Food Research 3 (5) : 530 - 536 (October 2019)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Ultrasound-assisted extraction of medicinal plants and evaluation of their biological activity

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Article history:
Received: 26 March 2019
Received in revised form: 6 April 2019
Accepted: 8 April 2019
Available Online: 12 April 2019

Keywords:
Biological activity,
Medicinal plants,
Ultrasound-assisted extraction,
Antioxidant potential

DOI:
https://doi.org/10.26656/fr.2017.3(5).128

Abstract

Nowadays, medicinal plants used in folk medicine are being increasingly studied and used in pharmaceutical, food and nutraceutical fields. Ultrasound-assisted procedure is extensively used recently for the extraction of valuable molecules. The study objective was to investigate total phenolic content using Folin-Ciocalteu's method and determine the antioxidant capacity in *Centaurium erythraea* Pers., *Glycyrrhiza glabra* L., *Polygonum hydropiper* L., *Silene vulgaris* L., *Aspalathus linearis* L., *Helichrysum arenarium* (L.) Moench., *Sambucus nigra* L. and *Echinacea purpurea* M. In order to evaluate the antioxidant activity various *in vitro* methods such as Fe^{3+} reducing power by FRAP method, 2,2-diphenyl-1-picryl-hydrazyl free radical ($DPPH^{\cdot}$) scavenging activity, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical ($ABTS^{\cdot+}$) scavenging activity and Cupric reducing antioxidant capacity (CUPRAC) were performed separately. Total phenolic content ranged from 4.08±0.03 to 20.48±0.13 mg GAE/g DW. The established antioxidant potential of the studied plant extracts correlated significantly with total phenolic content. Both *S. nigra* flowers and *A. linearis* leaves extracts revealed to be the most potential. The results demonstrated the investigated ultrasound extracts as potential sources of useful properties and could contribute to different benefits in fields like pharmacy, food preparation and cosmetics.

1. Introduction

Despite the wide use of medical drugs, modern people still look back and use a wide range of natural products by resolving medicinal issues, maintaining their health status or just for every day well- being a concept. Medicinal plants are traditionally used from decades in the folk medicine for treatment of health problems: *Centaurium erythreae* Pers. - anti-inflammatory, anti-pyretic, hypoglycemic, antioxidant, antimicrobial, hepatoprotective, gastroprotective, etc. (Božunović et al., 2018), *Glycyrrhiza glabra* L. - anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities (Dhingra *et al.*, 2004), *Polygonum hydropiper* L. - pain-relieving and haemorrhagic, against low blood pressure, for weight loss (Tao *et al.*, 2016), *Silene vulgaris* (*Moench*) *Garcke* - good for bronchitis and asthma (Tardio *et al.*, 2005), *Aspalathus linearis* L. - provide relief for allergies, dermatological problems, asthma, infantile colic and other gastrointestinal complaints, such as nausea and heartburn (Joubert *et al.*, 2008; Van Wyk *et al.*, 2009), *Helichrysum arenarium* L. - treatment of urinary disorders, snake bites, sciatica and hernias (Quer, 1993), *Echinacea purpurea* M. - respiratory infections, urinary tract infections, skin disorders, etc. (Hobbs, 1990) and *Sambucus nigra* L. - beneficial effects on blood pressure, glycaemia reduction, immune system stimulation, antitumor potential, increase in the activity of antioxidant enzymes in the blood plasma, including also glutathione, and the reduction of uric acid levels (Sidor and Gramza-Michałowska, 2015).

Oxidative stress is involved in the pathogenesis of numerous diseases such as cardiovascular diseases (Mangge *et al.*, 2014; He and Zuo, 2015), diabetes mellitus, Alzheimer disease (Luca *et al.*, 2015), inflammatory diseases (Mangge *et al.*, 2014), carcinogenesis (Li *et al.*, 2015), neurodegenerative diseases (Gandhi *et al.*, 2012), pulmonary and hematological diseases (Imbesi *et al.*, 2013). Studies of the toxicity exerted by synthetic antioxidants, support the regulation of these compounds use in foods, by agencies as European Food Safety Authority (EFSA) and Food and Drug Administration (FDA) (Banerjee *et al.*, 2017), among others (IARC, 1986; EFSA, 2011, 2012). Their undesirable long-term possible toxicological effects on humans has produced, in recent years, an increasing, on

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eISSN: 2550-2166

the demand for natural antioxidants, in particular in plants, with application in the food, pharmaceutical and cosmetic sectors, since they can be used as substitutes for synthetic antioxidants (García-Alonso *et al.*, 2004; Ferreira *et al.*, 2006).

Phenolic acids, flavonoids, and tannins are the most commonly found polyphenolic compounds in plant extracts (Naik *et al.*, 2006). Phenolic compounds along with plant pigments (carotenoids, chlorophylls) have proven significant antioxidant activity, which is directly correlated with removal of free radicals and superoxide (Hsu *et al.*, 2013; Sinkovič *et al.*, 2015). Therefore, it is important to emphasize the need to increase the consumption of foodstuffs rich in phenolic compounds aimed at preventive action on human health from cancer disease prevention to the occurrence and prevention of cardiovascular diseases (Barba *et al.*, 2014). However, phenolic compounds exhibit a high degree of degradation in terms of technological processes and show distinct thermolability, sensitivity to light, the impact of pathogens, mechanical damage to plant tissue cells (Ross *et al.*, 2009). Lately modern, non-invasive extraction techniques of plant metabolites with the main objective of increased extracted compound yield and a shortened period of extraction have been increasingly popularized. High-intensity ultrasound treatment does not show any degradation rates on the content of bioactive compounds in the treated food products (Koubaa *et al.*, 2015; ŠicŽabur *et al.*, 2015; Zinoviadou *et al.*, 2015). In addition, the technique is applicable for the extraction of compounds with various chemical structures and is characterized by non-invasive temperatures (Koubaa *et al.*, 2015; ŠicŽlabur *et al.*, 2015).

The proposal of a green extraction approach using an ultrasound extraction with water will meet the challenges of the 21st century, such as to protect both the environment and people and to enhance the competitiveness of industries to be more ecologic, economic and innovative. Water has been certified with GRAS (Generally Recognised as Safe) status by the United States Food and Drug Administration and is therefore appropriate for the manufacture of nutraceuticals along with ethanol, isopropanol and their combinations (Wang and Weller, 2006). In addition, the use of a green extraction is a part of the sustainable development and industrial strategy (Chemat *et al.*, 2012; Rombaut *et al.*, 2014).

The aim of the present study was the quantification of the respective antioxidant activity, emphasizing the traditional daily use of the studied plants. The ultrasound extracts obtained in accordance with the novel trends of green chemistry were analyzed and compared in respect to the presented biologically active substances.

2. Materials and methods

2.1 Plant material

The plant materials of *C. erythraea*, *Gl. glabra*., *P. hydropiper, S. vulgaris, A. linearis, H. arenarium*, *S. nigra* and *E. purpurea* were obtained from a herbal drugstore in Plovdiv (Bulgaria) and then was air-dried, grounded in laboratory homogenizer and stored in darkness at room temperature (Table 1).

Table 1. Bulgarian plants investigated in the present study

Latin name	Common name	Plant part
Centaurium erythraea Pers.	Centaury	aerial parts
Glycyrrhiza glabra L.	Licorice	roots
Polygonum hydropiper L.	Marsh pepper	aerial parts
Silene vulgaris L.	Bladder Campion	leaves
Aspalathus linearis L.	Rooibos	leaves
Helichrysum arenarium (L.) Moench	Everlasting Flower	flowers
Echinacea purpurea M.	Purple coneflower	aerial parts
Sambucus nigra L.	Elder, elderberry, black elder	flowers

2.2 Preparation of plant extracts

The extracts were prepared by weighing 1 g of dry grounded plant, added to 20 mL of water and placed in a 250 mL Erlenmeyer conical flask. The ultrasonic bath (UST 5.7150 Siel, Gabrovo, Bulgaria) was operated at a frequency of 35 kHz with a maximum input power of 240 W, for 30 mins, at 60°C. All obtained extracts were filtered after incubation and stored at 4°C without adding any preservatives until analyses.

2.3 Total polyphenol content analysis (TPC)

The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala *et al.* (2000) with some modifications. Each sample extract (1 mL) was mixed with 5 mL of Folin-Ciocalteu`s phenol reagent and 4 ml of 7.5% Na_2CO_3. The mixture was vortexed well and left for 5 mins at 50°C. After incubation, the absorbance was measured at 765 nm. The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g dry weight (DW).

2.4 Antioxidant activity (AOA)

2.4.1 DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams *et al.* (1995). Freshly prepared $4x10^{-4}$ M methanolic solution of DPPH was mixed with the samples in a ratio of 2:0.5 (v/v). The

light absorption was measured at 517 nm at room temperature after 30 mins incubation. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox having equivalent AOA expressed as the μM Trolox per g DW.

2.4.2 ABTS radical cation decolorization assay

The radicals scavenging activity of the extracts against radical cation ($ABTS^{\bullet+}$) was estimated according to a previously reported procedure with some modifications (Re *et al.*, 1999). The results were expressed as μM TE/g DW.

2.4.3 Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie and Strain (1999). The FRAP reagent was prepared fresh daily and was warmed to 37° C prior to use. The absorbance of the reaction mixture was recorded at 593 nm after incubation at 37°C for 4 mins. The results were expressed as μM TE/g DW.

2.4.4 Copper reduction assay (CUPRAC)

CUPRAC assay was performed according to the method of Apak *et al.* (2004). To a test tube were added 1 mL of $CuCl_2$ solution (1.0×10^{-2} M), 1 mL of neocuproine-methanolic solution (7.5×10^{-3} M), and 1 mL NH_4Ac buffer solution (pH 7.0), and mixed; 0.1 mL of herbal extract (sample) followed by 1 mL of water were added (total volume of 4.1 mL), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 mins. Trolox was used as standard and total antioxidant capacity of herbal extracts was expressed as μM TE/g DW.

2.5 Statistical analysis

The results obtained are average from two independent experiments carried out in triplicates. The values were expressed as mean ± SD, analyzed using MS Excel 2007 software.

3. Results and discussion

3.1 Total polyphenol content

Plant polyphenols are a significant group of compounds acting as free radical scavengers or primary antioxidants. The results regarding the total phenolic content of the studied water ultrasound extracts are presented in Table 2. The established total phenolic content varied from 4.08±0.03 to 20.48±0.13 mg GAE/g DW. The highest values were determined for *S. nigra* and *A. linearis* (20.48±0.13 and 12.65±0.03 mg GAE/g DW, respectively) and the lowest result was established in the *C. erythraea ultrasound extract* – 4.08±0.03 mg GAE/g DW.

Oniszczuk *et al.* (2016) conducted ultrasonic extraction of *S. nigra* at 60°C and established the presence of various phenolic acids and flavonoids. According to Viapiana and Wesolowski (2017), the TPC of *S. nigra* flowers infusions ranged from 15.23 to 35.57 mg GAE/g DW, whereas Veljković *et al.* (2013) detected TPC of 42.67 g GAE/kg DW. In comparison, Boukhira *et al.* (2015) established TPC of 3.35±0.12 mg GA/g of extract in the ultrasound-assisted extract of *S. vulgaris* from Morocco and Kiselova *et al.* (2006) established TPC for infusion of *A. linearis* of 437.90±1.11 μM QE. Compared to the report by Petkova et al. (2017) TPC for *E. purpurea* microwave extract (24.1±1.1 mg GAE/g DW), our findings are lower - 8.02±0.06 mg GAE/g DW. This could be due to the evaluation procedure differences and the plant sample variations. The phenolic compounds present in plant material may range from simple to highly polymerized. The different types and quantities of phenolic compounds also differ among plant types, and these may interact with proteins and carbohydrates to form insoluble complexes. The complete recovery of phenolics from plant material is therefore not always possible (Takeuchi *et al.*, 2009).

3.2 Antioxidant activity

The antioxidant ability and radical scavenging properties of plants are commonly associated with their medicinal value. The studied in the present research plants were widely used in traditional medicine. In accordance with the recommendations for at least two (Schlesier *et al.*, 2002) methods for antioxidant activity assessment, four reliable methods were employed in the present study. However, it is very difficult to select the most suitable antioxidant assay method due to the various mechanisms of antioxidant action (Badarinath *et*

Table 2. Total phenol content (mg GAE/g DW) and *in vitro* antioxidant activity (μM TE/g DW) of ultrasound-assisted extracts

Sample/assay	TPC	DPPH	ABTS	FRAP	CUPRAC
C. erythraea	4.08 ± 0.03	41.16 ± 0.40	27.37 ± 0.12	50.99 ± 0.30	43.92 ± 0.41
G. glabra	6.18 ± 0.01	61.32 ± 0.76	29.59 ± 0.29	34.16 ± 0.55	34.91 ± 0.69
P. hydropiper	5.80 ± 0.03	85.22 ± 0.26	70.00 ± 1.67	107.53 ± 0.58	108.75 ± 1.20
S. vulgaris	6.36 ± 0.06	63.24 ± 1.39	73.16 ± 1.80	75.96 ± 0.80	84.54 ± 1.26
A. linearis	12.65 ±0.03	166.64 ± 1.07	83.01 ± 1.36	222.32 ± 0.87	263.72 ± 3.85
H. arenarium	8.31 ±0.03	79.98 ± 0.60	34.54 ± 0.21	86.14 ± 0.62	124.55 ± 0.48
E. purpurea	8.02 ±0.06	70.03 ± 0.89	18.33 ± 0.33	93.94 ± 1.27	196.88 ± 2.88
S. nigra	20.48 ± 0.13	281.42 ± 1.63	144.54 ± 2.21	273.60 ± 2.81	407.96 ± 6.96

eISSN: 2550-2166

al., 2010).

According to the DPPH assay (Table 2) the highest results were recorded for *S. nigra* extract followed by *A. linearis* extract - 281.42±1.63 and 166.64±1.07 μM TE/g DW, respectively. The lowest value was established for *C. erythraea* - 41.16±0.40 μM TE/g DW. The results were in accordance with the total phenolic content established. Other authors determined the antioxidant activity of *S. nigra* flower infusions from 0.57±0.07 to 0.92±0.01 mM TE/g DW toward DPPH (Viapiana and Wesolowski, 2017). According to the ABTS assay (Table 2), the highest results were established for *S. nigra* and *A. linearis* - 144.54±2.21 and 83.01±1.36 μM TE/g DW, respectively and the lowest was evaluated in *C. erythraea* 27.37±0.12 μM TE/g DW. Mikulic-Petkovsek *et al.* (2016) reported significant variation in ABTS scavenging activity among Sambucus species and hybrids in a range from 44.87 to 118.26 mM TE/kg DW. The results, according to the conducted FRAP test, were in accordance with already established (Table 2). In particular, the highest antioxidant activity was evaluated in the *S. nigra* extract followed by *A. linearis* extract - 273.60±2.81 and 222.32±0.87 μM TE/g DW, respectively. Among the investigated ultrasound extracts the lowest values were established in *G. glabra* and *C. erythraea* samples- 34.16±0.55 and 50.99±0.30 μM TE/g DW, respectively. In comparison Marhev *et al.* (2013) established in ultrasound acetone extracts of *S. nigra* values between 13.58±0.10 and 31.29±0.37 mM TE/g dw. In respect of CUPRAC assay, *S. nigra* extract (407.96±6.96 μM TE/g DW) followed by *A. linearis* extract (263.72±3.85 μM TE/g DW) were established with the highest antioxidant potential. The lowest values were established in *G. glabra* and *C. erythraea* extracts. Marhev *et al.* (2013) established in ultrasound acetone extracts of *S. nigra* flowers values between 49.88±1.23and 108.54±1.43 mM TE/g dw according to CUPRAC assay.

Table 3. Correlation coefficients (r) for relationships between assays

Correlation coefficients	DPPH	ABTS	FRAP	CUPRAC
TPC	0.9823	0.8318	0.9332	0.9595
CUPRAC	0.9409	0.7743	0.9551	
FRAP	0.9580	0.8632		
ABTS	0.8968			

Correlation analysis between total phenolics and antioxidant capacity are shown in Table 3. The established coefficients varied between 0.7743 and 0.9823, which mean significant correlation between assays conducted. However, the strongest correlation was established between TPC and DPPH assay (0.9823). It has to be noted that a weaker correlation was observed when involving the ABTS assay. This indicates the fact that single assay may not be used to assess comprehensively the antioxidant activity and confirmed the trend for using at least three different assays.

4. Conclusion

Nowadays, the ultrasound-assisted method is extensively used for the extraction of valuable molecules. The present paper evaluates the biological activity resulted from applying this green chemistry approach. Among the studied plants, *S. nigra* flowers and *A. linearis* leaves extracts revealed to be the most effective in respect of total polyphenolic content and antioxidant capacities. The extracts could be used as potent sources of biologically active substances. However, the exact mechanism of action of the extracts *in vivo* definitely must be conducted as further research in order to interpret their functionality.

Conflict of Interest

The authors declare no conflict of interest.

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eISSN: 2550-2166