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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THREE ENDEMIC PLANTS FROM ALGERIAN SAHARA

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ABSTRACT

Background. Saharan plants are known by their high content of antioxidant products like phenolic compounds due to the extreme climatic conditions. They constitute the basis of treatments used by local population for various diseases. The purposes of this study were to measure the total phenolic compounds and total flavonoid compounds, to determine antioxidant capacity, and to evaluate the antibacterial activity of three wild Saharan medicinal plants.
Material and methods. Hexane and ethyl acetate fractions of ethanol:water extract and the residu of the extracted aqueous layer of *Ferula vesceritensis* fruits, *Genista saharae* aerial parts and *Zilla macropterae* fruits were assayed to determine their antibacterial activity using the disc diffusion method against: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853). In addition, the total phenolic compounds and total flavonoids and antioxydant activity using DPPH test of ethyl acetate fractions (EAF) of plant parts studied were investigated. Gallic acid, quercetin and vitamin C were used for these parameters.
Results. Among the extracts tested, ethyl acetate fractions of all plants and hexane fraction of *F. vesceritensis* showed activity against *S. aureus*. Good activity was shown by EAF of *G. saharae*. According to the results, it is observed that *Z. macropterae* fruits possess a good antioxidant activity.
Conclusion. The results indicate that the ethyl acetate fraction of *G. sahara* Aerial parts possesses a good antibacterial activity against *S. aureus*, which justifies its use in traditional medicine for treating respiratory diseases. Furthermore, evaluation of in vitro antioxidant capacity of Ethyl acetate fractions of these plants, particular *Z. macroptera* fruits, has also provided interesting results. *Zilla macroptera* fruits may therefore be a good source of antioxidants.

Key words: antibacterial activity, DPPH test, VCEAC, *Ferula vesceritensis*, *Genista saharae*, *Zilla macropterae*

INTRODUCTION

Saharan plants are known by their resistance to several stress factors. Under extreme climatic conditions, Saharan plants could constitute a reservoir of new natural, safe and effective biomolecules potentially useful as antioxidants [Bouaziz et al. 2009]. Antioxidants retard or inhibit the oxidation possibly by reactive radicals including ROS in a biological system [Kim et al. 2003].

Ferula vesceritensis, *Genista saharae* and *Zilla macropterae* are Saharan endemic medicinal plants used by local population for various diseases. It is reported that the fruits of *F. vesceritensis* are used

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in traditional medicine to treat headaches, fever and throat infections [Oughlissi-Dehak et al. 2008]. Few previous works reported on chemical composition of roots [Ahmed et al. 2007] and aerial parts [Oughlissi--Dehak et al. 2008], but there are still no phytochemical and biological studies about fruits of *F. vesceritensis*. According to ethnobotanical investigation, Arial parts of *G. saharae* are traditionally used for treating respiratory diseases and possess diuretic property. Some previous studies have interested in chemical composition of this species [Mekiou et al. 2005, Lograda et al. 2009]. However, there is no literature data about the antibacterial and antioxidant activities for its aqueous alcoholic extract. As for *Z. macropterae*, the aerial parts are used for treating rheumatism. This species has not been chemically or biologically investigated. Therefore, the aims of the present study were to estimate phenolic and flavonoid contents and to evaluate the antibacterial and antioxidant activities of these plants collected from the Algerian Sahara.

MATERIAL AND METHODS

Plant materials. Plants were collected from Gardaya, approximately 600 km south of Algiers and they were identified by Dr. A. Chehma (Department of Biology, University of Ouargla, Algeria). Fruits of *Ferula vesceritensis* and aerial parts of *Genista saharae*, during the flowering stage, were collected in May 2010. Fruits of *Zilla macropterae* were collected in July 2009.

Chemicals. Folin-ciocalteu's phenol reagent, aluminum chloride, quercetin, gallic acid and vitamin C were purchased from Across Organics. Sodium carbonate, ethanol, methanol, hexane, ethyl acetate and DPPH were obtained from Sigma and Rhot (France).

Extraction procedures. Plants were dried in the shade. Dried and powdered material plants were macerated with ethanol:water (70:30 v/v) for 24 h three times. The aqueous ethanol extract was filtered, concentrated under reduced pressure and diluted with distilled water. The aqueous solution was extracted successively with hexane and ethyl acetate. The solvents were completely removed under vacuum. The extracted aqueous layer was concentrated under reduced pressure to dryness. Plant extracts were dissolved in ethanol for hexane and ethyl acetate fractions and in water for extracted aqueous layer at 50 mg/ml before use in the antibacterial assay.

Dosage of phenolic compounds

Total phenolic compounds. The Folin-Ciocalteu method was used to measure the total phenolic compounds [Kim et al. 2003]. Briefly, an aliquot (1 ml) of standard solutions of gallic acid at different concentrations or appropriately diluted extracts was added to a 25 ml volumetric flask containing 9 ml of ddH_2O. A reagent blank using ddH_2O was prepared. One millilitre of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume 25 ml with ddH_2O and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 750 nm. Total phenolic content was expressed as mg gallic acid equivalents (GAE)/g plant extract. Samples were analyzed in three replications.

Total flavonoid compounds. The flavonoid contents were measured according to a colorimetric assay reported by Adedapo et al. [2008]. A 1 ml of standard solution of quercetin at different concentrations or appropriately diluted samples was added to 1 ml of 2% $AlCl_3$ methanol solution. After incubation for 60 min at room temperature, the absorbance was read at 420 nm. Total flavonoid content was expressed as mg quercetin equivalent (QuE)/g plant extract. Samples were analysed in three replications.

DPPH Radical Scavenging Test. The DPPH radical scavenging activity was evaluated according to the method described by Adedapo et al. [2008]. 1 ml of 135 M DPPH solution in methanol was mixed with 1 ml of plant extract. The reaction mixture was incubated in dark for 30 minutes, and the optical density was recorded at 517 nm against the blank. For the control, 1 ml of DPPH solution in methanol was mixed with 1 ml of methanol, and optical density solution was recorded after 30 minutes. The DPPH radical scavenging activity of plant extracts was expressed in terms of mg of Vitamin C Equivalents (VCE)/g plant extract. Samples were analysed in three replications.

Antibacterial assay. The following bacteria were used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853).

The antibacterial activity of the extracts was carried out by the disc diffusion method. A population of approximately 10^8 CFU/ml of each strain was inoculated on the surface of Mueller-Hinton agar. Sterile filter paper discs of 5 mm diameter impregnated with 10 µl of each plant extract (50 mg/ml) were placed on the agar. The concentration of each plant extract was 0.5 mg/disc. Sterile water and ethanol were used as negative controls, while Enroflaxacine was used as positive control. The plates were then incubated at 37°C for 24 h. The diameter of the inhibition zone around each disc was then measured. Each experiment was carried out in triplicate.

RESULTS AND DISCUSSION

Determination of total phenolic compounds and total flavonoids

Results obtained in the present study and shown in Table 1 revealed that Ethyl acetate fraction (EAF) of *G. sahara* was found to have the highest content of both total phenolic compounds and total flavonoids with 459.28 mg GAE and 242 mg QuE per g of plant extract, respectively. The lowest amounts of total phenolic compounds and total flavonoids were recorded in EAF of *F. vesceritensis* with 61.91 mg GAE and 10.57 mg QuE/g plant extract, respectively.

Antioxidant activity

The radical scavenging activity of the EAF of studied plants, determined by DPPH and expressed as mg of Vitamin C Equivalent (VCE) per g of plant extract is shown in Table 1. The DPPH test revealed that the EAF of *Z. macroptera* fruits possessed the highest antioxidant capacity of 37.2 mg VCE/g plant extract, followed by EAF of *G. sahara* aerial parts of 26.5 mg VCE/g plant extract.

The total phenol and flavonoid contents in the EAF of *G. sahara* aerial parts were found to be higher than in the EAF of *Z. macroptera* fruits. On the other hand, the DPPH antiradical test showed that *Z. macroptera* EAF possessed significantly higher activity compared with *G. sahara* EAF. Thus, there is no correlation between recorded antioxidant activity and total phenolic compounds and flavonoid contents.

Antibacterial test

The results for the general screening for antibacterial activity are shown in Table 2. A total of seven extract fractions belonging to three plant species were investigated. The three plants showed some level of antibacterial activity only against *S. aureus*. Among the extract fractions of plants tested, Ethyl acetate fraction (EAF) of *G. sahara* exhibited the highest antibacterial activity. None of the plant extract fractions showed activity against the gram (-) organisms; *E. coli* and *P. aeruginosa*.

CONCLUSION

The aim of this study is to evaluate antibacterial and antioxidant proprieties of three endemic plants from Algerian Sahara. The results indicate that the Ethyl acetate fraction of *G. sahara* Aerial parts

Table 1. Total phenolic compounds, total flavonoids and vitamin C Equivalent Antioxidant Capacity (VCEAC) of EAF of plants

Plant	Total phenolics* mg of GAE/g extract	Total flavonoids* mg of QuE/g extract	VCEAC* mg of VCE/g extract
F. vesceritensis (fruits)	61.91	10.57	19.2
G. sahara (aerial parts)	459.28	242	26.52
Z. macroptera (fruits)	423.94	119.48	37.2

* Average of two determinations.

Table 2. *In vitro* antibacterial activity of the different extract fractions of aqueous ethanol extract from three Algerian Saharan plants

Plant	Plant part extracted	Extract fractions	Extract yield %	Inhibition zone diameter, mm*		
				E. coli	*S. aureus*	*P. aeruginosa*
Ferula vesceritensis	fruits	hexane	4.5	na	15	na
		ethyl acetate	32.3	na	12	na
		water	4	nt	nt	nt
Genista saharae	aerial	hexane	0.7	na	na	na
	parts	ethyl acetate	2	na	19	na
		water	31.2	na	na	na
Zilla macroptera	fruits	ethyl acetate	1	na	11	na
		water	9.4	na	na	na
Enrofloxacine				33	30	18

*Average of three determinations, nt – not tested, na – not active.

possesses a good antibacterial activity against *S. aureus*, which justifies its use in traditional medicine for treating respiratory diseases. Furthermore, evaluation of in vitro antioxidant capacity of Ethyl acetate fractions of these plants, particular *Z. macroptera* fruits, has also provided interesting results. Further chemical and pharmacological investigations should be carried out to confirm our results and to isolate and identify active compounds in the interesting extracts.

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AKTYWNOŚĆ PRZECIWBAKTERYJNA I PRZECIWUTLENIAJĄCA TRZECH ENDEMICZNYCH ROŚLIN Z REJONU SAHARY ALGIERSKIEJ

STRESZCZENIE

Wstęp. Rośliny pochodzące z rejonu Sahary są znane z dużej zawartości związków fenolowych, będących wynikiem ekstremalnych warunków klimatycznych panujących w tym regionie. Są one szeroko wykorzystywane w lokalnej medycynie naturalnej. Celem badań było oznaczenie zawartości fenoli ogółem i flawonoidów oraz aktywności przeciwbakteryjnej i przeciwutleniającej ekstraktów wybranych roślin – trzech dziko rosnących roślin leczniczych.

Materiał i metody. Ekstrakt etanolowo-wodny poddano frakcjonowaniu z użyciem heksanu i octanu etylu. Uzyskaną frakcję wodną owoców *Ferula vesceritensis*, *Zilla macropterae* oraz napowietrznej części *Genista saharae* poddano analizom pod kątem ich aktywności przeciwbakteryjnej z wykorzystaniem metody dyfuzyjno-krążkowej w obecności: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) i *Pseudomonas aeruginosa* (ATCC 27853). Dodatkowo dla frakcji roślin ekstrahowanych z wykorzystaniem octanu etylu (EAF) oznaczono zawartość związków fenolowych i flawonoidów ogółem oraz określono aktywność przeciwutleniającą z użyciem metody DPPH. Jako standardy wykorzystano kwas galusowy, kwercetynę i witaminę C.

Wyniki. Największą aktywność przeciwbakteryjną wobec *S. aureus* stwierdzono dla wszystkich frakcji otrzymanych z użyciem octanu etylu, natomiast z zastosowaniem heksanu tylko dla owoców *F. vesceritensis*. Dużą aktywność stwierdzono dla EAF *G. saharae*. Na podstawie uzyskanych wyników stwierdzono dobrą aktywność przeciwutleniającą owoców *Z. macropterae.*

Podsumowanie. Wyniki wskazują na dobrą aktywność przeciwbakteryjną wobec *S. aureus* frakcji octanu etylu *G. sahara*, co potwierdza celowość ich tradycyjnego wykorzystania w leczeniu chorób układu oddechowego. Stwierdzono także, że aktywność przeciwutleniająca *in vitro* frakcji octanu etylu, szczególnie owoców *Z. macroptera*, będących dobrym źródłem przeciwutleniaczy, może być interesującym kierunkiem dalszych badań.

Słowa kluczowe: aktywność przeciwbakteryjna, test DPPH, VCEAC, *Ferula vesceritensis*, *Genista saharae*, *Zilla macropterae*

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