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RESEARCH ARTICLE

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 Hespirtin (39.233 mg/100g), Hespiridin (34.589 mg/100g), Luteolin-6- arbinose-8- glucose (25.344 mg/100g), Apigenin-6- arbinose -8- glactose (23.049 mg/100g) and Apignin-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, hespiridin, 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 (Tâkholm, 1974). Previous chemical investigation on the genus *Echinops* demonstrated the presence of thiophenes (Hymete 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 arial parts of *Echinops spinosissium*. 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-Ghazali (2013) were examined the methanolic extract of *Echinops spinosissium* 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 Mattruh, 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 Csomos 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 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 hight (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 (R_f 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 (R_f 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).

Table 1:- Relative percentage of free and combined sugars.

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).

Table 2:- Relative percentage of total amino acids.

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
Arachidic acid	C20:0	16.03
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 Hespirtin (39.233 mg/100g), Hespiridin (34.589 mg/100g), Luteolin-6- arbinose-8- glucose (25.344 mg/100g), Apigenin-6- arbinose -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- arbinose-8- glucose	25.344
2	Luteolin-6- glucose -8- arbinose	2.004
3	Apigenin-6- arbinose -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	Narengin	12.89
8	Rutin	2.326
9	Hespiridin	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	Quercetrin	1.262
16	Quercetin	0.604
17	Naringenin	1.154
18	Hespirtin	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:- R_f 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 ^{1}H-NMR (Table 6).

Ferulic acid:- R_f 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), ^{1}H-NMR (Table 6) and MS [M^+ 194 and fragments at m/z 179 and 77].

Apigenin:- R_f 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 ^{1}H-NMR (Table 6) and the results were agreement with the reported data of apigenin (Mabry, et al., 1970).

Kaempferol:- R_f 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 ^{1}H-NMR (Table 6) and the results were agreement with the reported data of kaempferol (Mabry, et al., 1970).

Hesperidin:- R_f 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 R_f – 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), ^{1}H-NMR (Table 6) and ^{13}C-NMR (Table 7).

Hesperitin:- R_f 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), ^{1}H-NMR (Table 6) and ^{13}C-NMR (Table 7).

Rutin (quercetin-3-O-α L-rhamnoside (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 R_f – values with authentic markers). UV spectral data at Table (5) 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 revealed M/Z (rel-int %): 610 (M^+, glucose, 60%), M/Z 464 (M^+, rhamnose, 191.1%), 302 (quercetin, 100%). UV analysis (Table 5), ^{1}H-NMR (Table 6) and ^{13}C-NMR (Table 7).

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

No.	Compounds	Reagents					
		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

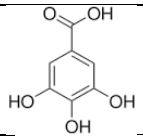
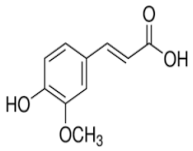
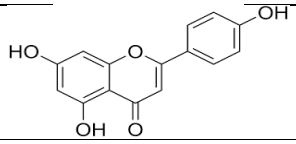
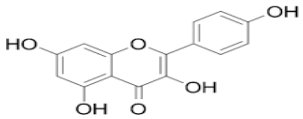
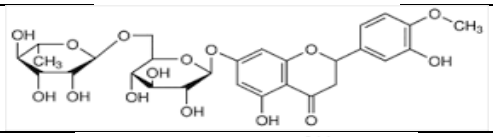
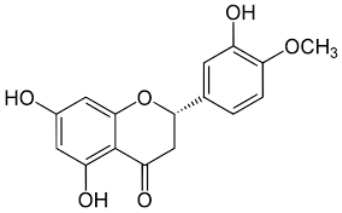
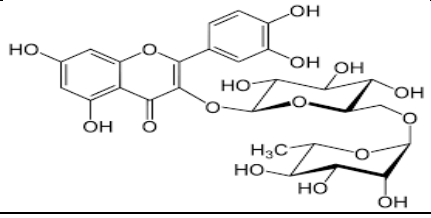
Table 6:- ^{1}H-NMR spectral data of the isolated flavonoid and phenolic acid compounds.

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,OCH_3).
3	Apigenin	7.92 (d, J=7.5 H_Z,H2, H6`), 6.93 (d, J=7.5 $H_Z$, H3`, H5`), 6.75 (s, H3), 6.54 (d, J=2.5 H_Z, H8) and 6.14 (d, J =2.5 H_Z H6).
4	Kampferol	8.2 (2H,d, J=8 H_Z, H-2` and H-6`), 7.1 (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	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	13.01 (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, 2.5H_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).

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

No.	Compounds	δ (ppm)
1	Gallic acid	----------
2	Ferulic acid	----------
3	Apigenin	----------
4	Kampferol	----------
5	Hesperidin	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).
6	Hesperitin	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).
7	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'), 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'").

Table 8:- The structure of the isolated flavonoid and phenolic acid compounds.

No.	Compounds	Structure
1	Gallic acid	
2	Ferulic acid	
3	Apigenin	
4	Kampferol	
5	Hesperidin	
6	Hesperitin	
7	Rutin	

References:-

1. Abdallah, E. and El-Ghazali, G. (2013): Screening for antimicrobial activity of some plants from Saudi folk medicine. Global J Res. Med. Plants & Indigen. Med. 2(4): 210–218.
2. Boulos, L. (1995): Flora of Egypt Checklist. Al Hadara publishing. Cairo, Egypt, p 69.
3. Chaplin, M. F. and Kennedy, J. F. (1994): Carbohydrate Analysis. A Practical Approach. Oxford University Press, Oxford, New York, Tokyo, 2nd Ed, pp. 324.
4. Csomos, E. and Simon-Sarkadi, L. (2002): Characterisation of tokaj wines based on free amino acid and biogenic amine using ion-exchange chromatography. Chromatographia Supplement. 56:185−188.
5. Dawidar, A. M., Metwally, M. A., Abou-Elzahab, M. and Abdel-Mogib, M. (1990): Sesqui-terpene lactones from *Echinops spinosissimus.* Pharmazie 45, 70–71.
6. Dong, M., Cong, B., Yu, S. H., Sauriol, F., Huo, C. H., Shi, Q. W., Gu, Y. C., Zamir, L .O. and Kiyota, H. (2008): Echinopines A and B: sesquiterpenoids possessing an unprec-edented skeleton from *Echinops spinosus.* Org. Lett. 10, 701–704.
7. El Sayed, K.A. (2001): Pseudoguaiane sesquiterpene xylopyranoside from *Echinops* hussoni. Pharmazie 56, 415–417.
8. Farag, R. S., Ahmed, A. I., Rashad, S. E. and Ewies, M. W. (1986): Unsaponifiable matter of six pollens collected by honey bees in Egypt. J. Agric. Res., 19(4): 52-58.

9. Fokialakis, N., Cantrell, C. L., Duke, S. O., Skaltsounis, A. L. and Wedge, D. E. (2006): Antifungal activity of thiophenes from Echinops ritro. J. Agric. Food Chem. 54, 1651–1655.
10. Hamed, H. B. and Eisa, E. S. (2011): Biochemical studies on *Echinops spinosissimus*. plant. Journal of Agricultural Science,31:969-81.
11. Harborne, J. B. (1984): Phytochemical Methods. published in U.S.A by Chapman and Hall London, 287 pp.
12. Hymete, A., Rohloff, J., Kjøsen, H. and Iversen, T. H. (2005): Acetylenic thiophenes from the roots of *Echinops ellenbeckii* from Ethiopia. Nat. Prod. Res. 19, 755–761.
13. James, C. S. (1995): Analytical Chemistry of Foods. Blackie Academic and Professional Publisher, an imprint of Chapman and Hall, pp. 178.
14. Koike, K. K., Jia, Z. H., Nikaido, T., Liu, Y., Zhao, Y. Y. and Guo, D. A. (1999): Echinothiophene, a novel benzothiophene glycoside from the roots of *Echinops grijissii*. Org. Lett. 1, 197–198.
15. Li, L.B., Ren, J., Cheng, Z. M. and Zhu, H. J. (2010): Three new sesquiterpenoids from *Echinops ritro* L. Helv. Chim. Acta 93, 1344–1349.
16. Lin, C. C. and Lin, C. H. (1993): Pharmacological and pathological studies on Taiwan folk medicine (IX): the hepatoprotective effect of the methanolic extract from *Echinops Grijisii*. Am. J. Chin. Med. 21, 33–44.
17. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970): The Systematic Identi0fication of Flavonoids. Springer-Verlag, New York, Heidlberg, Berlin, pp 354.
18. Mattila, P., Astola, J. and Kumpulainen, J. (2000): Determination of flavonoids in plant material by HPLC with diode-array and electro- array detections. J. Agric. Food Chem.48: 5834-5841.
19. Metwally, M.A. (1987): Egyptian *compositae*. Part 10. Triterpenes from *Echinops spinosissimus*. Pak. J. Sci. Ind. Res. 30, 18–19.
20. Nakano, H., Cantrell, C. L., Mamonov, L. K., Osbrink, W. L. and Ross, S. A.(2011): *Echinops acetylenes* A and B, new thiophenes from *Echinops transiliensis*. Org. Lett. 13, 6228–6231.
21. Papadopoulou, P., Couladis, M. and Tzakou, O. (2006): Essential oil composition of two Greek *Echinops* species: *Echinops graecus* Miller and *Echinops ritro* L. J. Essent. Oil Res. 18, 242–243.
22. Radwan, H. M. and Hassan, R. A. (2006): The flavonoids, lipids and protein contents of *Limoniastrum monopetatum* L. growing in Egypt and their biological activity. Bull. Fac. Pharm. Cairo Univ., 44 (1): 93-101.
23. Ram, S. N., Roy, R., Singh, B., Singh, R. P. and Pandey, V. B. (1996): An acylflavone glucoside of *Echinops echinatus* flowers. Planta Med. 62, 187.
24. Rustan, A. C. and Drevon, C. A. (2005): Fatty Acids: Structures and Properties. Encyclopedia of life Science.1-7.
25. Singh, S., Upadhyay, R. K., Pandey, M. B., Singh, J. P. and Pandey, V. B. (2006): Flavonoids of *Echinops echinatus*. J. Asian Nat. Prod. Res. 8, 197–200.
26. Su, Y. F., Luo, Y., Guo, C. Y. and Guo, D. A. (2004): Two new quinoline glycoalkaloids from *Echinops gmelinii*. J. Asian Nat. Prod. Res. 6, 223–227.
27. Tâckholm, V. 1(974): Students Flora of Egypt. 2nd Ed. Published by Cairo University, Printed by Cooperative printing compnay Beirat, pp. 888.
28. Tene, M., Tane, P., Sondengam, B. L. and Connolly, J. D. (2004): Lignans from the roots of *Echinops giganteus*. Phytochemistry 65, 2101–2105.
29. Van Wyk, B. E. and Wink, M. (2015): Phytomedicines, Herbal drugs and Poisons; Briza, Kew Publishing, Cambridge University Press: Cambridge, UK.
30. Yadava, R. N. and Singh, S. K. (2006): New anti-inflammatory active flavanone glycoside from the *Echinops echinatus* Roxb. Indian J. Chem. B 45, 1004–1008.
31. Yasukawa, K., Akihisa, T., Oinuma, H., Kasahara, Y., Kimura, Y., Yamanouchi, S., Kumaki, K., Tamura, T. and Takido, M. (1996): Inhibitory effect of di- and trihydroxy triterpenes from the flowers of *Compositae* on 12-O-tetradecanoylphorbol-13- acetate-induced inflammation in mice. Biol. Pharm. Bull. 19, 1329–1331.
32. Zielinski, A. A. F., Braga, C. M. I., Demiate, M., Beltrame, F. L., Nogueria, A. and Wosiacki, G. (2014): Development and optimization of a HPLC-RI method for the determination of major sugars in apple juice and evaluation of the effect of the ripening stage. Food Sci. Technology, 34(1): 38-43.