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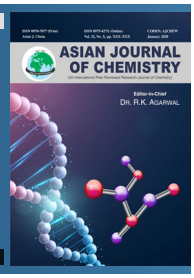

Chemical Composition Analysis and Evaluation of Antibacterial Activities of Essential Oil from Clove (*Syzygium aromaticum*) Buds

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Essential oils from *Syzygium aromaticum* (Myrtaceae) were extracted through hydrodistillation method, which applied in different fields such as relieve pain, promote healing, and aromatherapy industry. In this study, essential oil of clove buds was obtained using hydrodistillation method and evaluated for chemical compositions and physico-chemical characteristics. The averaged physico-chemical parameters were determined as specific gravity (1.0203 g/cm^3), acid index (21.984), ester index (34.92). Moreover, the hydrodistillation process performance achieved by 6.85%. The composition of essential oils was analyzed by GC-MS, revealing a total of 15 volatile constituents existing in the *Syzygium aromaticum* oil. The major components were eugenol (76.542%), caryophyllene (4.319%), α-caryophyllene (0.456%), acetegenol (18.11%), caryophyllene oxide (0.192%), 4,4-dimethyl tetracyclo[6.3.2.0.(1,8)]tridecan-9-ol (0.12%). *Syzygium aromaticum* essential oil in this study was found to inhibit five bacteria strains including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The highest antimicrobial activity of clove essential oil was found for *Bacillus cereus* strain (14 mm).

Keywords: Clove buds, *Syzygium aromaticum*, Chemical composition, Antibacterial activities.

INTRODUCTION

In recent years, natural herbs have been receiving a great deal of public attention due to their benefit in human activities. Essential oils could be obtained from different parts of plant material such as peels, seeds and leaves. Essential oils have received economic benefits primarily in applications to pharmaceuticals, cosmetics, food and antioxidant properties of natural origin, often at a lower risk of harm environmental and health hazards [1-4].

Clove (*Syzygium aromaticum*) is a plant in the Myrtaceae family, native to India, Indonesia, Zanzibar, Mauritius and Ceylon [5,6]. Cloves are evergreen trees that can be as high as 10-20 m, with large oval leaves and dark red flowers growing in clusters at the ends of branches. The flowers are harvested when they are about 1.5-2.0 cm long, including long calyx, stretched into four sepals and four non-blooming petals forming small round members in the center. Flower buds are picked by hand, the shoots are dried until they turn brown. The shoots are then left raw, ground into spices or steam distilled to produce clove oil. On the island, Zanzibar (part of Tanzania) is the world′s largest producer of cloves, in the world's leading producers including Indonesia and Madagascar [7]. The dried flower buds are then crushed to make spices, or steam distilled to produce clove essential oil. Clove essential oil is evenly dispersed in flower buds, although a tiny part also contains essential oils, usually it accounts for 14 to 20%. The main chemical component is eugenol, which is also responsible for the strong scent of cloves. Scientific research proves that clove′s ability is very effective in dental care as an analgesic and antiseptic [8,9].

Clove essential oil has oral antimicrobial activity related to tooth decay and periodontal disease [10] and is effective

against different bacteria strains: *Salmonella enteric*, *Escherichia coli* [11] and *Staphylococcus aureus* [12-14]. Previous studies illustrated antifungal agents [15], anticarcinogenic [16], antioxidant [17] and insecticidal [18,19]. The purpose of this study is to evaluate the physico-chemical properties and determine the chemical composition of clove essential oil. The analysis in this study is carried out through GC-MS. Moreover, the antibacterial effect of clove oil on Gram-positive and Gram-negative bacteria has also been studied.

EXPERIMENTAL

Plant material: In this study, the flower buds of cloves were selected from the Indian market in March 2019. Common parts used were flower buds, which harvested when starting to turn red, flower buds are dried under the sun or lightly dried.

Extraction of clove essential oil: First, the clove flower buds are harvested by hand. Next, the buds were pretreated and removed impurities. Then, clove buds were pureed and then put into the tank of the steam distillation device. The essential oil obtained by steam distillation system, with 1300 g of raw material extracted in 3 h [20]. The essential oil obtained after distillation is dehydrated and anhydrous with Na_2SO_4 to obtain the essential oil product. Essential oils are stored in dark bottles before analysis.

Physico-chemical analysis of extract: Some basic physical and chemical parameters of the raw materials have been identified including some criteria for finished oil products analyzed by TCVN: acid index (TCVN 8450: 2010), sensory index (TCVN 8460: 2010) and density of essential oils.

Density: The proportion of essential oils is the ratio of essential oils at 25 ºC with the mass of the oil. The same volume of distilled water was also at 25 ºC.

Acid index: The acid index is the number of milligrams of KOH needed to neutralize free acids in 1 g of fat.

Ester index: The soap index is the number of milligrams of KOH needed to neutralize all free acids and acid combined as esters in 1 g of fat.

Determination of constituents of essential oil by GC-MS: Chemical composition of the clove fruit oil was determined by GC-MS analysis using GC Agilent 6890 N instrument coupled with HP5-MS column and MS 5973 inert. The pressure of the head column was 9.3 psi. Essential oil (25 µL) was added with 1.0 mL *n*-hexane and dehydrated with Na_2SO_4. The flow rate of was constant at 1 mL/min. Injector temperature was 250 ºC and the rate of division was 30.

Antibacterial activity: Five bacterial species *viz. S. aureus*, *B. cereus*, *E. coli*, *S. enterica* and *P. aeruginosa* were used in this study. The concentrations was 1×10^8 CFU/mL (bacterial density was determined by optical density (OD) method at a wavelength of 625 nm. Antibacterial activity of the obtained essential oil was evaluated by the agar-well diffusion assays. First, the agar plates were prepared with 10 mL of LB solution. Following that, 3 mL of liquid cultures were incubated at 37 ºC with aeration (150 rpm) overnight on LB. On the surface of the LB agar, 5 mm wells were loaded with 20 µL of essential oils and then incubated at 37 ºC for overnight. In this case, amoxicillin (100 µg/mL) and sterile water act as the positive and negative control, respectively.

RESULTS AND DISCUSSION

Performance and physico-chemical properties of clove bud essential oil: In this study, essential oils of clove buds are pale yellow, obtained by steam distillation with a yield of 6.85%. However, in the report of Alitonou *et al*. [21] indicated that essential oil yield was 0.18% with raw material from fresh flower buds in Nigeria by hydrodistillation method. Besides, the same source of materials from dried flower buds in Egypt, El-Mesallamy *et al*. [22] conducted steam distillation with a yield of 9.5% as pale-yellow oil, which is much higher than the essential oil sample in this study. Another study by Lee *et al*. [23] also conducted distillation of clove essential oil from dried clove flower buds collected from Terengganu market, Malaysia. Table-1 shows the physico-chemical properties of clove bud essential oil as a volatile, transparent liquid, colored from light yellow to bright yellow, spicy, typical flavour and has a strong antiseptic effect. At room temperatures, the liquid has a density (1.0203) which is higher than 1, so clove oil is heavier than water. Clove essential oil is insoluble in water or slightly soluble but dissolves well in organic solvents.

TABLE-1
PHYSICO-CHEMICAL INDEXES OF CLOVE ESSENTIAL OIL

Physical parameters	Clove essential oil
Aspect	Liquid
Colour	Clear liquid, light yellow
Odour	Specific
Density	1.0203
Acid index	21.984
Ester index	34.92

There are differences in the method of extracting essential oils, which leads to the difference in the composition ratio in essential oils. The location where the tree develope can also contribute to the content and quality of the essential oil that has been confirmed. The effect of harvesting time on oil production and chemical composition is significantly crucial.

Chemical composition of clove bud essential oils: GC-MS is the best method due to its simplicity, quickness and efficiency for both the determination and quantification of essential oil components and component variations. More than 99% of the volatile compound in clove oil is determined by GC-MS method. Through the chromatogram results in Fig. 1, it was found that the time of occurrence of volatile compounds in essential oils mainly concentrated in the range of 27-35 min and there were seven different retention time values. Corresponding to this is in essential oil samples containing seven compounds. The components in the 27,454, 32,107, 29,074 peaks have a relatively large intensity, proving that these are high-density constituents. The remaining constituents have relatively low intensity, so the content of essential oils is negligible. As shown in Table-2, seven compounds representing about 99% volatile component content of essential oils from clove buds were identified. The chemical composition of clove bud essential oil is eugenol (76.542%), caryophyllene (4.319%),

TABLE-2
CHEMICAL COMPOSITION OF CLOVE ESSENTIAL OILS

Peak	Compound	This study	Bangladesh	Malaysia	Portugal	Togo
1	Eugenol	76.542	49.71	49	85.3	82.95
2	Eugenyl acetate	–	–	–	–	5.01
3	Caryophyllene	4.319	18.94	7.5	0.9	–
4	α-Caryophyllene	0.456	–	1.4	–	–
5	β-Caryophyllene	–	–	–	–	3.14
6	Aceteugenol	18.111	–	–	–	–
7	Unknown	0.259	–	–	–	–
8	Caryophyllene oxide	0.192	1.53	–	0.1	0.1
9	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	0.12	0.67	–	–	–
10	Benzoic acid, 3-(1-methylethyl)	–	8.95	–	–	–
11	Benzene, 1-ethyl-3-nitro	–	11.12	–	–	–
12	2-Propanone, methylhydrazone	–	–	5.6	–	–
13	Cyclopentane, methyl	–	–	4.0	–	–
14	Furan, tetrahydro-3-methyl	–	–	2.5	–	–
15	α-Humulene	–	–	–	6.8	–

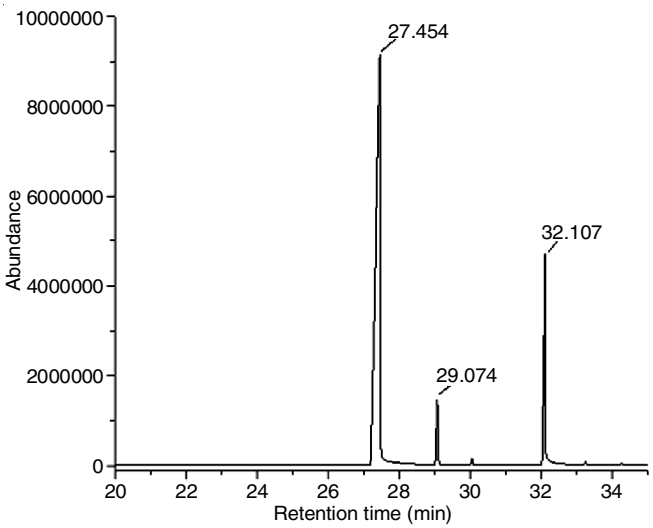

Fig. 1. Gas chromatogram of clove essential oil

α-caryophyllene (0.456%), aceteugenol (18.11%), caryophyllene oxide (0.192%), 4,4-dimethyl tetracyclo[6,3.0(1,8)]tridecan-9-ol (0.12%) and an unspecified component (0.259%). Table-2 also shows that aceteugenol is a notable ingredient and it occupies a significant amount (18.111%) in the evaporation component of clove oil, while this component is almost absent in the remaining areas. In contrast, eugenyl acetate (5.01%) is only available in the composition of clove bud oil in the area of Togo. The previous study found that eugenol was lower in the young bud stage (39.66%) and raised in the subsequent stages to reach the maximum during the entire fruiting period (94.89%). In contrast, eugenyl acetate was higher in young shoots (56.07%), then gradually decreased to a minimum during the full fruiting period (2.01%) [24].

In other studies (Table-2), the content of clove buds essential oil reported by Koba *et al.* [25] and Machado *et al.* [26] indicated that the concentration of eugenol (85.3% and 82.95%) correspond, and a higher concentration in this study. Similarly, compare with the results of clove buds essential oil analysis of Lee *et al.* [23] and Mohammad [27] showed a similarity in a percentage of the major components of eugenol (49.71% and 49%), respectively, and lower than the content in this study.

Eugenol isolated from *Syzygium aromaticum* is the main chemical component of clove oil and have relatively high in content [28]. It is a colorless or light yellow liquid and turns dark yellow when exposed to air. It smells strong phenolic and sharp acrid [29]. Proven pharmacological properties of eugenol include strong fungicides, bactericidal, anesthetic, analgesic, antioxidant, anticancer, antiallergy as well as match killing insects [30,31].

Antimicrobial activity of clove bud essential oil: The antimicrobial results in clove essential oil are shown in Table-3. The antimicrobial properties for bacterial strains expressed in ascending order include (*Staphylococcus aureus*, *Escherichia coli*) < (*Samonella typhimurium*, *Pseudomonas aerugin*) (*Bacillus subtilis*) corresponds to 10 mm < 12 mm < 14 mm. Moreover, the highest level of activity observed against five strains of bacteria is *Bacillus subtilis* with 14 mm inhibition. In addition, the antibacterial properties of clove essential oil are almost better than amoxicillin in *B. subtilis* and *E. coli* bacteria strains. This can be explained by the strong diffusion ability of the essential oil on the surface of the agar plate along with the volatile properties of the essential oil at room temperature and vapour of pure essential oil without escaping from the petri dish, completely inhibits the growth of most of the bacteria examined. Through analytical data, GC-MS shows the main

TABLE-3
ANTIBACTERIAL ACTIVITY OF CLOVE ESSENTIAL OIL COMPARED WITH SELECTED ANTIBIOTIC TESTED ON FIVE STRAINS OF BACTERIAL PATHOGENS

Tested bacteria	Zone of inhibition (mm)		
	EO	NC	PC
Bacillus subilits	14	6	13 (AMX400)
Staphylococcus aureus	10	6	17 (AMX400)
Escherichia coli	10	6	9 (AMX400)
Salmonella typhimurium	12	6	18(AMX400)
Pseudomonas aeruginosa	12	6	15(AMX400)

EO = Essential oil of clove; NC = H_2O as a negative control (6 mm size of disk from filter paper); PC = positive control: AMX400 = Amoxicillin (400 µg/disk).

ingredients in clove oil, including eugenol and aceteugenol, which are essential ingredients with high antibacterial properties through inhibiting their growth.

Conclusion

In this study, the clove bud was used for extraction of essential oil using hydrodistillation method. The obtained oil was evaluated for chemical composition and physico-chemical characteristics. The extraction process achieved the yield of 6.85%. The analyzed physico-chemical parameters were specific gravity (1.0203 g/cm^3), acid index (21.984 mg KOH/g), ester index (34.92 mg KOH/g). Result of GC/MS revealed six predominant components existing in the clove essential oils. Abundant component is eugenol (76.542%), followed by caryophyllene (4.319%), α-caryophyllene (0.456%), aceteugenol (18.11%), caryophyllene oxide (0.192%), 4,4-dimethyl tetracyclo[6.3.2.0.(1,8)]tridecan-9-ol (0.12%). Among the tested bacterial strains, the highest antimicrobial activity of clove essential oil was found for *Bacillus cereus* strain (14 mm).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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