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Analysis of composition and antioxidant activity of essential oil obtained from AG-5 herbal dietary supplement

Analiza składu i aktywności antyoksydacyjnej olejku eterycznego otrzymanego z suplementu diety AG-5

Free radicals induce many types of diseases such as various types of cancer, cardiovascular disease, atherosclerosis, stroke, obesity, cataracts and diabetes. Natural substances in plants include a wide variety of antiradicals which prevent against such diseases [3,8,14]. Antioxidants delay the oxidation process, they suppress the free radicals by donating them a hydrogen atom [9].

AG-5 dietary supplement contains numerous medical plants: cayenne pepper (*Capsicum frutescens*), ginger (*Zingiber officinalis*), rosemary (*Rosmarinus officinalis*), cardamom (*Ellettaria cardamomum*), lavender sage (*Lavandula officinalis*), angelica (*Archangelica officinalis*), curcuma (*Curcuma longa*), clove tree (*Syzygium aromaticum*).

Previously, AG-5 dietary supplement was tested *in vivo* against 12 reference strains of microorganisms. The test showed antibacterial activity against Gram-positive bacteria [7]. AG-5 is recommended prophylactically, to prevent heart and cardiovascular diseases, as well as atherosclerosis and Alzheimer disease, also in the adjuvant treatment of these diseases.

The purpose of this work was to examine the composition of essential oil obtained from AG-5 drug and to test antioxidant activity.

MATERIAL AND METHODS

Essential oil from AG-5 dietary supplement was isolated according to the method of Polish Pharmacopoeia no. VI [12]. The samples after steam water distillation were placed in small glass vials, dried over anhydrous sodium sulphate, and stored at 4°C until further analysis.

G C - M S a n a l y s i s. MS analysis was carried out with GC/MS method using GCQ spectrometer (Thermo-Finnigan, USA) on Restek RT-5 capillary column (20 m x 0.18 i.d. coated 0.2 μm film thickness). The analysis was carried out in the programmed mode with the temperature gradient of 50°C–280°C at 10°C/min. In our study we used a mass selective detector with the electron impact (EI) ionization mode (70 eV). The qualitative analysis was carried out on the basis of MS spectra

which were compared with the spectra of the NIST library [10], and data available in literature [1,6]. Identity of the compounds was confirmed by their retention indices taken from literature and own data [1,6]. The composition of essential oil was determined by GC/MS, by assuming the total of all particular oil to be 100%.

D P P H m e t h o d. Antioxidant activity of oil obtained from AG-5 drug was determined according to the method of Brand-Williams et al. [2]. For this research, we used the Cary 50 Scan, Varian, UV-visible spectrophotometer and disposable cuvettes (1 x 1 x 4.5 cm) for visible absorbance measurements. DPPH (2,2– diphenyl-1-picryl-hydrazyl-hydrate) was used as a free radical. The different concentrations of antioxidant solution in methanol were tested (ranged 0.0187mg/ml – 0.9125mg/ml) and then 0.1 ml was mixed with 3.9 ml of a 6x10-5 mol/l methanol DPPH solution. The mixture were incubated in darkness at room temperature, the absorbance was measured every 5 min. for 30 min. at 517 nm until the reaction reached a plateu. Spectrophotometer plotted each kinetic reaction for different concentration. Using these graphs we transferred the values onto another graph showing the function of the mg/ml ratio of antioxidant to $DPPH^{\cdot}$. Antioxidant activity was defined with parameter EC50 (concentration, at which the sample compound shows 50% radical - scavening activity). Percentage of inhibition was calculated using the following formula [5]:

$$\% \text{ inhibition} = [(A_b - A_a)/A_b] \times 100$$

where, A_a is the absorbation of tested solution, A_b is the absorbation of blank sample

RESULTS AND DISCUSSION

DPPH is a free radical, which produces a deep violet colour solution in methanol. Because of antioxidant molecule, it is reduced giving rise to light-yellow methanol solutions [9,14]. Kinetic behavior of different antioxidants is an important factor in the evaluation of antioxidant activity [13].

Using the FP VI method [12] 0.788 g/ml essential oil of AG-5 was isolated. Through GC-MS analysis we identified 41 compounds. The major component was eugenol (52.9%). Other ingredients were in much smaller percentage: β-caryophyllene (12.7%), 1,8-cineole (5.8%), α-zingiberene (3.2%), α-terpinyl acetate (2.7%). Identified compounds, their retention time and percentage composition are shown in Table 1. A chromatogram from GC-MS analysis of essential oil from AG-5 is shown in Figure 1.

Essential oil reacted rapidly with the DPPH, the highest used concentration (0.9125%) reached a steady state in less than 2 min (Fig. 2). The DPPH method has shown that AG-5 drug has high antioxidant properties. The percentage of inhibition is 80.6% (Fig. 3). The parameter EC50 is 0.04 mg/ml (Fig. 4) and 0.095 mM of Trolox.

We suspect that eugenol (52.9%) is responsible for so high antioxidant activity of isolated essential oil (0.04 mg/ml). Despite the availability of only one hydrogen on a hydroxyl group, one eugenol molecule reduces two $DPPH^{\cdot}$ molecules [2,4,16]. Brand-Williams et al. [2] suggest 3 hypotheses to explain the antiradical efficiencies of eugenol. The first one involves the donation of a second hydrogen following electron delocalization onto the para-substituted group, the second hypothesis involves a dimerization between two phenoxyl radicals and the last hypothesis shows a complex of one $DPPH^{\cdot}$ molecule with one acryl radical [2,11,15].

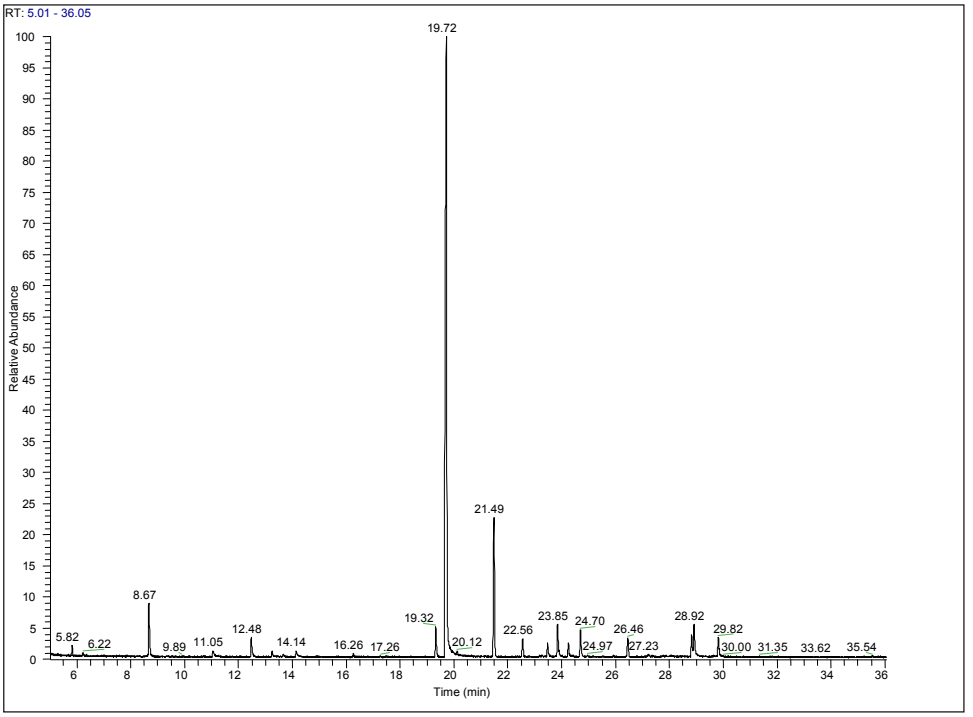

Fig. 1. GC-MS chromatogram of chemical compounds present in essential oil of AG-5. Compounds are marked in accordance with Table 1

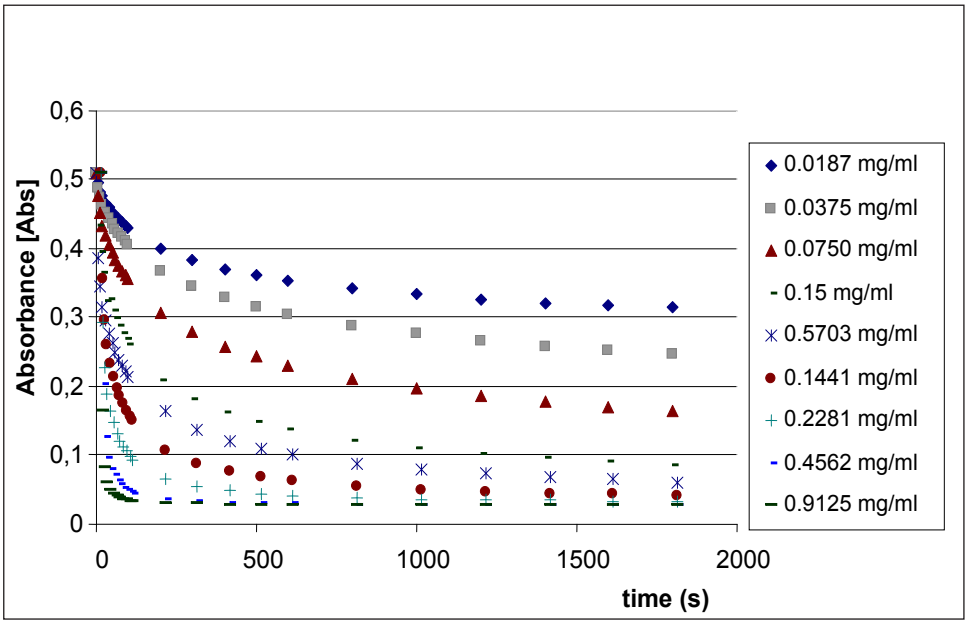

Fig. 2. The example of kinetic behavior of essential oil obtained from AG-5 dietary supplement. Concentrations of antioxidant ranged from 0.0187 mg/ml to 0.9125 mg/ml

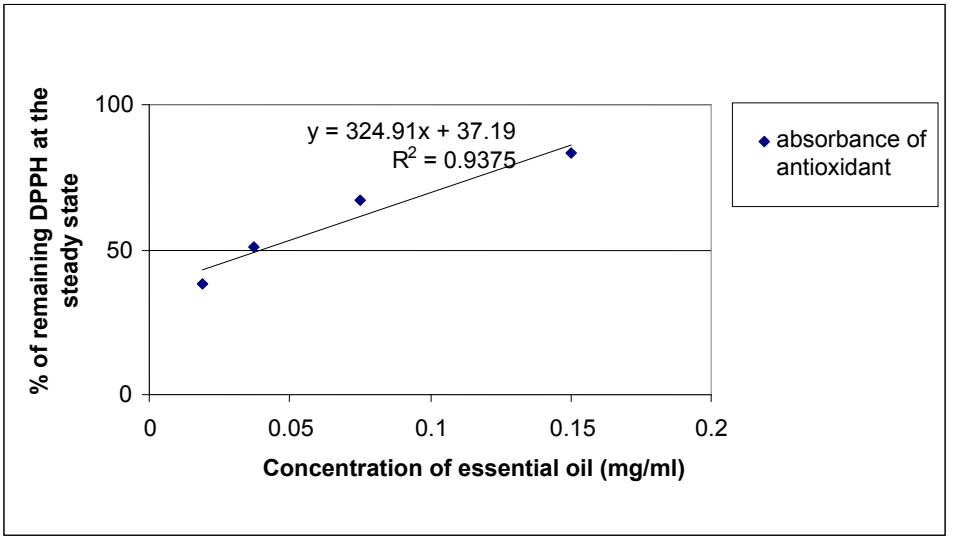

Fig. 3. Parameter EC_{50} of essential oil isolated from AG-5 dietary supplement

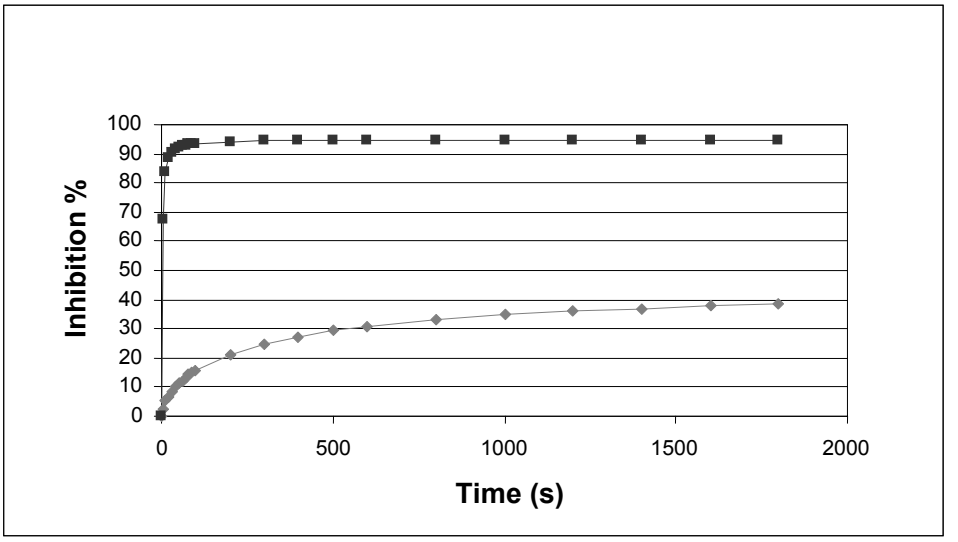

Fig. 4. Kinetic curves obtained for the reaction of the essential oil with DPPH for different concentrations of essential oil; ■ 0.9125 mg/ml and ♦ 0.0187 mg/ml

Table 1. Composition of the essential oil obtained from AG-5 using distillation method described in Polish Pharmacopoeia no. VI

RI	RT	Compounds	Percentage content (%)
935	5.82	α-pinene	0.7
950	6.22	camphene	0.3
980	6.77	β-pinene	0.1
1008	7.66	α-phellandrene	0.1
1029	8.35	p-cymene	0.1
	8.44	limonene	0.2
1033	8.67	1,8-cineole	5.8
1106	11.05	linalool	1.5
1148	12.48	camphor	2.2
1170	13.15	borneol	0.5
1201	14.14	δ-terpineol	0.1
	14.45	isopinocamphone	0.2
1183	14.56	terpinen-4-ol	0.4
1197	14.64	α-terpineol	1.0
1219	14.78	verbenone	0.1
1260	16.26	Linalyl acetate	0.3
1290	17.22	Bornyl acetate	0.1
1353	19.32	α-terpinyl acetate	2.7
1367	19.72	eugenol	52.9
1378	20.12	α-copaene	0.3
1421	21.49	β-caryophyllene	12.7
1456	22.56	α-humulene	1.6
1479	23.27	y-Amorphene	0.1
1486	23.46	ar-Curcumene	1.3
1489	23.56	α-selinene	0.1
1498	23.85	α-zingiberene	3.2
1511	24.24	β-bisabolene	1.3
1517	24.41	γ-cadinene	0.1
1527	24.70	β-sesquiphellandrene	2.4
1535	24.97	cadina-1,4-diene	0.1
1554	25.49	elemol	0.1
1568	25.9	(E)-nerolidol	0.1
	26.32	spathulenol	0.1
1587	26.46	Caryophyllene oxide	1.3
1614	27.23	1,2-humulenepoxide	0.2
1670	28.82	ar-Turmerone	1.4
1674	28.92	α-Turmerone	2.1
1699	28.92	trimethoxyacetophenone isomer	0.1
1706	29.82	β-Sesquiphellandrone turlone	1.2
1782	31.35	(E)-α-Atlantone	0.1
1929	35.54	methyl palmitate	0.2

RI – retention index, RT – retention time

CONCLUSIONS

The DPPH method is an easy and rapid way to test the antiradical activities of antioxidants. The results obtained in the presented study show that it is evident that AG-5 has antiradical activity, so it is opening new possibilities for applications in medicine. It would be interesting to study antiradical activity of each compound from the isolated essential oil of AG-5.

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SUMMARY

Essential oil was isolated from AG-5 drug using the method described in Polish Pharmacopoeias VI. GC-MS analysis of essential oil was made. In essential oil 41 compounds were identified, in which the major component was eugenol (52.9%). The antiradical activity was tested by popular DPPH method. The tested essential oil showed high antioxidant activity EC_{50} = 0.04 mg/mL and 0.095 mM of Trolox.

STRESZCZENIE

Z suplementu diety AG-5 wyizolowano olejek eteryczny metodą opisaną w Farmakopei Polskiej VI. Przeprowadzono analizę jakościową olejku przy użyciu metody GC-MS. Zidentyfikowano 41 składników, z czego dominujący był eugenol (52,9%). Olejek eteryczny przebadano również spektrofotometrycznie pod kątem aktywności antyoksydacyjnej, wykorzystując popularną metodę DPPH. Analizowany olejek wykazał znaczną aktywność antyoksydacyjną EC50 = 0,04 mg/ml oraz 0,095 mM w przeliczeniu na Trolox.