HUNGARIAN JOURNAL
OF INDUSTRIAL CHEMISTRY
VESZPRÉM
Vol. 36(1-2) pp. 39-42 (2008)

INVESTIGATION OF *SATUREJA HORTENSIS L.* AS A POSSIBLE SOURCE OF NATURAL ANTIOXIDANTS

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Natural antioxidants play important roles as health-protecting factors. Antioxidants are also widely used as additives in fats and in food processing to prevent or delay spoilage of foods. Spices have received an increased attention as natural sources of many effective antioxidants. In this study *Satureja hortensis L.* (summer savory) was examined as a potential source of natural antioxidant compounds. For the isolation of the active components two extraction methods were investigated: conventional Soxhlet extraction and supercritical fluid extraction. Conventional Soxhlet extraction was carried out with organic solvents with different polarities. Supercritical fluid extractions were performed with neat CO_2 at two different pressures (300 and 450 bar) at 40 °C. To estimate the antioxidant activity of the extracts, 1,1-diphenyl-2-picrylhydydrazyl (DPPH) assay was used. The results were reported as IC 50%, where IC 50% was defined as the extract concentration required decreasing the initial DPPH concentration by 50%. The antioxidant activity of the extracts obtained with organic solvents decreased in the following order: ethanol 50% > ethanol 96% > isopropanol > ethanol 100% > acetone > ethyl acetate > pentane. The highest antioxidant activity exhibited the extract obtained with ethanol 50% (with an IC 50% value of 14.48 ± 0.02 μg/ml), while the extract obtained with pentane showed the lowest antioxidant activity (with an IC 50% of 98 ± 0.1 μg/ml). The antioxidant activity of the extracts was also compared with the antioxidant activity of butylated hydroxytoluene (BHT). The extract obtained with ethanol 50% showed approximately similar antioxidant activity as BHT (with an IC 50% of 12.86 ± 0.19 μg/ml). Although in the case of the supercritical extraction the antioxidant activity increased with increasing the pressure, it was lower than the antioxidant activity of the extracts performed with organic solvents.

Keywords: summer savory, extraction, antioxidant activity

Introduction

Recently the interest in natural antioxidants has increased dramatically due to: (1) concerns regarding the safety of the chronic consumption of synthetic antioxidants (butylated hydroxyltoluene and butylated hydroxylanisole), (2) the antioxidant efficiency of a variety of phyto-chemicals, (3) the consensus that foods rich in certain phytochemicals can affect the aetiology and pathology of chronically diseases and the ageing process and (4) the public's conceived belief that natural compounds are innately safer than synthetic compounds and are thus more commercially acceptable [1]. Herbs, spices and teas are the most important targets in research for natural antioxidants from the point of view of safety.

Satureja hortensis L. is an annual culinary herb belonging to the family Labiatae. It is known as summer savory, native to southern Europe and naturalized in parts of North America [2]. The leaves, flowers and stems of summer savory are frequently used as additives in commercial spice mixtures for many foods to confer aroma and flavour. This plant is also used in the traditional medicine to treat various ailments as cramps, muscal pains, nausea, indigestion, diarrhoea, and infection diseases [3].

Besides, it was demonstrated that extracts from *Satureja hortensis L.* exhibited antimicrobial, antioxidant, sedative, antispasmotic and antidiarrheal properties [2-8].

The objectives of the present study were first of all to select the plant material, and then to identify the most suitable solvent to recover the antioxidant compounds from this plant. In order to select the raw material a preliminary investigation on the quality was carried out.

The antioxidant activity of natural extracts has been found to depend on the active components of the raw material, the type and polarity of extraction solvent and the isolation procedure [9]. In our study two extraction methods were compared: conventional Soxhlet extraction and supercritical fluid extraction.

Materials

Solvents and reagent

For the laboratory extraction, CO_2 used was of 99.5% (w/w) purity and was supplied by Linde Gas Hungary Co. Ltd. All other solvents (pentane, ethyl-acetate, isopropanol, ethanol 100%, ethanol 96%, acetone) used for the conventional Soxhlet extractions were purchased from Molar Chemicals Ltd, Hungary. The ethanol 50% (50% water) used also for the conventional Soxhlet extraction was prepared from ethanol 96%. 1,1pdipheny-2-picryl-hydrazyl (DPPH) free radical used for the estimation of the antioxidant activities of the extracts and BHT used as a standard were purchased from Fluka, Switzerland.

Plant material

Four samples of dried summer savory plant *(Satureja hortensis L.)* were bought from three different companies Fitodry KFT, Rózsahegyi KFT, Bio- Drog-Berta KFT in Hungary. In our work the samples are noted with savory 1, savory 2, savory 3 and savory 4, respectively. A preliminary investigation on the quality of the samples was carried out. The moisture content of every sample was measured. The moisture content decreased as follows: savory 1 (14.37%) > savory 2 (11.42%) > savory 3 (11.25%) > savory 4 (8.81%). For the characterization of the rubbed raw material sieving was performed using a vertical vibratory sieve shaker (Labortechnik Gmbh, Ilmenau) for 20 min. The particle size of the rubbed raw material was approximately 0.8–1.2 mm.

Methods

Soxhlet extraction

Extractions with organic solvents of different polarities (pentane, acetone, ethyl-acetate, isopropanol, ethanol 100%, ethanol 96% and ethanol 50%) were carried out. Samples about 15–20 g raw material were extracted in a Soxhlet apparatus with 250 ml solvent, until totally depleted. The whole process took 22–24 h. After extraction the solvent was removed under vacuum using a rotator evaporator Rotadest, Type 2118. Two parameters were measured: the yield% (w/w) (which was determined as the amount of the extract/100 g of dry material) and the antioxidant activity. Every extraction was carried out in triplicate.

Supercritical fluid extraction

The extraction experiments were performed in a high pressure pilot plant equipped with a 5 L volume extractor vessel (delivered by NATEX, Austria). Two extractions with neat CO_2 at two different pressures (300 and 450 bar) at 40 °C were performed. For each extraction about 1000 g rubbed savory plant was weighted accurately and filled into the extractor. The desired temperature and pressure were adjusted, and the CO_2 feed was started. The accumulated product samples were collected and weighed at certain time intervals. The CO_2 flow rate was measured with a Micro Motion RFT 9729 type mass flow meter and it was about 7 kg/h in both cases. The extractions were carried on until the amount of the last product sample decreased for one hour under 0.1% of the raw material. A more detailed description of the equipment is given extensively elsewhere [10].

Estimation of antioxidant activity by DPPH assay

To estimate the antioxidant activity of the extracts DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay was used. DPPH is a stable free radical which is often used as an indicator in testing hydrogen-donation capacity and thus antioxidant activity. The DPPH assay was carried out following the same method as reported elsewhere [11].

Different concentrations of various extracts dissolved in methanol were added to 2.5 ml methanol solution of DPPH. After 30 min incubation period at room temperature, the absorption was read against a blank at 517 nm using a UV/VIS spectrophotometer M501 Single Beam – Camspec. The inhibition of the free radical DPPH was calculated in percent (I%) in the following way:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \cdot 100$$

where:

A_{blank} – is the absorbance of the control reaction (containing all reagents except the test compound),

A_{sample} – is the absorption of the test component.

Results were reported as IC 50%, where IC 50% was defined as the extract concentration required decreasing the initial concentration by 50%.

Results and discussion

Selection of plant material

In order to select the plant material for our experiments a preliminary investigation on the quality of four different samples of summer savory was performed.

Antioxidants are known to interrupt the free radical chain of oxidation by donating hydrogen from phenolic hydroxy groups and to form stable products, which do not initiate or propagate further oxidation [12].

The concentration of an antioxidant needed to decrease the DPPH concentration by 50% is a parameter widely used to estimate antioxidant activity [13]. The

lower the IC_{50} value, the higher is the antioxidant activity [14].

The results of the extraction yield and antioxidant activity of the ethanol and pentane extracts are shown in *Table 1* and *2*.

Table 1: Yield and antioxidant activity of different samples of *Satureja hortensis L.* extracted with ethanol

raw material	96% ethanol extract	
	[a]Yield (%)	[a]IC 50% (µg/ml)
savory1	28.96 ± 0.41	40 ± 0.1
savory2	24.83 ± 0.22	27 ± 0.3
savory3	17.92 ± 0.93	80 ± 0.1
savory4	15.27 ± 1.09	50 ± 0.6

[a]Mean value of three measurements ± SD (standard deviation)

It can be observed that the ethanol extracts showed both antioxidant activities and the yields higher than the extracts obtained with pentane.

Among the extracts obtained with ethanol, the savory 2 extract exhibited the highest antioxidant activity (with an IC 50% of 27 ± 0.3 µg/ml), while the savory 3 extract showed the lowest antioxidant activity (with an IC 50% of 80 ± 0.1 µg/ml). In the ethanol extracts no correlation could be observed between the antioxidant activity and the yield.

Table 2: Yield and antioxidant activity of different samples of *Satureja hortensis L.* extracted with pentane

raw material	pentane extract	
	[a]Yield (%)	[a]IC 50% (µg/ml)
savory1	3.19 ± 0.19	160 ± 0.2
savory2	3.51 ± 0.12	160 ± 0.2
savory3	2.27 ± 0.09	185 ± 0.8
savory4	2.44 ± 0.11	180 ± 0.3

[a]Mean value of three measurements ± SD (standard deviation)

In the case of the extractions performed with pentane the extracts of savory 1 and savory 2 manifested a higher antioxidant activity (with an IC 50% of 160 ± 0.2 µg/ml) and higher yield than the extracts of savory 3 and savory 4 but less than the antioxidant activity of the same samples obtained with ethanol. It can be concluded that the best quality exhibited the extract of savory 2. This sample was used in our further experiments.

In the attempt to increase the yield and the antioxidant activity, the savory 2 was subjected to the extraction with three different organic solvents in milled form and without milling. Influence of the milling on the yield and antioxidant activity of different extracts of savory 2 is represented in *Table 3*.

Although by the milling of the plant material the yield increased, the antioxidant activity decreased.

Therefore for the next experiments it was decided to use the plant material (savory 2) without milling.

Table 3: Extraction Yield and antioxidant activity of different extracts of savory 2 with and without milling

S	[a]Yield (%)		[a]IC 50% µg/ml	
	with milling	without milling	with milling	without milling
1	26.91 ± 0.50	25.36 ± 1.07	35 ± 0.1	24 ± 0.1
2	25.77 ± 0.35	18.67 ± 0.90	60± 0.1	53 ± 0.7
3	11.48 ± 0.54	8.48 ± 0.44	90 ± 0.2	80 ± 0.3

[a]Mean value of three measurements ± SD (standard deviation)
S - solvent; 1: ethanol 96%; 2: ethanol 100%; 3: ethyl acetate.

Selection of the solvent

In order to isolate the active compounds two extraction methods were investigated: conventional Soxhlet extraction and supercritical fluid extraction. *Fig. 1* shows the effect of the polarity of the solvents on the antioxidant activity of different extracts of savory 2.

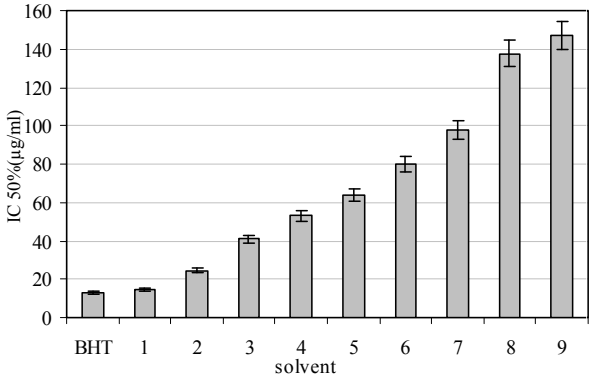

Figure 1: Antioxidant activity of different extracts of savory 2 and BHT
1: ethanol 50%, 2: ethanol 96%, 3: isopropanol, 4: ethanol 100%, 5: acetone, 6: ethyl acetate, 7: pentane, 8, 9: supercritical fluid extracts performed at 450 and 300 bar, respectively at 40°C

In the case of the extraction performed with organic solvents the antioxidant activity of the extracts decreased as follows: ethanol 50% > ethanol 96% > isopropanol > ethanol 100% > acetone > ethyl acetate > pentane. The extract obtained with ethanol 50% exhibited both the highest antioxidant activity (with an IC 50% of 14.48 ± 0.02 µg/ml) and the highest yield (34.67 ± 1.57 w/w) whereas the extract performed with pentane showed the lowest antioxidant activity (with an IC 50% of 98 ± 0.1 µg/ml) and the lowest yield (3.08 ± 0.1 w/w). The extraction yield of different extracts of savory 2 is represented in *Fig. 2*. A correlation between the antioxidant activity and the extraction yield was found.

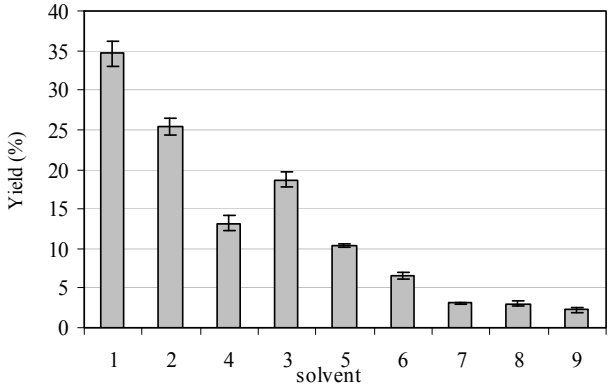

Figure 2: Yield of different extracts of savory 2. 1: 50% ethanol; 2: 96% ethanol; 3: isopropanol; 4: 100% ethanol; 5: acetone; 6: ethyl acetate; 7: pentane; 8, 9: supercritical fluid CO_2 at 450 and 300 bar, respectively at 40 °C

Supercritical fluid extraction was carried out with neat CO_2 at two different pressures, 300 and 450 bar at 40 °C. It was observed that by increasing the pressure both the antioxidant activity (with an IC 50% from 147.3 to 137.6 µg/ml) and the yield (from 2.23 to 3.02 w/w) increased.

However, the antioxidant activity of the extracts performed with supercritical fluid CO_2 was lower than the antioxidant activity of the extracts obtained with organic solvents.

The explication can be found in the polarity of the solvents, because the active compounds are usually polar compounds. Since the CO_2 is non-polar solvent more non-polar compound can be extracted. More experiments with supercritical fluid CO_2 in present of different concentrations of a modifier are in progress in order to concentrate the active compounds. We assume that the maximum antioxidant activity was recovered with ethanol 50%.

Conclusions

Satureja hortensis L. was investigated as a potential source of natural antioxidant compounds. To recover the antioxidants two isolation methods, conventional Soxhlet extraction and supercritical fluid extraction were compared. The best organic solvent to recover the antioxidant compounds was found to be ethanol 50% (with an IC 50% of 14.48 ± 0.02 µg/ml). The extracts obtained by using supercritical fluid extraction with neat CO_2 at two different pressures (300 and 450 bar) at 40 °C showed approximately 10 times lower antioxidant activity then the extracts obtained with organic solvents. To increase the polarity of the active compounds by using supercritical CO_2 a modifier is required. More extraction experiments with different concentrations of an entrainer are in progress.

ACKNOWLEDGEMENTS

This research was financially supported through a European Community Marie Curie Fellowship (Project MEST-CT-2004-007767). For further information: http://www.cordis.lu/imprcong.

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