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## STUDY OF CHEMICAL COMPOSITION AND MEDICINAL PROPERTIES OF VOLATILE OIL FROM CLOVE BUDS (*EUGENIA CARYOPHYLLUS*)

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**ABSTRACT:** The aim of this study was to analyze the chemical composition of dried clove buds volatile oil in Bangladesh by Gas Chromatography-Mass Spectrometry (GC-MS) technique and also show their medicinal properties according to the characteristics of the constituents present in that essential oil. In this experiment the clove buds essential oil was extracted by hydro distillation method. The major constituents obtained from the oil includes 3-Allyl-6-methoxyphenol i.e. m-Eugenol (69.44%), Eugenol acetate (10.79%), 4-hydroxy-4-mehtylpentan-2-one i.e. Tyranton (7.78%) Caryophyllene (6.80%), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-and trace amounts (<1%) of other constituents. Eugenol is present in concentration of 69.43% which is the major volatile constituent of clove bud essential oil. It has wide range of medicinal value such as antiseptic and anaesthetic analgesic, antioxidant, anti-inflammatory and antimicrobial activities. Clove oil is used in dental care and it can relieve toothache temporarily. So, from these chemical constituents it is clear that clove buds essential oil of Bangladesh has medicinal properties because of the medicinal characteristics of these constituents.

**INTRODUCTION:** Cloves are the aromatic flower buds of a tree in the family Myrtaceae, *Eugenia caryophyllus*. They are native to the Maluku Islands in Indonesia, and it is mainly used as a spice. Cloves are commercially harvested primarily in India, Pakistan, Indonesia, Madagascar, Zanzibar, Sri Lanka and Tanzania. But, Indonesia and Madagascar are the main clove buds oil producer <sup>1</sup>.

There are three types of clove oil; bud oil, leaf oil and stem oil. Bud oil is derived from the flower-buds of *Eugenia caryophyllus*. It contains mainly of 60-90% eugenol, eugenyl acetate, caryophyllene and other minor constituents. Leaf oil is derived from the leaves of *Eugenia caryophyllus*. It consists of 82-88% eugenol, little amount of eugenyl acetate, and other minor constituents. Stem oils are evolved from the twigs of *Eugenia caryophyllus*.

It consists of 90-95% eugenol, and some other minor constituents. A major component of clove taste is imparted by the chemical eugenol. Eugenol is the component which is most responsible for clove aroma and comprises 72-90% of the essential oil extracted from cloves <sup>2</sup>.

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Other important essential oil constituents of clove oil are acetyl eugenol, beta-caryophyllene and vanillin, cratogeomycetic acid, tannins such as bicornin<sup>3-4</sup>, methyl salicylate (painkiller), gallic acid, the flavonoid eugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids such as oleanolic acid, campesterol and stigmasterol, and several sesquiterpenes<sup>5-8</sup>.

It is now believed to the FDA that there are not enough evidence indicates clove oil or eugenol is effective for toothache pain or other types of pain<sup>9</sup>. Studies to determine its effectiveness for fever reduction, as a mosquito repellent, and to prevent premature ejaculation have been inconclusive<sup>9-10</sup>. Clove may reduce blood sugar levels but it is not proven yet and studies going on to determine this. Furthermore, clove oil is used in preparation of some Clovacaïne solution, and toothpastes which are the local anesthetic used in oral ulceration and inflammation. Eugenol and zinc oxide are mixed together to form a temporary tooth cavity filling. Clove oil can be used to anesthetize of fish. It also can be considered as a humane means of euthanasia with higher doses and the recommended dose is 400 mg/l<sup>11</sup>. Eugenol is a colorless or pale yellow oily liquid which are extracted from different

essential oils especially from clove oil, nutmeg, basil, cinnamon and bay leaf. In clove bud oil it is present in concentrations of about 80-90% where clove leaf contain 82-88% of eugenol. Eugenol is used as the perfumeries, flavorings, essential oils and also in the medicine as a local antiseptic and anaesthetic<sup>12</sup>.

Eugenol can be produce zinc oxide eugenol by mixing with zinc oxide which is used in dentistry as the restorative and prosthodontics application. For root canal sealing, zinc oxide eugenol is used. It is also used in some mousetraps and kills particular human colon cancer cell lines *in vivo* and *in vitro*<sup>13</sup>. Eugenol may have potential therapeutic effects against diseases characterized by excessive osteoclast activity<sup>14</sup>.

#### MATERIALS AND METHODS:

**Plant materials:** Healthy and mature buds of clove (*Eugenia caryophyllus*) were purchased from local market of Kushtia, Bangladesh. The cleaned and dust free samples were dried in air and at 30°C in a ventilated drying oven and stored in plastic bags at ambient temperature. Then the samples were ground by blender machine into a finely grounded form (**Fig. 1**). Exposure to direct sunlight was avoided to prevent the loss of active components.



FIG. 1: A. DRIED CLOVE BUD, B. GROUNDED CLOVE BUDS POWDER

**Essential oil isolation:** The dried and ground clove buds (200 g) were taken in a 2-litre round bottom

flask. Then distilled water was added according to the ratio 2:1 (400 ml distilled water for 200 g

ground clove buds) and subjected to hydro distillation for 3 h using a modified Clevenger type glass apparatus. The volatile distillate was collected until no oil drop out. By using anhydrous  $\text{Na}_2\text{SO}_4$  the oil was dried and stored in an air tight container at 2 °C before GC- MS analysis.

**Gas Chromatography-Mass Spectroscopy (GC-MS) analysis:** Essential oil constituents from clove buds were analyzed by GC-MS. The GC-MS was carried out using total ion monitoring mode on a Varian 3800 gas chromatograph interfaced to a Varian Saturn ion trap 2200 GC-MS spectrometer. The temperatures of transfer line and ion source were 280°C and 275°C respectively. Ions were obtained by electron ionization mode. The VF-5 capillary column (30 m length, 0.25 mm I.D. and 0.25  $\mu\text{m}$  film thickness) was used. A 20% split injection mode was selected with a solvent delay time of 3 min with injection volume 0.2  $\mu\text{l}$ .

The initial column temperature was started at 50°C for 1 min, programmed at 8°C/min to 200°C and heated until 280°C at 10°C/min. Injection port was set at 250°C. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. Molecular ions

(mass range: 40-500 m/z) were monitored for identification. The relative percentage of the oil constituents was expressed as percentage by peak area normalization. Identification of components of the essential oil was based on their retention indices, relative to a homologous series of *n*-alkane ( $\text{C}_8 - \text{C}_{20}$ ) on the VF-5 capillary column under the same operating conditions and computer matching with the GC-MS spectra from the Wiley 6.0 MS data and literature data<sup>15-16</sup>.

## RESULTS AND DISCUSSION:

**Chemical composition of the essential oil:** The GC-MS analysis of clove buds (*Eugenia caryophyllus*) essential oil led to identification of 13 different compounds (**Fig. 2**). The major compounds are 3-Allyl-6-methoxyphenol; Eugenol acetate; 2-Pentanone, 4-hydroxy-4-methyl and Caryophyllene which given in **Table 1**. The minor compounds are 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-; Toluene; 2-Pentanone-3-methylene; Copaene; alpha-Farnesene; 2,4,4,6-Tetramethyl-4,5-dihydro-1,3-oxazine; m-Dioxan-4-ol, 2,6-diethyl-5methyl acetate and m-Dioxan-4-ol, 2,6-diethyl-5methyl acetate.

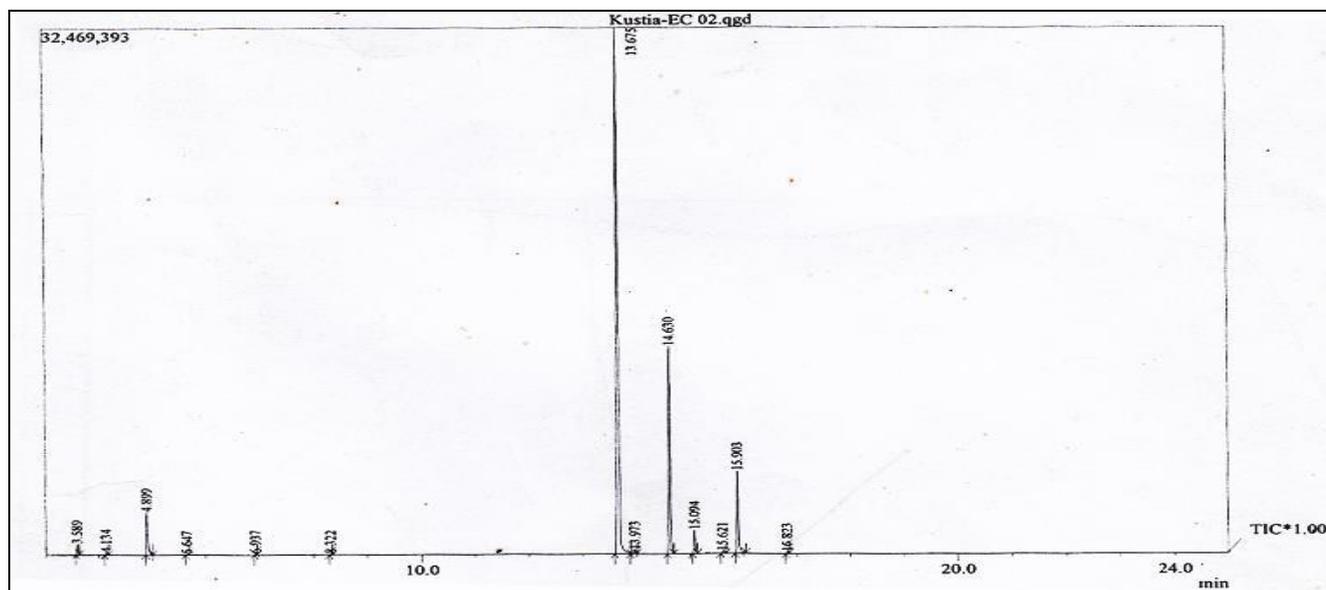


FIG. 2: GC-MS ANALYSIS OF CLOVE BUDS (*EUGENIA CARYOPHYLLUS*) ESSENTIAL OIL

TABLE 1. CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF BUDS FROM *EUGENIA CARYOPHYLLUS*

Peak no.	Retention time (min)	Conc. %	Name of the compound	Formula
1	3.589	1.873	Toluene	$\text{C}_7\text{H}_8$
2	4.134	0.286	2-Pentanone-3-methylene	$\text{C}_6\text{H}_{10}\text{O}$
3	4.899	7.785	2-Pentanone, 4-hydroxy-4-methyl	$\text{C}_6\text{H}_{12}\text{O}_2$
4	5.647	0.066	m-Dioxan-4-ol, 2,6-diethyl-5methyl acetate	$\text{C}_{11}\text{H}_{20}\text{O}_4$
5	6.937	0.15	2,4,4,6-Tetramethyl-4,5-dihydro-1,3-oxazine	$\text{C}_8\text{H}_{15}\text{NO}$
6	8.322	0.118	2,4,4,6-Tetramethyl-4,5-dihydro-1,3-oxazine	$\text{C}_8\text{H}_{15}\text{NO}$

7	13.675	69.436	3-Allyl-6-methoxyphenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
8	13.973	0.219	Copaene	C <sub>15</sub> H <sub>24</sub>
9	14.63	6.803	Caryophyllene	C <sub>15</sub> H <sub>24</sub>
10	15.094	2.253	1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-	C <sub>15</sub> H <sub>24</sub>
11	15.621	0.163	alpha-Farnesene	C <sub>15</sub> H <sub>24</sub>
12	15.903	10.798	Eugenol acetate	C <sub>12</sub> H <sub>14</sub> O <sub>3</sub>
13	16.823	0.048	Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O

### Determination of medicinal properties:

According to the chemical composition of the essential oil of *Eugenia caryophyllus* given in **Table 1**, it has been seen that thirteen major constituents are present in that sample. For analysis of medicinal values, the essential constituents are m-Eugenol *i.e.* 3-Allyl-6-methoxyphenol (69.43%), Eugenol acetate (10.78%), Caryophyllene (6.80%), 2-Pentanone (7.78%) etc. Eugenol is present in concentration of 69.43% which is the major volatile constituent of clove bud essential oil. It has wide range of medicinal value such as antiseptic and anaesthetic <sup>12</sup> analgesic, antioxidant, anti-inflammatory and antimicrobial activities. Clove oil is used in dental care. It can relieve toothache temporarily <sup>17</sup>. As an important ingredient, it is used in manufacturing some toothpastes, mouth wash and oral care medications. To relieve general pain and skin problem clove oil is used. Clove oil is also used in the treatment of fever, headache, cough, asthma, indigestion, depression etc.

**CONCLUSION:** The chemical composition of dried clove bud in Bangladesh was investigated. The essential oil composition was identified by Gas Chromatography- Mass Spectrometry (GC-MS) technique and also showed their medicinal properties according to the characteristics of the constituents present in the essential oil. In this experiment the clove buds essential oil was extracted by hydro distillation method. The major constituents obtained from the oil includes 3-Allyl-6-methoxyphenol *i.e.* m-Eugenol (69.44%) and Eugenol acetate (10.79%) which have wide range of medicinal values such as antiseptic and anaesthetic, analgesic, antioxidant, anti-inflammatory and antimicrobial activities.

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**CONFLICT OF INTEREST:** Authors have no conflict of interest.

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