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Original Article

Phytochemical Analysis of Selected Indigenous Plants and Essential Oils by Gas Chromatography/Mass Spectrometry Dedicate Anti-bacterial, Anti-Proliferative and Anti-Inflammatory Activity

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ABSTRACT

Background: Natural compounds from medicinal plants have received much attention and research as resources in developing of new drugs. The challenge of for developing antibacterial agent from natural products for the treatment of MERSA, fungal and viral disease. **Materials and Methods:** This study analyzed the chemical composition of *Artemisia judaica* and *Juniperus procera* methanol extracts and three essential oils include lemongrass (*Cymbopogon citratus*), clove (*Syzygium aromaticum* (L.) merr and perry) and tea tree (*Melaleuca alternifolia*). **Results:** The obtained results revealed the presence of bioactive functional molecules that exert several biological properties like antibacterial, anti-proliferative and anti-inflammatory activities such as caryophyllene, eugenol, palmitic acid and limonene. **Conclusion:** It was concluded that, it is promising in development of a novel plant-based drugs for MERSA, anti-inflammatory and antioxidant activities for mangment of different pathological conditions

Keywords: *Artemisia judaica*, *Juniperus procera*, Essential oils, GC/MS active compounds.

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INTRODUCTION

Plants have been used for thousands of years as medicines for treating a variety of different diseases and medical complaints. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases.[1] Saudi Arabia is rich in medicinal plants due to biodiversity and climatic variation; with approximately 2,250 wild plant species.[2] Many of these have been used by the local communities for the treatment of many ailments. All plants contain primary metabolites that play a crucial role in the fundamental metabolic processes of constructing and maintaining plant cells. In contrast, secondary metabolites aid specific plant species in their interactions with the environment. These secondary metabolites, including volatile oils, are particularly important for the medicinal properties of plants, mainly contributing to their antimicrobial activity.[3] The antimicrobial effectiveness of plants is attributed to various mechanisms of action, some mechanisms involve the destruction of microbial membranes, while others disrupt cellular metabolism; others can modify the biofilm formation, while others exhibit an inhibitory effect on the production of bacterial capsules; and some can reduce the virulence of microbes.[4]

Extraction is the main step for the recovery and isolating bioactive phytochemicals from plant materials prior to component analysis.[5] GC-MS method used for the analysis of the obtained extracts and it can be an interesting tool for testing the amount

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of some active principles in herbs used in cosmetic, drugs, pharmaceutical, food industry, environmental and forensic applications.[6] It combines two analytical techniques to a single method of analyzing mixtures of chemical compounds, gas chromatography separates the components of the mixture and mass spectroscopy identifies each of the components separately.[7]

Bioactive components including phenolics, flavonoids, terpenoids, alkaloids and their derivatives are important attributable to their applications in various fields including medicine, pharmacology, agriculture and food industry.[8] The active compounds differ from plant to plant due to their biodiversity and they produce a specific physiological response on the human body.[9] Some of the selected herbs include *Juniperus procera* Hoechst. Ex Endl. (*J. procera*, hereafter), belongs to the Cupressaceae family, is an evergreen, highland, tropical and dioecious tree species spanning from Yemen and Saudi Arabia to southern and eastern Africa.[10] *J. procera* serves as a natural source of photochemical components that possess potential antibacterial, antioxidant, insecticidal and anticancer properties.[11] *Artemisia judaica* a perennial aromatic small shrub with pubescent leaves, is a member of the Asteraceae family, which grows widely in the southern desert of Algeria, in Egypt and in the Middle East (Sinai Peninsula, Jordan and Saudi Arabia).[12] Numerous studies investigated the biological importance of *A. judaica* in Saudi Arabia; for instance, the volatile oil contents of *A. judaica* grown in the northern region of Saudi Arabia have demonstrated the presence of diverse phytoconstituents that exhibit antimicrobial properties.[13]

This research presents a comprehensive Gas Chromatography/Mass Spectrometry (GC/MS) analysis of the herbal mixture extract including *Juniperus procera* (leave and berries) and *Artemisia judaica* extracts. Additionally, the study examines the essential oils mixture, which includes lemongrass (*Cymbopogon citratus*), clove (*Syzygium aromaticum* (L.) Merr and Perry) and tea tree (*Melaleuca alternifolia*) oils.

MATERIALS AND METHODS

Chemicals

All the chemicals including methanol, chloroform and acetonitrile of analytical grade were purchased from Fisher Chemicals, Loughborough, U.K.

Collection and preparation of plant extracts

The plants were collected from two Saudi Arabian locations (Table 1). The plants were washed to remove impurities and dried under shade for three days, then were grounded to powder by blender and stored. The dried *Artemisia judaica* sample was extracted with methanol while the *Juniperus procera* was extracted by chloroform both in ratio of 1:20. The different extracts were filtered by Whatman filter paper#1 and then allowed to evaporate using rotary evaporators at 60°C until the solvent evaporates.[14] All extracts were stored at -20°C until used.

Extraction of mixture of essential oils

The three essential oils include lemongrass (*Cymbopogon citratus*), clove (*Syzygium aromaticum* (L.) *merr* and *perry*) and tea tree (*Melaleuca alternifolia*) oils were obtained from iHerb, a supplier of medicinal herbs in the United States, Korea and Hong Kong. The mixture of essential oils was prepared according 1:1:1 percentage. The oils were dissolved in acetonitrile (HPLC grade) for preparation (1%).

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The samples were first centrifuged at 3000 RPM for 10 min. 100 µL of each sample was dissolved in 0.5 mL acetonitrile (purity 99.9%, high grade). 10 µL was injected to GC/MS. The flow rate of carrier gas helium was 1 mL min^{-1}. The instrument was equipped with Agilent 5975C-VL MSD mass spectrometer with Agilent 7693A automatic liquid sampler was used for analysis of plant extracts. Agilent HP-5MS (30 m in length x 0.25 mm id x 0.25 µm thickness stationary phase film, Agilent, USA) was used for separation. Data acquisition was employed via Agilent ChemStation for GC/MS software version.[15,16]

RESULTS AND DISCUSSION

Gas Chromatography and Mass Spectroscopy (GC-MS) has been validated to be an important tool for bioprospecting of bioactive compounds in plants. The GC/MS analysis of plant extracts as shown in (Table 2) and essential oils as indicated in (Table 3) reveals the existence of various components linked to diverse secondary metabolites. The results revealed the existence of caryophyllene in both plant extracts and essential oils, is a common sesquiterpene among plant species. The antibacterial and antifungal activities of caryophyllene were shown in previous

Table 1: The plants and its location in Saudi Arabia.

Plants	Family	Part used	Location
Artemisia judaica	Asteraceae	Areal part	Collected from Unaizah city (26°05'17"N 43°58'59"E).
Juniperus procera	Cupressaceae	Leave	Collected from El-Shefaa region of Al-Taif city (21°04'18.4"N 40°18'53.7"E).
		Berries	

Table 2: The main constituents of *Artemisia judaica* and *Juniperus procera* methanol extracts plant extracts detected by GC/MS.

Peak No.	Retention time	Compound name	Molecular formula	Match	Retention match	CAS Library
1	13.762	Dodecane, 2,7,10-trimethyl-	$C_{15}H_{32}$	842	846	74645-98-0 mainlib
2	16.658	Isocaryophillene	$C_{15}H_{24}$	916	920	mainlib
3	17.273	α-Caryophyllene	$C_{15}H_{24}$	917	932	6753-98-6 replib
4	17.713	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	855	867	31295-56-4 mainlib
5	18.679	γ -Elemene	$C_{15}H_{24}$	871	900	339154-91-5 mainlib
6	19.224	Ledene oxide-(II)	$C_{15}H_{24}O$	832	836	mainlib
7	19.604	Calarene epoxide	$C_{15}H_{24}O$			
8	19.704	Heptacosane	$C_{27}H_{56}$	825	848	1560-84-5 mainlib
9	20.554	5,8,11-Eicosatriynoic acid, methyl ester	$C_{21}H_{36}O_2$	676	679	mainlib
10	21.335	Myristic acid P1035Univ Homburg/Saar	$C_{14}H_{28}O_2$	808	898	544-63-8 Pfleger
11	21.685	(-)-Spathulenol	$C_{15}H_{24}O$	750	754	77171-55-2 mainlib
12	23.631	Heptacosane	$C_{27}H_{56}$	832	835	593-49-7 replib
13	24.451	Palmitic acid	$C_{16}H_{32}O_2$	P1210	862	57-10-3 replib
14	27.067	Phytol	$C_{20}H_{40}O$	793	808	150-86-7 replib
15	27.292	Nonadecane, 2-methyl-	$C_{20}H_{42}$	827	842	1560-86-7 mainlib
16	28.042	Nonadecane	$C_{19}H_{40}$	773	848	629-92-5 replib
17	29.108	4,14-Retro-retinol	$C_{20}H_{30}O$	702	737	16729-22-9 mainlib
18	29.168	Trans-Dehydroandrosterone, pentafluoropropionate octahydrophenanthrene	$C_{22}H_{27}F_5O_3$	602	608	mainlib
19	30.013	Androst-5-en-7-one, 3-(acetyloxy)-, (3β)-	$C_{21}H_{30}O_3$	724	765	25845-92-5 mainlib
20	30.418	4,14-Retro-retinol	$C_{20}H_{30}O$	732	769	16729-22-9 mainlib
21	30.553	Methandriol	$C_{20}H_{32}O_2$	669	679	521-10-8 replib
22	30.854	5-Androstene, 4,4-dimethyl-	$C_{21}H_{34}O$	731	734	mainlib
23	31.049	Pentacosane	$C_{25}H_{52}$	805	814	629-99-2 replib
24	31.264	Androst-2-en-17-one, 3-hydroxy-, (5β)-	$C_{19}H_{28}O_2$	692	699	57289-70-0 mainlib
25	31.404	9(11)-Dehydrotestosterone	$C_{19}H_{30}O_2$	653	668	2398-99-4 mainlib
26	31.564	4,14-Retro-retinol	$C_{20}H_{30}O$	760	800	16729-22-9 mainlib
27	31.804	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	512	527	55124-79-3 mainlib
28	32.134	Retinol	$C_{20}H_{30}O$	780	804	68-26-8 replib

Peak No.	Retention time	Compound name	Molecular formula	Match	Retention match	CAS Library
29	33.044	Abietic acid	$C_{20}H_{30}O_2$	807	836	514-10-3 mainlib
30	33.190	Methylprednisolone Acetate	$C_{24}H_{32}O_6$	625	663	53-36-1 replib
31	33.455	Trans-Dehydroandrosterone, trifluoroacetate	$C_{21}H_{27}F_3O_3$	661	681	mainlib
32	33.720	Retinal, 9-cis-	$C_{20}H_{28}O$	639	674	514-85-2 mainlib
33	34.250	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	626	732	23470-00-0 mainlib
34	34.865	Eicosane, 7-hexyl-	$C_{26}H_{54}$	746	764	55333-99-8 mainlib

Table 3: The main constituents of mixture essential oils detected by GC/MS.

Peak No.	Retention time	Compound name	Molecular formula	Match	Retention match	CAS Library
1	6.339	Limonene	$C_{10}H_{16}$	924	925	138-86-3 replib
2	6.409	α-Phellandrene	$C_{10}H_{16}$	862	889	99-83-2 replib
3	6.494	Eucalyptol	$C_{10}H_{18}O$	903	904	470-82-6 mainlib
4	7.669	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}O$	935	950	99-85-4 replib
5	8.054	4-Nonanone	$C_9H_{18}O$	891	970	4485-09-0 replib
6	8.774	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	930	938	586-62-9 mainlib
7	9.275	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{11}H_{18}O_2$	932	933	78-70-6 mainlib
8	10.145	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-methylethyl)-	$C_{10}H_{18}O$	879	886	29803-82-5 replib
9	10.690	Terpineol, cis-β-	$C_{10}H_{18}O$	825	840	7299-41-4 mainlib
		trans-Chrysanthemal	$C_{10}H_{16}O$	787	805	20104-05-6 mainlib
10	10.845	6-Octenal, 3,7-dimethyl-	$C_{10}H_{18}O$	845	849	106-23-0 mainlib
11	10.940	6-Octenal, 3,7-dimethyl-	$C_{10}H_{18}O$	843	843	106-23-0 replib
12	11.241	Bicyclo [3.1.1] hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1α,2β,5α)]-	$C_{10}H_{16}O$	852	860	18881-04-4 replib
13	12.881	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	837	847	106-25-2 replib
14	13.286	2,6-Octadienal, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	918	920	106-26-3 replib
15	13.416	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	898	898	106-25-2 replib
16	14.042	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(1-methylethenyl)-	$C_{10}H_{16}O$	756	785	1195-92-2 replib
17	15.817	Eugenol	$C_{10}H_{12}O_2$	925	930	97-53-0 mainlib
18	15.992	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	$C_{12}H_{20}O_2$	894	895	105-87-3 mainlib
19	16.803	Caryophyllene	$C_{15}H_{24}$	946	947	87-44-5 mainlib
20	17.978	γ-Elemene	$C_{15}H_{24}$	892	900	339154-91-5 mainlib

research.[17,18] The effectiveness of terpenes would be expected to relate to both of their structural configuration and functional groups. The anti-fungal and anti-bacterial efficacy against *Aspergillus niger, Staphylococcus aureus*[19] and *Bacillus cereus* were attributed to the existence of active ingredient in the tested *Juniper procera* extract such as α-Humulene (α-Caryophyllene).[20] The obtained results indicated the presence of bioactive compound spathulenol (an oxygenated sesquiterpene) which is used in aromatizing compositions for food and as a flavoring agent in the food and cosmetics industries as well as it has antibacterial property.[21] Research on essential oils with spathulenol as a major component has documented various biological effects including anti-inflammatory properties[22] and antimicrobial activities.[23] Eugenol (phenylpropanoid) is an aromatic compound that belongs to the group of phenols. Eugenol exhibits various biological properties including antioxidant, antimicrobial,[24] anti-proliferative and anti-inflammatory effects.[25] It has demonstrated antibacterial characteristics against numerous species like *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* and this potential has been attributed to the presence of free OH group in its structure.[26] A study assessed the antimicrobial efficacy of clove essential oil (containing 68.52% of eugenol, 19% of β-caryophyllene, 10.15% of 2-methoxy-4-[2-propenyl]phenol acetate and α-caryophyllene) and rosemary essential oils against numerous American Type Culture Collection (ATCC) standard bacteria and fungi using agar diffusion and broth microdilution methods, clove essential oil exhibited the most effective inhibitory activity against *Proteus vulgaris* with an inhibition zone of (18.2±1.3 mm) and *S. epidermidis* with an inhibition zone (16.8±1.2 mm).[27] Palmitic acid is the primary saturated fatty acid have been reported to inhibit the growth of many oral microbes.[28] Antifungal activity is also reported for palmitic acid against several fungal strains.[29] Fatty acids can act as anionic surfactants and have antibacterial and antifungal properties at low pH.[30] The obtained results of GC/MS showed the existence of Limonene in the essential oils, a compound of the terpene family. Limonene is popular for its various properties like antibacterial, antifungal, antiviral and anti-biofilm.[31] An earlier investigation demonstrated that limonene interacts with the cytoplasmic membranes of bacteria leading to loss of membrane integrity, dissipation of proton-motive forces and inhibition of the respiratory enzymes.[32] However, antimicrobial effectiveness of plant extracts and essential oils are due to the nature and content of these various constituents that may act synergistically. It was concluded that, it is promising in development of a novel plant-based drugs for MERSA, anti-inflammatory and antioxidant activities for management of different pathological conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC/MS: Gas Chromatography-Mass Spectrometry; **ATCC:** American Type Culture Collection; **MERSA:** Methicillin-resistant Staphylococcus aureus.

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