INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF AQUEOUS EXTRACT OF LEMONGRASS (*CYMBOPOGON CITRATUS*) UNDER SOME PARAMETERSHajarat O. Yusuf ^{*1} and Joshua Olu²

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ABSTRACT

The antimicrobial effect of aqueous extract of lemongrass (*Cymbopogon citratus*) on some isolated microorganisms under varying parameters was investigated. Aqueous extract of lemongrass was prepared and its antimicrobial effect was evaluated against isolated bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*) and fungi (*Aspergillus flavus* and *Candida albicans*) at varying temperature (40, 60 and 80°C) and concentration (10^{-1} , 10^{-2} , 10^{-4} , 10^{-8} and 10^{-16} mol/L) of the lemongrass aqueous extract shows that the extract was resisted by *Escherichia coli*, *Bacillus cereus*, *Aspergillus flavus* and *Candida albicans*. The aqueous *C. citratus* was able to inhibit the growth of *Staphylococcus aureus* and this inhibition increases as the concentration of the aqueous extract increase irrespective of the temperature of extraction. The aqueous extract of *C. citratus* is a tremendous antimicrobial substance especially towards *Staphylococcus aureus*.

Keywords: Lemongrass, *Cymbopogon citratus*, Antimicrobial, Aqueous extract, *Staphylococcus aureus*

INTRODUCTION

Lemongrass scientifically is known as *Cymbopogon citratus* and it belongs to the Poaceae family which counts more than 635 genera and 9000 species (Majewska *et al.*, 2019). *C. citratus* is widely spread globally. More than 140 species of *Cymbopogon*, 52 of them grow in Africa, 45 in India, 6 in Australia, 6 in South America, 4 in Europe (only in Montenegro), 2 in North America, and the others in South Asia (Majewska *et al.*, 2019). The genus *Cymbopogon* has been reviewed comprehensively in several articles (Avoseh *et al.*, 2015; Haque *et al.*, 2018). Nambiar and Matela (2012) reported that lemongrass grows well in both tropical and subtropical climates with around 900m elevation. Ideal conditions for growing *C. citratus* are warm and humid climate with enough sunshine with temperature ranging from 20-30°C and rainfall between 250 and 330 cm annually. Moderately fertile soils and moderate irrigation has been proving to aid the growth of *C. citratus* and can also be grown on different kind of soils from loam to poor laterite (Nambiar and Matela, 2012).

In lemongrass pattern of growth shows cell division occurs at the apical meristem which usually encourage tiller growth, production of axillary buds follow and the emergence of new tillers. Tillering increases in a sigmoidal-shaped curve until the maximum tiller number is reached where the main culms may be difficult to distinguish from the tillers (Tajidin *et al.*, 2012). *C. citratus* biological activity could be attribute to the essential oil and phenolic compounds such as phenolic acids, avonoids and tannins (Olorunnisola *et al.*, 2014; Roriz *et al.*, 2014; Tavares *et al.*, 2015). Various chemical compounds of *C. citratus* essential oil has been to show some antimicrobial characteristics (Bassolé *et al.*, 2011), deplete oxidants (Mansour *et al.*, 2015), antiparasitic (Kpoviesi *et al.*, 2014), insecticidal, and insect repellent actions (Brugger *et al.*, 2019). Ekpenyong & Akpan, (2017) has reported that lemongrass antimicrobial and antioxidant properties has been used for preservation of food as an alternative to chemical products. Avoseh *et al.*, (2015) reported that *C. citratus* is applied in Nigeria for stomach upset, malaria treatment, insect repellent and as an antioxidant. In Eastern and Western India it is used for insecticides, cosmetics products, and for gastrointestinal disorders and fevers therapy (Desai. and Parikh, 2012). This study investigated the antimicrobial effect of aqueous extract of lemongrass (*C. citratus*) on some isolated microorganisms under varying parameters

MATERIALS AND METHODS

Plant Material

Lemongrass (*Cymbopogon citratus*) was obtained from the premises of Ahmadu Bello University post office main campus, Samara-Zaria and it was identified in Biological Sciences Herbarium Laboratory Department in Faculty of Science Ahmadu Bello University (ABU), Zaria.

Figure 1 Lemongrass

Source of microbes

The microbial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Aspergillus flavus* and *Candida albicans*) used in this study were obtained from the Department of Microbiology ABU, Zaria.

Proximate Analysis

Moisture and volatile matter determination

20g of freshly cut lemon grass was weighed in clean dry and pre-weighed crucible. The fresh sample was oven dried at 70°C until a constant weight was attained. The drying sample is placed in a dessicator containing silica gel to cool before every successive weight remained constant.

$$\% \text{ Ash and moisture matter} = \frac{\text{loss in weight} \times 100}{\text{Initial weight of sample}}$$

Ash and organic dry matter determination

Exactly 10g of dried lemon grass was weighed in a crucible; it was then heated in a muffle furnace pre-set at 300°C for 2 hours and gradually rises to 500°C for 3 hours. The sample was removed and cooled in a dessicator after it has been completely ashed. Its weight was determined and percentage ash and organic dry matter was calculated.

$$\% \text{ Ash and organic dry matter} = \frac{\text{loss in weight} \times 100}{\text{Initial weight of sample}}$$

Preparation of Aqueous extract of lemon grass

20g grounded oven-dried lemon grass was poured in a pre-weighed thimble which was covered with a cotton wool and was dropped into the column of the soxhlet extractor. The extractor has been coupled to the pre-weighed 300ml round bottom flask known as quick fit containing 300ml sterile water. The quick fit and its content was placed on a heating mantle. The extraction was allowed to run for 6 hours at different temperatures of 40°C, 60°C, and 80°C respectively in a given 3 samples and it was later autoclaved at 110°C for 20 minutes for sterility.

Media preparation

The media used to culture the organisms include nutrient agar (N.A) and potato dextrose agar (PDA) were prepared according to the manufacturer's specifications and sterilised by autoclaving at 121°C for 15 minutes. The media were cooled to 45°C and then poured into sterile petri-dishes and allowed to gel.

Antimicrobial activity determination:

Bactericidal

Three bacterial isolates: *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* were used and another three sets of plates in duplicates containing nutrient agar were inoculated with *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* using the spread plate technique.

A suction pump device was used to bore wells (six wells) according to the dilution of the extracts. Therefore 0.1ml of the serially diluted extracts with varying concentration in mol/L was added to the wells as follows:

Well 1 = 10^{-1} Well 2 = 10^{-2} Well 3 = 10^{-4} Well 4 = 10^{-8} and Well 5 = 10^{-16}

While well 6 = control filled with only sterile distilled water. They were then incubated for 2 hours to allow diffusion to take place and finally incubated at 37°C for 24 hours.

Fungicidal activity

Two fungal isolated: *Aspergillus flavus* and *Candida albicans* were used and two sets of plates in duplets containing potato dextrose agar were inoculated with *Aspergillus flavus* and *Candida albicans* using the spread plate technique.

A suction pump device was used to bore wells (six wells) according to the dilution of the extracts. Therefore 0.1ml of the serially diluted extracts was added to the wells as follows:

Well 1 = 10^{-1} Well 2 = 10^{-2} Well 3 = 10^{-4} Well 4 = 10^{-8} and Well 5 = 10^{-16}

While well 6 was set as a control which was filled with sterile distilled water of 0.1ml. The plate was left at room temperature for 24-48 hours.

RESULTS AND DISCUSSION

Proximate composition of lemongrass

Table 1 Moisture and volatile matter determination:

Determination	% of moisture content	% of dry matter
1	68.00	32.00
2	66.00	34.00
3	65.70	34.30
Mean $\pm$ S.D	66.56 $\pm$ 1.76	33.43 $\pm$ 1.76

Table 1 shows the moisture content of the lemongrass with 66.56%, this simply explained that the plant contain high level of moisture

Table 2 Ash and organic dry matter determination:

Determination	% of Ash dry matter	% of organic dry matter
1	3.23	96.77
2	2.00	98.00
3	3.30	96.70
Mean $\pm$ S.D	2.84 $\pm$ 1.03	96.16 $\pm$ 1.03

Table 2 indicate that Ash content of the plant is 2.84% and dry organic matter as 96.16%

Cymbopogon citratus has been found to contain bioactive compounds that have been proven to have anti-oxidant, anxiolytic, anti-obesity, antihypertensive, bactericidal, fungicidal, anti-inflammatory, and antinociceptive (Moore-Neibel *et al.*, 2012; Olorunnisola *et al.*, 2014).

Antimicrobial activity determination

Table 3 Extract at temperature of 40°C, Concentration of extracts (double serial dilution method):

		10^{-1}	10^{-2}	10^{-4}	10^{-8}	10^{-16}	Control
Bacterial							
1	<i>Escherichia coli</i>	R	R	R	R	R	RR
2	<i>Staphylococcus aureus</i>	SS	S	SX	SX	SX	RR
3	<i>Bacillus cereus</i>	R	R	R	R	R	RR
Fungal							
1	<i>Aspergillus flavus</i>	R	R	R	R	R	RR
2	<i>Candida albicans</i>	R	R	R	R	R	RR

Keys: R = Resistant, S = Sensitive, RR = highly resistant, SS = highly sensitive, SX = slightly sensitive

In this study, the evaluation of the antimicrobial effect against isolated bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*) and fungi (*Aspergillus flavus* and *Candida albicans*) at varying temperature and concentration of the lemongrass aqueous extract shows that the extract was resisted by *Escherichia coli*, *Bacillus cereus*, *Aspergillus flavus* and *Candida albicans* at temperature 40, 60 and 80°C. Also these five (5) microorganisms were resistance to the antimicrobial effect of the lemongrass aqueous extract at 10^{-1} , 10^{-2} , 10^{-4} , 10^{-8} and 10^{-16} mol/L concentrations.

Table 4 Extract at temperature of 60°C, Concentration of extracts (double serial dilution method)

		10^{-1}	10^{-2}	10^{-4}	10^{-8}	10^{-16}	Control
Bacterial							
1	<i>Escherichia coli</i>	R	R	R	R	R	RR
2	<i>Staphylococcus aureus</i>	SS	S	S	SX	SX	RR
3	<i>Bacillus cereus</i>	R	R	R	R	R	RR
Fungal							
1	<i>Aspergillus flavus</i>	R	R	R	R	R	RR
2	<i>Candida albicans</i>	R	R	R	R	R	RR

Keys: R = Resistant, S = Sensitive, RR = highly resistant, SS = highly sensitive, SX = slightly sensitive

Table 3, 4, and 5 shows that at different temperatures i.e. 40, 60 and 80°C, the aqueous *C. citratus* was able to inhibit the growth of *Staphylococcus aureus* and this inhibition increases as the concentration of the aqueous extract increase irrespective of the temperature of extraction. In like manner with this study, Nyamath and Karthikeyan (2018) among the six bacterial cultures tested with cold water, hot water and different solvents like ethanol and methanol extracts of lemongrass the maximum zone of inhibition was observed in *staphylococcus aureus*.

Table 5 Extract at temperature of 80°C, Concentration of extracts (double serial dilution method)

		10^{-1}	10^{-2}	10^{-4}	10^{-8}	10^{-16}	Control
Bacterial							
1	<i>Escherichia coli</i>	R	R	R	R	R	RR
2	<i>Staphylococcus aureus</i>	SS	SS	SS	S	S	RR
3	<i>Bacillus cereus</i>	R	R	R	R	R	RR
Fungal							
1	<i>Aspergillus flavus</i>	R	R	R	R	R	RR
2	<i>Candida albicans</i>	R	R	R	R	R	RR

Keys: R = Resistant, S = Sensitive, RR = highly resistant, SS = highly sensitive, SX = slightly sensitive

It had been proposed that the phytochemicals such as alkaloids, tannis, flavanoids, terpenes and phenolic compounds. Phenols and flavonoids found in *C. citratus* causes disruption of the membrane and affect their hereditary information (Adegbeji *et al.*, 2012; Bhoj *et al.*, 2011; Gopinath *et al.*, 2013; Jayashree *et al.*, 2013).

CONCLUSION

Cymbopogon citratus is no doubt of a plant that has been shown to possess antimicrobial values by different studies and of which this study has joined in upholding this claim. The aqueous extract of *C. citratus* is very active in inhibiting the growth of *Staphylococcus aureus* as the concentration of the extract increases.

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