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Exploitation of the biological potential of *Satureja thymbra* essential oil and distillation by-products

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ABSTRACT

Dried leaves of *Satureja Thymbra* were subjected to water-steam distillation to recover the essential oil (EO) and the water extract (Aq). The solid by-product was subjected to sequential Soxhlet extraction with ethyl acetate (EAc) and ethanol (EtOH). The obtained extracts were analysed by HPLC-DAD-ESI–MS/MS and the main flavonoids and phenolic acids were identified and quantified. The high antiradical activity of the extracts, as estimated by the DPPH• assay, was correlated to their total phenol content. Moreover, the antioxidant activity of the extracts was tested in bulk palm oil and palm oil-in-water emulsions. EAc extract prolonged the induction period and reduced by 42% the rate of peroxide formation in palm oil, while EtOH extract was the most effective in emulsions. Additionally, the EtOH and EAc extracts depressed the growth of *Listeria monocytogenes* in emulsions deliberately spiked with 100 cfu/mL.

The antimicrobial properties of EO, Aq, EAc, and EtOH extracts were assayed and the minimum inhibitory and non-inhibitory concentration values were determined. EO was effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Pseudomonas fragi*, *Saccharomyces cerevisiae* and *Aspergillus niger*. In contrast, EAc and EtOH extracts were active only against the bacteria species, but not against *S. cerevisiae* and *A. niger*, while no antimicrobial activity was observed for Aq extract.

1. Introduction

Currently, there is a strong debate and interest regarding the safety aspects of chemical preservatives added widely in many food products to prevent mainly growth of spoilage and pathogenic microbes. The synthetic compounds are considered responsible for many carcinogenic and teratogenic attributes and residual toxicity. To remedy the aforementioned problems, consumers and the European authorities increased the pressure on food manufacturers to substitute the harmful artificial additives with alternative natural substances. In this context, the use of natural compounds with antimicrobial activity presents an intriguing case. Moreover, natural compounds, like polyphenols, exhibit antiradical and antioxidant activities, and a certain body of research has been focused on their potential use in lipid food to retard oxidation.

Herbs of the Lamiaceae family are well known raw materials that contain substances with bioactive, antimicrobial, or antioxidant properties. The current commercial exploitation of these herbs is limited to the recovery of the essential oil, while the remaining solid waste is discarded, often creating an adverse environmental impact. Among the *Lamiaceae* herbs, *Satureja thymbra* is wildly grown and also cultivated in Mediterranean counties. The essential oil of the herb has been analysed and tested for antimicrobial activity by several researchers (Gören et al., 2004; Fleisher and Fleisher 2005; Chorianopoulos et al., 2006b; Karabay-Yavasoglu et al., 2006; Giweli et al., 2012; Öztürk, 2012; Tepe and Cilkiz, 2015). However, the essential oil consists a minor fraction of the plant, while the solid waste remaining after the recovery of essential oil contains polyphenolic compounds that could be used as antimicrobial or antioxidant agents. Skoula and Grayer (2005) detected some flavonoids in the aerial part of the herb, while there is no literature report about a systematic analysis of other phenolic compounds.

Abbreviations: Aq, water extract; EAc, ethyl acetate extract; EO, essential oil; EtOH, ethanol extract.

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Flavonoids, flavonoid glycosides and phenolic acids may be fractionated and recovered from aromatic plants by successive extractions with organic solvents. Research on the component characterization, the antimicrobial and antioxidant properties of *S. thymbra* extracts rich in polyphenolic substances is rather limited. Therefore, the present study focused on recovery of the essential oil and the polyphenolic fractions of *S. thymbra,* analysis of their components and determination of the antimicrobial, antiradical, and antioxidant properties. The obtained results might help in exploitation of the herb, and further its use beyond the essential oil recovery.

2. Materials and methods

2.1. Reagents and standards

Dried leaves of *Satureja thymbra* were obtained from the Institute of Plant Breeding and Genetic Resources" – Hellenic Agricultural Organization DEMETER. The plant was cultivated in the experimental field of the Institute, harvested in early May 2014, and provided to our Laboratory just after drying. The extractions were performed with ethyl acetate (Fisher Scientific, Loughborough, UK) and ethanol 96°. The reagents included DPPH (Sigma-Aldrich, Steinheim, Germany), Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) and sodium carbonate (Mallinckrodt, St. Louis, Missuri). The standard compounds used in the study were quercetin dihydrate and rosmarinic acid, products of Sigma-Aldrich (Steimheim, Germany), as well as luteolin, apigenin, eriodictyol, and naringenin (Extrasynthese, Genay, France), carvacrol (≥97%, Merck, Darmstadt, Germany), γ-terpinene (≥97%, Fluka, St Louis, USA) and gallic acid (98% w/w, Acros Organics, Fair Lawn, New Jersey). Water, acetonitrile, and methanol were used for chromatography analyses (HPLC and MS grade) (Fisher Scientific, Loughborough, UK), while glacial acetic acid (HPLC grade) (PanReac, Barcelona, Spain) was used for the acidification of HPLC solvents.

2.2. Microbial strains

Salmonella enterica subsp. *enterica* ser. Enteritidis FMCC B56 PT4 (kindly provided by Prof. Nychas G.J.E., Agricultural University of Athens, Greece), *Salmonella enterica* subsp. *enterica* ser. Typhimurium DSMZ 554, *Listeria monocytegenes* NCTC 10527 serotype 4b, *Escherichia coli* ATCC 25922, *Staphylocccus epidermidis* FMCC B-202 C5M6 (kindly provided by Dr. Nisiotou A., Athens Wine Institute, ELGO-DIMITRA, Greece), *Staphylococcus aureus* ATCC 25923, *Pseudomonas fragi* 211 (kindly provided by Prof. Nychas G.J.E.), *Saccharomyces cerevisiae* uvaferm NEM (Lallemand, Canada) and *Aspergillus niger* 19111 (kindly provided by Prof. Nychas G.J.E.) were used in the present study.

2.3. Extraction procedure

S. thymbra was subjected to a series of processes for the recovery of the bioactive compounds. The flow diagram of Fig. 1 depicts the sequence of treatments, the obtained products and their coded names. Dry leaves (500 g) of the plant were initially subjected to water-steam distillation to recover the essential oil (EO) and the water extract (Aq). An open type pilot scale distiller, made of copper (Chalkos, Greece) was used for the distillation. The apparatus was equipped with inner perforated grid to hold the plant material (net vol. for plant 10 L) above the boiling water (3.5 L). The headspace between the level of boiling water and the plant material was 3.5 L at the start of distillation, while the distillation procedure was carried for 3 h. The distilled water (hydrosol or floral water) was not recycled and, therefore, it was collected together with the EO. It amounted to 460 mL and was not further studied. The EO was kept in sealed glass vial in the refrigerator until used. Water-soluble compounds of the herb were partially extracted by the condensed steam and accumulated in the boiling water of the distiller. The remaining water phase at the end of distillation amounted to approximately 1.9 L. It was collected, filtered, diluted to 2 L and comprised the Aq. Aq was stored in a plastic container at the refrigerator, until used.

The wet herbal residue (~1.6 kg) was further dried, in a ventilated oven (Function Line UT20, Heraeus Instruments GmbH, Hanau, Germany) at 35 °C for 24 h, ground in a laboratory mill (Retch ZM 1; Haan, Germany), equipped with a 0.5 mm sieve, divided to batches of 50 g, and subjected to Soxhlet extractions sequentially with ethyl acetate and ethanol to obtain the EAc and

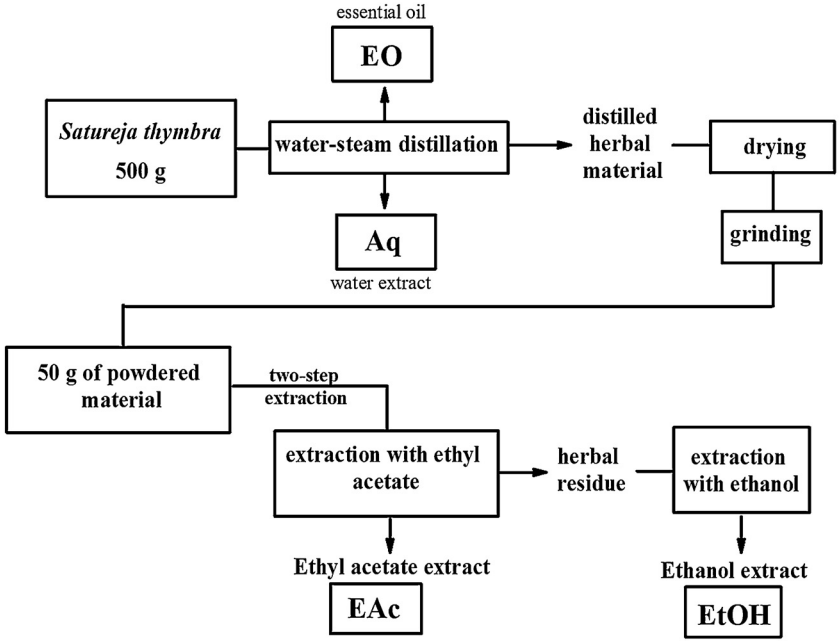

Fig. 1. The flow diagram of the extraction procedure and the coded names of the essential oil (EO), water (Aq), ethyl acetate (EAc) and ethanol (EtOH) extracts obtained from *S. thymbra*.