Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 9–13
Food Chemistry
www.elsevier.com/locate/foodchem

Screening of antioxidative properties of the methanolic extracts of *Pelargonium endlicherianum* Fenzl., *Verbascum wiedemannianum* Fisch. & Mey., *Sideritis libanotica* Labill. subsp. *linearis* (Bentham) Borm., *Centaurea mucronifera* DC. and *Hieracium cappadocicum* Freyn from Turkish flora

Bektas Tepe [a,*], Munevver Sokmen [b], H. Askin Akpulat [a], Onder Yumrutas [a], Atalay Sokmen [a]

[a] *Cumhuriyet University, Faculty of Science and Literature, Department of Biology, 58140 Sivas, Turkey*
[b] *Cumhuriyet University, Faculty of Science and Literature, Department of Chemistry, 58140 Sivas, Turkey*

Received 7 March 2005; received in revised form 17 May 2005; accepted 17 May 2005

Abstract

Five extracts from five plants, of which four are endemic to Turkish flora, were screened for their possible in vitro antioxidant activities by two complementary test systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid. In the first case, *Pelargonium endlicherianum* extract exerted greater antioxidant activity than those of other plant extracts studied with an IC_{50} value of 7.43 ± 0.47 μg/ml, followed by *Hieracium cappadocicum* (30.0 ± 0.14 μg/ml). When compared to the synthetic antioxidant BHT (18.0 ± 0.40 μg/ml), the methanolic extract of *P. endlicherianum* exhibited more than two fold greater antioxidant activity. In the β-carotene/linoleic acid test system, the most active plant was *P. endlicherianum* with 72.6% ± 2.96 inhibition rate, followed by *H. cappadocicum* (55.1% ± 2.33) and *Verbascum wiedemannianum* (52.5% ± 3.11). Antioxidant activities of curcumin and ascorbic acid were also determined as positive controls in parallel experiments.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Turkish medicinal plants; Antioxidant activity; Methanolic extract; BHT; Curcumine; Ascorbic acid

1. Introduction

A number of reports concerning the antioxidant activities of plant extracts of medicinal plants have appeared in the literature, but most have not been adequately evaluated (Balandrin, Klocke, Wurtele, & Bollinger, 1985). This is also particularly valid for Turkey, which has one of the most extensive floras in continental Europe (Sezik et al., 1991) with more than 9000 flowering plant species (Davis, 1982). Owing to its strategic position, the accumulation of the knowledge of traditional medicine from the West and East has enabled this region to have a rich tradition in terms of the uses of medicinal plants (Gozler, 1993). Previous reports on the Turkish medicinal plants have appeared as a list of plants and their uses (Cubukcu & Ozhatay, 1987; Tuzlaci & Erol, 1999; Tuzlaci & Eryasar Aymaz, 2001; Tuzlaci & Tolon, 2000), or the local use of the plants (Yildirimli, 1985) or an encyclopedic book (Baytop, 1984) in the literature. Screening of some Turkish medicinal plants for several purposes is also reported in the

* Corresponding author. Tel.: + 90 346 219 10 10x2907; fax: + 90 346 219 11 86.
E-mail address: bektastepe@yahoo.com (B. Tepe).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.05.046

literature (Ahmad, Rashid, Bingol, & Sener, 1993; Sener, 1994; Sokmen, Jones, & Erturk, 1999).

The genus *Pelargonium* is represented by only *Pelargonium endlicherianum* in Turkish flora (Davis, 1967, 1988). There are several studies concerning various biological activities of *Pelargonium* species in the literature (Demo, Petrakis, Kefalas, & Boskou, 1998; Jeyabalan, Arul, & Thangamathi, 2003; Matthys, Eisebitt, Seith, & Heger, 2003; Seidel & Taylor, 2004). As far as our literature survey could ascertain, there are no reports concerning the antioxidant activity of *P. endlicherianum*.

The genus *Verbascum* is represented in Turkish flora by 232 species, of which 185 are endemic (Davis, 1982, 1988). Antimicrobial (Barbour et al., 2004), antiviral (McCutcheon et al., 1995) and cytotoxic activities (Afifi, Ahmed, Pezzuto, & Kinghornt, 1993) of some members of this genus have been reported elsewhere. Some members of this genus are also used for liquor production (Cemek & Kucuk, 2001). We could not find any report in the literature dealing with the antioxidant properties of *Verbascum wiedemannianum*.

The genus *Sideritis* is represented in Turkish flora by 40 species, of which 31 are endemic (Davis, 1982, 1988). Anti-inflammatory and analgesic (De las heras, Vivas, & Villar, 1994; Hernandez-Perez, Sanchez, Montalbetti-Moreno, & Rabanal, 2004; Hernandez-Perez & Rabanal, 2002) activities of some members of this genus have been investigated. Moreover, antioxidant activities of *S. raeseri* and *Sideritis javalambrensis* were reported elsewhere (Gabrieli, Kefalas, & Kokkalou, 2005; Rios, Manez, Paya, & Alcaraz, 1992). Antioxidant activity of *Sideritis libanotica* subsp. *linears* was reported previously with other members of this genus by Tunalier et al. (2004).

The genus *Centaurea* is represented in Turkish flora by 177 species, of which 109 are endemic (Davis, 1975, 1988). Various biological activities of the members of this genus were reported elsewhere; cytogenetic (Radic, Prolic, Pavlica, & Pevalek-Kozlina, 2004), antimicrobial (Barbour et al., 2004), antifungal (Barrero et al., 2000), anti-inflammatory (Garbacki et al., 1999), anti-ulcerogenic activities (Yesilada, Gurbuz, & Shibata, 1999). There is no report concerning the antioxidant properties of *Centaurea mucronifera* in the literature.

The genus *Hieracium* is represented in Turkish flora by 99 species, of which 66 are endemic (Davis, 1975, 1988). Antimicrobial activity of some members of this genus was reported by Barbour et al. (2004). But we could not find any information on the antioxidant activity of *Hieracium cappadocicum* in the literature.

The literature outlines different approaches for determination of the antioxidant activities of the plant extracts. Therefore, different methodological approaches lead to scattered results, which are hardly comparable and often conflicting (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002; Mantle et al., 1998; Ruberto & Baratta, 2000; Zygadlo, Lamarque, Maestri, & Grosso, 1995). A plethora of different antioxidant assays in available and, because results rely on different mechanisms, they strictly depend on the oxidant/antioxidant models employed and on lipophilic/hydrophilic balance (Frankel, Huang, Kanner, & German, 1994). A single/substance/single-assay produces relative results and it is perceived as a reductive approach whenever a phytocomplex is involved. Therefore, antioxidant activities of the plant extracts studied here were determined by two complementary test-systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid systems.

The aim of the present work is to study in vitro antioxidant activities of the methanolic extracts of *P. endlicherianum*, *V. wiedemannianum*, *S. libanotica*, *C. mucronifera* and *H. cappadocicum*. All of the plants presented here are endemic to Turkish flora except *P. endlicherianum*.

2. Materials and methods

2.1. Collection of plant material

Localities and collection periods of the species studied are as follows:

1. *P. endlicherianum*: Gurun-Pinarbasi road, Sivas, Turkey; 7th July, 2004
2. *V. wiedemannianum*: Sivas-Celalli road, Yeniyol village, Sivas, Turkey; 20th July, 2002
3. *S. libanotica* subsp.*linearis*: Fish production farm, Ulas-Sivas, Turkey; 18st July, 2002
4. *C. mucronifera*: Bogrudelik, Gurun-Sivas, Turkey; 2nd July, 2002
5. *H. cappadocicum*: Taslidere, Domuzlukici district, Sivas, Turkey; 13th August, 2003

The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey (CUFH – Voucher No: 1, AA3412; 2, AA3042; 3, AA3022; 4, AA2999 and 5, AA3367, respectively).

2.2. Preparation of the methanol extracts

The air-dried and finely ground samples were extracted by using the method described elsewhere (Sokmen et al., 1999). Briefly, the sample, weighing about 100 g, was extracted in a Soxhlet apparatus with methanol (MeOH) at 60 °C for 6 h. The extract was then filtered and concentrated in vacuo at 45 °C, yielding a waxy material (8.45%, 6.94%, 7.28%, 6.03% and 6.91% w/w, respectively). Finally, the extracts were then lyophilized and kept in the dark at +4 °C until tested.

2.3. Antioxidant activity

2.3.1. DPPH assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple-coloured methanol solution of DPPH. This spectrophotometric assay (Pharmacia, Uppsala, Sweden), LKB-Novaspec (II) uses stable radical diphenylpicrylhydrazyl (DPPH$^{\cdot}$) as a reagent (Sigma–Aldrich) (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Fifty μl of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of the free radical DPPH$^{\cdot}$ in percent (I%) was calculated as follows:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate and butylated hydroxytoluene (BHT) and ascorbic acid (AA) were used as positive controls.

2.3.2. β-Carotene-linoleic acid assay

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Barriere et al., 2001).

A stock solution of β-carotene/linoleic acid (Sigma–Aldrich) was prepared as follows: first, 0.5 mg of β-carotene was dissolved in 1ml of chloroform (HPLC grade), then 25 μl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator (Büchi, Flawil, Switzerland). Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) were added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 μl portions of the extracts (2 g/l in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 μl of ethanol. After the incubation period, the absorbances of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

3. Results and discussion

In the light of the differences among the wide number of test systems available, the results of a single-assay can give only a reductive suggestion of the antioxidant properties of extracts toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behaviour, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among the plethora of methods that can be used for the evaluation of the antioxidant activity (TEAC, TRAP, LDL, DMPD, FRAP, ORAC, DPPH, PCL, and β-carotene bleaching), very few of them (TEAC, DPPH, PCL) are useful for determining the activity of both hydrophilic and liphophilic species, thus ensuring a better comparison of the results and covering a wider range of possible applications (Sacchetti et al., 2005). Taking this into account, the in vitro antioxidant activity of the five extracts tested, compared to that of BHT, ascorbic acid and curcumin, were assessed by two different tests: DPPH free radical-scavenging and β-carotene/linoleic acid systems.

Extracts obtained by Soxhlet extraction, from the aerial parts of the plants studied, were individually assessed for their possible antioxidative activities by employing two complementary tests: DPPH free -radical scavenging and β-carotene/linoleic acid assays. Free radical-scavenging capacities of the corresponding oils were measured by DPPH assay and the results are shown in Table 1.

As shown in Table 1, free radical-scavenging activity of *P. endlicherianum* (7.43 ± 0.47 μg/ml) was superior to the other plant extracts studied. IC_{50} value of *H. cappadocicum* was also noteworthy (30.0 ± 0.14 μg/ml) when compared to the synthetic antioxidants used in this study as positive controls. On the other hand, *V. wiedemannianum* extract exerted the weakest antioxidant activity in this system (117 ± 0.56 μg/ml).

In the β-carotene/linoleic acid system (Table 2), oxidation of linoleic acid was effectively inhibited by *P. endlicherianum* extract (72.6% ± 2.96), followed by *H. cappadocicum* (55.1% ± 2.33) and *V. wiedemannianum*

Table 1
Free radical-scavenging capacities of the extracts measured by DPPH assay[a]

Extracts	IC_{50} (μg/ml)
Pelargonium endlicherianum	7.43 ± 0.47
Verbascum viedemannianum	117 ± 0.56
Sideritis libanotica subsp.*linearis*	109 ± 0.43
Centaurea mucronifera	67.8 ± 2.16
Hieracium cappadocicum	30.0 ± 0.14
BHT	18.0 ± 0.40
Curcumin	7.80 ± 0.32
Ascorbic acid	3.80 ± 0.17

[a] Results are means of three different experiments.

Table 2
Inhibition percentages of the linoleic acid oxidation by the extracts[a]

Extracts	Inhibition (%)
Pelargonium endlicherianum	72.6 ± 2.96
Verbascum viedemannianum	52.5 ± 3.11
Sideritis libanotica subsp.*linearis*	38.5 ± 2.33
Centaurea mucronifera	35.2 ± 3.04
Hieracium cappadocicum	55.1 ± 2.33
BHT	96.6 ± 1.29
Curcumin	89.3 ± 2.14
Ascorbic acid	94.8 ± 1.86

[a] Results are means of three different experiments.

(52.5% ± 3.11). Methanolic extract of *C. mucronifera* exhibited the weakest antioxidant activity (35.2% ± 3.04).

As mentioned in the first section of this paper, all of the plants presented here are endemic to Turkish flora except *P. endlicherianum*. Antioxidant activity of *S. libanotica* subsp. *linearis* was reported before by Tunalier et al. (2004). From this point of view, the results presented here could be considered as the first information on the antioxidant activities of the plants studied. Results obtained from the natural sources can be used in food industries for preservation and/or extension of the shelf-life of raw and processed foods. The results in the present study also support the use of *P. endlicherianum* and *H. cappadocicum* as additives in foods, and traditional for anti-aging remedies.

References

Afifi, M. S. A., Ahmed, M. M., Pezzuto, J. M., & Kinghornt, A. D. (1993). Cytotoxic flavonolignans and flavones from *Verbascum sinaiticum* leaves. *Phytochemistry, 34*, 839–841.

Ahmad, F., Rashid, S., Bingol, S., & Sener, B. (1993). Screening of some Turkish medicinal plants for their analgesic activities. *Journal of the Faculty of Pharmacy of Gazi, 10*, 37–46.

Balandrin, M. F., Klocke, J. A., Wurtele, E. S., & Bollinger, W. H. (1985). Natural plant chemicals: Source of industry and medicinal materials. *Science, 228*, 745–747.

Barbour, E. K., Sharif, M. A., Sagherian, V. K., Habre, A. N., Talhouk, R. S., & Talhouk, S. N. (2004). Screening of selected indigenous plants of Lebanon for antimicrobial activity. *Journal of Ethnopharmacology, 93*, 1–7.

Barrero, A. F., Oltra, J. E., Alvarez, M., Raslan, D. S., Saude, D. A., & Akssira, M. (2000). New sources and antifungal activity of sesquiterpene lactones. *Fitoterapia, 71*, 60–64.

Barriere, C., Centeno, D., Lebert, A., Leroy-Setrin, S., Berdague, J. L., & Talon, R. (2001). Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *FEMS Microbiology Letters, 201*, 181–185.

Baytop, T. (1984). Türkçe Bitki Adlari Sö2zlüğu (*A dictionary of vernacular names of wild plants of Turkey*) (No. 578, 512 pp). Ankara: Publication of the Turkish Language Society.

Burits, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotheraphy Research, 14*, 323–328.

Cemek, M., & Kucuk, M. M. (2001). Liquid products from *Verbascum* stalk by supercritical fluid extraction. *Energy Conversion and Management, 42*, 125–130.

Cubukcu, B., & Ozhatay, N. (1987). Anadolu halk ilaclari hakkinda bir arastirma (A survey on folk medicine in Turkey). III. Milletlerarasi Turk Folklor Kongresi, Bildiriler Kitabi. In *Proceedings of 3rd international symposium of Turkish folklore* (pp. 103–115). Ankara: Basbakanlik Basimevi.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Davis, P. H. (1967). *Flora of Turkey and the East Aegean Islands* (Vol. 2). Edinburgh: Edinburgh University Press.

Davis, P. H. (1975). *Flora of Turkey and the East Aegean Islands* (Vol. 5). Edinburgh: Edinburgh University Press.

Davis, P. H. (1982). *Flora of Turkey and the East Aegean Islands* (Vol. 7). Edinburgh: Edinburgh University Press.

Davis, P. H. (1988). *Flora of Turkey and the East Aegean Islands* (Vol. 10). Edinburgh: Edinburgh University Press (Supplement I).

De las heras, B., Vivas, J. M., & Villar, A. (1994). Anti-inflammatory activity of *Sideritis javalambrensis* extracts. *Journal of Ethnopharmacology, 41*, 15–17.

Demo, A., Petrakis, C., Kefalas, P., & Boskou, D. (1998). Nutrient antioxidants in some herbs and Mediterranean plant leaves. *Food Research International, 31*, 351–354.

Frankel, E. N., Huang, S. W., Kanner, J., & German, J. B. (1994). Interfacial phenomena in the evaluation of antioxidants: Bulk oils versus emulsions. *Journal of Agricultural and Food Chemistry, 42*, 1054–1059.

Gabrieli, C. N., Kefalas, P. G., & Kokkalou, E. L. (2005). Antioxidant activity of flavonoids from *Sideritis raeseri*. *Journal of Ethnopharmacology, 96*, 423–428.

Garbacki, N., Gloaguen, V., Damas, J., Bodart, P., Tits, M., & Angenot, L. (1999). Anti-inflammatory and immunological effects of *Centaurea cyanus* flower-heads. *Journal of Ethnoparmacology, 68*, 235–241.

Gozler, B. (1993). Recent studies on Turkish traditional drugs of plant origin. In B. Mukherjee (Ed.), *Traditional medicine* (pp. 36–45). New Delhi: Oxford and IBM Publishing.

Hernandez-Perez, M., Sanchez, C. C., Montalbetti-Moreno, Y., & Rabanal, R. M. (2004). Studies on the analgesic and anti-inflammatory effects of *Sideritis candicans* Ait. var. *eriocephala* Webb aerial part. *Journal of Ethnopharmacology, 93*, 279–284.

Hernandez-Perez, M., & Rabanal, R. (2002). Evaluation of the antinflammatory and analgesic activity of *Sideritis canariensis* var. pannosa in mice. *Journal of Ethnopharmacology, 81*, 43–47.

Jeyabalan, D., Arul, N., & Thangamathi, P. (2003). Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. *Bioresource Technology, 89*, 185–189.

Koleva, I. I., van Beek, T. A., Linssen, J. P. H., de Groot, A., & Evstatieva, L. N. (2002). Screening plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis, 13*, 8–17.

Mantle, D., Anderton, J. G., Falkous, G., Barnes, M., Jones, P., & Perry, E. K. (1998). Comparison of methods for determination of total antioxidant status: Application to analysis of medicinal plant essential oils. *Comparative Biochemistry and Physiology Part B, 121*, 385–391.

Matthys, H., Eisebitt, R., Seith, B., & Heger, M. (2003). Efficacy and safety of an extract of *Pelargonium sidoides* (EPs 7630) in adults with acute bronchitis: A randomised, double-blind, placebo-controlled trial. *Phytomedicine, 10*, 7–17.

McCutcheon, A. R., Roberts, T. E., Gibbons, E., Ellis, S. M., Babiuk, L. A., Hancock, R. E. W., et al. (1995). Antiviral screening of British Columbian medicinal plants. *Journal of Ethnopharmacology, 49*, 101–110.

Radic, S., Prolic, M., Pavlica, M., & Pevalek-Kozlina, B. (2004). Cytogenetic effects of osmotic stress on the root meristem cells of *Centaurea ragusina* L. *Environmental and Experimental Botany* (in press).

Rios, J. L., Manez, S., Paya, M., & Alcaraz, M. J. (1992). Antioxidant activity of flavonoids from *Sideritis javalambrensis*. *Phytochemistry, 31*, 1947–1950.

Ruberto, G., & Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry, 69*, 167–174.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., et al. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry, 91*, 621–632.

Seidel, V., & Taylor, P. W. (2004). In vitro activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. *International Journal of Antimicrobial Agents, 23*, 613–619.

Sener, B. (1994). Recent results in the search for bioactive compounds from Turkish medicinal plants. *Pure and Applied Chemistry, 66*, 2295–2298.

Sezik, E., Tabata, M., Yesilada, E., Honda, G., Goto, K., & Ikeshiro, Y. (1991). Traditional medicine in Turkey I. Folk medicine in Northeast Anatolia. *Journal of Ethnopharmacology, 35*, 191–196.

Sokmen, A., Jones, B. M., & Erturk, M. (1999). The in vitro antibacterial activity of Turkish medicinal plants. *Journal of Ethnopharmacology, 67*, 79–86.

Tunalier, Z., Kosar, M., Ozturk, N., Baser, K. H. C., Duman, H., & Kirimer, N. (2004). Antioxidant properties and phenolic composition of *Sideritis* species. *Chemistry of Natural Compounds, 40*, 206–210.

Tuzlaci, E., & Erol, M. K. (1999). Turkish folk medicinal plants, part II: Eğirdir (Isparta). *Fitoterapia, 70*, 593–610.

Tuzlaci, E., & Eryasar Aymaz, P. (2001). Turkish folk medicinal plants, Part IV: Gönen (Balıkesir). *Fitoterapia, 72*, 323–343.

Tuzlaci, E., & Tolon, E. (2000). Turkish folk medicinal plants, part III: Sile (Istanbul). *Fitoterapia, 71*, 673–685.

Yesilada, E., Gurbuz, I., & Shibata, H. (1999). Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity. *Journal of Ethnoparmacology, 66*, 289–293.

Yildirimli, S. (1985). Munzur daglarinin yerel bitki adlari ve bunlardan bazilarinin kullanilislari (Local names and usages of plants on Munzur Mountains). *Doga Bilimleri Dergisi, 9*, 593–597.

Zygadlo, J. A., Lamarque, A. L., Maestri, D. M., & Grosso, N. R. (1995). Use of essential oils as natural antioxidants. *Grasasy Aceites, 46*, 285–288.