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# Effect of ultrasonic treatment on total phenolic extraction from *Lavandula pubescens* and its application in palm olein oil industry

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ABSTRACT

The aims of the current study were to evaluate the best technique for total phenolic extraction from *Lavandula pubescens* (Lp) and its application in vegetable oil industries as alternatives of synthetic food additives (TBHQ and BHT). To achieve these aims, three techniques of extraction were used: ultrasonic-microwave (40 kHz, 50 W, microwave power 480 W, 5 min), ultrasonic-homogenizer (20 kHz, 150 W, 5 min) and conventional maceration as a control. By using the Folin–Ciocalteu method, the total phenolic contents (TPC) (mg gallic acid equivalent/g dry matter) were found to be 253.87, 216.96 and 203.41 for ultrasonic-microwave extract, ultrasonic-homogenizer extract and maceration extract, respectively. The ultrasonic-microwave extract achieved the higher scavenger effect of DPPH· (90.53%) with $EC_{50}$ (19.54 μg/mL), and higher inhibition of β-carotene/linoleate emulsion deterioration (94.44%) with $IC_{50}$ (30.62 μg/mL). The activity of the ultrasonic-microwave treatment could prolong the induction period (18.82 h) and oxidative stability index (1.67) of fresh refined, bleached and deodorized palm olein oil (RBDPOo) according to Rancimat assay. There was an important synergist effect between citric acid and Lp extracts in improving the oxidative stability of fresh RBDPOo. The results of this work also showed that the ultrasonic-microwave assisted extract was the most effective against Gram-positive and Gram-negative strains that were assessed in this study. The uses of ultrasonic-microwave could induce the acoustic cavitation and rupture of plant cells, and this facilitates the flow of solvent into the plant cells and enhances the desorption from the matrix of solid samples, and thus would enhance the efficiency of extraction based on cavitation phenomenon.



## 1. Introduction

Ultrasound-assisted [1] and microwave-assisted [2] techniques are very important in improving the extraction of bioactive compounds from plant sources. The promotion of extraction of phenolic compounds by ultrasound and microwave energy can be attributed to an intensification of mass transfer, due to the phenomenon of cavitation bubble collapse produced by the sonication and radiation shocks and this would facilitate the flow of solvent inside the plant tissue, thus enhancing the efficiency of extraction [3,4]. Likewise, these techniques are able to employ the physical and chemical phenomena of plant cell (like osmotic pressure and water activity etc.…) that are principally different compared with those applied in conventional extraction techniques like maceration and hydrodistillation. Therefore, the ultrasound-assisted and microwave-assisted techniques are characterized as being able to increase the yield of extraction, reduce the chemical hazards and shorten the time for processing [1,3,4].

The uses of phenolic compounds as antiradicals are one of the most effective and convenient strategies to retard or prevent lipid oxidation [5] for common vegetable oils used, which include olive, sunflower, rapeseed, soybean and palm oil [6]. Palm oil occupy a considerable importance in the trade of world's vegetable oils and widely uses in the cooking oil, frying and margarine industries [7].

Polyphenolic compounds and their derivatives are normally found in most plant sources, especially those that belong to Lamiaceae [2,8,9], and they have promising potentials to be used as antioxidants [9–12] and antibacterial [9,13] in food industries.

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There are many rare species of medicinal and aromatic plants in Yemen, whose chemical and functional characteristics have not yet been studied until now. The Yemeni Lp Dencne (Lamiaceae), is one of the rare wild herbaceous shrub that grows in the rainy seasons in the highlands, and locally known under the common name "Sunaib" [14]. Yemeni Lp "Sunaib" has been utilized in a variety of food products commonly used as food additives herb to improve the flavor of tea, coffee, some of the local cuisine and dairy products and protect them for a longer period. However, there is limited information about the phenolic extract of Lp and its biological activities in the previously published studies.

The present study is an attempt to focus on the effectiveness of the ultrasonic technology in the extraction of total phenolic compounds from the plant sources through the comparison between the ultrasonic-microwave and ultrasonic-homogenizer techniques with the maceration method as a conventional technique (control). As well as study some of the biological properties of the Lp extract, to better contribute to the promotion of the use of natural compounds as an important and safer alternative of synthetic additives in the food industries.

## 2. Materials and methods

### 2.1. Plant material

The aerial parts of the Lp were collected during the flowering season (August–September 2013) from Al-Khatwah (a mountainous region) in Ta'izz city located in the Southern Highlands of Yemen at geographic coordinates: Latitude (13°34′46″ N) and Longitude (44°01′15″ E) Elevation above sea level: 4317 ft. [15]. Botanical identification was made by Dr. Abdulhabib Mahiuob Al-Kadasi (Head of the Forests and Grasslands Department with Public Authority for Agricultural Research – Ta'izz), according to "A handbook of the Yemen flora" [14]. The aerial parts of the Lp were dried for 10 days at room temperature (23–25 °C) in a shaded place and milled using an electrical mill. Moisture contents (%) of the Lp powder were measured in triplicate according to the American Association of Cereal Chemists (AACC) method [16], using a laboratory oven at 105 °C until constant weight was achieved (6.8%, w/w). The powdered samples were kept in a dry, cool and dark place.

### 2.2. Ultrasonic equipments

#### 2.2.1. CW-2000 ultrasonic-microwave cooperative extractor/reactor

CW-2000 Ultrasonic-microwave Cooperative Extractor/Reactor, Shanxi, Xi'an, (China) with a microwave power of 10–800 W (adjustable), Microwave frequency of 2450 MHz, Ultrasonic power 50 W and Ultrasonic frequency 40 kHz, equipped with a digital screen monitor 4″ LCD and with a reactor volume of 500 ml was used.

#### 2.2.2. Ultrasound-homogenizer

JY98-III DN Ultrasound Homogenizer, Nanjing FeiQi industry & trade Co., Ltd. Nanjing, (China), the maximum ultrasonic power of 1200 W at ultrasonic frequency of 20 kHz, equipped with an LCD digital screen monitor, thermometer, jacketed beaker volume of 100 mL and a circulating water bath was used.

### 2.3. RBDPOo

Fresh RBDPOo sample was obtained from Yemen Company for Ghee & Soap Industry (YCGSI) in Ta'izz (Yemen). It was free of food additives and the color was 5.25″ Lovibond Cell/2.9 Red according to AOCS Official Method Cc 13e-92 [17].

### 2.4. Chemicals

All chemicals and solvents used were of analytical grade. Folin–Ciocalteu reagent, 3,4,5-Trihydroxybenzoic acid (Gallic acid), beta,-beta-Carotene (β-Carotene), (9Z,12Z)-9,12-Octadecadienoic acid (linoleic acid) and (2R)2,5,7,8-Tetramethyl-2-[(4R,8R)-(4,8,12-titramethyltridecyl)]-6-chromanol (α-tocopherol) procured from Sigma Chemicals Company (Germany). Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), 2,6-Bis(1,1-dimethylethyl)-4-methylphenol (BHT), 2-(1,1-Dimethylethyl)-1,4-benzenediol (TBHQ) obtained from TCI EUROPE N.V. Belgium. Tween 80, sodium carbonate, citric acid, Nutrient Broth and Nutrient agar procured from Sinopharm Chemical Reagent Co. Ltd. (SCR), China. Streptomycin and penicillin procured from Amersco, Biochemicals and Life Science Research Products by VWR, Shanghai, Co., Ltd.

### 2.5. RBDPOo analysis

#### 2.5.1. The initial characteristics of fresh RBDPOo

The analysis of the initial characteristics of fresh RBDPOo were carried out according to AOCS Official Method [17]: Cd 1-25 [Iodine Value (g of $I_2$/100 g oil)], Ca 5a-40 [free fatty acid (%)], Cd 8-53 [Peroxide Value (meq $O_2$/kg oil)], Cd 18-90 (*p*-Anisidine Value), Cc 7–25 [Refractive Index (at Nd 40 °C)] and Cc 3–25 [Slip Melting Point (°C)], the values were as follows: (56.88 ± 0.11), (0.027 ± 0.002), (0.33 ± 0.01), (Nil), (1.4586 ± 0.000) and (23.0 ± 0.0), respectively. The previous values indicated that the oil sample was of good quality and the values of the properties were found to be within the range imposed by Codex Alimentarius standards [18].

### 2.6. Extraction procedures

#### 2.6.1. Maceration extraction

The maceration extract (MAC-E) was carried out as described by Upson et al. [19] with some modification. Briefly, 10 g of dried Lp powder were extracted with 100 ml of aqueous methanol MeOH·$H_2O$ at ratio 70:30 (v/v). The mixture was incubated in a thermostatic shaking water bath at 40 °C for 5 min. Excess chlorophyll was removed using petroleum ether. Then, it was allowed to cool to room temperature and then centrifuged at 4500 rpm for 15 min. The supernatant solution was filtered under vacuum through No.1 Whatman paper by Buchner funnel [20], and the solvents were removed by rotary evaporator at 55 °C to dryness. The absolute methanol was added to the final volume 25 mL and kept at −4 °C.

#### 2.6.2. Ultrasonic-microwave assisted extraction

To get the ultrasonic-microwave extract (USM-E), the operating conditions were set as follows: ultrasonic power/frequency at 50 W/40 kHz, microwave power input 480 W. 10 g of Lp powder was placed in a 500 mL amber glass bottle containing 100 mL of MeOH·$H_2O$ 70:30 (v/v), and connected to a condenser. In this technique, the ultrasonic energy was 50 W/10 g of the Lp powder at frequency 40 kHz and microwave energy was 480 W/10 g of the Lp powder and the mixture temperature was set at 40 ± 1 °C for 5 min with a continuous exposure to acoustic waves. Then, filtration and solvent removal from USM-E were done as described in the previous item (2.6.1).

#### 2.6.3. Ultrasonic-homogenizer assisted extraction

To prepare the ultrasonic-homogenizer extract (USH-E), the operating conditions were set as follows: ultrasonic power/frequency at 150 W/20 kHz. 10 g of Lp powder was placed in a glass jacketed vessel and dissolved in 100 ml of MeOH·$H_2O$ 70:30 (v/v) with magnetic stirring. The total programmed time was 12 min, the mixture was exposed during it to acoustic waves for a