Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Essential oils from Taiwan: Chemical composition and antibacterial activity against *Escherichia coli*

Po-Chen Lin [a], *Jason Jwo Lee* [b], *I-Jy Chang* [a,*]

[a] *Department of Chemistry, National Taiwan Normal University, Taipei, Taiwan*
[b] *Department of Biotechnology and Laboratory Science in Medicine, National Yang-Ming University, Taipei, Taiwan*

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form
4 September 2015
Accepted 25 December 2015
Available online 11 April 2016

Keywords:
antibacterial activity
chemical composition
Escherichia coli
essential oil
gas chromatography-mass spectrometry

ABSTRACT

The chemical compositions of seven essential oils from Taiwan were analyzed by gas chromatography-mass spectrometry. The eluates were identified by matching the mass fragment patents to the National Institute of Standards and Technology (NIST) 08 database. The quantitative analysis showed that the major components of lemon verbena are geranial (26.9%) and neral (23.1%); those of sweet marjoram are γ-terpinene (18.5%), thymol methyl ether (15.5%), and terpinen-4-ol (12.0%); those of clove basil are eugenol (73.6%), and β-(Z)-ocimene (15.4%); those of patchouli are carvacrol (47.5%) and *p*-cymene (15.2%); those of rosemary are α-pinene (54.8%) and 1,8-cineole (22.2%); those of tea tree are terpinen-4-ol (33.0%) and 1,8-cineole (27.7%); and those of rose geranium are citronellol (28.9%) and 6,9-guaiadiene (20.1%). These components are somewhat different from the same essential oils that were obtained from other origins. Lemon verbena has the same major components everywhere. Tea tree, rose geranium, and clove basil have at least one major component throughout different origins. The major components and their amounts in sweet marjoram, patchouli, and rosemary vary widely from one place to another. These results demonstrate that essential oils have a large diversity in their composition in line with their different origins. The antibacterial activity of essential oils against *Escherichia coli* was evaluated using the optical density method (turbidimetry). Patchouli is a very effective inhibitor, in that it completely inhibits the growth of *E. coli* at 0.05%. Clove basil and sweet marjoram are good inhibitors, and the upper limit of their minimum inhibitory concentration is 0.1%.

1. Introduction

Essential oils have a long history of being used by people in their daily lives for both spiritual and practical reasons. Plant products were the principal sources of pharmaceutical agents used in traditional medicine. Some medicinal plants are rich in antimicrobial reagents [1]. Several essential oils derived from varieties of medicinal plants are known to possess

* *Corresponding author*. Department of Chemistry, National Taiwan Normal University, 88 Tingchou Road, Section 4, Taipei 11677, Taiwan
E-mail address: changijy@ntnu.edu.tw (I.-J. Chang).

http://dx.doi.org/10.1016/j.jfda.2015.12.006

insecticidal, antifungal, anti-inflammatory, and antioxidant activities [2–5]. Using essential oils from flowering plants (angiosperms) as food preservatives can be traced back to the ancient Egyptians [6]. In modern society, pure chemicals are developed for use as insecticides, food preservatives, and antibacterial reagents. The practical functions of essential oils have been completely replaced by industrial products. However, in recent years, many concerns have been raised regarding the overuse of chemicals as preservatives and additives in food products. The urge to search for healthy chemical substitutes has drawn massive attention to essential oils [7]. Addition of essential oils into food, drugs, and cosmetics is now desired in various products. Many remarkable results have been made possible by the advent of essential oil products. It is therefore important to scientifically evaluate the possible applications of essential oils.

One of the intriguing properties of essential oils is their antibacterial property. Depending on the plants' growth condition, such as location and climate, the chemical composition of the same essential oils may vary. Hence, it is important to determine the chemical composition of each essential oil. Documentation of the contents of essential oils not only makes the investigation of the influence between essential oils and claims possible, but it also builds a common ground for discussion.

In this paper, we report the chemical compositions of seven essential oils that are extracted from the plants that were grown and harvested in Ji-an Town, Hualien, Taiwan, and the potential of these essential oils for inhibition against *Escherichia coli*.

2. Materials and methods

2.1. Chemicals

Ethyl acetate was ACS grade and was purchased from Mallinckrodt (Dublin, Ireland). Normal paraffin C7, C8, C9, C10 mix and C10, C12, C14, C16 mix were purchased from Aldrich (St. Louis, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). Tryptone (casein hydrolysate) enzymatic digest, yeast extract, and agar were purchased from USB Corporation (Cleveland, USA). All reagents were used as received without further purification.

2.2. Plant material

Fresh leaves of *Lippia citrodora* (Paláu) Kunth (lemon verbena), *Origanum majorana* L. (sweet marjoram), *Ocimum gratissimum* L. (clove basil), *Pogostemon cablin* Benth. (patchouli), *Rosmarinus officinalis* L. (rosemary), *Melaleuca alternifolia* Cheel (tea tree), and *Pelargonium graveolens* L'Hér. (rose geranium) were collected from Ji-an Town, Hualien, Taiwan. The plant samples were identified, and the voucher specimens were deposited at the Herbarium of the Department of Life Science, National Taiwan Normal University. The voucher numbers are as follows: *P. cablin* Benth (TNU055241); *P. graveolens* L'Hér (TNU055242); *O. majorana* L. (TNU055243); *O. gratissimum* L. (TNU055246); *M. alternifolia* Cheel (TNU055247); *L. citrodora* (Paláu) Kunth (TNU055248); *R. officinalis* L. (TNU055249). All seven essential oils were extracted from air-dried leaves of plants by hydrodistillation for 40 minutes using a Clevenger-type apparatus. The oil samples obtained were dried over anhydrous sodium sulfate and stored in sealed vials in a cool and dark place prior to analyses. Essential oils were volumetrically diluted to a thousand times in ethyl acetate prior to gas chromatography (GC) injection.

2.3. GC and GC-mass spectrometry analysis

GC was performed on Agilent Technologies 6850 Series II equipped with a flame ionization detector. The capillary column was HP-5MS cross bond (5% diphenyl-polysiloxane and 95% dimethyl-polysiloxane; 30 m × 250 μm × 0.25 μm). The injector and detector temperatures were both set at 250°C. Nitrogen was used as the carrier gas, and the flow rate was set to constant mode (1 mL/min). Injection volume was 1 μL and the injection mode was splitless. The temperature was raised by a step gradient that starts at 60°C for 15 minutes then quickly rises up to 80°C in 4 minutes, followed by a slow rise to 135°C in 55 minutes, and finally a rapid rise to 260°C in 8 minutes and hold for 2 minutes. GC-mass spectrometry (GC-MS) analysis was performed using a Hewlett-Packard 6890 Gas Chromatograph and Hewlett-Packard 5973 Mass Selective Detector. The capillary column was also HP-5MS cross bond. The injector and detector temperatures were set at 250°C and 230°C, respectively. Helium was used as the carrier gas, and the flow rate was set to constant mode (1 mL/min). The injection volume was 1 μL, and the injection mode was split with a 50:1 ratio. The temperature gradient was the same as in the GC condition. Ionization voltage was 70 eV by electron impact. The acquisition mass range was set to 30–650 amu. The mass spectra were searched and compared through the database of National Institute of Standards and Technology (NIST) 08 libraries for characterization (the matching quality between the experimental data and the database is more than 90%). For further confirmation, Kovats retention index (RI) was calculated relative to standard *n*-alkanes of *n*-paraffin mix C7, C8, C9, C10 and C10, C12, C14, C16. The calculated RI was compared with those reported by Pino et al [8] and Pitarokili et al [9]. The relative percentage amounts were calculated on the basis of peak areas for both GC and GC-MS analysis, and the results were very similar. To confirm the high percentage components, standards were used to spike into the sample.

2.4. Antibacterial test

Sterilized lysogeny broth (LB) was prepared for the growth of *E. coli*. Frozen stock of strain ATCC 13676 was grown at 37°C on a 1.5% agar plate overnight. An *E. coli* stock solution was prepared by suspending a selected colony to 1 mL sterilized LB [10]. For each test, tubes with 5 mL sterilized LB were inoculated with 40 μL of *E. coli* stock solution. Essential oils were added to the tubes directly to give the appropriate concentrations: 0.01%, 0.05%, and 0.1% (v/v). The antibacterial activity of the essential oils against *E. coli* was evaluated using the optical density method (turbidimetry) [11]. During the incubation (37°C and 150 rev/min), the optical density of the inoculated broths at 600 nm was measured using Amersham Pharmacia Biotech Novaspec II Visible Spectrophotometer