Phytochemical Constituents and Extraction of Essential Oil from *Cymbopogon citratus* Stapf. (Lemongrass) and Its Antimicrobial Activity

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Abstract

Cymbopogon citratus are commonly cultivated as culinary and medicinal herbs because of their scent, resembling that of lemons. Lemongrass oils are natural products obtained from plants. The essential oil of this plant is used in aromatherapy such as headache, rheumatism, abdominal pain and cold. The health benefits of lemongrass essential oil are fights against harmful microorganisms, reduces fever and pain, strengthens the nervous system, eliminate toxic substances in the body, combat cancer cell, support the digestive system, treat obesity and depression. In the present study, an attempt was to determine the phytochemical constituents by using test tube methods, the essential oil (0.71 %) by hydro-distillation method and antimicrobial activity of oil extract from *Cymbopogon citratus* by ager well diffusion method. Fourier Transform Infrared (FTIR) Spectrometry permitted to characterize the essential oil and revealed the chemical structure of the major component: citral (a mixture of neral and geranial aldehydes). The antimicrobial activity of essential oil extract were determined against different microorganisms such as *Bacillus subtilis, Staphylococcus, aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans, and Escherichia coli*. The measurable zone diameter, including well diameter, shows the degree of antimicrobial activity. The essential oil of lemongrass showed high activity (18-25 mm) on all tested microorganisms. The larger the zone diameter, the greater is the more activity on the test organisms.

Keywords: *Cymbopogon citratus*, essential oil, health benefits, phytochemical constituents, antimicrobial activity, ager well diffusion method

Introduction

Aromatic Plants

Aromatic plants are a particular group of plants that give out/ product fragrance and used for their aroma, used in perfumery and flavour. Here again, a number of aromatic plants and their essential oils are exclusively used also for medicine. Herbs refer to the leafy green or flowering parts of a plant (either fresh or dried), while spices are usually dried and produced from other parts of the plant, including, leaves, seeds, bark, roots, flowers and fruits (Ashish and Janesha, 2016).

Cymbopogon citratus (Lemongrass)

The name *Cymbopogon* is derived from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement (Gagan *et al*., 2011). Widely known as "Fever Grass", lemongrass is popular in the Asian countries since the primordial times for its ability to bring down fever and normalize the body temperature during hot weather. It is a tropical perennial plant, which yields aromatic oil. Lemongrass contains 1-2% of essential oil on a dry basis wide variation of chemical composition as a function of genetic diversity, habitat and agronomic treatment of culture (Lawrene *et al*., 2015).

Essential oil

Essential oils are defined as volatile substances of a complex mixture of chemical components (terpenes, monoterpenes, terpenoids, alcohols, aldehydes, and ketones) which evaporate when contact with air and are biosynthesized by plants (Parikh and Desai, 2011).

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They can be obtained from different parts of plants and are generally recognized as safe. Attention is now given to natural antimicrobial substances of plant origin since they could be a rich source of bioactive compounds (Burt, 2004; Garcia *et al*., 2008) and they might replace synthetic additives. The colour of essential oil is pale to bright yellow colour. The bright, clean, and robust aromatic top notes of lemongrass oil reflect its ability to sharpen mental clarity and elevate the mood.

Uses of lemongrass essential oil

As a vaporizer, the oil works as an effective panacea against bacteria, flu and cold. Lemon grass oil is a stimulant agent, tonic, aromas, diuretic, antispasmodic, antiseptic, febrifuge, carminative, diuretic, anti-inflammatory, anti-diabetic and is useful against rickets. People suffering from urine problems can apply lemon grass oil (Rubey, 1997).

The rich, robust, organic lemongrass essential oil works equally well as a mood elevator, a potent skin and body toner, and a natural promoter of comfort and ease of bodies. Pure lemongrass essential oil also does triple duty as an insect repellent and a key component in the culinary and perfume industries. Despite its ability to repel some insects, such as mosquitoes, its oil is commonly used as a 'lure' to attract honey bees.

On account of their diverse uses in pharmaceutical, cosmetics, food, toilet soaps, flavour and agriculture industries. It also used as a pesticide and a preservative (Mohamed Hanaa *et al*., 2012).

Aim and objectives

The aim of this study was to investigate the phytochemical constituents, extraction of essential oil and the effect of antimicrobial activity on the essential oil from lemongrass.

Materials and Methods

Experimental apparatus

Most of the chemicals and reagents were employed in the study, from internationally established companies such as BDH, Kento, Merck, Hopkin and Williams and also locally from the commercial chemicals stores in Yangon. The following instruments were used: Electronic Balance, Oven, Grinding Machine, Distillation set, Condenser, FT IR Spectrometer, Hot-air sterilizer, Autoclave, Clean bench, Water bath and Incubator.

Plants materials and identification

The fresh sample was collected from the farm located at Hlegu Township, Yangon Region, in June, 2018. The identification of the sample was conducted with the Department of Botany, Dagon University.

Sample preparation

The whole plant (leaf sheath and the blade) was used for phytochemical tests and extraction of the essential oil. Seven hundred grams above-ground parts of the plant samples were used for the process. The wilted leaves were removed and the plants were then washed with water. The fresh plants were cut into small pieces, about one and half cm or smaller and crushed using mortar and pastel to increase the surface area, then subjected to assays immediately. Fresh plants were used not more than three weeks after being collected and stored. Theese fresh samples were used for extraction of essential oil. Air-drying was carried out for 7 days under the shade at room temperature. The air-dried plants were chopped into small pieces and grind into purely fine powder and stored in airtight bottle containers (Fig. 1). The dried samples were used within three weeks after these were stored for phytochemical

test (Anggraeni *et al*., 2018).

Figure (1). Sample preparation of lemongrass

Phytochemical investigation

The phytochemical constituents test was performed to determine the type of active compounds contained in the extracts (Prashanth and Krishnaiah, 2014). The constituents in 1 % hydrochloric acid, ethanol and water extracts of lemongrass were preliminary investigated by the screening procedures. The resultants are presented in Table 1 and Figure 3.

Extraction of essential oil by steam distillation

Two hundred grams of the fresh sample and distilled water (500 mL) were placed in 1 liter round bottomed flask. The flask was subjected to steam distillation set which was joined to water condenser. The heating mantle was regulated to maintain a proper rate of condensation of the steam during the oil extraction at 70°C for 3 hours (Sukari *et al*., 2008). The lemongrass oil was extracted from the distillate with petroleum ether. The mixture were combined and dried over anhydrous sodium sulphate. Finally, the petroleum ether solution was evaporated or removed by using a rotatory evaporator to give a yellowish essential oil and stored in sealed vials at 4°C before analysis (Fig. 2) (Tajidin *et al*., 2012).

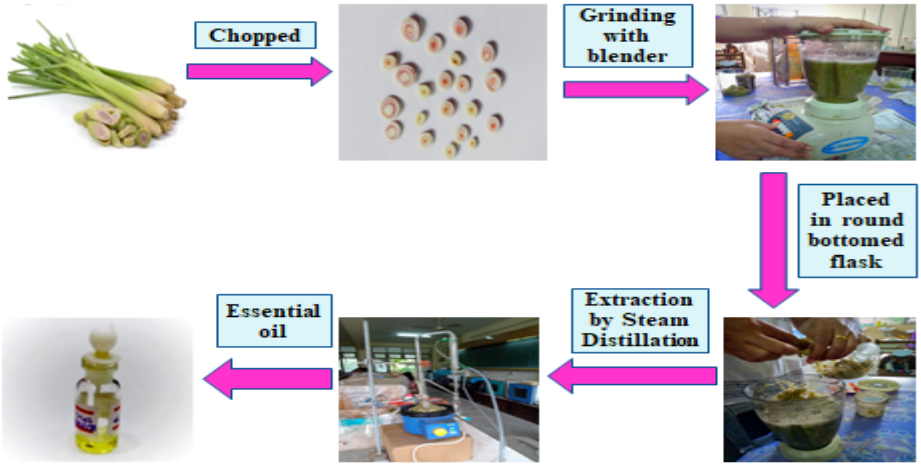

Figure (2). Sample preparation and extraction of essential oils by steam distillation

Yield percent of essential oil content

The samples were removed after freeze drying and then weighed with a balance. The percentage yield of essential oil was determined using the formula where the amount of essential recovered (g) was determined by weighting the oil after moisture was removed. The essential oil percentage was calculated by following formula.

$$\text{Percentage of essential oil} = \frac{\text{Essential oil weight}}{\text{}} \text{ X } 100$$

(Fresh sample weight) (air – dry sample factor)

Fourier Transform infrared (FTIR) spectrometry

Spectrometry of the essential oil of lemongrass was carried out with a Spectrum One FTIR Spectrometer, (Summit PRO, US). An essential oil sample was placed directly on the surface of the Everest ATR top plate at room temperature; measurements were performed in the IR region at 3937-400 cm^{-1}. The crystal used was diamond.

Antimicrobial examination of essential oil

Antimicrobial activity of the ethanol extract of the lemongrass oil was determined by using Agar-well diffusion method on six selected organisms in Central Research and Development Centre (CRDC), Insein, Yangon.

Antibacterial activity of essential oil

Nutrient agar (4.6 g) and agar (1 g) were dissolved in 50 mL distilled water. Nutrient agar was boiled and 20-25 mL of the medium was poured into the conical flask and plugged with cotton wool and autoclaves at 121°C for 20 min and cooled down to 30-35 °C. After cooling, bacteria suspension of each bacterial strain (0.02 mL) was added and poured into sterilized petri-dishes. The seeded plates were allowed to dry in room temperature for 20 min. A standard cork borner of 10 mm diameter was used to cut uniform wells on the surface of the solid medium. Essential oil (0.15 mL) were filled into each of the wells and incubated at 37°C for 24 hours. The inhibition zone which appeared around the agar well indicated the presence of antibacterial activity. The screening of antibacterial activity was assessed based on the diameter of the clear zone surrounding the disc in millimeter (mm) (Yagub *et al*., 2013).

Antifungal activity of essential oil

Potatoes (20 g) in 100 mL distilled water were heated on hotplate until boiling and filtered. Potato infusion was obtained. Dextrose (2 g) and agar (1.5 g) were added to potato infusion to obtain the potato dextrose agar medium. The resulting potato dextrose agar medium was autoclaved at 121 °C for 20 min and cooled down to 30-35 °C. After cooling, fungal suspension of each fungal strain (0.1 mL) was added and poured into petri-dishes. The seeded plates were allowed to dry in room temperature for 20 min. A standard cork borner of 10 mm diameter was used to cut uniform wells on the surface of the solid medium. Essential oil (0.15 mL) was filled into the well and incubated at 37°C for 72 h. Antifungal activities in terms of zones of inhibition was recorded (Ibrahim *et. al*., 2011).

Results and Discussion

Preliminary phytochemical investigation

Phytochemicals are chemical derived from plants and term is often used to describe the large number of secondary metabolic compounds found in plants. They are characterized by multilateral pharmacological activity and broad spectrum of therapeutic actions. Phytochemical screening assay is a simple, quick and inexpensive procedure that gives the researcher a quick answer to various types of phytochemicals in a mixture and important tool in bioactive compound analyses.

Preliminary phytochemical investigation was performed to examine the different types of chemical constituents present in the powdered of lemongrass. Phytochemical tests

were carried out by test tube method. Ethanol and water extracts of lemongrass contain α-amino acid, flavonoids, glycoside, carbohydrates and phenolic compounds. Alkaloids, saponins, tannins, starch and reducing sugars were absent (Fig. 3 and Table 1).

Figure (3). Phytochemical tests of various extracts of Lemongrass

Table (1).Results of phytochemical composition in lemongrass

No	Test	Extract	Test reagent	Observation	Result
1	Alkaloids	1% HCl	Wagner's reagent	No brown ppt	–
			Mayer's reagent	No white ppt	–
2	α-amino acids	H_2O	Ninhydrin reagent	Purple spot	+
3	Carbohydrates	H_2O	10% α-Naphthol Conc: H_2SO_4	Red ring	+
4	Flavonoids	EtOH	Mg ribbon, Conc:HCl	Pink color	+
5	Glycosides	H_2O	10% lead acetate	White ppt	+
6	Phenolic Compounds	H_2O	5% $FeCl_3$, $K_3Fe(CN)_6$	Deep blue colour	+
7	Saponins	H_2O	Distilled water	Not frothing	–
8	Starch	H_2O	Iodine solution	No bluish black colour	–
9	Tannins	EtOH	5% $FeCl_3$	No green ppt	–
10	Reducing sugars	H_2O	Benedict's solution	No ppt	–

(+) = present, (-) = absent and (ppt) = precipitate

Essential oil yield

A pale yellow essential oil with yield of 0.71 % was obtained from fresh lemongrass plant. This yield agree with some works who reported that oil content of a normal cut should average 0.25 - 0.50 %, but with good management and selected strains could be yielded up to 0.66 - 0.90 % (Maiti *et al*., 2006). As observed in other works, when using a simple distillation procedure, sometimes the steam do not reach the center of the ground plant; the yield of the oil extraction is not optimized. Essential oil content was influenced by factors

such as temperature, light intensity, soil moisture, fertilizer, and maturity stage. The percent yield of essential oil is depend upon the sample condition such as fresh or dry or sample collecting period of season.

FTIR analysis

The essential oil was also confirmed by Infrared spectrum indicating the functional groups present in lemongrass oil. According to FTIR spectral data, the absorption band of the lemongrass essential oil were described in Fig. 4. In the spectrum of essential oil, the peaks were observed at 3455 cm^{-1} which may be due to the presence of O-H stretching bands, 2966 cm^{-1} represents asymmetric stretching of $–CH_3$ group, 2916 and 2856 cm^{-1}indicates symmetric and asymmetric stretching of $–CH_2$ groups, 1672 cm^{-1}also indicates vibration of C=C (cis and trans), confirming the presence of conjugated double bonds (C=C-CHO) in citral which are common in acyclic monoterpenes. The peak at 1442 cm^{-1}indicates bending of $-CH_2$ group. At 1376 cm^{-1} bending of $–CH_3$ group is observed. From 1194 to 1120 cm^{-1}, C-OH stretching of alcohol or phenolic group, stretching of –C-O and vibrations of the C=O and –CH skeleton are observed. At 984 cm^{-1}, OH bending of alcohol and at 841 cm^{-1}, substitution in 1, 3 and 1, 4 are observed. At 450 to 407 cm^{-1}, stretching of metal and functional group of organic compound are observed. It may be confirmed that the essential oil contains geranial, nerol and other alcohols in small proportions. These peaks are in agreement with the results of literature (Vazquez-Briones *et al*., 2015)

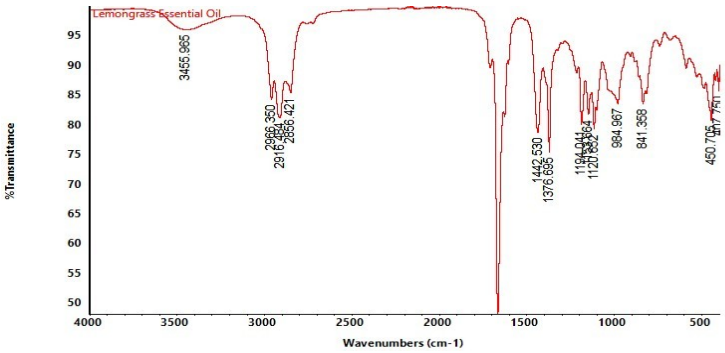

Figure (4). FT IR spectrum for essential oil of lemongrass

Antimicrobial activity

Bacterial, small microorganisms are widely distributed in soil, air, water, milk dust on the surface of fruit. Antimicrobial means, which kills or stops the spread of microorganisms.

In this present work, antimicrobial activity of essential oil extract was screened on six different strains of microorganisms *Bacillus subtilis*, *Straphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli*, by agar diffusion method. The measurable zone diameter, including well diameter, shows the degree of antimicrobial activity. The well diameter is 10 mm in this experiment (Fig. 5). The larger the zone diameter, the greater is the more activity on the test organisms.

The results of the assay of *C.citratus* essential oil against six microorganisms were shown in Table 2 and Fig. 6. It was observed that essential oil of lemongrass showed high activity (18-25 mm) on all tested microorganisms. Therefore, from this observation, it can be inferred that essential oil of lemongrass may be effective in the medicinal formulation for the treatment of some disease caused by the above organisms.

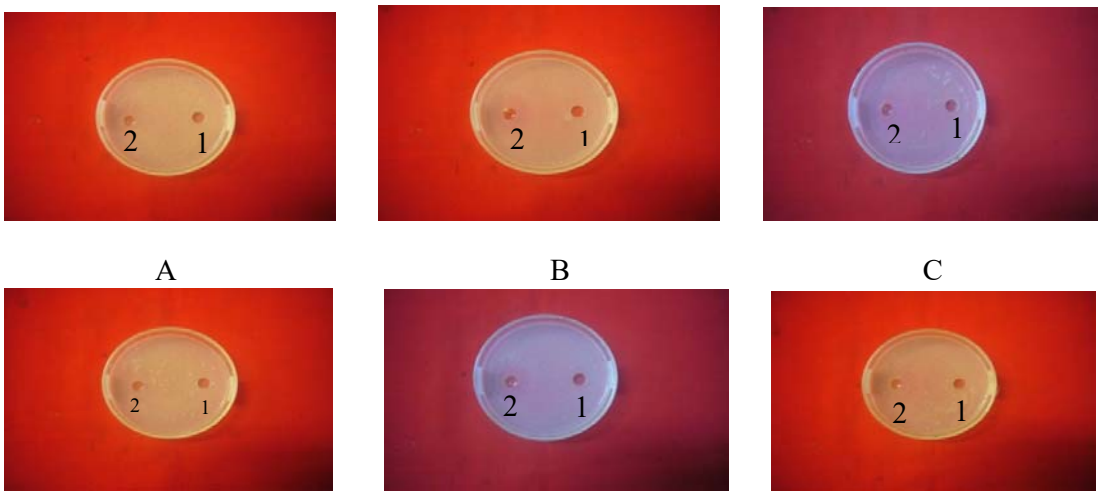

Figure (5). Inhibition zone for antimicrobial activity screening of lemongrass (1) Control and (2) Essential oil against *Bacillus subtilis* (A), *Staphylococcus aureus* (B), *Pseudomonas aeruginosa* (C), *Bacillus Pumilus* (D), *Candida albican* (E) and *Escherichia coli* (F)

Table (2). Inhibition zone diameters of lemongrass essential oil

No	Microorganisms	Inhibition zone diameter (mm)	
		Essential oil	Control
1	*Bacillus subtilis*	18 (++)	-
2	*Straphylococcus aureus*	24 (+++)	-
3	*Pseudomonas aeruginosa*	25 (+++)	-
4	*Bacillus pumilus*	20 (+++)	-
5	*Candida albicans*	18 (++)	-
6	*Escherichia coli*	20 (+++)	-

Agar well – 10 mm
10 mm~14 mm (+) (low)
15 mm~19 mm (++) (medium)
20 mm ~ above (+++) (high)

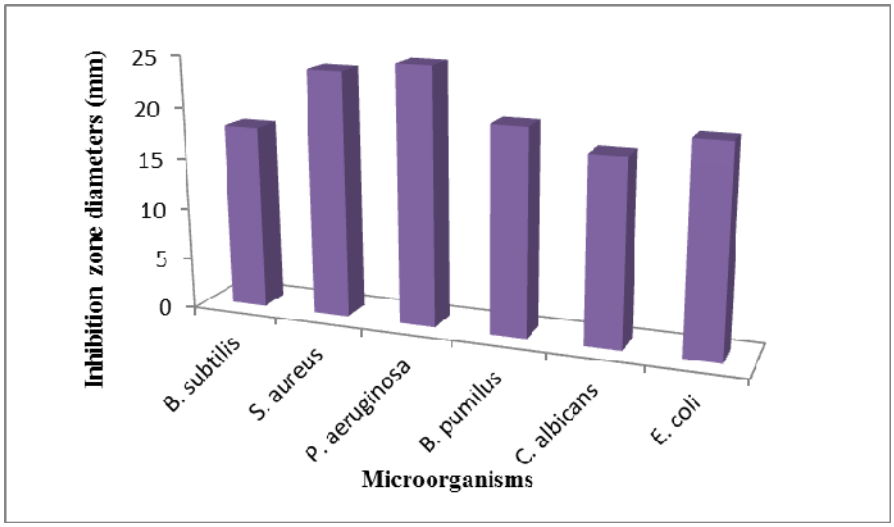

Figure (6). Histogram of inhibition zone diameters for ethanol extract of lemongrass oil

Conclusion

Lemongrass oil has variety of uses and its shows number of biological activities also. It is used in antimicrobial agent, preservative and pesticide. This study demonstrates the extract of essential oil from the *Cymbopogon citratus* by using steam distillation. Essential oil (0.71 % yield) extracted from the fresh lemongrass. In this results, essential oil exhibited antimicrobial activity with inhibition zone diameters ranged between 18 ~ 25 mm. According to the results of the screening of antimicrobial activity of lemongrass oil, it may be possible to use the oil in formulations against some medically important pathogens that are implied in various infections and used for the formulation of antimicrobial drugs.

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