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Original Paper

Bioactive compounds of methanolic extract of *Helianthemum lippii* grows in Hafr Al-Batin region, northeastern Saudi Arabia

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Helianthemum lippii (L.) Dum. Cours species of Saudi Arabia are a potential source of unique bioactives and chemical compounds due to the extreme environmental conditions under which the plants adapted to grow. These phytochemicals make the native *Helianthemum lippii* possibly interesting plant to investigate. In this study GC-MS analysis has been conducted to identify the most abundant phytochemical compounds in *H. lippii* methanolic extract. In total, 53 compounds have been identified in the studied extract. The identified phytochemicals included 6 compounds with eminent pharmatheotical importance recorded in relatively high percentage in the studied extracts, including Chloroform (0.29%), Benzeneethanol-Phenylethyl Alcohol (5.94%), 4-(1,1-dimethylethyl) 2,2-Dimethylcyclopropane carboxylic acid (1.04), Mebutamate (3.40%), Spermatheridine (1.17%) and Ethyl isoallocholate (1.13%), Oxirane, [4-(1,1-dimethylethyl) phenoxy]methyl] (5.0%). The resault of this study could provide additional clues about figuring out the formula of biomolecular therapy in future drug studies.

Keywords: *Helianthemum lippii*, GC-MS, Saudi Arabia, phytochemical compounds

1 Introduction

Saudi Arabia is an arid desert country with a few exceptional subhumid regions on the northeastern part, located in the with an area of about 2250,000 sq kms covering the major part of the Arabian Peninsula (Mohammed-Ibtisam et al., 2018). This study was carried out in Hafr Al-Batin region (28° 26′ 3″ N, 45° 57′ 49″ E), which is located in the Northeastern Province about 430 km north of Riyadh. This area is generally an arid desert lies in the dry valley of Wadi Al-Batin. The wild plants in this erea are highly adapted to the local environmental and utilize by people especially during the raining season. Very little is known about wild plants chemical composition in Hafr Al-Batin area, except few studies (Mohammed-Ibtisam et al., 2018; Zaman et al., 2008; Mandaville, 1990). There are approximately 100 taxa in the *Helianthemum* genus around world. *Helianthemum lippii* plant reported to be distributed in small patches in northern central and southern part of Kuwait (Zaman et al., 2008). *Helianthemum lippii* (L.) Dum. Cours is commonly known in Saudi Arabia as Raqruq that belongs to the family Cistaceae (Zaman et al., 2008). *H. lippii* is a small shrubby plant that grow up to 10–45 cm tall (Zaman et al., 2009). Seeds are very tiny and brown in color and mature during the raining season in February to April and then dispersed by wind during summer. The economic important of *H. lippii* is for being the main host plant associates for desert truffles (Tirmania and Terfezia) locally known as Fuq'a or Kamaa (Bokhary 1987; Omar et al., 2000; Bermaki et al, 2017). *Helianthemum* sp. is a valuable medicinal herb with a wide range of uses. Diarrhoeal and epigastric discomfort have been treated with this plant in the past (Meckes, 1999). *Helianthemum* plants are also used for the treatment of stomach illnesses, injuries, and burns around the world due to their anthelmintic, anti-inflammatory, antiulcerogenic, antiparasitic, antibacterial, analgesic, and vasodilating properties (Benitez et al., 2010 and Rubio-Moraga et al., 2013). Furthermore, the leaves and stems of the *H. syriacum* plants are used in drinks in Spain (Tardío et al., 2006). The chemical composition of this genus was studied previously. It has antioxidant compounds (Chemam et al., 2017); flavonoids, tannins, glycosides, simple phenolics, free reducing sugars, and

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saponines (Ermeli et al., 2012). Some of *Helianthemum* species are essential medicinal herbs that are utilized for a variety of purposes in a variety of places. However, there are currently no research addressing the biological activity or phytochemical constituents of many of these plants. Therefore, the purpose of the current study is to screen the abundant phytochemical compounds in the ethnolic extracts of *H. lippii* by gas chromatography-mass spectrometry (GC-MS). The result of this study would provide additional clues about figuring out the formula of biomolecular therapy in future drug studies.

2 Material and methods

2.1 Sample Coolection and processing

The present study was carried out at the Biology Department, College of Sciences, Hafr Al-Batin University (Saudi Arabia) from February to May, 2021. The plant samples were collected from Hafr Al-Batin region which is located in the northeastern province of the Kingdom of Saudi Arabia. The *Helianthemum lippii* plant materials (leaves and stems) were repeatedly washed by hand using distilled water so that any dust or other residues were removed. Then, the plant airdried at room temperature in a shady spot for around three weeks. They were then ground to a fine powder using an electric grinder and stored in tightly sealed bottles.

Twenty-five grams of fresh Plant powder sample were soaked in 250 ml of 96 percent methanol and shaken at 150 rpm for five days at room temperature before being kept in the refrigerator for one day. The extract was then vacuum-filtered through a Buchner funnel. The filtrate was centrifuged for 10 minutes at 3000 rpm, as well as the extract was concentrated in a rotary vacuum evaporator According to Tofighi et al. (2015), the crude was left in unsealed jars in the fume hood for 2 days at ambient temperature before being stored at 4 °C in a glass container before further usage.

The extracted sample was subsequently subjected to GC and GC-MS analysis. Components were discovered using the MS library NBS or their mass spectral data were compared with the documented ones (Stenhagen et al., 1974). The retention index was then used to help determine chemical identification (Sadtler,1986); some standard structural samples were injected for more confirmation.

2.2 Gas chromatography (GC) and GC-mass spectroscopy (GC-MS)

2.2.1 GC analysis

A HewlettPackard model 5985 B with a flame ionisation detector was used to evaluate the *Helianthemum lippii* extract (FID). A fused silica capillary column with a diameter of 60 m 0.32 mm and a carbowax coating of 60 m 0.32 mm was used.

The oven was set to increase from 50 to 200 degrees Celsius at a pace of 2 degrees Celsius per minute. The injector and detector were both set at 250 degrees Celsius. At a rate of 0.7 ml min^{-1}, helium has been used as a carrier gas. The data presented are the average of two assessments. Hydrocarbons (C8-C30 Aldrich Chemical Company) were used as references to compute the linear retention indices.

2.2.2 GC-MS analysis

This experiment was carried out on a Hewlett-Packard 5985B computer with an HP MS instrument system. The temperature of the ion source was 200 °C and the ionisation voltage was 70 eV. The GC-MS analysis was conducted at central Lab in National Research Centre, Egypt.

3 Results and discussion

The taxa of *Helianthemum* utilized in traditional medicine did not form a distinct group. There is a close correlation between methanol extract composition from *Helianthemum* leaves and their therapeutic applications. *Helianthemum* is a genus with over 100 species. In many nations, they are essential medicinal herbs that have multiple uses. It's worth noting that there is currently no research on the biological or phytochemical activity of several of these species (Rubio-Moraga et al., 2013). The GC-MS chromatogram analysis of the methanolic extract of *H. lippii* showed thirty-five major peaks (Figure 1). Also, the major phytochemical corresponding to the peaks were shown in (Table 1). The chemical compounds, molecular formula, and molecular weight were shown in (Table 1). From GC-MS analysis, extract of *H. lippii* contains 6 compounds that reported had biological activities as shown in (Table 2). These compounds such as Chloroform (RT, 5.45) which acts as liniment counterirritant for relief of deep seated pain (Rivera et al., 2005 and Tardio et al., 2006), Phenethyl alcohol (RT, 19.01) is reported to act as an antimicrobial, antiseptic and disinfectant that is used also as an aromatic essence and preservative in pharmaceutics and perfumery (Baudouin, 1976). Oxirane, [4-(1,1-dimethylethyl)phenoxy]methyl], RT (36.94) has an anti-inflammatory, analgesic and antipyretic). Oxirane effects (Baudouin, 1976). Mebutamate (RT, 41.36) has a sedative and anxiolytic drug with anti-hypertensive activity (Morin et al., 1963). Spermatheridine (RT, 46.69) act as an Anticancer, antimicrobial and an antifungal agent (Shakhatreh et al., 2016). Ethyl isoallocholate (RT, 60.63) was reported to inhibit dihydropteroate synthase (Kargutkar et al., 2018). Others have found similar findings

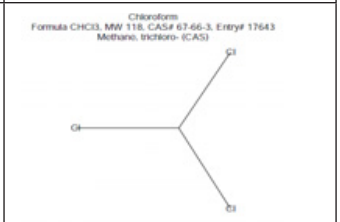
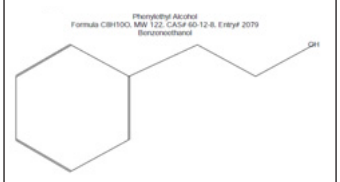
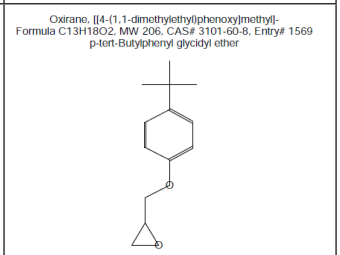
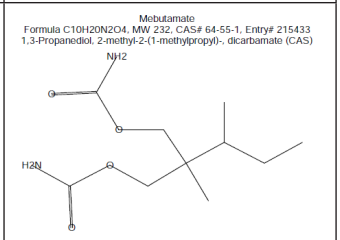
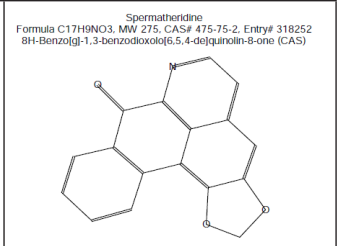
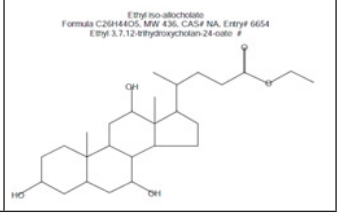
Table 1 GC-MS analysis of bioactive components of extract of *Helianthemum lippii*

S.NO.	RT	Area	Molecular wight	Compound name	Molecular female
1	5.14	4.97	130	4,4-dimethyl-3-hexanol	C8H18O
2	5.40	0.21	176	dichloromethyl ethyl sulfone	C3H6Cl2O2S
			229	7 phenyl trans bicyclo[4.3.0]non oxime	C15H19NO
			263	2,2-dimethyl-1-oxo-3,5-diphenyl-2H-1lambda~5~-pyrrole	C18H17NO
3	5.45	0.29	118	Chloroform	CHCl3
4	5.50	0.39	176	dichloromethyl ethylsulfone	C3H6Cl2O2S
			118	Chloroform	CHCl3
			229	7 phenyl trans bicyclo[4.3.0]non oxime	C15H19NO
5	5.80	0.51	176	dichloromethyl ethylsulfone	C3H6Cl2O2S
			229	7 phenyl trans bicyclo[4.3.0]non oxime	C15H19NO
			263	2,2-dimethyl-1-oxo-3,5-diphenyl-2H-1lambda~5~-pyrrole	C18H17NO
6	19.01	5.94	122	Phenethyl alcohol	C8H10O
7	19.08	2.00	122	Benzeneethanol (CAS)	C8H10O
8	19.12	2.47	122	Benzeneethanol (CAS)	C8H10O
9	19.20	5.32	122	Benzeneethanol (CAS)	C8H10O
10	19.94	0.27	260	Benzaldehyde, 3-benzyloxy-2-fluoro-4-methoxy	C15H13FO3
			122	Benzeneethanol (CAS)	C8H10O
11	21.20	0.42	122	Benzeneethanol (CAS)	C8H10O
12	21.27	0.82	122	Benzeneethanol (CAS)	C8H10O
13	24.69	1.12	168	Jasmonol	C11H20O
			224	1-Propene, 2-methyl-, tetramer (CAS)	C16H32
			196	Cyclohexane, 2,4-diisopropyl-1,1-dimethyl	C14H28
14	25.15	0.33	154	5-Isopropenyl-2-deutero-2-methylcyclohexanol	C10H17DO
			138	3,10-Dioxatricyclo[4.3.1.0(2,4)]dec-7-ene	C8H10O2
15	25.39	1.34	168	1-Propene, 2-methyl-, trimer (CAS)	C12H24
			224	1-Propene, 2-methyl-, trimer (CAS)	C16H32
			168	Jasmonol	C11H20O
16	27.72	1.78	168	7-Oxabicyclo[4.1.0]heptane, 2-methyl-l4(2-methyl oxirane) (CAS)	C10H16O2
			280	3-Methy-l4-(phenylthio), 2-propeny-l2,5-dihydrothiophene -1,1-dioxide	C14H16O2S2
17	32.39	1.55	206	Phenol, 4-(1,1,3,3-tetramethylbutyl) (CAS)	C14H22O
18	34.15	2.61	234	1-[3(2,6,6-Trimethylcyclohex-2-EN-1-YL)-4,5-dihydro-3-Hpyrazolyl] ethanone	C14H22N2O
19	35.76	0.50	304	3-[3-(butyl)dimethylsilyloxy-2-methyl-2-propenylidene] 2,4,4-trimethylcyclohexene	C16H20N2O2S
			382	6-Oxa-3-thia-2,4-diazadecanoic acid, 2,4-dimethyl-5-oxo-, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl ester	C18H26N2O5S
20	35.88	7.26	163	9-hydroxypyrimido[1,6-a]pyrimidin-4-one	C7H5N3O2
			280	(3,5-dimethyladamantyl)-phosphonic-acid,dichloride	C12H19Cl2OP
21	36.38	5.54	266	(2-Dodecen-1-yl)succinic anhydride	C16H26O3
			278	Decane, 5,6-bis(2,2-dimethylpropylidene)	C20H38

Continuation of table 1

S.NO.	RT	Area	Molecular wight	Compound name	Molecular female
22	36.94	5.09	262	Dodecylphenol (CAS)	C18H30O
			191	6-tert-Butylpyrano [2,3c] pyrrole2(6H)one	C11H13NO2
			206	Oxirane, [[4-(1,1-dimethylethyl)phenoxy]methyl]	C13H18O2
23	37.72	4.63	192	Alpha-ionone	C13H20O
24	38.43	1.04	192	1-(3,3-Dimethyl)-2,2-dimethylcyclopropane-3-carboxylic acid	C12H16O2
			218	2,2,7,7-tetramethyltricyclo[6.2.1.01,6]undec-5-en-4-one	C15H22O
25	41.36	3.40	232	Mebutamate	C10H20N2O4
			278	Decane, 5,6-bis(2,2-dimethylpropylidene)- (E,Z)	C20H38
26	41.58	5.62	262	Sulfurous acid, cyclohexylmethyl hexyl ester	C13H26O3S
			276	Sulfurous acid, cyclohexylmethyl hexyl ester	C14H28O3S
			140	4,4-dimethyl-1-octene	C10H20
27	41.78	1.18	207	Methyl 2-oxo-2,3-dihydro-1,3-benzoxazole-6-carboxylate	C9H9N3O3
			250	Acetic acid (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methyl ester	C16H26O2
28	42.41	7.58	232	Mebutamate	C10H20N2O4
			266	(2-Dodecen-1-yl)succinic anhydride	C16H26O3
29	43.22	3.57	247	[(Cyano-2-cyclopent1yl) amino]-2N,N-(pentamethylene) propanamide	C14H21N3O
			318	2-(Iodomethyl)-5-(4-methylphenyl)oxolane	C12H15IO2
			270	Phenylacetic acid, 2-(1-adamantyl)ethyl ester	C18H22O2
30	44.30	14.15	247	[(Cyano-2-cyclopent1yl) amino]-2N,N-(pentamethylene) propanamide	C14H21N3O
			247	6-Methyl-3-(methoxyphenyl)-2-aza-4,7-dioxabicyclo-[3.3.0] octane	C13H13NO4
			262	Allyl-3-Dimethyl(phenyl)silylbutyrate	C15H22O2Si
31	44.56	0.39	261	1-Cyclopropyl-3-hydro methylindole-4,7-dione	C14H15NO4
			388	4-Picolylamine, N,N-dinonyl	C26H48N2
32	45.25	4.86	276	chrysene-5,6:4b,10b:11,12-trioxide	C18H12O3
			277	3,6-Diphenyl-3-H5H1,7-àdihydropyrrolo[1,2c]oxazo	C18H15NO2
			326	7-Bromobicyclo	C15H11BrN4
33	45.73	0.58	460	Dimethoxyglyc erol docosyl ether	C27H56O5
			204	17-(1,5-Dimethylhexyl)-10,13-dimethylhexadecahydrocyclopent a[a]phenanthren-7-ol	C27H46O2
			204	2-alpha Hydroxy-5-alpha cholest an,3-one	C27H46O2
34	46.69	1.17	275	12-hydroxy-N-methyl benzo[b]phenanthridi none	C18H13NO2
			275	Spermatheridine	C17H9NO3
			332	5,7-Dimethoxy-6-(2-methylbutanoyl)-4-propyl-2H, 8H chromen-2-one	C19H24O5
35	60.63	1.13	594	Flavone. 4'oh,5oh,7dioglucoside	C27H30O15
			436	ethyl iso allocholate	C26H44O5

Table 2 Reported biological activities of compounds present in *Helianthemum lippii* extract

S.NO	RT	Area	Molecular wight	Compound name	Molecular female	Reported bioactivity	Structure
1	5.50	0.39	118	Chloroform	CHCl3	liniment counterirritant for relief of deep seated pain	
2	19.01	5.94	122	Phenethyl alcohol	C8H10O	an antimicrobial, antiseptic, and disinfectant that is used also as an aromatic essence and preservative in pharmaceutics and perfumery	
3	36.94	5.09	206	Oxirane, [[4-(1,1-dimethylethyl) phenoxy]methyl]	C13H18O2	anti-inflammatory, analgesic, and antipyretic effects	
4	41.36	3.40	232	Mebutamate	C10H20N2O4	sedative and anxiolytic drug with anti-hypertensive	
5	46.69	1.17	275	Spermatheridine	C17H9NO3	anticancer, antimicrobial and an antifungal agent	
6	60.63	1.13	436	thyl iso-allocholate	C26H44O5	inhibitor for dihydropteroate synthase	

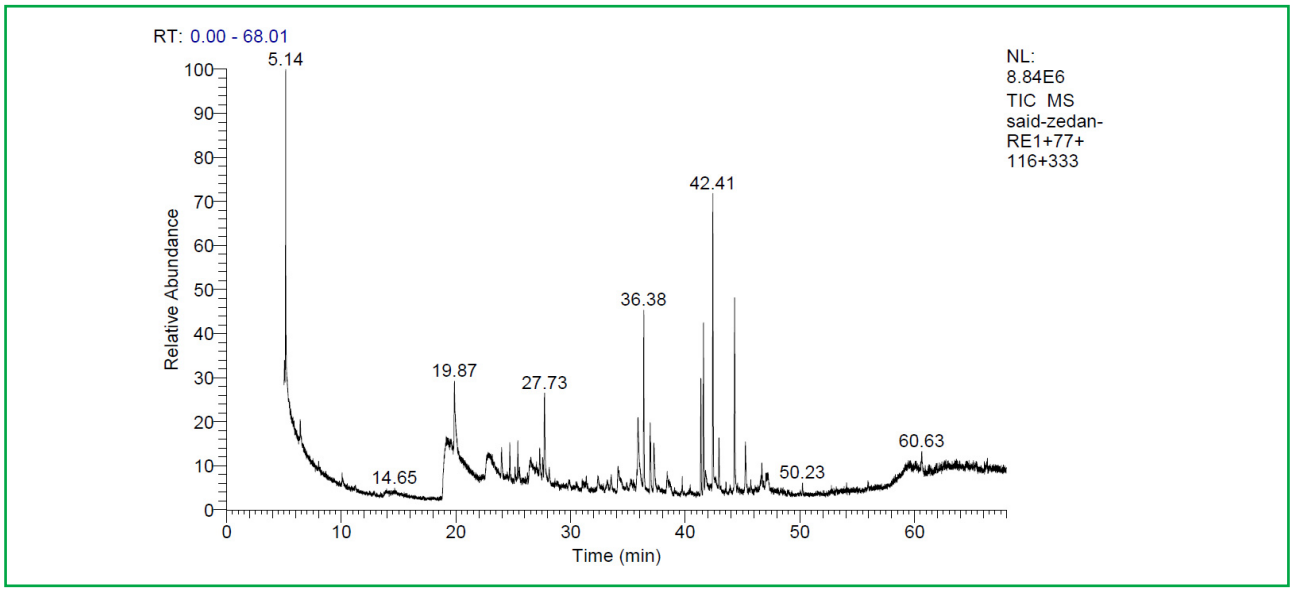


Figure 1 GC-MS chromatogram of methanolic extract of *Helianthemum lippii*

on *H. lippii* leaves and roots. In Algeria, a decoction of leaves is used for diabetic treatment (Hamza et al., 2019). Roots decoction is used in Saudi Arabia to cure camel colic (Sher and Aldosari, 2013). Camel food in the Sahara Desert (Volpato and Puri, 2014). In the Saharan region of Algerian, leaves powder or a compress were used to treat wounds and skin conditions (Lakhdari et al., 2016).

The presence of the six chemicals stated before in *H. lippii* was consistent with major investigations on *Helianthemum* species (Calzada and Alanis, 2007; Benabdelaziz, 2015; Calzada et al., 1995). Accordingly, study of the chemical constituents of *H. lippii* extract using GC-MS analysis would be the first step to identifying of the bioactive compounds in this plant. This study would be helpful to investigate *H. lippii* materials as a new supplies of natural antioxidants and acceptable commercial food enhancers or pharmaceutical components.

4 Conclusions

In the present study, fifty-three compounds extracted from *H. lippii* were identified by GC-MS analysis. The biological activities of each of the identified phytocomponents range from antimicrobial, antioxidant, anti-inflammatory, an antifungal and anticancer. These behaviors could be a result of its individual or group contents.. In conclusion, The efficacy of plants is demonstrated in this study. However, more research is needed to identify the ingredients that are responsible for the antibacterial, antioxidant, anti-inflammatory, antifungal, and anticancer properties. These findings provide new insights into the potential use of this plant not only as a therapeutic agents but also as a source of the economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of this plant. Further study is required to determine the constituents responsible for antibacterial, antioxidant, anti-inflammatory, antifungal, and anticancer activities..

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