GLECHOMA HEDERACEA AS NATURAL LIPID ANTIOXIDANT FOR FAT AND SUNFLOWER OIL

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Abstract

Ethanolic and ethyl acetate extracts of aerial parts of Serbian origin of *Glechoma hederacea* L. (familu Lamiaceae) were found to possess antioxidant activities, when tested at 60°C by Schall-Oven test and at 120°C by Rancimat method. Antioxidant activities of tested samples were determined in different concentrations, such as: 0.02%, 0.04% and 0.05%. Tests were performed with three substrates separately: prime steam lard, edible sunflower oil and C-active treated edible sunflower oil. The addition both of extracts to edible oil has shown a little effect on elongation of induction time and significance effect when tested in lard. In contrast, these extracts possess major antioxidant activities when tested with C-active treated edible oil. It seems that C-active treated sunflower edible oil can be used as a very suitable substrate for evaluation of plant extracts antioxidant activity.

Key words. *Glechoma hederacea*, lipid antioxidant, substrates.

INTRODUCTION

Over the past couple of years research has emerged in the application of well-known and lesser-known natural lipid antioxidants. Among naturally occurring substances of potential antioxidant activity flavonoids are of particular interest (SIX, 1994).

Preliminary studies have also been made on the content of fatty acids (OKUDA et al., 1986 and VAVILOVA et al., 1988), triterpenoids and flavonoids heterosides (ZIEBA, 1973). On the contrary, we isolated and identified the aglycones of the already reported heterosides, flavone luteolin and apigenin, flavonol quercetagetin and its 7-O-glucoside, which have not hitherto been isolated from this plant species. Our chemical research of domestic plant species *Glechoma hederacea* has showed the high content of flavonoid compounds, what promoted us to investigate their potential use as natural lipid antioxidants in food (MILOVANOVIC et al.,1995). Alcoholic extract of this origin are used in folk medicine as creams

against itching and aqueous extracts for treatment of indigestion and urinal diseases (SERBIAN ACADEMY OF SCIENCE AND ARTS, 1983). The present report is concerned with the isolation and characterization of antioxidative extracts of *Glechoma hederacea* on several substrates at different temperatures.

MATERIALS AND METHODS

Plant material. *Glechoma hederacea* L. or *Nepeta glechoma* Benth, (Lamiaceae) (TUTIN et al, 1976), air-dried plant material (2 kg) was collected in may 1991, in the vicinity of Belgrade. A herbarium specimen is deposited at Botany Department, University of Belgrade.

Extraction of Samples

Ten grams of dried and ground plant material were extracted in Soxlet extractor with 300ml of petroleum ether for 24 hours. The residue was reextracted in ethanol for 48 hours. The ethanolic extract was concentrated *in vacuo* at 40 °C yielded 16.09% of this extract. The crude ethanolic extract was extracted in the next step with ethyl acetate and ether, separately and 11.52% and 0.71% of these extracts were obtained.

Antioxidant Activity Analysis

Tests for antioxidant activity were conducted on lard, edible sunflower oil and C-active treated oil. The antioxidant activity of each extracts tested was based on its ability to prevent the formation of peroxides in lard and oils. The samples were kept at 60°C without the light according to the Schall test (SCHERWIN, 1985) in the concentrations of 0.02-0.05% comparison with γ-tocopherol and butulated hydroxyanisole (BHA). A tocopherol mixture consisting of 12% α-tocopherol, 1% β-tocopherol, 61% γ-tocopherol and 26% δ-tocopherol (Coviox-T, Germany) was used as a positive control. Changes in peroxide value (POV) were determined by Official Method Cd 8-53 of the American Oil Chemist's Society (AOCS, 1980). All Rancimat analyses were performed with a 617 Rancimat (Metrohm AG, CH-9100 Herisau, Switzerland). Samples were investigated at temperature of 120°C, air flow was 18-20 ml/min and sample size was 2.5g.

Statistical analysis of experimental data

All experiments were repeated in duplicate and typical results are shown from one of the two independent experiments. Significance of treatments was estimated by the Student's test (SNEDECOR et al., 1967).

RESULTS, DISCUSSION AND CONCLUSION

The selection of *Glechoma hederacea* L. (familu Lamiaceae) was based on its easy accessibility, spread and nontoxicity. The results of this research have showed that ethanolic and ethyl acetate extracts possess potent antioxidant activities. The other extracts were not further investigated and also, they did not give a positive test treated with $FeCl_3$ and α,α'-dipyridyl reagents, respectively (BARTON, 1965). The inhibitory effect on the oxidation of these extracts was copared with that of commercial antioxidants, BHA and some tocopherols. Table 1 shows the effect of ethanolic and ethyl acetate extracts on the peroxide formation of lard during 3 days storage at 60°C. As the storage days increased POV of lard increased from 0 to 32 meq/kg lard. The POV of lard containing ethanolic and ethyl acetate extracts in 0.05% concentrations, after 3 days of storage were 14.22 and 11.05meq/kg ($P< 0.05$ compared with γ-tocopherol), respectively. The antioxidant effect of 0.02% γ-tocopherol mixture was 15.42 meq/kg ($P<0.001$). These results strongly suggest that these extracts of *Glechoma hederacea* contain antioxidative components. Flavonoids could be possible antioxidative components because ethanol is a good solvent for extracting these compounds from plant material (SIX, 1994). The presence of other phenolic compounds in these extract, which were not eluted under the chromatographic conditions of the method used, may be significant to the antioxidant effect. Table 2 presents the effect of the adding ethanolic and ehyl acetate extracts to edible sunflower oil as control in concentrations of 0.02% and 0.04% on the peroxide formation during 5 days of storage at 60°C. As it may be seen the POV of the both of extracts at the 0.02% and 0.04% levels are the same just as the POV of control values and with the same effect as 0.01% BHA. A probable reason for these results is higher stability of the domestic sunflower edible oil, which contains high amount some tocopherols mixture, such as: 600mg/kg of α-isomer and around 90 mg/kg of other tocopherols mixture.

On the contrary, both of extracts showed especially strong antioxidative effect on the oxidation, when tested on C-active treated edible oil as control (Table 3). The control, which contained no herb extract, reached a POV of 199.45 meq/kg for 3 days. However, all the samples containing some extracts of herb developed POV ranging from 29.42 meq/kg after 3 days ($P< 0.05$ compared with BHA) to 35.84 meq/kg ($P< 0.05$ compared with BHA) for ethyl acetate and ethanolic extracts in 0.04% concentrations. These results indicated strong antioxidant activities which were consistent with the results presented in Table 1. Because the POV of BHA and γ-tocopherol after 3 days were 64.94 and 15.42 meq/kg, respectively both of extracts are stronger antioxidants than the commercially used antioxidants.

The Rancimat method is based on conductometric determination of volatile degradation secondary product of oxidation and features automatic plotting of the conductivity against time (LAUBLI and BRUTTEL, 1986). Antioxidant activities of different extracts at 120°C were measured by Rancimat method (Fig. 1). Ethanolic extract of 0.04% (curve 2) possessed low activity with induction period (I.P.) of 1.88 hours ($P< 0.01$) better than C-treated oil, as control (curve 1). The pattern of the curve 3, as ethanolic extract of 0.06%, showed that no secondary products were formed since no volatile products were detected by 5 hours. The I.P. values of ethyl acetate extracts in 0.02, 0.04 and 0.06% were 2.37, 2.48 and 2.32 hours (see curves 4, 5 and 6) and clearly showed that the antioxidative effect under these experimental conditions was not concentration-dependent ($P< 0.05$).

The results demonstrated that ethanolic extracts of plant species *Glechoma hederacea* acted as a potent antioxidant, especially in stabillizing C-treated sunflower oil, which is better substrate than steam lard for the evaluation of antioxidant activity. The results of these research strongly suggest that ethanolic extract of this herb can be successfully applied to lard and oil to prevent oxidative deterioration. Nevertheless, the presented data provide important information for new source of known antioxidants.

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FIGURE 1. Effect of the antioxidants on rate of autoxidation of C-treated edible oil by Rancimat method at 120°C, 1) control, 2) ethanolic extract (0.04%) 3) ethanolic extract (0.06%), 4) ethyl acetate extract (0.02%), 5) and 6) ethyl acetate extracts (0.04% and 0.06%).

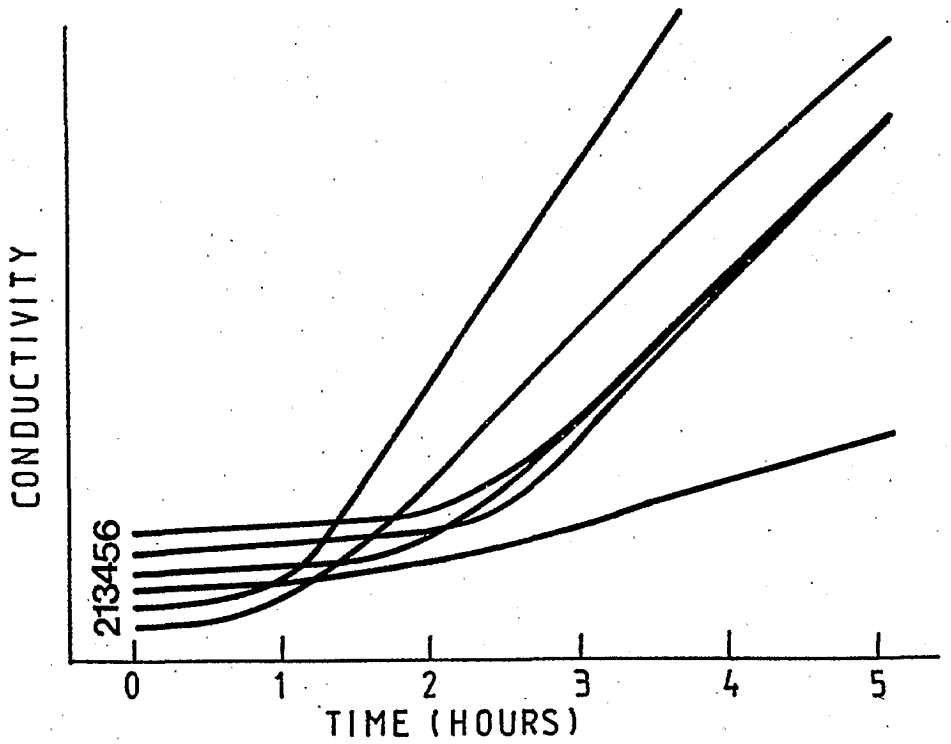

TABLE 1. POV in meq/kg of *G. hederacea* of lard in the Schall test at 60°C, C) prime steam lard as control, EtOH) ethanolic extract (0.05%), EtOAc) ethyl acetate extract (0.05%) , EtOAc) ethyl acetate extract (0.05%) and Tch) γ-tocopherol (0.02%).

time (hr)	24	48	72
C	2.0	12	32
EtOH	3.1	7.08	14.22
EtOAc	1.4	4.88	11.05
Tch	3.15	8.12	15.42

TABLE 2. POV in meq/kg of *G. hederacea* extracts of edible oil in Schall test at 60°C, C_1) sunflower edible oil as control, EtOH-1) ethanolic extract (0.02%), EtOH-2) ethanolic extract (0.04%), EtOAc-1) ethyl acetate extract (0.02%), EtOAc-2) ethyl acetate extract (0.04%) and BHA) butylated hydroxyanisole (0.01%).

time (hr)	23	45	76	117
C_1	7.45	16.54	26.30	46.64
EtOH-1	9.21	17.49	27.67	46.88
EtOH-2	6.69	15.15	26.21	44.55
EtOAc-1	7.72	16.30	27.12	46.25
EtOAc-2	7.45	16.83	29.13	48.48
BHA	8.40	17.25	27.97	44.02

TABLE 3. POV in meq/kg of *G. hederacea* extracts of C-active treated sunflower edible oil in the Schall test at 60°C, C_2) C-active treated oil as control. Symbols as in Tab. 2.

time (hr)	23	45	76	117
C_2	26.23	39.94	190.45	-
EtOH-1	18.75	28.25	35.50	50.51
EtOH-2	18.29	28.78	35.84	50.70
EtOAc-1	18.43	27.56	32.94	46.11
EtOAc-2	16.24	24.07	29.42	41.35
BHA	27.81	41.80	64.94	-