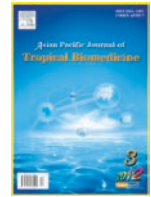




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Constituents of the essential oil from different brands of *Syzygium caryophyllatum* L by gas chromatography–mass spectrometry

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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 *Syzygium caryophyllatum* (clove) samples using gas chromatography–mass spectrometry (GC–MS). **Methods:** The two essential oils were isolated by hydrodistillation from two different brands of *Syzygium 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%), acetyleugenol (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 known as Kronfol. This plant belongs to the genus *Syzygium*, family Syzygiaceae, 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 are 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 clove 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 of 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°C 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 μ m) 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 °C, respectively. The oven temperature was programmed from 60 °C (hold 2 min) to 270 °C at 4 °C/min, then held isothermal for 20 min and finally raised to 290 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 μ L 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

The GC–MS total ion chromatogram (TIC) of each isolated essential oil from the Guzal and Shahi brands is shown in Figure 1 and Figure 2. The chemical constituents of both essential oils were identified by comparing their retention times, mass fragmentation patterns with those data of the available reference samples and GC–MS spectral database. The percent composition of components in each essential oil was determined by computerized peak area measurements using an internal normalization method. The two brands (Guzal and Shahi) clove samples yielded 1.2% and 0.79% v/w essential oils, respectively. Qualitative and quantitative differences in the components of the two essential oils

are compiled in Table 1. The essential oils were isolated from the two different brands of clove samples separately by hydrodistillation using Clevenger apparatus and the oil samples were analysed by using GC–MS leading to the characterization of 11 and 22 different organic compounds, representing 99.03% and 99.73% of the total oils from two brands of clove samples, respectively. The identified chemical compounds inside the essential oils are listed in Table 1 according to their elution order on Rtx® –5MS fused silica capillary column. Both essential oil contain a mixture of different chemical constituents consisting of mainly oxygenated mono and sesquiterpene hydrocarbons and their derivatives. The major organic chemical compounds isolated and identified in Guzal clove oil were eugenol (51.51%), caryophyllene (36.20%), α – caryophyllene (4.26%), acetyleugenol (2.64%), carvacrol (2.42%), α – cubebene (0.77%)

Table 1

Percentage composition of the volatile Guzal and Shahi clove oils–.

Compound Name	M.W.	Molecular Formula	Retention time (min.)	Guzal oil (%)	Shahi oil (%)
2–Heptanol	116	C ₇ H ₁₆ O	4.729	–	0.01824
Hexanoic acid, Ethyl ester	144	C ₈ H ₁₆ O ₂	7.34	–	0.02292
2–Heptanol, acetate	158	C ₉ H ₁₈ O ₂	8.741	–	0.15359
Cis– β –Ocimene	136	C ₁₀ H ₁₆	8.986	–	0.05334
2–Nonanone	142	C ₉ H ₁₈ O	10.562	–	0.11752
Benzoic acid, Methyl ester	136	C ₈ H ₈ O ₂	10.717	–	0.03687
2–Ethenyl–1,1–dimethyl–3–methylcyclohexane,	150	C ₁₁ H ₁₈	11.512	–	0.04108
Acetic acid, Phenylmethyl ester	150	C ₉ H ₁₀ O ₂	13.423	–	0.04046
Benzoic acid, Ethyl ester	150	C ₉ H ₁₀ O ₂	13.683	–	0.03695
Gaultheriaol	152	C ₈ H ₈ O ₃	14.668	–	0.21227
2–Acetoxydodecane	228	C ₁₄ H ₂₈ O ₂	16.394	–	0.0112
Chavicol	134	C ₉ H ₁₀ O	17.129	0.45546	0.05436
2–Undecanone	170	C ₁₁ H ₂₂ O	18.75	–	0.02534
Eugenol	164	C ₁₀ H ₁₂ O ₂	21.671	51.5156	46.53
Copaene	204	C ₁₅ H ₂₄	22.187	–	0.80475
Caryophyllene	204	C ₁₅ H ₂₄	24.057	36.2089	43.0359
α –Caryophyllene	204	C ₁₅ H ₂₄	25.288	4.26567	4.6159
Germacrene D	204	C ₁₅ H ₂₄	26.353	–	0.43876
α –Farnesene	204	C ₁₅ H ₂₄	27.399	–	0.72349
δ –Cadinene	204	C ₁₅ H ₂₄	28.004	0.22505	0.27148
Aceteugenol	206	C ₁₂ H ₁₄ O ₃	28.184	2.64891	2.54059
Caryophyllene Oxide	220	C ₁₅ H ₂₄ O	30.235	0.20731	0.13
Carvacrol	150	C ₁₀ H ₁₄ O	19.06	2.41017	–
Thymol	150	C ₁₀ H ₁₄ O	18.65	0.42274	–
α –Cubebene	204	C ₁₅ H ₂₄	22.117	0.76575	–
α –Farnesene	204	C ₁₅ H ₂₄	27.334	0.34262	–

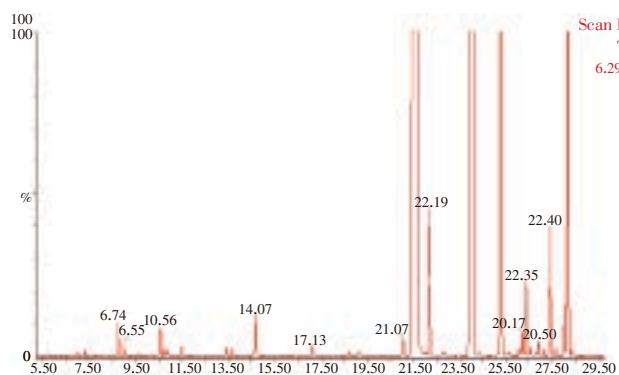


Figure 1. A typical chromatogram of the constituents of essential oil from the Shahi brand clove.

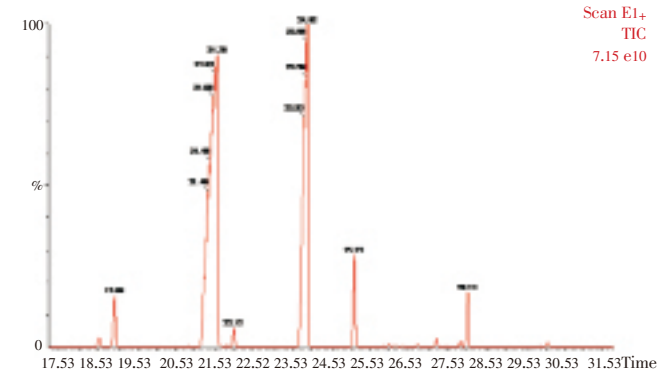


Figure 2. A typical chromatogram of the constituents of essential oil from the Guzal brand clove.

and thymol (0.42%). The other minor chemical constituents also isolated from the same were chavicol, caryophyllene oxide, (-)- β -cadinene, α -farnesene and α -cubebene. Whereas the major organic chemical compounds isolated and identified in the Shahi clove oil were eugenol (46.53%), caryophyllene (43.0%), α -caryophyllene (4.61%), acetugenol (2.54%), copaene (0.80%), α -farnesene (0.72%), germacrene (0.43%) and δ -cadinene (0.27%) along with 2-heptanol, hexanoic acid ethyl ester, 2-heptanol acetate, cis- β -ocimene, 2-nonanone, benzoic acid methyl ester, 2-ethenyl-1,1-dimethyl-3-methylcyclohexane, acetic acid phenylmethyl ester, gaultheriaol, 2-acetyldodecane and chavicol. Some minor chemical constituents were also obtained from the Shahi clove oil.

4. Discussion

The major chemical components in both the clove oils are eugenol and caryophyllene. The other seven organic chemical constituents such as chavicol, eugenol, caryophyllene, α -caryophyllene, δ -cadinene, acetugenol, caryophyllene oxide were obtained and identified as common in both the essential oils with a difference in percent age content (Table 1). The rest other volatile constituents were not found in the Guzal oil imported from Indonesia in this study (Table 1). The Sultanate of Oman is very hot country and low molecular weight constituents in the Guzal oil evaporated during the sample processing and isolation or the samples may have been imported long time ago. This present study reveals that eugenol and caryophyllene are as major components and the composition of other chemical constituents in both essential oils differ from earlier reports [3–7] and may, therefore be treated as different chemotypes. On the basis of the above chemical ingredients listed in both essential oils, it may be concluded that clove can be cultivate any place in the world or may be utilized as a major source for the isolation of natural eugenol.

The very high concentration of eugenol and caryophyllene in both brands of essential oil makes it potentially useful for the preparation of both herbal and modern medicines because they exhibit antibacterial, antifungal, anti-inflammatory capacities, insecticidal and antioxidant potential, and are also used traditionally as a flavouring agent and antimicrobial material in food industry[5–9]. It is also used in traditional medicine for the therapeutic treatment, including antiphlogistic, antivomiting, analgesic, antispasmodic, anticarminative, kidney reinforcement, antiseptic, diuretic, odontalgic, stomachic, tonicardiac. It is known for its aromatic condiment value as well as for its carminative and simulative activity.

Essential oils were isolated from the two brands of imported clove samples by hydrodistillation method, and their chemical organic volatile constituents were determined and compared by GC–MS. The results indicated that both the essential oils mainly contain a very high concentration of eugenol and caryophyllene.

Conflict of interest statement

We declare that we have no conflict of interest.

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