




Biological activities and chemical composition of essential oil isolated from *Artemisia herba-alba*

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ABSTRACT

Artemisia herba-alba (AHA), known as sheeh in Jordan, is recommended by regional traditional healers for the treatment of a variety of diseases. AHA has been used in folk medicine to treat colds, coughing, bronchitis, intestinal disturbances, diarrhea, neuralgias, arterial hypertension, and diabetes. The objectives of the current study were to identify the chemical compositions of the essential oil extracted from dried leaf powder of AHA cultivated in Jordan and investigate its antibacterial and antioxidant activities. The essential oil was isolated using hydro distillation, and the identification of *artemisia herba-alba* essential oil (AHEO) composition was performed using validated gas chromatography-mass spectrometry (GC-MS). The antibacterial activity of AHEO was assessed against *escherichia coli*, *pseudomonas aeruginosa*, *klebsiella pneumonia*, and *staphylococcus aureus* and two clinical isolates (methicillin-resistant *staphylococcus aureus* and methicillin-resistant *staphylococcus epidermidis* [MRSE]) using a disc diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values, using the micro-dilution broth method. Additionally, antioxidant activities were determined using DPPH and ABTS radical scavenging assays. The results revealed that the yield of AHEO was 4.41% v/w, with nearly 22 identified compounds, constituting approximately 96.80% of the total mass of essential oils. Monoterpenoids was the major compounds (71.90%), with alpha pinene being the major component, accounting for 17.20% of the composition. The total phenolic and flavonoid contents were 43.97 mg GAE/g and 30.11 mg CE/g, respectively. The antibacterial activity of AHEO against MRSE exhibited the highest inhibitory effect, while *E.coli* showed the highest MBC value. Furthermore, AHEO demonstrated significant antioxidant activity (IC_{50} = 64.57 and 34.01 for DPPH and ABTS, respectively). The results indicate that AHEO possess good antioxidant and antibacterial properties, suggesting that they may be used as a supplementary food and antimicrobial agent.

Keywords: *Artemisia herba-alba*, essential oil, antibacterial, antioxidant, GC-MS

INTRODUCTION

Antibiotic resistance has been a complicated problem for many years. Antimicrobial resistance has grown over time, rendering many current medications ineffective, which has brought about a lot of focus to the development of antibiotics in recent years. Health services are challenged by the growing resistance of dangerous bacteria and fungi against commercial antimicrobial medications, thus it's critical to produce effective antimicrobial agents [1, 2]. Since thousands of years ago, people have utilized plants or parts of them like roots, stems, flowers, and fruits for medical and therapeutic purposes because some plants have demonstrated their ability to alleviate ailments [3]. Due to the presence of bioactive chemicals such flavonoids, tannins, saponins, alkaloids, and

terpenoids, medicinal plants exhibit biological properties such as antibacterial, antioxidant, and anticancer activity [4, 5].

Aromatic herbs are among the therapeutic plants that have biologically active substances that may be applied to both agriculture and medicine [6]. The volatile molecules known as essential oils are obtained from plants and have antibacterial and antifungal activities, particularly against diseases that may be resistant to conventional medicines [7-10]. Different activities were documented for essential oils including antiviral, control plaque [11], apoptosis [12, 13], herpes simplex virus (type III) [14], and central nervous system [15], menopausal disorder, reduced levels of cholesterol and triglycerides while raising the level of high-density lipoproteins in patients with coronary heart diseases [16]. It is possible for essential oils to have entirely diverse odors and qualities when

they are extracted from different regions of the same plant. For example, geranium produces oil from both the flowers and the leaves, and the composition, aroma, and other qualities of the two types of oils are entirely diverse [17, 18].

In addition, essential oils exhibited its efficacy against many types of bacteria because its ability to extract the effective compounds, which could lead to inhibition of bacteria and fungi [9, 19-24]. For example, clove, thyme and cinnamon isolated from some spice plants showed good inhibition effect against *listeria monocytogenes* through reducing the production of listeriolizin O [19].

Also, it was reported that eugenol exerts the same effect on *listeria monocytogenes* [20], carvacrol have the ability to prevent the production of toxins in *bacillus cereus* and *clostridium botulinum* [21] and oregano essential oil showed its efficiency in decreasing the production of enterotoxin by *s. aureus* [22]. Treatment of *aspergillus flavus* cells with lime (*citrus aurantifolia* and *cytrus hirtus*) essential oils had a significant impact on the amount of aflatoxin produced in the cells [23]. Researchers are still searching for alternatives that have a high antibacterial impact against many different types of bacteria with few adverse effects due to the spread of resistant bacteria.

Artemisia genus is one of the different species in the Asteraceae family, which has a variety of secondary metabolites and essential oils [25]. Many researchers showed that the plants belonging to the genus artemisia are rich in terpenoids, sesquiterpenes, flavonoids, and coumarins [25]. One of the most famous medicinal plants, *artemisia herba-alba* (AHA), also known as sheeh in Jordan, is recommended by regional traditional healers for the treatment of a variety of diseases [26]. This plant has a variety of therapeutic benefits including antioxidant [27], antibacterial [28], anticancer [29], anti-inflammatory [30], antifungal [28], and antidiabetic [31]. It can be found in Middle East (Jordan, Saudi Arabia, Turkey, Sinai Desert), North America, Asia, and Europe (Spain), as well as in deserts of North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt) [32].

In the present study, essential oils was extracted from AHA plant to be examined for its antibacterial and antioxidant activities. In addition, chemical constituents of *artemisia herba-alba* essential oil (AHEO) were identified using gas chromatography-mass spectrometry (GC-MS).

MATERIALS & METHODS

Source of Plant Material

In June 2022, AHA (Figure 1) was collected from Karak-South Jordan. The plant was identified by the Department of Biology, Faculty of Science, Mut'ah University, Karak, Jordan. The collected materials were dried at 25 °C in the shade for 12 days. Then, dried leaves were ground into a fine powder and kept in glass jars for further use [33, 34].

Essential Oil Extraction

About 200 grams of dried leaf powder of AHA plant were steam-distilled for six hours in an essential oil steam distiller (modified cleverger apparatus) for obtaining AHEO. The essential oil layer obtained on top of the aqueous phase was separated by *n*-hexane, dried by Na₂SO₄ and stored at 4 °C for further analysis [33, 34].



Figure 1. Image of AHA plant from Karak City (South Jordan)

Validation & Identification of EOA Using Gas Chromatography-Mass Spectrometry

Development & validation of GC-MS method

In this study, Shimadzu qp2010 plus, Japan GC/MS-200 equipped with split-splitless inlet (S/SL), injector was used. The extracted components were separated on a DB-SMS GC column and the mass detector was set to scan ions between 40-400 m/z using full scan mode and electron impact (EI, 70 eV). The temperature of injector was set at 250 °C with a split ratio of 1:10. Detector and transfer-line temperatures were 160 °C and 230 °C, respectively. For separation of the different oil components, a linear temperature program was used. The heating rate was programmed at 4 °C/min starting from 50 °C (initial temperature) to 290 °C (final temperature) and held at 50°C for five minutes with a total run time of 68 minutes [35, 36].

Identification of isolated compounds using GC-MS

Based on the percentage composition of the essential oil, which was determined using GC-MS peak area, isolated compounds from AHEO were identified and characterized. Interpretation of the mass spectrum was conducted using the database of the National Institute of Standard and Technology (NIST).

The identification of compounds was made by comparing the retention time with the authentic samples, based on their linear indices relative to a series of n-alkanes (C8-C20) at the same chromatographic conditions and mass spectra by matching of essential oil constituents with of NIST library and published reports. Whenever possible, co-chromatography for certain standard compounds was performed under similar chromatographic condition [36].

In Vitro Evaluation of Antimicrobial Activity & Growth Inhibition

Source of microorganisms

Essential oil antibacterial efficacy was assessed against six pathogenic bacterial strains: four standard strains were used, as follows: *Escherichia coli* ATCC 25922, *pseudomonas aeruginosa* ATCC 25853, *klebsiella pneumonia* ATCC 200603, and *staphylococcus aureus* ATCC 29213, and two clinical isolates were used, as follow: methicillin-resistant *staphylococcus aureus* (MRSA, OQ568766), methicillin-resistant

staphylococcus epidermidis (MRSE, OQ568719). The reference strains (ATCC: American Type Culture Collection Center), were provided by the Jordan University Hospital, Amman, Jordan.

Preparation of bacterial culture

Fresh bacterial cultures were prepared by sub-culturing stock bacterial cultures into freshly prepared nutrient agar and incubating at 37 °C for 24 hours. These microbial cultures were transferred into the freshly prepared nutrient broth and standardized using the method of (0.5 McFarland turbidity standards) using the spectrophotometer (600 nm) to obtain the desired cell density of 1.5×10^8 (cells/ml).

Determination of antibacterial activity using Kirby-disk diffusion method

A disc diffusion method was used to test antibacterial activity of AHEO against six bacteria species. DMSO was used to dilute the essential oil to the following concentrations: 7.5, 15, 30, 70, and 100 µL/mL (v/v).

Approximately 25 µL from each concentration was impregnated into a six mm diameter paper disk, which was then inoculated on the surface agar with 100 µL of 10^6 cfu/mL suspension for each tested bacterium, and then placed onto Mueller-Hinton agar plates. The petri-dishes were incubated at 35 °C for 24 hours, then the inhibition zone was measured as mm in diameter. By working in a laminar flow, aseptic conditions were maintained [37].

Evaluation of Minimum Inhibitory Concentration & Minimum Bactericidal Concentration of AHEO

The broth dilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of AHEO for each bacterial species according to the study in [38] with some modifications. Stock solution of essential oil was prepared in DMSO and the microdilution of AHEO was carried out using sterile 96-well microtiter plates to produce a concentrations equal to 30, 15, 7.5, 3.75, 1.88, 0.94, and 0.47 µL/mL.

Then, 10 µL of bacterial suspension containing 5×10^4 CFU/well was transferred to each tested well. The positive control was also prepared containing 90 µL of Mueller Hinton broth and 10 µL of the inoculum. After that, the optical density was measured at 600 nm by Eliza reader. The plate was then incubated at 37 °C for 24 hours and the optical density was measured at 600 nm.

MIC₉₀ was reported as a concentration of the essential oil that inhibited 90% of bacterial growth (MIC₉₀). MBC values were assigned by culturing the content of the wells with concentrations equal to or greater than MIC on agar plates. MBC was defined as the lowest concentration of AHEO that kills the tested bacteria (no growth on the agar plate) after 24 hours at 37 °C.

Determination of Total Phenolic, Flavonoid, & Tannins Contents

Folin-ciocalteu reagent was used to determine the total phenolic amount in AHEO according to [39]. The results were given as mg of gallic acid/g of the dried AHEO. Total flavonoid content of AHEO was measured by modified aluminium chloride technique [34]. Tannins concentration was measured based on the method in [40].

DPPH Antioxidant Activity

DPPH assay, AHEO was diluted to prepare a range of concentrations from 0.94 to 30 µl/mL. In a brief, two milliliters of DPPH (0.2 mmol) ethanolic solution was mixed with one milliliter of each sample concentration. The mixtures were maintained at room temperature in the dark. After 30 minutes of incubation, the mixture's absorbance at 517 nm was determined using a spectrophotometer in comparison to a blank solution (one ml of ethanol + two ml of DPPH• solution). As a standard reference that demonstrated strong antioxidant activity, gallic acid was employed. At concentrations between 10 and 100 µg/ml, a gallic acid standard calibration curve for the DPPH radical was measured. All the determinations were performed in three replicates. Eq. (1) was used to determine the percentage inhibition of the DPPH radical:

$$\% \text{ inhibition} = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \times 100, \quad (1)$$

whereas Abs C is absorbance of the control and Abs S is the absorbance of the sample.

ABTS Radical Scavenging Assay

ABTS radical scavenging activity of AHEO was assayed using method of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS, 7 mM) and potassium persulfate (K₂S₂O₈, 2.45 mM) were dissolved to produce ABTS radical cation, which was then stored at room temperature for 16 hours in the dark. Using a spectrophotometer, the mixture was diluted with PBS to obtain absorbance of 0.700 ± 0.02 units at 734 nm for the evaluation of ABTS radical scavenging activity. The diluted AHEO or trolox solution (10-100 µg/mL) was then combined with the 180 mL ABTS solution in 200 mL. A microplate reader was used to measure the absorbance at 734 nm six minutes after the first mixing.

RESULTS

Chemical Compositions of Artemisia Herba-Alba Essential Oil

The hydrodistillation technique of dried leaf powder of AHA produces pale yellow oil with a pleasant odor and yield of 4.41% v/w based on the fresh weight (200 grams). The results of chemical profiles are displayed in **Table 1**.

As indicated in **Table 1** and **Figure 2**, nearly 22 compounds were identified to constitute about 96.80% of the total mass of essential oils. The majority of the identified components were monoterpenoids (71.90%), while hydrogenated and oxygenated monoterpenes account for 35.60% and 36.30% of the total composition of essential oil, respectively. Hydrogenated and oxygenated sesquiterpene represented 17.40% and 0.90% of the total composition of AHEO, respectively.

Table 1 shows that AHEO was very rich in oxygenated monoterpene (36.30%), being 1,8-cineole (10.40%) as the main component. Hydrocarbon monoterpene (35.60%) was the second most abundant chemical class, largely represented by Alpha pinene (17.20%). Hydrocarbon sesquiterpene was present (17.40%) as sabinene (8.40%), whereas oxygenated sesquiterpene was present in lower amounts (0.90%).

Table 1. Chemical composition of essential oil identified from *artemisia herba-alba* essential oil using GC-MS

No	KI cal	KI let	Compounds	%
1	933	930	Alpha thujene	0.40
2	941	940	Alpha pinene	17.20
3	975	975	Sabinene	8.40
4	982	980	Beta pinene	6.20
5	1,030	1,029	1,8 cineole	10.40
6	1,031	1,031	Limonene	1.30
7	1,068	1,062	Gamma terpinene	2.10
8	1,072	1,070	Artemisia ketone	0.20
9	1,086	1,086	Artemisia alcohol	5.20
10	1,102	1,102	Alpha thujone	11.40
11	1,117	1,116	Beta thujone	2.30
12	1,155	1,156	Sabina ketone	3.80
13	1,157	1,157	Viridene	3.70
14	1,168	1,163	Chrysanthenol	0.90
15	1,198	1,194	Alpha terpinol	2.10
16	1,425	1,419	Beta caryophyllene	8.30
17	1,446	1,441	Aromandendrene	2.20
18	1,483	1,482	Alpha curcumene	1.80
19	1,487	1,484	Germacrene D	5.10
20	1,652	1,653	Alpha cadinol	0.90
21	1,703	1,700	Caryophyllene acetate	2.30
22	3,380	3,381	Artemetin	0.60
Oxygenated monoterpene				36.30
Hydrocarbon monoterpene				35.60
Hydrocarbon sesquiterpene				17.40
Oxygenated sesquiterpene				0.90
Others				6.60
Total				96.80

Antibacterial Effect of AHEO Using Kirby-Disk Diffusion Method

The results of antimicrobial effects of AHEO on six types of microbial strains were presented in **Table 2**.

The results revealed that the highest resistance to AHEO was observed on gram-negative *K. pneumonia*, while *P. aeruginosa* was the most sensitive strain. Moreover, this study demonstrated that AHEO at concentration 100 μ L/mL exhibited the largest inhibitory zone diameter with 13.67 ± 1.52 mm against *P. aeruginosa*. On the other hands, the antimicrobial activity was reduced at concentrations of 15 and 7.5 μ L/mL, the inhibitory zone was not apparent for any strains, with the exception of *P. aeruginosa*, which showed an inhibition zone of 9.33 ± 0.58 and 7.33 ± 0.52 mm, respectively (**Table 2**). From the most sensitive strains to the most resistant, the sensitivity of the studied microbes was, as follows: *P. aeruginosa* > MRSA > *S. aureus* > *E. coli* > MRSE > *K. pneumonia*.

In comparison to a positive control (erythromycin = $11.33 \text{ mm} \pm 0.58$), the best activity of various concentrations (100-7.5 μ L/mL) of AHEO was expressed against *P. aeruginosa* with inhibition zone 13.67 ± 1.52 , 11.66 ± 0.58 , 10.00 ± 0.57 , 9.33 ± 0.58 , and 7.33 ± 0.52 mm, respectively, as shown in **Table 2**.

Minimum Inhibitory Concentration & Minimum Bactericidal Concentration of AHEO

MIC and MBC values of AHEO are presented in **Table 3**. Analysis of MICs led to the identification of MIC₉₀. According to the findings of the present study, MIC₉₀ values for six bacterial strains assessed ranged from 2.10 to 2.78 mg/mL and the control cefoxitin varied from <0.0038 to 0.125 mg/mL. As illustrated in **Table 3**, AHEO showed the best activity against MRSE, MRSA and *S. aureus* with an MIC₉₀ values at 2.10, 2.12,

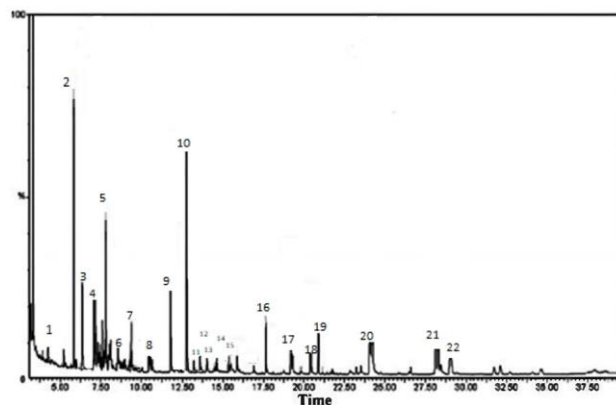


Figure 2. GC-MS spectrum of AHEO (Source: Authors' own elaboration, using Shimadzu qp2010 plus, Japan GC/MS-200 equipped with split-splitless inlet (S/SL))

and 2.16 mg/mL, respectively. While MBC values of AHEO is varied between 7.5 and 30 mg/mL and the control cefoxitin varied between <0.0038 and 0.25 mg/ml. In particular, AHEO showed the lowest MIC values of 2.10 mg/mL against MRSE. Whereas MIC and MBC values toward MRSE changed from 2.10 to 30 mg/mL, respectively. For MRSA, MIC and MBC values for AHEO were 2.12 and 30 mg/mL, respectively. For *S. aureus*, MIC and MBC values for AHEO were 2.16 and 15 mg/mL, respectively. MIC and MBC of AHEO against *E. coli* were 2.37 and 7.5 mg/mL, respectively. MIC and MBC values for *P. aeruginosa* were determined to be 2.49 and 15 mg/mL, respectively, while those for *K. pneumonia* were 2.78 and 30 mg/mL (**Table 3**).

Phenolic, Flavonoid, & Tannins Contents

Secondary metabolites such as flavonoids, phenolic, and tannins compounds are frequently abundant in medicinal plant tissues. On the basis of the absorbance values of AHEO and in comparison, with the equivalent standard solution described above, the results for the determination of total polyphenols, total flavonoids, and total tannins are summarized, as follows:

Phenolic compounds are the main class of naturally occurring antioxidants compounds found in plants, which are usually quantified using Folin-Ciocalteu method. In this work, AHEO had a total phenolic content of 43.97 ± 0.11 mg GAE/g dry of plant. Whereas using the aluminum chloride colorimetric method, the total flavonoid concentration of AHEO was determined to be 30.11 ± 0.27 mg CE/g dry extract. Moreover, AHEO has a total tannin concentration of 9.91 ± 0.50 mg QE/g dry extract, as indicated in **Table 4**.

DPPH and ABTS Scavenging Activities

The antioxidant capacity of AHEO was assessed using DPPH and ABTS free radical scavenging assays. The scavenging activity was calculated based on its percentage of DPPH and ABTS inhibition and expressed as IC₅₀ values, as shown in **Table 4**.

Based on DPPD and ABTS techniques, AHEO demonstrated a significant antioxidant activity when compared to gallic acid and trolox. The scavenging activity increased as AHEO concentrations increased. From the results obtained, AHEO demonstrated IC₅₀ values of 64.57 ± 8.74 μ g/mL for DPPH and 34.01 ± 0.65 μ g/mL for ABTS. The antioxidant potential's capacity to scavenge free radicals increases as IC₅₀ value decrease. In contrast to the standards include gallic acid,

Table 2. Antibacterial effect of *artemisia herba-alba* essential oil using disc diffusion method

Bacteria strains	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumonia</i>	<i>P. aeruginosa</i>	MRSA	MRSE
Concentrations ($\mu\text{L/mL}$)						
100	8.33 \pm 0.58	6.66 \pm 0.58	NA	13.67 \pm 1.52	NA	NA
70	6.33 \pm 0.29	6.66 \pm 0.58	NA	11.66 \pm 0.58	NA	NA
30	5.00 \pm 0.00	NA	NA	10.00 \pm 0.57	NA	NA
15	NA	NA	NA	9.33 \pm 0.58	NA	NA
7.5	NA	NA	NA	7.33 \pm 0.52	NA	NA
Erythromycin	24.00 \pm 1.73	NA	NA	11.33 \pm 0.58	18.67 \pm 1.15	22.33 \pm 0.58

Note. Results are presented as mean \pm standard deviation & NA: No activity

Table 3. Minimum inhibitory & minimum bactericidal concentrations of AHEO, cefotaxime, & cefuroxime controls

	Essential oil (mg/mL)		Cefotaxime (mg/mL)		Cefuroxime (mg/mL)		
	MIC ₉₀	MBC	MIC	MBC	MIC	MBC	
<i>S. aureus</i>	2.16	15	<0.004	0.008	<0.003	0.019	$y=-16.864x+127.71$ & $R^2=0.912$
<i>E. coli</i>	2.37	7.5	<0.004	<0.004	0.019	0.039	$y=-11.526x+119.02$ & $R^2=0.8911$
<i>K. pneumonia</i>	2.78	30	0.016	0.125	0.625	1.250	$y=-10.656x+119.41$ & $R^2=0.864$
<i>P. aeruginosa</i>	2.49	15	<0.004	0.0157	0.078	0.625	$y=-12.345x+124.08$ & $R^2=0.9135$
MRSA	2.12	30	0.625	0.625	0.039	0.078	$y=-12.345x+124.08$ & $R^2=0.9135$
MRSE	2.10	30	0.125	0.250	1.250	2.500	$y=-17.023x+126.24$ & $R^2=0.8842$

Table 4. Total phenolic, flavonoids, tannins, & antioxidant activity of AHEO

Test	Values
Total phenols (mg GAE/ g dry extract)	43.97 \pm 0.11
Total flavonoids (mg CE/ g dry extract)	30.11 \pm 0.27
Tannins (mg QE/g dry extract)	9.91 \pm 0.50
DPPH (IC ₅₀)	64.57 \pm 8.74 $\mu\text{g/mL}$
ABTS (IC ₅₀)	34.01 \pm 0.65 $\mu\text{g/mL}$
Trolox	5.80 \pm 0.45 $\mu\text{g/mL}$
Quercetin	3.20 \pm 0.32 $\mu\text{g/mL}$
Gallic acid	3.65 \pm 0.74 $\mu\text{g/mL}$

Note. GAE: Gallic acid equivalent; CE: Catechin equivalents; & QR: Quercetin equivalents

quercetin, and trolox (IC₅₀ 3.65 \pm 0.74, 3.20 \pm 0.32, and 5.80 \pm 0.45 respectively) (Table 4).

DISCUSSION

Essential oils, volatile and pungent byproducts of secondary plant metabolism are widely used in food flavoring and preservation [41]. Also, monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives, which make up most essential oils, may exhibit antimicrobial properties [42]. The results of this study were strongly supported because AHEO also contained these components, which confirmed its efficacy as a natural antimicrobial. In this study, the yield of the hydro distilled volatile oil from AHA leaves component was 4.41%. In addition, monoterpenes made up the majority of the contents (36.30%), with 1,8-cineole and viridene serving as the principal compounds (10.40% and 3.70%, respectively). In agreement with the study in [30], the chemical components of AHA, which grows in Buseirah, south Jordan, revealed that oxygenated monoterpenes were the principal oil constituents with 1,8-cineole (20.10%) as the main ingredient.

The yield of the extracted oil from AHA in the present study was found to be 4.41% (v/w), which is higher than 0.24% from Mutah, Alkarak, South Jordan [43], 0.49% from Al Qalibah (Tabuk Region), from Saudi Arabia [44], 0.86% from Azzemour region, Southwest Morocco [45], 1.30% from the Middle Atlas area of Morocco [28], and 1.93% [46]. Numerous factors, including variations in the ambient components, extraction

technique, and other relevant environmental conditions, could be the cause of variations in the yield percentages of essential oils [17, 18].

Numerous studies have shown that EO has potent antioxidant properties that may aid in countering and preventing numerous diseases [47]. Since these conditions are typically brought on by the cellular oxidative damage caused by free radicals, recent research has focused heavily on the antioxidant potential of essential oils and suggested that these compounds may play a significant role in the prevention of other chronic conditions as well as the treatment of diabetes, inflammation, hyperlipidemia, hypertension, and other conditions [48-50].

The chemical constituents of essential oils and the concentration of the principal single components have a considerable impact on their antibacterial activities [51]. Under certain situations, these chemical substances are released by a sequence of molecular interactions [52]. Different mechanisms of action against microorganisms may be displayed by various compounds. In general, a series of biochemical processes in the bacterial cell mediate the antibacterial effect, and these processes depend on the type of chemical elements found in the essential oil [51]. Additionally, the bacterial architecture of gram-positive and gram-negative bacteria differs in the composition of their cell membranes, which impacts how effective essential oils are against bacteria [51, 53, 54].

Different mechanisms of essential oils' antimicrobial action have been put suggested. Essential oils affect several cellular processes, including membrane-coupled energy production, membrane transport, and other metabolic regulatory processes by primarily destabilizing cellular architecture, breaking down membrane integrity, and increasing permeability [51, 53]. Essential oil may induced cell membrane disruption may facilitate a number of crucial biological activities, including the conversion of energy, the breakdown of nutrients, the production of structural macromolecules, and the release of growth regulators in addition, the outer layer of the cell's membrane and its cytoplasm may be damaged by the essential oils [51, 55]. Essential oils are readily able to pass through the bacterial cell membranes due to their lipophilic nature. It has been observed that the essential oils of several MAPs increase the permeability of bacterial cell membranes,

causing cellular components to flow out and ions to be lost [51, 53, 55, 56].

In addition, reduced membrane potentials, the interference with proton pumps, and the depletion of ATP are additional factors that contribute to essential oils' antibacterial effects [57]. This change in the structure of the cell could have a cascading effect on other cell organelles [54]. For instance, Tea tree oil has been shown to stop the growth of *S. aureus* and *E. coli* by affecting cell permeability, causing an increase in the leakage of intracellular K⁺ ions, and interfering with cell respiration [58, 59]. Also, essential oils can disturb the arrangement of molecules with different fatty acids, phospholipid bilayers, and polysaccharides because they pass through the cell wall and cytoplasmic membrane [53, 60]. All these events could be behind the cytoplasmic coagulation of internal cellular components and the dissolution of the connections between the lipid and protein layers [61].

The most prevalent compounds in AHEO oil in the present study was α -pinene (17.20%), which is compatible with a prior study in [62]. However, AHA collected from four separate locations revealed varying amounts of α -pinene, ranging from 17.20%, 14.10%, 11.30%, and 8.20%. [62]. Another study conducted showed less amount of α -pinene (1.50%) [27]. Also, the percentages of 1,8-cineole was found to be 10.40%, which is less than 26.00% [46], 20.10% [30], and 13.30% [63], but it is higher than 8.90% [64] and 3.40% [27]. It was reported that α -pinene have antimicrobial activity against *C. albicans*, *C. neoformans*, *R. oryzae*, and MRSA [65].

In the current study, AHEO has been extensively studied for its antimicrobial activity and several studies have reported the effectiveness of AHEO against various microorganisms. The antimicrobial activity of AHEO has been evaluated using agar paper disc method, which was used by Amor and his colleges to determine the growth inhibiting activities of AHEO on various microorganisms [45]. It was also tested antimicrobial activity of AHEO against gram-negative and gram-positive bacteria using the agar diffusion method [66]. It was found that AHEO exhibited antimicrobial activity against *S. aureus*, *E. coli*, *Bacillus cereus*, *K. pneumoniae*, *Listeria monocytogenes*, *Vibrio cholerae*, and *Salmonella typhimurium* [45]. The antimicrobial activity of AHEO can be attributed to its content of oxygenated monoterpenes, which constitute a significant portion of the EO. It was mentioned that the antimicrobial activity of AHEO is related to its high content of oxygenated monoterpenes [45, 66]. Additionally, camphor, the most prevalent component in AHEO, was shown to have bacteriostatic action against *P. aeruginosa* [66]. Overall, the available literature supports the antimicrobial activity of AHEO against a wide range of microorganisms. Its effectiveness can be attributed to its high content of oxygenated monoterpenes, particularly camphor. Further research is needed to explore the potential applications of AHEO in various fields, including medicine and food preservation.

AHA also known as white wormwood or desert wormwood is a plant species that is native to North Africa, particularly the countries of Morocco, Algeria, Tunisia, and Libya. It is also found in some parts of the Middle East, including Egypt, Jordan, and Saudi Arabia. These regions have arid or semi-arid climates, which are suitable for the growth of AHA. Regarding the effect of different origins on the activity of AHEO, the provided studies do not directly address this aspect. Therefore, there is no specific information available on how the origin of

AHEO may affect its antibacterial activity. Further research would be needed to investigate this aspect.

The antioxidant activity of AHEO has been investigated in several studies. It was reported that AHEO exhibited high antioxidant activity in the DPPH, FRAP, and ABTS assays [67]. The high antioxidant activity in the DPPH and FRAP assays may be attributed to the relative abundance of camphor in AHEO. Camphor has been reported to have favorable antioxidant power in other artemisia species [46]. Additionally, the higher antioxidant activity in the ABTS assay may be due to the predominance of oxygenated terpenes in AHEO, which are known to be effective in neutralizing free radicals and decomposing peroxides [46]. Regarding the effect of different origins on the antioxidant activity of AHEO, it was shown that the chemical composition of the essential oil, which can vary depending on the origin, may influence its antioxidant activity. For example, the relative abundance of oxygenated terpenes in AHEO has been linked to its antioxidant power [67]. Therefore, it is plausible that the antioxidant activity of AHEO may vary depending on its origin. These chemicals have antioxidant and antibacterial capabilities because of their chemicals and redox characteristics [47].

Therefore, at the end of this study, it can be concluded that AHEO exhibited a strong antibacterial and antioxidant activities, which could be attributed to the high contents of phenolic, flavonoid, and tannins compounds. Also, the major compounds identified from AHEO were α -pinene, α -thujone, and 1,8 cineole exhibited strong antibacterial effect even at low doses. To demonstrate the safety of the oil and encourage its usage in food and natural products, we advise further research that considers organoleptic properties and toxicological examinations.

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