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Phytochemical and Biological Studies on *Ziziphora bungeana*

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The genus *Ziziphora* L. (Lamiaceae) comprises about 30 species wide spread all over Asia, Africa, and Europe. *Ziziphora bungeana* Juz. is distributed mainly in Kazakhstan, China, Central Asia, and Mongolia. It is used for the treatment of hypertension, and cardiovascular diseases [1]. It is used as infusion, decoction and maceration for various purposes such as sedative, stomach tonic, common cold, inflammation, coughing, antiseptic, migraine, fever, diarrhea, expectorant, depression, carminative and gastrointestinal diseases [2, 3]. Also it is used as a traditional medicine in Kazakhstan and only few bioactive compounds were reported from this plant [4, 5]. This gained our attention to study the chemistry and pharmacology of this plant.

The aerial parts of *Z. bungeana* were collected in summer 2014 in the flowering stage at the foothills of the Dzungarian Alatau, Republic of Kazakhstan and identified by Dr Gemejiyeva N.G. (Institute of Botany and Phytointroduction, Science committee-Ministry of Education and Science of the Republic of Kazakhstan). A voucher sample (№01-04/257) has been deposited in the herbarium of the Institute of Botany and Phytointroduction, Almaty, Republic of Kazakhstan.

Gas chromatographic analysis was performed on an Agilent 7890 GC instrument equipped with an Agilent 5975C mass specific detector and an Agilent 7693 auto-sampler. A fused silica capillary column (30 m, 0.25 mm i.d.) coated with a 0.25 μ m film of crosslinked 5% phenyl methyl silicone (J&W HP-5MS) was used with helium as the carrier gas at a flow rate of 1 mL/min. In a typical analysis, the oven was held for 2 min at 45 °C, and then programmed at 1.5 °C/min to 100°C, 2 °C/min to 200 °C. In the cleanup step, the oven temperature was programmed at 10 °C/min to 280 °C and held for 30 min. The injector temperature was 250 °C. The split ratio was set to 25:1. Duplicate injections were made for each sample. Mass spectra were recorded at 70 eV from m/z 40 to 550. Compound identification involved comparison of the spectra with the databases (Wiley and NIST) using a probability-based matching algorithm. Further identification was based on the relative

retention indices (RRI) compared with literature and the standard references isolated in-house or purchased from commercial sources. ^1H and ^{13}C NMR spectra were obtained on Bruker model 400 NMR spectrometers with standard pulse sequences, operating at 400 MHz in ^1H and 100 MHz in ^{13}C , respectively. CDCl_3 was used as solvent, and TMS was used as internal standard. High-resolution mass spectra (HRMS) were recorded on a Micromass Q-ToF Micro mass spectrometer with a lock spray source. Column chromatography was carried out on silica gel (70–230 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F254).

Fresh plant material (250 g) was subjected to hydrodistillation to afford 1.2 g of essential oil (0.5%, w/w) [6]. GC-MS analysis revealed the presence of eight major components (represent 94.41% of the oil) were identified: pulegone (**1**, 58.30%), isomenthone (**2**, 13.49%), menthone (**3**, 6.71%), α -limonene (**4**, 5.79%), isopulegone (**5**, 5.01%), isomenthol (**6**, 2.63%), β -pinene (**7**, 1.44%), and α -pinene (**8**, 1.04%). Pulegone **1**, can be considered a marker of essential oil *Z. bungeana* and regulate the determination of its quantitative content of the design parameters of standardization of raw materials [7].

Subcritical fluid (CO_2) extraction of *Z. bungeana* has done in LLP "Fito-aromat" to install the flow of carbon dioxide extraction with a capacity of 5 liters, according to the enterprise standard ST 27658-1910-LLP-02-2011 in different conditions (Table 1) [8]. The yield of extract was maximum (50g, 2.17%) at a pressure of 62 atm, temperature 22 °C.

Subcritical fluid (CO_2) extract of areal parts of *Z. bungeana* was fractionated over silica gel column. Repeated silica gel chromatographic purification eluting with gradient ethyl acetate-hexane yielded six compounds (**9–14**). Compounds (**9–14**) were identified and confirmed by comparing the literature data with their ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT, HMBC, HSQC, and mass spectra as: 3 β -acetoxyolean-11-en-28,13 β -olide (**9**, 5mg) [9], 3 β -acetoxy-11 α ,12 α -epoxy-oleanan-28,13 β -olide (**10**) [9], betulin (**11**, 5mg) [10], β -sitosterol (**12**, 15 mg) [11], oleic acid (**13**, 3mg) [12], and glyceryl 1,3-dilinoleate (**14**, 7mg) [13].

Extracts and isolated compounds were submitted for cannabinoid and opioid receptors assay. The affinity of the total extracts and isolated compounds towards cannabinoid and opioid receptors were carried out according to the published method and the results were tabulated in Table 2 [14].

In conclusion, GC-MS analysis indicated the presence of eight compounds (represent 94.41% of the oil) and Pulegone **1** is the major component (58.30%) in the volatile oil of *Z. bungeana*. Compounds 3 β -acetoxyolean-11-en-28,13 β -olide (**9**) and 3 β -acetoxy-11 α ,12 α -epoxy-oleanan-28,13 β -olide (**10**) were isolated for the first time from this plant besides four known compounds (**11–14**). Compound **9** has shown moderate Delta [δ] opioid receptor activity (41.5% displacement). Compounds **12**, **13** and **14** showed moderate CB1 activity (56.8%, 52.8% and 56.6% displacement respectively).

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TABLE 1

Extraction results in different conditions.

The list of experiments	Weight of plant material	Working pressure	Extraction temperature	Extraction time	Yield extraction
1st extraction	2300g.	73 atm.	28 °C.	16h	5g. (0.2%)
2nd extraction	2295g.	70 atm.	26 °C.	10h.	3g. (0.13%)
3rd extraction	2292g.	68 atm.	25 °C.	12h.	3g (0.13%)
4th extraction	2289g.	62 atm.	22 °C.	10h.	50g (2.17%)

TABLE 2CB1, CB2, Opioid receptor activity of *Z.bungeana* (% displacement at 10 µg/mL)

Extract/ Compound	CB1	CB2	Delta [δ]	Mu [μ]	Kappa [κ]
Volatile oil	32.1	20.6	12.0	26.3	31.9
Super critical fluid extract	16.8	16.1	18.9	25.4	3.5
9	8.4	2.2	41.5	2.5	9.7
11	56.8	30.9	14.0	22.0	-
12	52.8	17.6	19.7	33.0	9.2
13	24.2	7.6	18.2	18.9	0.8
14	56.6	22.5	15.2	18.9	7.7