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Research Article

Chemical profiling and bioactivity studies on aerial parts *Ammoides atlantica* (Coss. et Durieu) H. Wolff

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Abstract: The Algerian endemic plant *Ammoides atlantica* (Coss. et Durieu) H. Wolff was studied for the chemical profiling and biological activities of its essential oil (EO) and ethanolic extract (EE). The chemical analysis by GC/MS and HPLC/DAD/UV revealed, respectively, the major compounds thymol (39.46%), γ-terpinene (31.74%), and p-cymene (19.01%) in the EO, and apigenin (33.58%), luteolin 7-*O*-glucoside (20.09%), and luteolin (14.39%) in the EE. The EO exhibited strong antioxidant activity, with a significant $ABTS^{\bullet+}$ scavenging capacity (IC_{50} = 2.79 µg/mL) compared to EE, Trolox, and BHT. The EE showed comparable effects to BHT in DPPH scavenging and reducing power tests. Moreover, the EO demonstrated noteworthy antibacterial activity against *S. aureus, E. coli,* and *P. aeruginosa*, with inhibition zone diameters ranging from 32.1 to 70 mm and MICs below 0.3 to 5 mg/mL. Furthermore, the EE exhibited strong anti-inflammatory activity by inhibiting hemolysis of red blood cells >70% at a concentration of 20 µg/mL.

1. INTRODUCTION

In recent years, the world has witnessed a tremendous development of effective synthetic drugs and new generations of antibiotics that have saved the lives of millions of people. Synthetic drugs, on the other hand, are experiencing difficulties in the treatment of some diseases caused by organisms or cells being resistant to drugs, such as multidrug-resistant bacteria and some chemotherapy-resistant tumor cells. Furthermore, drugs designed to cure certain ailments seem to be toxic or harmful to some other body organs. As a result, there is currently a strong trend toward researching natural sources for biologically active extracts that can be combined with synthetic drugs to create more effective and safer medications (Kieliszek *et al.*, 2020). Statistics on the sources of new drugs from 1981 to 2007 indicate that almost half of the drugs approved since 1994 are based on active metabolites from natural sources (Akoto *et al.*, 2021). Medicinal and aromatic plants contain the chemical constituents first used by humans as medicines for healing, as flavoring agents for food and drink, and as mental stimulants for mystic interactions with super natural gods. These plant materials continue to play positive roles in human life, as

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sources of modern pharmaceuticals to treat medical problems, as herbs and spices to tempt the palate, and in a multitude of other applications (Inoue & Craker, 2014).

Moreover, herbal medicines have, nowadays, received great scientific interest because they provide both important biomolecules, which are used in the treatment of several diseases, as well as a broad spectrum of long-term use and safety. Nevertheless, plant secondary metabolites are excellent candidates for developing new phytopharmaceuticals with various biological activities, including antioxidant, antimicrobial, and anti-inflammatory (Toiu *et al.*, 2018; Gunathilake *et al.*, 2018).

The genus *Ammoides* belonging to the Apiaceae family (Umbelliferae) is represented in Algeria by two species: *A. pusilla* (Brot.) Breistr. and *A. atlantica* (Coss. et Dur.) Wolf. *Ammoides atlantica* is an endemic species of Algeria found in mountain grasslands above 1000 m (Quezel & Santa, 1962). In Algerian folk medicine, the species is widely used as an infusion to treat headaches, fever, and diarrhea. It is also used in compresses, alone or soaked in alcohol or vinegar and mixed with henna, to treat children with mental deficiency. This plant also has interesting digestive properties and is used as a spice in certain recipes (Laouer *et al.*, 2003; Laouer *et al.*, 2008). Most of the previous studies on this plant were focused on volatile fraction while the non-volatile fraction and their biological effects have not yet been fully investigated, especially their anti-inflammatory activity. The purpose of this study is to assess the antibacterial, antioxidant, and anti-inflammatory properties of the essential oil and ethanol extract of *A. atlantica.*

2. MATERIAL and METHODS

2.1. Plant Material

The aerial parts of *A. atlantica*, were harvested in the region of "Amira Arrès" located in the Mila province (east of Algeria, 36° 32′ 15″ N 6° 03′ 55″ E) at 1000 m of altitude, during the flowering period (March 2021). The taxonomic identity of the plant was confirmed Prof. H. Abdelkrim by comparing it with specimens of known identity (ID: 052_64) already deposited in the herbarium of the National Superior School of Agronomy (ENSA), Algiers.

2.2. Isolation of Essential Oil

The essential oil was isolated by hydrodistillation in a Clevenger-type apparatus according to the protocol of the European Pharmacopoeia (2007). The traces of water in the collected oil were removed using anhydrous sodium sulfate (Na_2SO_4). The oil was then kept at 4 °C in dark vials hermetically sealed until analysis.

2.3. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of the Essential Oil

The essential oil was analyzed with a Perkin-Elmer CLARUS 500 model GC/MS device. Analyzes were performed on an Elite Series 5-MS gas chromatograph fitted with a fused silica capillary column with an apolar stationary phase HP 5MS (30 m x 0.25 mm x 0.25 µm film thickness), with a stationary phase nonpolar, directly connected to a Hewlett-Packard 6890 quadrupole mass spectrometer. The chromatographic conditions were as follows: a heated oven with a temperature setting of 70 to 220°C for 15 min at a rate of 4°C/min and maintenance of the final temperature for 56.5 min. Essential oil diluted in hexane (1/10, v/v) was injected by splitting (split ratio 1/25). The injector and transfer line were maintained at a temperature of 250°C. For each sample, the analysis was carried out by electron impact (EI) at 70 eV or by chemical ionization (CI) with methane as the pressurized gas used. The temperature of the ion source was maintained at 250°C. The carrier gas is helium (He). The gas chromatograph was operated in scan mode between 20 and 550 atomic mass units

2.3. Identification of the Essential Oil Compounds

The identification of the compounds was carried out by comparing the mass spectra of the EO compounds with those of computerized commercial libraries (NIST, PFLEGER, NBS and WILEY) and those of the database developed by the laboratory from authentic substances. The

modified Van den Dool and Kratz formula (Tranchant et *al.*, 1995) was used to calculate the retention indices. The column and analysis conditions used for the determination of the retention indices are those described above in the section on gas chromatography. Confirmation was carried out by comparing the retention indices of the separate products with those described in the Adams Library (Adams, 2001).

2.4. Preparation of Ethanolic Extract

The ethanolic extract was prepared using ethanol as a solvent by submitting 20 g of dried powdered plant to extraction with 200 mL of absolute ethanol in a Soxhlet apparatus. The ethanolic solution obtained was concentrated using a rotary evaporator under vacuum, lyophilized to obtain a dry extract (4.6 g), and then stored at 4 °C until analysis.

2.5. HPLC/UV/DAD Analysis and Identification of Ethanolic Extract Compounds

The ethanolic extract was analysed using an Agilent 1100 series HPLC device equipped with a UV/DAD detector, a quaternary pump, an in-line degasser, and an automatic injector. The diode array detector (DAD) with several wavelengths of maximum absorption (chosen according to the maximum absorbance (ʎmax) of the researched molecules), is fixed with the analytical column Hypersil (BDS-C18, 5 µm, 250 × 4.6 mm) constituting the stationary phase. The mobile phase used is a mixture of two solvents, the first is a mixture of water and acetic acid (0.2%) at pH = 3.1 (solvent A) and the second is acetonitrile (solvent B). The two solvents were used in a linear gradient elution for 30 min at 1 mL/min, starting with 95% of solvent A and ending with 100% of solvent B. The flow rate is 1.5 mL/min, the injection volume of 20 µL and the detector wavelength is fixed at 220 nm, 255 nm, 280 nm, 300 nm, and 355 nm, chosen according to the maximum absorbance of the molecules identified. The identification of the compounds was carried out by comparing the retention times and the UV spectra of the peaks of the sample's chromatogram with those of the standards previously analysed under the same operating conditions and recorded in the database of the HPLC chromatograph (Tzima *et al.*, 2018).

2.6. Total Phenolic and Flavonoid Content Determination

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1999). 1 mL of the Folin-Ciocalteau reagent solution diluted 10 times was added to 0.25 mL of ethanolic extract. Then, 1 mL of 7.5% sodium carbonate solution (75 g/L) was added after 3 minutes of reaction. Afterward, the reaction mixture was incubated for 30 minutes in the dark at room temperature. Finally, the absorbance was measured using a spectrophotometer at 765 nm against a blank, which was prepared under the same conditions by replacing the extract with methanol. The samples were analyzed in triplicate. A calibration curve established with gallic acid was used to determine the amount of total phenols (TP) in mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW) ($Y = 0.0111X - 0.0067$, where X is the absorbance and Y is the GAE (mg/g), R^2=0.99). The total flavonoid content (TFC) was determined according to the aluminium chloride method (Lamaison & Carnet, 1990). 1 mL of $AlCl_3$ was added to 1 mL of the extract solution dissolved in methanol. After incubation for 60 minutes in the dark at room temperature, the absorbance of the reaction mixture was measured using a spectrophotometer at 510 nm against a blank prepared under the same conditions by replacing the extract with ethanol. The content of total flavonoids (TF) was estimated using a calibration curve of quercetin and the result was expressed in mg quercetin equivalent per gram of dry weight (mg QE/g DW) ($Y = 0.0344X + 0.008$, where X is the absorbance and Y is the QE (mg/g), R^2=0.99).

2.7. Antibacterial Activity

2.7.1. *Bacterial strains*

The bacterial strains used in this study were: a Gram-positive bacterium: *Staphylococcus aureus* (ATCC 25923) and two Gram-negative bacteria: *Escherichia coli* (ATCC 25922),

Pseudomonas aeruginosa (ATCC 27853). All the strains were grown on Mueller-Hinton agar (MHA) at 37 °C.

2.7.2. *Susceptibility test by agar disk diffusion method*

The agar disk diffusion method was employed to determine the susceptibility of bacteria according to CLSI standards (2012a). Briefly, isolated colonies of each strain from an 18–24-h agar plate were suspended in 5 mL of sterile saline to achieve a turbidity equal to the 0.5 McFarland standard ((1–2)×10^8 CFU/mL). Then, the inoculum suspension was spread on the solid media plates using a sterile cotton swab. Filter paper discs (6 mm in diameter) individually impregnated with 15 µL of the diluted oil (or extract) (with DMSO at 10% w/v) were placed on the incubated plates. The plates were placed at 4°C for 2 h and then incubated at 37°C for 16–18 h. The diameters of the inhibition zones were measured and expressed in millimeters. DMSO and gentamicin (8 µg/mL) were used as negative and positive controls, respectively.

2.7.3. *Determination of Minimal Inhibition and Bactericidal Concentration (MIC & MBC)*

The minimum inhibition concentration was determined using the agar dilution method according to CLSI standards (2012b). Mueller-Hinton agar plates containing serial twofold dilutions of each extract from 0.3 to 20 mg/mL and gentamicin from 0.125 to 512 µg/mL were prepared. Then inoculation of the medium with a standardized suspension adjusted to 0.5 McFarland standard containing a concentration of 5×10^8 CFU/mL was conducted. The bacteria are diluted to around 10^7 CFU/mL (0.5 McFarland suspension 1:10 in sterile saline), and 2 µL are spotted with a multi-point into agar plates that contain approximately 10^4 CFU/spot. One agar plate was used as a control and seeded without an antibacterial agent. The inoculated plates were allowed to stand at room temperature until the moisture in the inoculum spots had been absorbed into the agar, and then incubated at 37 °C for 16 to 20 h. The MIC was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation. Minimal Bactericidal Concentration (MBC) was determined by taking streaks from the plates exhibiting invisible growth and subcultured onto sterile MHA plates. The plates were incubated at 37 °C for 16 to 18 h and then examined for bacterial growth. MBC was defined as the concentration of an antimicrobial that did not exhibit any bacterial growth on the freshly inoculated agar.

2.8. Antioxidant Activity

2.8.1. *DPPH radical scavenging activity*

The scavenging activity of DPPH radicals was determined according to the method described by Hazzit *et al*. (2009). Briefly, 975 µL of ethanolic solution of 2,2-diphenyl-1- picrylhydrazyl (DPPH) (0.0024%) and 25 µL of a sample at final concentrations were mixed and the resulting solution was left at room temperature for 30 min. The absorbance was measured at 517 nm against ethanol as blank. BHT and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used as positive controls. The scavenging activity of the $DPPH^{\bullet}$ radical expressed as percentage inhibition was calculated as follows:

$$\text{DPPH scavenging (\%)} = \left[\frac{\text{Ab - As}}{\text{Ab}}\right] x100$$

Where, Ab is the absorbance of the control reaction; As is the absorbance of the test compound. The concentration providing 50% inhibition of the $DPPH^{\bullet}$ radical or IC_{50} was calculated from the graph giving the percentage of inhibition in relation to the concentration of the sample. A low IC_{50} value indicates high antioxidant activity.

2.8.2. *$ABTS^{\bullet+}$ radical scavenging activity*

The scavenging activity of $ABTS^{\bullet+}$ radicals was evaluated following the method of Re *et al.* (1990). The $ABTS^{\bullet+}$ cationic radical was produced by reacting 7 mM of $ABTS^{\bullet+}$ solution with 2.45 mM of potassium persulfate ($K_2S_2O_8$), then the reaction mixture was kept in the dark and at room temperature for 18 h before use. Aliquots of 25 µL of each sample at different

concentrations were mixed with 975 µL of diluted $ABTS^{\bullet+}$ in ethanol whose absorbance should be equal to 0.7 ± 0.02 at 734 nm. After 7 min of reaction in the dark, the absorbance was measured using a spectrophotometer at 734 nm against ethanol as blank. BHT and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used as positive controls. The percentage of inhibition and IC_{50} were calculated as described in the DPPH assay.

2.8.3. *Ferric reducing power*

The ferric reducing power (FRP) was evaluated using the method of Oyaizu (1986). A volume of different concentrations of the samples (0.125 mL) was added to 2.5 mL of phosphate buffer (0.1 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1.0%, w/v). Each mixture was incubated at 50 °C for 20 min, and afterwards 2.5 mL of trichloroacetic acid (10%) was added. The mixture was shaken vigorously, and the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%, w/v). After 30 min of incubation at 50 °C, absorbance was read at 700 nm. BHT was used as a positive control. Increased absorbance of the reaction mixture indicates stronger reducing power. The IC_{50} values corresponding to the concentration of the reducing agent that allows obtaining an absorbance of 0.5 were calculated.

2.9. Anti-inflammatory Activity

The method of membrane stabilization of erythrocytes was used whose principle is based on the ability of the ethanolic extract of *A. atlantica* to prevent hemolysis of human red blood cells (HRBC) induced by hypotonia and heat and therefore prevent the release of hemoglobin (Thenmozhi *et al.,* 1989; Oyedapo *et al.,* 2004). The erythrocyte suspension was prepared according to the method described by Shinde *et al.* (1999) with some modifications. Whole human blood was collected from a healthy human subject. Blood in heparinized tubes was centrifuged at 3000 rpm for 5 min, and washed three times with an equal volume of normal saline (0.9% NaCl). After centrifugation, blood volume was measured and reconstituted as a 10% (v/v) suspension with an isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). The composition of the buffer solution (g/L) used was NaH_2PO_4 (0.2), Na_2HPO_4 (1.15) and NaCl (9.0). An Alsever solution is prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in distilled water, then sterilized. A volume of 1 mL of ethanol extract or aspirin at different concentrations (10-200 µg/mL) was mixed with 1 mL of phosphate buffer (pH-7.4), 2 mL of hypo saline (0.45%), and 0.5 mL of red cell suspension. The reaction mixture (4.5 mL) was incubated in a water bath at 54°C for 20 min and centrifuged again at 2500 rpm for 5 min and the absorbance of the released hemoglobin was measured at 560 nm. In parallel, the control was carried out under the same conditions, replacing the extract with distilled water (positive control corresponding to 100% hemolysis) and the phosphate buffer as blank. The level of hemolysis was calculated using the following equation (Okoli *et al.,* 2008):

$$\text{Percentage of inhibition of hemolysis (\%)} = \left[\frac{1 - A_2}{A_1}\right] x100$$

Where A_1 = absorbance of the control and A_2 = absorbance of test sample mixture

2.10. Statistical Analysis

In this study, three analyses of each sample were carried out and each experiment was carried out in triplicate (n = 3). The mean value and the standard deviation were calculated. The bioassay data was analyzed by ANOVA using IBM SPSS Statistics version 26.0 software, followed by the Tukey test. The significance level was set at $p < 0.05$.

3. RESULTS

3.1. Essential Oil and Ethanolic Extract Chemical Profiling

GC-MS analysis of *A. atlantica* essential oil revealed the presence of 14 constituents. In total, six compounds were identified accounting for 96.02% of the overall composition, most of

which are monoterpenes (Table 1). Thymol represented the major compound with a content of 39.46% followed by γ-terpinene and *p* -cymene with contents of 31.74 and 19.01% respectively. This composition is in agreement with those reported by some authors. In fact, Laouer *et al.* (2008) identified thymol (53.2%), γ-terpinene (19.4%), and *p*-cymene (10.6%) as main constituents in the EO of *A. atlantica* from the Djebel Megress (Setif region). Similarly, Latreche-Douar (2019) identified, in addition to thymol (48.5%), p-cymene (20.4%) and γ-terpinene (6.5%) as major compounds, limonene (8.9 %) and carvacrol (8.5%). However, Boudiar et al. (2011) reported a very different composition of the essential oil of *A. atlantica* collected in the Jijel region where safranal (17.9%), endoborneol (17.6%), chrysanthenone (15.5%), filifolone (12.1%) and camphor (11. 8%) represented the main constituents.

Table 1. Chemical composition (%) of the essential oil of *A. atlantica.*

N°	Compound[a]	RT[b]	CRI[c]	LRI[d]	(%)
	Monoterpene hydrocarbons				50.75
1	p-cymene	8.40	1022	1020	19.01
2	γ-terpinene	9.690	1060	1059	31.74
	Oxygenated monoterpenes				46.97
3	Thymol methylether	15.352	1229	1232	6.61
4	Thymol	17.98	1290	1289	39.46
5	Carvacrol	20.484	1296	1298	0.68
6	Methyleugenol	23.41	1405	1403	0.22
	Total (%)				97.72

[a] Compounds listed in order of elution from HP5MS column
[b] Retention time (min)
[c] Calculated retention indices relative to *n*-alkanes C6–C19
[d] Literature retention indices.

HPLC-DAD-UV analysis of the ethanolic extract revealed seven phenolic compounds (Table 2) including 4 phenolic acids (isovanillic, caffeic, sinapic and 3-hydroxy-4-methoxycinamic), and 3 flavonoids (luteolin 7-*O*-glucoside, apiginin and luteolin). Apiginin represented the most abundant phenolic compound with a percentage of 33.58 followed by luteolin 7-*O*-glucoside and luteolin (20.09 and 14.39% respectively). These compounds were previously reported. In fact, Louaar *et al.* (2008) isolated and identified 4 compounds from the extract of *Ammoides atlantica* namely apigenin, luteolin, luteolin 7-*O*-glucoside and apigenin 7-*O*-glucoside. Recently, Benteldjoune *et al.* (2019) characterized 45 constituents in the ethanolic extract of *A. atlantica* by RP-UHPLC-ESI-QTOF-MS including luteolin, apigenin, luteolin *O*-glucoside and caffeic acid.

The polyphenols and flavonoids contents for the ethanolic extract were 148.89 mg GAE/ g DW and 33.95 mg QE/g DW respectively. These values are more or less comparable to previously reported: 371.57 - 141.74 ± 0.44 mg GAE/g DW (Benteldjoune *et al.*, 2019) and 41.02 - 61.87 ± 6.7 mg QE/g DW (Loucif *et al.*, 2020).

Table 2. HPLC-DAD-UV chromatographic profile of *A. atlantica* ethanolic extract.

N°	Compound	Retention time (min)	Wavelenght (nm)	%
1	Isovanilic acid	6.989	255	2.35
2	Caffeic acid	7.626	300	2.73
3	Sinapic acid	8.706	230	2.12
4	Luteolin 7-*O*-glucoside	9.194	355	20.09
5	3-hydroxy-4-methoxycinamic acid	9.679	300	5.05
6	Apigenin	10.321	355	33.58
7	Luteolin	12.761	355	14.39

3.2. Antibacterial Activity

From the results summarized in Table 3, EO was found to be more active than EE. *E. coli* and *Staphylococcus aureus* were the most sensitive to the essential oil (70 and 30 mm diameter, respectively) compared to *Pseudomonas aeruginosa* (48 mm diameter). Unlike the EO, the strains tested were much less sensitive to the EE (13.5 - 16.5 diameters) (Figure 1). Moreover, the lowest MICs and MBCs and therefore the most important inhibitory activities were noted for EO against *S. aureus* (MIC < 0.3 mg/mL). While the *E. coli* and *P. auroginosa* strains were found to be less sensitive (MIC= 1.25 and 5 mg/mL, respectively). In addition, the EO were bactericidal against *S. aureus* and *E. coli* at 10 mg/mL. Contrarily, *P. aeruginosa* seemed to be more resistant. It should be noted that the strains tested were resistant to the EE (MBC>20 mg/mL). These findings are superior to those obtained by Laouer *et al.* (2008), who showed that the oil of *A. atlantica* possessed significant antibacterial activity against all Gram-positive and Gram-negative strains with MIC>25 mg/mL.

Table 3. Antibacterial activity expressed in DZI (mm), MIC and MBC (mg/mL).

	Microoganism	DZI[a] (mm)	MIC[b] (mg/mL)	MBC[c] (mg/mL)
Essential oil	*S. aureus*	70.0 ± 0.7	<0.3	10
	E. coli	48.2 ± 0.28	1.25	10
	P. aeruginosa	32.1 ± 0.14	5	> 20
Ethanolic extract	*S. aureus*	13.5 ± 0.5	20	> 20
	E. coli	15 ± 0.56	20	> 20
	P. aeruginosa	16.5 ± 0.28	> 20	> 20
Gentamicin	*S. aureus*	31 ± 0.0	4*	8*
	E. coli	24.1 ± 0.1	16*	64*
	P. aeruginosa	22 ± 0.0	16*	64*

[a] Diameter of zone of inhibition
[b] Minimal inhibitory concentration
[c] Minimal bactericidal concentration
*µg/mL

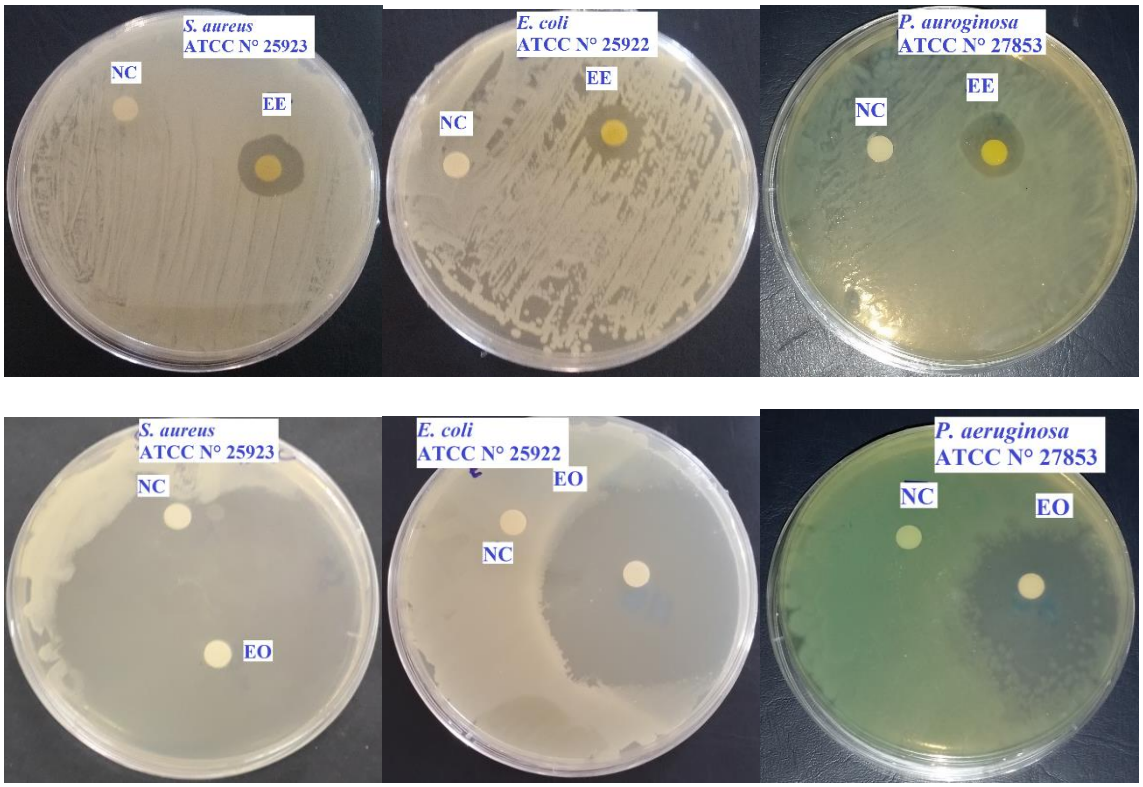

Figure 1. Petri plates showing the antibacterial activity expressed in zones of inhibition.

3.3. Antioxidant Activity

In the $ABTS^{\bullet+}$ test, the essential oil showed the highest scavenging activity with an IC_{50} of 2.79 µg/mL. This activity is superior to those of standard Trolox and EE whose IC_{50} are respectively 3.69 and 6.9 µg/mL (Table 4). However, in the DPPH test, the ethanolic extract and the essential oil showed a scavenging activity comparable to that of BHT with IC_{50} values of 33.78 and vs 22.32 µg/ml respectively, on the other hand, a very low activity for the essential oil (IC_{50} >1000 µg/mL). In addition, EO and EE demonstrated moderate iron-reducing capacities with IC50 values of 145.72 and 152.45 µg/mL respectively compared to 62.83 for BHT and 9.0 µg/mL for ascorbic acid. The latter is known as a very powerful reducing antioxidant of natural origin. These results are relatively in agreement with those previously reported on the hydro-alcoholic extract of the same species (Benteldjoune *et al.*, 2019). These authors indicated BHT/extract IC_{50} ratios of 0.55 versus 0.66 for this study in the DPPH.

Table 4. Antioxidant activity of *A. atlantica* EO and EE expressed in IC_{50} (µg/mL).

Sample	$ABTS^{+\bullet}$	$DPPH^{\bullet}$	FRP
EE	6.9 ± 0.11^{c}	33.78 ± 1.28^{a}	73.45 ± 4.51^{c}
EO	2.79 ± 0.04^{a}	150.0 ± 2.7^{b}	145.72 ± 6.82^{d}
BHT	13.01 ± 0.21	24.23 ± 1.28^{a}	65.38 ± 1.65^{b}
Trolox	3.81 ± 0.1^{b}	511.43 ± 2.51^{d}	–

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test (p<0.05)

3.4. Anti-inflammatory activity

Table 5 shows the effect of *Ammoides atlantica* and Aspirin on HRBC membrane stabilization expressed in percentage of inhibition of hemolysis (%). The highest inhibition percentages were recorded for the standard aspirin, a non-steroidal anti-inflammatory known for its activity, compared to the ethanolic extract of *A. atlantica*. A moderate activity was noted at a low concentration of 10 µg/mL with an inhibition of 38% against 87.5% for aspirin. This activity increased significantly to give an inhibition of 71.25% at 20µg/mL up to 87.5% at 200µg/mL, which ensures better protection of the human erythrocyte membrane against the lysis induced by the hypotonic solution and therefore against inflammation.

Table 5. Effect of *A. atlantica* and Aspirin on HRBC[a] membrane stabilization.

Concentration (µg/mL)	HRBC membrane stabilization (%)	
	AAEE	Aspirin
10	38.54 ± 1.47	87.5 ± 0.2
20	71.25 ± 1.18	88.3 ± 0.05
50	75.01 ± 1.16	90.42 ± 0.05
100	78.96 ± 0.88	90.83 ± 0.1
200	87.5 ± 0.4	95.83 ± 1.2

[a] Human Red Blood Cells

4. DISCUSSION and CONCLUSION

Considering EO composition, strong antibacterial activity was expected since monoterpenes like thymol, γ-terpinene and *p*-cymene dominated. Several studies concluded that, as lipophilic agents, these compounds execute their action at the level of the membrane and membrane-embedded enzymes (Sikkema *et al.* 1994). Juven *et al.* (1994) and Lambert *et al.* (2001) explained the action of thymol by the fact that it binds to the membrane protein and increases the permeability of the bacterial cell membrane. In addition, *p*-cymene causes swelling of the cytoplasmic membrane (Ultee *et al.*, 2002).

In the antioxidant activity, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging reaction and ferric reducing power (FRP) assays mainly attributed to hydrogen atom donation and single electron transfer

(SET) reaction were used in the present study (Bondet *et al.,* 1997; Baczek *et al.,* 2017). In the DPPH and ABTS assays, such reaction results in the decrease in the absorbance of free radical species, visible as the change of color from purple-blue to yellow-transparent, respectively. In turn, FRP assay relies on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of ferricyanide, which results in the formation of an intense prussian blue complex. From the results found, it seems that the antioxidant potential of the oil could be attributed mainly to the high content of thymol, while luteolin, apigenin, luteolin 7-*O*-glycoside are responsible for that of the extract. Several authors reported that the ABTS scavenging activity of EO was generally significantly higher than for DPPH (Özgen *et al.,* 2006; Bendjabeur *et al.,* 2018; Aebisher *et al.,* 2021). In this sense, Salamone *et al.* (2012) and Özturk (2012) explain that despite the fact that the initial reaction of antioxidants with DPPH is, in fact, much slower than $ABTS^{\bullet+}$ reactions due to reduced access to phenols like thymol at the site of the $DPPH^{\bullet}$ radical due to steric hindrance. Moreover, the high activity of the essential oil compared to the extract could be due to their lipophilic nature and their ability to access the $ABTS^{\bullet+}$ radical.

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, which is frequently linked with pain and involves many biological occurrences, such as an increase of vascular permeability, an increase of protein denaturation, and membrane alteration (Ferrero-Millani *et al.,* 2007). Non-steroidal anti-inflammatory drugs (NSAIDs) are used for the treatment and management of inflammation, pain and fever. However, adverse effects associated with NSAIDs can lead to ulcers and hemorrhage (Hajhashemi *et al.,* 2009 Gunathilake *et al.,* 2018). NSAID acts by inhibiting the function of prostaglandin, an autocoid that is released extracellularly and initiate pain, whose synthesis is blocked by anti-inflammatory agents via either inhibiting cyclooxygenase (COX) or protecting lysosomal membrane from breakdown (Yusuf *et al.,* 2009). According to Chippada *et al.* (2011), stabilization of the lysosomal membrane is vital in controlling the inflammatory response by inhibiting the release of lysosomal constituents from activated neutrophils, and given the similarities between the 2 membranes, the effect of the extract on the stabilization of red blood cells could be extrapolated to the stabilization of the lysosomal membrane and this could be considered as a factor of anti-inflammatory activity (Murugasan *et al.,* 1981; Kumar *et al.,* 2011). The protective effect against heat-induced erythrocyte lysis can be explained by the interaction of the extract with membrane proteins, thus inhibiting their denaturation (Lepock *et al.,* 1989). Phenolic compounds, in particular flavonoids, in the membrane of erythrocytes improve their stability against hypotonic lysis. This would be due to the increase in the volume/surface ratio of the cells either by the expansion of the membrane or the shrinkage of the cell. In addition, the deformability and cell volume of erythrocytes are closely related to the intracellular calcium content. Therefore, we can think that the protective effect of the extract would be due to its ability to modify the influx of calcium into erythrocytes (Chopade *et al.,* 2012).

Our results showed that extracts (EO and EE) from *A. atlantica* aerial parts possess interesting antioxidant properties acting as free radical scavengers and reducing agents. Moreover, the EO exhibited a potent antibacterial effect against ATCC Gram positive and negative bacteria due to its high content in monoterpenes. In addition, the anti-inflammatory effect of the EE has also been demonstrated *in vitro* by its protective role against hemolysis of human lysozyme-like erythrocyte membranes. These findings revealed the medicinal and pharmacological potential of this plant, which could be an excellent candidate for the treatment of inflammation and pain-related illnesses as well as infections or as a food preservative. Therefore, this medicinal plant could be potentially exploited for the development of new drugs and have potential applications in pharmaceuticals, nutraceuticals and cosmetics. As it is the first contribution which concerns both the evaluation and the comparison of the bioactivities of EO and EE, this study opens the way to investigate more deeply the highlighted activities and to treat other aspects as well as explore other biological properties and the chemical biodiversity of the compounds of this plant.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

The first author carried out the biological activities, compound identification of HPLC analysis, and writing of the article. The second author contributed to the GC/MS analysis and provided laboratory assistance for antioxidant activity as well as a review of the article.

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