EXTRACTION, DENTIFICATION AND ANTIMICROBIAL SCREENING OF ESSENTIAL OILS FROM *Cymbopogon citrates* STAPF. (LEMONGRASS)

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Abstract

Cymbopogon citratus stapf .(Lemongrass) is the popular plant in Myanmar. It is used as the food as well as traditional medicine. Especially, the essential oil extracted from the leaves is commonly used in insect repellents, perfumes and soaps. Therefore, the essential oil of lemongrass was extracted by steam distillation. Then, the essential oil was analyzed by Gas Chromatrography Mass Spectrometry. According to the GC-MS conditions, the two main constituents essential oils of lemongrass could be deduced as citronellal (2-isopropenyl-5-methyl-4-hexenal) (RT: 6.52 min), and citral (RT: 6.11 min). Moreover, the functional groups of essential oils could be assigned by FT IR analysis. *In vitro* antimicrobial activity screening of lemongrass oil 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 *Escherichia.coli* species were used. According to the antimicrobial screening, the lemongrass oil was found to possess antimicrobial activities against *C.albicans*, *B.subtilis, P.aeruginosa* and *B.pumilus*.

Keywords*;* Lemongrass, steam distillation, GC-MS analysis, essential oil, antimicrobial activity

Introduction

***Cymbopogon citratus* stapf.(** Lemongrass)

Cymbopogon citratus stapf (Lemongrass) is a perennial grass in the family Poaceae grown for its fragrant leaves and stalks which are used as a flavoring. The grass grows in dense clumps and has several stiff stems and slender blade-like leaves which droop towards the tips. The leaves are blue-green in color, turning red in the Fall and emit a strong lemon fragrance when damaged. Lemongrass produces large compound flowers on spikes when grown in the tropics, but rarely flowers when grown in more Northern latitudes. Lemongrass can reach a height of 1.8 m (6 ft) and will grow for several years, typically its economical lifespan is 4 years. Lemongrass may also be referred to as ginger grass or citronella grass and likely originates from Sri Lanka or Malaysia although a wild form of the plant is not known (CABI, 2013).

Lemongrass (Figure 1) is a genus of Asian, African, Australian, and tropical island plants in the grass family. Some species are commonly cultivated as culinary and medicinal herbs because of their scent, resembling that of lemons (*Citrus limon*). Common names include lemongrass, barbed wire grass, silky heads, citronella grass, fever grass, amongst many others (Soenarko, 1977).

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(A) (B)

Figure 1 (A) Lemongrass plant and (B) stem of lemongrass

Scientific Classification

Kingdom	: Plantae
Order	: Poales
Family	: Poaceae
Genus	: *Cymbopogon*
Species	: *C. citratus*
Botanical name	: *Cymbopogon citratus* stapf.
English name	: Lemongrass
Myanmar name	: Sabalin

Uses of Lemongrass

Lemongrass is widely used as a culinary herb in Asian cuisines and also as medicinal herb in India. It has a subtle citrus flavour and can be dried and powdered, or used fresh. It is commonly used in tea, soups, and curries. It is also suitable for use with poultry, fish, beef, and seafood. It is often used as a tea in African countries such as Togo, south eastern GhanaVolta Region and the Democratic Republic of the Congo and Latin American countries such as Mexico. Lemongrass oil is used as a pesticide and a preservative. Research shows that lemongrass oil has antifungal properties (Shadab *et al*., 1992).

Despite its ability to repel some insects, such as mosquitoes, its oil is commonly used as a "lure" to attract honey bees. Lemongrass works conveniently as well as the pheromone created by the honeybee's Nasonov gland, also known as attractant pheromones. Because of this, lemongrass oil can be used as a lure when trapping swarms or attempting to draw the attention of hived bees (Blanco *et al*., 2009)..

Lemongrass Oil

Lemongrass oil, used as a pesticide and preservative, is put on the ancient palm-leaf manuscripts found in India as a preservative. It is used at the Oriental Research Institute Mysore, the French Institute of Pondicherry, the Association for

the Preservation of the Saint Thomas Christian Heritage in Kerala, and many other manuscript collections in India. The oil also injects natural fluidity into the brittle palm leaves, and the hydrophobic nature of the oil keeps the manuscripts dry so the text is not lost to decay due to humidity (Bleasel *et al*., 2002).

East Indian lemongrass also called Cochin grass or Malabar grass, is native to Cambodia, Vietnam, Laos, India, Sri Lanka and Thailand, while West Indian lemongrass is native to South Asia and maritime Southeast Asia. While both can be used interchangeably, *C. citratus* is more suitable for cooking. In India, *C. citratus* is used both as a medical herb and in perfumes. *C. citratus* is consumed as a tea for anxiety in Brazilian folk medicine, but a study in humans found no effect. The tea caused a recurrence of contact dermatitis in one case. Lemongrass is also used as an addition to tea, and in preparations such as *kadha*, which is a traditional herbal brew used in Ayurvedic medicine (Bleasel, *et al*., 2002).

Aim

To study the identification and antimicrobial activity of lemongrass essential oil

Materials and Methods

Lemongrass plant samples were collected from Hlaing Thar Yar Township, Yangon Region. Firstly, the essential oil of lemongrass aerial parts wasextracted by steam distillation method. Then, the extracted essential oil (1%) was analyzed by GC-MS Autosampler (Trace 1300, ISQ QD,Germany). The antimicrobial activity of extracted essential oil on six test organisms, namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* was examined by agar well diffusion method (Mar Mar Nyein *et al*., 1991) .

Results and Discussion

Sample

Lemongrass aerial part

The Essential Oil of Lemongrass

The collected lemongrass aerial part samples were used to extract the essential oil by steam distillation method.

The extracted essential oil of lemongrass is shown in Figure 2.

Figure 2 Extraction of essential oil from lemongrass by steam distillation

GC-MS Analysis of the Extracted Essential Oil of Lemongrass

The extracted essential oil of lemongrass 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) were arranged in 15 to 250 amu (m/z) and assigned retention time (min) in the range of 2 to 20.

From the GC-MS analysis, only two retention time and two compounds from the GC chromatogram were observed at 6.11 and 6.52 min of different run times. The mass fragmentation patterns (m/z values) of each compound were matched with those of reference compounds, citral and citronellal from GC-MS Libraries. By using GC-MS, each chromatogram of different run times could be deduced as citral (RT: 6.11 min), and citronellal (2-isopropenyl-5-methylhex-4-enal) (RT: 6.52 min). GC-MS analyzed data are shown in Figure 3 (a), and 3 (b) and 3 (c).

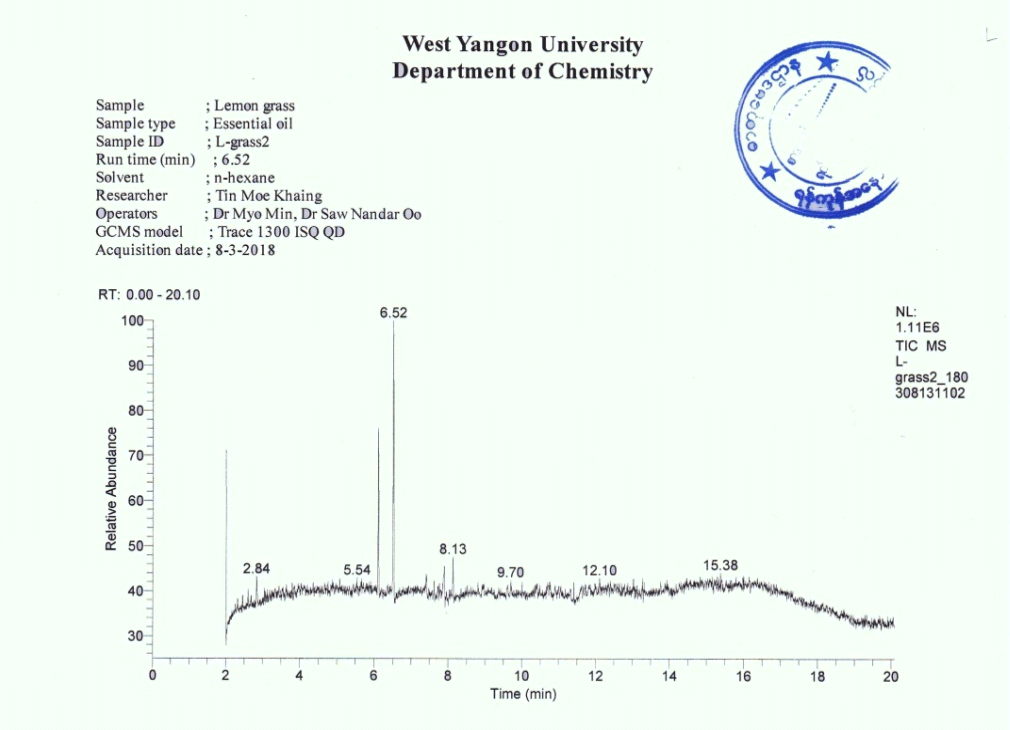


Figure 3(a) chromatogram of the essential oils from *Cymbopogon citratus* stapf.(Lemongrass)

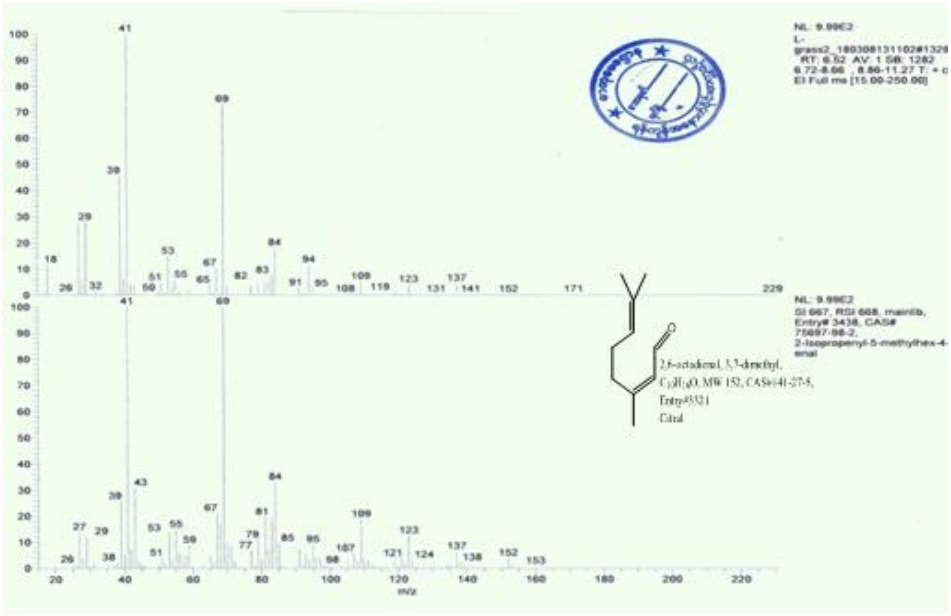


Figure 3 (b) Fragmentation patterns of Citral from extracted essential oil atretention time 6.11 minand MS(Nist)library

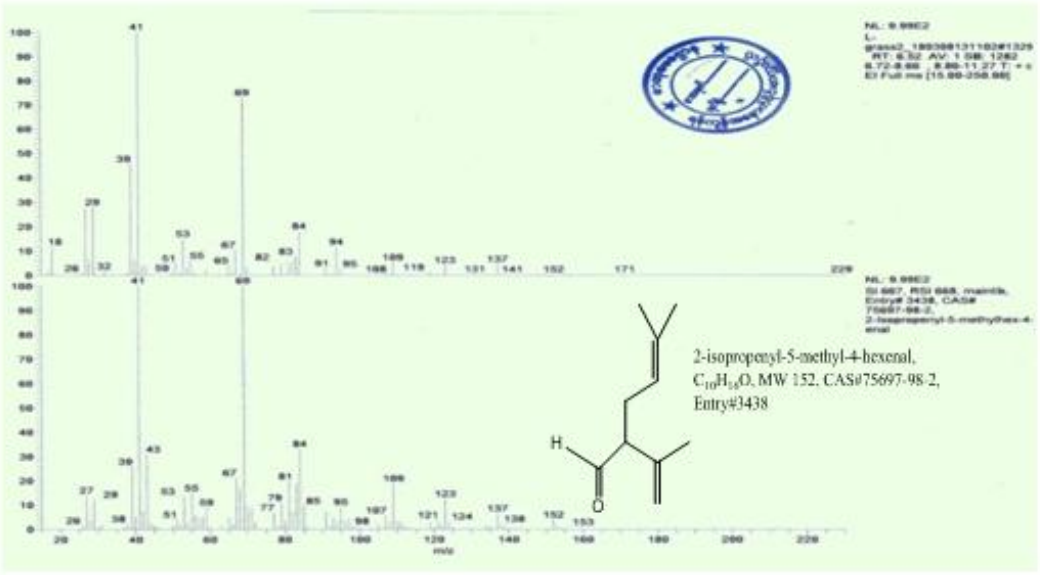


Figure 3 (c) Fragmentation patterns of citronellal(2-isopropenyl-5-methyl-4-hexenal) from extracted essential oil at retention time 6.52 min andMS(Nist) library

FTIR Analysis of the Extracted Essential Oils of Lemongrass

According to the FTIR analysis (Figure 4), the various functional groups of essential oils of lemongrass could be assigned with the various frequencies. C-H stretching frequencies (aldehyde and aliphatic protons) occur at 3386 and 2918 cm^{-1}. Moreover, C=O, and C=C stretching frequencies appear at 1735, and 1645 cm^{-1}, respectively. The bending frequencies at 1415 and 1375 cm^{-1} indicate the presence

of $–CH_2$ and $–CH_3$ group. According to the observed functional groups, it could be deduced as the presence of the characteristics of essential oils.

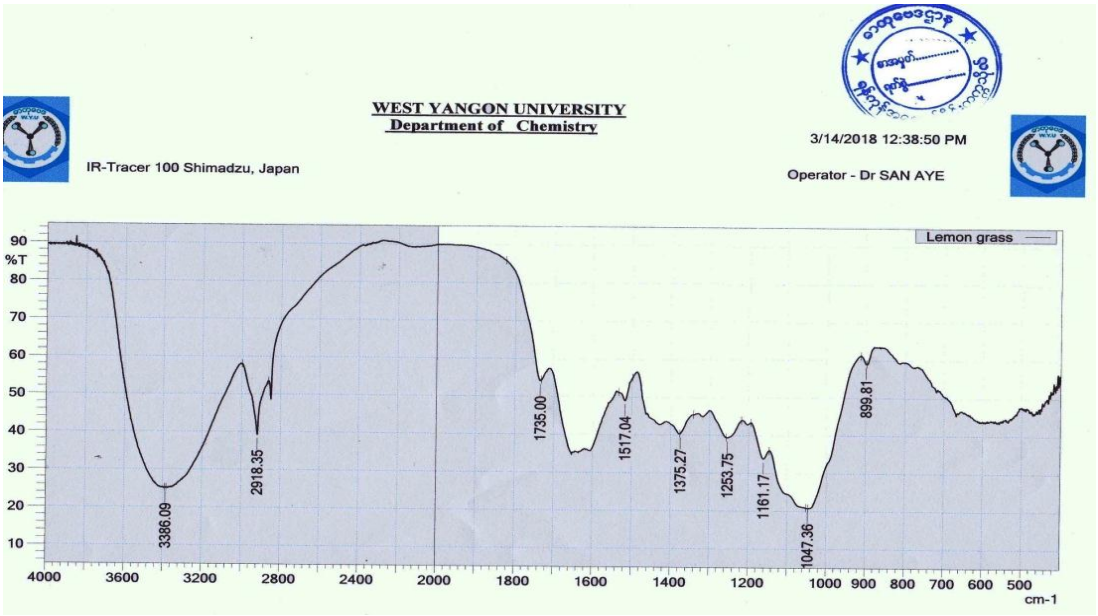


Figure 4 FTIR spectrum of the extracted essential oils of lemongrass

Screening of Antimicrobial activity

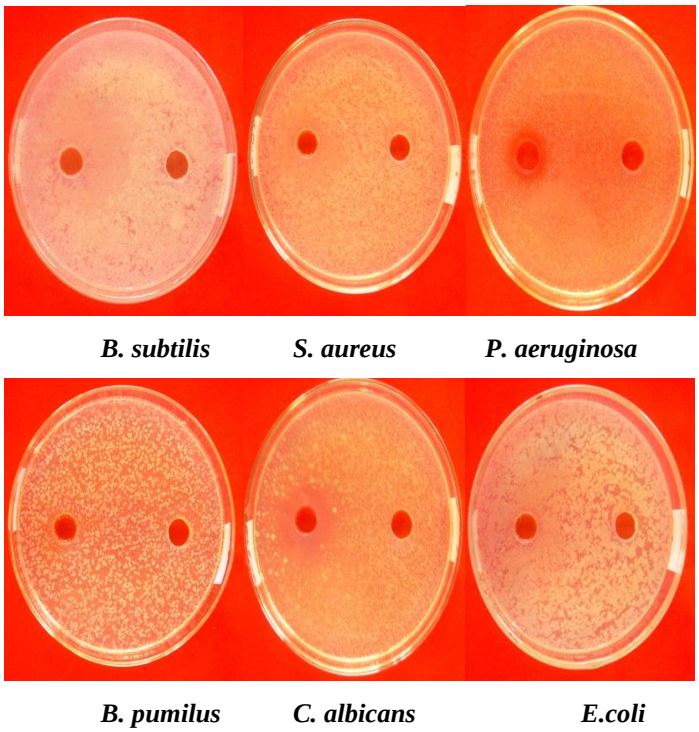
Antimicrobial activities of essential oil from lemongrass were screened by agar well diffusion method (Table 1). In this screening, the extracted essential oil was tested on six species of microorganisms; *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* species.

In the screening, the antimicrobial activity on *C. albicans* 22 mm(+++) showed the highest activity. The activities on *B.subtilis* (15 mm(++), *P.aeruginosa* 16 mm (++)and *B.pumilus*15mm (++) were observed as the medium activity. With other test organisms such as *S.auress*14 mm (+) and *E.coli* 12 mm (+) it showed the lowest activity.

Table 1 Results of Antimicrobial Screening of Lemongrass Essential Oil

Sample	**Inhibition for diameter(mm)**					
	B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E.coli***
Lemongrass oil	15 mm (++)	14 mm (+)	16 mm (++)	15 mm (++)	22 mm (+++)	12 mm (+)
Control	-	-	-	-	-	-

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



(A = Lemongrass essential oil, B = Control)

Figure 5 Antimicrobial activities of lemongrass essential oil

Conclusion

This research is concerned with the GC-MS analysis and antimicrobial screening of extracted essential oils from lemongrass sample. Firstly, the extraction of essential oil from lemongrass sample (1 % yield) 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 as citral (6.11 min), and citronellal (2-isopropenyl -5-methyl 4-hexenal) (6.52 min). The citronellal (2-isopropenyl-5-methyl 4 hexenal)was observed as the major constituent from the extracted essential oils. Moreover, the various functional groups of essential oils could be assigned by FT IR analysis.

Then, the antimicrobial activity of essential oil from lemongrass was studied by agar well diffusion method. The extracted essential oil was tested against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli* species. In the screening, the antimicrobial activity on *C. albicans* showed the highest activity. The activities on *B.subtilis, P.aeruginosa* and *B. pumilus* were observed as the medium activity. With other microorganisms such as *S.aureus* and *E.coli*, it showed the lowest activity. According to the antimicrobial screening, the essential oil of lemongrass may be used in the medicinal formulation of antimicrobial drugs on *B.subtilis, P.aeruginosa* and *B. pumilus*. Especially, lemongrass oil may be used in the antifugal drugs due to the highest activity on *C. albicans*.

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