# Quntative Analysis and Isolated Main Active Material of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus*

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**Abstract:** The percentage of total phenolic acids, flavonoids, tannins, saponins and alkaloids of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* aerial parts (flower, leaf and stem) were calculated by specificity methods. Chromatographic methods revealed the separation of twenty eight biologically active constituents from *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus*. Identification of the chemical composition as well as the physico-chemical properties of the twenty eight active substances was carried out using ultraviolet, $^{1}$H-NMR and $^{13}$C-NMR spectral data , the separated compounds are; Gallic acid, Vitexin, Robinin (kamferol 3-O-Robinoside-7-O- rhamnoside, 7- hydroxyl flavone, 5- hydroxyl flavone, Alizarin, Chrysopharol, Emodin, Cumaric acid, 2-naphthalene carboxylic acid, Isorhamnetin 3-O-galactoside, *P*- coumaric acid, Apigenin 7-O- glucoside, 4\, 7- dihydroxy flavone, Apigenin, Ferulic acid, Querstein, Syringic acid, 3\-hydroxy 4, 4\, 6- trimethoxy aurone, Rutine, Coutaric acid, Benzoic acid, Baicalin, Rosmarinic acid, Isovitexin, Flavone, Syringetin 3- glucose, Luteolin -7, 3\, 4\ tri-O-glucuronate.
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**Key words**: *Convolvulus althaeoides*; *Convolvulus stachydifolius* var. *villosus*; total active constitutes; U.V.; $^{1}$H NMR; $^{13}$C NMR

## 1. Introduction

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The chemical constituents present in the plant play a significant role in the identification of crude drugs (Akindele & Adeyemi, 2007).

Medicinal plants play a major role in the treatment of human diseases and have various effects on living systems. *Convolvulus* is a genus of about 250 species of flowering plants of family *Convolvulaceae*, (Boulos, 2009), Kampferol and Scopoletin were isolated from the leaves of *Convolvulus pluricaulis* by (Agarwa *et al.,* 2014). Two aglycones flavonoid compounds were isolated from Convolvulus fatmensis Ktz. using column and preparative paper chromatography and identified by using $^{1}$HNMR, $^{13}$CNMR and UV shift reagent, these compounds were kaempferol and quercetin. Four coumarin compounds were also isolated from Convolvulus fatmensis Ktz. and identified as umbelliferone, scopoletin, asculetin and scopoline for the first time by (Atta *et al.,* 2007).

So it is of interest to choose *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* as herbal plants belongs to this family. *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* are a perennial shrubs growing in north coast habitats. The aim of the study was evaluated some metabolomics parameter also biologically evaluation activity of the methanol extracts (70%) of both plants under investigation.

## 2. Materials and Methods

### 2.1. Plant Materials:

Fresh flower, leaf, stem and total aerial part of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus*.

### 2.2. Methods

#### 2.2.1. Investigation of Total Active Materials

##### 2.2.1.1. Total Phenolic Content (TPC)

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as µg/mg gallic acid equivalent to (GAE). Concentrations of 10, 20, 30, 40 and 50 µg/ml of gallic acid were prepared in methanol. Concentration of 1mg/ml of plant extract was also prepared in methanol and 0.5 ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold dilute Folin Ciocalteu reagent and 2ml 0f 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760 nm spectrophotometric all determination was performed in triplicate. The Folin Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue color was measured spectrophotmetrically (Chun *et al.,* 2013, Maurya and Singh. 2010). Line of

Regression from Gallic acid was used for estimation of unknown phenol content. From Standard curve of Gallic acid line of Regression was found to be

$$y = 0.0021x + 0.0755$$
$$R_2 = 0.9805$$

(**y**) was the absorbance and (**x**) was the µg GAE/mg of the extract, Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned GA.)

**2.2.1.2. Total flavonoid content**

The amount of Total Flavonoid content in extracts was determined aluminum chloride assay through Colorimetric. A 0.5ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15ml of a 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total Flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. All samples were analysed in three replications. (Samatha *et al.,* 2012 and Han *et. al.,* 2012*)*

$$y = 7.958x - 0.074$$
$$R_2 = 0.9985$$

(**y**) was the absorbance and (**x**) was the µg rutine/mg of the extract Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned rutin.

**2.2.1.3. Estimation of Total Tannins {Gravimetric Method (Copper Acetate Method)}**

This method depends on quantitative precipitation of tannin with copper acetate solution, igniting the copper tannate to copper oxide and weighing the residual copper oxide (Ali *et al.,* 1991).

Two grams of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* aerial parts were separately extracted for about one hour with two successive quantities, each of 100ml of acetone-water (1:1) and then filtered. The combined extract, in each case, was separately transferred into 250ml volumetric flask and adjusted to volume with distilled water. Each extract was quantitatively transferred to a 500ml beaker and heated till boiling, then 30ml of 15% aqueous solution of copper acetate was added with stirring. The precipitate of copper tannate was collected on ashless filter paper and the precipitate was ignited in a porcelain crucible (the crucibles were previously ignited to a constant weight at the same temperature). Few drops of nitric acid were added to the residue and reignited to constant weight. The weight of copper oxide was determined and the percentage of tannin was calculated according to the following correlation: Each 1g of Cuo = 1.305g tannins.

**2.2.1.4. Estimation of Total Saponins**

20g of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* aerial parts (flower, leaf and stem) were dispersed in 200 ml of 20 % ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorous. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage according to Obadoni & Ochuko (2001) and Okwu & Ukanwa (2007).

**2.2.1.5. Estimation of Total Alkaloids (Gravimetric Method)**

About (10 g) of the plant powders of *Convolvulus althaeoides and Convolvulus stachydifolius* var. *villosus* were extracted with 90% ethanol till exhaustion (tested with Mayer's reagent). The alcoholic extract of the plant was concentrated under reduced pressure until dryness at a temperature not exceeding 40°C, acidified with HCl (3 %), and filtered; the filtrate obtained was extracted with chloroform to remove acid alkaloid poration. The acidic aqueous layer was adjusted to alkaline media with ammonia and the liberated alkaloid bases poration were extracted with chloroform till exhaustion (tested by Mayer and Dragendorrf's reagents). The chloroform extract was filtered over anhydrous sodium sulphate and evaporated under reduced pressure till dryness, then weighed it to calculate the percent w/w (Woo *et al.,* 1977.

## 3. Results and Discussion

### 3.1. Total Active Constituents

#### 3.1.1. Total Flavonoids

Table (1) showed that the percentages of total flavonoids has significantly increased value in flower (190.4, 200.7) mg/gm rutin and significantly decreased in stem (76.9 mg/gm rutin of *Convolvulus althaeoides*

and *Convolvulus stachydifolius* var. *villosus* respectively.

### 3.1.2. Total phenolic acid

The percentages of total total phenolic acid has significantly increased in flower (114.4, 148.1) mg/gm rutin and significantly decreased in stem (69.6 and 99.5) mg/gm rutin of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* respectively, phenolic acid similar to flavonoids in antioxidant behavior so that total phenolics can play a major role in the antioxidant activity of plant materials (Velioglu *et al.,* 1998).

### 3.1.3. Total tannin

The percentages of total tannins (Table 1) showed that the total tannins has significantly increased in leaf (3.3% and 3.5%) and have significantly decreased in stem (1.8% and 2.3%) for *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* respectively.

### 3.1.4. Total saponin

Table (1) demonstrate that The percentages of total saponnin has maximum value in leaf (1.4%, 1.5%) and lower value in stem (0.77% and 0.78%) of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* respectively,

### 3.1.5. Total alkaloid

The percentages of total alkaloid determination by acid base extraction, table (1) showed that the total alkaloid have maximum values in leaf (3.12% and 4.01%) and have minimum value in stem (1.5% and 2.3%) for *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus,* respectively alkaloids (e.g. harmine) has various type of pharmacological activities such as antimicrobe, antitumor, cytotoxic, antiplasmodial and antioxidant (parcel *et al.,* 2012).

Table 1. Total active materials in aerial parts of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus*

| Plant parts | Item | Total active materials in different parts | | |
|---|---|---|---|---|
| | | Flower | Leaf | Stem |
| *Convolvulus althaeoides* | Total flavonoids (mg/gm rutin) | 190.4±0.5 | 160.4±0.7 | 76.9±0.12 |
| | Total phenolic acids (mg/gm Gallic acid) | 114.4±1.09 | 85.9±0.17 | 69.6±2.1 |
| | Percentage of Total Tannins (%) | 2.2±0.03 | 3.3±0.02 | 1.8±0.06 |
| | Percentage of total Saponins (%) | 1.1±0.08 | 1.4±0.13 | 0.77±0.04 |
| | Percentage of total Alkaloids (%) | 2.4±0.02 | 3.12±0.03 | 1.5±0.11 |
| *Convolvulus stachydifolius* var. *villosus* | Total flavonoids (mg/gm rutin) | 200.7±0.63 | 170.2±0.39 | 93.8±0.86 |
| | Total phenolic acids (mg/gm gallic acid) | 148.1±0.88 | 112±0.86 | 99.5±1.3 |
| | Percentage of Total Tannins (%) | 2.38±0.11 | 3.5±0.37 | 2.3±0.01 |
| | Percentage of total Saponins (%) | 1.3±0.02 | 1.5±0.02 | 0.78±0.007 |
| | Percentage of total Alkaloids (%) | 3±0.1 | 4.01±0.08 | 2.4±0.14 |

## 3.2. Phenolic Compounds

The dried powder of *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus* total aerial parts (1 kg for each) in studied habitats, when extracted successive with hexane, petroleum ether, chloroform, ethyl acetate, methanol and distal water in soxhlet apparatus concentrated to very small volume by used rotavapour under reduce pressure at 40C$^{o}$ to yield (7 & 9.6), (16 &16.8), (12 & 18.3), (40 & 38.7), (57 & 51) and (61 &54) gm. respectively., all fraction in both plants under investigation were examined by using AcOH-15% and BAW, dried chromatograms examined under UV light, re-examined after exposure to ammonia then spread $AlCl_3$ to complete evaluated phenolic compounds and chosen the best method for isolation and purification.

### 3.2.1. Isolation and Identification of Phenolic Compounds in *Convolvulus althaeoides*

Hexane fraction after examination by using AcOH-15% and BAW, was applied on the top of silica gel column (7cm X 60cm, 140 gm. silica jell, 20 drops per minutes and 50 ml per fractions) which started from hexane and increased in polarity., revealed the presence of one main compound (hexane: petroleum ether 9:1), which were subjected to spotting by paper chromatography to using solvent AcOH-15% and BAW revealed the presence of one spot of phenolic acid in nature, compound 1 95 mg., table2. Petroleum ether fraction after examination by using AcOH-15% and BAW was preparative application in paper chromatography 3MM (30 sheet) in BAW as a mobile

phase, the presence of two main fractions (Band I & Band II), Band I (2.6 gm.) was applied on the top of silica gel column (5cm X 30cm, 40 gm. silica jell, 30 drops per minutes and 25 ml per fractions ) which started from hexane and increased in polarity., revealed the presence of one main compound (hexane: petroleum ether 2:8), which were subjected to spotting by paper chromatography to using solvent AcOH-15% and BAW revealed the presence of one spot of flavonoid in nature, compound 2 48 mg., table 2, band II (3.3 gm.) was applied on the top of silica gel column (5cm X 40cm, 60 gm. silica jell, 30 drops per minutes and 25 ml per fractions) which started from hexane and increased in polarity., revealed the presence of one main compound (petroleum : chloroform 9:1), which were subjected to spotting by paper chromatography to using solvent AcOH-15% and BAW revealed the presence of one spot of flavonoid in nature, compound 3 54.3 mg, table 2. Chloroform fraction after examination by using AcOH-15% and BAW, subjected to spotting by paper chromatography 3MM (20 sheet) to using solvent AcOH-15% to obtained two majors band which related to isolation two pure compound by cutting this band and soaked in pure ethanol to obtain compound 4 48.7 mg., 2 and compound 5 53.4 mg., table 2. Ethyl acetate fraction after examination by using AcOH-15% and BAW, was preparative application in TLC (12 sheet) to obtain 4 major band this band after exanimate by U.V. lamp and cutting this band and soaked in pure ethanol to obtain compound 6 36.1 mg., table 2, compound 7 38.3 mg., table 2, compound 8 31.6 mg., table 2 and compound 9 26.4 mg., table 2, Methanol fraction after examination by using AcOH-15% and BAW, was applied on the top of silica gel column (10cm X 125cm, 600 gm. silica jell, 20 drops per minutes and 50 ml per fractions) which started from ethyl acetate and increased in polarity., revealed the presence of three main fractions, fraction I was eluted by ethyl acetate as mobile phase and produce 3 main sub fraction, sub fraction 1 when applied in chromatogram in (acetone: distal water 1:1) as a descending mobile phase produce compound 10 21 mg., table 2, sub fraction 2 applied in paper chromatography 3MM (10 sheet) ascending mobile phase produce compound 11 33.6 mg., table 2 and sub fraction 3 applied in chromatogram in (acetone: petroleum ether : distal water 3:1:6) as a descending mobile phase produce compound 12 22 mg., table 2, fraction II was eluted by (ethyl acetate: methanol 7:3) as mobile phase and produce two main sub fraction, sub fraction 1 was preparative application in paper chromatography 3MM (12 sheet) in BAW as a mobile phase to produce compound 13 33.4 mg., table 2, compound 14 62.2 mg., table 2 and compound 15 41.2mg., table 2 and sub fraction 2 was preparative Band III was preparative application in paper chromatography 3MM (10sheet) in BAW as a mobile application in paper chromatography 3MM (10 sheet) in BAW as a mobile phase to produce compound 16 50.2 mg., table 2 and compound 17 42.3 mg, table 2. Final water fraction have trace amount of phenolic compounds.

### 3.2.2. Isolation and Identification of Phenolic Compounds from *Convolvulus stachydifolius* var. *villosus*

Petroleum ether fraction after examination by using AcOH-15% and BAW showed the presence of trace amount of phenolic acids and flavonoids and were ignored. Chloroform fraction after examination by using AcOH-15% and BAW, subjected to spotting by paper chromatography 3MM (25 sheet) to using solvent AcOH-5% to obtained two majors band which related to isolation two pure compound by cutting this band and soaked in pure ethanol to obtain compound 18 65 mg., table 2 and compound 19 50 mg., 2. Ethyl acetate fraction after examination by using AcOH-15% and BAW, was preparative application in Precoated thin layer chromatography (15 sheet) to obtain 2 major, band I were preparative again by use precoated TLC in TEF ascending system and exanimate by U.V. lamp and cutting this band and soaked in pure ethanol to obtain compound 20 32.2 mg, table 2 and compound 21 62.7 mg, table 2, band II was applied on the top of silica gel column (1cm X 20cm, 12 gm. silica jell, 20 drops per minutes and 50 ml per fractions) which started from ethyl acetate and increased in polarity., revealed the presence of two main compound, compound 1 (ethyl acetate : methanol 9.5:0.5), which were subjected to spotting by paper chromatography to using solvent AcOH-15% and BAW revealed the presence of one spot of phenolic acid in nature, compound 22 28.5 mg., table 2 and compound 23 31.2, table 2 in (ethyl acetate : methanol 6:4), which were subjected to spotting by paper chromatography to using solvent AcOH-15% and BAW revealed the presence of one spot of phenolic acid in nature. Each of methanol and water fractions contain narrow compound in nature so I was collected this fraction together and complete process, methanol and water fractions were preparative application in paper chromatography 3MM (40 sheet) in BAW as a mobile phase, the presence of four main fractions, Band I was preparative again by use precoated TLC (6 sheet) in TEF ascending system and exanimate by U.V. lamp and cutting this band and soaked in pure ethanol to obtain compound 24 71 mg, table 2. Band II was preparative again by use precoated TLC (5 sheet) in (chloroform : methanol 9:1) ascending system and exanimate by U.V. lamp which obtained two main compounds, cutting this bands and soaked in pure ethanol to obtain compound 25 58.5 mg, table 2, and compound 26 59.4 mg, table 2. phase, the presence of compound 27 84.7 mg, table 2. Band IV was preparative again by use precoated TLC (6

sheet) in TEF ascending system and exanimate by U.V. lamp, cutting this band and soaked in pure ethanol to obtain compound 28 65.4 mg (Table 2).

In the present studies, Vitexin was sepration in pure form and identification by use U.V. and $^1$H- NMR, which clear that the Vitexin was content three hydroxyl group attached with heterocyclic ring and four hydroxyl group attached to aliphatic sugar chain, this hydroxyl group refer to polar behavior, "polar solvent dissolved polar solute and non-polar solvent dissolved non-polar solute" this is a rule in solubility search, the result in this search and rule of solubility was conflict, To explain this contrast is possible to say that the **Vitexin** attached with non polar compound e.g. lipid by bond and this bond was break by mobile phase with high elute strength according to that the rule may be modification by adding Condition that we should note conjugated compound attached by bonds.

Table 2. Spectra data for main active constitutes isolated from *Convolvulus althaeoides* and *Convolvulus stachydifolius* var. *villosus*

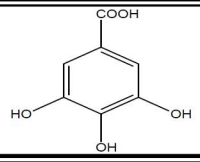
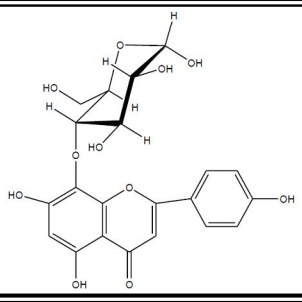
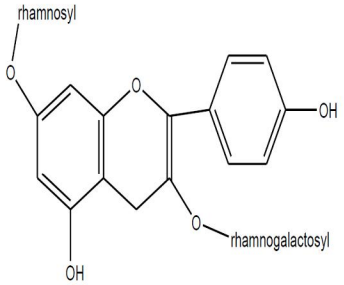
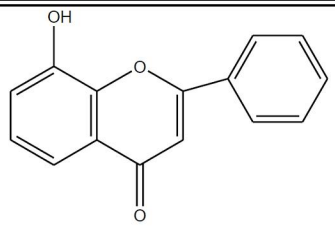
| NO. | SPECTRA DATA | STRUCTURE |
|---|---|---|
| 1 | **Gallic acid** (3, 4, 5-trihydroxy benzoic acid), blue salt, U.V. $\lambda_{max}$ **MeOH**: 273, 334, **NaOMe**: ▲276, ▲344, **$^1$HNMR**. (DMSO-$D_6$), δ: 3.0 (s, 3H, OH), 6.99 (s, 2H, $C_6H_5$), 11.0 (s, 1H, COOH). |  |
| 2 | **Vitexin**, yellow salt, U.V. $\lambda_{max}$ **MeOH**: 282, 341 **NaOMe**: ▲289, ▲381, **$AlCl_3$**: 280, ▲374, **$AlCl_3$+ HCl:** 283, ▲380, **NaOAc:** ▲291, ▲384, **NaOAc+ $H_3BO_3$:** ▲281, ▲350, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(s, 4H, ali-OH), 3.62 (d, 1H, J= 8.1Hz , ali-OH), 3.92 (d, 2H, J= 2.7Hz, ali-OH ), 4.38 (s, 1H, , ali-OH), 5(s, 1H, Ar-OH), 5.41 (d, 2H, J= 10.8Hz, Ar-OH), 5.84 (s,2H, C=C), 6.68 (d, 2H, J= 2Hz, C=C), 6.7 (s, 1H, Ar-OH), 7 (d, 2H, J= 16.2Hz, C=C). |  |
| 3 | **Robinin** (Kaemforol 3-O-robinoside 7-O rhaminoside), yellow salt, U.V. $\lambda_{max}$ **MeOH**: 274, 292 sh, 382, **NaOMe**: ▲292, 303sh, 382, **$AlCl_3$:** ▲281, 301 sh, ▼344, **$AlCl_3$+ HCl:** 282, 300 sh, ▼362, **NaOAc:** 274, 306 sh, ▼362, **NaOAc+ $H_3BO_3$:** 274, 309 sh, ▼347, **$^1$HNMR,** (DMSO-$D_6$), δ: 1.24 (s, 3H, $CH_3$), 2.04 (s, 10H, aliph-H 3.37 (m, 3H, = 2.7Hz, aliph-H), 3.40 (s, 2H, aliph-H), 3.49 (d, 2H, J= 5.4Hz, aliph-H), 3.61 (s, 1H, aliph-H), 3.71 (s, 1H, aliph-H), 3.75 (s, 1H, aliph-H), 3.85 (s, 1H, aliph-H), 3.91 (s, 1H, aliph-H), 5 (d, 2H, J= 2Hz, Ar-OH), 5.65 (s, 3H, aliph-H), 5.88 (s, 1H, aliph-H), 6.68 (d, 2H, J= 8.1Hz, Ar-H), 7.13 (d, 2H, J= 2Hz Hz, Ar-H). |  |
| 4 | **7-hydroxy flavone,** yellow salt, U.V. $\lambda_{max}$ **MeOH**: 282, 313, **NaOMe**: 280, ▼307, **$AlCl_3$:** ▼273, 312, **$AlCl_3$+ HCl:** ▲280, 311, **NaOAc:** ▲295, 312, **NaOAc+ $H_3BO_3$:** 280, ▲315 **$^1$HNMR,** (DMSO-$D_6$), δ: 5.19(s, 1H, OH), 6.25 (s, 1H, Ar-H), 6.33 (m, 1H, J= 5.4 Hz, Ar-H), 6.71 (m, 1H, J= 2.75 Hz, Ar-H), 7.21 (d, 2H, J= 7.1 Hz, Ar-H), 7.3 (d, 2H, J= 13.5 Hz, Ar-H), 7.58 (s, 1H, Ar-H). |  |

Table 2. Continue

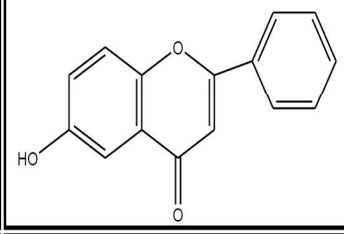
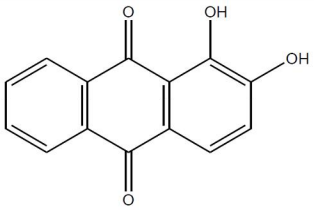
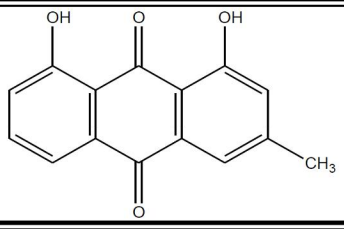
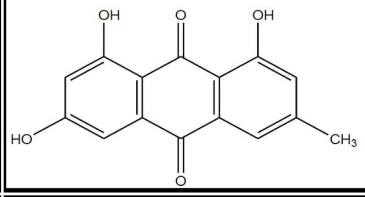
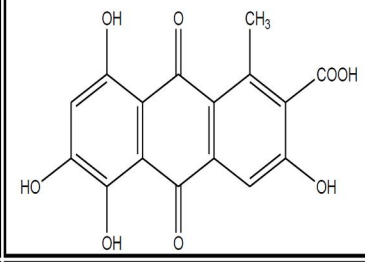
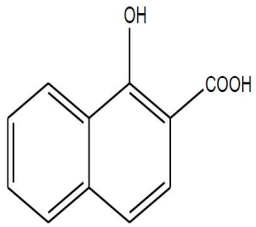
| NO. | SPECTRA DATA | STRUCTURE |
|---|---|---|
| 5 | **5-hydroxy flavone**, purple salt, U.V. $\lambda_{max}$ **MeOH**: 281, 305, **NaOMe**: ▼277, ▼299, **$AlCl_3$:** 280, 304, **$AlCl_3$+ HCl:** 282, 307, **NaOAc:** ▼277, 304, **NaOAc+ $H_3BO_3$:** 280, 306, **$^1$HNMR,** (DMSO-$D_6$), δ: 5(s, 1H, OH), 6.71(m, 1H, J=21.6 Hz, Ar-H), 6.75(m, 1H, J=18.6 Hz, Ar-H), 6.84(d, 1H, J=19.74 Hz, Ar-H), 7.11 (m, 1H, J=27 Hz, Ar-H), 7.14 (m, 1H, J=19.89 Hz, Ar-H), 7.21 (d, 2H, J=12.3 Hz, Ar-H), 7.30(m, 2H, J=15.32 Hz, Ar-H) |  |
| 6 | **Alizarin,** red crystal, U.V. $\lambda_{max}$ **MeOH**: 234, 251, 273, 312, 435, **$^1$HNMR,** (DMSO-$D_6$), δ: 5.17(d, 2H, J=3.75Hz, OH), 6.85(d, 1H, J=4.85Hz, A-H), 7.19(m, 1H, J=6.78Hz, Ar-H), 7.55(d, 2H, J=3.75Hz, Ar-H), 7.8(m, 2H, J=4.18Hz, Ar-H), **$^{13}$CNMR**, (DMSO-$D_6$):δ: 120.7(1), 123.5(1), 127.7(1), 130.0(2), 132.2(2), 133.7(2), 150.7(1), 152.5(1), 182.2(2). |  |
| 7 | **Chrysopharol**, red salt, U.V. $\lambda_{max}$ **MeOH**: 235, 268, 278, 289, 441, **$^1$HNMR,** (DMSO-$D_6$), δ: 2.5(s, 1H, $CH_3$), 5(d, 2H, J=2.7Hz, OH), 6.82(s, 1H, Ar-H), 7.02(m, 1H, J=4.68Hz, Ar-H), 7.16(d, 1H, J=5.14Hz, Ar-H), 7.38(d, 2H, J=10.5Hz, Ar-H), **$^{13}$CNMR**, (DMSO-$D_6$): δ: 24.6(1), 118(1), 119.1(1), 122.1(1), 133.6(1), 135.1(2), 143.2(1), 161(2), 182.2(2). |  |
| 8 | **Emodin,** red crystal, U.V. $\lambda_{max}$ **MeOH**: 233, 261, 272, 294, 443, **$^1$HNMR,** (DMSO-$D_6$), δ: 2.42(s, 1H, $CH_3$), 5.26(s, 3H, OH), 6.49(d, 1H, J=2.7Hz, Ar-H), 6.83(d, 2H, J=6.68Hz, Ar-H), 7.16(s, 1H, Ar-H), **$^{13}$CNMR**, (DMSO-$D_6$): δ: 24.6(1), 107.0(1), 108.4(1), 114.1(1), 118.1(1), 119.1(1), 122.9(1), 135(1), 136.5(1), 143.2(1), 161.8(1). |  |
| 9 | **Carminic acid**, colourless salt, U.V. $\lambda_{max}$ **MeOH**: 231, 236, 254, 289, 453, **$^1$HNMR,** (DMSO-$D_6$), δ: 2.35(s, 1H, $CH_3$), 5.21(m, 4H, J=2.7Hz, OH), 6.32(s, 1H, Ar-H), 7.29(s, 1H, Ar-H), 11(s, 2H, COOH), **$^{13}$CNMR**, (DMSO-$D_6$): δ: 11.1(1), 108.4(1), 112.7(1), 116.1(1), 118.3(1), 124.9(1), 127.1(1), 140.2(1), 145.1(1), 145.7(1), 152.1(1), 155.9(1), 165.5(1), 169.1(1), 182.2(2). |  |
| 10 | **2-naphthalene carboxylic acid**, colourless salt, U.V. $\lambda_{max}$ **MeOH**: 233, 279, 291, 345, 475, **$^1$HNMR,** (DMSO-$D_6$), δ: 5.22(d, 1H, J=2.7Hz, OH), 7.38(m, 2H, J=14.52Hz, Ar-H), 7.52(d,1H, J=12.1Hz, Ar-H), 7.68(s, 1H, Ar-H), 8.26(m, 2H, J=9.75Hz, Ar-H), 11.16(s, 1H,COOH), **$^{13}$CNMR**, (DMSO-$D_6$): δ: 107.1(1), 121.2(1), 123(1), 124.9(2), 127(1), 127.6(1), 129.1(1), 136.2(1), 163.8(1), 169.1(1). |  |

Table 2. Continue

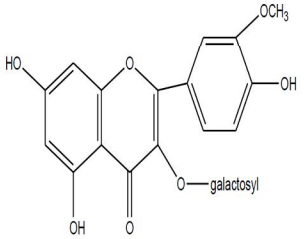
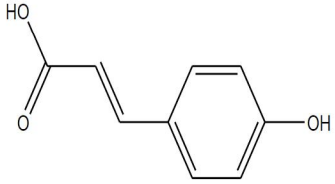
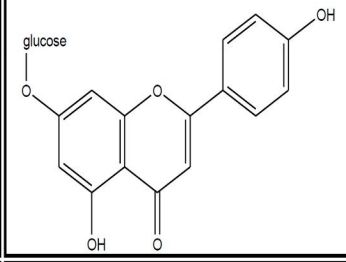
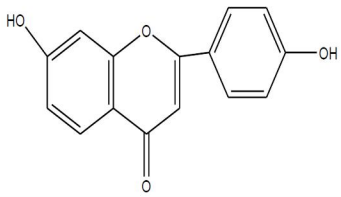
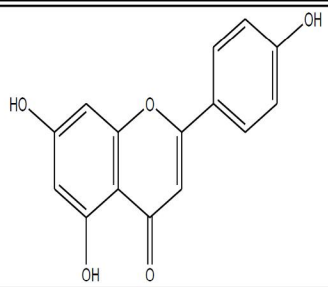
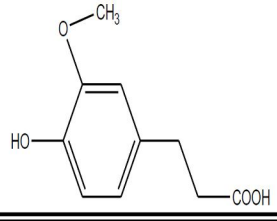
| NO. | SPECTRA DATA | STRUCTURE |
|---|---|---|
| 11 | **Isorhamnetin 3-O-galctoside**, yellow salt, U.V. $\lambda_{max}$ **MeOH**: 255, 268sh, 358, **NaOMe**: ▲284, 327sh, ▲429, **$AlCl_3$:** ▲270, 300sh, ▲401, **$AlCl_3$+ HCl:** ▲269, 301sh, ▲398, **NaOAc:** ▲277, ▲382, **NaOAc+ $H_3BO_3$:** ▲277, ▲382, **$^1$HNMR,** (DMSO-$D_6$), δ: 2.18(m, 4H, J–2.7Hz, aliph-OH), 3.41(s, 1H, Ar-H), 3.56(s, 1H, aliph-H), 3.73(s, 1H, $CH_3$), 3.76 (s, 1H, alip-H), 3.84 (s, 1H, alip-H), 3.9(m, 1H, J=3.5Hz, alip-H), 3.99(d, 1H, J=4.5Hz, alip-H), 4.24 (m, 1H, J=12.75Hz, Ar--H), 5.18 (s, 3H, OH), 5.56 (m, 1H, J=12.6Hz, alip-H), 5.64 (m, 2H, J=2.7Hz, Ar-H), 6.57 (s, 1H, Ar-H), 6.69 (m, 2H, J=21.5Hz, Ar-H) |   |
| 12 | ***P*- Coumaric acid** (3-(4 hydroxy-phenyl)-acrylic acid), colorless salt, U.V. $\lambda_{max}$ **MeOH**: 273, 311, **NaOMe**: ▲279, ▲337, **$^1$HNMR,** (DMSO-$D_6$), δ: 5.14(d, 1H, J=4.25Hz, OH), 6.41(d, 1H, J=15.5Hz, alip-H), 6.68(d, 2H, J=7.5Hz, Ar-H), 7.31(d, 2H, J=2.7Hz, Ar-H), 7.93(d, 1H, J=24.1Hz, alip-H), 11(s, 1H, COOH). |   |
| 13 | **Apigenin-7-O- glucoside,** yellow salt, U.V. $\lambda_{max}$ **MeOH**: 227 sh, 273, 335, **NaOMe**: 275, 306 sh, ▲389, **$AlCl_3$:** 277, 330 sh, ▲380, **$AlCl_3$+ HCl:** 279, 302 sh, ▲383, **NaOAc:** 280, 340, ▲390, **NaOAc+ $H_3BO_3$:** 276, 342, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(d, 4H, J=3.5Hz, alip-H), 3.26(s, 1H, alip-H), 3.43(s, 1H, alip-H), 3.61(s, 1H, alip-H), 3.72(s, 1H, alip-H), 3.81(s, 1H, Ar-H), 5(s, 2H, Ar-H), 5.85(m, 1H, J=5.7Hz, Ar-H), 6(s, 2H, Ar-H), 6.7(m, 3H, J=17.2Hz, Ar-H), 7.11(m, 2H, J=10.5Hz, Ar-H). |   |
| 14 | **4\, 7 dihydroxyflavone,** yellow salt, U.V. $\lambda_{max}$ **MeOH**: 285 sh, 328, **NaOMe**: 280, ▲397, **$AlCl_3$:** 278 sh, ▲394, **$AlCl_3$+ HCl:** 273 sh, ▲401, **NaOAc:** 291, ▲354, **NaOAc+ $H_3BO_3$:** 290sh, 328, **$^1$HNMR,** (DMSO-$D_6$), δ: 5.37(d, 2H, J=5.7Hz, Ar-OH), 6.39(d, H, J=2.7Hz, Ar-H), 6.48(d, 1H, J=3.6Hz, Ar-H), 6.71(m, 2H, J=4.8Hz, Ar-H), 7.31(m, 1H, J=19.2Hz, Ar-H), 7.47(s, 1H, Ar-H) |   |
| 15 | **Apigenin,** yellow salt, U.V. $\lambda_{max}$ **MeOH**: 271, 340, **NaOMe**: ▲279, ▲400, **$AlCl_3$:** 280, 301 sh, 391, **$AlCl_3$+ HCl:** 275, ▲394, **NaOAc:** 271, ▼382, **NaOAc+ $H_3BO_3$:** 270, 342, **$^1$HNMR,** (DMSO-$D_6$), δ: 5(s, 3H, Ar-OH), 6(s, 2H, Ar-H), 6.65(m, 2H, J=14.9Hz, Ar-H), 6.73(m, 1H, J=3.5Hz, Ar-H), 7.16(m, 2H, J=10.7Hz, Ar-H) |   |
| 16 | **Ferulic acid**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 283 sh, 319, **NaOMe**: 284 sh, 319, **$^1$HNMR,** (DMSO-$D_6$), δ: 3.7(s, 3H, $CH_3$), 5 (s, 1H, Ar-OH), 6.45(d, 1H, J=7.8Hz, Alip-H), 6.55(m, 1H, J=10.5Hz, Ar-H), 6.65(m, 1H, J=4.8Hz, Ar-H), 6.7(d, 1H, J=2.7Hz, Ar-H), 7.6 (d, 1H, J=8.5Hz, alip-H), 11(s, 1H, COOH). |   |

Table 2. Continue

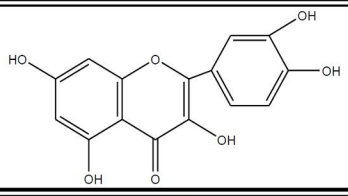
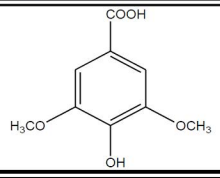
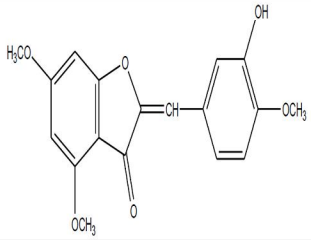
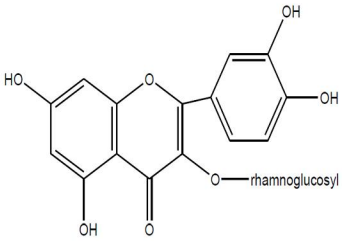
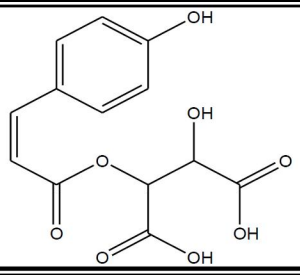
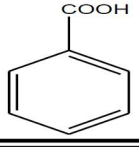
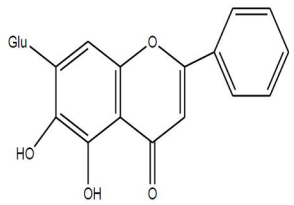
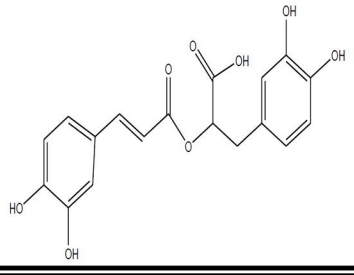
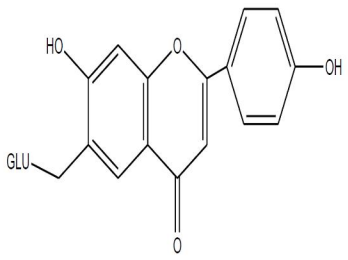
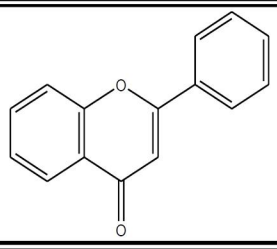
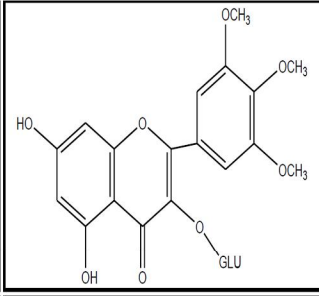
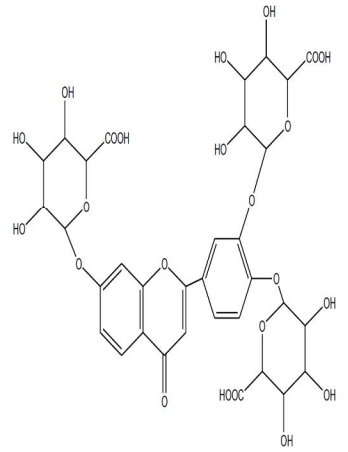
| NO. | SPECTRA DATA | STRUCTURE |
|---|---|---|
| 17 | **Quercetin,** yellow salt, U.V. $\lambda_{max}$ **MeOH**: 261, 302 sh, 375, **NaOMe**: 290, ▼453, **$AlCl_3$:** ▲275, ▲450, **$AlCl_3$+ HCl:** 276, ▼428, **NaOAc:** 264, ▲385, **NaOAc+ $H_3BO_3$:** 265, 302 sh, ▲380, **$^1$HNMR,** (DMSO-$D_6$), δ: 5(s, 4H, Ar-OH), 6(s, 1H, Ar-H), 6.5(s, 1H, Ar-H), 7.1(s, 1H, Ar-H), 7.8(s, 1H, Ar-H), 14.95(s, 1H, Ar-OH) |  |
| 18 | **Syringic acid**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 275, 284 sh, **NaOMe**: ▲295, ▲305sh, **$^1$HNMR,** (DMSO-$D_6$), δ: 3.7(s, 2H, $CH_3$), 5(s, 1H, Ar-OH), 7(d, 2H, J=2.7Hz, Ar-H), 11(s, 1H, COOH). |  |
| 19 | **3\-hydroxy-4.4\.6-trimethoxy aurone**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 274, 328 sh, 389, **NaOMe**: ▲289, 337 sh, ▲430, **$AlCl_3$:** 274, 335 sh, ▲391, **$AlCl_3$+ HCl:** 275, 329 sh, 390, **NaOAc:** 273, 327 sh, 390, **NaOAc+ $H_3BO_3$:** ▼262, 329 sh, 391, **$^1$HNMR,** (DMSO-$D_6$), δ: 3.5(s, 3H, CH3), 5(s, 1H, Ar-OH), 6(s, 1H, Ar-H), 6.06(s, 1H, Ar-H), 6.6(d, 1H, J=2.7Hz, Ar-H), 6.7(d, 1H, J=3.5Hz, Ar-H), 6.8(d, 1H, J=5.6Hz, Ar-H). |  |
| 20 | **Rutin**, yellow crystal, U.V. $\lambda_{max}$ **MeOH**: 250, 273 sh, 292 sh, 359, **NaOMe**: ▲266, 324, ▲405, **$AlCl_3$:** ▲268, 298 sh, ▲429, **$AlCl_3$+ HCl:** ▲268, 300, ▲404, **NaOAc:** ▲266, 330, ▲394, **NaOAc+ $H_3BO_3$:** ▲262, 303, ▲381, **$^1$HNMR,** (DMSO-$D_6$), δ: 1.25(d, 3H, J=7.3Hz, CH3), 2(s, 7H, alip-OH), 3.4(s, 2H, alip-H), 3.45(s, 1H, alip-H), 3.52(s, 2H, alip-H), 3.64(s, 1H, alip-H), 3.75(s, 1H, alip-H), 3.81(s, 1H, alip-H), 5(s, 4H, Ar-OH), 5.7(d, 1H, J=6.2Hz, Ar-OH), 6(d, 2H, J=8.7Hz, Ar-H), 6.6(d, 1H, J=5.7Hz, Ar-H), 6.64(d, 1H, J=3.5Hz, Ar-H), 6.75(d, 1H, J=6.9Hz, Ar-H). |  |
| 21 | **Coutoric acid**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 275 sh, 283 sh, 310, **NaOMe**: 276 sh, 284 sh, ▼302, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(d, 1H, J=4.9Hz, alip-OH), 5(d, 1H, J=3.8Hz, Ar-OH), 5.21(d, 1H, J=3.6Hz, alip-H), 5.34(d, 1H, J=6.1Hz, alip-H), 5.96(m, 1H, J=9.8Hz, alip-H), 6.68(d, 2H, J=4.8Hz, Ar-H), 7.09(m, 1H, J=12.4Hz, alip-H), 7.11(m, 2H, J=15.6Hz, Ar-H), 11(d, 2H, J=3.9Hz, COOH). |  |
| 22 | **Benzoic acid**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 276, 283, **NaOMe**: ▲282, **$^1$HNMR,** (DMSO-$D_6$), δ: 7.4(d, 2H, J=5.5Hz, Ar-H), 7.7(s, 1H, Ar-H), 8.15(m, 2H, J=2.7Hz, Ar-H), 11(s, 1H, COOH) |  |
| 23 | **Baicalin**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 275, 280 sh, 317, **NaOMe**: 275, 281 sh, ▲356, **$AlCl_3$:** 280, 284 sh, ▲346, **$AlCl_3$+ HCl:** 279, 292, ▲341, **NaOAc:** ▲307, ▲390, **NaOAc+ $H_3BO_3$:** ▲311, 320 sh, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(s, 4H, alip-H), 3.40(s, 1H, alip-H), 3.46(s, 2H, alip-H), 3.54(s, 1H, alip-H), 5(d, 2H, J=3.6Hz, Ar-OH), 5.41(d, 1H, J=2.7Hz, alip-H), 5.78(s, 1H, Ar-H), 6.71(s, 1H, Ar-H), 7.21(s, 1H, Ar-H), 7.3(s, 2H, Ar-H), 7.46(s, 1H, Ar-H) |  |

Table 2. Continue

| NO. | SPECTRA DATA | STRUCTURE |
|---|---|---|
| 24 | **Rosmarinc acid**, colorless salt, U.V. $\lambda_{max}$ **MeOH**: 276, 283, **NaOMe**: ▲282, **$^1$HNMR,** (DMSO-$D_6$), δ: 7.4(d, 2H, J=5.5Hz, Ar-H), 7.7(s, 1H, Ar-H), 8.15(m, 2H, J=2.7Hz, Ar-H), 11(s, 1H, COOH). |  |
| 25 | **Isovitexin**, yellow salt, U.V. $\lambda_{max}$ **MeOH**: 275, 281 sh, 329, **NaOMe**: 281, ▲392, **$AlCl_3$:** 271, 307, ▲380, **$AlCl_3$+ HCl:** 283, 345, ▲377, **NaOAc:** 281, ▲380, **NaOAc+ $H_3BO_3$:** 276, ▲348, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(s, 4H, alip-OH), 3.06(s, 1H, alip-H), 3.24(s, 1H, alip-H), 3.5(s, 1H, alip-H), 3.7(s, 1H, alip-H), 3.84(s, 1H, alip-H), 4.63(s, 2H, Ar-$CH_2$) 5(s, 2H, Ar-OH), 5.41(d, 1H, J=2.7Hz, alip-H), 6.32(s, 1H, Ar-H), 6.68(m, 2H, J=6.5Hz, Ar-H), 6.71(m, 1H, J=3.6Hz, Ar-H), 7.31( m, 2H, J=12.5Hz, Ar-H), 7.4(s, 1H, Ar-H). |  |
| 26 | **Flavone**, yellow salt, U.V. $\lambda_{max}$ **MeOH**: 251, 272, 291, **NaOMe**: 249, 273, 292, **$AlCl_3$:** 250, 272, 292, **$AlCl_3$+ HCl:** 251, 275, 292, **NaOAc:** 250, 274, 291, **NaOAc+ $H_3BO_3$:** 250, 275, 290, **$^1$HNMR,** (DMSO-$D_6$), δ: 6.71(s, 1H, Ar-H), 6.92(s, 1H, Ar-H), 7.08(s, 1H, Ar-H), 7.24(s, 1H, Ar-H), 7.61(d, 2H, J=4.6Hz, Ar-H), 8.03(s, 2H, Ar-H), 8.57(s, 1H, Ar-H). |  |
| 27 | **Syringetin 3-O- glucoside,** yellow salt, U.V. $\lambda_{max}$ **MeOH**: 268, 359, **NaOMe**: ▲284, ▲409, **$AlCl_3$:** 288, 446, **$AlCl_3$+ HCl:** 279, ▲404, **NaOAc:** 281, ▲385, **NaOAc+ $H_3BO_3$:** ▲274, ▲ 379, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(s, 4H, alip-H), 3.24(s, 1H, alip-H), 3.44(m, 2H, alip-H), 3.53(s, 1H, alip-H), 3.73(d, 9H, J=5.1Hz, Ar-CH.), 3.86(s, 1H, alip-H), 5(s, 2H, Ar-H), 5.44(s, 1H, alip-H), 5.95(s, 2H, Ar-H), 6.3(s, 1H, Ar-H). |  |
| 28 | **Luteliolin-7, 3\', 4\', tri-O- glucuronide,** colorless salt, U.V. $\lambda_{max}$ **MeOH**: 274, 281 sh, 292 sh, 318, **NaOMe**: 281, 315 sh, ▲375, **$AlCl_3$:** ▲291, 314 sh, ▲398, **$AlCl_3$+ HCl:** 276, 305 sh, ▲340, **NaOAc:** 277, 290 sh, ▲351, **NaOAc+ $H_3BO_3$:** 279, ▲342, **$^1$HNMR,** (DMSO-$D_6$), δ: 2(d, 9H, J=5.6Hz, alip-H), 3.5(d, 3H, , J=2.7Hz, alip-H), 3.75(d, 3H, J=6.4Hz, alip-H), 3.9(m, 3H, J=9.5Hz, alip-H), 4.5(m, 3H, J=5.8Hz, alip-H), 5.9(d, 3H, J=4.6Hz, alip-H), 6.4(m, 1H, J=2.7Hz, Ar-H), 6.53(d, 1H, J=2.7Hz, Ar-H), 6.6(d, 1H, J=3.5Hz, Ar-H), 6.7(d, 1H, J=3.8Hz, Ar-H), 6.8(d, 2H, J=10.5Hz, A-H),7.6(d, 1H, J=4.3Hz, Ar-H), 11(s, 3H, COOH). |  |

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