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Component composition and biological activity of essential oil of plant *Zinnia elegans*

Abstract. By the method of steam distillation, samples of essential oil were obtained from a plant cultivated in the East Kazakhstan region. The essential oil was obtained with a yield in terms of air-dry raw materials. By the gas liquid chromatography investigated a quantitative analysis of the essential oil components. Essential oil components of quantitative content calculated from the areas of gas chomatographic peaks. From plant of genus *Zinnia elegans* obtained main compounds of essentiol oil such as α-terpineol, β-myrcene, camphene and myrtenal. Terpineol has nice smell of lilac and is a sought-arter substance in the production of perfumes, fragrances and cosmetics. Pleasant smelling oily liquid. Myrcene has a large number of beneficial properties for the body, whether pure or in aromatherapy through the essential oils that contain it: Sedative and muscle relaxant, anti-inflammatory, interferon inhibition, gastric and duodenal ulcers, antalgic and antinociceptive, reduces sensitivity to pain by self-healing endogenous derivatives of morphine. Myrtenal – has an anti-inflammatory, resolving effect on acne, infiltrates. Increases the protective barrier of the epidermis. Antioxidant, rejuvenating. Camphene components are effective in the treatment of fungal, viral and bacterial infections that affect the respiratory system.
Key words: *Asteraceae, Zinnia elegans,* chemical composition, essential oils, *α*-terpineol, *β*-myrcene, gas chromatography-mass spectrometry (GC/MS), antioxidant activity.

Introduction

Zinnia elegant annual herbaceous plant, species of the genus *Zinnia* of *Asteraceae* families, it consists of species grown all over the world for their decorative role. Genus *Zinnia* there are about 20 species of herbaceous and semi-shrub annual and perennial plants from the family *Asteraceae*. In nature, the culture grows in the regions of South and Central America. The genus is named after the German professor of botany and pharmacology, director of the Botanical garden in Göttingen Johann Gottfried Zinn [1].

Nothing its predominant use as an ornamental plant, many studies have been devoted to the analysis of secondary metabolites identified in the plant in correletion with the therapeutic potential of the plant. Many studies have revealed the presence of several classes of biological active (natural) compounds in some plants organs. Studies of ethanol-water extracts obtained from the whole leaf or plant have revealed the presence of flavonoids, saponins, steroids, polyphenols and glycosides [2].

Certain spesies within the *Zinnia* genus have undergone extensive research due to their potential biological effects. These studies have uncovered various properties, including antifungal, antioxidant, hepatoprotective, antibacterial, antiviral, antimalarial, cytotoxic (evident in cancer cell lines), and insecticidal activities. Furthermore, the literature contains specific investigations into the antioxidant, hepatoprotective, antifungal, and antimalarial capabilities of these species [3].

Considering the constant requirement to explore novel plants and indirectly discover new sources of secondary metabolites with therapeutic potential for the treatment of inflammatory diseases and cancer, our focus was directed towards analyzing an excessively cultivated ornamental plant. Previous identification of certain classes of compounds, particularly polyphenols, within this plant suggested possible therapeutic benefits. Additionally, the ethnopharmacological data surrounding this plant highlighted its traditional usage as an infusion for pain relief. Therefore, this study aimed to conduct

a comprehensive phytochemical analysis of the hexane extract derived from the inflorescences of *Zinnia elegans*, along with its respective fractions. Furthermore, we aimed to evaluate the antioxidant activity of the extract though two distinctive mechanisms – inhibition of lipoxygenase and cheletion of iron [4].

Romanian scientists conducted a comprehensive study on the component composition and biological activity of the essential oil derived from plants belonging ti the *Z. elegans* species. The phytochemical analysis of *Zinnia elegans* plants grown in East Kazakhstan had not been previously explored. Hence, the study of the chemical composition of Kazakhstan's *Zinnia* species is of great significance in terms of discovering new medicinal recources [5].

Materials and methods

One commonly used method obtaining essential oil from dried and crushed aboveground parts of plants, steam distillation was carried out with hexane as a trap in a Clevenger apparatus for 2 hours. The essential oil's component composition

The component composition of essential oil of plants of the genus *Zinnia* was determined on a gas chromatograph with a mass spectrometric detector (GC/MS) using a RestekRxi®-1ms capillary column (0.25mm × 30 m × 0.25 μm), sample volume 1.0 μl, carrier gas flow rate 1 ml/min, scanning time from 4 to 120 min, ion scanning mode 39-500 m/z [6,7]. The components were identified by comparing retention time, mass spectral fragments using the NIST library. Table 1 shows the GC/MS analytical results for *Z.elegans* essential oil. *Z.elegans* essential oil contained 43 components, which accounted for 99.46% of the total number of oil components, the main volatile components of which were: β-myrcene (14.07%), α-terpineol (9.45%), myrtenal (7.87%) and camphene (5.36%). (presented on Figures 1 and 2, and Table 1).

Results and discussion

The antioxidant activity of fractions isolated from the essential oil of *Z. elegans* inflorescences was determined using two established methods: the iron chelating activity test and the 15-LOX inhibition test. The effectiveness of the test samples in chelating iron irons and inhibiting lipoxygenase was quantified using EC50 and IC50 values (as presented in Table 2). The obtained results were also compared to those of positive controls (terpinol and ethylenediaminetetraacetic acid EDTA) in order to assess their efficacy [8].

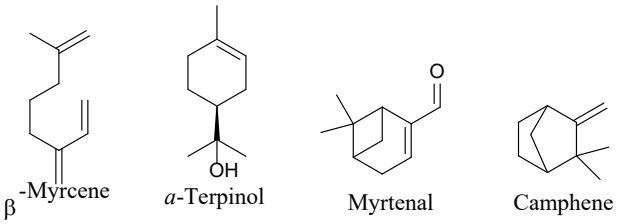

Figure 1 – Structural formulas of essential oils identified in *Zinnia Elegans*

As for the activity of inhibition of lipoxygenase, Fraction 2, where contains α-terpineol common compounds, showed the most promising activity (18.98 ± 0.22 micrograms / ml of the final solution), similar to the activity of positive control (terpineol). Other fractions, such as Fraction 3 and Fraction 4, have also exhibited significant inhibitory activity against the enzyme. In contrast, Fraction 5, which consists of fewer polar compounds compared tothe previous fractions, displayed a similar level of activity to that of the original extract. It is worth nothing that the fractions obtained generally demonstrate improved IC50 values for the analysis of lipoxygenase inhibition when compared to the total extract [9]. Interestingly, while the iron chelating activity was most promising in the initial essential oil, with a final solution concentration of 0.615± 0.001 mg/ml, this activity was not observed to the same extent in its selective fractions. However, it is noteworthy that the calculated value was found to be 10 times higher than that obtained for EDTA, a well-known metal chelator. This suggests the presence of a lower concentration of an effective iron chelator in the essential oil. Overall, these findings highlight the potential of different fractions in exhibiting inhibitory activity against the targeted enzyme. Moreover, the initial essential oil demonstrated remarkable iron-chelating properties, surpassing the efficacy of a widely recognized metal-chelating agent. There results suggest the presence of novel bioactive compounds within the fractions and essential oil, which can be further explored for potential therapeutic applications.

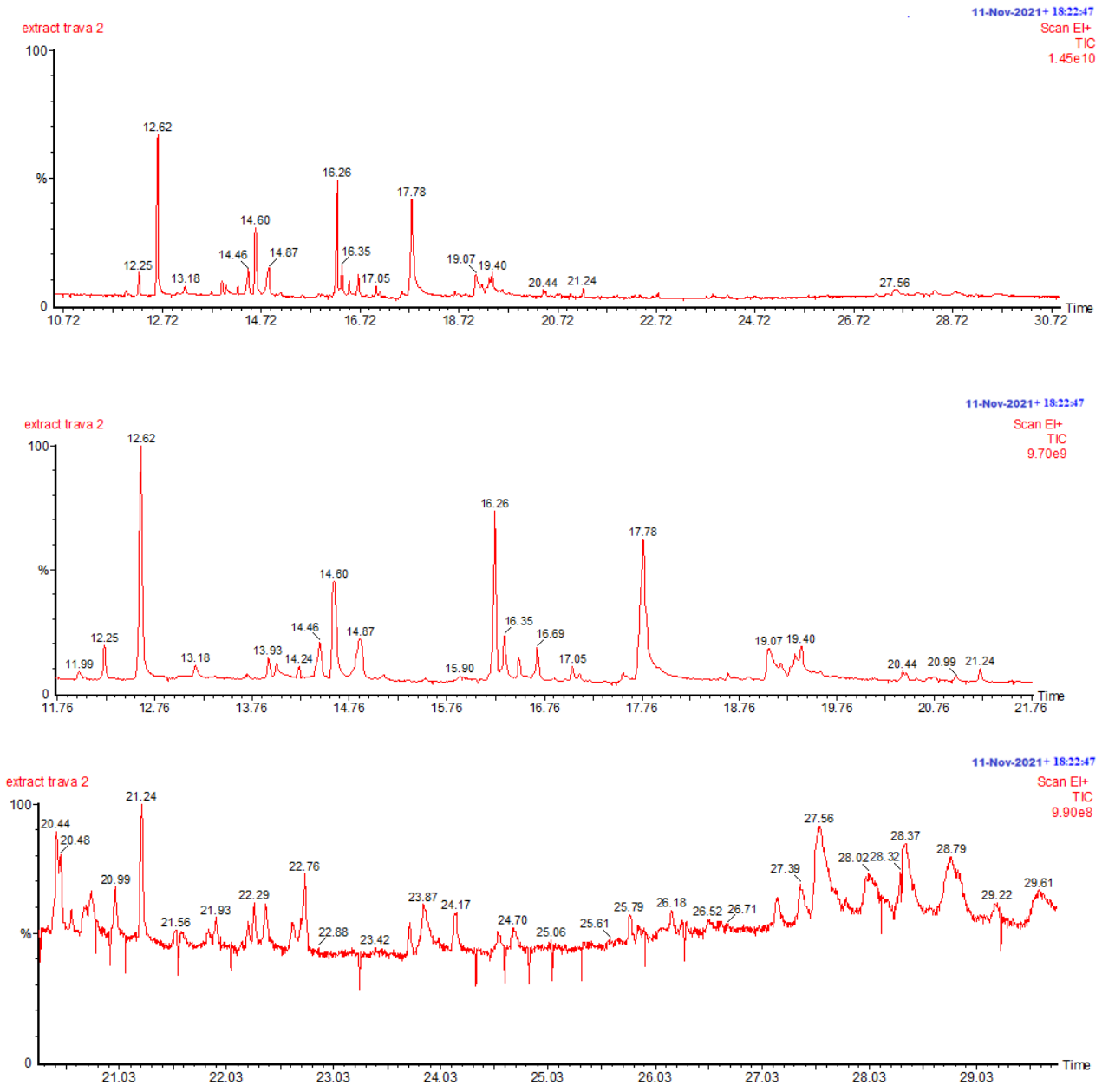

Figure 2 – GC/MS analytical results for *Zinnia elegans* essential oil

Table 1 – Component composition of *Zinnia Elegans* essential oil,%

№	RT	Component name	Content, %
	11,9843	*α* -Bulnesene	0,18
	12,2497	4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane	1,42
	12,6185	*α* -terpineol	9,45
	13,1776	1 α,2,3,5,6,7,7 α,7 β -octahydro-1,1,7,7 α -tetramethyl-, [1 α R-(1 α α,7 α,7 α α,7b α)]-1H-Cyclopropa[a]naphthalene	0,24
	13,927	3,7,11-trimethyl-, (E,E)-2,6,10-Dodecatrien-1-ol	0,09
	14,2403	Diepicedrene-1-oxide	0,12

Table continuation

№	RT	Component name	Content, %
1	14,4594	*α* -Bisabolol	1,61
2	14,5978	1-ethylideneoctahydro-7 α -ethyl-, (1E,3 α α,7 α β)-1H-Indene	1,65
3	14,8747	1,2,3,4,4 α,7,8,8 α -octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1a,4 β,4 α β,8 α β)]-1-Naphthalenol	1,63
4	16,2586	23-dien-3-ol, (3 β)-26,27-Dinorergosta-5	2,16
5	16,3507	6-Methyl-2-tridecanone	0,78
6	16,6854	2,6,10-Trimethylundeca-1,3-diene	1,45
7	17,0465	6-Methyl-2-tridecanone	0,76
8	17,7795	1,5-diethenyl-3-methyl-2-methylene-, (1 α,3 α,5 α)-Cyclohexane	1,99
9	19,0688	3,7,11-trimethyl-, acetate, (E,E)-2,6,10-Dodecatrien-1-ol	1,52
10	19,397	3-(3-methyl-1-butenyl)-(E)-Cyclohexene	1,48
11	19,9334	6-Isopropenyl-4,8 α -dimethyl-4 α,5,6,7,8,8 α -hexahydro-1H-naphthalen-2-one	0,45
12	20,4435	Camphene	5,36
13	20,4843	4,8,12-Trimethyltridecan-4-olide	3,07
14	20.9851	tetrahydro-6-octyl-2H-Pyran-2-one	1,97
15	21,2390	*β*-myrcene	14,07
16	21,5640	Nickel tetracarbonyl	1,76
17	21,9353	Adipic acid, 2-ethylhexyl octyl ester	1,80
18	22,7563	Dodecanoic acid, ethyl ester	2,18
19	22,8788	Tridecanoic acid, methyl ester	1,68
20	23,4241	Hexacosanoic acid, methyl ester	1,70
21	23.8689	9-Octadecyne	1,94
22	24,1717	methyl ester, (E)-2-Hexadecenoic acid	1,86
23	24,7086	Undecanoic acid, ethyl ester	1,78
24	25,0634	Benzoic acid, nonadecyl ester	1,71
25	25,6143	Benzoic acid, heptadecyl ester	1,74
26	25,7912	Benzoic acid, pentadecyl ester	1,84
27	26,1823	Benzoic acid, tridecyl ester	1,87
28	26,5256	Benzoic acid, undecyl ester	1,82
29	26,7134	Benzoic acid, octyl ester	1,81
30	27,3967	n-Hexadecanoic acid	1,98
31	27,5567	myrtenal	7,87
32	28,0227	3-hydroxy-6-isopropenyl-4,8 α -dimethyl-1,2,3,5,6,7,8,8 α -octahydronaphthalen-2-yl ester Acetic acid	2,20
33	28,3278	6-Isopropenyl-4,8 α -dimethyl-4 α,5,6,7,8,8 α -hexahydro-1H-naphthalen-2-one	2,21
34	28,3719	4,14-dimethyl-, acetate, (3б,4 α,5 α)-9,19-Cycloergost-24(28)-en-3-ol	2,42
35	28,7987	Benzoic acid, tetradecyl ester	2,00
36	29,2225	Eicosanoic acid, ethyl ester	1,88
37	29,6154	Acetic acid, 3-hydroxy-6-isopropenyl-4,8 α -dimethyl-1,2,3,5,6,7,8,8 α -octahydronaphthalen-2-yl ester	1,96
Total			99,46

Table 2 – *Zinnia elegans's* fractions antioxidant activity results

	Inhibition of lipoxygenase	Iron chelating activity
Name of sample	IC 50 (mg / ml)	EC 50 (mg / ml)
Fr 1	65,65 ±0,70[a*]	0,719 ±0,001[a]
Fr 2	19,99 ±0,23[d]	1,036 ±0,003[d]
Fr 3	31,65 ±0,74[c]	1,634 ±0,006[c]
Fr 4	43,54 ±1,47[b]	1,199 ±0,011[a]
Fr 5	69,43 ±6,78[a]	1,665 ±0,011[b]
The original extract	69,95 ±0,89[a]	0,645 ±0,001[f]
Positive control	20,45 ±0,44[d]	0,078 ±0,003[g]

* The values are the average values ± standard deviation, n = 3. a-g The average values in the column without a common uppercase letter differ ($p < 0.05$), as indicated by a one-factor analysis of variance.

Six strains were used in the study, which included four Gram-positive cocci strains, three of which were reference strains; *Staphylococcusaureus* ATCC 6538 (used in the determination of disinfectant – test), *Staphylococcusaureus* ATCC 25923 (used in the determination of antibiotic susceptibility – test), *Staphylococcusaureus* TS 77 (QacA/B harbor and disinfectant resistant gene and vancomycin resistant *Enterococcus* (VRE). In addition, three gramm-negative bacilli, which included 2 reference strains: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 (both used for antibiotic susceptibility testing) and one carbapenem-resistant strain of Klebsiella pneumoniae (CRKP). All initial and clinical strains were obtained from the Department of Medical Microbiology, Faculty of Medicine, Istanbul University.

All strains of bacteria were cultivated on yryptic soy agar, supplied by OXOID in Turkey. The agar plates were incubated aerobically at a temperature of 35ºC for a period of 24 hours. After the incubation period, the bacterial cultures were suspended in sterile saline solution containing 0.85% NaCl. The suspension was carefully adjusted to a concentration of 108 colony-forming units per milliliter. A 96-well round bottom microtiter was used, including a negative (plant extract only medium) and positive control (bacteria only medium) and 10 serial two-fold dilutions of each of the two plant extracts ranging from 9.765 to 5000 µg/mL with a final concentration a suspension of bacterial cells equivalent to $1*10^5$ cfu/ml. All grafted plates were incubated as above. The microphones were evaluated after 24 hours. Minimum bactericidal concentration (MBC) was performed by subculturing 10 ml from all wells in which no visible growth was observed (concentration equal to or higher than MIC) on a plant extract without Muller Hinton agar (OXOID, Turkey) and incubated as above [10]. (Table 3).

Table 3 – Minimum inhibitory concentration (MIC) for various plant extracts against various strains

Bacterium	Minimum inhibitory concentration (MIC), mcg/ml	
	T1	T2
S.aureus ATCC 6538	R	625
S.aureus ATCC 25923	R	R
S.aureus TS 77	R	R
E.coli ATCC 25922	1250	1250
P.aeruginos ATCC 27853	625	R
B.subtilis ATCC 6633	625	625
Carbapenem resistant *K.penumoniae* (CRKP)	625	625
Vankomycin resistant Enterococci (VRE)	312,5	312,5

Abbreviation: R – the strain is resistant to the high concentration of the tested plant extract of 5000 µg/ml.

The tests were repeated twice or more and averages were calculated. In the study, two plant extracts (T1 and T2) had different effects on the strains. T1 microphones for *E. coli* ATCC 25922, *P. aeruginos* ATCC 27853, *Vankomycin resistant Enterococci* (VRE) and *B. subtilis* ATCC 6633 cells producted 625 mg/ml, 321.5 mg/ml and 1250 mg/ml, 625 mg/ml, respectively. The extract exhibited a remarkable capability to eliminate all strains mentioned above, as evidenced by its minimum bactericidal concentration (MBC) of 250 mg\ml, 625 mg/ml, and 1250 mg/ml. On the other hand, T2 microflora for *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *B. Subtilis* ATCC 6633 and *Vankomycin resistant Enterococci* (VRE) were 625 μg/mL, 1250 μg/mL, 625 μg/mL and 312.5 μg/mL, respectively

Table 4 – Minimum bactericidal concentration (MBC) of various plant extracts against various strains

Bacterium	Minimum inhibitory concentration (MIC), μg/ml	
	T1	T2
S.aureus ATCC 6538	NT	5000
E.coli ATCC 25922	2500	2500
P.aeruginosa ATCC 27853	625	NT
B.subtilis ATCC 6635	625	2500
Vankomycin resistant Enterococci (VRE)	1250	>5000

Abbreviation: NT – not tested because the MBC value was not available as it was resistant to high tests at an herbal extract concentration of 5000 μg/mL.

The MBC T2 values for the aforementioned strains were 5000 mg/ml, 2500 mg/ml, 2500 mg/ml, >5000mg/ml, respectively. Comparison of MBC of these two extracts showed the superiority of T1 (625 μg/ml) in the fight against the *P. aeruginosa* strain and T2 (5000 μg/ml) in the destruction of S. aureus strains (Table 4).

Conclusions

It is shown that *Zinnia Elegans*, growing in the East Kazakhstan region, is a promising source of essential oils. Essential oil's of plant main components are monoterpenes, contained 43 components, which amounted to 99.46% of the total number of oil components, the main volatile components of them were: *β*-myrcene (14.07%), *α*-terpineol (9.45%), myrtenal (7.87%) and camphene (5.36%). This fraction of hydrocarbons of essentiol oil has a wide spectrum of pharmacological action, mainly determining the biological effects. It is concluded that all essential oils have a weak antioxidant effect, and this is one of the facets of their biological activity. Of all the studied in the work, *Z. elegans* essential oil, which has the highest content of *β*-myrcene (14.07%), shows great antioxidant activity. The research results indicate the pharmacological potential of *Z. elegans* essential oil, which grows in the East Kazakhstan region, which allows it possible to consider it as a promising component for obtaining phytopreparations and natural medicinal products.

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