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2005

Introduction

1.1. Falvonoids:- are ubiquitous group of polyphenolic substances which are present in all parts of plants[1] . Flavonoids are present in most edible fruits and vegetables, however the type of flavonoids obtained from different sources varies . The main dietary flavonoids and their source are listed in Table [١]. Flavonoid subclass such as flavonols, flavones and isoflavonones[2].

There are thousands of flavonoids in nature, and to aid in classification, they have been divided into eight major groups. These include the flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones[3,4]. Flavonoids that have received recent attention in the field of human nutrition include the proanthocyanidins in grape seeds, the flavanones (e.g. hesperetin) in citrus , the flavonols (e.g. quercetin) in onion and other vegetables, the catechins in green tea, the anthocyandin in bilberry, and the isoflavones in soybean[٣,٤]. That were shown in figure [4] .

A great number of medicinal plants contain flavonoids, having antioxidant, antibacterial, anti – inflammatory, anti-allergic, antimutagenic, antiviral, anti neoplastic, anti – thrombotic, and vascodilatory . The structure components common to these molecules include two benzene rings on either side of 3 carbon ring (ring C).

Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, super oxide anions, lipid peroxy radicals and hydrogen peroxide. Then and have been implemented in a number of disease including asthma[٥,٦], cancer[٧], cardiovascular[٨,٩], cataracts[١٠,١١], diabetes[12,13], gastrointestinal inflammatory[14,15], liver[16], mascular degeneration[١٧,18], periodontal disease[19] .

Table [1]: Main flavonoids and their sources in the diet [4]

Flavonoids	source	Content of aglycone (mg/kg)
Flavonol		
Quercetin-3,4-glucoside	onion	284- 486
Querectin-3-glucoside		
Querectin-3-rhamnoglucoside	black tea	10-25
Quercetin-3-galactoside	apple	21-72
Quercetin-3-rhamnoside		
Quercetin-3-glucoside		
Quercetin-3-rhamnoglucoside	black currant	44
Quercetin-3-rhamnoside		
Quercetin-3-galactoside		
Myrisetin-3-glucoside		
Flavone		
Luteolin-7-glucoside	red pepper	7-14

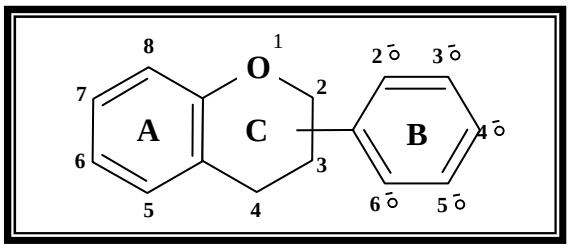
Flavanone		
Hesperetin-7-rhamnoglucoside (hesperidin)	orange juice	116-201
Naringenin-7-rhamnoglucoside (naringin)		15-42
Naringenin-7-rhamnoglucoside (naringin)	grapefruit juice	68-302
(+) -Catechin	apple	4-16
(-)- Epicatechin		67-103
(+) -Catechin	red wine	16-53
(-)- Epicatechin		9-42
Anthocyanins	black currant	760
Isoflavones		
Genistein-7-glycoside	soy bean	480
Daidzein-3-glycoside		330

The antioxidant effect of flavonoids can reside both in their radical-scavenging activity or in their metal-chelating properties, of which the former may dominate[20]. The antioxidant activities of flavonoid components synergize the antioxidant of vitamin C, vitamin E, and carotenoids. As such flavonoids represent an important nutrition component in the body's defenses against free radical damage[21].

Quercetin has been reported to block the "Sorbitol Pathway" that linked to many problems associated with diabetes. Rutin and several other bioflavonoids may also protect blood vessels. As antioxidant, some bioflavonoids, such as quercetin protect LDL-cholesterol from oxidative damage. Others, such as the anthocyanidins from bilberry, may help protect the lens of the eye from cataracts. Research on animal suggested that naringenin may have anticancer activity[22]. The link between flavonoids and arteriosclerosis was based partly on the evidence that some flavonoids possess antioxidant properties and have been shown to be potent inhibitors of LDL oxidation in vitro[23]. The phenolic substances in red wine inhibit oxidation of human LDL[23]. Flavonoid have also been shown inhibit platelet aggregation and adhesion[24]. Which may be another way to lower the risk of heart disease. Isoflavones in soybean foods have been reported to lower plasma cholesterol and also to have estrogen like effects[25].

1.2. Structures of Flavonoids:

The basic flavonoids structure consist 15 carbon atoms arranged in three rings (C_6-C_3-C_6) labeled A, B and C[26]. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of C ring .The benzene ring (A) is condensed with six member ring (C) and in the 2 or 3-position carries a phenyl benzene ring (B) as a substitute[27] .



Figure[1]:Flavonoids structure

Flavonoids containing a hydroxyl group in position 3 of the C ring are classified as 3-hydroxy flavonoids (flavonols, anthocyanidins, leucoanthocyandins and catechin), those lacking it are named or classified 3-desoxy flavonoids (flavanones and flavones)[27].

Classification within the two families was based on whether and how additional hydroxyl or methoxy groups and their position in the structure flavonoid[28]. Isoflavonoids differ from the other groups in which the B ring is bound to C-3 of ring C instead of C-2. anthocyanidins and catechins lacking the carbonyl group on C-4 of ring C[28]. Some of these structures are shown in figure [2]

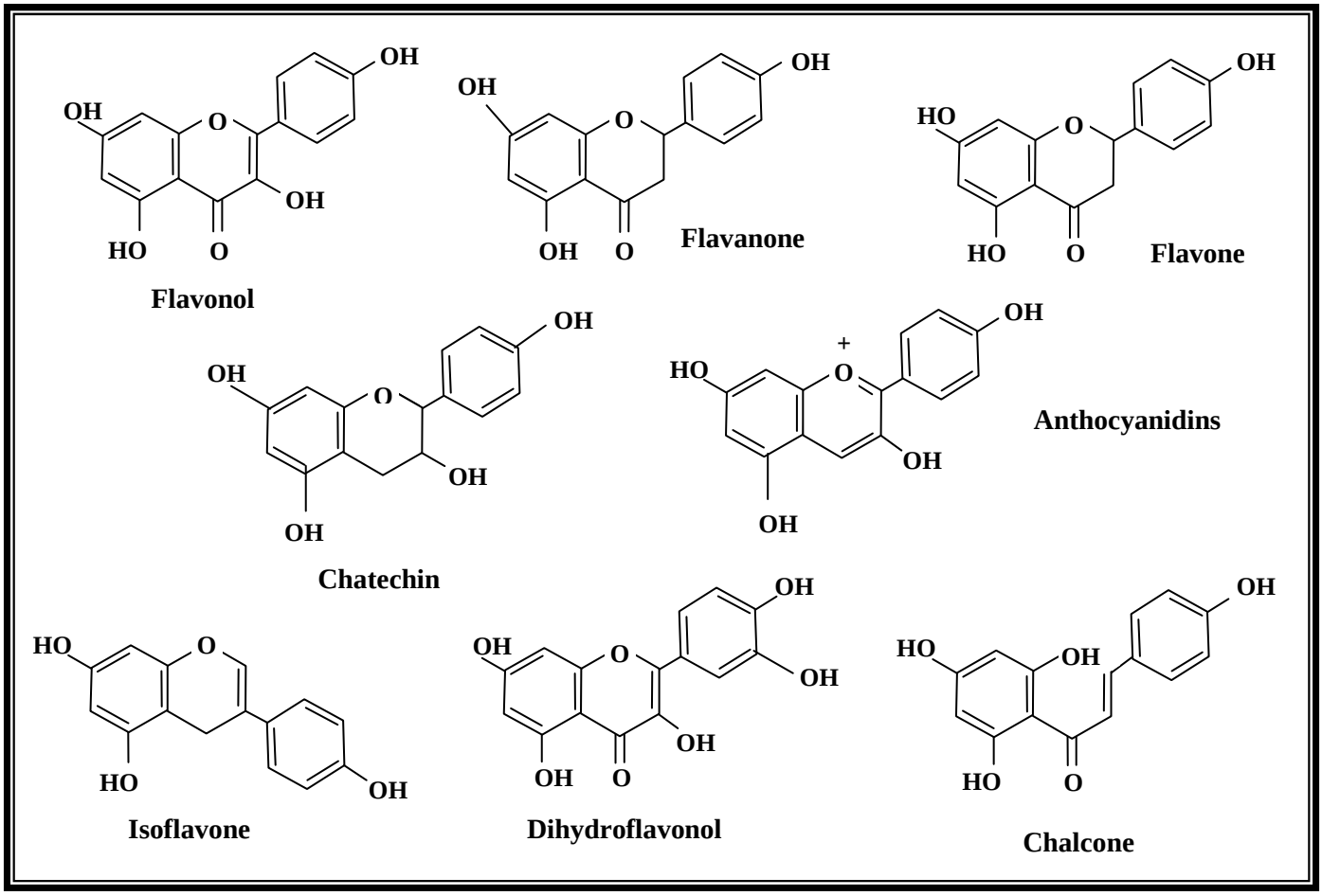


Figure [2]: Classes of Flavonoids

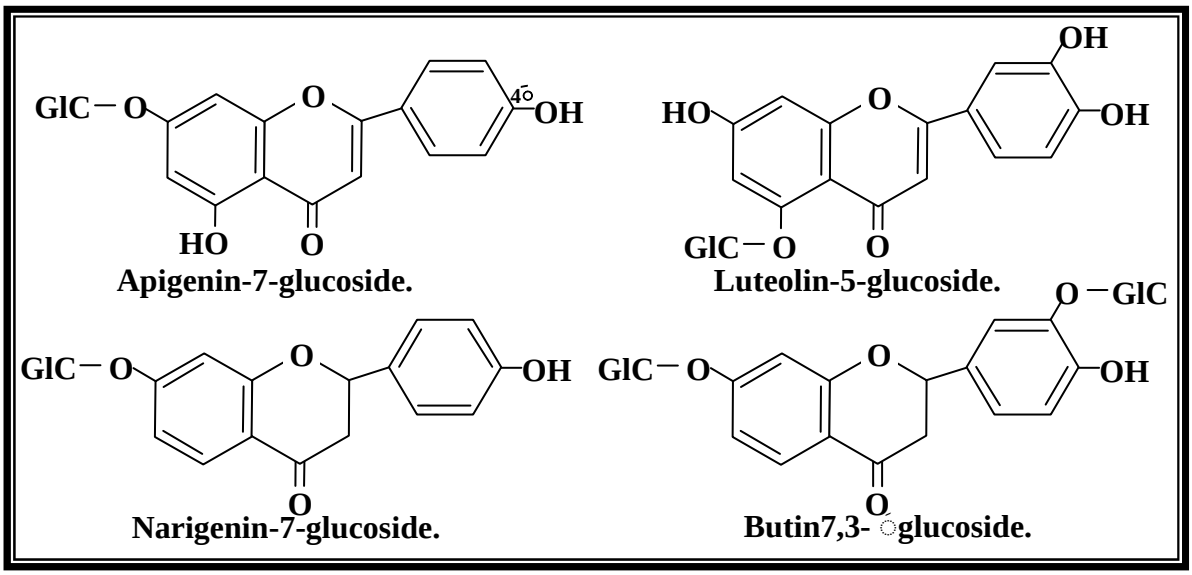
1.3. Chemistry of flavonoids:-

The chemical structure of flavonoids are based on a C_{15} skeletal on with chromone ring (ring A and ring C) bearing second aromatic ring B in position 2,3. Showed in figure1. In few cases, the six membered heterocyclic ring C occurs in a two isomers form or is replaced by a five-membered where the resulting heterocyclic is of the furan type[29 – 31].

Various subgroups of flavonoids are classified according to the substitution patterns of ring C. both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification[29 – 31].

Flavonoids are mainly present in plant as glycosides. Aglycones (the form lacking sugar moieties) occur less frequently. At least of the eight different mono saccharides or combinations of these (di-or trisaccharides) can bind to the different hydroxyl groups of the flavonoid aglycone[32].

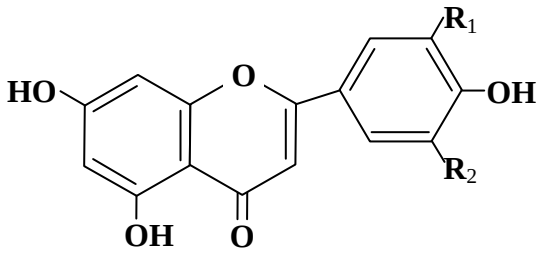
The most common sugar moieties are D-glucose and L-rhamnose. The glycosides, are usually O-glycoside with sugar moiety found in edible plants or food , the sugar moiety bound to the hydroxyl group at the position 3 and 7 on the ring C[32]. Some example of flavonoids glycosides are shown in figure[3]



Figure[3]: Flavonoids glycosides

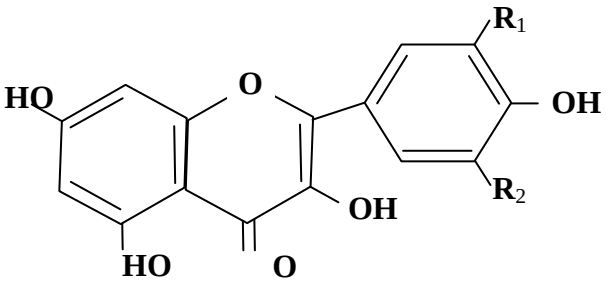
1.4. Classification of Flavonoids:-

1- Flavone



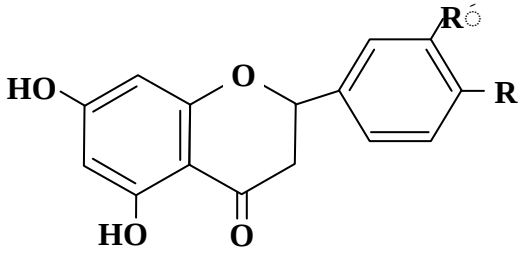
Examples
Apigenin $R_1 = R_2 = H$
Luteolin $R_1 = OH, R_2 = H$
Tricin $R_1 = R_2 = OMe$

2- Flavonol



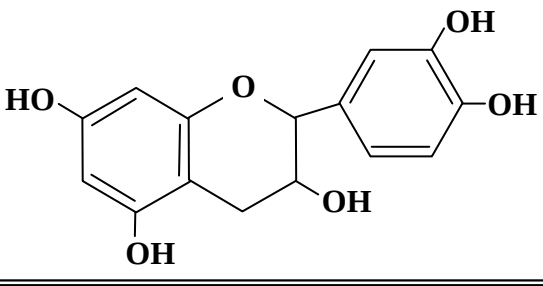
Kaempferol ($R_1 = R_2 = H$,)
Quercetin (R_1 =, R_2=H)
Isorhamnetin(R_1=OMe, R_2=H)
Myricetin ($R_1 = R_2$=OH)
Rutin ($R_1 = R_2$=OH, O-$C_{12}H_{21}O_9$ attached to OH group in position 3 of C ring

3- Flavanone

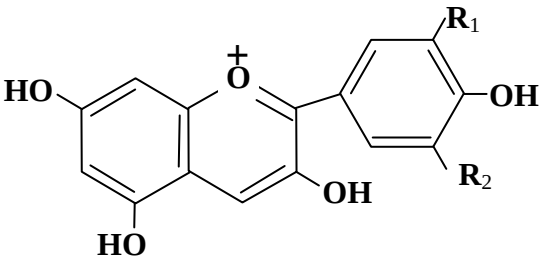


Pinocembrin (R = R = H)
Naringenin (R = OH, R = H)
Hesperetin (R_1=H , R_2= OCH_3)
Eriodictyol (R = R = OH)

4- Catechin

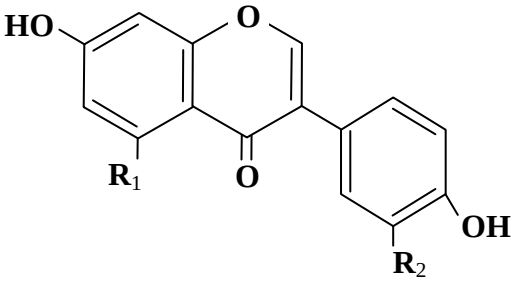


5- Anthocyandin



Pelargondin ($R_1 = R_2 = H$)
Cyanidin ($R_1 = OH, R_2 = H$)
Peonidin ($R_1 = OMe, R_2 = H$)
Malvidin ($R_1 = R_2 = OMe$)

6- Isoflavonoids



Daidzein ($R_1 = R_2 = H$)
Genistein ($R_1 = OH, R_2 = H$)
Orobol ($R_1 = R_2 = OH$)

Figure[4]:Types of Favonoids and their conman names

1.5. *Flavonols, Flavones and Flavanones:-*

Flavonols are simply flavones in which having a hydroxyl group at position 3 [(33)]. Flavonol formation in plants was both associated with lignifications in leaves and wood[(34)] and with UV absorption by flowers[(35)].

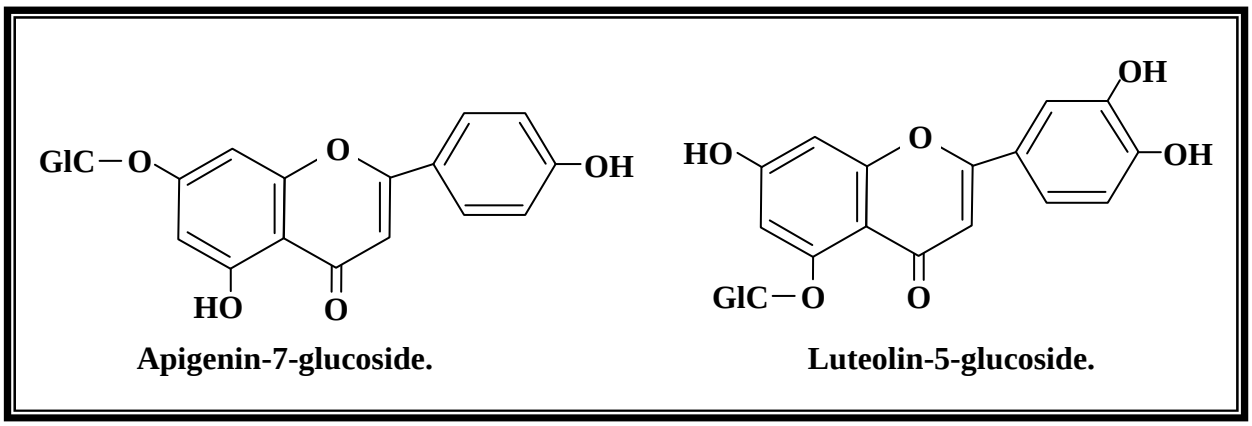
The most common flavonol in the diet is quercetin, It is present in various fruits and vegetables, but the highest concentration was found in onion[(36)]. Quercetin is present in plants in many glycosidic forms[(28)] with quercetin-3- rutinoside which is also called quercetin-3- rhamnoglucoside or rutin, being one of the most widespread forms. In onion, the compound is bound to one or two glucose molecules (quercetin- 4 -glucoside , quercetin-3,4 -glucoside).

Other quercetin glucosides present in the diet are, for instance, quercetin galactosides (apples) and quercetin arabinosides (berries) .

Quercetin is known to exhibit biological effects such as antioxidant[37,38], anticarcinogenic[39], anti-inflammatory and antiaggregatory effects[40]. The other flavonols in the plant include kaempferol (broccoli), myricetin (berries) and isorhamnetin (onion). Flavones differ from flavonols in lacking a 3-hydroxy substitution, the main flavones in the plants are apigenin and Luteolin. They are present in rather low concentration in red pepper[36] and celery[41].

Apigenin and Luteolin, occur frequently in the angiosperms[34]. These two compounds occur sporadically in the leaves of dicotyledons; they probably occur more regularly in flowers. Tricin, which might be expected to occur with it's close biogenetic relationship to sinapic acid, is definitely rare[42].

About twelve different classes of flavon glycoside have been isolated. The sugar is attached most commonly at the position 7 (e.g. apigenin-7-glucoside) as shown in figure [5] and much less often at the position 5. Flavone-7- glucosides and 7-rutinoside are also a common type[42].

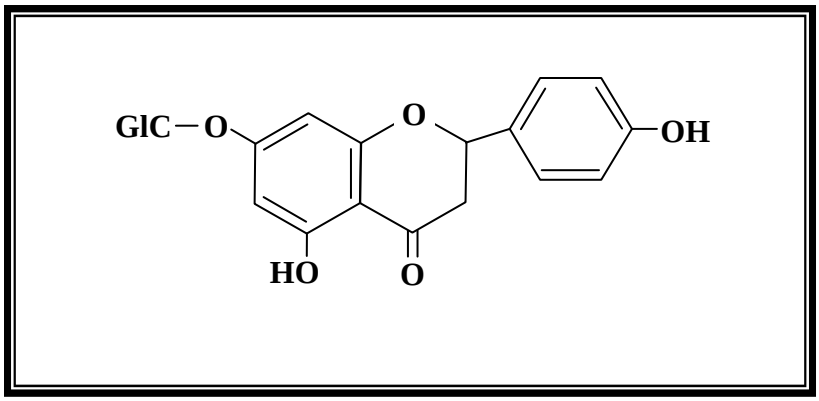


Figure[5]: Flavones glycosides

Flavanones differ from flavones in having single bond in the 2,3-position instead of the double bond . Naringenin and eriodictyol, which correspond to apigenin and luteolin, are, like two flavones, relatively common[(42)] .

Flavanones occur almost in the solid tissue of plant, but concentration of several hundred milligram per liter present in it's juice as well[(43)]. Low concentrations of narengenin are also found in tomatoes. Fresh tomatoes, especially tomato skin, contain naringenin chalcone, which is converted to naringenin during processing to tomato ketchup[(44)].

The glycosidic pattern of flavanones is rather similar to that of flavones and eight classes of glycoside have been found[(45)] naringenin-7-glucoside shown in figure 6. Pruninl common constituent of prunes wood[(45)].



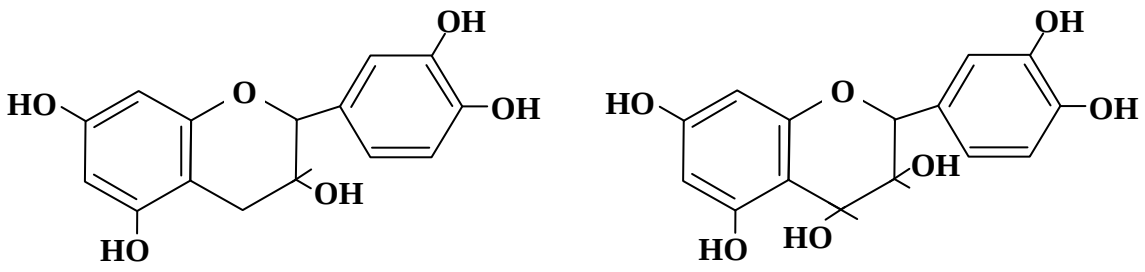
Figure[6]: Naringenin-7-glucoside structure

1.6. Catechin :-

Catechins usually occur as aglycones or esterified with gallic acid. The(+)-catechin and (-)-epicatechin are found in various fruits and vegetables such as apples, pears, grapes and peaches[46]. The highest concentration of catechins are found in tea and red wine[47].

Catechins are structurally closely allied to the leucoanthocyanidins and are also optically active (+)-catechin itself is the quercetin /cyanidin analogue[47]. Catechin itself, 3,5,7,3ó 4-ópentahydroxy flavan, is one of the most widely occurring flavonoids and optically active. The isomers may be separated by paper chromatography with water[48].

Two optically active catechins and their gallate esters present in tea leaves were separated on paper chromatography and the gallate esters have also been separated by column chromatography on cellulose powder[49]. The structure of these two compounds are present in figure [7]



Figure[7]: Catechin structure

1.7. Functions of Flavonoids:-

1-) Flavonoids as Antioxidant:-

Mechanisms of antioxidant action includes (1)suppressing ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production, (2) scavenging ROS, and (3) regulating or protecting antioxidant defenses. Flavonoids fulfill most of the criteria required for antioxidant action [50,51]. Thus , the effect is of two-fold action :

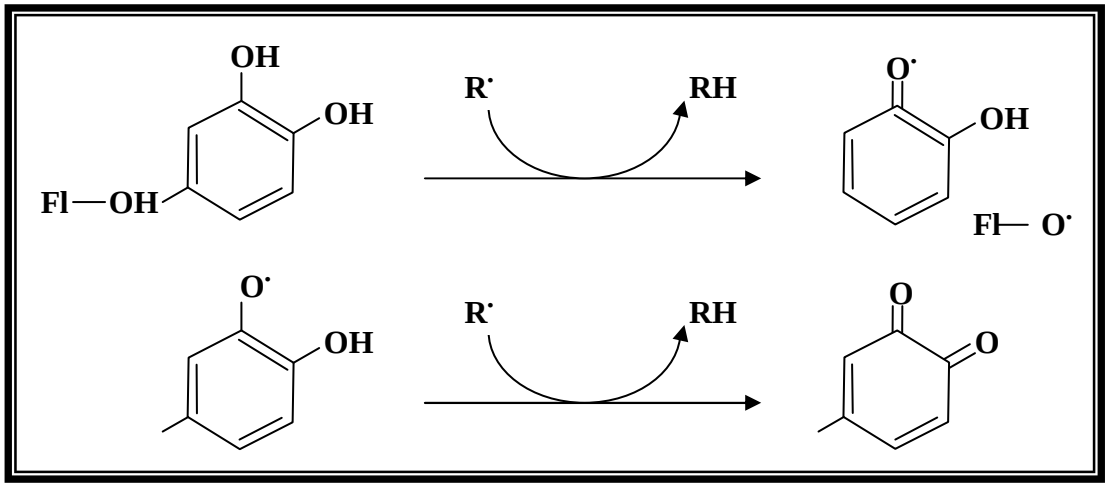
1-) Flavonoid inhibit the enzymes responsible for superoxide anion production, for instance xanthine oxidase and protein kinase C[52]. Flavonoids reduce the super oxide radicals in the pH range from 7 to 10, depending on their redox properties . In addition to redox properties, the reactivity of superoxide also depends on the charge[52].

The rate constant for reaction of superoxide with uncharged catechin at pH 7 (determined by pulse radiolysis) is approximately 4 times higher than the corresponding rate at pH 10, K = 1.8×10^4 $M^{-1}S^{-1}$ where catechin is doubly negatively charged[52].

Furthermore, flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism (as shown in equation 1)[50]. Free iron and copper help in formation of reactive oxygen species (ROS), exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical[50]. However, these metal ions are essential for many physiological functions, constituents of hemoproteins and cofactors of different enzymes, including those involved (e.g., iron for catalase, Cu and Zn for super oxide dismutase) in the antioxidant defense.

$$H_2O_2 + Fe^{2+}(Cu^{+}) \rightarrow O^{\bullet}H + O\bar{H} + Fe^{3+}(Cu^{2+}) \quad \text{--------(1)}$$

2) Due to their lower redox potentials ($0.23 < E_7 < 0.75$ V), which is the same compared to other antioxidizing species like 4-methoxyphenol (E_7=0.73V)[52], flavonoids (F1-OH) are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 0.13-1.0 V such as superoxide radicals by hydrogen atom donation, as described in figure 8 below. Where, $R^{\bullet}$ represents superoxide anion. The aroxyl radical (Fl-O$^{\bullet}$) may react with second radical, acquiring a stable quinine structure[52].



$$Fl-OH+R^{\bullet} \rightarrow Fl-\dot{O}+RH \quad \text{--------}(2)$$

Figure[8]: Scavenging of ROS by Flavonoids [50]

The aroxyl radicals could interact with oxygen, generating quinines and super oxide anion, rather than terminating chain reaction. The last reaction may take place in the presence of high levels of transient metal ions and is responsible for the undesired pro oxidant effect of flavonoids[50].

Thus the overall capacity of flavnoids to act as antioxidants depends not only on the redox potential of the couple *Fl-O•/Fl-OH* but also on possible side reaction of the aroxyl radical [50].

2-)Flavonoids as Enzyme Inhibitor:-

Flavonoids inhibit the enzyme responsible for peroxide anion production, such as xanthine oxidase and protein kinase C. Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, in which all involved in reactive oxygen species generation[50].

The enzymes cyclooxygenase and lipoxygenase act on arachidonic acid in cell membranes, oxidizing arachidonic acid and forming potent pro-inflammatory metabolites including prostaglandins, leukotrienes and thromoboxanes. Many flavonoids, including quercetin, rutin, baicalein, kaempferol, curcumin, silymarin and green tea polyphenols exhibit inhibition of cyclooxygenase and lipoxygenase in vitro[53,54].

Flavonoids may inhibit enzymes by non-specific binding, competing or reacting with substrate. Specific oxidation of sulphhydryl groups control tertiary enzyme structure, or through complexing metallic prosthetic groups[55]. Flavonoids have been added to isolated enzyme system at levels of 1 to 4 μmole[55]. Malvidin -3-glucoside inhibits malate dehydrogenase and glutamate decarboxylase isolated from the bacterium salmonella enteritidis. Malate dehydrogenase was inhibited non-competitively while glutamate decarboxylase showed a competitive or non-competitive inhibition depending on a number of experimental factor[56].

Protein synthesis may be inhibited by flavonoids, at least in vitro, found that hesperitin, naringenin, quercetin, and their glycosides, phloridzin, inhibited incorporation of C^{14}-leucine into tuber slices of solanum tuberosum or cell free extracts of Escherichia Coli. In all cases, the aglycons were much more effective inhibitors than their glycoside[57] .

3-) Flavonoids as Vitamine C:-

Flavonoids have been associated with vitamin C , flavonoids in citrus fruits, then referred to as "Vitamin P" were known to enhance vitamin C activity[58] .

- Flavonoids posses vitamin C-stabilizing and antioxidant-dependent vitamin C-sparing activities.
- Both flavonoids and vitamin C have complementary roles in protecting the stomach and the intestine from food born substances which can cause cancer[58].
- Vitamin C has been shown to enhance the ability of flavonoids to inhibit tumor growth[58].
- Flavonoids and vitamin C occur together in plants.
- Vitamin C can protect flavonoids from oxidation.
- Flavonoids enhance vitamin C absorption or via versa [59].

4-) Flavonoids as Pigments:-

The pigments found in plants play important roles in plant metabolism and visual attraction in nature[60,61]. Major plant pigments include carotenoids, anthocyanins, other flavonoids, betalains, and chlorophylls. Flavonoids include red or blue anthocyanins and white or pale yellow compounds such as rutin, quercitin and kaempferol[62].

Flavonoids in flowers and fruit provide visual cues for animal pollinators and seed disperses to locate their target. They also occur in most other plant parts and in most genera. Flavonoids are located in the cytoplasm and plastids[63]. The flavonoids function include attraction for insects to distribute pollen and seeds; flavonoids are thought to act as sunscreens, protecting the plant against damaging ultraviolet radiation[63].

Flavonoids have a high absorbance in the 250 to 270 nm range, flavones and flavonols absorb significantly in the range from 330 to 350 nm and anthocyanins strongly absorb in the range from about 520 to 560 nm[64].

The type of compound attached to the basic structure greatly influences the color of the anthocyanins. For example the attachment of methyl group can result in reddish color and the presence of flavones or flavonols can cause a more bluish color[65].

Plant pigments are importance cues to humans and other herbaceous animals in helping identify plants, find plant parts such as fruit, leaves, stems, roots, or tubers, and determine stages of plant development such as fruit ripeness or overall senescence[65].

5) Flavonoids as Antibiotics:-

Most of the anthocyanins and leucoanthocyanins inhibited respiration and reproduction in some type of bacteria at the level of one or two μmoles if glucose were present in the medium. In the absence of glucose the flavonoid were metabolized[66]. More than 20 flavonoids were investigated, no compound was devoid of inhibitory activity toward one or more of the ten bacteria studied[66].

Flavonoids of many types have antiviral effect. In human cell lines (Hela Cells) herpesvirus hominis is inhibited by qurecetin at levels of 300 mg ml^{-1}[67] but not by rutin or dihydroquercetin[68]. When quercetin was added to cultures of several viruses associated with human maladies, viruses with an envelope were inhibited while those lacking such an envelope were moderately, or completely resistant[67].

Jain,[69] was found that quercetin and some of it's metallic complexes of it exhibited antibacterial and antifungal activities. Hg and Pb complexes were the most effective antimicrobial agents, but they are of limited therapeutic value because of their high toxicities. However they may be useful for external application.

El-Gammal[70] had tested six flavonoid compounds namely kaempferol, quercentin, myricetin, rutin, naringin and morin which were separated from different plant materials, for their antimicrobial property against bacterial and fungal strains as test organism. All compound inhibited yeast and fungi. Only four flavonoids (Kaempferol, quercetin, myricetin and morin) possessed antibacterial activity. Antihelmitic activity have been reported for flavonoids[71]. The chalcones were generally effective, especially those with few hydroxyl substituents.

1.8. Extraction, Isolation and Identification of Flavonoids:-

Flavonoids occur in virtually all parts of the plant, method of isolation depended on the type of flavonoids would been 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[72].

The possibility of enzymes action occurring during their early period of isolation, leading in particular to hydrolysis of glycosides[73], may be avoided by plunging of the plant material into boiling solvent or by rapid drying prior to extraction[74]. Pre-drying of plant material generally appears to increase the yield of extracts, 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 flavonoids aglycones, whilst the more polar solvents are used if flavonoid glycosides or anthocyanins are sought. The less polar aglycones, such as isoflavones, flavanones, and dihydro flavonols or flavones and flavonols which are highly methylated, are usually extracted with solvents such as benzene, chloroform, ether or ethyl acetate[75,76]. A pre-extraction with light petroleum ether or hexane is frequently carried out to rid plant material of sterols, carotenoids, chlorophylls, etc[77].

Flavonoid glycoside and the polar aglycones such as hydroxylated flavones, flavonols, bioflavonyls, aurones and chalcones are generally isolated from plant material by extraction with solvents like acetone, alcohol, water or a combination of them[78,79]. Perhaps the most useful solvent for the extraction of this group of compounds is 1:1 mixture of water methanol. Traces of acid are occasionally incorporated in the solvent for the extraction of flavonoid glycosides[80], although this practice is normally reserved for the extraction of anthocyanin and anthocyanidins[74,81]. The use of acid, however, can lead to hydrolysis of glycosidic materials.

TLC is a technique which has developed rapidly during the last decade and to a limited extent it has replaced by paper chromatography in analytical and small scale separation of flavonoids[82,83] .

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 a wider variety of detecting reagents [82,83] .

Flavonoids are not sufficiently colored to be visible to the eye on a thin layer plate; thus some form of visualization is necessary for spot detection. In many cases this was achieved by viewing the plate in UV-light (366 nm) either in the presence or absence of ammonia vapor[84]. Flavonoids some times appeared as dark spots against a fluorescent green background .

Another useful method of detection was brief exposure of the plate to iodine vapour which produced yellow-brown spots against a white background with most flavonoids. Flavonoids were chromotographed in solvents such as toluene-ethyl formate-formic acid (5:4:1)[85] and benzene–pyridine–formic acid(36:9:5)[86].Chloroform-methanol(96:4) was found useful for distinguishing flavones such as apigenin, chrysoeriol and luteolin[87] and have also been used with flavones and flavonols. Isoflavones, flavonones, and dihydro flavonol were generally chromotographed using less polar solvents. The isoflavones daidzein, genistein and biochanin have been separated using chloroform-methanol (92:8)[88] and ethyl acetate-light petroleum ether (3:1 and 1:1)[88]. Dihydro flavonols were separated[89] with chloroform-methanol-acetic acid (7:2:1)[89].

Flavanones such as naringenin, hesperetin have been identified using benzene-acetic acid–water (125:72:3)[90], chloroform-acetic acid-water (2:1:1) and by using benzene-pyridine-formic acid (6:9:5)[91]. Separation of flavonoid glycosides using alcoholic solvent n- butanol-acetic acid-water 4:1:5 (BAW), t-butanol-acetic acid water, 3:1:1 or water saturated with butanol) which produced separations based largely on partitioning[48].

More polar flavones and flavonols required more polar solvents. Thus apigenin luteolin, galanin, kaempferol, quercetin, myricetin, isorhamnetin, datiscetin and morin were separated well in toluene-chloroform-acetone (8:5:7). Similar flavonod mixtures were separated [91] using benzene-pyridine-formic acid (36:9:5).Although paper chromatography or TLC-cellulose was the method of choice for the separation of anthocyanins and anthocyanidins[8١,82] Other number of separation have been achieved on silica gel[81].

Ethyl acetate-formic acid- 2N HCl (85:6:9) was recommends for separation of malridin and peonidin which were difficult to distinguish by paper chromatography. Solvents such as n-butanol-formic acid-water (3:4:1) were satisfactory used for the separation of both anthocyanin and leucocyanidin[82].

1.9. Separation of flavonoid by High Performance Liquid Chromatography:-

HPLC is technique, somewhat similar to GLC except that the carrier gas is replaced by one solvent or solvent mixtures. The poly phenols in beers and red wines were separated using silica gel column (1mm x 2mm) with a UV detector was reported[92]. The solvent system was: hexane followed by methanol-chloroform-acetic acid (30:70:1) and hexane-chloroform (1:1), followed by methanol –chloroform-acetic acid (50:50:1), were found suitable for the separation of quercetin, kaempferol, catechin, catechin gallate, caffeic acid and coumaric acid . Separation of phenolic compounds using this method was carried out using different columns like ODS or Novapack or Lichrosorb Bondapack C_{18} [93] .

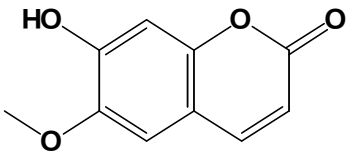
Bilk [94] separated qurectin and kaempferol using methanol: acetic acid : water (42:8:50) with flow rate of 1ml / min , using bounda pack C_{18} column, the solvent systems were used 10% acetic acid in water, at flow rate of 0.8 ml / min[95]. Teisserre[93] used two types of HPLC system, semi preparative HPLC and HPLC analyzer, by Novapack column, using two types of solvent systems one is dichloro acetic acid and the second is methanol. Flavonol, quercetin, rutin, kaempferol were separated from ginko leaf by HPLC using two mobile phases one is acetonitrile the other is 0.3% formic acid using minibore phenomenex luna 5µm C18 column with 400µl /min flow rate. Advantages claimed for HPLC analyzer included 1) short analysis time, 2) high resolution, 3) no derivatization required, 4) no risk of thermal decomposition and 5) easy quantitable[96].

1.10. Coumarins :-

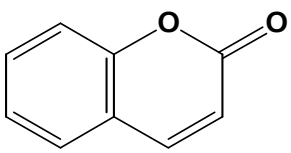
The most widespread plant coumarin is the parent compound, coumarin itself, which occurs in over twenty-seven plant families. It is common in many grasses fodder crops and is familiar as the sweet-smelling volatile material released from hay. Hydroxy coumarins were also found in many different plant families; the common ones were based on umbelliferone (7-hydroxycoumarin), scopoletin (6-hydroxycoumarin) were generally lipid soluble and be isolated by extraction of dried plant material with ether or light petroleum ether or ethyl acetate[(96)].

Coumarins were class of polypropanoids compounds which were naturally occurring phenolic compounds having aromatic ring to which a three-carbon side chain was attached . They were derived biosynthetically from aromatic amino acid phenylalanine and they may contain one or more C6-C3 residues[(96)].

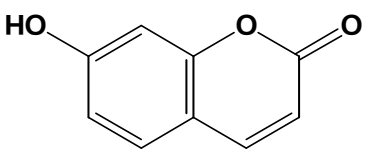
The hydroxycinnamic acid includes importance not only as providing the building block of lignans, but also in relation to regulation and to disease resistance[(96)]. The phenylpropanoids were included hydroxycoumarins, phenypropenes and lignans[(96)]. They occasionally occurred in bound form as glycosides and have then to be released by prior acid hydrolysis. TLC on silica gel was most commonly used for their separation[(96)].



Scopoletin



Coumarin



Umbeliferone

Coumarins is a lacton for hydroxy cinnamic acid and found as a colorless crystals, aromatic odor, bitter test, which is soluble in alcohols[97,98]. The other hydroxyl groups undergo methylation pathway by mean of addition methyl group before isomerisation and cyclic rearrangement like ferulic acid which gave scopoletin[97,98]. The first step for coumarins synthesis is O-hydroxylation group which means the addition of hydroxyl group to cinnamic acid derivatives so phenolase enzyme would prevent this step. Coumarin and coumarin derivatives play important role in inhibition of many fungals growth, so protect plant from virus and fungi accruing. [98]

1.11. Tannic acid:-

Tannic acid, a commercial form of tannin , is not a true acid but an acid-like substance called a polyphenols. Tannic acid is a basic ingredient in the chemical staining of wood. The tannic acid or tannin is already present in wood like oak, walnut, and mahogany. Tannic acid can be applied to woods that is low in tannin so chemical stains that require tannin content will react[99]. Tannic acid is the most common mordant for cellulose fibers such as cotton.

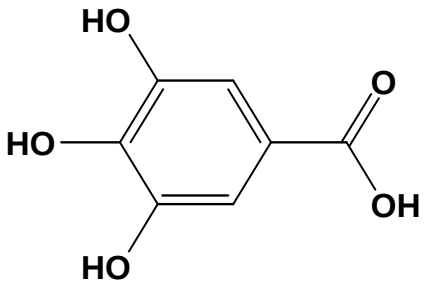
Tannin is often combined with aluminum and / or iron . Poly(phenols) tannin was used to convert animal skin into leather. Molecular formula of acid is ($C_{76}H_{52}O_{46}$), with molecular weight of 1701.18 g/mol. Tannic acid is yellowish to brown powder, odor characteristic, taste strongly astringent. soluble in 1 part of water or alcohol, soluble in acetone, almost insoluble in chloroform or ether[99].

Tannic acid used for the extraction of germanium, the production of ink, rust presenter of metals and as raw material for pharmaceutical industry. In printing and dyeing of textiles, it is used as color fixer, polygenetic dye and fiber deodorizer. It is also used in clearing and stopping dysentery and also for synthesizing sulphanilamide synergist [(99)]

1.12. Gallic acid:-

Gallic acid, chemically 3,4,5-trihydroxybenzoic acid, $C_6H_2(OH)_3COOH$, is a clear crystalline compound found in gallnuts, sumach, tea leaves, oak bark, and many other plants, both in its free state and as part of the tannin molecule. It can be prepared commercially by the hydrolysis of tannic acid with sulfuric acid[(100)].

Gallic acid has two functional groups in the same molecule, hydroxyl groups and a carboxylic acid group two molecules of it can react with one another to form an ester, digallic acid . They can yield various kinds of esters and salts when heated above 220°C, gallic acid loses carbon dioxide to form 1,2,3-trihydroxybenzene, $C_6H_3(OH)_3$, which is widely used in the azo dyes and photographic developers and in treating certain skin diseases. Gallic acid and its derivatives were used as photographic developers and used as astringents in medicine. Some gallates are used as antioxidants in foods, and in laboratories for absorbing oxygen [(100)].



Gallic acid

1.13. Medicinal Plants :-

Medicinal plants a gift of nature are being used against various infections and diseases in the subcontinent since past history. Herbs were used as food (vegetables) and flavors medicine for hundred of years in many parts of world. Whereas number of herbs have also been traditionally regarded as natural remedies for common ailments of human population. Furthermore some herbal plants are considered as house of medicines and played an important role in nearly every culture on earth, including Asia, Africa, Europe and the Americas [(101)].

The herbs chemical components provide important role in Pharmaceuticals companies. Furthermore interest of people in plant made medicines are increasing due to their concern about the side effects of powerful synthetic drugs and high prices of these medicines.

It is expected that raw materials obtained from plants will not only reduce the prices of medicines in the pharmaceutical market but also provide rapid and reliable system of treatment of various infections and diseases in human population[(101)]. Herbs can be used to make tea, perk up cooked such as meats , vegetables, sauces and soups, or to add flavor to vinegars, or mustards.

Many herbs are grown for their fragrance and used in potpourris, sachets, and nosegays; or to scent bath water candle, oil, or perfumes. More of 25% of our modern drugs came from plant extracts as active ingredients ; and researchers continue to isolate valuable new medicine from plants and confirm the benefits of those used in traditional folk medicine[(102)]. Herbs can be classified as either annual, biennial, or perennial.

Some herbs such as borage, anise, caraway, chervil, coriander, cumin, dill, and fennel, should be direct – seeded, because they grow easily from seed or do not transplant well. Other herbs, such as mints, oregano, rosemary, thyme, and tarragon, should be purchased as plants and transplanted or propagated be cutting to ensure production of the desired plant (do not come true from seeds)[102].

1.14. Chamomile plant:-

Chamomile is one of the widely used and well-documented medicinal plants in the world .It is included in the pharmacopoeia of 26 countries. In Germany, more than 4,000 tons of chamomile are produced yearly. The use of chamomile as a medicinal plant dates back to ancient Grece and Rome[103-105].

The name (chamomile) comes from Two Greek words meaning (ground apple) for it's apple-like smell. In Europe it is considered as "cure all" and meaning " capable for anything". Chamomile grows indigenously in Europe, N.W. Asia, N. Africa , and N. America and many other parts of the world .

The Egyptians considered the herb a sacred gift from the sun god, and used to alleviate fever and sun stroke[105]. This herb has been believed by Anglo-Saxons as one of nine sacred herbs given to humans by the lord[106].

The main medicinal part of the herb is the flower . The composite flower is white in color with a yellowish orange center. There are numerous varieties of chamomile, the most popular are Roman chamomile (Anthemis nobilis) and German chamomile (Matricaria chamomile) ; both are from composite family[104] .

German chamomile is considered as the more potent of the two. It has received more scientific evaluation, and is more widely cultivated than Roman chamomile .German chamomile possess anti-inflammatory, vulnerary, deodorant, bacteriostatic, antimicrobial, and anticatarrhal, carminative, sedative, antiseptic, and spasmolytic properties[107,108]. Chamomile products can be administrated in many forms. The common ones are: oral, topical, inhalation, solution for bath and infusion[107].

Chamomile is used both internally and externally to treat an extensive list of conditions . It is used externally for wounds, ulcers, eczema, gout, skin irritations, neuralgia, sciatica, rheumatic pain, poison ivy and conjunctivitis, and as hair tint and conditioner [107]. Chamomile used internally to treat hysteria, nightmares, and other sleep problems. One of chamomile main roles is as multipurpose digestive aid to treats gastrointestinal disturbance including flatulence, indigestion, diarrhea, anorexia, nauses, and vomiting. In children it is used to treat colic, croup, and fever. In woman's health, it is used an uterine tonic. Chamomile essential oil is also used for treatment of malaria and parasitic worm infections, cystitis, cold , and flu[109,110].

Chemical components of chamomile plant :-

The most active chemical constituents found in chamomile are:

1- Terpenoids: α-bisabolol , α-bisabolol oxide A and B, chamozulene sesquiterpens.

2- Flavonoids: apigenin , luteoline , quercetin and others .

3- Coumarins: umbelliferone.

4- Spiroethers: en-yn dicycloether.

One hundred twenty chemical constituents have been identified in chamomile including Terpenoids, Flavonoids, Coumarins and others, such as anthemic acid, choline, tannin and polysaccharides. German chamomile oil comprises of 0.5% to 1.5% in flower head[103,107]. The essential oil of both German and Roman chamomile is a light blue color due to the terpenoids chamazulene, it is artifact formed during heating and comprises about 5% percent of essential oil. It has antiallergic and antispasmodic properties. Bisabolol comprises 50% percent of German chamomile oil and a spasmolytic for intestinal smooth muscle[١١,١١٢].

The flavonoids apigenin and luteolin possess anti-inflammatory, carminative, and antispasmodic properties. Apigenin binds to GABA receptor and has a mild sedative effect. Apigenin reduces the latency of picrotoxin – induced convulsions[١١٣].

1.15. Toxicity and side effects of the chamomile plant:-

All herbal products carry the potential for contamination with other herbal products, pesticides, herbicides, heavy metal and pharmaceuticals. Allergic reaction can occur to any natural product in sensitive persons. Allergic reaction to chamomile are rare[114]. It 's allergic properties have been attributed to anthecotulid, a sesquiterpene lactone and matricarin, a proazulene[107,115]. Some individuals allergic to other members of the composite family are allergic to chamomile. Due to the presence of lacton in chamomile it can cause allergic reaction in sensitive individuals. Conjunctivitis and eye-lid angioedema, contact dermatitis and eczema and emesis in administrations of higher doses have been reported[115].

Hypersensitivity reactions include contact dermatitis, dyspnea, asthma, bronchitis and conjunctivitis[١٠٩]. Consumption of chamomile tea may exacerbate existing allergic conditions, and there is a report of asthma and urticaria in one patient after a chamomile enema [١٠٧].

The use of chamomile products are in patients with known sensitivities or allergies to plants of asteraceae (Composite) such as ragweed, asters, and chrysanthemums, and in patient with a topic hay fever or asthma. It is contraindicated in woman during their early pregnancy due to it's teratogenic effects[١١٦].

1.16. Aim of the work:-

Due to the importance of flavonoids and poly (phenolic) compounds in medicinal treatment and the importance of flavonoids in plant extract capable plant to use as drugs in the treatment of many diseases therefore the aim of this work is extraction and isolation of some flavonoids like querctin and rutin and others from Matricaria chamomile flowers, at the same time involved separation of some poly (phenolic) compounds like coumarin, tannic acid, gallic acid and other from this plant flowers .

Chapter Two

Experimental

2.1. Chemicals:-

The following chemicals were supplied from different companies:

Compounds	***Supplied by***
Acetic acid	Fluka
Bismuth nitrate	BDH
Butanol	BDH
Chloroform	BDH
Ethyl acetate	Fluka
Ether	Fluka
Ethanol 95%	BDH
Formic acid	Fluka
Hexane	BDH
Hydrochloric acid	Fluka
Lead acetate	Fluka
Methanol	BDH
Mercury chloride $HgCl_2$	Fluka
Methyl ethyl ketone	BDH
Petrolium ether (60-80°)	Fluka
Phenol	Fluka
Potasium hydroxide	BDH
Potasium iodide	BDH
Sodium hydroxide	Fluka
Toluene	Fluka

Standard compounds	Supplied from
Apigenin	Fluka
Catachin	Fluka
Coumarin	Fluka
Gallic acid	Fluka
Kaempferol	Fluka
Myrectin	Fluka
Quercetin	Fluka
Rutin	Fluka
Scopoletin	Fluka
Tannic acid	Fluka
Umbelliferone	Fluka

2.2. Instruments:-

1- Melting points :- were determined for the two separated compounds using Gallen Kamp (Japan) melting point apparatus.

2- Ultraviolet absorption spectra :- UV–Visible were measured using Shimadzu 1085 (Japan) spectrophotometer and wavelength scanned between190-500 nm.

3- Infrared absorption: - FTIR of the separated compounds measured using KBr disk by FTIR model 8300 shimadzu (Kyoto, Japan).

4- Atomic – absorption spectrophotometer model 5000 American Perkin-Elmer company.

5- TLC glass (Merk), Micropipette (Brand,Germany), Hot blower (China)

2.3. Plant material:-

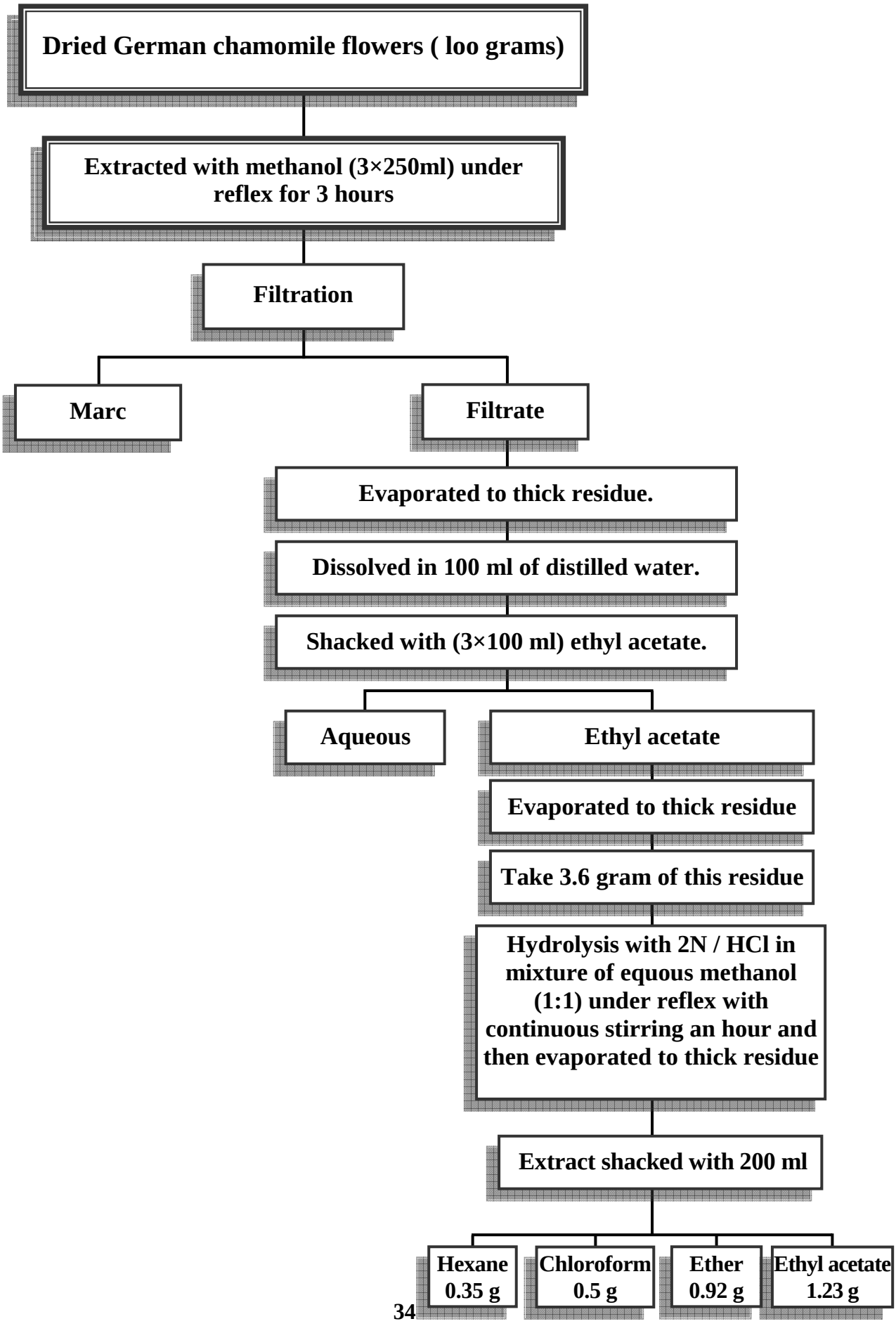
The flower of German chamomile (*Matricaria Chamomile*) plant were collected from the botanical garden in college of Agriculture in Baghdad – Abu Ghrabe during the flowering season between May and June. In which the plant head flowers were cut and separated from another parts of plant and then dried in a dark place for 4 day's before use .

2.4. Extraction, Hydrolysis of German Chamomile Flowers:-

One hundred grams of the dried German chamomile flowers were crushed using small electric blender and then extracted with 750 ml of methanol by heating under reflux with continuous stirring for three hour. The total methanolic extract was filtered using watman filter paper No.2.

The filtrate was evaporated under reduced pressure in rotary evaporator at 50° to obtain thick residue, then washed with petroleum ether (b.p. 60-80°) to remove carotenoids and chlorophylls. Then evaporate to obtain thick extract and transferred to a reparatory funnel and add to it about (100) ml distilled water. The mixture was then shaken three times with (100) ml ethyl acetate each time. The organic layer was separated and evaporated using the rotary evaporator at 30° to a thick extract with brown color crystal composition. Portion of 3.6 grams of this extract was hydrolyzed with (2N) hydrochloric acid in a mixture of (water/methanol) (1:1) under reflux for one hour and evaporated solution to thick extract. Extract was divided and shaked with 200 ml of each the following solvents hexane, chloroform, ether and ethyl acetate separately . Each divide was collected and evaporated to give 0.35 gram hexane fraction, 0.5 gram chloroform fraction, 0.92 gram ether fraction and 1.23 gram ethyl acetate fraction[(11ٰ)].

Scheme: Extraction and hydrolysis of German chamomile flowers [11 ٧]



2.5. Chemical Tests for some plant components:-

1- Flavonoids Test:-

This test was done using two solutions; the first one was prepared by mixing 10 g plant material in 5 ml of 95% ethanol, and the second solution was prepared by addition of 10 ml from 50% ethanol to 10 ml 0f 50% potassium hydroxide, equal amounts from the two solutions were mixed together in which a yellow color was obtained which indicated the presence of flavonoids in plant[١١٨].

2- Alkaloids Test:-

Preparation of Dragendoff's reagent:-

Dragendorff Test :- Two solutions were prepared; one from the addition of 20 grams of bismuth nitrate $Bi(NO_3)_3$ to 80 ml distilled water. The second solution was prepared by dissolving 16 grams of potassium iodide KI in 30 ml distilled water. The two solutions were mixed in 1:1 portions[١١٩].

10 g's plant material was heated with 50 ml of distilled water acidified with 5% HCl until boiling . The solution was filtered and let stand to cool. Dragendorff reagent was added to 1 ml of the cold filterate in a test tube. The absence of the orange color indicated that plant was free from alkaloid compounds[١١٩].

3- Tannins Test:-

10 gram plant material was mixed with 50 ml distilled water. The solution was heated until boiling then filtrated and cooled. This solution was divided into two parts in two test tubes, 1% $(CH_3COO)_2Pb$ was added to the first part, gel composition precipitate indicated the presence of tannins in plant, and 1% ferric chloride $FeCl_3$ was added to 10 ml from the second part of the solution. Blue –green color confirm presence of tannins[١٢٠].

4- Resins Test:-

This test was performed by addition 50 ml 95% ethanol to 5 gram plant flowers powder and heated in water bath for 2 minutes. The solution was filtrated and added to it about 100 ml distilled acidified water with 5% HCl. Trepid solution formed was confirm the presence of resins in plant[120].

5- Saponins Test:-

The aqueous solution of plant was prepared by addition 50 ml distilled water to 10 gram plant material and the solution was heated until boiling. To the cold 2 ml aqueous plant solution 5 ml mercury chloride solution was added. The absence of White precipitate indicating plant was free from saponin[١٢٠].

6- Coumarin Test:-

A 1 gram of plant material was dissolved in a 2 ml of 80% methanol in test tube. The tube was covered with watman filter paper No.1 saturated with diluted sodium hydroxide. The tube was heated in water bath for 5 minute, then the filter paper was viewed under UV-light, in which the appearance of green-yellow color indicates the presence of coumarin[١٢١].

7- pH for the plant Extract:-

A 10 grams of plant flowers powder was taken and mixed with 50 ml distilled water with continuous shaking for 15 minute at room temperature, then the solution was filtered and the pH was determined using pH-meter[١٢٠].

2.6. Thin layer Chromatography (TLC) Separation of Flavonoids:-

Separation of flavonoids was carried out using 0.25 mm thick (20x20)cm glass TLC plates. The plates were developed with solvent systems and allowed to dry overnight at room temperature and then activated by heating at 120° for an hour before it used. Solvent system was placed in glass tank (22.5cm x 22cm x 7.0cm), 100-120 ml of solvent was placed in the tank each time. Spots were lined as a bands on the plates using Micropipette and these bands were dried using hot blower, then plates were placed in the glass tank was well covered with glass cover and the plates allowed to stand for an hour .

After development, plates were dried at room temperature. The separated bands were determined according to the values of R_f of separated compounds and compared with R_f of the authentic samples. Then two separated bands were removed from the plates and purified by washing with 15 ml ethyl acetate to dissolve the separated compounds and then filtrated to remove silica gel and obtained purified compounds after evaporating the solvent.

2.7. Solvent System Used in TLC and paper Chromatography Separations :-

Different solvent systems were prepared for TLC and paper chromatography separations of the interested compounds. The solvent prepared according to Stahl (1965)[12٢].

1- Butanol: Acetic acid: Water (BAW) (4:1:5).

2- Toluene : Ethyl acetate : Ethanol (TEE) (2:1:1).

3- Ethyl acetate : Methyl ethyl ketone : Formic acid : Water (EMFW) (5:3:1:1).

4- Forestal solvent = Acetic acid : concentrated HCl : Water (30:3:10).

5- Phenol saturated with water.

2.8. Separation using High Performance Liquid Chromotography/ HPLC:-

The separation of flavonoids from plant extract from each fraction was performed on HPLC model 10 AVT gradient model 10 A-SPD. The flavonoids were separated on reversed phase column shim pack C-18 particle size 5 nm (250 × 4.6 mm l.d), using mobile phase: (acetic acid: deionized water, methanol) (1%: 40 :60) V/V, the flow rate was 1 ml/min 30°. The eluted compounds were monitored at 280 nm. The measurement carried out by taking 0.1 gram from each fraction of plant extract and add to it 1 ml from mobile phase, then mixing by vortex and 5µl from solution was injected into instrument and the separated compound retention time was compared with that of standard compound.

Other phenolic compounds were separated on HPLC under the same conditions, and these compounds are: Tannic acid, Gallic acid, Scopoletin ,Umbelliferone and Coumarin and were compared with standard compounds.

2.9. Determination of metals concentration in plant:-

The concentrations of the following metals Mn, Cu, Cd, K, Ca, Mg, Fe and Zn, were determined using atomic –absorption spectrophotometer model 5000 American Perkin-Elmer company. 2 grams of plant powder was placed in crucible, then the crucible was transferred to the muffle furnace at 500° for 3 hours. The residue was dissolved in 5 ml of 20 % HCL and the solution was remained (30-60) minutes. The solution was filtered using (Watman filter paper No.1, then the volume was completed to 50 ml volumetric flask with deionized water[١٢٣].

3.1. Chemical constituents found in plant :-

German chamomile flowers were examined using many chemical tests, to show if plant flowers contain flavonoids , alkaloids , tannins and coumarin. Flavonoids test gave a yellow precipitate which indicated the presence of flavonoids. The result agreed with the same result obtained by Salamon[(103)].

Alkaloids test gave no orange precipitate which indicated that German chamomile flowers were free from alkaloid compounds. No white precipitate was formed in Saponins test referred that plant was free from Saponin compounds. While in the case of tannins test blue – green color was appeared which represented a positive result for tannins. In Coumarin test the green – yellow color that formed on the filter paper when it was viewed under UV- light indicated the presence of Coumarin in plant extract, which agreed with other studies[(103)]. The Resin test showed unclear solution after the addition of acidified distilled water to the filtrate which indicated the presence of resins in plant[(124)] .

These chemical constituents were found in German chamomile plant give the plant a medicinal importance and make plant posses significant pharmacological activity[(125)]. The pH of the plant also was determined for the plant flowers powder solution and was found 4.35. Table[2] summarized the results of the above tests .

Table [2] : Tests carried on German chamomile plant

Compounds	*Results*	*Color reaction*
Flavonoids	+	yellow
Alkaloids	–	orange
Saponins	–	No white color
Tannins	+	Blue-green
Coumarins	+	Green-yellow
Resins	+	Trepid solution

3.2. Separation and Identification using Thin-layer Chromatography (TLC) and Paper Chromatography (PC) :-

The results in this study appeared that the German chamomile flowers that grown in Iraq contain 2.53g/Kg total extract of dry weight plant flowers. Thin-layer chromatography was qualitative screening survey for the presence of flavonoids like quercetin and it's Querectin-3-rhamnoglucoside (rutin) in German chamomile flowers grown in Iraq. Table [3] shows the results of TLC separation using four solvent systems like Butanol – Acetic acid – Water (BAW) (4:1:5), Toluene – Ethyl acetate – Ethanol (TEE) (2:1:1), Ethyl acetate –Methyl ethyl keton – Formic acid – Water (EMFW) (5:3:1:1) and phenol saturated with water. All these solvent systems were indicated the presence of flavonols quercetin and rutin, the R_f values for the separated quercetin and rutin were the same or near to that R_f values for authentic samples. At the same time colors of separated compounds were identified under UV-light which have the same colors of standard quercetin and rutin compounds.

Table[3] : R_f values and color reactions for standards & separated quercetin & rutin from German chamomile flowers extract on paper chromatography (PC)

Compounds	*BAW*	*TEE*	*EMFW*	*Phenol/Water*	*Color reactions/UV+NH_3*
Standard Quercetin	0.84	0.73	0.86	0.42	yellow
Standard Rutin	0.5	0.2	0.41	0.44	Bright yellow
Separated Quercetin	0.85	0.7	0.86	0.4	yellow
Separated Rutin	0.48	0.22	0.4	0.45	Bright yellow

TLC chromatography was indicated the presence of these two flavonols quercetin and rutin in German chamomile flowers that grown in Iraq using two solvent systems Butanol – Acetic acid – Water (BAW) (40:10:50) V/V, forestal solvent: acetic acid – concentrated HCl – Water (30:3:10). Table [4] listed the R_f values and colors for two separated flavonols which were found near to the R_f values and colors of standards querectin and rutin. The weight of separated compounds was found to be 0.035% rutin and 0.023% quercetin from the total weight of the plant .

Table [4] : R_f values & color reactions for the standards & separated quercetin & rutin from German chamomile flowers extract on TLC chromatography

Compound	BAW	Forestal	Color reactions UV+NH_3
Standard Quercetin	0.82	0.43	yellow
Standard Rutin	0.45	0.29	yellow
Separated Quercetin	0.8	0.44	yellow
Separated Rutin	0.47	0.31	yellow

Separation using TLC and paper chromatography showed that there were other separated spots for different compounds, but these separated spots could not be identified because of their little amounts which can not be separated completely. These spots may be belong to the compounds that were separated using HPLC method. In TLC and paper chromatography noted that the best solvent systems used in the separation of quercetin and rutin was butanol – acetic acid – water(40:10:50) V/V, because the two separated flavonols bands and spots appeared clear.

HPLC method was used to separate other flavonoids like kaempferol, myricetin, catechin and other poly phenolic compounds like coumarin, scopoletin, umbelliferone, gallic acid, tannic acid that were not separated by TLC and paper chromatographic methods. The local market chamomile flowers were extracted by the same method and examined by TLC and paper chromatography using same solvent systems, it was found that the local market chamomile flowers was free from quercetin and rutin.

3.3 Identification of Separated compounds:-

The two separated flavonols quercetin , rutin from German chamomile flowers grows in Iraq were purified and identified using melting points, UV- Visible spectra and infrared spectra (FTIR).

Melting point measurements of quercetin and rutin from German chamomlile flowers extract were (317-318) ° and (190-193)°, and for the standards (317-319)° and (192-194)° respectively.

The isolated quercetin absorbed at 256 nm and 372 nm where that of standard quercetin appeared at 255 nm and 374 nm . while isolated rutin absorbed at 257nm and 385nm compared to 259 nm and 363 nm for the standard solutions. This indicated that the UV- Visible spectra of the isolated quercetin and rutin were similar to the UV-Visible spectra of the standards quercetin and rutin . as shown in figure [9] and [10] below:

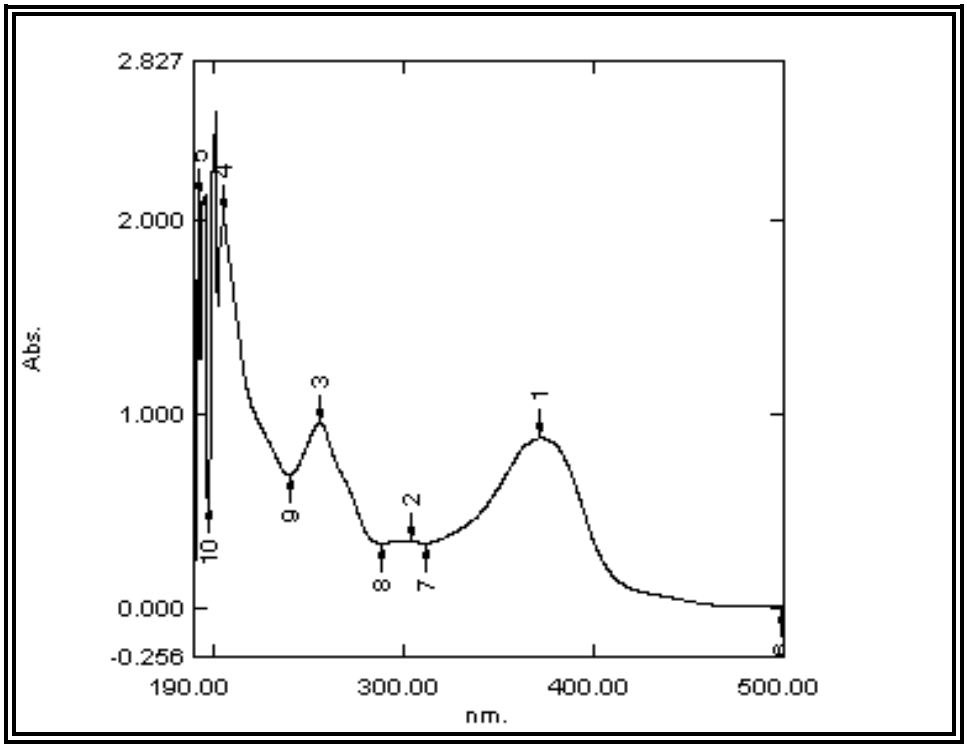


Figure [9] : UV-Visible Spectrum for Separated Quercetin from German chamomile flowers extract

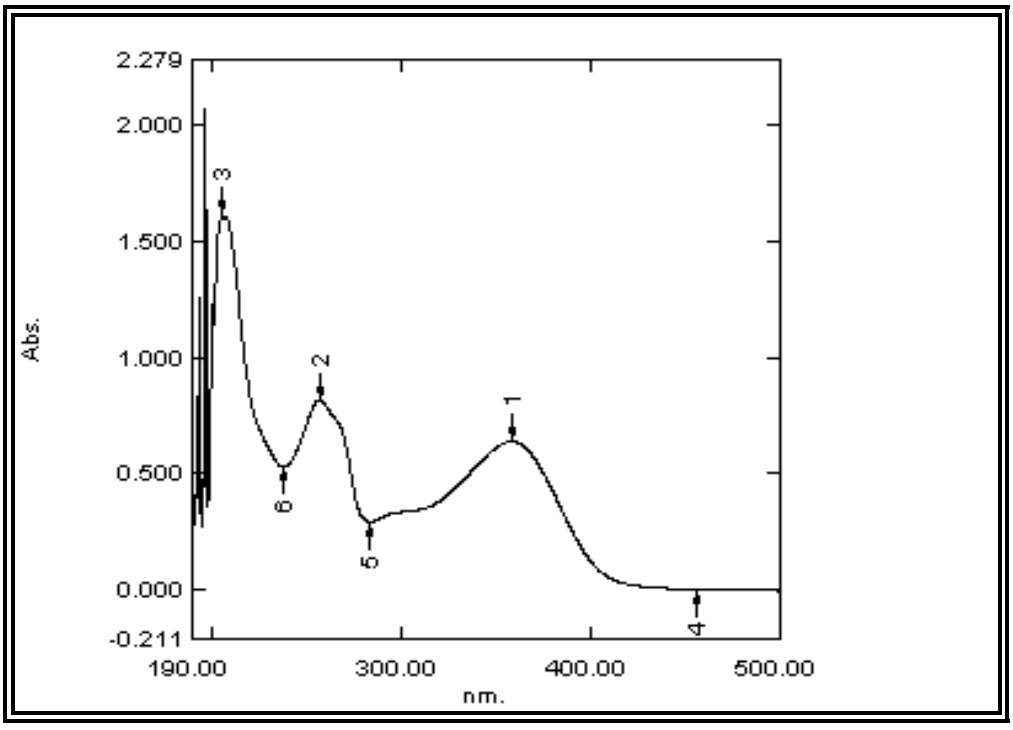


Figure [10] : UV-Visible Spectrum for Separated Rutin from German chamomile flowers extract

At the same time the two isolated flavonols quercetin and rutin were identified through infrared spectra (FTIR), in which spectral properties of the separated querectin and rutin were similar to infrared spectral of standard compounds as shown in figure[11-12]. Table [5] listed the importance bands for these compounds.

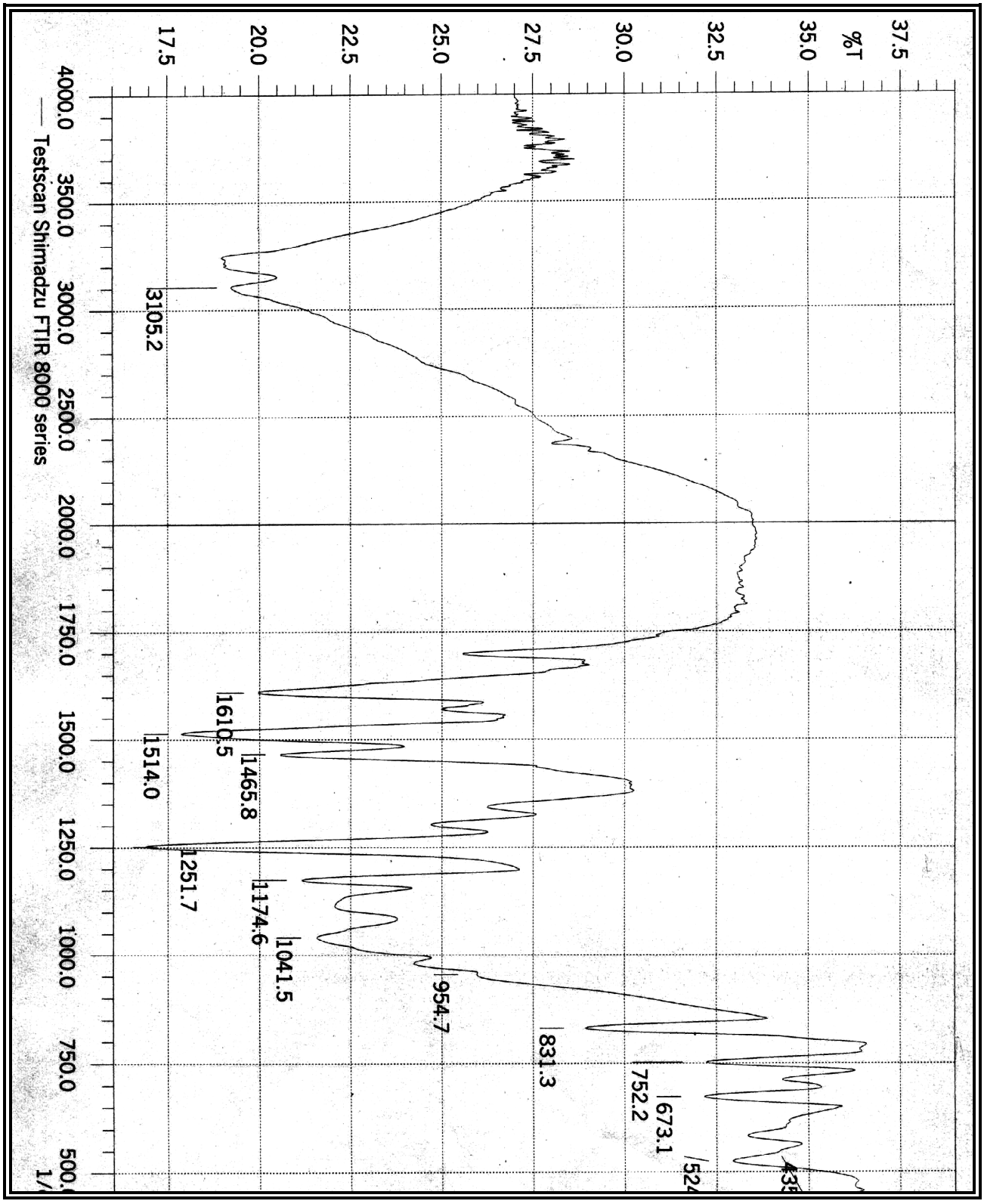
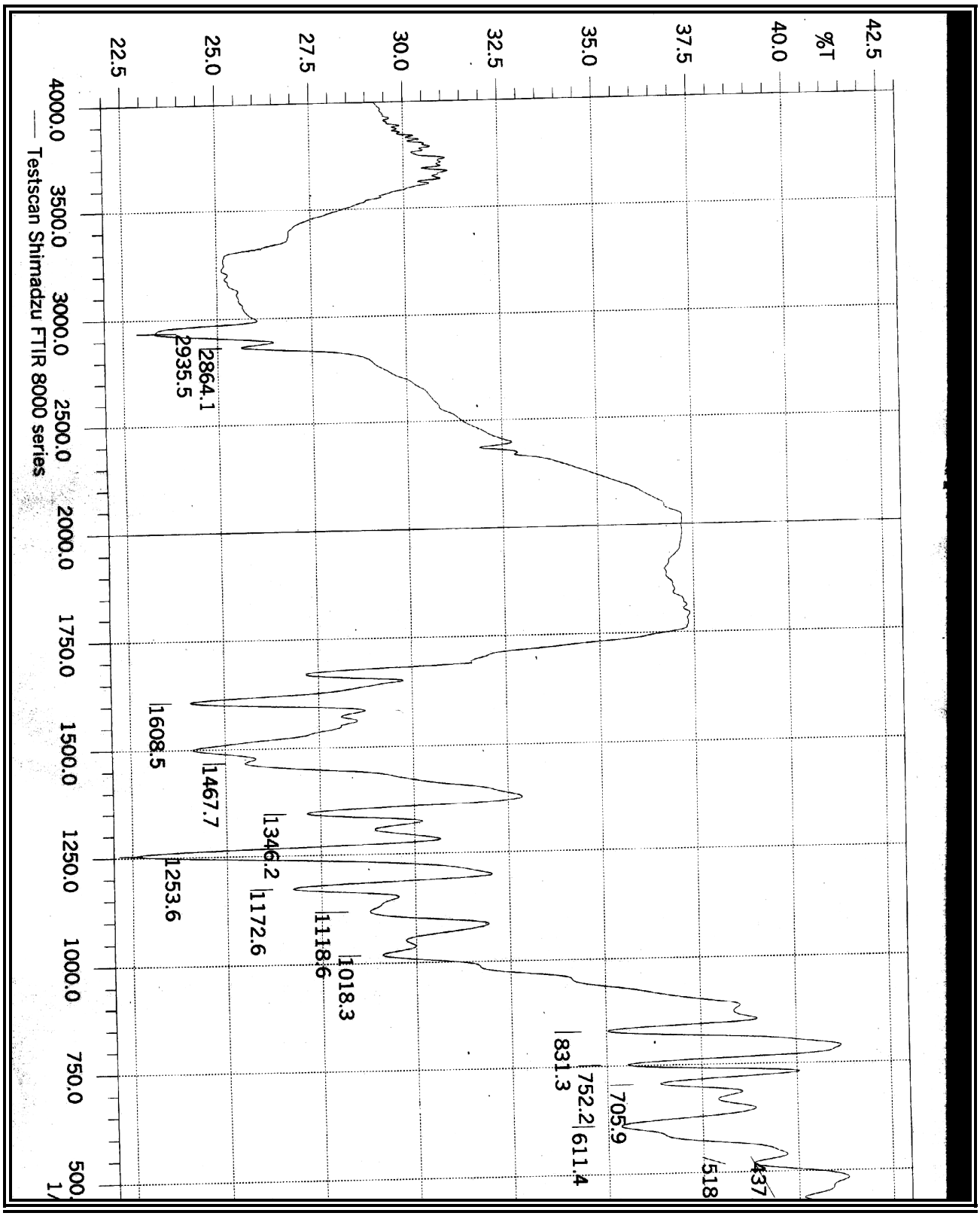
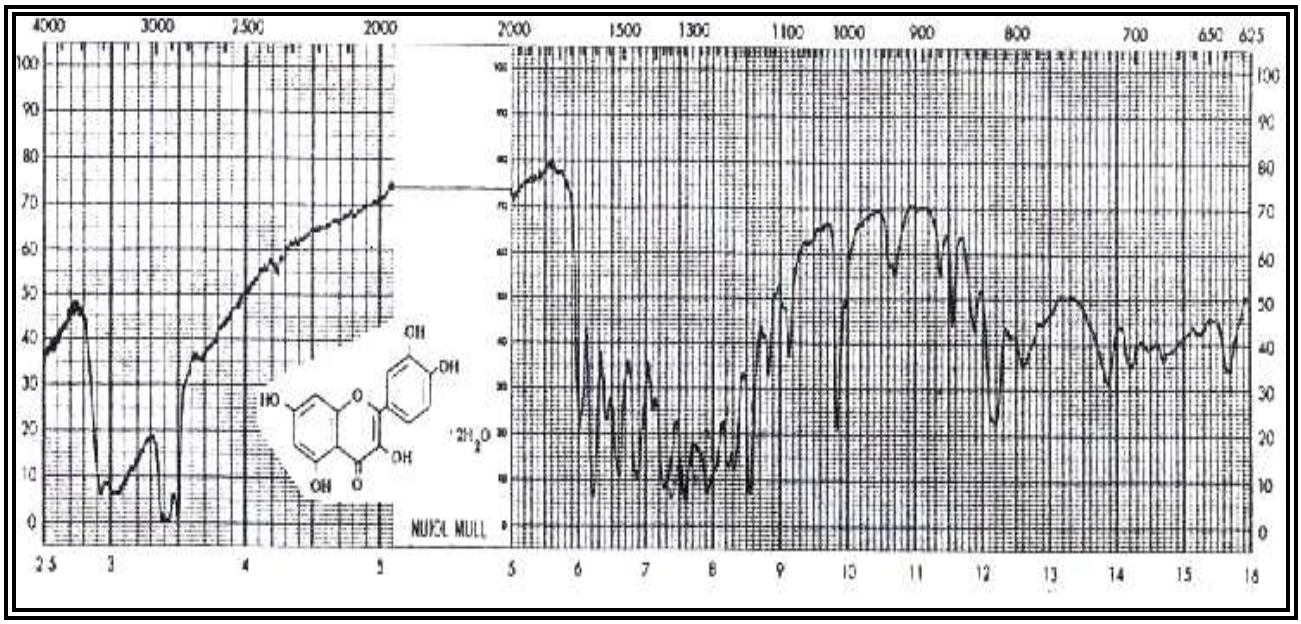


Figure [11] : FTIR Spectrum for the separated Quercetin from German chamomile flowers extract



Figure[12] : FTIR Spectrum for the separated Rutin from German chamomile flowers extract



Figure[13] : FTIR Spectrum for Standard Quercetin compound

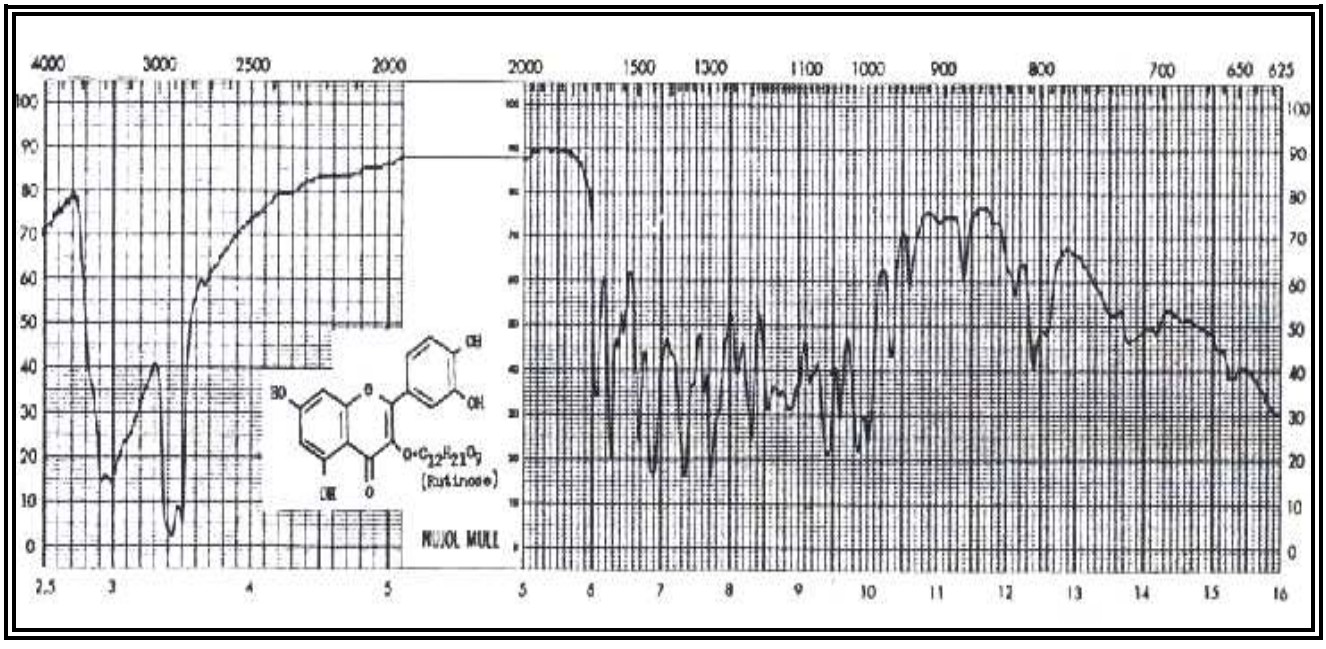


Figure [14] : FTIR Spectrum for Standard Rutin compound

Table [5] : FTIR Spectral properties of Separated and Standards Quercetin and Rutin

Compounds	**O-H**	**C—H aromatic**	**C═O**	**C═C**	**—C—O—C— asy**	**Sy**	**—C—H aliphatic**
Separates Quercetin	3250.–3200.0	3105.2	1700.5	1610.5	1251.7	1041.5	
Separated Rutin	3300.–3100.6	Overlapped with OH stretching	1650.3	1608.5	1253.6	1018.3	asym 2935.5 sym 2864.1
Standared Querectin	3600-3100	3050	1697	1600	1250	1024	
Standard Rutin	3600-3000	Overlapped with OH stretching	1652.4	1600.8	1296.1	1064.6	asym 2925.8 sym 2862.2

3.4. Separation and Identification of Flavonoids using high performance liquid chromatography HPLC :-

In this work HPLC method was used in separation of other flavonoids like kaempferol, myricetin, catechin and other polyphenolic compounds like coumarin, scopoletin, umbelliferone, gallic acid, tannic acid. In addition of the two flavonols querctin and rutin that were separated in TLC and paper chromatographic methods.

The results of the separation by HPLC from each fraction of plant extract, hexane, chloroform, ethyl acetate and ether were found to contain flavonoids such as quercetin, rutin, kaempferol, myricetin, by as comparing the retention time of these compounds with that for the standard compounds. At the same time other flavonoids were separated by HPLC like apigenin and catechin.

Hexane fraction of plant extract was found to contain compounds like tannic acid, catechin, rutin, querectin and kaempferol, as shown in figure [15]. The chloroform fraction was found to contain tannic acid, catechin, gallic acid and apigenin. While ethyl acetate fraction contained myricetin, scopoletin, umbelliferone and querectin. However the ether fraction contained compounds like Tannic acid and Catechin, as shown in figures [15-18] below. The retention times for these separated compounds were compared and found similar to that of standard compounds as listed in tables [6-9] for each of the above extract.

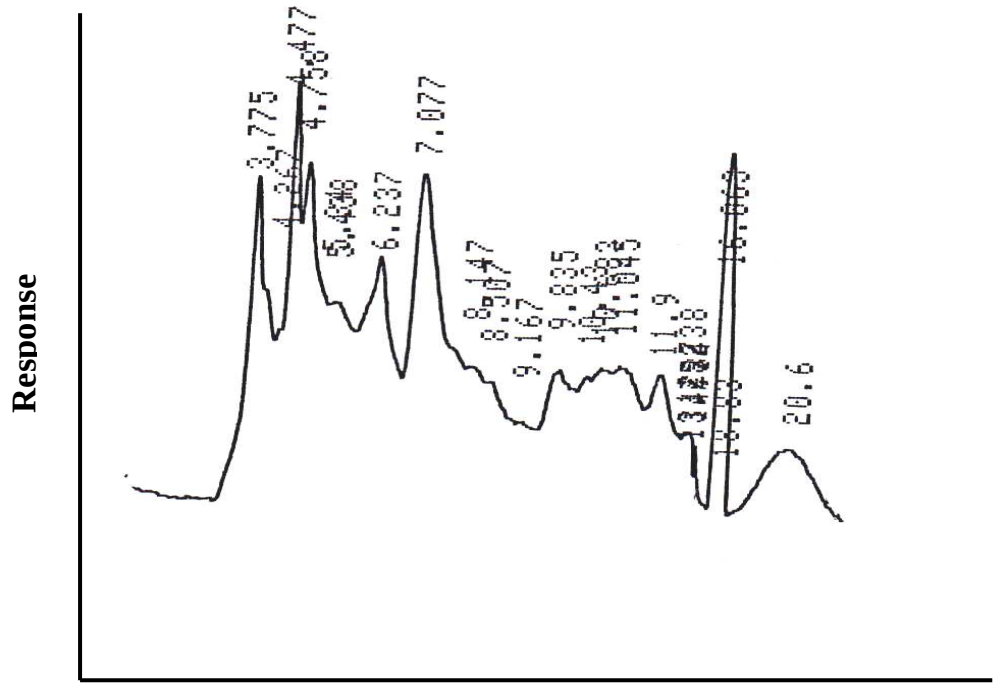


Figure [15] : HPLC chromatogram for German chamomile flowers extract (Hexane fraction) using methanol: acetic acid : de-ionized water (60: 1%:40) V/V

Table[6]: Retention time for standards and separated compounds from German chamomile flowers extract (Hexane fraction)

Compounds	***Retention time for Standards***	***Retention time for Separated compounds***
Tannic acid	3.8	3.77
Catechin	4.5	4.48
Rutin	6.24	6.24
Quercetin	16.11	16.06
Kaempferol	20.5	20.6

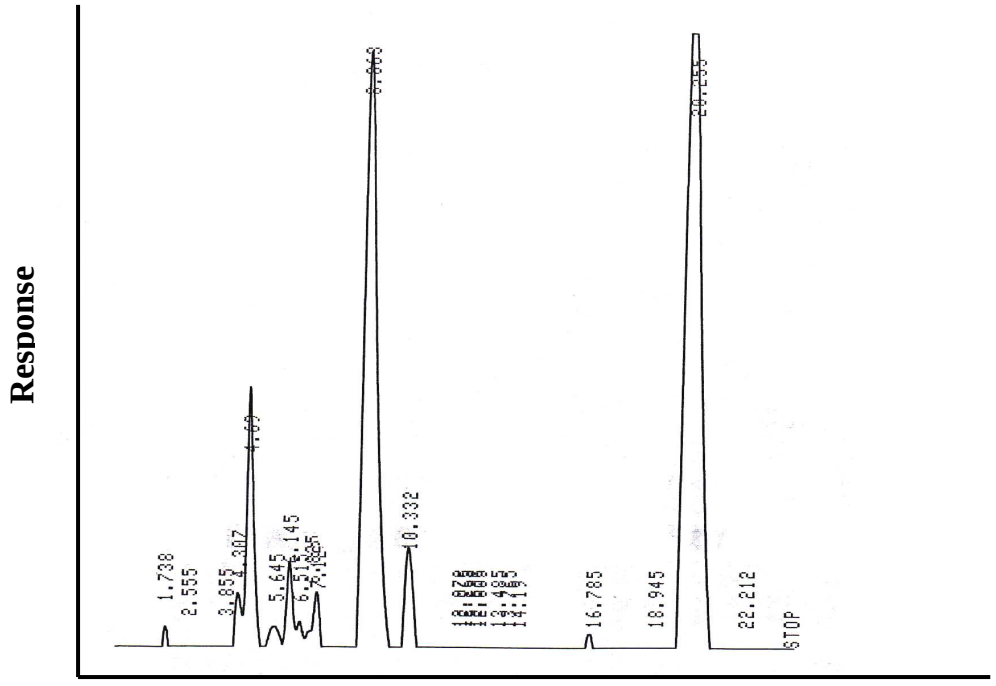


Figure [16] : HPLC chromatogram for German chamomile flowers extract (Chloroform fraction) using methanol : acetic acid : de-ionized water (60:1%:40) V/V

Table [7] : Retention times for standards and separated compounds from German chamomile flowers extract (Chloroform fraction)

Compounds	Retention time for Standards	Retention time for Separated compounds
Tannic acid	3.8	3.855
Catechin	4.5	4.69
Gallic acid	5.6	5.645
Apigenin	7.12	7.123

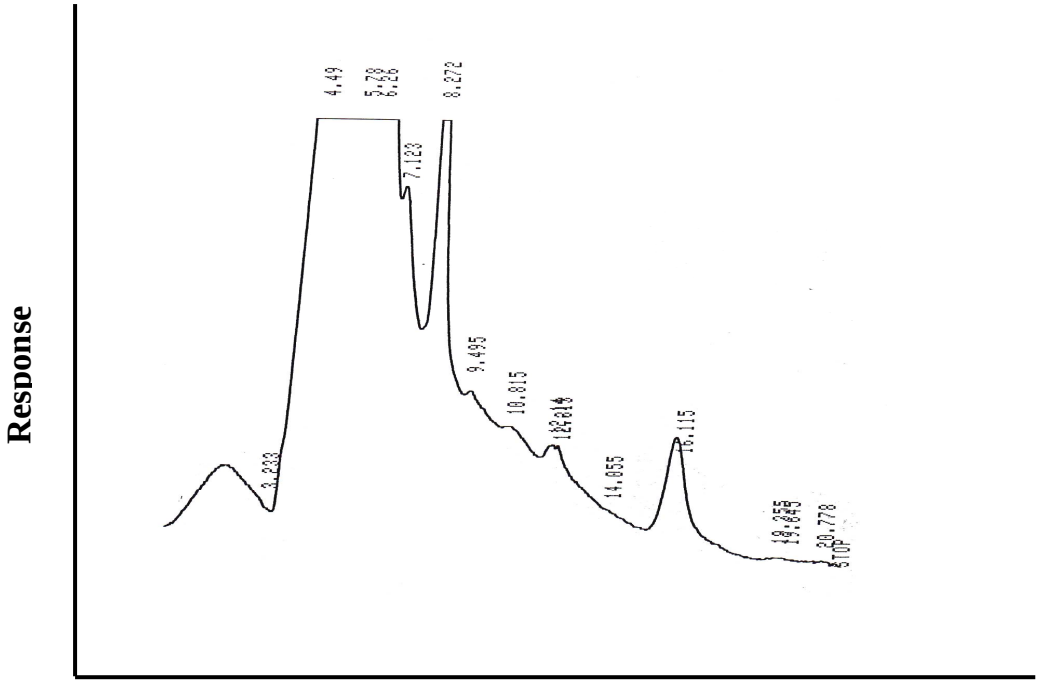


Figure [17] : HPLC chromatogram for German chamomile flowers extract (Ethyl acetate fraction) using methanol : acetic acid : de-ionized water (60:1%:40)

Table[8]: Retention time for standards and separated compounds from German chamomile flowers extract (Ethyl acetate fraction)

Compounds	***Retention time for Standards***	***Retention time for Separated compounds***
Myricetin	8.27	8.272
Scopoletin	9.48	9.495
Umbelliferone	10.8	10.815
Quercetin	16.11	16.115

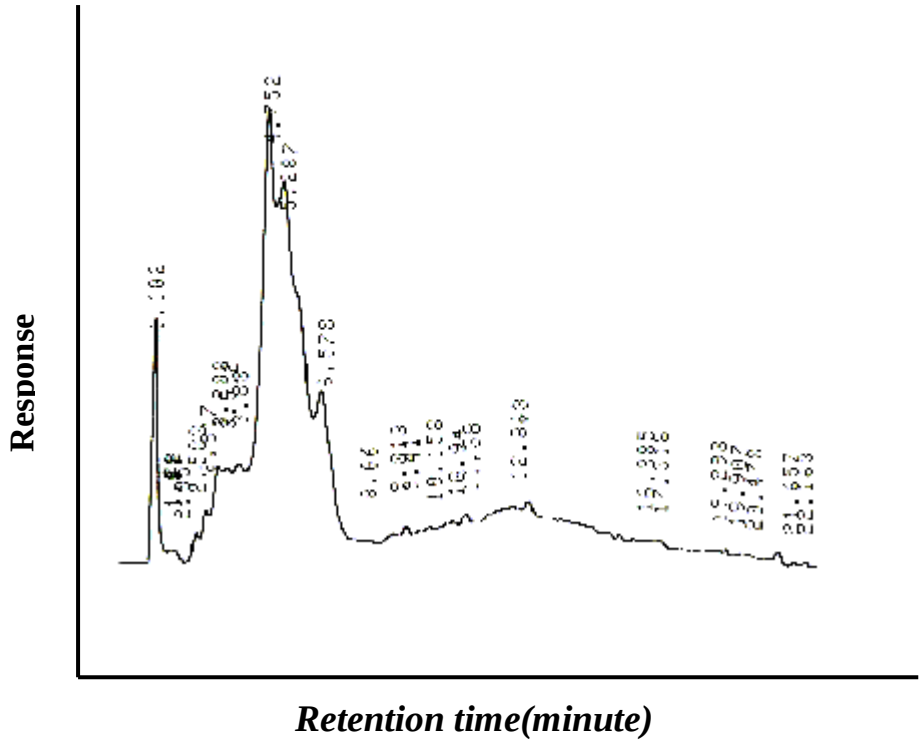


Figure [18] : HPLC chromatogram for German chamomile flowers extract (Ether fraction) using methanol : acetic acid : de-ionized water (60:1%:40)

Table [9] : Retention times for standards and separated compounds from German chamomile flowers extract (Ether fraction)

Compounds	*Retention time for Standards*	*Retention time for Separated compounds*
Tannic acid	3.8	3.88
Catechin	4.5	4.752

3.5. Nutrition metals found in plant:-

Table [10] listed the concentrations of minerals, nutrition and toxic metals were found in German chamomile flowers extract. The highest concentrations were found for potassium and calcium and the smallest metal concentration was that of zinc and cadmium. The presence of potassium and calcium would increased the importance of plant in enhancing the strength of the body and bones in addition to the iron. The presence of Pd and Cd gave the plant it's importance role in medicinal treatments.

Table[10]: Concentration of metals found in German chamomile flowers plant grown in Iraq

Metals	*Concentration (ppm)*
K	500
Ca	450
Mg	50
Fe	46
Cu	0.9
Mn	1.0
Zn	0.8
Cd	0.47
Pd	3.63
S	4.94

3.6. Conclusion:-

The study of matricaria chamomile flowers that grown in Iraq. In which the extract was appeared to consist flavonoid compounda like quercetin, rutin, kaempeferol, apigenin, myrectin and other polyphenolic compounds like coumarin, scopoletin, umbelliferone, tannic acid, gallic acid. TLC chromatography for two isolated flavonols querctin and rutin and were identified using infrared and UV-Visible-spectrophotometers. At the same time local market chamomile flowers were examined searching for quercetin and rutin using TLC and same solvent systems, the result has been noted that plant flowers was free from these two flavonols.

These results agreed with Angal[117] which found that Roman chamomile flowers (Anthemis nobilis) that grown in Iraq were free from querctin and kaempferol compound. Herisset & Chaumont[126] were found that chamomile plant contain many types of flavonoid glucosides such as apigenin-7-glucoside and luteolin-7-glucoside and free luteolin.

Mady in 1999 [127] reported the presence of flavonoids, coumarins in matricaria chamomile flowers. Avallone[113] identified flavone like apigenin in methanolic extract of matricaria chamomile plant, from the previous review we can say that difference in chemical composition of the various chamomile may be return to the difference in environment and agriculture conditions.

3.7. Suggestion for further work:-

Extraction and isolation of other type of chemical constituents from matricaria chamomile plant grown in Iraq, and try to isolate these compounds using TLC,PC,HPLC in separation, extracted and separated flavonoid compounds from other herbs using different solvent in extraction process in addition to water, and try to study antitumer activity for the flavonoid compounds as strong antioxidant in addition to the antibacterial & antifungal properties .

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Experimental

Introduction

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Extraction, Isolation, and Characterization of Medicinal Components from Flowers of *Matricaria Chamomile* Plant

A Thesis
Submitted to the College of Science of
Al-Nahrain University
In Partial fulfillment of the requirements for
The Degree of Master of Science In Chemistry

By
Reem Ibraheem Qurban Ali
(B.Sc. Al-Nahrain University, 2002)

May 2005 Rabeeh Al-Thanee 142٦

Results & Discussion

Summary

This study included extraction of total flavonoids compounds from Matricaria chamomile flowers that grown in Iraq. The extract was brown color crystals composition. Querecetin and rutin were isolated by TLC, HPLC and identified by using UV-Visible spectra and FTIR spectra .

HPLC method used to separated and identified another flavonoid compounds such as apigenin, catechin, myrectin, other poly(phenolic) compounds were separated such as coumarin, scopoletin, umbelliferone, tannic acid, gallic acid .

Also the local market chamomile flowers extract examined by TLC method using same solvent systems, viewed that local market flowers extract was free from flavonols querectin and rutin.

The concentration of metals found in plant material was determined using atomic absorption spectrophotometer .

Supervisors certification

We certify that this thesis was prepared under supervision at the Department of Chemistry, College of Science, Al-Nahrain University as a partial fulfillments of the requirement for the degree of master of science in chemistry.

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Examining Committee's Certification

We, the Examining Committee, certify that we have read this thesis and examined the student Reem Ibraheem Qurban, in it's contents and that, according to our opinion, is accepted us a thesis for the degree of Master of Science in Chemistry .

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I hereby, certify a bone the decision of the examining committee .

Signature:
Name: Dr. Laith Abd Al-Aziz
Title: Assistant Professor
Address: Dean of College of Science
Date:

الإهداء

إلى من أمدني بعونه وسنده ..... والدي العزيز

إلى من غمرتني بحنانها وحبها ..... والدتي الغالية

إلى مثلي الأعلى في الحياة ..... أخي الحبيب

إلى روح التعاون والوفاء ..... أخواتي العزيزات

مع حبي وتقديري أهدي ثمرة جهدي المتواضع

ريم

الخلاصة

في هذه الدراسة تم استخلاص مركبات الفلافونيدات من أزهار نبات البابونج Matricaria Chamomile الذي ينمو في العراق وقد تم الحصول على مستخلص بشكل بلورات ذات لون بني مصفر .

وقد تم عزل وتشخيص مركبات فلافونيدية على شكل أكلايكون (Aglycone) وهو عبارة عن أحد كلايكوسيدات الكورستين سكر $C_{12}H_{21}O_9$ وقد لوحظ أن نسبة مركبي الروتين والكورستين في مستخلص نبات البابونج الألماني النامي في العراق هي 0.035% و 0.023% من وزن النبات المستخدم . لقد تم عزل هذه المركبات باستخدام كروماتوكرافي الطبقة الرقيقة TLC وكروماتوكرافي الورقة PC ، وقد شخصت باستخدام طرق التحليل الطيفية مثل الأشعة فوق البنفسجية UV-Spectra والأشعة تحت الحمراء FTIR-Spectra بالإضافة إلى تقنية السائل عالي الأداء HPLC . وقد تم أيضا استخدام تقنية السائل عالي الأداء لكشف عن مركبات متعددة الفينول في مستخلص أزهار البابونج النامي في العراق. حيث فصلت مركبات فلافونيدية أخرى بالإضافة إلى مركبي الكورستين والروتين مثل الكاتيكينCatechin والمايرستين Myricetin وفلافون الابجينين Apigenin . وكذلك مركبات الكومارين Coumarin والسكوبوليتين Scopoletin والامبليفيرون Umbelliferone بالإضافة إلى فصل مركبات التانين مثل Tannic acid ومركب Gallic acid بتقنية كروماتوكرافي السائل عالي الأداء.

ومن الجدير بالذكر انه تم اختبار مستخلص نبات البابونج التجاري في احتوائه على فلافونول الكورستين والروتين باستخدام كروماتوكرافي الطبقة الرقيقة باستخدام نفس نظام المذيبات ، وجد انه مستخلص أزهار البابونج التجاري لا يحتوي على فلافونول الكورستين والروتين . كما تم تعيين تراكيز المعادن الموجودة في نبات البابونج باستخدام طيف الامتصاص الذري .

بسم الله الرحمن الرحيم

قالُوا سُبحانك لا عِلم لَنا إلا ما عَلْمتَنا انْك أنْتَ العَليم الحَكيم

صدق الله العظيم

(سورة البقرة الآية ٣٢)

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

استخلاص، عزل، وتشخيص بعض المكونات الطبية من أزهار نبات البابونج *Matricaria Chamomile*

رسالة

مقدمة إلى كلية العلوم – جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير

في الكيمياء

من قبل

ريم إبراهيم قربان علي

بكالوريوس ٢٠٠٢ (جامعة النهرين)

أيار-٢٠٠٥م ربيع الثاني-١٤٢٥

Dictionary for some words in thesis

Antioxidant	مضاد الأكسدة
Anti-inflammatory	مضاد الالتهابات
Anti- allergic	مضاد الحساسية
Antiviral	مضاد الفيروسات
Anti-neoplastic	مضاد السرطان
Anti-thrombotic	مانع لتخثر الدم
Anti helmitic	مضاد الديدان
Angiosperms	كاسيات البذور
Astringent	مادة قابضة
Annual	سنوي
Anorexia	فقدان الشهية
Anise	يانسون (نبات)
Alleviate	يسكن، يهدئ
Anti catarrhal	مضاد النزلة الشعبية
Antiseptic	مضاد التعفن
Asters	زهرة النجمة
Bilberry	التوت البري (نبات)
Broccoli	قرنبيط(نبات)
Bark	لحاء
Biennial	يعيش مدة سنتين
Borage	لسان الثور(نبات)
Cardiovascular	أوعية قلبية
Cataracts	اعتمام عدسة العين
Carminative	طارد للغازات
Celery	الكرفس(نبات)
Citrus	الليمون (نبات)
Caraway	كراويا(نبات)
Chervil	مقدونس(نبات)
Coriander	كزبرة (نبات)
Cumin	كمون (نبات)
Chrysanthemums	زهرة الأقحوان
Croup	سعال
Colic	مغص

Diet	وجبات
Dicotyledons	نباتات ذوات الفلقتين
Diarrhea	الإسهال
Dermatitis	التهاب الجلد
Dyspnea	عسر التنفس
Eye-lid	جفن العين
Fodder	علف
Fennel	شمار (نبات)
Flatulence	غازات
Gastrointestinal	اضطرابات معدية
Gallnuts	العفص (نبات)
Gout	داء المفاصل (النقرص)
Herb	عشب
Herrpesvirus hominis	الفيروسات المسببة لمرض القوباء الجلدي
Herbicides	مادة ضارة بالأعشاب
Hay	قش أو تبن
Insects	حشرات
Infection	عدوى
Inhalation	استنشاق
Infusion	يعطى عن طريق الأشربة
Indigestion	عسر الهضم
Irritation	تهيج
Lignifications	واقع ضمن النسيج الخشبي
Muscular degeneration	انحلال عضلي
Mahogany	خشب الماهوغاني
Mordant	محرق،لاذع
Mustard	خردل(نبات)
Mint	نعناع(نبات)
Nosegay	باقة صغيرة
Neuralgia	ألم عصبي
Nauses	غثيان
Oral	فموي

Peel	قشرة
Platelet aggregation	تجمع لويحات الدم
Peaches	خوخ (نبات)
Pears	أجاص (نبات)
Pigments	أصباغ
Pollen	لقاح
Perennial	دائم طوال السنة
Poison ivy	اللبلاب السام
Parasitic worm	الديدان الطفيلية
Pesticides	مبيدات الحشرات
Patient	معالجة طبية
Red wine	النبيذ الأحمر
Red paper	الفلفل الأحمر
Remedies	علاج
Ragweed	عشبة الرجيد
Rosemary	إكليل الجبل
Soybean	فول الصويا
Sun stroke	ضربة شمس
Stomach	معدة
Steam	سويق
Sapwood	خشب النسغ
Sachets	معطر
Sciatica	مرض عرق النسا
Thyme	زعتر (نبات)
Tarragon	طر خون(نبات)
Topical	موضعي
Urticaria	طفح جلدي
Ulcers	قرحة
Vasodilatory	الأوعية القلبية
Vomiting	تقئ
Wounds	جروح

Abbreviations

TLC	Thin layer chromatography
PC	Paper chromatography
HPLC	High performance liquid chromatography
ROS	Reactive oxygen species
NADH	Nicotin amide adenine dinucleotide hydrogenase
GABA	γ- amino butyric acid

معلومات شخصية

الاسم :- ريم إبراهيم قربان علي الدلوي

الكلية :- العلوم

القسم :- كيمياء

الدرجة :- ماجستير

اسم البحث :- استخلاص،عزل، وتشخيص بعض المكونات الطبية من أزهار نبات البابونج *Matricaria Chamomile* .

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