Industrial Crops and Products 99 (2017) 49–59

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

# Utilization of sage by-products as raw material for antioxidants recovery—Ultrasound versus microwave-assisted extraction

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ARTICLE INFO

*Article history:*
Received 29 July 2016
Received in revised form 18 January 2017
Accepted 19 January 2017
Available online 31 January 2017

*Keywords:*
*Salvia officinalis* L.
Ultrasound-assisted extraction (UAE)
Microwave-assisted extraction (MAE)
Polyphenols
Antioxidant activity
Optimization

ABSTRACT

In this work, sage (*Salvia officinalis* L.) by-products from filter tea factory, i.e. sage herbal dust, was valorized as raw material for extraction of phenolic antioxidants. Ultrasound-assisted (UAE) and microwave-assisted extraction (MAE) of polyphenols from sage herbal dust were separately optimized by simultaneous maximization of total phenols (TP) and total flavonoids (TF) yields. Box-Behnken experimental design and response surface methodology (RSM) was used for extraction optimization. In case of UAE, temperature (40, 60 and 80 °C), extraction time (40, 60 and 80 min) and ultrasonic power (24, 42 and 60 W/L) were independent variables, while optimized MAE parameters were ethanol concentration (40, 60 and 80%), extraction time (10, 20 and 30 min) and liquid to solid ratio (20, 30 and 40 mL/g). Antioxidant activity of sage extracts was determined by DPPH•, FRAP and superoxide anion radical neutralization assays, and good correlation between polyphenols content and antioxidant activity was observed. According to results, it could be concluded that novel extraction techniques (UAE and MAE) provided significant advantages for recovery of sage polyphenols comparing to traditional methods.



## 1. Introduction

Sage (*Salvia officinalis* L.) is a valuable medicinal plant from Lamiaceae family, which has been recognized for many medicinal plants with designated radical scavenger activity (Babovic et al., 2010). Traditionally, it has been widely used as herbal tea, spice and food flavouring agent, while industrially it found application as fragrance agent in cosmetics, perfumery and pharmaceutical industry. Up to date, sage has been utilized in production of various pharmaceutical formulations, due to wide range of biological activities, namely: antimicrobial (Bozin et al., 2007), preservative (Hayouni et al., 2008), immunomodulatory (Capek and Hříbalová, 2004), antioxidant (Babovic et al., 2010) and anticancer (Sertel et al., 2011) properties. Different functional preparations have been prepared from sage such as essential oil, and both lipophilic and hydrophilic extracts. Content of volatile fraction in sage, isolated by, either, solvent extraction, hydrodistillation, supercritical fluid extraction or subcritical water extraction, varies from 0.7 to 5.2% (Glisic et al., 2010), while α-thujone, β-thujone and camphor are its most abundant terpenoid compounds (Aleksovski and Sovová, 2007). Moreover, sage represents a good source of polyphenols, particularly phenolic acids derivatives (rosmarinic, carnosic, caffeic, ferulic, cinnamic and chlorogenic acid) (Hossain et al., 2010; Dragovic-Uzelac et al., 2012) and certain flavonoids (Roby et al., 2013), which has been recognized as bioactive compounds with high antioxidant activity.

Nowadays, people are returning to the use of traditional herbal preparations, rather than synthetic drugs, for treatment of various medicinal conditions. Therefore, herbal tea is rapidly becoming more and more popular beverage worldwide. During the production of filter tea, plant material is subjected to various unit operations, such as drying, cutting, grinding, fractionation, etc., described in details by Vidović et al. (2013). During hammer mill grinding, certain amount of fine powder (approx. 20%) is being produced, which has been recognized as herbal dust and has been considered as by-product. This fraction could not be used for filter tea packing since its particle size (<0.315 mm) is lower that filter pore size, therefore, this fraction is usually being discarded

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http://dx.doi.org/10.1016/j.indcrop.2017.01.028
0926-6690/

from the factory as by-product (Ramić et al., 2015). Although, processing of plant material during the production of filter tea causes loss of bioactive compounds (essential oil, polyphenols, ascorbic acid, etc.) (Vidović et al., 2013), this material still possesses certain amount of health benefit compounds and could be potentially utilized as raw material for extraction. Volatile compounds from essential oils rather easily evaporate from the herbal dust, however, significant amount of low-volatile compounds such as polyphenols is being retained in plant matrix. Moreover, solid-liquid extraction from herbal dust occurs rather quickly due to particularly low mass-transfer limitations, since particle size of plant material is rather small. Ultrasound and microwave-assisted extraction of polyphenols from tea factory by-products, i.e. herbal dust, has been recently performed from various plant materials such as horsetail (Milutinović et al., 2014), yarrow (Milutinović et al., 2015) and chokeberry (Ramić et al., 2015). The most commonly used filter tea in Balkan countries is being produced from aromatic medicinal plants (chamomile, mint, lemon balm, sage, etc.), or fruit (apple, rose hip, etc.), and amount of herbal dust generated from these plants is particularly high. Therefore, there is an initiative to valorize utilization of herbal dust as raw material for extraction of various bioactive compounds.

Classical extraction techniques commonly used for chemical standardization of botanicals and herbal preparations have been overcome by emerging green extraction technologies such as ultrasound-assisted (UAE), microwave-assisted (MAE) and pulsed-electric fields assisted extraction, as well as, high pressure techniques such as accelerated-solvent (ASE), subcritical water (SWE) and supercritical fluid extraction (SFE) (Heng et al., 2013). All these techniques have the same goals: 1) increase of yield of target compounds, 2) reduction of time, solvent and energy consumption, 3) minimization of environmental impact using green solvents. UAE and MAE have been particularly useful for extraction of polyphenols from various plant materials (Chan et al., 2011; Roselló-Soto et al., 2015). Recently, MAE has been utilized for the recovery of polyphenols from sage (Putnik et al., 2016). According to Tiwari (2015), the most important parameters influencing UAE are ultrasonic power and frequency, temperature, extraction time, matrix and solvent properties. Whilst, solvent polarity (dielectric constant), extraction time, irradiation power, temperature and contact surface area have been recognized as the main MAE parameters affecting the polyphenols extraction (Routray and Orsat, 2012).

In this work, the most important UAE and MAE parameters affecting the polyphenols extraction from sage herbal dust have been identified and these extraction techniques have been optimized separately. Response surface methodology (RSM) was used for optimization of UAE and MAE of polyphenols extraction from sage herbal dust, which was valorized for utilization as raw material for extraction of polyphenols. Influence of UAE (temperature, extraction time and ultrasonic power) and MAE (ethanol concentration, extraction time and solid to liquid ratio) parameters on sage polyphenols extraction was evaluated by RSM influence analysis. Moreover, antioxidant activity of obtained extracts was evaluated by DPPH•, FRAP and superoxide anion radical neutralization assays.

## 2. Materials and methods

### 2.1. Plant material

Sage (*Salvia officinalis* L.) originated from Montenegro was kindly donated by domestic filter tea factory, Fructus (Bačka Palanka, Serbia). Dry plant material was subjected to processing in the filter tea factory and production of herbal dust (by-product) has been described elsewhere (Ramić et al., 2015). Herbal dust fraction with <0.315 mm mean particle size was discarded as by-product, was used as raw material for present study.

### 2.2. Chemicals

Following reagents were purchased from Sigma-Aldrich Chem, Steinheim, Germany: Folin-Ciocalteu reagent, (±)-catechin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT), phenazine methosulfate (PMS), 2,3,5-triphenyltetrazolium chloride (TPTZ, Tetrazolium Red) and β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADH). Nitroblue tetrazolium (NBT) was purchased from Alfa Aesar (Karlsruhe, Germany), while ferric chloride-6-hydrate and ascorbic acid were purchased from Centrohem (Stara Pazova, Serbia). All other reagents used in this study were of analytical reagent grade.

### 2.3. Conventional solid-liquid extraction (CE)

Conventional solid-liquid extraction was performed in order to determine experimental domain for the ethanol concentration used in optimization study and to compare yield of polyphenols obtained by conventional and novel extraction techniques. In each experimental run, 5.0 g of sample was extracted with different solvent (100 mL). Extractions were performed at room temperature for 24 h with 150 rpm shaking speed. Water and following mixtures of water and ethanol were used: 20%, 40% 60%, 80% and 96% ethanol. After extraction, obtained extracts were filtrated through filter paper. Extracts were collected into glass vials and stored at 4 °C prior analysis.

### 2.4. Novel extraction techniques

#### 2.4.1. Ultrasound-assisted extraction (UAE)

In all UAE experimental runs, 5.0 g of sample was mixed with 100 mL of 60% ethanol in 250 mL glass flasks. Ultrasound-assisted extraction was performed in sonication water bath (EUP540A, Euinstruments, France) with frequency fixed at 40 kHz. Temperature (40, 60 and 80 °C), extraction time (40, 60 and 80 min) and ultrasonic power (24, 42 and 60 W/L) were independent variables which were set by the control panel of the instrument. In order to prevent evaporation of the extraction solvent, condenser was added on the flask during extraction. Flasks were always positioned in the position of the ultrasonic bath in order to provide constant ultrasonic power. After extraction, extracts were filtered through filter paper, collected into glass vials, sealed and stored at 4 °C prior analysis.

#### 2.4.2. Microwave-assisted extraction (MAE)

Mono-mode microwave-assisted extraction (MAE) was performed in experimental setup described in details by Zeković et al. (2016). In all MAE experimental runs, certain mass of sage herbal dust (depending on applied liquid to solid ratio) was mixed with 100 mL of extraction solvent (40, 60 and 80% ethanol) in 250 mL round glass flasks. Selection of experimental domain for the ethanol concentration was based on results of CE, and optimal ethanol concentration obtained in CE was chosen as the middle level of this variable for MAE. Extractions were performed at fixed microwave frequency and irradiation power (600 W), always positioned at the same distance from the magnetron. Ethanol concentration (40, 60 and 80%), extraction time (10, 20 and 30 min) and liquid to solid ratio (20, 30 and 40 mL/g) were independent variables. After the extraction, crude extracts were filtered through filter paper under vacuum, collected into glass vials, sealed and stored at 4 °C prior analysis.