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Antibacterial Efficacies of Extracts of Lemon Grass (*Cymbopogon citratus*) on Some Clinical Microbial Isolates

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Abstract

The spread of new infections and the emergence of multidrug resistant strains of microorganisms necessitate the search for new antimicrobials with focus on plant extracts. In this study, the antimicrobial activity of the extracts (aqueous and ethanolic) of lemon grass known usually for bioactive essential oil (*Cymbopogon citratus*) was investigated in-vitro, against selected clinical isolates (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans*). Antimicrobial efficacy of the extracts was assessed using the agar-well diffusion and broth-dilution techniques. The aqueous extract of *C. citratus* was more efficacious as it showed higher antimicrobial activity against all the test isolates at all tested concentrations. In contrast, only two of the test isolates; *P. aeruginosa* and *S. aureus* were susceptible to the ethanolic extract with minimum inhibitory (MIC) concentrations of 2 mg/ml (against *S. aureus*) and 12.5 mg/ml (against *P. aeruginosa*). The MIC of the aqueous extract were (mg/ml): 2, 6.25, 12.5, 25 and 50 against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli* and *C. albicans* respectively. Meanwhile, both the aqueous and ethanolic extracts of *C. citratus* exerted only a bacteriostatic effect against the five test isolates at all concentrations. Quantitative phytochemical screening of the two extracts revealed the presence of more concentration of active compounds in the aqueous extract than the ethanolic extract which plausibly accounts for its greater antimicrobial activity. This study has shown that, in difference to the use of its conventional essential oil, solvent extraction of *Cymbopogon citratus* is also a potent source of efficacious antimicrobials. Further works to determine the respective mechanisms of action(s) of the extracts' active principle is being projected.

Keywords: Lemon grass, clinical isolates, antimicrobial efficacies, bioactive compounds

Introduction

Antibiotics are regarded as “miracle drugs” (Levy, 2013) because of their ability to greatly reduce mortality and morbidity due to infectious diseases (Zaffiri *et al.*, 2012). Infectious diseases are known

life-threatening ailment (Leeb, 2004) with overall impact on the society (Zaffiri *et al.*, 2012).

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Infectious diseases are known life-threatening ailments (Leeb, 2004) with overall impact on the society (Zaffiri *et al.*, 2012). However, this success is recently being reversed due to increased antibiotic resistance *in vivo*. Antibiotic resistance is, mostly, due to human anthropogenic activities ranging from the over-use (Llor & Bjerrum, 2014) and misuse (El Salabi *et al.*, 2013) of antibiotics which often lead to treatment failure (Woolhouse *et al.*, 2016) as direct consequence (Llor & Bjerrum, 2014). Misuse and over-use are the major vehicles driving antibiotic pressures which ultimately accelerates the mechanisms of resistance in previously susceptible bacteria and/or the emergence of a new resistant strains. The problem of antibiotic resistance is of immense magnitude that it is now recognized as belonging to the category of critical dangers to humanity (Llor & Bjerrum, 2014). This is not overly exaggerated as it ranks according to Santajit & Indrawattana (2016) in the top three global public health threat. The significance of the problem and the urgency of solutions therefore necessitate a search for alternative sources of antimicrobials from verifiable ancient therapeutic system which employ plant sources often referred as nature's pharmacy (Pouliot, 2011). Plants, has throughout human history provided drugs to cure ailments (Cragg & Newman, 2013) and supported healthy growth of man and animals (Hasan & Sadiq, 2020). Rural dwellers and about 34 % of the world's population, even now, largely depend on plants (Pouliot, 2011). Lemongrass, *Cymbopogon citratus*, is one such potent alternative source of antimicrobials (Ekpenyong *et al.*, 2015) which is widely cultivated globally (Francisco *et al.*, 2014). It has been reported for wide range of activities against several diseases-causing organisms (Figueirinha *et al.*, 2008; Francisco *et al.*, 2014; Francisco *et al.*, 2011; Naik *et al.*, 2010; Shah *et al.*, 2012). Researches on the use of lemon grass as potential antimicrobial agent have been focused majorly on the use of its essential oils (Adukwu *et al.*, 2016; De Silva *et al.*, 2017; Naik *et al.*, 2010). While studies on the use of its other equally potent but extractible components are, despite high potency of reported activities, very few or relatively insignificant. For instance, respective reports by Adeneye & Agbaje

(2007) and Ekpenyong *et al.* (2015) have shown the activities of different extracts of *Cymbopogon citratus* as antibacterial, antimalarial, antifungal, and antiprotozoal. On the other hand, details of the components and mode of actions of the essential oil components of *C. citratus* have been well elucidated (Adukwu *et al.*, 2016; Katsukawa *et al.*, 2010). For instance, Katsukawa and co-researchers (Katsukawa *et al.*, 2010) established that essential oil from lemon grass acts on microorganism by suppressing the protein expression. Through cell-based transfection assay, they postulated that citral, a major component of lemon grass essential oil suppressed cyclooxygenase (COX-2) which is a key enzyme for prostaglandin synthesis and also an activator of peroxisome proliferator-activated receptor (PPAR). While prostaglandins are group of **lipids** made at sites of tissue damage or injurious microbial infection for quick repairs, PPAR is a molecular target for "lifestyle-related" diseases. They further argued that citral also suppressed both LPS-induced COX-2 mRNA and protein expression, albeit dose-dependently. However, there is scarcely a report on the determination of the exact active principles of the non-oil extract of this plant; and where exist, there is no report on its mechanism of action, a basis to understand the logical therapeutic and nutritional uses of the drug. This study thus investigated the phytochemical composition and the antimicrobial activity of aqueous and ethanolic extracts of *C. citratus* leaves against selected clinical isolates (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Candida albicans*) which currently form some of the most formidable multidrug resistant pathogens (Santajit & Indrawattana, 2016).

Materials and Methods

The *C. citratus* leaves were obtained from a local herb store in Ilorin, Kwara state, Nigeria (8.4799° N, 4.5418° E). The plant sample was identified visually and confirmed at the Herbarium unit of Department of Plant Biology, University of Ilorin and registered under the voucher number UILH/001/2019/949. The leaves were sun-dried, grounded into a fine powder and thereafter kept in

sterile plastic containers until further use at room temperature (28 ± 1 °C).

Test Organisms

Five test isolates viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Candida albicans* were obtained from the stock culture of the Department of Microbiology, University of Ilorin Teaching Hospital, Ilorin, Nigeria. Before use, each organism was grown on respective selective media and were all gram-stained (except *Candida albicans*) to confirm the respective identity as follows: *Staphylococcus aureus* (yellow colonies on Mannitol Salt Agar), *Escherichia coli* (green metallic sheen on Eosin Methylene Blue), *Pseudomonas aeruginosa* (green colonies on CLED Agar), *Enterococcus faecalis* (black zones around colonies on Bile Esculin Agar) and *Candida albicans* (cream pasty convex on Sabouraud Dextrose Agar). Purity of the culture was checked at regular intervals according to standard protocols.

Preparation of Extracts

Aqueous extract of lemongrass was prepared following the protocol described by Balakrishnan *et al.* (2014). On the other hand, the ethanolic extract was prepared by soaking 50 g of finely cut lemongrass leaves in 500 mL of ethanol (95 %) at room temperature and kept on a shaker (150 rpm) for 36 hours in an air-tight container as earlier described by Ajijolakewu & Awarun (2015). The aqueous and ethanolic extracts obtained were filtered at first using clean muslin cloth and afterwards with Whatman No 1 filter paper. Both filtrates were respectively evaporated to dryness and stored in sterile, air-tight cap bottles at room temperature ($29^\circ\text{C} \pm 1^\circ\text{C}$) until further use.

Reconstitution and Preparation of Stock Concentration

Dimethyl sulfoxide (DMSO) was used to prepare stock concentration from all extracts. Two grams of extracts were dissolved in 10 mL of solvent to obtain the general stock at a concentration of 200 mg/ml. Varying concentration were prepared from the main stock in the following proportions 100, 50, 25, 12.5, 6.25 and 2 mg/ml by diluting with

appropriate volume of DMSO. The different concentrations were stored in air-tight bottles after proper agitation until used.

Determination of Antimicrobial Activity

Inocula of the test isolates were standardized as described by Singh *et al.* (2010) to quantitatively assess the antimicrobial activity of the extracts. Inoculum standardization was done by making up each microbial suspension to equal the absorbance (530 nm) of 0.5 McFarland standard (300×10^6 cfu/ml) on UV spectrophotometer. The agar well diffusion technique as described by Ajijolakewu & Awarun (2015) was adopted to determine the antimicrobial activities of the extracts. In this method, 0.1 mL of the respective standardized inocula was spread on sterile Mueller Hinton agar plate with using sterile glass spreader. Agar plates were allowed to dry and agar wells (6.0 mm diameter) were bored aseptically using sterile cork-borer. The wells were bored according to the number of intended extract concentrations in addition to an extra used as negative control. Subsequently, 0.1 mL of each extract concentration was introduced into the wells and thereafter incubated at 37 °C for 24 hours. Streptomycin was used as positive control. Antimicrobial activity of the extracts was determined by measuring the zones of inhibition produced around the wells. The diameter of the zones indicated the degree of susceptibility of the test organisms.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was determined following the protocol described by Ajijolakewu & Awarun (2015) with slight modification. One milliliter (1.0 mL) of all extracts at concentrations 200, 100, 50, 25, 12.5, 6.25 and 2 mg/mL were added into 9 mL of sterile nutrient broth in different test tubes. Subsequently, 0.1 mL of an 18-hour old culture broth adjusted to 0.5 McFarland turbidity standard was inoculated into each test tube. Two controls (one consisting of a combination of plant extracts at various concentrations plus the nutrient broth without inoculum in the same proportion as the experiment and a blank tube consisting of only

sterile broth) were set. The tubes were incubated at 37 °C for 24 hours and observed. Upon observation, the tube with the lowest concentration (highest dilution) that had no detectable growth when compared with the control tubes using both visual and spectrophotometer observations was taken according to the protocol of Dahiya & Purkayastha (2012) as the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined as described by Ajijolakewu & Awarun (2015). The Minimum Bactericidal Concentration of the extracts was evaluated by sub-culturing test solutions which showed no turbidity after 24hrs incubation (i.e. no detectable growth) onto a recovery medium (a fresh Nutrient Agar plates) and incubated further for 24 hours. Appearance or absence of growth on the fresh nutrient agar plates suggested either bacteriostatic or bactericidal effect respectively

Phytochemical Screening of Extracts

The extracts were screened for the presence of tannins, saponins, flavonoids, steroids, terpenes, terpenoids, glycosides, alkaloids and phenol as described by Madhu *et al.* (2016).

Statistical Analysis

Data were expressed as mean and standard deviation of three independent replicates based on statistical analysis using IBM SPSS Statistics V22.

Results and Discussion

This study investigated the antimicrobial properties of aqueous and ethanolic extracts of *Cymbopogon citratus* leaves against selected clinical isolates involving a fungus and four bacterial species so as to generate data to be used as a logical leap for the determination of the plant's most potent active principle(s) and for a subsequent elucidation of their various modes of action against test organisms which, as far as we know, have not been previously described in any report. Findings in this study are hereby presented below:

Quantitative Analysis of Phytochemical Constituents

Table 1 shows the quantitative ranking of the phytochemicals present in both aqueous and ethanolic extracts of *Cymbopogon citratus* respectively. Except for glycosides, the generally known and important plant's bioactive compounds such as, tannins, saponins, alkaloids and terpenoids were present in both the aqueous and ethanolic extracts albeit in different amounts. The aqueous extracts showed preponderance of the important components (e.g. terpenoids, triterpenes and flavonoids) as against the ethanolic extract which was only able to extract more of the inconsequential saponins and steroids on a comparative scale with aqueous extract (Table 1). The reasons for the obvious disparity are immediately unknown but may be due to differences in the polarity of the two solvents. Water is more polar than ethanol, hence the greater amounts of component amounts. Another reason could be the insufficiency in the volume or quantity of ethanol used during extraction, a factor which is being considered for further research. Generally, these bioactive compounds have been demonstrated to be responsible for the antimicrobial activity of medicinal plants. For instance, phenolics generally classified into tannins, phenolic acids, flavonoids among other categories (Tacouri *et al.*, 2013) exerts antioxidant effects which are known to disrupt cell enzyme activities and to cause disintegration of the outer membrane of microorganisms (Lacombe *et al.*, 2010). Phenolics have also been shown (Espín *et al.*, 2017) to modulate healthy gut microbiota thereby establishing a scientific basis for the oral administration of lemongrass decoctions as used in folk medicine. Saponins are a category of glycosides with fat-soluble core (aglycone) in the form of a triterpenoid (C-30) or alkaloid steroid (C-27) (Hassan *et al.*, 2010b). Although, with yet unclear mechanism of antibacterial activity, saponins are known to generally express a dose-response effect on all bacterial species even though some glucosidases produced by Gram negative bacteria have been reported to be capable of saponins degradation (Hassan *et al.*, 2010a).

Table 1: Quantification of Phytochemical Constituents of the Aqueous and Ethanolic Extracts of *Cymbopogon citratus*

Phytochemicals	Aqueous Extract (mg/mL)	Ethanolic extract (mg/mL)
Phenolics	0.78 ± 0.003	0.94 ± 0.001
Flavonoid	1.45 ± 0.000	0.79 ± 0.000
Saponins	0.08 ± 0.000	1.33 ± 0.000
Glycoside	0.00 ± 0.000	0.00 ± 0.000
Alkaloid	1.49 ± 0.004	1.46 ± 0.026
Steroids	1.47 ± 0.000	2.19 ± 0.059
Tannin	0.33 ± 0.016	0.20 ± 0.004
Triterpene	7.13 ± 0.170	4.07 ± 0.270
Terpenoids	3.90 ± 1.530	2.17 ± 0.650

Data are expressed as mean + standard deviation of three independent replicates.

As shown in Table 1, although in different quantities, both the aqueous and ethanolic extracts exhibited similar fractional components to enhance the modulation of their respective antimicrobial activities.

Antimicrobial Activity of Extracts

Tables 2a & 2b shows the results of the antimicrobial efficacies of the aqueous (Table 2a) and ethanolic (Table 2b) extracts of *C. citratus*. Varying degrees of growth inhibition were expressed by the respective extracts against the test isolates.

Table 2: Antimicrobial Activity of Aqueous and Ethanolic Extracts of *Cymbopogon Citratus* against Selected Clinical Isolates

a. Aqueous Extract of *Cymbopogon citratus*

Concentration (mg/ml)	Diameter of Zones of inhibition (mm) on the representative clinical isolates				
	<i>Candida albicans</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
2.00	-	-	-	-	2±0
6.25	-	7±2	7±2	-	3±0
12.50	-	8±0	9±1	5±0	5±1
25.00	-	15±2	10±2	9±1	6±0
50.00	4±0	17±1	10±0	13±2	15±2
100.00	10±1	19±2	14±1	14±0	15±1
200.00	20±2	21±2	19±2	21±2	25±2

b. Ethanolic Extract of *Cymbopogon citratus*

Concentration (mg/ml)	Diameter of Zones of inhibition (mm) on the representative clinical isolates				
	<i>Candida albicans</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
2	-	-	-	-	-
6.25	-	-	-	-	-
12.5	-	3±0	-	-	-
25	-	8.5±1	-	-	-
50	-	12±2	-	-	-
100	-	16±1	-	-	-
200	-	19±2	-	-	11±2

As shown in Table 2a, *S. aureus* was the most susceptible organism to the aqueous extract of *C. citratus* expressing different degree of sensitivity to various concentrations of the extract used. All other species were also susceptible to a fair concentration (6.25 mg/ml) of aqueous extract except *E. coli* whose susceptibility was observed at concentration 12.5 mg/ml of the extract. On the other hand, the unicellular fungus *Candida albicans* is the least susceptible organism eliciting noticeable susceptibility at 50 mg/ml of the aqueous extract. Table 2b shows the susceptibility patterns of test isolates to ethanolic extracts of *C. citratus*. The isolate *P. aeruginosa* was the only susceptible organism at concentration between 12.5 and 200 mg/ml of the extract. All other isolates showed resistance except *S. aureus* which was only susceptible at higher concentration of 200 mg/ml. Shah *et al.* (2011) had earlier reported the efficacy of aqueous and ethanolic extracts of *C. citratus* against several medically important microorganisms including species of those used in this study.

Comparatively, the aqueous extract was most potent against all the tested isolates (Table 2a) compared to the ethanolic extract (Table 2b) of Lemon grass (*Cymbopogon citratus*). The broader spectrum of activity of the aqueous extract is most probably due to the preponderance or higher amount of the its component phytochemicals (Table 2) compared to the ethanolic extract. This finding is however, in contrary to the report of Dahiya & Purkayastha (2012) who reported a consistent and potent activity for ethanolic and methanolic extracts over extracts of hexane, chloroform, methanol, ethanol and aqueous. Other researchers (Fagbemi *et al.*, 2009; Joshua *et al.*, 2012) had also observed higher potency of the ethanolic extracts of *C. citratus* compared with other forms of extraction. However, this puzzling observation in this study could justifiably be due to the higher polarity of water in comparison to the less polar organic ethanol. Moreover, the variation could also be due to difference in geographic and climatic conditions which is established to affect phytochemical composition of plants as earlier observed and reported by Shah *et al.* (2011). Moreover, Since the quantity of phytochemicals

present is a function of its efficacy for antimicrobial effect, it is plausible to tie the better antimicrobial effect of aqueous to flavonoids, glycosides, alkaloids, triterpenes, and terpenoids which were higher in the aqueous compared with the ethanolic extracts.

Results of antimicrobial susceptibility testing of the aqueous extract (Table 2a) and the ethanolic extract where applicable (Table 2b) established an effect of concentration gradient on the susceptibility pattern in all test isolates. In Table 2a, the rate of microbial inhibition (represented by the zone of inhibition) is proportional to the concentration of the extract. The highest concentration (200 mg/ml) was the most efficacious against all the organism while the lowest effective concentration was organism-dependent. The least susceptible organism was *C. albicans* with the lowest effective concentration 50 mg/ml. *Staphylococcus aureus* was most susceptible such that a 2mg/ml concentration of the aqueous extract successfully inhibited its growth to a certain degree i.e. 2mm (Table 2a). On the other hand, nearly all the tested isolates were resistant to the ethanol extract of *C. citratus* (Table 2b). Even though, *Staphylococcus aureus* was also susceptible (only at 200 mg/ml); it was only *Pseudomonas aeruginosa* which showed obvious susceptibility pattern at extract concentrations between 25 mg/ml & 200 mg/ml. Others, *C. albicans*, *Enterococcus faecalis*, and *Escherichia coli* are entirely resistant across concentrations.

Comparatively, the data presented in Tables 2a & b show that the aqueous extract of *C. citratus* is generally more efficacious performing better than the ethanolic extract. Also, independent of the type of susceptible organisms, both extracts exerted a dose-response susceptibility (Table 2a); and only *Pseudomonas aeruginosa* was the only major organism susceptible to the ethanolic extract (Table 2b). This is spectacular since previous report by Naik *et al.* (2010) has concluded that (essential) oil extract from lemon grass was ineffective against *P. aeruginosa*. However, Relative to the finding here, diseases to which *P. aeruginosa* has been implicated can easily be treated using the aqueous extract from ABU.

Further, as shown in Table 2a, *Candida albicans*, a fungus, requires particularly higher extract

concentration before inhibition. This was followed by all Gram negatives isolates, while *S. aureus* was particularly susceptible at all concentrations tested. This hierarchy in susceptibility may be attributed to the distinct difference in the cell types. Naik *et al.* (2010) has also reported that gram positive organisms are more susceptible to lemon grass oil than gram negatives. This is more so that clinical fungal isolates, Gram negative bacteria, and then Gram positive pathogens rank in virulence in descending order (Abe *et al.*, 2010).

Minimum Inhibitory Concentrations (MIC) and Maximum Bactericidal Concentrations

Table 3 show the minimum inhibitory (MIC) and maximum bactericidal (MBC) concentration values of the respective extracts. As shown, both extracts showed no bactericidal property on all the tested isolates. Based on the larger inhibitory activities, MIC values were demonstrated and recorded for the aqueous extract on all the tested isolates but were recorded only for two isolates on which the ethanolic extract was effective. The MIC values were organism as well as extraction-type-dependent. For instance, MIC value of *S. aureus* using aqueous extract was 2 mg/ml but was 150 mg/ml using ethanolic extract. On the other hand, the MIC on *C. albicans* is 50 mg/ml against the 2 mg/ml or 6.25 mg/ml exhibited by *S. aureus* and *E. faecalis* (Table 3). No MBC value was recorded as there was regrowth across all the concentrations tested as shown in Table 3.

Minimum inhibitory Concentration (MIC) of the extracts of *Cymbopogon citratus*

Organism	MIC (mg/ml)		MBC (mg/ml)	
	Aqueous Extract	Ethanolics Extract	Aqueous Extract	Ethanolics Extract
<i>S. aureus</i>	2	150	-	-
<i>P. aeruginosa</i>	12.5	12.5	-	-
<i>E. faecalis</i>	6.25	-	-	-
<i>E. coli</i>	25	-	-	-
<i>C. albicans</i>	50	-	-	-

The absence of biocidal effect points to the presence of resistance mechanisms within the test isolates that overturns the inhibitory activity of the extracts. In previous studies, drug inactivation

/alteration of target sites, limited intracellular concentration of antimicrobial, agent binding site modification, biofilm formation (Santajit & Indrawattana, 2016), ribosomal protection-encoding genes, efflux pump (Castanheira *et al.*, 2014), alteration of responses towards cellular stress (Shapiro *et al.*, 2011) have been reported as resistance mechanisms commonly exhibited by most the organisms used in this study. In further studies, the specific resistance mechanism will be studied in addition to knowing the mechanism of actions of the active phytochemicals of the lemon grass. Other extraction methods (non-polar) will also be tried in subsequent researches to determine the likely presence of other important phytochemicals.

Conclusion

Aqueous and ethanolic extracts of lemon grass (*Cymbopogon citratus*) have been used to demonstrate its potency against selected clinical isolates thus justifying its use in traditional medicine, as its essential oil, for treating different diseases associated with the tested susceptible isolates of both superficial and enteric origins. While both extracts exerted a dose-response susceptibility the susceptible organisms, the aqueous extract of *C. citratus* is generally more efficacious than the ethanolic extract of same. Work is still on to optimize concentration towards the determination of the biocidal concentration, the mechanism of action of synergistic effect of the plant's extract active principles and to understand the mode of microbial resistance. Further toxicological, purification and identification studies would be carried out to investigate the general effects of the use of lemon grass for drug development.

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