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# Solvent-free microwave extraction of essential oil from pigeon pea leaves [*Cajanus cajan* (L.) Millsp.] and evaluation of its antimicrobial activity

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ABSTRACT

Solvent-free microwave extraction (SFME) of essential oil from pigeon pea leaves and its antimicrobial activity were investigated. The process of SFME was optimized by a central composite design (CCD) and response surface methodology (RSM). The optimal parameters were extraction time 44 min, irradiation power 660 W, and humidity 68%, with extraction yield of 0.330 (%, w/w). The main constituents were sesquiterpenes (72.89%), including α-copaene (5.89%), β-caryophyllene (7.46%), α-himachalene (12.97%), α-humulene (17.43%), alloaromadendrene (8.45%), and α-bisabolene (12.64%). The essential oil showed stronger antimicrobial activity against *Bacillus subtilis* and *Propionibacterium acnes* with MIC and MBC values 1.06 mg/mL and 2.12 mg/mL, 0.13 mg/mL and 0.26 mg/mL, respectively. These results indicated that SFME method was an outstanding alternative for the extraction of essential oil from pigeon pea leaves, and the essential oil was a potential source of natural antimicrobial.



## 1. Introduction

Pigeon pea [*Cajanus cajan* (L.) Millsp.], is a perennial leguminous plant. The cultivation of the pigeon pea dates back at least 3500 years, and the center of origin is the eastern part of peninsular India. Nowadays, pigeon pea is extensively cultivated in semitropical and tropical areas of the world. Pigeon pea has been an important food crop consumed as dried peas, farina, and green vegetable peas. In addition, pigeon pea leaves have been widely used in traditional Chinese medicine (TCM). It has been used to treat wounds, diabetes, hepatitis, measles, jaundice, dysentery, and blood disorders. (Fu et al., 2006; Fu et al., 2007; Zu et al., 2006; Rao et al., 2003). Chemical investigations showed the major active components in pigeon pea leaves were flavanoids, stilbenes, and coumarins, which possessed notable anti-inflammatory, anti-microbial, antioxidant, antitumor, and antivirus (Wu et al., 2009, 2011). Nevertheless, the research on essential oil from pigeon pea leaves has not been found.

Essential oil has been utilized for many years for food flavorings, pharmaceuticals, and non-traditional medicine (Lis-Balchin and Deans, 1997). Plant essential oil has been proved to have a wide range of pharmaceutical activities, such as anticancer, antimicrobial, insecticidal, and anti-parasitic properties (Lang and Buchbauer, 2012; Pilau et al., 2011; Vale-Silva et al., 2012). Many methods are applied to extract essential oil, such as classical hydrodistillation (HD), solvent extraction, and supercritical fluid extraction (Riela et al., 2008). However, these methods have various disadvantages, such as high volume solvent, rigorous heat, and toxic organic solvents, as well as expensive electronic equipment problems (Reverchon, 1997; Vinatoru, 2001). Solvent-free microwave extraction (SFME), a new extraction method, has been defined as an effective tool for the extraction of essential oil (Sacchetti et al., 2005). The SFME apparatus, a combination of microwave heating and dry distillation, is carried out at atmospheric pressure (Lucchesi et al., 2007). It has a lot of advantages, such as effective heating, fast energy transfer, time-saving, and low operating costs.

Up to our knowledge, the SFME was applied to extract essential oil from pigeon pea leaves has not been reported yet. In this study, the process of SFME of essential oil from pigeon pea leaves was optimized by a central composite design (CCD) and response surface methodology (RSM). The chemical compositions of essential

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oil were analyzed by GC–MS. Moreover, the antimicrobial activity of the essential oil was evaluated.

## 2. Materials and methods

### 2.1. Plant materials and chemical reagents

Pigeon pea leaves were collected from Hainan province, China, and authenticated by Professor Shao-quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, PR China. The samples were dried to constant weight, pulverized into a uniform size by a disintegrator (HX-200A, Yongkang Hardware and Medical Instrument Plant, China), sieved (40 mesh) and stored in the shade.

Erythromycin was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Erythromycin solution was prepared with sterile distilled water and stored at −20 °C. n-Alkanes ($C_{10}$–$C_{25}$) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Other analytical reagents were purchased from Beijing Chemical Reagents Co. (Beijing, China). Ultrapure water was prepared using a Milli-Q Water Purification system at the 18.25 MX resistance (Millipore, Billerica, MA).

### 2.2. Microorganisms and culture conditions

*Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacilus subtillis* (ATCC 6633), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 49132), *Candida albicans* (ATCC 10231), and *Propionibacterium acnes* (ATCC 6919) were purchased from the Institute of Applied Microbiology, Heilongjiang Academy of Science (China). The bacterial strains were cultivated on an agar plates at constant 4 °C. They were incubated on nutrient agar at constant 37 °C for 24 h (Demirci et al., 2008; Du et al., 2009).

### 2.3. Solvent free microwave extraction (SFME)

The microwave-accelerated reaction system (NEOS, Milestone, Italy) was utilized for the experiment. This is a 2.45 GHz multimode microwave reactor with a maximum power of 900 W delivered in 10 W increments. This instrument is equipped with an infrared temperature sensor, an electromagnetic stirrer, a time controller, and a circulating water-cooling system. For SFME procedure, an aliquot of 200 g plant materials were wetted before extraction by soaking in certain proportion of water for 1 h, and then removal the excess water. The wetted material was placed inside the reaction flask, and connected to a glass reaction flask. Essential oil was condensed in the receiving flask with circulating water-cooling system. The extraction time, extraction temperature, and irradiation power can be controlled by an electronic control panel. The anhydrous sodium sulphate was used for drying the isolated essential oil. The extraction yield of essential oil was calculated according to the equation given:

$$\text{Extraction yield}\,(\%,\ \text{w/w}) = \frac{\text{Mass of extracted essential oil}}{\text{Mass of dried material}} \times 100$$

### 2.4. Hydrodistillation (HD)

Compared HD experiment was conducted as follows. An aliquot of 200 g materials were hydrodistilled with 1.5 L of water in a glass reaction flask for 5 h (until no more essential oil obtained). The anhydrous sodium sulphate was used for drying the isolated essential oil.

### 2.5. Experimental design and statistical analysis

The three main independent variables studied were extraction time ($X_1$: 20–40 min), irradiation power ($X_2$: 300–700 W), and humidity ($X_3$: 60–80%), and the response surface method (RSM) was used for optimizing the operating conditions. Simultaneously, the central composite design (CCD) software was employed in data processing. Based on the preliminary experiments, the range of these three factors and central point were chosen. As shown in Table 1, the effect of three main factors on the extraction of essential oil was investigated at five levels (−1.68, −1, 0, +1, +1.68). In this CCD, these 20 different experiments together with six replicates at center point were employed to fit the full quadratic equation model. The equation had the following form:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_{ii}X_i + \sum_{i=1}^{k}\beta_{ii}X_i^2 + \sum_{\substack{i=1\\ i<j}}^{k-1}\sum_{j=2}^{k}\beta_{ij}X_iX_j$$

where $Y$ represented the response variable, $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ were the regression coefficients of variables for intercept, linearity, square, and interaction terms, respectively; $X_i$ and $X_j$ were the independent coded variables influencing the response variable $Y$ and $k$ represents the quantity of variables. Data obtained from the CCD for the optimization of SFME experiments were analyzed statistically with the Design-Expert 8.0.5. Analysis of variance (ANOVA) was used for calculating and modeling of the optimum conditions for SFME of essential oil from pigeon pea leaves.

### 2.6. Scanning electron micrographs (SEM)

In order to study the morphological changes of samples with different treatment method, a Hitachi S-520 field emission scanning electron microscope (Hitachi, San Jose, CA) was used. The tested samples were air dried, and fixed on a specimen holder with aluminum tape and marked, then sputtered with gold. At an acceleration voltage of 15.0 kV, treated samples were observed under high vacuum conditions.

### 2.7. Constituent analysis of essential oil by GC–MS

Essential oil was immediately analyzed by gas chromatography coupled to mass spectrometry (GC–MS) with an Agilent 7890A gas chromatograph connected to an Agilent 7000B mass spectrometer, using a HP-5MS column (30.0 m × 250 μm × 0.25 μm film thickness). The GC–MS was performed using the following operating conditions: carrier gas helium; flow velocity 1.0 mL/min; split ratio 1:10; injection volume 1 μL; injection temperature 200 °C; oven temperature progress from 80 °C to 135 °C at 5 °C/min, from 135 °C to 185 °C at 2 °C/min, and keeping at 185 °C for 4 min; the ionization mode used was electronic impact at 70 eV, and spectra were acquired from $m/z$ 30 to 400. The percentage composition of essential oil was produced by the GC peak areas. Identification of essential oil components was performed by comparing their retention indices and mass spectral fragmentation patterns with those stored in the NIST mass spectral library.

### 2.8. Antimicrobial activity

Bacterial cells ($10^5$ CFU/mL) were inoculated into a nutrient broth at 0.1 mL/well, in 96-well microtiter plates. Minimum inhibitory concentration (MIC) was determined using a serial 2-fold dilution according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006). After incubation at constant 37 °C for 24 h, the minimum concentration of