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Studies on the Antibacterial Effects of Leaf Exracts of Lemon Grass (*Cymbopogon citratus*) on Some Selected Microorganisms obtained from the University of Ilorin Teaching Hospital (UITH)

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ABSTRACT

This Experiment was carried out to find outthe efficacy of two solvent extracts of *Cymbopogoncitratus* against some test bacteria.. The solvents used were ethanol and water.The investigation was carried out using completely randomised design. The ethanolic and aqueous extracts were tested using the agar well diffusion method. The test bacteria were:*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae*. The minimum

inhibitory and bactericidal concentrations of the extracts were also determined.. The ethanolic extract was active against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae* at concentrations of 100mg/ml to 400mg/ml, but inactive against *Klebsiella pneumoniae* at concentration of 100mg/ml, while the aqueous extract was active against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae* at concentrations of 100mg/ml to 400mg/ml, but inactive against *Staphylococcus aureus* and *Klebsiella pneumoniae* at concentration of 100mg/ml. Results showed that the plant contain various compounds including carbohydrate, tannins, polyphenol, lipid/fats, alkaloid, steroid, flavonoid, saponins, coumarin and cardiac glycosides. The crude extract was observed to inhibit the growth of tested bacteria. The presence of some secondary metabolites in these extracts on the bacterial isolates support the ethno medicinal uses of this plant in treatment of various diseases such as diabetes, veneral disease and malaria.Further screening, purification, and toxicological studies can be carried to improve the use of the extracts to treat various bacterial diseases.

Keywords: *Cymbopogoncitratus* , antibiotic, crude extract, tannins, alkaloid, ethanolic.

INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action in human body. The most important of these bioactive constituents are alkaloids, flavonoids, phlobotannins, saponins and cardiac glycoside (Edeoga, *et al*., 2005; Abubakar, *et al*., 2008). Traditional medicine has a long history. It is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as

in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2004). It also comprises therapeutic practices that have been in existence often for hundreds of year before the development of modern scientific medicine and is still in use today without any documented evidence of adverse effects (Elujoba*et al*., 2005).There are different types of medicinal plants which can be used as antimicrobial, antiviral, antifungal, antimalarial, anticancer, antistress etc. and in promoting man's health. The common examples of medicinal plants include:*Eucalyptusglobulus*, *Cymbopogoncitratus*, *Vernoniaamygdalina*, *Ocimumgratissimum*, *Carica papaya*, *Azadirachtaindica*, Cinchona, Opium, Alfalfa etc. (Odugbemi and Ayoola, 2008).Lemon grass belongs to the section of *Andropogan*called *Cymbopogon*of the family Poaceae (Grarmineae). A very large genus of the family, including about 500 described species out of which eight species occur in Iraq. Due to the production of lemon grass oil as major component, two of the species i.e. *Cymbopogoncitratus*and *C. flexuosus*are generally called Lemongrass (Anonymous, 2005). Research shows that lemongrass oil has antifungal properties (Shadab*et al*., 1992). The antimicrobial effect of these plants extracts also help to prevent diseases in many forms (Bibitha*et al*., 2002).

*Cymbopogoncitratus*is found to be effective against various Gram positive and Gram negative bacteria as well as *Plasmodium falciparum* due to presence of some chemical substance like saponins, tannins, flavonoids alkaloids etc. (Gills, 1992) Medicinal use of lemongrass is known to mankind since antiquity. Its oil has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for

the toothache and swollen gums. It is also been claimed to be stimulating, diuretic, anti-purgative and sudorrific to reduce fever. To cure cholera, colic and obstinate vomiting only 3-6 drops of the oil are effective medicine of choice (Stadtman, 1996). *Cymbopogoncitratus*(lemon grass) plant generally, is used traditionally for the treatment of malaria and typhoid fever. Concoction prepared from the combination of the leaves and grass of these plants or the boiling of the individual plant leaves have been used in the treatment of ailments like typhoid fever, stomach ache etc., (Udeh *et al*., 2001).

Antibiotics and the chemotherapeutic agents have been of value in controlling many infections, but they depend on judicious use to minimize the incidence of resistant forms (Danso, 2002). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological testing, and a substantial number of new antibiotics introduced to the market are obtained from natural or semi synthetic resources (Mothana and Lindequist, 2005).

The aims of this research were (i) To evaluate the antibacterial activities and phytochemical constituents of aqueous and ethanolic extracts of *Cymbopogoncitratus*on some selected organisms which include: *K. pneumoniae, E. coli, S. aureus*and *P.aeruginosa.* (ii) To compare the antibacterial activity of the extracts to some standard antibiotics.

MATERIALS AND METHODS

Sterilization of Materials

All glass wares were sterilized in model DHG-9023A hot air oven at 170^{o}C for 1hour. All the media used were autoclaved at 121^{o}C for 15minutes at 1.8kg/cm^{2}. Laboratory workbench was disinfected by swabbing with cotton wool soaked in 70% ethanol.

Collection and maintenance of test organisms

Pure culture of selected organisms were obtained from the Microbiology Laboratory of University of Ilorin Teaching Hospital (U.I.T.H) and preserved by inoculating into sterile agar slants, and kept in the refrigerator to serve as stock culture. The following organisms were employed as test organism for this work, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae.*Physiological and biochemical tests were carried out on these organisms to confirm their identity. These organisms were further confirmed from the

Culture collection unit of the Department of Microbiology of University of Ilorin

Collection of Plant Materials

Fresh leaves of *Cymbopogoncitratus* (lemon grass) was collected from a residential compound in Okeodo, Ilorin, Kwara state. It was identified at the herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria.

Extraction of Plant

The leaves were air dried in a dehumidified room for two weeks at 25^0C, and pulverized into powdery form using an electric blender. The powdered leaves were then weighed and stored in clean air tight bottle jars until use.

Cold Water extraction

Ground leaves of *Cymbopogoncitratus* (lemon grass) (256.25g) was weighed and extracted with 750ml of sterile distilled water and placed on a shaker for 48hours.It was filtered using Whatman filter paper and the extracts were further stored in a covered sterile beaker.

Alcoholic extraction

Ground leaves of *Cymbopogoncitratus* (lemon grass) (256.25g) was weighed and extracted with 850ml of 70% ethanol and placed on a shaker at 190rpm for 48hours and it was filtered using Whatman filter paper**.** The extracts were decanted, filtered,

concentrated using rotary evaporator and subjected to dryness on water bath. The crude extracts were then transferred into a small reagent bottle and labelled appropriately.

Test for Sterility

The extracts were plated on sterile nutrient agar to test for contaminants and incubated at 37^{o}C for 24hours. Observation was carried out on the plates to detect any visible growth. No growth on the plates showed that the extracts were sterile.The extract was then assessed for antimicrobial activity.

Standardization of inoculum

The bacterial cultures were prepared by transferring 2 to 3 colonies into bacterial growth medium (Luria broth agar) and incubated at 37^{o}C for 14hours before use as described by (Eloff, 1998). Preparation and standardization of each isolate was done using the modified method described by Bauer *et al.*, (1966). This was carried out by picking test organism growing on agar slant and then transferred into sterile nutrient broth and incubated for 18-24 hours to grow. Each standardized inoculum was used for antimicrobial test.

Antimicrobial assay of the extracts

The agar diffusion method of Irobi*et al.*, (1994) was employed to determine the antimicrobial activities of the extracts. Sterile Mueller Hinton agar was dispensed into Petri dishes at 45^{o}C and allowed to solidify. The test organisms were then swabbed onto the surface of the solid agar using sterile swab stick. After inoculation, sterile cork borer (6mm) was used to dig four wells on the surface of inoculated agar plates. This process was done for each extract at different concentrations against each test organisms. All the plates were labelled and the extract allowed to diffuse before incubation at 37^{o}C for 24hours.After incubation, the zones of inhibition around each well was measured using a metric ruler and diameter of the cork borer was

subtracted from the mean reading to obtain the zones of inhibition.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts.

The MIC of the aqueous and ethanolic extracts was determined using the method of Adegboye*et al.,* (2008). Extracts with concentrations from 100mg/ml to 400mg/ml were dispensed into each of the test tubes after which 1ml each of the extract at different concentrations were introduced and shaken to mix properly. A loopful of the test organism culture prepared was inoculated into each test tube and incubated at 37^{o}C for 24hours. After 24hours of incubation, each of the test tubes was examined for the presence or absence of growth by checking its turbidity against the control. The test tubes that produced no growth were selected and inoculated onto Nutrient agar plate and incubated for 24hours at 37^{o}C. The least concentration of the extract that prevented visible growth was made the minimum bactericidal concentration

Antimicrobial susceptibility test

The antibiotic sensitivity test was carried out using the method described by Irobi*et al.,* (1994). The bacterialisolates were streaked on Mueller Hinton agar using a sterile swab. The sensitivity disc containing Ofloxacin(5µg), Amoxycillin (25µg),Ciprofloxacin (10µg), Gentamycin (10µg) Cotrimoxazole (25µg) and Pefloxacin (5 µg) was placed on the agar and sterile forceps was used to place the disc against the agar tightly. The plates were incubated for 24 hours, and zones of inhibition were observed and recorded (Owoseni*et al*., 2010). Mueller Hinton agar was used for the sensitivity tests. Overnight broth cultures of the organisms were swabbed on the sterile Mueller Hinton agar plate using sterile swab sticks. The plates were allowed to diffuse for few minutes.

The multiple antibiotic disc were then placed on the agar surfaces and pressed using sterile forceps to ensure complete contact with the agar.

Phytochemical screening

Phytochemical screening of the crude extracts was carried out using standard methods of Akinyemi*et al.,* (2005); Parekh *et al*, (2006); Kumar *et al.*,(2007); and Sofowora, (2008).

Test for anthraquinone: Anthraquinone was tested using the methods of Kumar *et al.*,(2007).

Test for carbohydrate: Carbohydrate was tested for using the method of Sofowora, (2008).

Test for tannin: Tannin was tested for using the method of Kumar *et al.*,(2007).

Test for alkaloid: Alkaloid was tested for using the method of Kumar *et al.*,(2007).

Test for flavonoid: Flavonoid was tested for using the method of Akinyemi*et al.*,(2005).

Test for cardiac glycosides: Cardiac glycosides were tested for using the method of Parekh*et al*, (2006).

Test for saponin: Saponin was tested for using the method of Parekh *et al*, (2006).

Test for reducing sugar: Reducing sugar was tested for using the method of Akinyemi*et al.*,(2005).

Test for fat: Fat was tested for using the method of Dahiru*et al.*,(2006).

Test for steroids: Steroids was tested for using the method of Kumar et al., (2007).

Test for coumarin: Coumarin was tested for using the method of Edeoga*et al.*, (2005).

RESULTS AND DISCUSSION

The antibacterial activities of the aqueous extracts of *Cymbopogoncitratus*(Lemon grass) showed inhibitory activities against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, at concentrations of 100mg/ml to 400mg/ml; *Escherichia coli* and *Staphylococcus aureus* at concentrations of 100mg/ml to 400mg/ml, but inactive against

Klebsiella pneumoniae and *Staphylococcus aureus*concentration of 100mg/ml (Table 1).

Table 1: Antibacterial activities of the aqueous extracts of *Cymbopogoncitratus*against different test organisms

	Diameter of zone of inhibition (*mm)			
Extracts concentration (mg/ml)	*Klebsiella pneumoniae*	*Pseudomonasaeruginosa*	*Escherichia coli*	*Staphylococcusaureus*
100	-	2.0	3.5	-
200	3.0	4.0	5.0	4.0
300	4.0	5.0	7.0	6.0
400	10.5	7.0	8.5	11.0

Key:

(-) No inhibition,

mg/ml- milligramme per millilitre

(*) – mean values of replicates.

Also, the antibacterial ethanolic extracts of *Cymbopogoncitratus*(Lemon grass) showed inhibitory activities against *Klebsiella pneumonia* with no inhibition at concentration of 100mg/ml but inhibition at concentrations 200mg/ml to 400mg/ml; *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* at concentrations of 100mg/ml to 400mg/ml (Table 2).

Table 2: Antibacterial activities of the ethanolic extracts of *Cymbopogoncitratus* against different test organisms

	Diameter of zone of inhibition (*mm)			
Extracts concentration (mg/ml)	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*
100	-	1.5	5.0	3.0
200	3.0	4.0	7.0	5.0
300	5.5	7.0	10.0	9.0
400	10.0	11.0	13.0	12.0

Key:

(-) No inhibition

mg/ml- milligramme per millilitre

(*) – mean values of replicates

The minimum inhibitory concentration (MIC) for ethanolic extracts were 170mg/ml, 60mg/ml, 70mg/ml and 160mg/ml for *Klebsiella pneumoniae, Pseudomonasaeruginosa*, *Escherichia coli* and *Staphylococcus aureus* respectively, while the minimum inhibitory concentration for aqueous extracts were 160mg/ml, 80mg/ml, 90mg/ml for *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* respectively but *Pseudomonas aeruginosa* showed no turbidity in the aqueous extracts of the

Cymbopogoncitratus(Lemon grass) (Table 3).

The ethanolic and aqueous extracts showed inhibitory activities against some of the test organisms, though in varying degrees with the ethanol extracts showing more inhibitory activities. From Tables 2 and 3 above, the ethanol extract inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*and *Pseudomonas aeruginosa* at concentrations of 100mg/ml to 400mg/ml but inactive against *Klebsiella pneumonia* at concentration of 100mg/ml. The aqueous extract inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at concentrations of 100mg/ml to 400mg/ml but was inactive against *Staphylococcus aureus*and *Klebsiellapneumoniae* at concentration 100mg/ml. This is due to the presence of higher or less amount of active components of the extract. The minimum inhibitory concentration (MIC) of lemon grass ranged from 60mg/ml to 170mg/ml on all extracts. It was observed that not all the concentrations of the extracts were able to inhibit the growth of the test organisms on the culture plate. This was attributed to insufficient concentration for the permeability of cell membrane of the test organism or modification of the chemical components of the extracts.

The inhibitory activities exhibited by the extracts was in agreement with an earlier report by Elmahmood*et al.*(2008) that the antimicrobial properties of plants can be linked to the presence of bioactive secondary metabolites like alkaloids, tannins, saponins, flavonoids, phenols, glycosides and diterpenes. These metabolites present in the extract enable the portion of the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable and susceptible to lysis. Also, a

study by Gills (1992) revealed that *Cymbopogoncitratus* contains alkaloid which is in line with the observation in this study, despite the climatic differences. It was observed in this study that *Cymbopogoncitratus* contain steroids which are important compounds of interest in Pharmacy due to their various uses in inducing the production of sex hormone in humans and animals. The presence of Cardiac glycosides and Flavonoids in *Cymbopogoncitratus* also revealed the usefulness of these plants in the Pharmaceutical industry in production of drugs that can be used to treat cardiac failure. More of the bioactive principles were extracted when water was used. Ethanol has antimicrobial properties, so using it as an extraction solvent increased the potency of the ethanol extract against these organisms. This was achieved when the ethanol dissolved the lipid content of the membrane thereby increasing its permeability for the entrance of the bioactive components of the extract which destroy the cell. The efficiency of the aqueous extract compared to the ethanol extract against the test organisms used in this study was low, and this is in agreement with previous works by Koduru*et al.*, (2006) which showed that aqueous extracts of plants generally showed little antimicrobial activities.

Table 3: Minimum Inhibitory Concentration (MIC) of extracts of *Cymbopogoncitratus* against different test organisms

Test organisms	Minimum inhibitory concentration (mg/ml)	
	Ethanol extract	Aqueous extract
K. pneumonia	170	160
P. aeruginosa	60	-
E. coli	70	80
S. aureus	160	90

Key:

(-) No inhibition

mg/ml- milligramme per millilitre

The minimum bactericidal concentration (MBC)for ethanol, aqueous cold, aqueous hot extracts ranges from 50mg/ml to 150mg/ml for the different test organis

Table 4: Minimum Bactericidal Concentration (MBC) of the extracts of *Cymbopogoncitratus*

Test organisms	Minimum bactericidal concentration (mg/ml)	
	Ethanol extract	Aqueous extract
K. pneumonia	150	130
P. aeruginosa	50	-
E. coli	50	60
S. aureus	150	70

Key:

(-) No inhibition

mg/ml- milligramme per millilitre

In Table 5 the susceptibility and resistance of test bacteria to various antibiotics is presented. *Klebsiella pneumoniae* was resistant to ofloxacin, amoxicillin, gentamycin, cotrimoxazole and pefloxacin but susceptible to ciprofloxacin. *Escherichia coli* was resistant to ciprofloxacin and cotrimoxazole but susceptible to ofloxacin, amoxicillin, gentamycin and pefloxacin. Staphylococcus aureus was all susceptible to ofloxacin, amoxicillin, ciprofloxacin, gentamycin, cotrimoxazole and pefloxacin. *Pseudomonas aeruginosa* was resistant to all the antibiotics used in this study. The standard antibiotics used in this experiment, gentamycin, cotrimoxazole, ofloxacin, amoxycillin, ciprofloxacin, pefloxacin, displayed greater potency when compared to the extracts. This might be due to the fact that these conventional antibiotics are

refined and purified products, while extracts of plants are a mixture of various constituents some of which might interfere with antimicrobial activities and are subject to degradation and decomposition on storage.

Table 5: Antibiotic sensitivity test on microorganisms

Antibiotics (ug)	*K. pneumonae*	*E. coli*	*S. aureus*	*P. aeruginosa*
Ofloxacin (5)	-	++	++	-
Amoxycillin (25)	-	++	+	-
Ciprofloxacin (10)	+	-	++	-
Gentamycin (10)	-	++	++	-
Cotrimoxazole (25)	-	-	++	-
Pefloxacin (5)	-	++	++	-

Key:

Zones of inhibition present;

(++), 9mm-15mm

(+), 3mm-9mm

(-), 0-3mm [Resistant]

The phytochemical screening carried out on the extracts of *Cymbopogoncitratus*, showed the presence of carbohydrates, tannins, lipids, alkaloid, steroid, flavonoid,saponin and cardiac glycosides as presented in Table 6

Table 6: Phytochemical constituents of the crude extracts of *Cymbopogoncitratus*

COMPOUND TEST	INFERENCE
Anthraquinone	–
Carbohydrate	+
Tannins/Polyphenol	+
Lipid/Fats	+
Alkaloid	+
Steroid/Triterpenes	+
Flavonoid	+
Saponin	+
Reducing Sugar	–
Coumarin	–
Cardiac Glycosides	+

Key:

(-) Absent

(+) Present

These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. The medicinal value of these plants lies in these phytochemical compounds and as such produce definite physiological actions in the human body.

CONCLUSION:

This research has shown that extracts of lemon grass (*Cymbopogoncitratus*) has

antibacterial effects against some selected microorganisms. This might be due to the presence of phytochemicals found in this plant. Though, the efficacy of these extracts when compared to standard antibiotics is lesser in antibacterial action. This may be improved if the extracts are subjected to further pharmacological and toxicological studies. This may promote its use in clinical trials.

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