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# ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF THE PARASITIC PLANTS *OROBANCHE FOETIDA* AND *OROBANCHE CRENATA* COLLECTED ON FABA BEAN IN TUNISIA

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## ABSTRACT

The antioxidant activity of the methanolic and aqueous extracts of two parasitic plants *Orobanche crenata* and *Orobanche foetida* collected from faba bean fields was investigated with 2 complementary test systems, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ) radical scavenging activities. The *O. crenata* methanol extract showed the highest level of DPPH and ABTS free radical scavenging activities, with $IC_{50}$ values of 2.76 µg/ml and 7.96 µg/ml respectively. The amount of total polyphenol and tannins varied in the different plant extracts and ranged from 3.02 to 19.99 mg GAE/g DW for polyphenol contents and from 0.09 to 0.32 mg EC/g DW for tannins contents. Antimicrobial activity was investigated with the disc diffusion method. The methanol extract of *O. foetida* showed activity against all tested bacterial strains, except *S. aureus* ATCC 6538, by forming clear inhibition zones with diameters between 12 and 30 mm whereas methanol extracts of *O. crenata* inhibits only *L. monocytogenes* and *S. enteredis* ATCC 502 with an inhibition zone of 10 and 25 mm respectively. Aqueous extracts of the two Orobanche species were not active against any of these bacterial isolates. These results implied that these two Orobanche species collected from infested faba bean fields might be potential resources of antioxidant and antibacterial activities and can be used in human nutrition and some industrial and pharmaceutical products.

**Keywords:** antioxidant, antibacterial, *O. foetida, O. crenata,* polyphenol.

## INTRODUCTION

The free radicals as superoxide anions, hydrogen peroxide, hydroxyl radicals induce oxidative damage to biomolecules which eventually causes many diseases such as ageing, inflammation and tissue injury and infection (Aruoma, 1994). The use of plant extracts as an alternative solution to these diseases has become very important (Arshad *et al*., 2012; Prakash *et al*., 2007). Thus, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects (Papuc *et al*., 2008). Many studies focused on medicinal plants containing significantly high levels of phenolic compounds and exhibiting strong antioxidant and antibacterial activities (Kaya *et al*., 2010; Silva *et al*., 2012). Other studies indicated the presence of these compounds and biological activities in many other plants such as the parasitic plants Striga and Orobanche (Sharaf and Youssef, 1971; Saadoun and Hameed, 1999; Badami *et al*., 2003; Saadoun *et al*., 2008; Nagaraja *et al*., 2010).

Despite the large amount of research on screening for active compounds from plants in different parts of the world, to date little is known about the possible active compounds from the parasitic plants *O. crenata* and *O. foetida*. However, the antioxidant and antibacterial activities of *O. foetida* plants have not been previously studied.

The goals of this study were to quantify polyphenolic and tannins contents of methanolic and aqueous extracts of two Orobanche species (*O. crenata* and *O. foetida*), to test their antibacterial activities and to emphasize their scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS).

## MATERIALS AND METHODS

**Plants Sampling and Extraction:** The aerial parts of *O. foetida* and *O. crenata* grown on faba bean were collected from Beja and Ariana research stations (Tunisia) in June, 2010. The freshly cut plants were sorted out, dried in a drying room with active ventilation at ambient temperature and stored until use. The dried powder of each plant materials (9 g) was extracted with 250 ml of methanol or water in a soxhlet apparatus, according to the method described by Kalia *et al*. (2008), with some modification. The extracts were then filtered and

concentrated with rotary evaporator at a constant temperature of 40°C.

**Determination of Total Phenolic Contents:** The total phenolics content was determined according to Velioglu *et al*. (1998). 100 µl of each diluted extract (dilution factor of 1/50 for *O. crenata* extract and 1/25 for *O. foetida* extract) was mixed with 100 µl of distilled water and 0.5 ml of 10% Folin-Ciocalteu reagent. Then, 1 ml of sodium carbonate solution (7.5%) was added. The mixture was incubated at room temperature in the dark for 60 min. The absorbance was measured at 725 nm against a blank using a spectrophotometer. The results were expressed as milligram gallic acid equivalents per gram of dry weight of plant material (mg GAE/g DW). The assay was performed in triplicate for each extract.

**Total Condensed Tannin Assay:** The analysis of condensed tannins was carried out according to Sun *et al*. (1998). To 50 µl of properly diluted sample (dilution factor of 1/2), 1.5 ml of 4% vanillin solution in methanol and 750 µl of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min, and the absorption was measured at 500 nm against methanol as a blank. Results are expressed as mg (+)-catechin/g DW. The calibration curve range of catechin was established between 0 and 400 µg/ml. All samples were analysed in triplicate.

**Antioxidant Activity:**

- *DPPH radical scavenging assay:* Radical scavenging activity was determined by spectrophotometry at 517nm according to Brand-Williams *et al*. (1995). Briefly, sample extracts at different concentrations (1, 10, 100 and 200 µg/ml) were mixed with the same volume of 0.2 mM methanolic DPPH solution. Samples were kept in the dark for 30 min at room temperature and absorption decrease was then measured. The percent inhibition was calculated using formula:

$$\% \text{ Inhibition} = [(AB-AA)/AB]\times 100;$$

where AB is absorption of blank sample at t=0min and AA is the tested sample absorption at t=30min.

The antioxidant activity was also expressed as $IC_{50}$, which was defined as effective concentration of sample (in µg/ml) at which 50% of DPPH radicals are scavenged. The synthetic antioxidants 2,6-di-tert-butyl-4-methylphenol (BHT) and ascorbic acid (AA) were used as positive controls. Each assay was repeated three times (Aslim and Ozturk, 2011).

- *ABTS activity:* ABTS radical-scavenging activity of Orobanche extracts was determined according to Re *et al*. (1999). ABTS was dissolved in deionized water to 7 mM concentration, and potassium persulfate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature for 24 h in the dark before the use. Each sample extract, at different concentrations (0.5, 1, 5, 10, 100 and 200µg/ml), was gotten as described above. The ABTS solution was diluted with ethanol, to an absorbance of 0.7 ± 0.02 at 734 nm. After the addition of 950 µl of diluted ABTS solution to 50 µl of plant extract, the reaction mixture was incubated at 37°C for 10 min and then the absorbance was measured at 734 nm. AA and BHT were used as a standard. All determination was carried out in triplicate.

**Antimicrobial Activity:** The Orobanche extracts were tested against a large panel of microorganisms obtained from international culture collections; ATCC, and the local culture collection of Pasteur Institute of Tunis. They included Gram-positive and Gram-negative bacteria (Table 1). The bacterial strains were cultivated in Luria Bertani Medium (LB) at 37°C except for Bacillus species which were incubated at 30°C. The paper disc-diffusion method was employed for the determination of antimicrobial activities (NCCLS, 1999). Briefly, suspension in LB of the tested microorganism (0.1 ml of $10^7$–$10^8$ cells per ml) was spread on the solid LB media plates. Paper disc (9 mm in diameter) were impregnated with 12 µl of the Orobanche extract and placed on the inoculated plates. The concentration of methanolic extracts were 10 mg/ml for *O. foetida* and 60 mg/ml for *O. crenata*, however the concentration of water extracts were 50 mg/ml and 40 mg/ml respectively for *O. foetida* and *O. crenata*. These plates, after remaining at 4°C for 2 h, were incubated at 37°C for 24 h. The diameter of the inhibition zones were measured in millimetres. All the tests were performed in duplicate and repeated twice. Streptomycin B (10 µg/ml) and Chloramphenicol (30 µg/ml) were used as positive controls.

**Statistical Analysis:** Data from the experiments were subjected to analysis of variance (ANOVA) using SPSS 15.0 for Windows. Means were separated at the 5% significance level by the least significant difference (LSD) test. Data were expressed as mean ± SD.

## RESULTS AND DISCUSSION

**Total Polyphenol and Tannins Contents:** Phenolic compounds are receiving increasing attention because of their health promoting effects**.** The amount of total polyphenol and tannins varied in the different plant extracts and ranged from 3.02 to 19.99 mg GAE/g DW for polyphenol contents and from 0.09 to 0.32 mg EC/g DW for tannins contents (Figures 1 and 2).

With respect to the total polyphenol contents, the *O. crenata* methanol extracts have higher content of polyphenol than that of water. While for *O. foetida* the two extracts have similar contents. The phenol content of the *O. crenata* methanol extract is higher than that of *O. foetida* at 19.99 and 3.42 mg/EAG respectively. The aqueous extract had similar phenol content relative to *O. foetida* and *O. crenata* at 4.24 and 3.02 mg/EAG respectively (Figure 1). In the case of total condensed

tannins, *O. crenata* methanol extracts also have higher contents than aqueous extracts i.e. 0.32 and 0.09, respectively. With *O. foetida*, total condensed tannins are higher in aqueous extracts than in methanol ones i.e. 0.26 and 0.13 respectively. Unlike aqueous extracts, *O. crenata* methanolic extracts contained more condensed tannins than *O. foetida* ones (Figure 2).

The results obtained with *O. foetida and O. crenata* are slightly low than those obtained by other authors for other plants (Kähkönen *et al*., 1999; Kaya *et al*., 2010). Sharaf and Youssef (1971) indicated that *P. aegyptiaca* proved to contain alkaloids at 0.156 g/g of the dried plant.

This study shows for the first time the polyphenol and tannins contents in these two parasitic plants. The differences observed between the two Orobanche species might be due to environmental factors or due to genome. Indeed, it has been reported that the amount of total phenolics vary with respect to families, varieties and also the different parts of the same plant (Kaur and Kapoor, 2002; Romani *et al*., 2003). More analysis on these Orobanche extracts is recommended in order to give a full picture of the quality and quantity of the phenolic constituents.

**Antioxidant Activity:**

- *DPPH activity:* DPPH analysis is one of the tests used to prove the ability of the components of the different orobanche extract to act as donors of hydrogen atoms. The obtained results are shown in Figure 3. The different Orobanche extracts showed a significant effect in inhibiting DPPH, reaching up to 91.97% at a concentration of 200 µg/ml (*O. foetida* methanol extract) and its $IC_{50}$ was 7.19 ± 1.75 µg/ml compared with the $IC_{50}$ of BHT of 65. 48 ± 1.44 µg/ml and AA of 0.93 ± 0.07 µg/ml.

At 1 µg/ml, *O. crenata* methanol extract, *O. crenata* water extract, *O. foetida* methanol extract, *O. foetida* water extract, BHT and ascorbic acid caused DPPH radical scavenging activity at 19.49, 18.26, 5.86, 14.66, 13.35 and 53.95% respectively while at 200 µg/ml, the results were 88.11, 76.98, 91.97, 86.09, 85.02 and 86.32% for *O. crenata* methanol extract, *O. crenata* water extract, *O. foetida* methanol extract, *O. foetida* water extract, BHT and ascorbic acid respectively.

- *ABTS activity:* The second test used in this study to determine antioxidant activities of the different orobanche extract is the ABTS test. The obtained results for Orobanche extracts and synthetic antioxidants BHT and AA are shown in Figure 4. The annihilation of ABTS radical, expressed in % inhibition at 0.5µg/ml, was 4.04% for *O. crenata* methanol extract, 1.64% for *O. crenata* aqueous extract, 1.28% for *O. foetida* methanol extract and 2.34% for *O. foetida* aqueous extract. The scavenging effect of synthetic antioxidants was 9.98% for BHT and 23.08% for AA (Figure 4).

At 200 µg/ml, the results were 99.57, 99.49, 94.82, 95.34, 98.46 and 99.34% for *O. crenata* methanol extract, *O. crenata* aqueous extract, *O. foetida* methanol extract, *O. foetida* aqueous extract, BHT and ascorbic acid respectively. These values of the different Orobanche extracts were found to be less than those obtained for the reference standards, BHT and ascorbic acid at a concentration less to 100.

No study on the antioxidant activity of Orobanche plants was shown in the literature. However, another parasitic plant Striga exhibited strong antioxidant activity with $IC_{50}$ values of 18.65 ± 1.46 and 11.20 ± 0.52 µg/ml respectively in the DPPH and nitricoxide radical inhibition assays. These values were found to be less than those obtained for the reference standards, ascorbic acid and rutin (Badami *et al*., 2003). Striga extracts seems to have also antibacterial activities (Hiremath *et al*., 1996).

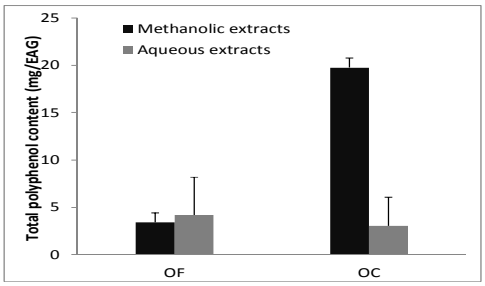


**Figure 1: Total polyphenol content in methanolic and aqueous extracts of Orobanche species. Data expressed as mean ± SD. OF: *O. foetida*, OC: *O. crenata*.**

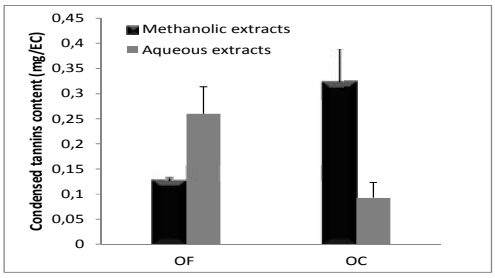


**Figure 2: Condensed tannins content in methanolic and aqueous extracts of Orobanche species. Data expressed as mean ± SD. OF: *O. foetida*, OC: *O. crenata*.**

**Antibacterial Activity:** Orobanche extracts were evaluated for their inhibitory activity against a large number of bacterial strains (Table 1). The methanol extract of *O. foetida* showed activity against all bacterial strains, except *S. aureus* ATCC 6538, by forming clear inhibition zones with diameters between 12 and 30 mm

whereas methanol extracts of *O. crenata* inhibits only *L. monocytogenes* and *S. enteredis* ATCC 502 with an inhibition zone of 10 and 25 mm respectively. Results showed that methanol extracts are more active than aqueous extracts. Thus, aqueous Extracts of the two Orobanche species were not active against any of these bacterial isolates (Table 1). The same observation was shown with Striga, a parasitic plant, where petroleum ether, chloroform and ethanol extracts showed antibacterial activity against most of the organisms, whereas the aqueous extracts were found to have no effect against most bacteria (Hairemath *et al*., 1996).

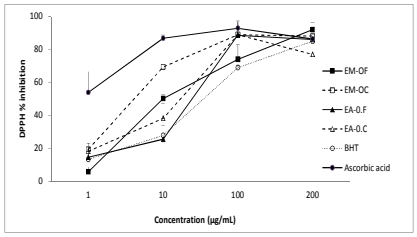


**Figure 3: Orobanche species tested and BHT and AA at different concentrations (µg/ml). Data expressed as mean ± SD. EM-OF: *O. foetida* methanol extract, EM-OC: *O. crenata* methanol extract, EA-OF: *O. foetida* aqueous extract, EA-OC: *O. crenata* aqueous extract, BHT: 2,6-di-tert-butyl-4-methylphenol.**

Recently, Saadoun *et al*. (2008) showed that *O. cernua* extract has an effective inhibitory action against local isolates of crown gall (*Agrobacterium*) and soft rot (*Erwinia*) phytopathogens. Saadoun and Hameed (1999) demonstrated that *O. cernua* extract displays also remarkable activity against some Gram-positive and Gram-negative bacteria. Similarly, Nagaraja *et al*. (2010) demonstrated that alcoholic and acetone extracts of *P. aegyptiaca* are found to possess potential antifungal property.

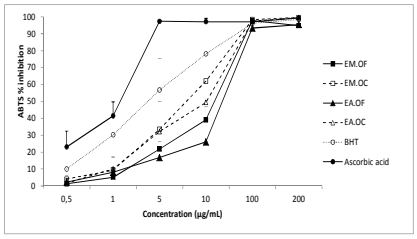


**Figure 4: ABTS free radical scavenging activities of methanolic and aqueous extracts of Orobanche species tested and BHT and AA at different concentrations (µg/ml). Data expressed as mean ± SD. EM-OF: *O. foetida* methanol extract, EM-OC: *O. crenata* methanol extract, EA-OF: *O. foetida* aqueous extract, EA-OC: *O. crenata* aqueous extract, BHT: 2,6-di-tert-butyl-4-methylphenol.**

**Table 1: Antimicrobial activity of Orobanche species as mean of inhibition diameter zone (mm).**

| Strains | Diameters of inhibitions zones (mm) (DD) | | | | | |
|---|---|---|---|---|---|---|
| | Methanol extracts | | Aqueous extracts | | Antibiotics | |
| | *O. crenata* | *O. foetida* | *O. crenata* | *O. foetida* | a | b |
| *Pseudomonas aeruginosa* ATCC 27853 | 20 | NA | NA | NA | 21 | 12 |
| *Pseudomonas aeruginosa* ATCC 9027 | 15 | NA | NA | NA | 19 | 13 |
| *Escherichia coli* ATCC 25922 | 19 | NA | NA | NA | NA | 12 |
| *Enterococcus faecolis* ATCC 11700 | 18 | NA | NA | NA | 20 | 14 |
| *Enterobacteter cloacae* ATCC 13097 | 19 | NA | NA | NA | 18 | 13 |
| *Salmonella typhi* ATCC 14028 | 18 | NA | NA | NA | 22 | 15 |
| *Salmonella enteredis* ATCC 502 | 28 | 25 | NA | NA | 21 | 13 |
| *Salmonella salamae* ATCC 6633 | 18 | NA | NA | NA | 22 | 15 |
| *Shigella flexenerie* ATCC 29903 | 16 | NA | NA | NA | 18 | 15 |
| *Staphylococcus aureus* ATCC 2592 | 16 | NA | NA | NA | 20 | 22 |
| *Staphylococcus aureus* ATCC 6538 | NA | NA | NA | NA | 23 | NA |
| *Streptococcus pyogenes* ATCC 12344 | 30 | NA | NA | NA | 21 | NA |
| *Listeria monocytogenes* ATCC 19118 | 13 | 10 | NA | NA | 23 | 16 |
| *Yersinia enterocolitica* ATCC 23715 | 12 | NA | NA | NA | NA | NA |
| *Proteus mirabilis* ATCC 29906 | 13 | NA | NA | NA | NA | NA |
| *Bacillus cereus* ATCC 11768 | 15 | NA | NA | NA | 20 | 16 |
| *Bacillus cereus* (food isolate) | 30 | NA | NA | NA | NA | NA |
| *Bacillus subtilis* (food isolate) | 25 | NA | NA | NA | 15 | 5 |

DD: Disc-diffusion method. Inhibition zone in diameter around the discs impregnated with 30 µl of the extract. The diameter (9 mm) of the disc is included.

The concentration of methanolic extracts were 10 mg/ml for *O. foetida* and 60 mg/ml for *O. crenata*, however the concentration of water extracts were 50 mg/ml and 40 mg/ml respectively for *O. foetida* and *O. crenata*.

a: Chloramphenicol (30 µg/µL) b: Sreptomycine B (10 µg/µL) NA: Not active

**Conclusion:** Our results suggest that these two Orobanche species might be potential resources of antioxidant and antibacterial activities and can be used in human nutrition and some industrial and pharmaceutical products. However, *in vivo* tests are necessary to confirm the use of these species in medical practice. Much research work will need to be conducted on these plants in order to determine other specific functions.

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