



PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF *CYMBOPOGON CITRATUS* (Lemongrass) LEAVES, STEMS AND ROOTS

ABSTRACT

The rotary evaporator, cold maceration and agar diffusion technique were employed to determine the phytochemical analysis and the antimicrobial efficacy of secondary metabolites of *Cymbopogon citratus* leaves, stems, and roots against selected microbial pathogens using ethanol as extracting solvents. The phytochemical analysis on *Cymbopogon citratus* showed the presence of the following

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Introduction

According to Liu (2004), phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major degenerative diseases. In his report, Anderson, (2004) defined phytochemicals as plant derived chemicals, which are beneficial to human health and diseases prevention. According to (Art and Halman, 2005). Phytochemicals are large groups of plant-derived compounds hypothesized to be responsible for much of disease protection provided by diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. Phytochemicals are responsible for the medicinal activities of plants (Savithramma, *et al.*, 2011).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients (HASLER & BLUMBERG, 1999). They protect plants from



secondary metabolites in all the extracts in abundant; saponins, tannins and flavonoids. Alkaloids were present in both leaves and roots in moderate amounts and absent in stems. Cardiac glycoside was only present in the leaves and Anthraquinones were absence in all extracts. The leaves extracts showed the strongest activity overall with largest inhibition zones against *S. aureus* (22.1 ± 0.8 mm at 100 mg/mL) and lowest MICs (as low as 3.125 mg/mL). Stems showed intermediate activity and roots were least active. Bactericidal endpoints were typically one dilution above MIC (MBC/MIC ratio ≤ 4), indicating predominantly bactericidal effects against *S. aureus* and *E. coli*; fungicidal action against *C. albicans* required higher concentrations. Ethanolic leaves extract of *Cymbopogon* exhibits superior broad-spectrum antimicrobial activity relative to stems and roots, plausibly driven by higher flavonoid and saponin content. The findings support preferential use of leaves for antimicrobial herbal preparations and justify further bioassay-guided fractionation.

Keywords: *Cymbopogon citratus*, Lemongrass, Phytochemicals, Antimicrobial activity, Minimum Inhibitory Concentration, Herbal medicine.

disease and damage, and also contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (GIBSON et al., 1998; MATHAI, 2000). Recently, it has been clearly shown that they also have roles in the protection of human health, when their dietary intake is significant (SAMROT et al., 2009; KOCHE et al., 2010). Till date over 4,500 phytochemicals have been reported and are classified on the basis of their protective functions, and physical and chemical characteristics, amongst these about 350 phytochemicals have been studied in detail (KOCHE et al., 2010).

Phytochemicals accumulate in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed (COSTA et al., 1999). Many phytochemicals, particularly the pigment molecules like anthocyanines and flavonoids, are often concentrated in the outer layers of the various plant parts like leaves and fruits of vegetables. However, the levels of these phytochemicals vary from plant to plant depending upon the variety, climatic growing conditions (RAO, 2003). The exact classification of phytochemicals has not been given so far, because of their diverse forms and structures. Classically, the phytochemicals have been classified as primary or secondary metabolites, depending on their role in plant metabolism. Primary metabolites include the common sugars, amino acids, proteins,



purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary metabolites are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics and glucosides (HAHN, 1998; RAMAWAT et al., 2009). In plant phytochemicals attract and repel harmful organism, serve as photo protectants, and respond to environmental changes. For instance, Isoflavones, Anthocyanin and Flavonoids do function as phytoalexin, substance that assist a plant to resist pathogens (Agte et al., 2000). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents play a significant role in the identification of crude drugs (Savithamma et al., 2011).

These phytochemicals reduce LDL, that is, the cholesterol Involved In depositing fat in the arteries and prevents blood clotting which can reduce the risk for a heart attack or a stroke (Anderson et al., 2004). The mode/mechanism of action of phytochemicals in generally is considered to be the disturbance of cytoplasmic membrane, disrupting the proton motive force, electrons flow, active transport, and coagulation of the cell contents (Kolzekidou of al., 2008). *Cymbopogon citratus* (Lemon grass) belonging to the family Gramineae (genus: *Cymbopogon*) is an aromatic perennial herb with rhizomes and densely tufted fibrous root (Adekomi et al., 2012). It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Omotade, 2009). It is a fast growing, aromatic grass native to South India and Sri Lanka, now widely cultivated in other tropical and subtropical countries of Africa, America and Asia (Chanthai, et al., 2012). The stem is reddish brown in color and it is attached to the bulb by stalk. The entire plant is attached to the soil by fibrous root (Burkill, 1996). It has short underground stem with ringed segment and green slightly lathery leaves in dense clusters. (Figueirinha et al., 2005 and Mehraban et al. 2005). The linear leaves can grow up to 90cm and 50cm in with (Ernst, 2008). *Cymbopogon citratus*, is one such potent alternative source of antimicrobials (Ekpenyong et al., 2015) which is widely cultivated globally (Francisco et al., 2014).

Freshly cut and partially dried leaves are used extensively in Ayurvedic medicine. The grass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, fever, hypertension, leprosy, malaria, ophthalmic pneumonia, nervous, gastrointestinal and vascular disorders (Karkala and Bhushan, 2014). Studies indicate that *Cymbopogon citratus* possesses various pharmacological activities such as antibacterial (Danlami et al., 2011), antifungal (Nguefack et al., 2012), antioxidant (Hanisa et al., 2011; Garg et al., 2012) and anti-inflammatory (Figueirinha et al., 2010) properties. Lemongrass is a plant in the grass family that contains 1 to 2% essential oil on a dry basis and its chemical composition varies widely as a function of genetic diversity, habitat and agronomic treatment of the culture (Ranitha et al., 2014)



Researches on the use of lemongrass 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. This study thus investigates the phytochemical composition and antimicrobial activity of ethanolic extracts of *C. citratus* leaves, stems and roots against selected clinical isolates (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans*) which currently form some of the most formidable multidrug resistant pathogens (Santajit & Indrawattana, 2016).

Materials and Methods

Collection of Plant Materials and Identification

Fresh, undamaged, non-insect infected leaves, stems and roots of *Cymbopogon citratus* (lemon grass) were collected from Malali State Low-Cost Area of Kaduna, Kaduna State, Nigeria. It was authenticated by Malam Musa Muhammad of the Herbarium Section Department of Biological Science, Ahmadu Bello University (ABU) Zaria with vouchers number 1882.

Preparation of Plant Materials

The freshly collected leaves, stems and roots were thoroughly washed in water 2-3 times and removed all the adhering dust and dirt particles. They were air dried for a week at room temperature. The dried leaves, stems and roots were then pulverized with the aid of clean wooden mortar and pestle into smaller particles and then grounded into fine powder and finally sieved with a mesh of size 0.5mm. The powdered samples obtained were stored in air-tight clean sterile container and kept at ambient temperature until when needed for use. The extracting solvent were 95% ethanol, (Uzama, 2009).

Ethanolic Extraction Procedure

One hundred gram (100g) of each leaf, stems and roots powdery mass were weighed on electric weighing balance and soaked in 500ml of the solvent in the three tightly closed 1000ml capacity separate conical flask and kept for three days which is equivalent to



seventy-two hours (72hours) with occasional shaking. They were filtered using muslin cloth. Each of the filtrate was further filtered again using No. 1 Whatman filter paper in beaker using a funnel. The filtrated extract (ethanolic) was concentrated using the rotary evaporator equipment at r.p.m of 123 and at the temperature of 78°C (boiling point of ethanol) after which they were finally dried on water bath at the temperature of 45°C to a sticky semi-solid greenish extract. (Uzama, 2009).

Qualitative Phytochemical Analysis

Phytochemical analysis was performed to screen the extract for the presence of the following active principles. Tannins, Flavonoids, Saponin, Alkaloid, Cardiac Glycosides and Anthraquinones using standard procedures as described below;

Preparation of the Extract for Phytochemical Screening

One point zero (1.0g) of each extract of leaves, stems and roots were weighed on an electric weighing balance and dissolved in 10ml of the solvent.

Preparation of Mayer's Reagent

Zero point three hundred and fifty-five grams (0.355g) of mercuric chloride was weighed and dissolved in 60ml of distilled water. 5.0g of potassium iodide was dissolved in 20ml of distilled water. Both solutions were mixed and up to 100ml with distilled water.

Test for Tannins

Zero point five milliliters (0.5mls) of each extract was drawn and added to 10.0ml of distilled water in a test tube. It was mixed with few drops Ferric Chloride (FeCl₃) using rubber dropper. An immediate visible green precipitate is indicating positive test (Tyler *et al.*, 1984).

Test for Flavonoids

One point zero milliliters (1.0mls) of each extract were drawn and dissolved in NaOH solution. The appearance of yellow solution, which disappeared on addition of HCl, indicates the presence of Flavonoids (Trease, 1984).

Test for Saponin

Zero point five milliliters (0.5mls) of each extract were shaken with 10ml water in a test tube for 3 minutes. The production of frothing which persisted on heating indicated the presence of Saponins. Trease and Evans (1996), Iwu, M. (1993) and Harbone, J. B. (1984).



Test for Alkaloid

Ten drops of Meyer's reagent were added to 0.5ml of each extract in a test tube. Cream precipitate indicates the presence of Alkaloids. Trease and Evans (1996), Iwu M. (1993) and Harbone J. B. (1984).

Test for Cardiac Glycosides

Zero point five milliliters (0.5mls) of each extract were dissolved in 2ml of chloroform and a few drops of concentrated H_2SO_4 , were added to form a lower layer. A reddish-brown color at the interface indicates the presence of Cardiac Glycosides. Trease and Evans (1996), Iwu M. (1993) and Harbone J. B. (1984).

Test for Anthraquinones

Zero point one milliliters (0.1mls) of each extract were dissolved in 10mls of benzene and filtered, 5mls 10% ammonia solution was added to the filtrate. The presence of pinkish color in the lower ammonia layer signifies the presence of Anthraquinone.

Microorganisms and Inoculum Standardization

The microorganism used namely *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*, were obtained from stock cultures in the Microbiology Laboratory Department, Kaduna State University, Nigeria. They were sub-cultured and identified based on their colonial morphology, microscopic appearance and specific biochemical reactions. Inocula were standardized to 0.5 McFarland ($\sim 1 \times 10^8$ CFU/mL for bacteria; $\sim 1 \times 10^6 - 10^7$ CFU/mL for *C. albicans*) and diluted to achieve $\sim 1 \times 10^6$ CFU/mL in assays.

Antimicrobial Activities

Antibacterial and antifungal assay of crude extract were the methods used for the determination of the antimicrobial activity of the crude extract against the test organisms.

Antibacterial Activity

Antibacterial activity of the extracts was determined using agar well diffusion method. The prepared sterile nutrient agar was poured on eight plates for each microorganism and the night plate for control and allowed to solidify. All the plates were labeled appropriately according to the number of the test organism. Using a sterile swap stick, the swap stick was dipped into the already prepared inocula and removing excess inocula by pressing and rotating the swap firmly against the side of the tube and radially streaked all over the surface of the solidified nutrient agar plates aseptically. The inoculated plates



Were allowed to dry for a few minutes at room temperature with the lid closed to enable the organism stick properly to the surface of the nutrient agar. Wells were then bored aseptically in the inoculated palates with the help of a sterilized cork borer of 6mm in diameter. In a drop – wise manner, 1ml of the prepared extracts was introduced into each well using a sterile dropper and the cultures were allowed to stand for 30minutes for per-diffusion of the extracts into the medium before they were transferred into the incubator. The cultures were incubated for 24hours at 37°C before final readings were taken. Control plates were also prepared for each test organism without the addition of extracts. Zone of Inhibition were measured to the nearest millimeter (Cheesbrough M. 2002) and (Esimone *et al.*, 1998)

Antifungal Activity

The agar diffusion method was employed. (Babayi *et al.*, 2004). The test organism *Candida albican* was inoculated into test tubes containing Potatoes Dextrose Broth and incubated at room temperature for 48hours. The organism was sub-cultured into Potatoes Dextrose Agar by the pour plate method. A sterile cork borer (6mm) was used to bore holes in the culture media. In a drop-wise manner, 1ml of the extract was added into each of the well and the cultures were allowed to stand for 30minutes before inoculation a room temperature for 48hours. After 48hours of active growth, the zones of inhibition were measured with the aid of a meter rule considering the diameter of the crock borer. A control plate was also prepared for the test organism without the addition of extracts.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the plate extract can be defined as the lowest concentration of the plant extract (Antimicrobial) that will inhibit the growth of microorganisms after overnight incubation period, (Jenifer and Andrew., 2001). Four sterilized (4) test tubes were dispensed with 2ml of ethanol. To the first test tubes 2ml of 50% extract was added and doubly diluted into the rest of the three (3) test tubes followed by adding 2ml of Muller Hinton Broth (MHB) to each tube. Each of the tubes containing MHB and the extract was then inoculated with a loop full of standardized organism. The tubes were incubated at 37°C for 24hours. The concentration of the least clear tubes after incubation that shows no visible turbidity was recorded as MIC (NCCLS, 2002).

Determination of Minimum Bacterial Concentration (MBC)

The minimum bacterial concentration was determined from broth dilution test resulting from the MIC tubes as described by Vollekova, *et al.*, (2001) and Usman *et al.*, (2005) by sub-culturing the clearest tubes that show no visible turbidity on a fresh nutrient agar



plate and incubating for 18-24 hours. The concentration of the tube dilution that yielded no single microbial colony on the agar plate was recorded as the MBC of the extract.

Data analysis

All assays were performed in triplicate. Results are expressed as mean $\pm$ SD. One-way ANOVA with Tukey's post-hoc test compared zones/MICs between plant parts ($\alpha = 0.05$). Pearson correlations explored relationships between metabolite abundance scores and antimicrobial indices.

Results and Discussion

Table 1. Phytochemical Constituents of Ethanolic Extracts

Phytochemicals	Leaves	Stems	Roots
Alkaloids	+	-	+
Flavonoids	+++	++	++
Tannins	+++	++	++
Saponins	++	+	++
Anthraquinones	-	-	-
Cardiac glycoside	+	-	-

Key: + = present, ++ = highly present, +++ = strongly present

Table 2. Antimicrobial activity by agar-well diffusion

Zones of inhibition (mm, mean $\pm$ SD; n=3)

Organisms /Concentration	Leaves 100mg/ml	Stems 100mg/ml	Stems 100mg/ml	Positive Control
<i>S. aureus</i>	22.1 $\pm$ 0.8	8.2 $\pm$ 0.7	15.3 $\pm$ 0.5	Gent 24.5 $\pm$ 0.6
<i>E. Coli</i>	18.0 $\pm$ 0.6	16.2 $\pm$ 0.5	13.6 $\pm$ 0.5	Gent 23.1 $\pm$ 0.5
<i>S. typhi</i>	16.4 $\pm$ 0.5	14.8 $\pm$ 0.5	12.0 $\pm$ 0.4	Gent 22.7 $\pm$ 0.5
<i>C. albicans</i>	19.1 $\pm$ 0.6	17.0 $\pm$ 0.	14.2 $\pm$ 0.5	Fluc 21.9 $\pm$ 0.6

Table 3. MIC/MBC (mg/mL)

Organisms	Leaves MIC	Leaves MBC/MFC	Stems MIC	Stems MBC/MFC	Roots MIC	Roots MBC/MFC
<i>S. aureus</i>	3.125	6.25	6.25	12.5	12.5	25
<i>E. Coli</i>	6.25	12.5	12.5	25	25	50
<i>S. typhi</i>	12.5	25	25	50	50	100
<i>C. albicans</i> (MFC)	6.25	12.5	12.5	25	25	50

Interpretation: MBC/MIC ratios ≤ 4 across organisms indicate predominantly bactericidal/fungicidal action at higher concentrations, with leaves most potent.



Discussion

This study demonstrates that ethanolic leaf extract of *Cymbopogon* possesses superior antimicrobial activity compared with stem and root extracts against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*, *S. typhi*), as well as *C. albicans*. The trend aligns with phytochemical screening: leaves contained abundant flavonoids and saponins, classes known to disrupt microbial membranes, inhibit nucleic acid synthesis, and chelate vital ions. Gram-negative organisms showed comparatively higher MICs, consistent with outer-membrane barriers. The relatively modest activity of roots, despite high tannins, may reflect polymeric tannins' lower diffusion in agar and reduced bioavailability. The MIC and MBC/MFC values (3.125–12.5 mg/mL for leaves across organisms) suggest practical potency for crude extracts. MBC/MIC ratios ≤ 4 indicate bactericidal rather than purely static effects at higher doses, notably against *S. aureus*. Compared with positive controls (Gentamicin/Fluconazole), crude extracts are less potent on a mass basis, but their broad activity and phytochemical richness justify fractionation to identify active principles (e.g., citral-rich fractions, flavone glycosides, saponins).

Conclusion

Ethanolic extracts of *Cymbopogon* leaves exhibit the highest and broadest antimicrobial efficacy among the plant parts tested, with low MIC/MBC/MFC values particularly against *S. aureus*. Preferential use of leaves in herbal antimicrobial formulations is supported, while bioassay-guided isolation of active constituents is warranted.

Recommendations

1. Pharmaceutical potential: Further studies should focus on isolation and characterization of the active compounds, particularly from the leaves, for possible drug development.
2. Synergistic studies: Combining lemongrass extracts with conventional antibiotics could be explored to combat resistant strains.
3. Toxicological studies: Safety profiling in animal models and clinical trials is recommended before large-scale application.
4. Alternative medicine: Lemongrass leaf extract may be promoted as a natural antimicrobial agent in herbal formulations.

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