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ALKALOID CONTENTS AND ANTI-OXIDANT EFFECTIVENESS OF EXTRACTS OF THE *Orobanche* aegyptiaca L. AND *Orobanche ramosa* L. (OROBANCHACEAE)

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ABSTRACT

Total alkaloids content of both *Orobanche aegyptiaca* and *Orobanche ramose* Was detected using High-performance liquid chromatography (HPLC). The different kinds and concentration of alkaloids were also studied. Anti-oxidant effectiveness of both species was investigated using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). In *O. ramose* five alkaloids were identified (Lupanin, Sparteine, Oxoretamine, Isospartein and Oxosparteine), in case of *O. aegyptiaca* only four alkaloids were identified (Lupanine, Spartein, Isosparteine and Oxosparteine). The highest level of alkaloids was found in *O. aegyptiaca* (138.93 µg/gm) and the rate inhibition of free radicals was (85.31%).

Keywords: Phytochemistry, Anti-oxidant activity, Alkaloids, *Orobanche aegyptiaca* and *Orobanche ramose*

INTRODUCTION

The medical plants were used for the medicinal purpose in various ancient civilizations. According to scientific reports that 60-80% of the world's people rely on integrity and primary health on alternative medicine (traditional medicine), and 80% of the people of the developed countries depend on medicinal plants and the origin of 25% of the widely used modern drugs and medicine that uses for diseases treatment are traditional medicine plants (WHO, 2003, Mahmoud and Gairola, 2013).

Studies indicates that the plant species with medical important is about more than 30% of the total known species in the world (8000 species of medical plant of total 250,000 identified species in the world) (Joy et al, 1998), the isolated of secondary metabolites from natural sources is estimated at less than 10% (about 12,000 compounds) (Savadi, 2009), which refers that there is fertile ground for scientific research on the plant and their active substances and using them in the therapeutic range, perhaps the most important of these plants is the genus *Orobanche* (Orobanchaceae) includes holoparasitic plants 170 species Uhlich, et al. (1995) parasites on the host plant roots, Abbes *et al* (2014) refered to the *Orobanche* with a highly effectiveness in the inhibition of free radicals during their studies, which included two species of *Orobanche*. Gevezova *et al* (2012) isolated the chemical compound of *Orobanche aegyptiaca*, which is an important compound of *Orobanche* spp.

MATERIALS AND METHODS

1 - Collection of Plants and Diagnosis

The plant samples were collected in flowering period (March-Jun) through field trips (2015) in mountain and plain areas, the fresh specimens were kept and labeled (collection place, date, collector name, ...) and then transferred to the laboratory there were dried, the remains Plant diagnosing based on the Sirwan, 2002.

2- Extraction and Chemical Analysis:

Alkaloids of entire plant (flowers and stem) in *O. aegyptiaca* and *O. ramosa* were estimated by a technique of (HPLC) High-Performance Liquid Chromatography as follows:

- **Extraction**:

The extraction alkaloids was conducted according to Kamada *et al*, (1986), this method provides a good amount of alkaloids derived from the use of a small amount of the sample (about 20 gm), the steps are according to the following scheme

Crushed plant material
▼
Me OH-NH4OH (25%) (15:5:1) - Extraction with CHCl3
(10 ml per 100 mg of sample) sonicated for 10 minutes
▼
Leave at room temperature for 1 hour
▼
Filter through paper filter and wash 2× with 1 ml CHCl3
▼
Dryness by evaporate
▼
Add 5ml CHCl3 and mix thorough 2ml 1 N H2SO4
▼
Remove CHCl3 fraction and adjust to Ph. 10 with 28% NH4OH on ice
▼
Extract alkaloids once with 2 ml CHCl3 and twice with 1 ml CHCl3
▼
Filter after the addition of anhydrous Na2SO4 and wash the residue with 1-2 ml CHCl3
▼
Evaporate and dissolve the sample in 1ml Me OH

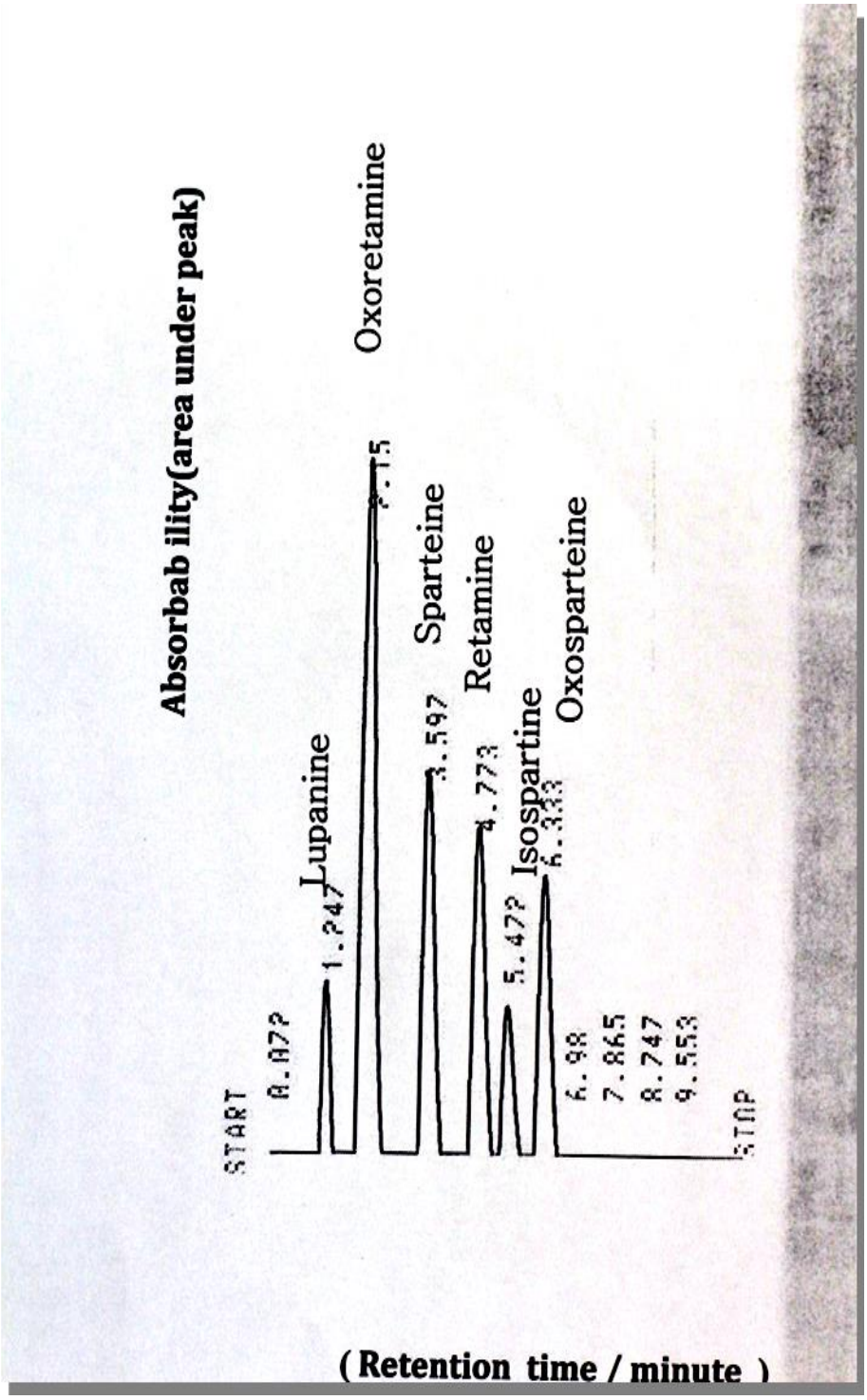

Figure1. The standard alkaloid peaks by HPLC

To calculate the concentrations of unknown diagnosed alkaloids of both species as well as the percentage of the diagnosed compound, the following equations was used, (Sawhney and Singh, 20

$$\text{Unknown specimen concentration µgm/gm} = \frac{\text{Specimen area under the curve}}{\text{Standard compound area}} \times \text{Standard concentration} \times \text{times of dilution}$$

$$\text{Concentration \%} = \frac{\text{Diagnosed compound concentration}}{\text{Total concentration of the diagnosed compound}} \times 100$$

Table1. The standard analysis of alkaloids with retention time, area and concentration

Series	Alkaloids	Retention time	minute	Area	Concentration 25 µgm \ml
1	Lupanine	1.24		62525	25
2	Oxoretameine	2.15		207390	25
3	Sparteine	3.59		134651	25
4	Retamine	4.77		104204	25
5	Isosparteine	5.47		80599	25
6	oxosparteine	6.33		100392	25

A- Anti-oxidant Effectiveness:

La bioactivité de l'anti-oxydant a été testé par la mesure de capacité à la répression sans usine radicaux avec à l'aide d'un spectrophotomètre et style de contrôle 2,2-diphenyl-1-picrylhydrazyl (DPPH) et à l'aide de l'acide ascorbique comme un contrôle de comparer la capacité de l'acide ascorbique avec capacité d'échantillons végétaux pour réprimer les radicaux libres en se fondant sur les antioxydants capacité à l'électron don et transformer les radicaux libres dans des composés stables incapable d'interagir avec bimolecules dans le corps afin d'éliminer ainsi l'activité nocive de radicaux libres. Les mesures ont été fondées comme Stojicevic et al, (2013), Brand- Williams, 1995, Shih et al, (2002), avec quelques modifications, comme nécessaires comme suit :Preparation of standard curve for ascorbic acid:

Dissolving of 10 mg of ascorbic acid in 1 ml of absolute ethanol, the final condensation is 10 mg/ml, five concentrations were used they are 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml they were tested with the preparator by dissolving 0.002 mg of DPPH in 100 ml of ethanol, samples conserved in the dark at 25 C° for 30 minutes and the wavelength measured at 517 nm at the inhibition proportion of using aqueous extract of plant in the same concentrations but the plant extract has been used instead of ascorbic acid, the 0.5 ml of the plant sample was added to 2.5 ml of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), the mixture was incubated for 30 minutes in a dark place, and absorbance were measured at a wavelength of 517nm.

The ratio of inhibition of free radicals measured by using the following equation:

$$\text{The Inhibition \%} = \frac{\text{Absorption of the specimens -}}{\text{Absorption of control or (ascorbic acid)}} \times 100$$

The figure 2 shown the inhibition ratio has reached to 94.09% at the concentrate of 10mg/ml

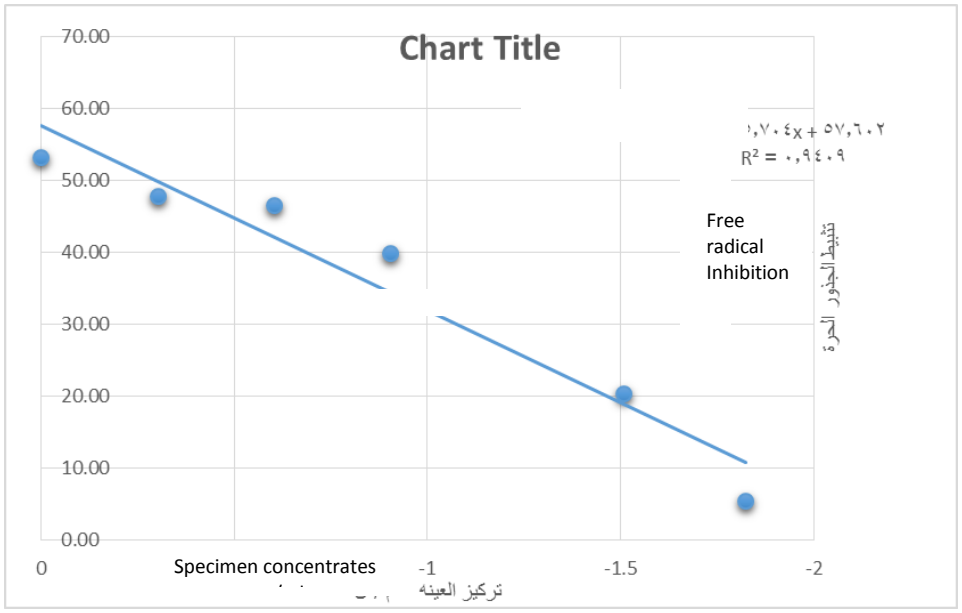

Figure 2. Percentage of free radicals suppression by ascorbic acid at different concentrations between 2 - 10 mg / ml.

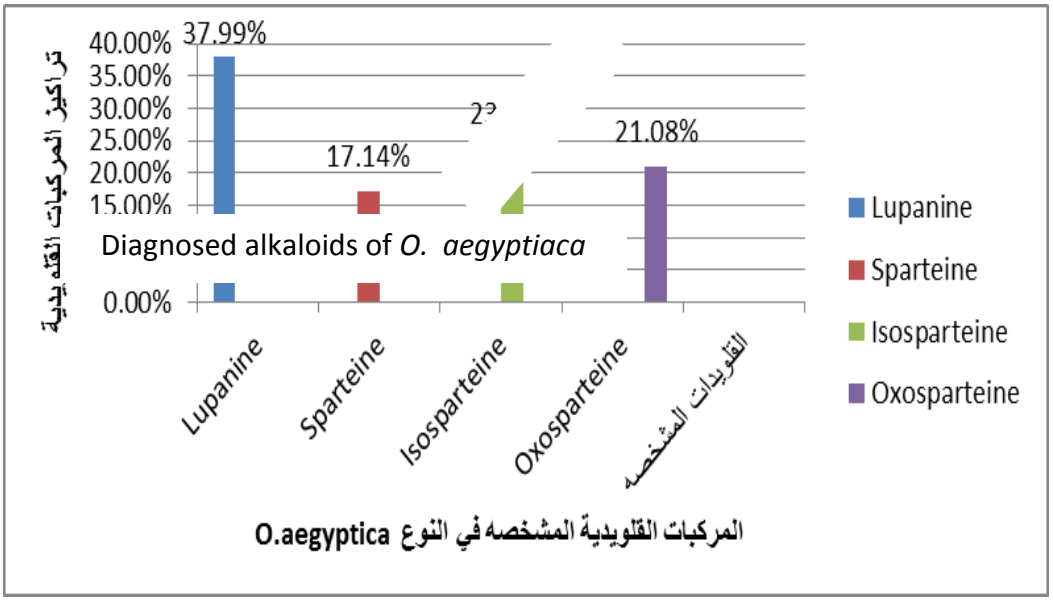

Figure 3. Diagnosed alkaloids of *O. aegyptiaca*

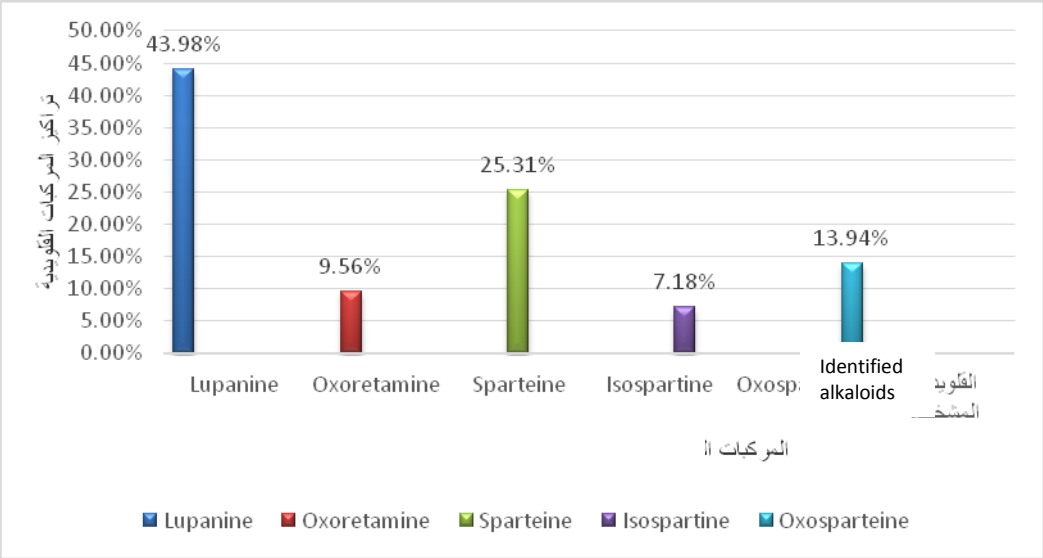

Figure 4. Diagnosed alkaloids of *O. ramosa*

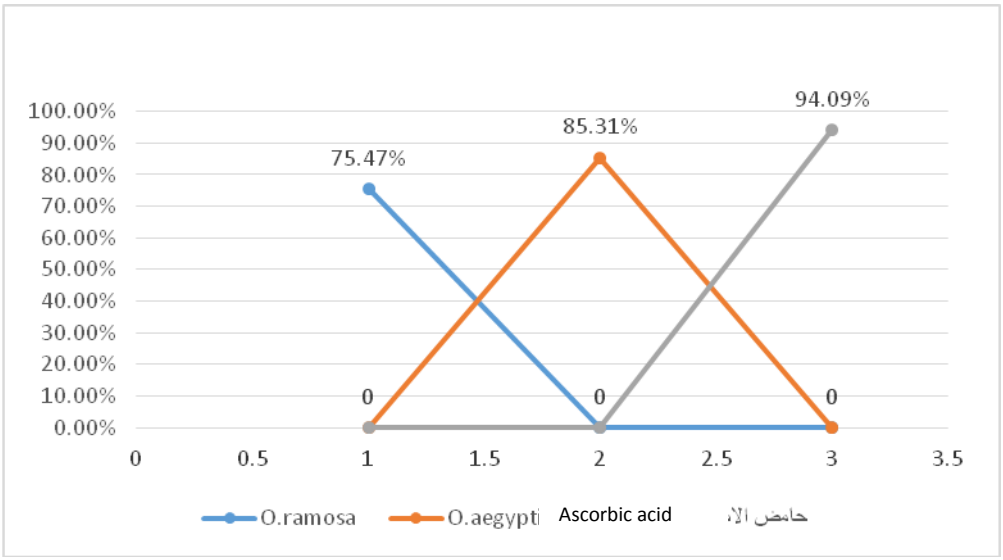

Figure 5. Anti-oxidant effectiveness percentage of the specie *O .aegyptiaca and O. ramosa*

RESULTS AND DISCUSTION

Alkaloid Contents:

Results of HPLC showed that the *O. aegyptiaca* contained four types of alkaloids they are: Lupanine, Sparteine, Isosparteine and Oxosparteine, the alkaloid Lupanine showed superiority to the remain alkaloids with concentrations of 37.99%, while the Sparteine is least concentrated among alkaloids that have been diagnosed in the entire flowering plant above the ground surface for the species *O. aegyptiaca* reached to 17.14% and the concentrations of the two compounds Isosparteine and Oxosparteine are 33.78% and 21.08% respectively, as shown in figure 4 the diagnosed alkaloids in the entire flowering plant above the ground in *O. ramosa* are five includes Lupanine, Sparteine, Isosparteine, Oxosparteine and Oxoretamine and appeared that the alkaloid Lupanine seemed superiority to the rest of alkaloids with concentrations of 43.98% however the Isosparteine with the least concentration among alkaloids 7.18%, the other diagnosed alkaloids concentrations varying, Sparteine was 25.31%, Oxosparteine 13.94% and Oxoretamine 9.57%.

Results seems that the chemical content varies in different plant species, as well as both of species differed in the total content of alkaloids where outweigh the *O. aegyptiaca* 138.93 mg/g on the species *O. ramose* 98.8 mg /g, the all diagnosed alkaloids belonging to the Quinolizidine alkaloids group (QA), widespread group in many plant families such as families Fabaceae and

Malvaceae which accounted about 2% of the total diagnosed alkaloids in plants (Petruczynik, 2012). there are parts of the plant contains two alkaloids Lupanine and Sparteine they has industrial importance in terms of the first alkaloid is toxic which is of secondary metabolites produced by the plant for the purpose of defense against herbivores and insects either compound sparteine with a medical significance it use in the treatment of heart disease Ruano et al (2012).

Antioxidant Effectiveness of the entire flowering plant above ground (stem and flowers) of the *O. aegyptiaca* and *O. ramosa:*

The Aqueous extracts of the entire flowering plant above the ground of both studied species showed the anti-effectiveness to control free radicals with obvious varying in anti-oxidant effectiveness percentage between them, the inhibition rate to free radicals in *O. aegyptiaca* was reached 85.31% it has been close to the standard antioxidant effectiveness (ascorbic acid) 94.09%, the aqueous extract of *O. ramosa* showed the apparent anti-oxidant effectiveness in compared to the standard, but with a lower percentage than the anti-effective of free radicals in *O. aegyptiaca*, the inhibition proportion of free radicals by aqueous extracts of the *O. ramosa* reached 75.47% compared to the standard 94.09%. The results consistent with the results of Abbes et al (2014), which indicated the existence of anti-oxidant effectiveness of two *Orobanche* species (*Orobanche crenata* and *Orobanche foetida*)

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المحتوى القلويدي والفعالية المضادة للأكسدة لنوعين من جنس الهالوك *Orobanche aegyptiaca and Orobanche ramose* (Orobanchacae)

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تم دراسة المحتوى القلويدي و الفعالية المضادة للاكسدة في نوعين من جنس الهالوك هما *Orobanche ramose* و *Orobanche aegyptiaca* لكامل النبات المزهر فوق سصح الارض باستخدام تقنية High-performance liquid chromatography (HPLC) وتم تحديد تراكيزها و طريقة الجذر الحر الثابت 2,2-Diphenyl-1- picrylhdrazyl (DPPH) حيث شخصت خمسة انواع من المركبات القلويدية في النوع *Orobanche ramose* (Oxospartine و Lupanine, Sparteine, Oxoretamine Isosparteine) و اربعة قلويدات في النوع *Orobanche aegyptiaca* (Oxosparteine و Isosparteine, Sparteine, Lupanine) و قد اظهر النوع *Orobanche aegyptiaca* اعلى مستوى من القلويدات الكلية (138.93 µg/gm) و كذلك في تثبيط الجذور الحرة بنسبةالتثبيط 85.31%