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Antibacterial and Antioxidant Activities of Various Extracts and Essential Oil from Dried Leaves of *Artemisia herba-alba* Asso of Tamanrasset (South Algeria)

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ABSTRACT

The objective of this study was to evaluate the antibacterial and antioxidant activities of two extracts (aqueous and ethanolic) and essential oil from dried sheets of Artemisia herba alba collected in southern Algeria. The extracts were prepared separately with different polarity solvents (water and ethanol). Total phenolics, flavonoids and tanins contents were evaluated. The essential oil was isolated using hydrodistillation. Two tests were established to assess the antioxidant activity (DPPH and FRAP), agar-well diffusion method was used to evaluate the antibacterial effect: Escherichia coli, Enterococcus faecalis, Staphylococcus aureus and Bacillus cereus. The yield of the aqueous extract is higher than that of the ethanolic extract. The phytochemical study revealed the presence of phenolic compounds, flavonoids and tannins. The aqueous extract contains higher amounts of total phenolics (97.17 ± 1.06 mg/g DM), flavonoids (35.61 ± 0.39 mg/g DM) and tannins (46.58 ± 0.91 mg/g DM) compared to the ethanolic extract, 28.69 ± 0.99, 10.98 ± 0.64 and 15.11 ± 0.49 mg/g DM respectively. Antioxidant activity (IC50) of aqueous, ethanolic extracts and essential oil were 2.02, 0.753 and 1.088 mg/ml, respectively. Analysis of the antibacterial activity showed that aqueous extract exhibited much higher activity that the ethanolic extract and essential oil. RP HPLC analysis of aqueous extract show the presence of certain compounds that belong to flavonoids (cathecine and apigenin) and others to phenolic acids (caffeic acid and ferulic acid). The results of this study demonstrated that the essential oil and extracts can be used as antioxidant and antimicrobial agents.

***Keywords*:** Antibacterial activity, antioxidant activity, *Artemisia herba alba*, phytochemical screening

INTRODUCTION

Nowadays, herbal medicine is booming with a growing interest in the use of medicinal plants as palliative treatments to conventional medicine. Indeed, synthetic drugs are very expensive with undeniable adverse effects on human health (Nair and Chandra, 2006; Rahman and Fakir, 2015; Mirihagalla and Fernando, 2021). Several studies have demonstrated that medicinal plants are the core of many bioactive phyto-chemicals that possess the antimicrobial potential and have the ability to protect the human body from stress arises due to free radicals that might cause heart and neurodegenerative disorders, joints inflammation, cancer and several malfunctions.

Artemisia (A) herba alba is widespread in the semi-arid and arid steppes of North Africa, including Algeria. It is a medicinal and aromatic plant, rich in phytochemicals (phenolic compounds, flavonoids, sterols, tannins and essential oil) which are known for their antioxidant activities (Yoon *et al*., 2011; Dif *et al*., 2016; Laouini *et al*., 2018). Many works have demonstrated that it has several pharmacological properties: Antidiabetic (Al-Khazraji *et al.*, 1993), antispasmodic (Goze *et al*., 2009), antimicrobial (Zouari *et al*., 2010), antimalarial and antioxidant (Bourgou *et al*., 2016).

The aim of this work is based on the quantification and phyto-chemical screening (total phenols, total flavonoids and condensed tannins), as well as the study of antioxidant activities (DPPH radical scavenging and power iron-reducing) and antibacterial (*Escherichia coli* ATCC 25922, *Enterococcus faecalis WDCM 009*, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579) of the extracts (aqueous and ethanolic) and essential oil of *A. herba alba* collected in southern Algeria.

MATERIALS AND METHODS

Plant materiel

The plant, *A. herba alba*, was collected in December 2015 and comes from the Assekrem area of the southern Algerian town of Tamanrasset (the Ahaggar Mountains) (Figure 1). This is the main town of the Tuaregs and is located at an altitude of 1,320 meters.

Preparation of the extracts

The leaves were dehydrated in the dark at room temperature, crushed and stored in glass bottles, protected from light and moisture. Aqueous and ethanolic extracts are prepared by maceration of 10 % (w/v) leaf powder in distilled water and ethanol, respectively. The mixtures were then filtered and the filtrates were lyophilized (Ingel *et al*., 2017).

Essential oil extraction

The essential oil was extracted from leaves powder (0.05%) by hydrodistillation for 3h using a Clevenger apparatus type. The oil was stored at + 4 °C (Bruneton, 1999).

Phytochemical Screening

Secondary metabolites were revealed following the methods described by Ayoola *et al*. (2008) and Ravalison *et al*. (2015).

Estimation of total phenolics content

The amount of total phenolics was determined using the Folin-Ciocalteu (Singleton and Rossi, 1965). A calibration curve of gallic acid was prepared and the results were expressed as mg gallic acid equivalents per gram of the sample (mg EAG/g). In this method, 0.2 mL of sample was mixed with 1 ml of Folin-Ciocalteu reagent. Then, 800 µL of 7.5% sodium carbonate solution is added. The mixture is allowed to stand for 30 min and absorbance was measured at 700 nm using a spectrophotometer.

Estimation of flavonoids content

The concentration of flavonoids was quantified according to the colorimetric assay of Kosalec *et al*. (2004). One milliliter of sample was made up to 3 ml of methanol (95 %), mixed with 5.6 ml of distilled water and then 0.2 ml of potassium acetate (1 M), 0.2 ml of 10% $AlCl_3$ solution was added. The mixture was allowed to stand for a further 30 min, and absorbance was measured at 420 nm. The total flavonoids content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight.

Estimation of tannins content

Tannin contents were determined by spectrophotometry (λ = 700 nm) as described by Makkar *et al*. (1993). The results were expressed in terms of mg tannic acid equivalents (TAE) per gram of dry matter (mg TAE/g) using the tannic acid calibration curve (0.5-1 mg/ml).

Evaluation antioxidant activities

Antioxidant activity by DPPH assay

This activity measures the free radical scavenging capacity by reduction of the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) as described by Brand Williams *et al*. (1995). Absorbance at 517 nm was used to calculate radical scavenging activity (% of inhibition) with the formula:

$$DPPH\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Abs control: Absorbance of DPPH solution mixtures without extract. Abs sample: Absorbance of DPPH mixtures containing extract.

Ascorbic acid was used as a reference. The results obtained allow us to calculate the IC_{50} (concentration in which the 50 % of the free radical DPPH is reduced).

Ferric reducing assay

The antioxidant capacity was also evaluated using ferric reducing antioxidant power (FRAP) assay. FRAP assay was performed based on the methods of Oyaizu, 1986. The reducing power was expressed by the increase in optical density of the sample measured at 700 nm, using ascorbic acid as a standard.

Antibacterial test

Antimicrobial testing and minimum inhibitory concentration were performed by agar well diffusion (Nalawade *et al*., 2016) against *E. coli* ATTCC 25922, *S. aureus* ATTCC 25923, *B. cereus* ATCC 14579, and *E. faecalis* WDCM 009. 1 ml of

each microbial suspension to be tested was incorporated into Mueller Hinton agar. Then, 6-mm-diameter wells were perforated in the medium, filled with 20 µl (1.5 mg/ml) of sample and incubated at 37°C/24 hours. DMSO (10 %) and standard antibiotics were used as negative and positive controls, respectively. After incubation, activity was assessed by measuring the diameter of the growth inhibition zone.

Chromatographic analysis

RP-HPLC was performed using AGILENT Ultimate 1100 system equipped with degasser, quaternary pump, auto sampler, column oven and ultraviolet (UV) detector. Separation was done using Hypersil BDS C18 column (250 mm×4.6 mm). Column temperature was maintained at + 30 °C, injection volume was 5 ìl, and a gradient mobile phase was used which comprised of acetonitrile (A), 0.2 % acetic acid in water (B). Flow rate was maintained at 1.5 ml/min. The retention times of the different standards were used to identify the composition of the aqueous extract.

Statistical Study

The data are presented as the means ± standard deviations from the three replicates. Calculations were carried using the SAS v. 9.1.3 program.

RESULTS AND DISCUSSION

Extraction

The yield of the ethanolic extract is higher than that of the aqueous extract (Table 1). The solvents used for extraction influence the composition and quantity of secondary metabolites (phenolic and flavonoid) and/or their biological activity (Rebey *et al*., 2012; Ngo *et al*., 2017). Dirar *et al*. (2019) reported that the aqueous and ethanolic extracts showed high contents of phenolic compounds. The essential oil yield obtained is lower than the results found by Esam *et al*. (2016) and Ezzoubi *et al*. (2018) for the aerial part of the same plant collected in Jordan and Morocco.

Phytochemical screening

The results of phytochemical screening are mentioned in Table 2. These results show the presence of flavonoids, terpenoids, phenols, tannins and reducing compounds. Moreover, the tests performed do not detect any presence of alkaloids, free quinines, glycosides and saponins. The same results were reported by Dif *et al*. (2016).

RP HPLC

Table 3 shows the results of the chromatography analysis. RP HPLC analysis of aqueous extract show the presence of certain compounds that belong to flavonoids (cathecine, apigenin, lutelin) and others to phenolic acids. In addition, the extract was characterized by the presence of caffeic acid in higher amounts. In concordance of our results, apigenin was also detected in the aerial parts of *A. herba alba* collected from Egypt and Tunisia (Bourgou *et al*., 2016).

Total polyphenols, flavonoids and tannins contents

Total polyphenols, flavonoïds and condensed tannins were estimated in two extracts. The results are given in Table 4. Significantly different contents of phenolic compounds, flavonoids and tannins were found in the two extracts. The highest amounts of these compounds are shown in aqueous extract. The results obtained in this study are similar to those reported by Shahid *et al*. (2012), who showed that water is more efficient than ethanol in the extraction of polyphenols. The influence of the solvent used on the extraction yield of polyphenols shows a correlation with its polarity (Dixon *et al*., 2011). Compared to our results, Sekiou *et al*. (2018) reported lower total phenolics, flavonoids and condensed tannins amount in Algerian *A. herba alba* aqueous extract. The quantitative and qualitative composition of polyphenols depends on the origin of the plant, the nature of the extraction solvent and the method used (Zhao *et al*., 2006; Ashraf *et al*., 2015; Mohammed *et al*., 2021). In addition, adverse environmental conditions such as water deficit cause physiological stress in the plant, which leads to the synthesis and accumulation of phenolic compounds (Ashraf *et al*., 2017).

Antioxidant activities (DPPH radical scavenging activity and FRAP)

Table 5 shows the results of the antiradical activity (DPPH). The antiradical activity is more important when the IC_{50} or EC_{50} is lower. The ethanolic extract had the highest antioxidant activity, followed by essential oil and aqueous ex-

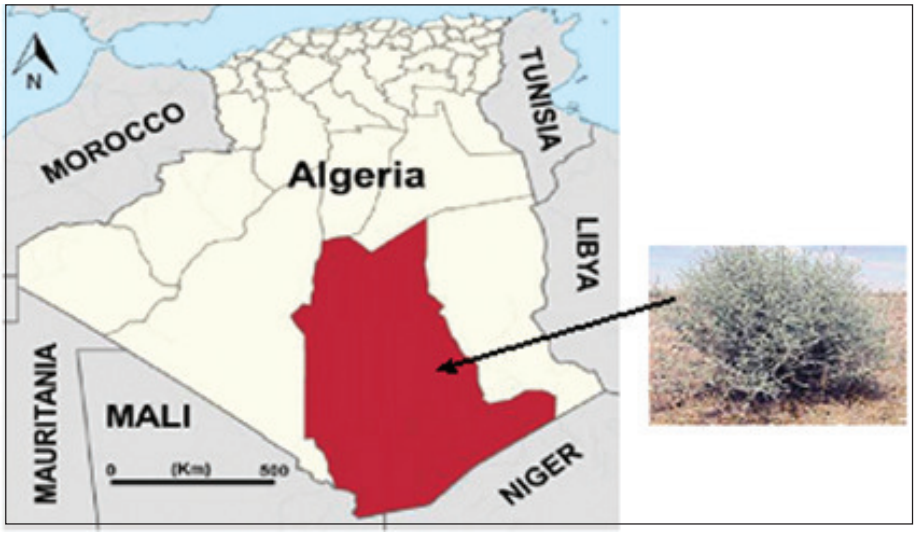

Figure 1: Location of the Wilaya of Tamanrasset in south Algeria.

tract. Ethanol extract has the highest scavenging activity (DPPH) with the lowest IC_{50} value of 0.735 mg/ml. The comparative study of the antiradical activity of the tested samples is weaker than that of ascorbic acid. On the other hand, the antioxidant power of the essential oil is higher than the extracts. These results correlate with those reported by Dirar *et al*. (2019) and El-Massry *et al*. (2002), who showed that the nature of the solvents used significantly influences the composition of extracted polyphenols as well as their antioxidant effect. However, the antioxidant power of the aqueous extract is higher than that reported by Khilifi *et al*. (2013).

The results in Table 5 revealed that the essential oil of the studied plant was characterized by slightly lower EC_{50} values than those of the positive control (EC_{50} = 0.029 mg/ml). In our study both extracts (aqueous and ethanolic) have a low reducing power compared to the standard. However, the antioxidant activity of the essential oil of the studied plant could be associated with its high concentration of oxygenated monoterpenes (Griep *et al*., 2007; Lou *et al*., 2011).

The release of electrons or hydrogen atoms from polyphenols was considered to be the most important element of the structure-antioxidant activity relationship. The redox characteristics of these compounds can explain the different antioxidant mechanisms (*viz*., adsorption, neutralization of free radicals, chelation of metal ions, termination of autoxidative chain reactions, decomposition of peroxides, and quenching of singlet or triplet oxygen) (Dorma and Deans, 2000). So, the antioxidant activity of phenolic compounds is related to their ability to release electrons. The antioxidant power of flavonoids depends mainly on their ability to eliminate free radicals. Phenolic compounds were reported to be very strong antioxidants. The chemical structure and polarity of the antioxidant are crucial to its ability to trap free radicals. Synergistic but also antagonistic effects are observed in model solutions that contain several functional compounds with antiradical activity (Zaouali *et al*., 2010).

Antimicrobial Activity

The *in vitro* activity of extracts and essential oil from leaves of *A. herba alba* is determined. The antibacterial activity of the essential oil was very low against all bacteria tested. The results obtained indicated that Gram-positive *Staphylococcus aureus* was the most resistant strain tested to the essential oil and extracts, with a low zone of inhibition. An inhibition zone of 6.5 ± 0.7mm for *Enterococcus feacalis* and 7 ± 0.41mm for *Escherichia coli* and *Staphylococcus aureus* then 9 ± 0.7mm for *Bacillus cereus.* The aqueous extract has an interesting antibacterial activity against all strains tested. The aerial parts aqueous extract exhibited the highest activity (20.5 ± 3.53 mm) against *Enterococcus faecalis* followed by

Table 1: Percentage yield of extracts and essential oil from *A. herba alba*

Extraction	Yield extract (%)
Water extraction (Aqueous extract)	15.68 ± 0.4
Solvent extraction (Ethanolic extract)	30.03 ± 2.07
Essential oil	0.18

Table 2: Phytochemical screening of extracts from of *A. herba alba*

Compounds	Aqueous Extract	Ethanolic extract
Flavonoids	+	+
Saponins	-	-
Reducing compounds	+	+
Phenols	+	+
Terpenoids	+	+
Glycosides	-	-
Catechic tannins	+	+
Tannins	+	+
Alkaloids	-	-
Free quinones	-	-

(+): Present; (-): Absent

Table 3: Compounds identified in the *Artemisia herba alba* extract by RP HPLC

Compounds	T_R (min)	Area of the peak (%)
Catherine	5.865	1.302
caffeic acid	7.155	13.268
p-OH benzoic acid	6.423	3.846
Rutin	8.284	6.209
ferulic acid	9.360	3.754
3-Hydroxy-4-methoxycinamic acid	9.738	7.295
Luteolin	13.087	2.662
Apigenin	14.714	1.183
Isoramenitin	15.064	3.011

Table 4: Total polyphenols, flavonoids and condensed tannins contents (mgE/gDW) from *A. herba alba*

	Aqueous extract	Ethanolic extract
Polyphenols	97.17 ± 1.06	28.69 ± 0.99
Flavonoids	35.61 ± 0.39	10.98 ± 0.64
Condensed tannins	46.58 ± 0.91	15.11 ± 0.49

E: Equivalent; DW: Dry Weight

Table 5: DPPH test (IC_{50} mg/mL) and reducing power (EC_{50} mg/mL)

	Ascorbic acid	Essential oil	Aqueous extract	Ethanolic extract
IC_{50} (mg/ml)	0.123	1.088	2.02	0.735
EC_{50} (mg/ml)	0.029	0.030	0.508	0.416

Table 6: The Minimal inhibitory concentration (MIC) values (mg/ml) of the extracts and essential oil

Bacteria species	Aqueous extract	Ethanolic extract	Essential oil
S. aureus	0.29 ± 0.13	0.75 ± 0	0.46 ± 0
E. coli	0.38 ± 0	0.46 ± 0	0.75 ± 0
E. faecalis	0.12 ± 0	0.12 ± 0	0.46 ± 0
B. cereus	0.05 ± 0	0.29 ± 0.13	0.05 ± 0.02

Bacillus cereus (17 ± 2.82 mm), *Escherichia coli* (12 ± 1.41 mm) and *Staphylococcus aureus* (10 mm). The ethanol extract exhibited 15±0.7 mm zone for *Bacillus cereus*, which is the maximum followed by 11.5 ± 0.7 mm zone for *Escherichia coli* and *Enterococcus faecalis* then10 ± 1.41mm for *Staphylococcus aureus*. Overall, the ethanolic extract of *A. herba alba* leaves was strongly bactericidal with larger inhibition zones and low MIC values (0.05-0.038 mg/ml) (Table 6). The richness of the essential oil of A. herba alba in oxygenated monoterpene compounds could explain the antimicrobial effect of this oil (Bertella *et al*., 2018; Amor *et al*., 2019).

Phenolic acids and flavonoids are known to be responsible for strong antimicrobial activity against a wide spectrum of microorganisms, through different processes such as substrate reduction, binding with polypeptides exposed on the microbial cell surface, formation of complexes with the microbial wall, disruption of the microbial cell membrane (Cox *et al*., 2000). Antibacterial activities of flavonoids were described previously. According to Cowan (1999), the antimicrobial activity of flavonoids without free hydroxyl groups is greater because the chemical affinity for membrane lipids is increased.

Various terpenoïds or phenolic components may be primarily responsible for this activity (Nedorostova and Kloucek, 2009; Pavela, 2014). Thus, to better understand the biological function of phenolics and terpenoids, it is therefore necessary to test these compounds separately and subsequently evaluate the structure-activity relationship. The antimicrobial power of the aqueous extract of the leaves of this plant could be attributed to its high content of phenolic compounds and flavonoids. In addition, the synergistic effect of various compounds could be an important factor of its antimicrobial activity.

The monoterpenes of essential oils can diffuse into cell membranes and damage them. These compounds are also capable of destroying cell integrity, inhibiting respiration and thus altering their permeability (Bertella *et al*., 2018). In fact, the variability in chemical components of essential oils can explain the biological effects (*viz*., antibacterial, antioxidant, anti-inflammatory, anticarcinogenic, antifungal) of these oils.

CONCLUSION

Phytochemical screening of leaves of *A. herba alba* Asso reveals the richness of the aqueous extract in polyphenols well known for their antioxidant property. The aqueous extract and essential oil of *Artemisia herba alba* exhibited interesting biological activities. The results of this study suggest that *Artemisia herba-alba* may be a potential source of biomolecules that can be used for applications such as food, cosmetics, pharmaceuticals and other related fields.

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