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GC-MS Analysis and Antimicrobial Activity of *Artemisia Herba Alba* Plant from Libya

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ABSTRACT

Artemisia Herba-alba plant is well known in Libya as a folk medicine treatment for parasitic worms. Identification of biologically active constituents was necessary and guide for useful research. Primarily phytochemical screening was performed on ethanolic extract and revealed presence of most phytochemicals such as alkaloids, carbohydrates, saponines, glycosides, phenols, flavonoids, proteins and amino acids, phytosterols and diterpenoids. More advanced analysis tool, GC-MS, was also used identifying many biologically active compounds including fatty acids and their esters, long chain alcohols, sterols, terpenoids, heterocyclic compounds and peptides. The highest concentration in the chromatogram were 5,5-Dimethyl-1-ethyl-1,3-Cyclopentadiene, 1,6-Dimethylhepta-1,3,5-triene, (-) Spathulenol. Antibacterial assay of the extract was accomplished showing significant activity against two pathogenic organisms, Escherichia coli (Gram negative bacteria) and Staphylococcus aureus (Gram positive bacteria) comparing to control compound, gentamycin.

Graphical abstract:

Artimisia Herba Alba plant.

Keywords: *Artemisia Herba Alba,* Biological activity, Antioxidant activity, Phytochemical screening.

INTRODUCTION

Artemisia herba-alba plant belongs to Asteraceae family, is a short perennial green bush (Figure 1) growing in the grasslands and deserts of the Middle East and North Africa [1]. The plant is widely used in the folk medicine to treat diabetes, bronchitis, diarrhea, hypertension and neuralgias [2].

Figure 1. *Artimisia Herba Alba* plant.

Also, reports of using the plant for treating the cough, stomach and intestinal upset, the common cold, measles was also demonstrated, in addition to the using in treatment of different kinds of worms such as roundworms, pinworms, tapeworms and hookworms [3]. Hydro distilled essential oil of the plant showed antimicrobial and antioxidant [4]. In Libya, the plant is widely distributed in the country and commonly used in treatment of parasitic worm [5].

MATERIALS AND METHODS

Plant Material: *Artimisia Herba Alba* plant material (aerial part) was collected from rocky region south of Gaser Khiar city (32°38′44.0″ N 13°50′44.3″ E) in March 2024, the plant was identified by plant taxonomist from Biology department, Science Faculty, Elmergib University. The whole material was cleaned and dried in the shade till complete dryness then cut to small pieces before grinding to fine powder using electrical blender, the powder was stored in a sealed bottle at room temperature until use in analysis.

Phytochemical Screening: 15 g of the plant fine powder was stirred in 250 mL flask with 150 mL of the desired solvent for 48 hrs. using Jenway 1002 Stirrer machine at room temperature, the solvents were; ethyl acetate, ethanol and water. The extracts were filtered then concentrated to dryness using rotary evaporator and kept in cool place until using in phytochemical screening tests [6]. Aqueous extract was prepared separately by heating 10 g of the powder in 150 mL of water at 70°C for 30 min., after cooling, the mixture was filtered and kept in fridge.

In vitro antimicrobial activity test: The antimicrobial activity of the plant extract was determined using the widely used procedure, Agar well diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. A hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [7]. The well method employs an inoculum suspension adjusted as described for the broth dilution standard method, and agar plates should be inoculated within 15 min after adjusting the suspension. The entire dried agar surface is evenly streaked in three different directions. Allowing the agar surface to dry for no more than 15 min, Then, a hole with a diameter of 6 to 8 mm is punched aseptically with

a sterile cork borer or a tip, and a volume (20-100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. After placing the extract solution, plates should be incubated within 15 min. after it's have been disposed. After incubation times of 48 hrs., the resulting inhibition zone diameters (in mm) surrounding the wells should be measured to the nearest whole millimeter at the point at which there is prominent reduction in growth. If growth is insufficient at the recommended times, the plates should be re-incubated and read later [8].

GC-MS Analysis: About 10.0 ml of methanol was added to 2.0 g of a homogenized powder sample, the mixture was shaken vigorously for 60 min. The extract was centrifuged and the supernatant was collected and filtered through 0.20 µm syringe to remove particulate matter. The filtered extract was concentrated using rotary evaporation. The dried concentrated extract was dissolved in 5.0 ml ethanol, then, 1.0 µL of reconstituted sample was injected into the GC injection port using a microliter syringe [9]. The chemical composition of roots powder ethanolic extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5 °C /min to 230 °C hold for 2 min. increased to the final temperature 290 °C by 30 °C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min. and diluted samples of 1 µL were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

RESULTS AND DISCUSSION

GC-MS Analysis: Extensive analysis of ethanol extract of aerial part of *Artemisa Herba Alba* produced comples spectrum of peaks with different areas and peak heights. The chromatogram (Figure 2), shows the retention time which lasts 82.45 mins., (X axis) and relative abundance (Y axis), the ethanol solvent is a very strong polar solvent and could extract most of the polar and nonpolar components of the plant material even with small concentrations. The identified compounds were included wide diverse of chemical classes; fatty acids and their esters, alkyl halides, pentacyclic triterpenes derivatives, long chain alcohols, amides and interesting alkaloids.

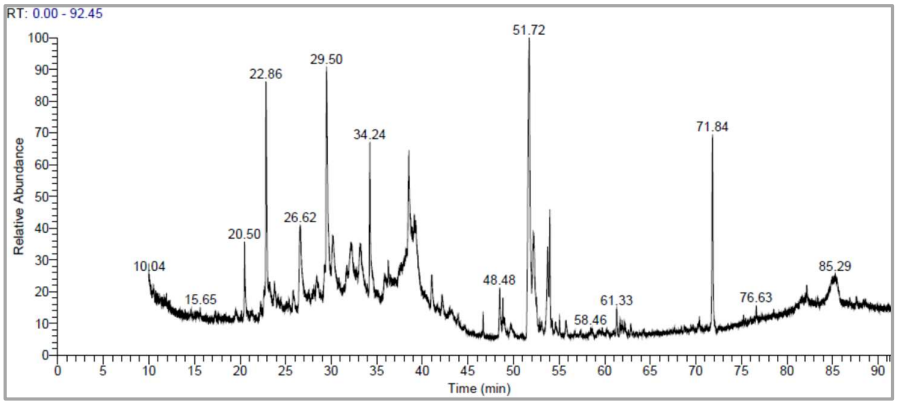

Figure 2. GC-MS chromatogram of *Artemisia Herba Alba* plant ethanolic extract.

Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) library. The spectrum of the unknown component was linked with the spectrum of the known components stored in the NIST library. The retention times, names, peak

areas, molecular weights and molecular formulas of highest peak area and most important components are shown in table 1.

Table 1. Phytochemicals identified in ethanolic extract of *Artemisia Herba Alba* plant

No.	RT	Compound Name (Class)	Peak Area	MW	MF
1	10.50	Atriopeptin I	0.41	2081	$C_{83}H_{135}N_{29}O_{30}S_2$
2	10.50	Secretin	0.41	3036	$C_{130}H_{220}N_{44}O_{40}$
3	20.50	3-Methyl-6-(1-methylethyl)-2-Cyclohexen-1-one	2.61	152	$C_{10}H_{16}O$
4	22.86	5,5-Dimethyl-1-ethyl-1,3-Cyclopentadiene	7.81	122	C_9H_{14}
5	22.86	1,6-Dimethylhepta-1,3,5-triene	7.81	122	C_9H_{14}
6	23.78	2-Ethylidene-6-methyl-3,5-Heptadienal	1.22	150	$C_{10}H_{14}O$
7	23.78	Apamin	1.22	2025	$C_{79}H_{131}N_{31}O_{24}S_4$
8	24.17	Ascaridole epoxide	0.37	184	$C_{10}H_{16}O_3$
9	25.36	2-Methylene-Cholestan-3-ol	0.50	400	$C_{28}H_{48}O$
10	25.81	Serotonin	0.82	176	$C_{10}H_{12}N_2O$
11	25.81	3-Phenylthioacrylic acid, S-benzoxazol-2-yl ester	0.82	281	$C_{16}H_{11}NO_2S$
12	26.60	2,2-Dimethyl-3-(2-methyl-1-propenyl) Cyclopropane carboxylic acid	3.43	168	$C_{10}H_{16}O_2$
13	27.52	(*Z*)-9-Octadecenoic acid	0.13	282	$C_{18}H_{34}O_2$
14	28.12	10-Undecenoyl chloride	0.36	202	$C_{11}H_{19}ClO$
15	28.12	2-(1-Methyl-2-Nitroethyl) Cyclohexanone	0.36	185	$C_9H_{15}NO_3$
16	28.12	(Z)- 9-Tetradecenal	0.36	210	$C_{14}H_{26}O$
17	28.37	10-Methyl-8-tetradecen-1-ol acetate	0.84	268	$C_{17}H_{32}O_2$
18	28.37	6-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one	0.84	297	$C_{16}H_{27}NO_4$
19	28.37	Methyl-6-oxoheptanoate	0.84	158	$C_8H_{14}O_3$
20	29.49	3-Phenyl-2-Propenoic acid, ethyl ester	7.60	176	$C_{11}H_{12}O_2$
22	30.18	1-Heptadec-1-ynyl-cyclopentanol	2.20	320	$C_{22}H_{40}O$
23	30.18	10,10-Dichloro-bicyclo[6.2.0]decan-9-one	2.20	220	$C_{10}H_{14}C_{l2}O$
24	30.84	(*E*)-9-Tetradecenoic acid	0.38	226	$C_{14}H_{26}O_2$
25	30.84	*(Z)*-9-Pentadecenol	0.38	226	$C_{15}H_{30}O$
26	30.84	*Cis*-13-Eicosenoic acid	0.38	310	$C_{20}H_{38}O_2$
27	32.19	1-Chloro-7-Heptadecyne	1.68	270	$C_{17}H_{31}Cl$
28	32.19	Bicyclo[2.2.1]heptane-2-carboxylic acid,3,3-dimethyl, methyl ester	1.68	182	$C_{11}H_{18}O_2$
29	33.18	5-Oxo-Prolyl-L-Histidinamide	2.19	265	$C_{11}H_{15}N_5O_3$
30	34.24	(-)-Spathulenol	4.92	220	$C_{15}H_{24}O$
31	35.83	6-Chloro-*N*-Ethyl-1,3,5-Triazine-2,4-Diamine	0.83	198	$C_5H_8ClN_5$
32	36.26	Jasmonic Acid	0.90	201	$C_{12}H_{18}O_3$
33	39.19	Gala-l-ido-octonicamide	0.19	255	$C_8H_{17}NO_8$
34	38.51	(3*E*,10*Z*)-Oxacyclotrideca-3,10-diene-2,7-dione	3.75	208	$C_{12}H_{16}O_3$
35	38.51	Butyl-6,9,12-hexadecatrienoate	3.75	306	$C_{20}H_{34}O_2$
36	39.28	2-Amino ethanethiol hydrogen sulfate	1.22	157	$C_2H_7NO_3S_2$
37	39.28	4-O-β-D-Galactopyranosyl- β -D-Glucopyranose,	1.22	342	$C_{12}H_{22}O_{11}$
38	42.20	2-Acetyl-3-(2-Cinamido) ethyl-7-methoxy indole	0.24	362	$C_{22}H_{22}N_2O_3$
39	42.20	*Cis*-Vaccenic acid	0.24	282	$C_{18}H_{34}O_2$
40	46.67	Hexadecanoic acid, methyl ester	0.66	270	$C_{17}H_{34}O_2$
41	46.67	Pentadecanoic acid, 14-methyl, methyl ester	0.66	270	$C_{17}H_{34}O_2$
42	48.48	Hexadecanoic acid	2.00	256	$C_{16}H_{32}O_2$
43	48.84	Hexadecanoic acid, ethyl ester	1.01	284	$C_{18}H_{36}O_2$
44	48.99	β-Santonin	0.77	246	$C_{15}H_{18}O_3$
45	51.72	Pallensin	14.15	264	$C_{15}H_{20}O_4$
46	51.72	Acepromazine	14.15	326	$C_{19}H_{22}N_2OS$
47	53.09	Corymbolone	0.39	236	$C_{15}H_{24}O_2$
48	53.75	*Trans*-13-Octadecenoic acid	2.47	282	$C_{18}H_{34}O_2$
49	53.99	Ethyl oleate	3.35	310	$C_{20}H_{38}O_2$
50	55.75	Gibberellic acid	0.65	346	$C_{19}H_{22}O_6$
51	71.84	(*Z*)-9-Octadecenamide (Oleamide)	7.28	281	$C_{18}H_{35}NO$
52	71.84	(*Z*)-13-Docosenamide	7.28	337	$C_{22}H_{43}NO$

The most abundant compounds in the spectrum came out at 51.72 min. with 14.15 % peak area, and were; Acepromazine and Pallensin. The next high peak area group of compounds came out at 22.86 min. with peak area of 7.86 %, were; 5,5-Dimethyl-1-ethyl-1,3-Cyclopentadiene and 1,6-Dimethylhepta-1,3,5-triene. The third major component in the chromatogram was (*E*)-3-phenyl-2-Propenoic acid ethyl ester, which appeared at 29.49 min. with 7.60% peak area. The tricyclic sesquiterpene alcohol, (-) Spathulenol eluted at 34.24 min. with concentration of 4.92 % peak area, spathulenol was isolated for the first time in 1975 with an earth-aromatic odor and bitter-spicy taste, the compound was already isolated from our plant family members, *Artemisia vulgaris* and *Artemisia dracunculus* [10]. In table 2, a list of the interesting components identified in the extract and their biological importance.

Table 2. Classes and importance of selected compounds from *Artemisia Herba Alba*

Compound Name	Compound class	Importance
Atriopeptin I	Poly Peptide	Regulation of fluid and electrolyte balance [11].
Secretin	Poly Peptide	Regulation of fluid balance and pH levels [12].
Apamin	Poly Peptide (in bee venom)	Research on SK channels activation [13].
Ascaridole epoxide	Mono terpene	antimalarial activity [14].
2-methylene-Cholestan-3-ol	Phytosterol	Antioxidant [15].
Serotonin	Indole derivative	Regulates numerous biological processes including cardiovascular function, bowel motility, ejaculatory latency, and bladder control [16].
3-Phenylthio acrylicacid, S-benzoxazol-2-yl ester	Multifunction group	Acidifier, arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity, inhibit the production of uric acid. [17]
(*Z*)-9-Octadecenoic acid (Oleic acid)	Unsaturated fatty acid	Help boost memory, may also be responsible for the hypotensive (blood pressure reducing) effects of olive oil [18].
(*Z*)-9-Tetradecenal	Fatty acid aldehyde.	Increase zinc bioavailability [19].
Methyl-6-oxoheptanoate	Oxo ester	Anticancer activity [20].
Spathulenol	Tricyclic sesquiterpene	Anticholinesteras, antinociceptive, anti-hyperalgesic, anti-mycobacterial, antioxidant, anti-proliferative, anti-oedematogenic, cytotoxicity [21].
Jasmonic acid	Cyclopentanone substituted acetic acid.	Improves plant growth and development by regulating root development [22].
Cis-Vaccenic acid	Mono unsaturated fatty acid	Antibacterial activity and hypolipidemic effect in rats [23].
Hexadecanoic acid methyl ester	Fatty acid methyl ester (methyl palmitate)	Antimicrobial effect against clinical pathogenic bacteria [24].
β-Santonin	Sesquiterpene	Inhibited the growth of the breast cancer cells [25].
Acepromazine	Phenothiazine derivative	Sedative and antiemetic drug for animals. [26].
Corymbolone	Sequiterpene	Exhibited significant anti-plasmodial properties [27].
Trans-13- Octadecenoic acid	Fatty acid	Anti-inflammatory, antiamdrogenic, dermatitigenic, anaemiagenic, insecticides, flavor [19].
Gibberellic acid	Multifunction pentacyclic compound	Plant growth regulator, improve germination, plant development, productivity, and the quality of food [28].
Oleamide	Amide of oleic acid	Participates in the biochemical mechanisms underlying the drive to sleep, thermoregulation, and antinociception [29].
(Z)-13-Docosenamide	Amide of Docosenoic acid	Antinociceptive and anti- inflammatory activities [30].

Phytochemical Screening: Phytochemical screening of *Artemisia Herba Alba* using polar solvents, table 3, revealed presence of different useful phytochemicals including; alkaloids, carbohydrates, saponines, glycosides, phenols, flavonoids, proteins and amino acids, phytosterols and diterpenoids. Tannins, phlopatannins and anthraquinones were absent in all extracts.

As expected, both Aqueous and ethanolic extracts were richer with phytochemicals than ethyl acetate extract. Biological activity of the phytochemicals found in the plant are well documented, exhibit antioxidation, antibacterial, anticancer and antimalarial properties [31].

Table 3. Phytochemical screening of *Artemisia Herba Alba*

Test	Aqueous	EtOAc	EtOH
Alkaloids	+	-	+
Carbohydrates	+	+	+
Saponines	+	-	+
Glycosides	+	-	+
Phenols	+	-	+
Tannins	-	-	-
Phlopatannins	-	-	-
Falvonoids	+	-	+
Proteins and amino acids	+	+	+
Phytosterols	+	+	+
Diterpenoids	+	-	+
Anthraquinones	-	-	-

APPLICATION

Antimicrobial Activity: The antibacterial behavior of *Artemisia herba alba* areal part ethanolic extract was assessed by Agar well diffusion method. The pathogenic selected were; *Escherichia coli* (Gram negative bacteria) and *Staphylococcus aureus* (Gram positive bacteria). The results were promising in terms of inhibition level observed as the extract was notably active against the assayed pathogens. The inhibition zones were 26 ±0.2 (mm) and 25±0.2 (mm) for *Escherichia coli* and *Staphylococcus aureus* respectively comparing to 20±0.1 (mm) and 18±0.1(mm) for control sample as shown in table 4.

Table 4. Antibacterial activity of studied plant extract

Pathogenic microorganism	Plant extract	Gentamycin
Escherichia coli (Gram negative bacteria)	26±0.2 (mm)*	20±0.1 (mm)
Staphylococcus aureus (Gram positive bacteria)	25±0.2 (mm)	18±0.1(mm)

* Inhibitions zones were represented as mm±SD.

CONCLUSION

Artemisia herba alba plant is well known in folk medicine in Libya and middle east region, and the results obtained from this study were very promising in terms of important compounds identified in GC-MS analysis and good biological effect against pathogenic organisms.

Conflict of Interest: Author declares no conflict of interest.

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