Polyphenol-, Mineral Element Content and Total Antioxidant Power of Sage (*Salvia officinalis* L.) Extracts

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Abstract

The alcoholic extracts of *Salvia officinalis* L. leaves were studied for element composition, polyphenol content and antioxidant property. The procedure of the alcoholic extract preparation was different from the conventional tincture preparing and it was amplified with extraction in ultrasonic water bath. The plant material was treated (infused) with the water proportion of the tincture-solvent (alcoholic solution of 20 %), then the alcohol-part (of tincture-solvent) was added to the cooled aqueous extract. Element concentrations of the samples were determined by inductively coupled plasma atomic emission spectrometry. The phenol carbonic acid (rosmarinic-, caffeic-, chlorogenic- and ferulic acid) content was measured by HPLC. The highest amount of caffeic acid (5.3 µg 100mL^{-1}) and ferrulic acid (2.6 µg 100mL^{-1}) were observed in macerated extracts made from 5 g leaves with 20 % alcohol, while the highest amount of rosmarinic acid (25.2 µg 100mL^{-1}) was measured in ultrasonic extract. The mineral element content showed significant differences in the extracts prepared in diverse ways. The macerated extract, prepared by 40 % alcohol, showed the highest tannin- (266 mg 100mL^{-1}) and polyphenol content (394 mg 100mL^{-1}), the conventional tincture contained 166 mg 100mL^{-1} of tannin and 200 mg 100mL^{-1} of polyphenol. The flavonoid content was 58 mg/ 100mL in conventional alcoholic extract and 136 mg 100mL^{-1} in infused extract. The total antioxidant power was found to be significantly different in the different extracts which was measured by the FRAP assay.

INTRODUCTION

Salvia officinalis L. (sage) is one of the most popular medicinal drug in Hungary and it is also well known as a spice. The preparation of dried leaves of sage (*Salviae officinalis folium*) is registered as a medicinal drug. It has been used for thousands of years in folk medicine for the treatment of inflammatory processes (gingival haemorrhage, tonsillitis, sore throat, etc.), common cold, state of exhaustion and nervosity. It is applied both externally and internally as an antiphlogistic and astringent drug (e.g., for curing inflamed wounds, gastric ulcer). Volatile oil of sage is used by the food industry, perfume industry and pharmaceutical industry as well (Millet et al., 1980; Karakaya and El, 1999; Hodisan et al., 1985).

The most effective bioactive components of sage are monoterpenes: borneol, bornyl acetate, α-pinene, β-pinene, α-thujone, β-thujone, eucalyptol, myrcene etc. (Bernáth et al., 1991; Di Cesare et al., 2001, Couladis et al., 2002), flavonoids [Cuvelier et al., 1994; 1996, Wang et al., 1998; Miura et al., 2002) and other polyphenolic components (Adzet et al., 1987; Lu and Foo, 1999, 2000, 2001, Ho et al., 2000). Hardly any investigations were made to determine the metal ion content of sage (Azhabov et al., 1998; Then and Szentmihályi, 1998), although a closer knowledge of the quality and quantity of the metal content would be essential, with special regard to the application of

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sage in medicine and in cosmetics.

The importance of sage extract in cosmetic industry is growing. While concentrate alcoholic extracts (tinctures: 70 % and 96 % macerated extracts in room temperature for 6 days) of sage is used for medicinal purposes, max. of 20 % alcoholic extract may be applied for cosmetics although 40 % tincture may be also used in some cases. Since biological activity of extracts depends not only on the components of the drug but the extraction method used, therefore, 20 % and 40 % alcoholic extracts were made from sage leaves by different modified extraction methods using the tea making in the first step of extraction and ultra sound extraction to yield tincture richer in bioactive compounds. For the investigation and comparison of the bioactive components in the extracts, the analytical methods applied were ICP for elements, spectrophotometry for polyphenols, tannins and flavonoids, HPLC for polyhydroxylic compounds.

MATERIALS AND METHODS

The leaves of *Salvia officinalis* L. *(sage No.:* 965/b) originates from the Botanical and Economical Research Institute of the Hungarian Academy of Sciences, Vácrátót, 2000.

Tinctures of 20 % and 40 % alcoholic extracts were made from sage leaves. The procedure of the extract preparation was different from the conversational tincture preparing. The authors alloyed the tincture and the infusion preparation method according to the Hungarian Pharmacopoeia (Ph.Hg.VII.1992).

Macerated extract was made from the plant material (5 g dried leaves) by soaking in alcoholic solution (100 ml, 20 and 40 v/v %) in room temperature for 6 days. After filtration, the extract was completed to 100 ml with alcoholic solution.

Infused extract was made by pouring the leaves (5 g) with hot water (80 ^{o}C, 80 ml) and after having cooled, the alcohol quantity (20 ml) of the tincture was added and the suspension was allowed to stay at room temperature for 6 days. After filtration, the extract was completed to 100 ml with alcoholic solution.

Extract in ultrasonic water bath was made from plant material (5 g) with alcoholic solution (20 ml alcohol and 80 ml water) by amplified for 30min. The suspension was filtered and the extract was completed to 100 ml with alcoholic solution.

Concentrations of the elements of extracts were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash Co.), a sequential plasma emission spectrometer. The samples (10 ml of evacuated extract) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (3 ml) in teflon vessels. After digestion the samples were diluted to 25 ml, from which the following elements were determined in three parallel measurements: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Hg, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Ti, V, Zn.

The total polyphenol- and tannin content of the extracts were determined according to the Hungarian Pharmacopoeia (Ph. Hg.VII, 1992) by spectrometry. The determination of tannin content is an indirect method. The tannin content is equivalent with the difference between the total polyphenol content remained back after the the tannins were adsorbed by hide power. The tannin content is calculated for pyrogallol as reference material.

The total flavonoid content of the extracts was determined according to the German Pharmacopoeia (DAB 10, 1996). After acidic hydrolyses of flavonoid glycosides, flavonoid aglycons were complexed with aluminum chloride, measured at 420 nm and the result were calculated in hyperoside.

The phenol carbonic acid (chlorogenic-, caffeic-, ferrulic- and rosmarinic acid) content was measured by HPLC. The evaporated residues were redissolved in 10 ml HPLC grade methanol and injected (20 µl) on the normal phase HPLC column (Spherisorb ODS-2, 5µm, 250 x 4.6 mm) which was eluted with methanol- 0.02 mol l^{-1} KH_2PO_4 (30 % and 70 %, pH = 3.00) as the mobile phase. Eluent speed was 1 ml min^{-1}. The separated compounds were detected fluorometrically, using a Shimadzu RF-535 fluorescence detector 330 nm as extinction and emission wavelengths, respectively

(sensitivity: 0.16 AUFS). BORWIN™ integrator software program was used for qualitative and quantitative evaluation of chromatograms.

Antioxidant property was measured by FRAP method (ferric reducing ability of plasma) according to Varga and coworkers (1998). 1.5 ml of FRAP reagent (25 ml acetate buffer: 300 mmol l^{-1}, pH = 3.6; 2.5 ml 2,4,6-tripyridyl-S-triazine: 10 mmol l^{-1} TPTZ in 40 mmol l^{-1} HCl) and 50 µl of plant extract were measured up to 5 min at 593 nm. The relative activities of the samples were assessed by comparing their activities with that of ferrulic and chlorogenic acid.

Mean values and standard deviations (SD) were calculated from the results. For comparison of the means one way analysis of variance (ANOVA) was used by GraphPAD software version 1.14 (1990). Significance limit was $p < 0.05$

RESULTS AND DISCUSSION

Sage tincture extracts contain mineral elements and their concentrations are not negligible (Table 1). Concentration of As, Cd, Co, Cr, Hg, Li, Mo, Ni, Pb, Ti and V was below the detection limit, therefore, these elements are missing from the table. The mineral element content in extracts prepared in diverse ways showed significant differences at $p< 0.05$. Relatively high amount of K, Ca and S was detected in macerating and infusing (20 % alcoholic water) extracts (38500-47983, 28071-19010 and 3726-4600 µg $100ml^{-1}$, respectively). The mineral element content of extract decreased with the increasing of the alcohol content in tincture.

Inquiring measurements were made in extracts for compounds containing phenolic groups by spectrophotometry. The results are summarized in Table 2. The 40 % alcoholic extract prepared by macerating showed the highest polyphenol- (394 mg 100 ml^{-1}) and tannin content (266 mg 100 ml^{-1}). Infused and macerated extracts (20 % alcohol) contained also relatively high amount of polyphenols (253 and 200 mg $100ml^{-1}$). Some part of polyphenols are flavonoids and since Salvia species are rich in flavonoids, we were interested in the distribution of flavonoids in the extracts. The flavonoid content was 136 mg $100ml^{-1}$ in infused extract in 20 % alcohol followed by macerated extract in 40 % alcohol (116 mg $100ml^{-1}$). From these results it can be seen that the richest extract in phenolic compounds is the macerated extract in 40 % alcohol.

The results for HPLC determination of phenolic compounds are summarized in Table 3. The highest amount of rosmarinic acid was observed in ultrasonic extracts made with 20 % alcohol and the caffeic acid content of extract was also relatively high. Since the extract have significant rosmarinic acid content, it may be effective for medicinal purpose e.g., in cases of inflammation. For cosmetic applications, extracts made with 20 % alcohol may be used, especially the ultrasound extract seems suitable (Fig.1.).

Antioxidant activities of the extracts are shown in Table 4. The ultrasonic extract has the highest ferric reducing ability followed by the infused extract and the macerated extract in 20 % alcohol shows the lowest antioxidant power.

CONCLUSIONS

Since the antioxidant power and biological activity of the medicinal plant extract depends on the polyphenol- and element content (Szentmihályi et al., 2001; Bandoniene et al., 2002; Lu and Foo, 2002; Szentmihályi et al., 2002), the element composition and polyphenol content in the alcoholic extracts of *Salvia officinalis* leaves were studied.

The mineral element content showed significant differences in the extracts prepared in diverse ways. It is favourable in the case of use that no concentration in extracts higher then the detection limit was found for toxic elements, while the concentrations of alkaline and alkaline earth metals were relatively high. The 40 % alcoholic extract, prepared by maceration showed the highest polyphenol- (394 mg mL^{-1}) and tannin content (266 mg $100mL^{-1}$), while the highest flavonoid content was 136 mg 100 mL^{-1} in infused extract.

The extract prepared by maceration in 40 % alcohol contains the highest amount of polyphenols and tannins. Since the antioxidant and biological activity is closely

connected with the polyphenol content (Lugasi, 2000, Miura et al., 2002), the macerated extract in 40 % alcohol seems to be good for medical purposes. However the antioxidant activity of it was not the best. For cosmetic use the infused extract and the ultrasonic extract in 20 % alcohol would be better since their have significantly high antioxidant activities. The ultrasonic extract contains relatively low amount of antioxidant polyphenol with low amount of metallic elements, while infused extract has relatively high amount of heavy metal (Fe, Mn) and other element (Ca, K, Mg, P, S) content with relatively high polyphenol content comparing with other extracts (Szentmihályi and Then, 2000).

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Tables

Table 1. Element content of sage tinture extracts and ± standard deviations (μg $100ml^{-1}$, n=3). The concentration of elements are significantly different at $p<0.05$.

Elements	Maceratum in 20 % alcohol	Infused in 20 % alcohol	Ultrasonic in 20 % alcohol	Maceratum in 40 % alcohol	Significance
Al	167 ± 13	128.7 ± 4.7	47.8 ± 3	84.9 ± 3.8	p<0.05
B	5.1 ± 0.8	9.4 ± 0.4	3.4 ± 0.8	5.6 ± 0.4	p<0.05
Ba	1.0 ± 0.1	1.89 ± 0.2	5.1 ± 0.1	3.4 ± 0.4	p<0.05
Ca	19010 ± 150	28071 ± 142	1933 ± 15	5595 ± 67	p<0.05
Cu	8.6 ± 0.7	1.45 ± 1.7 8	3.8 ± 0.2	4.6 ± 0.6	p<0.05
Fe	44.7 ± 0.7	44.4 ± 0.6	14.5 ± 0.5	27.6 ± 1.0	p<0.05
K	38500 ± 30	47983 ± 190	3333 ± 11	18410 ± 340	p<0.05
Mg	1460 ± 121	1478 ± 60	1256 ± 46	1119 ± 45	p<0.05
Mn	5.3 ± 0.1	5.8 ± 0.5	3.5 ± 0.6	8.7 ± 0.4	p<0.05
Na	2120 ± 48	1816 ± 35	281 ± 6	587 ± 8	p<0.05
P	3019 ± 8	3539 ± 5	168 ± 5	656 ± 8	p<0.05
S	4600 ± 68	3726 ± 8	654 ± 9	3022 ± 48	p<0.05
Zn	54.1 ± 0.8	1.66 ± 0.2	20.3 ± 0.4	50.1 ± 0.3	p<0.05

Table 2 . Bioactive material content of sage extracts (mg $100ml^{-1}$) and the statistical data (± standard deviation in mg $100ml^{-1}$, n=3) measured by spectrophotometry.

Extracts	Total polyphenol	Tannin	Total flavonoid
Macerated in 20% alcohol	200 ± 0.12	166 ± 5	58 ± 2
Infused with 20% alcohol	253 ± 9	124 ± 7	136 ± 8
Ultrasonic in 20% alcohol	188 ± 10	121 ± 8	66 ± 3
Macerated in 40% alcohol	394 ± 18	266 ± 10	116 ± 5
p	< 0.05	< 0.05	< 0.05

Table 3. Content of polyphenolic compounds in sage tinctures (μg $100ml^{-1}$) and the statistical data (± standard deviation in mg $100ml^{-1}$, n=3) measured by HPLC.

Extracts	Chlorogenic acid	Caffeic acid	Ferulic acid	Rosmarinic acid
Macerated in 20% alcohol	0.6 ± 0.1	5.3 ± 0.3	2.6 ± 0.1	10.5 ± 0.2
Infused with 20% alcohol	1.9 ± 0.2	2.6 ± 0.2	1.5 ± 0.1	22.6 ± 0.2
Ultrasonic in 20% alcohol	1.4 ± 0.1	2.2 ± 0.2	1.8 ± 0.1	25.2 ± 0.3
Macerated in 40% alcohol	0.8 ± 0.1	3.0 ± 0.2	1.9 ± 0.1	8.8 ± 0.2
p	< 0.05	< 0.05	< 0.05	< 0.05

Table 4. Antioxidant activities of sage extracts measured by FRAP method.

Extracts	Antioxidant activity ($\mu mol\ l^{-1}$)
Ferrulic acid	1025 ± 13
Chlorogenic acid	391 ± 9
Macerated in 20% alcohol	3928 ± 15
Infused with 20% alcohol	5328 ± 25
Ultrasonic in 20% alcohol	5661 ± 21
Macerated in 40% alcohol	4394 ± 16

Figures

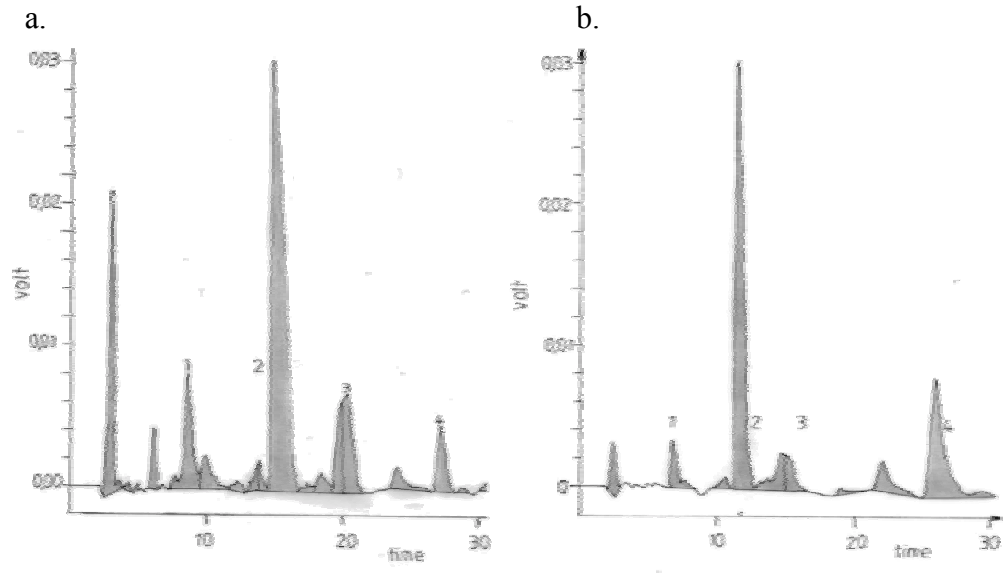

Fig. 1. HPLC chromatogram of infused (a) and macerated (b) extract (5 g drug in 100ml of 20 v/v % alcoholic water) of *Salvia officinalis* leaves.1. chlorogenic acid, 2. caffeic acid, 3. rosmarinic acid, 4. ferrulic acid.