African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 5, Issue 1, 2026
Page No: 264-273
Website: https://aaasjournals.com/index.php/ajapas/index
ISI 2025: 1.126 **SJIFactor 2024: 6.752** **معامل التأثير العربي: 1.62**

Effect of aqueous and alcoholic extracts and essential oil of *Artemisia herba alba* on some types of bacteria resistant to antibiotics

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تأثير المستخلص المائي والكحولي والزيوت الاساسية لنبات الشيح على بعض أنواع البكتيريا المقاومة للمضادات الحيوية

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Received: November 21, 2025	Accepted: January 31, 2026	Published: February 11, 2026

Abstract
This study aimed to evaluate the effect of *Artemisia herba alba* extracts (aqueous, alcoholic and essential oil) on antibiotic-resistant bacteria. In which four types were tested, namely *Staphylococcus aureus* (MRSA) *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, using the diffusion disk method and concentrations (100, 200, 300 mg/ml) for the aqueous and alcoholic extract, while the essential oil was used in its pure form. The most effective antibacterial activity was observed for the essential oil of plant with an inhibition diameter from 8.00±0.00 to 11.33±1.52 mm, and its aqueous extract effect on bacteria MRSA and *S. epidermidis* was 8.66±1.52 and 8.33±0.57 mm, respectively, at 300 mg/ml. The inhibitory effect of alcoholic extract was 10.00±0.00 mm at 300 mg/ml for MRSA as for *S. epidermidis* at the same concentration had high inhibition diameter 13.66±1.15 mm. Phytochemical screention of aqueous extract revealed the presence of phenols and saponins, whereas, alcoholic extract revealed the presence of phenols, tannins, terpenoids and saponins. The chemical components of the essential oils were determined by GC-MS device. The main compounds of *A. herba alba* were Camphor (30.527%), Thujone (22.471%) and Camphene (10.291%). These results suggest that essential oil extract can be considered as a potential source of antibacterial compounds.

Keywords: Artemisia herba alba, aqueous extract, alcoholic extract, essential oil, resistant bacteria.

الملخص
هدفت هذه الدراسة إلى تقييم تأثير المستخلصات النباتية المائية، الكحولية والزيوت الأساسية لنبات الشيح *Artemisia herba alba* على البكتيريا المقاومة للمضادات الحيوية، حيث تم اختبار أربعة أنواع من البكتيريا، باستخدام طريقة قرص الانتشار، وطبقت التركيزات للمستخلص المائي والكحولي (100 ، 200 ، 300 مجم/مل)، بينما تم استخدام الزيت العطري في شكله النقي .أظهرت نتائج دراسة الفعالية الحيوية لزيوت الأساسية بأن أفضل الكفاءة في التثبيط ضد البكتيريا. تم تحديد المكونات الكيميائية للزيوت الأساسية بأستخدام جهاز GC-MS، المركبات الرئيسية لنبات الشيح كانت الكافور 30.52 ٪،ثوجون 22.471 ٪. أما بالنسبة للمستخلصات المائية و الكحولية لنبات الشيح فكان تأثيره علي البكتيريا الموجبة الجرام عند تركيز (300 مجم/مل) فكانت بكتيريا *S. aureus* أكثر حساسية للمستخلص المائي بقطر تثبيط 8.66 ±0.52 مم، و كان أفضل تأثير مثبط للمستخلص الكحولي بقطر 13.6±1.15 مم لبكتيريا *S. epidermidis* . وأظهرت نتائج الفحص الكيميائي أن كلا المستخلصين لنبات يحتويان على مركبات الفينولات والصابونيات ذات النشاط الفعّال .تشير هذه النتائج إلى إمكانية اعتبار مستخلص الزيت العطري مصدرًا واعدًا للمركبات ذات النشاط المضاد للبكتيريا.

الكلمات المفتاحية: الشيح، المستخلص المائي، المستخلص الكحولي، الزيوت الأساسية، البكتيريا المقاومة.

Introduction

Medicinal plants are defined as plants that possess therapeutic properties, in one or more of its various organs one or more active chemical substances are presentin low or high concentrations, and have the physiological ability to treat a specific disease or reduce the symptoms of infection with this disease by using the plant in its natural or dried form or by using the active substances extracted from it [1]. According to the World Health Organization (WHO), which formulated this definition, this description distinguishes medicinal plants as those whose therapeutic properties and components have been scientifically identified [2].

Medicinal plants have healing properties when used in a specific dose and in a specific way [3]. This is due to their availability, ease of access, low cost, and avoidance of the negative effects resulting from the use of chemical drugs [4]. They are used in two forms: raw form, as infusions, essential oils, or dye extracts, pure form in which the active ingredient (active substance) responsible for the therapeutic effect is identified and chemically defined. Pure compounds are generally used when the active ingredients have a strong and specific effect [5]. These plants are a source for the production of these biologically active compounds of pharmacological importance for the production of drugs that have therapeutic efficacy, as they are used as antioxidants, antibacterials and antivirals [6]. They are classified into different groups based on functional groups and chemical structure, including phenols, alkaloids, flavonoids, saponins, tannins, terpenes, and volatile oils [7].

Artemisia belongs to the Asteraceae family, which includes 1,600 genera and more than 25,000 species [8]. It is distributed worldwide, as it is found in North Africa, the Middle East, Europe, southwest Asia, and northeastern America. Its common name is Wormwood, its Arabic name is Chih, and its scientific name is *Artemisia*. This genus includes about 500 speciesaround the world, including *A. herba alba*, *A. afra*, *A. annua*, *A. judaica*, and *A. campestris* [9]. *A. herba alba* is used by the people of North Africa to treat diabetes and high blood pressure. Chemical analysis has found that it is rich in large quantities of minerals, especially potassium and calcium, which are the predominant elements in the medicinal plant. There are also other elements such as iron, zinc, chromium, sodium, and cobalt [10]. In addition to containing oils, the essential oil in the date plant represents 0.33% of the plant and is rich in monoterpenes and sasquiterpenes. The most important substances are *Artemisia* Ketone 68%, Cineole 51.5%, Camphor 48%, in addition to phenolic compounds, flavonoids, tannins, saponins and alkaloids [11].

Antibiotics are compounds that inhibit or kill bacteria through a specific interaction with a specific target in the bacterial cell. Since antibiotics were widely introduced in the late 1940s to treat human bacterial infectious diseases, there has been a steady selection and increase in the frequency of antibiotic-resistant bacteria [12]. The development of resistance is a complex process driven by the interaction of a number of biotic and abiotic factors. The main factors underlying this dynamic are the rates of emergence and persistence of resistant bacterial clones, the temporal and spatial gradients of antibiotics and other foreign substances, and the rates of transmission of infection within human populations and between humans and various other sources including animals, the environment, food, etc [13].

The World Health Organization announced what are known as pathogens (ESKAPE) on the basis of their clinical significance and levels of resistance **E**: *Enterococcus faecium*, **S**: *Staphylococcus aureus*, **K**: *Klebsiella pneumoniae*, **C**: *Clostridioides difficile*, **A**: *Acinetobacter baumannii*, **P**: *Pseudomonas aeruginosa*, and **E**: *Enterobacteriaceae* [14]. The most common and dangerous antibiotic-resistant listed by the World Health Organization are methicillin-resistant *Staphylococcus aureus* (MRSA), which may cause nosocomial infections or have individual complications, carbapenem-resistant Enterobacteriaceae (CRE), and various strains of antibiotic-resistant Mycobacterium tuberculosis [15].

The aim of this study is evaluate the effectiveness of the aqueous and alcoholic extracts and essential oils of *A. herba alba* on the activity of bacteria resistant to traditional antibiotics in the laboratory. Identifying active compounds of plant capable of killing or inhibiting the growth of antibiotic resistant bacteria.

Materials and Methods

Plant material

Plants were collected from the city of Zintan, in the Al-jabal Al- Gharpi (Nafusa mountain), from the Ouled Belhol area at site (8F3JW6WV+CG) 31°56'45.8"N, 12°14'37.7"E during October 2024, The botanical identification of plant was carried out by Dr. Abdul Hmid Giweli, Department of Ecology, Faculty of Science, University of Zintan. The plant was washed under tap water and left to dry naturally in a dark place away from sunlight and at room temperature. The leaf parts were collected and stored until used.

Aqueous extraction

The mixing and soaking method was described by Khanzada et al. [16], where 50 g of plant was weighed, placed in a conical flask with a tight plastic cover, added 1000 ml of distilled water, then the flask was placed in a dark place at room temperature for 24 hours, the extract was filtered and check for colour, measured pH using pH meter. Then evaporated in an oven at 37°C for 24 hours, the dry extract was collected, weighing and stored at 4 °C until use.

Percentage yield = weight of extract / weight of the sample × 100

Alcoholic extraction

The alcoholic extract was prepared according to the method of Hail & Jiru [17], using methyl alcohol at a concentration of 75%, weigh 50 g of plant leaves, placed a conical flask with a plastic cover, added 1000 ml of 75% diluted alcohol and place it in a dark place for 3 days. The extract was filtered and check for color, measured pH using pH meter. Then evaporated by air oven at 37°C for 24 hours, the dry extract was collected, weighed and kept in the refrigerator at 4 °C until use.

To prepare the basic stock solution of the aqueous and alcoholic extracts, 2.4 g of the extract mass was weighed and dissolved in 4 ml of sterile distilled water using the w/v ratio, from this solution we prepared (100, 200, 300 mg/ml), using the following equation: $C1\ V1 = C2\ V_2$.

Phytochemicals screening of aqueous and alcoholic extracts, to detect phenolic compounds used ferric chloride test, for flavonoids compounds used sodium hydroxide test, for alkaloids compounds used picric acid test [18]. Detection of saponins compounds by foam test [19], tannins compounds by lead acetate test [20], and terpenoids compounds by salkowiski test [21].

Essential oil extraction

Essential oils was extracted by weighing 50 g of plant leaves and placed in a flask Clevenger apparatus containing 500 ml of distilled water at 60 °C for 3 hours [22]. The obtained oil was measured to calculated the percentage yield, and check for color, measured pH using pH meter. The oil was stored in a sterile, opaque glass tube and kept at 4°C until use. The quantitative and qualitative analyzes of oils were carried out using the gas chromatography-mass spectrometry (GC-MS) device at the institute of arid zones in Medenine, Tunisia (central laboratory) according to the method [23].

Collection and identification of bacterial samples

Bacterial samples were collected from 1/11/2024 to 1/1/2025, from patients with tonsillitis, urinary tract infection and dermatitis by cotton swab and preliminary diagnosis was made at the National Research Center for Tropical and Transboundary Diseases (NRCTTD) in Zintan city. The samples were cultured on nutrient agar, mannitol salt agar (MSA), macConkey agar, blood agar, and CLED agar and incubated for 24 hours at 37 °C. Initial identification was based on colony morphology, hemolytic patterns on blood agar, and reactions on selective media, such as MSA and CLED. Then followed by gram staining [24], and biochemical tests, including oxidase reagent [25], catalase reagent [26], coagulase reagent [27] and enterosystem 18 R [28]. Fiinally, the automated MA120 identification system (Render, China), was used for identification the species of bacteria following the company, s operational protocol [29]. The isolates used in this study were confirmed as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Staphylococcus aureus* (MRSA), and *Staphylococcus epidermidis*.

Antimicrobial effectiveness of plant extracts

Antibacterial activity of plant extracts were estimated by used disc diffusion method [30]. After preparing bacterial suspension from fresh 24-hour cultures and adjusted to a 0.5 McFarland standard, 100 µl aliquot of each suspension was spread on Nutrients agar plats, leave the plates for 15 minutes at room temperature. The sterile filter papers (6mm diameter) containing 10 µl of each plant extracts; aqueous and alcoholic extracts with concentrations (100, 200, 300 mg/ml), for essential oil used in its pure form, were placed on the surface of inoculated agar plate in addition to a negative control (distilled water). The plates were incubated at 37°C for 24-hour and the diameter zone of inhibition (IZD) was measured.

Statistical analysis

Data were analyzed with a statistical software program (SPSS 20). The one-way ANOVA followed by Least Significant Difference (LSD) test was used for statistical analysis. Results are expressed as mean±SD, and statistical significance was accepted ($P<0.05$).

Results

The results showed that the yield of plant extracts from the mass of dry plant material was obtained, where the ratio of *A. herba alba* to the alcoholic extract was the highest, 20.76% and the lowest ratio for essential oil 2%. The characteristics of plant extracts in terms of the solvent used, color, pH and the quantity or volume extracted are presented in Table 1.

Table 1: Yield % and properties of *A. herba alba* extracts and essential oil.

Extract	Yield %	Color	PH	Wight, volume
Alcoholic	20.76	Dark brown	5.52	10.86 g
Aqueous	7.3	Orange	4.86	3.65 g
Essential oil	2	Light yellow	5	1 ml

The composition of the hydrodistilled essential oil from the aerial (leaf) parts of plant was shown in Table 2, the compounds of *A. herba alba* oil were identified, which contains 24 compounds of the oil mass. The main compounds were Camphor at 30.527%, Thujone at 22.471%, Camphene at 10.291%, and 3-Carene at 7.322%.

Table 2: Chemical composition of *A. herba-alba* essential oil using GC-MS from Zintan, Libya.

No	Identified compound	Composition (%)	Retention time (RT)	Retention index (RI)
1	α –Pinene	0.369	2.557	932
2	3- Hexene-1-ol, (E)	0.313	5.597	857
3	Camphene	10.291	6.809	953
4	β –Pinene	2.934	7.46	976
5	3-Carene	7.322	7.836	1009
6	trans-β -Ocimene	4.791	8.418	1036
7	α –Terpinolene	0.397	9.452	1087
8	p-Cymene	0.945	9.641	1024
9	Sabinene	0.227	9.698	969
10	γ-Terpinene	0.461	9.755	1059
11	Eucalyptol	3.047	9.828	1031
12	Terpinen-4-ol	2.253	10.694	1176
13	Linalool	1.428	11.485	1096
14	Camphor	30.527	11.964	1143
15	p-Menth-8-en-1-ol, stereoisomer	0.268	12.053	1169
16	Thujone	22.471	12.644	1428
17	Borneol	1.029	12.835	1165
18	Dihydrocarveo	4.238	13.329	1289
19	β –Elemene	0.168	13.452	1390
20	α –Terpineol	0.693	13.593	1189
21	cis-3-Hexenyl isovalerate	0.949	14.418	1261
22	Trimethadione	0.450	15.311	1233
23	Bornyl acetate	0.497	15.692	1314
24	β–Bisabolene	0.154	19.055	1335

For detecting the active compounds present in the studied plant through the qualitative examination of the aqueous and alcoholic extracts using the reagents specific to each group of active compounds, which form a white precipitate as in the case of alkaloids and tannins, or a change in color as in the case of phenol, which gives a blue-green color, flavonoids give a yellow color, while terpenes show the appearance of a reddish-brown layer, and soaps form a foam for the extract (Table 3).

Table 3: Some active groups of *A. herba alba* leaves extracts.

Composite	Aqueous extract	Alcoholic extract
Phenol	+	+
Alkaloids	-	-
Flavonoids	-	-
Tannins	-	+
Terpenoids	-	+
Saponins	+	+

(-) Negative detected, (+) Positive detected.

Biochemical tests were performed as shown in Table 4. The result of the catalase test was positive, which distinguishes it from streptococci. The oxidase test is also used to distinguish the first from Micrococci. *S. aureus* was cultured on MSA agar and it was mannitol fermenting and the medium changed from red to yellow. On blood agar, it was completely hemolysis for red blood cells, which was of the β- hemolysis type. A coagulase test was performed and the test was positive for *S. aureus.* Whereas, it was negative *S. epidermidis*, which was confirmed by the MA 120 device. The genus of bacilli was cultivated on CLED agar and blood agar, which gave isolates with a yellow color on CLED agar as a result of fermentation of lactose sugar in it, while on blood agar it did not give hemolysis of red blood cells. Biological tests were negative for oxidase. To distinguish between them *(K. oxytoca and K. pneumoniae*), the Enterosystem 18 R system was used, as one of the isolates was positive for the indole test (*K. oxytoca*).

Table 4: Biochemical tests of bacteria.

Bacteria / Test	*K. pneumoniae*	*K. oxytoca*	*S. epidermidis*	*S. aureus*
Gram stain	-	-	+	+
Oxidase reagent	-	-	-	-
Catalase reagent	+	+	+	+
Coagulase test	*	*	-	+
Indole test	-	+	*	*
Hemolysis	-	-	-	+
MSA agar	*	*	-	+

(-) Negative test, (+) Positive test, *Not applied.

The results of aqueous and alcoholic extracts against both *K. pneumoniae* and *K. oxytoca* bacteria showed no sensitivity to all concentrations used and did not inhibit their growth. As for *S. aureus* and *S. epidermidis* bacteria, the aqueous extract was affected at 300 mg/ml with inhibition diameter 8.66±1.52 and 8.33±0.57 mm, respectively, (P<0.05). The alcoholic extract had inhibitory activity against *S. aureus* bacteria at concentration 300 mg/ml with 10.00±0.00 mm) while, *S. epidermidis* bacteria were affected at 200 mg/ml with 10.00±0.00 mm, and at 300 mg/ml, was 13.66±1.15 mm, (P<0.05). Whereas, the average values of the inhibition zone diameters (IZD) for the antimicrobial activity of *A. herba alba* essential oil were between 8.00±0.00 to 11.33±1.52 mm. The average IZD for *S. aureus* was (10.00±1.73 mm) and *K. oxytoca* bacteria was (8.66±0.57 mm) thus it was sensitive to plant essential oil compared to *K. pneumoniae* bacteria which was less inhibited with an average IZD of (8.00 ±0.00 mm). The highest average IZD was for *S. epidermidis* bacteria (11.33±1.52 mm) compared to *K. pneumoniae* bacteria, which was less inhibited, which means that there are significant differences between the bacteria at the probability level (P<0.05), as shown in Table 5.

Table 5: Effect of aqueous and alcoholic extracts and essential oil of *A. herba alba* leaves against bacteria.

Bacteria	Aqueous extract			Alcoholic extract			Essential oil
	Concentration. (mg/ml)						
	100	200	300	100	200	300	
K. pneumoniae	-	-	-	-	-	-	8.00 ±0.00*
K. oxytoca	-	-	-	-	-	-	8.66±0.57*
S. aureus	-	-	8.66±1.52*	-	-	10.00±0.00	10.00±1.73
S. epidermidis	-	-	8.33±0.57*	-	10.00±0.00*	13.66±1.15*	11.33±1.52*

Values represent Mean±SD of IZD for triplicates, * (L.S.D) at $P<0.05$. (-) No inhibition zone.

Disscussion

Medicinal plants are considered the best source for obtaining a variety of medicines and many medicinal plants have been used for their antimicrobial properties. The main strength of plant natural products lies in their rich and unique chemical diversity that is produced in the secondary metabolism of the plant, their global distribution, ease of access, diversity of antimicrobial modes of action and proven clinical efficacy of plant extracts isolated from them and found in different parts of plants [31]. Rich in a wide range of secondary metabolites such as phenols, alkaloids, terpenes, tannins, saponins and other components, these substances are biologically active as innovative disease-preventing agents including antimicrobial, antiviral, antioxidant and anti-inflammatory properties [32].

Through the results of this study the yield of plant essential oils was 2%, which was lower than the found in *Artemisia* Jordan (karak) 4.41%, and thus our results do not agree with the study of Dmour et al. [33]. The aqueous extract was 7.3%, which was close to the results of Benmeziane et al. [34], in their study with 12.2% and for alcoholic extract was 20.76% in this study, which is not consistent with the results of Benmeziane et al. [34] for *Artemisia* collected from (Amman) Jordan. Many factors, including environmental differences, extraction technique, and environmental conditions, may be related to the reason for the difference in the production rates of essential oil [33]. There are also differences in the extracted oil, both quantitatively and qualitatively, for different stages of the plant during vegetative growth, the beginning of flowering, full flowering, and during the fruit and seed formation stage [35].

When examining the properties of the extracts, it was found that the pH value of the alcoholic extracts is higher than the aqueous extracts, and that the high pH helps to increase the solubility of the active ingredients [36]. When chemically examining the aqueous extract of *Artemisia* plant, which contained phenolic and saponin compounds, while for the alcoholic extract, the compounds were phenolics, tannins, terpenes and saponins. Our results were agreement with the results of Benmeziane et al. [34]. Through the results obtained for bacteria from cultural diagnosis, microscopic examination and biological tests for the genus *Staphlyococcus*, our results were positive

and agreement with the study of Rasigade & Vandenesch [37]; AL-Joda & Jasim [27], and the genus *Klebsiella* were consistent with the study of Rawy et al. [38]; Abd Al-Hassan et al. [39]. As for the diagnosis in the MA120 device for bacterial isolates, it has been shown that it is a fast and accurate technique that must be used in the laboratory diagnosis of microorganisms. It can also diagnose the appropriate antibiotic for each pathogenic microorganism through an automatic sensitivity test, thus saving time in determining the identity and sensitivity to antibiotics at the same time.

There are four main sites of action of antibiotics: they may be inhibitors of cell wall synthesis, such as penicillins, cephalosporin, and vancomycin, or they may work to destroy the permeability of the cytoplasmic membrane, such as polymyxin, or they may be inhibitors of protein synthesis, such as amikacin and gentamicin, or they may be inhibitors of DNA synthesis, such as ciprofloxacin [40]. The results of this study of the shared antibiotic resistance of both species of the genus *Klebisella* were the following antibiotics: moxifloxacin, ampicillin, amoxicilline, and trimethoprim, which were agreement with the study of Rawy et al. [38]; Mustafa et al. [41]. The reason for multi-resistance is attributed to the presence of resistance genes located on the bacterial chromosome, as well as a change in the permeability barrier, which makes it difficult for the antibiotic to pass through and reach its site of action, which is specific to gram-negative bacteria, as the outer membrane contains protein channels called porin, which work to prevent the entry of antibiotics into the bacterial cell. The production of biofilms is one of the most important mechanisms of resistance, and thus it is a major source of concern, and often leads to therapeutic failure, as it provides protection for bacteria [42].

Our results for the genus *Staphylococcus* reported that they are resistant to antibiotics common to both species, namely oxacillin and penicillin, and thus are resistant to methicillin (MRSA). The reason for the high resistance of the genus *Staphylococcus* is attributed to the bacteria possessing beta-lactamase enzymes that degrade the penicillin group, whose genes are either chromosomal or plasmid in origin. These bacteria also produce penicillin-binding proteins (PBPs) located in the cytoplasmic membrane that is linked to the cell wall. These proteins are a target for both penicillin and cephalosporin antibiotics, as they change the target site of beta-lactam antibiotics, which results in bacterial resistance to them [43].

The effect of *A. herba alba* essential oil was more effective on antibiotic-resistant bacteria, the result for *K. pneumoniae* bacteria (8.00±0.00 mm) was due to the presence of thujone, camphor, and camphene, close to a previous study by Sara et al. [44], with an inhibition diameter of 9 mm, while the study of Ouguirti et al. [45], differed from our study with an inhibition diameter of 21 mm due to the difference in the concentration of active ingredients, as the α-thujone compound was 48%. The inhibition diameter for *K. oxytoca* bacteria (8.66±0.57 mm) did not match what was indicated by Bertella et al. [46], which was an inhibition diameter of 31 mm, and the aerial parts were collected in October during the flowering stage from eastern Algeria. *S. arueus* has an inhibition diameter (10.00±1.73 mm) in agreement with the study of Bouhouia et al. [47], which was collected at the flowering stage in July from Souk Ahras, Algeria. While *S. epidermidis* has a large inhibition diameter (11.33±1.52 mm) in agreement with the study of Bertella et al. [46].

Artemisia plant, when analyzed by a quantitative and qualitative GC-MS device, the result of the Camphor compound was 30.527% in our study close to the study Mohyeldin et al. [48], which was 34.84%. The Thujone compound in our study had a high percentage of 22.471% compared to what was reached Bouhouia et al. [47], which was 0.16%. while it differed with the Camphene compound in our study, which was 10.291%, while the study of Mohyeldin et al. [48], showed a small percentage of 1.97%, while the 3-Carene compound was 7.322% which does not agree with the study of Bouhouia et al. [47], in the city of Bejaia, Algeria, which was 0.16%, and it was close in the study of Bekka-Hadji et al. [49], in Bejaia, Algeria, for the Camphor compound 32%, while the following compounds were at a small percentage compared to our study: Camphene 3.6% and the 3-Carene compound 0.2%.

When testing the effect of aqueous and alcoholic extracts of *A. herba alba*, no effect was shown on the growth of Gram-negative bacteria (*K. pneumonia, K. oxytoca*). This effect was consistent with the study of Abdel-Monem et al. [50], for the aqueous extract, while in a study conducted in northeastern Algeria in which *A. herba alba* was collected in November by Saida et al. [51], it gave an inhibition with a diameter of 6 mm. Our results also agree with the alcoholic extract, which had no effect, with Jasim & El-Zayat [52]. However when comparing our results with the study of Hafidh et al. [53], for the methanol extract, the inhibition diameter was 11 mm, in which the plant was collected in January in Algeria. This discrepancy is due to the nature of the bacteria, the extraction method, and the concentration of active substances in the extract [54].

Regarding positive bacteria, the aqueous and alcoholic extracts of *A. herba alba* showed a clear inhibitory activity against bacteria (*S. aureus* & *S. epidermidis*). The results for *S. aureus* bacteria for the aqueous extract were weak at 300 mg/ml, which was not consistent with what Hassan & Hadi [11] indicated, as the aqueous extract contains saponins and phenols that have an antibacterial effect [55]. As for the alcoholic extract, its highest inhibition at same concentration, due to the presence of phenols, terpenes, saponins and tannins that have an important role in inhibiting bacteria. The results were close to the study of Benmeziane et al. [34] as the plant was collected in May from Amman, Jordan, and its inhibition was 8 mm. As for *S. epidermidis* bacteria, the highest inhibition was in

the alcoholic extract at 300 mg/ml, with 13.66±1.15 mm). These results are agreement with what was indicated by Jasim & El-Zayat [52].
The most important aspect that must be taken into account is the extraction efficiency, as it is affected by solvents, their chemical composition, concentration, polarity, pH, temperature, time, and the ratio of solvent to sample, because one solvent cannot extract all active substances reliably [56]. The molecular affinity between the solute and the solvent may also affect the extractability. Traditional extraction methods are considered to require a long time, a large amount of solvent, low extraction yield, and reduced selectivity, they are sometimes exposed to excess oxygen, heat, and light, which leads to their subsequent deterioration [57]. Climate changes and environmental stresses such as salinity and drought also affect the active ingredients. Therefore, it can be concluded that the composition of the soil changes the pressures on the physical and chemical processes of plant compounds [58]. The quality of the active ingredients is also affected by the age of the plant, the harvest period, and the drying and extraction techniques [59].

Conclusion

In conclusion, the extracts (aqueous, alcoholic and essential oils) of *A. herba-alba* from Zintan, Libya, was effected on antibiotic-resistant bacteria under study. The greatest effect was achieved by the essential oils compared to the aqueous and alcoholic extracts, due to specific chemical profile (a high-potency camphor-thujone chemotype, with these two compounds constituting over half of its total composition) underpins its notable antibacterial activity against multidrug-resistant pathogens. Future studies should focus on the biological activity and successful extraction methods of active ingredients using advanced devices for purifying active ingredients that affect bacteria, which play an important role in traditional medicine in Libya.

Acknowledgments: The authors extend their appreciation to the National Research Center for Tropical and Transboundary Diseases (NRCTTD) in Zintan city, Libya, for funding this work through grant no. NBC, 016.P.25.01. Also, the authors wish to thank the Institute of Arid Regions (Médenine, Tunisia) for providing laboratory facilities and technical support during quantitative and qualitative analyzes of essential oils.

Compliance with ethical standards

Disclosure of conflict of interest

The author(s) declare that they have no conflict of interest.

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