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# Evaluation of chemical composition and assessment of antimicrobial activities of essential oil of lemongrass (*Cymbopogon citratus* (dc.) stapf)

**U. G. A.T Premathilake[1], D. L. Wathugala[2*], R. M. Dharmadasa[3]**

*[1]Department of Export Agriculture, Uva Wellassa University of Sri Lanka,*
*[2]Department of Crop Science, University of Ruhuna, Sri Lanka,*
*[3]Herbal Technology Division, Industrial Technology Institute, Colombo, Sri Lanka*

***Email: lakmini077@yahoo.com***

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**ABSTRACT**

*Use of synthetic antimicrobial agents raises harmful effects for environment and human health. Therefore, this study focused on analysis of the chemical composition and assessment of the antibacterial and antifungal activities of essential oil of lemongrass (Cymbopogon citratus (DC.) Stapf). The essential oil was extracted from fresh leaves of lemongrass by hydro-distillation technique. Chemical composition of essential oil was analyzed by Gas chromatography and Mass spectroscopy (GC-MS) and Citral (35.97%), â- Citral (26.5%), cis – Verbenol (26.3%) and Citral diethyl acetal (19.58%) were identified as main chemical compounds. Antifungal activity of essential oil was tested using Poisoned Food Technique. Essential oil of lemongrass possessed promising growth inhibitory effect on tested fungal strains. Total growth inhibition was observed in Fusarium spp., Penicillium spp. and Crysosporium spp. respectively for all four different concentrations (1,000, 5,000, 10,000 and 15,000 ppm) of essential oil and Colletotricum truncatum at 10,000 ppm and 15,000 ppm concentrations. Disk diffusion method was applied to assess the antibacterial activity of essential oil (25, 50, 75, and 100%) against 3 different pathogenic bacteria strains (Escherichia coli, Bacillus cerveius and Staphylococcus aureus). Essential oil had significant (pd"0.05) growth inhibitory effect against tested pathogenic bacterial strains compared to the control. Therefore, it can be concluded that essential oil of Cymbopogon citrates (DC.) Stapf exhibited strong antimicrobial activity against tested pathogenic fungi and bacterial strains and there is a high potential to use as a natural antimicrobial agent.*

***Key words:*** Lemon grass, chemical composition, anti-microbial activity, essential oil

## INTRODUCTION

The essential oils are natural products obtained from plants. These are formed by heterogeneous and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehyde, alcohols and ketone which are deposited in several structures of the plant (Linares *et al*., 2005). Lemongrass (*Cymbopogan citratus* (DC.) Stapf) belongs to family *Poaceae* and great interest is due to its commercially valuable essential oils, it is ranked among the top ten oil bearing crops in the world (Ravinder *et al.*, 2010). Essential oils of lemongrass widely used in pharmaceutical, cosmetics, food, flavor, and agriculture industries.

The interest on bioactive potential of *Cymbopogan* essential oils and their constituents have been rapidly increased in last few years. There were a number of studies carried out to prove the antioxidant, antibacterial, antifungal and antiviral activities of lemongrass (Oloyede, 2009; Pereira *et al*., 2004; Matasyoh *et al*., 2011; Bankoel and Joda 2004; Minami, 2003). Plant pathogens including fungi, nematodes, bacteria and viruses can cause diseases or damage in plants. They cause yield losses in numerous economically important crops (Fletcher and Bender, 2006).

Different chemicals and synthetic compounds have been used as antimicrobial agents for many years. Benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors are often used in control of plant diseases in agriculture (Zhang *et al*., 2009). However, there is a risk of developing resistance towards the antimicrobial agent and high level toxic residues in the agricultural products, which harmfully affect human health. *Cymbopogon citratus* (DC.) Stapf. contains 1 to 2% essential oil on a dry basis and non-phytotoxic in nature (Paranagama, 2003). Many researches explained that citral is the chemical constituent responsible

antimicrobial properties of the lemongrass oil (Paranagama *et al*., 2003; Kakarla and Ganjiwala, 2009). Therefore, this research was carried out to analyze the chemical composition and assess the antibacterial and antifungal activities of essential oil of *Cymbopogon citratus* (DC.) Stapf.

## MATERIALS AND METHODS

### Extraction of essential oil

Lemongrass grown in Uva Wellassa University, Sri Lanka research field during March, 2015 to June, 2016 was used for this study. Essential oil of *Cymbopogon citratus* (DC.) Stapf was extracted by hydrodistillation method (Guenther, 1950). Fresh leaves were harvested and dried in shade in room temperature for 24 hours. Leaves were cut into small pieces (1 cm x 1 cm) and 200 g of each sample was put into 2 L round bottom flask and covered with distilled water. Distillation process was carried out for 2.5 hrs. The distillate was collected into a separating funnel to separate oil from water and oil was dried over anhydrous sodium sulfate to remove existing moisture from the oil.

### Analysis of the chemical composition of essential oil

Chemical composition of essential oil was analyzed by Gas chromatography and Mass spectroscopy (GC-MS). A Thermoscientific TRACE 1300 GC-MS was used and RTX WAX was used as capillary column. The operating conditions were: Injection Mode: Split (1:50). GC-MS analysis was done at 1700 eV. Helium was used as the carrier gas. Oven temperature program: 60˚C (0.00 min.), 60˚C to 240˚C (@ 5˚C/min.), 240˚C (10.00 min.) Quad temperature was 250˚C. MS Source temperature was 250˚C. Scan parameters: 50-450 (amu). Library Search: NIST.

### Antifungal activity of essential oil of *Cymbopogon citratus* (DC.) Stapf

#### Propagation and maintenance of test organisms

*Colletotricum truncatum*, *Fusarium spp*, *Penicillium spp* and *Crysosporium spp* were used as test organisms. The tested fungal strains were inoculated into Potato Dextrose Agar (PDA) medium. Plugs of mycelium were removed with a cork borer, inverted and placed in PDA plates and plates were kept in room temperature. The pure cultures were kept under refrigerated conditions (4˚C) and they were sub cultured after every fourteen days.

#### Antifungal Assay

The different concentrations (v/v) of essential oil (1,000, 5,000, 10,000 and 15,000 ppm) of *Cymbopogon citratus* (DC.) Stapf were prepared aseptically diluting in tween 20 (10%). Tween 20 (10%) alone used as the control. Poisoned Food Technique (Trivedi and Singh, 2014) was used to assess antifungal activity.

The different concentrations (v/v) of essential oil were mixed with 15mL of cooled molten PDA medium and allowed to solidify at room temperature for thirty minutes. Mycelia discs (6 mm diameter), cut out from periphery of five day old fungal cultures using a 6 mm diameter cork borer, inverted and placed in the center of each agar plate containing the essential oil. Tween 20 (10%) along used as the control. Inoculated plates were kept in room temperature after sealed and labeled properly. Diameters of mycelia were recorded after 3 days. The rate of mycelia growth inhibition (GI %) was calculated by the following formula.

$GI\% = dc\text{-}dt/dc \times 100$

Where dc, is mean colony diameter of control sets and dt, is mean colony diameter of treatment sets (Amini *et al*., 2012). The experiment was conducted in a Completely Randomized Design (CRD) with three replicates.

### Antibacterial activity of essential oil of *Cymbopogon citratus* (DC.) Stapf

#### Propagation and maintenance of test organisms

Four different pathogenic bacteria strains (*Escherichia coli, Bacillus cerveius and Staphylococcus aureus)* were used for the experiment. The test organisms were streaked on the Nutrient Agar plates and were incubated overnight at 37°C. The pure cultures were kept under refrigerated conditions (4˚C) and they were sub cultured after every fourteen days.

#### Antibacterial assay

The different concentrations (v/v) of essential oil (25, 50, 75 and 100%) of *Cymbopogon citratus*

(DC.) Stapf were prepared aseptically by diluting in EthylacetateEthylacetate alone used as the control. Disc diffusion method (Bauer and Kirby, 1966) was used to assess the antibacterial activity.

Nutrient Agar plates were prepared and allowed to solidify under aseptic conditions. Inoculums were prepared by suspending the organism in 2 mL of sterile saline solution and mixed well to create smooth suspension. Turbidity of this suspension was adjusted to a 0.5 McFarland standard. The suspension was evenly distributed on the Nutrient Agar medium. Sterilized filer paper disks were impregnated with 10 µL of essential oil in different concentrations (25, 50, 75 and 100%) and Ethylacetate as the control. Each disk was placed on the medium with a forcep and slightly pressed down to ensure complete contact with the agar surface and incubated at 37°C inside an incubator for 24-48 hours.

The zone of inhibition (mm) was measured after the period of incubation. The experiment was conducted in a Completely Randomized Design (CRD) with three replicates.

**Statistical Analysis**

Data were statistically analyzed using Analysis of variance (ANOVA) and means were compared using Tukey test. Statistical analysis was performed with Minitab 17 software.

## RESULTS AND DISCUSSION

It has been reported that lemongrass possesses strong lemony odour due to its high content of aldehyde citral, which has two geometric isomers, geranial (citral A) and neral (citral B) (Shahi et al., 2005). It has also been reported that the essential oil content of lemongrass is 1-2% on dry basis (Carlson et al., 2001). However it is obvious that the chemical composition of essential oil is varying widely upon genetic diversity, habitat and agronomic practices. Chemical composition of essential oil of *Cymbopogon citrates* (DC.) Stapf analyzed by Gas chromatography and Mass spectrometry (GC-MS) in this study is summarized in table 1.

**Table 1: Essential oil composition of *Cymbopogon citrates (*DC) Stapf**

| Chemical compound | Retention Time (RT) | Percentage (%) |
|---|---|---|
| Citral | 20.54 | 35.97 |
| β- Citral | 19.17 | 26.50 |
| Citral diethyl acetal | 18.82 | 19.58 |
| α Pinene | 5.60 | 2.00 |
| cis - Verbenol | 19.16 | 26.30 |
| Epoxy-linalooloxide | 25.68 | 0.56 |
| Geraniol | 23.55 | 1.16 |
| (R) – lavanduly acetate | 21.33 | 1.13 |

Citral (35.97%), β- citral (26.5%), cis-verbenol (26.3%) and citral diethyl acetal (19.58%) were identified as main chemical constituents in essential oil of *Cymbopogon citrates* (DC.) Stapf.

Aromatic plants are the source of secondary metabolites with biological activities. The interest on bioactive potential of lemongrass essential oils and their constituents have been rapidly increased in last few years. There were number of studied carried out to prove the antioxidant, antibacterial, antifungal and antiviral activities of lemongrass. The antifungal assay of this study clearly showed that essential oil of *Cymbopogon citrates* (DC.) Stapf possessed promising growth inhibitory effect on tested fungal strains. 100 % growth inhibition was observed in *Fusarium spp., Penicillium spp.* and *Crysosporium spp.* respectively for all four different concentrations (1,000, 5,000, 10,000 15,000 ppm) of essential oil and *Colletotricum truncatum* at 10,000 ppm and 15,000 ppm concentrations (figure 1).

Recent studies indicated that *Cymbopogon citrates* (DC.) Stapf essential oil has the potential for fungi control. Kumar *et al*. (2009) found that essential oil of *Cymbopogon citrates* (DC.) Stapf exhibited broad fungitoxic activity against *Aspergillus flavus.* Soares *et al*. (2013) have found that *Cymbopogon citrates* (DC.) Stapf essential oil is effective against *Candida albicans* as well as the emerging *Candida parapsilosis* and *Candida tropicalis* pointing to its usefulness as an antifungal agent. Tzortzakis and Economakis (2007) proved the antifungal activity against *Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger.* Helal *et al*. (2006) demonstrated

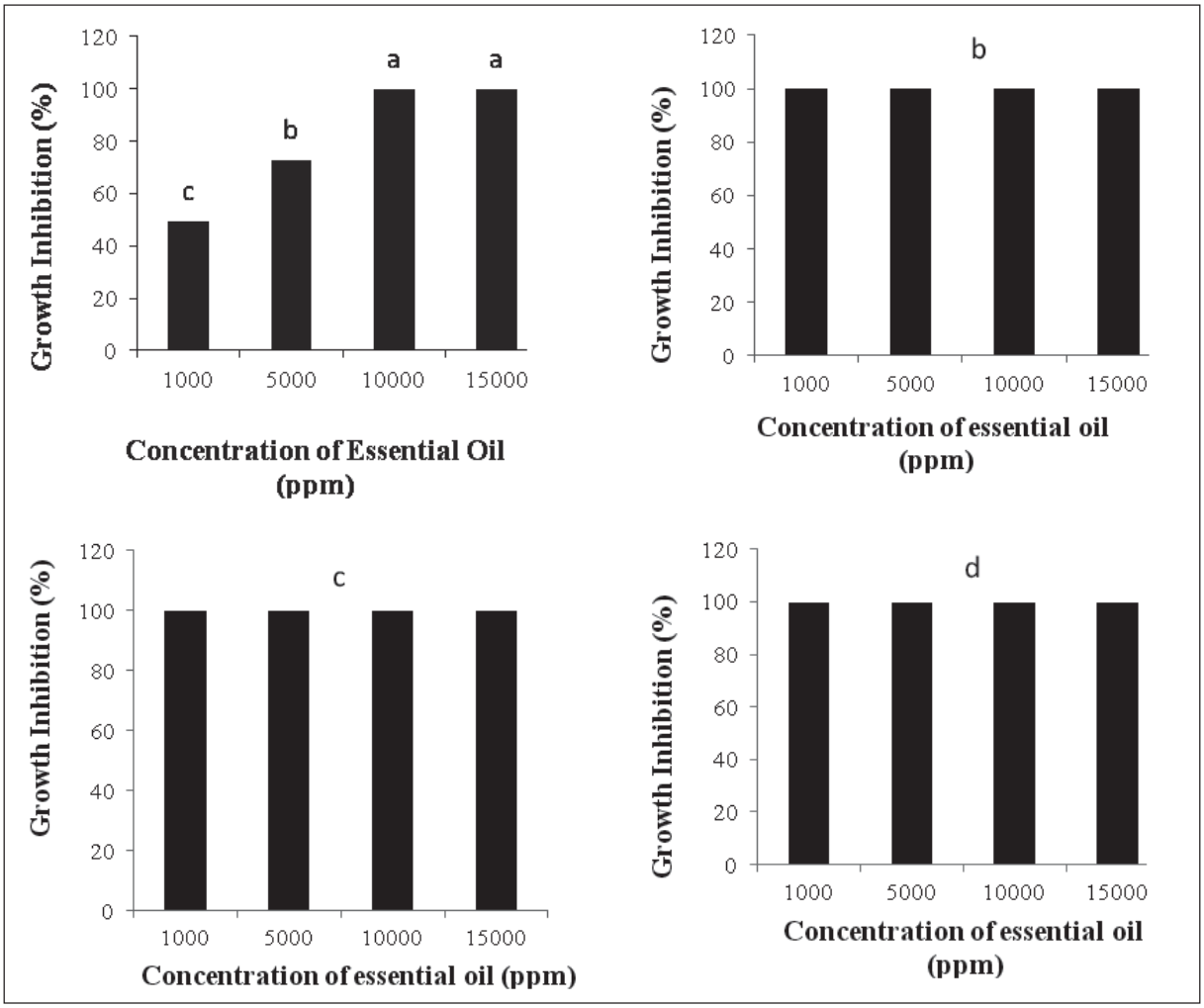


**Figure 1:** Effect of essential oil on growth inhibitions of (a) *Colletotricum truncatum* (b) *Fusarium spp.* (c) *Penicillium spp.* (d) *Crysosporium spp.*

the antifungal activity of essential oil of *Cymbopogon citrates* (DC.) Stapf against *Aspergillus niger* ML2-strain. Farhang *et al*. (2013) indicated that essential oil of *Cymbopogon citrates* (DC.) Stapf effectively controls the mycelium growth of three species of *Phytophthora* including *P. capsici, P. drechsleri and P. melonis*. Yousef (2013) studied the antifungal activity of volatiles of *Cymbopogon citrates* (DC.) Stapf against *Aspergillus niger*, *A. flavus* and A. *fumigates* and results proved that lemongrass oil produces a fungi toxic effect.

Paranagama *et al*. (2003) and Gupta *et al*. (2011) explained that citral is the chemical constituent responsible antifungal properties of the lemongrass oil. In addition to that, Palhano *et al*. (2003) mentioned that citral has proved effective in controlling mycelia growth and conidia germination of *Colletotricum gloeosporioides.*

The antibacterial assay of this study revealed that essential oil of *Cymbopogon citrates* (DC.) Stapf had significant (pd”0.05) growth inhibitory effect against tested pathogenic bacterial strains when compared to the control (figure 2).

The antibacterial activity was found progressively increasing with the increase in concentration of essential oil. Gram positive bacterial strains were more sensitive to essential oil of *Cymbopogon citrates* (DC.) Stapf with respect to all concentrations of oil than gram negative strain (*Escherichia coli*). Antibacterial activity of the essential oil of lemongrass has been studied by

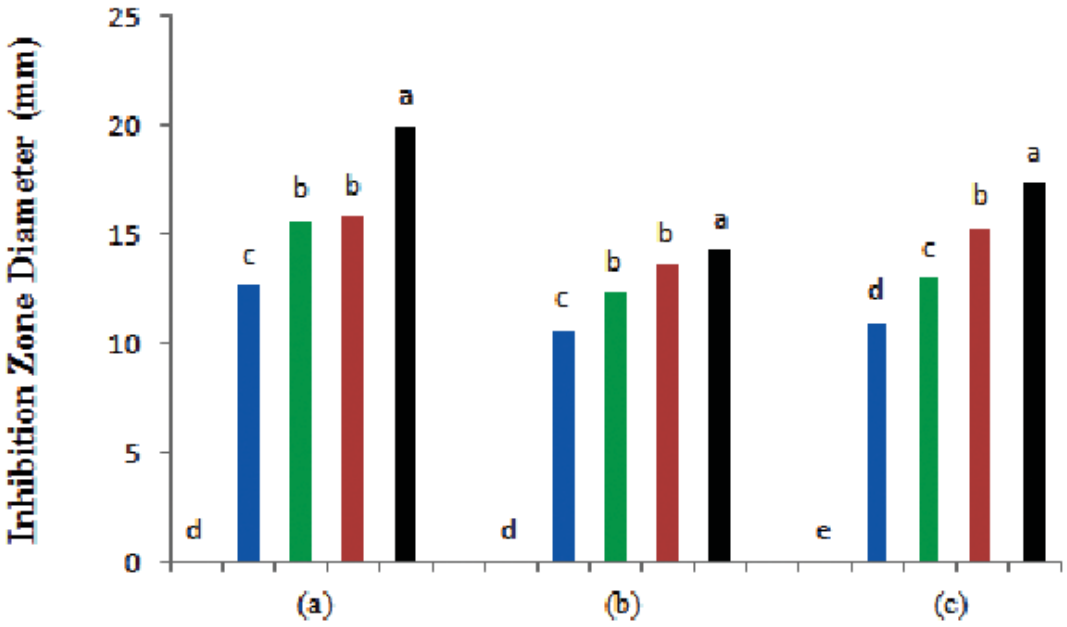


**Figure 2:** Growth inhibitions of essential oil of *Cymbopogon citrates* (DC.) Stapf on (a) *Staphylococcus aureus*, (b) *Escherichia coli*, (c) *Bacillus cerveius*

many researches. As an examples Naik *et al*. (2010) investigated antibacterial activity of lemongrass oil against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, Klebsiella *pneumoniae and Pseudomonas aeruginosa*. Lemongrass oil was found effective against all the test organisms except *P. aeruginosa.* In this study, gram positive organisms were found more sensitive to lemon grass oil as compared to gram negative organisms. Similar results were reported by Soares *et al*. (2013), Pereira *et al*. (2004), Marta War *et al*. (2004) and Alam *et al.* (1994). Nikaido (2003) suggested that higher resistance pattern of gram negative bacteria could be due to the constitution of the outer membrane that acts as a relatively effective permeability barrier. Gram-negative bacteria are inherently resistant to hydrophobic antibiotics, as their outer membrane limits the entry of these antibiotics into the cell (Pool, 2002). Saha *et al*. (2008) hypothesized that the essential oil of *Cymbopogon citrates* (DC.) Stapf is less effective against gram-negative bacteria because of the out membrane barrier that these bacteria present to hydrophobic molecules.

Numerous studies have been conducted to assess antibacterial activity of *Cymbopogon citrates* (DC.) Stapf against diverse range of gram-positive and gram-negative bacteria. Naik *et al*. (2010) reported that essential oil of *Cymbopogon citrates* was found effective against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia.* Ethanol extract exhibited high inhibitory activity against all the tested bacteria in order of sensitivity as *Staphylococcus aureus>Salmonella typhi>Bacillus aureus>Escherichia coli*, while aqueous extract was more active against *Salmonella typhi*, at the tested concentrations (Oloyede, 2009).Chemical costituents present in oil must be resposible for these antimicrobial properties. Results of Kakarla and Ganjiwala (2009) reported that citral is the main chemical constituent present in lemongrass oil reposible for antibacterial activity.Onawunmi *et al*. (1984) and Bibiana (2012) concluded that, antimicrobial activity of the oil is exhibited by Geraniol.

## CONCLUSION

Essential oil of *Cymbopogon citrates* (DC.) Stapf exhibited strong antifungal activity against *Colletotricum truncatum*, *Fusarium* spp., *Penicillium* spp. and *Crysosporium* spp. and high antibacterial activity against *Staphylococcus aureus, Escherichia coli*, *Bacillus cerveius.* Further

studies should be conducted to find out the minimum inhibitory concentrations of essential oil against tested organisms. It can be concluded that *Cymbopogon citrates* (DC.) Stapf oil is a good alternative to the synthetic chemical antimicrobial agents and can be effectively used as natural antimicrobial agent.

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