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LC-ESI-MS profile, antioxidant activity and cytotoxic screening of *Oligomeris linifolia* (Vahl) Macbr. (Resedaceae)

Ali M. El-Hagrassy[1], Ahmed Elkhateeb[1*], Sameh R. Hussein[1], El-Sayed S. Abdel-Hameed[2,3], Mona M. Marzouk[1]

[1]Department of Phytochemistry and Plant Systematics, National Research Centre, 33 El Bohouth St., Dokki, Giza, Egypt.
[2]Laboratory of Medicinal Chemistry, Theodor Bilharz Institute, Giza, Egypt.
[3]Chemistry Department, Faculty of Science, Taif University, Saudi Arabia.

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ABSTRACT

LC-ESI-MS analysis was investigated for the n-butanol, methanol and water fractions of *Oligomeris linifolia*, which leads to the identification of twenty-one compounds, among them sixteen compounds were firstly reported from this plant and five compounds were identified previously. Butanol and methanol fractions showed higher antioxidant activity with IC_{50} values of 10.37µg/ml and 31.07µg/ml, respectively, compared with that of the standard; Trolox (33.55µg/ml) using DPPH method. However, water fraction showed lower activity (IC_{50}: 91.86 µg/ml). The three fractions were also screened for their cytotoxicity and showed weak effect against HCT116, A549 and MCF7 at 100 mg/ml and no effect against HEPG2.

INTRODUCTION

Resedaceae family contains about 52 species of six genera divided into three tribes, according to the position of ovary, number of carpels and the placentation types (Martín-Bravo *et al*., 2007). Astrocarpeae; with the genus *Sesamoides*, is characterized by apocarpous ovary and marginal placentation, Cayluseae; with the genus *Caylusea*, showing semiapocarpous ovary and basal placentation, while the remaining 4 genera were included within Resedeae (syncarpic ovary and parietal placentation) (Martín-Bravo *et al*., 2007). On the basis of the position of calyx, corolla and stamens, the genera of tribe Resedeae were distributed into two sub tribes (Martín-Bravo *et al*., 2007). *Randonia* belongs to Randoninae as characterized by the perigynous flowers while *Oligomeris*, *Ochradenus* and *Reseda* are belonging to Resedinae which has hpogynous flowers. *Oligomeris linifolia* (Vahl) Macbr. (Resedinae: Resedeae: Resedaceae) is one of four *Oligomeris* species, commonly named as lineleaf whitepuff. It is native to some parts of the Middle East India, Southern Europe, North Africa and North America. The plant grows in many habitat types including deserts, saline soils, plains, coastline, and other places. It is an annual herb, up to 50 cm tall, with a linear leaves and white flowers. We have previously reported the first phytochemical investigation of *O. linifolia* which leads to the isolation of eleven compounds; one sterol, five flavonols, one nucleotide, two phenolic acids and two free sugars (Hussein *et al*., 2013). To the best of our knowledge, except the above mentioned report, there are no reports about this plant. Therefore, more investigation was of a great importance. In the current study, for a detailed phytochemical investigation, we have used the LC-ESI-MS analysis for butanol, methanol and water fractions of *O. linifolia* and checking their cytotoxic and anti-oxidant activities.

* *Corresponding Author*
Ahmed Elkhateeb, Department of Phytochemistry and Plant Systematics, National Research Centre, 33 El Bohouth St., Dokki, Giza, Egypt.
E mail: elkhateeb.ahmed @ gmail.com, Tel: +20 111 6757 520

EXPERIMENTAL

LC-ESI-MS analysis

LC-ESI-MS analysis: HPLC (Waters Alliance 2695) & MS spectrometry (Waters 3100). The mobile phase was freshly prepared by filtering through membrane disc filter (0.45 µm) then degassed by sonication. For gradient elution, the mobile phase consists of solvent A (0.1% formic acid (FA) in H_2O) and solvent B (0.1% FA in CH_3CN/MeOH (1:1; v/v)). The linear gradient profile was as follows: 95% A (5 min), 95-90% A (10 min), 90-50% A (55 min), 50-95% A (65 min), and 95% A (70 min). The injection volume was 10 µL. The flow rate (0.6 mL/min) was split 1:1 before the MS interface with negative ion mode parameters (source temperature 150 °C, desolvation temperature 350 °C, cone gas flow 50 L/h, cone voltage 50 eV, capillary voltage 3 kV, and desolvation gas flow 600 L/h). Spectra were recorded in the ESI negative mode between 50-1000 *m/z*. The peaks and spectra were processed using the Maslynx 4.1 software. The flavonoids isolated from *O. linifolia* in our previous study (Hussein *et al*., 2013), together with other pure flavonoids, obtained from Phytochemical and Plant Systematic Department, were used as reference samples. Known compounds were confirmed by comparing their retention times and mass spectra with standards. Unknowns were tentatively identified by comparing their mass fragmentation pattern with literatures.

Plant Material

O. linifolia was collected in March 2010 from Cairo-El Fayium desert road (73 km), and identified by Dr. Sameh Reda Hussein. A voucher specimen (s.n.820) was deposited in the herbarium of the National Research Center (CAIRC).

Cell culture and sample treatment

The cell line under investigation were human breast adenocarcinoma (MCF7), human hepatocellular carcinoma cell line (HEPG 2), human lung carcinoma (A549) and human colon cell line (HCT116). They were purchased from American Tissue Culture Collection. HEPG2, MCF7 and HCT116 cells lines were cultured in RPMI 1640 medium while A549 cell line was cultured in DMEM media. Media were prepared as a mixture of 1% antibiotic antimycotic (10,000 Uml^{-1} potassium penicillin, 10,000 μgml^{-1} streptomycin sulphate and 25 μgm^{-1} amphotericin B), 1% L-glutamine and 10% fetal bovine serum. According to the cells growth profile, cells were seeded with a density of 1×10^4 cell per well. This number was sufficient to give a reliable reading with the MTT assay, which corresponded well with the cell number and was the one that gave exponential growth throughout the incubation period with the tested sample (Ibrahim *et al*., 2013).

Free radical scavenging activity (DPPH: 1,1-diphenyl -2-picryl hydrazyl)

DPPH in methanol (0.1mM) solution was prepared and then 1 ml was added to 3 ml of the three tested extracts and standard solutions, separately. Trolox was used as reference. Four concentrations of tested samples (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml) and standard (1.0 µg/ml, 2.5 µg/ml, 5.0 µg/ml) were prepared using methanol. Solutions were kept for 30 min in dark and their absorbances were measured at 517 nm. 3ml of methanol were used as blank. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH Scavenged (%) = $\{(A_0-A_1)/A_0\}\times100$, where A_0 is the absorbance of the blank and A_1 is the absorbance of test sample (Sharma *et al*., 2004). The antioxidant activity of was expressed in IC_{50} values (concentration in µg/ml of test sample which scavenges free radicals by 50%).

RESULTS AND DISCUSSION

Identification of phenolic compounds.

In the present study, the LC-ESI-MS analysis was investigated for the n-butanol, methanol and water fractions of *O. linifolia* using negative ion mode technique due to its high sensitivity in the analysis of different polyphenols (Cuyckens and Claeys, 2004). Twenty-one compounds were identified; sixteen for the first time from this plant and five were previously reported (Hussein *et al*., 2013). The HPLC chromatograms of the different extracts are shown in Figure (**1**) and the tentative identification of phenolic compounds is shown in Table (**1**).

In this study three compounds were identified as phenolic acids, (compounds **1**, **11** and **19),** and eighteen compounds were identified as flavonoids. The previously isolated flavonoids were detected at peaks **6**, **12**, **17**, **18** and **21** and confirmed by comparing their retention times and mass spectra with the authentic samples (Hussein *et al*., 2013). Compound **1** was tentatively identified as dihydroxybenzoyl caffeic acid which showed a pseudo-molecular ion at *m/z* 315 and a fragment ion at *m/z* 153 [M-(caffeic acid-H_2O)-H]$^-$ (Zamboni *et al*., 2010). The ESI-MS fragmentation pattern of compound **11** showed a molecular anion peak at *m/z* 341 and three fragments at *m/z*: 179 [caffeic acid-H]$^-$, 161 [caffeic acid-H-H_2O]$^-$, and 135 [M-H-hexose-CO_2]$^-$. This suggests that compound **11** is caffeic acid-*O*-hexoside (Munekata *et al*., 2016). Compound **19** showed the same fragments of compound **11** in addition to a molecular ion peak at *m/z* 683 [M-H]$^-$ which revealed the tentative identification of compound **19** as caffeic acid-*O*-hexoside dimer (Chen *et al*., 2011). Compound **2** showed a deprotonated molecular ion [M-H]$^-$ at *m/z* 609. The predominant fragment at *m/z* 447 is corresponding to loss of hexose [M-H-162]$^-$ as ether linkage, while that at *m/z* 357 [M-H-hexose-90]$^-$, suggested the mono-*C*-glycosylation. Therefore, compound **2** was tentatively identified as kaempferol-*O*-hexoside-*C*-hexoside (Vallverdú-Queralt *et al*., 2011). In their ESI-MS spectra, compounds **3** and **10** showed the characteristic pattern of acylated flavonoid-hexoside. They showed caffeic acid peak at *m/z* 179 and caffeic acid-hexoside peak at *m/z* 341. Compound **3** showed a deprotonated molecular ion at *m/z* 639 and fragment ion at *m/z* 447 [M-H-caffeoyl]$^-$, while **10** showed a deprotonated molecular ion at *m/z* 609 and fragment ion at *m/z* 477 [M-H-caffeoyl]$^-$. Aglycones of **3** and **10** were suggested to be isorhamnetin (*m/z* 315) and kaempferol (*m/z* 285), respectively.

Therefore, compounds **3** and **10** were tentatively identified as isorhamnetin-*O*-caffoyl hexoside and kaempferol-*O*-caffeoyl hexoside, respectively (Parejo *et al*., 2004). Compound **4** displayed $[M-H]^-$ ion at *m/z* 595 and showed the characteristic fragments of flavonoids-di-*C*-glycoside at *m/z*: 505 $[M-H-90]^-$, 475 $[M-H-120]^-$, 385 $[272+113]^-$ and 355 $[272+ 83]^-$, with 272 is corresponding to the molecular weight of naringenin leads to its identification as naringenin-6,8-di-*C*-hexoside (Llorent-Martínez *et al*., 2016). Compound **5** showed a deprotonated ion $[M-H]^-$ at *m/z* 887 which further fragmented giving three fragments at *m/z*: 725 $[M-H-162]^-$, 609 $[M-H-162-116]^-$, 447 $[M-H-2(162)-116]^-$. Finally, the aglycone unit was characterized as kaempferol from the fragment at *m/z* 285 $[M-H-3(162)-116]^-$. The neutral loss of 278 Da is characteristic for the loss of hexose-malic acid moiety (Abu-Reidah *et al*., 2015). Consequently, compound **5** was identified as kaempferol-*O*-trihexoside malic acid. By the same manner compound **8** was identified as kaempferol-*O*-dihexoside malic acid. Also, compound **9** is a flavonoid conjugated with glycoside-malic acid. It showed a molecular anion peak $[M-H]^-$ at *m/z* 871 which gave fragment at *m/z* 725 due to loss of rhamnose unit $[M-H-146]^-$, followed by fragment at *m/z* 609 due to loss of malic acid unit $[M-H-146-116]^-$. Finally, it gave two fragments at *m/z* 447 and 285 due to the successive loss of two hexose units leads to its tentative identification as kaempferol-*O*-dihexoside-rhamnoside malic acid.

Compound **7** was characterized as isorhamnetin 3,7-di-*O*-glucopyranoside by comparing its retention time and mass spectrum with the authentic. Compound **13** with a molecular anion peak $[M-H]^-$ at *m/z* 609 gave fragment at *m/z* 447 due to loss of hexose unit $[M-H-162]^-$ and obtained another fragment at *m/z* 285 due to loss of another hexose moiety which characterized the *O*-glycosylation on phenolic hydroxyls. Based on the above mentioned data, compound **13** was tentatively identified askaempferol-*O*-di-hexoside. Similarly, compound **14** was tentatively characterized as isorhamnetin-di-*O*-hexoside. Compound **15** showed a pseudo molecular ion peak (*m/z* 447) and a fragment (*m/z* 285) due to loss of hexose unit $[M-H-162]^-$ confirmed its identification as kaempferol-*O*-hexoside. By the same manner, **16** was characterized as isorhamnetin-*O*-hexoside. Compound **21** was identified as kaempferol by comparing its mass spectra with standard.

Antioxidant activity and cytotoxic screening

The results of antioxidant activity (DPPH assay) of the three fractions were recorded scavenging properties as ordered in ascending: butanol > methanol > water with IC_{50} values of 10.37, 31.07 and 91.86 µg/ml, respectively, compared with that of the standard; Trolox (33.55 µg/ml). The sequence of these activities could be due to the quantity of the detected compounds of the three successive fractions (Table. 1, Figure. 1).

The cytotoxic screening showed weak effect for butanol, methanol and water fractions against HCT116, A549 and MCF7 at 100 mg/ml with (11.3, 6.8, 15.3) %, (2.9, 0, 14.8) % and (0, 5.6, 8.3) %, respectively, as well as no effect against HEPG2.

Table 1: Phenolic compounds identified from *O. linifolia*.

Peak No.	R_t (min)	M	$[M-H]^-$	*m/z* Fragments	Identification	BuOH	MeOH	H_2O
1	13.94	316	315	153, 179, 135, 107	Dihydroxy benzoyl caffeic acid	-	-	+
2	21.79	610	609	447, 357	Kaempferol-*O*-hexoside-*C*-hexoside	+	+	+
3	23.04	640	639	477, 315, 179	Isorhamnetin-*O*- caffoyl hexoside	+	+	-
4	25.1	596	595	385, 355, 313	Naringenin-6,8-di-*C*-hexoside	+	+	+
5	26.13	888	887	725, 609, 447, 285	Kaempferol-*O*- tri-hexoside malic acid	+	+	+
6	27.22	610	609	447, 285	Kaempferol 3,7-di-*O*-glucopyranoside [a]	+	+	+
7	27.72	640	639	477, 315	Isorhamnetin 3, 7-di-*O*-glucopyranoside [b]	+	+	+
8	31.14	726	725	609, 447, 285	Kaempferol-*O*- di-hexoside malic acid	+	-	-
9	31.31	872	871	755, 725, 609, 447, 285	Kaempferol-O-di-hexoside-rhamnoside malic acid	+	+	+
10	32.8	610	609	447, 285, 179	Kaempferol -*O*- caffoyl hexoside	+	+	+
11	33.98	342	341	179, 161, 135	Caffeic acid-O-hexoside [b]	+	+	+
12	34.5	462	461	315	Isorhamnetin 4´-*O*-rhamnopyranoside [a]	+	-	-
13	36.4	610	609	447, 285	Kaempferol di-*O*-hexoside	-	+	-
14	36.56	640	639	477, 315	Isorhamnetin-di-*O*-hexoside	-	+	-
15	39.41	448	447	447, 285	Kaempferol -*O*-hexoside	+	+	+
16	39.6	478	477	315	Isorhamnetin-*O*-hexoside	+	+	+
17	43.4	448	447	447, 285	Kaempferol 3-*O*-glucoside [a]	+	+	+
18	43.7	478	477	315	Isorhamnetin 3-*O*-glucoside [a]	+	+	+
19	44.8	684	683	341, 179, 161, 135	Caffeic acid-O-hexoside dimer	+	-	-
20	47.01	286	285	179, 151	Kaempferol [b]	+	-	+
21	53.8	316	315	179, 151	Isorhamnetin [a]	+	-	-

[a] Compound isolated previously from *O. liniofolia*, (Hussein *et al*., 2013), and confirmed by comparing their retention times and mass spectra with the authentic, [b] Compounds identified by comparing their retention times and mass spectra with the authentic, R_t Retention time.

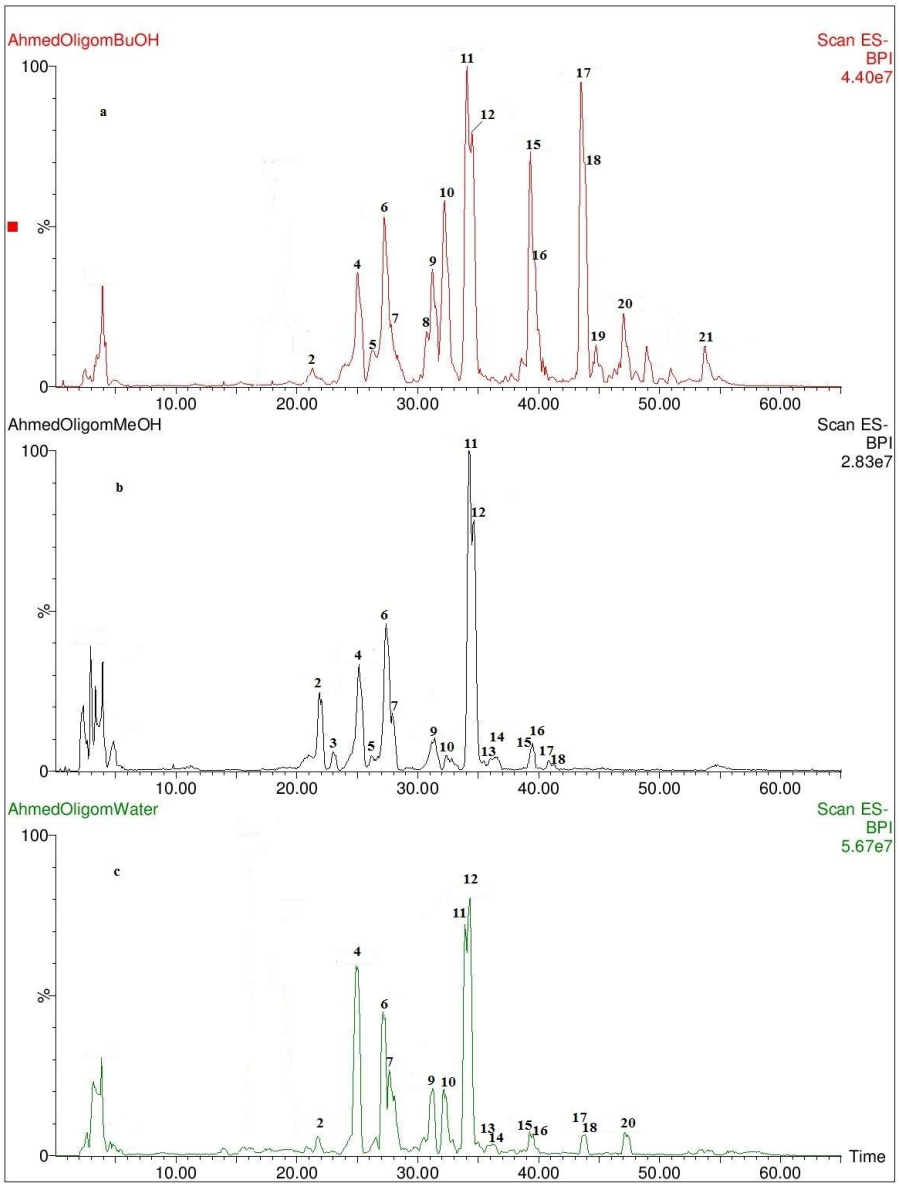

Fig. 1: LC-ESI-MS chromatogram of *Oligomeris linifolia*; a: n-butanol, b: methanol and c: water fractions.

Chemosystematic significance

From the chemosystematic point of view, the flavonoids detected in the present study provide additional data to further our knowledge on the infrageneric relationship of *O. linifolia* within the tribe Resedeae (family Resedaceae). Eighteen flavonoids were identified or tentatively identified from three successive fractions of *O. linifolia*; five of them were pervious reported from its aqueous methanol extract. They are belonging to flavonol *O*-glycosides (kaempferol and isorhamnetin), flavonol *C*-glycosides (kaempferol *C*-hexoside-*O*-hexoside), flavonol *O*-acyl glycosides (*O*-malic and *O*-caffeic acid hexosides of kaempferol), and dihydroflavones (naringenin di-*C*-hexoside). Either flavonol mono-*O*-hexoside (3 and/or 7-glucoside) or flavonol di-*O*-hexosides (3-diglucosides, 3,7-diglucosides and/or 3,4´-diglucosides) of kaempferol and/or isorhamnetin were reported in the most species of tribe Resedeae (Berrehal *et al*., 2006; Makboul *et al*., 1989). The acylated flavonols were also reported in *Ochradenus baccatus* (Barakat *et al*., 1991), *Reseda muricata* and *Reseda alba* (El-Sayed *et al*., 2001) of the same sub tribe; Resedinae. Additionally, the sub tribe Randoninae (*Randonia africana*) still characterized by the presence of methylated flavonol glycosides (Berrehal *et al*., 2010; Hussein *et al*., 2013). Since our previous study reported that *O. linifolia* is the only species of tribe Resedeae which characterized by its facility to produce a flavonol 4´-mono-*O*-glycoside (Hussein *et al*., 2013), however the present study also supported its characterization by the ability to synthesis the *C*-hexoside derivatives of kaempferol and naringenin.

CONCLUSION

The antioxidant and cytotoxic activities together with the LC-ESI-MS profile were investigated for the n-butanol, methanol and water fractions of *O. linifolia*. Twenty-one phenolic compounds, among them sixteen compounds were firstly reported giving more information about the phenolic content of this plant. Butanol and methanol fractions showed higher antioxidant activity than water fraction. The three fractions showed weak effect against HCT116, A549 and MCF7 and no effect against HEPG2. Our study revealed that *O. linifolia* can synthesizes flavonol 4′-mono-O-glycoside and the C-hexoside derivatives of kaempferol and naringenin.

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