1- Introduction

1.1-General approach

A natural product is a chemical compound or substance produced by a living organism that is, found in nature(1)(2). In the broadest sense, natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis (both semi synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets(3) (4).Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism(5). Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines(6).

Primary metabolites as defined by Kossel are components of basic metabolic pathways that are required for life, include carbohydrates, lipids, amino acids, and nucleic acids which are the basic building blocks of life(7).

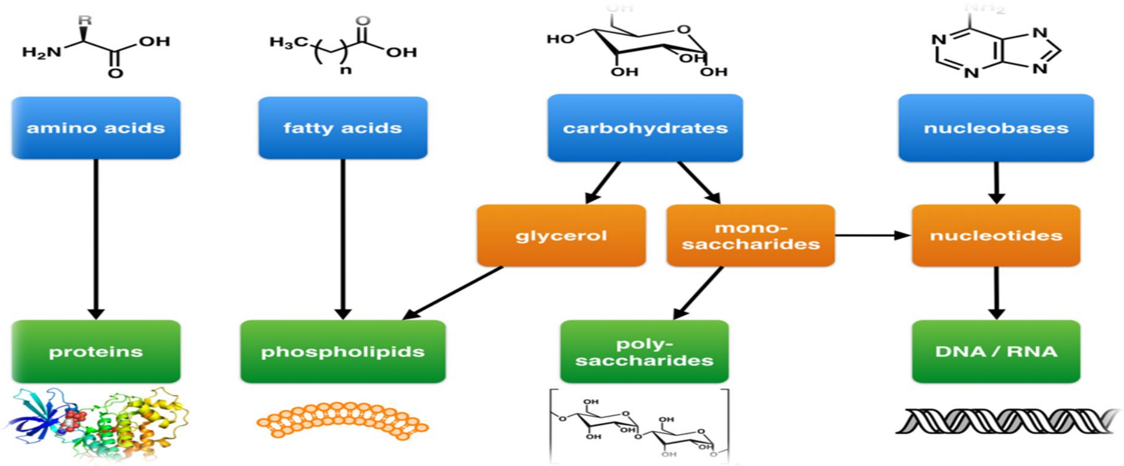


Fig (1.1) : Molecular building blocks of life

Secondary metabolites contrast to primary metabolites are dispensable and not absolutely required for survival. General structural classes of secondary metabolites include alkaloids, phenylpropanoids, polyketides, steroids, flavonoids, vitamins, and terpenoids.(5)

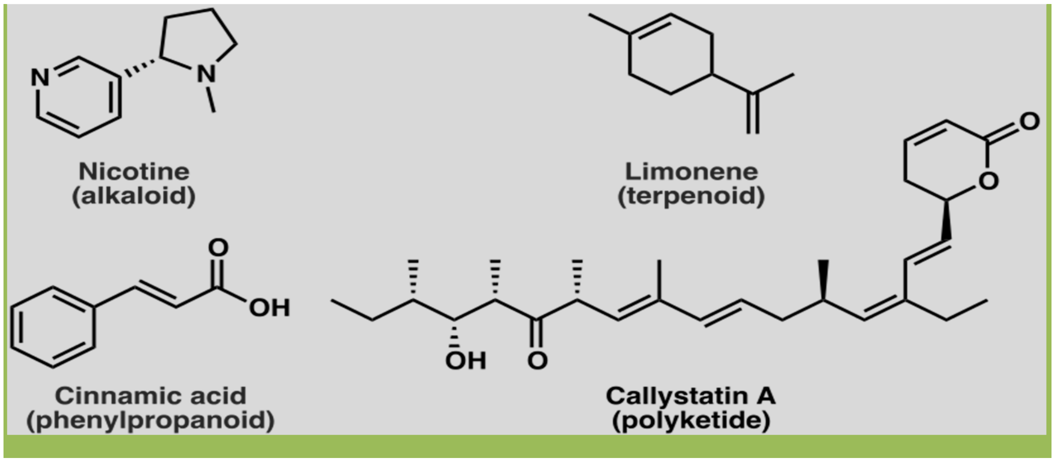
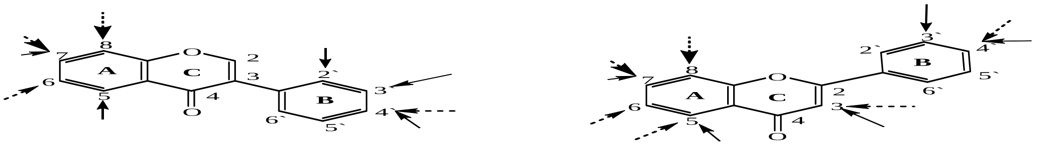


Fig (1.2) : Representative examples of each of the major classes of secondary metabolites

Flavonoids are natural phenolic compounds which appear as secondary metabolites of plants[6]. The name “Flavonoid” is derived from Greek word “Flafus” which means yellow [7]. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs(8). They act in the plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents and for light screening(9). They are generally water soluble compounds. The more conjugated compounds often are brightly colored .They are generally found in plants as their glycosides which can complicate structure determinations (10). This group contains more than 8000 known compounds, and this number is constantly growing because of the great structural diversity arising from the various hydroxylation, methoxylation, glycosylation, and acylation patterns. Flavonoids are the pigments responsible for the shades of yellow, orange and red in flowering plants. They are also important factors for plant growth, development, and defence(11). Many studies have shown that flavonoids exhibit biological and pharmacological activities, including antioxidant ,cytotoxic, anticancer ,antiviral ,antibacterial cardioprotetive, neuroprotective ,antimalarial, antileshmanial ,antitrypanosomal and antiambebial properties(12). The chemical structure of this class of compounds is based on the flavone nucleus which consist of 15 carbons atoms derived from a C6-C3-C6 skeleton. They differ in the saturation of the heterocyclic ring C, in the placement of the aromatic ring B at the positions 2, 3 and 4 of ring C, and in the overall hydroxylation patterns$^{(13)}$ (Fig: 1.3).



(1) (2)

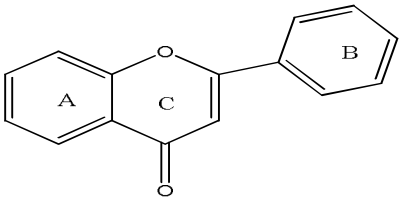
Fig; 1.3 Flavonoid structures ring labeling, and carbon atom numbering**.**

1.2- Properties and Classification of flavonoids

Flavonoids are a large family of compounds comprising about 12 classes, different from each other by the degree of oxidation of the C ring (viz. flavones, flavonols, flavans, flavanones, flavanols, chalcones, dihydrochalcones, isoflavones, aurones, anthocyanins , anthocyanidins, catechins)$^{(14)}$. Both the oxidation state of heterocyclic ring and the position of ring B are important in the classification [15].

1.2.1- Flavones

Flavones are a class of polyhdroxy derivatives based on flavone itself, the numbering of flavone (3) skeleton is shown below:



(3)

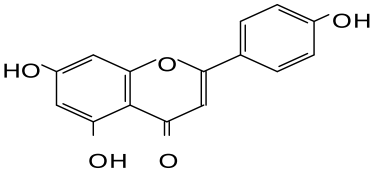
flavone

Flavones are closely related to the flavonols (or hydroxyl -3-ol) but differ in their spectroscopic and chromatographic properties and can readily be distinguished by these means.

Chemically, flavones can be classified into groups according to whether they are: (a) hydroxylated; (b) O-methylated; (c) C-methylated; (d) methylenedioxy substituted; and (e) isoprenylated. Flavones occur in the free state as lipophilic components of leaves and in bud exudates. They occur much more frequently in polar forms glycosides or other conjugates. Two main glycosides are known; O-and C-glycosides (16).

Flavones constituent one of the major classes of naturally occurring products(17). Flavones occur as glycosides, a common type is the 7-glycoside exemplified by luteotlin 7-glycoside. Flavones unlike flavonols also occur remarkably, with sugar bound by a carbon-carbon bond. A carbon-carbon bound is a very resistant to acid hydrolysis so that it is relatively easy to distinguish C-glycosides from O-Glycosides which are more readily hydrolysed [18]. A Special group of flavone –based C-glycosides occur in plants [19, 20].

C -glycosides of flavones commonly occur as such or with further O-glycolization. These glycosides readily lose their O-linked sugars on acid hydrolysis .Such O-glycosidic residues may be attached either to the hydroxyl of sugar carbon or directly linked to one of the free phenolic groups [21]. Flavone is the root from which the word flavonoid is derived [12]. The commonest flavones are apigenin (4) and luteolin (5). Flavones are common in angiosperms. 22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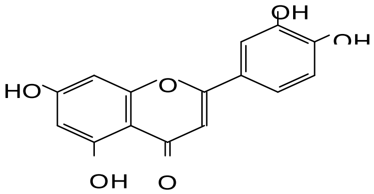
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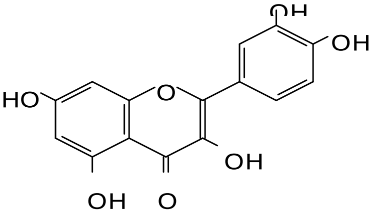
1.2.2- Flavonols

Flavonol are very widely distributed in plant, both as co pigment to anthocyanins in petal and also in leaves of higher plant .They occur most frequently in glycosides combination. Flavonols are limited natural occurrences (17). Flavonol are flavones with a 3-hydroxy substituent and they share the same nomenclature (23).

The most widely distributed Flavonol in nature are quercetin (6) kaempferol (7), myricetin (8) and methylated derivative isorhamnetin (9) [17].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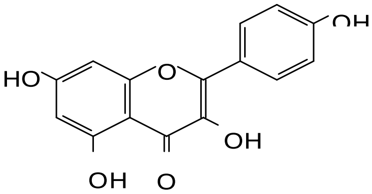
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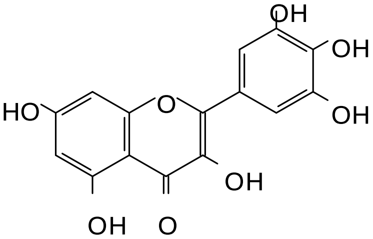
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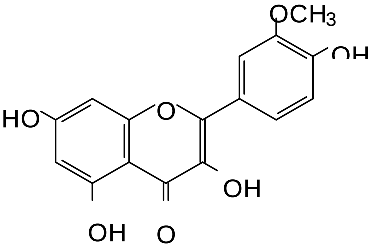
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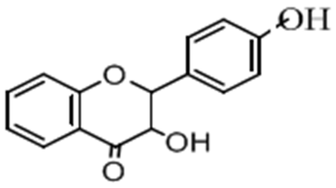
Flavonol, quercetin is the most abundant flavonol. It is present in many fruits, vegetables, and herbs, especially in onions, apples, berries, tea, or wine. Quercetin may be present either as the aglycone or as glycosides (rutin, quercitrin, isoquercitrin, hyperoside) (24). Quercetin (6) is also often added as a soluble derivative to multi-vitamin tablets, with other common flavonols like myricetin and kaempferol.

Flavonols have a wider range of biological activities than flavones. Quercetin, for example, is an effective inhibitor of many enzymes activities. Chrysosplenol C is an antiviral agent, while gossypetin is a yellow flower pigment in cotton plant and the primrose [25]. Flavonol play important role in the protection of plant from UV damage. They also play role in regulation of stem elongation; dormancy and fruit maturation [26]. Flavonols appear to be important in regulating control of growth in the pea plant [27].

1.2.3- Flavanones

Flavanones also called dihydroflavone lack the double bond between carbons 2 and 3, 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 stereo isomeric forms of each flavanone structure are possible, since C-2 is a centre 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)-, because the enzymatic reaction catalysing the conversion of chalcones to flavanones is stereospecific [28].

Flavanones are mainly represented by narigenin (10), hespertin (11), and eriodictyol (12), while a number of minor compounds, including sakuranetin (13) and isosakuranetin (14) also occur (18). Flavanones are represented by the saturated heterocyclic ring (C) and a carbonyl functions at the 4 position. Flavanones are usually glycosylated at position 7 by a disaccharide (neohesperidose, rutinose) or, by a monosaccharide (glucose) (29).



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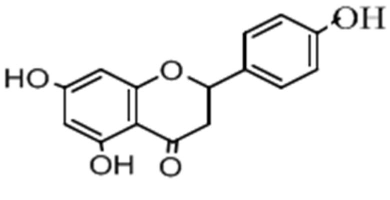

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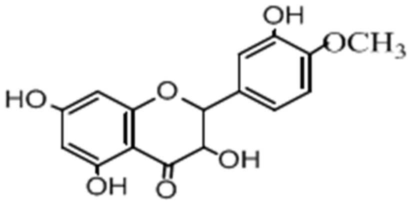

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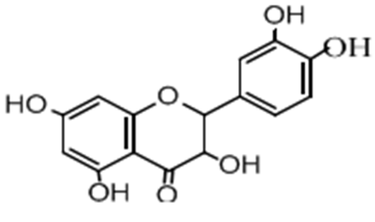

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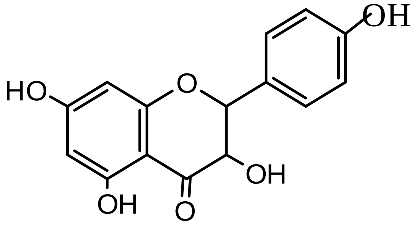
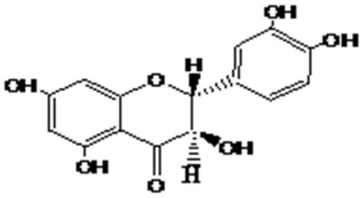


(14)

Dihydroflavonol or 3-hydroxyflavanones has wide distribution in the plant kingdom. The most common member of this group are: dihydroquercetin (15), dihydrokaempferol (16), dihydromyricetin (17) [30].

Dihydroflavonols have two asymmetric carbons at C-2 and C-3 .Most dihydroflavonols are polyhydroxylated and some are partially O-methylated. Dihydroflavonols have a very wide natural occurrence. Dihydroquercetin, for example, has been found in 50 angiosperm families. The most significant biological property is their antimicrobial activity [11].



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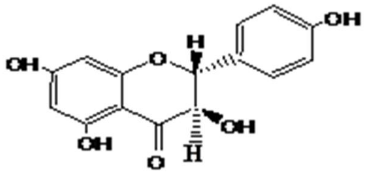

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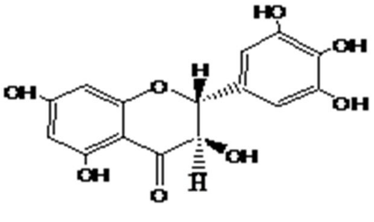


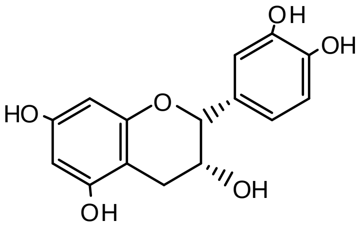
(17)

1.2.5 Flavan-3-ols or catechins

Flavan-3-ols comprise the largest class of monomeric flavans. The two compound Catechin and epicatechin are commonest flavonoids known. A notable feature of the naturally occurring flavan -3-ols is there occurrence in leaf of tea .They are also known to occur in glycosidic combination, with sugar attached to the 3ˋ, 5ˋ, or 7-hydroxyls. These compounds are used as a haemostatic drug in the treatment of liver disease (25).

Flavan-3-ols represent the most common flavonoid consumed in the American and most probably, the Western diet and are regarded as functional ingredients in various beverages, whole and processed foods, herbal remedies, and supplements. Their presence in food affects quality parameters such as astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation(31). Flavan-3-ols are structurally the most complex subclass of flavonoids ranging from the simple monomers (+)-catechin (18) and its isomer (-)-epicatechin (19) to the oligomeric and polymeric proanthocyanidins which are also known as condensed tannins(32).

Flavan-3-ols are found abundantly in fruits such as apricots (Prunus armeniaca), sour cherries (Prunus cerasus), grapes and blackberries (Rubusspp.) (20).



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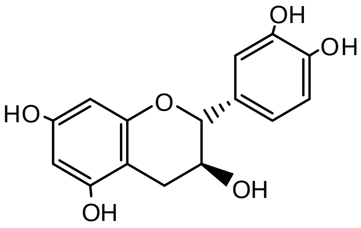

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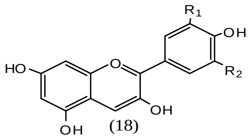
1.2.6 Anthocyanidins and anthocyanins

Anthocyanins are water-soluble plant pigments and are particularly evident in fruit and flower tissue where they are responsible for a diverse range of red, blue, and purple colors. They occur primarily as glycosides of their respective aglycone anthocyanidin (Fig. 1.2), with the sugar moiety typically attached at the 3-position on the C-ring or the 5-position on the A-ring. They are involved in the protection of plants against excessive light by shading leaf mesophyll cells. They also have an important role in attracting pollinating insects. There are about 17 anthocyanidins found in nature, but only 6 — cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin— are ubiquitously distributed and of dietary importance. The variation of anthocyanins are due to: (i) the number and position of hydroxyl and methoxy groups on the basic anthocyanidin skeleton; (ii) the identity, number, and positions at which sugars are attached; and (iii) the extent of sugar acylation and the identity of the acylating agent (33). Unlike other subgroups of flavonoids with the same C6–C3–C6 skeleton, anthocyanins have a positive charge in their structure at acidic pH.

The most widespread anthocyanin in fruits is cyanidin-3-glucoside (34). However, malvidin glycosides are the characteristic anthocyanins in red grapes and their derived products (35). Other anthocyanins that occur in grapes include petunidin-3-O-glucoside, malvidin-3-O-(6-O-p-coumaroyl) glycoside, malvidin-3-O-(6v-O-acetyl) glycoside, delphinidin-3-O-glucoside, and malvidin-3-, 5-O-diglucoside(36)



Anthocyanidin	R$_1$	R$_2$
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH$_3$	H
Petunidin	OCH$_3$	OH
Malvidin	OCH$_3$	OCH$_3$

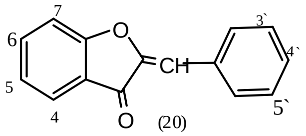
Fig. 1.4 : Structures of major anthocyanins

The anthocyanins constitute a major flavonoid group, which is responsible for colours ranging from salmon-pink through red and violet to dark blue in plant tissues (37).

The anthocyanins are the most important group of colouring matters in plants. They are all based chemically on a single aromatic structure, that of cyanidin, and all are derived from this pigment by addition or subtraction of hydroxyl group or by methylation or by glycosylation (18). Anthocyanins are those flavonoid pigment which provide intense cyanic colours from pink to blue in the flowering plant .Chemically ,anthocyanins are highly oxidised flavylium cation ,exemplified by the structure of the common anthocyanidin ,pelargonidin The anthocynidin are unstable to light and are water –insoluble so that they do not occur usually in the free state ,instead ,they are present in the cell vacuole linked to sugars ,which provide stability and water solubility. Some anthcyanidins have malonic acid (or other aliphatic dicarboxylic acid) residue linked through sugar and are zwitterionic in their properties(38).

1.2.7 Aurones

Aurones are small group of yellow pigment related to flavonoid(39).Aurones have a limited occurrence, and the first aurones was discovered in 1943(40). Aurones are hydroxylated 2-benzylidenecoumaranones, the parent compound being (20).The normal numbering system applies to this group of compounds: positions on the A ring are identified by un primed numbers and B-ring positions by primed number .Note that in aurones position 4 corresponds biosynthetically to position 5 of other heterocyclic flavonoids(41)(42). The two most common structures are sulfretin, (6, `3, `4-triahydroxyaurone) (21) and aureusidin (4, 6, `3, `4-tetrahydroxaurones) (22).



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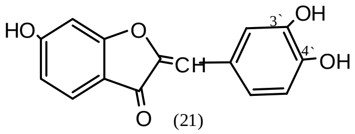

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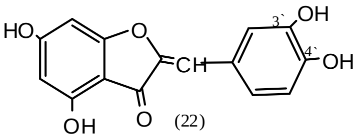

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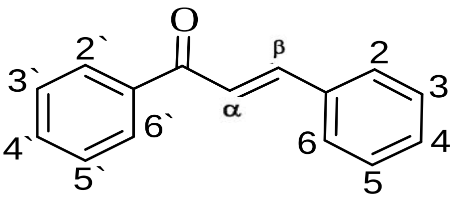
1.2.8 Chalcones

Chalcones (1, 3- diaryl-2-propen-1-ones) are a group of naturally or synthetic organic compounds, containing at least two aromatic rings, linked by ketoethylenic group (-CO-CH=CH-), or they are α, β- unsaturated ketones, belonging to the flavonoids (43). The general formula of chalcone is as illustrated below.

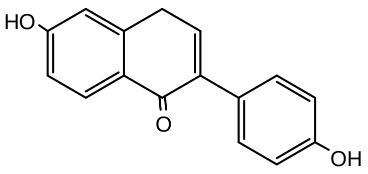


The chemistry of chalcones has generated intensive scientific studies throughout the world. Especial interest has been focused on the synthesis and biodynamic activities of chalcones. The name “Chalcones” was given by Kostanecki and Tambor**.** These compounds are also known as benzalacetophenone or benzylidene acetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcone bears a very good synthon so that variety of novel heterocycles with good pharmaceutical profile can be designed (44).

1.2.9 Isoflavones

Isoflavones differ from flavones in the position of the B-ring, linked to the C_3 position of the heterocycle instead of the C_2 position as in most flavonoids. Isoflavones are also termed phytoestrogens because of their estrogenic activity in mammals derived from the structural similitude between oestrogens and isoflavones. They occur almost exclusively in legumes, mainly in soy, although some isoflavones can also occur in nuts, grain products, etc. The most common isoflavones are daidzein (23) and genistein (24), together with their 7-O-glucosides daidzin and genistin, respectively(43).

In contrast to most other flavonoids, isoflavones are characterized by having the B-ring attached at C-3 rather than the C-2 position. They have a very limited distribution in the plant kingdom with substantial quantities being found only in leguminous species (44). Isoflavones are known for their estrogenic activity due to their ability to bind to estrogen receptor and have received much attention due to their putative role in the prevention of breast cancer and osteoporosis (45). In plants isoflavones occur predominantly as glucosides (genistin, daidzin, glycitin), or as acetyl-β-glucosides and malonyl-β-glucosides, and are therefore polar, water-soluble compounds (46). Isoflavones also undergo various modifications, such as methylation, hydroxylation, or polymerization, and these modifications lead to simple isoflavonoids, such as isoflavanones, isoflavans, and isoflavanols, as well as more complex structures including rotenoids, pterocarpans, and coumestans [47].



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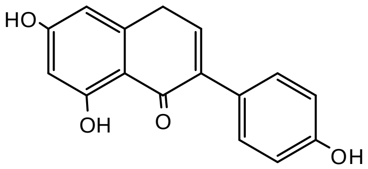
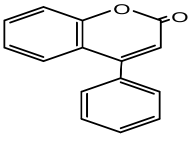


(24)

1.2.10 Neoflavonoids

The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2H-1-benzopyran-2-ones) (22), 3, 4-dihydro-4-arylcoumarins (25), and neoflavenes (24) [4].A typical neoflavonoids is melanin (25).(45)



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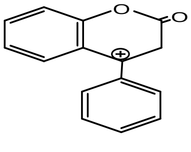

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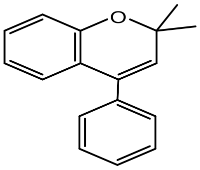

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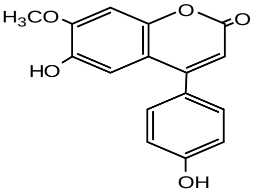

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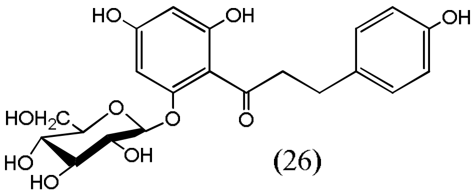
1.2.11 Dihydrochalcones

Dihydrochalcones are directly related to the chalcones and are derived from them by reduction of the chalcones α, β-double bond. The best known dihydrochalcone is phloridzin (26), which occurs in the skin of apples. When taken orally, it causes glycosuria by interfering with tubular read sorption of glucose in the kidney. It is therefore in demand in experimental physiology to study glucose transport.



(26)

Dihydrochalcones are relatively small group of flavonoids and they have a somewhat erratic distribution(44).

1.3 Health benefits of flavonoids

Flavonoids are important antioxidants, and promote several health effects. Aside from antioxidant activity, these molecules provide the following beneficial effects:

- Anti-viral
- Anti-cancer
- Anti-inflammatory
- Anti-allergic

One flavonoid called quercetin can help to alleviate eczema, sinusitis, asthma, and hay fever. Some studies have shown that flavonoid intake is inversely related to heart disease, with these molecules inhibiting the oxidation of low-density lipoproteins and therefore reducing the risk of atherosclerosis developing.

Many studies have also shown that one to two glasses of wine a day can help protect against heart disease. Some types of tea are also rich in flavonoids and their consumption is thought to lower levels of triglycerides and cholesterol in the blood. Soy flavonoids or isoflavones also lower cholesterol, as well as protecting against osteoporosis and alleviating the symptoms of menopause Certain drugs do interact with flavonoids. Studies have shown that the enzyme cytochrome P450, which is involved in the metabolism of drugs, is inhibited by flavonoids(48).Studies have found that flavonoids inhibit a variety of cancers in animals, but insufficient evidence exists to show that high flavonoid intake can reduce human cancer risk, according to the Linus Pauling Institute. However, researchers at Cornell University have found that extracts from flavonoid-rich onions provided strong anti-proliferation effects against liver and colon cancer cells(49).

1.4- Nomenclature

Individual flavonoids names may be assigned in three different ways:

I. Trivial names are employed extensively and sometimes indicate flavonoid compound class depending on:

(a) Names ending as in ‘nidin’ can denote an anthocyanidin. (e.g. pelargonidin) and the ending ‘etin’ a flavonol (e.g. quercetin).

(b) Position of bonding as glycosides of quercetin has related names such as quercitrin (the 3-rhamnoside), isoquercitrin (the 3-glucoside) and quercimeritrin (the 7- glycoside).

(c) Plant source - as many names have been derived from the generic or specific name of plant source (e.g. tricin from triticum corniculatusin)(50).

II- Semi – systematic chemical names are based on trivial names such as flavones or chalcone as the parent structure, giving precedence to substituents: e.g. 3, 5, 7, 3′, 4′-pentahydroxyflavone. In which the A- and B ring substituents precede C-ring substituents. In other cases the substituents are ordered numerically (e.g.3, 3′, 4′, 5, 7-pentahydroxyflavone)(50).

III- Systematic chemical names: - e. g. 3, 4- dihydro -2 –phenyl - 2H-1- benzopyran for flavan, but this method is cumbersome and rarely used as it’s easy to get wrong in cases of poly substitution .

There are two conventions for drawing flavonoid formulae:

(a) With the heterocyclic oxygen at the top.

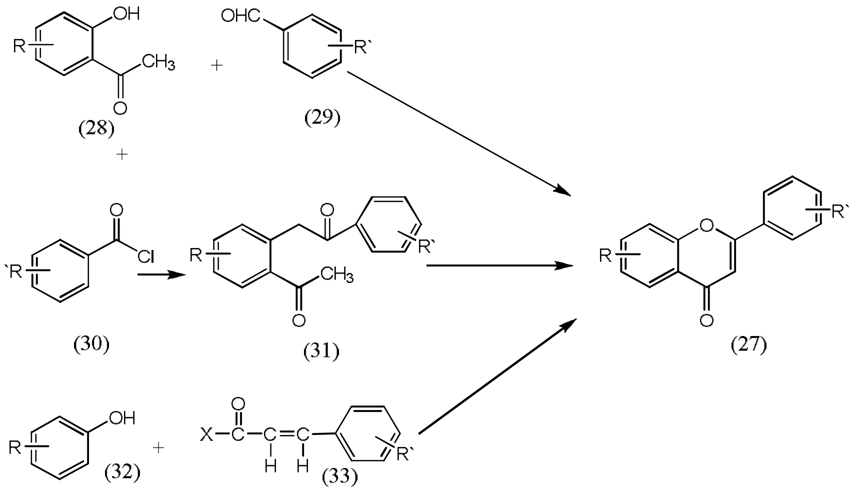
(**b)**With the heterocyclic oxygen at the bottom**.**

1**.5- Spectral characteristic of flavonoids**

Studies on flavonoids by spectroscopy have revealed that most flavones and flavonols exhibit two major absorption bands. Band I (320-385nm) represents the B ring absorption, while Band II (250- 285nm)corresponds to the A ring absorption. Functional groups attached to the flavonoid skeleton may cause a shift in absorption such as from 367nm in kaempferol (3,5,7,4,-hadroxyle groups) to 374 nm in myricetin (3,5,7,3,4,5, hydroxyl group)(51). Flavanones have a saturated heterocyclic C ring, with no conjugation between the A and B rings, as determined by their UV spectral characteristics(52).Flavanones exhibit a very strong Band II absorption maximum between 270and 295 nm, normally , with a shoulder at 300-340nm (taxifolin), and only one peak(270nm) in compounds with a mono substituted B ring. They exhibit two peaks or one peak (258nm) with a shoulder(272nm)-when a di, tri, or substituted B ring as present. As anthocyanins show distinctive Band l peak in the 450-560nm region due to cinnamoyl system of the B ring and Band (ll) peaks in the 240-280 nm region due to the benzoyl system of the A ring. The color of the anthocyanins varies with the number and position of the hydroxyl groups(52)

1.6.1 Synthesis of Flavonoids

The precursor of flavonoids can be synthesized through the following two strategies. The first synthesized flavonoid precursor(C_6-C_3 – C_6-)(**27**) was obtained through condensation of 2- hydroxyacetophenone derivatives (**28**) with benzaldehyde derivatives (**29**) or through the intermolecular reaction of ester (**31**), which was prepared from 2-hydroxyacetophenone derivative (**28**) and benzoyl chloride derivatives (**30)**. The second, flavonoid precursor could be prepared through acylation of phenol derivatives (**32**) with cinnamoyl chloride derivatives (**33**). Substituent on ring A, ring B, and ring C may have an effect in synthesis of specific compounds. Some modified approaches were also reported[46]. General synthetic routes of flavonoides are discussed below.



1.6.1 Syntheses of flavonols

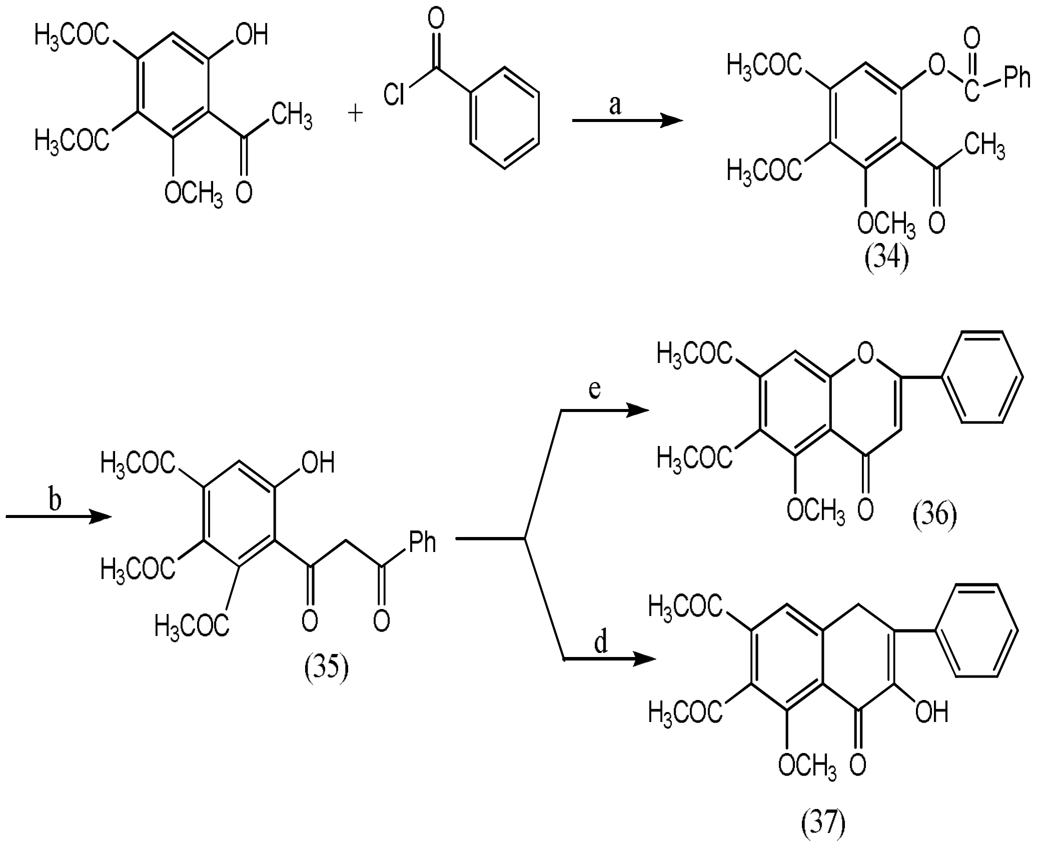
1.6.1.1Baker – Venkateremans method

The key step for the synthesis of baicalein is intermolecular Claisen rearrangement. Treatment of intermediate (**34**) with –KOH or NaOH in pyridine, benzene, or toluene give β –diketone intermediate

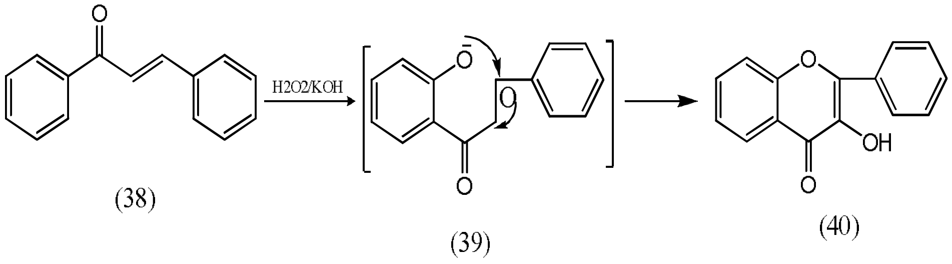
(**35**). Cyclization of (**35**) with acid (H_2SO_4/ETOH) or weak base (NaOAC/AcOH) gives bacalecin **(36**). cyclization and simultaneous hydroxylation of **(35)** with peroxyformic acid give 3-hydroxy bacalecin (**37**). This approach can also be applied to the synthesis of flavonols(53).

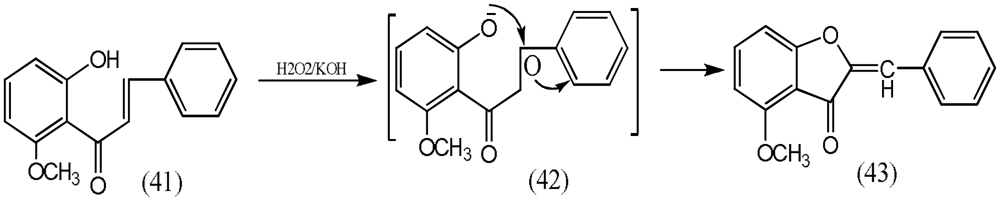
1.6.1.2 Algar-Flynn-Oyamadas method:

Oxidation of 2 –hydroxy chalcone (**38**) with H_2O_2 in basic condition gave corresponding flavonol (**39**) which is an epoxide derivative. Then, an intermolecular nucleophlic substitution of (**39**) at the benzylic carbon and a concomitant oxidation afforded favono l(**40**). In contrast, nucleophlic substitution on the other side of the epoxide, i.e., α position of ketone, gave (**43**) as a by-product.



K_2CO_3d) /Py (b) KOH/Py ; (c) H_2SO_4/EtOH ; (d) HCOOH

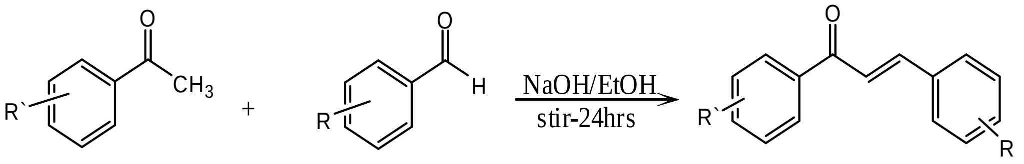




Substitution and reaction temperature are the major factors for this reaction. For chalcones which contains 2- or 4- hydroxyl and does contains 6`methyl, the major product is favonol. For a substrate chalcone which contains 6`methyl and does contain 2- or 4- hydroxyl, the product will be a mixture of (**43**) and favonol. High temperature is beneficial to the formation of favonol(54).

1.6.2- Synthesis of chalcones

The most convenient method is the one that involves the Claisen-Schemidt condensation of equimolar quantities of substituted acetophenones with substituted benzaldehydes in the presence of aqueous alcoholic alkali(55) . In the Claisen Schemidt reaction, the concentration of alkali used, usually ranged between 10- 60%.The reaction is carried out at about $50C^0$ for 12 -15 hours or at room temperature for 24 hours (56)

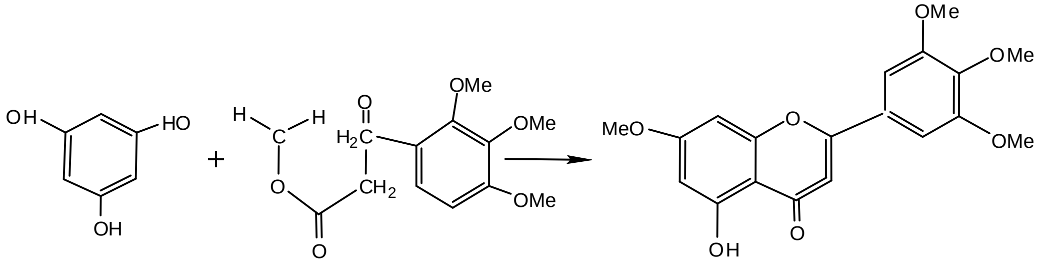


1.6.3 Synthesis of flavone

Flavones can be synthesized from chalcone or from simpler precursors by the condensation methods of Baker and Venkataraman or Allan and Robinson.

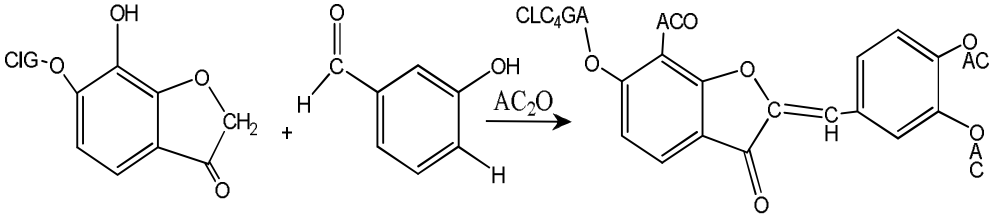
Allan—Robinson condensation

Flavones are obtained in one step by the condensation of an o-hydroxy acetophenone with the anhydride of an aromatic acid in the presence of trimethylamine or pyridine as a catalyst at oil bath temperature. Partially or completely alkylated flavones can be prepared by melting together phlorogluionl and benzyloxy or methoxy –benzoyl acetic ethyl ester .e.g. .the preparation of the `7-`4dibenzyl ether of luteolin(57).



1.6.4 Synthesis of aurones

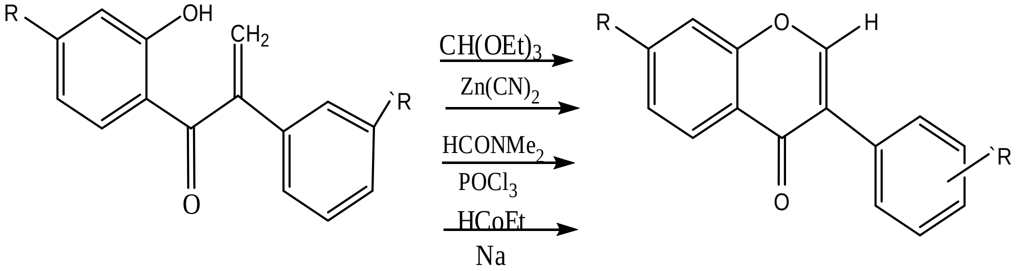
Aurones are prepared by the condensation of coumaran -3-ones with aromatic aldehydes using alkali or hydrochloric acid as condensing agent and these fails with 4-6-dihydroxy coumaran-o-ones type. The generally applied method is the condensation in boiling acetic anhydride without the use of a catalyst, and it has an advantage of giving leptosin .The natural aurone glycosides have been synthesized by condensation of the appropriately glucosylated aglycones in acetic anhydride(58).



Maintincin-heptaacetate

1.6.5 Synthesis of isoflavones

Methods for preparation of isoflavones often serve as key intermediates in the synthesis of isoflavanones .The 3-phenylbenzopyrone system of isoflavones can either be formed from C_{14}or C_{15} compound or by ring closure of phenyl benzyl ketones and oxidative transformation of chalcone by joining C_7 and C_8 units in the enamine acylation method. The ring closure of phenyl benzyl ketones is preferred(59).



1.7. Biological properties of flavonoids

Various classes of flavonoids have been investigated for different physiological activities. No universal function for the flavones and/or flavonols in plants has yet been established, in spite of the fact that these are the most common and widely distributed flavonoids. However, many functions in individual plants or plant groups have either been demonstrated or proposed (60).Willaman has reviewed the biological effects of the flavonoids and listed thirty-three different manifestations of activity under the heading “bioflavonoids”(61).

Rutin and hesperdin, also called vitamin P or permeability factors, are used in the treatment of various diseases, like capillary bleeding, increased capillary fragility, diabetes, allergic manifestation and hypertension. Claims have also been advanced for the citrus bioflavonoids in the treatment of symptoms of common cold (62). A number of flavonoids and chalcones have anti-protozoal activities (63).

Phenolic substances are well known to have anti-inflammatory activity. Some flavonoids like myricetin and kaempferoL-3-glucoside have an anti HIV-I potency at non-toxic concentration (64).

The position of the substitution also affects the properties. The flavonols containing two *ortho* or *para* hydroxyl in the 2-phenyl ring have anti-oxidant properties, while free hydroxyl at the 5, 7-positions have a pro-oxidant effect. Apigenin and genkwanin, which are present in the Chinese, drug “Yuen - hua” are believed to have diuretic and anthelmintic properties. These actions become more pronounced with an increasing number of hydroxyl groups. Fluka has reported the cardiac stimulation and vasoconstriction of the flavones and their glycosides [65].

Some flavones have the properties of prevention from anaphylactic shock, protection against X-rays and cure from frostbite. The simple isoflavone and coumestans have oestrogenic activities; rotenoids have insecticide properties while isoflavonoid phytoalexins have anti-fungal and anti-bacterial activities [66].

Some of the minor flavonoids have very interesting activities. They have anti-microbial, anti-fungal and cytotoxic properties. Anthocyanin peonin and isorhamnenetin of the algae Chlamydomonas are highly potent sex determining hormone.

Anthocyanins have wide applications in the food industry. It helps in determining wine quality. It is used as natural colorant to replace synthetic red dyes. Anthocyanins are also used for the inhibition of larval growth in insects.[67].

Chalcones have been thoroughly investigated by Michiro and it was found that they have anti-microbial activity, which is enhanced by the bromination. Dihydroxychalcones, which is a usual class of flavonoids are generally considered physiologically inactive but 2, 5-dihydroxy-4-methoxy-3, 5-dimethylhydrochalcone has anti-microbial and lipoxygenase inhibiting activity. Bioflavonoids functions as UV filters. They functions as guard on the leaf from insect and microbial attack and inhibit adhesion of blood platelets and blocking of inflammatory effects. These also act as heart stimulant (68).

A number of flavones, flavonols, flavanones, and isoflavones, as well as some of their methoxy, isoprenyl, and acylated derivatives, show antimicrobial activity(69).

The health effects of flavonoids have long been recognized for antioxidant, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic properties [70]. Medicinal activities shown for flavonoid compounds range from scavenging of harmful reactive oxygen species, enzyme inhibition, anti-inflammatory, vascular and estrogenic activities to cytotoxic antitumor activities. Anthocyanins and some flavonoid are UV protectants, signaling transmitters. Isoflavones, the bioactive ingredient in leguminous vegetables, not only cause a small reduction in blood cholesterol but also reduce blood pressure, arterial dimensions, and oxidative stress [71].

Antibacterial activity has been displayed by a number of flavonoids. Quercetin has been reported to completely inhibit the growth of *Staphylococcus aureus*. Most of the flavanones having no sugar moiety showed antimicrobial activities whereas none of the flavonols tested showed inhibitory activity on microorganisms [72].

A number of flavonoids isolated from the peelings of tangerine orange, when tested for fungistatic activity towards *Deuterophoma tracheiphila* were found to be active; nobiletin and langeritin exhibited strong and weak activities, respectively, while hesperidin could stimulate fungal growth slightly. Chlorflavonin was the first chlorine-containing flavonoid-type with antibacterial properties. It is produced by strains of *Aspergillus candidus*[73].

Naturally occurring flavonoids with antiviral activity have been recognized since the 1940s. Later attempts have been made to make synthetic modifications of natural compounds to improve antiviral activity. Quercetin, morin, rutin, dihydroquercetin (taxifolin), apigenin, catechin, and hesperidine have been reported to possess antiviral activity against some viruses[74]. The antiviral activity appears to be associated with the non-glycosidic compounds, and hydroxylation at the 3-position is apparently a prerequisite for antiviral activity. It has been found that flavonols are more active than flavones against *Herpes simplex* virus type I and the order of importance was galangin>kaempferol> quercetin[75]. Natural plant flavonoid polymers of molecular weight 2,100 daltons were found to have antiviral activity against two strains of type 1 *Herpes simplex* virus and type 2 *Herpes simplex* viruses[76]. Because of the worldwide spread of HIV since the 1980s, the investigation of the antiviral activity of flavonoids has mainly focused on HIV[77].

There have appeared several recent reports on the anti-AIDS activity of flavonoids. Out of twenty eight flavonoids tested, the flavans were generally more effective than flavones and flavanones in the selective inhibition of HIV-1 and HIV-2 or similar immunodeficiency virus infections [78].

Flavonoid glycosides of *Ocimum basilicum* (Labiatae) decreased ulcer index, and inhibited gastric acid and pepsin secretions in aspirin-induced ulcers in rats[79]. Quercetin, rutin, and kaempferoL administered intraperitoneally (25-100 mg/kg) inhibited dose-dependent gastric damage produced by acidified ethanol in rats[80].

The hepatoprotective activities of flavonoids were investigated. The liver is subject to acute and potentially lethal injury by several substances including phalloidin (the toxic constituent of the mushroom, *Amanita phalloides*), CCl_4, galactosamine, ethanol, and other compounds. Flavonoids have been found to possess hepatoprotective activity. In a study carried out to investigate the flavonoid derivatives silymarin, apigenin, quercetin, and naringenin, as putative therapeutic agents against microcrystin LR-induced hepatotoxicity, silymarin was found to be the most effective one [81]. The flavonoid, rutin and venoruton, showed regenerative and hepatoprotective effects in experimental cirrhosis [82].

Some flavonoids exerted significant anti-inflammatory activity in the animal model of both acute and chronic inflammation when given orally or topically [83, .84]. Hesperidin, a citrus flavonoid, possesses significant anti-inflammatory and analgesic effects [85]. Recently apigenin, luteolin and quercetin have been reported to exhibit anti-inflammatory activity [86].

A number of reports have been published which demonstrate that flavonoids can modulate arachidonic acid metabolism via the inhibition of cyclo-oxygenase (COX) and lipooxygenase activity (LO). Also, it has been speculated that the anti-inflammatory and antiallergic properties of flavonoids are the consequence of their inhibitory actions on arachidonic acid metabolism [87].

Flavones/flavonols glycosides as well as flavonoid aglycone have been reported to possess LO and COX inhibitory activities [88, 89].

1.8 Isolation techniques of flavonoids

Isolation of flavonoid compounds involves two separate procedures: (a) extraction and (b) isolation and separation into pure compounds.

1.8.1 Extraction of flavonoids

Solvents used for extraction are chosen according to the polarity of the flavonoids being studied. The less polar solvents are particularly useful for the extraction of flavonoid aglycones. Whilst the more polar solvents are used if flavonoid glycosides, sulphates or anthocyanins are involved [90, 91 ,92].

In general 70-80% ethanol is the solvent of choice for the extraction of the dried plant material of all classes of flavonoids. 50% ethanol is recommended when water soluble derivatives (glucuronides or potassium bisulphate`s) are present. For anthocyanins, hydrochloric acid (0.1%) should be present to prevent their conversion to the pseudobase form [93].

The crude extract is then subject to two dimensional papers or thin layer chromatography. This is followed by the separation of the flavonoids using the suitable method for each class [94, 95].

1.8.2 Separation techniques of flavonoids

When flavonoids of varying types are to be extracted from a single batch of plant, a method for preliminary separation is sequential extraction with a number of solvents of varying polarity. This can lead to the separation of glycosides from aglycones and to the separation of polar from non-polar aglycones. Although a certain degree of overlap could occur.More conventional methods are the use of column, paper and thin layer chromatography, high performance liquid chromatography (HPLC) and countercurrent chromatography (CCC) (96).

1.9 *Aristolochia bracteolate*

Aristolochia bracteolate

1.9.1 Scientific classification

Kingdom	Plantae
Family	Aristolochiaceae
Subfamily	Aristolochioideae
Genus	Aristolochia
Clade	Magnoliids
Order	Piperales
Species	A. pontica
Binomial name	Aristolochia bracteolata
Phylum	Tracheophyta

1.9.2 Description

Aristolochia is a large plant genus with over 500 species in the family Aristolochiaceae(97). Its members are commonly known as birthwort, pipevine or Dutchman's pipe and are widespread and occur in the most diverse climates. Some species, like *A. utriformis* and *A. westlandii*, are threatened with extinction.

Various *Aristolochia* species have been used in herbal medicines since antiquity in obstetrics and in treatment of snakebite(98), festering wounds, and tumors, and they remain in use particularly in Chinese herbal medicine(99). *Aristolochia bracteolata* is known as “worm killer” due to supposed anti helminthic activity and trypanocidal effect(100). *Aristolochia bracteolata* also possess a potent antiallergic activity(101) and has pronounced antibacterial and antifungal activities(102).

1.9.3 Medicinal uses of Aristolochia bracteolate

In Sudan, *Aristolochia* is traditionally used as an analgesic, antiscorpion, and antisnake. It is also used in the treatment of tumors, malaria and for fevers(103). The plant is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery. leaves extract of *Aristalochia bracteolata* has effect on diabetic rats(104) The root part of *Aristolochia bracteolate* has antifungal and antibacterial activity and was used to treat syphilis, gonorrhoea, and skin diseases (105). Its leaves are bitter and anti-helmintic, antiulcer, anti-plasmodial and are medicinally important. The whole plant is very bitter and has abortifacient, alterative, anthelmintic, antiperiodic, emmenagogue and purgative properties. These properties may help in further research for developing a novel herb based medicine against various disease(104)(106).

1.9.4. Chemical constituent of *Aristolochia bracteolate*

Ethanol extract of plant parts of *A. bracteolata* was the source of physiologically active compounds(104). The secondary metabolites from *Aristolochia* species cover 16 major groups classified by their chemical structures, including aristolochic acids and esters, aristolactams(107), isoquinolines, benzylisoquinolines, amides, lignans, biphenyl ethers, coumarins, tetralones, terpenoids, benzenoids and others. The Phytochemical analysis of this plant has revealed the presence of alkaloids, triterpenoids, steroids, sterols, flavonoids, tannins, phenolic compounds and cardio glycosides(104).

Considerable amounts of reducing sugars in free form are present in the roots. The chief active principle of the drug is aristolochic acid, though aristolic and p-coumaric acids also appear to contribute to the activities of the drug. Aristolochic acid is 8-methoxy-3; 4-methylenedioxy – 10 – nitrophenanthrene – 1 –carboxylic acid(108) (104).

Table 1 : Antibacterial activity of whole plant and Leaves extracts of Aristolochia bracteolate(109)

Plant extracts	Organisms	Zone of inhibition in mm			
		Ethanol	Chloroform	Aqueous	Standard
Whole plant	Staphylococcus aurous	7mm	8mm	8mm	7mm
	Bacillus subtilis	13mm	11mm	10mm	8mm
	E. coli	9mm	7mm	9mm	4mm
	Klebsiella pneumoniae	10mm	10mm	13mm	8mm
Leaves extract	Staphylococcus aureus	11mm	12mm	10mm	6mm
	Bacillus subtilis	12mm	11mm	13mm	9mm
	E. coli	8mm	10mm	8mm	3mm
	Klebsiella pneumoniae	10mm	2mm	12mm	8mm

Table 2 : Antifungal activity of whole plant and Leaves extracts of Aristolochia bracteolate(109)

Plant extracts	Organisms	Zone of inhibition in mm			
		Ethanol	Chloroform	Aqueous	Standard
Whole plant	Aspergillus flavus	10mm	8mm	9mm	8mm
	Aspergillus niger	10mm	11mm	12mm	7mm
	Fusarium solani	12mm	9mm	10mm	9mm
Leaves extract	Aspergillus flavus	8mm	9mm	7mm	6mm
	Aspergillus niger	7mm	10mm	9mm	8mm
	Fusarium solani	9mm	8mm	5mm	6mm

Table 3 : Previous studies of *Aristolochia bracteolate*

No	Tittle of previous studies	Author name	Source	Year
1	Wound healing studies of Aristolochia bracteolata Lam. with supportive action of antioxidant enzymes(110).	Shirwaikar A; Somashekar A P; Udupa A L;	Phytomedicine : international journal of phytotherapy and	2003

		Udupa S L; Somasheka	phytopharmacology, Volume: 10, Pages: 558-562	
2	Systematics of pipevines: combining morphological and fast-evolving molecular characters to investigate the relationships within subfamily Aristolochioideae (111)	Wanke, Stefan; Gonzalez, Favio; Neinhuis, Christoph	International Journal of Plant Sciences, Volume: 167, Pages: 1215-1227	2006
3	Antioxidant properties of some medicinal Aristolochiaceae species(112).	Thirugnanasampandan, R.; Mahendran, G.; Bai, V. Narmatha	African Journal of Biotechnology, Volume: 7, Pages: 357-361	2008
4	. Local uses of Aristolochia species and content of nephrotoxic aristolochic acid 1 and 2-A global assessment based on bibliographic sources(113)	Heinrich, Michael; Chan, Jennifer; Wanke, Stefan;	Journal of Ethnopharmacology, Volume: 125, Issue: 1, Pages: 108-144	2009
5	Antiallergic activity of Aristolochia bracteolata Lank in animal model(114).	Chitme H Malipatil MallikarjunChandrashekhaPrashant .	Indian journal of experimental biology, Volume: 48, Issue: 1, Pages: 46-52.	2010
6	Screening for feeding deterrent activity of herbal extracts against the larvae of malaria vector Anopheles subpictus	Elango Gandhi; Rahuman Abdul Abdul	Parasitology research, Volume: 109, Pages: 715-26	2011

	Grassi(115).			
7	Isolation, characterization and quantity determination of Aristolochic Acids, toxic compounds in Aristolochia bracteolate(116) .	Abdelgadir, Abdelgadir A.; Ahmed, Elhadi M.; Eltohami, Mahgoub	Environmental Health Insights, Volume: 5, Pages: 1-8	2011
8	Separation and quantification of a natural drug component against human pathogens from Aristolochia bracteolata Lam(117)	Jebakumar, Solomon R. D.; Vimalan, Jayaraj; Kumar, R. Senthil	Asian Journal of Chemistry, Volume: 23, Pages: 1535-1538	2011
9	In vitro rapid clonal propagation of Aristolochia bracteolata Lam. (Aristolochiaceae) - a valuable medicinal plant(118)	Sebastinraj, Joseph; Sidique, K. M. Inayath	World Journal of Agricultural Sciences, Volume: 7, Pages: 653-658	2011
10	Development of formulation containing extracts of Aristolochia bracteata and Cassia tora - evaluation of antimicrobial activity(119).	Monic, Josephine Nithila S.; Veerabathini, Rajinikanth	International Journal of Pharmacy and Pharmaceutical Sciences, Volume: 8, Pages: 348-350	2011
11	In - vitro propagation and antimycotic potential of extracts and essential oil of roots of Aristolochia bracteolata Linn. (Aristolochiaceae)(120).	Gbadamosi, I. T.; Egunyomi	African Journal of Traditional, Complementary and Alternative Medicines, Volume: 9, Pages: 50-55	2012
12	Formulation Aristolochia bracteolata and/or Clerodendrum multiflorumfor extracts for prevention and treatment of smut and process thereof (121).	Rathod, Bachubhai Lakshmanb	Indian Pat. Appl., 25pp., Patent, 2012, INXXBQ	2012

13	Production of aristolochic acid (AA) I and II from Aristolochia indica and Aristolochia bracteolate through in vitro culture(122)	Prabha, A. Lakshmi; Nandagopalan, V.; Vasanth, K.; Jayabalan.	Plant Cell Biotechnology and Molecular Biology, Volume: 4, Pages: 131-140	2012
14	Activity of Aristolochia bracteolata against Moraxella catarrhalis(123).	Suliman Mohamed Malik; Timan Idriss Mona; Khedr Amgad	International journal of bacteriology, Volume: 5 Pages: 481-686	2014
15	One-step preparative isolation of aristolochic acids by strong ion-exchange centrifugal partition chromatography(124)	Abdelgadir, Abdelgadir Boudesocque-Delaye, Leslie; Thery-Kone,	Separation and Purification Technology, Volume: 156, Issue: Part_2, Pages: 444-449	2015
17	Evaluation of Aristolochia bracteolata linn. for antimicrobial activity, α-glucosidase inhibition, and its phytochemical constituents(126).	Das, Trayee Sarkar; Latha, R.; Agastian,	Asian Journal of Pharmaceutical and Clinical Research, Volume: 9, Pages: 137-142	2016
18	In vivo, in vitro and in silico anti-dysmenorrhea studies of A. bracteolata Lam. leaf extract and its molecular docking(127).	Sowmya, G.; Varatharaju, G.; Balasar T.; Thangave, K.; Sharmila,	World Journal of Pharmacy and Pharmaceutical Sciences, Volume: 5 Pages: 1843-1856	2016

19	Osteogenic differentiation of human gingival mesenchymal stem cells by Aristolochia bracteolata supplementation through enhanced Runx2 expression.(128)	Murugan Girija Dinesh; Y Ranga Rao Suresh; Kalachaveedu Mangathayaru;	Journal of cellular physiology, Journal; Article	**2017**
20	A review on worm killer: Aristolochia bracteolate(129)	D Udhaya Nandhini, M Rajasekar and T Venmathi	Journal of pharmacognosy and photochemistry	**2017**

1.10 -Oils

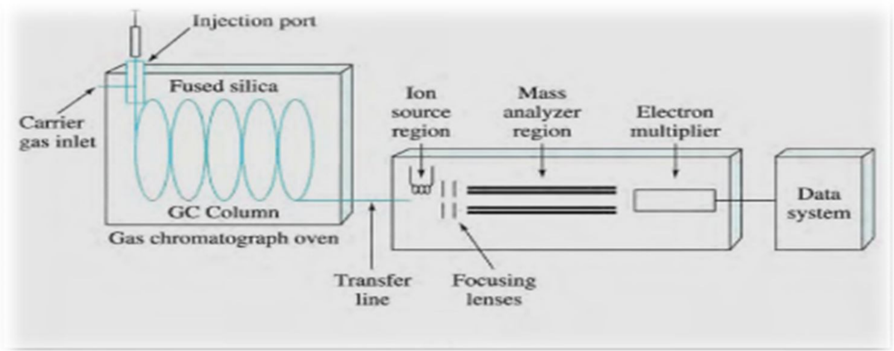
Essential oils are concentrated liquids of complex mixtures of volatile compounds. The term “essential oil” is reserved for a “product obtained from vegetable raw material(130). Aromatic oily liquids extracted from different parts of plants, for example, leaves, peels, barks, flowers, buds, seeds, and so on(131). They can be extracted from plant materials by several methods, steam distillation, expression, and solvent extraction(132) Essential oils are a good source of several bioactive compounds, which possess anti oxidative and antimicrobial properties. In addition ,some essential oils have been used as medicine(133) .Furthermore ,the uses of essential oils have received increasing attention as the natural additives for the shelf-life extension of food products, due to the risk in using synthetic preservatives, essential oils can be incorporated into packaging(134).

The first systematic investigations of constituents from essential oils may be attributed to the French chemist M. J. Dumas (1800–1884) who analyzed some hydrocarbons and oxygen as well as sulfur- and nitrogen-containing constituents. He published his results in 1833(135).

1.10.1 Biological Activities of Essential Oils :

The last few years more studies on the biological properties of essential oils have been published,. Many essential oils have been used for centuries in folk medicine and in recent years the biological properties of various essential oils have been proved by a number of studies. Their use in the treatment of pain, inflammation, viral diseases and cancer and their potential to enhance the penetration of other drugs, their insect repellent activity and their antioxidative effects were confirmed(136).

1.11 Gas Chromatography / Mass Spectrometry:



The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures by GC and identifies the components at a molecular level by MS. It is one of the most accurate tools for analyzing environmental samples(137). The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule(138). A "library" of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector(138). The components of GC/MS instrument are shown below(137):

1. Sample Introduction through the gas chromatograph.

2. The mass spectrograph components are kept under a vacuum,
the ion source, mass analyzer, and detector information
processed by a computer 3. Date Output.

1.12 - Aim of work

This work was aimed :

- To extract flavonoids from *Aristolochia bracteolate*.
- To extract fixed oil from some species.
- To isolate flavonoids from *Aristolochia bracteolate* using PC chromatography .
- To Identify isolates using: IR, UV, ^{1}H NMR,^{13} C NMR, mass spectroscopy.
- To analyze extracted oil by GC-MS.
- To evaluate antimicrobial potential of crude extract and oils.