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Research Article

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Antioxidant, Antimicrobial and Anti-Inflammatory Activities Valorisation of Methanol Extracts of some Species Growth in the Mountain of Megriss Setif Algeria

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ABSTRACT

In aiming to recover the floristic biodiversity in the mountain of Megriss, several species were chosen haphazardly and studied for their antioxidant, antimicrobial and anti-inflammatory activities. 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 three bacterial strains and one yeast (Staphylococcus aureus ATCC25923, Escherichia coli ATCC 25922, Bacillus subtilis ATCC6633 and Candida albicans ATCC1024) and the anti-inflammatory activity was evaluated with proteins denaturation test. The results show that Phlomis crinita was an excellent antioxidant extract compared to the used standard due to the high countenance of polyphenol, however Arabis pubescens (Desf.), Gagea granatellis subsp. Granatelli M and Rumex algeriensis Barr. Et Murb may better anti-inflammatory agents.

Keywords: Antioxidant; Antimicrobial; Anti-inflammatory; Megriss; Flora

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INTRODUCTION

The Mountain of Megriss is located in the northern part of high plains of Setif – Algeria at twenty kilometres of the city of Setif at the coordinates: X: 5° 18’ 20" and X’: 5° 24' 7”, Y: 36° 18' 30” and Y’:36° 21’ 54” (Figure 1) with the summit of 1737 m. The paleogeographic and structural evolution of the Mountain show the presence of three geologic units: the quaternary marked by different type of mass of fallen rocks, the Numidian nape and the tellian nape [1,2]. According to Boulaacheb, the mountain of Megriss is very rugged. The slopes are very strong, they reaching 90% at the cliffs [3]. However, the soils of the mountain Megriss are of two types: Vertisols and leached soils [4]. The hydrographic network of Megriss shows the richness of the water area. The water regime is quite variable throughout the year; it is linked to rainfall patterns. The strongest flow is recorded in winter and melting snow in early spring. The average annual rainfall is 503 mm and average annual temperatures are 10°C make the mountain of Megriss in the semi-continental climate. This climate classifies the mountain of Megriss, in bioclimatic sub humid conditions with cold winter [3]. The flora of Megriss contains 495 species and 22 sub-species belong to 271 genera and 69 families [3]. This vascular flora (phanerogams and vascular cryptogam) represent 15.7% of Algerian flora [5], 40% are Mediterranean origin; 24% are Nordic; 19% are a transitive species between the Mediterranean element and the others chorological ones; 10% are endemic; 5% cosmopolites and 2% are tropical [3]. The aims of this study were to valorise the phytochemical and therapeutical sides of Megriss flora to expand the Algerian pharmacopeia.

MATERIALS AND METHODS

Plant Material

The random sampling were used during the harvesting, the areal parts of *Arabis pubesc*ens (Desf.). , *Gagea grana*tellis subsp. *Granatelli* M., *Rumex algeriensis* Barr. Et Murb., *Phlomis crinita* Cav and *Tulipa silvestris* L. subsp. *australis* were chosen haphazardly from the mountain of Megriss Setif – Algeria in April 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 the chosen species 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 [6]. 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 [7]. 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 were 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 our extracts were estimated by the Aluminium chloride solution according to the method described by Bahorun et al. [8]. 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. [9]. One millilitre of the extracts at different concentrations was added to 0.5 mL of a DPPH-methanol solution. The mixtures were shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbances of the resulting solutions were 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:

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

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 [10].

Antimicrobial Activity

Bacteria Strains were obtained from the American Type Culture Collection: Gram-positive bacteria (*Staphylococcus aureus* ATCC25923), Gram-negative bacteria (*Escherichia coli* ATCC 25922 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 chosen species methanol extracts [11,12]. 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 (100 mg/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. [13]:

- <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 [11]. *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 (100 mg/mL) of each

sample. 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 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 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 [14]. The denaturation of protein inhibition by the extracts and standard was expressed as percentage using the formula:

Percentage of inhibition = [(Control – Test)/Control] × 100 [15].

Statistical Analysis

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

RESULTS AND DISCUSSION

Yield, polyphenols and flavonoids quantification are summarised in Table 1.

Table 1: Polyphenols and flavonoids quantification

	Yield (%)	Poly phenols (mg EAG/GE)	Flavonoids (mg EQ/GE)
Arabis pubescens (Desf.)	17.4	9.29 ± 0.21	5.32 ± 0.06
Gagea granatellis	29.48	5.62 ± 0.08	4.72 ± 0.14
Rumex algeriensis Barr. Et Murb	13.17	12.46 ± 2.67	6.16 ± 0.27
Phlomis crinita Cav	9	57.32 ± 0.70	23.88 ± 0.85
Tulipa silvestris L.	34.89	5.35 ± 0.38	3.63 ± 0.28

The antioxidant activities of extracts (*Arabis pubescens* (Desf.), *Gagea granatellis*, *Rumex algeriensis* Barr. Et Murb, *Phlomis crinita* Cav and *Tulipa silvestris* L) were determined using a DPPH method (Figure 1).

Free radicals, which are involved in the process of lipid peroxidation, are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others [16]. The DPPH• is considered to be a model of a stable lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid autoxidation. Antioxidants react with DPPH•, reducing a number of DPPH• molecules equal to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH• [17].

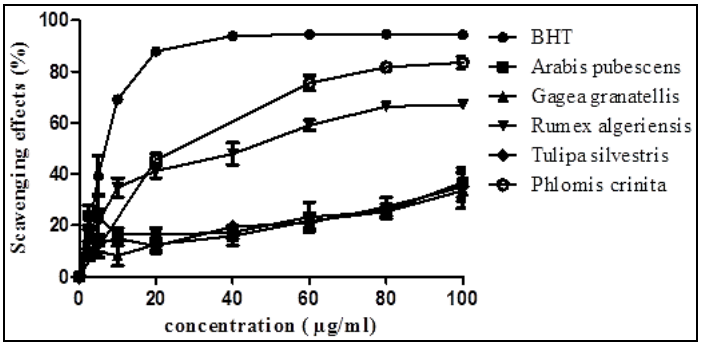

Figure 1: Scavenging effect of standards and the chosen species methanol extracts

The amount of plant extract needed to decrease the initial DPPH• concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity (Table 2).

Table 2: IC_{50} of standard and the chosen species methanol extracts for the DPPH test

	IC_{50} (µg/mL)
Arabis pubescens (Desf.)	185.60 ± 6.97***
Gagea granatellis	220.49 ± 11.38***
Rumex algeriensis Barr. Et Murb	36.30 ± 2.55**
Phlomis crinita Cav	18.74 ± 1.84
Tulipa silvestris L.	162.78 ± 18.49***
BHT	6.79 ± 0.94

Among the tested extracts, *Phlomis crinita* Cav had the highest activity (IC_{50} of 18.74 ± 1.84 µg/ml) followed by the extract of *Rumex algeriensis* Barr. Et *Murb* (IC_{50} of 36.30 ± 2.55 µg/ml**). Other tested extracts had weak DPPH scavenging activities with IC_{50} ranging from 162.78 ± 18.49 to 220.49 ± 11.38 µg/ml. Exposure to proton radical scavengers is known to significantly decrease the level of DPPH [18]. Therefore, free radical-scavenging activity has a marked impact on the phenolic composition of the sample [19]. The antimicrobial activities in various plants extracts of the chosen species were assessed by a paper disc diffusion assay. The results indicated a weak capacity in the antimicrobial properties of the plant extracts (Table 3).

Table 3: Antimicrobial activity of standards and the chosen species methanol extracts

	Staphylococcus aureus	***Escherichia coli***	***Bacillus subtilis***	***Candida albicans***
Arabis pubescens	-	6.10 ± 0.05	-	-
Gagea granatellis	-	6.86 ± 0.17	7.64 ± 0.11	-
Rumex algeriensis	-	-	7.33 ± 0.24	-
Phlomis crinita	-	-	7.33 ± 0.24	-
Tulipa silvestris L.	6.68 ± 0.31	7.10 ± 0.29	7.42 ± 0.22	-
Standard	27.67 ± 0.47	18.50 ± 0.41	23.83 ± 0.62	15.58 ± 0.12
Control	NI	NI	NI	NI

Antimicrobial activity in plant extracts depends not only on the presence of phenolic compounds but also on the presence of various secondary metabolites [20]. George et al. [21] explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities. Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis [22]. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of different plants extracts protein denaturation was studied (Table 4). Maximum percentage of inhibition 49.72 ± 0.37*% was observed from *Arabis pubescens* followed by *Gagea granatellis* 36.05 ± 4.78% at the concentration of 100 µg/ml, against 35.57 ± 12.26% in case of Diclofenac Sodium at the same concentration. However, *Phlomis crinite* show an inflammatory effect in dose dependent ability making -37.67 ± 3.53***% at the concentration of 800 µg/ml. Nevertheless, Diclofenac Sodium give the most important result by 90.13 ± 3.71% at the concentration of 800 µg/ml.

Table 4: Protein denaturation inhibition power of standards and the chosen species methanol extracts

Concentration	**Diclofenac Sodium**	***Gagea granatellis***	***Arabis pubescens***	***Rumex algeriensis***	***Tulipa silvestris L.***	***Phlomis crinita***
100	35.57 ± 12.26	36.05 ± 4.78	49.72 ± 0.37*	21.51 ± 3.41*	22.23 ± 3.45*	-12. 21 ± 6.66***
200	42.43 ± 10.62	27.79 ± 0.65**	-14.71 ± 10.74***	12.32 ± 1.75***	27.9 ± 3.24**	-25.82 ± 3.19***
400	82.84 ± 2.74	11.38 ± 5.09***	-14.04 ± 5.7***	29.74 ± 1.8***	18.03 ± 4.78***	-29.85 ± 4.74***
600	87.5 ± 2.4	19.36 ± 3.8***	-18.03 ± 5.47***	35.54 ± 1.3***	12.69 ± 4.59***	-26.74 ± 5.57***
800	90.13 ± 3.71	34.77 ± 3.5***	32.8 ± 0.51***	26.55 ± 0.27***	20.17 ± 12.18***	-37.67 ± 3.53***

Ours result demonstrate an important activity of *Arabis pubescens* methanol extract as an anti-inflammatory drug which represent a good alternative of NSAIDs. These lasts, despite are reported to possess prevention of the denaturation of proteins, which act as antigens and leads to auto-immune diseases [23], they have several adverse effects especially gastric irritation leading to formation of gastric ulcers [24].

CONCLUSION

Owing to high biogeographic value of their endemic plants and the various threats burdensome on the site, the mountain of Megriss was chosen to carry out a pharmacognosical study to contribute significantly of revealing exhaustive classification and organization of vascular phytocenosis and to enrich the Algerian pharmacopoeia.

*Phlomis crini*ta was the best antioxidant extract compared to the used standard due to the high countenance of polyphenol, however *Arabis pubesc*ens (Desf.), *Gagea granatellis* subsp. *Granatelli* M and *Rumex algeriensis* Barr. Et Murb may a better alternative to NSAIDs. Further studies involving the purification of the chemical

constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent antioxidant and anti-inflammatory agent with a low toxicity and better therapeutic index.

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