International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 09 Issue: 05 | May 2022 www.irjet.net p-ISSN: 2395-0072

Classification and Identification of Flavonoids from plant kingdom as Acacia species

Hassan .E . Elkhidr [(1)], Tayseir .M. Ahmed [(2)]

Shendi university ,faculty of science and technology ,department of chemistry ,shendi ,Sudan

Red sea University , Department of chemical engineering, Port Sudan , Sudan

---

Abstract

Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical (are products, nutrition are also used as medicine), pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function.

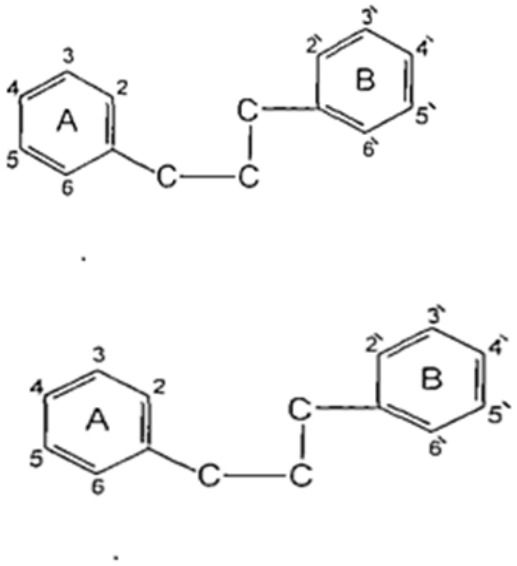
In this study we try to classify the flavonoids from higher plant specially Acacia species. Various sub groups of flavonoids are classified according to the substitution pattern of ring C. Flavonoids classified into: flavones, flavanols, flavanones, chalcones, aurones, isoflavonoids and anthocyanins .The chemical structure of the all types of flavonoids can be deduced on the basis of its Infra-red(IR),Ultra violet (UV), proton nuclear magnetic resonance (^{1}H NMR) Spectrophotometer.

***Key words**: Acacia species, Flavonoids, Spectrophotometer*

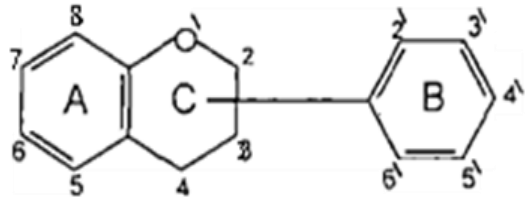
I. Introduction

The flavonoids are poly phenolic compounds their conjugates from a very large groups of natural products, they are found in many plants tissues, where they are present inside the cells or on the surfaces of different plant organs. Flavonoids possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain. The chemical structures of this class of compounds are based on a C_6-C_3-C_6 skeleton. They differ in the saturation of the hetero aromatic ring C, in the placement of the aromatic ring B at the position C_2 orC_3 of ring C (Figure 1).

Flavonoids constitute one of the most characteristic classes of compounds in higher plant and they are widely distributed through the plant kingdom[1]. Many flavonoids arc easily recognized as flower pigments in most angiosperum families (flowering plants) and they are usually known as plant pigments. However their occurrence is not restricted to flower but include all part of the plant[2]. The chemical structure of flavonoids arc Based on a C_{15} skelton with achromance ring bearing a second aromatic ring B in position 2, 3 to 4[3](Figure 2).

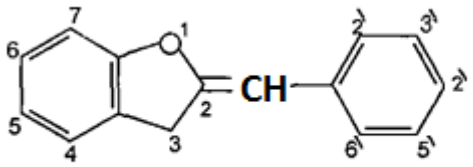


(Figure 1): the skeleton of the flavonoids



(Figure 2): skelton with achromance ring bearing a second aromatic ring B in position 2,3

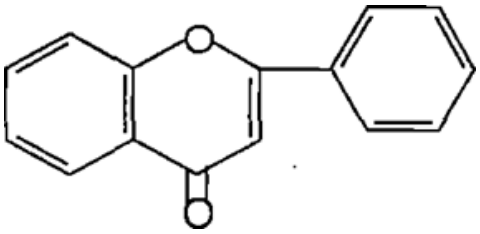
In few cases, the six-membered hetrocyelic ring C occurs in an isomeric open form or is replaced by a five-membered ring, giving aurones [4] (Figure 3)



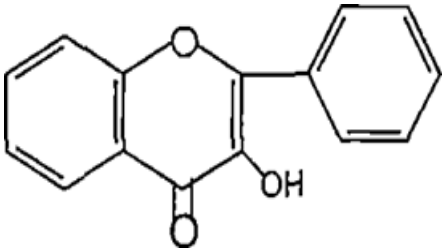
(Figure 3): the structure of aurones (five membered rings in C ring)

1.1Classification of Flavonoid

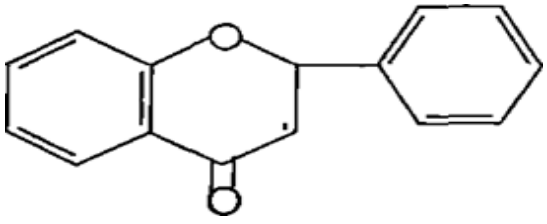
Various sub groups of flavonoids are classified according to the substitution patterns of ring C. Both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification. The flavonoids are classified into: flavones, flavonols , flvanones ,chalcones, aurones, isoflvonoids , and anthocyanins[5].(Figures 4-9)



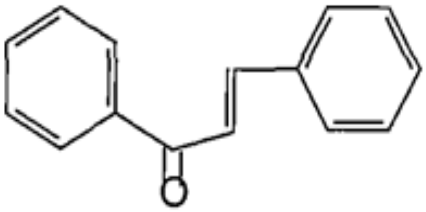
(Figure 4): the structure of Flavones



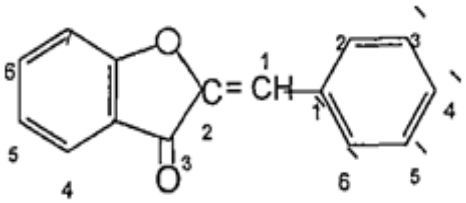
(Figure 5): the structure of Flavanols



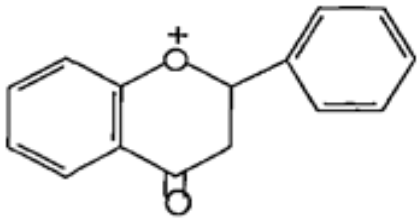
(Figure 6): The structure of Flavanones



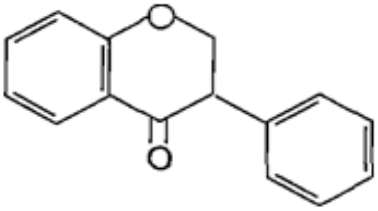
(Figure 7): The structure of Chalcones



(Figure 8): The structure of Aurones

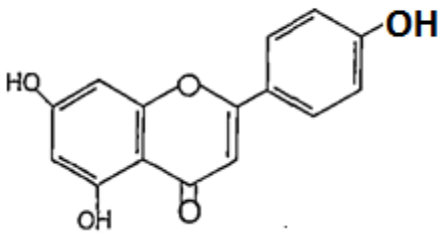


(Figure 9): The structure of Iso flavonoids

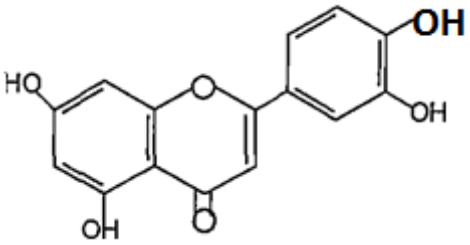


(Figure 10): the structure of Anthracyanins.

The flavones are generally found in herbaceous families' e.g labiatice, unbelliferae, compositae. Apigenin (Figure 11) and luteolin (Figure12) are examples.

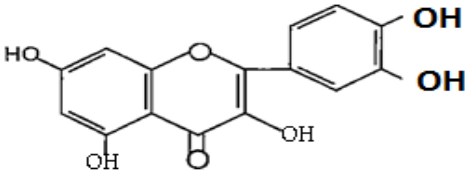


(Figure 11): The structure of Apigenin

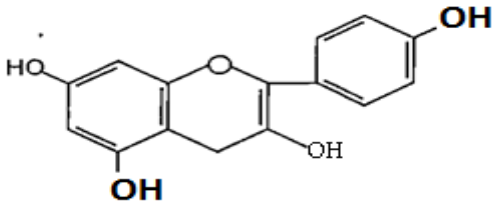


(Figure 12): The structure of Luteolin

Flavonols are generally found in woody angiosperum. Quercitol (Figure13) and kampherol (Figure14) are examples.

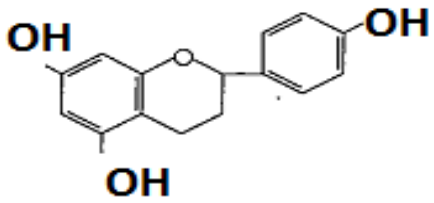


(Figure 13): The structure of Quercitol



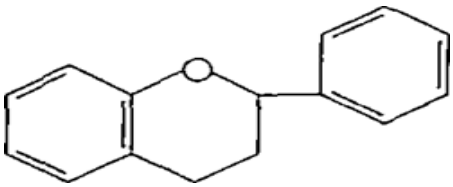
(Figure 14): The structure of kampherol

Flavanones are characterized by the absence of the double bond located between C_2 and C_3. Flavanones can be dehydrogenated to yield flavones or undergo hydroxylation at position-3 to yield dihydroflavonols (3-hydroxyf1avanones); an example of flavanone is naringenin (Figure 15)[6]

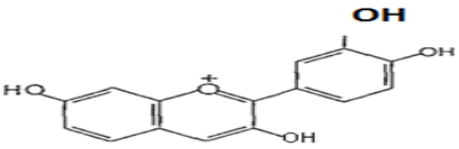


(Figure 15): The structure of Naringenin.

Anthocyanin are very similar to the flavan nucleus (Figure16), the difference is that the oxygen which is cited at the I. position in flavone in now a positive charge in anthocyanins the change is delocalized over whole structure. Cyaniding (Figure17) in an example of class.

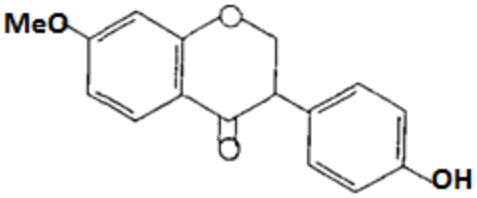


(Figure 16): the structure of flavan nucleus



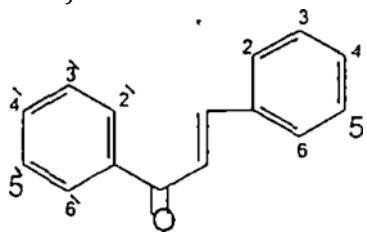
(Figure 17): The whole structure of Cyaniding.

In contrast to the large number of isoflavones encountered in nature, isoflavones can be distinguished from flavones and isoflavanones by UV and N NIR spectroscopy [7], an example is shown in (Figure 18).



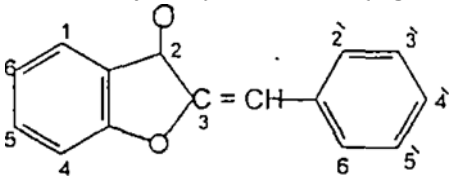
(Figure18): The structure of padmakastein

Chalcones are open chain flavonoid in which the two aromatic rings are joined by a three carbon α andβ, unsaturated carbonyl. Fundamentally they can be considered as derivatives of phenyl ketones shown below[8] (Figure 19).



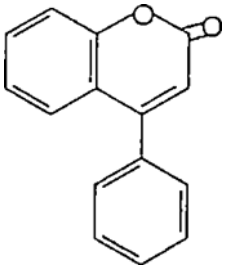
(Figure 19): The derivatives of phenyl ketones.

The aurones are based on the 2-benzylidenecoumaranone or 2-benzyliden 3-(2Hbenzofuranone system) as shown in (Figure20).[6]



(Figure 20): The structure of or 2-benzy liden3 (2H benzofuranone system).

Most of the flavonoids (Flavone, flavonols anal anthocyanins) bear ring B in position 2 of the Heterocyclic ring, but is iso flavonoids ring B occupies position 3. A group of chromane derivative with ring B in position 4 (4-phenyI coumarins) are termed neo flavonoids they are illustrated in (Figure 21). The neo flavonoids as well as the isoflavonoids are regarded as abnormal flavonoids [6].



(Figure21) The structure of Neoflavonoids.

Flavonoid compounds occur in all parts of plant: roots, stem, leave, flower, fruit, seed, wood and bark. However, some kinds of flavonoids are more characteristic of certain tissues[6].

1.2 The Flavones

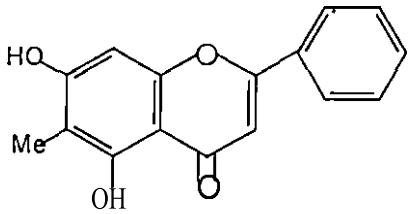
A large number of naturally occurring flavones is known now. The difference between flavones and flavonols residues in the presence o f a 3-hydroxy substituent in case of flavonols[6], and this effects their U V absorption, chromatographic mobility and color reactions and it is possible to distinguish simple flavones on these basic.

There are only two common on flavones apiginin and luteolin as we showed that in
(Figure 11, 12). Flavones have B-ring attached at C-2 and the carbonyl function is α, β-unsaturated. The parent un substituted flavone (Figure 4) produced apparently by an biosynthetic pathway, occurs in farina on species of primula and the closely related Dionysia[9]. 2'-hydroxyflavone and 5, 2'-diydroxy- flavone have been detected in the secretion of the glandular ce1ls of primulaflorinae flavones[10].
A pigenin and leuteolin free and as glycoside are the most widely occurring flavones. The A-ring of the great majority of flavones is derived from phloroglcinol and the B- ring is oxygenated in the 4' or 3', 4'. Or 3', 4', 5-positions as expected from their established acetate shikimate biosynthetic origin[7]. In the survey of twelve

Table (1) illustrates the color reaction of flavonoids.
Table (1) The color properties of flavonoid in UV-Vis light

Visible colour	Colour in UV light		Flavonoid present
	Alone	With ammonia	
Orange red mauve	Dull orange, red or mauve fluorescent yellow cerise or pink	Blue Blue	Anthocyanidin-3-glycoside most anthocyanidin 3,5 di glycosides
Bright yellow	Dark brown or black	Dark brown or black	6-hydroxylated flavonoid and flavone, some chalcone glycoside
		Dark and or bright orange	Mst chalcone
	Bright yellow or yellow green	Bright orange or red	Aurones
Very pale yellow	Dark brown	Bright orange yellow	Most flavonol glycosides, bidflavanyl and usually substitution flavonols
		Vivid yellow ,green, dark brown	Most isoflavones and flavonols
None	Dark mauve	Faint mauve	Most isoflavnes and flavonols
	Faint blue	Intense blue	5-deoxy isoflavones and 7-8 dihydroflavone
	Dark mauve	Pale yellow or yellow green	Flavanones flavanol 7-glycosides

highly specialized herbaceous families, harbone and Williams[9] found that 6-hydroxy leutolin is present in the majority of plant, as a lead constituents, accompanied occasionally by its 6-methyI and 6, 4'-dimethy1 ether. The ability of angiosperms to hydroxyl flavones in the 6-position apparently arose relatively late in evaluation anytime. Strobochrysin Figure22) (6-metliylchrysin) occur in heartwood of of pinus strobes and other pine species[11].

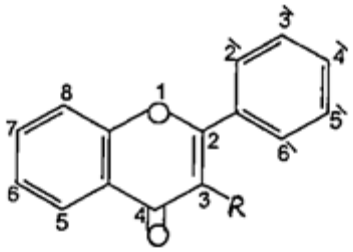


(Figure 22): The structure of Strochrysin.

It has been encountered in the fern lonchitistisserantii[12]. 5- hdroxy7, 4'-dimethoxy-6-methyl flavone and 6,8-dimethyl derivatives (eucalyptin) have been isolated from heart wood of eucalyptus trolliana (myrtace) and 5, 4'-dihydroxy-7-methoxy 6- 6-dimethy1 flavone (sideroxylin) from E sideroxiam[12].

1.3 The Flavonols

Since flavonol (I, R = OH) are simply flavone (I, R = H) in which the 3-position is substituted by a hydroxyl is shown in (Figure23).



(Figure 23): R=OH, flavonol and R=H, Flavone.

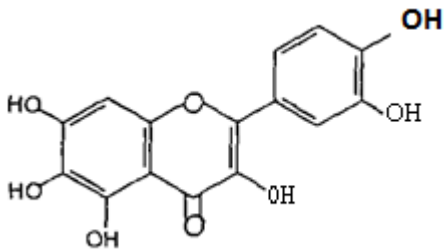
Both classes of pigments (flavonol or flavone) have so far been considered together, [13,14,15]. This practice is justified in much of their chemistry, analysis, synthesis, reactions have a common theoretical basis. However, it is apparent that this simple difference in structure is of considerable biosynthetic, physiological, phytogenic, chemo systematic, pharmacological and analytical significance[6].

Preliminary information on flavonol, present in plant extract can be obtained by two-dimensional paper chromatography[16] . Column chromatography though of inferior separation efficiency is the method of choice when larger quantities of flavonol are required[17].

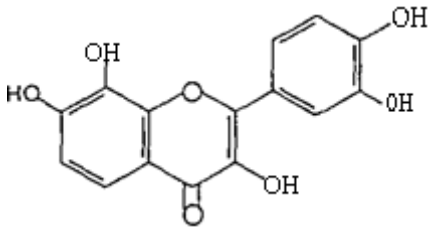
Of the various thin layers chromatography (TLC) procedures that can be used silica impregnated with lead acetate is of interest since it is based on the earlier isolation procedure for flavonol by precipitation of their lead salt[18 ' 19.] about 30 chromatograms obtained from 2-dimensiona1 paper chromatography yield sufficient quantities either of a pure flavonoid for UV spectral analysis, or of partially purified product for one dimensional paper chromatography separation.

Lists of Rt values are available for different developing system [20'21,22,23]. The yellow flower pigment gossypetin (Figure 24) is readily distinguished by its low R_f values. The dull black quercetagetin

(Figure25), has all most the same R_f value in range as solvents.



(Figure 24): The structure of gossypetin



(Figure 25): The structure of Quercetagetin

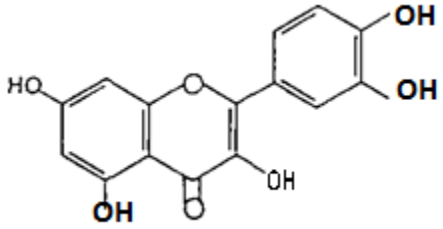
But can be distinguished in saturated aIcoholic sodium acetate and drying at room temperature, within 40 minutes, gossypetin (23) gives a blue-grey color, white auercetagenin (Figure25) remains yellow[24].

The two isomers also hay e different spectral properties, gossypetin (Figure24) has λ max 262,278,341 and 386nm; quercetaetin(Figure25) λmax9.272and 365 25. Gas-liquid chromatography of flavonol methyl ether on low loaded columns is feasible.

However, higher retention times are related to number of hydroxyls in ring B[26]. Gas-liquid chromatography is particularly useful when coupled with mass spectra analysis[27]. Paper chromatography in borate does not rapidly separate flavonols from their glycosides. But relative motilities may even reveal structural features[27]. lt must be remembered, however, that flavonols are oxidized very quickly at alkaline PHS[28]. Fractional

sublimation in vacuo may sometimes prove useful[29]. Co four reaction are important and they will even distinguish between gossypetin (Figure24) and queractagetin (Figure25), two isomers which are usually difficult to separate chromatographically [30].

Flavonols are widely distributed in plants as co-pi amends to anthocyanins in petals and in leaves of higher plants. they occur most frequently in glycosidic combinations. The most common flavonoIs are: kaempterol (Figure14) and querctin[6] (Figure 26).

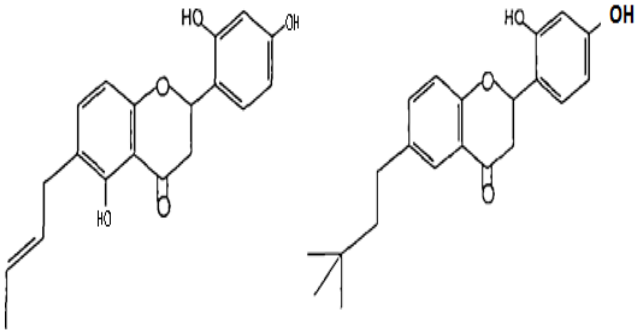


(Figure 26): The structure of querctin

Flavonols are more susceptible to oxidation than other flavonoids so that chemical methods of determination of structure rely on oxidants. The reaction of fiavonols with diazonium compounds give, as by-products, hydroxyl phenyl azo derivatives via displacement of the two phenyl group[6] Flavonols display arrange of colour when chromatographed on paper and viewed in UV light.Flavonols substituted in 3- positin appear as dark spot while derivatives which do not have a free hydroxyl at C-5 are generally distinguished by intense fluorescent colour[6].

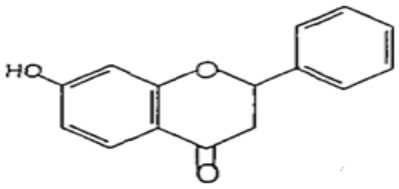
1.4 The Flavanones

The flavanones as we showed before in (figure 6) which are isomeric with chalcones (figure 7) are obtained from the latter by acid-or alkaline-catalyzed ring color of ring C. They cannot be detected during chromatographic survey unless chromatographic sprays are employed. It is true that some flavanones give bright yellow-green or light blue colours on paper when viewed in UV light with the help of ammonia vapour[7]. .An important color test in alcoholic solution is reduction with Mg powder and Conc. HCl. Only flavanones among the flavonoids give intense cherry-red colours[31]. This procedure can be applied to paper chromatography or thin layer chromatography plates by spraying first with alcoholic solution of sodium borohydride (Ca. 1%) and then later spraying with ethanolic aluminium chloride[31] . A suitable procedure for detecting 3-hydroxy flavanone or (di hydro flavonols) on paper is by use of zinc dust spread on the paper, followed by spraying with 2N HCL on chromatograms originally run in water, mauve spot will appear. Some flavanoncs beer prenyl side chains examples are preny lated flavanones artocarpesin and its oxydihydro cartocarpesin as we showed below in (Figure28) [32].

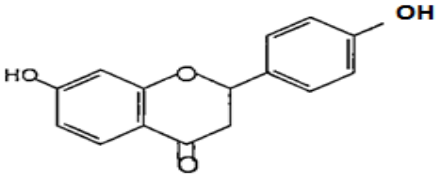


(Figure28): the structure of Artocarpesin and its Oxydihydrocarpesin.

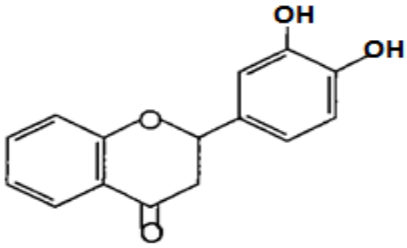
The naturally occurring fJavanones will be treated according to B-ring hydroxy lation pattern. Flavanone lacking B- ring hydroxyl groups are examplified by 7-hydroxy flavanones (Figure29) which was isolated from two legumes [33]. Flavanones having one B-ring hydroxyl group are exemplified by 7, 4'-di1iydroxy flavanone (31) .Flavanones having two B-ring hydroxyls are examplified by butin (31) [6].



(Figure29): The structure of 7- hydroxyl flavanones.



(Figure 30): the strucure of 7,4'-dihydroxy flavanone

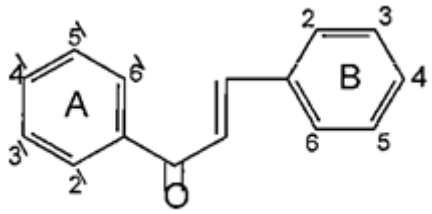


(Figure 31): The structure of Butin.

In alkaline solutions some flavanones undergo ring opening to the corresponding chalcone , such a change will be a cambanied by large bathochromic shifts in the UV spectrum.

1.5 Chalcones

Chalcones are described as open chain flavonoids in which the two aromatic rings are joined by a three carbon u, b- unsaturated carbonyl. It should be noted that the numbering of the position of substitution in the chalcone nucleus is reversed from that in most other flavonolds. The A-ring is numbered 2' — 6' and B-ring 2 — 6 as shown below (32)[6].



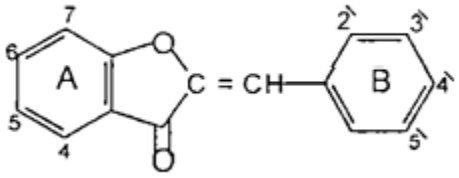
(Figure 32): The structure of Chacones.

Chalcones are yellow phenolic pigments which give intense deep brown UV colour when chromatographed on paper; on fuming the paper with ammonia, the color may change to a- rich deep -red, though few chalcones fail to respond in this way. Fundamentally chalcones can be considered as derivatives of phenyl styryl ketones[19].

Naturally occurring chalcones are all hydroxylated to greater or lesser extent6. The parent compound chalcone(Figure 32) itself is not known as natural product[6], Chalcones play ecological role in nature in relation to plant color. These brightly yellow colored compounds are found in many plant organs, but most conspicuonsly in flower. Chalcones are synthesized [34'35] utilizing aryl methyl ketones and aryl aldehydes as synthons. This will allow Perkin condensation with strong bases(e.g. NaOEt).

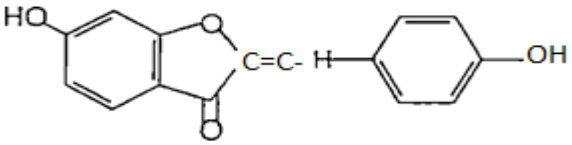
1.6 Aurones

Aurones have a limited occurrence and were discovered in 1943 front flowers of Coreopsis grandiflora [36'37] .Aurones like chalcones appear on paper chromatograms as yellow sptots in daylight. In the UV, they are very different, the color of aurones is intense bright yellow, changing with ammonia to bright orange —red. Auroncs are based on the 2-benzlidine coumaranone or 2benzylidine-3(2H)- benzofuranone system as showed in (Figure 33).



(Figure (33) The structure of 2-benzylidine coumaranone

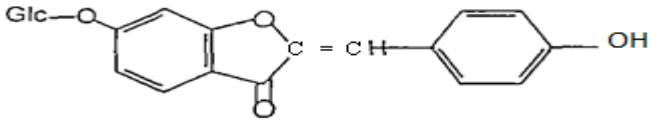
Aurones having one B-ring hydroxy I as hispidol (34) and its 6-O-glucoside (Figure35) occur in glycinemax seedlings[38].



(Figure 34): The structure of Hispidol

All the natural glycosides of aurones have been synthesized by condensation of the

Appropriately glycosidated components in acetic anhydride[39].



(Figure 35) The structure of 6-Oglycosides aurone.

1.7 Isoflavonoids

Isoflavonoids differ from other flavonoids in the position of the B-ring which is attached at 3-position.

Skeletal structures of the various classes of isoflavonoids showed before in (Figure10).

Many classes of isoflavonoids are known: isoflavones, isoflavanones, retonoids, pterocarpans, and coumestans[40].

Isoflavans, 3-aryI-4-hydroxycoumarins,chromones and hydroxyl- and de hydro variants of ptetrocarpans and retonoids have also been reported[7] .

1.7.1 Isoflavones

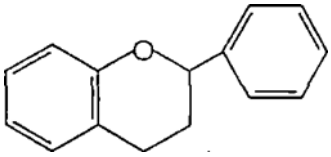
The isoflavones are very similar to flavones except that the B-ring is attached to 3-position of C-ring rather than the 2- position of flavones. There are many examples of this class differing from each other is the nature of

substituent groups and their location such as genistien daidzein and baptigenin[41-43].

Methods of preparation of isoflavones deserve a special attention since they often serve as key intermediates in synthesis of isoflavans and pterocarpans. The 3 –phenyl - benzopyrane ring system of isoflavones can be formed from C_{14} , or C_{15} , compounds . by ring closure of benzyl phenyl ketones and Oxidative transformation of chalcones or by Joining C7 an d C8, units as in the an amine acylation method [7].

1.8 Anthocyanins

AI2tliocyanins are very similar to flavan nucleus, the dÎ fferelice is that the oxygen cited in l -position in flavan is no iv carrying positive charge in the anthocyanins also there is a double bond in the C-ring of anthocyanins. The basic skeleton of anthocyanins shown in (Figure 9) and flavans are shown below in (Figure 35)



(Figure 35): The structure of Flavan.

The anthocyanins are water- soluble pigmens which are largely responsible for the attractive colors of lowers, leaves fruits and wines. Apart from their biological role, they are important aesthetically and economically since their stability is of significance in the marketability of plant products[7].

Anthocyanins occur not only as monomers, but as part of much larger system in loose association with or chemically bonded to other components. This has led to a desire to characterize anthocyanins as they actually exist in plant material using methods of extraction and examination designed to cause least interference or alteration in structure[7].

Anthocyanin are normally found as the aglycones (without glucose). They may al so occur as glycosides with glucose.

1.9 Flavonoid Glycoside

Flavonoid glycosides are generally soluble in water and alcohol but insoluble in organic solvents. They also dissolve in alkalis giving a yellow solution, which becomes colorless on addition of acid[44]. Flavonoids are present in plants bound to sugar a

glycosides and any flavonoid a glycone may occur in a single plant in several glycosides combinations[1,2].

1.10 Flavonoids of acacia species

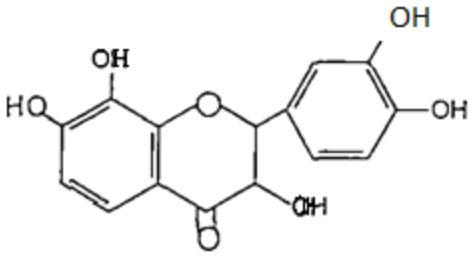
The genus Acacia has been classified into section[45], and this classification depends on morphological characteristic of foliage and inflorescence.

PhytochemicaI survey of the heartwood flavonoids of Acacia specics collected from Australia gave a number of flavonoids with similar hydroxylation pattern[46].

The heartwood of Acacia melanoxylon gave flavonoid possessing the 3', 4', 7, 8-hydroxylation pattern which is characteristic of a great numbering of Acacia species.

The heart wood also gave a pair of diastereomeric Leucoanthocyanidins [47'48].

7, 8, 3', 4'-tetrahydoxydihydroflavonol (Figure36) was found to occur in lequminoseae and especially in Acacia species[6].



(Figure 36): 7, 8, 3', 4'-tetrahydoxydihydroflavono

1.7. Conclusions

- The flavonoids are poly phenolic compounds. Flavonoids cane be obtained from plant as Natural products (organic compounds).

.Flavonoids arc easily recognized as flower pigments in most angiosperum families (flowering plants).

- The flavonoids are classified into: flavones, flavonols , flvanones ,chalcones, aurones, isoflvonoids , and anthocyanins.
- phytochemical screening of plant extracts give yellow colour evidence of presence of flavonoids.
- Spectroscopic data give strong evidence of the formation of flavonoids and its derivatives material.

ACKNOWLEDGENT

The authors would like to acknowledge of College of Graduate.

References:

1. Philip , M., "The chemotaxonomy of the Plants", Edward Arnold, London 1st, Edward (1976).
2. Harbone , J. B., "phytochemical methods "Chapman, and Hall, London (1969).

3.http ://www.friedi.com/bcrbs/phytochem/flavonoids.html.
4. Mastumura , F., "Toxicity of Insecticides", plenum Prcss, York(1985).
5. Pterson , J. and Dwyer, J. J. Am Dietet Assoc., 98 (1998).
6.Harbone,J.B., Mabry, T.J., Helga Mabry "The F!avonoids" part I, Academic press, NewYork (I975).
7. Harbone, J. B. and Williams, C. A., phytochemistry, 10, 2727 (1971).
8. Kumar, A. A., Pande, C. S. and Kaul, R. K., lithium J. Chem., 4, 640 (1966).
9.Bouillant, M. L.,Wollen Weber, E. and Chopin. J. C. r., *Acad. Sci*. **273**, 1629 (1971).
10.Harbone, J. B., "Comparative Biochemistry of Flavonoids", Academic P ress, York and London (1967).
11.Lebreton, P., Jay, M. and Voirin, B., *Chim.Analy* ., Paris. 49, 375 (1967).
12. Hillis,W,E and Iosi, K., phytochemistry, **4**, 451 (1965).
13. Venkalarmurn, K.,*Forisch*. *Chan*. *Org*. *Naturs* **17**, 1 4(1959).
14. Geissman, T. A., "The he chemistry of flavonoid compound", Pergaman press, Oxford (1902).
15.Dean, F. M., "Naturally occcurring oxygen ring compounds", Butter worth, London (1963).
16.Simon, J. P. and Goodull, D. W., *Aust* .J. Bol.. **16**, 89(1968).
17.Harbone J. B., Mabry T. J. and l4.. "The fJavonoid" part one, Claepman and Hall, London (1 975).
18.Pitteni,G.P.,*J.Chroma,***43**,539(1069).
19.Kumar, A. R., Punde, C. S. and KauI, R. K., Indian, J. Chem., 4, 640 (1966).
20.Egger, K., J. Chromat , 5,74 (196 I).
21. Wong. E., J. Chromat., 9. 449(1962).
22. Bianki, G. B., Fizoil,Rost., 11, 544 Chem.Absa.,61.. 61,7359(1964).
23. Harbone, J. B., "Comparative chemistry of flavonoids" Academic Press. London,((1967)
24.Pellizzari, E. D., Chuany, C. M., Kue., J. a nd Williams, E. B., J. Chromat., 40, 285 (1969).
25.Harbone, J. B., "*Phytochmical metthods*", Chapmpand and Hall, London (1984).
26.Furuga,T., *J. Chromat,* **19,** 607,(1965).
27.Paris, R. and Faugeras, G.*Ann,pharmy,*France,**81,**29(1960).
28.Kolasek, Z. 8nd 1nngmur. F., Veda avyzkum, V., Prumystu Kozeddnm, 6, 7,. J. *Chem abs*t. **57**, 13937 (l 962).
29. Sim,Y.K., *J.Chem Soc.,*(C) 976(1967)
30.Harbone, J. B., "*Phytochmistry*",, **8**, 1 77 (1 969).
31.Koppen, B. ll., J. C/ii omatoy. 8, 604 i 965).
32.Imamura, H.,Korosu,K. andTakahashi, T.,Nippon Mokuzai Gakkaishi, 13, 295 (1967).
33. Knekt, P., Jarvinen R. Sappaen, R., Heliavara M., Teppo! Pukkala, E., Aroma A."Dietary flavonoid and the risk of lung cancer and other malignant neoplasm American Journal of Epidemiology., 164, 223 (l 997).
34. Lunardi, L.,Guzela, M., et al., J. Antimicrobial Agent and Chromatography, 47, 1449 (2003). Pitteni, G. P., J. Chroma, 43, 530 [1969).
35.Correa, R., NI. A. S.. Pereria, D., Button. L. Santos, V., Cechinal Filho, A. R. S., Santos, and R. J. Nunes., J. Arch. Pharma. 33J, 332 (2001).
36. Giessman, T. A. and Heaton, C. D., J. Am.Soc., 65,677 (I943).
37. Harbone, J. B., "Phytochmical metthods", Chapmpand and Hall, London (1969).
38. Wong, E.Phytochemistry,5, 463(1966).
39.Asen,S. and Plimer,J.R., Phytochemistry, 11,2601(1972).
40.Farkas,L.,Palles. L., "In progress in the chemistry of organic natural products".,spring-verlage,New York, 25, 163,(1967).
41.Murakami,T.Nishikawa, Y. and Ando, T., *Chem,pharm.Bull.,Tokyo,**5,***688(1960).
42.Farkas,L., Varady,J. and Gottegen,A.,Chem.Ber., 46,1865(1963).
43. Francis,C.M., Millington,A.S. and Bailey, E.T., *Aust.J.ORGIC.Res.,***18,**47(1967).
44. Harbone, J. B., "Phytochmistry", Chapman and Hall, London(1 973).
45.Frakas, I.and Pollos,I., *Forischer.Chem.Org. Natures.,***25**,105,(1967).
46. Clark-Lewis, J. W. and Proter, L. J., *Australian journal of chemistry,***25,**1943(1972).
47. King, F.E and Clark-Lewis, *J.,Chem.Soc.*3384(1955).
48.Clark-Lewis, J.W. and Mortimer *J. ,Chem.Soc.,*4104(1960).