GENERAL INTRODUCTION

“Somewhere in the plant kingdom

There is remedy for everything

Evolution argues quietly for the natural drugs

While economics argues loudly for the synthetic drugs”.

The subject of phytochemistry, or plant chemistry, has developed in recent years as a distinct discipline, somewhere in between natural product, organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function\(^1\).

‘Natural products’, in the broadest sense, should cannote all the chemical compounds which occur in nature. However, by convention and practice, the term is now used to refer only to the organic compounds occurring in nature. The boundaries are further defined by restricting the term to the secondary metabolites, leaving out the aminoacids, proteins, carbohydrates, nucleic acids, and lipids, whose biochemical functions are more or less well known and therefore covered under biochemistry. By thus restricting the cannotation of the term ‘natural products’, it is possible, till recently, to keep the focus on the chemistry of these compounds, and thus keep the subject under the umbrella of organic chemistry\(^2\).

The flavonoid pigments, one of the most numerous and widespread groups of constituents, are of importance and interest to a wide variety of physical and biological function continues unabated\(^3\).

The flavonoid compounds can be regarded as C\(_6\)-C\(_3\)-C\(_6\) compounds, in which each C\(_6\) moiety is a benzene ring, the variation in the state of oxidation of the connecting C\(_3\) moiety determining the properties and class of each such compound.

A summary outline of the chemical classes can be emphasized as follows (Fig.I)\(^4\).
Flavonoids constitute the largest group of naturally occurring substance in plants. About 2% of the total carbon photosynthesized by plants is converted to flavonoids. Approximately 36% of the naturally occurring compounds of plants contain phenolic hydroxyls and of these about one-third are flavonoid types.

The term flavonoid (Lat, flavus = yellow) was coined by Geissman and Hinreiner. Kostanecki and Tambor suggested the name flavone first. Over 4000 structurally unique flavonoids have been identified in plant sources.

The flavonoids are a group of compounds that contain a C6-C3-C6 carbon skeleton in which the C3 unit links two aromatic groups. The C3 chain is essentially the key to the different major classes of flavonoids since these classes are recognized on the basis of the oxidation state of the C3 unit in addition to the mode of ring closure to form a heterocyclic middle ring (if ring closure ensues).

Flavonoids usually occur as glycosides and sometimes also as acylated compounds, the acyl group being in many cases a phenolic acid. Glycosides are mostly formed as esters at carbons 3, 5, or 7 but some carbon glycosides at position 8 are known. Classes of flavonoid compounds include anthocyanins (I), flavones (II), flavonols (III), flavanones (IV), flavanonols (V), isoflavones (VI), chalcones (VII), aurones (VIII), catechins (IX), leuco – anthocyanins (X) and biflavonyls (XI). Other common groups include the xanthones and the condensed tannins. The catechins and leuco anthocyanins are structurally very similar and only rarely exist as their glycosides. They polymerize to form condensed tannins which help give tea its colour. They also are sufficiently prevalent to darken the colour of streams and rivers in some woody areas.

The flavanones and flavanonols are fairly rare and normally exist as their phenolics. The flavones and flavonols are the most widely distributed of all the phenolics. The anthocyanins are the common red and rare blue pigments of flower petals and can make up as much as 30% of the dry weight of some flowers. They exist typically as glycosides. The chalcones, such as butene, lack the pyran-ring found in flavonoids, although this is often subject to pH – controlled equilibria. The chalcone is more fully conjugated and normally brightly coloured. The aurones are golden yellow pigments common in certain flowers.
Naturally occurring polyphenols have two alternative modes of sugar linkage, viz, O-glycosylation (XII) or C-glycosylation (XIII). The most significant features of C-glycosylated compounds is that a direct carbon to carbon bond links the sugar portion of the molecule to the aglycone or non – sugar moiety leading to a single stable carbon framework. Illustrative of this type are vitexin (XIVa)\textsuperscript{13} and orientin (XIVb)\textsuperscript{14} which contrast with the more common O-glycosides having sugar residues attached to phenolic oxygens by C-O-C hemiacetal linkage.
Fig I-PRODUCTS OF SECONDARY PLANT METABOLISM
VII Chalcone

VIII Aurones

IX Catechins

X Leuco-anthocyanins

XI Biflavonoids
XII O-Glycosylation

XIII C-Glycosylation

XIV (a) R = H, Vitexin
(b) R = OH, Orientin
(a) $R = CH_2OH, \beta$-D-Glucose
(b) $R = COOH, D$ Glucuronic acid

(a) $R = CH_2OH, \beta$-D-Galactose
(b) $R = COOH, D$ Galacturonic acid

$\alpha$-L-Rhamnose

$\beta$-D-Xylose

$\alpha$-L-Arabinose

D-Apiose
Glucose (XVa), galactose (XVIa), rhamnose (XVII), xylose (XVIII), arabinose (IX), apiose (XX), glucuronic acid (XVb) and galacturonic acid (XVIb) are the monosaccharides and rutinos e [α-L–rhamnosyl(1→6)glucose] (XXI), neohesperidose [α-L–rhamnosyl(1→2)glucose] (XXII) and sophorose [β-D-glycosy (1→2) glucose] (XXIII) are the disaccharides most commonly involved in glycosylation. Within a plant it has been observed that the glycosidic pattern may be relatively constant 15,16.

The monosaccharides are generally present in the expected pyranose form, although occasionally the less stable furanose forms have been reported 17. Earlier method of distinction of O – and C – glycosyl compounds depends on acid or enzymic hydrolysis which showed that C – glycosylated compounds remain unchanged under the conditions. In order to identify the sugar and aglycone moieties, it is necessary to bring about the cleavage of C – glycosyl compounds. Ferric chloride 18,19, ozone 20 and hydriodic acid 21,22 have been employed for the purpose.

Primarily recognized as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange and red in flower and food 23,24, the flavonoids are found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers as well as tea and red wine. They are prominent components of citrus fruits, and other food sources and are consumed regularly with human diet 25,26. They have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances and pigments and light screens. In addition, these compounds are also involved in photo sensitization and energy transfer, the actions of plant growth hormones and growth regulators, protein synthesis, the control of respiration, photosynthesis, morphogenesis, and sex determination as well as defense against infection 27,29.

Reports indicate that plant flavonoids cause the activation of bacterial (Rhizobium) modulation genes that involved in control of nitrogen fixation, which suggests important relationships between particular flavonoids and the activation and expression of mammalian genes 30-33.

Though the flavonoids were once considered as inert, their functions in the plants are well understood now. The role of flavonoids in the pollination process is very complex interesting. They are responsible for the preferential attraction of either birds or insects as pollinators towards the flowers 34. The flavonoid pattern in the orchid flowers changes after
pollination, which helps in guiding the pollinating insect, to visit unpollinated flowers of the same species\textsuperscript{35}.

Flavonoids can modulate the feeding behaviour of larvae and oviposition of adult insects\textsuperscript{36}. Flavonoid uptake and metabolism by insects is strongly dependent on the specific flavonoid pattern of their host plants\textsuperscript{37-40}. They act as antibiotic and antiviral agents in insects\textsuperscript{41}.

An ever-increasing number of pharmacological effects of flavonoids have become known over the years through the discovery of new plant flavonoids and their derivatives\textsuperscript{42,43}. An excellent review of flavonoids in health and disease have been published\textsuperscript{44}. The anticancer, antiviral, anti-oxidant, anti-microbial, anti-inflammatory, anti-allergie, hepatoprotective and anti-thrombotic effects\textsuperscript{45-56} of flavonoids are note-worthy.
**XXI**
Rutinose
\([\alpha-L-Rhamnosyl(1\rightarrow6)-\beta-D-glucose]\)

**XXII**
Neohesperidoside
\([\alpha-L-Rhamnosyl(1\rightarrow2)-\beta-D-glucose]\)

**XXIII**
Sophorose
\([\beta-D-glucosyl(1\rightarrow2)-\text{glucose}]\)
(a) $R_1 = R_2 = R_3 = R_4 = H$, Genistein
(b) $R_1 = R_2 = OMe$, Genistein-4', 5-dimethylether

(a) $R_1 = R_2 = R_3 = R_4 = H$, Quercetin
(b) $R_2 = R_3 = R_4 = H$, $R_1 = rut$, Rutin
(c) $R_2 = R_3 = R_4 = H$, $R_1 = glu$, Isoquercitrin
(d) $R_2 = R_3 = R_4 = H$, $R_1 = glu-6''-gal$, Isoquercitrin-6''-O-gallate
(e) $R_2 = R_3 = R_4 = H$, $R_1 = gal$, Hyperin
(f) $R_2 = R_3 = R_4 = H$, $R_1 = gal-(2''-rha)-6''-gallate$, 2''-O-rhamnosyl-
     hyperin-6''-O-gallate
(g) $R_2 = R_3 = R_4 = H$, $R_1 = gal-(2''-rha)$, 2''-O-Rhamnosylhyperin
(h) $R_2 = R_3 = R_4 = H$, $R_1 = ncohesp$, Quercetin-3-O-ncohesperidoside
(i) $R_2 = R_3 = R_4 = H$, $R_1 = glucur$, Miquelianin
(j) $R_2 = R_3 = R_4 = H$, $R_1 = rha$, Quercitrin
(k) $R_2 = R_3 = R_4 = Me$, $R_1 = H$, Quercetin-3,7,4'-methyl ether
(l) $R_2 = R_3 = R_4 = H$, $R_3 = Me$, Isorhamnetin
(m) $R_1 = rut$, $R_2 = R_4 = H$, $R_3 = Me$, Narcissin
(n) $R_2 = R_3 = R_4 = H$, $R_1 = glucur-(6''-methylster)$, Hyperoside
(o) $R_2 = R_3 = R_4 = H$, $R_1 = ara$, Quajaverin
(a) R₁, R₂, R₃, R₄ = OH, Taxifolin
(b) R₁, R₃ = H, R₄ = OH, R₂ = rut, Naringin
(c) R₁, R₃ = H, R₂, R₄ = OH, Naringenin
(d) R₁, R₃ = H, R₂ = OMe, R₄ = OH, 7-O-Methylnaringenin
(e) R₂, R₄ = OH, R₃ = H, R₅ = rha, Engelein
(f) R₁, R₃ = H, R₄ = OH, R₅ = glu-[6-O-p-(coumaryl)], Prunin
(g) R₁ = H, R₂, R₄ = OMe, R₅ = OH, Persicogenin
(h) R₂, R₅, R₄ = OH, R₁ = rha, Asilibin
Genistein (XXIVa) inhibits DNA topoisomerase and tyrosine protein kinase, as well as possessing antioxidant and cell cycle inhibitor activity. Davis et al., reported that quercetin (XXVa) suppressed thyroxine stimulation of human red blood cell Ca\(^{2+}\) - ATPase activity \textit{in vitro} and interfered with the binding of the hormone to red blood cell membranes. Silybin (XXVI), an antioxidant flavonoid from the European milk thistle, had a biphasic effect on secretion of steroids from adenomatous, hyperplastic, and atrophied adrenals. Ikeda et al., found that particularly purified catechin mixtures reduce cholesterol absorption from rat intestine due to reduction of cholesterol solubility in mixed bile salt micelles.

The relationship of the flavonoids to the human endocrine system has been reviewed by Michael and Bors. It is now well recognized that flavonoids can interact with some hormone-transporting proteins and inactivate enzymes, all of which can alter the tissue concentrations of hormones such as steroids, prostaglandins, thyroid and retinoids. Sequence analysis has revealed that dihydroflavonol - 4 - reductase share a common ancestor with human 3-β-hydroxy-steroid dehydrogenase. Other similar relationships have also been discovered.

Quercetin, morin(XXVII), rutin (XXVb), taxifolin (XXVIIIa), dihydrofisetin (XXIX), leucocyanidin (XXX), pelargonidin (XXXIa), apigenin (XXXIIa) catechin, hesperidin (XXXIIIa) have been reported to posess antiviral activity against 11 types of viruses. Ishitsuka and co-workers isolated 4',5-dihydroxy-3,3'7-trimethoxyflavone (XXXIV) from the Chinese medicinal herb \textit{Agastache folium} and detected antiviral activity against representatives of the picornavirus group. Naturally occurring 4'-hydroxy-3-methoxyflavones possessed antiviral activity against rhino – and poliomyelitis viruses. Comparison with synthetic derivatives indicated that high antiviral activity was associated with the 4'-hydroxyl and 3-methoxyl groups, a substituent in the 5-position and poly substituted A – ring.

Sanz et al., examined the influence of a series of natural flavonoids isolated from Indian medicinal plants for their effective on free radical generating systems and their oxidative effect. The flavonoids quercetin, kaempferol (XXXVI), catechin and taxifolin suppressed the cytotoxicity of O\(_2\) - and H\(_2\) O\(_2\) on Chinese hamster V79 cells, as assessed with a colony formation assay. Fuchs and Milbradt found subsequent intradermal application of apigenin-7-O-glucoside (XXXIIb) inhibited skin inflammation caused by xanthine oxidase.
and cumene hydroperoxide. Naringenin (XXVIIIc) was shown to have cytoprotective properties on mucosal injury induced in rats by ethanol\textsuperscript{71}.

Several flavonoid glycosides in orange were reported to have vasodilatory activity\textsuperscript{72}. Ning et al\textsuperscript{73}, reported that flavones administration markedly improved functional recovery in the reperfused rabbit heart after a bout of global ischemia. Two flavonoids, quercetin and silybin were reported to exert a protective effect by preventing the decrease in the xanthine dehydrogenase, oxidase ratio observed during ischemia reperfusion in the rat\textsuperscript{74}. The epidemiologic studies showed that dietary flavonoid intake was inversely associated with mortality from coronary heart disease\textsuperscript{75,76} and incidence of stroke\textsuperscript{77}. These results were supported by \textit{in vitro} studies, such as inhibition of LDL oxidation and platelet aggregation by flavonoids \textsuperscript{78,79}. Monforte et al., determined that hesperidin, an important citrus flavanone, increased HDL while it lowered LDL, plasma triglycerides and total lipids. Flavonoids may be protective against coronary artery disease by influencing several processes, such as 1) decrease in LDL oxidation, 2) increase in HDL levels, 3) reduction of cardiac mast cell mediator release and 4) decrease in cardiovascular inflammation\textsuperscript{80-84}. 

\[\text{equation}\]
(a) \( R = \text{OH}, \text{Dihydrofisetin (fustin)} \)
(b) \( R = \text{H}, \text{Garbanzol} \)

\[ \text{Leucocyanidin} \]
Flavonoids function as attractants as well as deterrents of insects. The presence of quercetin - 3 - glucoside is one of the factors which attract the silk worms to mulberry leaves\textsuperscript{85}. Quercetin-3-rhamnoside (XXVc) repels tobacco budworm\textsuperscript{86}. Such a protective action explains why the same flavonoid is absent in the flowers, but present in the leaves of the related family\textsuperscript{87}.

All flavonoids strongly absorb in the range 250-380 nm. Thus anthocyanin absorbs light and keeps the plant tissues warm in the cold regions\textsuperscript{88}. Metals like B and Al which can complex with quercetin but not with kaempferol derivatives have a role in the growth rate\textsuperscript{89}.

A number of reports relate the tendril coiling of the pea plant and the flavonoid content. It has been noticed that during the coiling process the concentration of the quercetin glycosides in pea tendril decreases by two thirds. The essential reaction in tendril coiling is associated with ATPase – ATP system. Hence, the glycosides may have some controlling action on this system\textsuperscript{90}.

The isolation of kaempferol – 3 – arabinoside from the chloroplasts of \textit{Impatiens balsamina}\textsuperscript{91} and quercetagenin from the chloroplasts of spinach\textsuperscript{92} suggests that flavonoids may play a role in the photosynthesis of plants, apart from UV screening function.

Flavonoids possess physiological effects in animals as well as in humans. The silkworm has discriminating receptor cell for flavonoids. Quercetin-3-rhamnoside is stimulating to silkworm feeding\textsuperscript{93}. The identification of flavonoid traces in the wings of butterflies\textsuperscript{94} and in the cocoons of the silkworm\textsuperscript{95} reveals their dietary habit in the larvae stage.
(a) $R_1, R_2, R_3, R_4, R_5 = H$, Apigenin
(b) $R_1, R_2, R_4, R_5 = H$, $R_3 = \text{glu}$, Apigenin-7-O-glucoside
(c) $R_1, R_2, R_4 = H$, $R_3, R_5 = \text{OMe}$, Apigenin-3, 5-dimethylether
(d) $R_1, R_4, R_5 = H$, $R_2 = \text{OMe}$, $R_3 = \text{glu}$, 6-Methoxyapigenin-7-O-glucoside
(e) $R_1, R_4, R_5 = H$, $R_2 = \text{OMe}$, $R_3 = \text{rha}$, 6-Methoxyapigenin-7-O-rhamnoside
(f) $R_1, R_4, R_5 = H$, $R_2 = \text{OMe}$, $R_3 = \text{rut}$, 6-Methoxyapigenin-7-O-rutinoside

(g) $R_1 = \text{OMe}$, $R_2, R_3, R_4, R_5 = H$, Thevitaflavone
(h) $R_1, R_2 = H$, $R_3 = \text{glu}$, $R_4 = \text{OMe}$, $R_5 = \text{Me}$, 8-Methoxyacetin-7-O-glucoside

(i) $R_1, R_3, R_5 = H$, $R_2, R_4 = \text{glu}$, Vicenin II
(j) $R_1, R_2, R_4, R_5 = H$, $R_3 = \text{Me}$, 4',5-Dihydroxy-7-methoxyflavone

(k) $R_1, R_2, R_4, R_5 = H$, $R_3 = \text{glucur}$, Apigenin-7-O-glucuronide

(a) $R_1 = \text{Rut}$, $R_2 = H$, $R_3 = \text{Me}$, Hesperidin
(b) $R_1 = \text{Me}$, $R_2$, $R_3 = H$, 3, 4', 5-Trihydroxy-7-methoxyflavonone
(c) $R_1, R_3 = H$, $R_2 = \text{Me}$, Homoeordictyol
(d) $R_1, R_2, R_3 = H$, Eriodictyol
(e) $R_1, R_2 = H$, $R_3 = \text{Me}$, Hesperitin
It has been reported that many flavonoids possess antibiotic effect on *Escherichia coli*, *Lactobacillus casei*, *Salmonella typhosa*, *S.enteritidis* and *Staphylococcus aureus* \(^{96}\). Chalcones and analogues especially with hydroxyl substituents have been reported to possess anti helmenthic properties \(^{97}\). The anti-viral effects of many types of flavonoids have been demonstrated. Herpes virus hominis could be inhibited in human cell lines (He La cells) by quercetin \(^{98}\). Anthocyanins show resistance to the tobacco mosaic virus in Nicotiana tabacum \(^{99}\).

Flavonoids also possess anti-cancer activity. Quercetin and its glycosides have been found to inhibit human brain tumour \(^{100}\). Isoquercitin (XXVc) and rutin were effective inhibitors of benzopyrene induced pulmonary adenoma in mice. It is believed that the flavonoids may induce in these cases, benzopyrene hydroxylase which may detoxify the carcinogen \(^{101}\).

Brain oedema, due to the increased permeability of blood – brain barrier in rats, develop due to a diet deficient in flavonoids. Treatment with flavonoids can overcome this \(^{102}\). Hesperidine (XXXIIIa), eriodictyol (XXXIIIb) rutin and quercetin have been found to decrease the capillary permeability \(^{103}\).

The prior administration of flavonoids can reduce significantly the dialation induced by UV or X-irradiation or the dialation induced by irritants like histamine, bradykinin and prostaglandin \(^{104}-^{108}\). It can be suggested that the flavonoids have a direct constrictor action on the capillary bed, thereby decreasing the permeability and fragility of the vessels \(^{109}\).

In general, ineffective doses, flavonoids do not produce any deleterious effects in humans or animals \(^{110}\). By acting on the aggregation of erythrocytes, flavonoids play an important role on the circulatory system also \(^{111}\). Histamine induced ulcers could be prevented by orange bioflavonoids in combination with vitamin-C \(^{112}\).

In Germany an extract of the plant *Crataegus monogyna* which contains a number of flavonoids have been used for curing cardiac ailments \(^{113}\). Isoflavone such as genistein possess oestrogenic activity. The molecular shape of genistein is similar to the animal oestrogen \(^{114}\).

Variation in structures, wide distribution in plants and rapid methods of characterization make flavonoids occupy an enviable position as reliable taxonomic markers \(^{115}\).
HPLC takes its place in the isolation of flavonoids from the plant material along with conventional methods like PC, TLC and CC, Desorption chemical ionization(DCI) and Fast Atom bombardment (FAB) with field desorption (FD) are the modern advancements in the mass spectroscopy which make the identification of the flavonoids are simple and accurate.

The site of glycosylation as also location of acyl / prenyl substituents etc., have become possible by employing these methods\textsuperscript{116}. Negative mode MS have also been adopted in recent years to characterize flavonoids particularly to distinguish between methylated flavones and the non-methylated ones\textsuperscript{117}.

Flavonoids are powerful antioxidant against free radicals and are described as free radical scavengers\textsuperscript{118}. High level of total phenolics and flavonoids in \textit{Halia bara} variety possesses antioxidant activities\textsuperscript{119}. It has been reported that flavonoids protect against gastric cancer. Similar to aspirin, acylated flavonoids may transfer their acyl group to the side chain hydroxyl group of serine in the active site of COX\textsuperscript{120}. 
XXXIV  4', 5-Dihydroxy-3,3',7-trimethoxyflavone

(a) \( R_1 = \text{Me}, \ R_2, R_3 = \text{OH}, \) Hispidulin
(b) \( R_1, R_2 = \text{Me}, \ R_3 = \text{H}, \) Cirsimaritin
(c) \( R_1, R_3 = \text{Me}, \ R_2 = \text{H}, \) Pectolinaringenin
(d) \( R_1 = \text{apio-gal}, \ R_2 = \text{H}, \ R_3 = \text{Me}, \) 4'-O-Methylscutellarein-6-O-apiosylgalactoside

XXXV

(a) \( R_1, R_2, R_3, R_4, R_5 = \text{H}, \) Kaempferol
(b) \( R_1, R_2, R_4, R_5 = \text{H}, R_3 = \text{OH}, \) 6-Hydroxykaempferol
(c) \( R_1, R_4, R_5 = \text{H}, R_2 = \text{Me}, R_3 = \text{OH}, \) Vogeletin
(d) \( R_1, R_4, R_5 = \text{H}, R_2 = \text{Me}, R_3 = \text{ara-rha}, \) Vogelin
(e) \( R_2, R_3, R_4, R_5 = \text{H}, R_1 = \text{glu}, \) Astragalin
(f) \( R_2, R_3, R_4, R_5 = \text{H}, R_1 = \text{glucur}, \) Kaempferol-3-O-glucuronide
(g) \( R_2, R_3, R_5 = \text{H}, R_1, R_4 = \text{rha}, \) Kaempferitrin
(h) \( R_2, R_3, R_4, R_5 = \text{H}, R_1 = \text{gal}, \) Trifolin
Flavonoid glycosides of *Ocimum basilicum*¹²¹ have been found to decrease ulcer index and thus inhibit gastric acids in aspirin–induced ulcers. Flavones are antithrombotic¹²² due to their ability to scavenge free radicals and are also used in the treatment of rheumatic arthritis¹²³,¹²⁴. Quercetin prevents immune cells¹²⁵ and inhibits both the production and release of histamine and is useful in allergic conditions like asthma, hayfever, etc¹²⁶. It has been reviewed that dietary flavonoids possess multiple neuroprotective action in central nervous pathophysiological conditions including depression. Naringenin has been found to have antidepressive like property via, central seotonergic and nonadrenergic system¹²⁷.

Soy is a rich source of non-steroidal estrogens of the isoflavone class¹²⁸. These compounds, which are structurally similar to estrogens, bind to the estrogen receptor and behave as partial estrogen antagonists¹²⁹. High intake of isoflavone rich soybean products is assumed to protect against cancer, especially estrogen-related cancers, such as breast, endometrial, ovarian, prostatic and colon cancer¹³⁰-¹³⁴.

The negative correlation of tumour formation with total dietary isoflavone concentration, and in particular with the dietary intake of genistein and the urinary isoflavone excretion has been well established¹³⁵,¹³⁶. Isoflavones have shown antioxidant activity and can inhibit the oxidative modification of isolated LDL¹³⁷,¹³⁸. The administration of two major isoflavones¹³⁹ daidzin (XXXVIIa) and daidzein (XXXVIIb), present in *Pueraria lobata* extracts have been found to reduce ethanol intake in Syrian Golden hamsters.
XXXIX
(a) $R_1 = R_2 = H$, 2,3,2",3"-Tetrahydroamontoilavone
(b) $R_1 = R_2 = Me$, 7,7"-Di-O-methyltetrahydroamontoilavone

XL
(a) $R_1 = R_2 = R_3 = H$, Myricetin
(b) $R_1 = H$, $R_2, R_3 = Me$, Syringetin
(c) $R_1$ glucur, $R_2, R_3 = H$, Myricetin-3-O-glucuronide

XLI Trilobatin
(a) $R_1, R_4 = H$, $R_2, R_3 = Me$, Velutin
(b) $R_1, R_2, R_5, R_6 = H$, Luteolin
(c) $R_1, R_3, R_4 = H$, $R_2 = glu$, Luteolin-7-O-glucoside (glucoluteolin)
(d) $R_1, R_2, R_4 = Me$, $R_3 = H$, Trimethyl-luteolin
(e) $R_1, R_3 = H$, $R_2 = glu$, $R_4 = O$-ang, 5,3'-Dihydroxy-4'-O-angeloxy-flavone-7-O-glucoside
(f) $R_1, R_3, R_4 = H$, $R_2 = glucur$, luteolin-7-O-glucuronide
(g) $R_1, R_3 = H$, $R_2 = (2",6"$-di-rha)-glu, $R_4 = Me$, Diosmetin-7-O-(2",6"$-$dirhamnosyl)-glucoside
(h) $R_1, R_3 = H$, $R_2 = rut$, $R_4 = Me$, Diosmin

XLIII Sulfuretin

XLIV
(a) $R_1, R_2, R_4, R_5 = H$, $R_3 = OH$, Fisetin
(b) $R_1, R_3, R_4, R_5 = H$, 3,7,4'-Trihydroxy-flavone
(c) $R_1, R_2, R_4 = H$, $R_3, R_5 = OH$, Robinetin
(d) $R_1, R_2, R_5 = H$, $R_3 = OH$, $R_4 = Me$, Fisetin-3'-methylether
A rapid and detailed survey of the distribution of flavonoids have become possible, mainly because of the presently available techniques such as PC, TLC, GLC, HPLC and refined physical tools like UV, IR, NMR and GC-MS.

Bignoniaceae is a predominately tropical family of trees and lianas with ca. 107 genera and ca. 900 species. Harborne investigated the flavonoid patterns in the Bignoniaceae and the Gesneriaceae, reported the common anthocyanin, cyaniding-3-rutinoside in Campsis radicans and Tecoma garrocha petals. Subramanian et al. examined the flavonoids of eight Bignoniaceous species comprising Bignonia gracilis and B. megapotamica Spreng which were found to contain quercetin-3-galactoside, investigation of other flavonoids in leaves and petals of bignonias showed that most species contained flavones rather than flavonols.

Luteolin is probably a common consistent of the Bignoniaceae. Its provisional identification in the leaf of Campsis radicans has been confirmed by the author and it has been found as the 7-glucoside in Catalpa bignonoides; an acylated derivative of luteolin-7-O-glucoside (XLIc) occurs in the latter plant.

The nectar structure and chemical nectar composition of 15 species of Bignoniaceae were analysed by Gralet. From the leaves of Mayodendron igneum, apigenin-7-O-glucoside, 6-methoxyapigenin-7-O-glucoside (XXXIId), 6-methoxyapigenin-7-O-rhamnoside(XXXIe),6-hydroxyapigenin-7-O-rutinoside (XXXIf) and genistein-5,4’-dimethylether (XXIV) have been isolated.

A new pigment 5,6,7,3”4”-pentahydroxyflavone (6-hydroxyluteolin) (LIVa) was identified in fresh leaves of Tecoma australis. Flavonoids isolated from Chaerophyllum hirsutum showed anti-oxidant activity when tested using the reaction with the stable free radical DPPH. The occurrence of thevetia flavone (XXXIIg) and carajuflavone (LIVb) has been identified in the leaves of Arraviddaea chicaf. Cuprea.

The wax of A.brachypoda has been found to contain cirsiliol (LIVc), cirsimaritin (XXXVb), hispidulin and 3’,4’-dihydroxy-5,6,7-trimethoxy flavone (LIVe). Pectolinarignenin (XXXVc) and related flavonoid have been reported from the leaves of Millingtonia hortensis. Cirsimartin and cirsileneol (LIVd) have been isolated from Tecoma undulata.
The flavones trimethyl-luteolin (XLIId) and 6-hydroxy-5,7-dimethoxy flavone (LVa) and a flavanone, 5-hydroxy-6,7-dimethoxy flavanone (LVla) have been reported from dichloromethane extract of Zeyheria montana\(^{154}\). The occurrence of 8-methoxyacacetin-7-O-glucoside (XXXIIh), 6-methoxyapigenin-7-0-glucoside, 4'-O-methyl scutellarein-6-O-apisoyl galactoside (XXXVd), acacetin-7-O-glucosyl-8-C-rhamnosyl-3-O-arabinoside (LVIIa), 4',6-dimethoxykaempferol -7-O-8-C-diglucoside (LVIIIb) and vicenin II (XXXIIIi) have been reported in ethyl acetate extract of aerial parts of Macfadyena unguis-cati L.\(^{155}\) and from the ethanol extract, 6-methoxy aceacetin – 7 – O – glucoside and quercitrin have been identified.

The plant Oroxyllum indicum contains flavonoids like chrysin (LVB), baicalein (LVC)\(^{156-158}\), oroxylin A (LVD)\(^{159}\), kaempferol\(^{160}\), 2’ 5 – dihydroxy– 6, 7–dimethoxyflavone (LVIIIa). 5-hydroxy-8-methoxy-7β-D-glucuronyl flavone (LVIIIb)\(^{161}\) and 4’, 5 – dihydroxy – 7 – methoxyflavone (XXXIIj)\(^{162}\).

Proteaceae is one of the popular families. It contains 80 genera and 1,600 species\(^{163}\) the Proteace family contains flavonol such as Kaempferol, kaempferol and quercetin or quercetin and myricetin. Quercetin 3– O– rutinoside has been found in the leaves of Protea concinnum\(^{164}\).

A flavonoid was isolated from Radal lomatia hirusta and its biological study was elaborately conducted\(^{165}\). The flowers, fruits of Grevillea robusta showed a weak inhibitory activity against HIV – I PR was identified\(^{166}\).

The family Anacardiaceae includes 75 genera and 800 species\(^{167}\). The compounds of the family are of chemical interest and they hold great promise in the search of new medicinal and commercial agents\(^{168}\). Chromatographic separation of aq.MeOH extract of the leaves of Schinus molle L.\(^{169}\) has yielded two new acylated quercetin glycosides, isoquercitrin(XXVc) and 2”O-α-L-rhamno pyranosyl, hyperin-6”-O-gallate (XXVf) together with 2”-O-L-rhamno pyranosyl, hyperin (XXVg), quercetin–3-O-β-D-galacturopyranoside, isoquercitrin (XXVc), hyperin (XXVe) isoquercitrin-6”-O-gallate (XXVd), hyperin -6”-O-gallate (XXVf).

From the fruits of S. molle, engeletin (XXVIIe), quercetin-3-rhamnoside and two biflavones, agathisflavone (XXXVIII) and 2,3,2”3”– tetra hydro amento flavone (XXXIa) have been isolated\(^{170}\).
The leaves of *Pistacia lentiscus* L. have found to contain myricetin (XLa) and quercetin glycosides, delphinidin-3-O-glucoside and cyanidin-3-O-glucoside\textsuperscript{171}. From *Mosquitoxylon jamaicense* Krug & Urb., trilobatin (XII) and quercetin-3-O-β-D-galactoside have been isolated\textsuperscript{172}. The isolation of persicogenin (XXVIIg) and homoeriodictyol (XXXIIIc) from *Rhus retinorrhoea*\textsuperscript{173} is reported.

From the bark of *R. verniciflua*\textsuperscript{174}, butein (LIIa) has been isolated. The heart wood and branches of the plant has been found to contain the compounds, garbanzol (XXIXb), sulfuretin (XLI), fisetin (XLIVa) and fustin with antimutagenic activity\textsuperscript{175,176}. Apigenin-dimethylether (XXXIIc) has been isolated from the roots of *R. undulata*\textsuperscript{177}.
XLVII
Volkensiflavone

XLVIII
Succedaneaflavone
(a) $R_1, R_3, R_5 = \text{Me}, R_2, R_4 = \text{H}$, Oxyayanin B
(b) $R_1, R_2, R_3, R_4, R_5 = \text{H}$, Quercetagetin
(c) $R_1, R_4, R_5 = \text{H}, R_2 = \text{Me}, R_3 = \text{glu}$, Patulitrin
(d) $R_1, R_5 = \text{H}, R_2, R_3, R_4 = \text{Me}$, Chrysospleninet
(a) $R_1, R_3, R_5 = \text{Me}, R_2, R_4 = \text{H}$, Oxyayanin B
(b) $R_1, R_2, R_3, R_4, R_5 = \text{H}$, Quercetagetin
(c) $R_1, R_4, R_5 = \text{H}, R_2 = \text{Me}, R_3 = \text{glu}$, Patulitrin
(d) $R_1, R_5 = \text{H}, R_2, R_3, R_4 = \text{Me}$, Chrysosplenetin
(a) \( R_1 = H, R_2 = \text{OH}, 3', 4', 7\)-Trihydroxyflavone
(b) \( R_1 = \text{OH}, R_2 = H, 3, 7, 4'\)-Trihydroxyflavone

LII Melacacidin

(a) \( R_1, R_2 = H, R_3 = \text{OH}, \text{Butein} \)
(b) \( R_1, R_2, R_3 = H, \text{Isoliquiritigenin} \)
(c) \( R_1 = H, R_2, R_3 = \text{OH}, \text{Okanin} \)
(d) \( R_1, R_2, R_3 = \text{OH}, \text{Neoplatyrrhenin} \)
(a) $R_1, R_3, R_4 = H, R_2 = OH$, 6-Hydroxyuteolin
(b) $R_1 = Me, R_2 = OH, R_3, R_4 = H$, Carajuflavone
(c) $R_1, R_4 = H, R_2 = OMe, R_3 = Me$, Cirsiliol
(d) $R_1 = H, R_2 = OMe, R_3, R_4 = Me$, Cirsileneol
(e) $R_1, R_3 = Me, R_2 = OMe, R_4 = H$, 3',4-Dihydroxy-5,6,7-trimethoxyflavone
(f) $R_1, R_3, R_4 = H, R_2 = C-glu$, Iso-orientin

(a) $R_1, R_3 = OH, R_2 = OH$, 6-Hydroxy-5,7-dimethoxyflavone
(b) $R_1, R_3 = OH, R_2 = H$, Chrysin
(c) $R_1, R_2, R_3 = H$, Baicalein
(d) $R_1, R_3 = OH, R_2 = OMe$, Oroxylin A

(a) $R_1 = OMe, R_2, R_3 = H$, 5-Hydroxy-6,7-dimethoxyflavanone
(b) $R_1 = H, R_2 = Me, R_3 = OH$, 5,4'-dihydroxy-8-C-methyl-7-methoxyflavanone
The leaves of *R. retinorrhoea*\(^{178}\) yielded 7-O methylnaringenin (XXVIIIb), eriodictyol and a biflavone(2S-2S), 7,7"-di-O-methyl tetra hydro amento flavone (XXXIXb). Fruits of *R. succedanea*\(^{179}\) have been recorded to contain biflavones, Viz., robustaflavone (XLV), amentoflavone (XLVI), agathisflavone, volkensiflavone (XLVII), succedaneaflavone (XLVIII) and rhusflavanone (XLIX).

The compositae (Asteraceae) give rise to a phenomenal array of flavonoids. It comprises 1,100 genera and 25,000 species\(^{180}\). The flavonols quercetin and kaempferol and common especially, the polymethylated type including compounds with 6– and 8-hydroxyl / methoxyl substitution. Similar pattern has been observed in flavones. Carbon – carbon linked glycosides are rare. Compositae is the only family to experiment, extensively with anthochlors.

In this respect compositae have concentrated ‘exploitation’ of flavonoids (many with biological activity), sesquiterpene lactones (also with biological activity), and polyacetylenes and in a few cases of alkaloids.

The earlier statement of Gornall et al.,\(^{181}\) that myricetin is almost absent from the compositae is no longer valid 5,7,4′ – OH – 6 – O – Me – hispidulin, dinatin were reported from the leaves of *Gaillardia spp.*, *Helenium spp.*, *HymenSoxis spp.*, *Plummera ambiguens*, *P. floribunda* and *Ratibida columnera*\(^{182}\). Myricetin and several methylated derivatives have been isolated in *Haploappus*\(^{183}\).

Convolvulaceae is a family of about 60 genera and more than 1,650 species of mostly herbaceous vines, but also trees, shrubs and herbs. It is commonly known as the bind weed or morning glory family\(^{184}\). Convolvulaceae can be recognized by their funnel-shaped, radially symmetrical corolla. The floral formula for the family has five sepals, five fused petals, five epipetalous stamens, and a two-part syncarpous and superior gynoecium. The stems of these plants are usually winding, hence their Latin name (from *convolvere*, "to wind"). The leaves are simple and alternate, without stipules. The fruit can be a capsule, berry, or nut, all containing only two seeds per one locule.

The leaves and starchy, tuberous roots of some species are used as foodstuffs e.g. sweet potato and water spinach, and the seeds are exploited for their medicinal value as purgatives. Some species contain ergoline alkaloids that are likely responsible for the use of these species
as ingredients in psychedelic drugs e.g. ololiuhqui. Members of the family are well known as showy garden plants (e.g. morning glory) and as troublesome weeds (e.g. bindweed).\textsuperscript{185}

In the present work, a few plants belonging to the Proteaceae, Bignoniaceae, Anacardiaceae, Convolvulaceae and Asteraceae have been examined with a view to isolate their flavonoids and investigate the pharmacological activity.
**Grevillea Robusta. A. Cunn. Exr. BR**

**INTRODUCTION**

*Grevillea robusta* A. Cunn. exr. Br commonly known as savuku\(^{186}\) in Tamil belongs to Proteaceae family. It is an Australian silk oak, commonly planted as ornamental in many warm–temperate and semi tropical climates. It is distributed in the tropical highlands of India\(^ {187}\). It has been planted extensively as shade for tea and coffee\(^{188-191}\). It is frequently used as a wind break although opinions differ as to its wind firmness and branch – shedding tendencies \(^ {192}\). The specimen was confirmed with reference to herbarium sheets available in Botanical Survey of India, Coimbatore. The accession number is being MH 45032.

Silk oak is an important honey tree in India where it is also regarded as a good fuel wood producer\(^ {193}\). The tree produces an attractively figured easily worked wood, which was once a leading face veneer in world trade, where it was marketed as “lace wood”. The wood contains an allergen that causes dermatitis for many people\(^ {194}\).

With a view to locate additional flavonoids, the flowers of *G. robusta* have been investigated and the results were presented here under.
EXPERIMENTAL

EXTRACTION AND FRACTIONATION

The flowers of G. robusta collected from the slopes of hills of Velagiri, Dharmapuri district, during May, were refluxed with MeOH (4x500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate successively fractionated with benzene (3x250 ml), peroxide–free Et₂O (3x250 ml) and EtOAc (4x250 ml). No isolable material obtained from the C₆H₆ and Et₂O fraction.

EtOAc fraction: (Kaempferol-5-O-β-glucoside):

The residue from EtOAc fraction afforded yellow crystals (on recrystallisation from MeOH) (m.p.184-86°C). It was soluble in acetone but insoluble in water, Et₂O and CHCl₃. It developed a red colour with Mg-HCl, brown colour with alc. Fe³⁺ developed a yellow colour when viewed under UV light with and without NH₃. It answered the Horhammer-Hansel¹⁹⁵ Gibb’s and Molisch’s¹⁹⁶ test but did not respond to Wilson’s boric acid¹⁹⁷ test. It had λ<sub>max</sub> nm 257, 271sh, 298sh, 306 sh, 371sh; +NaoMe 290sh, 340, 436; +AlCl₃ (with or without HCl) 272 sh, 316, 362; +NaOAc 274, 330, 398 and (+NaOAc-H₃BO₃) 258, 302 sh, 330, 384. It had Rf values as depicted in Table I-1. The ¹H-NMR of the glycoside were appended in Fig I-1. It is identified as kampferol on the basis of colour reactions, direct comparison and mixed – PC with an authentic sample of kaempferol as described under Maytenus emarginata¹⁹⁸.

HYDROLYSIS OF THE GLYCOSIDE:

The glycoside (0.05g, 0.2 mmole) was dissolved in hot aq.MeOH (2ml, 5%) and an equal volume of H₂SO₄ (7%) was added to it. The reaction mixture was refluxed under 100°C for 2 h, the excess of alcohol was distilled off from the hydrolysate and the resulting aq. solution was distilled with more water and left under chilled conditions for 2h.

IDENTIFICATION OF THE AGLYCON: (Flavonol-Kaempferol):

The residue from ether fraction of the hydrolysate was taken up in acetone and left under chilled conditions for few days.
Yellow colour solid was obtained and its colour reactions, chromatographic behavior and UV spectral data were found to be similar to those described under *Ipomoea aquatica*.

**IDENTIFICATION OF SUGAR: (Glucose):**

The aq. hydrolysate after the removal of the aglycone was cautiously neutralised with BaCO₃ and filtered. The concentrated filtrate on PC gave Rf values corresponding to those of glucose. The running properties of the glycoside was in favour of a monoside. The identity of the sugar was confirmed with an authentic sample of glucose.
**TABLE I-1**

Rf (x100) values of constituents of the flowers of *G. robusta*

(Whatmann No.1, Ascending, 30+ 2°C) DEVELOPMENT

SOLVENTS

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucoside from EtoAc fraction</td>
<td>10</td>
<td>23</td>
<td>34</td>
<td>52</td>
<td>58</td>
<td>26</td>
<td>45</td>
<td>70</td>
</tr>
<tr>
<td>Kampeferol 5-O-β-Glucoside</td>
<td>10</td>
<td>23</td>
<td>34</td>
<td>52</td>
<td>58</td>
<td>26</td>
<td>45</td>
<td>70</td>
</tr>
</tbody>
</table>
**Solvent Key:**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>H₂O</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>5% aq.HOAc</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>15% aq.HOAc</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>30% aq.HOAc</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>60% aq.HOAc</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>(b-BuOH, Acetic acid, water, BAW 4:1:5 Upper phase)</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>phenol saturated with water.</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>t-BuOH, HOAc : H₂O = 3:1:1</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>forestol HOAc ; Conc.HCl : H₂O = 30:3:10</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>n – BuOH ; 27% HOAc = 1:1.</td>
<td></td>
</tr>
</tbody>
</table>
FIG 1–1 ¹H NMR SPECTRUM OF THE GLYCOSIDE FROM EtOAc FRACTION OF G. ROBUSTA
FIG 1–2 $^{13}$C NMR SPECTRUM OF THE GLYCOSIDE FROM EtOAc FRACTION OF G. ROBUSTA
### TABLE 1-2

**$^{13}$C- NMR DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM **

**G. ROBUSTA**

<table>
<thead>
<tr>
<th>Compound</th>
<th>C₁'</th>
<th>C₂'</th>
<th>C₃'</th>
<th>C₄'</th>
<th>C₅'</th>
<th>C₆'</th>
<th>C₇'</th>
<th>C₈'</th>
<th>C₉'</th>
<th>C₁₀'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside from EtOAc fraction</td>
<td>121.5</td>
<td>128.2</td>
<td>115.8</td>
<td>159.8</td>
<td>114.6</td>
<td>125.5</td>
<td>103.0</td>
<td>73.7</td>
<td>76.4</td>
<td>70.1</td>
</tr>
<tr>
<td>Kaempferol 5-O-β Glucoside (from literature)</td>
<td>121.7</td>
<td>129.5</td>
<td>115.4</td>
<td>159.2</td>
<td>115.4</td>
<td>129.5</td>
<td>103.3</td>
<td>74.3</td>
<td>76.0</td>
<td>70.2</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The flowers of *G. robusta* have been found to contain Kaempferol-5-O-β-glucoside.

The UV spectrum of the aglycone exhibited two major peaks at 257 nm and 372 nm reveals a flavonol skeleton. A bathochromic shift of 64 nm (Band-I) noticed in its NaOMe spectrum indicated that the presence of a free OH at C-4’. The AlCl₃ spectra (with or without HCl) showed only two peaks and a shoulder, indicating the absence of free-OH at C-5. The presence of a free –OH at C-7 was evident from the bathochromic shift of 17 nm (band II) on the addition of NaOAc. The AlCl₃ spectrum was exactly the same as that of (AlCl₃–HCl) revealing the absence of catechol type of substitution in B-ring.

The most significant clue for 5-glycosylation in the glycoside was its rapid rate of hydrolysis (hydrolysis was completed within 5 minutes). Another characteristic feature was its ability to adsorb strongly on to cellulose and it proved very difficult to elute it from chromatography.

In the ¹H-NMR spectrum (300 MHz, DMSO₆, TMS) of the glucoside obtained from EtOAc fraction, the protons at C-6 and C-8 appear at δ 6.3 and 6.5 ppm respectively. The C-2’ and C-6’ protons appear as a doublet at δ 8.2 ppm and the C-3’ and C-5’ protons at δ 6.9 ppm. The ¹H-NMR of the glucose moiety appears at δ 5.4 ppm. The remaining glucosyl protons appear in the range δ 3.1 to 3.5 ppm.

Supporting evidence for the structure of the glycoside was provided by ¹³C-NMR (67.89 MHz, DMSO₆, TMS) data and the complete assignment of signals to various carbons is as given in TableI-2. As a result of glycosylation, the 5-OH, 4-keto hydrogen bond is broken and this has a profound effect on the electron density distribution in the molecule. The signal of C-4 appears at δ 166.7 ppm instead of at δ 175.8 ppm as in the case of the corresponding aglycone.
The signals of C-6 and C-8 appear downfield at δ 103.2 and 96.7 ppm respectively due to glycosylation at C-5. It also cause an upfield effect on the corresponding meta carbon i.e, C-7.

On this basis the identity of the pigment obtained from EtOAc fraction can be confirmed as Kaempferol 5-O-β-glucoside.
JIACARANDA MIMOSIFOLIA D. DON

INTRODUCTION

Jacaranda mimosifolia D. DON of Bignoniaceae is cultivated in the Indian gardens and also found in Brazil, Bolivia and Argentina. The specimen was confirmed with reference to herbarium sheets available in Botanical Survey of India, Coimbatore. The accession number is being MH 74293.

The Jacarandas are impressive trees when covered with cluster of blue tubular flowers. The flowers are used as a substitute for the Unani herb Gul-e-Gaozabaan in Pakistan. The bark of J. mimosifolia has been used in the treatment of wounds and dermatitis. Astringent and diuretic properties have also been assigned to the bark extracts. The plant has been attributed with properties to treat syphilis and disease related to urinary tract problems. The ground bark is used as a decoction against venereal diseases or as ethanolic maceration along with a small amount of Cordia alliodora against rheumatism and sciatica. The plant possesses antioxidant, anti hypertensive, antimicrobial and antitumour activities.

The leaves of J. mimosifolia found to contain jacaranone, verbacoside and the flavonoids scutellarin-7-O-glucosylmethylesters, apigenine-7-O-glucosyl-methylester, luteolin-7-O-glucoside and isovitexin. The flower extract of J. mimosifolia has been found to be potent indicator in all types of acid base titrations, and the activity is attributed to the flavonoids and anthocyanins present in it. With a view to locate additional flavonoids, the fresh flowers of J. mimosifolia have been investigated and the results are presented hereunder.
EXPERIMENTAL

EXTRACTION AND FRACTIONATION:

Fresh flowers of *J. mimosifolia* collected from Kolli hills of Namakal district during March were extracted with 80% MeOH (4x500 ml) under reflux. The alc. extract was concentrated *in-vacuo* and the aq. concentrate was successively fractionated with benzene (3x250 ml), peroxide free Et$_2$O and EtOAc (4x250 ml). The benzene and Et$_2$O fractions did not yield any isolable material.

**EtoAC fraction: (Flavonol glycoside: Isoquercitrin)**

The EtOAc fraction was concentrated *in-vacuo* and left in an ice chest for 2 days. A yellow solid that separated was filtered and studied. It was recrystallized from MeOH when it afforded yellow crystals, m.p. 229-30$^\circ$C (yield 0.1%). It was freely soluble in EtOAc and MeOH and sparingly soluble in water. It gave an olive green colour with alc. FeCl$_3$, deep pink colour with Mg-HCl, yellow colour with NaOH and appeared deep purple under UV that turned yellow on exposure to NH$_3$. It did not answered the Horhammer-Hansel test but responded to Wilson’s boric acid, Gibb’s and Molisch’s test. The pigment had Rf values as indicated in TableI-II-1and had $\lambda_{max}$ nm 257, 269sh, 299sh, 362; (+NaOMe) 272, 327, 409sh; (+AlCl$_3$) 275, 303sh, 333, 430; (+AlCl$_3$/HCl) 274, 303sh, 353, 401; (+NaOAC) 271, 320sh, 372; (+NaOAC/H$_2$BO$_3$) 265, 300 sh, 372. The $^1$H- and $^{13}$C-NMR of the glycoside were appended (Figs II-1 and II-2). The identity of the glycoside was confirmed by direct comparison with an authentic sample of the same from the seeds of *Bauhunia acuminata*.²⁰⁹

**Hydrolysis of the glycoside:(Flavonol:quercetin)**

To a solution of the glycoside (0.1g, 0.2 m mole) in hot aq. MeOH (10 ml, 50%) and an equal volume of H$_2$SO$_4$ (10%) was added and the mixture was refluxed at 100$^\circ$C for 2 h and the hydrolytic products were identified as described below.

**Identification of the aglycone:**

The Et$_2$O fraction from the hydrolysate was concentrated *in vacuo* and left in an ice chest for about a week. A yellow solid that separated was filtered and studied. It
came out as pale yellow needles m.p.316-18°C on recrystallisation from MeOH. It was soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, olive green with NH₃ and NaOH, yellow solution with a pale green fluorescence with conc. H₂SO₄ and appeared yellow under UV and UV/NH₃. It answered Wilson’s boric acid, Horhammer-Hansel and Gibb’s tests but did not respond to Molisch’s test. It had λ<sub>max</sub><sub>MeOH</sub> nm 255, 269 sh, 301 sh, 370; (+NaOMe) 247 sh, 321 (dec); (+AlCl₃) 272, 304 sh, 333, 458; (+AlCl₃/HCl) 265, 301 sh, 359, 428; (+NaOAc) 257sh, 274, 329, 390; (+NaOAc/H₃BO₃) 262, 304 sh, 388 and had Rf values as depicted in Table II-1. The <sup>1</sup>H and <sup>13</sup>C-NMR of the flavonol were appended. (Figs.II-1 and II-2). It was identified as quercetin and the same was confirmed by co-PC and mixed-PC and m.m.p with an authentic sample of quercetin from <i>Physalis minima</i>²¹⁰.

**Identification of the sugar:(glucose)**

The aq. solution from the above hydrolysate was neutralized with BaCO₃ and filtered. The concentrated filtrate on chromatographic examination (PC) gave Rf values corresponding to those of glucose. The running properties of the glycoside were in favour of a monoside. The identity of the sugar was also confirmed by direct comparison with an authentic sample of glucose.
TABLE II-1

Rf (X 100 ) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF

*J. MIMOSIFOLIA*

(Whatman No. 1, Ascending, 30±2°C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Developing Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Glycoside from EtOAc fraction</td>
<td>3</td>
</tr>
<tr>
<td>Isoquercitrin (Authentic)</td>
<td>3</td>
</tr>
</tbody>
</table>
FIG II – $^1$H – NMR SPECTRUM OF THE GLYCOSIDE FROM EtOAc FRACTION OF J. MIMOSIFOLIA
FIG II - 2 $^{13}$C– NMR SPECTRUM OF THE GLYCOSIDE FROM EtOAc FRACTION OF *J. MIMOSIFOLIA*
### TABLE II-2

**13 C-NMR SPECTRAL DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM THE FLOWERS OF J. MIMOSIFOLIA**

<table>
<thead>
<tr>
<th>Compound</th>
<th>C_2</th>
<th>C_3</th>
<th>C_4</th>
<th>C_5</th>
<th>C_6</th>
<th>C_7</th>
<th>C_8</th>
<th>C_9</th>
<th>C_10</th>
<th>C_1\textsuperscript{\textprime}</th>
<th>C_2\textsuperscript{\textprime}</th>
<th>C_3\textsuperscript{\textprime}</th>
<th>C_4\textsuperscript{\textprime}</th>
<th>C_5\textsuperscript{\textprime}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside (δ ppm)</td>
<td>156.385</td>
<td>133.383</td>
<td>177.518</td>
<td>161.303</td>
<td>98.724</td>
<td>164.164</td>
<td>93.568</td>
<td>156.385</td>
<td>104.055</td>
<td>121.666</td>
<td>115.274</td>
<td>144.868</td>
<td>148.519</td>
<td>116.281</td>
</tr>
<tr>
<td>Isoquerctrin (from literature) (δ ppm)</td>
<td>156.5</td>
<td>133.7</td>
<td>177.6</td>
<td>161.3</td>
<td>98.8</td>
<td>164.2</td>
<td>93.6</td>
<td>156.5</td>
<td>104.2</td>
<td>121.4</td>
<td>115.3</td>
<td>144.8</td>
<td>148.5</td>
<td>116.5</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>C_1\textsuperscript{\textprime}</th>
<th>C_2\textsuperscript{\textprime}</th>
<th>C_3\textsuperscript{\textprime}</th>
<th>C_4\textsuperscript{\textprime}</th>
<th>C_5\textsuperscript{\textprime}</th>
<th>C_6\textsuperscript{\textprime}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside (δ ppm)</td>
<td>100.920</td>
<td>74.154</td>
<td>76.555</td>
<td>69.984</td>
<td>77.620</td>
<td>61.031</td>
</tr>
<tr>
<td>Isoquerctrin (from literature) (δ ppm)</td>
<td>101.4</td>
<td>74.3</td>
<td>76.8</td>
<td>70.3</td>
<td>77.5</td>
<td>61.3</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The fresh flowers of *J. mimosifolia* have been found to contain isoquercitrin. The UV spectrum of the glycoside showed two major absorption peaks at 362 nm (band-I) and 257 nm (band-II) showing a flavonol skeleton. A bathochromic shift of 47 nm in band –I observed in its NaOMe spectrum indicated the presence of a free 4′-OH group. The AlCl₃–HCl spectra of the glycoside as well as its aglycone showed 3 absorption peaks and a shoulder indicating a free 5-OH group in both. The glycoside as well as its aglycone did not exhibit any intense UV fluorescence, ascertaining the presence of a free hydroxyl group at C-5 in both. A bathochromic shift of 39 nm and 58 nm respectively in AlCl₃–HCl spectra was yet another evidence for the same. The presence of an ortho di hydroxyl group in the B-ring could be inferred from a shift of +10 nm noticed in the glycoside and +18 nm noticed in case of the aglycone on the addition of H₃BO₃. In the AlCl₃ spectrum, an absorption peak was noticed at 430 nm (band-I) which on addition of HCl reduced by 29 nm. This was another evidence for the presence of a catechol type di-OH group in the B-ring. In the ¹H-NMR spectrum (400 MHz, DMSO-d₆, TMS) of the glycoside, the protons at C-6 and C-8 appear at δ 6.18 and 6.42 ppm respectively. The C-5′ proton appears as a doublet at δ 6.81 ppm. The 5-OH proton resonates at δ 12.64 ppm as distinct singlet. The OH protons at C-7, C-3′ and C-4′ show up to δ 9.7, 9.45 and 9.22 ppm respectively. The H-1″ signal of the flavonol-3-O-glucoside was found at δ 5.45 ppm. The remaining glycosyl protons appear in the range δ 3.4 to 3.8 ppm.

Supporting evidence for the structure of the glycoside was provided by the analysis of ¹³C-NMR (400 MHz, DMSO-d₆, TMS) data and a complete assignment was given (TableII-2). Due to glycosylation at 3-position, C-2 and C-4 carbons absorb at δ 156.3 and 177.2 ppm respectively. C-1″, absorbs at δ 100.9 ppm. The rest of the carbons of the sugar unit appear between δ 69.9 ppm and 77.6 ppm.

Based on this, the glycoside has been characterized as isoquercitrin (quercetin-3-O-glucoside).
ANACARDIUM OCCIDENTALE LINN.,

INTRODUCTION

Anacardium occidentale Linn., popularly known as Mundiri, in Tamil, is a small spreading, ever green tree sometimes reaching a height of 12m, native to tropical America, and naturalized in the warm parts of India especially near the sea. The specimen was confirmed with reference to herbarium sheets available in Botanical Survey of India, Coimbatore. The accession number is being MH 5248.

The fruit is a sub acid and astringent. The pericarp of the nut contains a black acrid oil, known as cardole, which is a powerful vesicating agent. It requires, however, to be cautiously used. It is applied to warts, corns and ulcers, but it is said that the vapour of the oil when roasting is apt to produce swelling inflammation.

The acrid oil stated above as cardole is often applied to floors or wooden rafters of house to prevent the attack of white ants and most effectively keeps them away. A transparent gum is obtained from the trunk of the tree, useful as a good varnish, and making a fair substitute for gum- Arabic. It should be collected while sap is raising. It is particularly useful when the depredations of insects require to be guarded against. For this purpose it is used in South America by the book binders, who wash their books with a solution of it in order to keep away moths and ants.

EXPERIMENTAL

EXTRACTION AND FRACTIONATION

Fresh flowers of A. occidentale collected from in and around Jayamkondam, Perambular district, during May were extracted with 85% MeOH (5x500ml) under reflux. The alc. extract was concentrated in-vacuo and the aq. concentrate was successively fractionated with petroleum ether (60-80°C) (4x250ml) peroxide-free Et$_2$O (3x250 ml) and EtOAc (4x250 ml). The petrol fraction did not yield any crystalline solid and could not be studied further.

Et$_2$O Fraction: (Flavonol:quercetin)

The Et$_2$O fraction was concentrated in-vacuo and left in an ice chest for a week. A yellow solid that separated was filtered and studied. On crystalllization from
MeOH, pale yellow needles were obtained (G, m.p. 313-15°C, yield-0.028%). It was readily soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, olive – green colour with alc. Fe $^{3+}$, golden yellow colour with NH$_3$ and NaOH, yellow solution with a pale green fluorescence with conc.H$_2$SO$_4$ and appeared yellow under UV and UV/NH$_3$. It reduced ammonical AgNO$_3$ in the cold and Fehling’s solution on heating. It answered the Horhammer-Hansel, Wilson’s boric acid and Gibb’s tests. It gave a penta acetate, m.p.200-01°C and a penta benzoate m.p. 188-90°C. It had $\lambda_{max}$ MeOH 255, 269 sh, 370; + NaOMe 262sh, 322, 420 (dec.); + AlCl$_3$ 267, 303, 458; + (AlCl$_3$. -HCl) 267, 303, 351, 428; + NaOAc 275, 328, 390 and + (NaOAc-H$_3$BO$_3$) 262, 303 sh, 386 nm and had Rf values depicted in Table III – 1 and III – 2. The $^1$H-NMR of the flavonol were appended. It was identified as quercetin and was confirmed by CO-and mixed –PC and m.m.p with an authentic sample of quercetin from Physalis minima.

**Ethyl acetate fraction:** (flavonol glycoside-isoquercitrin)

The EthOAc was concentrated in vacuo and left in ice chest for 2 days. A yellow solid that separated was filtered and studied. It was recrystallised from MeOH when it afforded yellow crystals, m.p. 229-30°C (yield 0.1%). It was freely soluble in EthOAc and MeOH and sparingly in water. It gave an olive – green colour with alc. FeCl$_3$, deep pink colour with Mg-HCl , yellow colour with NaOH and appeared deep purple under UV that turned yellow on exposure to NH$_3$. It did not answer the Horhammer-Hansel test but responded to the Wilson’s boric acid, Gibb’s and Molisch’s tests. The pigments was homogeneous on TLC and had Rf as indicated in Table III – 1 and III – 2 and had $\lambda_{max}$ nm 257, 269 sh, 299 sh, 362; +NaOMe, 272, 327, 409; +AlCl$_3$ 275, 303 sh; 333, 430; +(AlCl$_3$. -HCl) 274, 303 sh, 353, 401; +NaOAc 271, 320 sh, 372 and + (NaOAc-H$_3$BO$_3$) 265, 300 sh, 372. The $^1$H-NMR $^{13}$C-NMR of the glycoside were appended (Figs.III -1, III – 2, III – 3, III - 4). It was identified as isoquercitrin by comparing it with an authentic sample isolated from G. ulmifolia $^{213}$. 

**Hydrolysis of the glycoside:**

To a solution of the glycoside (0.1g 0.2 mmole) in hot aq. methonal (10 ml, 50%) an equal volume of H$_2$SO$_4$ (10%) was added to the mixture and refluxed at 100°C
for 2 h. The aq. hydrolysate was worked up in the usual way as mentioned under Et$_2$O fraction.

**Identification of aglycone: (Flavonol: quercetin)**

The yellow pigment from the above hydrolysate was identified as quercetin as described under Et$_2$O.

**Identification of sugar: (glucose)**

The aq. solution from the above hydrolysate was neutralized with BaCO$_3$ and filtered. The concentrated filtrate on chromatographic examination (PC) gave Rf values corresponding to those of glucose. The running properties of the glycoside was in favour of a monoside. The identity of the sugar was also confirmed by direct comparison with an authentic sample of glucose.
### TABLE III-1

**Rf (X 100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF ANACARDIUM OCCIDENTALE**

(Whatman No.1, Ascending, 30±2°C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Developing solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td><strong>Glycoside from</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. Occidentale</em></td>
<td>3</td>
</tr>
<tr>
<td><strong>Isoquercitrin (authentic)</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>Aglycone of the above Glycoside</strong></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Quercetin (authentic)</strong></td>
<td>-</td>
</tr>
<tr>
<td>Compound</td>
<td>Developing Solvents</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>e</td>
</tr>
<tr>
<td>Sugar from the hydrolysate of EtOAc fraction</td>
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</tr>
<tr>
<td>Glucose (authentic)</td>
<td>77</td>
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</table>

**TABLE III - 2**

Rf (X 100) VALUES OF THE SUGAR FROM THE GLUCOSIDE FROM *A. OCCIDENTALE*
FIG .III – 1H – NMR SPECTRUM OF Et2O FRACTION OF A. OCCIDENTALE
FIG. III – $^{13}\text{C}$ – NMR SPECTRUM OF Et$_2$O FRACTION OF *A. OCCIDENTALE*
FIG. III – $^1$H – NMR SPECTRUM OF EtOAc FRACTION OF *A. OCCIDENTALE*
FIG III – 4 $^{13}$C – NMR SPECTRUM OF EtOAc FRACTION OF A. OCCIDE.
**TABLE III - 3**

$^{13}$C NMR SPECTRAL DATA OF THE AGLYCONE *A. OCCIDENTALE* AND ASSIGNMENT OF SIGNALS TO VARIOUS CARBONS

<table>
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<tr>
<th>Compound</th>
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<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
<th>C-7</th>
<th>C-8</th>
<th>C-9</th>
<th>C-10</th>
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<tr>
<td>Quercetin (δ ppm)</td>
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<td>135.865</td>
<td>175.765</td>
<td>161.483</td>
<td>98.849</td>
<td>166.356</td>
<td>93.837</td>
<td>156.114</td>
<td>103.741</td>
</tr>
<tr>
<td>Quercetin (From Literature)</td>
<td>147.5</td>
<td>136.5</td>
<td>176.4</td>
<td>161.0</td>
<td>99.6</td>
<td>166.0</td>
<td>94.5</td>
<td>156.7</td>
<td>104.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-1’</th>
<th>C-2’</th>
<th>C-3’</th>
<th>C-4’</th>
<th>C-5’</th>
<th>C-6’</th>
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<tbody>
<tr>
<td>Quercetin (δ ppm)</td>
<td>122.007</td>
<td>116.057</td>
<td>145.405</td>
<td>148.101</td>
<td>116.057</td>
<td>121.594</td>
</tr>
<tr>
<td>Quercetin (From Literature)</td>
<td>123.0</td>
<td>116.0</td>
<td>145.7</td>
<td>148.1</td>
<td>116.5</td>
<td>121.0</td>
</tr>
</tbody>
</table>
### TABLE III-4

**13C-NMR SPECTRAL DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM THE FLOWERS OF A. OCCIDENTALE**

<table>
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<th>Compound</th>
<th>C&lt;sub&gt;2&lt;/sub&gt;</th>
<th>C&lt;sub&gt;3&lt;/sub&gt;</th>
<th>C&lt;sub&gt;4&lt;/sub&gt;</th>
<th>C&lt;sub&gt;5&lt;/sub&gt;</th>
<th>C&lt;sub&gt;6&lt;/sub&gt;</th>
<th>C&lt;sub&gt;7&lt;/sub&gt;</th>
<th>C&lt;sub&gt;8&lt;/sub&gt;</th>
<th>C&lt;sub&gt;9&lt;/sub&gt;</th>
<th>C&lt;sub&gt;10&lt;/sub&gt;</th>
<th>C&lt;sub&gt;1'&lt;/sub&gt;</th>
<th>C&lt;sub&gt;2'&lt;/sub&gt;</th>
<th>C&lt;sub&gt;3'&lt;/sub&gt;</th>
<th>C&lt;sub&gt;4'&lt;/sub&gt;</th>
<th>C&lt;sub&gt;5'&lt;/sub&gt;</th>
<th>C&lt;sub&gt;6'&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside (δ ppm)</td>
<td>156.3</td>
<td>133.3</td>
<td>177.5</td>
<td>161.3</td>
<td>98.7</td>
<td>164.1</td>
<td>93.5</td>
<td>156.3</td>
<td>104.0</td>
<td>120.6</td>
<td>115.2</td>
<td>144.8</td>
<td>148.5</td>
<td>116.2</td>
<td>121.2</td>
</tr>
<tr>
<td>Isoquerctrin (from literature)</td>
<td>156.5</td>
<td>133.7</td>
<td>177.6</td>
<td>161.3</td>
<td>98.8</td>
<td>164.2</td>
<td>93.6</td>
<td>156.5</td>
<td>104.2</td>
<td>121.4</td>
<td>115.3</td>
<td>144.8</td>
<td>148.5</td>
<td>116.5</td>
<td>121.6</td>
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</table>

<table>
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<th>Compound</th>
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<th>C&lt;sub&gt;2''&lt;/sub&gt;</th>
<th>C&lt;sub&gt;3''&lt;/sub&gt;</th>
<th>C&lt;sub&gt;4''&lt;/sub&gt;</th>
<th>C&lt;sub&gt;5''&lt;/sub&gt;</th>
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<td>Glycoside (δ ppm)</td>
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<td>77.1</td>
<td>76.5</td>
<td>69.9</td>
<td>77.6</td>
<td>61.0</td>
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<tr>
<td>Isoquerctrin (from literature)</td>
<td>101.4</td>
<td>74.3</td>
<td>76.8</td>
<td>70.3</td>
<td>77.5</td>
<td>61.3</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The flowers of *A. occidentale* have been found to contain quercetin and isoquercitrin. The fraction exhibited a maximum absorption ($\lambda_{\text{max}}$) at 370 nm (band I) and 255nm (band II) indicating a flavonol skeleton. Its NaOMe spectrum degenerated with time. Flavonols possessing free-OH groups at the 3-3’ and 4’ positions were known to be unstable in NaOMe. It could therefore be inferred that there are free-OH groups at C-3, C-3’ and C-4’ in the compound. A shift of +58 nm on the addition of AlCl$_3 \cdot$HCl was indicative of the presence of a free – OH at C-5 in the A-ring.

A comparison of AlCl$_3$ and AlCl$_3 \cdot$ HCl spectra revealed an additional bathochromic shift of 30 nm in the case of AlCl$_3$ spectrum (without acid), which again points to the presence of catechol type of B-ring. The presence of a free – OH at C-7 is evident from the bathochromic shift of 20 nm in band II on the addition of NaOAc. The presence of a catechol type of B-ring was also evident from the bathochromic shift of 16 nm noticed in band I on addition of H$_3$BO$_3$.

In the $^1$H-NMR spectrum (400 MHz, DMSO-d$_6$, TMS) Fig III – 1 and III - 3 of the aglycone the hydroxyl proton at C-5 shows up at $\delta$ 12.4799 ppm as a distinct singlet.

The signal of $\delta$ 9.469 ppm corresponds to –OH proton C-3. The doublet at $\delta$ 8.04 and 8.02 ppm accounts for the hydroxyl protons at C-3’ and C-4’ the C-5’ protons appears as a doublet $\delta$ 6.91 ppm (j=8.5 MHz). The signals due to the protons at C-2’ and C-6’ overlap at $\delta$ 7.53 ppm a ring protons at C-6 and C-8 could be respectively at $\delta$ 6.17 and 6.40 ppm. Based on these observation the aglycone has been unambiguously characterised as quercetin.

The fresh flowers of *A. occidentale* have been found to contain quercetin and isoquercitrin.
**IPOMOEA AQUATICA**

**Introduction**

*Ipomoea aquatica* Forsskal syn. reptans Poiret, belongs to Convolvulaceae family. They are found trailing on most soil or mud along the margins of stagnant streams, fresh water ponds, ditches, marshes and wet rice fields. It is sometimes found floating on water surfaces. It occurs both wild and cultivated and is easily propagated by cutting. It grows rapidly producing dense masses of foliage within a few weeks of planting. The specimen was confirmed with reference to herbarium sheets available in Botanical Survey of India, Coimbatore. The accession number is being 4477.

The plant serves as a green folder of nutritive value. It is relished by cattel and pigs, and produces no ill effects. It is also used as fish food. The roots are eaten by warthog’s; they taste sweet and are eaten in times of scarcity.

It is also used for piles in Cambodia. The plant is applied as a poultice in febrile delirium. The buds are used in the treatment of ringworm\textsuperscript{214}.

**EXPERIMENTAL**

**EXTRACTION AND FRACTIONATION**

Fresh flowers of *I. aquatica* collected from the banks of Cauvery in Kumbakonam during December were extracted with 85\% EtOH (4x500ml) under reflux. The alc. extract was concentrated *in-vacuo* and the aqueous concentrate successively fractionated with petrol (b.p.60-80\(^{0}\)C) (3x250 ml), peroxide-free Et\(_2\)O (3x250ml) and EtOAc (4x250ml). The petrol fraction did not yield any crystalline solid and could not be studied further.

**Et\(_2\)O fraction ( Flavonol – Kaempferol):**

The fraction subjected to PPC yielded yellow needles (MeOH), m.p. 278-280\(^{0}\)C (yield 0.02\%). It is soluble in organic solvents but insoluble in water. It developed a reddish orange colour in the Shinoda test and yellow colour with NaOH. It appeared pale yellow under UV as well as on exposure to NH\(_3\). It answered to Wilson’s boric acid, Horhammer-Hansel and Gibb’s tests, but did not respond to the Molisch’s test.
It had \( \lambda_{max} \text{MeOH} \) nm 256, 266, 294 sh, 322 sh, 367; +NaOMe 278, 316, 416; +NaOAc 274, 303, 387; + (NaOAc/ H\(_3\)BO\(_3\)) 267, 297 sh, 269, 303 sh, 348, 424 and had Rf values as depicted in Table IV-1, IV -2. The \(^1\)H and \(^{13}\)C-NMR spectra of the flavonol were appended. (FigIV-1, IV-2). It was identified as Kaempferol and identified was confirmed by Co-and mixed-PC and m. p. with an authentic sample of Kaempferol isolated from flowers of *Hydrangea macrophylla*\(^ {215}\).

**EtOAc Fraction: (Flavonol glucoside: Astragalin)**

The EtOAc fraction was concentrated *in-vacuo* and left in an ice chest for a day when a yellow colour solid separated which was filtered and studied. When crystalline from MeOH, it was soluble in aq. NaOH, hot water, EtOH and EtOAc but insoluble in Et\(_2\)O, Me\(_2\)CO and CHCl\(_3\). It gave a greenish brown colour with alc. FeCl\(_3\), an intense yellow colour with NaOH, red colour with Mg-HCl and yellow precipitate with aq. lead acetate. It appeared as a dark purple spot in UV light which turned yellow on fuming with NH\(_3\). It answered Wilson’s boric acid, Gibb’s and Molisch’s test but did not respond to the Horhammer-Hansel test. It had \( \lambda_{max} \text{MeOH} \) nm 264, 301 sh, 350, +NaOMe 273, 324, 398 +AlCl\(_3\) with and without HCl. 275, 304, 353, 397 sh +NaoAc 269, 305, 317 sh, 353 and +(NaoACH\(_3\) BO\(_3\) ), 265, 301 sh, 351. It had Rf values as depicted in Table IV-3.

**Hydrolysis of the glycoside:**

The glycoside (0.05g) dissolved in hot aq. MeOH (2ml, 50%) was hydrolysed with H\(_2\)SO\(_4\) (5%) at 100\(^{0}\)C for about 2 h and the hydrolytic products identified as described below.

**Identification of aglycone: (Flavonol: Kaempferol)**

The (yellow) aglycone on recrystallisation from MeOH afforded a yellow crystalline solid m.p. 278-80\(^{0}\)C which was identified as Kaempferol by colour reaction, behavior under UV and Rf values (Table IV-4) it had the same UV spectral values, mentioned under Et\(_2\)O fraction.
### TABLE IV -1
**Rf ( X 100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF *IPOMOEA AQUATICA***
(Whatman No.1, Ascending , 30± 2°C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Developing solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Aglycone from the Et₂O fraction</td>
<td>-</td>
</tr>
<tr>
<td>Kaempferol (authentic)</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside from the EtOAc fraction</td>
<td>13</td>
</tr>
<tr>
<td>Kaempferol-3-O-Glucoside (authentic)</td>
<td>13</td>
</tr>
</tbody>
</table>
Identification of sugar:(Glucose)

The aq. hydrolysate after the removal of the aglycone was neutralized with BaCO$_3$ and filtered. The concentrated filtrate on PC gave Rf values corresponding to those of glucose.

The glycoside was thus identified as astragalin and that was confirmed by co-PC with an authentic sample of astragalin, isolated from *H. macrophylla*. The $^1$H and $^{13}$C-NMR values of the glycoside are appended in fig IV-1 and 2.
FIG IV-1 ^1^H NMR OF THE GLYCOSIDE OF *I. AQUATICA*
FIG IV-2 $^{13}$C NMR OF THE GLYCOSIDE OF *I. AQUATICA*
### TABLE IV-2

13C-NMR SPECTRAL DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM THE FLOWERS OF

*I. AQUATICA*

<table>
<thead>
<tr>
<th>Compound</th>
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<th>C₈</th>
<th>C₉</th>
<th>C₁₀</th>
<th>C₁'</th>
<th>C₂'</th>
<th>C₃'</th>
<th>C₄'</th>
<th>C₅'</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Glycoside (δ ppm)</td>
<td>156.4</td>
<td>133.2</td>
<td>177.5</td>
<td>161.2</td>
<td>98.6</td>
<td>164.1</td>
<td>93.6</td>
<td>156.4</td>
<td>104.0</td>
<td>120.9</td>
<td>130.8</td>
<td>115.1</td>
<td>159.9</td>
<td>115.6</td>
<td>130.8</td>
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<tr>
<td>Glycoside (Authentic)</td>
<td>156.4</td>
<td>133.4</td>
<td>177.5</td>
<td>161.1</td>
<td>98.8</td>
<td>164.2</td>
<td>93.8</td>
<td>156.4</td>
<td>104.0</td>
<td>120.9</td>
<td>131.0</td>
<td>115.1</td>
<td>159.9</td>
<td>115.1</td>
<td>131.0</td>
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<th>Compound</th>
<th>C₁''</th>
<th>C₂''</th>
<th>C₃''</th>
<th>C₄''</th>
<th>C₅''</th>
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<td>Glycoside (δ ppm)</td>
<td>100.9</td>
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<td>77.4</td>
<td>69.9</td>
<td>76.4</td>
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<tr>
<td>Glycoside (Authentic)</td>
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<td>76.5</td>
<td>70.1</td>
<td>77.2</td>
<td>61.0</td>
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FIG IV-3 $^1$H NMR OF THE AGLYCON OF *I. AQUATICA*
FIG IV-4 $^{13}$C NMR OF THE AGLYCONE OF *I. AQUATICA*
### TABLE IV - 3

**13C-NMR SPECTRAL DATA AND THEIR ASSIGNMENT FOR THE AGLYCONES ISOLATED FROM THE FLOWERS OF *IPOMOEA AQUATICA***

<table>
<thead>
<tr>
<th>Compound</th>
<th>C_2</th>
<th>C_3</th>
<th>C_4</th>
<th>C_5</th>
<th>C_6</th>
<th>C_7</th>
<th>C_8</th>
<th>C_9</th>
<th>C_{10}</th>
<th>C_{11}</th>
<th>C_{12}</th>
<th>C_{13}</th>
<th>C_{14}</th>
<th>C_{15}</th>
<th>C_{16}</th>
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<tbody>
<tr>
<td>Aglycone isolated from Et_{2}O Fraction</td>
<td>146.8</td>
<td>135.7</td>
<td>175.8</td>
<td>160.7</td>
<td>98.1</td>
<td>163.8</td>
<td>93.4</td>
<td>156.1</td>
<td>103.0</td>
<td>121.6</td>
<td>129.5</td>
<td>115.4</td>
<td>159.1</td>
<td>115.4</td>
<td>129.5</td>
</tr>
<tr>
<td>Kaempferol from literature (δ ppm)</td>
<td>146.8</td>
<td>135.6</td>
<td>175.9</td>
<td>160.7</td>
<td>98.2</td>
<td>163.9</td>
<td>93.5</td>
<td>156.2</td>
<td>103.1</td>
<td>121.7</td>
<td>129.5</td>
<td>115.4</td>
<td>159.2</td>
<td>115.4</td>
<td>129.5</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The fresh flowers of *I. aquatica* have been found to contain Kaempferol and astragalin.

The UV spectrum of the flavonol aglycone obtained from the Et$_2$O fraction exhibited two major peaks at 367 (band II) and 266 nm (band II) which showed a flavonol skeleton, a bathochromic shift of 49 nm on the addition NaOMe revealed the presence of a free 4’ OH in the B ring. A shift of +57 nm on the addition of AlCl$_3$ – HCl showed the presence of a free 5-OH in the A – ring. The presence of a free OH at C-7 was ascertained by a shift +8 nm (Band II) on the addition of NaOAc. The AlCl$_3$ spectrum was exactly same as that of AlCl$_3$ – HCl, revealing the absence of catechol type of substitution in B-ring. The H$_2$BO$_3$ spectrum also confirmed it, as there was only +5 nm shift on the addition of NaOAc-H$_3$BO$_3$.

The band I UV absorption of the glucoside is at 350 nm which is again indicative of a flavonol skeleton. Comparison of band I absorption of the glycoside and the aglycone reveals that there may be 3-glycocylation in the flavonol. A bathochromic shift of 45 nm (band I) ascertained the presence of free OH at C-4’. The AlCl$_3$ spectra (with or without HCl) showed four absorption to reveal the presence of a 5-OH group. It was confirmed by the bathochromic shift of 47 nm on the addition of AlCl$_3$ – HCl. The presence of a free –OH at C-7 was evident from the +5 nm (band II) shift on the addition of NaOAc. The H$_2$BO$_3$ spectrum is exactly is same as that of MeOH, indicating the absence of catechol type of substitution in B-ring.

In the $^1$H-NMR spectrum (400 MHz, DMSO-d$_6$, TMS) the A-ring protons at C-6 and C-8 appear separately as at δ 6.4ppm δ 6.249 ppm and δ 6.420 ppm respectively. The 5-OH proton resonates at δ 12.609 ppm. In the B-ring, the protons at C-2’, 3’, 5’and 6’ due to the free rotation of the phenyl ring as two pairs of ortho coupled doublets at δ 6.944 and δ 8.017 ppm. H-3’, 5’ occurs upfield from the H-2’, 6’ doublet due to the shielding effect oxygenation at C-4’ as also due to the deshielding influence of C-ring operating on H-2’ and H-6’. The H-1 signal of the glucose moiety appears at δ 5.442 ppm found downfield from the bulk of the sugar protons. The remaining glucosyl
protons appear at $\delta$ 3.319 ppm. The $\beta$-linkage of the glucose to 3-OH is evident from the large coupling constant 7.28 MHz of H-1.

Comparing $^{13}$C-NMR (400 MHz, DMSO $d_6$, TMS) spectrum of the glycoside (recorded with AMx400 spectrometer) with that of the aglycone, the carbonyl carbon at C-4 of the glycoside appears at 1.6 ppm downfield to that of the aglycone. Due to the glycosylation at C-3, its ortho carbon of the glycoside C-4 appears at 2.4 ppm downfield to that of the aglycone and due to the ortho-effect C-2 appears 9.5 ppm downfield to that of the aglycone. All the other carbons of the glucoside at A and C-rings appear at downfield. In the B-ring the 1’, 3’and 5’carbons appear up field to that of the aglycone and the 2’, 4’and 6’carbons downfield. A complete assignment of the $^{13}$C-NMR spectrum of both the flavonol and its glycoside is available in table IV-2 and IV-3.

On this basis the identity of the pigments obtained from Et$_2$O and EtOAc can be confirmed as kaempferol and astragalin respectively.
**TITHONIA DIVERSIFOLIA (HEMSL) A.GRAY**

**INTRODUCTION**

*Tithonia diversifolia* (Hems). A. Gray of Asteracea\(^{216}\) helps to protect soil erosion, commonly known as Mexican sunflower is probably a building material and shelter for poultry\(^{217}\). It is introduced in West Africa as an ornamental plant\(^{218}\). The specimen was confirmed with reference to herbarium sheets available in Botanical Survey of India, Coimbatore. The accession number is being MH 80812.

It is distributed throughout the waste lands, water ways and on cultivated farm lands of Kenya, Malawi and Zimbabwe\(^{219}\).

*T. diversifolia* has high vegetative matter, makes it a candidate of research interest because of the relatively high species for soil rejuvenation\(^{220}\). After its introduction to Tiwan and southern China, the plant is incorporated into Chinese medicine, where it is still used to improve liver function, to treat hepatitis, jaundice, help with night sweats, reduce water retention, lower blood pressure, fight athlete’s foot and combot cystitis. The extraction of *T. diversifolia* showed promising results in the treatment of cancer\(^{221}\).

**EXPERIMENTAL**

**EXTRACTION AND FRACTIONATION:**

**Et\(_2\)O FRACTION:**

Fresh flowers (800 g) of *Tithonia diversifolia* were collected from the slopes of kodaikanal hills during May, were extracted with 90% MeOH (4X 500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aq. concentrate successively fractionated with petrol (b.p. 60-80\(^{0}\)C) (3X 250ml), peroxide – free Et\(_2\)O (4X250 ml). The petrol fraction did not yield any isolable material.
**EtOAc FRACTION: (FLAVONOL GLYCOSIDE: QUERCIMERITRIN)**

The EtOAc fraction was concentrated *in vacuo* and left in an ice-chest for a few days. A yellow solid that separated was filtered and studied. It came out as yellow plates, m.p. 249 – 250°C. It was almost insoluble in cold water and some what sparingly soluble in hot water. It was freely soluble in EtOH and Me₂Co. It gave a deep orange precipitate with lead acetate. It appeared yellow under UV which turned fluorescent yellow on exposure to ammonia. It responded to Wilson’s boric acid, Gibb’s, Horhammer – Hansel and Molisch’s tests. It had \( \lambda_{\text{MeOH}}^{\text{max}} \) nm 255, 375; + NaOAc 255, 455 (dec) ; + AlCl₃ 273, 339, 457; + AlCl₃ HCl, 268, 303 sh, 365, 426; + NaOAc 255, 268, 378,425 sh(dec); + NaOAc / H₃BO₃ 261, 288 sh, 387 and had Rf values as depicted in Table V -1.

The \(^1\)H and \(^{13}\)C NMR spectra of the glycoside were appended in Fig. V-1 and V-2. It was identified as quercimeritrin and the same was confirmed by CO – and mixed – PC and m.m.p. with an authentic sample of quercimeritrin from the flowers of *Gossypium herbaceum\(^{222}\).*

The glycoside dissolved in hot aq. MeOH was hydrolysed with H₂SO₄ (7 %) at 100°C for about 2 h and the hydrolytic products identified as described below.
**TABLE V -1**

**Rf ( X 100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF TITHONIA DIVERSIFOLIA**

(Whatman No.1, Ascending, 30± 2°C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Developing solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Aglycone (From ether fraction)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Quercetin (authentic)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06</td>
</tr>
<tr>
<td>Quercimeritrin from EtOAc fraction (authentic)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06</td>
</tr>
</tbody>
</table>
IDENTIFICATION OF AGLYCONES: (FLAVONOL: QUERCETIN)

The (yellow) aglycone on recrystallisation from MeOH afforded a yellow
crystalline solid, m.p. 316-18°C which was identified as quercetin by colour reactions,
behavior under UV and Rf values (Table V-1). It had the same UV spectral values,
mentioned under Et2O fraction.

IDENTIFICATION OF SUGAR : (GLUCOSE)

The aq. hydrolysate after the removal of the aglycone was neutralized with BaCo3
and filtered. The concentrated filtrate on PC gave Rf values corresponding to those of
glucose.
FIG V-1 $^1$H NMR OF THE GLYCOSIDE OF *T. DIVERSIFOLIA*
FIG V-2 $^{13}$C NMR OF THE GLYCOSIDE OF *T. DIVERSIFOLIA*
<table>
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<tr>
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<th>C-3</th>
<th>C-4</th>
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<th>C-6</th>
<th>C-7</th>
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<tr>
<td>Quercimeritin (δ ppm)</td>
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<td>136.68</td>
<td>176.03</td>
<td>160.40</td>
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<td>162.75</td>
<td>94.34</td>
<td>155.77</td>
<td>104.71</td>
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<tr>
<td>Quercimeritin (From Literature) (δ ppm)</td>
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<td>135.9</td>
<td>175.9</td>
<td>160.3</td>
<td>98.9</td>
<td>162.8</td>
<td>94.5</td>
<td>155.7</td>
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<table>
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<th>C-2’</th>
<th>C-3’</th>
<th>C-4’</th>
<th>C-5’</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Quercimeritin (δ ppm)</td>
<td>121.86</td>
<td>115.61</td>
<td>145.09</td>
<td>147.94</td>
<td>115.61</td>
<td>120.08</td>
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<tr>
<td>Quercimeritin (From Literature) (δ ppm)</td>
<td>121.9</td>
<td>155.5</td>
<td>145.0</td>
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<td>115.6</td>
<td>120.1</td>
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<th>C-2”</th>
<th>C-3”</th>
<th>C-4”</th>
<th>C-5”</th>
<th>C-6”</th>
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<tr>
<td>Quercimeritin (δ ppm)</td>
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<td>77.21</td>
<td>69.61</td>
<td>77.21</td>
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<td>100.3</td>
<td>73.2</td>
<td>77.2</td>
<td>69.9</td>
<td>76.5</td>
<td>60.9</td>
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Results and Discussion

The fresh flowers of *Tithonia diversifolia* have been found to contain quercetin and quercimeritrin.

The UV spectrum of the aglycone exhibited two major peaks at 370 nm (Band -I) and 255 nm (Band - II) to reveal a flavonol skeleton. Decomposition was observed on the addition of NaOMe to the aglycone. Since flavonols which have free hydroxyl groups at the 3, 3’ and 4’ positions are unstable in NaOMe and the absorption peaks in NaOMe spectrum degenerate in a few minutes, it was inferred that there was free – OH group at C-3, C-3’ and C-4’ in the compound. A shift of +58 nm on the addition of AlCl₃ – HCl showed the presence of free 5- OH in the A – ring. Comparing the AlCl₃ spectra +30 nm shift was observed in the case of AlCl₃ without acid which also revealed a B-ring orthodihydroxyl group. The presence of a free – OH at the C-7 was ascertained by a shift of +19 nm (Band - II) on the addition of NaOAc. The catechol type of dihydroxyl group in B-ring was further evidenced by bathochromic shift of +18 nm on the addition of H₃BO₃.

The UV spectrum of the glycoside from EtOAc fraction exhibited 2 absorption peaks at 375 nm (Band – 1) and 255 nm (Band – II) was indicative of the flavonols skeleton. Decomposition observed on the addition of NaOMe, indicated the presence of free – OH groups at C – 3, C – 3’ and C – 4’. The presence of a free 5-OH was evidenced from a shift of +51 nm on the addition of AlCl₃/HCl. No change was observed in band – II on the addition of NaOAc, which revealed the absence of free – OH at C-7. The corresponding aglycone however showed a bathochromic shift of + 19 nm, supporting the presence of a free 7 – OH as a result of hydrolysis. A bathochromic shift of 12 nm indicated the presence of an ortho dihydroxyl grouping in the B – ring (Viz., 3’ and 4’) on the addition of H₃BO₃.

In the ¹H – NMR spectrum (400 MHz, DMSO – d₆, TMS) of the flavonol glycoside, the C-5 proton appeared at δ 12.52 ppm. The protons at C₆ and C₈ showed up at δ 6.91 ppm (d, J= 8 MHz) and δ 6.84 ppm (d, J=11 Hz) respectively. The C-5’ proton appeared as a doublet at 7.04 ppm (J= 6 MHz). The signals of the C-2’ and C-
6’ protons occur at δ 7.56 and δ 7.70 ppm respectively. The – OH protons at C-3’ and C-4’ respectively appeared at δ 9.67 and δ 9.51 ppm. The rest of the sugar protons appeared at δ 3.35 ppm. The anomeric protons of the sugar moiety at 5.08 ppm (d, J = 7MHz). The large coupling constant showed the β - linkage of the sugar moiety.

Supporting evidence for the structure of the flavonol glycoside was provided by the analysis of 13C – NMR (400 MHz, DMSO – d6, TMS) data and a complete assignment is available in Table V-2. On this cases, the yellow pigments of the fresh flowers of Tithonia diversifolia have been identified as quercetin and quercimeritin.