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ORIGINAL ARTICLE

Phytochemical and biological studies of *Sisymbrium irio* L. Growing in Saudi Arabia

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KEYWORDS

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Sisymbrium irio;
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Apigenin;
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Abstract Ten flavonoids were isolated for the first time from the aerial parts of *Sisymbrium irio* L. grown in Saudia Arabia, using chromatographic methods, and identified as apigenin, apigenin-7-galactoside, apigenin-7-*O*-β-D-glucoside, luteolin-7-*O*-glucoside, apigenin-7-di-glucoside 5, apigenin-7-*O*-(6″acetyl) glucoside, apigenin-7-*O*-gluco(6″-1‴) rhamnoside, apigenin-7-*O*-gluco (6″-1‴)rhamnoside-5-methoxide, kampferol and kampferol-3-xyloside-7-galactoside. The median lethal dose of the extract was determined in mice and found to be greater than 5000 mg/kg body weight, suggesting that this plant is non-toxic. Antioxidant activities were assessed for all extracts and fractions (total, ether, chloroform, ethyl acetate and butanol) and isolated compounds, and showed variable antioxidant activity, with apigenin-7-*O*-galactoside being the most potent.

1. Introduction

Family cruciferae is one of the most wide spread plant kingdom. Most of the plants belonging to this family are of economical importance, being either very common food (Care, 1955) plants or constitute important articles in oil (Winton, 1946) production. Moreover, many members of the family have wide applications in folk medicine. *Sisymbrium* species is one of cruciferae members which have many uses in folk medicine in a treatment of inflammation and rheumatoid (Bolus, 1983). By reviewing the literature this genus was found to contain flavonoids (Khan et al., 1991; Itziar et al., 1982; Rizk et al., 1986; Lockwood and Fsharypuor, 1986), alkaloids, anthraquinones (Arayno and Zafor, 1983), oils, steroids (Soulier, 1994) and glycosides (Krets et al., 1987). Some workers mentioned that *Sisymbrium irio* has antipyretic, analgesic and anti-microbial activities (Vohora et al., 1980), while *Sisymbrium officinale* has a role in treatment of voice disorders (Mayer et al., 1994). On the other hand *Sisymbrium thellungii* showed antioxidant activity (Lindsey et al., 2002).

Sisymbrium irio L. (cruciferae) is widely distributed in Saudia Arabia (Migahed, 1996; Collenete, 1999). The seeds were used as expectorant and as febrifuge (Ghazanfar, 1994). Reviewing the current literature, few reports were found dealing with *Sisymbrium irio*. Some worker studied the fixed oil percent, fatty acid and oil composition of the seeds (Rauf and Ahmad, 2004; Al-Qudah and AbuZarga, 2010). Three

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flavonoids and two sitosterols were isolated from seeds and aerial parts of *Sisymbrium irio* L., (Khan et al., 1991). It was reported that *Sisymbrium irio* L. could be used for dietary purposes; due to the amount and diversity of nutrients and protein (35%) content (Guil et al., 1998). The ethanol extract from the seeds of *Sisymbrium irio* L. exhibited significant antipyretic and analgesic activities in addition to marked antibacterial action with no effect against fungi.

Due to the high nutritional value and the use of *Sisymbrium irio* L. in folk medicine with no published data about the Saudi Arabia species, we found that it's worthy to carry out phytochemical and pharmacological study on it.

2. Materials and methods

2.1. Plant

The aerial part of *Sisymbrium irio* L. was collected from the Najed Region during April 2008. Identified by Professor K. Al-Farhan, Professor of Botany, Botany Dept., Faculty of Science, KS University and by comparison with plant description in Flora of Saudi Arabia (Migahed, 1996; Collenete, 1999). A voucher specimen of the titled plant was kept in the herbarium of the Botany Dept. Faculty of Science. Plant sample was air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored for phytochemical and biological studies.

2.2. Phytochemical screening

Powdered samples of the aerial parts of *Sisymbrium irio* were subjected to preliminary phytochemical screening (Awaad, 2009).

2.3. Extraction

One kg of the air dried powder of the aerial parts of *Sisymbrium irio* were extracted by percolation in 90% ethanol at room temperature for two days. The ethanol extract was filtered and the residues were re-percolated four times. The total ethanol extract was concentrated under reduced pressure at a temperature not exceeding 35 °C to yield a dry extract of 190 g.

The total ethanol extract (50 g) was dispersed in 200 ml of distilled water and extracted successively with diethyl ether, chloroform, ethyl acetate, and *n*-butanol, respectively. Each extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure at a temperature not exceeding 35 °C to yield 8.5, 10.6, 15.6 and 19 g dry extracts, respectively.

2.4. Isolation of the phenolic compounds

TLC examination of the different extractives (Stahl, 1969) using two different solvent systems (chloroform–methanol (a) 98:2 and ethyl acetate–methanol–water (b) 60:5:4) and spray reagents revealed that both ether and chloroform extracts are identical (same numbers and color of spots) in addition both extracts exhibit the higher activity as hepatic protective. Therefore both extracts were collected together (15 g), fractionated on column packed with silica gel (450 g) and eluted gradually with benzene–ethyl acetate, 120 fractions were collected (60 ml each) and reduced to three main fractions, these sub-fractions were reapplied on preparative thin layer chromatography using system a. For final purifications, each compound was re-applied on column packed with Sephadix LH20 and eluted with methanol. The fractions were concentrated under reduced pressure to produce compounds **1**–**5** and **9**. Hepatoprotection was achieved in rats following ethyl acetate and butanol extracts medication but in less significant, accordingly they were subjected to fractionation as follows.

The ethyl acetate and butanol were found to be identical adopting solvent systems (ethyl acetate–methanol–water 30:5:4) (d), visualized by UV before and after spraying with aluminum chloride, accordingly. Both of them were collected together (20 g) and fractionated on column packed with silica gel (600 g) and eluted with ethyl acetate–methanol–water (120:5:4), 100 fractions were collected (50 ml each) and reduced to three main fractions in different yields 3.4, 2.7 and 1.6, respectively (according to the number, color and R_f values of the spots), these sub-fractions were reapplied on preparative paper chromatography using system acetic acid–water (85:15). For final purifications each compound was reapplied on column backed with sephadix LH20 and eluted with methanol. The fractions were concentrated under reduced pressure to produce compounds **6**–**8** and **10**.

2.4.1. Apigenin (*1*)

(33 mg), yellow crystals, R_f = 0.9 (system a) and 0.058 (system b), mp 348–349 °C. UV λ_{max} in MeOH: (nm) 266, 335; ($AlCl_3$) 277, 302, 348, 384; ($AlCl_3$/HCl) 277, 302, 338, 384; (NaOAc) 272, 300, 373; (NaOAc/H_3BO_3) 270, 300, 340; (NaOMe) 275, 321, 390. 1H NMR (DMSO-d_6) δ (ppm): 7.93 (1H, d, J = 8 Hz, H2′ & H6″), 6.92 (2H, d, J = 8 Hz, H3′ and H5′), 6.77 (1H, d, J = 2.5 Hz, H8), 6.47 (1H, S, H3), 6.21 (1H, d, J = 2.5 Hz, H6). ^{13}C NMR (DMSO): δ (ppm): 182.21 (C-4); 164.19 (C-2); 163.31 (C-7); 161.95 (C-5); 161.79 (C-4′); 157.86 (C-9); 128.98 (C-2′ & C-6′); 121.63 (C-1′); 116.51 (C-3′ & C-5′); 105.83 (C-10); 103.30 (C-3); 99.89 (C-6); 94.55 (C-8).

2.4.2. Apigenin-7-galactoside (*2*)

(25 mg) was obtained as yellow crystals with R_f = 0.71 (system a) and 0.22 (system b), mp 267–268 °C. UV λ_{max} in MeOH: (nm) 269, 335; ($AlCl_3$) 276, 302, 348, 388; ($AlCl_3$/HCl) 277, 300, 342, 384; (NaOAc) 256 sh, 269, 355, 388; (NaOAc/H_3BO_3) 265, 342; (NaOMe) 245 sh, 303 sh, 390. ^{1}H NMR (DMSO-d_6): δ 7.97 (1H, d, J = 8 Hz, H2′ & H6″), 7.05 (2H, d, J = 8 Hz, H3′ and H5′), 6.96 (1H, d, J = 2.5 Hz, H8), 6.56 (1H, S, H3), 6.32 (1H, d, J = 2.5 Hz, H6). 5.7 (1H, d, J = 2.5 Hz, H6), 5.4 (1H, d, anomeric sugar proton), 3–4 (m, remaining sugar protons). ^{13}C NMR (DMSO-d_6) δ (ppm): 182.45 (C-4); 164.24 (C-2); 163.83 (C-7); 162.03 (C-5); 161.99 (C-4′); 158.00 (C-9); 129.24 (C-2′ & C-6′); 122.13 (C-1′); 116.76 (C-3′ & C-5′); 106.21 (C-10); 103.73 (C-3); 100.04 (C-6); 95.15 (C-8); 102.5 (C-1″); 71.2 (2″); 73.2 (3″); 68.5 (4″); 75.5 (5″); 59.8 (6″).

2.4.3. Apigenin-7-*O*-β-D-glucoside (*3*)

(39 mg,) was obtained as yellow needle crystals with R_f = 0.69 (system a) and 0.23 (system b), mp 240–241 °C. UV λ_{max} MeOH: (nm) 266, 336; (NaOAc) 265, 350; (NaOAc + H_3BO_3) 266, 341; ($AlCl_3$) 274, 297, 343, 383; ($AlCl_3$/HCl) 275, 297, 340, 381. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.9 (2H, d, J = 8.4, H-2′, 6′), 6.8 (2H, d, J = 8.8, H-3′, 5′), 6.8 (1H, s, H-3), 6.77 (1H, d, J = 2.0, H-8), 6.38 (1H, d, J = 2.0,

H-6), 5.02 (1H, d, J = 7.2, H-1″), 3–4 (remaining sugar proton, m); ^{13}C NMR (DMSO-d_6) δ (ppm): 165.8 (C-2), 103.8 (C-3), 182.5 (C-4), 161.9 (C-5), 100.0 (C-6), 163.5 (C-7), 95.4 (C-8), 157.5 (C-9), 105.8 (C-10), 121.5 (C-1′), 129.2 (C-2′), 116.5 (3′), 161.6 (C-4′), 116.5 (C-5′), 129.2 (C-6′), 105.2 (C-1″), 73.6 (C-2″), 76.9 (C-3″), 69.9 (C-4″), 77.7 (C-5″), 61.1 (C-6″).

*2.4.4. Luteoline-7-O-glucoside (**4**)*

(21 mg) was obtained as yellowish brown particles with R_f = 0.55 (system a) and 0.1 (system b), mp 226–227 °C. UV λ_{max} in NaOMe: 262, 395; (NaOAc) 259, 402; (NaOAc/H_3BO_3) 258, 372; ($AlCl_3$) 272, 421; ($AlCl_3$/HCl) 271, 354, 383. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.39 (1H, q, J = 2, 7.6, H-6′), 7.3 (1H, d, J = 2.5, H-2′), 6.8 (1H, d, J = 8.4, H-5′), 6.6 (1H, s, H-3), 6.7 (1H, d, J = 2.0, H-8), 6.38 (1H, d, J = 2.0, H-6), 5.02 (1H, d, J = 7.6, H-1″), 3–4.01 (remaining sugar proton, m).

*2.4.5. Apigenin-7-O-diglucoside (**5**)*

(19 mg) was obtained as dark brown particles with R_f = 0.24 (system a) and 0.57 (system b). UV λ_{max} MeOH: 256, 267, 389; (NaOAc) 268, 395; (NaOAc/H_3BO_3); 268, 336; ($AlCl_3$) 277, 300, 345, 384; ($AlCl_3$/HCl) 277, 299, 342, 382. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.9 (2H, d, J = 8.8, H-2′, 6′), 6.8 (2H, d, J = 8.8, H- 3′, 5′), 6.8 (1H, s, H-3), 6.79 (1H, d, J = 2.0, H-8), 6.4 (1H, d, J = 2.0, H-6), 5.0, 5.2 (1H, d, J = 8, 2H-1″), 3-4 (remaining sugar proton, m); ^{13}C NMR (DMSO) (ppm): 164.3 (C-2), 103.5 (C-3), 182.4 (C-4), 160.9 (C-5), 100.4 (C-6), 162.6 (C-7), 94.1 (C-8), 160.2 (C-9), 105.2 (C-10), 121.3 (C-1′), 128.9 (C-2′), 116.3 (C-3′), 159.5 (C-4′), 116.3 (C-5′), 128.8 (C-6′), 102.3 (C-1″), 73.6 (C-2″), 74.0 (C-3″), 69.8 (C-4″), 81.2 (C-5″), 61.0 (C-6″), 101.4 (C-1‴), 72.8 (C-2‴), 77.5 (C-3‴), 69.8 (C-4‴), 76.2 (C-5‴), 61.0 (C-6‴).

*2.4.6. Apigenin-7-O-(6″ acetyl) glucoside (**6**)*

(55 mg), mp 214–217 °C was obtained as canary yellow needle crystals with R_f = 0.67 (system a) and 0.35 (system b). UV λ_{max} in MeOH: 265, 391; (NaOAc) 266, 390; (NaOAc/H_3BO_3) 267, 342; (AlCl3) 274, 298, 343, 384; (AlCl3/HCl) 275, 297, 341, 382. 1H NMR (DMSO-d_6) δ (ppm) 7.93 (2H, d, J = 8.4, H-2′, 6′), 6.91 (2H, d, J = 8.7, H-3′, 5′), 6.4 (1H, s, H-3), 6.84 (1H, d, J = 2.0, H-8), 6.79 (1H, d, J = 2.0, H-6), 5.04 (1H, d, J = 7.3, H-1″), 3–4 (remaining sugar proton, m), 2.04 (3H, sm CH_3); ^{13}C NMR (DMSO), δ (ppm) 164.76 (C-2), 103.63 (C-3), 182.52 (C-4), 161.63 (C-5), 100.02 (C-6), 163.46 (C-7), 95.36 (C-8), 157.47 (C-9), 105.85 (C-10), 121.52 (C-1′), 116.51 (C-2′), 129.15 (C-3′), 161.88 (C-4′), 116.05 (C-5′), 121.63 (C-6′), 100.38 (C-1″), 76.87 (C-2″), 73.65 (C-3″), 69.99 (C-4″), 77.67 (C-5″), 61.06 (C-6″).

*2.4.7. Apigenin-7-O-gluco(6″–1‴) rhamnoside (**7**)*

Powder with R_f = 0.82 (system a) and 0.1 (system b). (29 mg) was obtained as yellowish white UV λ_{max} in MeOH: 265, 387; (NaOAc) 229, 265, 388; (NaOAc/H_3BO_3) 266, 339; ($AlCl_3$) 274, 297, 342, 378; ($AlCl_3$/HCl) 274, 296, 339, 376. ^{1}H NMR (DMSO-d_6) δ (ppm) 7.9 (2H, d, J = 8.8, H-2′, 6′), 6.8 (2H, d, J = 8.8, H-3′, 5′), 6.8 (1H, s, H-3), 6.75 (1H, d, J = 2.4, H-8), 6.38 (1H, d, J = 2.4, H-6), 5.1 (1H, d, J = 7.2, H-1″), 4.3 (1H, d, J = 2.0), 3–4 (remaining sugar proton, m), 1.17 (3H, s, of rhamnose); ^{13}C NMR (DMSO-d_6) δ (ppm): 164.3 (C-2), 103.5 (C-3), 182.4 (C-4), 160.9 (C-5), 100.4 (C-6), 162.6 (C-7), 94.1 (C-8), 160.2 (C-9), 105.2 (C-10), 121.3 (C-1′), δ 128.9 (C-2′), 116.3 (C-3′), 159.5 (C-4′), 116.3 (C-5′), 128.8 (C-6′), 102.3 (C-1″), 73.6 (C-2″), 74.0 (C-3″), 69.8 (C-4″), 81.2 (C-5″), 61.0 (C-6″), 100.0 (C-1‴), 70.2 (C-2‴), 68.1 (C-3‴), 73.9 (C-4‴), 65.7 (C-5‴), 17.2 (C-6‴).

*2.4.8. Apigenin-7-O-gluco(6″–1‴)rhamnoside-5methoxide (**8**)*

(35 mg,) was obtained as light yellow crystals with R_f = 0.8 (system a) and 0.26 (system b), mp 199 °C. UV λ_{max} in MeOH: 265, 323, 388; (NaOAc) 266, 322, 385; (NaOAc/H_3BO_3) 266, 322, 331, 374; ($AlCl_3$) 274, 297, 343, 374; ($AlCl_3$/HCl) 272, 296, 341, 373. ^{1}H NMR (DMSO-d_6) δ (ppm) 7.9 (2H, d, J = 8.8, H-2′, 6′), 6.8 (2H, d, J = 8.8, H-3′, 5′), 6.8 (1H, s, H-3), 6.7 (1H, d, J = 2.5, H-8), δ 6.38 (1H, d, J = 2.4, H-6), δ 5.1 (1H, d, J = 7.3, H-1″), δ 4.3 (1H, d, J = 1.6), δ 3-4 (remaining sugar proton, m), 3.8 (3H, s, of rhamnose); ^{13}C NMR (DMSO-d_6) δ (ppm): 161.5 (C-2), 104.3 (C-3), 181.0 (C-4), 193.5 (C-5), 86.4 (C-6), 162.9 (C-7), 93.6 (C-8), 161.5 (C-9), 93.3 (C-10), 120.5 (C-1′), 128.5 (C-2′), 116.1 (C-3′), 159.3 (C-4′), 116.1 (C-5′), 128.3 (C-6′), 102.3 (C-1″), 73.6 (C-2″), 74.0 (C-3″), 69.8 (C-4″), 81.2 (C-5″), 61.0 (C-6″), 100.0 (C-1‴), 70.2 (C-2‴), 68.1 (C-3‴), 73.9 (C-4‴), 65.7 (C-5‴), 17.2 (C-6‴).

*2.4.9. Kaempferol (**9**)*

(130 mg) yellow crystals, R_f = 0.91 (system a), mp 277–279 °C, UV, λ_{max} in MeOH: (nm) 367, 268; ($AlCl_3$):265, 350, 420; ($AlCl_3$/HCl): 265, 350, 420; (NaOA): 275, 300(sh), 380; (NaOAc/H_3BO_3): 267, 319(sh), 380; (NaOMe): 285, 322, 430. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.0 (2H, d, J = 8 Hz, H2′ and H6′), 6.9 (2H, d, J = 8 Hz, H3′ and H5′), 6.4 (1H, d, J = 2.5 Hz, H8) and 6.2 (1H, d, J = 2.5 Hz, H6). EI-MS m/z (% re. lent): 285 (M^+) (100), 258 (15), 229 (16), 184 (8), 121 (22) and 93 (10).

*2.4.10. Kaempferol-3-O-xylosoid-7-galactoside (**10**)*

(140 mg) yellow crystals, R_f = 0.49 (system a), mp 388–389 °C, UV λ_{max} in MeOH: (nm) 257, 350; ($AlCl_3$): 257, 350, 400; ($AlCl_3$/HCl): 257, 350, 400; (NaOAc): 257, 260(sh), 355; (NaOAc/H_3BO_3): 257, 260(sh), 355; (NaOMe): 270, 420.^{1}H NMR (DMSO-d_6):δ (ppm) 7.2 (2H, d, J = 8 Hz, H2′ and H6′), 6.8 (2H, d, J = 8 Hz, H3′ and H5′), 5.8 (1H, d, J = 2.5 Hz, H8), 5.7 (1H, d, J = 2.5 Hz, H6), 5.1 (1H, d, J = 7 Hz, H1″ anomeric galactose proton), 4.27 (1H, d, J = 7 Hz, anomeric protons, H1″ xylose), 3-4 (m, remaining sugar protons). ^{13}C NMR (DMSO) δ (ppm) 153.3 (C-2); 132.6 (C-3); 174.3 (C-4); 159.6 (C-5); 98.6 (C-6); 160.4 (C-7); 95.5 (C-8); 153.2 (C-9); 103.4 (C-10); 121.1 (C-1′); 130.3 (C-2′); 114.8 (C-3′); 157.4 (C-4′); 114.8 (C-5′); 130.3 (C-6′);)102.5 (C-1″); 71.2 (2″); 73.2 (3″); 68.5 (4″); 75.5 (5″); 59.8 (6″); 106.34 (C-1‴); 73.82 (C2‴); 76.92 (C3‴); 68.10 (C4‴); 66.50 (C5‴).

2.5. Determination of median lethal dose (LD_{50})

LD_{50} of the total ethanol extract of *Sisymbrium irio* plant was estimated in mice by using the method of Lorke (1983). In a preliminary test, animals in groups of three, received one of 10, 100, or 1000 mg kg^{-1} of the tested extract suspended in the vehicle (3% v/v Tween 80). Animals were observed for 24 h for signs of toxicity and number of deaths. Depending on the results of the preliminary test, doses of 1250, 2500, and 5000 mg kg^{-1} of the tested extract were administered to fresh groups, each of six mice. Control animals received the

vehicle and were kept under the same conditions. Signs of toxicity and number of deaths per dose were recorded within 24 h and the LD_{50} was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no lethality at all (Awaad et al., 2006).

2.6. Antioxidant activity

All extracts, total alcohol, ether, chloroform, ethyl acetate and butanol extracts were centrifuged (2000×*g*, 10 min) to remove insoluble material and the supernatants retained. Antioxidant activity (Awaad and Al-Jaber, 2010) in the Supernatants in addition to the isolated compounds **1**–**10** were determined *in vitro* via scavenging of the $ABTS^{\cdot+}$ [(2,2′-azinobis(3-ethylbenzothiazolone-6-sulfonic acid)] radical, which was generated by a metmyoglobin/hydrogen peroxide system (Rece-Evans and Miller, 1994). Each plant extract (10 μl) was added separately to a 1 cm pathlength spectrophotometer cuvette (1 ml capacity) containing 20 μM phosphate buffered saline pH 7.4, 2.5 μM myoglobin and 150 μM ABTS. The reaction was initiated by addition of 75 μM hydrogen peroxide and the absorbance change at 734 nm monitored at 30 °C. A quantitative relationship exists between the absorbance at 734 nm measured after 6 min, and the antioxidant property of the plant extract, as determined relative to Trolox (a water soluble vitamin E analog) antioxidant standards, and expressed in terms of mM Trolox equivalent (mMTE). Samples of the plant extracts for antioxidant activity were dried to constant weight (80 °C for 20 h) and the final antioxidant activity was expressed in terms of m MTE/g dried tissue. A two-point calibration was used. In the first stage of the assay, myoglobin was reacted with hydrogen peroxide to produce the ferrimyoglobin free radical which was then incubated with a chromagen, 2,2-amino-di-(3-ethylbenzthiazole sophonate) to produce ATBSR +, a radical cation with a blue-green color measured at 600 nm. Antioxidants in the added plant aqueous extracts suppressed the blue-green color to a degree that was proportional to their concentration and antioxidant property. The absorbance of the resulting oxidized solution was compared to that of the calibrated standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Results were expressed as μmol Trolox equivalents per gram plant dry weight.

3. Results and discussion

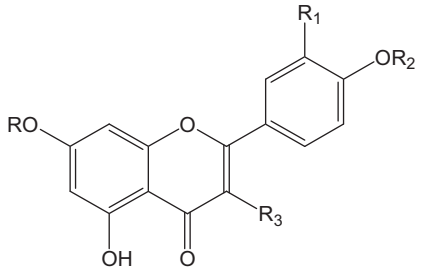
3.1. Phytochemical screening

Preliminary phytochemical screening of the plant revealed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes, proteins and/or amino acids, coumarines, tannins, flavonoids, alkaloids and/or nitrogenous bases and saponins.

3.2. Isolation of phenolic compounds

Ten flavonoidal compounds (Fig. 1) were isolated from *Sisymbrium irio* for the first time and identified as; Apigenin, apigenin-7-galactoside, apigenin-7-*O*-β-D-glucoside, luteolin-7-*O*-glucoside, apigenin-7-di-glucoside, apigenin-7-*O*-(6″acetyl) glucoside, apigenin.7-*O*-gluco(6″-1‴) rhamnoside, apigenin-7-*O*-gluco(6″-1‴) rhamnoside-5-methoxide, Kampferol and kampferol-3-xyloside-7-galactoside using different spectroscopic analysis such as; ^{1}H NMR, ^{13}C NMR DEPT, COSY, HMBC, HMQC and mass spectrometer.

Compounds **1** and **9** were identified as; Apigenin and Kampferol by comparing their TLC chromatograms, UV spectrum in methanol and with different shift reagents, EI-MS, ^{1}H



	Compound	R	R_1	R_2	R_3
1	Apigenin	H	H	H	H
2	Apigenin-7-galactoside	galactose	H	H	H
3	Apigenin-7-O-β-D-glucoside	glucose	H	H	H
4	Luteolin-7-O-glucoside	glucose	OH	H	H
5	Apigenin-7-di-glucoside	di-glucose	H	H	H
6	Apigenin-7-O-(6"acetyl) glucoside	acetyl glucose	H	H	H
7	Apigenin-7-O-gluco(6",1"') rhamnoside	gluco rhamnose	H	H	H
8	Apigenin-7-O-gluco (6",1"')rhamnoside-5-methoxide.	gluco,rhamnose	H	CH_3	H
9	Kaempferol	H	H	H	OH
10	Kaempferol-3-O-xylosoid-7-galactoside	H	H	H	O-Xyloso, galactose

Figure 1 Structure of isolated compounds from aerial parts of *Sisymbrium irio* L.

Table 1 Antioxidant activity of the tested extracts and isolated compounds of *Sisymbrium irio* L.

Extracts and isolated compounds	Antioxidant capacity (μmol Trolox equivalent/gram dry weight)
Total alcohol	952
Ether	132
Chloroform	392
Ethyl acetate	810
Butanol	640
Compound-**1**	785
Compound-**2**	695
Compound-**3**	632
Compound-**4**	720
Compound-**5**	410
Compound-**6**	372
Compound-**7**	453
Compound-**8**	410
Compound-**9**	572
Compound-**10**	653

NMR and ^{13}C NMR spectra with authentic samples and published data (Mabry et al., 1970).

Compounds **2**, **3**, **5**–**7** were found to be glycosides (positive molesh test), Acid hydrolysis of these compounds (Harborne et al., 1975) revealed the sugar galactose, glucose and rhamnose which were identified by PC and TLC (system e and f) and an aglycon which was found to be identical with compound **1** when compared with its TLC, UV shift reagents. There are substituted at position 7 as indicated by their UV spectra upon addition of diagnostic shift reagent. Accordingly, based on the results obtained and some of the published data (Geissman, 1962; Mabry et al., 1970; Harborne et al., 1975), this compound was identified as apigenin-7-galactoside, apigenin-7-*O*-β-D-glucoside, luteolin-7-*O*-glucoside, apigenin-7-diglucoside, apigenin-7-*O*-(6‘‘acetyl) glucoside, apigenin.7-*O*-gluco(6″–1‴) rhamnoside, apigenin-7-*O*-gluco(6″–1‴) rhamnoside-5-methoxide.

Hydrolysis of compound **10** revealed the presence of sugar xylose and galactose which identified by PC and TLC (system e and f) and an aglycon which was found to be identical with compound **9** when compared with its TLC, UV shift reagents. There are substituted at positions 3 and 7 as indicated by their UV spectra upon addition of diagnostic shift reagent.

3.3. Determination of median lethal dose (LD_{50})

The total ethanol extract of *Sisymbrium irio* did not produce any behavioral changes and mortality in mice in doses up to 5000 mg kg^{-1}. Accordingly, it suggested that oral LD_{50} of the total extract was higher than 5000 mg kg^{-1}. Therefore, the tested plant can be categorized as highly safe since substances possessing LD_{50} higher than 50 mg kg^{-1} are non-toxic (Buck et al., 1976).

3.4. Antioxidant activity

The present study revealed that the total antioxidant content ranged from 129 to 952 μmol Trolox equivalent/g dry weight. Total alcohol extract was 952 μmol/g both ether and chloroform extract showed the lowest amount of antioxidant (132 and 129 μmol/g, respectively), while butanol and ethyl acetate fraction were found to have the highest amount (843 and 810 μmol/g, respectively). On the other hand, all isolated compounds have closer activity to butanol but in lesser concentration (100 mg/kg) Table 1. So we can conclude that *Sisymbrium irio* (cruciferae), is a medicinal plant, as it possesses anti-inflammatory, analgesic (due to the high flavonoidal content; 6.25%) and anti-oxidant activities and can be used as a remedy for treatment of such diseases. Compound **2** (apigenin-7-*O*-galactoside) showed the highest effect in the mean time and other compounds possessed nearer effect. All of the seven isolated compounds have a synergistic activity. No antipyretic activity was shown for all the extracts.

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