

## PREPARATION AND IDENTIFICATION OF ESSENTIAL OIL FROM LEMON BASIL AND ITS ANTIMICROBIAL ACTIVITY

Myo Min<sup>1</sup>, Aung Zaw Myo Thant<sup>2</sup>

### Abstract

In Myanmar, lemon basil is commonly used as a fragrant spice in cooking. Volatile oils present in lemon basil, such as linalool, nerol and citral, which possess antibacterial and antimicrobial properties. Therefore, the essential oil of lemon basil was prepared by hydrodistillation. Then, the essential oil was analyzed by GC-MS method. In GC-MS analysis, GC oven temperatures were assigned by four levels in the range of 80° to 250 °C. The increasing temperature rates were controlled by 10° to 15 °C/min; carrier gas, helium at a constant flow 1.0 mL/min. The injector temperature and mass transfer line temperature were fixed at 270° and 280 °C, respectively. The molecular masses are arranged in 15 to 250 amu (m/z) and assigned retention time (min) in the range of 3 to 11. According to the GC-MS analysis, the essential oil could contain linalool, terpinen-4-ol, *cis*-geraniol, *cis*-verbenol, citral, 3,3,7-trimethyl-oct-6-enoic acid methyl ester, 1-methoxy-3,7-dimethyl-2,6-octadiene, caryophyllene, germacrene and 4-[(1E), 1,5-dimethyl-1, 4-hexadiene-1-yl]-1-methyl cyclohexene. The antimicrobial screening of the prepared essential oil from lemon basil was carried out by agar well diffusion method. In the screening, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *B. pumilus*, *C. albicans* and *E. coli* species were used. From the screening, the antimicrobial activities of essential oil of lemon basil showed the high activities against all microorganisms.

**Keywords:** Antimicrobial activity, essential oil, GC-MS method, hydrodistillation, lemon basil

### Introduction

#### Plant Description

Lemon basil (*Ocimum africanum*) (Figure 1) is a hybrid between basil (*Ocimum basilicum*) and American basil (*Ocimum americanum*). In Myanmar, it is commonly used as a fragrant spice in cooking. Lemon basil stems can grow to 20–40 cm (8–20 in) tall. It has white flowers in late summer to early fall. The leaves are similar to basil leaves, but tend to be narrower with slightly serrated edges. Seeds form on the plant after flowering and dry on the plant (Culloty, 2010).



#### Scientific Classification

Kingdom	: Plantae
Order	: Lamiales
Family	: Lamiaceae
Genus	: <i>Ocimum</i>
Species	: <i>O. africanum</i>
Botanical name	: <i>Ocimum africanum</i> Lour.
English name	: Lemon basil
Myanmar name	: Pin-sein

**Figure 1** Lemon basil plant

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## Health Benefits of Lemon Basil

Basil is used as in cooking and may have some health benefits. Basil's proposed benefits include reducing inflammation, and it is said to have anti-aging and antibacterial properties. Basil is used in traditional Tamil and Ayurvedic medicine, which is a form of traditional medicine popular on the Indian subcontinent. Alternatively, lime and lemon basil have a strong citrus scent due to their high concentration of limonene (Baliga *et al.*, 2013).

The basil contains a wide range of essential oils, rich in phenolic compounds, and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins. The basil possesses the potential for holy basil to prevent cancer and it contains, including eugenol, rosmarinic acid, apigenin, myretenal, luteolin,  $\beta$ -sitosterol, and carnosic acid, it may help prevent certain types of skin, liver, oral, and lung cancers. The beta-caryophyllene from basil has prominent anti-arthritis activity which may be attributed to its anti-inflammatory activity." Basil restricts the growth of numerous bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, and *Pseudomonas aeruginosa* (Baliga *et al.*, 2013).

A decoction of basil leaves is used against hepatic and gastritis disorders. Basil leaf juice is used to treat dysentery, night blindness and conjunctivitis. The essential oils of basil have larvicidal properties. Basil has excellent antimalarial properties and eugenol is the main constituent responsible for its mosquito-repellent properties. Basil leaf paste is effective against ringworm infection and to clear marks on the face. The occurrence of urosolic acid in the leaves helps to remove wrinkles and returns skin elasticity. Basil is highly beneficial in healing wounds, cuts and ulcers, and in removing parasites and worms (Bansod and Rai, 2008).

## Lemon Basil Oil

Basil oil is obtained through hydro distillation from fresh plant material. The Basil oil is thin transparent fluid with light yellow to yellow-green colour with aroma characteristic of Basil. A native of Africa and Seychelles, it is grown as popular culinary herb and has small white flowers. The essence is distilled from leaves and has light greenish yellow with sweet green overtones. The Basil oil derived is known for intensely fresh, sweet-spicy and vibrant aroma. Lemon Basil Essential Oil has a sweet lemon aroma with the characteristic basil herbal notes but the body notes linger with a complex lemon-clean scent. The citral content is a natural occurrence in this subspecies of Basil (Texas Natural Supply, 2019).

Basil oil is known to have strong antioxidant properties. Research has shown the oil contains potent anticancer, antiviral and antimicrobial properties. Antioxidants are an important part of maintaining a healthy and balanced lifestyle, and basil maybe a very important source of these essential compounds (Tilebeni, 2011). However, despite these reputed properties, it is important to be aware that basil contains estragole, which may be carcinogenic. In Germany, for example, basil is not considered safe for pregnant women or children (Meyers, 2003).

## AIM

To identify the chemical compounds of essential oil from lemon basil which possesses antimicrobial activity

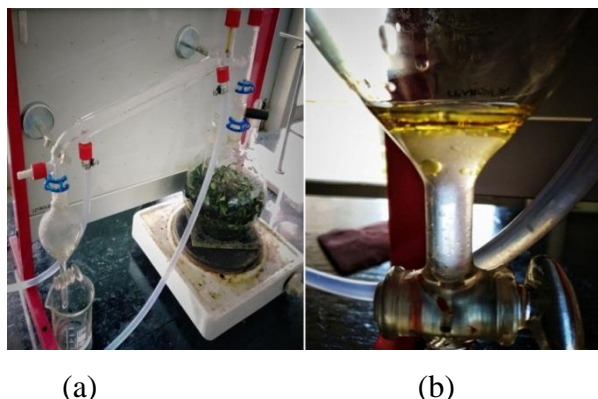
## Materials and Methods

The lemon basil samples were collected from Hlaing Thar Yar Township, Yangon Region. Firstly, the essential oil from fresh sample was extracted by hydrodistillation method. Then, the basil oil was analyzed by GC-MS Autosampler (Model: Trace 1300, ISQ-QD, Germany). The antimicrobial activity of basil oil was examined by agar well diffusion method (Mar Mar Nyein *et al.*, 1991).

## Results and Discussion

### The Basil Essential Oil

The collected fresh sample was used to prepare the essential oil by hydrodistillation method. 0.26 mL of basil oil was obtained from 100 g of fresh sample. The apparatus is shown in Figure 2.

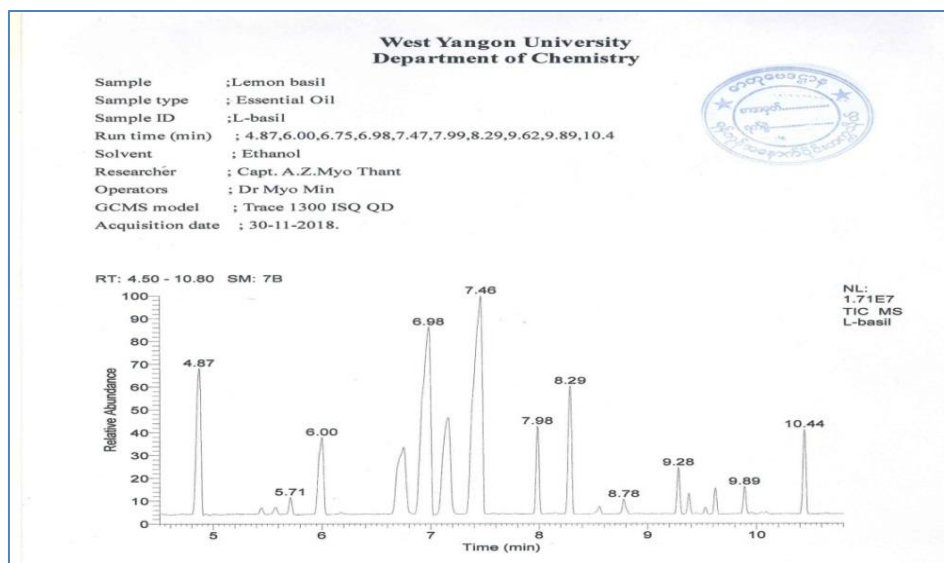


**Figure 2** (a) The preparation and (b) separation of basil essential oil by hydrodistillation

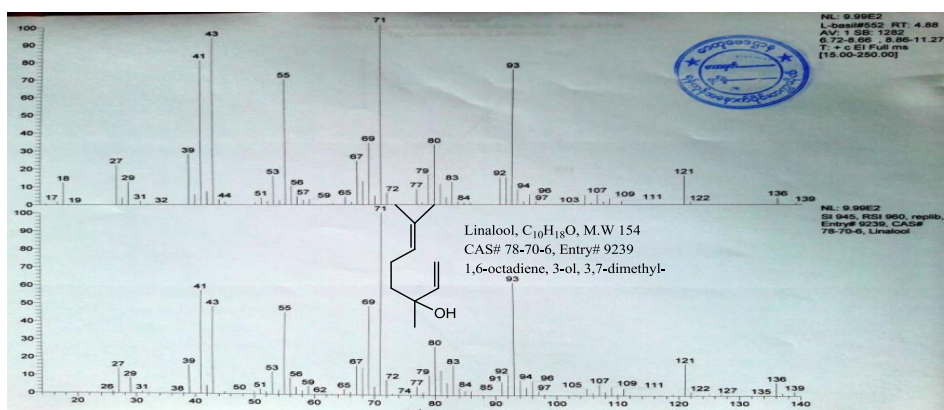
### GC-MS Analysis of the Lemon Basil Essential Oil

The prepared essential oil of basil was analyzed by GC-MS Autosampler (Trace 1300, ISQ-QD, Germany). In GC-MS analysis, GC oven temperatures were assigned by four levels in the range of 80 to 250 °C. The increasing temperature rates were controlled by 10 to 15 °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 270 and 280 °C, respectively. The molecular masses (mass fragmentations) are arranged in 15 to 250 amu ( $m/z$ ) and assigned retention time in the range of 3 to 11 min. From the GC-MS analysis, the peaks were observed at 4.8, 6.0, 6.7, 6.9, 7.4, 7.9, 8.2, 9.6, 9.8 and 10.4 min of different retention times in GC chromatogram. The mass fragmentation patterns ( $m/z$  values) of each compound were matched with that of reference compounds from GC-MS data library. By using GC-MS, each mass spectrum at different retention times could be deduced for linalool (4.8 min), terpinen-4-ol (6.0 min), *cis*-geraniol (6.7 min), *cis*-verbenol (6.9 min), citral (7.4 min), 3,3,7-trimethyl-oct-6-enoic acid methyl ester (7.9 min), 1-methoxy-3,7-dimethyl-2,6-octadiene (8.2 min), caryophyllene (9.6 min), germacrene (9.8 min) and 4-[(1E), 1,5-dimethyl-1,4-hexadiene-1-yl]-1-methyl- cyclohexene (10.4 min).

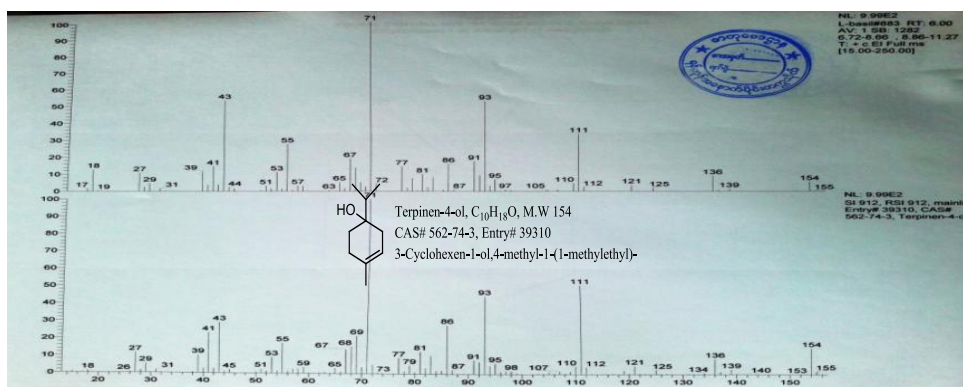
According to GC-MS analysis, citral was observed as the highest relative abundance of the essential oil from lemon basil. Other compounds such as linalool, verbenol, methyl ester compounds and caryophyllene were present in considerable compositions in the lemon basil oil. GC-MS analyzed data are shown in Figures 3 – 13.



**Figure 3** Gas chromatogram of the essential oils from lemon basil leaves

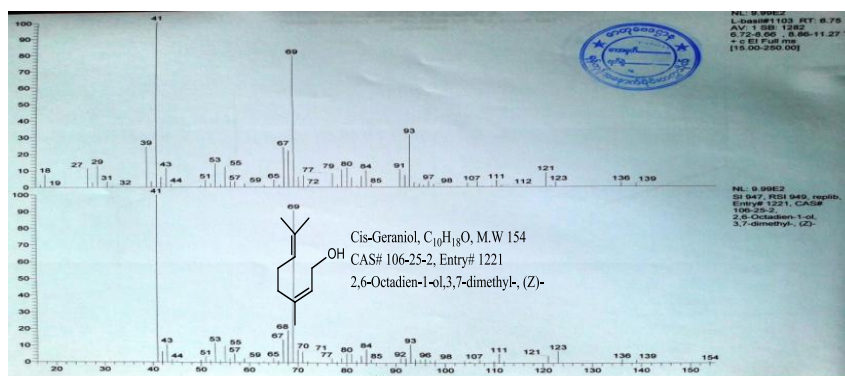


**Figure 4** Mass spectra of linalool from the prepared essential oil (4.8 min) by replib library

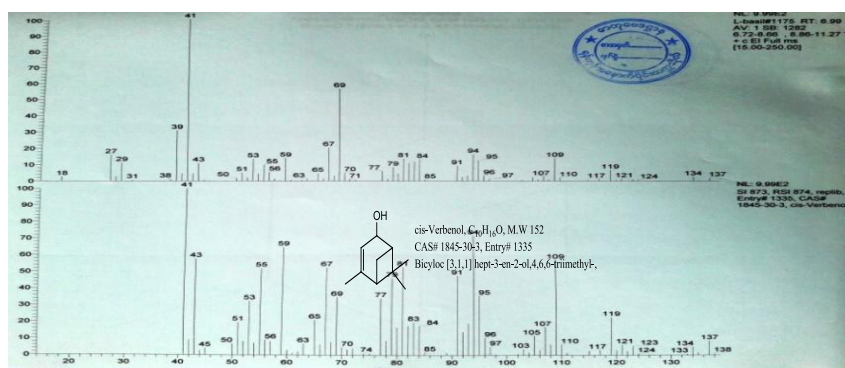


**Figure 5** Mass spectra of terpinen-1-ol from the prepared essential oil (6.0 min) by replib library

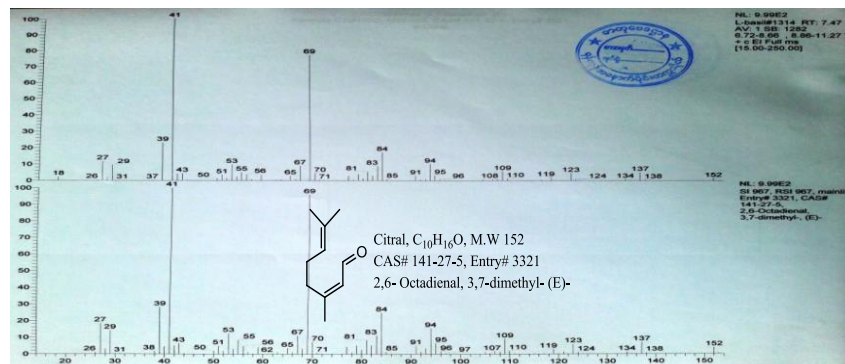




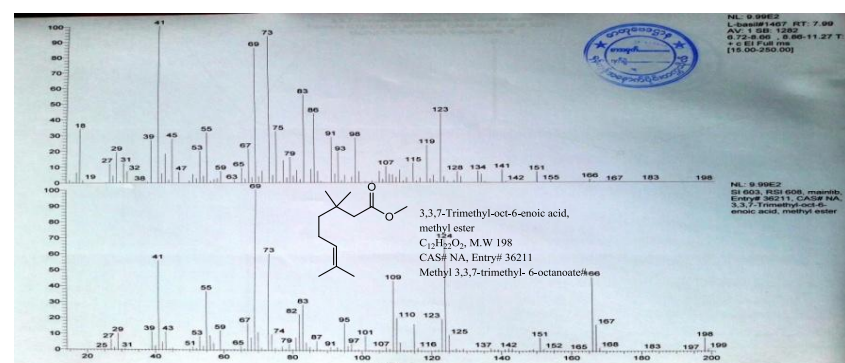
**Figure 6** Mass spectra of *cis*- geraniol from the prepared essential oil (6.7 min) by replib library



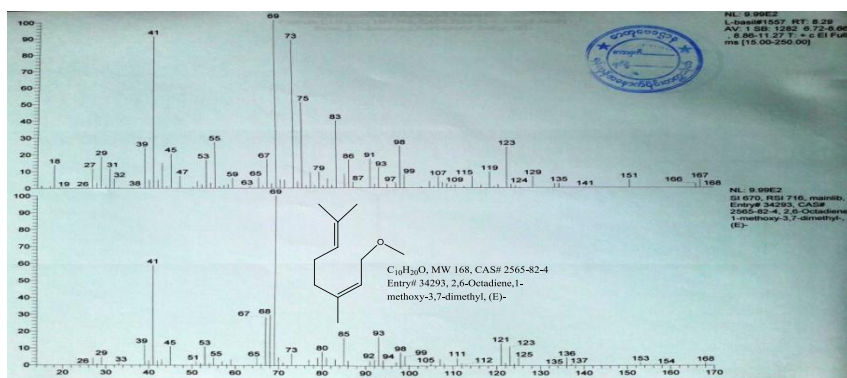
**Figure 7** Mass spectra of *cis*-verbenol from the prepared essential oil (6.9 min) by replib library



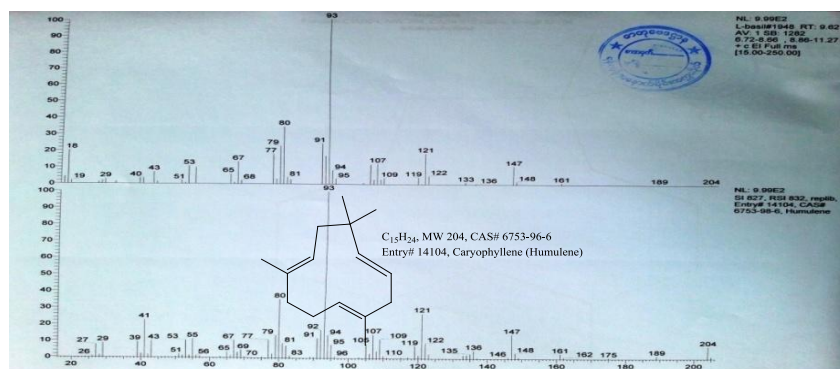
**Figure 8** Mass spectra of citral from the prepared essential oil (7.4 min) by replib library



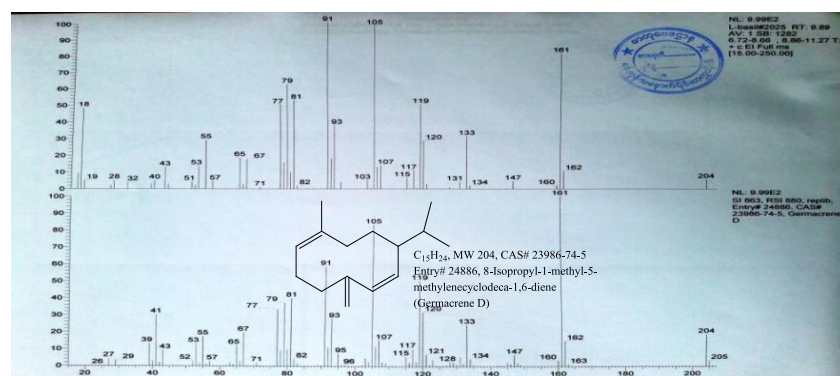
**Figure 9** Mass spectra of 3,3,7-trimethyl-oct-6-enoic acid, methyl ester from the prepared essential oil (7.9 min) by replib library



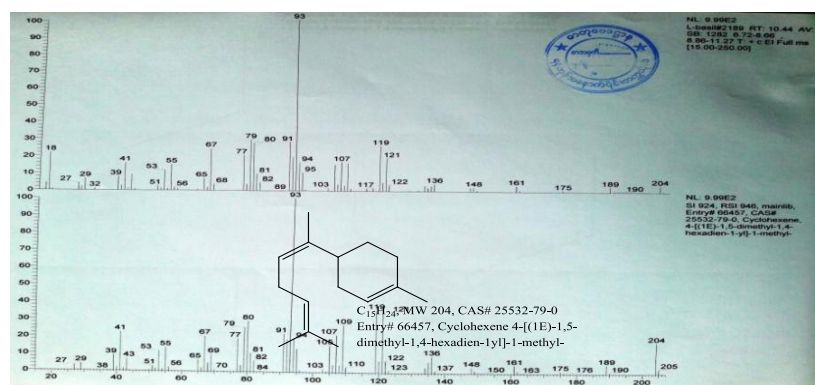
**Figure 10** Mass spectra of 1-methoxy-3,7-dimethyl-2,6-octadiene from the prepared essential oil (8.2 min) by replib library



**Figure 11** Mass spectra of caryophyllene from the prepared essential oil (9.6 min) by replib library



**Figure 12** Mass spectra of 8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene (germacrene D) from the prepared essential oil (9.8 min) by replib library

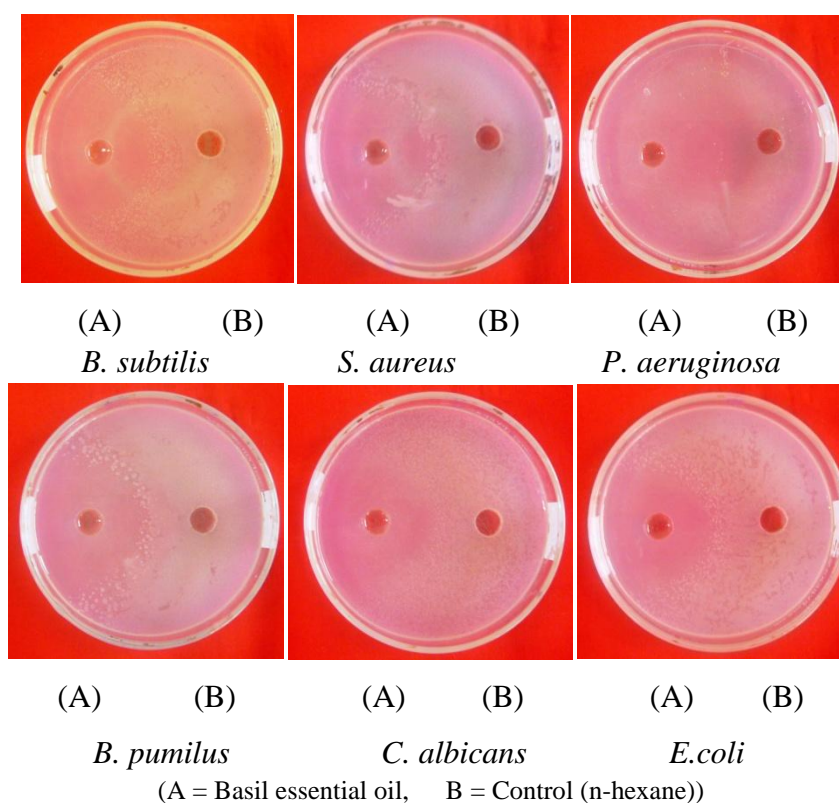


**Figure 13** Mass spectra of 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl cyclohexene from the prepared essential oil (10.4 min) by replib library

### Screening of Antimicrobial Activity

Antimicrobial activities of the essential oil from lemon basil were screened by agar well diffusion method and the results are shown in Figure 14 and Table 1. In this screening, the basil 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 are considerably high in the range of inhibition zone diameter 35 – 40 mm. The highest antimicrobial activities were observed at 40 mm with *B. pumilus* and *C. albicans* followed by *S. aureus*, *P. aeruginosa*, *B. subtilis* and *E. coli*. According to the antimicrobial screening, the basil oil may be used for the medicinal formulation of antimicrobial drugs.



**Figure 14** Screening of antimicrobial activities of basil essential oil

**Table 1** Results of Antimicrobial Activity Screening of Basil Essential Oil

Sample	Inhibition zone diameter (mm) against different microorganisms					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. pumilus</i>	<i>C. albicans</i>	<i>E. coli</i>
Essential oil	35 (+++)	38 (+++)	38 (+++)	40 (+++)	40 (+++)	35 (+++)
Control (n-hexane)	-	-	-	-	-	-

Agar well diameter: (10 mm) 10 mm - 14 mm (+) - mild activity  
 15 mm - 19 mm (++) - medium activity 20 mm - above (+++) - high activity



## Conclusion

This research concerns with the GC-MS analysis and antimicrobial screening of the prepared essential oils from lemon basil leaves. Firstly, the preparation of essential oil from basil sample was carried out by hydrodistillation method. Then, the prepared essential oil was analyzed by GC-MS method. From the analysis, the essential oil was found to contain linalool (4.8 min), terpinen-4-ol (6.0 min), *cis*-geraniol (6.7 min), *cis*-verbenol (6.9 min), citral (7.4 min), 3,3,7-trimethyl-oct-6-enoic acid methyl ester (7.9 min), 1-methoxy-3,7-dimethyl-2,6-octadiene (8.2 min), caryophyllene (9.6 min), germacrene (9.8 min) and 4-[(1E), 1,5-dimethyl-1,4-hexadiene-1-yl]-1-methyl cyclohexene (10.4 min). According to GC-MS analysis, citral was observed as the highest relative abundance of the essential oil from lemon basil. Other compounds such as linalool, *cis*-verbenol, methyl ester compounds and caryophyllene were present in considerable compositions from the lemon basil oil.

In the antimicrobial screening of basil oil, the activities on all of the tested microorganisms are considerably high in the range of inhibition zone diameter 35 – 40 mm. The highest antimicrobial activities were observed at 40 mm with *B. pumilus* and *C. albicans* followed by *S. aureus*, *P. aeruginosa*, *B. subtilis* and *E. coli*. Therefore, from these results, lemon basil oil has therapeutic potential on diseases related to microbial infections. It can be suggested that lemon basil leaves could be used as good sources of natural antimicrobial agents with possible application in food industry, cosmetics or medicine.

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