Research Article

Study of Chemical Constituents and Antibacterial Activity of Essential Oil of *Syzigium aromaticum*

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Received: 05-09-2018; **Revised:** 28-09-2018; **Accepted:** 10-10-2018.

ABSTRACT

The present study aims at evaluating *in-vitro* study of analyzing different chemical constituents by GC-MS and antibacterial activity of essential oil of *Syzigium aromaticum* (Clove). The major chemical constituents in *Syzigium aromaticum* (Clove) essential oil are eugenol (44.95%), β-caryophyllene (19.58%), δ-limonene (7.78%) and α-caryophyllene (7.41%) whereas acetyl eugenol (3%), copaene (2.6%), chavicol(1.6%), δ-cadinene (1.38%) , methyl salicylate (1.0%) and 4 (10) – thujene (0.98%) are present as the minor chemical constituents. The MIC of *Syzigium aromaticum* essential oil against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 0.71 µl/ml whereas for *Klebsiella pneumoniae* it was found to be 1.42µl/ml.

Keywords: Essential oil, MIC, *Syzigium aromaticum*, GC-MS.

INTRODUCTION

An essential oil is defined internationally as the product obtained by hydrodistillation or steam distillation or by a suitable mechanical process without heating (for citrus fruits) of a plant or some parts of it[1]. An essential oil is a liquid that is generally distilled (more frequent by steam or water) from the leaves and stem. Essential oils only represent a small fraction of plant's composition; they have the characteristics by which aromatic plants are used in the food, cosmetics and pharmaceutical industries[2].

Cloves (*Syzigium aromaticum,* syn .*Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae[3]. Clove is commercially cultivated in India, Madagascar, Srilanka, Indonesia and the south of China. Clove bud oil has biological activities such as antibacterial, antifungal, insecticidal and antioxidant properties and are used traditionally as flavouring agent and antimicrobial material in food. The high levels of eugenol in clove essential oil is responsible for strong antimicrobial activity. Clove oil is also widely used as perfume, food flavouring and as a general antiseptic in medical dental practices[4]. Cloves are used in Ayurveda, Chinese medicine and Western herbalism. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis[5]. It is also used in dentistry where the essential oil of clove is used as anadyne for dental emergencies[6]. In addition, the cloves are antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic[7]. The essential oil extracted from the dried flower buds of cloves is used for acne, warts, scars and parasites. Research has shown that clove oil is an effective mosquito repellent. The clove oil is also used as a topical application to relieve pain, to promote healing and in the fragrance and flavouring industries. However, clove oil is toxic to human cells[6], if ingested or injected in sufficient quantity, it has been shown to cause life-threatening complications, including acute respiratory distress syndrome and central nervous system disorder.

MATERIALS AND METHODS

The chromatographic procedure was carried out using IPC GCMSQQQ with autosampler. The sample was diluted 25 times with acetone and 1µl was injected into the column. A fused silica capillary column HP5-MS (30 m x 0.32 mm, film thickness 0.25 µm) was used. The oven temperature used was maintained at 60^0 C for 8 minutes .The temperature was then gradually raised at a rate of 3^0 C per min to 180^0 C for 5 minutes. The temperature at the injection port was 250^0 C. The components of the test solution were identified by comparing the spectra with those of known compounds stored in the NIST library (2005). The ion source temperature was set at 200^0 C. The fragmented ions were separated by the analyzer according to their mass to charge ratio[8].

The dilution test performed by Jorgensen method was used to determine minimum inhibitory concentration using the standard procedure[9].

Test tubes were taken and 1000 µl of BHI broth were added in each test tube. Then 1000 µl of stock solution of essential oil in DMSO (25µl in 975 µl DMSO) was added in first test tube and subsequently two fold serial dilution was done with BHI broth. The inoculum suspension (100 µl) of each bacterial strain was added to each test tube containing essential oil and BHI broth to get the final concentrations 22.72, 11.36, 5.68, 2.84, 1.42, 0.71, 0.35, 0.18 µl /ml respectively. The test tubes were incubated at 37^0 C for 18 hrs and observed after 18 hrs. MIC was determined by absence of turbidity and subculturing of bacteria for all the samples. The lowest concentration that inhibited visible growth of the tested organisms was reported as MIC.

RESULTS AND DISCUSSION

Gas chromatography/mass spectroscopy was done for *Syzigium aromaticum* (clove) essential oil to find out the chemical constituents present in them.

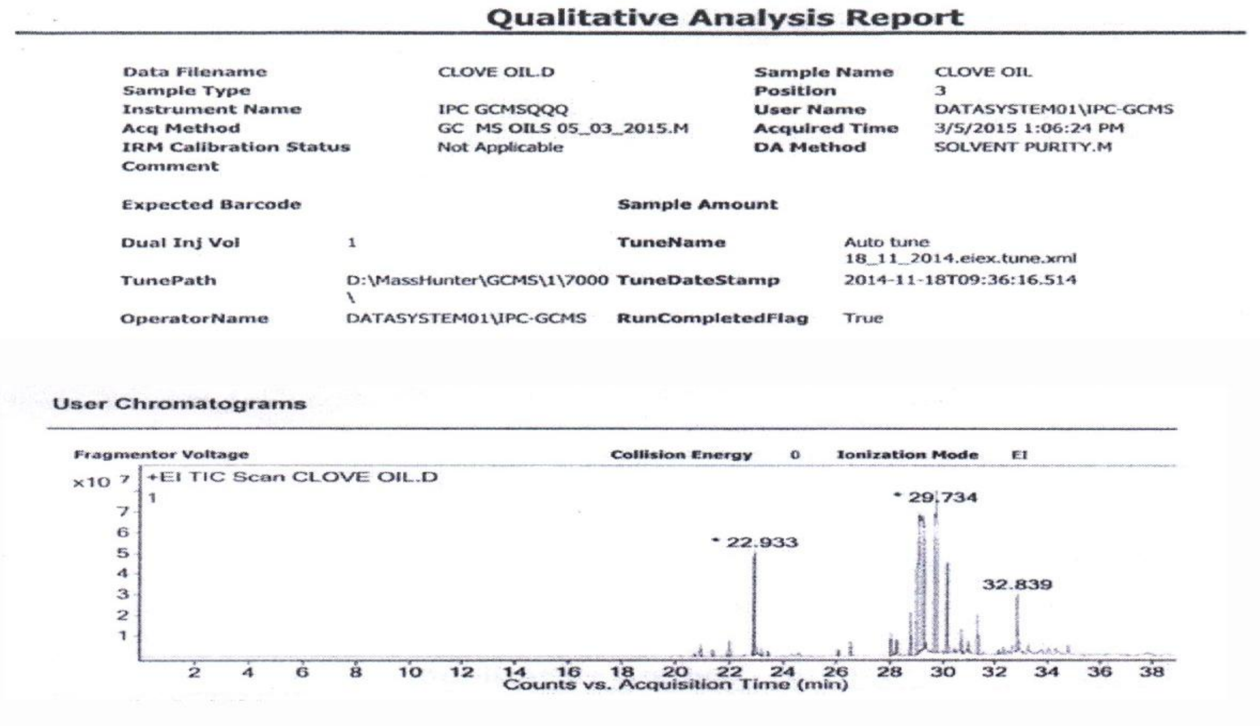
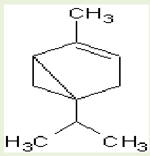
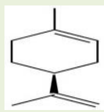
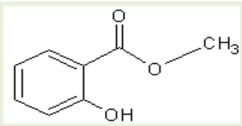
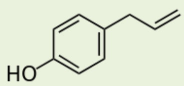
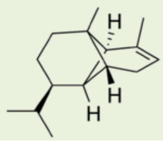
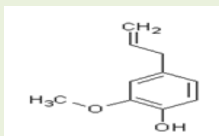
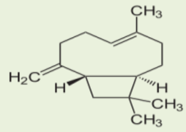
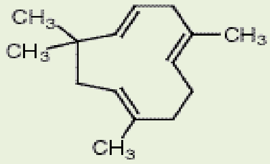
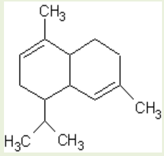
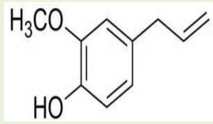


Figure 1: Chromatogram of *Syzigium aromaticum* essential oil by GC/MS

Table 1: GC/MS analysis of essential oil of *Syzigium aromaticum*

S. No.	Compounds	Area sum %	Retention time	Structure
1.	4 (10) - thujene	0.98	22.005	
2.	δ- Limonene	7.78	22.933	
3.	Methyl salicylate	1.0	26.564	
4.	Chavicol	1.6	28.065	
5.	Copaene	2.6	28.795	
6.	Eugenol	44.95	29.097	

7.	**β- Caryophyllene**	19.58	29.734	
8.	**α- Caryophyllene**	7.41	30.179	
9.	**δ - Cadinene**	1.38	30.733	
10.	**Acetyl eugenol**	3.0	32.839	

The GC/MS analysis of *Syzigium aromaticum* essential oil based on retention time and area sum % revealed that Eugenol (44.95%) , β-caryophyllene (19.58%), δ-Limonene (7.78%) and α-Caryophyllene (7.41%) are the major chemical constituents whereas acetyl eugenol (3%), Copaene (2.6%), Chavicol(1.6%), δ-cadinene (1.38%), methyl salicylate (1.0%) and 4(10) – thujene (0.98%) are minor chemical constituents.

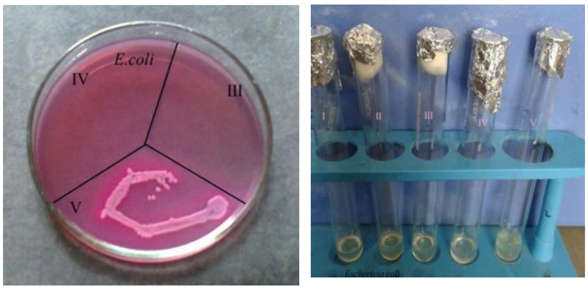
Minimum inhibitory concentration (MIC) of *Syzigium aromaticum* (Clove) essential oil

The lowest concentration of the plant essential oil that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC value of the sample[10]**.** Different concentrations ranged from 0.18 µl/ml to 22.72 µl/ml was prepared for clove essential oil and the MIC values against different pathogenic bacteria i.e *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* was observed. The lowest concentration of essential oil that still retained an inhibitory effect against the growth of a microorganism was known as MIC. The MIC values of *Syzigium aromaticum* (Clove) essential oil for different pathogenic bacteria are shown in Table 2.

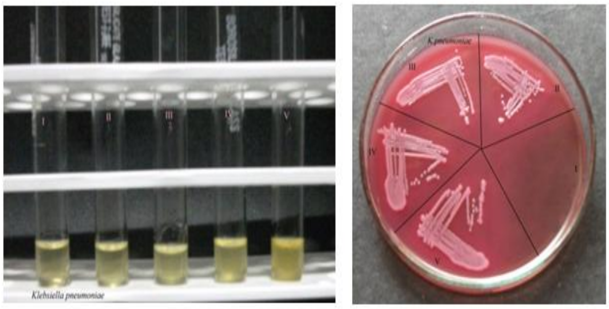
Table 2: MIC (µl/ml) of *Syzigium aromaticum* essential oil against selected bacteria.

Conc. (µl/ml)	*Syzigium aromaticum* (µl/ml) essential oil against bacteria			
	E.coli	*K.Pneumoniae*	*P.aeruginosa*	*S.aureus*
22.72	-	-	-	-
11.36	-	-	-	-
5.68	-	-	-	-
2.84	-	-	-	-
1.42	-	*	-	-
0.71	*	+	*	*
0.35	+	+	+	+
0.18	+	+	+	+
MIC	0.71	1.42	0.71	0.71

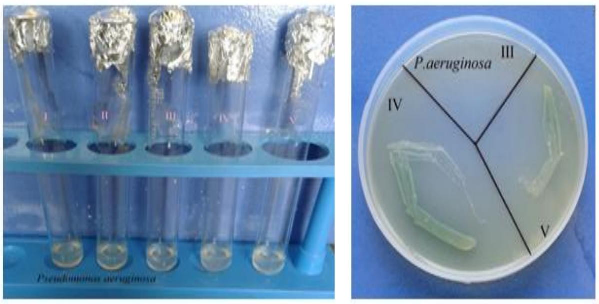
(+) Growth, (*) MIC, (-) No Growth



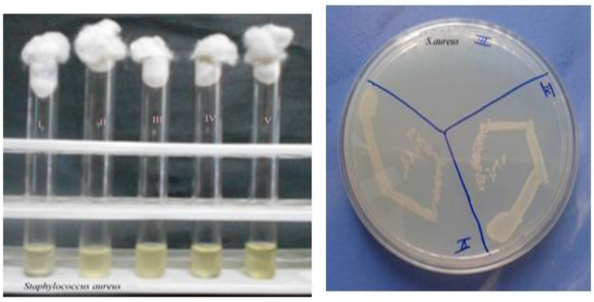
a) *Escherichia coli*



b) Klebsiella pneumoniae



c) Pseudomonas aeruginosa



d) *Staphylococcus aureus*

Figure 2: MIC (µl/ml) of *Syzigium aromaticum* (Clove*)* essential oil against selected bacteria

The MIC of *Syzigium aromaticum essential oil* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 0.71 µl/ml whereas for *Klebsiella pneumoniae* it was found to be 1.42µl/ml (table 2). The results have shown that the clove essential oil have a great potential to treat bacterial infectious diseases due to presence of bioactive compounds. These results were similar to the findings of Jagdeesh *et al*., (2011) who studied about antimicrobial activity and minimum inhibitory concentration of garlic, clove and cinnamon essential oils against various bacterial pathogens and concluded that the essential oil of clove was found to be most active against *S. aureus* followed by *E.coli* and *Campylobacter jejuni*[11]. Suree *et al*., (2005) reported about antibacterial activity of crude ethanolic extracts and essential oils of fourteen spices including *Syzigium aromaticum* (Clove)[12]. The zone of inhibition was tested by disc diffusion method and MIC by microbroth dilution test against twenty serotypes of *Salmonella* and five species of other *enterobacteria*. It was concluded that among all ethanolic extracts, clove extract had the most inhibitory effect on the growth of all bacterial strains tested. Oils of clove and Kaffir lime peels exhibited greater antibacterial activity against all tested strains compared to other spice oils. The antimicrobial activity may be attributed to the major compound i.e eugenol in the clove essential oil.

CONCLUSION

An important characteristic of essential oils and their components is their hydrophobicity which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and making the bacterial cell impermeable[13]**.** Leakage of ions and other cell contents may occur. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death of bacterial cell[14]. The essential oils containing a high percentage of phenolic compounds such as eugenol (2 – methoxy – 4 - (2-propenyl) phenol, carvacrol, thymol possess the strongest antibacterial properties. The mechanism of action may be attributed to the disturbance of cytoplasmic membrane, disruption of the proton motive force (PMF), electron flow, active transport and coagulation of cell contents.

The inhibitory effect of phenols could be explained by interactions with the cell membrane of micro-organisms and is often correlated with the hydrophobicity of the compounds. For instance, oregano essential oil was reported to induce permeability alteration in the micro-organisms membranes (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) with a consequent leakage of protons, phosphates and potassium[15]. Among phenolic compounds, carvacrol, an isoprenyl phenol, was reported to have one of the strongest antimicrobial activity[16].

With the recent trends in increase in resistance of microorganisms against various antibiotics, the potential of *Syzigium aromaticum* (clove) essential oil must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by bacteria.

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Source of Support: Nil, **Conflict of Interest:** None.