

# Anti-inflammatory, antibacterial and antioxidant activities of the medicinal species *Atractylis cancellata*

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## Abstract

This research is focused on the estimation of total bioactive contents and the evaluation of *in vitro* pharmacological activities of crude extracts (petroleum ether, ethyl acetate and *n*-butanol) obtained from the species *Atractylis cancellata*. The antioxidant activity was assessed by three different techniques. The antibacterial activity was determined using the agar disk diffusion assay against five bacterial strains. Furthermore, the anti-inflammatory activity was evaluated by the ovalbumin method. According to the results, *A. cancellata* extracts are rich in several classes of secondary metabolites, especially steroids, triterpenoids, flavonoids, and alkaloids. In addition, the tested extracts showed very interesting antioxidant activities in DPPH (2,2-Diphenyl-1-picrylhydrazyl) and FRAP assays and important correlation coefficients between the results of antioxidant activities and total phenolic and flavonoid contents were found. Moreover, all the tested extracts displayed an antibacterial effect at least against three bacterial strains. The petroleum ether extract inhibited the growth of all the tested bacteria in a dose-dependent manner except *Escherichia coli* ATCC 25922 and it revealed a strong anti-inflammatory activity (81.77±0.05%). We conclude that *A. cancellata* could be an important source of natural pharmacological candidates against oxidative stress, inflammatory and microbial diseases.

## Introduction

Human body is continuously in contact with bacteria that may be beneficial, harmless, or pathogenic. The exposure to pathogenic bacteria causes several infections and may aggravate the symptoms of several pathologies.<sup>1,2</sup> Despite their severe side effects, antibiotics constitute the most efficient treatment for these infectious diseases. However, the uncontrolled utilization and the overuse of these molecules cause the development of multidrug-resistant pathogenic bacteria, resulting in therapeutic failures.<sup>3</sup> In addition, it has been reported that bacterial infections trigger the production of reactive oxygen and nitrogen species. The excessive production of these pro-oxidant metabolites associated with the insufficient antioxidant defense induces oxidative stress, which in turn results in the structural alterations of many biomolecules and the establishment of many stress-oxidative related diseases such as rheumatoid arthritis, cancer, as well as neurodegenerative and cardiovascular diseases.<sup>4</sup>

Therefore, it is imperative to find new alternative candidates to the commercialized antibiotics that possess the same pharmacological effects and manifest additional advantages due to their light side effects and their antioxidant and anti-inflammatory activities.

In this context, we are interested in the biochemical study of the species *Atractylis cancellata*. The genus *Atractylis* (family Asteraceae) is represented by 30 species, distributed mainly in the Mediterranean region (Southern Europe, North Africa, Middle East) as well as in the Canary Islands.<sup>5</sup> Plants of this genus are very thorny annual, biennial or perennial herbaceous with spindly roots, the stems are branched from the base with purple flowers, the leaves are involucrel, the bracts carrying a long point and the hairy achenes are surmounted by a very white pappus.<sup>6</sup>

The objective of this study is to confirm the traditional use of this species for the treatment of skin disorders by the qualification and the quantification of the different secondary metabolites present in its crude extracts petroleum ether (PE), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH), and the evaluation of their anti-inflammatory, antioxidant and antibacterial activities. To the best of our knowledge, there are no previous reports on the anti-inflammatory and antibacterial activities of this species.

## Materials and Methods

### Plant material

The species *Atractylis cancellata* was collected in May 2021 from Kasrou mountain, Fesdis, Batna (Latitude: 35°36'16" N and Longitude: 6°9'23" E, Aures region, Algeria) and was identified by Professor Bachir Oudjehih, Agronomic Institute of the University of Batna 1, where a voucher specimen was deposited (No 1001/LCCE).

### Preparation of plant extract

The whole plant *A. cancellata* (200 g) was macerated twice with a solvent mixture of Ethanol-H<sub>2</sub>O (70:30; v/v) at room temperature. After filtration, the resulting solution was evaporated under vacuum to obtain a volume of 200 mL of aqueous extract. This latter was submitted to liquid-liquid fractioning using organic solvents PE, EtOAc and *n*-BuOH, successively. The obtained phases were dried over anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated to give 1.2 g of PE extract, 1.5 g of EtOAc extract and 2.4 g of *n*-BuOH extract.

### Phytochemical screening

In order to detect the different classes of bioactive molecules present in the crude extracts from the species *A. cancellata*, a phytochemical screening was performed using different methods based on the observation of color changes in the reaction mixture or the formation of a precipitate.<sup>7</sup>

### Detection of tannins

The diluted extracts (2 mL) were added to 1 mL of ferric chloride solution prepared in water (1 %). After agitation, the appearance of a greenish or bluish color showed the presence of tannins.

### Detection of steroids

An equivalent volume (2 mL) of each sample and chloroform (CHCl<sub>3</sub>) were added to 500 µL of acetic anhydride and 3 drops (0.15 mL) of concentrated sulfuric acid. After agitation, the appearance of a blue color indicated the existence of steroids.

### Detection of flavonoids

The tested extracts (500 µL) were added to 5 drops (0.25 mL) of acetic acid, 500 µL of distilled water, 500 µL of concentrated HCl, 5 drops (0.25 mL) of isoamlic acid and a piece of magnesium (0.1 mg). The resulting mixture was agitated for 1 min and the observation of a pink-orange or red-violet coloration in the supernatant layer showed the presence of flavonoids.

### Detection of triterpenoids

An equal volume of concentrated sulfuric acid, acetic anhydride and the tested samples were mixed. After agitation, the appearance of a blue-green ring indicated the presence of triterpenoids.

### Detection of saponins

A volume of the decoction (5 mL) prepared from the plant *A. cancellata* was added to 20 mL of distilled water. Then, the prepared mixture was stirred in a graduated cylinder for 15 min. The observation of foam revealed the existence of saponins.

### Detection of carotenoids

The saturated solution of antimony trichloride (SbCl<sub>3</sub>, 0.15 mL) prepared in CHCl<sub>3</sub> was added to 100 µL of samples. The change in color from blue-green to red indicated the presence of carotenoids.

### Detection of alkaloids

A volume of Dragendorff reagent (0.15 mL) was added to 1 mL of the tested extract. The appearance of orange-red precipitate suggested the presence of alkaloids.

### Detection of quinones

A volume of sample (1 mL) was mixed with 500 µL of sodium hydroxide (1%). After agitation, a red color showed the existence of quinones.

### Detection of fatty acids

A volume of the crude extracts (500 µL) was mixed with 5 mL of petroleum ether solvent. The obtained solution was filtered on a filter paper. After drying, the transparency noticed on filter paper supported the existence of fatty acids.

### Total bioactive contents

The total phenolic and flavonoid contents were assessed by Folin-Ciocalteu and trichloroaluminum methods, respectively.<sup>8</sup> The estimated phenolic and flavonoid contents of crude extracts were calculated according to the calibration curves established by gallic acid and quercetin, respectively.

### Antioxidant activity

The antioxidant activity of the crude extracts was carried out by three different methods including DPPH radical scavenging assay, ferric reducing antioxidant power and total antioxidant capacity.

### DPPH free radical scavenging activity

A volume of different concentrations (25 µL) of extracts (1 – 0.02 mg/mL) or standards (1 – 20 µg/mL) was added to 975 µL of DPPH solution (0.025 mg/mL) prepared in methanol. The obtained solutions were incubated for 30 min in the dark.<sup>8</sup> The absorbances were read at 517 nm and the percentage of DPPH radical scavenging activity of each sample was calculated according to the following formula:

$$\text{DPPH scavenging effect (\%)} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$$

Where:  $A_{\text{Control}}$  is the absorbance of blank;  $A_{\text{Sample}}$  is the absorbance of positive control or sample.

### Ferric reducing antioxidant power (FRAP)

A volume of the tested samples (500  $\mu\text{L}$ ) at different concentrations (2 – 0.2 mg/mL) was added to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1 %). The mixtures were incubated at 50°C for 20 min. Then, 2.5 mL of trichloroacetic acid (10 %) were added. The obtained solutions were centrifuged for 10 min at 3000 rpm. After centrifugation, 500  $\mu\text{L}$  of the supernatant was mixed with 500  $\mu\text{L}$  of distilled water and 100  $\mu\text{L}$  of ferric chloride (1 %) freshly prepared. The absorbance was measured at 700 nm and the antioxidant activity was estimated using a calibration curve of ascorbic acid and the results were expressed as  $\mu\text{g}$  equivalent of ascorbic acid per mg of dry extract ( $\mu\text{g}$  EAA/mg extract).<sup>8</sup>

### Total antioxidant capacity (TAC)

The reagent mixture (900  $\mu\text{L}$ ) consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was added to 100  $\mu\text{L}$  of samples. The resulting solutions were incubated at 95 °C for 90 min. After incubation, the prepared tubes were cooled at room temperature. The absorbance of each sample was read at 695 nm using a spectrophotometer. Ascorbic acid was used as a standard to determine the calibration curve from which total antioxidant capacity of the crude extracts was calculated and the result was expressed as microgram equivalents of ascorbic acid per milligram of dry extract ( $\mu\text{g}$  EAA / mg extract).<sup>8</sup>

### Antibacterial activity

The antibacterial activity of crude extracts (PE, EtOAc and *n*-BuOH) prepared from the plant *A. cancellata* was evaluated by using the agar disk diffusion assay,<sup>9</sup> against five bacterial strains, including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter koseri* and *Proteus mirabilis*.

### In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory effect of the crude extracts was evaluated by egg albumin denaturation method.<sup>10</sup>

### Statistical analysis

The experiments were carried out in triplicate and the results were given as a mean $\pm$ standard deviation ( $p < 0.05$ ) of three parallel measurements for each sample. Significant differences between means were analyzed by one-way ANOVA (GraphPad Prism 5) and by Tukey test.

## Results

### Phytochemical screening

The main compounds of the extracts have been previously detected by Badaoui *et al.*,<sup>11</sup> therefore, as we collected the plant from the same region and in the same period, we assumed that the chemical composition could be the same. For this reason, in the present study the chromatographic separation and isolation was not conducted. We were only interested in phytochemical screening of crude extracts to detect qualitatively the different classes of secondary metabolites like flavonoids, phenolics, alkaloids, fatty acids, steroids and triterpenoids (Table 1). Indeed, the PE and EtOAc extracts showed the presence of steroids, triterpenoids and flavonoids, while fatty acids were detected only in PE extract. In addition, the *n*-BuOH extract indicated the existence of flavonoids and alkaloids. Quinones, tannins, saponins and carotenoids were absent in all the tested extracts.

### Total phenolic and flavonoid contents

The results of total bioactive contents are illustrated in Table 2. The highest contents of flavonoids and phenols were found in the *n*-BuOH extract followed by the EtOAc and PE extracts, respectively.

### Antioxidant activity

The results of the antioxidant activity showed that all the tested crude extracts and references act as antioxidants (Table 3). In DPPH radical scavenging activity, the *n*-BuOH extract showed the highest antioxidant activity with an  $\text{IC}_{50}$  value of 35.43  $\mu\text{g}/\text{mL}$ . The PE and EtOAc extracts indicated a low antioxidant activity with inhibition percentages at 37.53 and 21.51%, respectively at the concentration of 500  $\mu\text{g}/\text{mL}$ . The results of total antioxidant capacity and reducing power assay showed that all the tested extracts exhibited moderate power.

**Table 1. Phytochemical screening of PE (petroleum ether), EtOAc (ethyl acetate) and *n*-BuOH (1-Butanol) extracts from *A. cancellata*.**

Phytochemicals	PE	EtOAc	<i>n</i> -BuOH
Tannins	-	-	-
Steroids	+	+	-
Flavonoids	+	+	+
Triterpenes	+	+	-
Saponins	-	-	-
Carotenoids	-	-	-
Quinones	-	-	-
Alkaloids	-	-	+
Fatty acids	+	-	-

+, presence of phytochemicals; -, absence of phytochemicals.

## Antibacterial activity

The antibacterial activity of PE, EtOAc and *n*-BuOH extracts was determined against five bacterial strains from different genus and the results were presented in Table 4. According to the results, Gram-negative strains were more sensitive to *A. cancellata* extracts

and antibiotics (Gentamicin and Penicillin) compared to Gram-positive bacteria. Indeed, all the tested crude extracts exhibit an antibacterial effect at least against three bacterial strains. The PE extract was the most active and inhibited the growth of all the tested bacteria in a dose-dependent manner except *E. coli* ATCC 25922. However,

**Table 2. Total phenolic and flavonoid contents of crude extracts from *A. cancellata*.**

Extracts	Total phenolics content <sup>a</sup>	Total flavonoids content <sup>b</sup>
PE	11.47±0.51	5.13±0.024
EtOAc	22.65±0.47	12.69±0.17
<i>n</i> -BuOH	93.89±0.93	66.14±0.022

PE, petroleum ether; EtOAc, ethyl acetate; *n*-BuOH, 1-Butanol. Results are expressed as means±standard deviation of three measures ( $p \leq 0.05$ ). <sup>a</sup>Total phenolic content was expressed as µg gallic acid equivalent/mg of plant extract. <sup>b</sup>Flavonoid content was expressed as µg quercetin equivalent/mg of plant extract.

**Table 3. Antioxidant activities of *A. cancellata* extracts by DPPH, FRAP and TAC.**

Extract and standards	DPPH assay <sup>a</sup> IC <sub>50</sub> (µg/mL)	FRAP assay <sup>a</sup> µg EAA/mg ex	TAC assay <sup>a</sup> µg EAA/mg ex
PE	>500	14.61±0.002	4.14±0.001
EtOAc	>500	27.47±0.005	4.90±0.05
<i>n</i> -BuOH	35.43±0.008	50.27±0.003	3.26±0.2
BHA <sup>b</sup>	6.82±0.49	NT	NT
BHT <sup>b</sup>	22.32±0.02	NT	NT
Tannic acid <sup>b</sup>	7.74±0.19	NT	NT
Ascorbic acid <sup>b</sup>	3.1±0.002	NT	NT
α-Tocopherol <sup>b</sup>	13.02±0.17	NT	NT

PE, petroleum ether; EtOAc, ethyl acetate; *n*-BuOH, 1-Butanol; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NT, not tested. <sup>a</sup>Values expressed are means±SD of three measurements ( $p < 0.05$ ); <sup>b</sup>Reference compounds.

**Table 4. Antibacterial activities of crude extracts from *A. cancellata*.**

Extracts/ Standards	Concentration (µg/mL)	Inhibition zone (mm)				
		<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. koseri</i>	<i>P. mirabilis</i>
PE	100	9±0.1	–	12±0.1	9±0.1	11±0.3
	50	9±0.1	–	10±0.25	8±0.1	9±0.15
	25	–	–	8±0.28	7±0.1	9±0.2
	12.5	–	–	–	–	–
	6.25	–	–	–	–	–
MIC (µg/mL)		50±0.2	NF	25±0.3	25±0.3	25±0.4
EtOAc	100	–	8±0.1	11±0.3	9±0.1	–
	50	–	8±0.1	9±0.15	8±0.1	–
	25	–	–	8±0.2	7±0.1	–
	12.5	–	–	–	–	–
	6.25	–	–	–	–	–
MIC (µg/mL)	25±0.2	NF	50±0.3	25±0.5	25±0.4	NF
<i>n</i> -BuOH	100	–	9±0.1	12±0.1	8±0.1	–
	50	–	8±0.1	10±0.3	8±0.1	–
	25	–	–	8±0.2	7±0.1	–
	12.5	–	–	–	7±0.1	–
	6.25	–	–	–	–	–
MIC (µg/mL)		NF	50±0.5	25±0.2	12.25±0.3	NF
Gentamicin	10 µg/disk	–	45±0.2	48±0.3	40±0.1	–
Penicillin	10 µg/disk	–	37±0.1	20±0.5	45±0.4	–

PE, petroleum ether; MIC, minimum inhibitory concentration; EtOAc, ethyl acetate; *n*-BuOH, 1-Butanol; NF, not found. Values expressed are means±SD of three measurements ( $p < 0.05$ ); –, No zones of inhibition around the discs.

EtOAc and *n*-BuOH extracts were less active compared to PE extract and reference antibiotics and inhibited the growth of the same bacterial strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *Citrobacter koseri*.

### Anti-inflammatory activity

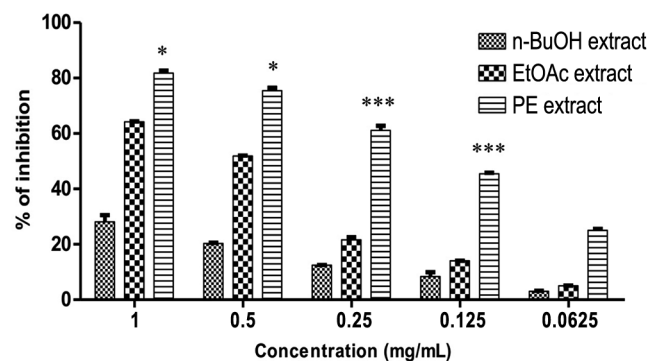
*In vitro* anti-inflammatory effects of crude extracts prepared from the species *A. cancellata* were tested through the assessment of their ability to inhibit ovalbumin denaturation and the results are illustrated in Figure 1 and Table 5. Based on the obtained results, all the crude extracts and the standard drug prevent proteins denaturation in concentration-dependent manner. At the concentration of 1 mg/mL, PE extract revealed the highest anti-inflammatory activity with a percentage of inhibition of 81.77%, followed by EtOAc extract (64.22%) and the *n*-BuOH extract which showed the lowest anti-inflammatory effect (28.15%). However, all the extracts were less active than the reference drug which totally inhibited proteins denaturation at the same tested concentration ( $IC_{50} = 139.44 \pm 0.13$   $\mu$ g/mL).

### Discussion

Many bioactive compounds were detected in *A. cancellata* crude extracts. Indeed, all the detected types of secondary metabolites (triterpenoids, steroids, flavonoids and alkaloids) were previously isolated from this species<sup>11</sup> and from other species of the same genus including *Atractylis gummifera*, *Atractylis carduus*, *Atractylis flava* and *Atractylis serratuloides*.<sup>12-16</sup>

High contents of phenolic and flavonoid were found in the *n*-BuOH and EtOAc extracts. These results were expected since many research indicated that phenolic compounds are more soluble in polar systems like water, methanol, ethanol and *n*-butanol due to the presence of hydroxyl groups.<sup>17</sup> Previous studies carried out on total phenolic and flavonoid contents of several species from the genus *Atractylis* including *Atractylis babelii*, *A. gummifera*, *A. cancellata* and *A. flava* showed low contents in phenols and flavonoids.<sup>11,18-20</sup> The observed variation in the amounts of phenols and flavonoids between species of the same genus could be related to many environmental factors such as temperature, humidity, light intensity, minerals, growing locations, storage and genetic factors.<sup>21</sup>

All the crude extracts prepared from *A. cancellata* species possess antioxidant activity in various tested assays. The previous study



**Figure 1.** *In vitro* anti-inflammatory activity of *n*-BuOH (1-Butanol), EtOAc (ethyl acetate) and PE (petroleum ether) extracts from *A. cancellata*.

conducted on crude extracts from the same species revealed a weak scavenging antioxidant capacity against DPPH free radical associated to very low contents in phenols and flavonoids.<sup>11</sup> While other studies carried out on species of the genus *Atractylis* including *A. babelii*,<sup>18</sup> *A. gummifera*<sup>17</sup> and *A. flava*<sup>20</sup> showed powerful antioxidant activities compared to our results. The variation in the results of the antioxidant activity could be explained by the difference in reactivity of the crude extracts due to their chemical composition, their synergistic or antagonistic interactions and the polarity of the used systems.<sup>22</sup>

The observed antioxidant activity in all the tested systems could be related to the chemical profile of the species especially in phenolic compounds. For this reason, the relationship between total phenolic (TPC) and flavonoid (TFC) contents and the antioxidant activity was established by the calculation of correlation coefficients. For the *n*-BuOH extract, an important correlation coefficient between DPPH radical scavenging activity and TPC ( $r = 0.67$ ) and a low correlation with TFC ( $r = 0.45$ ) were observed. In fact, many phenolic compounds namely 4-*O*-caffeoyl-2-*C*-methyl-*D*-threonic acid, chlorogenic acid methyl ester and 5-*O*-caffeoylshikimic acid isolated previously from this species are well-known for their ability to scavenge DPPH free radical.<sup>11</sup> A strong correlation between the total antioxidant capacity and total flavonoid content was observed in all the tested extracts PE, EtOAc and *n*-BuOH with values of  $r = 0.99$ , 0.84 and 0.83, respectively. These results suggested the direct contribution of flavonoids in the antioxidant activity. Indeed, several flavonoids were previously purified and identified as chrysin, apigenin, tricetin, quercetin, quercetin 3-*O*- $\beta$ -*D*-glucopyranoside, isoorientin and diosmin from organic extracts of this species, these metabolites are well documented as great antioxidant molecules.<sup>11</sup> Great correlation factors between the results of FRAP assay and total phenolic content were found with PE and EtOAc extracts with coefficients of determination ( $R^2$ ) at 0.998 and 0.993, respectively. However, a weak correlation with TFC was observed ( $r = 0.263$  and 0.255, respectively). Indeed, phenolic compounds are considered as excellent single electron donors, and the extent of conjugation and hydroxylation constitutes the main factor of their reducing power.<sup>23</sup>

*A. cancellata* crude extracts exhibited antibacterial effects against all the tested bacteria. The strains *P. aeruginosa* ATCC 27853 and *C. koseri* showed sensitivity to all the tested extracts but the observed antibacterial activity was weak compared to the used antibiotics. According to previous reports, *P. aeruginosa* is considered as the major cause of nosocomial, urinary tract, respiratory, and blood stream infections in immunocompromised patients,<sup>24</sup> and *C. koseri* is a common cause of neonates and immunocompromised patients' meningitis. This results frequently in intracerebral abscesses associated with many other neurological complications that causes severe neurological sequelae and mental retardation in the surviving infants.<sup>25</sup> It has been reported also that infection with these pathogenic strains had a high rate of mortality. Indeed, *C. koseri* and *P.*

**Table 5.** Anti-inflammatory properties of *A. cancellata* extracts.

Extracts and standard	Albumin denaturation assay <sup>a</sup> $IC_{50}$ ( $\mu$ g/mL)
PE	177.51 $\pm$ 0.76
EtOAc	475.58 $\pm$ 0.49
<i>n</i> -BuOH	ND
Diclofenac <sup>b</sup>	139.44 $\pm$ 0.13

<sup>a</sup>Values expressed are means $\pm$ SD of three measurements ( $p < 0.05$ ); <sup>b</sup>Reference compounds; ND, not detected.

*aeruginosa* have gained resistance against several types of antibiotics due to the many mechanisms of resistance.<sup>26</sup> For this, *A. cancellata* species could constitute an excellent source of antibacterial compounds destined to bacterial infection associated with these pathogens. The strains *S. aureus* ATCC 25923 and *P. mirabilis* resisted to both EtOAc and *n*-BuOH extracts and all the used reference antibiotics, but they were sensible only to PE extract with values of MIC estimated at 50 and 25 µg/mL, respectively. These results are very beneficial since bacterial infections with these strains are associated with high morbidity and mortality rates and constitute a critical challenge for therapists due to their resistance to several antibiotics. *S. aureus* bacteremia may induce several consequences ranging from moderate skin infections to fatal sepsis. It has been well documented that the superficial colonization of this bacterium especially in surgical wounds and in dwelling medical devices followed by their dissemination to internal organs via the bloodstream causes infections of the skin, osteoarticular, pulmonary, and endovascular sites inducing in many cases septicemia, pneumonia, endocarditis, and ocular infections.<sup>27</sup> *P. mirabilis* is a Gram-negative bacterium capable of inducing many pathological complications for humans including ophthalmological and gastrointestinal tract infections. However, this strain frequently causes complicated urinary tract infections, especially for patients with renal functional and anatomical problems. These infections may be accompanied by moderate symptoms including urine retention associated with painful distension of the bladder, pyelonephritis, and urolithiasis. In addition, severe complications may be associated with this infection such as septicemia and endotoxic shock.<sup>28</sup> The observed antibacterial activity of the PE extract prepared from species *A. cancellata* could be related to the presence of fatty acids which were totally absent in the other crude extracts. Based on several reports, fatty acids could possess bactericide or/and bacteriostatic effects. Indeed, the latter affect several microbial cellular components and interfere in many mechanisms of virulence, by the prevention of biofilm formation and the production of many enzymes and toxins.<sup>29</sup> Furthermore, the presence of other types of secondary metabolites known for their antimicrobial effects in this plant including phenolic compounds, flavonoids, steroids and terpenoids may explain its antibacterial activity.<sup>30-32</sup> According to the results, the strain *E. coli* showed a very low sensitivity to both EtOAc and *n*-BuOH extracts and resisted to the PE extract. However, the growth of this strain was highly inhibited by gentamicin and penicillin with inhibition zones at 45 and 37 mm, respectively. The observed weak antibacterial effect against this bacterium was expected since the species of the genus *Atractylis* are known for their low inhibitory actions against this strain. It was reported that the antibacterial activity of the methanolic extract prepared from *A. carduus* tested on nine gram-positive and five gram-negative bacterial strains showed a moderate sensitivity against the strains *Klebsiella oxytoca*, *Bacillus cereus* and *S. aureus*.<sup>33</sup> The antibacterial activity of *A. gummifera* aqueous extract tested against eight bacteria displayed a low sensitivity only against *P. aeruginosa* and *S. aureus*.<sup>34</sup> While, organic extracts prepared from *Atractylis humilis* exhibited a low antibacterial effect against *E. coli*, *P. aeruginosa* and *S. aureus*.<sup>35</sup>

The anti-inflammatory effect of this species could be attributed to its chemical composition, especially to the presence of flavonoids and phenolic compounds. According to Badaoui and collaborators,<sup>11</sup> PE and EtOAc extracts of *A. cancellata* are rich sources of these molecules. Indeed, many reports indicated that chrysin, apigenin, isoorientin, quercetin, quercetin 3-*O*-β-D-glucopyranoside, and diosmin isolated previously from this species<sup>11</sup> may alleviate inflammation and regulate the anti-inflammatory response by different mechanisms of action. Chrysin is known for its immunoregulatory

and anti-inflammatory properties due to its excellent antagonist effect on NF-κB, which controls genes expression of several inducible enzymes, adhesion molecules, pro-inflammatory cytokines, and chemokines.<sup>36</sup> Apigenin is considered a potential therapeutic candidate against rheumatoid arthritis and many autoimmune disorders. It has been reported that the intake of this molecule decreases the production of pro-inflammatory cytokines including L-1β, IL-2, IL-6, IL-8, and TNF-α through the inhibition of cyclooxygenase-2 and NF-κB genes expression.<sup>37</sup> Quercetin and its derivatives ameliorate the anti-inflammatory response and the cell's antioxidant status. These metabolites decrease significantly the histological signs of acute inflammation by the suppression of leucocyte recruitment and the decrease of chemokine and malondialdehyde levels.<sup>38</sup> Diosmin is very beneficial against colon chronic inflammation, it has been reported that this molecule significantly decreases the levels of inflammatory and oxidative stress markers which results in the prevention of ulcerative colitis progression.<sup>39</sup> In addition, this compound suppresses the production of many pro-inflammatory mediators.<sup>40</sup> Also, the anti-inflammatory effect of the non-polar extract could be related to its richness in terpenoids and sterols which were purified previously and identified as lupeol, oleanolic acid, β-sitosterol, and β-sitosterol-3-*O*-β-D-glucoside.<sup>11</sup> Lupeol possesses interesting anti-inflammatory effects via several mechanisms of action. This molecule prevents the production of some pro-inflammatory mediators. Its topical application alleviates TPA-induced inflammation and reduces cell infiltration into the inflamed mice tissues. Furthermore, the oral administration of lupeol significantly reduces the levels of cytokines and increases the activity of lysosomal enzymes and glycoproteins in arthritic animals.<sup>41</sup> β-Sitosterol and its derivatives have the ability to alleviate inflammation due to the down regulation of many pro-inflammatory signal transduction pathways and their ability to attenuate the overproduction of free radicals, particularly NO.<sup>42</sup>

## Conclusions

The present study described the *in vitro* anti-inflammatory, antibacterial and antioxidant activities of crude extracts (PE, EtOAc and *n*-BuOH) prepared from the species *A. cancellata*. According to the results, the organic extracts of this species are rich in many classes of secondary metabolites known for their pharmacological properties. High to moderate levels of phenolic and flavonoid contents were observed. The *n*-BuOH extract showed interesting antioxidant activities in DPPH and FRAP assays that correlate reasonably to its phenolic content. All the tested extracts exhibit an antibacterial effect at least against three bacterial strains and have the ability to prevent proteins denaturation and autoantigens formation in a dose-dependent manner. Furthermore, it can be concluded that *A. cancellata* extracts could be used as a good source of alternative natural products helpful in the treatment of inflammatory, infectious and oxidative-stress associated diseases.

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