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Food Chemistry 90 (2005) 685–689
Food Chemistry
www.elsevier.com/locate/foodchem

# In vitro antioxidant activities of the methanol extracts of four *Helichrysum* species from Turkey

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Received 16 February 2004; received in revised form 30 April 2004; accepted 30 April 2004

**Abstract**

This study was designed to examine the in vitro antioxidant activities of the methanol extracts of four *Helichrysum* species (*Helichrysum noeanum* Boiss., *H. chionophilum* Boiss. & Bal., *H. plicatum* DC. subsp. *plicatum*, *H. arenarium* (L.) Moench. subsp. *aucheri* (Boiss.) Davis & Kuphicha). The extracts were screened for their possible antioxidant activitiy by two complementary test systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid systems. In the first case, non-polar subfractions of the methanol extracts of *Helichrysum* species studied did not show any antioxidant activity, while the most active one was *H. chionophilum* ($IC_{50}$ = 40.5 μg/ml) among the polar subfractions. In the β-carotene/linoleic acid test system, inhibition rates of the oxidation of linoleic acid of *H. noeanum* and *H. arenarium* were very close to each other. The inhibition rate of the synthetic antioxidant BHT was determined to be 96%. Since the polar extracts of *Helichrysum* species dealt with here exhibited interesting antioxidant activities when compared to BHT, we believe that it would be useful to take the results into consideration as an alternative for food processing industries.


*Keywords:* *Helichrysum*; Antioxidant activity; DPPH; β-Carotene/linoleic acid test

## 1. Introduction

It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events (Aruoma, 1998; Feher, Csomos, & Vereckei, 1987; Halliwel & Gutteridge, 1989). Lipids containing polyunsaturated fatty acids are readily oxidised by molecular oxygen and such oxidation proceeds by a free radical chain mechanism (Aruoma, 1998; De Groot & Noll, 1987). When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals and hydrogen peroxide, are generated (Aruoma, 1998). These kinds of radicals are known to take part in lipid peroxidation, which causes food deterioration, aging of organisms and cancer promotion (Ashok & Ali, 1999; Cerruti, 1994). Reactive oxygen species are also reported to be involved in asthma, inflammation, arthritis, neurodegeneration, Parkinson disease, mongolism, and perhaps dementia (Adams & Odunze, 1991; Perry et al., 2000). Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical mediated processess. Therefore, they are able to protect the human body from several diseases attributed to the reactions of radicals (Nizamuddin, 1987; Takao, Kiatani, Watanabe, Yagi, & Sakata, 1994).

In order to prolong the storage stability of foods and to reduce damage to the human body, synthetic antioxidants are used for industrial processing. But according to toxicologists and nutritionists, side effects of some synthetic antioxidants, such as butylated hydroxyanisole (BHU) and butylated hydroxytoluene (BHT), are of concern. For example, these substances can show carcinogenic effects in living organisms (Ames, 1983; Baardseth, 1989). From this point of view, governmental authorities and consumers are concerned

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0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.04.030

about the safety of food and about the potential effects of syhthetic additives on health (Reische, Lillard, & Eintenmiller, 1998).

The genus *Helichrysum*, belonging to the family Asteraceae is represented by approximately 300 species in the world. This genus is represented in Turkish flora by 18 taxa, of which 9 are endemic. The plants of this genus are notable for their involucre, made up of a large number of bracts located in several rows and which are yellow and shiny (Peyron & Roubaud, 1970). According to Litvinenko, Popova, Popova, and Bubenchikova (1992), some members of this genus has cholagogue and choleretic activity and stimulate the secretion of gastric juice. Moreover, *H. arenarium*, *H. angustifolium*, *H. italicum* and *H. stoechas*, are used in folk medicine for their anti-inflammatory and anti-allergic properties (Carini, Aldini, Furlanetto, Stefani, & Facino, 2001). The medicinal properties of this genus are mainly attributed to the presence of flavanoids, but they may be also influenced by other organic and inorganic components, such as coumarins, phenolic acids and antioxidant micronutrients, e.g. Cu, Mn, Zn (Dombrowicz, Swiatek, & Kopycki, 1994).

The aim of the present study was to examine the in vitro antioxidant activities of the four *Helichrysum* species (*Helichrysum noeanum* Boiss., *H. chionophilum* Boiss. & Bal., *H. plicatum* DC. subsp. *plicatum* and *H. arenarium* (L.) Moench. subsp. *aucheri* (Boiss.) Davis & Kuphicha). Except for *H. plicatum* subsp. *plicatum*, the plants dealt with here are endemic to Turkish flora.

## 2. Materials and methods

### 2.1. Collection of plant material

Collection information of the four plant species which are individually numbered are listed below:

1. *H. noeanum*. Ulaş lake, Tecer mountain, 1400m, Sivas-Turkey, 25th July, 2003.
2. *H. chionophilum*. Ulaş lake, Tecer mountain, 1400m, Sivas-Turkey, 25th July, 2003.
3. *H. plicatum* subsp. *plicatum*. Tecer fish production form, 1400m, Sivas-Turkey, 24th July, 2003.
4. *H. arenarium* subsp. *aucheri*. Özen village, Poğanşar, 1300–1400m, Sivas-Turkey, 24th July, 2003.

Voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: 1-AA3366; 2-AA3365; 3-AA3358; 4-AA3352, respectively).

### 2.2. Preparation of the methanol extracts

The air-dried and finely ground samples were extracted by using the method described elsewhere (Sokmen, Jones, & Erturk, 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 (20.11%, 18.59%, 11.29% and 21.64 w/w, respectively). Finally, the extracts were then lyophilised 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 uses the stable radical 2,2'-diphenylpicrylhydrazyl (DPPH) as a reagent (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 free radical DPPH in percent (I%) was calculated in the following way:

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

where $A_{\text{blank}}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{\text{sample}}$ is the absorbance of the test compound. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated form the graph of inhibition percentage against extract concentration. Tests were carried out in triplicate.

#### 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 (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen (30 min 100 ml/min.) were added with vigorous shaking. 2500 μl of this reaction mixture were dispensed in to test tubes and 350 μl portions of the extracts prepared (at 2 $g l^{-1}$ concentrations) were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant, butylated hydroxytoluene (BHT) as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities

of the extracts were compared with those of BHT and blank.

## 3. Results and discussion

The extracts obtained by Soxhlet extraction were screened for their possible antioxidant activitiy by two complementary test systems, namely DPPH free radical-scavenging and β-carotene/linoleic acid systems. Free radical-scavenging capacities of the corresponding extracts, measured by DPPH assay, are shown in Fig. 1. Non-polar (chloroformic) subfractions of the methanol extracts of *Helichrysum* species studied did not show any antioxidant activity. Among the polar ($H_2O$) subfractions, the most active one was *H. chionophilum* ($IC_{50} = 40.5$ μg/ml). As can be seen from Fig. 1, the weakest free radical-scavenging activity was exhibited by *H. plicatum* and *H. arenarium* ($IC_{50} = 48.0$ and 47.6 μg/ml, respectively). When compared with the synthetic antioxidant BHT, polar subfractions of the all extracts showed better radical-scavenging activity. In the β-carotene/linoleic acid system, inhibition rates of the oxidation of linoleic acid of *H. noeanum* and *H. arenarium* were very close to each other (Fig. 2). The inhibition rate of the synthetic antioxidant BHT was determined to be 96%.

Free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects on organisms (McCord, 2000). For example, oxygen radicals exert critical actions, such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells (Lander, 1997; Zheng & Storz, 2000). Also, NO is one of the most widespread signalling molecules and participates in virtually every cellular and organ function in the body (Ignarro, Cirino, Casini, & Napoli, 1999). However, free radicals and other reactive species cause the oxidation of biomolecules (e.g., proteins, amino acids, lipid, and DNA), which leads to cell injury and death (Freidovich, 1999; Zheng & Storz, 2000).

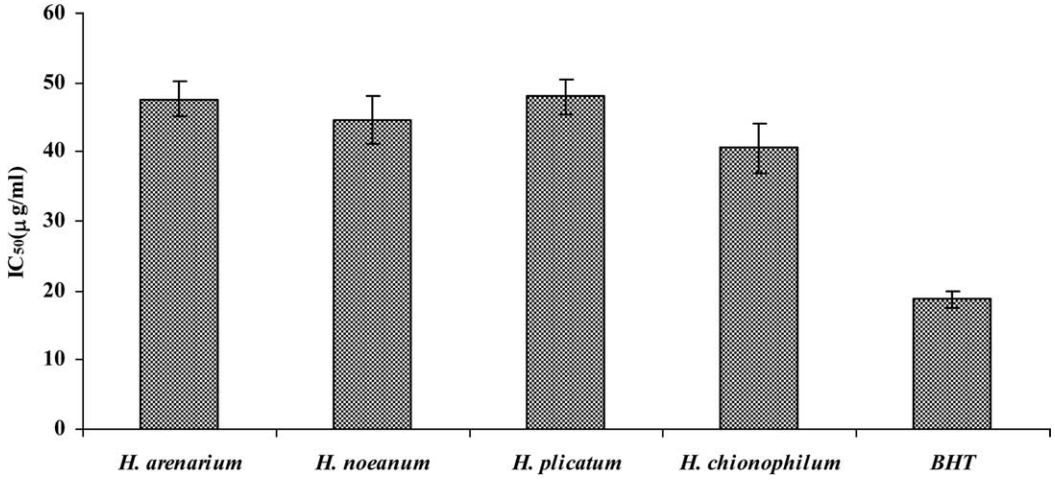


Fig. 1. Free radical-scavenging capacities of the extracts measured by DPPH assay (results are means of three different experiments).

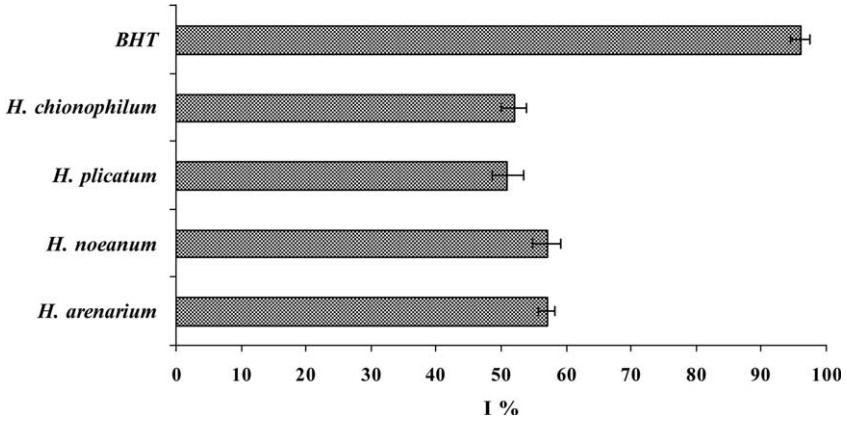


Fig. 2. Inhibition rate of linoleic acid oxidation by the extracts (results are means of three different experiments).

As far as our literature survey could ascertain, several studies were carried out with *H. arenarium* (Czinner et al., 2000; Czinner et al., 2001), *H. italicum* (Sala et al., 2003; Schinella, Tournier, Prieto, Mordujovich de Buschiazzo, & Rios, 2002) and *H. stoechas* (Carini et al., 2001). Based on the report of Czinner et al. (2000, 2001), *H. arenarium* has choleretic, hepatoprotective, cholagogue and detoxifying activities. Moreover, some phenolics (flavonoids, gnaphaliin and tiliroside) of *H. italicum* are capable scavenging free radicals effectively (Sala et al., 2003).

Except for *H. arenarium*, among plant species presented here, this may be the first report on the antioxidant activity of *H. plicatum*, *H. chionophilum* and *H. noeanum*. Especially in the case of the DPPH test system, polar extracts of the plants showed stronger free radical-scavenging activities than the synthetic antioxidant BHT. According to Czinner et al. (2001), the main phenolic compounds of the genus *Helichrysum* are flavonoids which have remarkable antioxidant activity. Based on this report, antioxidant potential of the extracts studied here could be attributed to flavanoids. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Madsen, Nielsen, Bertelsen, & Skibsted, 1996; Moller, Madsen, Altonen, & Skibsted, 1999).

In conclusion, our study provides evidence that water extracts of *Helichrysum* species dealth with here exhibited interesting antioxidant activities when compared to BHT. Because of the side effects of the synthetic agents used as antioxidants today, there is increasing interest in the use of natural products both in the pharmaceutical and food processing fields. Therefore, the results presented here could be useful for such industries.

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