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Original Article

Antioxidant, Antimicrobial and Anti-Inflammatory Activities Valorisation of Methanol Extract of Two *Geranium* Species Growth in Setif Algeria

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ABSTRACT

The present research reveals the antioxidant, antimicrobial and anti-inflammatory capacities of the methanol extracts of two *Geranium* species growth in Setif Algeria. The antioxidant properties were evaluated through the ability of the extract to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals, the antimicrobial activity was tested with four bacterial strains and one yeast (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC 25922 , *Pseudomonas aeruginosa* ATCC27853, *Bacillus subtilis* ATCC6633 and *Candida albicans* ATCC1024) and the anti-inflammatory activity was evaluated with proteins denaturation test. The two species gave an excellent antioxidant power better the standard, an interesting antimicrobial activity but a moderate to low anti-inflammatory effect.

Key words: Geranium, Antioxidant, Antimicrobial, anti-inflammatory, Setif

1. INTRODUCTION

Geranium genus is taxonomically classified within the family *Geraniaceae* Juss, which includes five to eleven genera, and in total near to 750 species. The genera best known are *Geranium* genus, as wild plants and *Pelargonium* genus, as garden plants. The names of these genera usually cause confusion because “geranium”, is the common name for certain species of Pelargonium. The names come from Greek and refer to the form that its fruits acquire, likes beaks. Thus, the word "Geranium" comes from “geranos" meaning crane, and "Pelargonium" derived from "Pelargos"

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meaning stork [1]. Within the classification of the *Geranium* genus, there are 423 accepted species that are distributed in three subgenera: *Erodioidea*, *Geranium*, and *Robertium* [2].

Some species of Geranium act as hypotensive agents, mild astringents, diuretics, hepatoprotective agents, antioxidants, anti-inflammatory agents, or antiviral agents. All phytochemical studies on these species indicate the presence of tannins, water-soluble polyphenolic compounds with molecular weights ranging between 500 and 30,000 g/mol and with special properties such as the ability to precipitate alkaloids, gelatine, and other proteins [3].

According to Quezel and Santa. (1962), *Geranium atlanticum* B. et R., or "*lbrat er raaï*" (figure 1) is a North African endemic species characterised by a red purple intense flowers, rarely white. A cylindrical knotty rhizome. A tall stem with leaves, ramified in general. Speared whitish dense pilosity of pedicels. The bracts inflorescence measure 3 – 5 mm with reddish membranes. This species can be found in mountainous areas of Algeria [4].

Fig 1: photo of Geranium atlanticum B. et R

Fig 2: photo of Geranium lucidum L

Geranium lucidum L., (figure 2) is characterised by leaves with a limb with rounded contour reniform, divides until the middle in 5 to 7 obtuse crenate lobes. Glabrous calyx, wrinkled across, with 5 projecting angles. Glabrous scentless plants can be found in mountainous forests and humid ravine [4].

The objectives of the present work were to investigate the medicinal side of two *Geranium* species growth in Setif Algeria devoted to scientific research in pharmacognosy.

2. MATERIAL AND METHODS

Plant material

The areal parts of *Geranium lucidum L* were harvested from the mountain of Boutaleb Setif – Algeria in April 2015 and *Geranium atlanticum* B. et R, from the mountain of Megriss - Setif -Algeria in July 2015, determined by Dr. Nouioua Wafa in Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natural Life and Sciences, University Ferhat Abbas Setif, Algeria.

Preparation of methanol extracts

The areal parts of two *Geraniums* were powdered and macerated in 80 % methanol for 24, 48 and 72 hours, at the laboratory temperature (1:10 w/v, 10 g of dried herb). After maceration, the extracts were collected, filtered and evaporated to dryness under vacuum [5]. The dry extracts were stored at a temperature of -18 °C for later use.

Determination of Total Phenolic Content

For total polyphenol determination, the Foline Ciocalteu method was used [6]. The samples (0.2 mL) were mixed with 1 mL of the Folin-Ciocalteu reagent previously diluted with 10 mL of deionized water. The solutions were allowed to stand for 4 minutes at 25 °C before 0.2 mL of a saturated sodium carbonate solution (75 mg/mL) was added. The mixed solutions were allowed to stand for another 120 minutes before the absorbances at 765 nm wre measured. Gallic acid was used as a standard for the calibration curve. The total phenolic compounds content was expressed as mg equivalent of Gallic acid per gram of extract (mg EAG/GE)

Determination of total flavonoids contents

The flavonoids content in ours extracts were estimated by the Aluminium chloride solution according to the method described by Bahorun et al., (1996) [7]. Briefly, 1 mL of the methanol solution of the extracts were added to 1 mL of 2 % $AlCl_3$ in methanol. After 10 minutes, the absorbances were determined at 430 nm. Quercetin (0 – 40 µg/ml) were used as a standard. Results were expressed as mg equivalent Quercetin per gram of extract (mg EQ/GE).

DPPH Assay

The donation capacity of extract was measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al.* , (1998) [8]. One millilitre of the extracts at different concentrations was added to 0.5 mL of a DPPH-methanol solution. The mixture was shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per millilitre). The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [(A_0 - A_1)/ A_0] \times 100$$

Where:

A_0: the absorbance of the control at 30 minutes

A_1: is the absorbance of the sample at 30 minutes. BHT was used as standard [9].

Antimicrobial activity:

Bacteria Strains were obtained from the American Type Culture Collection: Gram-positive bacteria (*Staphylococcus aureus* ATCC25923) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853 and *Bacillus subtilis* ATCC6633) and one yeast: *Candida albicans* ATCC1024. Muller Hinton agar was used for bacteria culture and Sabouraud for yeast.

Anti-bacterial Activity

Agar disc diffusion method was employed for the determination of antibacterial activities of the two *Geranium* methanol extracts [10, 11].

Briefly, a suspension of the tested microorganism (10^8 CFU / mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µL (100mg/mL) of the extracts and placed on the inoculated plates.

These plates were incubated at 37 °C for 24 hours. Gentamicin (10 µg/disc) was used as a standard and dimethylsulfoxide DMSO as a control.

The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by Alves *et al*. (2000) [12]:

- <9 mm, inactive ;
- 9–12 mm, less active ;
- 13–18 mm, active;
- >18 mm, very active.

Antifungal activity

The antifungal activity was tested by disc diffusion method with modifications [10]. *Candida albicans* ATCC1024 suspension was obtained in physiological saline 0.9 % of a culture in Sabouraud (24 hours at 37 °C), adjusted to 10^5 CFU / ml.

One hundred microliter of suspension was placed over agar in Petri dishes and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 µL of each sample (100mg/mL). Amphotericin 100 µg was used as standard and dimethylsulfoxide DMSO as control. Inhibition zones were determined after incubation at 27 °C for 48 hours.

Inhibition of proteins denaturation

The reaction mixture (5 ml) consisted of 0.2 mL of egg albumin taken from from fresh hen's egg, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of extracts to reach a final concentrations of 100, 200, 400, 500 and 800 µg/mL. A similar volume of double distilled water served as the control. Then, the mixtures were incubated at 37± 2ºC for 15 minutes and heated at 70ºC for five minutes. After cooling, the absorbances were measured at 660 nm using the vehicle as a blank. Diclofenac sodium in the final concentrations of 100, 200, 400, 500 and 800 µg/mL was used as the reference drug and treated similarly for the determination of absorbance [13]. The denaturation of protein inhibition by the extracts and standard was expressed as percentage using the formula:

Percentage of inhibition = [(Control – Test)/Control] × 100 [14].

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analysed using one-way ANOVA and Newman-Keuls Multiple Comparison to determine whether there were any significant with the criterion of P values < 0.05 between methanol extracts of the two *Geranium* species and standards, using Graphpad prism 5 Demo Software.

3. RESULTS AND DISCUSSION

Geranium atlanticum contain an important quantity of polyphenols and flavonoids in comparison of *Geranium lucidum* (table 1) and even the yield of extract.

Table 1: yield of the two Geranium methanol extracts and their quantification of polyphenols and flavonoids.

	Yield (%)	Poly phenols (mg EAG/GE)	Flavonoids (mg EQ/GE).
Geranium atlanticum B. et R	19.05 %	82,23±1,19	10,37±0,26
Geranium lucidum L	16.95 %	35,62±0,90	4,69±0,30

The free radical scavenging activities of the tested extracts was determined by DPPH method. The violet colour of the radical disappeared when mixed with the substances in the sample solution that donate a hydrogen atom. Antioxidant activities of the samples and BHT are presented in Figure 3 in which, lower IC_{50} values indicate higher antioxidant activity (Table 1).

Table 2: IC_{50} of standard and the two Geranium methanol extracts for the DPPH test.

	IC_{50} (µg/mL)
Geranium atlanticum B. et R	4,87±0,23***
Geranium lucidum L	1,86±0,22***
BHT	34,01±1,10

Up to date, there is no information about free radical scavenger of *Geranium atlanticum.* In this study, methanol extracts of the two *Geranium* exhibited a greater antioxidant activity, more than the standard. As seen, the extracts contained high levels of polyphenols showing a highly antioxidant activity.

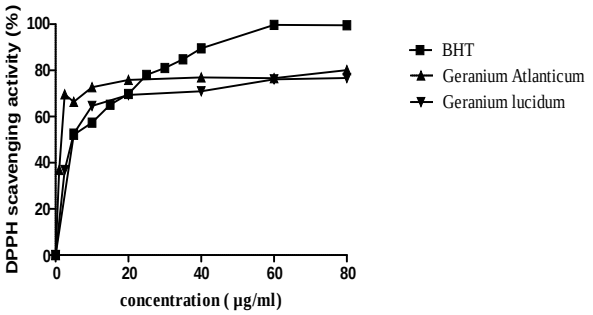

Fig 3: scavenging effect of standards and the two Geraniums methanol extracts

This were excellent results comparing with the literature data [15, 16]. According to Okuda et al., 1989, all phytochemicals studies described for the geranium species, indicates the presence of tannins [17], which are an excellent antioxidant and this may explain the strong antioxidant activity of the extracts.

The results of antimicrobial activity screening are summarized in Table 3. In general, *Geranium lucidum* extract showed the most important activity, more than *Geranium atlanticum. Staphylococcus aureus* was the most susceptible organism in the tested microorganisms.

Table 3: Antimicrobial activity of standards and the two Geranium methanol extracts

	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Candida albicans*
Geranium atlanticum B. et R	14,88±1,62***	-	7,50±0,24***	9,20±0,31***	-
Geranium lucidum L	16,37±0,22***	6,76±0,19**	6,84±0,14***	13,51±0,10**	-
Standard	27,67±0,47	18,50±0,41	18,53±0,41	23,83±0,62	15,58±0,12
Control	*NI*	*NI*	*NI*	*NI*	*NI*

The phenolic compounds often cited as antimicrobial and their antimicrobial effects may be associated with the presence of phenols in plant extracts as had been shown similarly in other studies [18]. In this study, analysis of these extracts showed the presence of phenols; thus, the methanol extracts of *Geranium atlanticum* B. et R and *Geranium lucidum* L displayed a an important antimicrobial activity against some microorganisms.

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors [19]. From the results of present study it can be stated that the extracts of *Geranium atlanticum* B. et R and *Geranium lucidum* L are effective in inhibiting heat induced albumin denaturation at small concentration (100 µg/mL) and provoke the opposite effect at the concentration of 800 µg/mL in the case of *Geranium lucidum* L, that mean that this species can be harmful in high concentration. The results are shown in Figure 4.

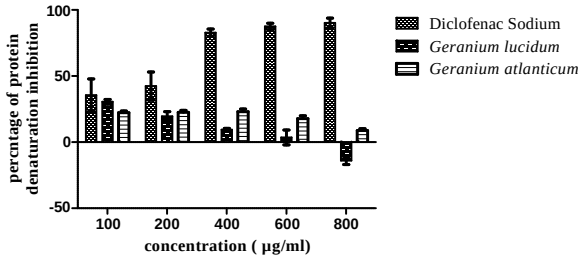

Fig 4: Protein denaturation inhibition power of standards and Geranium methanol extracts.

In the present study, results indicate that the extracts of *Geranium atlanticum* B. et R possess moderate anti-inflammatory properties at small concentration; however the *Geranium lucidum* L methanol extract presente an inflammatory activity at high concentration.

The anti-inflammatory activities may be due to the strong occurrence of polyphenolic compounds, serve as free radical inhibitors or scavenger or acting possibly as primary oxidant thereby inhibiting inflammation [20]. However, the inverse action at high concentration is probably due to the presence of tannins which may play a proxidant effect.

5. CONCLUSION

The results obtained in the present study showed that the extracts of the two *Geraniacean* species are promising sources of natural antioxidants. Also, the preservative potency of the two methanol extract suggests its use as antimicrobial agent. Anti-inflammatory studies using protein denaturation test showed that *Geranium atlanticum* B. et R extract has moderate anti-inflammatory activity.

In addition, these results form a good basis for selection of the *Geraniacean* species for further phytochemical and pharmacological investigations.

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