

Chromatography analysis, in vitro antioxidant and antibacterial activities of essential oil of *Artemisia herba-alba* Asso of Boussaâda, Algeria

MOUNIRA KADRI^{1,2,*}, ABDELOUAHAB YAHIA³, SANA GOUBI¹, NOUR ELHOUDA MEKHEDMI¹, MEHDI SELMANE¹, AHMED ELKHALIFA CHEMSA⁴

¹Department of Biology, Faculty of life sciences and Nature, University Echahid Hamma Lakhdar. El-Oued, Algeria.

Tel./fax.: +21358841710, *email: mounira-kadri@univ-eloued.dz

²Laboratory of Biology, Environment and Health Laboratory, Faculty of Nature and Life Sciences, University Echahid Hamma Lakhdar. El-Oued, Algeria

³Laboratory of Natural Sciences and Materials (LSNM), Abdelhafid Boussouf University Center. Mila, Algeria

⁴Laboratory of Biodiversity and Application of Biotechnology in Agriculture, University of Echahid Hamma Lakhdar. El-Oued, Algeria

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Abstract. Kadri M, Yahia A, Goubi S, Mekhedmi NE, Selmane M, Chems A. 2022. Chromatography analysis, in vitro antioxidant and antibacterial activities of essential oil of *Artemisia herba-alba* Asso of Boussaâda, Algeria. *Biodiversitas* 23: 4424-4431. *Artemisia herba-alba* Asso (Asteraceae family) is widespread in the semi-arid and arid steppes of North Africa. This plant is used for traditional treatment. The present study aims to investigate the chemical composition, antioxidant and antimicrobial activities of *A. herba-alba* essential oil found in southwest Algeria (Boussaâda region). *Artemisia herba-alba* essential oil was obtained by hydrodistillation method, and its chemical composition was identified by using GC/MS analysis. In addition, the antioxidant activity of the extracted essential oil was determined using the DPPH assay. The antimicrobial activity of the essential oil was determined by the agar disc diffusion method. The essential oil extracted from the aboveground portions by hydrodistillation was analyzed by GC/MS. 38 components were identified, making up 69.37% of the oil, the most important of which is thujone (9.875%), camphor (3.762%), cis-p-menthadien-1-ol (3.572%), and isoborneol (2.334%). The observed IC₅₀ values of the DPPH assay were 7.31 ± 0.088 mg/mL. On the other hand, this oil was active against all strains tested, this activity varied from 12.77 ± 0.510 mm on *Listeria innocua* CIP 74915. These results demonstrate that the plant tested could be a potential source of natural antioxidants and antimicrobial agents.

Keywords: Antibacterial activity, antioxidant activity, *Artemisia herba-alba* Asso, Boussaâda (Algeria), GC/MS

INTRODUCTION

Since antiquity, aromatic herbs have been used to preserve and flavor food, but in the last ten years, research has concentrated on their essential oils and natural extracts as potential sources of antibacterial and antioxidant compounds (Costa et al. 2015; Saad et al. 2022). Traditional medicinal plants have shown potential as sources of secondary metabolites, antimicrobials, and antioxidants for therapeutic interventions, which has paved the way for the creation of new plant-based antibacterial agents. Some of these chemicals, some of which have been isolated from plants, may be utilized to create novel medications that stop the spread of bacterial and fungal diseases (Aqil et al. 2005). Algeria is famous for its wealth of medicinal plants, many of which are used for many diseases (Aziz et al. 2012; Sarri et al. 2014; Benarba et al. 2015). There is a great diversity of flora related to the size and climatic diversity of Algeria. Between the north with a Mediterranean climate, the Atlas Mountains in the middle, and the Sahara in the south (Hamza et al. 2019). According to WHO statistics, about 80% of the African population uses traditional medicine for their primary health care (WHO 2002). In recent years, the use of medicinal plants

has increased significantly, likely due to their local abundance, cultural importance, and low cost of acquisition (Boukhris et al. 2012; Thomford et al. 2015; Lim et al. 2021).

The spread of the COVID-19 pandemic in Algeria has compelled the population to seek alternative therapies as preventatives and treatment options. The use of medicinal plants is a promising alternative solution for boosting immunity and preventing disease. COVID-19 (Beldi et al. 2021; Helali et al. 2020). In particular, especially the flora of the Algerian Sahara is highly abundant in medicinal plants that yield priceless natural products like essential oils. Due to their widespread consumer acceptance, relatively high level of safety, and potential for several uses, the essential oil and its components are attracting growing amounts of attention (Boukhris et al. 2012). Additionally, it has been demonstrated that several essential oils have antioxidant properties (Zhang et al. 2006), and anti-inflammatory properties (Asif et al. 2021). Antimicrobial activity (Imelouane et al. 2010; Lakehal et al. 2016; El Kamli et al. 2020). As essential oils have shown promise as antiviral agents against several pathogenic viruses (da Silva et al. 2020). Another study presents the very first report on the in vitro antiviral

activity of selected essential oils of Lamiaceae plant species and their monoterpenes against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Čavar Zeljković et al. 2022).

According to Brahmi et al. (2022) study, *Artemisia herba-alba* was among the most commonly used aromatic plants to counter the threat of the Corona virus (Asdadi et al. 2020; Anamul et al. 2022). According to the biological efficacy evaluation research of medicinal plants (Wang et al. 2004; Bouhouia et al. 2020), *A. herba-alba* (family Asteraceae) is common in the semi-arid and arid steppes of North Africa, Spain, the Middle East, and the North-Western Himalayas. This plant is used to heal exterior wounds and treat stomach problems such as diarrhea and abdominal pain. Its therapeutic benefits are due to the terpenoid sesquiterpene lactone dehydroleucodine, which is primarily present in the aerial sections of *A. herba-alba* (Abood et al. 2017). This type of essential oil is well-known for its therapeutic properties as a disinfectant, anthelmintic, and antispasmodic (Hatimi et al. 2001). The outcome of some work suggests that the essential oils of *A. herba-alba* can constitute a natural and environmentally friendly alternative to developing new bioinsecticides (Allali et al. 2022). Consumers are aware of the dangers posed by the use of synthetic antioxidants and antimicrobials in the food industry and are demanding safer

and 'greener' alternatives. In this study, the antioxidant activity of essential oils is analyzed by the DPPH method, their antimicrobial effects against bacterial strains and the chemical composition of the essential oil obtained from the aerial part of *A. herba-alba* originating from southwest Algeria (Boussaâda region). Maybe this minireview will be helpful for conquering COVID-19 in the near future.

MATERIALS AND METHODS

Study area

Aerial parts of *A. herba-alba* were collected during the flowering phase from Boussaâda which is located in the southwest of the Hodna region in the Hauts Plateaux, at the feet of the Ouled Naïl Range of the Saharan Atlas (Figure 1). The plant material was cleaned chopped into pieces and derided in air.

The climate of Boussaâda region is an arid climate characterized by summers with torrid heat reaching 35°C (Figure 2), and soft winters. The relative humidity is very low except for the winter months when 32% is common (Figure 3). Precipitation is low and less than 171 mm throughout the year (Figure 4). So, according to the Ombrothermic diagrams of BAGNOLS and GAUSSEN, Boussaâda is in conditions of permanent drought (Figure 5).

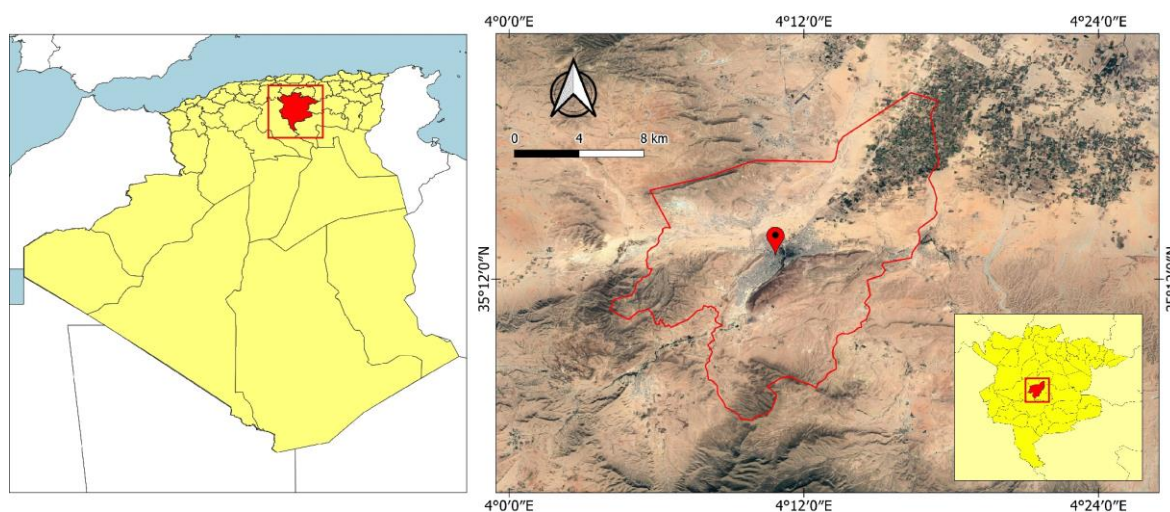


Figure 1. Location of Boussaâda, indicating the sampling sites of *Artemisia herba-alba*: point (35°08'37"N, 4°49'49"E)

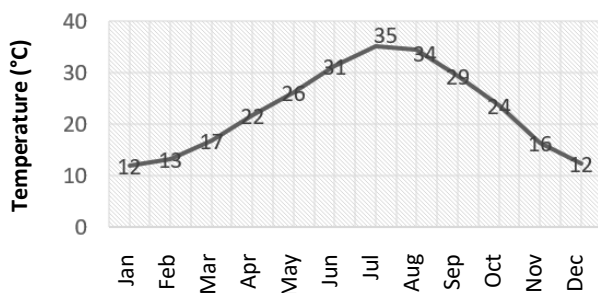


Figure 2. Annual monthly change in average temperature of Boussaâda during the period (2007-2016)

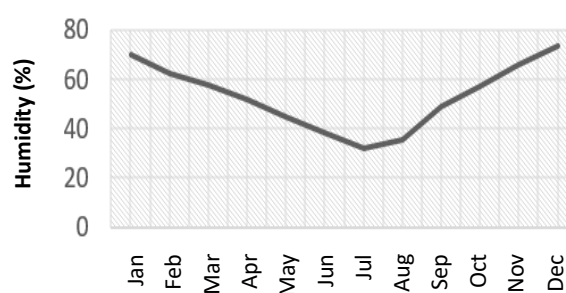


Figure 3. Annual monthly change in average humidity of Boussaâda during the period (2007-2016)

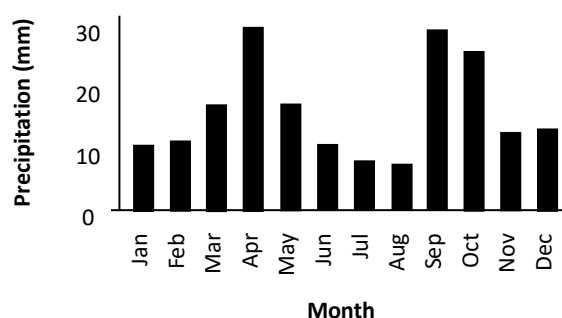


Figure 4. Annual monthly change in average Precipitation of Boussaâda during the period (2007-2016)

Procedures

Extraction of the essential oil

The essential oil was obtained by hydrodistillation from air-dried *A. herba-alba* parts using a Clevenger apparatus (3h). Each essential oil's average yield across the three replicates was computed. Until they were examined, the oils were kept at 4°C (Bruneton 1999).

Oil yield

The yield was calculated using the equation $Y\% = (M/Bm) \times 100$, where *M* is the mass of the extracted oil (g) and *Bm* is the initial plant biomass (g) (Falleh et al. 2008).

Antioxidant activity

The essential oil's radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and evaluated using the methodology described by (Blois 1958) as elaborated by (Elmastas et al. 2007). In dry test tubes, 200 µL of various essential oil dilutions (EO methanolic solutions) were mixed with 800 µL of 0.004% (w/v) DPPH methanol solution. The reaction mixture was vigorously stirred and incubated at room temperature and in the dark for 30 minutes. At 517 nm, absorbance was measured. The negative control contains 200 µL of methanol and 800 µL of DPPH.

The DPPH radical's scavenging capacity was calculated using the following formula: A control (*Ac*) is the absorbance of the control reaction, and A sample (*As*) is the absorbance when all extract samples and references are present. All tests were run in triplicate, and the results were averaged. Ascorbic acid was used as a standard:

$$\text{Inhibition\%} = \left(\frac{Ac - As}{Ac} \right) \times 100\%$$

The graph of the scavenging effect percentage against extract concentrations allowed for the calculation of the oil concentration that offers 50% inhibition (*IC*₅₀) (Shimada et al. 1992).

Gas chromatography-mass spectrometry (GC-MS)

Analysis by GC/MS was carried out using a Varian GC 3800 equipped with an SPB1 capillary column (30 mm, 0.25 mm, 0.25 mm) and a "Mass Selective MS Saturn Series 2200, column SPB-1." The temperature of the

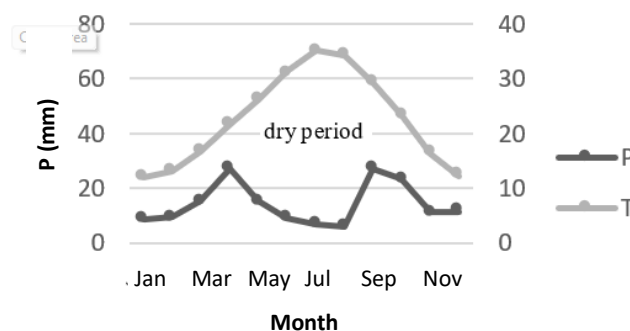


Figure 5. Ombrothermic diagrams of BAGNOULS and GAUSSEN the Boussaâda

detector was 250 °C and that of the injector was 210°C. The oven temperature was programmed as before, and the transfer line temperature was 280°C. Operating under the GC condition, programmed heating at 55°C for 1 min to 150°C for 3 min to 250°C for 8 min. The injector temperature was 250°C. Helium was the GC carrier gas.

Evaluation of antibacterial activity

In the current study, one strain of gram-positive bacteria, *Klebsiella pneumoniae* ATCC7000603, and four strains of gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* CIP 81-3, *Listeria innocua* CIP 74915, and *Escherichia coli* ATCC 25922, were donated by the hospital Elhakim Saadan Biskra (Algeria). With a small modification, the disc agar diffusion method was used to complete the research for this publication (Badlishah et al. 2014). After growing on Müller-Hinton agar for 18 hours at 37°C, the bacteria strains were suspended in a saline solution (0.9% NaCl). The turbidity was then corrected to 0.5 MacFarland standards (108 CFU/mL). The medium mentioned above was present in 90 mm diameter Petri plates that were inoculated with the suspension. After being sterilized, sterile paper disc No. 1 (6 mm in diameter) was put onto the surface of agar plates and soaked with 10 mL of essential oil. The incubation conditions for bacteria were 37°C for 24 hours prior to incubation. The widths of the inhibition zones were measured in order to assess antimicrobial activity. Chloramphenicol (C30), Céfixime (CFM), Gentamicin (GEN), Ofloxacin (OF), and Co-Trimoxazole Sulfamethoxazole (COT) were employed as positive controls to assess the sensitivity of gram-positive and gram-negative bacteria, respectively. The study was completed in aseptic circumstances (Schinor et al. 2007). The results of the inhibitory zone measured in millimeters were averaged across all tests carried out in triplicate for each strain of microorganism.

Preliminary screening

The methanol extracts were subjected to phytochemical tests for secondary metabolites, tannins, saponins, steroids, alkaloids and glycosides in accordance with (Trease and Evans 1987) and (Harbone 1998) with little modification.

Data analysis

The Co Stat-Statistics Software version 6.4 was used to perform an analysis of variance (ANOVA) on the data. The significance of differences between treated samples was assessed using the LSD test for mean standard deviation (SD) of inhibition diameter comparisons and yield. Each experiment had three replicates and three determinations, with the significance level for all measurements set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Oil yield

A Clevenger-type apparatus was used to hydrodistill the air-dried parts of *A. herba-alba*. Liquid, gilded yellow, and penetrating strong odor essential oil was obtained with a yield of $0.33 \pm 0.0291\%$ (w/w), based on the dry weight of the plants, but in another study, it was obtained with a yield of 0.72% in Boussaâda (Algeria) (Belhatab et al. 2014), 0.65% in Tunisia (Akrouit 2004) in Morocco, 1.2% (Zaim et al. 2012), and 0.95% (Bezza et al. 2010) in Biskra (Algeria), 1.02% in M'sila (Algeria) (Dob and Ben Abdelkader 2006).

Chemical composition of essential oils

GC/MS was used to analyze the chemical composition of essential oil extracted from the aerial parts of *A. herba-alba* via hydrodistillation. 38 constituents were identified, accounting for 69.37% of the oil, with the major ones being thujone (9.875%), camphor (3.762%), cis-p-menthadien-1-ol (3.572%), and isoborneol (2.334%) (Table 1) (Figure 6). According to Belhatab et al. (2014), the major compounds are composed of thujone (28.4%) and camphor (22.8%). Zaim et al. (2012) also concluded that the major compound is Chrysanthenone (28.10%). Camphor (49.3%), according to Dahmani-Hamzaoui and Baaliouamer (2010). The major compounds are composed of Cischrysanthenyl acetate (25.12%) (Bezza et al. 2010).

Phytochemical analysis

During the phytochemical screening of *A. herba-alba* plant extracts, the presence of alkaloids, saponin, flavonoids, steroid and triterpene, tannins, and reducing sugars was discovered (Table 2). These chemicals have been shown to have physiological activity. Our findings are very similar to those of Mouhamed et al. (2010).

Antioxidant activity

It is believed that antioxidants' ability to donate hydrogen is what causes them to affect DPPH (Shirwaikar et al. 2006). Activities that neutralize free radicals are crucial to preventing their harmful effects in conditions like cancer. Antioxidants are known to function to reduce lipid peroxidation by scavenging DPPH free radicals. The IC_{50} for the activity to scavenge DPPH radicals was 7.31 ± 0.088 mg/mL. The ascorbic acid IC_{50} values were 2.82 ± 0.06 µg/mL (Figure 7).

Antibacterial activity

The antibacterial activity of essential oil components is largely dependent on the lipophilic nature of the hydrocarbon skeleton and the hydrophilic nature of their functional groups (Griffin et al. 1999). Due to these data, we were interested to study the antimicrobial activity of the essential oil. The results were summarized in (Table 3), which showed that essential oil extracted from *A. herba-alba* prevented the growth of some tested microorganisms with an inhibition zone medium diameter. highest inhibition zone recorded for *Listeria innocua* at 12.77 ± 0.510 mm. It should be mentioned that there are no background antibacterial studies on *A. herba-alba*, while in *A. herba-alba* some studies have reported as the essential oil exhibited much higher antibacterial activity with 31.3 mm against *Klebsiella oxytoca* (Bertella et al. 2018).

Table 1. The composition of *Artemisia herba-alba* aerial part's essential oil

RT (min)	Compound	%
5,346	β-Pinene	0.329
5,452	1-Hexen-3-yne	0.373
5,604	Benzene	1.100
5,777	α -Phellandrene	0.219
6,269	cis-p-Menthadien-1-ol	3.572
6,794	Eucalyptol (1.8-Cineol)	0.012
7,127	1-Octene	0.06
7,343	3-Octyne	0.166
8,269	Thujone	9.875
8,934	Isocyclocitral	0.162
9,128	trans-Pinocarveol	0.24
9,218	Camphor	3.762
9,950	Isoborneol	2.334
11,383	D-Verbenone	0.434
11,825	Lenacil	5.355
12,275	Bornyl acetate	0.386
12,362	(-)-Myrtenyl acetate	0.202
12,542	Carveol (fr.1)	0.146
13,003	Thymol	0.179
14,078	2-Cyclopenten-1-one	0.267
14,211	Copaene	0.179
15,197	Humulen- (v1)	0.004
16,092	Isoaromadendrene epoxide	0.22
16,349	Azulene	0.025
16,401	Farnesene epoxide	0.022
16,806	γ -Elemene	0.036
16,933	Davana ether	0.053
17,965	Caryophyllene oxide	0.065
18,595	(-)-Spathulenol	0.482
18,830	Aristolene epoxide	0.098
18,918	1H-Cycloprop[e]azulen-4-ol,	0.096
19,656	Longipinocarvone	0.043
19,698	Androstan-3-one	0.036
19,813	γ-Gurjunenepoxide	0.057
19,925	Ledene alcohol	0.027
20,096	2-Naphthalenemethanol	0.072
25,293	Limonen-6-ol, pivalate	0.005
25,624	Phosphinous chloride	0.017
	NI	38.102

Note: a RT: Retention time of the compound in minutes

Chromatogram Plot

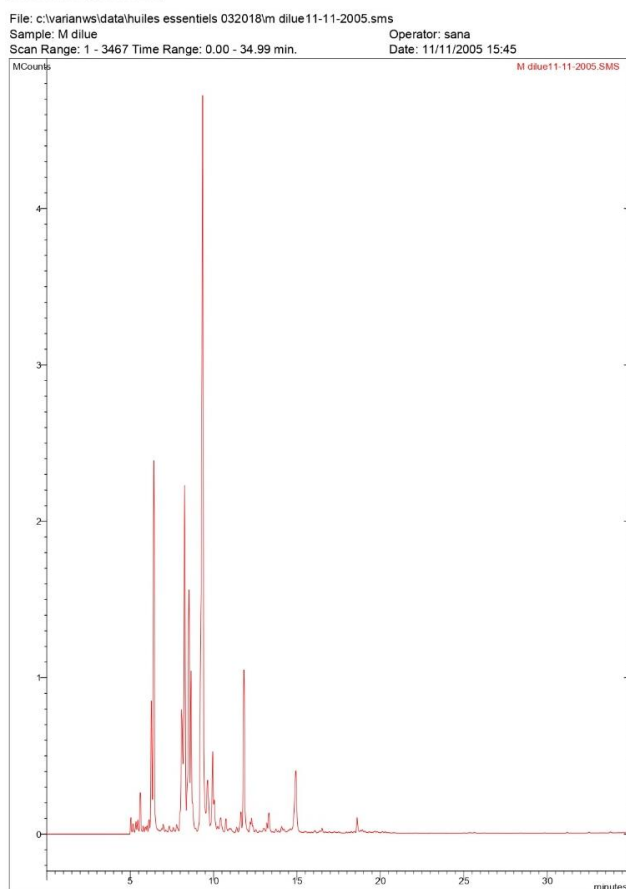


Figure 6. Chromatogram *Artemisia herba-alba* essential oil

Table 2. Phytochemicals found in methanolic extract of *Artemisia herba-alba*

Phytochemicals	Aerial part
Flavonoids	+
Saponin	+
Steroids	+
Reducing sugars	+
Tannins	++
Alkaloids-wagner's reagents	+
Alkaloids-Draghandroff-reagents	+
Volatile oils	+++

Note: a Key: + = present, - = absent

Discussion

This study was mainly carried out for *A. herba-alba* oil bioactivity research in southwest Algeria (Boussaâda region). This specific variability within the chemical composition of essential oils of *A. herba-alba* species can be attributed to geographical, genetic (Karousou et al. 2005), and seasonal factors. or even environmental (soil, humidity, etc.) (Ghanmi et al. 2010). The age of the plant

and the part of the plant that was studied could explain why the chemical analysis of *A. herba-alba* essential oils was different (El-Massry et al. 2002). according to the type of oil. Storage conditions can affect the chemical composition of the essential oil. This situation can be due to contact and reaction with oxygen, evaporation, and other undesirable variations in volatile oil components during the storage period (Jain and Sharma 2022).

In antioxidant activity, in fact, Akrou et al. (2011) discovered that, as compared to *Thymus capitatus*, the anti-free radical activity of essential oils from *Artemisia campestris*, which is of the Thujone ($\alpha + B$) type, is rather poor. On the other hand, they conducted a study on four species of *Artemisia* using DPPH, ABTS, and linoleic acid, and they found that *A. herba-alba* has low activity and that the activity of all the essential oils investigated remained lower than that of controls. This was validated in research on a few *Artemisia* species by (Lopes-Lutz et al. 2008). However, numerous studies have shown that the major compounds in essential oils are what give them their antioxidant properties, with essential oils rich in oxygenated compounds (such as linalool, eugenol, geraniol, borneol, and α -terpineol) having stronger antioxidant properties than those with hydrocarbon terpenes (Falleh et al. 2008). Based on this theory, we can explain the variation in antioxidant activity by looking at the chemical composition, which in turn can be explained by a several variables, including edaphic, climatic, and other ones. effect of extraction temperature, solvent type, extraction type (Teffane et al. 2021; Djousse et al. 2022).

The essential oil of *A. herba-alba* had a high antimicrobial influence. It is indeed possible that the essential oils' limited activity is due to their chemical makeup. Indeed, research on the antimicrobial effect of specific essential oil compounds has identified: Thymol and carvacrol, for example, are phenolic compounds with significant antibacterial action (Gergis et al. 1990; Cosentin et al. 1999). The constituents with a low antibacterial activity are pulegone, menthone, 1,8-cineole, p-cymene, iso menthone, myrcene, pinene, piperitone, limonene, linalool, terpinene, sesquiterpenes and terpenic (Lattaoui and Tantaoui-elarki 1994; Carson et al. 1995).

All of the essential oils' and their main constituents' modes of action appear to have an impact on the cytoplasmic wall or membrane. Contrarily, the variety of chemicals found in essential oils demonstrates the presence of substances that can work through novel biological mechanisms (Guinoiseau 2010; Arab et al. 2022). The hydrophobicity of the molecules found in essential oils is their primary quality. It permits their solubilization in the membranes, which leads to the structure becoming unstable and the membrane permeability increasing (Sikkema et al. 1994). As a result, we may explain the variance in antibacterial activity by the type of essential oils, which can vary based on edaphoclimatic conditions (Kadri et al. 2017).

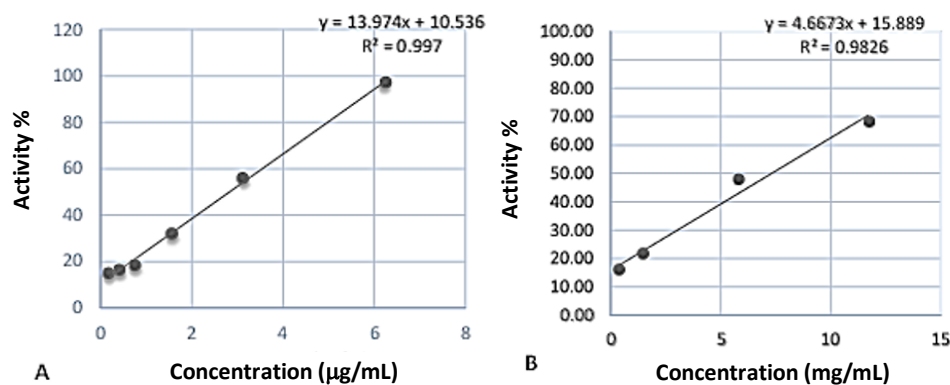


Figure 7. (A, B): Antioxidant activity Ascorbic acid (A) and essential oil of *Artemisia herba-alba* (B)

Table 3. Zone of Inhibition of bacterial strains against of essential oil *Artemisia herba-alba* and positive control

Bacteria strain	Zone inhibition (mm)						P
	Essential oils	C30	GEN	CFM	OF	COT	
<i>Escherichia coli</i>	10.22 ^d ±0.381	20±1 ^c	18±1 ^c	24±0.5 ^b	30±1 ^a	25±0.79 ^b	0.000
<i>Listeria innocua</i>	12.77 ^d ±0.510	26±0.7 ^b	30±3.46 ^{ab}	20 ^c	28±0.23 ^{ab}	32±0.6 ^a	0.000
<i>Klebsiella pneumoniae</i>	6 ^c	30±2.46 ^a	30±1.78 ^a	20±1 ^b	27±0.4 ^a	28 ^a	0.000
<i>Pseudomonas aeruginosa</i>	6 ^d	12±0.56 ^c	26±0.66 ^b	8 ^d	30±1.77 ^a	6 ^d	0.000
<i>Salmonella enterica</i>	6 ^c	20±0.87 ^b	18 ^c	20±0.4 ^b	15.30±1.13 ^d	29±0.50 ^a	0.000

Note: Means of three replicates ± SD (standard deviation) followed by at least one same letter are not significantly different according to LSD test at $p < 0.05$

In conclusion, the phytochemical analysis, antioxidant, and antibacterial of *A. herba-alba* growing in Algeria were reported to phytochemical screening of *A. herba-alba* Asso plant extracts revealed the presence of alkaloids, flavonoids, saponin, steroid and triterpene, tannins, and Reducing sugars. Air-dried parts of *A. herba-alba* were subjected to hydrodistillation using a Clevenger-type apparatus. Liquid, gilded yellow and penetrating strong odor essential oil was obtained with a yield of $0.33 \pm 0.0291\%$ (w/w), based on the dry weight of the plants. Chemical analysis of Essential oil extracted from the aerial parts of *A. herba-alba* by hydrodistillation was analyzed by GC/MS. 39 constituents, representing 69.37% of the oil, were identified, of which the major ones, Thujone (9.875%), camphor (3.762%), cis-p-menthadien-1-ol (3.572%), isoborneol (2.334%). For the antioxidant activity, IC₅₀ for DPPH radical-scavenging activity was 7.31 ± 0.088 mg/mL. The IC₅₀ values for Ascorbic acid were 2.82 ± 0.06 µg/mL. On the other hand, this oil was found effective against all tested strains, this activity was ranging from 12.77 ± 0.510 mm with *Listeria innocua* CIP 74915.

These interesting results show that the *A. herba-alba* grown in the southwest of Algeria has an important antioxidant and antimicrobial activity, which encourages further in-depth investigations on their pharmacological proprieties.

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