Extraction and Identification of Essential Oil from Cloves (*Syzygium aromaticum* L.) and Its Antimicrobial Activity

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Abstract

Cloves are used in medicine and dentistry for dental emergencies. The essential oil is also used in aromatherapy when stimulation and warming are needed. Therefore, the essential oil of cloves was extracted by steam distillation. Then, the essential oil was analyzed by GCMS method. In GCMS analysis, GC oven temperatures were assigned by four intervals in the range of 80 to 280°C. As the carrier gas, helium was used at a constant flow rate 1.0 mL/min. The injector temperature and mass transfer line temperature were fixed at 275 and 280 °C, respectively. The molecular masses are arranged in 15 to 250 amu (m/z) and assigned Run time (min) in the range of 2 to 20. According to GCMS method, the essential oil could be deduced as Eugenol, Caryophyllene, p-Mentha-1,8-diene and Phenol, 4-allyl-2-methoxy acetate. Moreover, the functional groups of essential oils could be assigned by FT IR analysis. *In vitro* antimicrobial screening of essential oil from cloves was carried out by agar well diffusion method. In the screening, six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and *E.coli*species were used. According to the antimicrobial screening, the essential oil of cloves possesses antimicrobial activities.

Keywords: Antimicrobial activity, Cloves, Essential oil, Steam distillation, GCMS method

Introduction

***Syzygium aromaticum* L. (Cloves)**

Cloves (Figure 1) are the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. They are native to the Maluku Islands in Indonesia, and are commonly used as a spice. Cloves are commercially harvested primarily in Bangladesh, Indonesia, India, Pakistan, Sri Lanka, Myanmar and Tanzania. Cloves are available throughout the year. The cloves tree is an evergreen that grows up to 8–12 m tall. Cloves are harvested at 1.5–2.0 cm long. Cloves may be used to give aromatic and flavor qualities to hot beverages, often combined with other ingredients such as lemon and sugar (Dorenburg *et al*., 2003).

Figure 1 Dried cloves

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Scientific Classification

Kingdom	: Plantae
Order	: Myrtales
Family	: Myrtaceae
Genus	: *Syzygium*
Species	: *S. aromaticum*
Botanical name	: *Syzygium aromaticum* L.
English name	: Cloves

Medicinal Uses

Cloves are used in Indian Ayurvedic medicine, Chinese medicine, and western herbalism and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. (Alqareer *et al*., 2012).

Generally, there is insufficient evidence that clove oil containing eugenol is effective for toothache pain or a variety of other types of pain, although one review reported efficacy of eugenol combined with zinc oxide as an analgesic for alveolar osteitis. It remains unproven whether using cloves or clove oil reduces blood sugar levels (Bensky *et al*., 2004).

Clove Essential Oil

Clove oil is very potent oil that should be used with great care in aromatherapy; it does have wonderful properties - from stimulating the mind and lifting depression, to aiding digestion, relieving pain in arthritis and rheumatism, easing respiratory problems and assisting leg ulcers. Clove oil can be extracted from the leaves, stem and buds. Clove oil is beneficial to the digestive system, effective against vomiting, diarrhea, flatulence, spasms and parasites. Clove oil is valuable for relieving respiratory problems, like bronchitis, asthma and tuberculosis. The disinfecting property is useful in cases of infectious diseases (Kamatou *et al*., 2012).

AIM

To apply the essential oil from cloves which possesses the antimicrobial activity

Materials and Methods

Experimental

The flower of cloves samples was collected from Hlaing Thar Yar Township, Yangon Region. Firstly, the essential oil of cloves flower was extracted by steam distillation method. Then, the cloves oil was analyzed by GC-MS Autosampler (Trace 1300, ISQ QD, Germany). The antimicrobial activity of cloves oil was examined by agar well diffusion method (Mar Mar Nyein *et al*., 1991).

Results and Discussion

The Essential Oil of Cloves

The collected cloves sample was used to extract the essential oil by steam distillation method. 0.5 mL of cloves oil was obtained from 50 g of cloves sample. The extractor and extracted essential oil of cloves are shown in Figure 2.

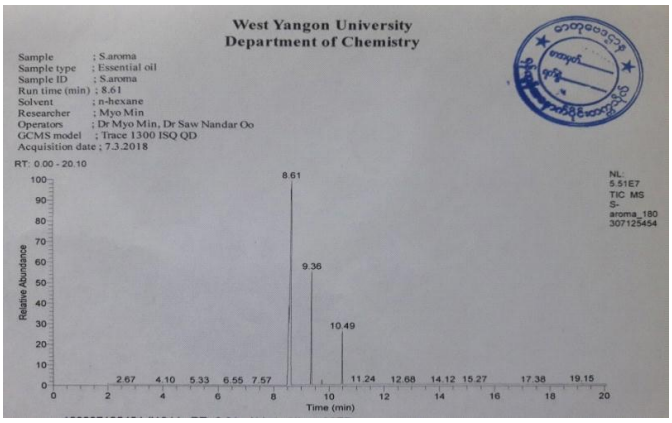
(a) (b)

Figure 2 (a) The extractor and (b) the extracted essential oil of cloves

GC-MS Analysis of the Extracted Essential Oil of Cloves

The extracted essential oil of cloves was analyzed by GC-MS Autosampler (Trace 1300, ISQ-QD, Germany) (Figure 3). In GC-MS analysis, GC oven temperatures were assigned by four levels in the range of 80 to 280 ^{o}C. The increasing temperature rates were controlled by 10 to 15 ^{o}C/min; carrier gas, helium at a constant flow rate 1.0 mL/min. The injector temperature and mass transfer line temperature were fixed at 275 and 280 ^{o}C, respectively. The molecular masses (mass fragmentations) are arranged in 15 to 250 amu (m/z) and assigned retention time (RT) in the range of 2 to 20 min.

From the GC-MS analysis, four peaks from the GC chromatogram were observed at 8.61, 9.36, 9.74 and 10.49 min of different retention times. The mass fragmentation patterns (m/z values) of each compound were matched with those of reference compounds from GC-MS data library. By using GC-MS, each chromatogram of different retention times could be deduced as eugenol (RT: 8.61 min), caryophyllene (RT: 9.36 min), p-mentha-1,8-diene (RT: 9.74 min) and phenol, 4-allyl-2-methoxy acetate (RT: 10.49 min). According to GC-MS analysis, eugenol was observed as the highest relative abundance of the essential oil. GC-MS analyzed data are shown in Figures 3 (a), 3 (b), 3 (c), 3 (d) and 3 (e).



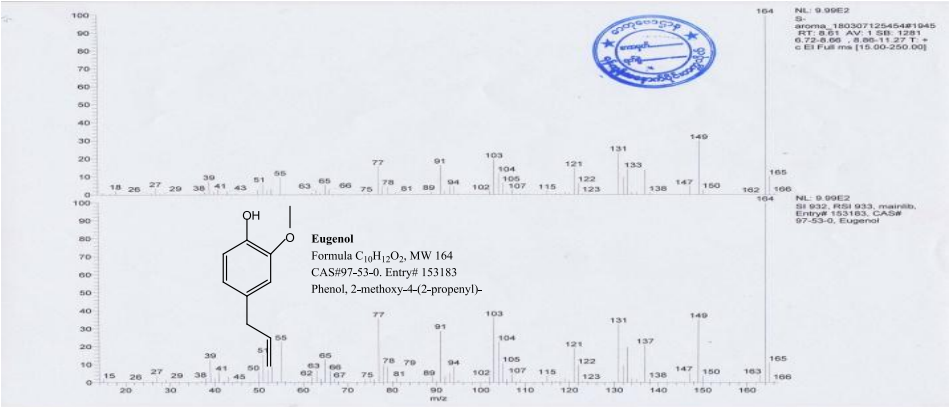


Figure 3 (b) GC-MS spectra of eugenol from the extracted essential oil (8.61 min) by replib library

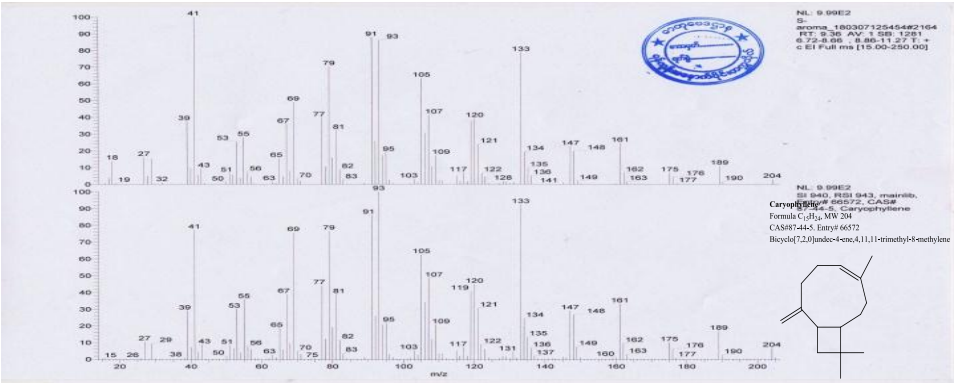


Figure 3 (c) GC-MS spectra of caryophyllene from the extracted essential oil (9.36 min) by replib library

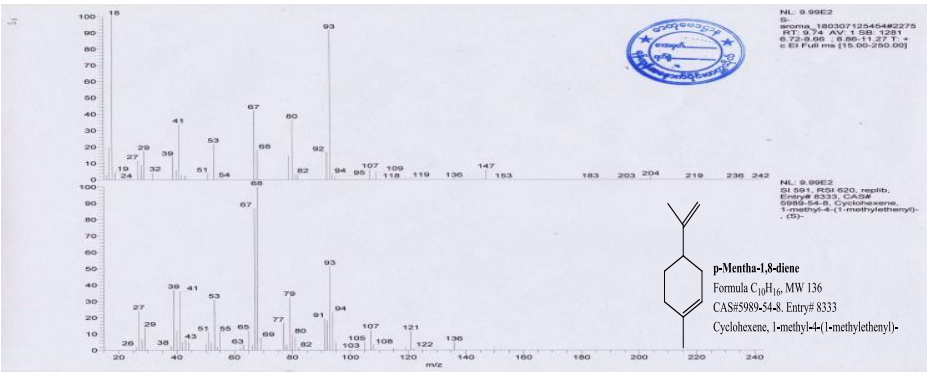


Figure 3 (d) GC-MS spectra of *p*-mentha-1, 8-diene from the extracted essential oil (9.74 min) by replib library

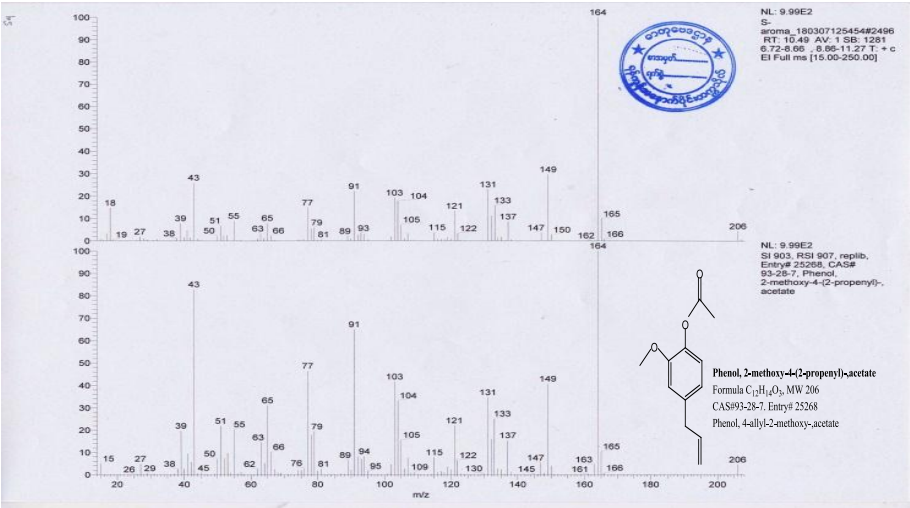


Figure 3 (e) GC-MS spectra of phenol, 4-allyl-2-methoxy-, acetate from the extracted essential oil (at retention time, 10.49 min) by replib library

FT IR Analysis of Extracted Essential Oils of Cloves

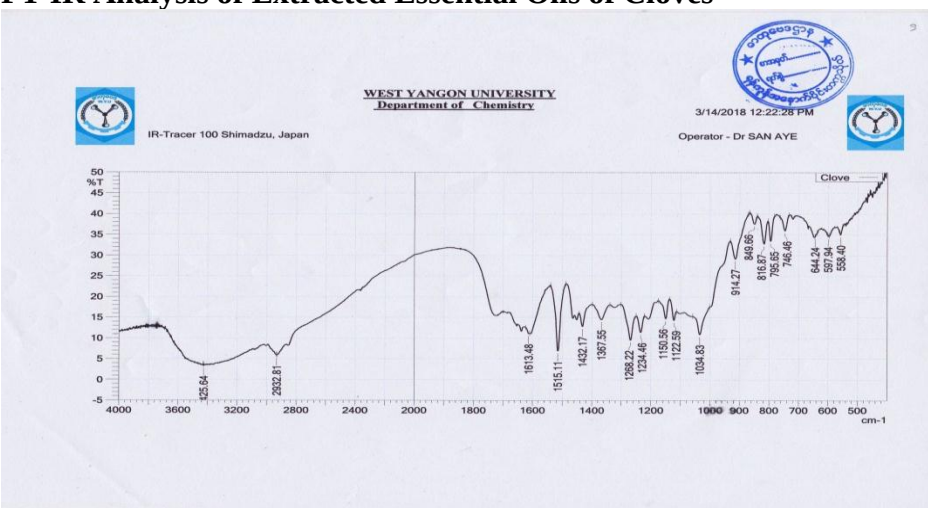


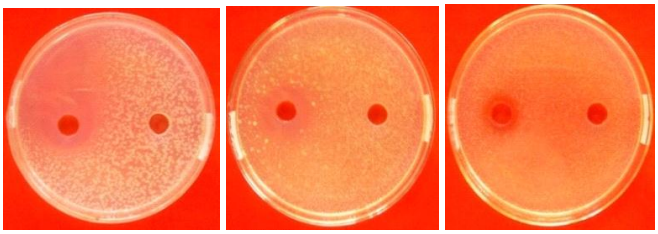
Figure 4 FT IR spectrum of the extracted essential oils of cloves

According to the FT IR analysis (Figure 4), the various functional groups of essential oils of cloves could be assigned with the various frequencies. O-H and C-H stretching frequencies occur at 3425 and 2932 cm^{-1}. Moreover, C=O, C=C, and C-O stretching bands appear at 1710, 1613 and 1268 cm^{-1}, respectively. The bending frequencies at 1432 and 1367 cm^{-1} indicated the presence of $–CH_2$ and $–CH_3$ groups. C-O-C functional group can be assigned by 1034 cm^{-1}. C-H out-of-plane bending can be observed at 644 and 597 cm^{-1}. According to the observed functional groups, it could be deduced as the presence of the characteristics of essential oils. (Silverstein and Webster, 1998)

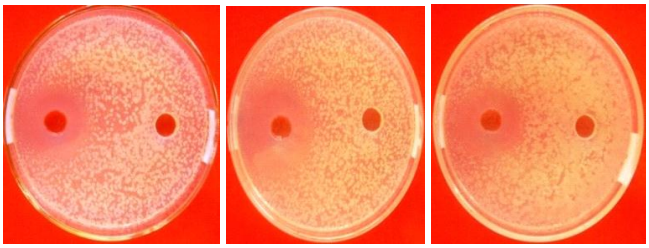
Screening of Antimicrobial Activity

Antimicrobial activities of clove essential oil were screened by agar well diffusion method and the results are shown in Figure 5 and Table 1. In this screening, the clove oil was tested on six species of microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli* species.

In the screening, the antimicrobial activities on all of the tested microorganisms with the exception of *P. aeruginosa* are considerably high. The activity with *P. aeruginosa* was observed as medium activity. According to the antimicrobial screening, the clove essential oil may be used for the medicinal formulation of antimicrobial drugs.



B. subtilis ***S. aureus*** ***P. aeruginosa***



B. pumilus ***C. albicans*** ***E.coli***

(A = Clove essential oil, B = Control (n-hexane))

Figure 5 Antimicrobial activities of clove essential oil

Table 1 Results of Antimicrobial Activity Screening of Cloves Essential Oil

Sample	Inhibition zone diameter (mm) of the sample against six types of microorganisms					
	B. subtilis	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*C. albicans*	*E. coli*
Cloves oil	24 mm (+++)	25 mm (+++)	16 mm (++)	25 mm (+++)	27 mm (+++)	25 mm (+++)
Control	-	-	-	-	-	-

Agar well : (10 mm)
10 mm - 14 mm (+)
15 mm - 19 mm (++)
20 mm - above (+++)

Conclusion

This research concerns with the GC-MS analysis and antimicrobial screening of the extracted essential oils from the clove flower. Firstly, the extraction of essential oil from cloves sample was carried out by steam distillation method. Then, the extracted essential oil was analyzed by GC-MS method. From the analysis, the essential oils could be deduced to contain eugenol (8.61 min), caryophyllene (9.36 min), p-mentha-1,8-diene (9.74 min) and phenol-4-allyl-2-methoxy acetate (10.49 min). The eugenol was observed as the major constituent from the extracted essential oils due to the highest relative abundance of the GC chromatogram. Moreover, the various functional groups of essential oils could be assigned by FT IR analysis.

Then, the antimicrobial activity of essential oil from clove flower was studied against six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli* species by agar well diffusion method.. In the screening, the antimicrobial activities on all microorganisms (exception of *P. aeruginosa*) are considerably high. The activity with *P. aeruginosa* was observed as medium activity.

According to the antimicrobial activity screening, the clove essential oil showed the strong potency of antimicrobial activity. Therefore, the clove oils may be used in the medicinal formulation of antimicrobial drugs.

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