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Chemical composition profile of the essential oil from *hymenocrater bituminous* and its health functionality

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ABSTRACT

Hymenocrater species are important medicinal and food plants. The aim of this work was to evaluate the potential of *Hymenocrater bituminous* Fisch. & C. A. Mey. for the management of public health problems such as Alzheimer's disease, obesity, diabetes mellitus, and skin diseases through inhibition of targeted enzymes. Essential oil composition, antioxidant activity, and the total bioactive contents of the plant were also determined. EO showed high α-glucosidase (40 mmol ACEs/g oil), α-amylase (9 mmol ACEs/g oil), acetylcholinesterase (3.8 mg GEs/g oil), butyrylcholinesterase (4.7 mg GEs/g oil), tyrosinase (45 mg KAEs/g oil), and lipase (1.5 mmol OEs/g oil) inhibitory activities. Methanolic extract exhibited strong antiradical (DPPH and ABTS) and reducing power (CUPRAC and FRAP) activities and high total phenolics content (120 mg GAEs/g extract). Gas chromatography/mass spectrometry analysis of EO showed the presence of α-pinene (18.2%), β-pinene (11.3%), trans-phytol (11.0%), and spathulenol (8.5%) as the major components. The results indicated that *H. bituminous* has promising potential for possible uses in food and pharmaceutical industries due to its valuable phytoconstituents and biological activities.

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Alzheimer's disease; Chemical fingerprints; Diabetes mellitus; Essential oil; Obesity

Introduction

Hymenocrater Fisch. & C. A. Mey. belonging to the Nepetoideae subfamily of the Lamiaceae family comprises 11 species distributed in Iran (*H. longiflorus* Benth., *H. calycinus* (Boiss.) Benth., *H. sessilifolius* Benth., *H. yazdianus* Rech.f., *H. platystegius* Rech.f., *H. oxyodontus* Rech.f., *H. incanus* Bunge, *H. bituminous* Fisch. & C.A. Mey., and *H. elegans* Bunge.), Afghanistan (*H. adenothrix* Rech.f., *H. sessilifolius*, and *H. altimuranus* Rech.f.), Western Pakistan (*H. sessilifolius*), Turkmenistan (*H. bituminous* and *H. elegans*), Eastern Turkey (*H. bituminous*), Northern Iraq (*H. longiflorus*), and Transcaucasia (*H. bituminous*). With nine species, the *Hymenocrater* genus is well represented in Iran, where most of them occur in the Iranian plateau.[1–3] Iran is the most important diversity centre of the genus followed by southern central Asia. The genus *Hymenocrater* by five endemic species (including *H. incanus, H. yazdianus, H. platystegius, H. oxyodontus*, and *H. calycinus*) shows nearly 55% endemism in Iran.[2–4] The members of the genus are herbaceous perennial or short shrubby plants, with usually colourful 15-veined membranous calyces expanded in fruits. The medicinal properties of *Hymenocrater* species are approximately unfamiliar. Nonetheless, they have been used by local people as medicine for the treatment of some health problems such as headache, wounds,

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giddiness, fever, skin allergies, and cardiac illnesses as well as an anti-inflammatory, anti-mosquito, house freshener, and sedative medicinal herb.[5–10]

Some biological activities such as antibacterial effects of *H. sessilifolius, H. elegans, H. yazdianus*, and *H. calycinus*,[5,9,11,12] cytotoxic, antioxidant, antibacterial, antifungal, and larvicidal activities of *H. longiflorus*,[6,7,13] anticancer and antioxidant properties of *H. platystegius*,[14] and antifungal, antibacterial, and antidiabetic activities of *H. bituminous*[15] have been reported.

All members of the genus are more or less aromatic and produce essential oil. 1,8-cineole, β-caryophyllene, α-pinene, spathulenol, and caryophyllene oxide are the most abundant volatile compounds in the genus.[9,16,17] Besides the aforesaid volatile constituents, flavonoids, alkaloids, saponins, and tannins are the other important groups of secondary metabolites in the *Hymenocrater* genus. Phytochemical studies on the genus have been resulted in identification of some important bioactive components such as rosmarinic acid, β-sitosterol, ursolic acid, quercetin-3-O-rutinoside, cirsimaritin, apigenin-7-O-glucoside, genistein, apigenin, acacetin, carnosic acid, caffeic acid, ferulic acid, and isorhamnetin.[6,15,18,19]

H. bituminous is an aromatic sturdy shrub growing in mountainous habitats from Turkmenistan to Turkey. This species as an Irano-Turanian element has distributed more extensive than any other *Hymenocrater* species in the region.[2,3,20] *H. bituminous* has been named as an ornamental plant. Also, this species has economic value because of its savoury lemon scent. Moreover, based on our survey research and unwritten date, foliage of *H. bituminous* is used as a sedative infusion or as herbal tea in local folk medicine of West Azerbaijan, Fars, and Northern provinces of Iran.

Alzheimer's disease, obesity, and Diabetes mellitus are considered as serious global health problems. The prevalence of these disorders is rising and it is estimated to increase significantly over the next decades. In this regard, many therapeutic methods have been developed during the last few years. Inhibition of key enzymes is an important strategy for the treatment of these health problems. Accordingly, in the present work, in continuation of our studies on Iranian Lamiaceae plants,[21–23] we aimed to evaluate the phytochemical profile and biological properties of *H. bituminous* as an important and uninvestigated medicinal plant for the first time. For biological properties, antioxidant activities and therapeutic target enzyme inhibitory effects of plant extracts/essential oil were investigated. For the phytochemical profile, the total bioactive components of extracts and chemical composition of essential oil were also determined. The present study may contribute to offer new insights into the biological and chemical fingerprint of *H. bituminous*.

Materials and methods

Plant materials

Plant aerial parts including flowers, leaves, and juvenile stems were collected during flowering season in early spring from Urmia, West Azerbaijan province of Iran and authenticated by Mr. Shahram Bahadori as *H. bituminous*. In addition, a voucher specimen was deposited in Herbarium of Urmia Pharmacy School (HUPS-366), Urmia, Iran.

Preparation of extracts

The studied extracts of the aerial parts of *H. bituminous* were obtained using maceration method. Fifty grams of the crushed dried material were extracted using 500 mL of dichloromethane (DCM) and methanol (MeOH) consecutively. The extractions were yielded by shaking at room temperature during 72 h. Afterwards, the extracts were passed through a paper filter and finally the filtrated solvent was evaporated by a rotary vacuum evaporator at 40 °C.

Isolation of essential oil

In accordance with the British pharmacopoeia, the essential oil was obtained by hydrodistillation of the dried aerial parts of the plant using a Clevenger-type apparatus for 3 h. The oil sample was stored at 4 °C in the dark until analysis.

Essential oil identification

Separation and analysis of essential oil components were achieved on an Agilent 6890N gas chromatograph coupled to a 5973N mass spectrometer and equipped with a HP-5 MS (5% phenyl methylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 µm film thickness; J & W Scientific, Folsom) capillary column. The used temperature program was as follows: 5 min at 60 °C then 4 °C/min up to 220 °C, then 11 °C min^{-1} up to 280 °C, held for 15 min. Injector and detector temperatures: 280 °C; carrier gas: He; flow rate: 1 mL/min; split ratio: 1:50; acquisition mass range: 29–400 m/z; mode: electron-impact (EI, 70 eV). The essential oil was diluted 1:100 in *n*-hexane and then 2 µL of the solution were injected into the GC-MS system. For identification of essential oil components, co-injection with available analytical standards was used whenever possible, together with correspondence of retention indices and mass spectra with respect to those occurring in ADAMS, NIST 08, and FFNSC2 libraries. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components. Percentage values were the mean of three chromatographic analyses.

Total phenolic and flavonoid contents determination

The total phenolics content was determined by the Folin–Ciocalteu method[24] with slight modification and expressed as gallic acid equivalents (GAEs/g sample). The total flavonoid content was determined according to $AlCl_3$ method[25] with some modifications and the results were expressed as rutin equivalents (REs/g sample).

Antioxidant assays

Several methods were used for measurement of antioxidant potential (DPPH and ABTS radical scavenging, ferric and cupper reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating activity (ferrozine method)) according to previously published procedures.[26]

Enzyme inhibitory assays

Enzyme inhibitory properties of *H. bituminous* against α-glucosidase, α-amylase, cholinesterases (AChE and BChE), and tyrosinase were investigated using previously published methods.[21] Also, porcine pancreatic lipase (type-II) inhibitory activity of the samples was determined using *p*-nitrophenyl butyrate (*p*-NPB) as substrate.[27] In brief, enzyme solution (1 mg/mL) was prepared in 50 mM Tris-HCl (pH 8.0). Test solution (25 µL) was mixed with lipase solution (50 µL) in a 96-well microplate and incubated for 20 min at 25 °C. The reaction was initiated by the addition of p-NPB (50 µL). Similarly, a blank was prepared for each sample (without enzyme) and analysed accordingly to this procedure. The enzyme inhibitory activities of the EO and extracts were obtained as equivalents of standard drugs per g of the plant sample (galantamine for AChE and BChE, kojic acid for tyrosinase, orlistat for lipase, and acarbose for α-amylase and α-glucosidase inhibition assays).

Statistical analysis

All experiments were carried out in triplicate. The results are expressed as mean value ± standard deviation (SD). Data analysis was performed using SPSS v.16.0. Differences between means were

determined by one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for multiple comparisons with control. A value of $p < 0.05$ was considered as indicative of statistical significance.

Results and discussion

Essential oil composition

The chemical composition of *H. bituminous* essential oil has not been investigated up to now. At the present work, the EO yield was 0.55% v/w. Chemical composition of the EO is shown in Table 1. Also, the chemical structures of the major volatile compounds are presented in Fig. 1. The EO was characterized by the presence of 51 volatile constituents, representing 94.1% of the total composition. α-pinene (18.2%), β-pinene (11.3%), *trans*-phytol (11.0%), and spathulenol (8.5%) were identified as the most abundant components. Monoterpene hydrocarbons (32.3%)

Table 1. Chemical composition of aerial parts of *Hymenocrater bituminous* essential oil.

No.	Compound [a]	Percentage[b]	RI[c]	RI lit[d]	Identification Method [e]
1	*α*-Thujene	0.7	920	924	RI,MS
2	*α*-Pinene	18.2	926	932	RI, MS, Co-I
3	Camphene	0.1	939	946	RI, MS, Co-I
4	Thuja-2,4(10)-diene	0.1	945	953	RI,MS
5	Benzaldehyde	0.1	953	952	RI, MS, Co-I
6	Sabinene	0.7	965	969	RI, MS, Co-I
7	*β*-Pinene	11.3	967	974	RI, MS, Co-I
8	Myrcene	0.1	989	988	RI, MS, Co-I
9	*p*-Cymene	0.4	1021	1020	RI, MS, Co-I
10	Limonene	0.7	1025	1024	RI, MS, Co-I
11	1,8-Cineole	4.1	1026	1026	RI, MS, Co-I
12	Linalool	0.4	1100	1095	RI, MS, Co-I
13	*α*-Campholenal	1.6	1122	1122	RI,MS
14	*trans*-Pinocarveol	1.0	1132	1135	RI, MS, Co-I
15	*cis*-Verbenol	0.2	1137	1137	RI,MS
16	*trans*-Verbenol	2.5	1140	1140	RI,MS
17	Pinocarvone	0.5	1157	1160	RI,MS
18	Terpinen-4-ol	0.2	1173	1174	RI, MS, Co-I
19	*p*-Cymen-8-ol	0.1	1183	1179	RI,MS
20	*α*-Terpineol	0.2	1187	1186	RI, MS, Co-I
21	(3*Z*)-Hexenyl butanoate	0.4	1188	1184	RI,MS
22	Myrtenal	0.8	1191	1195	RI, MS, Co-I
23	Myrtenol	0.8	1193	1194	RI, MS, Co-I
24	(3Z)-Hexenyl 3-methyl butanoate	0.6	1238	1232	RI,MS
25	*n*-Tridecane	0.4	1300	1300	RI, MS, Co-I
26	*α*-Copaene	0.2	1368	1374	RI,MS
27	*β*-Bourbonene	2.4	1375	1387	RI,MS
28	4a*α*,7*α*,7a*β*-Nepetalactone	1.1	1380	1386	RI,MS
29	*α*-Humulene	0.3	1443	1452	RI, MS, Co-I
30	Germacrene D	1.8	1471	1484	RI,MS
31	(*E*)-*β*-Ionone	0.8	1481	1487	RI, MS, Co-I
32	*n*-Pentadecane	0.5	1500	1500	RI, MS, Co-I
33	*trans*-Calamenene	1.9	1514	1521	RI,MS
34	Myristicin	1.8	1517	1517	RI, MS, Co-I

(*Continued*)

Table 1. (Continued).

No.	Compound [a]	Percentage[b]	RI[c]	RI lit[d]	Identification Method [e]
35	*α*-Calacorene	0.2	1534	1544	RI,MS
36	Spathulenol	8.5	1567	1576	RI,MS
37	Caryophyllene oxide	0.3	1571	1582	RI, MS, Co-I
38	Salvial-4(14)-en-1-one	0.3	1582	1594	RI,MS
39	Humulene epoxide II	0.9	1597	1608	RI,MS
40	*epi*-*α*-Cadinol	0.9	1632	1638	RI,MS
41	*α*-Cadinol	0.4	1646	1652	RI,MS
42	Eudesma-4(15),7-dien-1*β*-ol	1.0	1675	1687	RI,MS
43	Hexahydrofarnesyl acetone	0.7	1844	1845	RI,MS
44	*n*-Hexadecanoic acid	1.7	1966	1959	RI, MS, Co-I
45	Abietatriene	1.0	2036	2055	RI,MS
46	Abietadiene	1.2	2057	2087	RI,MS
47	*trans*-Phytol	11.0	2105	2104	RI, MS, Co-I
48	*n*-Tricosane	0.3	2306	2300	RI, MS, Co-I
49	*n*-Pentacosane	2.4	2500	2500	RI, MS, Co-I
50	*n*-Heptacosane	3.3	2700	2700	RI, MS, Co-I
51	*n*-Nonacosane	3.0	2900	2900	RI, MS, Co-I
Monoterpene hydrocarbons		32.3			
Oxygenated monoterpenes		13.5			
Sesquiterpene hydrocarbons		6.8			
Oxygenated sesquiterpenes		13.0			
Diterpenes		13.2			
Alkanes		9.9			
Others		5.4			
Total		94.1			

[a]Compounds are listed in order of their elution from a HP-5MS column. [b]Relative percentage values are means of three determinations with a RSD% in all cases below 10%. [c]Linear retention index on HP-5MS column, experimentally determined using homologous series of C_8–C_{30} alkanes. [d]Linear retention index taken from Adams (2007) and/or NIST 08 (2008). [e]Identification methods: Co-I, based on comparison with authentic compounds; MS, based on comparison with WILEY, ADAMS, FFNSC2 and NIST 08 MS databases; RI, based on comparison of calculated RI with those reported in ADAMS, FFNSC 2 and NIST 08.

represented the main fraction of the oil, followed by similar amounts of oxygenated monoterpenes (13.5), diterpenes (13.2%), and oxygenated sesquiterpenes (13.0%). Alkanes (9.9%) and sesquiterpene hydrocarbons (6.8%) gave a minor contribution. The results show that the EO of *H. bituminous* contains pharmacologically useful components. There are some similarity and also some differences between *H. bituminous* EO composition and previously investigated *Hymenocrater* species. α-Pinene and β-pinene together with other monoterpenoids are widely founded in the EOs of this genus.[9,28,29] Also, oxygenated and hydrocarbon sesquiterpene compounds are present in *Hymenocrater* species.[7] But, presence of *trans*-phytol as a major compound in the genus *Hymenocrater* has not been previously reported. Moreover, presence of alkane derivatives with 9.9% in *H. bituminous* EO is another observed difference. Morphologically, *H. bituminous* is a diverse species. Also, some hybrids between *H. bituminous* and either *H. elegans* or *H. calycinus* have been reported. A comparative study on the botanical description of *H. bituminous* growing wild in Turkey with those described in the floras of Turkey, Iran, and USSR indicated that the morphological characteristics differ inside the specimens studied from the different distribution localities.[2,20] Such variation observed in morphology as well as the influence of various ecological conditions of the habitats may result in different amount or probably type of chemical constituents and subsequently different bioactivity of the EOs and extracts of these species.

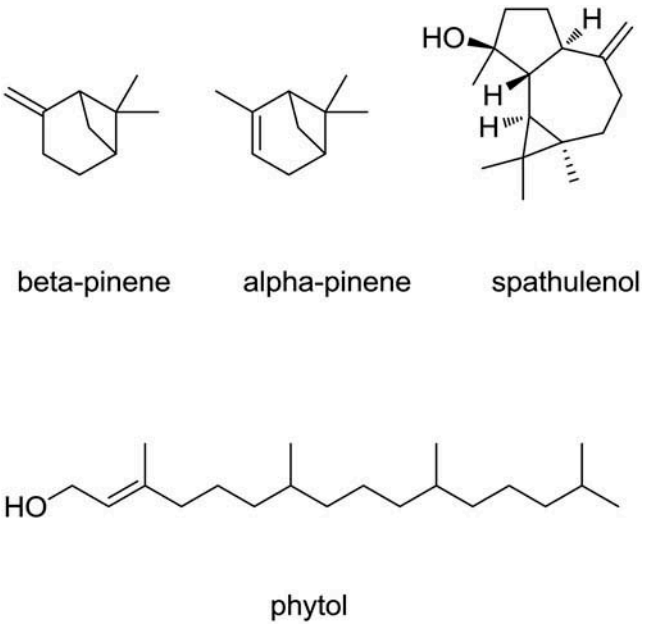

Figure 1. Chemical structures of the major components of *Hymenocrater bituminous* essential oil.

Antioxidant activity

Oxidant compounds such as reactive oxygen (ROS) and nitrogen (RNS) species are responsible for oxidative stress which plays an important role in many human disorders. In this work, several methods were used to evaluate the antioxidant potential of *H. bituminous*. As shown in Table 2, radical scavenging activity analysis revealed that EO is inactive but the MeOH extract has promising antiradical activity against DPPH and ABTS radicals (1.78 and 4.29 mmol TEs/g extract, respectively). Similarly, EO and DCM extract showed low reducing power, while MeOH extract exhibited high reducing power activity in CUPRAC (2.78 mmol TEs/g extract) and FRAP (1.93 mmol TEs/g extract) assays (Table 3). Both of antiradical and reducing activities of MeOH extract could be due to its high total phenolics content (120 mg GAEs/g extract) (Table 4) which are well-known for their antioxidant ability. In the total antioxidant activity and metal chelating assays, inverse results were obtained and EO exhibited the highest antioxidant potential (Table 4 and Table 3). These observations may be interpretable by antioxidant abilities of oxygenated monoterpenoids and sesquiterpenoids found in *H. bituminous* EO composition such as 1,8-cineol and spathulenol.

Enzyme inhibitory activities

Discovery of enzyme inhibitors is an important strategy to find more effective drugs for treatment of many diseases such as obesity (lipase), Alzheimer's diseases (cholinesterases), inflammation (cyclooxygenases),

Table 2. Radical scavenging activity of *Hymenocrater bituminous*.

	Radical scavenging activity	
Samples	DPPH radical (mmol TEs/g extract)[a]	ABTS radical cation (mmol TEs/g extract)[a]
EO	Na	na
DCM	0.14 ± 0.01*[a]	0.57 ± 0.02[a]
MeOH	1.78 ± 0.01[b]	4.29 ± 0.13[b]

[a]TEs: trolox equivalents. na: not active. *Values expressed are means ± S.D. of three parallel measurements. Data marked with different letters indicate significant difference ($p < 0.05$).

Table 3. Reducing power and metal chelating activity of *Hymenocrater bituminous*.

	Reducing power activity		Metal chelating activity
Samples	CUPRAC (mmol TEs/g oil or extract)[a]	FRAP (mmol TEs/g oil or extract)[a]	Chelating effect (mg EDTAEs/g oil or extract)[b]
EO	0.25 ± 0.01*[a]	0.19 ± 0.01[a]	40.52 ± 1.30[a]
DCM	0.46 ± 0.04[b]	0.31 ± 0.01[b]	20.26 ± 0.90[b]
MeOH	2.78 ± 0.05[c]	1.93 ± 0.03[c]	2.19 ± 0.29[c]

[a]TEs: trolox equivalents. [b]EDTAEs: EDTA equivalents. *Values expressed are means ± S.D. of three parallel measurements. Data marked with different letters indicate significant difference ($p < 0.05$).

Table 4. Total bioactive components and total antioxidant activity of *Hymenocrater bituminous*.

	Total bioactive compounds		Total antioxidant activity
Samples	Total phenolic (mg GAEs/g extract)[a]	Total flavonoid (mg REs/g extract)[b]	Phosphomolybdenum (mmol TEs/g oil or extract)[c]
EO	–	–	3.84 ± 0.12[a]
DCM	29.90 ± 0.66*[a]	14.31 ± 0.01[a]	1.82 ± 0.05[b]
MeOH	120.88 ± 0.82[b]	22.01 ± 0.14[b]	2.67 ± 0.18[c]

[a]GAEs: gallic acid equivalents. [b]REs: rutin equivalents. [c]TEs: trolox equivalents. * Values expressed are means ± S.D. of three parallel measurements. Data marked with different letters indicate significant difference ($p < 0.05$).

Table 5. Therapeutic target enzyme inhibitory activity of *Hymenocrater bituminous*.

	Anti-Alzheimer's disease effects		Anti-diabetic effects		Skin-care effects	Anti-obesity effects
Samples	AChE Inhibition (mg GEs/g oil or extract)[a]	BChE Inhibition (mg GEs/g oil or extract)[a]	α-amylase inhibition (mmol ACEs/g oil or extract)[b]	α-glucosidase inhibition (mmol ACEs/g oil or extract)[b]	Tyrosinase inhibition (mg KAEs/g oil or extract)[c]	Lipase Inhibition (mmol OEs/g oil or extract)[d]
EO	3.83 ± 0.01*[a]	4.71 ± 0.01[a]	0.91 ± 0.09[a]	40.17 ± 1.40[a]	45.41 ± 4.27[a]	1.51 ± 0.01[a]
DCM	2.08 ± 0.01[b]	1.78 ± 0.01[b]	0.57 ± 0.01[b]	4.32 ± 0.15[b]	26.01 ± 2.57[b]	0.57 ± 0.01[b]
MeOH	1.49 ± 0.04[c]	0.71 ± 0.06[c]	0.28 ± 0.04[c]	11.45 ± 0.18[c]	4.96 ± 0.45[c]	0.08 ± 0.01[c]

[a]GEs: galantamine equivalents. [b]ACEs: Acarbose equivalents. [c]KAEs: kojic acid equivalents. [d]OEs: orlistat equivalents. *Values expressed are means ± S.D. of three parallel measurements. Data marked with different letters indicate significant difference ($p < 0.05$).

skin disorders (tyrosinase), and diabetes mellitus (amylase and glucosidase). In this regards, at the present study, *in vitro* enzyme inhibitory potential of the EO, dichloromethane, and methanol extracts of *H. bituminous* were evaluated against acetylcholinesterase, butyrylcholinesterase, α-amylase, α-glucosidase, tyrosinase, and lipase. The results are expressed as equivalents of reference drugs (Table 5). Generally, the EO demonstrated the highest activity against all tested enzymes. The DCM and MeOH extracts showed moderate inhibitory effects against AChE and BChE. There are several reports in the literature indicating that alkaloids and flavonoids have strong cholinesterases inhibitory activities.[30–32] Accordingly, alkaloid and flavonoid rich *Hymenocrater* species could be considered as potent AChE and BChE inhibitors.

Anti-diabetic potential of *H. bituminous* were determined by its inhibition potential against α-amylase and α-glucosidase (Table 5). All samples had low α-amylase inhibitory and moderate to high α-glucosidase inhibitory activity. EO showed excellent activity against α-glucosidase (40 mmol ACEs/g oil). These results indicated that responsible compounds for anti-diabetic properties of *H. bituminous* have a selective effect and α-glucosidase is more sensitive than α-amylase. The tyrosinase inhibitory activity of the EO and extracts of *H. bituminous* varied from 4.9 to 45.4 mg KAE/g. As shown in Table 5, EO exhibited promising tyrosinase inhibitory effect and could be considered for possible uses in cosmetic industries as a skin-care agent.

Anti-obesity potential of *H. bituminous* was also evaluated by its inhibitory effect on porcine pancreatic lipase (type-II). The MeOH extract was inactive, while DCM extract showed moderate

activity. EO exhibited a prominent inhibitory potential (1.5 mmol OEs/g oil) and may be considered as a natural lipid absorption inhibitor in food and pharmaceutical industries.

Conclusion

The *Hymenocrater* genus has traditional uses as a food and medicine in Middle East countries but there is limited information on its phytochemistry and biological activities. In the present work, enzyme inhibitory activities (linked to diabetes mellitus, Alzheimer's disease, skin disorders, and obesity) of *H. bituminous* were evaluated for the first time. Moreover, chemical compositions of essential oil together with its antioxidant potential and bioactive compounds were investigated in this study. The results showed that EO and extracts of *H. bituminous* have remarkable ability for treatment of public health problems. In this sense, this species could be considered as a valuable source for preparing new functional foods, cosmetics, and pharmaceuticals.

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