CHEMICAL CONSTITUENTS OF ECHINOPS SPINOSISSIMUS TURRA.

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Abstract

Investigation of the primary products showed that the carbohydrates of Echinops spinosissimus, aerial parts were 23.23%, while the detected free and combined sugars were 9 free sugars and 13 combined sugars. The detected values of total nitrogen and total protein were 1.79% and 11.2%, respectively. Amino acid analyzer of total amino acids revealed the presence of 15 amino acids. The detected percentage of total lipid was 3.45%. Meanwhile GLC analysis revealed the presence of 6 saturated fatty acids and 2 unsaturated fatty acids. Further flavonoids of Echinops spinosissimus using HPLC revealed the presence of 22 compounds, with the major compounds Hesperidin (39.233 mg/100g), Hesperidin (34.589 mg/100g), Luteolin-6- arabinose-8- glucose (25.344 mg/100g), Apigenin-6- arabinose -8- glucose (23.049 mg/100g) and Apigenin-6- glucose -8- rhamnose (20.083 mg/100g). While on using paper and TLC chromatographers revealed the separation and identification of 5 pure flavonoid compounds (apigenin, kampferol, hespirtin, hespirtin and rutin) and 2 phenolic acids (gallic acid and ferulic acid).

Introduction:-

Echinops spinosissimus syn. Name Echinops viscosus DC. belong to family Asteraceae (Boulos, 1995). The genus Echinops belongs to family Asteraceae and comprises over 120 species of which five were known to grow in Egypt (Tåkholm, 1974). Previous chemical investigation on the genus Echinops demonstrated the presence of thiophenes (Hyeme et al., 2005; Koike et al., 1999; Nakano et al., 2011), quinoline alkaloids (Su et al., 2004), sesquiterpene hydrocarbons (Dawidar et al., 1990; Dong et al., 2008; El Sayed, 2001; Li et al., 2010) triterpenes (Metwally, 1987; Yasukawa et al., 1996), flavonoids (Ram et al., 1996; Singh et al., 2006), volatile oil (Papadopoulou et al., 2006) and lignans (Tene et al., 2004). Several members of genus Echinops showed hepatoprotective (Lin and Lin, 1993), anti-inflammatory (Yadava and Singh, 2006), anti-fungal (Fokialakis et al., 2006). Plants produce and store several carbohydrates, most of which must be regarded as primary metabolites. Several carbohydrates, such as glucose, galactose or fructose are used to form glycosides with secondary metabolism and are thus participants of both primary and secondary metabolism. In addition, a number of plants produce specific storage products, such as inulin in Asteraceae and Campanulaceae which can be used medicinally for patients with diabetes (Van Wyk and Wink, 2015). Fatty acids play a key role in metabolism: as a metabolic fuel, as a necessary component of all membranes, and as a gene regulator. In addition, fatty acids are frequently used in cosmetics such as soaps, fat emulsions and liposome (Rustan and Drevon, 2005). Echinops spinosissimus contained terpenoids, flavonoids and traces of alkaloids, on the other hand four flavonoids compounds (apigenin, hispidulin, 5,4dihydroxy flavone and apigenin 7-0 glucoside) were isolated and identified from the aerial parts of Echinops spinosissimus. These flavonoid compounds have been investigated as antiseptics against 4 bacterial strains, two pathogenic fungi strains and cotton leafworm (Hamed and Eisa, 2011). Abdallah and El-Hazali (2013) were examined the methanolic extract of Echinops spinosissimus against seven standard bacteria (Proteus vulgaris NCTC 8196, Escherichia coli ATCC 25922, Bacillus cereus NCTC 8236, Salmonella typhi NCTC 0650, Klebsiella pneumonia ATCC 53651, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923) and one standard fungus (Candida albicans ATCC 7596).
The phytochemical analysis showed presence of some active principles which correlates with the antimicrobial activity of plant extracts, hence we decide estimate and detect some active components of the plant.

**Materials and methods:-**

**Plant material:-**
*Echinops spinosissimus* aerial parts were collected from Mersa Maṭrūḥ, Egypt at March (2015). The aerial parts of *Echinops spinosissimus* were cleaned, dried in an oven at 40°C, ground to fine powder for investigations.

**Investigation of the primary product:-**
**Investigation of carbohydrates:-**
**Determination of total carbohydrates:** (Chaplin and Kennedy, 1994).
**Identification of free sugars and combined sugars** by HPLC according to Zielinski et al. (2014).

**Investigation of nitrogen:-**
**Determination of total nitrogen:** using Kjeldahl method (James, 1995).
**Determination of total protein:** (James, 1995).
**Investigation of total amino acids:** according to Csinos and Simon-Sarkadi (2002), using Amino Acid Analyzer.

**Investigation of lipids:-**
**Determination of total lipids:** Farag et al. (1986).
**Determination of saponifiable matter (fatty acids)**: They were determined using GLC according to Farag et al. (1986).

**Investigations of flavonoids:-**
**Qualitative and quantitative analysis of the flavonoids by HPLC:**
The ethanol extracts of *Echinops spinosissimus* were analyzed 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 35°C.

Flavonoid separation was done by ODS-3 (4.0 × 150 nm, 3µm) column with a C-18 guard column, with temperature set at 35°C. 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 height (EC). (Mattila et al., 2000).

**Separation and identification of flavonoid and phenolic compounds**: (Radwan and Hassan, 2006)
About 2 kg of defatted dried powder of *Echinops spinosissimus* was macerated with 80% ethanol. The combined aqueous alcoholic extracts were evaporated in vacuo 45°C. The residue (53 g) was dissolved in hot distilled water (400 ml) and left over night. The aqueous filtrate was extracted with successive portions of chloroform (3 x 300 ml), followed by ethyl acetate (3 x 300 ml) and finally with n-butanol (3 x 300 ml). Each fraction was subjected to preparative paper chromatography [3MM, Butanol: Acetic acid: Water (BAW) 4:1:5]. The chloroform fraction gave bands (Rf 0.78 and Rf 0.88) were cut and eluted separately by 70% methanol, while the ethyl acetate fraction gave bands (Rf 0.87 and 0.88) were cut and eluted separately by 70% methanol. On other hand other impure bands were further purified using preparative Thin Layer Chromatography (TLC) with methanol/ chloroform (9.5:0.5), where the flavonoidal zones (Rf 0.71 and 0.72) was cut and eluted by 70% methanol . The butanol fraction gave band ( Rf 0.54 at BAW 4:1:5) were cut and eluted separately by 70% methanol.

**Results and discussion:-**
**Investigation of the primary product:-**
**Investigation of carbohydrates:-**
**Carbohydrates content:** The estimated percentage of total carbohydrates was 23.23%.
**Investigation of free sugars:** The separation of the free sugars contents achieved using High Pressure Liquid Chromatography (HPLC), where nine of free sugars were detected. It was noticed that, the highest concentration of the separated free sugars was inulin (22.06%) (Table 1).
**Investigation of combined sugars:** The separation of the hydrolyzed combined sugars were achieved using HPLC, where thirteen of combined sugars were detected. The highest percentage of the separated sugars was that of fructose (0.78%) Table (1).

| No. | Sugars       | Free sugar (%) | Combined sugar (%) |
|-----|--------------|----------------|-------------------|
| 1   | Inulin       | 22.06          | 0.45              |
| 2   | Glucuronic   | 0.13           | 0.12              |
| 3   | Stachyose    | -              | 0.23              |
| 4   | Galacturonic | 0.16           | 0.30              |
| 5   | Sucrose      | 0.04           | 0.33              |
| 6   | Maltose      | -              | 0.22              |
| 7   | Glucose      | 0.49           | 0.32              |
| 8   | Xylose       | 0.74           | 0.11              |
| 9   | Galactose    | -              | 0.15              |
| 10  | L- Rhaminose | -              | 0.25              |
| 11  | Fructose     | 1.98           | 0.78              |
| 12  | Sorbitol     | 0.01           | 0.13              |
| 13  | Ribose       | 0.02           | 0.03              |

**Investigation of nitrogen:**

**Total nitrogen and protein contents:** The estimated percentage of the total nitrogen was 1.79%, while the percentage of total protein was 11.2%.

**Investigation of total amino acids:** The investigation of hydrolyzed protein-amino acids, achieved using amino acid analyzer, where fifteen amino acids of different types were detected. The highest percentage of the separated amino acids was that of lysine (27.77%) (Table 2).

| No. | Compound name | Total amino acids (%) | No. | Compound name | Total amino acids (%) |
|-----|---------------|-----------------------|-----|---------------|-----------------------|
| 1   | Asparagine    | 10.43                 | 9   | Methionene    | 0.71                  |
| 2   | Threonine     | 1.98                  | 10  | Isoleucine    | 2.81                  |
| 3   | Serine        | 4.02                  | 11  | Leucine       | 6.75                  |
| 4   | Glutamine     | 10.36                 | 12  | Tyrosine      | 4.21                  |
| 5   | proline       | 0.35                  | 13  | Phenylalanine | 3.21                  |
| 6   | Glycine       | 10.30                 | 14  | Histidine     | 3.78                  |
| 7   | Alanine       | 8.10                  | 15  | Lysine        | 27.77                 |
| 8   | Valine        | 5.20                  |     |               |                       |

**Investigation of lipids:**

**Total lipids content:** The estimated percentage of total lipids of the aerial parts of *Echinops spinosissimus* was 3.45%.

**Investigation of saponifiable matter (fatty acids):** The fatty acid contents of the lipids were determined using GLC technique, where the obtained results revealed the presence of six saturated fatty acids and two unsaturated fatty acids with different range of concentrations. Pentadecylic acid (33.76%) and Palmitic acid (30.86%) were the highest percentages of fatty acids, while Lauric acid was the lowest percentage (2.01%) in the plant (Table 3).
Table 3: Relative percentage of fatty acids.

| Compound name     | No. of carbon atom | Fatty acids (%) |
|-------------------|--------------------|-----------------|
| Lauric acid       | C12:0              | 2.01            |
| Myristic acid     | C14:0              | 4.02            |
| Pentadecylic acid | C15:0              | 33.76           |
| Palmitic acid     | C16:0              | 30.86           |
| Stearic acid      | C18:0              | 5.21            |
| Myristic acid     | C14:0              | 4.02            |
| Oleic acid        | C18:1              | 3.94            |
| Linoleic acid     | C18:2              | 4.12            |

Qualitative and quantitative analysis of the flavonoids by HPLC
Investigation of flavonoids by HPLC revealed the presence of 22 compounds of the aerial parts of Echinops spinosissimus, where the major compounds were Hespírtin (39.233 mg/100g), Hesperidin (34.589 mg/100g), Luteolin-6- arabinose-8- glucose (25.344 mg/100g), Apigenin-6- arabinose -8-glactose (23.049 mg/100g) and Apignin-6- glucose -8- rhamnose (20.083 mg/100g) (Table 4).

Table 4: HPLC analysis of the flavonoids of Echinops spinosissimus aerial parts.

| No. | Flavonoids                        | Mg/100g  |
|-----|-----------------------------------|----------|
| 1   | Luteolin-6- arabinose-8- glucose  | 25.344   |
| 2   | Luteolin-6- glucose -8- arabinose | 2.004    |
| 3   | Apigenin-6- arabinose -8-glactose | 23.049   |
| 4   | Apignin-6- rhamnose -8- glucose   | 0.743    |
| 5   | Apignin-6- glucose -8- rhamnose   | 20.083   |
| 6   | Luteolin-7- glucose               | 4.189    |
| 7   | Naringen                          | 12.89    |
| 8   | Rutin                             | 2.326    |
| 9   | Hesperidin                        | 34.589   |
| 10  | Quercetin-3-O-glucoside           | 0.724    |
| 11  | Rosmarinic                        | 0.898    |
| 12  | Apigenin-7-O- neohespiroside      | 0.480    |
| 13  | Kampferol-3,7-dirhamoside         | 1.424    |
| 14  | apigenin-7- glucose               | 3.902    |
| 15  | Quercetin                          | 1.262    |
| 16  | Quercetin                          | 0.604    |
| 17  | Naringen                          | 1.154    |
| 18  | Hesperidin                        | 39.233   |
| 19  | Kampferol                          | 0.336    |
| 20  | Rhamnetin                         | 0.468    |
| 21  | Apignin                           | 0.360    |
| 22  | Acacetin                          | 10.168   |

Identification of phenolic acid and flavonoid compounds:
Two phenolic acids were isolated from the chloroform fraction, while four flavonoid compounds were isolated from the ethyl acetate fraction and one flavonoid glycoside was isolated from the butanol fraction.

Gallic acid: Rf 0.78; isolated from the chloroform fraction by preparative PC. (3MM, BAW) and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 90% methanol a single phenolic acid compound (no.1) (Table 8) corresponding that of gallic acid (13 mg) which identified by PC, UV (Table 5) and ¹H-NMR (Table 6).

Ferulic acid: Rf 0.88; isolated from the chloroform fraction by preparative PC. (3MM, BAW) and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 90% methanol a single phenolic acid compound (no. 2) (Table 8) corresponding that of ferulic acid (12 mg) which identified by PC, UV (Table 5), ¹H-NMR (Table 6) and MS [M+ 194 and fragments at m/z 179 and 77].
Apigenin: Rf 0.87; isolated from the ethyl acetate fraction by preparative PC. (3MM, BAW) and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no.3) (Table 8) corresponding to that of apigenin (13 mg) which was identified by PC, UV (Table 5) and El-mass [M+ 270 and fragments at m/z 242, 153, 121 and 118]. Further confirmation was performed by caring out 1H-NMR (Table 6) and the results were agreement with the reported data of apigenin (Mabry, et al., 1970).

Kaempferol: Rf 0.88; isolated from the ethyl acetate fraction by preparative PC. (3MM, BAW) and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no.4) (Table 8) corresponding to that of kaempferol (14 mg) which identified by PC, UV (Table 5) and Ms [M+ 286 and fragments at m/z 285, 258, 229,121 and 93]. Further confirmation was performed by caring out 1H-NMR (Table 6) and the results were agreement with the reported data of kaempferol (Mabry, et al., 1970).

Hesperitin: Rf 0.71; isolated from the ethyl acetate fraction by preparative TLC. [methanol/ chloroform (9.5:0.5)] and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 60% methanol a single flavonoidal compound (no.5) (Table 8) corresponding to that of flavonoid glycoside (15 mg) (Harborne, 1984). Complete acid hydrolysis gave an hesperitin : and two sugar residues identified as glucose and rhamnose (comparative Rf – values with authentic markers). UV spectral data at Table (5) showed that the compound is a flavanone with 7-OH substitution. The remaining UV spectral data were found to be similar to that of hesperitin type compound. UV analysis (Table 5), 1H-NMR (Table 6) and 13C-NMR (Table 7).

Hesperidin: Rf 0.72; isolated from the ethyl acetate fraction by preparative TLC. [methanol/ chloroform (9.5:0.5)] and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 60% methanol a single flavonoidal compound (no.6) (Table 8) corresponding to that of hesperitin (17 mg) (Harborne, 1984). UV analysis (Table 5), 1H-NMR (Table 6) and 13C-NMR (Table 7).

Rutin (quercetin-3-O-α-L-rhamnose (1-6) β-D-glucoside): Rf 0.54; isolated from the butanol fraction by preparative PC. (3MM, BAW) and eluted by 70% methanol, gave after purification on Sephadex LH-20 column using 60% methanol a single flavonoidal compound (no.7) (Table 8) corresponding to that of flavonoid glycoside (14 mg) (Harborne, 1984). Complete acid hydrolysis gave an aglycone quercetin and two sugar residues identified as glucose and rhamnose (comparative Rf – values with authentic markers). UV spectral data at Table (5) showed that the compound is a flavanol with 3-OH substitution. The remaining UV spectral data were found to be similar to that of quercetin type compound. Mass spectrum revealed M/Z (rel-int %): 610 (M*, glucose, 60%), M/Z 464 (M*, rhamnose, 191.1%), 302 (quercetin, 100%). UV analysis (Table 5), 1H-NMR (Table 6) and 13C-NMR (Table 7).

Table 5: Ultra-violet spectral data (nm) of the isolated flavonoid and phenolic acid compounds.

| No. | Compounds          | MeOH | NaOMe | NaOAc | NaOAc + H3BO3 | AlCl3 | AlCl3 + HCl |
|-----|--------------------|------|-------|-------|---------------|-------|-------------|
| 1   | Gallic acid        | 270,335 | 273,346 |       |               |       |             |
| 2   | Ferulic acid       | 285,314 | 251 (sh), 288,320 |       |               |       |             |
| 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          | 268,342 (sh), 367 | 281,318, 421 | 257,302 (sh), 384 | 268,296 (sh), 320,372 | 267,305 (sh), 350,422 | 266,305 (sh), 350,422 |
| 5   | Hesperidin         | 290,315, 345 | 245,292,354 | 292,320 | 292,320 | 300,380 | 304,374 |
| 6   | Hesperitin         | 283,310 (sh), 345 | 240,295, 355 | 292,320 | 292,320 | 300,380 | 304,374 |
| 7   | 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 |
Table 6: ¹H-NMR spectral data of the isolated flavonoid and phenolic acid compounds.

| No. | Compounds       | δ (ppm)                     |
|-----|-----------------|-----------------------------|
| 1   | Gallic acid     | 6.97 (s, H2, H6)            |
| 2   | 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.OCH3). |
| 3   | Apigenin        | 7.92 (d, J=7.5 Hz Z, H6'), 6.93 (d, J=7.5 Hz Z, H3', H5'), 6.75 (s, H3), 6.54 (d, J=2.5 Hz, H8) and 6.14 (d, J=2.5 Hz, H6). |
| 4   | Kampferol       | 8.2 (2H, d, J=8 Hz, H-2^ & H-6^), 7.1 (2H, d, J=8 Hz, H-3^ and H-5^), 6.4 (1H, d, J=2.5 Hz, H-8), 6.2 (1H, d, J=2.5 Hz, H-6). |
| 5   | Hesperidin      | 12.26 (1H, br, s, 5-OH), 6.95 (1H, d, J=2.0 Hz, H-2'), 6.89 (1H, J=8.0 Hz, H-5'), 6.83 (1H, dd, J=8.0, 2.0 Hz, H-6'), 6.14 (1H, d, J=2.0 Hz, H-8), 6.13 (1H, d, J=2.0 Hz, H-6), 5.50 (1H, dd, J=11.0, 5.0 Hz, H-2), 4.97 (1H, d, J=7.2 Hz, H-1'), 4.54 (1H, br, s, H-1), 3.78 (3H, s, 4-OCH3), 3.20–3.60 (6H, m, H-2^ to H-6^), 3.20–3.60 (3H, m, H-2 to H-6), 3.11 (1H, dd, J=17.0, 11.0 Hz, H-3a), 2.76 (1H, dd, J=17.0, 5.0 Hz, H-3b), 2.54 (1H, d, J=6.0 Hz, H-5), 1.08 (3H, d, J=6.0 Hz, H-6). |
| 6   | Hesperitin      | 3.01 (1H, br, s, 5-OH), 8.01 (2H, dd, J=9.0, 2.0 Hz, H-6' and H-2'), 7.27 (2H, dd, J=9.0, 2.0 Hz, H-3' and H-5'), 7.01 (1H, s, H-3), 6.82 (1H, d, J=2.0 Hz, H-8), 6.37 (1H, d, J=2.0 Hz, H-6), 5.05 (1H, J=7.2 Hz, H-1'), 4.60 (1H, br, s, H-1), 3.91 (3H, s, 7-OCH3), 3.21–3.60 (6H, m, H-2' to H-6'), 3.21–3.60 (3H, m, H-2 to H-6), 2.50 (1H, d, J=6.0 Hz, H-5), 1.13 (3H, d, J=6.0 Hz, H-6). |
| 7   | Rutin           | 7.8 (1H, d, J=2.5 Hz, H2), 7.5 (1H, dd, J=8.5, 2.5Hz, 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, CH3 rhamnose). |

Table 7: ¹3C-NMR spectral data of the isolated flavonoid and phenolic acid compounds.

| No. | Compounds       | δ (ppm)                     |
|-----|-----------------|-----------------------------|
| 1   | Gallic acid     | 196.7 (s, C-4), 166.2 (s, C-7), 164.2 (s, C-5), 161.5 (s, C-9), 148.4 (s, C-4'), 146.9 (s, C-3'), 132.1 (s, C-1'), 118.8 (s, C-6'), 114.8 (d, C-2'), 112.4 (d, C-5'), 103.5 (s, C-10), 100.8 (d, C-1), 99.6 (d, C-1''), 96.1 (d, C-6), 95.5 (d, C-8), 79.5 (d, C-2), 76.5 (d, C-5'), 75.9 (d, C-3'), 73.2 (d, C-4), 72.2 (d, C-2''), 70.9 (d, C-4''), 70.5 (d, C-3), 69.7 (d, C-2), 68.4 (d, C-5), 66.2 (t, C-6'), 54.9 (q, 4-OCH3), 42.5 (t, C-3), 18.2 (q, C-6). |
| 2   | Ferulic acid    | 187.3 (s, C-4), 165.2 (s, C-7), 164.2 (s, C-2), 162.7 (s, C-9), 161.4 (s, C-5), 157.5 (s, C-4'), 128.8 (d, C-2' and C-6'), 124.0 (s, C-1'), 115.6 (d, C-5' and C-3'), 105.7 (s, C-10), 104.1 (d, C-3), 100.8 (d, C-1''), 100.1 (d, C-1), 99.9 (d, C-6), 95.1 (d, C-8), 76.9 (d, C-5'), 75.9 (d, C-3'), 73.4 (d, C-4), 72.3 (d, C-2''), 71.0 (d, C-4''), 70.6 (d, C-3), 69.9 (d, C-2), 68.6 (d, C-5), 66.4 (t, C-6'), 54.9 (q, 7-OCH3), 18.2 (q, C-6). |
| 3   | Apigenin        | 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'), 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''). |
| 4   | Kampferol       | 196.7 (s, C-4), 166.2 (s, C-7), 164.2 (s, C-5), 161.5 (s, C-9), 148.4 (s, C-4'), 146.9 (s, C-3'), 132.1 (s, C-1'), 118.8 (s, C-6'), 114.8 (d, C-2'), 112.4 (d, C-5'), 103.5 (s, C-10), 100.8 (d, C-1), 99.6 (d, C-1''), 96.1 (d, C-6), 95.5 (d, C-8), 79.5 (d, C-2), 76.5 (d, C-5'), 75.9 (d, C-3'), 73.2 (d, C-4), 72.2 (d, C-2''), 70.9 (d, C-4''), 70.5 (d, C-3), 69.7 (d, C-2), 68.4 (d, C-5), 66.2 (t, C-6'), 54.9 (q, 4-OCH3), 42.5 (t, C-3), 18.2 (q, C-6). |
Table 8: The structure of the isolated flavonoid and phenolic acid compounds.

| No. | Compounds     | Structure       |
|-----|---------------|-----------------|
| 1   | Gallic acid   | ![Gallic acid](image) |
| 2   | Ferulic acid  | ![Ferulic acid](image) |
| 3   | Apigenin      | ![Apigenin](image) |
| 4   | Kampferol     | ![Kampferol](image) |
| 5   | Hesperidin    | ![Hesperidin](image) |
| 6   | Hesperitin    | ![Hesperitin](image) |
| 7   | Rutin         | ![Rutin](image) |

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