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Phytochemical Constituents and Biological Prospects of Clover Broomrape

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Abstract

Up to the present moment, scores of parasitic plants are still uninvestigated for their chemical composition and biological activities. In this study, our objective is to supplement the recognition of the parasitic plant *Orobanche minor* by screening the phytochemical outline of its ethanol extract by UPLC-ESI-MS^2 analysis. The assesment contributed to detection and tentative identification of twelve compounds; including phenylethanoid glycosides, aromatic acids, sterols and a flavonoid. MTT assay revealed that the plant is fairly safe and has no *in vitro* cytotoxicity on normal human skin fibroblast cells (NHSFbs). The assessment of the extract's antioxidant capacity was carried out comparatively using three different methods; DPPH radical scavenging assay, FRAP assay and superoxide radical scavenging activity. The extract displayed a strong antioxidant activity when compared with ascorbic acid. Furthermore, the plant extract possessed a significant *in vitro* skin photoprotecting and anti-inflammatory activities on ultraviolet exposed NHSFbs diminishing MMP-1 the enzyme which induces collagen lyses and reducing the cytokine which induces inflammation IL-6 respectively.

Keywords: Chromatography; Phenylethanoid glycosides; Cytotoxicity; *Orobanche minor*; Antioxidant

1. Introduction

Orobanchaceae (broomrapes) can be considered as the only family that comprises all major nutritional types of parasitic weeds; facultative, hemiparasitic and holoparasitic which draw some or all of their nutrients and water from their host plants [1-2]. Parasitic weeds are difficult to eradicate because of that a single *Orobanche* species plant can produce more than 200,000 tiny microscopic seeds that continue to exist viable in soil for many years. These parasitic weeds attack different economic host plants causing serious problems for farmers all over the world. The damage due to these parasitic weeds sometimes reaches up to 100%. Presently, there are no powerful techniques for elimination of parasitic broomrape plants [3-4]. *Orobanche minor* Smith is one of Orobanchaceae plants which is interrelated with *Trifolium alexandrinum* (Egyptian Clover) Family Fabaceae, and does not live in the wild flora [5]. A number of important phytoconstituents were isolated or identified from *Orobanche* plants such as flavonoids, lignans, iridoides, phenylethanoid glycosides, phenolics, sterols, alkaloids and triterpenoids [6-8]. As far of our knowledge, constituents of *O. minor* have not been fully studied yet. Many biological activities had been reported for extracts of Orobanchaceae plants and their isolated compounds; including antioxidant, hepatoprotective and neuroprotective and anti-inflammatory activities, as far as the traditional Chinese medicine considered plants of this family as pharmaceutical awards [9-10]. Accumulating evidences during the last years have reported the implication of free radical-mediated processes in a large number of human diseases as a result of a condition called "oxidative stress" meanwhile the free radical load which causes general aging of human cells. It can also be the cause of certain diseases associated with the elderly. Although our body has effective defence mechanisms that protect it against oxidative stress, the potential of these protective mechanisms fade by age. Therefore, it is necessary to supply the human body with a sustained stream of antioxidants such as phenolic compounds through dietary supplements especially plants. The power of plant extracts containing phenolic compounds is mighty at small doses. For this reason, studies for the activity evaluation of the plant extracts as antioxidants could help to provide sources of powerful antioxidants. Several *Orobanche* species such as *O. crenata, O. lavandulacea* and *O. foetida* were listed to be useful resources of antioxidant constituents and could be listed in some pharmaceuticals [9, 11-12]. In a previous work we investigated the macro and micro-morphological characters of the plant to identify it in its entire and powdered form [5]. Based on the importance of sustainability all over the world, with the difficulty of eradicating the parasitic weed *O. minor*, the objective of this research is to look for the hopeful prospects of this noxious weed by identification of the chemical constituents using UPLC-ESI-MS/MS, determination of the antioxidant

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capacity and profiling the biological activities of the of *O. minor*. Additionally, we aspired to study the cytotoxicity of the extract to appraise its safety profile on normal human skin fibroblasts.

2. Experimental

Plant material and extraction: *Orobanche minor* Smith (*O. minor*) was obtained from Ezbet Almokabla, Benha, Qualyobia, Egypt. The plant material was gathered in February 2020 during its flowering stage. The plant was authenticated by Prof. Dr. Ahmed Abd Al-Razik Mubarak (Professor of Taxonomy, Botany Department, Faculty of Science, Benha University), and a sample was kept in the herbarium of Pharmacognosy, Faculty of Pharmacy, Zagazig University (OR-13).

4 kg of the fresh plant were cut into small pieces and boiled with 90 % Ethanol (ADWIC, Egypt) on water bath for 1 hour, the extract was left to cool, then blended till form a smooth paste. The paste was macerated for 7 days. The maceration was repeated three times till exhaustion and the collective extract was filtered and concentrated using vacuum evaporation at 45-50 ^{o}C to yield a highly viscous dark brown liquid of total ethanol extract (330 g).

UPLC-ESI-MS/MS analysis: The study employed UPLC-ESI-MS^2 to analyze plant ethanol extracts. Samples were dissolved in HPLC-grade methyl alcohol, filtered, and stored at 4°C. The analysis was conducted on a XEVO TQD mass spectrometer with a reversed-phase C-18 column, using gradient elution at 0.2 ml/min with two eluents: water-acetonitrile (90:10) with 0.1% formic acid and methanol with 0.1% formic acid. Operating conditions included a source temperature of 150°C, desolvation temperature of 400°C, cone voltage of 30 eV, and m/z range of 100–1000. Data were processed with MassLynx 4.1 software using retention time and mass spectra for identification [13-14].

The DPPH radical scavenging assay: This assay, as described by Desmarchelier et al. [18], assessed the ability of extracts to donate protons, measured through the bleaching of 2,2-diphenyl-1-picrylhydrazyl (DPPH). In a methanol solution, DPPH exhibits a violet color that shifts to yellow in the presence of antioxidants. For the experiment, 2.4 ml of 0.1 mM DPPH in methanol was combined with 1.6 ml of the extract at various concentrations (100–1000 µg/ml). After thorough vortexing, the mixture was incubated at room temperature for 30 minutes, protected from light. Absorbance was then measured at 517 nm using a spectrophotometer, with vitamin C serving as the standard and % DPPH• scavenging activity was calculated using the formula:

$$\% \text{ DPPH radical scavenging activity} = [(A_0 - A_1)/A_0] \times 100.$$

A_0: absorbance of standard and A_1: absorbance of *O. minor*. Inhibition% was planned to concentration, and IC_{50} was computed as the efficient sample conc. at which 50% of DPPH• were scavenged [15-17]. The experiment was replicated 3 times at each conc. and the results showed mean ± standard deviation.

Ferrous reducing antioxidant capacity assay: By using the Oyaizu technique [18], the ferric reducing antioxidant power (FRAP) of the samples was assessed. By tracking the production of Prussian blue color at 700 nm, the Fe^{2+} may be observed. The test tubes were filled with 0.25 ml of different conc. of *O. minor*/standard sol. (100-1000 ug/ml), 0.6 ml pot. buffer (0.2 M), and 0.6 ml of 1% pot. ferri-cyanide, [$K_3Fe(CN)_6$] sol. The mixture was incubated for 22 min. at 50 ^{o}C. Tubes were full of 0.6 ml 10% trichloroacetic acid (TCA) sol.. After centrifuging, 1.7 ml of supernatant was withdrawed and admixed with 1.7 ml of dist. H_2O and 0.4 ml of a 0.1% ferric chloride ($FeCl_3$) solution. An identical solution mixture was used to prepare the blank solution, which was incubated under the same conditions but without the plant extract or the standard. At 700 nm, the blank solution's absorbance was calculated. The reaction mixture's increased absorbance is an indication of its high reducing power [19-20]. The experiment was replicated 3 times for each conc.

Superoxide radical scavenging assay: In accordance with the established methodology [21], the superoxide anion scavenging activity was assessed using a phenazine methosulfate-nicotinamide adenine dinucleotide system (PMS-NADH). The superoxide radicals were created by the oxidation of nicotinamide adenine dinucleotide (NADH) and measured by reducing nitroblue tetrazolium (NBT). Oxidizing radicals were produced in 4 ml of 100 mM of pH 7.4 monosodium phosphate buffer containing 1 ml of 150 mM NBT sol., 1 ml of 468 mM NADH, and different conc. of *O. minor* extract. 1 ml of 60 mM phenazine methosulfate (PMS) solution was added to the reaction product, and incubated for 5 minutes at 25 ^{0}C, and then absorbance was calculated in comparison to the blank solution. Ascorbic acid was used as +control. The *O. minor* extract ability to scavenge superoxide radicals is correlated with a reduction in NBT as shown by the absorbance of the reaction product.

MTT- Cytotoxicity assay: Cell lines were obtained from American Type Culture Collection and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 µg/ml insulin, and 1% penicillin-streptomycin. Chemicals for the assay were procured from Sigma (In Vitro Toxicology Assay Kit, Stock No. TOX1). Cells were seeded in 96-well plates in 100 µL of growth medium and treated with 100 µl of the test compound one day prior. The assay is based on the reduction of the yellow MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan by metabolically active cells. The resulting color intensity, which corresponds to cell viability, was measured spectrophotometrically at 500–600 nm [22].

Measurement of MMP-1 and IL-6 production: NHSFbs were exposed to UV radiation at 144 mJ cm^{-2} using a UV radiation machine -Bio-Link BLX-312; Vilber Lourmat GmbH, Marne-la-Vallee, France. The production of MMP-1 in was analyzed by ELISA kits - ab187394 SimpleStep ELISA as specified by manufacturer. On the other hand, the production of IL-6 was explored using reverse transcription-polymerase chain reaction analysis (RT-PCR) - iScriptTM One-Step RT-PCR Kit with SYBR Green, Bio-Rad as indicated by Adams et al. [23]. Total RNA was taken out from NHSFbs using TRIzol reagent (acid-guanidinium-phenol based reagent) (Invitrogen Life Technologies, Carlsbad, CA). IL-6 mRNA expression was measured via RT-PCR as explained by [24]. The resulting products, stained with ethidium bromide, were separated on 2% agarose gel electrophoresis under UV luminence. Each experiment was conducted 3 times.

3. Results and Discussion

3.1. Ultra Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometric Analysis (UPLC-ESI-MS^2):

LC-MS approach had been confirmed to be a quick and high-powered analytical mechanism for identification of compounds in plant extracts [25]. The power of this technique comes from its accuracy to provide data about the structure of the samples. The recognition of the analyzed compounds was attained by UPLC-ESI-MS^2 technique in both negative and positive modes of ionization. Tentative identification of the secondary metabolites was built on comparing their Rt, molecular ions, MS^2 fragmentation figures with the formerly stated data. Twelve compounds were detected and tentatively identified in the *O. minor* total ethanol extract in both negative and positive ionization modes (10 in negative, 2 in positive and 3 in both negative and positive ionization modes respectively) as shown in Fig.1. The pinpointed compounds were arranged according to their retention times (Rt) and results were summarized in Table 1.

Identified compounds by LC-ESI-MS/MS (Fig. 2) are classified and discussed as follows:

Aromatic acids: Two compounds were tentatively identified in negative mode as aromatic acids. Compound (1) was identified as caffeic acid derivative with molecular ion peak at m/z 377 ($[M-H]^-$). It is characterized by the ion fragment m/z 341 which corresponds to a caffeoyl glucose moiety and the ion fragment at m/z 179 for caffeoyl moiety after losing glucose residue [13]. On the other hand, compound (2) (A, Fig. 2) was recognized as caffeic acid [26].

Phenylethanoid glycosides: Seven compounds (3, 4, 5, 6, 7, 8 and 9) were identified as phenylethanoid glycosides (PEGs). Compound (3) was identified as cistanoside F (B, Fig. 2) with molecular ion peak m/z at 487 ($[M-H]^-$) and the ion fragment base peak m/z at 179 corresponding to caffeic acid, the ion fragment at m/z 161 equaling caffeic acid after loss of carbon dioxide and m/z 135; suggesting the elimination of caffeoyl and pentosyl units, following the loss of H_2O [7]. Compound (4) was identified as β-hydroxyverbascoside (orobanchoside) (C, Fig. 2), with molecular ion peak at m/z 639. This compound was characterized by presence of the ion fragments at m/z 621[(M-H-water)]$^-$, 477 [(M-H -caffeoyl)]$^-$, 459 [(M-H -water-caffeoyl)]$^-$, 179 [(caffeoyl-H)]$^-$, 161 [(glucose-H-H_2O)]$^-$, 145 [(rhamnose-H-water)]$^-$ and m/z 135 which is corresponding to [(caffeoyl-H-CO_2)]$^-$ [27]. Compound (5) was observed in negative and identified as Decaffeoyl verbascoside (D, Fig 2) with molecular ion fragment at m/z at 461 ($[M-H]^-$). It is characterized by particular ion fragments at m/z 315, 161 and 135 which pursuant to literature [28]. Compound (6) was identified as β-methoxylverbascoside (E, Fig 2**)** with molecular ion peak at m/z 653 ($[M-H]^-$) which is characterized by fragments at m/z 621, 487, 453, 179 and 161[27]. Compound (7) was detected as the major component of the extract which was observed in both ionization modes. It was identified as verbascoside (acteoside) (F, Fig. 2) with molecular ion peak at m/z 623 ($[M-H]^-$). Verbascoside is characterized by the base peak at m/z 461 which referred to the deprivation of hexose sugar unit from the parent ion, m/z 179 and m/z 161 which were produced from the cleavage of the caffeoyl moiety followed by a further loss of H_2O [29-30]. Compound (8) with molecular ion peak at m/z 621 ($[M-H]^-$), detected also in both negative and positive mode, was identified as crenatoside (oraposide) (G, Fig. 2). It is characterized by fragment ions at m/z 179, 161, and 113 [31]. Compound (9) was observed in both negative and positive modes. It was tentatively identified as isocrenatoside with molecular ion peak at m/z 621 ($[M-H]^-$) [32]. In preliminary data, the two isomers were found to have the same MS fragmentation pattern (H, Fig. 2).

Flavonoids: One compound; compound (10) was pointed out in negative mode. Tricin flavonoid (I, Fig. 2) displayed a molecular ion peak at m/z 329 ($[M-H]^-$). It is characterized by the ion fragments at m/z 314, 299, 271 and 227 [33].

Sterols: Two compounds were detected in positive mode as sterols: Compound (11) was identified as β-sitosterol with molecular ion peak at m/z 415 ($[M+H]^+$) and was characterized by an ion fragment at m/z 397 $[M+H–H_2O]^+$ which is corresponding to β-sitosterol after losing H_2O residue, and the ion fragments at m/z 161 and 147 [34]. Additionally, compound (12) was recognized as stigmasterol. It was characterized by the molecular ion 0.peak at m/z 413 ($[M+H]^+$) and the ion fragments at m/z 395, 297 and 147 [M+H–H_2O] (J and K, Fig. 2) [35].

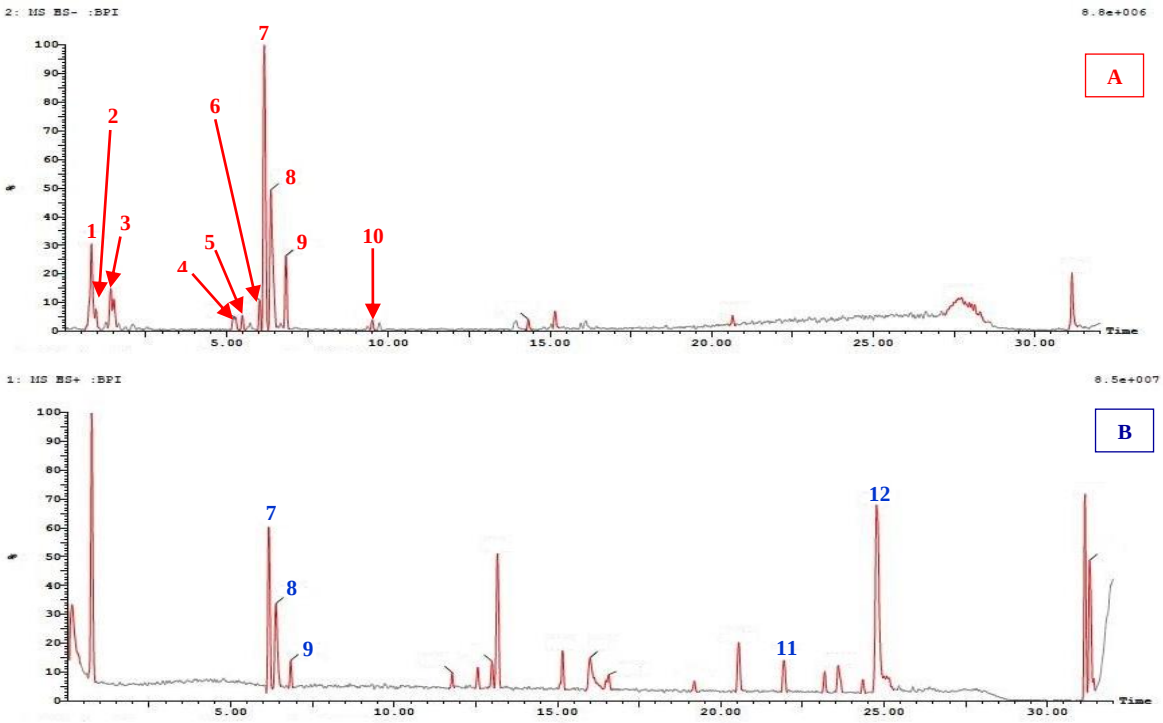

Fig. 1: LC-MS chromatograms of *O. minor* total ethanol extract. A: Negative ESI-MS mode, B: Positive ESI-MS mode.

Table (1): LC-ESI-MS/MS Analysis of *O. minor* ethanol extract

No.	Rt	$[M-H]^-$	$[M+Na]^+$/ $[M+H]^+$	MS^2 fragments	Compound name	Reference
1	0.74	377	-	*341, 179, 161, 133	Caffeic acid acid derivative	[13]
2	0.96	179	-	*135, 119, 91	Caffeic acid	[26]
3	1.41	487	-	*178, 161, 135	Cistanoside F	[7]
4	5.26	639	-	*621,477, 459, 179, 161, 145, 135	β-Hydroxyverbascoside (orobanchoside)	[27]
5	5.49	461		*315, 161, 135	Decaffeoyl verbascoside	[28]
6	6.02	653	-	*621, 487, 453, 179, 161	β-Methoxylverbascoside	[27]
7	#6.17	623	@647	*461, 179, 161	Verbascoside (Acteoside)	[29-30]
8	#6.38	621	@645	*179, 161, 113	Crenatoside	[31]
9	#6.83	621	@645	*179, 161, 113	Isocrenatoside	[32]
10	9.5	329	-	*314, 299, 271, 227	Tricin	[33]
11	21.93	-	@@415	**397,161, 147	β-Sitosterol	[34]
12	24.76	-	@@413	**395,297, 147	Stigmasterol	[35]

*MS^2 fragments in negative mode
**MS^2 fragments in positive mode
#Retention time in negative mode
@$[M+Na]^+$
@@$[M+H]^+$

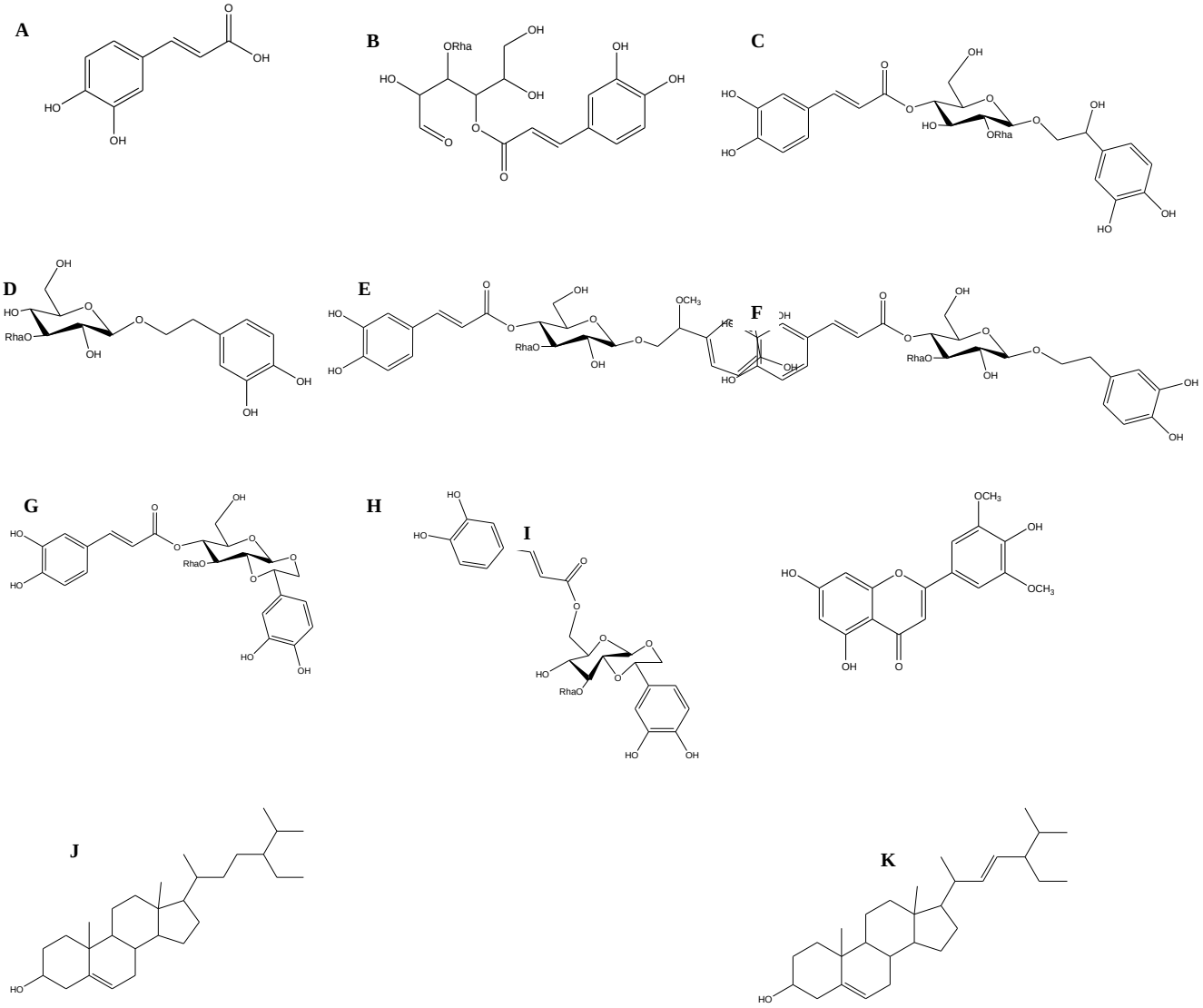

Fig. 2: Chemical structures of identified compounds. A: Caffeic acid, B: Cistanoside C: Orobanchoside, D: Decaffeoyl verbascoside, E: β-methoxylverbascoside, F: Verbascoside, G: Crenatoside, H: Isocrenatoside, I: Tricin, J: β-Sitosterol, K: Stigmasterol.

3.2. Antioxidant Activity:

DPPH scavenging assay: This assay is based on measuring the switch in color and the decrease in the absorbance of the deep purple colored DPPH$^{\bullet}$ radical to the pale yellow colored non-radical format of 517 ηm [36], which is the product of its reaction with antioxidants in the ethanol extract of *O. minor* different concentrations, using ascorbic acid as reference. *O. minor* ethanol extract showed remarkable antioxidant activity when compared with vitamin C as indicated by its high DPPH scavenging mean percentage 76.24 % at concentration of 100 µg/ml. An ascending correlation in a concentration-dependent manner was observed between the concentrations 100, 200 and 400, 800 and 1000 µg/ml of the plant extract and the DPPH scavenging capacity (A, Fig. 3). Additionally, the scavenging capacity value (SC_{50}) of *O. minor* ethanol extract was 18.32±2.01 µg/ml, while that of ascorbic acid was 11.35±1.12 µg/ml as shown in Table 2. The present results were compatible with previous results which proved that extracts of *O. foetida* and *Cistanche violacea* belonging also to family Orobanchaceae that showed strong antioxidant activity [11, 37].

FRAP assay: Ferric Reducing Antioxidant Power (FRAP) assay is based on the reducing potential of *O. minor* ethanol extract for potassium ferricyanide (Fe^{3+}) to $[K_3(FeCN)_6]^{2+}$, which reacts with ferric chloride producing ferric–ferrous Prussian blue complex which could be calculated at λ_{max} at 700 ηm [18, 38], using vitamin C as positive control. O. minor ethanol extract showed a strong reducing power activity in comparison with ascorbic acid as revealed by its higher Fe^{3+} reducing

percentage 69.12, 80.11, 86.11, 91.23 and 96.6 % at concentrations 100, 200, 400, 800 and 1000 µg/ml subsequently (B, Fig. 3). SC_{50} value was 22.57 ± 1.39 µg/ml, while that of ascorbic acid was 9.63 ±0.84 µg/ml as shown in Table 2. Results revealed that at the concentration 1000 µg/ml O. minor ethanol extract showed percentage free radical scavenging activities 94.4 and 96.5 % which were higher than those of ascorbic acid at the same concentration (91% and 94% respectively). However, the FRAP assay showed higher SC_{50} value than DPPH for O. minor ethanol extract. This might be because of the different affinities between the two radicals ($DPPH^{\bullet}$ and Fe^{3+}) and the constituents of *O. minor* ethanol extract (B, Fig 3).

Superoxide anion scavenging capacity assay: This method discovers the power of antioxidants to break the radical chain. This was carried out by calculation of the occlusion of superoxide radical created oxidation [39]. As shown in C, Fig 3, concentrations of 100, 200, 400, 800, 1000 µg/ml of *O. minor* extract showed superoxide anion scavenging activities 55.46, 63.59, 71.23, 79.32, 84.35% respectively with SC_{50} value 17.89± 0.79 µg/ml, in comparison with SC_{50} of ascorbic acid (10.37± 0.79 µg/ml). Although, at the concentration 800 µg/ml, *O. minor* ethanol extract showed almost the same free radical scavenging activity as ascorbic acid (C, Fig. 3).

All methods actively showed antioxidant activity for *O. minor* ethanol extract in concomitant with each other, which proves the strong antioxidant capacity of the extract. This strong antioxidant capacity could be attributed to the presence of PEGs (acteoside and crenatoside), that were detected by UPLC-ESI/MS^2 analysis of *O. minor* ethanol extract, and have been previously reported to exhibit high free radical scavenging capacity [40].

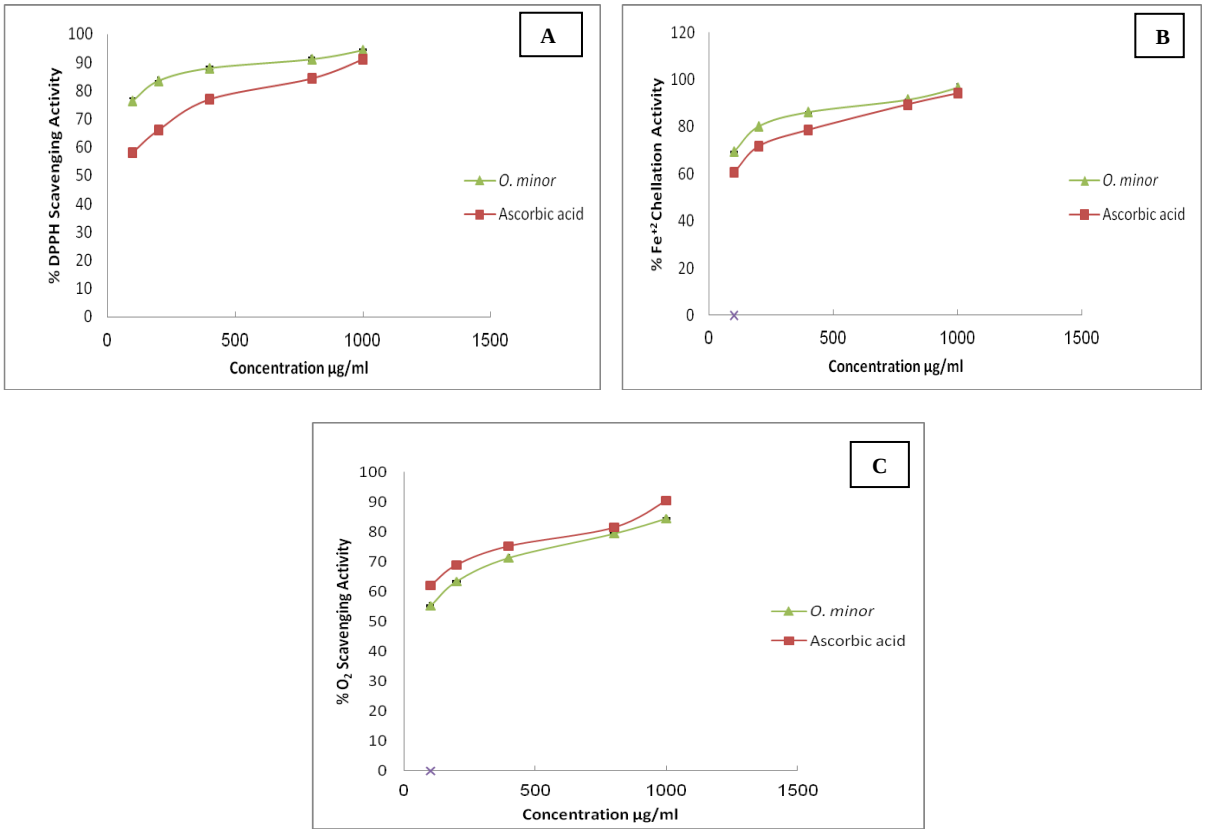

Fig. 3: Antioxidant activity of *O. minor* ethanol extract and ascorbic acid. A: DPPH assay, B: FRAP assay, C: Superoxide scavenging activity. ^{ns} Non-significant, * Significant *P*-value < 0.05, ** Significant *P*-value > 0.01

Table (2): SC_{50} of *O. minor* extract and ascorbic acid using the three different assay methods

	DPPH SC_{50}	FRAP SC_{50}	Superoxide anion SC_{50}
***O. minor* ethanol extract**	18.32±2.01 µg/ml	22.57 ± 1.39 µg/ml	17.89 ± 1.31 µg/ml
Ascorbic acid " standard"	11.35 ±1.12 µg/ml	9.63 ± 0.84 µg/ml	10.37± 0.79 µg/ml
***P*-value**	0.039	0.012	0.0492

3.3. Cytotoxic Activity:

The MTT cytotoxicity assay was done to estimate the viability of all cells in culture which is used to evaluate the metabolic activity of cells as a mark for cytotoxicity of the samples under investigation. This assay is based on the reduction of the yellow MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan by the metabolically active living cells. The resulting colour intensity, which corresponds to cell viability, was measured spectrophotometrically at 500–600 nm [41-42]. Total ethanol extract of *O. minor* was tested for its cytotoxicty on normal human skin fibroblasts (NHSFbs) cell lines. Our research was established to profile the toxicity of *O. minor* on the normal cells of the human skin aiming to confirm its safety for dermal use (Fig. 4). A graph was plotted expressing the relation between logarithm the average of *O. minor* total extract concentration and the percentage of the cell viability. The 50% inhibitory concentration (IC_{50}) was calculated according to the linear regression equation:

$$Y = -19.58 X + 115.92$$

Where Y is the percentage inhibition of half of the NHSFbs=50 and X is the logarithm of *O. minor* ethanol extract concentration [43].

It was found that incubation of NHSFbs with different concentrations of *O. minor* ethanol extract reduced the viability of these cells in a concentration dependent way (Fig. 4), with IC_{50} of 2326 ± 92 µg/ml. Following the guidelines of the American National Cancer Institute (NCI) [44] as a standard to evaluate the cytotoxicity of *O. minor* ethanol extract, cytotoxicity results can be expressed as: strong (IC_{50} value less than 20 µg/ml), moderate (IC_{50} value 20-50 µg/ml) and no cytotoxicity (IC_{50} value more than 50 µg/ml). Therefore, our obtained results indicated that the ethanol extract of *O. minor* Smith has no cytotoxicity on normal human skin fibroblasts, which ensure the high safety of the extract.

Table (3): Effect of different concentrations of *O. minor* ethanol extract on cell viability of NHSFbs

Log. Conc.	% Viability
3	52.89
2.398	70.74
1.796	86.57
1.193	92.8
0.591	100.8

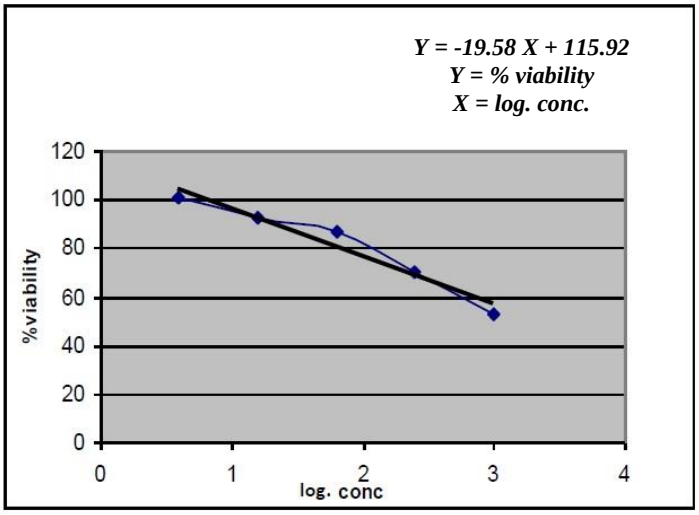

Fig. 4: Cell viability of NHSFbs treated with different concentrations of *O. minor* ethanol extract.

3.4. Photoprotecting Activity:

Scientists considered Ultraviolet (UV) light as an extremely dangerous factor that intimidate the human skin which can be considered as the first line of defence against UV rays [45]. Sustained exposure to UV radiation causes production of large amount of reactive oxygen species (ROS) that initiates matrix metalloproteinase expression and collagen lyses leading to premature vulnerability of the skin (photoaging), in addition to production of the inflammatory cytokine interleukin-6 (IL-6) leading to inflammation of the skin [46]. Photoaging looks as skin dehydration, lack of the flexibility and appearance of

wrinkles. Matrix metalloproteinase-1 (MMP-1) is considered as the main enzyme accounted for collagen decay as it had been announced to begin the break of collagen. From this point of view, the use of natural antioxidants as photoprotective agents has become an important topic for research [47].

The present study revealed that the *O. minor* ethanol extract have a significant antioxidant activity with high *in vitro* safety profile. As no scientific data exist regarding the photoprotective action of *O. minor*, our study was performed to explore the photoprotective activity of *O. minor* ethanol extract on NHSFbs through measuring the production of both major collagenolytic enzyme (MMP-1) and the inflammatory cytokine (IL-6) in UVB-exposed NHSFbs. To demonstrate the effects of *O. minor* on the damage induced by UV, MMP-1 level was measured by ELISA kits and the concentration of the pro-inflammatory cytokines IL-6 was measured using RT-PCR analysis; using the pre UV-irradiated NHSFbs as a control. Our findings indicated that *O. minor* inhibited the production of MMP-1 and the secretion of IL-6 in NHSFbs. *O. minor* ethanol extract (100 µg/ml) suppressed MMP-1 levels by 429.5 pg/ml (A, Fig. 5). Meanwhile, *O. minor* lowered the excretion of IL-6 by 1.671 RT-PCR fold change; compared with UV-irradiated control groups (B. Fig. 5). These results demonstrated that the *O. minor* total ethanol extract significantly reduced the levels of MMP-1 and IL-6 compared to the non- UV- treated normal cells.

A previous study reported that verbascoside inhibited the angiogenesis caused by tumors through the suppression of oxygen free radicals and MMP expression [48]. However, no statements are available regarding effect of *O. minor* on MMP-1 production. Our outcomes implied that the MMP-1 protein and IL-6 cytokine were sharply diminished after treatment with *O. minor* suggesting that *O. minor* ethanol extract can be effective as protecting agent from the UV-induced skin damage and anti-inflammatory activities. During our phytochemical study of *O. minor*, we have previously demonstrated that the plant contains mainly phenolic compounds such as phenylethanoid glycosides (verbascoside as a major component *O. minor* extract) and caffeic acids; the compounds which were reported previously to possess antioxidant and anti-inflammatory activities [49-50].

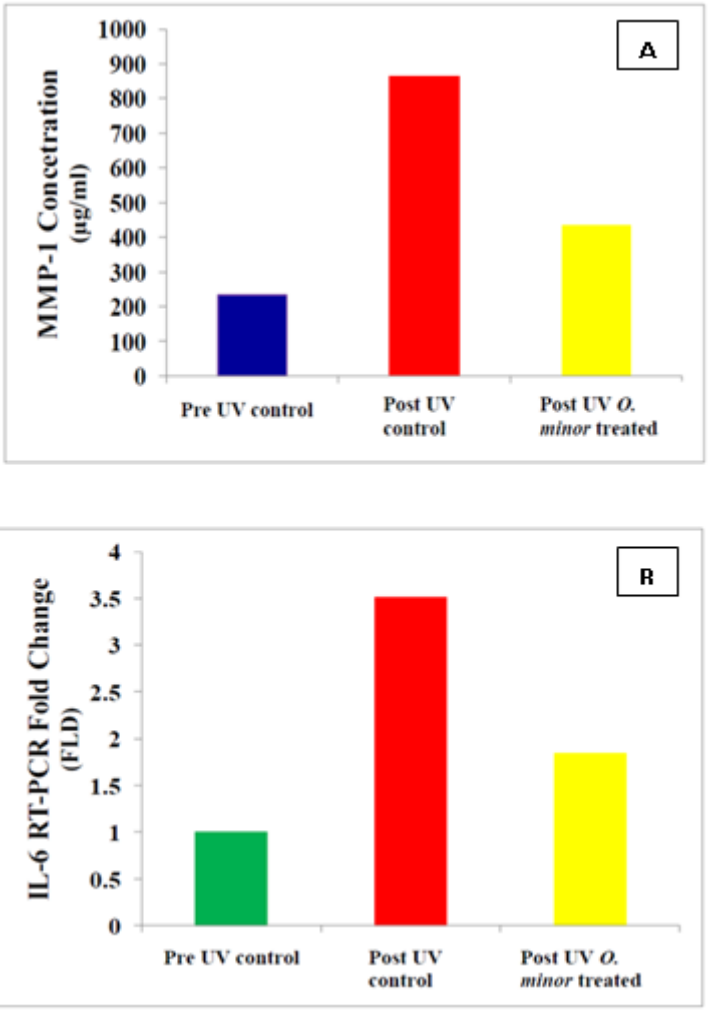

Fig. 5: Effect of *O. minor* ethanol extract on MMP-1 and IL6 expression in UV- exposed NHSFbs. A: Production of MMP-1, B: Secretion of IL-6.

4. Conclusion:

Finally, this study could be useful in valorization of *O. minor* by declaring its main phytochemical profile which included mainly phenylethanoid glycosides. After assaying with three different methods, our results showed that the plant extract possessed a strong antioxidant activity. Furthermore, the *O. minor* extract showed high *in vitro* protective and anti-inflammatory effects on UV irradiated normal human skin fibroblast cells by inhibiting secretion of MMP-1 and decreasing IL-6. Moreover, it showed no *in vitro* cytotoxicity on normal skin fibroblasts. Such results could highlight different beneficial uses of this parasitic weed.

5. Conflicts of Interest

We have no conflicts to declare.

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