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EVALUATION OF QUALITATIVE PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF THE ESSENTIAL OIL OF *Cymbopogon citratus* LINN

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Abstract

Antibiotic resistance has become a significant public health problem; thus, it is highly essential that new antibiotics continue to be developed. Recently,

Keywords:
Cymbopogon citratus, Antimicrobial Resistance, Essential Oil, Infectious Disease, Pathogenic Organisms.

INTRODUCTION

Essential oils are aromatic and volatile liquids obtained from plant material, including flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plants (Hyldgaard *et al*., 2012). Throughout history, these oils have been regarded with great interest, although many of their uses have been lost with time. The applications of essential oils for different purposes are varied and include not only their use in cooking to enhance the taste and health benefits of food, but also their application in the manufacture of perfumes and cosmetics.

Essential oils are more than just highly concentrated plant extracts. Most possess potent medicinal qualities, and many are valued for their exceptional cosmetic qualities. While the whole plants or plant parts they are derived from possess beneficial qualities, essential oils

studies have been carried out to ascertain the antimicrobial benefits of essential oils and how they can be incorporated into the fight against infections and infectious diseases. This research was carried out with the purpose of verifying the effectiveness of the essential oil of lemongrass for the treatment of pathogenic organisms. Lemongrass oil was investigated for activity against two gram positive organisms (*Streptococcus pneumonia* and *Staphylococcus aureus),* two gram negative organisms (*Escherichia coli and Salmonella typhi)* and fungi (*Candida albicans)* using Agar Diffusion Method and Broth Dilution Method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the Broth Dilution Method. Lemongrass was found to be effective against all the test organisms. The results depicted that Lemongrass extracted oil generated different inhibition zones diameters (IZDs) that ranged from 6-19.75mm, which were dose independent. The tested organisms were found to be inhibited by lemongrass oil even at low concentrations. Also, the test organisms were inhibited by Lemon grass oil at lower concentrations in Broth Dilution Method as compared to Agar Diffusion Method. Thus, it is concluded that lemongrass oil would be helpful in the treatment of emerging infections caused by susceptible pathogenic organisms.

are much more powerful. Sometimes referred to as ethereal oils or volatile oils, essential oils carry the actual essence or fragrance of the plants from which they are extracted (Guenther, 1950).

Essential oils are odiferous, highly volatile substances present in plants. Due to their volatility, these substances can be isolated by means of steam distillation from an aromatic plant of a single botanical species and can be detected by both smell and taste (Koul, *et al*, 2004). Individual essential oils are known by the name of the plant from which they are derived and the odor is similar to that of the part of the plant from which they are obtained, although the aroma is generally more intense. There are various definitions of essential oils, but the most accurate is probably that proposed by Schilcher, Hegnauer, and Cohn-Riechter (1987) "Essential oils are products or mixtures of products, which are formed in cytoplasm and are normally present in the form of tiny droplets between cells. They are volatile and aromatic". They are composed of "mixtures of fragrant substances or mixtures of fragrant and odorless substances", where a fragrant substance is defined as a "chemically pure compound which is volatile under normal conditions and which owing to its odor can be useful to society". For its part, the International Organization for Standardization (ISO) has defined essential oils as "product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase—if any— by physical processes", going on to specify that "the essential oil can undergo physical

treatments, which do not result in any significant change in its composition" (ISO 9235,2013)

As defined above, essential oils are complex mixtures, usually formed by numerous constituents, generally liquid, but sometimes solid. At room temperature, these mixtures usually range from colorless to slightly yellowish in color when freshly distilled, with an aromatic smell, very clean to the touch, and easily absorbed by the skin. In contrast with fatty vegetable, animal or mineral oils, a drop of essential oil on paper disappears quickly, taking between a few minutes and a few days, depending on the temperature (Dijilani and Dicko, 2012),

Cymbopogon citratus, popularly known as lemon grass or citronella grass is a member of the Poaceae family and belongs to the genus Cymbopogon. The genus Cymbopogon constitutes of approximately 140 species that show widespread growth across the semi-temperate and tropical regions of Asian, American and African continents. Australia and Europe are home to only a few species of lemon grass.

The members of the Cymbopogon genus produce volatile oils and thus are also known as aromatic grasses. A strong lemon fragrance, a predominant feature of this grass, is due to the high citral content of its oil. The redolence of the oil enables its use in soaps, detergents, etc. As a good source of citral, it finds an application in the perfumery as well as food industries. It is also the starting material for the manufacture of ionone's, which produce Vitamin A. Lemon grass, contains several bioactive compounds that impart medicinal value to it. Considerable evidence is available for its ethno-pharmacological applications (Adjorjan and Buchbauer, 2010). According to the WHO, herbal medicine is considered an important part of the healthcare industry by more than two-thirds of the population in developing countries. Apart from an overall description of lemon grass, this review article also highlights its medicinal properties that make it a potent herb for pharmacognostic applications.

The use of lemon grass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmic, pneumonia and vascular disorders. Researchers have found that lemon grass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties.

The development of bacterial resistance to presently existing antimicrobials has stimulated the search for other substances with antimicrobial properties.

MATERIALS AND METHODS

MATERIALS

Chemicals and reagents

- *Cymbopogon citratus*-leaves- 3kg
- Blood- 50mls
- Microbial isolates of: *Streptococcus pneumonia*, *Salmonella typhi*, *Escherichia coli,*
- *Staphylococcus aureus* and *Candida albicans.*

- Lemon grass essential oil
- Nutrient agar (Himedia)
- McConkey agar (Himedia)
- Potato Dextrose Agar (PDA) (TM media)
- Tween-80 (Polysorbate 80)

Materials:

- Petri dishes
- Test tubes
- Autoclave
- Incubator

METHODS

Sample collection

Lemon grass (*Cymbopogon citratus*) leaves grown in Abuja, Nigeria-Collected and identified in the herbarium of Bingham University, Karu were used for this work.
Microbial isolates were procured from the pharmaceutical microbiology lab in Bingham University.
All glass wares used in the study are of standard quality supplied by authorized dealers. The glass wares used in the experiment were sterilized in the autoclave at 121℃, 15 lbs pressure for 30 minutes.
All chemicals used during the investigation are of analytical grade (AR) and obtained from standard suppliers

Extraction of essential oil

The lemongrass essential oil was extracted by steam distillation which was conducted in an industrial steam distillation for 3hours, according to the method recommended by Koul *et al,* 2004.Three kilogram of fresh lemongrass leaves were washed clean and placed in the raw material chamber of the steam distiller, water was added into the water chamber and it was heated up to 100℃ for 3 hours. The steam condensed and the essential oil suspended above the water at the separating funnel. The distillate is collected and then separation of the oil phase from the water phase of the distillate was done using a separating funnel. Calculation of the percent yield of the extraction

$$\text{Yield\%} = \text{weight of the dry extract} \times \frac{100}{weight\,of\,the\,dry\,plant}$$

$$= \frac{25 \times 100}{4000}$$

$$= \frac{2500}{4000}$$

$$= 0.625$$

$$= \sim 0.63\%$$

Preparation of essential oil

The essential oil was diluted to five different concentrations. Each tube was a 10-fold dilution starting from the undiluted tube. The dilution liquid used is Tween-80.

All five test tubes were filled with 9ml of Tween-80. The first tube was a 1:10 dilution, the second a 1:100, the third 1:1000, the fourth 1:10000 and the fifth 1:100000 that is; 10%, 1%, 0.1%, 0.01% and 0.001%.

A test tube containing 2mls of the undiluted essential oil was prepared, 1ml of undiluted essential oil was drawn from it with a pipette and transferred into the test tube labeled 1:10 which already contains 9ml of the diluting liquid. After which it was thoroughly mixed together.

1ml of solution was then taken from tube 1:10 and added to the second tube 1:100. It is also thoroughly mixed. This process was repeated until the last test tube 1:100000.

Calculation of the final dilution ratio (D_t)

$Dt = D1 \times D2 \times D3 \times D4 \times D5$

$D_t = 10 \times 10 \times 10 \times 10 \times 10 = 100000$

This means that the concentration of the essential oil is now 100,000 times less than the original undiluted solution,

Phytochemical Screening

Standard preliminary phytochemical qualitative analysis of the LGEO was carried out for the presence or absence of biologically active compounds or secondary metabolites using standard procedures by Banu, and Cathrine, 2015, Harborne, 1998.

Preliminary Qualitative Analysis

Test for alkaloid

Mayer's test

To a few ml of sample extract, two drops of Mayer's reagent were added along the sides of the test tube. An appearance of white creamy precipitate indicated the presence of alkaloids.

Test for flavonoids

The extract (1ml) was taken in a test tube and added few drops of dilute NaOH solution were added. An intense yellow color appeared in the test tube. It became colorless on addition of a few drops of dilute acid which indicated the presence of flavonoids.

Test for saponins

The extract (1 ml) was diluted with distilled water and made up to 20 ml the suspension is shaken in a graduated cylinder for 15 minutes. A 2cm layer of foam indicated the presence of saponins.

Test for steroids

The extract 1ml was taken in a test tube and dissolved with chloroform (10ml), then added equal volume of concentrated sulfuric acid to the test tube by the sides. The

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upper layer in the test tube was turned into red and the sulfuric layer showed yellow with green fluorescence. It showed the presence of steroids.

Test for tannins

The extract (3 ml) was taken in a test tube and diluted with chloroform and added acetic anhydride
(1 ml). Finally, sulfuric acid (1 ml) was added carefully by the side of the test tube to the solution.
A green color was formed which showed the presence of tannins.

Test for terpenes

Exactly 0.1g of the plant extract was added into 1ml of chloroform. Acetic anhydride (1ml) and 2 drops of conc. H_2SO_4 were added. A pink colour which changed to bluish green on standing indicated the presence of terpenes.

Test for cardiac glycoside

Exactly 0.1g of the plant extract was dissolved in 1ml glacial acetic acid containing one drop of ferric chloride solution. Conc. H_2SO_4(1ml) was added and the presence of a brown ring indicated the presence of deoxy sugar characteristic cordenolides.

Antibacterial screening

Preparation of media

Nutrient agar (NA), MacConkey agar and Potato Dextrose Agar were prepared according to manufacturer's instructions and blood agar was prepared by adding human blood that has been warmed to room temperature into nutrient agar that had cooled to 40°C.

Culturing of *Streptococcus pneumonia*

Using a sterile inoculating loop, a single colony of *Streptococcus pneumonia* picked up from the agar plate and streaked across the entirety of a fresh plate which was incubated at 37°C with 5% CO^2 overnight. A sterile inoculating loop was then used to inoculate bacterium from the lawn of the plate into 5ml of nutrient broth.

Culturing of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*.

The surface of frozen *E. coli*, *S. aureus* and *S. typhi* isolates were lightly scratched with a sterile inoculating loop which were used to streak across the entirety of already prepared McConkey agar plate (for *E.coli)* and nutrient agar plates (for *S. aureus* and *Salmonella typhi*). The plates were inverted and incubated at 37°C overnight. After which a sterile inoculating loop was used to inoculate bacterium from the lawn of the plates into 5ml of nutrient broth.

Culturing of *Candida albicans*.

Potato dextrose agar (PDA) plate was prepared according to manufacturer's instruction. The isolates were picked up with a sterile inoculating loop and streaked

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across the PDA plate. The plates were then inverted and incubated at 30° C for 72 hours.

Sensitivity testing Agar well diffusion method

Antimicrobial activity of lemongrass leaves essential oil was tested against the organisms by agar well diffusion method. Nutrient agar media was prepared and added into individual sterilized petridishes separately on a level plate form and allowed to solidify. Exactly 1 mL of active cell suspension of organisms was spread with the help of sterilized swabs on the agar surface uniformly.

After culturing the organisms on agar plates, wells of 6mm size were made with sterile borer on the agar plates and 100µl of different concentration of the oil was dispensed. Tween-80 (Polysorbate 80) was used as diluent to make different concentrations of the oil. Tween-80 negative control was put. The in-vitro antibacterial activity was evaluated against the pathogen by measuring the diameter (mm) of zone of inhibition (i.e. no microbial growth produced by the sample) against the test organisms using graduated scale.

The plates were incubated at 37±1 °C for 24 hours for bacteria and 30°C for fungi for 72 h. The diameters of the inhibitory zones were measured in millimeters and mean value was calculated.

The procedure was performed aseptically in duplicates.

Before conducting experiments all the conditions were standardized to determine MIC and MBC values in vitro.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The determination of MIC of the essential oil of lemongrass on the test bacterial strains was done using broth dilution method as explained by Hammer *et al.,* (1999) with different concentrations of oil. The cultures of the test strains were prepared by inoculating the test strain in sterilized test tube containing 5ml nutrient broth which was then adjusted to the 0.5 McFarland standard. The tubes were incubated overnight at 37°C. The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of the test compound to kill the microorganisms.

The test tubes containing 10ml of sterilized nutrient broth with 0.5% Tween-80 were inoculated with different concentrations of lemongrass oil ranging from 0.001% - 10% (v/v), nutrient broths with Tween-80 without oil were used as negative control. An aliquot of bacterial suspension (25μl) from the overnight culture was measured with a micropipette and added to each test tube uniformly. The tubes were observed for turbidity after 24 hours.

The lowest concentration at which no visible growth occurred in either culture tubes was taken as MIC. Then the tubes showing no increase in the turbidity at each time interval 24-48 hours were streaked on nutrient agar plates to check the bacterial growth. Each trial was repeated twice.

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Results

A pale yellow essential oil with yield of 0.63% (v/v) was obtained from fresh lemongrass plant.

Antimicrobial activity

The antimicrobial activity of essential oil revealed a higher activity against bacteria than fungi at higher concentrations as shown in Table 1 below.

Table 1: Antibacterial activity of lemon grass oil against various selected pathogenic bacteria

ORGANISM	Zone of inhibition (mm)				
Bacteria	**10%**	**1%**	**0.1%**	**0.01%**	**0.001%**
Streptococcus pneumonia	17.00	16.00	11.50	11.25	10.25
Staphylococcus aureus	18.75	10.25	6.00	6.00	6.00
Salmonella typhi	19.75	11.25	11.00	7.25	6.25
Escherichia coli	16.00	15.25	14.75	12.25	11.25
Fungi					
Candida albicans	**14.75**	**10.5**	**10.00**	**10.00**	**7.00**

Note: Cork borer size is 6mm and 100μl of different concentrations of lemongrass essential oil were added into the wells

Table 3: The MIC of lemongrass essential oil against pathogenic bacteria

Test organism	10%	1%	0.1%	0.01%	0.001%
Escherichia coli	–	–	+	+	+
Salmonella typhi	–	–	–	+	+
Staphylococcus aureus	–	–	+	+	+
Streptococcus pneumoniae	–	–	–	+	+

(+ Means there's growth), (–means no growth)

Table 4: The MBC of lemongrass essential oil against pathogenic bacteria

Test organism	MBC (%)
Salmonella typhi	10
Staphylococcus aureus	-
Streptococcus pneumonia	10
Escherichia coli	1

(-) means there's growth even at that concentration

Table 5: The Phytochemical screening results

Parameters	Present (+) / Absent (-)
Tannins	**+**
Saponins	**+**

Steroids	**+**
Alkaloids	**+**
Flavonoids	**+**
Cardiac glycosides	**+**

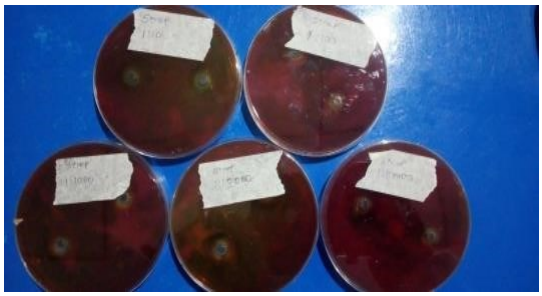

Figure 1: Image showing zone of inhibitions of the various concentrations of the essential oil on Streptococcus pneumoniae.

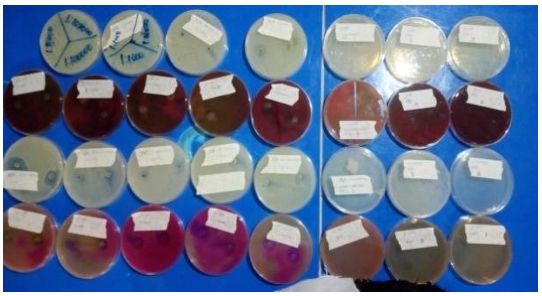

Figure 2: Image showing IZDs of selected pathogenic organisms

Figure 3: MIC of organisms in test tubes

DISCUSSION

The result of extraction of essential oil from *Cymbopogon citratus* yielded 0.63% (v/v) which agrees with some works reported that oil content of normal cut should be at of average 0.25-0.50%, but with improve techniques, it could be yield up to 0.66-0.90% (Weiss, 1997; Maiti *et al*, 2006). From the present study, it is clear that lemongrass oil possess a promising antimicrobial activity against the tested organisms. LGEO was found effective against all the test organisms. The antibacterial activity was found progressively increasing with the increase in concentration of oil. The maximum effect was found at 10% concentration and minimum effect was observed

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at 0.001% concentration of oil (Table 1). The result of phytochemical screening of the extract revealed the presence of the following bioactive compounds: flavonoids, tannins, alkaloids, steroids and cardiac glycosides. LGEO having phenolics compound (flavonoids) is the major player in its effectiveness as antimicrobial agents against pathogenic bacteria and deteriorative bacteria (Balakrishnan *et al*., 2014).

The test organisms were found inhibited by LGEO at very low concentration in broth dilution method as compared to agar diffusion method; this is in accordance with the results of Tortorano *et al*., 1998. The results obtained by each of these methods differ due to many factors between assays (Janssen *et al*., 1987 and Hili *et al*., 1997). These include differences in microbial growth, exposure of microorganisms to the oil, the solubility of oil or oil components and the use and quality of an emulsifier etc.

Several reports indicate that the antimicrobial activity of an EO is related to its chemical composition (Dorman et al., 2000). In the present work, the antifungal property of LGEO could be due to two major monoterpenic aldehydes (geranial and neral). Other grass oils rich in these oxygenated compounds were previously found to have effective antifungal actions (Negrelle *et al*., 2007, Silva *et al.,* 2008 and Boukhatem *et al*., 2014). Furthermore, previous studies also suggest that the fungicidal action of volatile oils could be explained by the presence of high amounts of oxygenated monoterpenes (Dorman *et al*., 2000). Geranial and neral are the most active components of LGEO and have a wide spectrum of antimicrobial properties against Gram-positive and Gram-negative bacteria and fungi. However, it is difficult to link the antifungal activity to particular compounds in a complex mixture of an EO. Some authors suggest that the strong inhibitory effect of LGEO against *Candida species* may result from synergism among the monoterpenes and other important compounds present in the EO, such as cymene, terpinene, and linalool (Bakkali *et a*l., 2008, Tyagi et al., 2010 and Inouye *et al*., 2001).

CONCLUSION

In this present era of emerging multidrug resistance among gram positive and gram negative organisms, aromatic oil from lemongrass is a potential remedy for the treatment of those resistant strains and infectious diseases.

In addition, it is endowed with interesting biological activities and has a therapeutic potential. This can be seen in the antimicrobial activities it exhibits which can be tagged as broad spectrum as it has activity against both gram positive and negative organisms. LGEO may be useful as natural remedies and it seems that it can be used as a suitable therapy for many pathologies.

Therefore, economic importance of LGEO is indisputable.

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