Pacific Science Review A: Natural Science and Engineering 18 (2016) 103–106

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Contents lists available at ScienceDirect

Pacific Science Review A: Natural Science and Engineering

journal homepage: www.journals.elsevier.com/pacific-science-review-a-natural-science-and-engineering/

In vitro antimicrobial potential of crude extracts and chemical compositions of essential oils of leaves of *Mentha piperita* L native to the Sultanate of Oman

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ARTICLE INFO

Article history:
Received 12 August 2016
Received in revised form
6 September 2016
Accepted 8 September 2016
Available online 29 November 2016

Keywords:
Mentha piperita
Essential oil
Hydrodistillation
Clevenger apparatus
Antimicrobial capacity
GC–MS analyses

ABSTRACT

Volatile oils of *Mentha piperita* L (*M. piperita*) contain various chemical constituents. The aim of this work is to isolate essential oil from leaves of *M. piperita*, which is native to the Sultanate of Oman, by steam distillation and to analyse chemical constituents by gas chromatography–mass spectroscopy (GC–MS). Fourteen main chemical compounds, representing 91.11% of the leaf oils, were identified in essential oils as carvone (34.94%), pulegone (14.89%), methyl petroselinate (15.51%), D-limonene (11.20%), ρ-cineole (5.70%), methyl isoheptadecanoate (2.35%), 1-tridecene (2.21%), methylene-6,10,10-trimethylbicyclo (7.2.0), undec-5-ene (0.91%) and isopulegone (0.89%). Some minor chemical compounds were also obtained (verbenone (0.72%), β-myrcene (0.57%), β.-pinene (0.73%), sabinene (0.49%) and α-pinene (0.49%)). The extracted methanol extract of *M. piperita* and its fractions of hexane, chloroform, ethyl acetate, and butanol extracts as well as essential oil were tested for antimicrobial activity via disc diffusion. The crude extracts and essential oils exhibited moderate antibacterial activity against the tested bacteria (e.g., Gram positive *Staphylococcus aureus* (*S. aureus*), Gram negative *Escherichia coli* (*E. coli*) and *Xanthomonas campestris* (*X. campestris*)) within a range of 0–14%. Our findings demonstrate that leaf extracts and essential oils of *M. piperita* exhibit excellent antimicrobial activity and thus have strong potential as a source of natural antibiotics.

1. Introduction

Mentha piperita L is a popular folk medicine used in Oman that is a cross between watermint and spearmint and that belongs to the Lamiaceae family. There are many species available worldwide including applemint, water mint, horsemint, pineapple mint, orange mint, pennyroyal and spearmint. *M. piperita* is indigenous to Europe and is now found throughout the world [1]. Presently, it is widely cultivated all over the world, and even in Oman. Pennyroyal is an organic compound obtained from *M. piperita*. Pennyroyal produces a toxic compound after being metabolized when it is taken internally. This toxic compound is damaging to the liver [2–4]. However, the crude extract can also be rubbed onto the skin as an insect repellent [2–4]. Traditionally, the leaves and crude extracts of this plant are widely used as medicine for the treatment of various medical aliments [5].

Essential oils of *M. piperita* contain menthol and menthone together with other minor chemical constituents, including pulegone, mentho furan and limonene [6,7]. The chemical composition of this plant can vary with plant maturity, across geographical regions and depending on processing conditions [8]. Commercially, it is widely used for the manufacturing of new flavours, foods and medicine owing to its aromatic and flavourful qualities [2–4]. Traditionally, it is used in Oman for the preparation of various commercial products such as toothpaste, chewing gum, mouthwash, soap, sweets, balms, creams and cough medicine [2–4]. The Sultanate of Oman has a rich heritage of traditional herbal treatment use, including the use of various mint species. Thus far, no information is available on either the constitutional analysis of essential oils of the Omani variety of *M. piperita* or on its potential antimicrobial properties in protecting against bacterial strains. More advanced research must be conducted to evaluate the

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Peer review under responsibility of Far Eastern Federal University, Kangnam University, Dalian University of Technology, Kokushikan University.

http://dx.doi.org/10.1016/j.psra.2016.09.005

phytochemical constituents and other elements of these traditional Omani medicines and particularly of the *M. piperita* species. Due to the medicinal value of the selected plant, we isolate and identify the chemical compounds of essential oils and *in vitro* antimicrobial activities of various crude extracts and essential oils from the leaves of *M. piperita* plants native to the Sultanate of Oman.

2. Materials and methods

2.1. Plant sample

Healthy leaves of *M. piperita* were collected for the extraction of essential oils and methanol extracts from Nizwa, Sultanate of Oman. Initially, plant samples were identified by morphological features listed in a database available on the following website: https://en.wikipedia.org/wiki/Peppermint. The collected samples were transported to a research laboratory and stored at room temperature for necessary processing.

2.2. Sample collection

Fresh and healthy leaf samples of *M. piperita* were collected on April 15, 2012 at 10.00 am from Nizwa, Sultanate of Oman. After harvesting, the leaves were immediately packed in sealed plastic bags, which were stored in a refrigerator at 4 °C for the isolation of essential oils.

2.3. Preparation of crude extracts

Half of the collected leaf samples were rinsed with water, and healthy rinsed leaves were separated from the stems. The separated leaf samples were dried in the shade at room temperature (25 ± 1 °C) for 7 days. Approximately 50 gm of shade-dried leaves was ground in a blender (Jaipan, Super Deluxe, India) for 20 s. The 102-gm powder sample was extracted with methanol (500 ml) for 72 h using a Soxhlet extractor. The methanol was evaporated using a rotary evaporator to create semi-solid masses (30.89 gm). The methanol extract (30 gm) was dissolved in water (100 ml) and extracted twice with hexane, chloroform, ethyl acetate and butanol to yield hexane (6.68 gm; yield 22.26%), ethyl acetate (4.40 gm; yield 18.29%), chloroform (7.100 gm; yield 29.34%), and butanol (2.58 gm; yield 15.78%) fractions, respectively [9].

2.4. Isolation of essential oil

The other half of the fresh and healthy leaf material (100 g) was subjected to 3 h of hydro distillation using a Clevenger-type apparatus. After extraction was complete, the collected essential oil was re-extracted with dichloromethane and dried over anhydrous sodium sulphate [10]. The pure essential oil was preserved in a sealed amber-coloured vial at 4 °C until further analysis.

2.5. GC–MS analysis

An analysis of the isolated essential oil was performed using a Perkin Elmer GC–MS system. The GC system was equipped with an Rtx®-5MS fused silica capillary column (30 m × 0.25 i.d. film thickness 0.25 μm). It was coupled with a Perkin Elmer MS. Helium was used as a carrier gas at a constant flow rate of ±1 ml/min. The mass transfer line and injector were set at 220 and 290 °C, respectively. The oven temperature was programmed at 60 °C (hold 2 min) to 270 °C at 4 °C/min, then set as isothermal for 20 min and then finally increased to 300 °C at 10 °C/min. The essential oil was diluted with methanol (1/100, v/v, in methanol) and 1 μl was injected into the split mode at a split ratio of 120:1. The percentage of each peak of essential oil was expressed as a percentage of the peak area [10].

2.6. Essential oil identification

Chemical compounds of the essential oils were identified based on GC retention times and by matching mass spectra with standard ones (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC–MS systems) [10].

2.7. Antibacterial activity assay

An antibacterial potential test was carried out via agar disc diffusion with modifications [11]. An antimicrobial assay of different crude extracts and essential oils was carried out using one gram (+) of *Staphylococcus aureus* bacteria and two grams (−) of *Escherichia coli* and *Xanthomonas campestris* bacteria. Each extract and essential oil was serially diluted using dimethyl sulphoxide (DMSO) to create 2.5, 1.25, 0.675 and 0.325 mg/ml solutions. Sterile Whatman filter paper was used as disc material for this experiment (5 mm diameter). The filter paper discs were separately impregnated with each of the above concentrations of crude extract and essential oil and placed on an inoculated agar plate. Negative controls were prepared using DMSO alone without extracts. All plates were then incubated micro-aerobically at 37 °C for 24 h. Inhibition zones were measured as differences between disc diameters and the diameters of inhibitions. Each assay was performed in triplicate.

3. Results and discussion

3.1. Chemical composition of the essential oil

Essential oils isolated from leaves of the selected plant by hydro distillation were yellowish in colour. A total ion chromatogram (TIC) of isolated essential oil drawn from fresh and healthy *M. piperita* leaves is shown in Fig. 1. The main chemical constituents of the essential oil are oxygenated monoterpenes, sesquiterpenes, hydrocarbons and their derivatives. All of these monoterpenes, sesquiterpenes hydrocarbons and their oxygenated derivatives have organic volatiles of low molecular weight [10].

The essential oil was found to include 14 different volatile organic compounds representing 91.11% of the sample. Chemical compounds identified in the essential oil are listed in Table 1 by elution order in an Rtx®-5MS fused silica capillary column. The essential oil contains a mix of compounds consisting of mainly oxygenated mono and sesquiterpene hydrocarbons and their derivatives. Most researchers have reported that the main chemical constituents of essential oils of medicinal and aromatic plant origin are monoterpenes and sesquiterpenes hydrocarbons and their oxygenated derivatives [2–4].

The main volatile compounds detected in the essential oil were carvone (34.94%), pulegone (14.89%), methyl petroselinate (15.51%), D-limonene (11.20%), ρ-cineole (5.70%), methyl isoheptadecanoate (2.35%), 1-tridecene (2.21%), methylene-6,10,10-trimethylbicyclo (7.2.0), undec-5-ene (0.91%) and isopulegone (0.89%). Minor chemical compounds found included verbenone (0.72%), β-myrcene (0.57%), β-pinene (0.73%), sabinene (0.49%) and α-pinene (0.49%) (Table 1). Almost all chemical constituents identified in essential oils from leaves of *M. piperita* exhibited very high levels of potent biological activity [2–4]. The essential oil contains a mixture of compounds consisting mainly of oxygenated mono and sesquiterpene hydrocarbons and their derivatives. Most researchers have reported that the main chemical constituents of essential oils of medicinal and aromatic plant origin include monoterpenes and