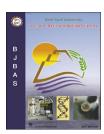


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Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria



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ABSTRACT

The aim of this work to isolate and identify two essential oils by hydro distillation method from two imported lemon fruits samples collected from local supermarket and evaluate their antimicrobial activity against pathogenic bacteria through disc diffusion method. The essential oil was obtained from Turkish and Indian lemon fruits samples by hydro distillation method using Clevenger type apparatus. Both isolated essential oils were identified by GC-MS and determine their in vitro antimicrobial activity against pathogenic bacteria through agar gel method. Twenty two bioactive ingredients with different percentage were identified based on GC retention time from Turkish and Indian lemon collected from local supermarket. The predominant bioactive ingredients with high percentage in Turkish essential oil were DL-limonene (78.92%), α-pinene (5.08%), L-α-terpineol (4.61%), β-myrcene (1.75%), β -pinene (1.47%) and β -linalool (0.95%) and in Indian essential oil were pL-limonene (53.57%), L- α -terpineol (15.15%), β -pinene (7.44%), α -terpinolene (4.33%), terpinen-4-ol (3.55%), cymene (2.88%) and E-citral (2.38%) respectively. Both isolated essential oils by hydro distillation were used for the study of antimicrobial activity against four pathogenic bacterial strains such as Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) and Proteus vulgaris (Pseudomonas vulgaris). Almost all bacterial strains did not give any activity against the employed essential oils at different concentrations. Therefore, the obtained results show that both essential oils could be needed further extensive biological study and their mechanism of action.

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1. Introduction

Normally the essential oils were obtained from the natural sources such as herbs, fruits, roots, barks, stems and medicinal plants by hydro distillation, expression or solvent extraction methods. All these methods are considered as wellknown methods for the isolation of essential oils in traditional medicine. Using these methods for the production of essential oils that chemical ingredients showed a potential source of antimicrobial agents. The isolated essential oils from the natural sources are showed broad-spectrum activity, including antifungal, antibacterial and antiviral activities. Besides that the essential oils from natural sources are used for food preservation, flavoring agent, pharmaceuticals formulation, cosmetics as fragrances and the preparation of alternative medicine (Hussain et al., 2010). The percentage of major and minor chemical constituents are considered as chemical composition of each essential oil. Furthermore, chemotypes of essential oils are depends on the levels of the major chemical components. The biological activities such as antimicrobial activity are not only depends on the chemical component but also depends on the structure activity relationship might be occur by the other chemical components (Faleiro et al., 2003). Essential oils from the natural sources are more potent than various antimicrobial agents which are used for air disinfection. Because the essential oils have low toxicity level and high volatile properties which is not obtained in antimicrobial agents (Inouye et al., 2003). In addition, essential oil as natural food preservatives has been used widely and accepted by the consumers all over the world (Militello et al., 2011).

The lemon is a small evergreen tree native to Asia. The lemon has several varieties and it has ellipsoidal yellow fruits (Frederick and Xulan, 1990). It is the major species of citrus fruits after oranges and mandarins (Rainer, 1975). The "word" lemon originates from old French known as "limon". It has also different name in Italy "limone", Arabic "laymūn" or "lmūn" and Persian "lmūn". The generic term for citrus fruit which is a related of Sanskrit nimbū, "lime" (Giacosa, 1994).

The lemon is a flowing plant belongs to the family Rutaceae. The genus citrus comprises of about 140 genera and 1300 species (Kamal et al., 2011). It is first originated from Southeast Asia then it is spread to Northeast India, Burma and China. Since ancient times citrus fruit has been cultivated all over the world (Andrews, 1961). The tree is small with alternate, usually evergreen, leaves, which are shiny and leathery and dotted with oil glands. Most of the time, the stems are mostly winged and jointed with the leaves. The flowers smell is strong sweet and have five petals. The fruit is spherical or eggshaped. It has 8-14 juicy sections containing large, white or greenish seeds (Freitas et al., 2003). Traditionally it is used lemon to soothe sore throats, and as an additive for flavoring to our foods (Nicolosi et al., 2000). The lemon is also well known for its alkaloid properties. The crude extracts of different parts of lemon showed anticancer and antibacterial properties against clinically significant bacterial strain has been reported (Saidan et al., 2004). It is mainly used to reduce high blood pressure, mental health, respiratory problems, arthritis and rheumatism (Saidan et al., 2004; Silalahi, 2002). It

is also used to prevents kidney stones. In addition, lemon fruit and vegetable are used to wash for oral health to freshen your breath and to treat flaky dandruff, headaches and reduce asthma symptoms (Saidan et al., 2004; Silalahi, 2002; Reichling et al., 2009). Lemon crop is one of the famous crops in the Sultanate of Oman. Especially it is completely different from other varieties of lemon. The Omani lemon variety contains large content of acid and juice. The most famous varieties are available in Batinah region, Sohar and Saham mandate etc (Saidan et al., 2004; Silalahi, 2002). The chemical composition of lemon juice apart from water is contains certain acidic substances called citric acid and carboxylic acid (Saidan et al., 2004; Silalahi, 2002). The literature search reveals that still no works has been done on essential oil from lemon imported from other countries. Keeping in mind, the aim of this present work was to isolate and identify the chemical constituents of essential oils from imported Turkish and Indian lemon fruits by GC-MS and determine their antimicrobial activity against pathogenic bacterial strains.

2. Materials and methods

2.1. Materials

Dimethyl sulphoxide (DMSO) and dichloromethane (DCM) were used in this present study from Fisher Scientific Company, UK. Sodium sulfate was purchase from Scharlau, European Union. Filter paper were used as disc from Whatmann (GE Healthcare Company, China). The bacterial strains such as Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) and Proteus vulgaris (P. vulgaris) were obtained from Nizwa Hospital, Nizwa, Sultanate of Oman. Clevenger apparatus was used for extraction of essential oil from lemon.

2.2. Fruit samples collection

Lemon fruits samples such as Turkish and Indian were collected from local Al Seeb Supermarket, Muscat, Sultanate of Oman. The fruits were collected from the Supermarket on February 19, 2014 at 9.00 am. Morphological identification of the fruits samples was carried out by a Botanist. After collection, the fruits samples were packed instantly in separate polyethylene bags. Then the samples were transported to the Natural Products Laboratory, Nizwa University for further processing.

2.3. Preparation of samples

Both the lemon fruits samples were washed carefully with water to remove dust and foreign materials. Then the healthy fruits samples were separated from the affected fruits. The Turkish and Indian lemon fruits samples were ready for use to isolate of essential oil by using Clevenger type apparatus.

2.4. Extraction of essential oil by Clevenger apparatus

The clean lemon fruits samples were cut into small pieces by the knife and put into the round bottom flask. Then water

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