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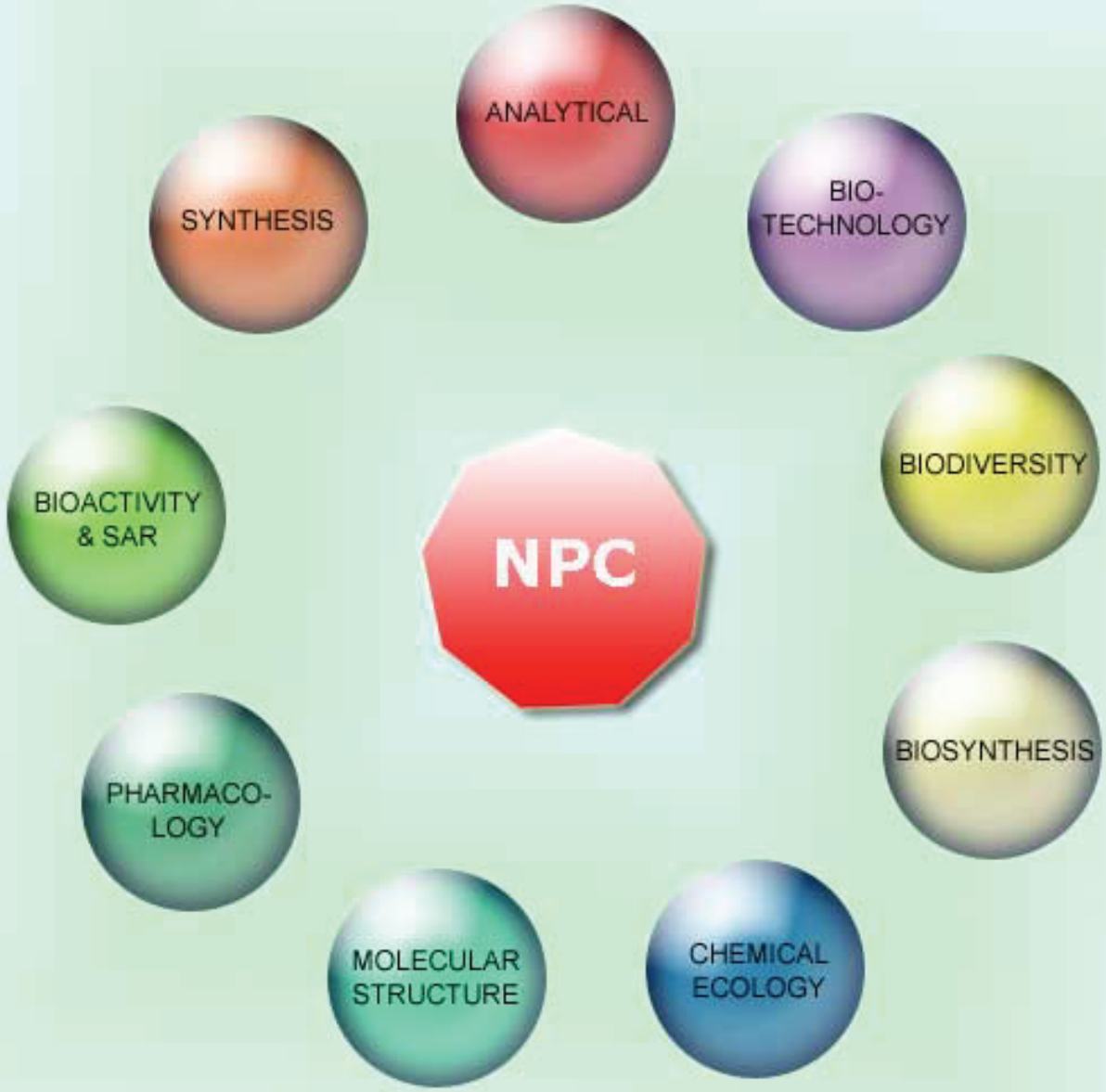

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Chemical Composition of Essential Oil, Antioxidant, Antidiabetic, Anti-obesity, and Neuroprotective Properties of *Prangos gaubae*

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Chemical composition of essential oil and the potential of *Prangos gaubae* for the management of public health problems such as Alzheimer's disease, obesity, Diabetes mellitus, and skin diseases were evaluated for the first time. In this direction, enzyme inhibitory effects, antioxidant activity, and total bioactive contents of the plant were determined. EO showed high acetylcholinesterase (2.97 mg GEs/g oil), α-amylase (1.35 mmol ACEs/g oil), α-glucosidase (38.84 mmol ACEs/g oil), and lipase (1.59 mmol OEs/g oil) inhibitory activities. Moreover, strong antioxidant effects were observed in antiradical (DPPH and ABTS), reducing power (CUPRAC and FRAP), total antioxidant, and metal chelating assays. Methanol extract exhibited promising DPPH radical scavenging activity (0.47 mmol TEs/g extract) and also high reducing power in CUPRAC (0.89 mmol TEs/g extract) and FRAP (0.52 mmol TEs/g extract) assays. All extracts showed low total flavonoid but high total phenolics content. Furthermore, they exhibited strong skin-care effect in tyrosinase inhibition assay. EO analysis showed the presence of germacrene D (26.7%), caryophyllene oxide (14.3%), (*E*)-caryophyllene (13.8%), and spathulenol (11.3%) as the major volatile components. Results indicated that *P. gaubae* has promising potential for possible uses in food, cosmetic, and pharmaceutical industries due to its valuable phytoconstituents and biological activities.

Keywords: *Prangos gaubae*, Diabetes mellitus, Alzheimer's disease, Obesity, Antioxidant, Essential oil.

The genus *Prangos* L. belonging to the Apioideae subfamily of the Apiaceae family contains around 45 herbaceous hemicryptophyte species worldwide. The members of the genus are mostly distributed in the southwest and central Asia. *Prangos* represents by 14 species in Iran, of which 5 species including *P. gaubae* (Bornm.) Herrnst. & Heyn are endemic to the country [1, 2].

The genus traditionally have been used as spice, food, fodder, and medicine for treatment of some health problems like seizures, headaches, leukoplakia, bleeding, and digestive disorders [3, 4]. Some pharmacological properties such as aphrodisiac, tonic, carminative, emollient, anthelmintic, antispasmodic, antiinflammatory, anti-hemorrhoid, abortifacient, antiflatulent, diuretic, and soothing effects have been also reported for *Prangos* species [5, 6]. In addition, scientific studies have been resulted in detection of some biological functions such antibacterial, allelopathic, cytotoxic, anti-oxidant and anti-fungal activities [7]. *Prangos* species are rich in coumarin compounds like osthol, oxypeucedanin and isoimperatorin which show wide range of bioactivities. Furthermore, flavonoids, alkaloids, terpenoids, and essential oils constitute other remarkable chemical content of the genus [8].

Prangos gaubae has not previously been subjected to any phytochemical or biological studies. So, at the present study, we aimed to investigate the bioactivities and phytoconstituents of *P. gaubae* for the first time. In this direction, antioxidant and enzyme inhibitory activities and the EO composition of the plant were evaluated using several bioassays. The fresh plant is not much pleasantly aromatic. The ripen fruits of the plant are edible. Unlike the other members of the genus, *P. gaubae* is not a polycarpic species. On the other hand, nutritional usages of fruits in the long term by the locals, has been one of the anthropological reasons of the restricted distribution of *P. gaubae* in the area.

The chemical composition of *P. gaubae* essential oil has not been investigated up to now. At the present work, the EO yield was 0.4% v/w. Chemical composition of the EO is shown in Table 1. The EO was characterized by the presence of 41 volatile constituents, representing 92.8% of total composition. Germacrene D (26.7%), caryophyllene oxide (14.3%), (*E*)-caryophyllene (13.8%), and spathulenol (11.3%) were identified as the most abundant components (Figure 1). Sesquiterpene hydrocarbons (48.5%) represented the main fraction of the oil, followed by oxygenated sesquiterpenes (32.0%). In comparison, the essential oil analyzing of some *Prangos* species such as *P. denticulate*, *P. cheilanthifolia*, *P. ferulacea*, *P. acaulis*, and *P. pabularia* indicated that the major volatile constituents in the genus are as followed: sabinene, *p*-cymene, δ-3-carene, (*Z*)-3,5-nonadiyne-7-ene, β-myrcene, camphor, *trans*-caryophyllene, α-pinene, *cis*-ocimene, spathulenol, α-bisabolol, caryophyllene oxide, linalool, 3-ethylidene-2-methyl-1-hexen-4-yne, α-terpinene, limonene, lavandulyl acetate, 1,8-cineole, and geranyl isobutyrate [9-13].

As could be seen in Table 2, all of the extracts showed moderate concentration of phenolic compounds (12-53 mg Gallic acid equivalents/g extract) and low concentration of flavonoid components (1.7-12.4 mg Rutin equivalents/g extract). This is in agreement with this fact which major compounds in the genus *Prangos* are coumarins [8]. Total phenolic content of methanolic and water extracts of 4 *Prangos* species (roots, leaves, and fruits) from Konya, Turkey showed the range of 37-140 mg GAEs/g extract [14].

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 [15-17]. In this work, several methods were used to evaluate the antioxidant potential of *P. gaubae*. As shown in Table 2, radical scavenging activity analysis revealed that EO has strong 2,2-azino-bis (3-ethylbenzothiazloine-6-sulfonic acid) radical cation (ABTS) scavenging activity (2.02 mmol Trolox equivalents/g oil) and the MeOH extract has promising antiradical activity against ,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (0.47 mmol TEs/g extract). Similarly, the MeOH extract exhibited high reducing power activity in the cupric ion reducing activity (CUPRAC) (0.89 mmol TEs/g extract) and the ferric reducing antioxidant power (FRAP) (0.52 mmol TEs/g extract) assays (Table 2). Phenolics together with coumarin compounds may be responsible for antioxidant capacity of MeOH extract. There some reports in the literature showing that these metabolites have strong antioxidant properties in the genus [5, 14, 18]. The EO exhibited the highest antioxidant potential in the total antioxidant (9.17 mmol TEs/g sample) and metal chelating (37 mg EDTAEs/g sample) assays (Table 2). These observations may be interpretable by antioxidant abilities of oxygenated sesquiterpenoids found in *P. gaubae* EO such as spathulenol and caryophyllene oxide.

Discovery of enzyme inhibitors is an important strategy to find effective drugs for treatment of many diseases such as obesity (lipase), Alzheimer's diseases (cholinesterases), inflammation (cyclooxygenases), skin disorders (tyrosinase), and diabetes mellitus (amylase and glucosidase) [19, 20]. In this regards, at the present study, *in vitro* enzyme inhibitory potential of *P. gaubae* was evaluated against acetylcholinesterase, butyrylcholinesterase, α-amylase, α-glucosidase, tyrosinase, and lipase. The results are expressed as equivalents of reference drugs (Table 3). The EO demonstrated the highest inhibitory activity against cholinesterases followed by DCM extract. There are several reports in the literature indicating that coumarins have strong cholinesterases inhibitory activities [21, 22]. All of the plant samples showed moderate α-amylase inhibition and strong α-glucosidase inhibition (7-38 mmol Acarbose equivalents/g sample). The tyrosinase inhibitory activity of the EO and extracts of *P. gaubae* varied from 16 to 36 mg Kojic acid equivalents/g sample. As shown in Table 3, Hex extract and EO exhibited promising tyrosinase inhibitory effects (36 and 29 mg KAEs/g oil or extract, respectively) and could be considered for possible uses in cosmetic industries as skin-care agents. Anti-obesity potential of *P. gaubae* was also evaluated by its inhibitory effect on porcine pancreatic lipase (type-II). The EO showed strong activity (1.59 mmol Orlistat equivalents/g oil) and may be

Table 1: Essential oil composition of aerial parts of *Prangos gaubae*.

No.	Compound [a]	Percentage [b]	RI [c]	RI lit [d]	Identification Method [e]
1	α-Pinene	1.4	926	932	RI,MS
2	Camphene	0.2	939	946	RI, MS, Co-I
3	Sabinene	0.2	965	969	RI, MS, Co-I
4	β-Pinene	0.1	969	974	RI,MS
5	Myrcene	0.2	989	988	RI, MS, Co-I
6	*p*-Cymene	1.0	1021	1020	RI, MS, Co-I
7	Limonene	2.8	1024	1024	RI, MS, Co-I
8	(*E*)-β-Ocimene	0.5	1046	1044	RI, MS, Co-I
9	γ-Terpinene	0.1	1055	1054	RI, MS, Co-I
10	Terpinolene	0.1	1084	1086	RI, MS, Co-I
11	*p*-Cymen-8-ol	0.2	1183	1179	RI, MS, Co-I
12	Carvacrol, methyl ether	1.6	1242	1241	RI, MS, Co-I
13	Bornyl acetate	0.1	1282	1287	RI,MS
14	Thymol	0.1	1294	1289	RI, MS, Co-I
15	*n*-Tridecane	0.1	1300	1300	RI,MS
16	Carvacrol	0.1	1302	1298	RI,MS
17	α-Copaene	1.1	1368	1374	RI,MS
18	β-Cubebene	0.7	1383	1387	RI, MS, Co-I
19	β-Elemene	2.1	1385	1389	RI,MS
20	(*E*)-Caryophyllene	13.8	1409	1417	RI, MS, Co-I
21	α-Humulene	1.0	1443	1452	RI,MS
22	Germacrene D	26.7	1472	1484	RI, MS, Co-I
23	(*E*)-β-Ionone	0.4	1481	1487	RI, MS, Co-I
24	Bicyclogermacrene	1.3	1487	1500	RI,MS
25	α-Muurolene	0.2	1494	1500	RI, MS, Co-I
26	*n*-Pentadecane	1.3	1500	1500	RI,MS
27	γ-Cadinene	0.3	1505	1513	RI,MS
28	δ-Cadinene	1.2	1517	1522	RI,MS
29	Spathulenol	11.3	1567	1576	RI, MS, Co-I
30	Caryophyllene oxide	14.3	1571	1582	RI,MS
31	Salvial-4(14)-en-1-one	0.2	1583	1594	RI, MS, Co-I
32	Humulene epoxide II	0.8	1597	1608	RI, MS, Co-I
33	epi-α-Cadinol	0.4	1632	1638	RI,MS
34	epi-α-Muurolol	0.4	1633	1640	RI, MS, Co-I
35	α-Cadinol	1.2	1646	1652	RI,MS
36	Eudesma-4(15),7-dien-1β-ol	3.0	1676	1687	RI,MS
37	*n*-Heptadecane	0.1	1699	1700	RI, MS, Co-I
38	Neophytadiene	0.4	1838	1846	RI,MS
39	Hexahydrofarnesyl acetone	0.2	1844	1845	RI,MS
40	*n*-Hexadecanoic acid	1.1	1965	1959	RI,MS
41	*trans*-Phytol	0.4	2104	2104	RI,MS
Monoterpene hydrocarbons		6.5			
Oxygenated monoterpenes		2.1			
Sesquiterpene hydrocarbons		48.5			
Oxygenated sesquiterpenes		32.0			
Diterpenes		0.4			
Others		3.3			
Total identified (%)		92.8			

[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] RI: Linear retention index on HP-5MS column, experimentally determined using homologous series of C_8-C_{30} alkanes. [d] RI lit: Linear retention index taken from Adams (2007) and/or NIST 08 (2008). [e] Identification methods: Co-I: Co-injection: 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.

Table 2: Total bioactive compounds and antioxidant properties of *P. gaubae*.

Assay	EO	Hex	DCM	MeOH
Total phenolic content (mg GAEs/g extract)[a]	-	12.22 ± 0.11	37.46 ± 1.18	53.85 ± 1.28
Total flavonoid content (mg REs/g extract)[b]	-	1.77 ± 0.14	1.80 ± 0.07	12.42 ± 0.16
Total antioxidant (mmol TEs/g sample)[c]	9.17 ± 0.20	1.35 ± 0.07	2.01 ± 0.12	1.47 ± 0.06
DPPH radical (mmol TEs/g sample)[c]	-	0.04 ± 0.01	0.12 ± 0.01	0.47 ± 0.01
ABTS radical cation (mmol TEs/g sample)[c]	2.02 ± 0.07	0.08 ± 0.01	0.76 ± 0.03	1.34 ± 0.05
CUPRAC (mmol TEs/g sample)[c]	0.47 ± 0.02	0.21 ± 0.01	0.64 ± 0.02	0.89 ± 0.01
FRAP (mmol TEs/g sample)[c]	0.37 ± 0.01	0.14 ± 0.01	0.41 ± 0.01	0.52 ± 0.02
Metal Chelating (mg EDTAEs/g sample)[d]	37.89 ± 0.95	18.91 ± 0.28	15.84 ± 0.84	11.60 ± 0.16

[a] GAEs: gallic acid equivalents. [b] REs: rutin equivalents. [c] TEs: trolox equivalents. [d] EDTAEs: EDTA equivalents.

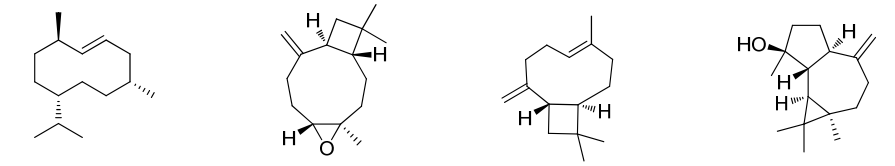

Figure 1. Chemical structures of the major compounds from *Prangos gaubae* essential oil.

Table 3: Enzyme inhibitory activities of *P. gaubae* linked to public health problems.

Sample	Neuroprotective effects		Antidiabetic effects		Skin-care effects	Anti-obesity effects
	AChE (mg GEs/g sample)[a]	BChE (mg GEs/g sample)[a]	α-amylase (mmol AEs/g sample)[b]	α-glucosidase (mmol AEs/g sample)[b]	Tyrosinase (mg KAEs/g sample)[c]	Lipase (mmol OEs/g sample)[d]
EO	2.97 ± 0.01	3.30 ± 0.10	1.35 ± 0.04	38.84 ± 1.20	29.24 ± 3.91	1.59 ± 0.03
Hex	1.55 ± 0.14	2.33 ± 0.25	0.64 ± 0.03	19.70 ± 0.29	36.33 ± 2.18	0.42 ± 0.03
DCM	2.62 ± 0.29	3.51 ± 0.24	0.93 ± 0.08	20.07 ± 0.54	27.82 ± 0.62	0.56 ± 0.03
MeOH	1.80 ± 0.03	1.26 ± 0.04	0.47 ± 0.02	7.42 ± 0.16	16.85 ± 2.79	0.23 ± 0.01

[a] GEs: galanthamine equivalents. [b] AEs: acarbose equivalents. [c] KAEs: kojic acid equivalents. [d] OEs: orlistat equivalents.

considered as a natural lipid absorption inhibitor in food and pharmaceutical products. This is the first report on the therapeutic target enzyme inhibitory potential of *Prangos* species against cholinesterases, amylase, glucosidase, tyrosinase, and lipase. So, this work may open a new window for possible uses of *Prangos* species for the management of some public health problems

Experimental

Plant material: The aerial parts of the plant 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 *Prangos gaubae*. In addition, a voucher specimen was deposited in Herbarium of Urmia Pharmacy School (HUPS-202), Urmia, Iran.

Extraction: The studied extracts of the aerial parts of *P. gaubae* were obtained using maceration method. Twenty g of the crushed dried material were extracted using 200 mL of *n*-hexane (Hex), dichloromethane (DCM), and methanol (MeOH) consecutively. The extractions were yielded by shaking at room temperature during 48 h. The extracts were passed through a paper filter and finally the filtrated solution 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 in 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 programme was as follows: 5 min at 60°C then 4°C/min up to 220 °C, then 11 °C/min 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 Folin-Ciocalteu method [23] with slight modification and expressed as gallic acid equivalents (GAEs/g sample). Total flavonoids content was determined according to $AlCl_3$ method [24] 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), total antioxidant (phosphomolybdenum assay) and metal chelating activity (ferrozine method)) according to previously published procedures [25].

Enzyme inhibitory* assays*: Enzyme inhibitory properties of *P. gaubae* against α-glucosidase, α-amylase, cholinesterases (AChE and BChE), lipase, and tyrosinase were investigated using previously published methods [26].

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.

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