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Original Article

Phytochemical Identification of Active Components of Some Medicinal Herbs and Oils Relative to its Biological Activities in Saudi Arabia

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ABSTRACT

Background: Herbal medicine and its bioproducts are rich in bioactive compounds that promising in drug development. These phytochemical constituents may be secondary metabolites that exert high potency. **Materials and Methods:** This study investigated the phyto-constituents bioactive compounds from the aerial parts of some Saudi medicinal plants using n-hexane extracts (*Trichodesma trichodesmoides, Baccharoides schimperi, Anabasis articulate* and *Achillea fragrantissima*) and a mixture of essentials oils in equal ratio (*Arnica montana*, Chamomile, *Salvia officinalis*, Oenothera, Walnut leaves and Flaxseed) using GC-MS. **Results:** The GC-MS analysis of plants and essentials oils revealed the presence of various compounds like terpenoids and vitamins related to its anti-microbial activity, antioxidant and anti-inflammatory activity. **Conclusion:** It was concluded that, the broad range of terpenoids and vitamins are the main components in the hexane extract. These possess different biological activities. Further studies will be carried out *in vivo* and *in vitro* to identify the mechanism of action via signaling pathway.

Keywords: Herbs, Oils, Bioactive Compounds, Terpenoids, Vitamins.

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INTRODUCTION

The use of herbal medicine as traditional alternative or complementary medicine is important and effective in several health complications.[1] The chemical components of medicinal plants, possess anti-bacterial, anti-fungal, antioxidant, anti-inflammatory and pain-relieving properties.[2] The oils extracted from plants contain a wide range of chemical components and are renowned for their varied medicinal importance. These oils from plants through both traditional and advanced methods.[3] Various methods have been employed to extract oils, such as hydro-distillation, steam-distillation, microwave and organic solvent extraction affect its quality.[4] Extraction with different solvents and conditions in recovering and isolating bioactive phytochemicals from plant materials, preceding the analysis of individual components.[5] Primary metabolites are present in all plants, whereas secondary metabolites enable a specific plant species to interact effectively with its environment.[6] The environmental in Saudi Arabia is a good source of traditional herbal medicine to develop adaptive mechanisms.[7] Gas Chromatography-Mass Spectrometry (GC-MS) is commonly employed for the direct analysis of chemical components in plant-based medicine.[8] The GC-MS analysis depend on solvent polarity, components, fatty acids, volatile essential oils and lipids.[9] Since different decades, medicinal plants were used in treatment of some chronic diseases, as diabetic, hypertension, wound healing, gastric irritability, antimicrobial and anti-inflammatory agents, the active pure compound released from plants is affected by purity of solvents, temperature, method of extraction. The rational of current study is to optimize the suitable conditions for maximum purity of extraction of components from different plants collected from different regions in Saudi Arabia and a mixture of essential oils that suitable for *in vitro* and *in vivo* study to evaluate its biological activity.

MATERIALS AND METHODS

Collection and preparation of plants extract

The target plants included: *Trichodesma trichodesmoides, Baccharoides schimperi, Anabasis articulate* and *Achillea fragrantissima* were collected from different regions in Saudi Arabia (Table 1). All the aerial parts of dried plant samples

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extraction by using n-hexane (Riedel-de Haen, Germany).[10] All freeze-dried extracts were stored at -20°C prior to further experiments.

Selection of essential oils with high anti-inflammatory effects

In addition, mixture of 6 essential oils in equal ratio (*Arnica montana*, Chamomile, *Salvia officinalis*, Oenothera, Walnut and Flaxseed) were purchased from (Piping Rock Health Products, USA).

Identification the bioactive compounds by GC-MS

The n-hexane extracts obtained were subjected to GC-MS analysis for the determination of various bioactive volatile compounds. The samples were first centrifuged at 3000 revolutions per minute 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 mass selective detector mass spectrometer with Agilent 7693 A automatic liquid sampler was used for analysis of plant extracts and essential oils. Agilent HP-5MS columns (30 m in length x 0.25 mm id x 0.25 µm thickness stationary phase film, Agilent, USA) was used for separation.[11,12] Data acquisition was employed via Agilent ChemStation for GC/MS software version.

RESULTS

The phytochemical constituents of plant extracts by GC-MS showed a potential anti-inflammatory properties as reported by previous studies. The most class of compound contains terpenoids in (Table 2 and Figure 1). In current study, *Achillea fragrantissima*, in agreement with a previous result when analyzed using GC-MS, showing presence of 35 compounds were monoterpenes (61.40%). Data obtained in (Table 3 and Figure 2) showed 24 bioactive content of mixture of 6 essentials oils as anti-inflammatory.

DISCUSSION

In vitro and *in vivo* study showed that, terpenoids exert a significant role in management of various diseases as anticancer agents, antimicrobial, anti-inflammatory, antioxidants, allergic, neuroprotective, anti-aggregation of platelets, anti-coagulation, sedative and analgesic through the activity of monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes and glycoside compounds.[13] In addition, the santolina alcohol as monoterpenes compound from extract *Achillea* flowers exert anti-inflammatory and antioxidant properties have restorative and neuroprotective effects on spinal cord alpha-motoneurons after lesion.[14,15] α-Thujone showed induction of cell death, reduced proliferation and invasive at human glioblastoma multiform and human glioblastoma.[16] On the other hand, phytol exhibits anti-inflammatory activity, possibly via inhibition of cyclooxygenase and nuclear factor kappa and interleukin-1β dependent pathways.[17] Recent experiments conducted on rats with induced colitis using intra rectal injection of 2,4,6-trinitrobenzene sulfonic acid have shown that treatment involving α-linolenic acid effectively minimizes mucosal damage. This was evaluated through various biochemical and histological inflammation markers.[18]

Presence of monoterpenes and sesquiterpenes prevailing.[19] In previous study indicated the limonene compound role in decreased the levels of (tumor necrosis factor-α, interleukin-6 and interleukin-1β and increased the level of interleukin-10) as marker pro and anti-inflammation at gastric ulcer in

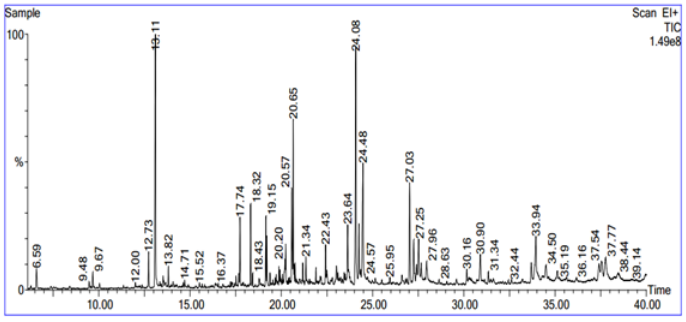

Figure 1: GC chromatogram of the four Saudi plants extracts.

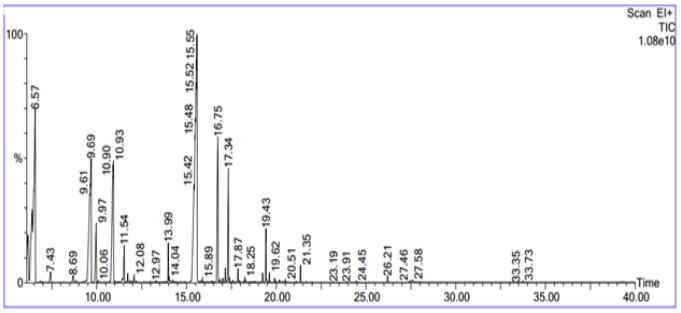

Figure 2: GC chromatogram of the six essential oils.

Table 1: Plant samples collected from different regions in Saudi Arabia.

Sl. No.	Scientific name	City	Global Positioning System
1	*Trichodesma trichodesmoides var. tomentosum*	Al-Namas	(19.0595990, 42.2297440)
2	*Baccharoides schimperi*	Fifa	(17.2541932, 43.0920545)
3	*Anabasis articulate*	Tabuk, Alqan	(29.1032810, 35.3974460)
4	*Achillea fragrantissima*	Al-Jawf	(30.2813680, 38.2318460)

Table 2: Identification of compounds by GC-MS analysis of the n-hexane extracts in four Saudi plants.

Sample No	Retention time	Name of the compound	Molecular formula	Class of compound	Retention Match	CAS Library
1	6.594	Santolina alcohol	$C_{10}H_{18}O$	Irregular monoterpenes	844	21149-19-9 mainlib
2	9.670	Thujone	$C_{10}H_{16}O$	A volatile monoterpene ketone	939	546-80-5 replib
3	12.726	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	$C_{10}H_{18}O_2$	Tertiary alcohols	722	13741-21-4 replib
4	13.106	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	$C_{10}H_{18}O_2$	Tertiary alcohols	747	13741-21-4 replib
5	13.822	Hexadecane	$C_{16}H_{34}$	Alkane	846	544-76-3 replib
6	17.738	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	Sesquiterpenoids	834	54833-48-6 mainlib
7	18.318	Cedrene	$C_{15}H_{24}$	Sesquiterpenoids	879	11028-42-5 mainlib
8	18.428	Decane,2,3,5,8-tetramethyl-	$C_{14}H_{30}$	Alkanes	778	192823-15-7 mainlib
9	19.154	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4methylene-,[1ar(1aà,4aà,7a,7aa,7bà)]-	$C_{15}H_{24}O$	Sesquiterpenoids	885	6750-60-3 replib
10	19.209	7-Tetracyclo [6.2.1.0(3.8)0(3.9)]undecanol,4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	Sesquiterpenoids	811	74842-43-6 mainlib
11	20.574	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$	An oxygenated terpenes	778	mainlib
12	20.654	Hexadecane, 2,6,11,15-tetramethyl-	$C_{20}H_{42}$	Acyclic diterpenoids	838	504-44-9 replib
13	20.754	6-(p-Tolyl)-2-methyl-2-heptenol	$C_{15}H_{22}O$	Alkane	740	39599-18-3 mainlib
14	21.180	Tetradecane,2,6,10-trimethyl-	$C_{15}H_{32}$	Sesquiterpenoids	706	14905-56-7 mainlib
15	21.345	Myristic acid P1035 Univ Homburg/Saar	$C_{14}H_{28}O_2$	Fatty acids	934	544-63-8 Pfleger
16	21.900	Octadecane	CH_3-$[CH_2]_{16}$-CH_3	Alkanes hydrocarbon	920	593-45-3 replib
17	22.425	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Isoprenoid hydrocarbon (terpenoids)	894	102608-53-7 mainlib
18	22.490	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	Sesquiterpene	757	502-69-2 mainlib
19	22.800	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	$C_{14}H_{26}O$	Diterpenoids	751	mainlib
20	23.020	Vitamin A aldehyde	$C_{18}CH_{28}O$	Vitamin	792	116-31-4 mainlib
21	23.105	Pentadecanal-	$C_{15}H_{30}O$	Fatty aldehydes	827	2765-11-9 mainlib
22	23.636	Eicosane, 2-methyl-	$C_{21}H_{44}$	Alkane	833	1560-84-5 mainlib

Sample No	Retention time	Name of the compound	Molecular formula	Class of compound	Retention Match	CAS Library
23	24.256	Cyclopropanepropionic acid,2-[(2decylcyclopropyl)methyl]-, methyl ester	$C_{22}H_{38}O_2$	Fatty acid ester	640	10152-67-7 mainlib
24	24.476	Palmitic acid (n-hexadecanoic acid)	$CH_3(CH_2)_{14}COOH$	A saturated fatty acid	910	57-10-3 replib
25	26.612	Sulfurous acid, octadecyl pentyl ester	$C_{23}H_{48}O_3S$	An organooxygen	846	mainlib
26	27.032	Phytol (3,7,11,15-tetram ethyl-2-hexadecen-1-ol)	$C_{20}H_{40}O$	Acyclic diterpene alcohol	933	102608-53-7 mainlib
27	27.252	Eicosane, 2-methyl-	$C_{21}H_{44}$	Alkane	838	1560-84-5 mainlib
28	27.527	α-Linolenic acid (9,12,15-Octadecatrienoic acid, (Z,Z,Z)-)	$C_{18}H_{30}O_2$	Unsaturated fatty acid	796	463-40-1 mainlib
29	27.662	Cyclohexanamine, N-cyclodecylidene-	$C_6H_{13}N$	Aliphatic amine	611	74810-29-0 mainlib
30	27.957	Stearic acid (octadecanoic acid)	$C_{18}H_{36}O_2$	Fatty acid	875	57-11-4 replib
31	29.598	Heptadecane, 2,6,10,15 tetramethyl-	$C_{21}H_{44}$	Alkane	764	54833-48-6 mainlib
32	30.163	1-Nonadecene	$C_{19}H_{38}$	Alkene	907	18435-45-5 replib
33	30.904	Eicosane,2-methyl-	$C_{21}H_{44}$	Alkane	837	1560-84-5 mainlib

Table 3: Identification of compounds by GC-MS analysis in essential oil mixture.

Sample No	Retention time	Name of the Compound	Molecular formula	Class of compound	Retention Match	CAS Library
1	6.404	Limonene	$C_{10}H_{16}$	Cyclic monoterpene	919	138-86-3 replib
2	6.574	Eucalyptol	$C_{10}H_{18}O$	Monoterpenoid	871	470-82-6 mainlib
3	6.859	1,3,6-Octatriene, 3,7-dimethyl-	$C_{10}H_{16}$	Cyclic monoterpenoids	776	13877-91-3 mainlib
4	7.429	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	C_7H_{10}	Monoterpene	945	99-85-4 replib
5	8.689	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	Monoterpene	940	586-62-9 mainlib
6	8.855	Benzene, 1-methyl-4-(1-methylethenyl)-	$C_{10}H_9F_3$	Monoterpene	928	1195-32-0 mainlib
7	9.690	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{16}O$	Terpenoids	951	1125-12-8 mainlib
8	9.970	Thujone	$C_{10}H_{16}O$	A volatile monoterpene ketone	933	546-80-5 mainlib
9	10.925	Camphor	$C_{10}H_{16}O$	Cyclic monoterpene ketone	928	76-22-2 mainlib

Sample No	Retention time	Name of the Compound	Molecular formula	Class of compound	Retention Match	CAS Library
10	13.326	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$	Phenol	871	501-92-8 mainlib
11	13.992	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	$C_{12}H_{20}O$	Bicyclic monoterpenoids	959	5655-61-8 mainlib
12	13.992	Bornyl acetate	$C_{12}H_{20}O_2$	Bicyclic monoterpenoids	949	76-49-3 replib
13	14.237	Thymol	$C_{10}H_{14}O$	Phenol	864	89-83-8 replib
14	15.892	Copaene	$C_{15}H_{24}$	Sesquiterpenoid	916	3856-25-5 replib
15	16.753	Caryophyllene	$C_{15}H_{24}$	Bicyclic sesquiterpene	951	87-44-5 mainlib
16	16.863	Bicyclo[2.2.1]heptan-2-one, 5-(acetyloxy)-4,7,7-trimethyl-, endo	$C_{10}H_{18}O$	Bicyclic sesquiterpene	746	55658-18-9 replib
17	17.023	1H-Cycloprop[e]azulene, decahydro-1,1,7-	$C_{15}H_{24}$	Sesquiterpenoids	936	72747-25-2 mainlib
18	17.163	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-,(E)-	$C_{15}H_{24}$	Terpenes	906	18794-84-8 mainlib
19	17.338	à-Caryophyllene	$C_{15}H_{24}$	Sesquiterpenoids	951	6753-98-6 replib
20	19.914	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	An oxygenated sesquiterpene	843	mainlib
21	20.514	Santalol, cis,à-	$C_{15}H_{24}O$	Sesquiterpene	771	19903-72-1 mainlib
22	27.582	Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	Fatty acids	901	13126-39-1 mainlib
23	33.365	Trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	Fatty acids	781	693-71-0 mainlib
24	39.327	ç-Tocopherol	$C_{29}H_{50}O_2$	Phenolic	837	7616-22-0 replib

rats.[20] A previous study reported that eucalyptol decreased cerulean-induced acute pancreatitis by preventing oxidative stress in mice and a clinical study in humans showed that inhalation of eucalyptus oil, which contains a considerable amount of eucalyptol, has an anti-inflammatory effect in patients after total knee replacement.[21] It has shown that thymol showed anti-inflammatory, antibacterial, antioxidant and healing properties.[22] Alpha-tocopherol prevents oxidative and inflammatory reactions triggered by ischemia/reperfusion injury, safeguarding cardiac function.[23] Plant extracted oils are becoming increasingly popular in pharmaceuticals, food, cosmetics, and perfumery. They are widely recognized for their antioxidant, antimicrobial, antifungal properties, as well as their ability to alleviate anxiety and pain.[24] It was concluded that, the broad range of terpenoids and vitamins are the main components in the hexane extract. These possess different biological activities. Further studies will be carried out *in vivo* and *in vitro* to identify the mechanism of action via signaling pathway.

CONCLUSION

In this study, analysis of a mixture of essential oils showed several components and the great majority of components identified in essential oils includes terpenes that exerted different biological effects.

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ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee of King Abdulaziz University Hospital, Jeddah, Saudi Arabia. The protocol was done according to the ethical guidelines of the 1975 Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

TAK, AA and EKB design protocol, TJA running experiments, TAK, SSM and AA analyzed data and interpretations. ALL authors revise manuscript and approve it.

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