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Assessment of Hot Water and Ethanolic Leaf Extracts of *Cymbopogon Citrates* Stapf (Lemon Grass) against Selected Bacteria Pathogens

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ABSTRACT

This study investigated the comparative activity of hot water and ethanol leaf extracts of Cymbopogon citratus (lemon grass) against some bacterial pathogens (viz: Escherichia coli, Staphylococcus aureus, Pseudomonas aerugionsa and Bacillus subtilis. The lemon grass samples were purchased from Etegwe market in Yenagoa, Bayelsa state, Nigeria. Ethanol and hot water was used for the extraction, and agar well diffusion sensitivity method was adopted for the study. The zone of inhibition for E. coli, Pseudomonas aerugionsa, Staphylococcus aureus and Bacillus subtilis were 9.33 mm, 9.33 mm, 11.33 mm and 9.67 mm respectively for hot water leaf extract, and 12.00 mm, 11.33 mm, 12.33 mm and 10.67 respectively for ethanolic leaf extract. There were significant variation (P<0.05) between hot water and ethanolic leaf extracts of Cymbopogon citratus for E. coli and Pseudomonas aerugionsa. Furthermore, apparent superior effects also occurred for Staphylococcus aureus and Bacillus subtilis but it was not significantly different (P>0.05) between the two solvents. The activity of the leaf extract of Cymbopogon citratus against the tested microbes suggests its potential for broad spectrum antibiotics.

***Keywords:** Antibacterial, Cymbopogon citratus, Disease Control, Medicinal Plants, Solvents*

INTRODUCTION

The challenge of drug resistance, emerging and re-emerging diseases is a serious concern to the field of phytomedicine, pharmacognosy and pharmaceutical microbiology and chemistry (Izah et al., 2018a). Furthermore, plants have emerged as credible sources of new antimicrobials (Kigigha et al., 2018, 2016, 2015; Izah et al., 2018a-d; Izah and Aseibai, 2018). As a result, research on the efficacy of plants has increased. Several Plants have been widely reported to be effective against various disease conditions including those caused by microorganisms.

Medicinal plants are plants whose one or more parts have therapeutic effects (Izah et al., 2018a-d; Izah and Aseibai, 2018; Kigigha *et al*., 2015, 2016, 2018). Studies have indicated that significant number of global population use herbal medicine for the treatment of several diseases (Kalunta, 2017; Kigigha and Kalunta, 2017; Epidi *et al*., 2016a,b; Nyarko *et al*., 2012) especially individuals residing in rural areas in many developing countries. According to Nyarko *et al*. (2012), phytomedicine or herbal medicine is a major component in all indigenous peoples' tradition, a common element in ayurvedic, homeopathic, naturopathic, traditional oriental.

Cymbopogon citratus which is commonly known as lemongrass belongs to the grass family of Poaceae (Vyshali *et al*., 2016; Izah and Aseibai, 2018). Lemon grass is a fast growing, perennial aromatic grass native to South India and Sri Lanka, and now its commonly cultivated in the tropical areas of America, Asia (Manvitha and Bidya, 2014), Africa including Nigeria. Typically, Cymbopogon represents an important genus of about 120 species that grows in tropical and subtropical regions around the world (Hanaa *et al*., 2012).

Cymbopogon species is a coarse grass with a strong distinct lemon flavour and citrusy aroma

(Manvitha and Bidya, 2014; Naik *et al*., 2010). Manvitha and Bidya (2014) reported that lemon grass can grow up to 1 meter with numerous stiff leafy stems arising from short rhizomatous roots. *Cymbopogon citratus* has been cultivated over many years for medicinal purposes in different regions of the world (Naik *et al*., 2010) and food. According to Zulfa *et al*. (2016), probably due to the sharp lemon flavor, it's an essential ingredient in Asian cuisines.

In addition to its culinary usage, lemongrass offers an array of medicinal benefits. The plant is used extensively in Ayurvedic medicine (Manvitha and Bidya, 2014). The leaves are used to make tea which can relieve stomach and gut problems (Nyarko *et al*., 2012). Lemon grass also has anti-depressant and mood enhancer, anxiolytic, hypnotic and anticonvulsant properties (Nyarko *et al*., 2012). Several others studies have documented the medicinal potentials of lemon grass (Sherwani et al., 2013; Manvitha and Bidya, 2014). Most of the claims made by traditional medicine practitioners have been scientifically validated for anti-hepatoxicity, anti-microbial, anti-protozoan, anti-diarrheal, anti-amoebic, anti-inflammatory, anti-filarial, hypoglycemic and neurobehavioral potentials (Manvitha and Bidya, 2014).

Several studies have been conducted with regard to the antimicrobial potentials of lemon grass (Jafari *et al*., 2012; Naik et al., 2010; Vyshali *et al*., 2016; Kapilan, 2015; Zulfa *et al*., 2016; Izah and Aseibai, 2018). To these effects, several solvents including methanol, hexane, chloroform and ethanol have been used to extract active ingredients from the leaves of lemon grass for antimicrobial studies. But the use of hot water appears to be scanty in literature. Therefore, this study assessed comparative antibacterial activity of hot water and ethanol leaf extracts of *Cymbopogon citratus* against some selected pathogenic bacteria.

MATERIALS AND METHODS

Sample procurement and preparation

The leaf of *Cymbopogon citratus* used in this study was bought from Etegwe market in Yenagoa metropolis, Bayelsa state, Nigeria. The leaf samples of *Cymbopogon citratus* were dried at room temperature. Then after, it was cut in pieces and blended with bender.

Extraction method

The extraction was carried out using soaking method previously described by Doherty *et al.* (2010) and Chiejina and Ukeh (2012) with slight modifications. Hot and cold distilled water were used for the extraction. 40g of the blended samples were extracted using 100ml of ethanol and hot water (Kigigha et al., 2015). The sample was soaked for 3 days, thereafter they were filtered with muslin cloth and the extract was collected in a conical flask (Kigigha et al., 2015). The extracts were further filtered using Whatman filter paper. The resultant filtrates of the ethanolic extracts were concentrated in a rotary evaporator.

Source and Preparation of organisms

The microorganisms used for the investigation were obtained from Medical Microbiology Unit, Federal Medical Centre, Yenagoa, Bayelsa state, Nigeria. The purity of the bacteria was checked by subculturing, and then subjected to biochemical test following the guide of Cheesbrough (2004).

Antimicrobial screening of the extract

Agar diffusion method previously described by Lino and Deogracious (2006) with modifications by Kigigha *et al.* (2015, 2016, 2018), Epidi *et al.* (2016a,b), Doherty *et al.* (2010), Agu and Thomas (2012), Izah and Aseibai (2018) were employed. Nutrient agar was prepared according to the manufacturer's instruction. The resultant zone of inhibition after incubation for 24 hours was recorded.

Statistical analysis

SPSS software version 20 was used to carry out the statistical analysis. The data were expressed as Mean ± standard error. Student "t" test was carried out to compare between hot water and ethanolic extract for each of the bacteria isolates.

RESULTS AND DISCUSSION

The comparative zone of inhibition for 100% concentration of hot water and ethanolic leaf extract of *Cymbopogon citratus* is presented in Table 1. The zone of inhibition for *E. coli*, *Pseudomonas aerugionsa*, *Staphylococcus aureus* and *Bacillus subtilis* was 9.33 mm, 9.33 mm, 11.33 mm and 9.67 mm respectively for hot water, and 12.00 mm, 11.33 mm, 12.33 mm and 10.67 respectively for ethanolic extract. The comparison of both hot water and ethanolic extract with regard to each of the bacterium under study showed that there significant

variation (P<0.05) between hot water and ethanolic leaf extracts of *Cymbopogon citratus* for *E.* coli and *Pseudomonas aerugionsa*. In addition, *Staphylococcus aureus* and *Bacillus subtilis* revealed that there no significant variation (P>0.05) between hot water and ethanol leaf extracts of *Cymbopogon citratus.*

Table 1: *Comparative zones of Inhibition (mm) of hot water and ethanolic leaf extracts of Cymbopogon citratus*

Isolate	Hot water extract	Ethanolic extract	t-value	p-value	Statistical Implication
E.coli	9.33±0.33	12.00±0.58	-4.000	0.016	SD
Pseudomonas aerugionsa	9.33±0.33	11.33±0.33	-4.213	0.013	SD
Staphylococcus aureus	11.33±0.33	12.33±0.33	1.342	0.251	NSD
Bacillus subtilis	9.67±0.33	10.67±0.33	-2.121	0.101	NSD

Data is expressed as mean ±Standard Error; SD- significant difference (P<0.05), NSD - no significant difference

Cymbopogon citratus have been several reported to possess antimicrobial potentials (Izah and Aseibai, 2018; Vyshali *et al*., 2016; Zulfa *et al*., 2016; Naik *et al*., 2010; Manvitha and Bidya, 2014; Danlami *et al*., 2011; Ewansiha *et al*., 2012; Jafari *et al*., 2012; Kapilan, 2015). The presence of phytochemical and bioactive ingredients in plant have been attributed to its anti-microbial potentials (Kigigha *et al*., 2015, 2016; Kigigha and Kalunta, 2017; Kalunta, 2017; Epidi *et al*., 2016a,b; Izah *et al*., 2018). Several phytochemicals have been reported in *Cymbopogon citratus*. For instance, Ewansiha *et al*. (2012) reported the presence of tannins, flavonoids, phenols, carbohydrates and volatile oil in both the root and leaf parts of *Cymbopogon citratus*. Ekpenyong *et al*. (2014) reported tannins, saponins, flavonoids, phenols, anthraquinones, alkaloids, deoxysugars, and various essential oil in *Cymbopogon citratus*. Shah *et al*. (2011) reported flavonoids, phenolic compounds, terpenes, alcohols, ketones, aldehyde and esters, Citral α, Citral β, Nerol Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and Terpinol Methylheptenone. Ranitha (2012) also reported the presence of Citral, Geranic Acid, Geranyl Acetate, Linalool, Neric acid, (Z) Citral, β-mycrene and β-Thujene in *Cymbopogon citratus*. The authors in their studies indicated that different solvent have varying effects on the phytochemical constitutes of the plant.

In this study both ethanolic and hot water extracts of *Cymbopogon citratus* is active against *Staphylococcus aureus*, *Bacillus* subtilis., *Escherichia coli* and *Pseudomonas aerugionsa*. The clear zone of inhibition depends on the solubility and rate of diffusion in agar medium (Kapilan, 2015). The variation that exists among the efficacy of the extracts among the different isolates under study could be due to variation in metabolism, physiology, nutrition and biochemistry of the test isolates (Kigigha *et al*., 2016; Epidi *et al*., 2016a,b; Izah *et al*., 2018b). Furthermore, the age of the plant and the prevailing environmental condition that the plant were cultivated may also affect the bioactive constituent of the plants (Izah et al., 2018b). Again, the different solvent have varying efficacy on the zone of inhibition. This trend have been reported by Kigigha and Atuzie (2012), Epidi *et al*. (2016a,b). The possible reason to this effect could be associated to the difference in the polarity level of the solvents (Epidi *et al*., 2016a,b).

The zone of inhibition of this study is similar to values previously reported by authors. For instance, Zulfa *et al*. (2016) reported that methanolic *Cymbopogon citratus* extracts has positive efficacy toward food borne pathogens such as *Bacillus cereus*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Candida albicans*. Kapilan (2015) reported that *Bacillus* pumilus, *Bacillus* cereus, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* is sensitive to aqueous, ethanolic, methanolic and liquid nutrient extracts of *Cymbopogon citratus*. Naik *et al*. (2010) reported that essential oil of lemon grass is effective against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Jafari *et al*. (2012) reported that methanolic extracts of *Cymbopogon citratus* against *Staphylococcus aureus*, *Bacillus* cereus and Escherichia coli, but not effective toward *Pseudomonas auroginosa*. Vyshali *et al*. (2016) reported that *Cymbopogon citratus* is effective against *Enterococcus faecalis*, *Staphylococcus aureus, Shigella dysenteriae*, *Salmonella typhii*, *Trichophyton rubrum* and *Cryptococcus neoformans*. The authors further attributed the antimicrobial efficacy to its essential oil. The

variation in this study with previous study could be due to the concentration used.

CONCLUSION

The use of plants for medicine can be dated back to the history of man. A significant number of world populations still rely on herbs for medicine especially in rural areas in many developing countries. Probably due to the instances of drug resistance, emerging and re-emerging microbial strains, there has been an upsurge of research in the field of herbal medicine, pharmaceutical microbiology and phytomedicine. *Cymbopogon citratus* is one of the major food spices used in preparing several delicacies. Within the last few decades, the used of *Cymbopogon citratus* in preparing several tea/ beverages products have increased. This study evaluated the activity of hot water and ethanol leaf extracts of *Cymbopogon citratus* against *Staphylococcus aureus*, *Bacillus subtilis*., *Escherichiacoli*, and *Pseudomonas aerugionsa*. The results showed that both extract is active against the test organisms. However, the ethanolic extract has superior effects, indicating the effect of choice of solvent in extracting active ingredients of plant parts. The effectiveness of the plant against the microbes under study suggests its potentials for broad spectrum antibiotics.

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