NPC Natural Product Communications

2010
Vol. 5
No. 1
61 - 63

Antioxidant Effects of *Glechoma hederacea* as a Food Additive

Mirjana Milovanovic*, **Dusan Zivkovic and Biljana Vucelic-Radovic**

Faculty of Agriculture, Department of Food Technology and Biochemistry, University of Belgrade, 11081 Belgrade-Zemun, Nemanjina 6, Serbia

mmmilova@agrif.bg.ac.rs

Received: September 1st, 2009; Accepted: November 9th, 2009

The antioxidant properties of *Glechoma hederacea* L. (Lamiaceae), of Serbian origin, were studied in respect to its potential use in foodstuffs. Ethanol-water (8:2, v/v) and purified ethyl acetate extracts of the plant were found to possess significant antioxidant activity. Tests were performed on two different substrates, prime steam pork lard and active-carbon-treated edible sunflower oil, using Schaal oven test storage conditions at 60ºC. The ethanol-water and purified ethyl acetate extracts of *G. hederacea* showed strong concentration-dependent antioxidant activity. On the contrary, under the Rancimat method conditions at 120ºC, the ethanol-water extract showed significantly stronger antioxidant activity, in comparison with the other tested extracts. All activities were compared with commercial antioxidants, such as BHA and a tocopherol mixture, respectively. For the first time, the activity of the flavonol quercetagetin was determined.

Keywords: Antioxidants, *Glechoma hederacea*, food additive, quercetagetin.

Among naturally occurring substances with potential antioxidant activity, flavonoids are of particular interest. They are also important factors in biological interactions between living organisms [1]. The large variety of flavonoids occurring in the Lamiaceae and the wide-spread distribution of *Glechoma hederacea* L. (synonym *Nepeta glechoma* Benth.) in Serbia inspired us to investigate the flavonoids in this species. From its aerial parts, heterosides, such as the flavonol quercetagetin (Qu: 3,5,6,7,3',4'-hexahydroxy-flavone) and its 7-*O*-glucoside have been reported [2]. Also, several flavonoid heterosides have been identified from the whole plant [3]. The composition of *G. hederacea* essential oil has been reported recently [4], as has the isolation of new alkaloids [5]. Studies on the phenolic acids composition of the species have also been made [6,7].

Numerous biological properties of *G. hederacea* have been reported, including antihypertensive [8], hypoglycemic [9] and cytotoxic [10] activities. Alcoholic extracts have been used in traditional medicine in creams against itching, and aqueous solutions of these extracts for the treatment of indigestion, as a spasmolytic tonic, mild sedative and against different urinary diseases [11,12].

The selection of *G. hederacea* was based on its easy accessibility and non-toxicity. This study was undertaken in order to investigate the antioxidant properties of this species which might lead to its application as an additive to oils and food products.

The antioxidant activity was based on the peroxide-inhibiting capacity of plant extracts, following the method of Schaal [13]. Ethanol-water, crude ethyl acetate and especially the purified ethyl acetate extracts showed potent antioxidant activities. The first two extracts were not further investigated because they did not give positive tests with $FeCl_3$ and α,α'-dipyridyl reagents, which produce a colored reaction with polyphenolic compounds. Separation and identification of the antioxidant components by column chromatography demonstrated that the main flavonoid components were luteolin, whose antioxidant properties have been reported before [17], and quercetagetin [2], the activity of which has not been previously recorded; the compound was shown to have potent antioxidant activity. The inhibitory effects of these extracts and quercetagetin on lard and oil autooxidation were compared with some commercial antioxidants, such as BHA and a tocopherol mixture (Tch).

All extracts, at a concentration of 0.02%, inhibited lipid oxidation of steam lard in the following order: Tch (14.5 days) > Qu (10.1days) > purified ethyl acetate extract (9.0 days) > BHA (8.5 days) > ethanol-water extract = crude ethyl acetate extract (3.6 days). The

same concentrations of the ethanol-water extract and crude ethyl acetate extract showed significantly lower antioxidant effects than the commercial antioxidants. In contrast, the purified ethyl acetate extract showed a high antioxidant activity. Quercetagetin produced a significantly greater antioxidant effect than did BHA and all the examined extracts; quercetagenin exhibited only a slightly lower activity than that of Tch.

When 0.05% concentrations of the investigated antioxidants were tested, the following order of activity was observed: purified ethyl acetate extract (14.6 days) > Tch (14.5 days) > BHA (8.5 days) > ethanol-water extract (7.2 days) > crude ethyl acetate extract (6.7 days). The purified 0.05% ethyl acetate extract showed potent antioxidant activity, which was almost the same as that of the standard Tch, while the ethanol-water extract at the same concentration showed approximately two-fold lower antioxidant activity when compared with Tch. The purified ethyl acetate extracts exhibited strong antioxidant activities, displaying increased oxidation inhibition with concentration enhancement from 0.02% to 0.05%. Generally, the ethanol-water extract had almost the same effect as that of BHA, and the purified ethyl acetate extract was as effective as the Tch mixture.

The results obtained in the Schall oven test, with active-carbon-treated (C-treated) sunflower oil as a control, were in a good agreement with those obtained at 0.05% levels on lard: Tch (6.3 days) > purified ethyl acetate extract (6.0 days) > Qu (4.5 days) > ethanol-water extract (3.8 days) > BHA (3.0 days). The C-treated oil had an IP value of one day. The activity of BHA showed almost the same trend as it had on lard.

The Rancimat method is based on the conductometric determination of volatile secondary degradation products of oxidation and features automatic plotting of conductivity against time [14]. The relative antioxidant activity of the tested samples can be concluded based on the values of the induction times assessed by two different methods.

The IP value of the C-treated oil control which contained no antioxidants was 0.7 hours. All the investigated samples, at a concentration of 0.05%, inhibited lipid oxidation in the following order: Tch (5.20 h) > ethanol-water extract (5.05 h) > quercetagetin (4.20 h) > purified ethyl acetate extract (2.48 h) = crude ethyl acetate extract (2.32 h). The results also demonstrated that the ethanol-water extract had an activity similar to that of the tocopherol mixture, over five hours. Quercetagetin possessed a little lower activity in comparison with Tch. The other extracts had very low antioxidant effects. However, no pro-oxidant effect was noticed at the 0.05% level. Finally, the IP values of the ethyl acetate extracts showed that no secondary products were formed, since no volatile products were detected after two hours.

On the basis of these results, we suggest that an ethanol-water extract of *G. hederacea* might be successfully applied to lard or oil to prevent oxidative deterioration. The presented data provide important information on this new source of natural antioxidants.

Experimental

Plant material: *Glechoma hederacea* L. (synonym *Nepeta glechoma* Benth.), family Lamiaceae, is known under its traditional name of 'good-natured girl' or 'goodness' [11, 12]. The air-dried plant material was collected, at full blossom time, in May 2004, in the vicinity of Belgrade. The specimen was identified by a plant taxonomist in the Department of Botany, Faculty of Agriculture, University of Belgrade. A voucher specimen is lodged in the herbarium of this institution.

Preparation of extracts: Air-dried aerial parts (1.2 kg) were ground and extracted in a Soxhlet apparatus with light petroleum for 24 h. After removal of the solvent, the residue was re-extracted with 5 L of ethanol-water mixture (8:2, v/v) for 48 h at room temperature. The extract was concentrated *in vacuo* at 50ºC yielding 180 g of dry extract. In the next step, the ethanol-water extract was re-extracted with ethyl acetate (100%) and, after removal of the solvent, the crude dry extract was obtained in a yield of 11.2% (19.4 g). The brown, oily, crude ethyl acetate extract was purified on a silica gel column, starting with 100% ethyl acetate as the initial eluent. The polarity was gradually increased by addition of methanol. With 20% methanol in the eluent mixture, the purified ethyl acetate extract was collected in a yield of 6.3% (1.2 g). All extracts were concentrated using a rotary evaporator at 40ºC.

Antioxidant activity analysis: Tests for antioxidant activity were conducted on prime steam pork lard and active-carbon-treated sunflower edible oil, as controls with no antioxidants added. The antioxidant activity of each extract was based on its ability to prevent the formation of peroxide in lard and oil. The samples were kept at 60ºC in the dark in conditions of the Schaal oven test [13]. Extracts were tested on samples in concentrations of 0.02-0.05% and compared with either a tocopherol mixture (Tch) or butylated hydroxyanisole (BHA). For the Schaal oven test, glass tubes (100 mL, 15 cm i.d.) with a flat bottom, each containing 50.00 ± 0.01 g of either prime steam lard or C-treated oil and either 0.02 or 0.05% of one of the test samples were placed into an incubator and kept at 60 ± 1ºC in the dark. 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 control. Changes in peroxide values (PV) were determined according to the Association of Official Analytical Chemists method [14]. A sample size of 5.00 ± 0.01g was used in each PV analysis every 24 h. All Rancimat tests were performed with a 617 Rancimat (Metrohm AG, CH-9100 Herisau, Switzerland). Samples were investigated at 120ºC, air flow 18-20 mL/min and sample size 2.5 g [15].

Statistical analysis: The Schaal oven test and the Rancimat method were run in triplicate for each analyzed sample. The obtained results were analyzed using Student's t-test and a data analysis software system [16]**.**

Acknowledgements - The authors are grateful to Professor Kojic, Professor of Botany, Faculty of Agriculture, University of Belgrade, for the selection and identification of the plant material and the Serbian Ministry of Science for the provision of a research grant during these studies.

References

[1] Anderson Q, Markham K. (**2006**) *Flavonoids, Chemistry, Biochemistry and Applications*, CRC Press, Boca Raton FL.

[2] Milovanovic M, Stefanovic M, Djermanovic V. (**1995**) Flavonoids from *Glechoma hederacea* L. *Journal of Serbian Chemical Society*, ***60***, 467-469.

[3] Yamauchi H, Kakuda R, Yaoita Y, Machida K, Kikuchi M. (**2007**) Two new glycosides from the whole plants of *Glechoma hederacea* L.. *Chemical and Pharmaceutical Bulletin*, ***55***, 346-347.

[4] Mockute D, Bernotiene G, Judzentiene A. (**2007**) The essential oil of ground ivy (*Glechoma hederacea* L.) growing wild in eastern Lithuania. *Journal of Essential Oil Research*, ***19***, 449-451.

[5] Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. (**2003**) Isolation, structure elucidation and biological activity of hederacine A and B, two unique alkaloids from *Glechoma hederacea*. *Tetrahedron*, ***59***, 6403-6407.

[6] Komprda T, Stohandlova M, Foltyin J, Pozdisek J, Mika V. (**1999**) Content of *p*-coumaric and ferulic acids in herbs with potential grazing utilization*. Archives of Animal Nutrition*, ***52***, 95-105.

[7] Vavilova KN, Fursa NS, Oshmarina VI. (**1988**) Hydroxycinnamic acids of *Glechoma hederacea*. *Khimiya Prirodnykh Soedinenii*, 293-294.

[8] Watanabe T, Kawata A, Inoue M, Ishihara, S, Tsuji K. (**2007**) Antihypertensive effect of *Glechoma hederacea* extract in spontaneously hypertensive rats. *Nippon Shokuhin Kagaku Kogaku Kaishi*, ***54***, 415-418.

[9] Ishihara S, Kawata A, Inoue M, Watanabe T, Tsuji K. (**2007**) Hypoglycemic effect of *Glechoma hederacea* extract on blood glucose level in rats. *Nippon Shokuhin Kagaku Kogaku Kaishi*, ***54***, 412-414.

[10] Kumarasamy Y, Nahar L, Kong-Thu-lin P, Jaspars M, Sarker D. (**2006**) Hederacine A and hederacine B from *Glechoma hederacea* inhibit the growth of colorectal cancer cells *in vitro*. *Natural Product Communications*, ***1***, 33-35.

[11] Hegnauer R. (**1966**) *Chemotaxonomie der Pflanzen*, *VI.* Birkhäuser Verlag-Basel-Stuttgart..pp. 320-324.

[12] Josifović M. (**1983**) *Medicinal Plants of Serbia,* Serbian Academy of Science and Arts, Belgrade, 287-289.

[13] Sherwin ER. (**1985**) Synthetic antioxidants for fats and oils. In: *Flavor Chemistry of Fats and Oils*. Min DB, Smouse TH. (Eds) *American Oil Chemist's Society*, 163-173.

[14] *Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), International* 16th ed. Vol ***2***, Ch. 41, 9 (**1995**) Arlington, Virginia.

[15] Laubli MW, Bruttel PA. (**1986**) Determination of the oxidative stability of fats and oils: comparison between the active oxygen method (AOCS Cd 12-57) and the Rancimat method. *Journal of the American Oil Chemists Society*, ***63***, 792-795.

[16] StatSoft, Inc. Statistica (**2003**) version 6, www.statsoft.com.

[17] Milovanovic M, Picuric-Jovanovic K, Vucelic-Radovic B, Vrbaski Z. (**1996**) Antioxidant effects of flavonoids of *Anthriscus sylvestris* in lard. *Journal of the American Oil Chemist's Society*, ***73***, 773-776.