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# Flavonoid and Phenolic Acid Compounds in *Euphorbia bivonae* Steud. Roots

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## ABSTRACT

*Euphorbia bivonae* Steud. roots (Family: *Euphorbiaceae*) collected from Agiba Mersa Mattruh. Investigation of flavonoids by HPLC revealed the presence of 22 compounds of *Euphorbia bivonae* roots, where the major compounds were luteolin-6- arbinose-8- glucose (138.34mg/100g), apigenin-6-glucose-8-rhmnose (72.642 mg/100g), acacetin (41.878 mg/100g), luteolin-6- glucose -8- arbinose (40.025 mg/100g) and apigenin-6- arbinose -8-glactose (34.684 mg/100g). On investigation of flavonoids and phenolic acids using chromatographic methods eight pure flavonoid compounds (quercetin and kaempferol as flavonol, luteolin and apigenin as flavone, acacetin as O- methylated flavone, rutin, kaempferol-3-O-α-dirhamnoside-O-β-D-glucopyranoside and kaempferol-3-rutinoside as flavonoid glycosides) and two phenolic acid compounds (gallic acid and ferulic acid) were separated and identified.

***Key words:*** *Euphorbia bivonae* Roots, *Euphorbiaceae*, flavonoids and phenolic acids.

### Introduction

Flavonoids, are widespread in different plants. In some cases they present as flavonoid aglycones which are including one or more sugar molecules attached to the flavon nuclei as O-glycoside or C-glycoside (Stobiecki, 2000). Flavonoid glycosides and free aglycones are involved in interactions of plants with microorganisms, both pathogenic and symbiotic (Dixon *et al.* (1994), Spaink (1995) and Gianinazzi (1996)), which declared its wide antimicrobial and pharmacological activities (Wollenweber (1988) and (Dixon and Steele, 1999).

The family *Euphorbiaceae* is one of the largest families of flowering plants, composed of over 300 genera and 8,000 species, grown at wide range of habitats (Webster, 1994). Different *Euphorbiaceae* species are tend to manufacture a wide range of secondary metabolites to aid response to a disparity of stimuli in their particular habitat (Mwine and Van Damme, 2011).

Ethyl acetate extract of the aerial parts of *Euphorbia bivonae* plant have a potent cytotoxic activity in vitro against different human cell line at low concentration and showed a remarkable with dose (400 mg/kg. b. wt. orally for 10 days) against hepato and nephro toxic rats induced by over dose of paracetamol. This activity attributed with its high content of flavonoid compounds as kaempferol-3-O-α-dirhamnoside-O-β-D-glucopyranoside, kaempferol-3-rutinoside, isorhamentin -3- rutinoside, kaempferol -3,7- diglucopyranoside, kaempferol-3- rhamnopyranoside, kaempferol-3- glucopyranoside, kaempferol-7- rhamnopyranoside, quercetin -3- glucopyranoside, kaempferol -4`- methylether, 3`, 4` dihydroxyflavone and isorhamentin) (Ibrahim, 2008). Investigation of the root of the *Euphorbia bivonae* may evaluate our knowledge about the flavonoids of the plant as pharmacological bioactive agents. Hence we decide to take care with its flavonoids contents.

## Materials and Methods

*Plant material:*

*Euphorbia bivonae* was collected from Agiba 20 km west of Marsa Mattruh at end of April, 2011.The roots of *Euphorbia bivonae* were cleaned, dried in an oven at 40˚C, ground to fine powder and kept for investigations (Fig.1)

*Preparation of the extract:*

About 2kg of roots powder of *Euphorbia bivonae* was extracted with 80% aqueous ethanol and was processed to remove inorganic salts and non-phenolic compounds.

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***Fig. 1:*** *Euphorbia bivonae* *Euphorbia bivonae* root

*Investigations of flavonoids:*

*Qualitative and quantitative of flavonoids by HPLC:*

The ethanol extracts of *Euphorbia bivonae* roots were analysed using HPLC. The employed HPLC system consisted of HP 1090M Series II high performance liquid chromatography equipped with an HP 1090M Series II diode array and an eight-channel electrochemical coulometric array detector ((EC); Esa Inc., USA). The EC was operated using 100-800 mV potentials (100mV intervals). The detector array was housed in a temperature-regulated compartment at 35ºC.

Flavonoid separation was done by ODS-3 (4.0 × 150 nm, 3µm) column with a C-18 guard column, with temperature set at 35ºC. The flow rate of the mobile phase was 0.7mL/min, and the injection volumes were 10µL of the standards and sample extracts. All flavonoids were quantified using the external standard method. Quantification was based on peak area (DAD) or beak high (EC). (Mattila *et al*., 2000).

*Separation and identification of flavonoid compounds:*

*Reagents:*

*Reagents for phenolics:*

a) Ferric chloride, 1% ethanolic solution (Smith, 1960).
b) Gibb's reagent, freshly prepared (Neish, 1960).

*Reagents for carboxylic acids (Aniline/glucose):*

The chromatogram was sprayed with the reagent and heated for 5-10 min at 105°C, brown to red spots were detected (Smith, 1960).

*Reagents for hydroxy-flavonoids:*

*a) Aluminum chloride:*

The chromatograms were sprayed with 1% methanolic solution reagent, then heated at 110ºC for 10 minutes and observed under UV light (Markham, 1982).

*b) Tetra phenyl diboroxie ethanol amino complex:*

1 % ethanolic solution (Markham, 1980).

*Reagents for sugars:*
*Aniline/hydrogen phthalate reagents (Stahl, 1969):*

The chromatogram was sprayed with reagent and heated in an oven at 105 °C for 10 minutes for visualization of sugars and heated to 110°C for sucrose. The developed color ranged from brown/yellowish brown to red.

*Reagents for UV spectroscopic analysis* (Mabry *et al.,* 1970 and Markham, 1982):

*a) Sodium methoxide solution:*

2.5g metallic sodium was added cautiously to 100 ml dry methanol:

*b) Aluminum chloride:*

Anhydrous 5g of the reagent was added to 100ml of methanol.

*Solvent systems (Mabry et al., 1970) :*

n-Butanol : Acetic acid : water (4:1:5 v/v/v) (BAW) = $S_1$ and Acetic acid : water (15:85 v/v AcOH-15%) = $S_2$

*Chromatography:*

Ethanolic extract *Euphorbia bivonae* roots were separately chromatographed on Whatman No. 1 paper chromatography (PC) using the solvent system ($S_1$) for the first way and solvent system ($S_2$) for the second way, air dried, and examined under Ultraviolet (UV) light, then exposed to ammonia and re-examined under UV light. The concentrated ethanolic extract of the roots of *Euphorbia bivonae* were applied on the top of a polyamide column. Elution was started with pure ethanol followed by a mixture of ethanol/water and finally distilled water was applied. The received fractions were evaporated and subjected to PC where similar fractions were collected together (Liu *et al*., 1989). Each fraction was separated when subjected to preparative paper chromatography (PPC) Whatman No 3 PC using solvent system $S_1$ and $S_2$, where the developed chromatograms were air dried, examined under UV light. Phenolic compounds were detected by spraying the dried chromatograms with the corresponding reagents.

The flavonoids and phenolic acid compounds were separated by elution of the formed bands with 50% ethanol, where the separated flavonoid compounds were purified on sephadex LH-20 column chromatography. The pure flavonoids and phenolic acid compounds were identified by chemical method and confirmed by physical method.

*Chemical analysis:*

*2.6.1. Controlled (Mild) acid hydrolysis, complete (normal) acid hydrolysis according to Harborne et al. (1975):*

*Physical analysis:*

*Ultraviolet spectrophotometer analysis:*

Chromatographically pure materials were dissolved in pure methanol and subjected to ultraviolet spectrophotometric investigation using shimadzu UV-240 spectrophotometeric analysis and addition of the different reagents (Mabry *et al*., 1970).

*$^{1}H$- and $^{13}C$ -NMR:*

The NMR measurements were carried out on JEOL EX-270 NMR spectrometer apparatus (270 MHz for $^{1}H$- NMR and 67.5 MHz for $^{13}C$-NMR).

*Mass Spectrometric analysis (MS):*

Mass measurements were conducted using finning SSQ 7000 spectroscopy.

## Results and Discussion

**Qualitative and quantitative of flavonoids by HPLC:**

Investigation of flavonoids by HPLC revealed the presence of 22 compounds of *Euphorbia bivonae* roots, where the major compounds were luteolin-6- arbinose-8- glucose (138.34mg/100g), apigenin-6-glucose-8-

rhmnose (72.642 mg/100g), acacetin (41.878 mg/100g), luteolin-6- glucose -8- arbinose (40.025 mg/100g) and apigenin-6- arbinose -8-glactose (34.684 mg/100g) (Table 1).

**Table 1:** HPLC analysis of the flavonoids of *Euphorbia bivonae* roots.

| No. | Flavonoids | Mg/100g |
|---|---|---|
| 1 | Luteolin-6- arbinose-8- glucose | 138.34 |
| 2 | Luteolin-6- glucose -8- arbinose | 40.025 |
| 3 | Apigenin-6- arbinose -8-glactose | 34.684 |
| 4 | Apignin-6- rhamnose -8- glucose | 2.742 |
| 5 | Apignin-6- glucose -8- rhamnose | 72.642 |
| 6 | Luteolin-7- glucose | 3.104 |
| 7 | Narengin | 20.903 |
| 8 | Rutin | 3.066 |
| 9 | Hespiridin | 10.912 |
| 10 | Quercetin-3-O-glucoside | 0.983 |
| 11 | Rosmarinic | 0.876 |
| 12 | Apigenin-7-O- neohespiroside | 5.951 |
| 13 | Kampferol-3,7-dirhamoside | 5.171 |
| 14 | apigenin-7- glucose | 0.787 |
| 15 | Quercetrin | 1.466 |
| 16 | Quercetin | 1.609 |
| 17 | Naringenin | 1.815 |
| 18 | Hespirtin | 12.455 |
| 19 | Kampferol | 0.304 |
| 20 | Rhamnetin | 2.892 |
| 21 | Apignin | 0.489 |
| 22 | Acacetin | 41.878 |

**Identification of flavonoids and phenolic acid compounds:**

*Compound 1:*

Compound (1) was detected as yellow color compound under UV light changed to yellow fluorescence on exposured to ammonia with $R_f$ 0.70 and 0.28 in BAW and AcOH 15%, respectively, UV spectral data at Table (3) showed that the compound is flavonol compound with free-OH at position 3, 5, 7, 3` and 4` on addition of different reagents (Mabry *et al*., 1970) as illustrated at Table (3). EI-Mass of compound (1) showed the presence of molecular ion peak $M^+$ at 301.9 m/z. UV analysis (Table 3), $^1$H-NMR (Table 4), $^{13}$C-NMR (Table 5) and EI-Mass spectrum declared that the compound was quercetin.

*Compound 2:*

Compound (2) was detected as brown color compound under UV light changed to yellow on exposured to ammonia with $R_f$ 0.78 and 0.09 in BAW and AcOH 15%, respectively, which indicated that the compound is aglycone (Harborne, 1984) (Table 2). (Table 2). UV spectral data at Table (3) showed that the compound is a flavone and contained free OH at position 5, 7, 3`, 4` declared by the addition of different reagents (Mabry *et al*., 1970) as illustrated at Table (3). The EI-Mass spectrum of compound (2) showed molecular ion peak ($M^+$) at m/z 286 as the base peak. From UV analysis (Table 3), $^1$H-NMR (Table 4) and EI-Mass spectrum indicated that the compound (2) was luteolin.

*Compound 3:*

Compound (3) was detected as purple color compound under UV light changed to yellow on exposured to ammonia with $R_f$ 0.88 and 0.11 in BAW and AcOH 15%, respectively, which indicated that the compound is aglycone (Harborne, 1984) (Table 2). UV spectral data at Table (3) showed that the compound is a flavone compound with free OH at position 5, 7 and 4` declared by the addition of different reagents (Mabry *et al*., 1970) as illustrated at Table (3). Mass spectrum of compound (3) revealed a molecular ion peak ($M^+$) at m/e 270.UV analysis (Table 3), $^1$H-NMR (Table 4) and mass spectrum indicated that the compound (3) was apigenin.

*Compound 4:*

Compound (4) was detected as yellow color compound under UV light changed to yellow fluorescence on exposured to ammonia with $R_f$ 0.89 and 0.13 in BAW and AcOH 15%, respectively, which indicated that the compound is aglycone (Harborne, 1984) (Table 2). UV spectral data at Table (3) showed that the compound is a

flavonol compound with free OH at position 3,5, 7 and 4` declared by the addition of different reagents (Mabry *et al*., 1970) as illustrated at Table (2). Mass spectrum of compound (4) revealed a molecular ion peak ($M^+$) at m/e 286 and other important ions. m/e 285, 258, 229, 153, 121 and 93.UV analysis (Table 3), $^1$H-NMR (Table 4) and mass spectrum indicated that the compound (4) was kaempferol.

**Table 2:** $R_f$-values and color reactions of the isolated phenolic compounds

| Compound No. | Compounds | $R_f$-values | | Color reactions | |
|---|---|---|---|---|---|
| | | BAW | 15% AcOH | UV | $NH_3$+UV |
| 1 | Quercetin | 0.70 | 0.28 | Yellow | Yellow |
| 2 | Luteolin | 0.78 | 0.09 | Brown | Yellow |
| 3 | Apigenin | 0.88 | 0.11 | Purple | Yellow |
| 4 | Kaempferol | 0.86 | 0.14 | Yellow | Florescence yellow |
| 5 | Acacetin | 0.91 | 0.10 | Deep purple | deep purple |
| 6 | Rutin | 0.54 | 0.50 | Purple | Yellow |
| 7 | Kaempferol-3-O-α-dirhamnoside-O-β-D-glucopyranoside | 0.50 | 0.61 | Deep purple | Yellow |
| 8 | Kaempferol-3-rutinoside. | 0.55 | 0.51 | Deep purple | Yellow |
| 9 | Gallic acid | 0.78 | 0.59 | Blue | Blue |
| 10 | Ferulic acid | 0.88 | 0.56 | Blue | Fluorescence blue |

**Table 3:** UV-spectral data ($\lambda_{max}$, nm) of the isolated phenolic compounds.

| No | Compounds | Reagents | | | | | |
|---|---|---|---|---|---|---|---|
| | | MeOH | NaOMe | NaOAc | NaOAc+$H_3BO_3$ | $AlCl_3$ | $AlCl_3$+HCl |
| 1 | Quercetin | 255, 270 (sh), 300 (sh), 370 | 265, 238 (sh),440 | 258, 332 (sh), 385 | 260, 300 (sh), 384 | 217, 318, 446 | 265, 305 (sh), 355 (sh), 425 |
| 2 | Luteolin | 253, 267, 349 | 266, 330, 400 | 268, 384 | 262, 370, 426 | 272, 300,330, 422 | 272, 300, 380 |
| 3 | Apigenin | 266, 295(sh), 335 | 274, 324, 390 | 274,300,376 | 266,300,335 | 274,300,350,383 | 274,299,343,380 |
| 4 | Kampferol | 253 (sh), 268, 342 (sh), 367 | 280, 318, 420 | 257, 302 (sh), 385 | 267, 296 (sh), 320, 370 | 266, 305 (sh), 350, 422 | 266, 305 (sh), 350, 422 |
| 5 | Acacetin | 260, 300(sh), 325 | 277, 397(sh), 365 | 277, 298(sh), 359 | 260, 300(sh), 330 | 277, 298(sh), 301, 350,380 | 279, 298(sh), 301, 352,375 |
| 6 | Rutin | 257 (sh), 267, 295 (sh), 360 | 275, 320 (sh) 415 | 270, 320 (sh), 380 | 260, 300 (sh), 380 | 275, 305 (sh), 355 (sh), 430 | 275, 305 (sh), 345 (sh),420 |
| 7 | Kaempferol-3-O-α-dirhamnoside-O-β-D- glucopyranoside | 265, 350 | 273,310 (sh), 401 | 273, 390 | 273, 300(sh), 350 | 270, 302 (sh), 401 | 266, 302 (sh), 394 |
| 8 | Kaempferol-3-rutinoside | 265,350 | 272, 362 | 272, 362 | 266, 350 | 272, 309, 345, 390 | 272, 310, 344, 390 |
| 9 | Gallic acid | 272, 335 | 274, 346 | - | - | - | - |
| 10 | Ferulic acid | 284, 310 | 251 (sh), 290, 320 | - | - | - | - |

*Compound 5:*

Compound (5) was detected as deep purple compound under UV light no changed on exposure to ammonia with $R_f$ 0.91 and 0.10 in BAW and AcOH 15%, respectively, which indicated that the compound is aglycone occupied with $OCH_3$ at 4`position (Harborne, 1984) (Table 2). UV spectral data at Table (3) showed that the compound is a flavones compound with free OH at position 5, 7 and occupied with $OCH_3$ at 4`position declared by the addition of different reagents (Mabry *et al*., 1970) as illustrated at Table (2). UV analysis (Table 3), $^1$H-NMR (Table 4) and $^{13}$C-NMR (Table 5) indicated that the compound (5) was acacetin.

*Compound 6:*

Compound (6) was detected as purple color compound under UV light changed to yellow on exposure to ammonia with $R_f$ 0.54 and 0.50 in BAW and AcOH 15%, respectively, which indicated that the compound may be flavonoid glycoside (Harborne, 1984) (Table 2). Complete acid hydrolysis gave an aglycone quercetin and two sugar residues identified as glucose and rhamnose (comparative $R_f$ – values with authentic markers). UV spectral data at Table (3) showed that the compound is a flavonol with 3-OH substitution. The remaining UV spectral data were found to be similar to that of quercetin type compound. Mass spectrum of compound ($A_2$) revealed M/Z (rel-int %): 610 ($M^+$, glucose, 60%), M/Z 464 ($M^+$, rhamnose, 191.1%), 302 (quercetin, 100%). UV analysis (Table 3), $^1$H-NMR (Table 4),$^{13}$C-NMR (Table 5) and EI-Mass spectrum indicated that the compound is rutin (quercetin-3-O-α L-rhamnoside (1-6) β D-glucoside).

*Compound 7:*

Compound (7) was detected as deep purple color compound under UV light changed to yellow on exposure to ammonia with $R_f$ 0.50 and 0.61 in BAW and AcOH 15%, respectively, which indicated that the compound may be flavonoid glycoside (Harborne, 1984) (Table 2). Complete acid hydrolysis gave an aglycone kaempferol and three sugar residues identified as rhamnose and glucpyrane (comparative $R_f$ – values with authentic markers). UV spectral data at Table (3) showed that the compound is a flavonol with 3-OH substitution. The remaining UV spectral data were found to be similar to that of kaempferol type compound. UV analysis (Table 3), $^1$H-NMR (Table 4) and $^{13}$C-NMR (Table 5) indicated that the compound is kaempferol-3-O-α-dirhamnoside-O-β-D- glucopyranoside.

*Compound 8:*

Compound (8) was detected as deep purple color compound under UV light changed to yellow on exposure to ammonia with $R_f$ 0.55 and 0.51 in BAW and AcOH 15%, respectively, which indicated that the compound may be flavonoid glycoside (Harborne, 1984) (Table 2). Complete acid hydrolysis gave an aglycone kaempferol and two sugar residues identified as rhamnose and glucose (comparative $R_f$ – values with authentic markers). UV spectral data at Table (3) showed that the compound is a flavonol with 3-OH substitution. The remaining UV spectral data were found to be similar to that of kaempferol type compound. UV analysis (Table 3), $^1$H-NMR (Table 4) and $^{13}$C-NMR (Table 5) indicated that the compound is kaempferol-3-rutinoside (kaempferol-3-O-α-rhamnoside (1→6)-β-D- glucopyranoside).

**Table 4:** $^1$H-NMR spectral data of the isolated phenolic compounds.

| No | Compounds | δ (ppm) |
|---|---|---|
| 1 | Quercetin | 7.6 (1H, dd, J=8.5, 2.3 Hz, H2`), 7.5(1H, dd, J=8.5 Hz, H6`), 6.89 (1H, d, J=8.5 Hz, H5`), 6.4 (1H, d, J=2.5 Hz, H6) and 6.2 (1H, d, J=2.5 Hz, H8). |
| 2 | Luteolin | 6.19 (d, J=2.5 $H_Z$, H6), 6.46 (d, J=2.5 $H_Z$, H8), 6.85 (s, H3), 6.89 (d, J=8 $H_Z$, H5`), 7.4 (d, J =2.5 $H_Z$ and J=8 $H_Z$, H2`and H6`). |
| 3 | Apigenin | 7.96 (d, J=7.5 $H_Z$,H2, H6`), 6.9 (d, J=7.5 $H_Z$, H3`, H5`), 6.75 (s, H3), 6.5 (d, J=2.5 $H_Z$, H8) and 6.1 (d, J =2.5 $H_Z$ H6). |
| 4 | Kampferol | 8.0 (2H,d, J=8 $H_Z$, H-2` and H-6`), 6.9 (2H,d, J =8 $H_Z$, H-3` and H-5`), 6.4 (1H,d, J=2.5 HZ, H-8), 6.2 (1H,d, J=2.5 $H_Z$, H-6 ). |
| 5 | Acacetin | 12.92(1H, s, 5-OH), 10.85 (1H,br s, 7-OH), 8.01 (2H, d, J= 9.0 Hz, H-2,6′),7.09 (2H, d, J= 8.8Hz, H-3′,5′), 6.86 (1H, s, H-3), 6.50 (1H, d, J=1.8 Hz, H-8), 6.18 (1H, d, H-3,J= 2.4 Hz, H-6). |
| 6 | Rutin | 7.6 (1H, d, J=2.5 Hz, H2), 7.5 (1H, dd, J=8.5, 2.5$H_Z$, H6`), 6.8 (1H, d, J=8 Hz, H5`), 6.4 (1H, d, J=1.5 Hz, H8), 6.2 (1H, d, J=1.5 Hz, H6), 5.3 (1H, d, J=8 Hz, H1`` glucose), 4.5 (1H d, J= 2.5 Hz, H1''' rhamnose), 3.4 (m, remaining sugar protons) and 0.8 (3H, d, J= 6 Hz, $CH_3$ rhamnose). |
| 7 | kaempferol-3-O-α-dirhamnoside-O-β-D-glucopyranosid | 7.8 (2H, d, J=8.7 Hz, H2`, H6` ), 6.8 (2H, d, J=8.7 Hz, H3`, H5`), 6.4(1H, d, J=2.5 Hz, H8),6.12 (1H, d, J=2.5 Hz, H6), 5.49 (1H, d, J=7.4 Hz, H1`` of glucosyl), 5.05 (1H, s, J=4 Hz, H1`` rhamnosyl), 4.3(1H, s, J=4 Hz, H1```` rhamnosyl), 0.96 (3H, d, J=6 Hz, rhamnosyl $CH_3$ ), 0.8 (3H, d, J=6 Hz, rhamnosyl $CH_3$). |
| 8 | kaempferol-3-rutinoside | 7.9 (2H, d, J=7.8 Hz, H2`, H6` ), 6.83 (2H, d, J=7. 8 Hz, H3`, H5`), 6.33 (1H, d, J=2.5 Hz, H8),6.12 (1H, d, J=2.0 Hz, H6), 5.44 (1H, d, J=7.3 Hz, H1`` of glucosyl), 5.05 (1H, s, J=4 Hz, H1`` rhamnosyl), 4.3(1H, s, J=4 Hz, H1````` rhamnosyl), 0.96 (3H, d, J=5.5 Hz, rhamnosyl $CH_3$ ). |
| 9 | Gallic acid | 6.97 (s, H2, H6) |
| 10 | Ferulic acid | 8.9(s, OH), 7.4 (1H, d, J= 18 Hz, H7), 7.12 (1H, d, J= 7.5 Hz, H2), 7.0 (1H, dd, J=7.5, 2.5 Hz, H6), 6.9 (1H, d, J= 7.5 Hz, H5), 6.3 (1H, d, J= 18 Hz, H8), 3.84 (3H, s,$OCH_3$). |

*Compound 9:*

Compound (9) was detected as blue color compound under UV light changed to blue on exposure to ammonia with $R_f$ 0.78 and 0.59 in BAW and AcOH 15%, respectively, (Table 2). The $R_f$ value and color reactions of compound (9) showed that it phenolic nature. UV spectra of compound (9) showed the presence of two major bands at 272 and 335 nm, where on the addition of NaOMe gave a bathechromic shift indicating the presence of free OH groups (Table 3), $^1$H-NMR (Table 4) indicated that the compound is gallic acid.

*Compound 10:*

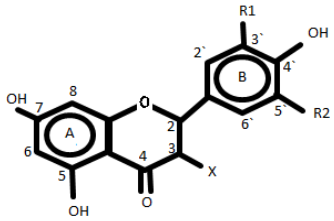
Compound (10) was detected as blue color compound under UV light changed to fluorescence blue on exposure to ammonia with $R_f$ 0.88 and 0.56 in BAW and AcOH 15%, respectively, (Table 2). The $R_f$ value and color reactions of compound (10) showed that it phenolic nature. UV spectra of compound (10) showed the

presence of two major bands at 284 and 310 nm, where on the addition of NaOMe gave a bathechromic shift indicating the presence of free OH groups (Table 3), $^{1}$H-NMR (Table 4) indicated that the compound is 4-hydroxyl-3- methoxy cinnamic acid (ferulic acid).

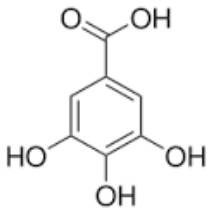
**Table 5:** $^{13}$C -NMR spectral data of the isolated phenolic compounds.

| No | Compounds | δ (ppm) |
|---|---|---|
| 1 | Quercetin | 175.7 (C-4), 163.9 (C-7), 160.7 (C-5), 156.2 (C-9), 146.9 (C-4`), 147.6 (C-2), 145.0 (C-3`), 135.6 (C-3), 122.0 (C-1`), 120.0 (C-6`), 115.6 (C-5`), 115.3 (C-2`), 103.0 (C-10), 98.2 (C-6), 93.4 (C-8) |
| 2 | Luteolin | - |
| 3 | Apigenin | - |
| 4 | Kampferol | - |
| 5 | Acacetin | 181.8 (C-4), 164.2 (C-7), 163.3 (C-2), 162.3 (C-4′), 161.4(C-5), 157.3 (C-9), 128.3(C-2′,6′), 122.8 (C-1′), 114.5 (C-3′,5′), 103.8 (C-10), 103.5(C-3), 98.9 (C-6),94.0 (C-8) |
| 6 | Rutin | 146.9 (C-2), 135.5 (C-3), 175.8 (C-4), 160.7 (C-5), 98.2 (C-6), 163.9 (C-7), 93.3 (C-8), 156.2 (C-9), 103.1 (C-10), 122.1 (C-1), 115.3 (C-2'), 145.0 (C-3), 147.6 (C-4'), 115.6 (C-5'), 120.0 (C-6'), sugar moiety, 101.5 (C-1''), 74.3 (C-2''), 75.9 (C-3''), 70.2 (C-4''), 76.2 (C-2'''), 71.0 (C-3'''), 72.2 (C-4'''), 69.1 (C-5'''), 181.0 (C-6''') |
| 7 | Kaempferol-3-O-α-dirhamnoside-O-β-D-glucopyranosid | 177(C-4), 163 (C-7), 161 (C-9), 159.2 (C-2), 156.8 (C-4'), 156.5 (C-5), 132.2(C-3), 130.3(C-2'), 130.5 (C-6'), 120 (C-1'), 115 (C-3'), 114 (C-5'), 104.2 (C-10), 98.5(C-8), 93.5 (C-6), sugar moiety, 100.7 (C- 1''), 100.7 (C- 1''''), 98.3 (C- 1''), 77.4 (C-3'''), 77 (C-2''), 75.5 ( C-5''), 71,8(C-4''''), 71.7 (C-4'''), 70.6 (C-3'''), 70.52 (C-3''''), 70.4 (C-2'''), 70.3 (C-2''''), 70.2 (C-4''), 68.27(C-5'''), 68.25(C-5''''), 66.85 (C-6''), 17.9 (C-6''''), 17.2 (C-6''') |
| 8 | Kaempferol-3-rutinoside | 157.8(C-2), 135 (C-3), 180 (C-4), 162.2 (C-5), 166.8 (C-7), 95.5 (C-8), 105.2(C-10), 127.5(C-1'), 115.5 (C-2'), 150 (C-3'), 149 (C-4'), 114 (C-5'), 124.2 (C-6'), sugar moiety, 100.5 (C- 1''), 70.7 (C-3'''), 77.3 (C- 3''), 71.4 (C-4''), 76 (C-5''), 68.5 ( C-6''), 101,8(C-1'''), 71.5 (C-2'''), 72 (C-3'''), 73.5 (C-4'''), 69.4 (C-5'''), 17.9 (C-6''''). |
| 9 | Gallic acid | - |
| 10 | Ferulic acid | - |

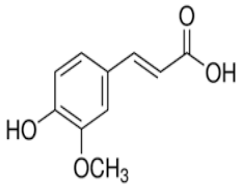
Flavonoid base structure




Quercetin: $R_1$=OH, $R_2$= H, X= OH  Luteolin: $R_1$=OH, $R_2$= H, X= H  Apigenin: $R_1$=H, $R_2$= H, X= H
Kaempferol: $R_1$= H, $R_2$= H, X= OH  Acacetin: $R_1$=H, $R_2$= H, X= H, OH (4') = $OCH_3$  Rutin: $R_1$=OH, $R_2$= H, X= O-rutinoside
Kaempferol-3-O-α-dirhamnoside-O-β-D- glucopyranoside = $R_1$= H, $R_2$= H, X= O- α-dirhamnoside-O-β-D-glucopyranoside.
kaempferol-3-rutinoside= $R_1$= H, $R_2$= H, X= O- rutinoside.




Gallic acid




Ferulic acid

## Conclusion

It was indicated from above concluded results, that the roots of *Euphorbia bivonae* contained the flavonoid compounds (quercetin, kaempferol, rutin, kaempferol-3-O-α-dirhamnoside-O-β-D- glucopyranoside and kaempferol-3-rutinoside) and two phenolic acid compounds (gallic acid and ferulic acid), which were also previously detected in the arial pats of the plant (Ibrahim, 2008). Meanwhile luteolin and apigenin and acacetin were detected only in the roots of *Euphorbia bivonae* only beside the other compounds which defined by HPLC.

Liu and Ng (2000), Halliwell and Gutteridge (1981), Keum *et al*. (2000) and Ghavami and Sajadi (2010) were demonstrated that, Kaempferol-3-O-rutinoside scavenging ability against the hydroxyl radical. Hydroxyl radicals are among the strongest free radicals; with damaging effects on living cells. They produce other kinds

of cell-damaging free radicals and oxidizing agents, which can attack DNA to cause strand scission. In biochemical systems, superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions such as iron or copper by UV photolysis. Simple derivatives of quercetin mono-glycosides such as 3-O-glucoside and 3-O-rhamnoside as well as diglycoside–rutin, have been best investigated to date.

A human body needs these substances to absorb and use vitamin C. Investigators have also found that quercetin 3-O-glucoside and rutin contribute to the relaxation of smooth muscles in mammals (Walton *et al*., 2006 and Materska, 2008 ). Apigenin has a variety of pharmacological activities, including antioxidant, anti-tumour, anti-inflammatory, anti-bacterial, anti-proliferative, oxygenase inhibitor induces apoptosis. It has protective effect on radiation-induced chromosomal damage in human lymphocytes (Patel *et al*., 2007 and Ghavami, 2008). Our concluded data indicated the importance of roots of *Euphorbia bivonae*.

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