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PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF DICHLOROMETHANE-METHANOL LEAVES EXTRACT OF *Cymbopogon citratus* (POACEAE) AGAINST SELECTED BACTERIA AND FUNGAL PATHOGENS

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ABSTRACT

The increasing incidence of microbial resistance to existing antimicrobial agents has intensified the search for novel therapeutic alternatives. This study evaluated the phytochemical composition and antimicrobial activity of a dichloromethane–methanol leaf extract of *Cymbopogon citratus* (Poaceae). Fresh leaves of *C. citratus* were collected from Lejja, Nsukka Local Government Area, Enugu State, Nigeria, and authenticated. One kilogram of the leaves was air-dried at 29–35 °C for three weeks, pulverized, and extracted by cold maceration using a 1:1 mixture of methanol (2.5 L) and dichloromethane (2.5 L) for 72 h with continuous agitation. The extract was filtered and concentrated *in vacuo* at room temperature to obtain the dry extract. Qualitative and quantitative phytochemical analyses were carried out using standard procedures. The antimicrobial activity of the extract was evaluated *in vitro* against nine human pathogenic microorganisms obtained from the clinical stock of the Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka, using agar well diffusion and broth microdilution methods. Phytochemical analysis revealed the presence of total phenolics (1897.84 ± 0.09 mg/100 g), alkaloids (476.67 ± 0.07 mg/100 g), terpenoids (203.36 ± 0.02 mg/100 g), flavonoids (119.00 ± 0.03 mg/100 g), reducing sugars (32.61 ± 1.02 mg/100 g), tannins (16.25 ± 0.05 mg/100 g), and steroids (4.77 ± 0.00 mg/100 g), while glycosides and saponins were absent. The extract exhibited antimicrobial activity against *Bacillus cereus* and *Aspergillus flavus*. A minimum bactericidal concentration (MBC) of 12.5 mg/mL was recorded against *B. cereus*, while a minimum fungicidal concentration (MFC) of 25 mg/mL was observed against *Candida albicans*. These findings demonstrate that the dichloromethane–methanol leaf extract of *C. citratus* possesses appreciable antimicrobial activity and is rich in bioactive phytochemicals with therapeutic potential.

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INTRODUCTION

Emerging and recurrent infectious diseases have contributed to the growing trend of microbial resistance to the currently available chemotherapeutic agents. The abuse and misuse of antibiotics in human healthcare and agriculture have sustained the surge in microbial resistance [1]. Incidentally, the processes of development of vaccines, phage therapy,

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monoclonal antibodies, lysin, probiotics, and antimicrobial peptides as other alternative are quite costly and may not be attractive to many pharmaceutical companies due to their low proceeds [1-4]. This worrisome development has necessitated a search from plants for newer moieties that will be effective, safe, and affordable in managing human health crises [5].

Various plants and plant products that abound in nature have been used in a traditional setting as a reliable source of care and cure for numerous diseases that afflict man's health over time. One such plant commonly used in trado-medicinal practices in many parts of the world is C. citrates[6-8]. It is also known in many places as lemongrass and belongs to the family Gramineae[8]. Citral is one of the major phytochemicals in *C. Citratus* and it is responsible for the perculiar lemon flavor common with lemongrass [9,10].

Lemongrass has been used in many traditional practices in the treatment of pneumonia, fever, malaria, sore throat, and cough [5,7,9,11]. It has also been utilized in herbal tea, and food industries where its characteristic lemon flavor is highly sought after [5,8,11]. The quality and quantity of the essential oils as well as other chemical constituents depend on the time, age of harvest, and geographical location [9]. The medicinal properties of lemongrass have been variously attributed to their secondary metabolites [6,12]. Terpenes, phenols, alkaloids, and essential oil among other secondary metabolites from C. citratus exert similar modes of action as other medicinal plants against many bacterial diseases through membrane-disruption mechanisms, protein binding, interference with intermediary metabolism, anti-quorum sensing, and anti-biofilm activities [4,12,13]. Endophytes that inhabit the tissues of these medicinal plants often produce metabolites that are similar or even more diverse in activity than that of the host plants. Given the regional variation of the active constituents of most medicinal plants, this study aims to evaluate and validate the phytochemical and antimicrobial activities of Dichloromethane-methanol leaves extract of *C. citratus* (Poaceae) from Nsukka, region of Southeastern Nigeria.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Cymbopogon citratus* were collected between March and April 2022 from Lejja, Nsukka Local Government Area, Enugu State, Nigeria. The plant material was identified and authenticated by a taxonomist in the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. A voucher specimen (No. PCG/UNN/0426) was prepared and deposited in the departmental herbarium for future reference.

Extraction of the phytochemicals

One kilogram (1000 g) of the harvested leaves was air-dried at room temperature (29–35 °C) for three weeks and then pulverized into a fine powder. The powdered sample was subjected to cold maceration using a 1:1 mixture of methanol (2.5 L) and dichloromethane (2.5 L) at room temperature for 72 h with continuous agitation. After extraction, the mixture was filtered, and the resulting filtrate was concentrated *in vacuo* at room temperature to obtain the dry extract. The percentage yield of the extract was subsequently determined using the formula

$$\%\,Yield\,(Recovery\,of\,extract) = \frac{Final\,weight\,of\,extract\,recovered\,after\,extraction\,(g)}{Initial\,weight\,of\,plant\,powder\,(g)} X\,100$$

… equation (1)

Qualitative and quantitative phytochemical analysis of the extract of *Cymbopogan citratus*

Qualitative phytochemical analysis of the methanol extracts and the fractions were done to determine the presence of phenols, tannins, terpenoids, alkaloids, steroids, saponins, flavonoids, and glycosides using standard methods described by [14] with slight modifications.The quantitative phytochemical analysis of *C. citratus* was carried out to determine the amount of phytochemicals present in the concentrated extract. Such phytochemicals analyzed include alkaloids, reducing sugar, flavonoids, steroids, tannins, glycosides, saponins, phenols, and terpenoids. The methods used in this analysis were adopted from [14,15].

Antimicrobial assay of the extract

Microorganisms

Twenty-four-hour cultures of nine human pathogenic microorganisms comprising bacteria (*Bacillus cereus, Escherichia coli, Klebsiella aerogenes, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Aspergillus flavus*) were employed for the *in vitro* antimicrobial evaluation. All test organisms were obtained from the clinical stock of the Department of Pharmaceutical Microbiology and Biotechnology,

Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

The antimicrobial activity of the extract was assessed using the agar well diffusion and broth micro-dilution techniques. The agar well diffusion assay was carried out to evaluate the susceptibility of the test microorganisms based on the ability of the antimicrobial agent to diffuse through the agar medium, specifically Mueller–Hinton agar (MHA). Fungal isolates were maintained on Sabouraud dextrose agar (SDA) at room temperature, while bacterial isolates were preserved on nutrient agar slants and stored at 4 °C. Prior to use, all microorganisms were reactivated by subculturing.

Inoculum preparation and standardization

Overnight cultures of the test bacteria on nutrient agar (28 g/L) plates and 48 – 72 h cultures of fungi on Saboraud dextrose agar (SDA, 65 g/L) plates were used for the agar well diffusion test. The test microorganisms were suspended in sterile normal saline solution (0.85% m/v) in separate test tubes and passed through a 0.45 µm Millipore filter (Millipore, India). The resulting filtrates were standardized using a UV–Visible spectrophotometer to an optical density of 1.0 ± 0.05 at 600 nm, in accordance with the 0.5 McFarland turbidity standard, corresponding to approximately 1.0×10^8 CFU/mL for bacteria and 5.0×10^6 CFU/ml for fungi.These standardized cultures were used for subsequent *in vitro* analysis.

Preparation and dilution of extract

The antimicrobial test compound was prepared by dissolution of 2000 mg of the compound in 5 ml sterile distilled water containing 10% DMSO (dimethyl sulfoxide). This resulted in 400 mg/ml of the extract stock concentration. Two-fold serial dilutions were further carried out to obtain concentration gradients of 100, 50, 25, 12.5, and 6.25 mg/mL.

Inoculation and incubation

The standardized microbial suspensions were separately seeded onto Mueller–Hinton agar (MHA) plates and allowed to stand for 15 minutes to ensure adequate absorption. Wells of 6 mm diameter were then aseptically punched into the agar using a sterile cork borer. Subsequently, 100 µL of different concentrations of the test compounds were dispensed into the wells with a micropipette. The plates were incubated at 37 °C for approximately 18 h for bacterial isolates, while fungal plates were incubated at 28 °C for 5 days. Gentamicin and fluconazole served as the positive controls for bacteria and fungi, respectively, while dimethyl sulfoxide (DMSO) was used as the negative control for both assays.

Determination of antimicrobial activity

The microbiological evaluation involved assessing the susceptibility of selected Gram-positive bacteria, Gram-negative bacteria, and fungi to the plant extract. The antimicrobial effects observed at different extract concentrations were compared with those produced by corresponding concentrations of standard antimicrobial agents. In addition, the minimum inhibitory concentration (MIC) as well as the minimum bactericidal and fungicidal concentrations (MBC/MFC) of the extract were determined for organisms that showed sensitivity.

Broth macro-dilution method for determination of MIC

This was done according to the Clinical and Laboratory Standard Institution [16] method. Two-fold serial dilutions from 6.25 to 100 mg/mLof the test extract were done in Eppendorf tubes containing 100 µL sterile Mueller Hinton broth. Each of the tubes was inoculated with equal volumes of the standard inoculum. The tubes were incubated for 18 h at 37 ºC for bacteria while plates for fungi were incubated at 28 ºC for 5 days. The lowest concentration of the test compound showing inhibition of the microorganisms was the minimum inhibitory concentration (MIC).

Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The test tubes indicating the MIC and other preceding tubes (also showing inhibition of the bioactive compound) were streaked on Mueller Hinton Agar (MHA) plates and incubated under the previous conditions. The absence of (or very scanty) growth after the incubation, was indicative of MBC/MFC of the bioactive compound.

Statistical Analysis

The data were statistically evaluated using descriptive statistics. Results are presented as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $P < 0.05$.

RESULTS

Percentage yield

Table 1 **shows t**he percentage yield of the crude extract of *C. citratus*

Table 1: The Percentage yield of the crude extract of *C. citratus*

The initial weight of the sample (g)	Yield (g)	Percentage yield (% w/w)
1000	400.85	40.09

Qualitative phytochemical analysis of the extract of *Cymbopogan citratus*

The result of the qualitative phytochemical analysis of *C. citratus*in Table 2 shows the presence or absence of the secondary metabolites and their corresponding relative abundance in *C. citratus* extract. It showed that phenols, steroids, tannins, and terpenoids were abundantly present; flavonoids were moderately in abundance; alkaloids and reducing sugar were low in abundance whereas glycoside and saponin were not detected in the extract.

Table 2: Qualitative Phytochemical analysis of the extract of *Cymbopogan citratus*

Constituents	Results
Alkaloids	+
Flavonoids	++
Glycoside	Nd
Phenol	+++
Reducing Sugar	+
Saponin	Nd
Steroids	+++
Tannins	+++
Terpenoids	+++

Key: '+++' = High in abundance, '++' = Moderate abundance, '+' = Low abundance, 'Nd' = Not detected

Quantitative phytochemical analysis of the extract of *Cymbopogan citratus*

The results of the quantitative phytochemical analysis of the extract of *C. citratus* are shown in Table 3. The quantitative phytochemical analysis of *C. citratus* was carried out to determine the amount of the phytochemicals present in the concentrated extract. The phytochemicals detected include: Alkaloids, reducing sugar, flavonoids, steroids, tannins, total phenolics, and terpenoids but glycosides and saponin were not detected.

Table 3: Results of the quantitative phytochemical analysis of the extract of *C. citratus*

Constituents	Quantity (mg/100g)
Alkaloids	476.67±0.07
Flavonoids	119±0.03
Glycoside	Nd
Total Phenolics	1897.84±0.09
Reducing Sugar	32.61±1.02
Saponin	Nd
Steroids	4.77±0.00
Tannins	16.25±0.05
Terpenoids	203.36±0.02

Key: Nd = Not detected

Antimicrobial activity of the extract of *Cymbopogan citratus*

The results of the antimicrobial susceptibility testing of the extract against the test organisms and the inhibition zone diameters (IZD) in (mm) of the various concentrations for each test organism are shown in Table 4. Results of the IZD of the plantextractof *C. citratus* against different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml), with their standard (gentamicin 10 mg/L) and

(fluconazole 40 mg/L). At 100 mg/ml concentrations, *Aspergillus flavus* recorded 9 ± 1.4 inhibitions, at 50 mg/ml concentration, it showed 9±1.4inhibitions while at 25 mg/ml, it showed 8 ± 1.4 inhibitions but gentamicin, the positive control showed27.5±2.12inhibitions. At the concentration of 100 mg/ml, 50 mg/ml, and 25 mg/m1, *Candida albican* had no inhibition zone diameter, whereas the positive control showed 37 ± 2.8 inhibitions. From 100 mg/m1 to 50 mg/ml concentrations, *Bacillus cereus* showed 9 ± 1.4 inhibitions each, at 25 mg/ml, there was no inhibition, while the standard drug showed 46 ± 4.2 inhibitions. *Escherichia coli* showed no inhibitions from 100 mg/m1 to 25 mg/m1 while the positive control showed 35 ± 7.0 inhibitions. *Klebsiella aerogenes, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhi,* and *Staphylococcus aureus* showed no inhibitions at concentrations of 100 mg/m1 to 25 mg/m1 while their positive control had inhibitions of 25 ±1.4, 41 ± 1.4, 46 ±1.4, 35 ± 0.0, and 50 ± 0.0, respectively.

Table 4. Determination of the antimicrobial susceptibility of the extract to the tested organisms

Test Organisms	Mean IZD) (mm ±SD)			Controls (mm ± SD mm)
	100 mg/ml	50 mg/ml	25 mg/ml	
Fungi				**Fluconazole (40 mg/L)**
Aspergillus flavus	9.0 ± 1.4	9.0 ± 1.4	8.0 ± 1.4	27.5 ±2.12
Candida albicans	-	-	-	37.0 ± 2.80
Bacteria				**Gentamicin (10 mg/L)**
Bacillus cereus	9.0 ± 1.4	9.0 ± 1.4	-	46.0 ± 4.20
Escherichia coli	-	-	-	35.0 ± 7.0
Klebsiella aerogenes	-	-	-	25.0 ± 1.4
Listeria monocytogenes	-	-	-	41.0 ± 1.4
Pseudomonas. Aeruginosa	-	-	-	46.0 ± 1.4
Salmonella typhi	-	-	-	35.0 ± 0.0
Staphylococcus aureus	-	-	-	50.0 ± 0.0

Key: (-) = **no inhibition**

The results of the minimum inhibitory concentration (MIC) using broth macro dilution of the active compound are shown in Table 5. The MIC of Aspergillus *flavus, Salmonella typhi,* and *E. coli* were recorded at 100 mg/ml, respectively. *Staphylococcus aureus, Klebsiella pneumonia, and Pseudomonas aeruginosa* had a MIC of 50 mg/ml each. *Candida albicans and Listeria monocytogenes* had a MIC of 25mg/ml each while *Bacillus cereus* had a MIC of 12.5 mg/ml.

Table 5. **Determination of the minimum inhibitory concentration (MIC) using broth macro dilution of the active compound.**

Test organism	MIC (mg/ml)
Aspergillus flavus	100
Candida albicans	25
Bacillus cereus	12.5
Staphylococcus aureus	50
Listeria monocytogenes	25
Klebsiella pneumonia	50
Pseudomonas aeruginosa	50
Salmonella typhi	100
E. coli	100

In Table 6, the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) which were determined using nine organisms comprising seven bacteria and two fungi were shown. *Bacillus cereus* gave an MBC of 12.5 mg/ml while *Candida albicans* had an MFC of 25 mg/ml.

Table 6. Determination of the minimum bactericidal concentration (MBC)/ Minimum fungicidal concentration (MFC)

Test organism	MBC/MFC (mg/ml)
Aspergillus flavus	-
Candida albicans	25
Bacillus cereus	12.5
Staphylococcus aureus	-
Listeria monocytogenes	-
Klebsiella pneumoniae	-
Pseudomonas aeruginosa	-
Salmonella typhi	-
E. coli	-

Key: (-) = no inhibition

DISCUSSION

The result of the phytochemical analysis conducted on the powdered leaves of C. citratus revealed that the plant contains the following metabolites: phenols, flavonoids, and tannins. This is in line with earlier reports by Valan *et al* [17]. Similarly, phenols, steroids, tannins, and terpenoids were abundantly present; flavonoids were moderately abundant; alkaloids and reducing sugars were low in abundance, whereas glycosides and saponins were not detected in the extract. It was also observed that the total phenolics possessed the highest quantitative phytochemical analyzed at 1897.84 ±0.09 mg/100g followed by Alkaloids (476.67 ±0.07 mg/100g), Terpenoids (203.36 ± 0.02 mg/100g), Flavonoids (119±0.03 mg/100g), Reducing sugar (32.61±1.02 mg/100g), Tannins (16.25±0.05 mg/100g), and Steroids (4.77±0.00 mg/100g) while Glycosides and Saponins were not detected.

It is, therefore, the findings of the present study that the antimicrobial properties demonstrated by lemongrass (*C. citratus*) samples were due to the presence of phytochemicals in the leaves. This has further validated earlier reports that the antibacterial activity of lemongrass was because the leaves had bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds[17]. In a related study, it was believed that flavonoids were capable of complexing with the bacterial cell wall, causing the death of the microorganism and tannins can inactivate enzymes, transport proteins, and microbial adherence [18]. The traditional medicinal uses of this plant in the management of various health conditions can again, be attributed to the presence of these phytochemicals and the synergy between the various chemical constituents. The tannic acid in tannin (16.25±0.05 mg/100g) which has been found abundant in the present study has been reported to act against some bacteria through the ability of these compounds to dissolve the fatty layer of the bacterial walls which causes leakage of cell fluid out the cell and destroys it[18]. The findings of this study indicate that the inhibitory activity of the lemon grass extract was highest against *B. cereus*, while its minimal activity was observed against *E. coli and Salmonella typhi*.

The antifungal and antibacterial activities of this plant extract suggest a potential source of antimicrobial agents that require further exploration. Comparatively, the Gram-positive bacteria like *Bacillus cereus* with MIC of 12.5 mg/ml, *Listeria monocytoenes* with MIC of 25 mg/ml, and *Staphylococcus aureus* with MIC of 50 mg/ml, were more susceptible to the extract than the Gram-negative bacteria such as *Salmonella typhi* with MIC of 100 mg/ml and the *Escherichia coli* with MIC of 100 mg/mL. Also, the yeast fungus, *Candida albicans* with a MIC of 25mg/ml was more susceptible to the extract than the mold fungus *Aspergillus flavus* with a MIC of 100mg/ml. This susceptibility pattern could further make the extract, a choice for targeted therapy. The MBC/MFC of the organisms showed that *Bacillus cereus* had an MBC of 12.5 mg/ml and *Candida albicans* had an MFC of 25 mg/ml. This is an indication that the extract has a high therapeutic index and is a suitable candidate for the formulation of human drugs. The leaf extract of *C. citratus* has demonstrated antimicrobial properties which could be harnessed for the control of pathogens tested.

CONCLUSION

The phytochemical and antimicrobial activities of the leaf extract of *C. citratus* were evaluated. The antimicrobial properties demonstrated by lemongrass (*C. citratus*) leaf extract in the present study were attributed to the presence of

phytochemicals in the leaves. The bioactive compounds such as alkaloids, flavonoids, tannins and phenolic compounds were detected. *C. citratus* possesses bioactive antimicrobial metabolites effective against *Bacillus cereus* and *Aspergillus flavus.* Further studies will be undertaken to purify and characterize these biologically active compounds.

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AUTHORS CONTRIBUTION

DPB and COU designed the work. JGN carried out the research under the supervision of DPB. DPB and IAO drafted the original manuscript. SAE, RCO, CAO, and DCO revised the manuscript and provided technical guidance. All authors read, made comments, and agreed on the final manuscript.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

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