**International Journal of Pharmacy and Natural Medicines**

Journal Home Page: www.pharmaresearchlibrary.com/ijpnm

**R E S E A R C H A R T I C L E**

# Antioxidant, antimicrobial and anti-inflammatory activities development of methanol extracts of some species growth in the massif of Boutaleb Setif Algeria

**Nouioua Wafa [a], Gaamoune Sofiane[b*]**

[a] *Faculty of Natural Life and Sciences, University Ferhat Abbas Setif, Algeria.*
[b]*National Institute of Agricultural Research – Setif – Algeria.*

**A B S T R A C T**

Boutaleb is one of the principal mountains in the Eastern side of the Hodna chains (Algeria). It rises between 980 and 1886 m. It soil is lime stoned. The bio-climate varies between semi-arid and sub-humid according to the height. The analysis of the floristic diversity has revealed the existence of 367 taxa, which belong to 56 families and 226 genera with 32 endemic taxa. The random sampling was used during the harvesting to choose nine species for testing their biological powers. The antioxidant properties were evaluated through the ability of the extract to scavenge DPPH 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 Ebenus pinnata was the most antioxidant extract with IC50 equal to 12, 25±2, 80 µg/mL, no interesting results were found concerning the antimicrobial activity but anti-inflammatory power of Asparagus altissimus in low concentrations was found.

**Key words:** Antioxidant, antimicrobial, anti-inflammatory, Boutaleb, flora.

**A R T I C L E I N F O**

***Corresponding Author**
Gaamoune Sofiane,
National Institute of Agricultural Research – Setif – Algeria.
MS-ID: IJPNM3616

**PAPER QR-CODE**

**A R T I C L E H I S T O R Y: Received** 21 March 2018, **Accepted** 29 April 2018, **Available Online**15 June 2018



**Citation:** Gaamoune Sofiane, *et al.* Antioxidant, antimicrobial and anti-inflammatory activities development of methanol extracts of some species growth in the massif of Boutaleb Setif Algeria. *Int. J. Pharm. Natural Med.*, 2018, 6(1): 15-20.

**C O N T E N T S**

1. Introduction . . . . . 15
2. Materials and Methods. . . . . 16
3. Results and Discussion. . . . . 17
4. Conclusion. . . . . .19
5. References. . . . . .19

## 1. Introduction

The forestry massif of Boutaleb constitute an important link well distinctive of the oriental part of assembly line of Hodna. It is situated between the Setifien high plain in North and Hodna basin in South [1]. The massif of Boutaleb is located at the coordinates:

X1 E: 5° 30’ 2.46”
Y1 N: 35° 44’ 41.74"
X4 E: 5° 18’ 45.10”
Y4 N: 35° 41’ 3.56" with the summit of 1886 m of the mountain of Afgnane.

According to Kanev (1972), the massif of Boutaleb is constituted of a big anticlinal [2], of which, the axis of the direction East – West of the occidental part of the massif curve toward the East – North – East in the central and oriental area. The soil of the massif is divided into parts: crude mineral and calcimagnesic soils [3]. From a climatic view, this region is characterised, in low altitudes, by semiarid bioclimate with cool winter in North and cold in South, and in high altitudes by sub –humid bioclimate with very cold winter. In general, it rain between 300 and 600 mm/year. The dry season is very long in low altitudes which may lasted five months, but in high altitudes, it should not exceed three months [4].

The analysis of the floristic diversity has revealed the existence of 367 taxa, which belong to 56 families and 226 genera with 32 endemic taxa [3]. The analysis of the floristic diversity has revealed the existence of 367 taxa, which belong to 56 families and 226 genera with 32 endemic taxa. The aims of this study was to valorise the phytochemical and therapeutical sides of the flora of the massif to expand the Algerian pharmacopeia.

## 2. Materials and Methods

**Plant material**

The random sampling were used during the harvesting, the areal parts of *Ebenus pinnata* L., *Hippocrepis atlantica* Ball. (= H. scabra DC.), *Hyoscyamus niger* L., *Asparagus altissimu*s Munby, *Festuca atlantica* Duv. Jouve, *Astragalus armatus* Willd, *Smyrnium olusatrum* L., *Asphodeline lutea* (L.) Rchb and *Arabis auriculata* Lamk were chosen haphazardly from the massif of Boutaleb Setif – Algeria in April 2015. Determined by Dr. Nouioua Wafa.

**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 each). 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 were measured at 765 nm. 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 the 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 was 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 mixtures were shaken vigorously and left standing at the laboratory temperature for 30 minutes in the dark. The absorbances of the resulting solutions were 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 [9].

**Antimicrobial activity:**

Bacteria Strains were obtained from the American Type Culture Collection:Gram-positive bacteria (*Staphylococcus aureus* ATCC25923), Gram-negative bacteria (*Escherichia coli* ATCC 25922and *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 speciesmethanol 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 (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*. (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 % from a culture in Sabouraud (incubated before 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 (100mg/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 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 70 ºC 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 thedetermination 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 mean ± standard deviation. Data were 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.

## 3. Results and Discussion

The results showed that total flavonoids content of various plantsdecreased in the following order *Arabis auriculata* > *Astragalus armatus*> *Smyrnium olusatrum* > *Asparagus latissimus.* Plants contained phenolic compounds in the following order*: Astragalus armatus* > *Arabis auriculata* > *Asparagus altissimus* > *Festuca atlantica*, as shown in Table 1, may explaned by the biotope conditions. DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole so that the molecules do not dimerise, like most other free radicals. The delocalisation also gives rise to the deep violet colour. The amount of each extract of different plants needed for 50 % inhibition ($IC_{50}$) Table *2*. In this assay, the scavenging of the DPPH radical is followed by monitoring the decrease in absorbance at 517 nm. Figure 1 shows the amount of each extracts of different plants needed for 50% inhibition ($IC_{50}$).The highest radical scavenging activity was showed by *Ebenus pinnata* and lower *Asparagus altissimus*. The antibacterial potency of each plants extracts was evaluated using three bacterial strains and *Candida albicans*, was recorded in Table 2.

The Agar diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts results revealed that all plant extracts were potentially effective in suppressing microbial growth.[15, 16]. The microorganism *E .coli* and *C. albicans*, which is already known to be multi-resistant to drugs,was also resistant to the plant extracts tested.On the other hand *Staphylococcus aureus* which is also resistant to different antibiotics, had its growth inhibited by the extract for *Hippocrepis atlantica (*11,67±0,26). In addition, *Bacillus subtilis*was the most susceptible strains to the extracted plants respectively. Moreover extracted of *Ebenus pinnata* was the most effective extract retarding (12,14 ±0,63).

One of the most common and essential properties of secondary metabolites is anti-inflammatory activity. Inflammation is caused by release of chemicals from tissues and migrating cells [17].The occurrence of inflammation has been regarded to be associated in a number of disorders and prominently related to the painful condition. Ability of different plants extracts protein denaturation was studied (Table 4). The *Asparagus altissimus* extract had shown the greatest inhibition capacity with 42,83±7,40% followed by *Hippocrep is atlantica* 35,10±1,01% at the concentration of 100 µg/ml, against 35,57± 12,26 in case of Diclofenac Sodium at the same concentration. However *Asphodeline lutea* show an inflammatory effect in dose dependent ability making -*53,09±9,72*% 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. Result demonstrates an important activity of *Asparagus altissimus* methanol extract. This assay had therefore provided another evidence for its promising anti-inflammatory properties. In the current study, diclofenac the one routinely used NSAIDs had been used as the reference compound anticipated.

**Table 1:** Yield, polyphenols and flavonoids quantification of the chosen species

| Plants | Yield (%) | Polyphenols (mg EAG/GE) | Flavonoids (mg EQ/GE). |
|---|---|---|---|
| *Ebenus pinnata* | 16.33 | 8,57±0,16 | 5,25±0,11 |
| *Hippocrepis atlantica* | 22.9 | 30,63±0,18 | 5,16±0,50 |
| *Asparagus altissimus* | 15.16 | 14,39±0,05 | 3,17±0,22 |
| *Festuca atlantica* | 13.42 | 5,52±0,20 | 4,27±0,23 |
| *Astragalus armatus* | 22.6 | 14,56±1,11 | 11,94±0,56 |
| *Smyrnium olusatrum* | 26.8 | 12,14±0,24 | 6,45±1,21 |
| *Asphodeline lutea* | 26.9 | 10,53±0,74 | 5,70±0,10 |
| *Arabis auriculata.* | 14.37 | 14,40±0,46 | 13,10±0,98 |
| *Hyoscyamus niger L* | 24,2 | 12,65±0,43 | 5,19±0,48 |

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

| | $IC_{50}$ (µg/mL) |
|---|---|
| BHT | *6,79±0,94* |
| *Ebenus pinnata* | 12,25±2,80 |
| *Hippocrepis atlantica* | 21,00±4,21 |
| *Asparagus altissimus* | 86,80±8,73*** |
| *Festuca atlantica* | 63,92±15,59*** |
| *Astragalus armatus* | 11,37±1,76 |
| *Smyrnium olusatrum* | 32,16±1,64** |
| *Asphodeline lutea* | 54,95±8,35*** |
| *Hyoscyamus niger L.* | 13,48±3,55 |
| *Arabis auriculata.* | 19,05 ±3,00 |

*******: highly significant difference; ****** very significant difference; ***** significant difference with P < 0,001

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

| | ***Staphylococcus aureus*** | ***Escherichia coli*** | ***Bacillus subtilis*** | ***Candida albicans*** |
|---|---|---|---|---|
| *Ebenus pinnata* | NI | NI | 12,14±0,63*** | NI |
| *Hippocrepis atlantica* | 11,67±0,26*** | 7,04±0,04*** | 8,63±0,27*** | NI |
| *Asparagus altissimus* | 6,90±0,28*** | NI | 9,78±0,29*** | NI |
| *Festuca atlantica* | 8,51±0,36*** | 8,51±0,36*** | NI | NI |
| *Astragalus armatus* | NI | 6,88±0,31*** | 9,30±0,10*** | NI |
| *Smyrnium olusatrum* | NI | NI | 7,35±0,17*** | NI |
| *Asphodeline lutea* | 7,81±0,21*** | 7,81±0,21*** | 6,43±0,04*** | NI |
| *Hyoscyamus niger.* | NI | NI | 7,37±0,59*** | NI |
| *Arabis auriculata* | 8,11±0,14*** | 8,11±0,14*** | NI | NI |
| *Standards* | 27,67±0,47 | 18,50±0,41 | 23,83±0,62 | 15,58±0,12 |
| *Control* | NI | NI | NI | NI |

*****:** highly significant difference; ****** very significant difference; ***** significant difference with P <0,001

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

| | **100** | **200** | **400** | **600** | **800** |
|---|---|---|---|---|---|
| *Diclofenac Sodium* | 35,57± 12,26 | 42,43± 10,62 | 82,84± 2,74 | 87,50±2,40 | 90,13± 3,71 |
| *Ebenus pinnata* | 19,25±5,26** | -2,70±8,96*** | 6,30±1,30*** | -20,89±6,07*** | -17,04±8,24*** |
| *Hippocrepis atlantica* | 35,10±1,01 | 16,41±4,32*** | 2,26±4,22*** | -7,28±2,92*** | -11,45±1,92*** |
| *Asparagus altissimus* | 42,83±7,40 | -0,48±2,42*** | -4,03±2,07*** | -51,75±4,52*** | -49,08±5,41*** |
| *Festuca atlantica* | 22,56±5,00* | 20,82±1,57*** | -29,70±24,52*** | -13,16±2,69*** | 2,16±3,44*** |
| *Astragalus armatus* | 26,87±3,88 | 7,99±0,37*** | 11,34±3,23*** | -6,22±0,62*** | 12,66±1,63*** |
| *Smyrnium olusatrum* | -11,03±6,82*** | -15,76±1,65*** | -14,48±3,72*** | -15,12±2,79*** | -24,48±1,13*** |
| *Asphodeline lutea* | -18,11±9,40*** | -21,03±11,41*** | -15,28±2,75*** | -18,43±6,56*** | *** |
| *Hyoscyamus niger* | 9,79±2,27*** | -0,41±4,95*** | -11,09±2,23*** | -19,19±2,57*** | -22,09±1,60*** |
| *Arabis auriculata* | 11,52±3,84*** | 12,90±2,18*** | -5,54±1,35*** | -25,43±2,65*** | -25,62±4,00*** |

*******: highly significant difference; ****** very significant difference; ***** significant difference with P <0,001

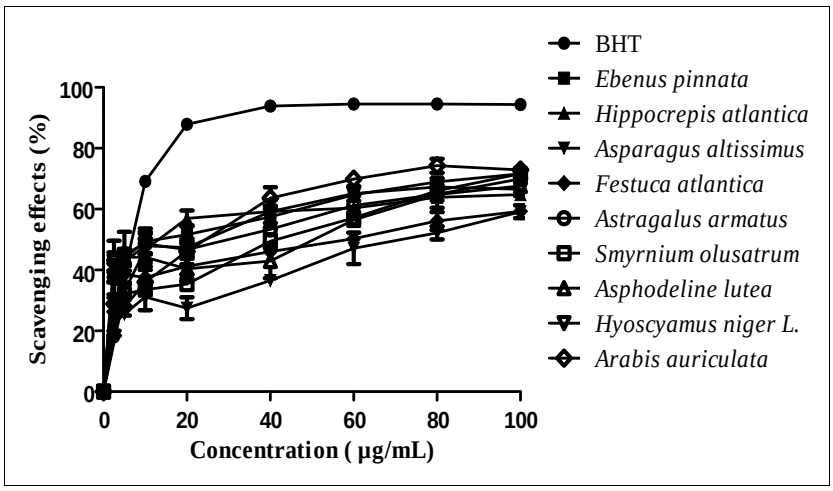


**Fig 1:** Scavenging effect of standards and the chosen species methanol extracts

## 4. Conclusion

In this study, antioxidant, antimicrobial and anti-inflammatory activities of methanol extracts of some plants growths in the massif of Boutaleb were evaluated. The results show an interesting antioxidants power of *Ebenus pinnata* and an important anti-inflammatory activity of *Asparagus altissimus* but a very weak to moderate activity of extracts were founds. The presence of flavonoids and related polyphenols may be responsible for the activities. Further investigations are required to find active component of the effectives extracts and to investigate the mechanism of action.

## 5. Reference

[1] Bertraneu J. Le massif du Boutaleb XIX , International Congress of Geology. Regional Monographic first series, Algeria, 1952, 5 :80 .

[2] Kanev D. Etude de la forêt domaniale du Boutaleb. Secrétariat d'état aux forêts et au reboisement (Mission bulgare), CNRF, 1972, 150.

[3] Sedjar A. Biodiversité et dynamique de la végétation dans un écosystème forestier - Cas de djebel Boutaleb [Magister Thesis]. Ferhat Abbas University Setif (2012): 91.

[4] Madoui A and Gehu J M. Etat de la végétation dansla forêt du Bou-Taleb Mont du Hodna, Algérie. forêt méditerranéenne, 1999, t. XX, n° 4.

[5] Neda S L, Neda M.M.D, Jelena M I and Biljana N B. Antioxidant properties of Galium verum L. (Rubiaceae) extracts. Cent. Eur. J. Biol , 2010, 331–337.

[6] Li WD, Wei C L, White P J and Beta T. High-amylose corn exhibits better antioxidant activity than typical and waxy genotypes. Journal of Agricultural and Food Chemistry, 2007, 55: 291-298.

[7] Bahorun T, Gressier B, Trotin F, Brunete C, Dine T , Vasseur J, Gazin J C, Pinkas M, Luycky M and Gazin M. Oxigen species scavenging activity of phenolic extract from howthorn fresh plant organs and pharmaceutical preparation. Arzneim Forsch / Drug Res, 1996 , 1-6.

[8] Hanato T, Kagawa H, Yasuhara T and Okuda T. Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. Chemical & Pharmaceutical Bulletin, 1998, 2090–2097.

[9] Bettaieb R I, Bourgou S, Ben Slimen Debez I, Jabri Karoui I, Hamrouni Sellami I, Msaada K, Limam F and Marzouk B. Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of Cumin (*Cuminum cyminum* L.) seeds. Techno Food Bioprocess Techno, 2011, 1007.

[10] NCCLS (National Committee for Clinical Laboratory Standards), Performance standards for antimicrobial susceptibility testing. Wayne Pa. 9th International Supplement, 1999, M100-S9.

[11] NCCLS (National Committee for Clinical Laboratory Standards)., Performance standards for antimicrobial disk susceptibility test. Wayne Pa. 6th ed. Approved Standard, 1997, M2-A6.

[12] Alves TMA, Silva AF, Brandão M, Grandi TSM, Smânia EFA, Smânia Jr A and Zani, CL, Biological screening of Brazilian medicinal plants. Memórias do Instituto Oswaldo Cruz, 2000, 95: 367–373.

[13] S. Chandra P, Chatterjee P, Dey S and Bhattacharya S. Evaluation of anti-inflammatory effect of ashwagandha: a preliminary study in vitro. Pharmacognosy Journal, 2012, 4: 47-9..

[14] Y. Mizushima M and Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharma Pharmacol, 1968, 20: 169-173.

[15] Magaldi S, Mata-Essayag S and Hartung de Capriles C. Well diffusion for antifungal susceptibility testingInt. J Infect. Dis, 2004, 8: 39-45.

[16] Valgas C, De Souza S.M and Smânia E F A. Screening methods to determine antibacterial activity of natural products Braz. J. Microbiol, 2007, 38 : 369-380.

[17] Vane J and Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J, 1987, 1:89–96.