Int J Biol Med Res. 2025; 16(1): 7970-7973

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Review Article

Antibacterial Activity of Cymbopogon citrus (Lemon grass) on Penicillin-Resistance Microorganisms Isolated from Garden Soils of Madonna University

A*Bibiana C. Uzor, Peace O. Ojezuabi-Imoode, and Convenant. T Udoette

Departmentof Microbiology,

Madonna University P.M.B 05Elele, Rivers State Nigeria

A R T I C L E I N F O

Keywords:
Antimicrobial activity,
extraction,
penicillin-resistant,
strains,
zone of inhibition

A B S T R A C T

The antibacterial activity of Cymbopogon citratus leaf extract were determined. The leaves of Cymbopogon citratus were extracted with petroleum ether and ethyl acetate and tested for antimicrobial activity using penicillin-resistant microorganisms isolated from the garden soil, through serial dilution obtaining the following organisms which include Micrococcus spp, Staphylococcus aureus and Staphylococcus intermidius.. The agar well diffusion method was employed and extracts were tested at 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml 62.5mg/ml and 31.25mg/ml concentrations. The results showed that petroleum ether had higher potency of extraction than ethyl acetate with a diameter zone of inhibition of 25mm at 1000mg/ml for Micrococcus sp. In ethyl acetate, Micrococcus spp was susceptible at a diameter zone of inhibition of 23mm at 1000mg/ml, while Staphylococcus aureus had a diameter zone of inhibition of 20mm at the same concentration. It was observed that the Minimum Inhibitory Concentration (MIC) value of petroleum ether for Micrococcus sppwas 31.25mg/ml while the ethyl acetate extracts had their MIC value for Micrococcus sp and S.aureus at 125mg/ml and 500mg/ml respectively. Micrococcus spp and Staphylococcus aureus were bactericidal for both petroleum ether and ethyl acetate extracts. The natural occurring substances in Cymbopogon citratus makes it effective against fever, achy joints and common cold.

© Copyright 2023 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685.

Introduction

Plants are used in the modern era for the extraction and development of many drugs which enhance the traditional use of herbal remedies. However, these plants with helpful nature could also be dangerous and detrimental to health if we neglect the fact that some of them that are helpful in healing or curing ailment could also have negative side effect either on the particular organ in which they act or to another organ in the body or even both. Medicinal plants are defined as any plant that can be put to culinary or medicinal use. Medicinal plants are the key sources of bio-active compounds for herbal medicine, antibiotics, antioxidant and pharmaceutical drugs [1].

The use of whole herbs and extractives has remained the main approach of folk medicine practitioners in the treatment of ailments and debilitating diseases. They usually claimed that such whole herbs and extractives are efficacious against several ailments and diseases without recourse to scientific proofs.

Corresponding author:

Dr. Bibiana C. Uzor
Departmentof Microbiology,
Madonna University P.M.B 05Elele, Rivers State Nigeria
Email: bibiana2uzor@gmail.com

© Copyright 2023 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685.

Increased cases of opportunistic diseases emanating from side effects associated with synthetic drugs continue to necessitate incremental efforts in searching for effective biological substitutes with little or no side effects. Therefore, efforts are being directed towards elucidating potential sources such as ethno-medicinal plants [2]. New, robust and less cumbersome extraction techniques assisted by recent developments in biotechnology have enhanced the investigation of natural compounds faster with more precision than before, leading to the isolation of bioactive compounds with intense health benefits [3].

According to folk medicine, several plants possess ethno-medicinal benefits, and Cymbopogon citratus also known as lemon grass remains one of them. Cymbopogon citratus is a perennial grass plant widely distributed worldwide and most especially in tropical and subtropical countries [4].

Several reports have linked its origin to Asia (Indochina, Indonesia and Malaysia), Africa and America. The plant could grow up to 6-inch-high and its bulb-like stems consist of terete and glabrous linearly venerated sheathed leaves with narrow base and acute apex. The leaf height is about 100cm in length and 2cm in width. When squeezed, the leaves usually produce yellow or amber colored, aromatic, essential oil [5]. Its aqueous extract is commonly used as an aromatic drink while the whole plant is well incorporated into traditional food for its lemon flavor. It also enjoys wide application in folk medicine [6].

Traditionally, tea made from lemongrass leaves is popular among

countries of South America, Asia and West Africa, having been widely utilized as antiseptic, anti-fever, anti-dyspeptic, carminative and anti-inflammatory effects. Others are febrifuge, analgesic, spasmolytic, antipyretic, diuretic, tranquilizer and stomachic agent [7, 5, 8].

Materials and Methods

Collection of samples

Cymbopogon citratus was obtained from a farmland in Akwa Ibom State. The plants was identified and authenticated at Micheal Okpara University of Agriculture, Umudike, Abia state.

Test organisms

The test organisms used were isolated from five different garden soil sites of Madonna University, Elele, and Rivers State. It was carried out aseptically; Ten grammes of soil sample were dissolved in 100ml of sterile water and serial dilution was carried out first of all before isolation of the organism. The procedure used was the ten-fold dilution of the soil sample. The dilution factor used was 10-6. Then the soil sample was plated on nutrient agar using the pour plate technique.

Identification of the isolate using colonial characterization

After pour plating the medium was allowed to solidify and it was incubated at 370C for 24 hours. After 24 hours the isolates were identified through their colonial morphology such as colour, size, elevation, edge and texture. The organism was subcultured and incubated at 370C for 24 hours, and kept on a slant for further analysis that involves the identification of penicillin-resistant microorganism

Identification of penicillin-resistant microorganisms

After the gram staining test, the identification of penicillin-resistant microorganism was the next procedure, this is known as Antimicrobial Susceptibility Testing. The microorganisms were subcultured on nutrient agar to obtain pure colonies and then incubated at 370C for 16 hours. After 16 hours the plates were removed from the incubator and standardized using the 0.5 McFarland Standards.

The standardized bacterial suspension of pure colonies was evenly inoculated on a sterile Muller Hinton agar medium and was allowed to dry for five minutes prior to the introduction of the antibiotics (penicillin) using agar well diffusion method. The inoculated plates were incubated for 24 hours at 370C. A clear zone of inhibition showed that the antibiotics had a positive effect on the organism which makes the organism sensitive to penicillin. When no zone of inhibition was seen around the organism, it was known to be resistant to penicillin. After the identification of the penicillin-resistant microorganisms, the next step that was taken was the biochemical test on the organisms that showed resistance to penicillin.

Identification of penicillin-resistant isolates using biochemical tests

After purification, isolates were identified by carrying out the biochemical tests which include, catalase test, coagulase, oxidase, citrate, urease, indole and methyl red. This biochemical tests were carried out for proper identification and confirmation of the isolates.

Preparation of extracts

About 50g of the powdered Cymbopogan citratus leaves were measured using the weighing balance and was introduced into a sterilized conical flask containing 500ml of the solvents ethyl acetate and petroleum ether respectively. It was shaken intermittently and was filtered using a beaker, funnel and Whatman Filter Paper No. 1 and it is later evaporated to dryness to obtain a thick concentration and prepare different concentrations with the extract. The different concentrations of the extracts that were prepared were 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml for the two different solvents (ethyl acetate and petroleum ether) respectively.

The test organisms (penicillin-resistant isolates) were subcultured to obtain pure cultures incubated at 370C for 16 hours. The isolates were inoculated into a test tube containing 10ml peptone broth and incubated for 4 hours before taken for evaluation, in order to ascertain the concentration of bacteria cells equivalent to 0.5 McFarland standards. Dimethylsulfoxide (DMSO) was used as a control.

Antimicrobial assay of the extracts

The modified agar well diffusion method (Collins, 1995), was employed to determine the antimicrobial activities of the leave extracts of Cymbopogan citratus. A different concentration of the extracts, 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml was prepared with a control, which was the DMSO. The Muller Hinton Agar (MHA) was used to carry out the antimicrobial assay of extracts.

The MHA was prepared under aseptic measures, then 20ml of the agar was dispensed in a bijou bottle. It was autoclaved and allowed to cool before introducing 0.5ml of the standardized 4 hours active cultures of the test organism in peptone broth, before dispensing inside the petri dish and allowing to set. With the aid of sterile cork borer, wells of about 6mm in diameter were bored on the plates. About 1ml of each concentration of the extracts was dispensed into the wells and then allowed to stand for about 15mins for pre-diffusion of the extracts to occur and then incubated at 37oC, as the agar was evaluated in mm [9].

Determination of the minimum inhibitory concentration (MIC) of Cymbopogon citratus leaf extract

Minimum inhibitory concentration of the plant extracts was determined by solution of the extracts to various concentrations of 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/l and 31.25mg/ml. 4ml of nutrient broth was dispensed into six sterilized test tubes, 5ml each of the extracts at different concentrations was introduced and mixed into the test tube, then 0.1ml of the standardized test organisms were added to each tubes and incubated at 37˚C for 10-16 hours.

Two control tubes were maintained for each tube batch. These include tube containing extracts and tubes containing nutrient broth and inoculums. The lowest concentration (higher dilution) of the extracts that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as Minimum Inhibitory Concentration.

Determination of minimum bactericidal concentration (MBC) of Cymbopogon citratus leaf extract

To determine the MBC, the negative test tubes were pour plated on nutrient agar plate and incubated for 24 hours at 37 . The plate with the least concentration of the extract that shows no growth was reported as the Minimum Bactericidal Concentration.

RESULTS

Results of the identification of the organisms through colonial morphology and gram staining

Table 1: Shows the identification and characterization of the organisms through colonial morphology and Gram staining. Micrococcus spp, Staphylococcus aureus and Staphylococcus intermedius were all gram-positive cocci except Chromobacterium violaceum which is a gram-negative rod.

Table 1: Organisms identified through colonial morphology and gram staining

Isolates	Colour	Size	Elevation	Edge	Texture	Gram reaction	Cell shape
Micrococcus spp	Yellow	Medium	Convex	Round	Smooth	Gram positive	Cocci
S.aureus	Golden Yellow	Medium	Convex	Round	Smooth	Gram positive	Cocci
S.intermedius	Creamy white	Medium	Convex	Round	Smooth	Gram positive	Cocci
C. violaecum	Violet	Pin-point	Convex	Round	Butyrous	Gram negative	Rods

Antibiotic susceptibility test on the isolated organisms using penicillin

Table 2 shows the shows the antibiotic susceptibility of isolates to penicillin

Table 2: Antibiotic susceptibility of isolated organisms to penicillin

Isolates	**Zone of inhibition (mm)**
Micrococcus spp	-
Staphylococcus aureus	-
Staphylococcus intermedius	28.0
Chromobacterium violaceum	27.0

key: - Negative (No inhibition)

Biochemical test results on penicillin-resistant microorganism

Table 3 shows the different biochemical tests carried out and their different results.

Table 3: Biochemical test result of isolated microorganisms

Biochemical tests	*Micrococcus spp*	*S.aureus*
Catalase	+	+
Coagulase	-	-
Oxidase	+	-
Oxidase	+	+
Urease	-	+
Methyl red	-	+
Indole	-	-

Zone of inhibition

Table 4: Zone of inhibition (mm) of leaf extracts of petroleum ether of Cymbopogon citratus against penicillin-resistant organisms.

Isolates	Concentration (mg/ml)						
	1000	500	250	125	62.5	31.25	Control
Micrococcus spp	23	20	19	17	15	14	-
Staphylococcus aureus	-	-	-	-	-	-	-

Key: — (No Inhibition)

Zone of inhibition

Table 5 shows the zone of inhibition in (mm) of leaf extract of ethyl acetate of Cymbopogon citratus against penicillin-resistant organisms.

Table 5: Zone of inhibition (mm) of leaf extracts of ethyl acetate of Cymbopogon citratus against penicillin-resistant organisms.

Isolates	Concentration (mg/ml)						
	1000	500	250	125	62.5	31.25	Control
Micrococcus spp	25	17	17	15	-	-	-
Staphylococcus aureus	15	-	-	-	-	-	-

Key: _ (No Inhibition)

Minimum inhibitory concentration (MIC) of leaf extract of petroleum ether and ethyl actate of Cymbopogon citratus against penicillin-resistant organisms.

Table 6 shows the minimum inhibitory concentration (MIC) of leaf extract of petroleum ether and ethyl acetate of Cymbopogon citratus against penicillin-resistant organisms.

Table 6: Minimum inhibitory concentration (MIC) of leaf extract of petroleum ether and ethyl acetate of Cymbopogon citratus against penicillin-resistant organisms.

Isolates	Concentration (mg/ml)	
	Petroleum ether	Ethyl acetate
Micrococcus spp	31.25mg	125mg/ml
Staphylococcus aureus	-	500mg/ml

Minimum bactericidal concentration (MBC) of leaf extract of petroleum ether and ethyl acetate of *Cymbopogon citratus* against penicillin-resistant organisms

Table 7 shows the minimum bactericidal concentration (MBC) of leaf extract of petroleum ether and ethyl acetate of *Cymbopogon citratus* against penicillin-resistant organisms.

Table 7: Minimum bactericidal concentration (MBC) of leaf extract of petroleum ether and ethyl acetate of C*ymbopogon citratus* against penicillin-resistant organisms

Isolates	Concentration (mg/ml)	
	Petroleum ether	Ethyl acetate
Micrococcus spp	31.25mg	125mg/ml
Staphylococcus aureus	-	500mg/ml

Discussion

Studies have been conducted with extracts of various plants of antimicrobial activity, as well as for the discovery of new antimicrobial compounds [10]. In this research, two extracts of Cymbopogon citratus were evaluated for exploration of their antimicrobial activities against penicillin-resistant microorganisms which include; Micrococcus sp, and Staphylococcus aureus. The ethyl acetate exhibited more antimicrobial effect against the test organisms than petroleum ether extract. It showed its highest inhibition of 25.0mm at a concentration of 1000mg/ml against Micrococcus spp, while petroleum ether extract showed inhibition of 23.00mm at the same concentration for the Micrococcus spp. This shows that more bioactive agents were extracted from ethyl acetate than petroleum ether.

The minimum inhibitory concentration of petroleum ether was recorded at 31.25mg/ml for Micrococcus spp, while ethyl acetate was recorded at 125mg/ml and 500mg/ml for Micrococcus spp and Staphylococcus aureus respectively as shown in Table 5. It was also observed that the extract exhibited bactericidal effect on the isolates. According to [11], phytochemical screening shows the presence of flavonoids, tannins, glycosides, saponins, alkaloids, phenols, terpenes and resins with absence of steroids. Presence of alkaloids, flavonoids and other phytochemicals in plant have amazing effect on man and has led to the development of powerful pain killer medications [12].

Hence, Cymbopogon citratus can be used for treatment of infection caused by Micrococcus spp and Staphylococus aureus. In conclusion it has been observed that Cympbopogon citratus (lemon grass) leaf extract are effective against penicillin resistant microorganism which include the Micrococcus spp and Staphyloccoccus aureus.

Conclusion

This study shows that the leaf extracts of Cymbopogon citratus (lemon grass) exhibit significant antimicrobial activity against penicillin-resistant microorganisms, particularly Micrococcus spp and Staphylococcus aureus. The research demonstrates that both petroleum ether and ethyl acetate extracts of the plant can inhibit the growth of these bacteria, with ethyl acetate showing slightly higher potency.

The minimum inhibitory concentrations (MIC) for the extracts were 31.25 mg/ml for petroleum ether and 125 mg/ml for ethyl acetate against Micrococcus spp, while Staphylococcus aureus had a higher MIC of 500 mg/ml with ethyl acetate. The bactericidal effects observed indicate that the plant extracts are effective in killing these resistant microorganisms. Therefore, Cymbopogon citratus holds potential as a natural treatment for infections caused by penicillin-resistant bacteria, with further research suggested for its application in medical and pharmaceutical contexts

Acknowledgements

We acknowledge useful advice from our colleagues and we declare that no grant was received for this research

References

[1] Hora, S. C. & Nair K. K. (2016). Medicinal plant and effect. National Institutue of Science Journal. India 10 (6): 143-147.

[2] Patil, A.S. (2010). Exploring Passiflora incarnata. A medicinal plants secondary metabolites as antibacterial agent. Journal of Medicinal Plants Research 4:1496-1501

[3] Wang, L. & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology17: 300-312.

[4] Franciso, V., Figueirinha, A., Neves, B. M., Garcia, R. C., Lopes, M.C., Cruz, M. T. & Batista, M. T. (2011). Cymbopogon citratus as source of new and safe anti- inflammatory drugs: bio-guided assay using lipopolysaccharide-stimulated macrophages. Journal of Ethnopharmacology 133:818-827

[5] Adejuwon, A. A. & Esther, O. A. (2007). Hypoglycemic and hypolipidemic of fresh leaf aqueous extract of Cymbopogon citratus. Journal of Ethnopharmacology. 112: 440-444.

[6] Figueirinha, A., Crus, M. T., Franciso, V., Lopes, M. C. & Batista, M. T. (2010). Anti-inflammatory of Cymbopogon citratus leaf infusion in lipopolysaccharide stimulated dendritic cells: contribution of the polyphenols. Journal of Medicinal Food 13: 681-690

[7] Viana, G. S., Vale, T. G., Pinho, R. S. & Matos, F. J. (2000). Antinociceptive effect of the essential oil from Cymbopogon citratus in mice. Journal of Ethnopharmacology 70:323-327.

[8] Tatiana, F. B. & Jose, M. S. (2011). Lemon grass and citral effect on cytokines production by murine macrophages. Journal of Ethnopharmacology 137:909-913.

[9] Janaid, S. A., Olabode, A. O., Onwuliri, F. C., Okwuri, A. E. J., & Agina, S. E. (2006). antimicrobial properties of Ocimum gratissimum extracts on some selected bacterial gastrointestinal isolates. African Journal of Biotechnology Vol 5 (22), pp 2315-2321

[10] Sharma, D., Kaur, T., Chadha, B. S., Manhas, R. K. (2011). Antimicrobial Activity of Actinomycetes Against Multidrug Resistant Staphylococcus aureus, E. coli and Various Other Pathogens. Tropical Journal of Pharmaceutical Research. 1010. 801-802. 10.4314/tjpr.v10i6.14.

[11] Amer, H.H., Alzobaay & Baidaa, H.K.(2018). Phytochemical screening, chemical composition and antibacterial activity of Lemon (Cymbopogon citratus) leave extracts.Indian. Journal of Natural Sciences. Vol 9: (51): 15306-15315.

[12] Kam, P. C., & Liew, S. (2002). Traditional Chinese herbal medicine and anaesthesia. Anaesthesia. 57 (11): 1083-9. 10.1046/j.1365-2044.2002.02823.x.

© Copyright 2023 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685.