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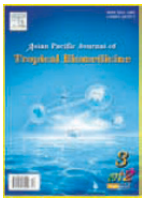

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Constituents of the essential oil from different brands of *Syzigium caryophyllatum* L by gas chromatography-mass spectrometry

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ABSTRACT

Objective: The aim of this present study was to isolate and analyze the chemical composition of essential oils from two different imported brands of *Syzigium caryophyllatum* (clove) samples using gas chromatography-mass spectrometry (GC-MS). **Methods:** The two essential oils were isolated by hydrodistillation from two different brands of *Syzigium caryophyllatum* (clove) such as Guzal and Shahi clove samples using Clevenger type apparatus. **Results:** Eleven chemical components were identified in the essential oil isolated from *Guzal clove* imported from Indonesia. The isolated components representing 99.03% of the Guzal clove oil were identified as eugenol (51.51%), caryophyllene (36.20%), α- caryophyllene (4.26%), acetyleugenol (2.64%), carvacrol (2.42%), α-cubebene (0.77%) and thymol (0.42%) were the major components with some other minor components isolated from the same. About twenty two components representing 99.73% were identified within the essential oil isolated from the Shahi brand clove which was imported from India with the main components being eugenol (46.53%), caryophyllene (43.03%), α-caryophyllene (4.61%), aceteugenol (2.54%), copaene (0.80%), α-farnesene (0.72%), germacrene (0.43%) and δ-cadinene (0.27%). **Conclusions:** Both the isolated essential oils were found to be rich in eugenol and caryophyllene. The clove essential oil from Guzal and Shahi was found to be comparable in terms of its eugenol and caryophyllene contents. According to the above findings, it is suggested that both brands of clove are of similar quality.

1. Introduction

Clove is one of the most important herbs in traditional medicine and its scientific name is *Syzygium aromaticum*. It is locally know as Kronfol. This plant is belongs to the genus *Syzygium*, family Syzygieae, and subfamily Myrtoideae of the family Myrtaceae. The cloves are classified according to Myrtales, which belongs to the superorder Rosids, under Eudicots of Dicotyledonae. Clove is an aromatic plant and belongs to division of Magnoliophyta in the kingdom Plantae [1]. Normally the morphology of clove tree is an evergreen that grows to a height ranging from 8-12 m, having large leaves and sanguine flowers in numerous groups of terminal clusters. In the beginning buds are lighter in colour and gradually change to green, after which they mature into a bright at which time they are ready for harvesting. Cloves samples are harvested when 1.5-2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals which form a small ball in the center.

Syzygium caryophyllatum L is commonly referred to as clove globally. It is an important aromatic spice. Since ancient times, clove has been cultivated in India, Madagascar, Sri Lanka, Indonesia and the south of China. Nowadays cloves is commercially cultivated and exported worldwide. Recently some other countries like Bangladesh, Burma, Thailand and Malaysia cultivate in a small scale. More recently middle east countries are trying to cultivate clove. Clove oil is very important and widely used in food items for flavouring pastry, special sauces and condiments. It is also used to prepare medicines, especially in the treatment for gum and teeth.

Oil of colve is comprised of various classes and groups of chemical compounds such as mono terpenes, sesquiterpenes, phenolics hydrocarbons compounds. Their derivatives result in biological benefits such as

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antibacterial, antifungal, insecticidal and antioxidant capacities. Traditionally clove oil is widely used all over the world to flavour food as an antimicrobial agent[2,3]. The increased concentration of eugenol and caryophyllene found in essential oil isolated from clove samples may be credited for strong antimicrobial and antifungal functions.

These phenolic compounds and other derivatives in clove essential oil can denature proteins and interact with cell membrane phospholipids changing their permeability[4]. Various active chemical constituents present in clove essential oil also have several therapeutic benefits, including anti-phlogistic, anti-vomiting, analgesic, antispasmodic, anti-carminative, kidney reinforcement, antiseptic and extracorporeal restraining effect[1].

In Korea, China and European therapy clove oil is mainly used in aromatherapy absorbed through the skin into the systemic circulation and is successfully used for asthma, arthritis, muscular disorders and various allergic disorders by oral administration[2]. Clove oil is also used in aromatherapy when stimulation and warming are needed, especially for digestive problems. Another popular benefit of clove essential oil is as a perfume, food flavour enhancer[5], and as a general antiseptic in medical dental practices[6]. Lee and Shibamoto[7] reported that oil of clove could be utilized as an anti-carcinogenic agent due to its antioxidant antimicrobial and antifungal capacities.

Results from different sources also suggest the essential oil from clove samples might be of use as a potential chemopreventative agent. Recently, lot of other high molecular weight compounds such as flavonoid triglycosides, terpenoids etc. have been isolated from clove[8]. The determination of antioxidant, antifungal and antimicrobial capacities of the raw and processed material allows the evaluation of its suitability as high quality food beneficial for human health. Therefore clove samples are of considerable importance for humans. The major chemical constituents in bud and leaf oils were reported to be eugenol and α-caryophyllene[8]. Kamel *et al*.[9] reported that the major chemical components f essential oil derived from clove flower buds are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde. Amla *et al*.[1] also reported that the major oil contained primarily eugenol, eugenyl acetate and α-caryophyllene. Therefore the aim of this work was to isolate the essential oil from two brands of clove samples and compare their chemical constituents obtained from two different countries. There are no previous references in literature about such kind of comparison results on clove essential oils.

2. Materials and methods

2.1. Plant Sample

Two brands imported clove samples such as Shahi and Guzal brand samples were collected as a sealed polyethylene packet from Lu Lu Supermarket, Nizwa, Sultanate of Oman. The two brand cloves samples were imported from outside of Oman. Shahi brand clove samples were imported from India and Guzal brand samples was imported from Indonesia. Both brand clove samples were collected on 8th March, 2012 and collected in the morning session (during 10.00 am to 12.00 pm). The samples will be transported to the laboratory and keep at room temperature for processing.

2.2. Preparation of samples

Approximately about 100 g of each cloves sample separately were ground separately by using a grinder for 20 s. The small pieces of the samples will be homogenised in a grinder for 3 min to 40-mesh size. The air-dried cloves samples of was pulverized into powdered form.

2.3. Isolation of the essential oils

The air-dried powder clove material (50 g) was subjected to hydrodistillation individually for 3 h using a Clevenger type apparatus. The essential oil part was carefully collected in a separated sealed container to avoid evaporation. The isolated essential oils were again reextracted with organic solvent dichloromethane. Finally, both the essential oils were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4℃ until further analysis.

2.4. GC-MS analysis

The GC-MS analysis of both essential oil isolated separately from two brands of imported clove samples were performed using a Perkin Elmer Clarus 600 GC system equipped with a Rtx® -5MS fused silica capillary column (30 m x 0.25 i.d., film thickness 0.25 μm) coupled with a Perkin Elmer Clarus 600C MS. Rtx® -5MS fused silica capillary column for gas chromatography-mass spectroscopic detection. An electron ionization system with ionization energy of 70 eV was used. 100% pure helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Mass transfer line and injector temperatures were set at 220 and 290 ℃, respectively. The oven temperature was programmed from 60 ℃ (hold 2 min) to 270 ℃ at 4 ℃/min, then held isothermal for 20 min and finally raised to 290 ℃ at 10 ℃/min. Diluted samples (1/100, v/v, in methanol) of 1 μL were injected in the split mode with a split ratio of 200:1. The relative percentage of the crude essential oil constituents was expressed as percentage by peak area normalization.

2.5. Identification of the compounds

The chemical compounds inside the essential oils were identified based on GC retention time on Rtx® -5MS fused silica capillary column, computer matching of mass spectra with those of standards (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems) and, whenever possible, by co-injection with authentic compounds [10-11].

3. Results

3.1. Chemical composition of essential oil