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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF ARTEMISIA HERBA ALBA GROWN WILD IN ALTIB, IRAQ

Areej Hasan S. Aldhaher

Department of Biology, College of Science, University of Basrah, Basrah, Iraq. e-mail : areej.saleem@uobasrah.edu.iq

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ABSTRACT : *Artemisia herba alba* has been used in modern and traditional medicine in different cultures over many years. The aim of present study was to explore the phytochemical composition, antibacterial and antifungal activity of various parts extracts of *A. herba alba* growing wild in Iraq. Methanol and water solvents were used to prepare leaves and roots extracts of *A. herba alba* and the essential oil was isolated by hydrodistillation and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The antimicrobial activities were assayed by using well diffusion method against two bacteria *Escherichia coli* and *Staphylococcus aureus* and two fungus, *Candida albicans* and *Aspergillus niger*. Forty-three3 compounds have been identified from the essential oil of the plant, and 2,4-pentadiynylbenzene (27.33%), capillene (24.33%), 2-methylnaphthalene (8.52%) were the main constituents of the essential oil. Homoaromatic compounds with unsaturated hydrocarbon chain represented the main chemical class of the oil (52.75%) followed by sesquiterpens (10.58%) and fatty acids (4.85%). The essential oil of *A. herba alba* showed strong antibacterial and antifungal activity in the range of 15-53 mm, whereas other extracts did not reveal antimicrobial activity. Thus, this study is the first chemical study of *A. herba alba* essential oil, growing wild in Iraq. It showed distinctive chemo style and effective essential oils against all microorganisms studied.

Key words : Artemisia herba alba, essential oil, chemical constituent, antimicrobial.

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INTRODUCTION

Artemisia herba alba Asso belongs to the one of the largest genera of the family compositae (Astraceae) (Bora and Sharma, 2011). This genus consists of more than 500 species widely distributed in the North America, Asia and the temperate zones of Europe (Bora and Sharma, 2011). Albermana and Hadi (2012) recorded 10 diverse species of Artemisia in Iraq and *A. herba alba* was found to be the most widely spread species at different environments around Iraq.

Different types of *Artemisia* sp. have been used in folk medicine, such as anthelmintic, antispasmodic, and stomachic, and to cure many conditions, such as colds, coughs, headaches, malaria, diabetes, dysmenorrhea bladder and kidney disorders and premenstrual syndrome (Bora and Sharma, 2011). *A. herba alba* has been reported to treat diabetes and hypertension in southeastern Morocco (Tahraoui *et al*, 2007). The antifungal, antibacterial, antispasmodic, antiproliferative, and pesticidal activities of *A. herba-alba* essential oil have been documented (Zouari *et al*, 2010; Tilaoui *et al*, 2015; Goudjil *et al*, 2016; Mahmood *et al*, 2020). Phenolic and flavonoids compounds of this species showed antioxidant activity and aqueous extracts are found to inhibit the hemolytic activities of both snake and scorpion venom anthelmintic activity and ethanol extract appeared as neurological activities (Mohamed *et al*, 2010; Twegh *et al*, 2020).

Many papers have covered the chemical constituents of *A. herba-alba* focusing on the essential oils extract due to its medicinal value. Tilaoui *et al* (2015) found that the differences of essential oil profiles depend on the used part of the plant in the study, while Mohamed *et al* (2010) reviewed the variability of essential oils composition at various sites of plants collection in both the same and in different countries with consideration as to the time of yield and weather status. For example, in Jordan, regular monoterpenes were major components, whereas ketones such as camphor were predominant in the oil of *A. herbaalba* in Morocco. Some studies in Spain revealed that oxygenated monoterpenes and hydrocarbons are the main components in *A. herba-alba* essential oil. New research has exhibited that the sesquiterpene davanone was the main component of the oil in Spain. Various studies in Algerian oil revealed different compounds to be the main oil skeletons, such as camphor and davanone. Camphor, 1,8-cineole, and chrysanthenone were found to be the principal constituents in the French oil, whereas artemisia ketone was predominant in Egyptian types.

In Iraq, Al-Ibrahemi and Hasan (2019) succeeded to identify artemisinin in the *A. herba-alba* by using GC-Ms and HPLC techniques, while Saleh and Numman, (2016) found the percentage of isolated proteins and minerals Ca⁺², Na⁺, K⁺, Mg⁺² from *A. herba-alba*. Hamid *et al* (2018) also showed the percentage of chemical contents in the species, such as proteins and carbohydrates, vitamins and minerals. However, no study has explored the chemical composition of essential oil of this species in Iraq. Therefore, this study aimed to discover the phytochemical profile of essential oil and ethanol extract of *A. herba-alba* growing wild in the east of Iraq, and to assay the antibacterial and antifungal activity of different plant extracts.

MATERIALS AND METHODS

Plant materials

The plant materials (leaves and roots) of *Artemisia herba alba* were collected in March 2019 from Altib, east of Iraq GPS location 32°26.497' N and 047°10.093' E. A specimen was deposited at the herbarium of the Department of Biology, College of Science, University of Basrah. The plant materials were washed, and some of it was air dried at room temperature, then the leaves and roots were homogenized separately into powder using an electric mill.

Preparation of organic solvents extracts

The ground materials were extracted on a shaker with methanol, for 72 h at room temperature. Filter papers (Whatman No.1) were used to filter the solvent, then the solvent was evaporated using rotary evaporator.

Extraction of essential oil

The essential oil of *A. herba alba* was extracted by hydrodistillation method, using a Clevenger apparatus, 500 g of fresh leaves were macerated in 1000 ml of distilled water for 6 hours. The obtained essential oil was stored in the dark at 4°C till analysis.

Preparation of water extract

The residue solution of hydro distilled leaves of *A*. *herba alb* was filtrated, then evaporated. The obtained extract was stored at 4° C till used.

GC-MS analysis

GC-MS analysis was carried out at the Nahr Bin Omar, Basrah Oil Company by using an Agilent Technologies 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI ion source, using HP-5MS 5% phenyl methyl siloxane (30m x 250 Um x 0.25 mm). The oven temperature was set at 40°C, held for 5 min then raised to 1 °C/min to 280°C for 1 min; the temperature was then held at 280°C for the remaining 20 min. Helium was used as the carrier gas at constant flow mode of 1 ml/min, and purge flow of 3 ml/min. The injection mode was pulsed splitless with injection temperature of 290°C and the injection sample volume was 1 µl. The mass spectrometer used Ion Source Temp, 230°C, with scan speed of 1562 (N2) and electron ionization was obtained over the mass range 35 - 650 m/ z. Data were run through the NIST 2014 library database as an additional tool to confirm identity of the compounds.

Test microorganisms

The following pathogen microorganisms were used to assay antimicrobial activity of various *A. herba alba* extracts: *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. All microorganisms were obtained from the Microbiology laboratory in the Department of Biology, College of Science, University of Basrah.

Antibacterial activity assay

Agar well diffusion method was used to evaluate the antibacterial activity of plant extracts. Tested bacterial were cultured overnight, then bacterial suspension was prepared by using sterile physiological solution equivalent to a McFarland standard 0.5. Then, 100 μ l of each bacteria suspension was spread on *Mueller Hinton Agar* (MHA) dishes then four wells (7 mm diameter) were made on each plate. Plant extracts were dissolved in dimethylsulfoxide (DMSO) and 50 μ l from 100 mg/ml concentration of each extract was added to each well. After incubation for 24 h at 36°C ± 1°C, inhibition of the bacterial growth was measured in mm. The experiment was repeated three times, and DMSO was used as control (Smâni *et al*, 1999).

Antifungal activity assay

Antifungal activity of plant extracts was assayed by the diffusion technique on PDA growth medium. The fungal suspension was prepared by using sterile saline solution (0.85%) equal 106 conidia/mL. Then, 100 μ l of suspension was spread on Petri dishes. A cork borer was used to make 6 mm diameter wells in the cultivated PDA agar; the wells were then filled with 50 μ l from 100 mg/ ml concentration of each extract. The inhibition of the fungi mycelial growth around the wells was measured after 72 h of incubation at (28 ± 2) °C. DMSO was used as control samples for each experiment and each extract form was evaluated with three repetitions (Talibi *et al*, 2012; Thangavelu *et al*, 2013)

RESULTS AND DISCUSSION

Various solvents were used to extract *Artemisia herba-alba* in this study. The percentage of oil yield, 0.9%, (v/w) was within ranges of the isolated *A. herba-alba* oils from different countries in the literature (Belhattab *et al*, 2014).

Over the past years, A. *herba-alba* oil has been examined in detail and numerous chemotypes have been

reported from plants grown- in the same country at different sites or various other countries (Mohamed *et al*, 2010). In present study, chromatogram of leaves *Artemisia herba alba* (Fig. 1).

Among the 43 compounds identified from the *A*. *herba-alba* oil, 10 compounds have been previously reported in different *Artemisia* species including *A*. *herba-alba*: acetophenone, methyleugenol, capillene, isospathulenol, 2,4-pentadiynylbenzene, aromadendrene, limonene derivatives, patchoulane, globulol, and n-hexadecanoic acid in addition to different ketons and phenols compounds (Table 1).

The major constituents of the essential oil were 2,4-

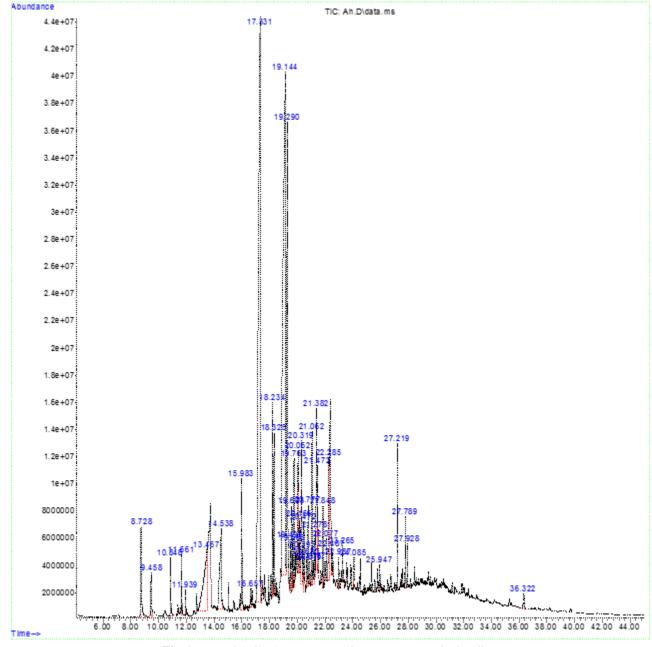


Fig. 1 : Essential oils chromatogram of leaves Artemisia herba alba.

 Table 1: Chemical composition of A. herba-alba essential oil using GC-MS.

n	RT	Area%	Library/ID	CAS	Formula	Synonames	Available	Categories ^a	Chemical structure
1	8,728	1.37	Benzaldehyde	000100-52-7	C ₇ H ₆ O	Phenylketone	Parsley leaf, almond bitter almond	Flavor and fragrance agents	
2	9,458	0.72	1-Propynyl-benzene	000673-32-5	C ₉ H ₈		1-Methyl-2 pheny lacetylenepropylb enzene	Lomatium dissectum	
3	10,848	0.68	Acetophenone	000098-86-2	C ₈ H ₈ O	1- Phenyl-1-ethanone	Artemisia herba- alba	Flavor and fragrance agents	i ()
4	11,661	0.48	Dimethyl acetal benzaldehyde	001125-88-8	C ₉ H ₁₂ O ₂	Alpha,alpha- dimethoxytoluene	Rhubarb, potato	Flavor and fragrance agents	
5	11,939	0.33	Benzyl methyl ketone	000103-79-7	C ₉ H ₁₀ O	2-Propanone	Jasminum sambac	Flavoring agents	-O
6	13,467	4.31	Benzoic acid	000065-85-0	C ₇ H ₆ O ₂	Phenylcarboxylic acid	Cassia, tea leaf cinnamon leaf	Antimicrobial, flavoring agents	H°
7	14,538	3.25	Benzeneacetic acid	000103-82-2	C ₈ H ₈ O ₂	Phenyl acetic acid	Pepper black pepper seed oil	Flavor and fragrance agents	но
8	15,983	1.82	Methyleugenol	000093-15-2	C ₁₁ H ₁₄ O ₂	4-Allyl veratrole	Artemisia variabilis Artemisia campestris	Fragrance agents antifungal, repel many insect	
9	16,657	0.31	trans-Cinnamic acid	000140-10-3	C ₉ H ₈ O ₂	Trans-3-phenyl-2- propenoic acid	Cassia bark, basil leaf oil	Flavor and fragrance agents	Ho
10	17,331	224.33	Agropyrene; Capillen; Capillene	000520-74-1	C ₁₂ H ₁₀	Hexa-2,4-diyn-1- ylbenzene	Artemisia capillaris, Triticum repens.	Natural substances and extractives	
11	18.00	2.02	Isospathulenol	006750-60-3	C ₁₅ H ₂₄ O	1H Cycloprop[e] azulen-7-ol, decahydr o-1,1,7-trimethyl-4- methylene-, [1ar-(1aα, 4aα,7β,7aβ,7bα)]-	Artemisia campestris Artemisia variabilis	Natural substances and extractives	

Table 1 continued....

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	, yo f	6	50	à	7500		\$	-8-	Δ	×,	111111	<i>4</i> 6	Å.
				Natural substances and extractives					Natural substances and extractives	Natural substances and extractives	Cosmetic, flavor and fragrance agents		
		Artemisia áracunculus	Johreniopsis seseloides	Gartic, leek			Artemisia abrotarum		Artemisia abrotarum	Artemisia abrotanum Artemisia argyi	Coriander seed oil		
	Ethenyi 4-tert-burylbenzoate	2,4.Pentadiynylbenzene	2-methylnaphthalene	Furan, 2,4-dimethyl-	Sopropyl styryl ketone	1,3-Heradienylbenzene	[laR-(lac,4ac,7c,7aß,7bc)]-Decahydro- l,1,7-trimethyl-4-methylene-1H- cycloprop[e]azulene	Guaia-4,11-diene	7-Oxabicyclo[4.1.0]heptane, 1-methyl- 4-(3-methyloxiranyl)-	1H-3a,7-Methanoazulene, octahydro- 1,4,9,9 tetramethyl-	myristic acid		1H-Cycloprop[e]azulen-4-οl, decabydro-1,1,4,7-tetramedhyl-, [1aR- (1aα,4β,4aβ,7α,7aβ,7box)]-
	C13H4O2	C ₁₁ H ₈	C ₁₁ H ₁₀	O'H'O	C ₁₂ H ₄ O	CoHu	C ₁₃ H ₄₄	C ₁₂ H ₂₄	CaH4O1	CIH	C ₁₄ H ₂₁ O2	C12HaM	C ₁₅ H ₄₀ O
	015484-80-7	041268-41-1	0091-57-6	003710-43-8	003160-32-5	041635-77-2	000489-39-4	087745-31-1	000096-08-2	025491-20-7	000544-63-8	065197-40-2	1000150-05-1
	benzoic acid, 4-(1,1- dimethylethyl)-, ethenyl ester	2,4-Pentadiynylbenzene	2-methylnaphthalene	2,4-Dimethylfuran	4-methyl-1-phenyl-1- Penten-3-one	Benzene, 1,3-benadienyl-	Aromandendrene	Aciphyllene.	limonene diepoxide	Patchoulane	Tetradecanoic acid	2-Ethyl-3-methylquinolin- 4-amine	Epiglobulol
pə1	1.57	27.33	8.52	15.0	0.39	0.37	125	1.76	0.41	0.41	2.21	0.37	0.94
Table I continued	18.325	19.144	19.29	19.492	19.603	19.666	19.763	20.062	20.166	20.166	20,319	20,395	20,472
Table	12	13	14	51	16	17	18	19	8	8	21	77	8

Table 1 continued....

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24	20,680	0.40	3,5-Diamino-4- phenylazopyrazole	003656-02-8	C ₂ H ₃₂ N ₄				ર્બ્યુ
25	20,777	0.64	(2E,4S,7E)-4-Isopropyl- 1,7-dimethy lcyclodeca- 2,7-dienol	198991-79-6	C15H24O	Germacrene-4-ol			-8
26	20,875	0.39	Isoaromadendrene epoxide	1000159-36-6	C12H24O				
27	21,062	1.23	6,10,14-trimethyl 2- Pentadecanone	000502-69-2	C11H16O	Hexahydrofamesyl acetone	Cerastium candidissimum	Flavor and fragrance agents	Lilili
28	21,167	0.35	Globulol	051371-47-2	C15H20	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl	Artemisia campestris Artemisia variabilis	Natural substances and extractives	
29	21.278	0.38	4-Methoxy-3- (phenoxymethyl)benzaldeh yde	1000273-81-3	C15H1605				2
30	21.382	2.63	Isopsoralen	000523-50-2	C11H4O2	Angelicin	Angelica root oil	Natural substances and extractives	3
31	21.472	1.24	Clovane diol	002649-64-1	C ₁₂ H ₂₆ O ₂	Clovanediol, (38,3a8,6R,78,9a8)-1,1,7- trimethyl-3,4,5,6,8,9,9a,10-octahydro- 2H-tricyclo[6.3.1.01,5]dodecane-3,6- diol	Salvia canariensis	natural substances and extractives	ţ,
32	21,848	0.61	Hexadecanoic acid, methyl ester	000112-39-0	C17H24O2	methyl palmitate	Salvia officinalis oil	flavor and fragrance agents	~~~~~
33	22,077	0.32	Isophytol	000505-32-8	C ₂₀ H ₄₀ O	1-Hexadecene-3-ol, 3,7,11,15- tetramethyl	jasmin	flavor and fragrance agents	
34	22,285	1.74	n-Hexadecanoic acid	000057-10-3	C16H22O2	Palmitic acid	Artemisia herba alba; A. verlotorum		·
35	22,487	0.3	Acetoxypentadecane	1000245-62-3	C1,H ₂₄ O ₂	Pentadecan-2-yl acetate			Y
36	22,987	0.38	Thunbergol	025269-17-4	C ₃₀ H ₃₄ O	Isocembrol			~3
38	24,085	0.29	Octadecanoic acid	000057-11-4	C11H201	Stearic acid	Flax seed oil	flavoring agents and adjuvants	~~~ J

Table 1 continued....

Table 1 continued....

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^ahttp://www.thegoodscentscompany.com/search2.html

Octateration: actu 0000/r-11-4 CaFH;NO; 5-(Dimethylamino)benzene-1,3-diol Frax seed out 1,3-Benzenediol, 5- 040248-00-8 CaFH;NO; 5-(Dimethylamino)benzene-1,3-diol Frax seed out (dimethylamino) (dimethylamino) 5-(Dimethylamino)benzene-1,3-diol Frax seed out Phthalic acid, di(2- 1000377-93-5 CaHi,NO; 5-(Dimethylamino)benzene-1,3-diol Frax seed out Phthalic acid, di(2- 1000377-93-5 CaHi,NO; 2,6-diphenyl-4-aminophenol Frax seed out 4-amino-2.6 050432-01-4 CaHi,NO; 2,6-diphenyl-4-aminophenol Frax seed out McCyclohenylbenzamide 1759-68-8 CaHi,NO; 2,6-diphenyl-4-aminophenol Earth seed out Nr-Cyclohenylbenzamide 1759-68-8 CaHi,O; 3,7-dimethyloctahydorolff- Earth seed out Nr-Cyclohenylbenzamide 023445-02-5 CaHi,O; 3,7-dimethyloctahydorolff- Earth seed out Outbebol 023445-02-5 CaHi,O; 3,7-dimethyloctahydorolff- Earth seed out Tubebol 023445-02-5 CaHi,O; 3,7-dimethyloctahydorolff- Earth seed out	2	Table 1 continued	ned	Ī	1 1 200000	0.0.0	Sterrit and	5		
0.28 1,3-Benzenediol, 5- (dmethylamino) 040245-00-8 C_iH_i,NO ₂ 5-(Dimethylamino)benzene-1,3-diol 1 1.11 Phthalic scid, di(2- propyipentyl) ester 1000377-93-5 5 5 1		C80,42	62.0	Octadecanoic acid	+-11-/ S0000	CirHirO.	Steanc acid	Flax seed oil	tiavonng agents and adjuvants	3
1.11 Phthalic acid, di(2- propy/pentyf) ester 1000377-93-5 C CitH2:NO CitH2:NO <t< td=""><td></td><td>25,947</td><td>0.28</td><td>1,3-Benzenediol, 5- (dimethylamino)</td><td>040248-00-8</td><td></td><td>5-(Dimethylamino)benzene-1,3-diol</td><td></td><td></td><td>-</td></t<>		25,947	0.28	1,3-Benzenediol, 5- (dimethylamino)	040248-00-8		5-(Dimethylamino)benzene-1,3-diol			-
0.7 4-amino-2,6 050432-01-4 C ₁₃ H ₁₃ NO 2,6-diphenyl-t-aminophenol 0.36 N-Cyclobenylbenzamide 1759-68-8 C ₁₂ H ₂₄ O ₃ 0.35 Cubebol 025445-02-5 C ₁₂ H ₂₄ O ₃ 0.35 Cubebol 025445-02-5 C ₁₂ H ₂₄ O ₃ 0.35 Cubebol 025445-02-5 C ₁₂ H ₂₄ O ₃ 0.35 Cubebol 025445-02-5 C ₁₂ H ₂₄ O ₃ 0.35 Cubebol 025445-02-5 C ₁₂ H ₂₄ O ₃		27,219	1.11	Phthalic acid, di(2- propylpentyl) ester	1000377-93-5					<u> </u>
0.36 N-Cyclohenylbenzamide 1759-68-8 C ₁₃ H ₂₀ O; 0.35 Cubebol 023445-02-5 C ₁₃ H ₂₄ O 1 3,7-dimethyloctahydro-1H- Lavandin oi flavoring agents 1 3,7-dimethyloctahydro-1H- cyclopropa[1,2]benzen- 5,7-dimethyloctahydro-1H-		27,789	0.7	4-amino-2,6 diphenyiphenol	050432-01-4	1	2,6-diphenyl-4-aminophenol			3
0.35 Cubebol 023445-02-5 C ₁₃ H ₂₄ O (3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl- Lavandin oi 3,7-dimetryloctahydro-1H- cyclopenta[1,3]cyclopropa[1,2]benzen- 3-ol		27,928	0.36	N-Cyclohexylbenzamide	1759-68-8	C ₂₃ H ₂₆ O ₃				040
		36,322	0.35	Cubebol	023445-02-5	C15H10	(3S, 3aR, 3bR, 4S, 7R, 7aR) -4-Isopropyl- 3, 7-dimethyloctahydro-1H- cyclopenta[1,3]cyclopropa[1,2]benzen- 3-ol	Lavandin oi	flavoring agents	ξ×.

pentadiynylbenzene (27.33%), capillene (24.33%), 2methylnaphthalene (8.52%), benzoic acid (4.31%), benzeneacetic acid (3.25%), isopsoralen (2.02%), isospathulenol (2.02%), myristic acid (2.21%) and methyleugenol (1.82%). Previous studies also reported that 2,4-Pentadiynylbenzene was a major component of the root oil of A. dracunculus cultivated in Iran with approximately similar area percentage, 22.2% (Haghi and Ghasian, 2010), whereas capillene was documented as a main component of A. capillaris oil with high percentage (32.7%) (Cha et al, 2005). Polyanskaya et al (2007) also found that the main constituents of the essential oil from glauca capillene (11–60%) Α. were and benzyldiacetylene (1-31%), which agrees with our study. 2-methylnaphthalene, benzoic acid and myristic acid were found in the chemical composition of essential oils of different plants, such as Johreniopsis seseloides (1.70%), Crinum latifolium (0.70%) and Thevetia peruviana (3.10%), respectively (Maia et al, 2000; Tram et al, 2003; Masoudi et al, 2005). Isospathulenol was found in two types of Artemisia with different percentage areas, A. verlofiorum (0.1%) and A. lobelii (0.4%) (Carnat, 1998; Polina et al, 1998). Methyleugeno was documented in the chemical profile of essential oil of Drimys brasiliensis (0.70%) (Limberger et al, 2008).

The chemical classification of *A. herba alba* essential oil in this study was separated into five main classes: homoaromatic compounds (70.25%), terpenes (11.69%), fatty acids (4.85%), heteroaromatic compounds (3.51%), and others (8.92%) (Table 2).

Homoaromatic compounds with unsaturated hydrocarbon chain represented 52.75% of the total essential oil constituents, and they were represented by 2,4-pentadiynylbenzene (27.33%), capillene (24.33%), 1,3-hexadienylbenzene (0.37%) and benzene, 1-propynyl «beta»-methylstyrene (0.72). Sesquiterpens constituted the second subclass of essential oil (10.58%) and they were represented by 11 compounds, among which were isospathulenol (2.02%), aciphyllene (1.76), aromandendrene (1.25), clovane diol (1.24%), epiglobulol (0.94%) and patchoulane (0.41%). Zouari et al (2010) analyzed the essential oil of Tunisian A. herba alba and found oxygenated monoterpenes constituted the main chemical class of the oil (50.53%), whereas oxygenated sesquiterpenes represented 15.65% and sesquiterpenes hydrocarbons were 3.06%. In Iraq, the GC-Ms analysis of methanolic extract of A. herba alba found fatty acids were the predominant compounds (Al-Ibrahemi and Hasan, 2019). In Jordan, the chemical analysis of A. herba alba oil showed that monoterpenes were the main components (39.3%) and thujones represented 27.7%

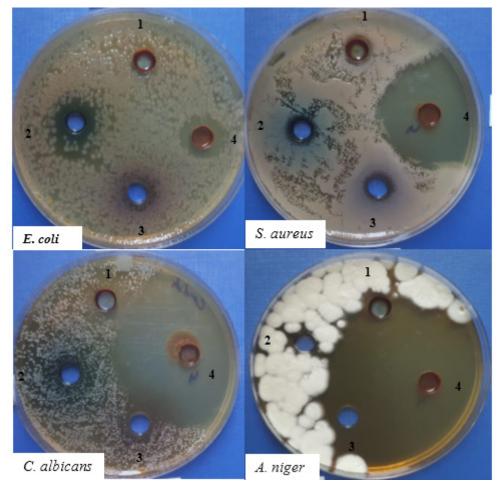


Fig. 2 : Antimicrobial activity of different *A. herba alba* extracts against pathogenic microorganisms. 1. Methanol root extract; 2. Methanol leaves extract; 3. Water leaves extract; 4. Essential oil.

followed by sabinyl acetate (5.4%) and germacrene D (4.6%) (Mohamed *et al*, 2010). Although, Al-Ibrahemi and Hasan (2019) detected artimisinin flavonoid in *A. herba alba* obtained from Karbalaa City, the present study could not identify it.

The antimicrobial activities of different extracts of A. herba alba were evaluated against two pathogenic bacterial and two pathogenic fungi. The result showed that the essential oil of A. herba alba had a broad spectrum effect on all tested microorganisms (Table 3). The inhibition zones were in the range of 15-53 mm, Gram-Negative bacteria (E. coli) appeared to be less sensitive (15mm) to the A. herba alba oil than Gram-Positive bacteria and both fungi (C. albicans and A. niger) were more sensitive to the oil than both bacteria types with 51 and 53 mm inhibition zone, respectively. No antimicrobial activity was recorded to the water and methanol extracts of root of A. herba alba against all tested microbials, whereas the methanol extract of leaves exhibited activity against E. coli only (Fig. 2). All results matched with the other findings that investigated the antimicrobial activity of A. herba alba, as it was reported

potential antimicrobial activity of essential oil of *A. herba alba* and absence of activity of water and alcoholic extracts (Mohamed *et al*, 2010; Zouari *et al*, 2010). Zouari *et al* (2010) also found similar results with 11.3 mm inhibition zone against *E. coli* and 51.0 mm against *A. oxysporum* by using the same experimental method and 10 μ L of *A. herba alba* essential oil. Goudjil *et al* (2016) also reported strong antifungal activity of oil *A. herba alba et al* (2018) found that ethanol extract of *A. herba alba* grown in Iraq had antibacterial activity against different species, which does not match with the present study.

The antimicrobial activity of *A. herba alba* essential oil could belong to specific compounds detected in essential oil in the present study, for example, previous study showed that capillene has antimicrobial activity against oral bacteria (Cha *et al*, 2005), while capillene represents 24.33% of total essential oil in this study. In addition, benzoic acid was used as preservative, antimicrobial and flavoring agent in foodstuffs (Lu *et al*, 2013). Also, Methyleugenol was reported to have antimicrobial activity (Medina *et al*, 2005; Marchese,

N.	Main chemical class	Subclass chemical type	Area %
1.	Homoaromatic compounds		70.25
		Unsaturated hydrocarbon chain	52.75
		Carboxylic acid	7.87
		Ester	2.98
		Ether	2.3
		Ketone	1.9
		Aldehyde	1.75
		Phenol	0.7
		Aniline	0.64
2.	Terpenes		11.69
		Sesquiterpen	10.58
		Diterpenes	0.7
		monoterpene	0.41
3.	Fatty acids	,	4.85
4.	Heteroaromatic compounds		3.51
5.	Others		8.92
		Protein kinase	0.4
		Indene	8.52
	Total	1	99.86

 Table 2 : The main chemical classes of A. herba alba oil.

Table 3: Diameters of inhibition zones through agar diffusion method of different A. herba alba extracts against pathogenic microorganisms.

	Types of Extracts	Inhibition Zone Diameters in mm						
	-, , , , , , , , , , , , , , , , , , ,	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger			
1	Methanol root	0.0	0.0	0.0	0.0			
2	Methanol leaves	14	0.0	0.0	0.0			
3	Water leaves	0.0	0.0	0.0	0.0			
4	Essential oil	15	37	50	53			

2017). However, the activity might be synergistic effects belonging to mixture constituent and not to particular compounds. The activity of essential oils against microorganisms was associated with cyclic monoterpenes compounds and it was noticed that monoterpenes were able to enlarge membrane fluidity and cause inhibition of a membrane enzyme. In yeast cells, monoterpene compounds cause inhibition of respiration and increasing of membrane permeability (Cox *et al*, 2000).

CONCLUSION

This study gives a first database of chemical constituents of *Artemisia herba alba* growing wild in Tib, Iraq, and found to be a new chemo type for *A. herba alba* oil. Bioassays confirmed the potential of essential oils against microorganisms studied, especially against fungus. Consequently, work must be undertaken for further investigation of novel anti-fungal agents from *A. herba alba* essential oil.

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