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Research Article

ANTIMICROBIAL ACTIVITY OF LEMON GRASS (*CYMBOPOGON CITRATUS*) ON CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA, SALMONELLA TYPHI, STREPTOCOCCUS PYOGENES* AND *CANDIDA ALBICANS*

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ABSTRACT

Cymbopogon citratus, commonly known as lemongrass due to its lemon fragrance when crushed. Due to the presence of many phytochemicals in lemongrass, their use in the treatment of illnesses has increased. The antimicrobial activity of Cymbopogoncitratus (Lemongrass) was evaluated on Pseudomonas aeruginosa, Salmonella typhi, Streptococcus pyogenes and Candida albicans. The extract was obtained using two solvents; ethanol and methanol. The antimicrobial activity of the extracts was tested for by the agar well sensitivity diffusion test method. The efficacy of the extract on the inhibition of bacteria was most prominent on S. typhi, S. pyogenes and C. albicans. This inhibitory effect was not observed on P. aeruginosa. The methanolic extract showed 4mm zone of inhibition on the bacteria and 5mm zone of inhibition on the fungus, thus, showing more activity than the ethanolic extract with 1mm zone of inhibition on the bacteria, and resistance on fungi. The Minimum Inhibitory Concentration (MIC) of the methanolic extract was at 1000µg/ml and that of the ethanolic extract was at 2000µg/ml. The Minimal Lethal Concentration (MLC) of the lemongrass extract was at 4000µg/ml. Lemongrass is non toxic to humans and have been used as medicinal drink.

INTRODUCTION

Exploration into the world of plants has given rise to the medicinal use of their antioxidant and antimicrobial properties. Plants are potential source of medical compounds in the world with. These plants traditionally are used in oral health drinks and in the treatment of many diseases especially infectious diseases including diarrhea, fever and cold, in addition, many organic compounds used in traditional medicine have plant root components (Dean *et al*, 1990). According to World Health Organization (WHO) definition- a medicinal plant is a plant that can be used for therapeutic purposes and or its compounds be used as a pioneer in the synthesis of semi-synthetic chemical drugs (World Health Organization, 1979). With increasing number of bacterial strains being resistant to various antibiotics, attempts to use antimicrobial potential of plants are under studies. On the other hand emergence of resistant bacterial strains among Gram negative bacilli and Gram positive cocci such as *Pseudomonas, Klebsiella, Enterobacter, Staphylococcus* and *Enterococcus* has caused problems in treating infections caused by these bacteria (Oussalah *et al*, 2007). Plant extracts have been used for centuries in traditional medicine to alleviate inflammatory diseases, with little knowledge about their mechanisms of action. The understanding of molecular mechanisms behind the healing properties of natural products is crucial to finding compounds that could be useful as templates to new therapeutic molecules. Owing to the presence of many phytochemicals in plants, their use in the treatment of illnesses has increased. Indeed, most of the drugs actually available are derived from natural products (Newman and Cragg, 2007).

The research according to Asaolu *et al* (2009) has shown that many biologically active substances have been identified to be present in *Cymbopogon citratus.* A very important one being citral (biologically active), which helps in digestion as well as spasm relieve, muscle cramps, rheumatism, and headache. In Southern parts of Nigeria, Lemon grass is used as tea by boiling its leaves and drinking the solution. A tea made from the leaves of *Cymbopogon citratus* has been used to treat fever, cold, cough, and stomach upset. The tea possesses diuretic properties and can help patients with urinating difficulties and water retention (Stehmann and Brandaw, 1995). Other cases like nervous condition and inflammation, headache, chest pain can be cured by drinking tea made from leaves of *Cymbopogon citratus.*

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MATERIALS AND METHOD

Plant Materials

The leaves and roots of *Cymbopogon citratus* (Lemon grass) were collected from a vegetable garden in Trans Ekulu in Enugu. The dried leaves and root were blended into powdery form and extracted using methanol and ethanol through room evaporation.

Test Organisms

Three (3) bacteria species - *Pseudomonas aeruginosa, Streptococcus pyogenes, Salmonella typhi,* and Fungi - *Candida albicans* were obtained from stock cultures in the Microbiology Laboratory of the University of Nigeria Teaching Hospital, Enugu, Nigeria. They were confirmed by their re-culturing and biochemical tests carried out on them. They were standardized using McFarland 0.5 turbidity standard (NCCL, 2003 and Vandepitte, *et al*, 2003).

Antimicrobial Screening of Extract

The antimicrobial activity of the extract was determined using agar diffusion method. The test organisms were inoculated into test tubes containing Sabouraud Dextrose Broth and Mueller Hinton Agar media and incubated at room temperature for 72 and 24 hours for fungi and bacteria respectively. Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) tests were carried out on them. (Cheesbrough, 2002).

Standard Antibiotic Susceptibility Test

Disc diffusion method was used in this test as described by Vandepette, *et al* (1991). Six millimetre (6mm) commercially prepared antibiotic paper discs were used. The antibiotic discs used and their concentrations are Augumentin (30µg), Ofloxacin (10mg), Levofloxacin (20mg) and Gentamycin (10mg) for *Pseudomonas aeruginosa*, Ketoconazole (25mg) for *Candida albicans,* a Gram negative antibiotics paper disc set for *Salmonella typhi,* and a Gram positive antibiotics paper disc set for *Streptococcus pyogenes*. The discs were applied in accordance to the National committee for clinical laboratory standard (2003).

RESULTS AND DISCUSSION

After Phytochemical analysis, the phytochemical components identified in *C. citratus* were Tannins, Flavonoids, Alkaloids and Volatile oil as against seven (7) phytochemicals tested for.
The Agar Susceptibility Test was carried out on the extracts, with four (4) clinical isolates at 50mg/ml concentration. The two (2) extracts showed no zone of inhibition on the clinical isolates of *Pseudomonas aeruginosa, Streptococcus pyogenes, Salmonella typhi* and *Candida albicans*. The Agar Susceptibility test was carried out again at 100mg/ml concentration of the extracts and there was still no zone of inhibition on the test organisms. The Agar Susceptibility Test was carried out for the third time at 200mg/ml; This time zones of inhibition were observed, Methanolic extract showed one (1) mm zone on *P. aeruginosa* and *S. pyogenes,* two (2) mm zone on *S. typhi,* and none on *C. albicans,* while the Ethanolic extract showed no zone of inhibition on the isolates(Table 1a). The final Susceptibility test was carried out on the isolates at 400mg/ml concentration of the extracts with increased zones of inhibition.

Table 1a Agar Susceptibility Test (200µg/ml conc.)

Test Organism	Zone of Inhibition (mm)	
	Methanolic Extract	Ethanolic Extract
Bacteria		
• *Pseudomonas aeruginosa*	1	NA
• *Streptococcus pyogenes*	1	NA
• *Salmonella typhi*	2	NA
• Fungi		
• *Candida albicans*	NA	NA

No Activity = NA

The Methanolic extract showed 4mm zone on *P. aeruginosa,* 4mm on *S. pyogenes,* 2mm on *S. typhi,* and 5mm on *C. albicans*. The Ethanolic extract showed inhibitions zones of 1mm on *P. aeruginosa, S. pyogenes, S. typhi,* and no inhibition on *C. albicans* as shown in (Table 1b).

Table 1b Agar Susceptibility Test (400µg/ml conc.)

Test Organism	Zone of Inhibition (mm)	
	Methanolic Extract	Ethanolic Extract
Bacteria		
• *Pseudomonas aeruginosa*	4	1
• *Streptococcus pyogenes*	4	1
• *Salmonella typhi*	2	1
Fungi		
• *Candida albicans*	5	NA

No Activity = NA

In the sensitivity testing, antibiotics like Ofloxacin, Augmentin, Ciprofloxacin and Gentamycin showed no inhibition on *P. aeruginosa* but sensitive with Levofloxacin inhibition zoneof 4mm. Ten Gram +ve antibiotics disc were tested on *S. pyogenes with* the following zones of inhibition, Levofloxacin 10mm, Erythromycin 5mm, Gentamycin 8mm, Streptomycin 5mm and Ciprofloxacin 8mm, the rest showed no inhibition. In relation testing the sensitivity parthern on *S. pyogenes* with Gram +ve antibiotics, Ten Gram –ve antibiotics disc were used on *S. typhi* (a Gram positive organism) with the following results, Pefloxacin 10mm, Clarithomycin 10mm and Gentamycin 8mm, the rest showed no inhibition. Ketoconazole was tested on *C. albicans* and showed 4mm zone of inhibition. Table 2 showed the result for the MIC test carried out on the isolates using the concentration (4000µg/ml) of the extracts for Agar Susceptibility test.

Table 2 Minimum Inhibitory Concentration (MIC) Test

Conc. (µg/ml)	Turbidity (Growth) in Tubes of Organisms			
	P. aeruginosa	*S. pyogenes*	*S. typhi*	*C. albicans*
Methanolic Extract				
4000	+	-	-	-
2000	+	-	-	-
1000	+	+	+	-
500	+	+	+	+
250	+	+	+	+
Ethanolic Extract				
4000	+	+	-	-
2000	+	+	+	+
1000	+	+	+	+
500	+	+	+	+
250	+	+	+	+

Turbidity (growth) = +, No Turbidity (No growth) = -

The Methanolic extract inhibited growth at dilution of 1(4000µg/ml) and 2(2000µg/ml) using*C. albicans, S. pyogenes,* and *S. typhi* tubes but showed no inhibition on *P. aeruginosa* tubes. The Ethanolic extract only inhibited *C. albicans* and *S. typhi* at 1(4000µg/ml) dilution. The rest of the dilution concentrations of the two extracts were turbid.

Table 3 shows the results of the Minimal Lethal Concentration (MLC) test, with only plate from the MIC tube of *C. albicans(+)* showing positive result at dilution 1(4000µg/ml) of the methanolic extract. Other plates showed no growth at 24hours of incubation but growth after 24-30hours of incubation.

Table 3 Minimum Lethal Concentration (MLC)

S/N	MIC Tubes	Organism Growth
	Candida albicans	
1.	Ca1E	+
2.	Ca1M *	--
3.	Ca2M	+
4.	Ca3M	+
	Salmonella typhi	
5.	St1E	+
6.	St1M	+
7.	St2M	+
	Streptococcus pyogenes	
8.	Sp1M	+
9.	Sp2M	+

* Positive growth observed after over 24 hours of incubation, Ca(1,2,3)E = MIC tube of *Candida albicans*+ Ethanolic extract, Ca(1,2,3)M = MIC tube of *Candida albicans*+ Methanolic extract, St(1)E = MIC tube of *Salmonella typhi*+ Ethanolic extract, St(1,2)M = MIC tube of *Salmonella typhi*+ Methanolic extract, Sp(1)M = MIC tube of *Streptococcus pyogenes*+ Methanolic extract, Sp(1)E = MIC tube of *Streptococcus pyogenes*+ Ethanolic extract. Where, 1 = 4000µg/ml, 2 = 2000µg/ml, 3 = 1000µg/ml, Turbidity (Growth) = --, No Turbidity = +

Statistical Analysis

No statistical tool was used due to the irregularity of the results. Most of the incubations of organisms had no measurable diameter zone of inhibition

CONCLUSION

Lemon grass is efficacious on microorganisms and was found to be bactericidal once the concentration is high. Comparing the results (by diameter zones of inhibition) from this research and that of other researchers it can be concluded that the reason for the low antimicrobial sensitivity on the clinical isolates may be due to the fact that there may have been some resistant microbial species. This is because most of the standard antimicrobial drugs at their right concentrations were found inactive in inhibiting the growth of the isolates, which is not the expected result with antimicrobial agents.

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