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Full Length Research Paper

Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston)

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Essential oil obtained by hydrodistillation from fresh leaves and dry buds of *Syzigium caryophyllatum* were analyzed by gas chromatography mass spectrometer (GC-MS). Thirty eight components were identified in the leaf oil. The main components were eugenol (74.3%), eucalyptol (5.8%), caryophyllene (3.85%) and α-cadinol (2.43%). Thirty one components were identified in bud oil with the main components being eugenol (49.7%), caryophyllene (18.9%), benzene,1-ethyl-3-nitro (11.1%) and benzoic acid,3-(1-methylethyl) (8.9%). The clove oil from Bangladesh was found to be comparable in terms of its eugenol content. It is suggested that clove can be grown as an economically viable crop in Bangladesh.

Key words: *Syzygium caryophyllatum*, bud and leaf oils, essential oil composition, gas chromatography mass spectrometer (GC-MS), eugenol.

INTRODUCTION

Syzygium caryophyllatum (L.) Alston, (syn. *Syzygium aromaticum* (L.) Merr. & Perry.) commonly called clove belongs to the family Myrtaceae and is an important aromatic spice. Clove is commercially cultivated in India, Madagascar, Sri Lanka, Indonesia and the south of China. Now a day it is cultivated in Bangladesh at a small scale. Clove oil is widely used for flavouring pastry, special sauces and condiments. It is also used in medicines, especially in the preparations for gum and teeth. The tinctures, extracts and oleoresins are also used (Atal and Kapur, 1982). Clove bud oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food (Lee and Shibamoto, 2001; Huang et al., 2002; Velluti et al., 2003). The high levels of eugenol contained in clove essential oil are responsible for strong antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids, changing their permeability (Briozzo, 1989; Deans and Ritchie, 1987). Clove oil also has several therapeutic effects, including antiphlogistic, antivomiting, analgesic, antispasmodic, anticarminative, kidney reinforcement, antiseptic, uman cytomegalovirus (HCMV) extracorporeal restraining effect (Liu et al., 1987). In Korea, clove oil is used in aromatherapy for asthma and various allergic disorders through oral administration (Kim et al., 1998). Clove oil is also widely used as a perfume and food flavor (Cai and Wu, 1996), and as a general antiseptic in medical dental practices (Baytop, 1999). Importantly, Lee and Shibamoto (2001) reported that clove oil might also be used as an anti-carcinogenic agent due to its antioxidant properties. Their results suggested that clove oil might be of use as a potential chemopreventative agent. Clove oil is used in the traditional blend of choji (1% clove oil in mineral oil) and is applied to Japanese sword blades to prevent tarnishing the polished surface (Cai and Wu, 1996; Baytop, 1999). *Syzygium* species have been reported to possess antibacterial (Shafi et al., 2002) and anti-inflammatory activity (Muruganadan et al., 2001). Boulos (1983) reported the use of buds of clove in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment and stimulant. Several compounds from *S. aromaticum* (namely 5, 7-dihydroxy-2-methylchromone-8-C-β- D-glucopyranoside, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid) have been found to possess growth inhibitory activity against oral pathogens (Cai and Wu, 1996). Also, an orsellinic acid glucoside has been isolated from *S. aromaticum* (Charles et al., 1998).

Table 1. Chemical constituents of the essential oil from clove leaves and buds.

Peak no.	Name of constituents	Quantity (%)	
		Leaf oil	Bud oil
1	α-Pinene	0.33	-
2	β-Phellandrene	0.12	-
3	β-Pinene	0.45	-
4	α-Phellandrene	0.09	-
5	α-Terpinene	0.31	-
6	m-Cymene	0.16	-
7	Limonene	2.08	0.05
8	Eucalyptol	5.78	0.06
9	γ-Terpinene	0.17	-
10	Linalool	0.14	-
11	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	0.04	-
12	2-Heptanol acetate	-	0.05
13	4-Terpineol	0.45	-
14	Methyl Salicylate	0.20	0.25
15	Terpinyl acetate	0.59	-
16	Chavicol	0.08	0.30
17	Eugenol	74.28	49.71
18	Benzyl acetate	-	0.07
19	4-Terpineol	0.45	-
20	Caryophyllene	3.85	18.94
21	Copaene	0.17	0.39
22	α-Caryophyllene	1.52	-
23	Alloaromadendrene	0.05	0.30
24	α-Cubebene	0.02	-
25	Germacrene D	0.38	0.08
26	α-Guaiene	0.06	0.02
27	γ-Elemene	0.21	-
28	β-Bisabolene	0.06	-
29	Benzoic acid, 3-(1-methylethyl)	-	8.95
30	δ-Cadinene	0.21	0.17
31	Benzene, 1-ethyl-3-nitro	-	11.12
32	Guaiene	0.09	-
33	Caryophyllene oxide	0.78	1.53
34	Globulol	0.38	-
35	Ledol	0.16	-
36	Humulane-1,6-dien-3-ol	0.51	-
37	Cedr-9-ene	0.16	-
38	Cubenol	0.19	-
39	Elixene	-	3.87
40	α-Cadinol	2.43	-
41	Megastigma-4,6(E),8(Z)-triene	-	0.05
42	Juniper camphor	0.17	-
43	Kauran-18-al, 17-(acetyloxy)-	0.13	-
44	Alloaromadendrene oxide-(1)	0.11	-
45	α-Amorphene	-	0.05
46	Germacrene D	0.38	0.08
47	(+)- Cycloisosativen	-	0.16
48	Nerolidyl acetate	0.06	
49	α-Farnesene	-	1.11

Table 1. Contd.

50	δ-Cadinene	-	0.05
51	Naphthalene,1,2,2,4,4a,7-hexahydro-1,6-dimethyl-4(1-methyethyl	-	0.19
52	Cyclohexane,1,2-dimethyl-3,5-bis(1-methylethenyl)-	-	0.22
53	9β-Acetoxy-3,5α,8-trimethyltricyclo[6.3.1.0(1,5)]dodec-3-ene	-	0.06
54	Cycloheptane,4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl	-	0.08
55	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-,	-	0.11
56	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol,4,4-dimethyl	-	0.67
57	2',3',4, Trimethoxyacetophenone	-	0.10
58	Benzyl benzoate	-	0.12
59	Squalene	-	0.69

Recently, flavonoide triglycosides have been isolated (Nassar, 2006). The evaluation of antioxidant properties of the raw material allows the determination of its suitability as high quality food beneficial for human health and therefore is of considerable importance. The major constituents in bud and leaf oils were reported to be eugenol and β-caryophyllene (Srivastava et al., 2003; Raina et al., 2001; Wenqiang et al., 2007). Kamel et al. (2007) observed that the main constituents of flower buds of clove essential oil are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde. Amla et al. (2007) reported that the bud oil contained primarily eugenol, eugenyl acetate and β-caryophyllene. This paper reports the results of chemical constituents on clove leaf and bud oils obtained from trees grown in Bangladesh.

MATERIALS AND METHODS

Plant material

The plant materials of clove were collected from the plants grown in the campus of BCSIR (Bangladesh Council of Scientific and Industrial Research) Laboratory, Chittagong in June 2009. The specimen was identified by Dr. M. Yusuf (Taxonomist). One-voucher specimen (N-99) was deposited in the herbarium of BCSIR Laboratory, Chittagong.

Extraction of essential oil

Leaves and buds were harvested from healthy, well-grown plants. The leaves weighing 400 g and dried buds weighing 300 g were ground in a blender seperately. The ground leaves and buds were subjected to hydrodistillation using Clevenger apparatus for 4 h in order to isolate oils (Clevenger**,** 1928). The oil samples were dried over anhydrous sodium sulfate, filtered and stored at 0℃ in air-tight containers. Analysis was done using gas chromatography mass spectrometry (GC-MS).

GC-MS analysis

The essential oils were analyzed by GC-MS electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu) coupled to a GC-MS QP 5050A Mass Spectrometer (Shimadzu); fused silica capillary column (30 m × 0.25 mm; 0.25 µm film thickness), coated with DB-5 ms (J&W); column temperature 100℃ (2 min) to 250℃ at the rate of 3℃/min; carrier gas, helium at constant pressure of 90 Kpa. Acquisition parameters full scan; scan range 40 to 350 amu. Samples were injected by splitting and the split ratio is 1:20.

Identification of the compounds

Compound identification was done by comparing the National Institute of Standards and Technology (NIST) library data of the peaks with those reported in literature, mass spectra of the peaks with literature data. Percentage composition was computed from GC peak areas on BD-5 ms column without applying correction factors.

RESULTS

The compounds were identified by hydro distillation of the leaves and buds of *S. caryophyllatum* yielded clear essential oils. Table 1 shows the relative percentages as single components of oils. There were 38 (96.3%) and 31 (98.5%) compounds characterized from the leaf and bud, respectively. The leaf oil contained eugenol, eucalyptol, aryophyllene, α-cadinol, limonene and α-caryophyllene. Oil from buds contained eugenol, caryophyllene, benzene, 1-ethyl-3-nitro, benzoic acid, 3-(1-methylethyl), elixene, caryophyllene oxide and α-farnesene. The oils were complex mixture of numerous compounds; many of which were present in trace amounts. There was slight variation in the chemical composition of leaf and bud oils. Eugenol was the main component found in both oils. Eugenol, caryophyllene, limonene, eucalyptol, methyl salicylate, chavicol, copaene, alloaromadendrene, germacrene D, α-guaiene and δ-cadinene were observed as the eleven versatile common components present in both the oils with variations in percent content.

DISCUSSION

This paper presents the qualitative and quantitative composition of *S. caryophyllatum* leaves and buds

essential oil isolated by hydrodistillation and of the volatile compounds of *S. caryophyllatum* leaves and buds, respectively. Our findings indicated that both of the essential oils mainly contain eugenol. The chemical constituents of the Bangladeshi clove leaf and bud oil were found to be comparable to those from clove trees naturally grown in its native regions. Recent studies have indicated that eugenol is the main constituent of the essential oils from leaves, and buds of *S. caryophyllatum*. Our study provides useful information for commercial cultivation and potential medicinal uses of *S. caryophyllatum* in Bangladesh.

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