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Evaluation of the antibacterial activity of *Cymbopogon citratus* in bacterial gastroenteritis

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Abstract
The emergence of antimicrobial resistance has led to the need for newer classes of antimicrobial agents. Herbal medicines provide one of the preferred route to drug discovery due to their chemical diversity. This study aims to evaluate the activity of *Cymbopogon citratus* in the treatment of bacterial gastroenteritis. Aqueous and methanol extracts of *C. citratus* were used in the study. Ciprofloxacin (a broad spectrum antibiotics and a drug of choice in bacteria gastroenteritis) was used as the standard drug. Multi-drug resistant clinical strains of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* were used in the study. The aqueous extract of *C. citratus* showed no antibacterial activity against any of the test organisms. However, all the organisms were susceptible to the methanol extract and the standard drug. Inhibition Zone Diameters and Minimum Inhibitory Concentrations were used in the evaluation. *C. citratus* could serve as a source of antimicrobial agent.

Keywords: Bacterial gastroenteritis, herbal extracts, antimicrobial resistance, *Cymbopogon citratus*, antimicrobial agents, inhibition zone diameters

1. Introduction
Herbal medicine is the use of plants (herbs) for the prevention and treatment of diseases, ranging from traditional medicines to the more standardized plant extracts, phytonutrients and nutraceuticals [1]. About 80 percent of the world population rely on herbal medicines for their healthcare needs [2].

Herbs are natural products that contain secondary metabolites which find use in the field of medicine. An herb is a plant or plant part used for its scent, flavor, or therapeutic properties. Herbal medicines are one type of dietary supplement. They are sold as tablets, capsules, powders, teas, extracts, and fresh or dried plants. People use herbal medicines to try to maintain or improve their health [3].

The earliest evidence of humans' use of plants for healing dates back to the Neanderthal period. In the 16th century, botanical gardens were created to grow medicinal plants for medical schools. Herbal medicine practice flourished until the 17th century when more "scientific" pharmacological remedies were favored [2].

The issue of antimicrobial resistance has given rise to the dire need for isolation of newer antibiotics particularly from natural sources, due to their chemical diversity. Multidrug-resistant bacteria are a serious problem which have rapidly spread worldwide. Health care-associated infections (HAIs) caused by MRSA, MRSE, and resistant gram negative bacteria have become global threats with high fatality rates [4].

Cymbopogon citratus, Stapf (Lemon grass) is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in aromatherapy. The compounds identified in *Cymbopogon citratus* are mainly terpenes, alcohols, ketones, aldehyde and esters. Some of the reported phytoconstituents are essential oils that contain Citral α, Citral β, Nerol, Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and Terpinol Methylheptenone. The plant also contains reported phytoconstituents such as flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apiginin. Studies indicate that *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties. Various other effects like antimalarial, antimutagenicity, antimycobacterial, antioxidants, hypoglycemic and neurobehaviorial have also been studied.

These results are very encouraging and indicate that this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects [5]. Lots of studies has been done on the effectiveness of *Cymbopogon citratus* oil in the prevention of microbial contamination in food products and the treatment of bacterial infections in humans. Lemon grass essential oil has been shown to be effective in the preservation of food and animal products by preventing contamination by either fungal or bacterial pathogens [6]. Another study demonstrated the effectiveness of *Cymbopogon citratus* oil in the control of Salmonella Heidenburg infection in poultry products [7].

The antibacterial activity of *Cymbopogon citratus* oil in humans has also been extensively studied. Lemon grass oil has antibacterial activity against some pathogen including *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae) and Pseudomonas aeruginosa (P. aeruginosa*) [8]. Nevertheless, studies on the antibacterial activity of the aqueous and methanol extracts of *Cymbopogon citratus* are limited.

Bacterial gastroenteritis is a disease that is pervasive in both the developing and developed worlds. While for the most part bacterial gastroenteritis is self-limiting, identification of an etiological agent by bacterial stool culture is required for the management of patients with severe or prolonged diarrhea, symptoms consistent with invasive disease, or a history that may predict a complicated course of disease [9].

Diarrheal diseases are a global public health problem causing considerable morbidity and mortality among infants and children especially in the developing countries [10].

Bacteria gastroenteritis has been linked to several pathogenic organisms including but not limited to *Salmonella enterica* species, *Campylobacter spp*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Escherichia coli*. Such pathogens are usually transmitted through consumption of raw or undercooked meat and poultry products, consumption of unpasteurized milk products and possible outbreaks [11].

Staphylococcus aureus is a gram positive spherical organism belonging to the Staphylococcus genus with diameter of 0.5 – 1.5 µm. *S. aeurus* is one of the most pathogenic species. It produces staphylococcal endotoxin responsible for almost all staphylococcal food poisoning [12].

Escherichia coli is a gram negative spherical shaped bacterium that lives normally in the intestine of healthy people and animals especially cattle. Most strains are generally harmless. However, some strains of E. coli can cause diseases. In March 2012, the CDC identified a strain of *E. coli* 0157: H7 which caused an outbreak of hemorrhagic colitis (severe bloody diarrhea) [13].

Salmonella typhi is a gram-negative, flagellated, non-spore forming, facultative anaerobic enteric bacteria belonging to the family Enterobacteriaceae. It was first identified in 1880 by Karl J. Elbert. *S. typhi* is an enteric pathogen that causes enteric fever. It is one of the most common infective bacteria pathogens in developing countries with poor sanitation and poor use of antibiotics especially in Asia, Latin America and Africa [14].

2. Materials and Method

2.1 Collection and Drying of Plant Material

The medicinal plant was collected from Bio-resources Research Centre, Nsukka and identified by a plant taxonomist. The plant was dried at room temperature for 21 days after which it was ground into fine powder using an herb grinder.

2.2 Test Microorganisms

Clinical multi-drug resistant strains of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* were used in the study. These isolates were obtained from the stock culture of the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

2.3 Standardization of Stock Microbial Cultures

To purify the stock cultures, subcultures were prepared by streak plate technique and incubated for 24 hours before use. The organisms were harvested with sterile water and dilution was done to obtain a microbial population of 1.0×10^8 CFU/ml using 0.5 McFarland standard.

2.4 Extraction of Plants

100 g of the fine lemon grass powder was weighed separately for both the distilled water and methanol solvents respectively. The extraction was done using cold maceration for 24 hours. The filtrate was concentrated using a rotary evaporator.

2.5 Sterilization of Materials

All glass wares (Petri dishes, test tubes and bijou bottles) were sterilized in the hot air oven at 160 °C for 1 hour. All liquid preparations (Mueller Hinton agar, Nutrient agar) were sterilized in the autoclave at 121°C for 15mins.

2.6 Preparation of Culture Media

The media used in the test were Nutrient agar and Mueller Hinton agar. Nutrient agar was used in the preparation of the agar slant used for the storage of the stock cultures and the Mueller Hinton agar was used for the sensitivity tests and determination of the Minimum Inhibitory Concentration (MIC).

2.7 Nutrient Agar Preparation

The nutrient agar media was prepared by suspending 28g of nutrient agar powder in a liter of distilled water. This was allowed to soak for 10 minutes and then homogenized by heating. It was dispensed in 20 ml volumes using bijou bottles then, sterilized in an autoclave at 121 °C for 15 minutes then allowed to cool to 45 °C before aseptically pouring into petri dishes.

2.8 Mueller Hinton Agar Preparation

The Mueller Hinton agar media was prepared by suspending 38 g of Mueller Hinton agar powder in 1 liter of distilled water. This was allowed to soak for 10 minutes and then homogenized by heating. It was dispensed into 20ml volumes using bijou bottles then, sterilized in the autoclave at 121°C for 15 minutes then allowed to cool to 45 °C before pouring into plates.

2.9 Preparation of the Stock Solution of the Plant Extract

The aqueous and methanol extracts of *Cymbopogon citratus* were prepared by dissolving 200 mg of each of the dry extracts in 2 ml of the diluents (distilled water and DMSO respectively). And agitating to complete dissolution of the extract to obtain a stock concentration of 100mg/ml for both extracts.

2.10 Preparation of the Stock Solution of the Standard drug (Ciprofloxacin)

1mg of ciprofloxacin powder was dissolved in 5ml of distilled water to obtain a stock concentration of 200 µg/ml.

2.11 Antimicrobial Screening Test

2.11.1 Preliminary Sensitivity Test of the Extracts

The preliminary sensitivity test of the aqueous and methanol extracts of *C. citratus* was done using the agar well diffusion method. 20ml of sterile molten Mueller Hinton agar was poured into each of 6 petri dishes and allowed to solidify. The plates were labelled appropriately (3 plates for the methanol extract and 3 plates for the aqueous extract). Each plate was divided into 4 quadrants with the aid of a marker. 0.1ml each of the freshly prepared bacteria stock cultures were spread on the surface of the agar using sterile cotton swab and allowed to dry for about 5 minutes (each organism seeded on one plate labelled aqueous extract and one plate labelled methanol extract). Using a cork borer of 10mm diameter, 4 wells were bored into each plates.

5 drops of the 100mg/ml stock solution of the aqueous and methanol extracts were added to each well according to the labelling. The plates were left to allow for diffusion of the extract into the agar. The plates were incubated at 37°C for 24 hours. The inhibition zone diameters (IZDs) were observed for each organism. The IZDs of each plate were taken and averages taken.

2.11.2 Preliminary test of the Standard Antibacterial Agent (Ciprofloxacin)

The preliminary sensitivity test of the standard drug was done using the agar well diffusion method. 20ml of sterile molten Mueller Hinton agar was poured into each of 3 petri dishes and allowed to solidify. 0.1ml each of the freshly prepared bacteria stock cultures were spread on the surface of the agar using sterile cotton swab and allowed to dry for about 5 minutes. Using a cork borer of 10mm diameter, 4 wells were bored into each plates. 5 drops of the stock solution were added into each well accordingly and allowed to diffuse into the agar. The plates were incubated at 37 °C for 24 hours. The inhibition zone diameter (IZDs) were observed for each organism. The inhibition zone diameters (IZDs) were observed for each organism. The IZDs of each plate were taken and averages taken.

2.11.3 Minimum Inhibitory Concentration (MIC) Determination of the Plant Extracts

The MIC of the methanol extract using the broth macro-dilution method. 10 concentration of the methanol extracts were used in the study (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 and 0.39 mg/ml). A negative control was also added. Mueller Hinton broth was used in the study. 0.1ml of freshly prepared bacteria stock culture was introduced into test tubes containing 9ml of sterile Mueller Hinton broth and 1ml of each concentration of the extract and incubated for 24 hours. The least concentration that showed no turbidity was taken as the MIC [15].

2.11.4 Minimum Inhibitory Concentration (MIC) Determination of Ciprofloxacin

The MIC of ciprofloxacin was determined using macro-dilution broth method. Serial dilution of the stock concentration of the ciprofloxacin was done to obtain 10 concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, and 0.39 µg/ml). A negative control was also added. Mueller Hinton broth was used in the study. 0.1ml of freshly prepared bacteria stock culture was introduced into test tubes containing 9ml of sterile Mueller Hinton broth and 1ml of each concentration of the ciprofloxacin and incubated for 24 hours. The least concentration that showed no turbidity was taken as the MIC [15].

3. Result

Table 1: Extraction yield of the Aqueous and Methanol extracts of *C. citratus*

Extract	Weight Of Plant Powder Used (G)	Weight of Extract (G)	Percentage Yield (%)
Aqueous extract	100	7.5	7.5
Methanol extract	100	6.0	6.0

Percentage yield

$$(weight\ of\ extract\ \div weight\ of\ plant\ powder\ used)\ \times 100\%$$

Table 2: Sensitivity Test

Test organism	Sensitivity of methanol extract	Sensitivity of aqueous extract	Sensitivity of the standard drug (Ciprofloxacin)
Staphylococcus aureus	+	_	+
Escherichia coli	+	_	+
Salmonella typhi	+	_	+

+ = Sensitive
- = Not sensitive

Table 3: Concentration and IZD of the Methanol Extract of *Cymbopogon citratus*

Test Organism	Concentration (mg/ml)	IZD (mm)
Staphylococcus aureus	100	9
	50	7
	25	6
	12.5	2
	6.25	1
	3.125	---
Escherichia coli	100	10
	50	6
	25	5
	12.5	4
	6.25	1
	3.125	---
Salmonella typhi	100	9
	50	8
	25	7
	12.5	5
	6.25	3
	3.125	1

Table 4: MIC of the methanol extract and standard drug (Ciprofloxacin)

Test Organism	MIC of Ciprofloxacin (mg/ml)	MIC of Methanol Extract (mg/ml)
Escherichia coli	0.003125	3.125
Staphylococcus aureus	0.00078125	6.25
Salmonella typhi	0.003125	3.125

4. Discussion

The aqueous extract of *Cymbopogon citratus* showed no activity against the organisms used in the study. All the organisms were susceptible to the methanol extract. Gram negative organisms (*E. coli* and *S. typhi*) showed the highest

inhibition with an MIC of 3.125mg/ml compared to the gram positive organism (*S. aureus*) with an MIC of 6.25mg/ml. Ciprofloxacin a broad spectrum antibiotic commonly used as a drug of choice in gastroenteritis expressed a strong inhibitory effect on all the organisms tested. The MIC of ciprofloxacin was also over 1000 times that of the methanol extract. This is because extracts are not pure forms, further isolation and purification of the extract could give better results.

The yields of the extracts were substantial despite the crude method (Cold maceration) used in the study with the aqueous solvent yielding more than the methanol solvent. Extraction using more advanced methods such as Soxhlet extraction could have yielded better results and increase the economic feasibility of producing these extracts commercially.

Previous studies done with various extracts of lemon grass on these organisms have varying results. Works described by [16], stated that the chloroform extract of lemon grass showed activity against *S. aureus*, *E. coli* and *S. typhi* while the methanol extract showed no activity. This could be due to bacterial strain to strain variation.

However, works described independently by [17] and [18] showed that *S. aureus* and *E. coli* were susceptible to methanol extract of *Cymbopogon citratus*.

Larger studies involving more strains of these organisms are needed to give a more holistic view of the antibacterial activity of methanol extracts of *Cymbopogon citratus*. Studies involving isolates and purer forms of the extract are also needed. However, *Cymbopogon citratus* shows promise as an alternative medicine for use in bacterial induced gastroenteritis.

5. Conclusion

The methanol extract of *Cymbopogon citratus* could serve as an important source of pharmaceutically active ingredient(s) for the treatment of bacterial gastroenteritis and possibly, other bacterial infections.

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