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Biological activities of phenolic extracts from *Artemisia herba-alba* Asso grown in western Algeria

Noura Ayad [1,2,*], Rachida Benaraba [1,2], Houari Hemida [3], Fatiha Abdellah [1,3]

[1] Laboratory of Research on Local Animal Products University of Tiaret, 14000, Algeria

[2] Faculty of Natural and Life Sciences - University of Tiaret, 14000, Algeria

[3] Institute of Veterinary Sciences, University of Tiaret, 14000, Algeria

[4] Farm Animal Laboratory, Institute of Veterinary Sciences - University of Tiaret, 14000, Algeria

* Corresponding author: E-mail: noura.ayad@univ-tiaret.dz

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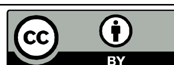

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ABSTRACT: The aim of this study was to determine the phenolic compounds from *Artemisia herba-alba* Asso, in order to evaluate their antioxidant and antibacterial activities, *in vitro*. The extraction of phenolic compounds was carried out by the maceration technique using absolute ethanol, absolute methanol, and distilled water. The quantification of polyphenols and flavonoids was performed using the Folin-Ciocalteu reagent and the aluminum trichloride method, respectively. The evaluation of the antioxidant activity of the extracts was carried out by the FRAP, the DPPH• radical trapping, and the neutralization of the hydrogen peroxide technique. The lipid peroxidation was assessed by thiobarbituric acid reactive substances. In addition, the antibacterial activity of the three extracts was tested on *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 33862, *Escherichia coli* ATCC 2592, and *Pseudomonas aeruginosa* ATCC 27853 bacteria using agar diffusion and agar incorporation methods. The results showed that the methanolic extract was highly rich in polyphenols and flavonoids. Also, the reducing power CE_{50} = 249.88 ± 6.07 µg/ml and the inhibition capacity of the DPPH• radical CI_{50} = 34.71 ± 0.96 µg/ml were significantly higher ($p<0.05$) than the ethanolic and aqueous extracts. Also, a highly significant inhibitory potential of lipid peroxidation was obtained with the methanolic extract (MDA = 66.97 ± 3.61 µmol/g tissue). However, a highly significant hydrogen peroxide scavenging effect was obtained from the ethanolic extract. A better antibacterial activity was obtained with the methanolic and ethanolic extracts.

Keywords: *Artemisia herba-alba* Asso; Antibacterial activity; Antioxidant activity; Flavonoids; Lipid peroxidation; Polyphenols.

1. INTRODUCTION

Currently, the increase of pathological conditions associated with oxidative stress, antibiotic resistance, and adverse effects of some drugs, leads researchers to turn to the plant world seeking for herbal alternatives. The study of secondary metabolism in plants is an important source for the discovery of bioactive compounds with a wide range of applications. Today these bioactive compounds derived from plants are important drugs such as antibiotics and agrochemical substitutes. They also have been economically important as flavors and

fragrances, dyes and pigments, and food preservatives. Many of the drugs sold today are synthetic modifications of naturally obtained substances [1]. Among polyphenols, flavonoids are gaining increasing attention for their powerful antioxidant and antimicrobial properties [2]. However, it has been established that the phytotherapeutic and/or pharmaceutical effectiveness of medicinal plants is mainly based on the qualitative and quantitative profile of their extracts [3]. *Artemisia herba-alba* Asso known as "White desert wormwood" and called "Shih" in the Algerian vernacular, a species of the Asteraceae family, grows spontaneously in the arid and semi-arid zones of the Mediterranean basin, and even extends as far as the north-western Himalayas; this plant has been used in traditional medicine by many cultures since antiquity as a hemostatic, analgesic, antibacterial and antispasmodic [4], anti-inflammatory [5], hypocholesterolemic and hypo-triglyceridemic agents [6-9]. In folk medicine, this herb is used to treat several digestives (diarrhea and stomach ache) and respiratory (bronchitis and cough) problems [10]. Nowadays, the prevention and management of oxidative stress disorders, such as diabetes and cardiovascular diseases, have become a priority and a sanitary and socio-economic issue for public health authorities. *Artemisia herba-alba,* a medicinal plant widely used in Algerian pharmacopeia and traditional medicine, could be proposed as a pharmaceutical or nutraceutical preventive formula against these conditions. During this study, particular attention was given to the process and the type of solvents used for the extraction of these active compounds in order to screen the phenolic extract with an optimal efficiency towards the oxidative stress, particularly the lipidic peroxidation, the free radicals scavenging and the antibacterial activity of Algerian *Artemisia herba alba* extracts.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *Artemisia herba-alba* (White Wormwood) were collected during May 2017 from Rechaïga, Tiaret, Algeria (35°24'29.09"N, 1°58'24.31"E). The samples were carefully cleaned and aired, ground into a fine powder using a mechanical grinder (Fritsch, Germany) and carefully stored in glass jars for analysis.

2.2. Animals

Six healthy male Wistar rats (252 ± 6.71 g), obtained from the Pasteur Institute of Algiers (Algeria) were used to evaluate the protective effect of extracts against lipid peroxidation. The animals were kept in individual polystyrene cages under animal’s house conditions (temperature 22 ± 1°C, 12/12 hours light-dark cycle, and relative humidity 60 ± 10%) for a two weeks period at the Veterinary Sciences Institute, Tiaret University. A standard pellet and clean drinking water were provided ad libitum. Animals were then sacrificed, subjected to a full gross examination and specific organs (liver) were obtained for further analysis. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

2.3. Bacterial strains

The bacterial strains; *Escherichia coli* ATCC 2592, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33862 were kindly provided by the university hospital Mustapha Pasha of Algiers, Algeria.

2.4. Extraction of phenolic compounds

The extraction of phenolic compounds was performed using the maceration method. Five g samples of *Artemisia herba-alba* powder were macerated in 50 mL of absolute methanol, absolute ethanol and distilled water at room temperature at 600 rpm for 24 h. After filtration of the obtained mixture, the solvent was evaporated at 50 °C to obtain a dry extract which was stored at -20°C for further analysis [11]. The yield of extraction is expressed as a percentage and it is calculated using the following equation 1:
Yield (%) = [W_1 extract/ W_0 powder] x 100

2.5. Determination of total polyphenols

The total phenol content of the extracts was determined by the Folin-Ciocalteu method [12]. A quantity of 250 µL of the extracts was mixed with 2 mL of distilled water and 250 µL of freshly prepared Folin-Ciocalteu reagent (0.2 N). After 2 min of incubation, 500 µL of 7.5% w/v sodium carbonate (Na_2CO_3) was added to the previous mixture. The resulting mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured using a UV-V spectrophotometer (Shimadzu Corporation, Japan) at 760 nm wavelength. The results were expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

2.6. Determination of total flavonoids

The total flavonoid content of the extracts was determined by the colorimetric method described by Bahorun et al. [13]. One milliliter of the extracts was mixed with 1 mL of a 2% w/v aluminum chloride ($AlCl_3$) solution, incubated for 10 min, then the absorbance was measured at 430 nm [13]. The results were expressed in mg quercetin equivalent per g dry matter (mg QE/g DM).

2.7. Ferric Reducing Antioxidant Power (FRAP) Test

The ferric reducing antioxidant power of the extracts was determined according to the method described by Yen et Duh [11]. A 500 µL volume of each extract (different concentrations) was mixed with 500 µL of a phosphate buffer solution (0.2 M, pH 6.6) and 500 µL of a 1% w/v potassium ferricyanide $K_3Fe(CN)_6$ solution. The total mixture was incubated at 50°C for 20 min, then 500 µL of 10% w/v trichloroacetic acid was added to stop the reaction. From the previous reaction mixture, an aliquot of 1 mL was combined with 1 mL of distilled water and 500 µL of 0.1% w/v $FeCl_3$ aqueous solution. The colorimetric measurement was performed at 700 nm [11]. Three standard antioxidant solutions were used as positive controls; gallic acid, quercetin, and ascorbic acid. The reducing power of extracts and standards was represented by the values of the median effective concentrations (EC_{50}).

2.8. DPPH$^•$ radical trapping test

This method was based on measuring the ability of antioxidants to trap the free radical 2,2-diphenyl-1-picrylhydrazil (DPPH$^·$). The effect of each extract on DPPH$^•$ was measured by the procedure described by Que et al. [14]. A volume of 750 µL of different concentrations of each extract and antioxidant standards (gallic acid, quercetin and ascorbic acid expressed in µg/mL) was added to 750 µL of the freshly prepared 4 mg/mL DPPH$^·$ solution. The reaction mixture was incubated at room temperature and in the dark for 50 min and the absorbance was read at 517 nm. The median inhibition concentration (IC_{50}) values were determined graphically by the exponential regression of extracts and solutions, and the antiradical activity % was calculated according to the following formula 2:

AA (%) = $[(A_0 - A_1) / A_0)] \times 100$

Where: AA %: Antiradical activity; A_0: The absorbance of DPPH$^{\bullet}$ radical; A_1: The absorbance of the sample.

2.9. Neutralization of hydrogen peroxide (H_2O_2)

The hydrogen peroxide (H_2O_2) scavenging capacity of extracts was determined by using the method of Ruch et al. [15]. Two milliliters of each extract at different concentrations were added to 1.2 mL of a H_2O_2 solution (4 mM in phosphate buffer 0.1 M at pH 7.4). The blank was prepared in the same way by replacing the H_2O_2 solution with phosphate buffer. After 15 min incubation period, and the absorbance was measured at 230 nm. Gallic acid and ascorbic acid (expressed in µg/mL) were used as standards [15]. The IC_{50} values were calculated from the linear regression curves. The H_2O_2 inhibition percentage was calculated according to the following formula 3:

H_2O_2 (%) = $[(A_0 - A_1) / A_0)] \times 100$

Where: A_0: The absorbance of H_2O_2; A_1: The absorbance of the sample.

2.10. Anti-lipid peroxidation activity

2.10.1. Liver homogenate preparation

At necropsy, the liver of six Wistar rats was carefully removed and rinsed with 9% NaCl. A 10 g liver pooled sample was added to 100 mL (0.1 M, pH 7.4 having 0.15 M KCl) phosphate buffer, ground by ultraturax T25 (Janke & Kunkel GmbH & Co kg; Ika Labortechnik Staufen Germany) and centrifuged at 4000 rpm for 20 min at 4°C, the resulting supernatant was recovered and incubated for 1 h in ice and then stored at -20°C [16].

2.10.2. Lipid peroxidation and thiobarbituric acid reactions

Lipid peroxidation assay was performed by a formerly described protocol by Gupta and Sharma [17]. Phosphate buffer 580 µL (0.1 M; pH 7.4), 200 µL of extract or standard, 200 µL liver homogenate and 20 µL ferric chloride (100 mM, H_2O_2 0.50% prepared in phosphate buffer 0.1 M, pH 7.4) [18], were combined to form a mixture that was placed in a shaking water bath for 1 h at 37°C. The assessment of malonic dialdehyde (MDA) content was performed according to the protocol described by Yagi [19], a volume of 800 µL of a 0.375% (w/v) TBA, TCA (20%), BHT (0.01%), and HCL (1N) mixture was added to 200 µL of the previously prepared solution. After shaking for 2 min, the mixture was incubated in a water bath at 100 °C for 10 min. During this step, the aldehyde functions of MDA were released by acid hydrolysis at 100 °C. They react with TBA forming a pink-colored complex (MDA-TBA). To stop the reaction, the tubes were placed in ice, the complex thus formed is extracted with 2 mL of 1-butanol for 2 min. After centrifugation at 4000 rpm for 10 min at 4 °C (Sigma, 3K10, Laborzentrifugen, Germany), the supernatant was collected and the absorbance of the pink chromogen obtained was measured at 532 nm using a spectrophotometer (Shimadzu 1240, Japan). The tissue concentration of malondialdehyde (MDA) was calculated using a linear PET curve. The percentage of MDA inhibition was determined according to the following formula 4:

MDA (%) = $[(C_0 - C_1) / C_0)] \times 100$

Where: C_0: MDA concentration without protection; C1: MDA concentration with protection.

2.11. Antibacterial activity

2.11.1. Agar diffusion method

A bacterial suspension was prepared in sterile physiological water (0.9%) for each strain. The turbidity

of this suspension was adjusted to 0.5 Mac Farland. This inoculum was spread on the surface of the Mueller-Hinton agar plate. Sterile filter discs (6 mm diameter) were impregnated with 20 µL of each extract solution and then deposited on the surface of the inoculated agar. The plates were incubated at 37°C for 24 h [20]. Antibiotic discs of tetracycline, amikacin, and erythromycin (Merck, Germany) served as positive controls and discs impregnated with 50% methanol, 50% ethanol and dimethyl sulfoxide (DMSO) served as negative controls. Antibacterial activity was determined by measuring the diameter of the inhibition zone around each disc.

2.11.2. Incorporation method

The MIC and MBC of the extracts were determined using an agar incorporation technique [21]. The different extracts were added in increasing amounts (v/v) to the Mueller-Hinton media for a final volume of 5 ml. The mixture was poured into plates, then each inoculum standardized to 10^6 cells/mL was deposited on the agar plate and incubated at 37°C for 24 h. MIC is determined as the lowest concentration of the extract that inhibits visible bacterial growth. However, MBC is the lowest concentration of the extract that killed 99% of the bacteria in the initial inocula within 24 h.

2.12. Statistical analysis

The results were expressed as mean ± standard error (M ± SE). The data analysis was performed using the Statistica StatSoft software (version 6.1, Statsoft, Tulsa, UK). The one-factor ANOVA was used to compare the means, followed by Duncan's post-hoc test. Differences were considered statistically significant at a p-value of less than 0.05 across all statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Extraction yield, quantification of phenolic and flavonoid compounds

Results of extraction yield and phenolic compound content are shown in Table 1. The total polyphenol (TP) and flavonoid (TF) content of *Artemisia herba-alba* are determined from the linear regression equation of the calibration curve using different concentrations of gallic acid and quercetin. The results are expressed in mg gallic acid equivalent (GAE) and mg quercetin equivalent (QE)/g dry matter.

Table 1. Extraction yield and, content of phenolic and flavonoid compounds.

Extracts	Yield (%)	Total polyphenols (mg GAE/g DM)	Total flavonoides (mg QE/g DM)
MEA	7.89 ± 0.00	154.06 ± 1.70	47.97 ± 0.32
EEA	6.75 ± 0.004^{b}	118.28 ± 2.31^{c}	18.22 ± 0.31^{c}
AEA	$12.14 \pm 0.003^{c, c}$	$52.44 \pm 0.99^{c, c}$	$31.86 \pm 0.80^{c, c}$

The values represent the means ± SE (n = 6). Values with a superscript are significantly different from those in the MEA. (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$).

Aqueous extraction of *A. herba alba* has yielded a very important amount of 12.14 ± 0.003% (w/w) compared to the methanolic and ethanolic extraction with 7.89 ± 0.004% (w/w) and 6.75 ± 0.004% respectively. A highly significant ($p<0.001$) amount of total polyphenols was obtained with the methanolic extract of *A. herba-alba* (MEA) compared to both ethanolic (EEA) and aqueous (AEA) extracts (154.06 ± 1.70, 118.28 ± 2.31 and 52.44 ± 0.99 mg GAE/g DM, respectively). However, the quantification of total

flavonoids revealed a high content in the MEA (47.97 ± 0.32 mg QE/g DM) followed by the AEA (31.86 ± 0.80 mg QE/g DM) and the EEA (18.22 ± 0.39 mg QE/g DM) (Table 1).

In the present study, the aqueous extract showed the highest extraction yield compared to the alcoholic extracts (methanol and ethanol); these results are similar to those reported by Al-Kharabsheh et al. [22] that the aqueous extract of *A. herba alba* gives the highest yield (13.783 ± 0.210 g/100 g DW) compared to other extracts. These findings are best illustrated by Lim et al. [23] who showed that best results are obtained using a more polar solvent aqueous ethanol 50% (v/v) with a yield of 15.8% compared to ethanol 100% and 70% (v/v) of 10.4% and 15.2% respectively. It is well documented that the variations in extraction yield and polyphenol content depend on the experimental conditions, essentially the method, the solvents used and the extraction temperature [24]. It is also widely accepted that variations in extraction yields could be attributed not only to the difference in the polarity of the solvent used, which plays a key role in increasing the solubility of phenolic compounds, but also to the polarity of the phenolic compounds that make up the extract [25,26].

The results of total polyphenols and flavonoids quantification in the extracts of *A. herba alba* showed that high amounts were found with the methanolic extract compared to ethanolic and aqueous extracts, these results are highly correlated to those obtained by Megdiche-Ksouri et al. [27] with high contents of total polyphenols and flavonoids of *Artemisia campestris* were found in the crude methanolic extract compared to the ethyl acetate fraction and water fraction and accounting for (158. 23 ± 7.2 mg EAG/g DM versus 94.17 ± 12.14 and 10.63 ± 2.16 mg EAG/g DM) total polyphenol and (175.23 ± 7.2 mg EC/g DM versus 67.45 ± 2.28 and 63.81 ± 0.52 mg EC/g DM) flavonoids, respectively. Same findings were reported in another study investigating the antioxidant activity of *Artemisia sp.* showing high content of total polyphenols 120 mg GAE/g DW where 111 mg EC/g DW (about 92.5%) are flavonoids recorded in the methanolic extract of *Artemisia capillaires*, in the other extracts of *Artemisia sp.* total polyphenols content accounted for 66-101 mg GAE/g DW from which 77-93% were flavonoids [28]. However, Abdallah et al. [29] have reported that the extraction of *A. herba alba* using 70% ethanol showed a high total polyphenol content (248.6 ± 20.4) mg GAE/g dry extract and flavonoids (62.15 ± 5.8) mg rutin/g dry extract. Studies have shown that polar solvents such as methanol and ethanol allow better extraction of phenolic compounds from plant materials than less polar solvents [30,31] and that phenolic compounds are more soluble in methanol than in water [32], which may explain the low quantity of phenolic compounds obtained from the aqueous extract of *A. herba-alba* in this study.

Touil and collaborators reported that *A. herba-alba* is a rich source of polyphenolic compounds and that the levels of phenolic compounds, including flavonoids, vary in quantity and quality depending on harvest time; with the highest content of phenolic compounds obtained with the July's harvest during the vegetative stage (515 ± 142 mg/g DMW), and the lowest content obtained in November's harvest (265 ± 48 mg/g DMW) [33]. The same findings were reported in previous studies with the highest content of phenolic compounds obtained at the flowering stage. Given the variations in the accumulation of secondary metabolites in *A. herba-alba*, it could be concluded that the physiological stage of the plant help choosing the suitable harvesting period [34,35].

3.2. Reducing power (Ferric Reducing Antioxidant Power)

The reducing power of *A. herba-alba* extracts, gallic acid, quercetin and ascorbic acid (used as standard antioxidants), are represented in Fig. 1.

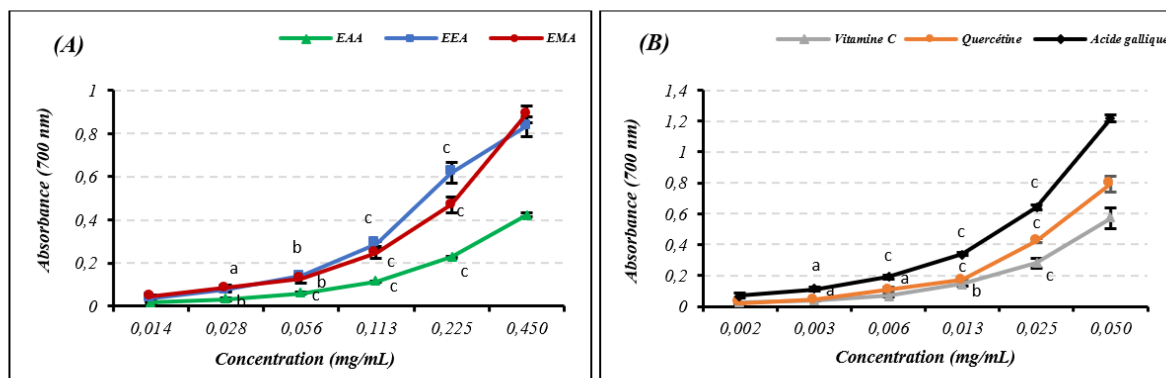

Figure 1. Reducing power of different concentrations of *Artemisia herba-alba* Asso extracts (A), gallic acid, quercetin and ascorbic acid (B) by spectrophotometric detection of Fe^{3+} transformation to Fe^{2+} (Mean ± standard error, n=6). Values with an exponent are significantly different from those with different concentrations (a, P < 0.05; b, P < 0.01; c, P < 0.001).

Figure 1 shows the results of the reducing power of iron at different concentrations of the extracts and standards. The results show the highest reducing power of standards were obtained with gallic acid and quercetin with (OD = 1.21 ± 0.02 and 0.79 ± 0.004, respectively) at the highest concentration (0.050 mg/ml), at the same concentration the reducing power of ascorbic acid was showed the lower effect with (OD= 0.57 ± 0.06). we have registered EC_{50} of the following order (25.24 ± 1.19 for gallic acid; 32.12 ± 2.01 for quercetin and 56.55 ± 1.91 for ascorbic acid µg/mL) in Table 2. Both alcoholic extracts recorded highest reducing power with (OD = 0.88 ± 0.04 for MEA and 0.83 ± 0.03 for EEA) at the highest concentration (0.450 mg/ml). However, at the same concentration the aqueous extract was recorded a lower reducing power (OD = 0.42 ± 0.009). Calculated EC_{50} values of three extracts were 249.88 ± 6.07, 261.59 ± 8.55 and 532.36 ± 2.58 µg/ml for EMA, EEA and EAA, respectively. In addition, we found a strong and positive correlation between the total polyphenols content and the reducing power of iron for all extracts. Recorded respective correlation coefficients were r = 0.9866, r = 0.9559 and r = 0.9854 for the methanolic, ethanolic and aqueous extracts. This indicates that 99% of the antioxidant capacity of the extracts, are due to the contribution of phenolic compounds which are the dominant antioxidants in these extracts [36].

Table 2. EC_{50} and IC_{50} values (µg/mL) of *Artemisia herba-alba* Asso extracts and standard antioxidants in FRAP, $DPPH^{•}$ and H_2O_2 tests.

Extracts	FRAP (CE_{50})	$DPPH^{•}$ (CI_{50})	H_2O_2 (CI_{50})
MEA	249.88 ± 6.07	34.71 ± 0.96	128.41 ± 1.40
EEA	261.59 ± 8.55	53.29 ± 1.89^c	96.54 ± 1.64^c
AEA	532.36 ± 2.52^c	97.65 ± 3.42^c	187.08 ± 7.95^c
Gallic acid	25.24 ± 1.19^c	1.82 ± 0.16^c	3.7 ± 0.036^c
Quercetin	32.12 ± 2.01^c	4.47 ± 0.57^c	-
Ascorbic acid	56.55 ± 1.91^c	5.34 ± 0.18^c	11.31 ± 0.80^c

The EC_{50} values are expressed as mean ± SE (n = 6). Values with a superscript are significantly different from those in the MEA (a, P < 0.05; b, P < 0.01; c, P < 0.001).

The biological efficacy of extracts depends on the experimental method, nature and concentration of the extracts [37,38]. In this study the antioxidant activity of the different *A. herba-alba* extracts was assessed using three complementary tests. The obtained results are in correlation with those reported by Hodzic et al. using the FRAP assay to evaluate the antioxidant capacity, that is reproducible and correlated to the concentration of antioxidants compounds found in the samples [39]. The phenolic compounds of the extracts of *A. herba-alba* produce a dark blue color complex via the reduction of ferric iron (Fe^{3+}) to ferric complex (Fe^{2+}) which has a maximum absorption at 700 nm [40], plants work as electron donors because of their content of phenolic compounds [18]. These findings are similar to the research achieved by Abdul Qadir and his collaborators, they reported an increase in reducing power with an increase in the concentration of antioxidant compounds [41]. Another study found that the crude methanolic extract of *A. campestris* had a high reducing power compared to the other two extracts; ethyl acetate fraction and water fraction with EC_{50} of 110 ± 2.01 µg/ml vs 230 ± 5.22 and 340 ± 7.51 µg/ml, respectively [27]. However, Lee found that methanolic extract of *A. japonica* has a reduction capacity 3.83 times higher than that of *A. montana* extract, they thought that the free radical scavenging activity and the reducing power of *Artemisia sp*. extract was not as high as that of *A. montana* extract [28]. Exercise electron donation can react with free radicals to convert them into more stable products and stop free-radical chain reactions that reduce inflammatory symptoms caused by harmful radical compounds. According to Younsi et al. the methanolic extract showed lower antioxidant activity than the essential oil of *A. herba-alba* using the FRAP assay (EC_{50} of 372 ± 6.0 and 79 ± 1.0 µmol Fe^{2+}/g, respectively) [38].

3.3. DPPH• radical scavenging activity

DPPH free radical scavenging activity (%) of the various extracts is shown in Fig. 2. The test was performed using six increasing concentrations of standards and extracts. All standards and extracts have recorded a concentration-dependent scavenging effect of DPPH radical with the highest activities obtained at the highest tested concentrations of extracts and standards, respectively. Similar scavenging activities were registered with the MEA (94.44 ± 0.51%) and EEA (92.74 ± 1.02%) followed by the AEA (77.67 ± 0.93%).

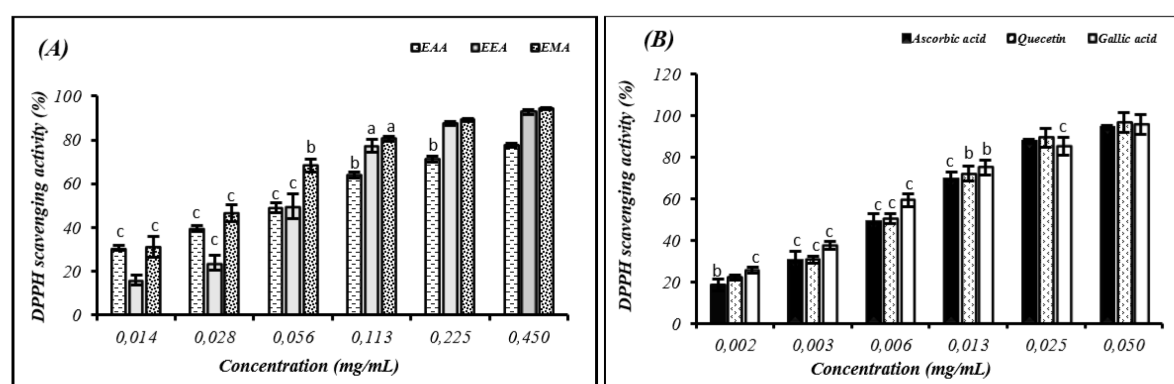

Figure 2. Scavenging activity of the free radical DPPH• at different concentrations of extracts of *Artemisia herba-alba* Asso (A), gallic acid, quercetin, and ascorbic acid (B) by the 2,2-diphenyl-1-picrylhydrazyl radical (Mean ± standard error, n=6). Values with an exponent are significantly different from those with different concentrations (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$).

To better assess the antioxidant activity, IC_{50} values for standards and extracts were calculated (Table 2). Results showed a highly significant ($P < 0.05$) antioxidant activity for the MEA (34.71 ± 0.96 µg/ml), compared to the EEA (53.29 ± 1.89 µg/ml) and the AEA (97.68 ± 3.42 µg/ml). Nearly similar scavenging activity values were recorded with all standards; gallic acid (96.04 ± 0.28%), quercetin and ascorbic acid with an inhibition rate of, 95.98 ± 1.28% and 94.61 ± 0.56%, respectively), these activities have been confirmed with IC_{50} of 1.82 ± 0.17 µg/ml for gallic acid; 4.47 ± 0.57 µg/ml for quercetin and 5.34 ± 0.18 µg/ml for ascorbic acid. Thus, this trapping activity is significantly ($P < 0.05$) affected by the concentration of the extracts with a positive correlation coefficient ($r = 0.7992$ for MEA; $r = 0.8197$ for EEA; $r = 0.8691$ for AEA). It was very evident that the antioxidant activity of the standards was remarkably higher than that of the extracts.

Our results are approximately similar to those of Seddik et al., who reported an IC_{50} of the $DPPH^{\bullet}$ radical of 32.9 ± 0.036 and 154 ± 0.014 µg/ml for ethyl acetate and aqueous extract of *A. herba-alba* respectively [42]. The trapping potential increased with the increasing concentration of the solutions studied. It is well established that the high scavenger capacity of the $DPPH^{\bullet}$ radical is significantly related to an increase in the concentration of trapped antioxidant substances [43,44].

3.4. Neutralization of hydrogen peroxide (H_2O_2)

The H_2O_2 scavenging ability of extracts and standards (gallic acid and ascorbic acids) is summarized in Fig. 3. Both extracts and standards showed a dose dependent ability to scavenge the H_2O_2. Almost similar scavenging activity percentages of trapping activity 84.36 ± 0.5% and 82.91 ± 0.34% were obtained using the EEA and the MEA, respectively, followed by the AEA (75.66 ± 0.60%). on the other hand, the two standards showed a very high H_2O_2 inhibition percent with 99. 87 ± 0.034% for gallic acid and 99.69 ± 0.09% ascorbic acid. In terms of $IC_{50,}$ the highest H_2O_2 scavenging effect ($P < 0.001$) was obtained with EEA (96.54 ± 1.64 µg/ml) compared to MEA (128.41 ± 1.41 µg/ml) and the AEA (187.08 ± 7.95 µg/ml). Comparatively, a very high scavenger effect of hydrogen peroxide was obtained with both standards 3.7 ± 0.03 µg/ml for gallic acid and 11.31 ± 0.80 µg/ml for ascorbic acid. However, the H_2O_2 scavenging capacity of an extract may be related to the natural structural properties of their active components, which determine their ability to donate electrons. The H_2O_2 scavenging of various extracts of *A. herba-alba* may be related to their phenolic compounds, which can donate electrons to H_2O_2 and thus neutralize it into water.

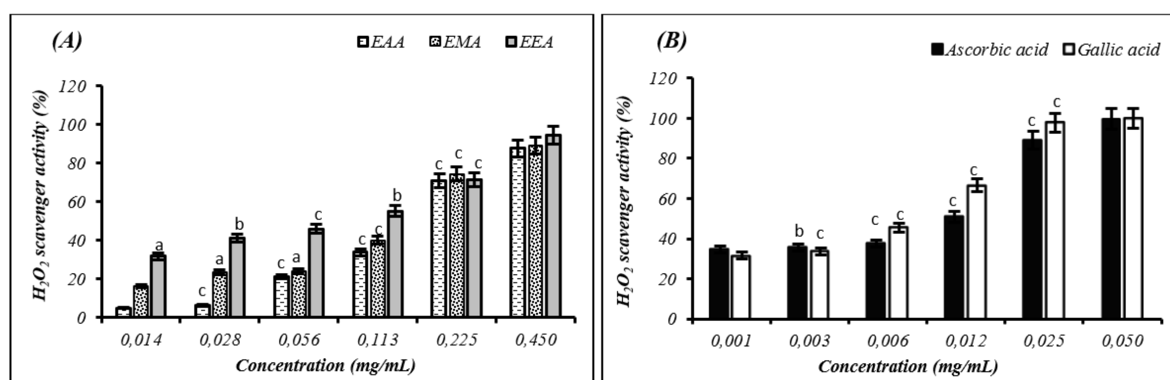

Figure 3. Hydrogen peroxide scavenging activity (%) against increasing concentrations of *Artemisia herba-alba* Asso extracts (A), gallic acid and ascorbic acid (B) (Mean ± standard error, n=6). Values with an exponent are significantly different from those with different concentrations (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$).

Our results are different to those found by Ruwali et al. they indicated that the methanolic extract of *Artemisia indica* showed a maximum activity of 68.3% inhibition comparable to that of quercetin with an activity of 77.7% at the same concentration of 200 µg/ml, while the ethanolic and 50% hydromethanolic extract showed a much lower inhibition of 39.8% and 25%, respectively [45]. However, according to our results, we confirm the above that the scavenger effect of H_2O_2, the uptake effect of the free radical DPPH$^•$ or the reducing power of iron, are proportional to the concentration of polyphenols which plays a role of scavenging and antioxidant substances.

3.5. Protective effect of *Artemisia herba-alba* extracts against in vitro lipid peroxidation

The results of the protective activity of standards and phenolic extracts of *A. herba-alba* against lipid peroxidation illustrated in Fig. 4 showed a highly significant increase (P = 0.00002) of MDA^+ level (with stress) 136.76 ± 1.85 µmol/g compared to MDA^- level (without stress) 36.53 ± 0.89 µmol/g.

Better protection against lipid peroxidation was obtained with the two standards showing MDA content similar to MDA^- group, accounting for 37.05 ± 0.50 µmol/g for gallic acid, 42.48 ± 0.70 for ascorbic acid and 36.53 ± 0.89 µmol/g for negative control; this is equivalent to 72.90 ± 0.36% and 68.93 ± 0.51% inhibition of MDA for gallic and ascorbic acid, respectively. However, a low protective activity was obtained with all extracts revealed by a highly significant decrease (P < 0.001) of MDA content. Among the tested extracts, 66.97 ± 3.61 was obtained with the MEA; 76.19 ± 3.02 with the EEA and 81.79 ± 2.30 µmol/g with the AEA. This was associated with 51.02 ± 2.64%, 47.17 ± 3.2% and 40.19 ± 1.68% MDA inhibition for the MEA, EEA and AEA, respectively. It's well known that the protective effect of *A. herba-alba* extracts against lipid peroxidation is achieved via a decrease of the oxidative cell damage caused by H_2O_2 and $HO^·$ and mediated by the Fenton reaction.

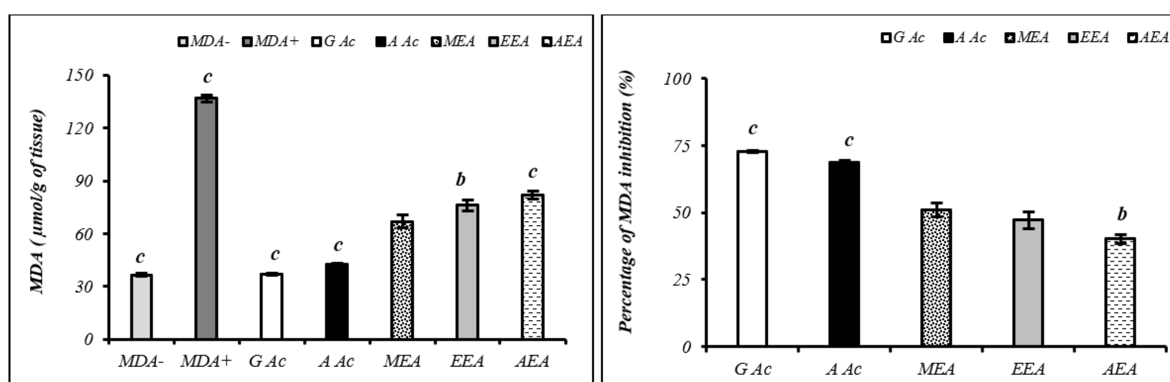

Figure 4. Protective properties of extracts of *Artemisia herba-alba* Asso, gallic acid and ascorbic acid against lipid peroxidation expressed in MDA µmol/g of tissue (A), and the percentage of inhibition of peroxidation (%) (Mean ± standard error, n=6). Values with a superscript are significantly different from those in the MEA (a, P < 0.05; b, P < 0.01; c, P < 0.001). MDA^-: negative control; MDA^+: positive control.

Our results are highly superior to those obtained by Seddik et al. they found a 45.7 ± 5.6% inhibition of MDA with the ethyl acetate extract of *A. herba-alba* Asso versus a 43 ± 5.12% reduction with the aqueous extract knowing that they tested a concentration of 50 mg/ml and they used homogenate of rabbit brain [42]. Effective antioxidants can protect against lipid peroxidation by different modes of action; they can act indirectly, by neutralizing one of the initiators of lipid oxidative damage, or directly, by neutralizing lipid

radicals, thus stopping the propagation reactions typical for lipid peroxidation. Thus, complex natural extracts will present a combined lipid peroxidation inhibitory potential (PIPL), depending on their content in antioxidants capable stopping the chain reaction of the propagation of lipid radicals by neutralizing oxidative stress [46].

3.6. Evaluation of antibacterial activity

The results of the antibacterial sensitivity test for three phenolic extracts of *A. herba-alba* are summarized in Table 3. Although they have reacted positively to all strains, the obtained results revealed a significant difference in antibacterial activity of *A. herba-alba* extracts against the four tested strains at a dose-dependent way. A significant inhibitory effect of bacterial growth was obtained with the ethanolic and methanolic extracts compared to the aqueous extract against *E. coli*, *P. aeruginosa*, *B. cereus and S. aureus* that could be related to their richness in bioactive compounds (polyphenols and flavonoids).

Table 3. Diameters of inhibition zones (mm) of *Artemisia herba-alba Asso.* extracts and antibiotic discs against the tested bacteria.

Extracts	**Bacteria**			
MEA (mg/mL)	*E. coli*	*P. aeruginosa*	*B. cereus*	*S. aureus*
250	11.25 ± 0.23	11.76 ± 0.32	14.78 ± 0.50	14.01 ± 0.61
125	9.94 ± 0.26	10.21 ± 0.22	11.90 ± 0.32	11.58 ± 0.19
62.5	8.23 ± 0.41	9.01 ± 0.16	9.59 ± 0.23	10.48 ± 0.25
31.25	6.39 ± 0.08	8.15 ± 0.13	7.81 ± 0.24	8.44 ± 0.21
EEA (mg/mL)				
250	13.56 ± 0.56[c]	13.92 ± 0.40[c]	16.89 ± 0.77[a]	15.63 ± 0.40[a]
125	11.54 ± 0.20[c]	11.98 ± 0.21[c]	13.77 ± 0.49[a]	11.88 ± 0.35
62.5	10.02 ± 0.36[b]	10.52 ± 0.45[b]	11.51 ± 0.23[c]	9.28 ± 0.18[c]
31.25	7.54 ± 0.18[c]	8.80 ± 0.53	9.18 ± 0.57[a]	7.41 ± 0.31[b]
AEA (mg/mL)				
250	10.53 ± 0.25	10.18 ± 0.32[b]	11.93 ± 0.44[b]	11.20 ± 0.28[c]
125	8.43 ± 0.17[c]	8.31 ± 0.09[c]	9.65 ± 0.55[b]	8.72 ± 0.25[c]
62.5	6.98 ± 0.19[a]	7.63 ± 0.19[b]	7.86 ± 0.24[c]	7.70 ± 0.16[c]
31.25	6.17 ± 0.05	6.54 ± 0.14[b]	7.18 ± 0.32	6.50 ± 0.09[c]
Antibiotics (µg/dics)				
Tetracycline (30 µg/dics)	15.30 ± 0.45	11.08 ± 0.47	10.91 ± 0.47	16.45 ± 0.31
Amikacin (30 µg/dics)	24.07 ± 0.45	21.27 ± 0.21	21.10 ± 0.41	26.95 ± 0.20
Erythromycin (15 µg/dics)	15.07 ± 0.61	-	-	21.11 ± 0.49

The values shown are the mean ±SD (n = 6). Values with a superscript are significantly different from those in the MEA (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$).

Gram-positive bacteria were more sensitive (*S. aureus* and *B. cereus*) compared to Gram-negative bacteria (*E. coli* and *P. aeruginosa*). The incorporation of EEA showed a strong antibacterial effect with MIC values of 6, 8, 10 and 10 mg/mL against *S. aureus, B. cereus, E. coli* and *P. aeruginosa,* respectively, and MBC values of 14,16, 15 and 22 mg/mL against *S. aureus*, *B. cereus, E. coli* and *P. aeruginosa*. Almost similar MIC values were recorded for all tested strains when the plates were incorporated with MEA, but the MBC values were slightly higher than those obtained with the EEA (Table 4). However, the aqueous extract

(AEA) has shown a very low antibacterial efficacy against all tested strains with MIC values of 75 mg/mL for *S. aureus*, 80 mg/ml for *B. cereus* and *E. coli* and 90 mg/mL for *P. aeruginosa*. Although, Gram-positive bacteria *S. aureus* and *B. cereus* were more sensitive compared to Gram-negative bacteria *E. coli* and *P. aeruginosa,* and this may be related to the difference in wall structure between Gram-positive and Gram-negative bacteria [47-49].

Table 4. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of *Artemisia herba-alba* Asso extracts against the bacteria tested.

Strains	EEA		MEA		AEA	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	10	*15*	16	*24*	80	Nd
P. aeruginosa	10	*22*	18	*25.5*	90	Nd
B. cereus	8	*16*	15	*20*	80	Nd
S. aureus	6	*14*	14	*18*	75	Nd

Nd: not determined.

In their study of the antimicrobial activity of extracts of the aerial part of 23 medicinal plants including *A. campestris*, Sassi et al. found that the acetone extract exerts an inhibitory effect among the three extracts (hexane and methanol extract) [50]. Our results corroborate those of Naili et al., the results obtained in this study show that the methanolic extract of *A. campestris* leaves has an inhibitory effect on all the studied bacteria, including *S. aureus, E. coli*, and *P. aeruginosa* [51]. Younsi et al., confirmed the substantial antimicrobial activity exhibited by the essential oil and extract of *A. herba-alba*, suggesting that the bioactivity of C-glycosyl flavonoids and caffeoylquinic acids is responsible for antibacterial activity [38].

Previous studies have identified major phenolic compounds such as 1,8-cineole, camphor, α-thujone and β-thujone, aglycone flavonoids and glycosyl flavonoids to be the main responsible for antibacterial activity [52,53].

4. CONCLUSION

The results of this study showed the aqueous extraction yield was greater than the alcoholic extracts and that the methanolic extract gave better content of polyphenolic compounds and flavonoids compared to both ethanolic and aqueous extracts. The antioxidant activity was the most powerful with the iron reducing power (FRAP) and antiradical activity (DPPH) of methanolic extract ($P< 0.05$) compared to ethanolic and aqueous extract, however the scavenger activity of hydrogen peroxide (H_2O_2) was more powerful with the ethanolic extract of *Artemisia herba-alba*. All extracts reacted positively in a dose-dependent way to all tested bacteria, confirming that the *Artemisia herba-alba* plant has strong antimicrobial properties against bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and less inhibiting properties against bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The extracts from *Artemisia herba-alba* are good candidates as part of new pharmaceutical or nutraceutical's formulations, and can be considered in preventive strategies for many metabolic disorders induced by oxidative stress.

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