Evaluation of Antimicrobial Properties of *Cymbopogon citratus* and *Momordical charantia*

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ABSTRACT: *Cymbopogon citratus* (Lemon grass) and *Momordical charantia* (Bitter melon) has been in use over time for medical purposes. In this study, aqueous and methanol extracts of both plants were tested for antibacterial activity against control strains of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* using broth dilution technique. *Cymbopogon citratus* was found to be inhibitory to *Proteus mirabilis* at 79mg/ml and 39.5mg/ml for others (aqueous extract); 19.75mg/ml for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and 39.5mg for others (methanol extract) and bactericidal at 79.0mg/ml for *Pseudomonas aeruginosa* and 158mg/ml for others (aqueous extract); 79.0mg/ml for *Staphylococcus aureus* and *Pseudomonas aeruginosa* and 158mg/ml for others (methanol extract). *Momordical charantia* was inhibitory at 20mg/ml for *Staphylococcus aureus* and 40mg/ml for others (both aqueous and methanol extract) while it was not bactericidal except at 80mg/ml to *Pseudomonas aeruginosa* (both extract) and to *Proteus mirabilis* (methanol extract only) It is recommended therefore that various plants alike) should be explored for their antibacterial activities as this could open ways for combating fast appearing resistant strains of pathogenic organisms.
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1. INTRODUCTION

The use of plants for healing is as ancient and universal as the medicine itself. Plants acts generally to stimulate and supplement the body's healing forces, they are the natural food for human beings (Hamburger and Hostettmaun, 1991). Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. During the last century, the practices of herbalism became mainstream throughout the world. This is due to the recognition of the value of traditional medicine system and the identification of medical plants from indigenous pharmacopoeias, which have significant healing power (Lewis, 2001). Till today plant materials continues to play a major role in primary health care as therapeutic remedies in many developing countries (Jonathan and Fasidi, 2003).

Plants still continue to be almost the exclusive source of drugs for the majority of the world's population and about 10% of these plants are consumed as food by humans and animals alike (Moerman, 1996). *Cymbopogon citrate* (lemon grass) has since been used in the treatment of malaria, cough, sprains, lumbago, cold and ringworm. It has also been used as stomach tonic, stimulant, diaphoretic, diuretic and refrigerant (Odugbemi and Akinsulire, 2006). It has also been found as an effective antimicrobial believed to dispel bacterial infections (Omoregbe, et.al., 1996). *Momordical charantia* (bitter melon) on its part is used against diabetes, piles, convulsions, jaundice, nervous disorders, night blindness, dysmenorrhoea and as antimicrobials (Odugbemi and Akinsulire, 2006).

Since both plants, i.e. lemon grass and bitter melon, has been found to be medicinal. The aim of this study is to determine the antibacterial activity or sensitivity of both plants using leaf extracts; their minimum inhibitory concentrations and their minimum bactericidal concentrations to the organisms.

2. MATERIALS AND METHOD

The procedures in carrying out this study involves extraction of the plants, collection of control strains of the required organisms, confirmation of the control strains by biochemical tests, preparation of

inoculums, determination of the Minimum Inhibitory Concentration of each plant extract and the determination of their Minimum Bactericidal Concentration on each organisms used.

2.1. COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The leaves of *Cymbopogon citrates* and *Momordical charantia* were obtained from a vegetable garden at Siun, Obafemi Owode, Ogun State and authenticated at the Department of Pharmacognosy, College of Medicine of the University of Lagos.

2.2. PREPARATION OF EXTRACTS

The leaves of *Cymbopogon citrates* and *Momordica charantia* were chopped into smaller pieces, dried in a hot air oven at 40^0C for about 3 hours, grinded into powder form and the weight noted. *Cymbopogon citrates* weighed 158g while *Momordica charantia* weighed 80g. The weight of each plant was divided into two equal parts (i.e. 79g each for *Cymbopogon citrates* and 40g each for *Momordical charantia*) and extracted in soxhlet extractor using 500ml of methanol for one part of each plant and 500ml of distilled water for the second part. The resultant extract which are 79g/500ml each of aqueous and methanol extract of *Cymbopogon citrates* and 40g/500ml each of aqueous and methanol extract of *Momordica charantia* were stored in sterile universal bottles in a refrigerator until needed (Vitturo et. al., 1998 and Huang, et. al., 1990). Concentration in g/ml of *Cymbopogon citrates*: 79g/500ml = 0.158mg/ml. In mg/ml: 0.158g/ml x 1000 = 158mg/ml. Concentration in g/ml of *Momordica charantia*: 40g/500ml = 0.08g/ml. In mg/ml = 0.08g/ml x 1000 = 80mg/ml. Neat concentrations of each extracts are: Aqueous extract of *Cymbopogon citrates*: 158mg/ml. Methanol extract of *Cymbopogon citrates*: 158mg/ml. Aqueous extract of *Momordica charantia*: 80mg/ml. Methanol extract of *Momordical charantia*: 80mg/ml.

2.3. COLLECTION OF CONTROL ORGANISMS

The organisms used in this study were control organisms from the National Institute of Medical Research, Yaba, Lagos. They are: *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus mirabilis* (ATCC 12453).

2.4. PREPARATION OF INOCULUM

All agar and broth used were prepared according to manufacturer's instructions. The inoculums were prepared using Kirby-Bauer's method. A sterile Nutrient broth was inoculated aseptically with a control organism and was incubated at 37^oC overnight prior to testing. The resultant broth culture was then diluted with sterile nutrient broth such that its turbidity matches that of MacFarland's opacity of 0.5 Standard (Cheesbrough, 2000).

2.5. MINIMUM INHIBITORY CONCENTRATION

The Minimum Inhibitory Concentration for each extract against each organism were determined by inoculating the standardized organisms into various concentration of the extract in sterile nutrient broth and incubating it at 37^oC overnight after which they were observed for turbidity or any sign of growth.

2.6. MINIMUM BACTERICIDAL CONCENTRATION

This was done by culturing the broth /extract/organism mixture of the Minimum Inhibitory Concentration tubes showing no sign of growth unto a solid media.

3. RESULT ANALYSIS

Preliminary test of the aqueous and methanol extract of *Cymbopogon citrates* showed that all the organisms used were susceptible. Table 1 shows the zone diameters of the extracts on each organism, the methanol extract being more sensitive than the aqueous extract.

Table 1: Zone Diameters from the Preliminary Test of *Cymbopogon citrates* (mm)

Organisms	Aqueous Extract	Methanol Extract	Distilled Water	Methanol
S. aureus	10	13	-	-
K. pneumonia	10	15	-	-
P. aeruginosa	8	12	-	-
P. mirabilis	6	10	-	-

For most of the organisms, the aqueous extract was inhibitory at ¼ dilutions with its equivalents concentration as 39.5mg/ml except for *Proteus mirabilis* which was inhibited at ½ dilutions i.e. 79.0mg/ml concentration as seen in Table 2. Its methanol extract has an inhibitory concentration of 39.5mg/ml (1/4 dilutions) for *Staphylococcus aureus* and *Proteus mirabilis* and 19.75mg/ml (1/8 dilution) for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Table 2: Minimum Inhibitory Concentrations of *Cymbopogon citratus* (mg/ml)

Organisms	Aqueous Extract	Methanol Extract
S. aureus	39.50 (1/4)	39.50 (1/4)
K. pneumonia	39.50 (1/4)	19.75 (1/8)
P. aeruginosa	39.50 (1/4)	19.75 (1/8)
P. mirabilis	79.00 (1/4)	39.50 (1/4)

Key: Dilutions in Parenthesis.

Table 3 Shows the Minimum Bactericidal Concentrations of both extracts on the control organisms used. The aqueous extract was bactericidal to all the organisms except *Pseudomonas aeruginosa* at it neat concentration (158mg/ml). It was bactericidal to *Pseudomonas aeruginosa* at a lower concentration of 79.0mg/ml (1/2 dilution). The methanol extract on its part was bactericidal to *Klebsiella pneumoniae* and *Proteus mirabilis* at the neat concentration (158mg/ml) and to *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 79.0mg/ml i.e. ½ dilution.

Table 3: Minimum Bactericidal Concentrations of *Cymbopogon citrates* (mg/ml)

Organisms	Aqueous Extract	Methanol Extract
S. aureus	158.0 (neat)	79.0 (1/2)
K. pneumoniae	158.0 (neat)	158.0 (neat)
P. aeruginosa	79.0 (1/2)	79.0 (1/2)
P. mirabilis	158.0 (neat)	158.0 (neat)

Key: Dilutions in Parenthesis.

The aqueous and methanol extract of *Momordica charantia* in its preliminary test showed no clearly defined zone of 6mm diameter for *Staphylococcus aureus* and 8mm diameter for *Pseudomonas aeruginosa* as seen in Table 4.

Table 4: Zone Diameters from the Preliminary Test of *Momordica charantia* (mm)

Organisms	Aqueous Extract	Methanol Extract	Distilled Water	Methanol
S. aureus	6	6	-	-
K. pneumonia	No cdz	No cdz	-	-
P. aeruginosa	8	8	-	-
P. mirabilis	No cdz	No cdz	-	-

Key: cdz= clearly defined zone

The Minimum Inhibitory Concentration of both extract (i.e. aqueous and methanol) was similar Table 5, being inhibitory to *Staphylococcus aureus* at 20mg/ml (i.e. ¼ dilutions) and to the others at 40mg/ml (i.e. ½ dilutions).

Table 5: Minimum Inhibitory Concentrations of *Momordica charantia* (mg/ml)

Organisms	Aqueous Extract	Methanol Extract
S. aureus	20.0 (1/4)	20.0 (1/4)
K. pneumoniae	40.0 (1/2)	40.0 (1/2)
P. aeruginosa	40.0 (1/2)	40.0 (1/2)
P. mirabilis	40.0 (1/2)	40.0 (1/2)

Key: Dilutions in Parenthesis.

Both extract were not bactericidal to *Staphylococcus aureus* and *Klebsiella pneumoniae* but bactericidal to *Pseudomonas aeruginosa* at its neat concentration i.e. 80mg/ml Table 6. The methanol extract was also bactericidal to *Proteus mirabilis* at 80mg/ml.

Table 6: Minimum Bactericidal Concentrations of Momordica *charantia* (mg/ml)

Organisms	Aqueous Extract	Methanol Extract
S. aureus	158.0 (neat)	79.0 (1/2)
K. pneumoniae	158.0 (neat)	158.0 (neat)
P. aeruginosa	79.0 (1/2)	79.0 (1/2)
P. mirabilis	158.0 (neat)	158.0 (neat)

Key: Dilutions in Parenthesis.

4. DISCUSSION

The development of resistance to antibiotics by pathogenic bacteria has put various pharmacopoeias to search for more active antimicrobials and this search is carried out mostly by screening plans for their antimicrobial activities (Martinez et. al., 1996). *Cymbopogon citrates* and *Momodica charantia* from this study showed that it is sensitive to commonly occurring pathogens recently showing resistance to readily available antibiotics especially those of the penicillin. *Cymbopogon citratus* with has since been used as antimicrobials and for the treatment of stomach discomfort, cold, sore throat, flu etc was both inhibitory and bactericidal to all the organisms used for this study which is confirmatory of its use as antimicrobials (Menut et. al., 2000). Although in the search carried out for the purpose of the study, no work was found to have been carried out using *Cymbopogom citrates* against organisms such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis,* the result from this study has proven that this plant needs no "synergism" from any other plant extract to be active against the listed organisms. Though reports have been made concerning its activity against organisms such as *Escherichia coli* and *Salmonella species* (Dubey et. al., 1997), its activities against the organisms used in this study is an added advantage.

Momordica charantia on its part was found from this study to be inhibitory to all the organisms used but not bactericidal except to *Pseudomonas aeruginosa* (both aqueous and methanol extract) and *Proteus mirabilis* (methanol extract only). Although *Momordica charantia* has been reported to be bactericidal to a wide range of organisms which includes *Escherichia coli, Staphylococcus aureus, Salmonella species, Shigella species* (Huang et. al., 1990) and even the stomach ulcer causing bacteria, *Helicobacter pylori* (Khan et. al., 1998) this study shows no confirmation of its bactericidal activity. This may be due to the reduced concentration (80mg/ml of neat extract) at which it was used for this study. *Momordica charantia* has also been found by some researchers to enhance immunity (Khan, et, al., 1998), this an added advantage to its use as antimicrobial even if it is not bactericidal. Its inhibitory effect can be assisted by its ability to enhance immunity in mopping up inhibited organisms in the host.

One interesting discovery about this study is that both *Cymbopogon citrates* and *Momordica charantia* (aqueous and methanol extract) were found to be both inhibitory and bactericidal to *Pseudomonas aeruginosa*, an organism that has a report of being resistant to majority of antibiotics, especially the hospital strains (Cheesbrough 2000). Note should also be taken of that methanol extract of *Cymbopogon citrate* is more active than its aqueous counterpart while for *Momordica charantia,* both extracts have the same activity.

5. CONCLUSION

The search for antimicrobials among plants is becoming more successful and encouraging as most plants tested for this purpose yields positive results. *Cymbopogon citratus* and *Momordica charantia* are not left out as they have been proven from this study to be active against all the control organisms used especially *Pseudomonas aeruginosa*. We therefore recommend the further search for antimicrobials in or among plants as this study has shown that there is hope in combating the increasing rate of resistant strains of organisms appearing from day to day. This search should not only be carried out on plants found out to be in use by herbal medical practitioners but should also be extended to plants which may be regarded as ornamentals. They could be goldmine of antimicrobials.

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