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Evaluation and improvement of antioxidant and antibacterial activities of supercritical extracts from clove buds

Jasna Ivanovic[a,*], *Suzana Dimitrijevic-Brankovic*[b], *Dusan Misic*[c], *Mihailo Ristic*[d], *Irena Zizovic*[a]

[a]*Department of Organic Chemical Technology, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia*

[b]*Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia*

[c]*Department for Microbiology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobodjenja 18, 11000 Belgrade, Serbia*

[d]*Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia*

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ABSTRACT

For the first time, extracts from clove buds obtained by supercritical carbon dioxide extraction were screened for antioxidant and antibacterial activities. Additionally, antioxidant and antibacterial activities of extracts obtained by the supercritical extraction of the clove bud–oregano leaf mixtures were studied. Supercritical extract of pure clove had the highest eugenol (64%) and total phenolic content (530.56 mg GAE/$g_{extract}$). All extracts had antioxidant activity comparable to synthetic antioxidants against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and formation of peroxides. Presence of 0.6% and 5% of oregano extract in the clove extracts obtained from the clove–oregano plant mixtures improved their antioxidant activity with respect to the extract from pure clove. Clove extract showed moderate antibacterial activities against selected *Staphylococcus* and *Enterococcus* bacterial strains. Presence of 50% of the oregano extract improved antibacterial activity of clove extract against all tested strains and resulted in a synergistic antibacterial activity against Methicillin-resistant *Staphylococcus haemolyticus* strain (MIC $\leqslant$ 1.25 μg/mL). Study demonstrated great potential of supercritical clove extract as natural functional ingredient and the possibility of increasing its antioxidant and antibacterial efficiencies in order to apply lower concentrations and to reduce undesirable flavour notes and toxicological effects in final products.

1. Introduction

Growing interest in functional food ingredients that may impart health-promoting effects other than general nutrition and increasing concern about potentially harmful synthetic additives led to the increasing interest in the use of natural extracts and their components as functional ingredients in foods, drinks, toiletries and health-care products (Sacchetti et al., 2005; Siriamornpun, Kaisoon, & Meeso, 2012; Vulić et al., 2012). Extracts from herbs and spices with a pleasant taste or smell combined with a preservative action against lipid oxidation and spoilage by microorganisms are commonly needed for application within a wide range of the above mentioned products (Dziri et al., 2012; Sacchetti et al., 2005).

* *Corresponding author*. Address: Karnegijeva 4, P.O. Box 3503, 11000 Belgrade, Serbia. Tel.: +381 113303709; fax: +381 113370387.
E-mail address: jasnai@tmf.bg.ac.rs (J. Ivanovic).

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In terms of world trade value, flower buds of clove trees (*Syzygium aromaticum*, syn. *Eugenia caryophyllata*) today represent one of the most important spices used primarily in food industry and though less frequently, in pharmaceutical industry and perfumery. Clove buds comprise around 20% of volatile oil rich in eugenol (85–95%) (Hemaiswarya & Doble, 2009). The United States Food and Drug Administration (FDA) categorizes clove oil and eugenol as generally recognized as safe (GRAS) for use as a food additive or in a dental cement (U.S. Code of Federal Regulations, 21CFR184.1257). Clove bud essential oil obtained by hydrodistillation, extracts isolated from clove buds using organic solvents and their major component eugenol still remain a research priority due to their wide range of pharmacological and biological activities such as antioxidant (Gülçin & Aboul-Enein, 2012; Mastelić et al., 2008; Politeo, Jukic, & Milos, 2010), antibacterial (Hemaiswarya & Doble, 2009; Hoque, Bari, Juneja, & Kawamoto, 2008; Michiels, Missotten, Fremaut, De Smet, & Dierick, 2007), antifungicidal (Omidbeygi, Barzegar, Hamidi, & Naghdibadi, 2007), antiviral, anticarcinogenic and antimutagenic, anesthetic, repellent (Chaieb et al., 2007) and antiprotozoal (Machado et al., 2011) effects.

Nowadays, food and pharmaceutical industries are challenged to detect and eliminate adulterants from foods, food ingredients including functional foods, dietary supplements, drugs, and excipients (Griffiths, Abernethy, Schuber, & Williams, 2009). In this context, supercritical fluid extraction (SFE) with carbon dioxide as environmentally friendly "green" technique has received particular attention in new and improved applications in food and pharmaceuticals processing. Reportedly, the SFE is considered to be the optimal process for isolation of high quality essential oil from clove buds with many important advantages over traditional methods including the absence of traces of organic solvents, higher extraction yields and possibility of concentrating the active principles such as eugenol and eugenol acetate (Della Porta et al., 2007; Ivanovic, Zizovic, Ristic, Stamenic, & Skala, 2011; Wenqiang, Shufen, Ruixiang, Shaokun, & Can, 2007). However, none of the existing studies on SFE from clove buds considered investigation of biological activities of obtained extracts and to the best of our knowledge there are no data in the available literature on the antioxidant and antibacterial activities of clove bud extracts obtained by SFE. Therefore, the primary goal of the present study was to investigate antioxidant and antibacterial activities of supercritical extracts from clove buds for their possible application as functional ingredient in food, drinks and health-care products.

A recent study (Ivanovic et al., 2011) demonstrated that the extraction rate of the SFE from clove buds can be doubled when small and defined amounts of oregano leaves are added to the clove buds prior to extraction. Thereby, the extraction yield and chemical composition of the obtained clove extracts were negligibly affected. As an extension to the aforementioned study, the second task of the present work was to investigate the influence of adding of oregano leaves to clove buds on antioxidant and antibacterial properties of the supercritical extracts obtained from the clove bud–oregano leaf mixtures.

2. Materials and methods

2.1. Plant material

Dried clove buds (*Eugenia caryophyllata*) originated from Canary Islands (Spain) and leaves of oregano (*Origanum vulgare* L.) grown in Zrenjanin-Čenta (northern Serbia) were purchased from the local markets in Belgrade (Serbia). Air-dried plant material was ground and sieved and fraction with average particle diameter of 0.40 ± 0.10 mm was used for the experiments. The moisture content of the clove buds and oregano leaves determined by Karl Fischer volumetric titration was 8.78% and 9.70%, respectively.

2.2. Chemicals and reagents

The compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were acquired from Sigma–Aldrich Chemie GmbH (Taufkirchen, Germany). Folin–Ciocalteu reagent was purchased from Merck and Co., Inc. (New York, NY, USA). All other chemicals and solvents used for analytical and antioxidant activity assays were of the highest commercial grades bought from Lachema Ltd. (Brno, Czech Republic) and Fluka Chemie GmbH (Buchs, Switzerland).

2.3. Supercritical fluid extraction (SFE) procedure

The SFE procedure and laboratory scale extraction unit have recently been described elsewhere (Ivanovic et al., 2011). All extractions were performed under the 10 MPa and 40 °C. Simultaneous SFE of clove buds–oregano leaves mixtures comprising 10%, 50% and 95% of oregano were used to obtain extracts with 0.6%, 5% and 50% of oregano extract ($C_{0.6}$, C_5 and C_{50}, respectively). The calculation was conducted on the basis of experimentally determined extraction yields from pure clove and oregano at the same conditions (Ivanovic et al., 2011). The process of simultaneous SFE was chosen due to the effect of extraction rate enhancement during the extraction from the mixture due to the cosolvent effect of carvacrol on the eugenol extraction rate (Ivanovic et al., 2011). Otherwise, mixing of the calculated quantities of the clove and oregano supercritical extracts would give the extracts of similar chemical compositions. All experiments were performed until the plant material was exhausted and the maximal extraction yields were determined after consumption of 94.3 kg CO_2/$kg_{plant\ material}$ (5.0 ± 0.5 h of extraction). The mass flow rate of the CO_2 was 0.62 ± 0.06 kg/h. All the experiments were carried out in triplicate.

2.4. Determination of total phenolic content (TPC)

The total phenolic content (TPC) in the extracts was determined by a modified version of the method of Folin–Ciocalteu (Singleton & Rossi, 1956). Methanol solutions (100 μL) of the investigated extracts (1 mg oil per 1 mL methanol) were shaken for 1 min on the Vortex mixer with 500 μL of Folin–Ciocalteu reagent and 6 mL of methanol. After the mixture was shaken, 2 mL of 15% Na_2CO_3 were added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 mL by adding distilled water. After 2 h, the