostettmann 1991). The solvent system selection is the first and most important step in performing (HSCCC) separation (Lin, Lee and Kim 2002).

Conventional methods such as column chromatography require several steps resulting in low recoveries of the product. (HSCCC) is a liquid-liquid partition chromatographic technique with excellent sample recovery compared to some conventional methods, and is widely used for separation and purification of various natural and synthetic products.

### **1. 3.6 Column chromatography**

Column chromatography remains one of the most useful techniques for the isolation of flavonoids from crude plant extract.

Adsorbents commonly used for the separation of flavonoids include silica gel, kieselguhr, magnesol, cellulose, alumina, polyamides, sephadex and ion exchange resins. The adsorbents of choice have generally been silica gel, cellulose and polyamide (Gamache and Ryan 1993).

Silica gel has traditionally been used for the separation of isoflavones, flavanones, di hydro flavanols and highly methylation flavones and flavonol. Occasionally, even flavonoid glycosides have been purified on silica, for example, the C- and O-glycosides of daidzein were separated on silica using ethyl acetate and ethyl acetate : methanol (19:1) as solvents (Mabry, Markham and Thomas 1970), and glycosides of the flavone naringenin were eluted from silica using benzene : ethanol (9:1) (Jared 1962).

It is clear that silica gel is a useful adsorbent for the separation of flavonoids of quite a wide range of polarities. In general terms, this may be extended to include many of the more polar flavonoids simply by deactivation through the addition of water. Many of the variable chromatographic properties observed with silica adsorbents from different sources are undoubtedly attributable to the water content of the gel (Gamache and Ryan 1993).

An additional factor is the presence of metal ions in silica. It was observed that a number of the commercially available silica gels contain iron causing the flavonoids to adhere strongly to the column. This impurity is removed by treatment of the silica with warm, conc. HCL thus making the adsorbent much more useful for the separation of polar compounds

Magnisol, a dehydrated magnesium acid silicate, and florisil, a 15:5:84 mixture of magnesium oxide and silica are somewhat more basic than silica. They have not been used extensively for the separation of flavonoids probably because they offer little or no advantage over silica. Separation of a number of simple mixtures of flavones, flavonols, flavanones and their glycosides was achieved using magnisol (Morgan and Orslser 1973). In particular, good separation of flavonoid aglycones from the more strongly adsorbed glycosides was obtained. Water- soluble flavonoids could be extracted by water containing up to 5% of an organic solvent such as ethanol, acetone or ether. However, individual flavonoids were not separated by this procedure (Seshadri 1962).

As with silica gel, alumina may be deactivated by addition of water. Alumina has generally found little use in the separation of flavonoids, largely because of the problem of complex formation.

Aluminum( III )ion are known to complex strongly with the 4- keto -5- hydroxyl and 4- keto -3- hydroxyl systems found in most flavones and flavonols.

Natural alumina has been used successfully for separation of completely methylated and fully substituted flavonols. It is clear that alumina is best suited to the purification of fully derivatized flavonoids (Gessman, Reding and Austin 1987).

In principle flavonoids which form only acid – labile complexes with aluminum, should be selectively removed from alumina with acid- containing solvents. Cellulose column chromatography can be considered as a scaled-up form of paper chromatography. As such, it is suited to the separation of all classes of flavonoids and their glycosides. It is used for separations based on both adsorption and partition, though a distinction between the two is often difficult to make. Cellulose powder has a low capacity (Horhammey 1964) and limited resolving power and though this may be compensated for, to some extent, by tight column packing, many workers favor scaling up of paper chromatography itself for large scale separations. As in paper chromatography, the solvents most favored for use with cellulose columns are of the aqueous alcohol and acid types.

Cellulose column chromatography has been used for the separation of Anthocyanins in the past, but variable results, limited column capacity and production of dilute elutes, led to the conclusion that the method offered little, if any advantage, over paper chromatography (Gamache and Ryan 1993).

Polyamides commercially available for chromatography are mainly of the perlon – type (polycaprolactam). Nylon – type (polyhexamethylenediamine) adiabatic, or polyvinylpyrrolidone (PVP) all have a high capacity for phenolic materials and all form strong hydrogen bonds with phenolic hydroxyl group via their amide function (Mizelle, Dunlap and Hagan 1965).

Like cellulose, polyamide is suitable for the separation of all types of flavonoids (Smith 1960). However, it has advantage over cellulose of higher capacity or higher resolution.

Sephadex is a highly cross – linked dextran on which separations are ideally obtained on the bases of molecular size. Adsorption on dextran gels is known to occur with aromatic compounds and phenols in particular, and it is thus not surprising that this is commonly encountered with flavonoids especially the aglycones (Gamache and Ryan 1993).

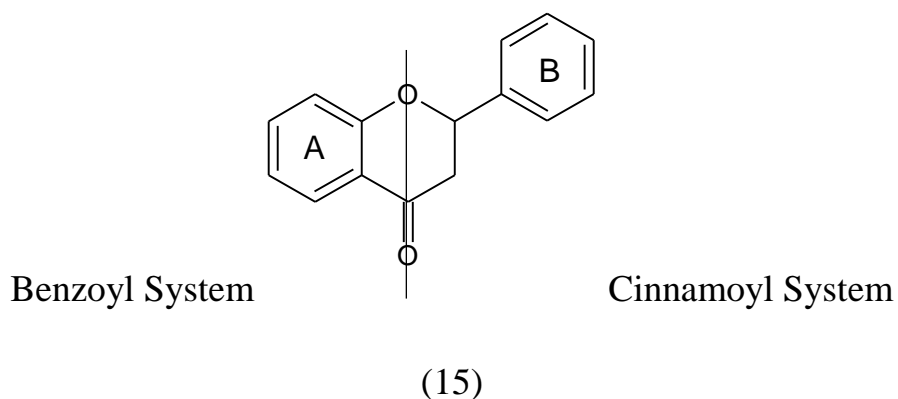
Ion – exchange resins have been used very little for the isolation of flavonoids. Early work (Clark-lewis and Dainis 1976), which has been thoroughly reviewed, is involving the use of cation – exchange resins entirely. These resins were generally used in preliminary clean – up procedure in which flavonoids were held on the column while other water – soluble impurities were washed off with water.

## **1.4 Spectroscopic methods**

In identifying a plant constituent, once it has been isolated and purified, it is necessary first to determine the class of compound and then to find out which particular substance is within that class. It should travel as a single spot in several TLC and or PC systems. The class of compound is usually clear from its response to colour test, its solubility and  $R_f$  properties. Complete identification within that class depends on measuring other properties and then comparing these data with those in the literature. These properties include melting point, boiling point,  $R_f$ , and optical rotation. However, equally informative data in plant substances are its spectral characteristics. The techniques are ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopes (MS). A known plant compound can usually be identified on the above basis. If a new compound is present all the above data should be sufficient to characterize it (Harbone and Williams 2000).

### **1.4.1 The Ultraviolet \ visible spectroscopy (UV\Vis)**

The UV spectra of most flavonoids consist of two major absorption maxima, one of which occurs in the range of 240-285 nm (band II) and other in the range 300-400 nm (band I). Band II occurs from A-ring benzoyl system, while band I originates from B-ring cinnamoyl system (15).



Flavonoids containing conjugated aromatic systems show intense absorption bands in UV region of spectrum (Harbone 1984) (table 3).

**Table 3: Spectral Characteristics of Main Flavonoid Classes**

<b>Class of Flavonoids</b>	<b>Band I <math>\lambda</math> \nm</b>	<b>Band II <math>\lambda</math> \nm</b>
<b>Flavones</b>	<b>300-350</b>	<b>250-285</b>
<b>Flavonol</b>	<b>330-385</b>	<b>240-280</b>
<b>Flavanone</b>	<b>300-330 (sh)</b>	<b>275-295</b>
<b>Isoflavones</b>	<b>310-330 (sh)</b> <b>320 (one peak)</b>	<b>270-280</b>
<b>Anthocyanins</b>	<b>465-550</b>	<b>270-280</b>
<b>Chalcone</b>	<b>340-390</b>	<b>220-270 (low intensity)</b>
<b>Aurone</b>	<b>390-430</b>	<b>230-270 (low intensity)</b>

UV spectroscopy has become a major technique for the structure analysis of flavonoids for two reasons: the first is that only a small amount of pure material



is required, often a single flavonoid spot on paper chromatogram will yield sufficient compound to several UV studies. The second reason is that the amount of structural information gained from a UV spectrum is considerably enhanced by the use of specific reagents (shift reagents), which react with one or more functional group on the flavonoid nucleus.

The addition of each of these reagents separately to an alcoholic solution of the flavonoid induced structurally significant shifts in the UV spectrum. Shifts are commonly induced by the addition of sodium methoxide, sodium acetate, sodium acetate\boric acid, aluminium chloride and aluminum chloride\hydrochloric acid (Porter and Markham 1970).

Sodium methoxide is a strong base ionizes to some extent all hydroxyl groups on flavonoid nucleus. However, use has been made of the effect of ( $\text{NaOCH}_3$ ) on the UV spectra of flavones and flavonols for detection of 3 and for 4'-hydroxyl groups, The addition of sodium methoxide to flavones and flavonols in methanol usually produces bathochromic shifts in all absorption bands( Jared and Harowitz 1961).

The absence of shift in the major band indicates the absence of isoflavones of A-ring hydroxylation, while the presence of 5, 6,7 and 5,7,8-hydroxyl system is evidenced by degeneration of sodium methoxide spectra with time(Harborne, Gotlied and Magalhães 1963).

Flavanones and dihydroflavonols with 5, 7-dihydroxyl system exhibit a consistent 35-40 nm bathochromic shift of band (II). Flavanones in particular, those lacking a free 5-hydroxyl group, isomers to chalcones and have band (I) peak in 400 nm region(Jared. and Harowitze 1961).

4'-hydroxyl groups in aurones and 4'-hydroxyl groups in chalcones are evidenced, by band (I) bathochromic shift of 80-96 nm and 60-100 nm respectively each with an increase in intensity, while 6-hydroxy-aurones give a similar shift (60-70 nm) than 4'-hydroxyaurones. Chalcones give a 60-100 nm bathochromic shift(Geissmann and Harbone 1956).

The only anthocyanidine that give stable spectra in sodium methoxide are the 3-deoxyanthocyanidins, the bathochromic shift in band (I) being 50-60 nm.

Sodium acetate is weaker base than sodium methoxide, and as such, ionizes only the more acidic hydroxyl groups. In flavones and flavonols it is diagnostic of the 3, 7- and 4'-hydroxyl groups. Ionization of the 7-hydroxyl groups mainly effect (band II), whereas ionization of 3- and or 4'-hydroxyl groups mainly effect (band I). in the presence of sodium acetate the UV spectra of flavones and flavonols containing free 7-hydroxyl groups, with few exception exhibit a diagnostic 5-20 nm bathochromic shift. Sodium acetate is particularly useful diagnostic reagent for the specific detection of 7-hydroxyl group. If within few minutes of flavonoid has decomposed then this is due to the presence of an alkali-sensitive grouping (Jared and Harowitze 1957).

The presence of 7-hydroxyl group in isoflavones is evidenced by a band (II) bathochromic shift 6-20 nm, in 5, 7-hydroxyl flavanones and dihydroflavonols by a shift of 35 nm. Alkali-sensitive grouping in the A-ring cause the spectrum to degenerate with time.

Hydroxyl groups at position 4'- and/or 4- in chalcones and at 4'- and/or 6- in aurones are evidenced by a bathochromic shift of band (I) or by the appearance of a long wavelength shoulder.

In the presence of sodium acetate, boric acid will chelate with ortho-dihydroxyl groups at all location on the flavonoid nucleus, except at C-5, C-6. Flavones and flavonols containing ortho-dihydroxyl groups show a consistent 12-30 nm bathochromic shift of band (I) in the presence of (NaOC<sub>2</sub>H<sub>5</sub>/H<sub>3</sub>PO<sub>3</sub>). A-ring catecholes at C-6, 7 and C-7,8 in flavonoids are also detectable by the effect of (NaOC<sub>2</sub>H<sub>5</sub>/H<sub>3</sub>PO<sub>3</sub>) on the UV spectra. A band (I) bathochromic shift of 5-10 nm observed (Arkham and Mabry 1968).

Isoflavones, flavanones and dihydroflavonols exhibits a bathochromic shift of 10-15 nm for band (II). Aurones and chalcones give bathochromic shift of 28-36 nm band (I), ortho-dihydroxyl groups in the A-ring give rise to a similar shift.

With aluminum chloride, flavones and flavonols which contain hydroxyl groups at C-3 or C-5. Form acid – stable complexes; in addition AlCl<sub>3</sub> forms acid-labile complexes with flavonoids which contain ortho-dihydroxyl system. The complex

formed between  $\text{AlCl}_3$  and the A- and B-ring ortho-dihydroxyl group; decompose in the presence of acid. In contrast, the  $\text{AlCl}_3$  complex between the C-4 keto function and either 3- or 5-hydroxyl group is stable in presence of acid. The presence of ortho-dihydroxyl group in the B-ring of flavones and flavonols can be detected by a comparison of the spectrum of the flavonoids in the presence of ( $\text{AlCl}_3$ ) with that obtained in ( $\text{AlCl}_3 \setminus \text{HCL}$ ).

The presence of 5-hydroxyl group is revealed by a 10-14 nm band (II) bathochromic shift in the spectra of flavones 20-26 nm in the spectra of flavonones and di hydro flavones. Ortho-dihydroxyl group are detectable only where present in the A-ring, and the spectrum shows a band (II) bathochromic shift of 11-30 nm.

The presence of 2'-hydroxyl group in chalcones and a 4'-hydroxyl group in aurones is evidenced by band (II) bathochromic shift of 48-64 nm.

B-ring ortho-dihydroxyl groups give rise to a 40-70 nm bathochromic shift of band (I) with  $\text{AlCl}_3$ . A-ring ortho-dihydroxyl groups give rise to a smaller shift.

Anthocyanidins and Anthocyanins containing ortho-dihydroxyl groups give band (I) bathochromic shift of 25-35 nm, longer shifts are observed with 3-deoxyanthocyanidins (Porter and Markham 1970).

#### **1.4.2 Mass Spectroscopy (MS)**

The value of the technique is that requires only microgram amounts of material. It can provide an accurate molecular weight and may yield a complex

fragmentation pattern, which is often characteristic of that particular compound (Harbone and Williams 2000)..

Mass spectra has been applied successfully to all classes of flavonoid aglycones, and more recently to a number of different types of glycosides including mono- and di-c glycosyl flavones and mono- to tetra-o-glycosides (Weiss Lundin and stern 1964). Electron impact spectroscopy of both flavonoids aglycones and glycoside serve as an available aid in determined other structures especially when only small quantities (i.e. less than 1 mg) of the flavonoid are available.

Most flavonoids glycone yield intense peaks for the molecule ion  $[M]^+$  and indeed this is often the base peak (Fool, Newman and Waghorn 1996). In addition to the molecule ion, flavonoid glycones usually afford a major peak for  $[M-H]^+$  and when methoxylated  $[M-CH_3]^+$ . Perhaps the most useful fragmentation in term of flavonoid identification is those which involve cleavage of intact A-ring and B-ring fragments (Agrawal 1989).

#### **1.4.3 Nuclear magnetic Resonance (NMR)**

The major use of proton NMR is for structural determination, in combination with other spectral techniques. It is used for determine the class of compound (Harbone 1984).

The application of NMR spectroscopy to the structure analysis of flavonoid is well established. Most naturally occurring flavonoids, including all of the flavonoid glycosides, have low solubility in deuteriochloroform ( $CDCl_3$ ). The

dimethyl sulfoxide (DMSO) has been used as solvent for number of extensive investigations of flavonoid structure by NMR spectroscopy (Batterham and Higher 1964). Some of the advantages of this method are that most flavonoid glycones and glycosides are sufficiently soluble in DMSO. Its signal occurs in a narrow band between  $\delta$  2.4 - 2.6 ppm, outside the region where most flavonoid protons absorb. DMSO can be used for observing protons in phenolic hydroxyl groups. It is anhydrous; the hydroxyl proton signals are readily distinguishable. Water in the solvent, however, causes the flavonoid hydroxyl proton signals to broaden, thus making their detection difficult (Mabry and Kagane 1965).

Protons of B-ring usually appear in the range  $\delta$  6.7 – 7.6 ppm, which is downfield from the region where the A-ring protons absorb. Considerable variation is found in the chemical shift of C-ring protons among the different flavonoid classes depending upon the oxidation level of the C-ring.

The chemical shift of the proton of sugar directly attached to the flavonoid hydroxyl group depends both on the nature of the flavonoid and on the position and stereochemistry of attachment. Methyl proton signals with few exceptions appear in the region  $\delta$  3.5 – 4.1 ppm. While most aromatic acetoxy proton signals occur in the range  $\delta$  2.25 – 2.5 ppm (Horowitz 1967).

### **1.5 Work problem**

The total area devoted to the cultivation of onions in Sudan 22,000 acres and produces 40 jwal per acre .there has been the development of techniques this year, where production reached 115-300jawal per acre. Red onion is grown in large quantities because it can be stored for long periods and can be stored for up to six months, after the end of the storage period is cleaned inventory and get rid of the crust (waste), which may be up to one-third of the stock ,the higher the storage period the greater the amount of waste and this waste contain Quercetin which a kilogram of it between 100 to1000\$ depending on its quality, and the question of why the Sudanese government does not use this waste to produce money(unpublished).

### **1.13 Objectives of study**

- Extraction of quercetin from allium cepa skin (onion skin).
- Identification of quercetin.

# Chapter2



## **2- Material and methods**

### **2.1 Materials**

#### **2.1.1 Chemicals and Instruments**

Analytical grade reagents were used. The UV-Visible spectra were recorded on a Perkin- Elmer Lambda -2 UV- Visible Spectrophotometer. The IR spectra were run on a Perkin- Elmer 1310 Infrared Spectrophotometer.

#### **2.1.2 Plant material**

The allium cepa skin was collected from al-lafah market.

### **2.2 Methods**

#### **2.2.1 Preparation of test reagents for phytochemical screening**

##### **2.2.1.1 Flavonoid test reagents**

(i) Aluminum chloride solution

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

(ii) Potassium hydroxide solution

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

(iii) Ferric chloride solution

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

### **2.2.2 Preparation of allium cepa skin extract for screening**

(500g) of powdered air – dried allium cepa skin were extracted with (3.5 liter ) absolute methanol for 24 hr. the chlorophyll and waxy materials were removed from methanolic extracts by adding (4g) of activated carbon . The alcoholic extract was filtered .the solvent was removed from the crude extract by vacuum distillation at 35°C .This prepared extract (PE) was used for the following tests.

#### **2.2.2.1 Test for flavonoids**

(0.5g) of prepared extract was dissolved in (30 ml) 95% ethanol and filtered .The filtrate was used for the following tests.

- (i) To (3 ml) of the filtrate few drops of methanolic aluminum chloride were added. A dark yellow colour soon developed.
- (ii) To (3 ml) of the filtrate few drops of potassium hydroxide solution were added. A dark yellow colour was observed.
- (iii) To (3 ml) of filtrate few drops of ferric chloride solution were added. A blue coloration was observed.

### **2.2.3 Extraction of flavonol aglycones from the allium cepa skin**

(4.2g) of prepared sample were hydrolyzed by refluxing at 90°C with (42ml) of HCL (6M) and (200ml) pure ethanol for 2 h .the resulting aglycones were extracted 3 times with ethyl acetate .the ethyl acetate extract evaporated by vacuum distillation at 35°C giving a brown solid (3.2g). Thin layer chromatography was then employed for fractionation.

### **2.2.4 Thin layer chromatography of the crude ethyl acetate extract**

Part of the brown solid ( 0.1 g) was dissolved in acetate (2 ml) and applied as concentrated spots on silica gel coated (0.2-0.3mm) and glass plates(20×5 cm) .these plates were developed in an organic solvents mixture of benzene ,acetic acid and water (125:72:3) , air dried and visualized under ultraviolet (UV) light . Three spots were observed which were further confirmed by spraying the plates with 5% ethanolic ferric chloride solution.a few other solvent system were used, but in investigation the solvent system of benzene ,acetic acid and water (125:72:3) gave excellent results. The spot at RF value 0.78 was eluded from plate by ethanol. Removal of the solvent gave compound I .The IR and UV spectra of compound I was then recorded. The melting point of compound I was recorded (313°C).

### **2.2.5 UV shift reagents**

- Stock solution of sodium methoxide and aluminum chloride

- I. Sodium methoxide: 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 tightly fitting plastic stopper.
  - II. Aluminum chloride stock solution:( 5g) of fresh anhydrous aluminum chloride were added cautiously to spectroscopic methanol (100ml)
- Sodium acetate ( $\text{NaOC}_2\text{H}_5$ ): Anhydrous powdered  $\text{NaOC}_2\text{H}_5$  is placed in crucible and heated slowly in a mineral oil bath until fused. Let cool and pulverize.
  - Boric acid ( $\text{H}_3\text{BO}_3$ ): Anhydrous powdered reagent grade  $\text{H}_3\text{BO}_3$  is used

#### **2.2.6 The UV spectra of compounds I in presence of sodium methoxide**

Three drops of sodium methoxide were added to a solution of compound I (in methanol (2 ml) and the UV spectrum was immediately recorded.

#### **2.2.7 The UV Spectra of compounds I presence of aluminum chloride**

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

### **2.2.8 The UV spectra of compounds in presence of sodium acetate\boric acid**

Excess of powdered anhydrous  $\text{NaOC}_2\text{H}_5$  and  $\text{H}_3\text{BO}_3$  was added with shaking to a cuvette containing (2 ml) of the solution of compound I in methanol and the UV spectrum was recorded after two minutes

# Chapter 3

### **3-Results and Discussion**

#### **3.1 Thin layer chromatography of crude ethyl acetate extract**

Plate size:-20×5 cm

Silica gel:-0.2-0.3 mm

Solvent system: -benzene: acetic acid: water (125:72:3)

Developing reagent: - 5% ethanolic ferric chloride solution



### 3.2 Thin layer chromatography of isolated compound

Plate size:-20×5 cm

Silica gel:-0.2-0.3 mm

Solvent system: -benzene: acetic acid: water (125:72:3)

Developing reagent: - 5% ethanolic ferric chloride solution

$R_f = 0.78$

$R_f$  of standard quercetin= 0.78

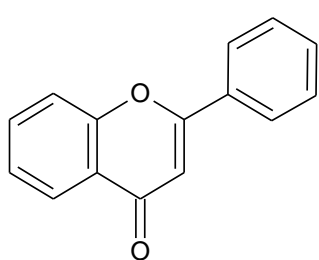




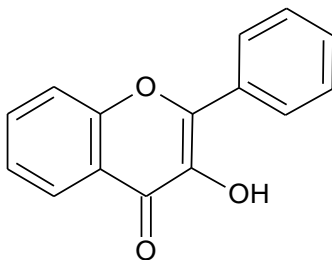
### 3.3 Spectral data of compound 1

The IR spectrum of compound (1) (Fig.1) gave  $\nu$  (KBr)  $3294\text{ cm}^{-1}$  (OH),  $2934\text{ cm}^{-1}$  (C-H,Ar),  $1734\text{ cm}^{-1}$  (C=O),  $1503\text{ cm}^{-1}$  and  $1608\text{ cm}^{-1}$  (C=C aromatic) and  $1061\text{ cm}^{-1}$  (COC ether).

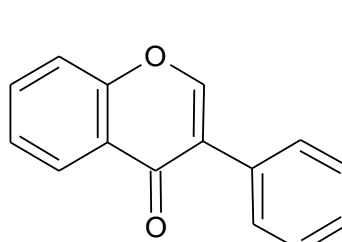
Since the infrared showed carbonyl absorption then the possibility that compound is neither an anthocyanin nor a catechin. It is probably a flavone, flavonol, isoflavone, flavanone or aurone.



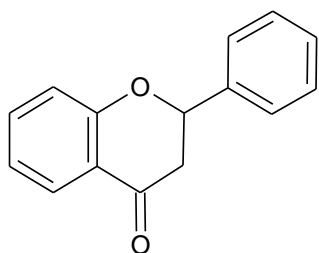
Flavone



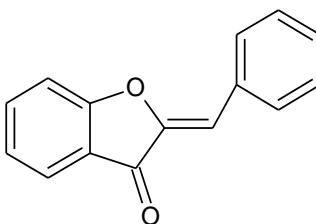
Flavonol



Isoflavone



Flavanone



Aurones

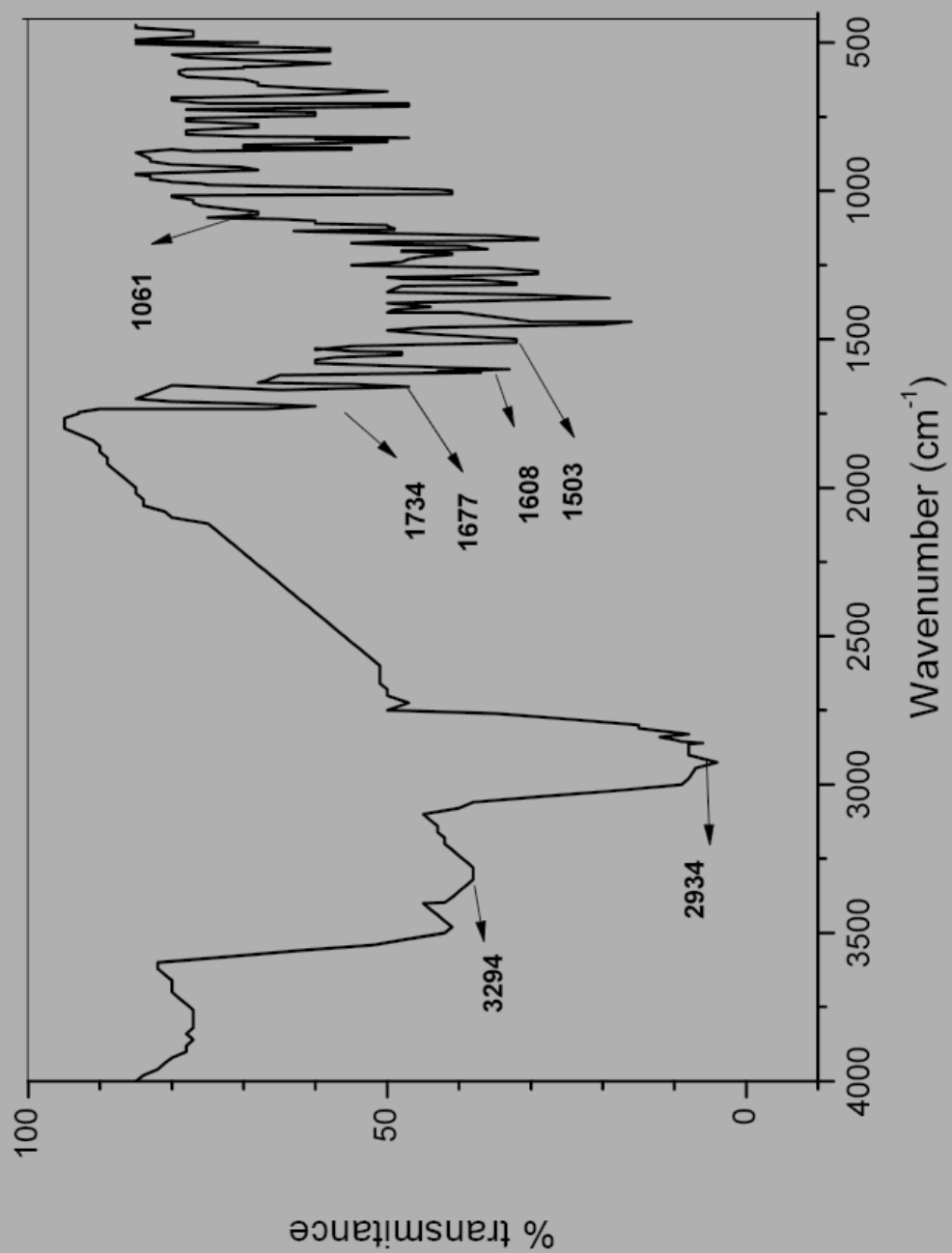
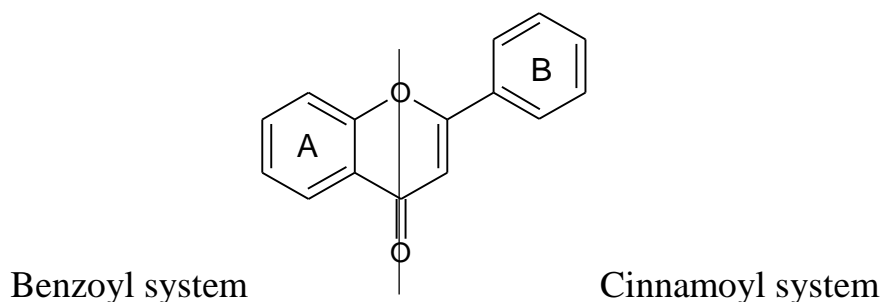


Fig (1): IR spectrum of compound 1

Considerable structural features have also been obtained using the UV spectroscopy to differentiate between flavonoids with double bond in position 2-3 and those lacking such bond. The UV spectra of most flavonoids consist of two major absorption maxima, band I in the range 300-400 nm arising from B-ring, and band II in the range 240-285 nm arising from A-ring. The appearance of both bands in the UV spectrum demonstrates conjugation between the benzoyl and cinnamoyl systems as in flavones, flavonols, chalcones and aurones.



(Fig.2). shows UV-Vis spectra for compound 1 exhibits two absorption bands at 255 and 373 nm referring to conjugation in the B-ring and A-ring and hence the compound 1 is neither flavanones, flavan-3-ols or dihydroflavonols



**Fig.(2): UV spectrum of compound 1 in Methanol**

Sodium methoxide is a strong base and ionizes to some extent all hydroxyl groups on the flavonoid nucleus. Sodium methoxide is, however, diagnostic of free 3 – and/or 4' –hydroxy groups. It gives bathochromic shift in presence of such functions, but with decrease in intensity in case of a free 3- OH.

When a methanolic solution of compound 1 was treated with a few drops of the shift reagent NaOCH<sub>3</sub> (Fig3) exhibited two absorption peaks, one maximum at 260 nm with shoulder peak at 329 nm. This indicated the presence of 3, 4' - hydroxy groups. NaOCH<sub>3</sub>/methanol solution spectrum showed shoulder peak at 239 nm which was an indicative of presence of 7-hydroxy group. This clearly indicates that compound 1 is flavonols.

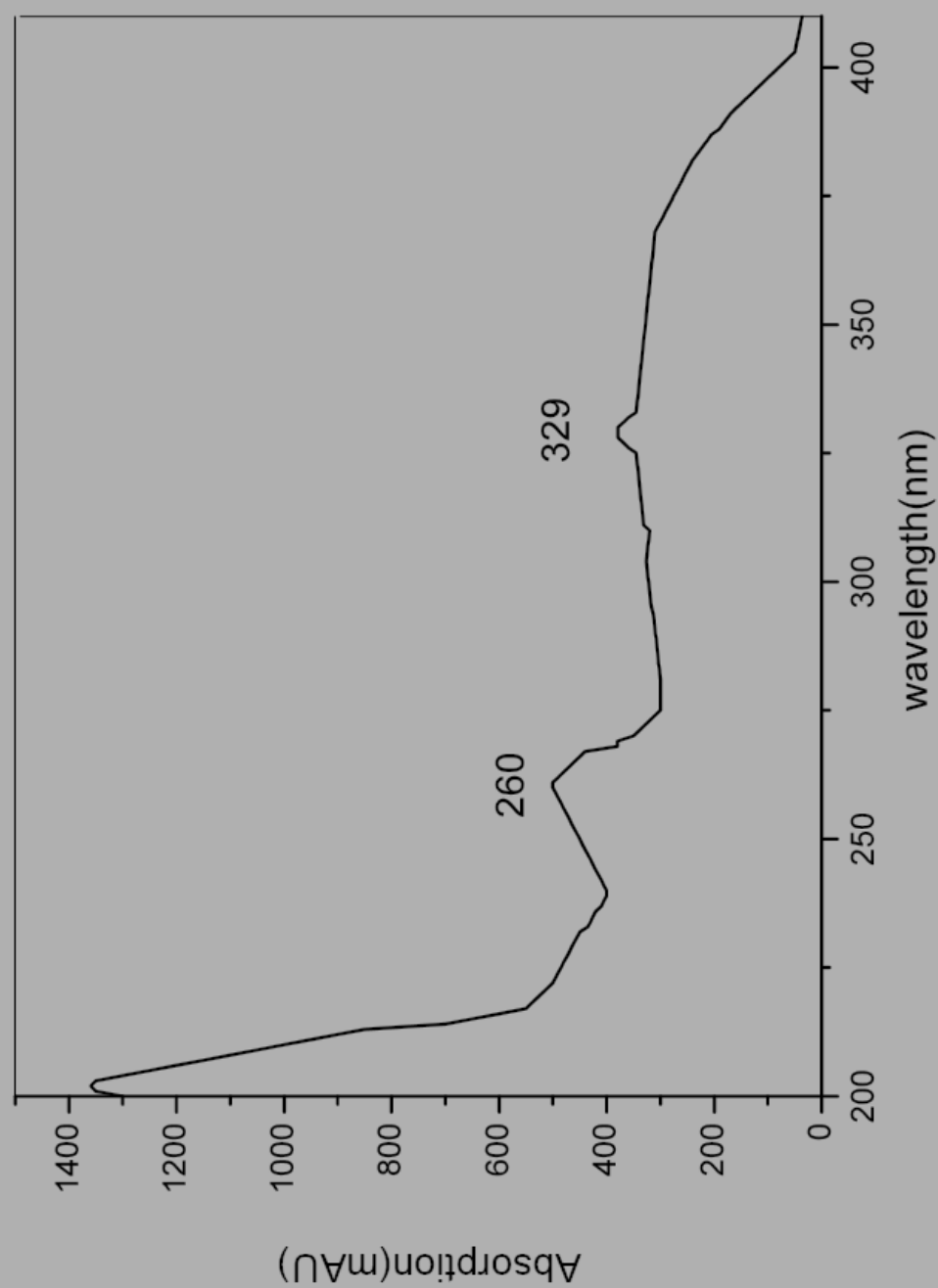
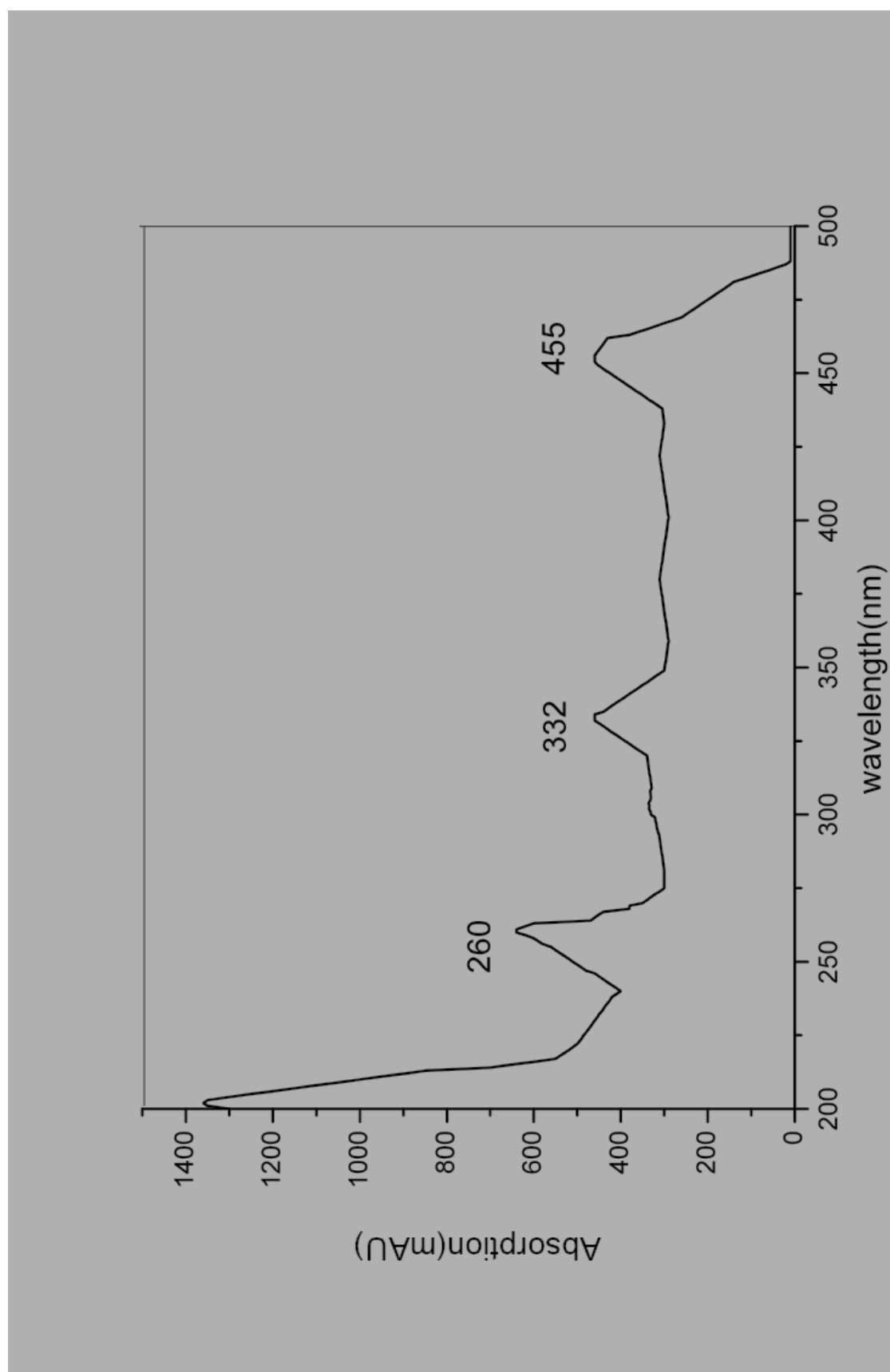


Fig.(3): UV spectrum of compound 1 in NaOCH<sub>3</sub>

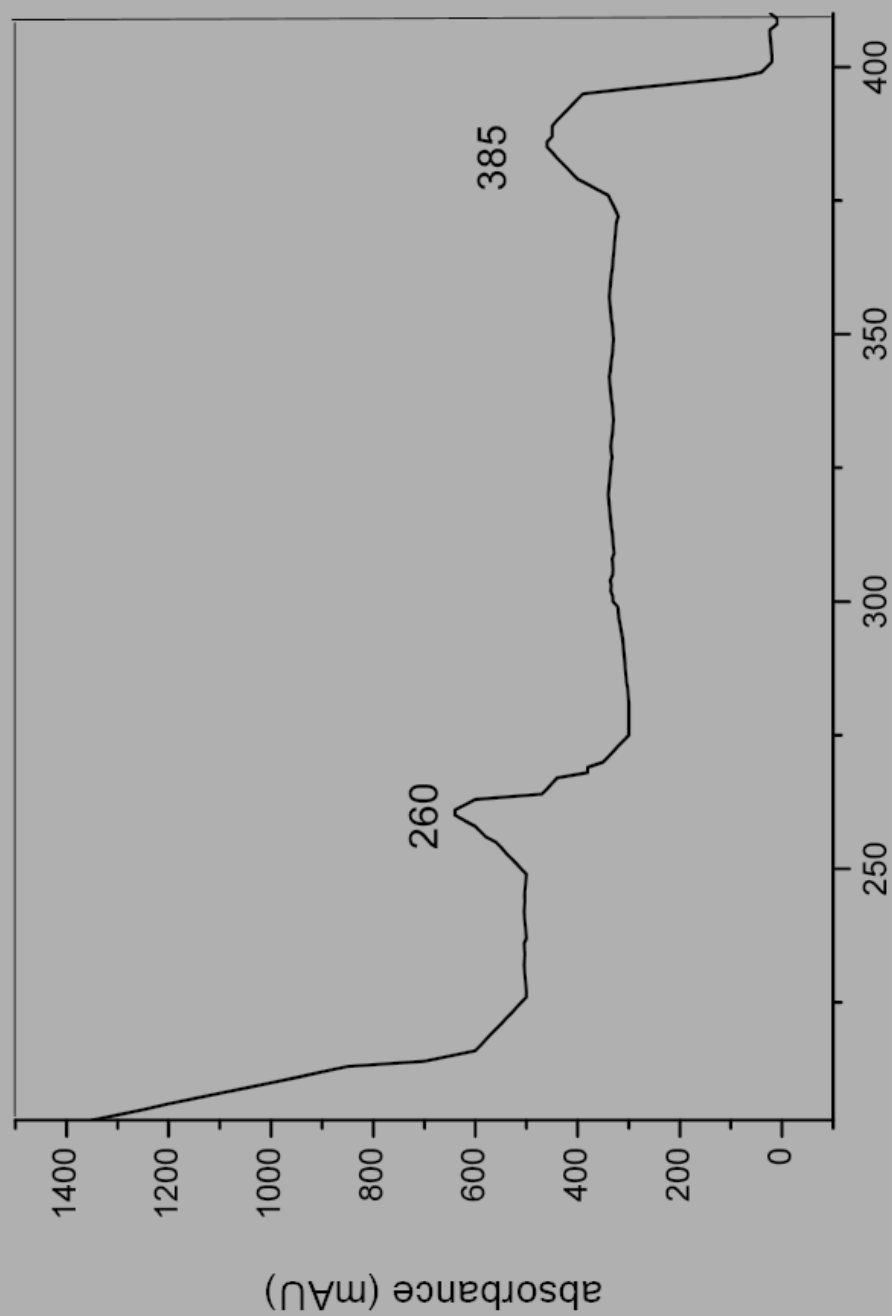
The bathochromic at band I upon addition  $\text{AlCl}_3$  Fig (4) witch was indicating the presence of 5-hydroxy group.



**Fig (4): UV spectrum of compound 1 in  $\text{AlCl}_3$**



The bathochromic at band I upon addition  $\text{NaOC}_2\text{H}_5/\text{H}_3\text{BO}_3$  Fig (5) which was indicating the catechol system at B-ring (3', 4'-hydroxy groups).



**Fig.(5): UV spectrum of compound 1 in NaOAc/H<sub>3</sub>BO<sub>3</sub>**

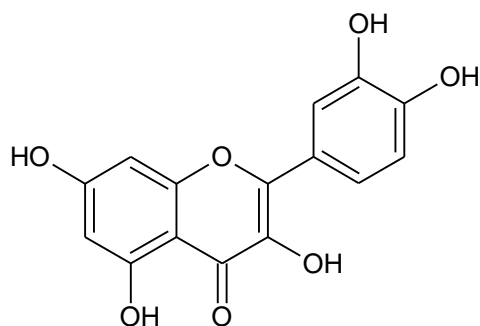
# Chapter4

## 4- Conclusion and Recommendations

### 4.1 Conclusion

Alcoholic extractive of allium cepa skin was isolated by thin layer chromatography by using solvent system: -benzene: acetic acid: water (125:72:3).

Three compounds were isolated. The structure of the compound 1 was suggested on the basis of its UV and IR spectral data (5, 7, 3', 4'-tetrahydroxy flavonol).



5, 7, 3', 4'-tetrahydroxy flavonol

### 4.2 Recommendations

The structures of the isolated flavonoids may further be elucidated by employing  $H^1$  NMR,  $^{13}C$  NMR and MS spectroscopy.

The isolated phytochemical may be evaluated for its ant inflammatory, antibacterial, antifungal, antimalarial and antioxidant potential.

Build factories to produce quercetin and disposal from onion waste.

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