



Demonstration of the Minimum Inhibitory Concentration (Mic) and Minimal Bactericidal Concentration(Mbc) of Both *Moringa oleifera* and *Gongronema latifolium* Extracts Mixture against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AAO and IOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AAO managed the analyses of the study. Authors AAO, IOO, OO, EUU and NAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Moringa oleifera* and *Gongronema latifolium* have many nutritional values that contain bioactive components such as alkaloids, saponin, tannin, steroids and terpenoids, as antimicrobials.

Objective: The general purpose of this study was to evaluate the minimum inhibitory concentration (MIC) and Minimal bactericidal concentration(MBC) of both *Moringa oleifera* and *Gongronema latifolium* extracts against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*.

Method: This is laboratory experimental research involving post test only control group design using the Kirby Bauer dilution method. With treatment combination of *Moringa oleifera* and *Gongronema latifolium* extracts concentration ranging from 50-200 mg/ml and positive control (gentamycin).

Result: Minimum bacterial concentration was found between 6.25 mg /ml – 12.5mg /ml for *Moringa oleifera* and *Gongronema latifolium* extracts while minimum inhibitory concentration of *Moringa oleifera* and *Gongronema latifolium* was found between 12.5 mg / ml – 25.0 mg/ml respectively. U-Mann whitney results showed that the value of $p < 0.05$ were significant. In this study, the *Staphylococcus aureus* showed the highest inhibition zone diameter compared to other isolates.

Conclusion: The results from this work affirms the use of *Moringa oleifera* and *Gongronema latifolium* as antimicrobials and explores the attention of pharmaceutical companies to exploit production of antibiotics for treatment of infections resulting from *Salmonella typhi* and *Staphylococcus aureus* isolates.

Keywords: *Moringa oleifera*; *Gongronema latifolium*; *Salmonella typhi*; *Staphylococcus aureus*.

1. INTRODUCTION

Plant based medicine is widely used and forms an integral part of primary health care in many developing countries across the globe. Recently plants have been explored to obtain crude natural extracts for testing and further refinement to develop effective antimicrobial drugs. Antimicrobial activities are evaluated with diverse set of methodologies, degree of sensitivity, amount of compounds and microbial strain [1]. Results obtained from antimicrobial activities are profoundly influenced by not only the method selected, but also by micro organisms used to carry out the test and by the degree of solubility of each test compound [1].

Bioautographic and diffusion methods are known as qualitative techniques since these methods will only give an idea of the presence or absence of substances with antimicrobial activity [1]. On the other hand, dilutions method are considered quantitative assays once they determine the minimum inhibitory concentration [1]. Plants are sources of potential therapeutic agent against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites [2]. The essential oil as well as aqueous and ethanolic extract of *Gongronema latifolium* were found to be active against *Staphylococcus* spp, *Escherichia coli*, *Shigella* spp, *Salmonella* spp and *Klebsiella pneumonia* [3]. Antimicrobial components of *Moringa* have been validated after the discovery of inhibitory activity against several microorganisms [4]. Secondary metabolites from *M. oleifera* leaves have shown antimicrobial effects against various human

pathogenic bacteria, including the genera *Shigella*, *Pseudomonas*, *Salmonella* and *Bacillus*.

Beside antibacterial activity of *Moringa* oils, it also possesses anti-fungal activity. Study comparing relative antimicrobial activity of seed extracts against bacteria (*Pasturella multocida*, *E. coli*, *B. subtilis* and *S. aureus*) and fungi (*Fusarium solani* and *Rhizopus solani*) revealed that *P. multocida* and *B. subtilis* were the most sensitive strains and their activity was influenced by cations (Na, K, Mg and Ca). Another relative comparison of antibacterial and antifungal efficacy of *Moringa* steam distillate observed more inhibition for *E. coli* followed by *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa* and *B. subtilis*. The use of medicinal plants in management of diseases is as old as mankind and is still an important alternative therapy widely employed in developing countries. Udoh et al. [5] reported the antimicrobial activity of *G. latifolium* extract against *S. aureus* in their research on the hopping potentials of *V. amygdalina*, *G. latifolium* and *Garcinia kola* in sorghum larger beer brewing. In a recent study, aqueous extracts of *Moringa* was found to be inhibitory against many pathogenic bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* dose dependent [6]. Therefore, the main objective of this study is to determine the minimum inhibitory concentration (MIC) and Minimal bactericidal concentration(MBC) of both *Moringa oleifera* and *Gongronema latifolium* extracts against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* respectively.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study was performed using randomly selected isolates from Medical Microbiology Laboratory of Namadi Azikiwe University Teaching hospital Nnewi. These isolates were subjected to biochemical test for identification.

2.2 Sample Collection

Isolates were obtained from the culture plates in Laboratory unit of Microbiology department Nnamdi Azikiwe University Awka Teaching hospital Nnewi on march, 2018. These isolate were preserved in Nutrient agar for further processing by subcultures. Isolates from urine cultures were subcultured in Blood agar, CLED agar and MacConkey agar while isolates from stool culture plates were subcultured onto MaConkey agar, Deoxycholate citrate agar and to Salmonella shigella agar using spread plate method. These isolates were however subjected to biochemical tests for further identification. Three major isolates *Staphylococcus aureus*, *Salmonell typhi* and *Escherichia coli* were obtained and used for the antibacterial activity test. *Gongronema latifolium* and *Moringa oleifera* fresh plant leaves were obtained from Onitsha market in Anambra State of Nigeria. These plants leaves were processed by Soxhlet extraction to obtain crude form for antimicrobial activities. The crude form was further subjected to liquid liquid fractionation method to extract fractions of it for antimicrobial activities. Phytochemical analysis was determined using [7], and proximate nutritional composition was determined biuretttest and molish test method.

2.3 Preparation of Extract and Fractions

The pulverized leaves were dissolved in 250mls of distilled water and partitioned exhaustively with Methanol, distilled water, H-Hexane, Butanol and Ethyl Acetate using Soxhlet extraction method [8], and liquid liquid fractionation. 800g of weighed plant powder was wrapped in Whatman' sfilter paper and placed in a soxhlet extractor, using Soxhlet extraction method [8]. Five hundred milliliters capacity soxhlet extractor was used for the extraction. Methanol and distilled water were used as solvent for extraction. This was done for 72 hours to obtain methanol and water extract separately. Each pulverized leaf

(*G. latifolium* and *M. oleiferas*) was placed in as thimble and loaded into the main chamber of the soxhlet extractor equipped with a condenser. The solvent was heated to reflux while the vapour travelled up the distillation arm and flooded into the chamber housing the plant extract. The condenser ensured that solvent vapour cools and drips back down into the chamber housing the plant extract. The chamber containing the plant extracted slowly filled the warm solvent. The desired compound dissolved in the warm solvent. When the soxhlet chamber was almost full the chamber automatically emptied with the solvent running back down to the distillation flask. This was allowed to repeat many times for several days. After each cycle the of methanol and aqueous crude extract were concentrated by evaporation at reduced pressure using rotary evaporator to a thick, dark brown gummy. The non-soluble portion of the extracted remained in the thimble. Both extract were dewaxed, removing all typane, fats and oil three times with N- Hexane since it was most polar. They were further partitioned with Ethyl Acetate up to four times and lastly Butanol two times while the remaining fraction was water. Each fraction obtained was concentrated individually.

2.4 Standardization of Test Organisms

In vitro antibacterial test of the bacteria isolates was carried out using standard agar well diffusion method. Sterile Mueller Hinton Agar plates were inoculated with the test culture using the spread plate method.

2.5 Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined by adopting the technique used by [8]. A two folds serial dilution of the reconstituted extract was prepared. Each dilution was seeded with bacterial suspension and incubated for 24hours at 37°C. MIC was determined as the highest dilution (the lowest concentration of the extract with no visible growth. Minimum inhibitory concentration for crude leaf extract of *G. latifolium* and *M. oleifera* was determined for each isolate using dilution susceptibility test this ranged from 0.785mg/ml-200mg/ml.

2.6 Minimum Bactericidal Concentration

Culture from Minimum inhibitory concentration (MIC) was sub-cultured unto sterilized Mueller Hinton Agar and incubated for 24hours at 37°C. Minimum Bacterial Concentration was

determined as the least concentration showing no visible growth on subculture [9]. Minimum Bacterial concentration for leaf extract of *G. latifolium* and *M. oleifera* ranged from 3.125mg/ml—50.00mg/ml.

2.7 Combination Studies

Five milliliters of (200mg/ml) crude concentration of *Moringa oleifera* leaf extract and 5ml of 50mg/ml of *Gongronema latifolium* leaf extract were measured and poured into the same plate to make up 10mls of the combined extract. Into a second petri dish 5ml of 200mg/ml Ethyl Acetate fraction of *Moringa .oleifera* and 5ml of 50mg/ml of *Gongronema latifolium* fraction was poured into the same plate. Filter paper disc (6mm) were immersed in each plate for 60 seconds to ensure complete impregnation of the extract on the filter paper disc. 10mg/ml of Gentamycin was used as positive control and 50% Dimethyl sulfoxide (DMSO) was used as negative control.

2.8 Preparation of Stock Solutions of Leaf Extract

Crude extracts with a yield of 155g for *Gongronema latifolium* and 158g of *Moringa oleifera* at 40 degree centigrade were obtained. Both extracts were stored at 4 degree centigrade in a refrigerator for use during the experiment. Stock solution of both methanolic and aqueous extracts were prepared by dissolving 10g of methanol extract of *G. latifolium* and *M. oleifera* in 10ml of 50% DMSO to give a stock solution of 1g/ml respectively.

2.9 Statistical Analysis

The data were subjected to Mann-Whitney U test to test if there was a significant difference between the plants extract, their different solvents and the method of extraction.

3. RESULTS AND DISCUSSION

Mixture of crude and fraction from *Moringa oleifera* and *Gongronema latifolium* showed poor susceptibility pattern on *Staphylococcus aureus*,

Salmonella typhi and *Escherichia coli*. Methanol crude extract combination of *Monriga oleifera* and *Gongronema latifolium* showed inhibition zone diameter of 3mm, 2mm and 0mm for *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* respectively (Table 1). In addition, mixture of Ethyl acetate fraction from *Moringa oleifera* and *Gongronema latifolium* on the respective isolates *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were showed resistance. However, Etim et al. [10], in their worked on Guava leaf extract and Utazi leaf extract produced better susceptibility pattern this can be attributed to the method of extraction adopted, the isolates worked on and the concentration of extract constituted for antimicrobial activities.

Minimum Bacterial concentration was found between 6.25 mg/ml—12.5 mg/ml for *Gongronema latifolium* and *Moringa oleifera* while minimum inhibitory concentration of *Gongronema latifolium* and *Moringa oleifera* was found between 12.5mg/ml-25.0mg/ml. *Gongronema latifolium* was most sensitive to *Staphylococcus aureus* and *Salmonella typhi* was least sensitive to the plant extract. Ethyl acetate fraction and aqueous fractions where the dominant fractions with the most antimicrobial activity. Combination of crude and fractions of *Gongronema latifolium* and *Moringa oleifera* on *S.aureus*, *S.typhi* and *E.coli* showed lower inhibition zone diameter signifying that when both are combined for treatment they do not produce good effect. It was observed that most studies done on plant extract adopted cold maceration for its extraction and ethanol as its major solvent. U-Mann whitney results showed that the value of $p < 0.05$ were significant, so it could be suggested that there was a significant difference in the inhibition zone diameter of *Staphylococcus auerus* than any other isolates (Table 2). This is because Gram-negative bacteria are known to be more resistant to inactivation by medium and long chain fatty acids of *Gongronema latifolium* than Gram-positive bacteria because of their impermeability to hydrophobic compounds [11].

Table 1. Combination of crude and fraction from *Moringa oleifera* and *Gongronema latifolium* leaf extract showing inhibition zone diameter

Test Organism	Concentration (mg/ml)	Methanol crude extract IZD(mm)	Ethylacetate fraction IZD(mm)(soxhlet)
<i>Staphylococcus aureus</i>	200 <i>Moringa oleifera</i>	3	0
	50 <i>Gongronema latifolium</i>		
<i>Salmonella typhi</i>	200 <i>Moringa oleifera</i>	2	0
	50 <i>Gongronema latifolium</i>		
<i>Escherichia coli</i>	200 <i>Moringa oleifera</i>	0	0
	50 <i>Gongronema latifolium</i>		
<i>Staphylococcus aureus</i>	10 Gentamycin	0	0
<i>Salmonella typhi</i>	10 Gentamycin	0	0
<i>Escherichia coli</i>	10 Gentamycin	0	0
<i>Staphylococcus aureus</i>	50% DMSO	0	0
<i>Salmonella typh</i>	50% DMSO	0	0
<i>Escherichia coli</i>	50% DMSO	0	0

Table 2. Mann-whitney u test showing antimicrobial activity at the same concentrations of crude leaf extract and fraction of *Moringa oleifera* and *Gongronema latifolium* on *Staphylococcus aureus*

Extracts	50mls	25mls	12.5mls	6.25mls
Crude	8.25	8.50	8.50	7.50
Fraction	5.63	5.50	5.50	6.00
U-Mann Whitney (mean Rank)	9.00	8.00	8.00	12.00
p-value	0.12	0.04*	0.04*	0.16

*P-value <0.05 was considered as a significant

Table 3. Mann-whitney u test showing antimicrobial activity at the same concentrations of crude leaf extract and fraction of *Moringa oleifera* and *Gongronema latifolium* on *Salmonella typhi*

Extracts	50mls	25mls	12.5mls	6.25mls
Crude	1.00	7.25	7.38	7.38
Fraction	6.25	6.13	6.06	6.06
U-Mann Whitney (mean Rank)	14.00	13.00	12.5	12.5
p-value	0.72	0.54	0.48	0.48

*P-value <0.05 was considered as a significant

Table 4. Mann-whitney u test showing antimicrobial activity at the same concentrations of crude leaf extract and fraction of *Moringa oleifera* and *Gongronema latifolium* on *Escherichia coli*

Extracts	50mls	25mls	12.5mls	6.25mls
Crude	7.13	6.63	6.88	6.00
Fraction	6.19	6.44	6.31	6.75
U-Mann Whitney (mean Rank)	13.50	15.50	14.50	14.00
p-value	0.66	0.92	0.70	0.48

*P-value <0.05 was considered as a significant

4. CONCLUSIONS

The plant part works best with fractions which makes available the phytochemicals specifically responsible for its antimicrobial activities. Ethyl acetate fractions did show good antimicrobial activity on *Staphylococcus aureus* better than other fractions. Methanol crude extract did show better antimicrobial activity on *Staphylococcus aureus* compared to the aqueous crude. These solvent should be exploited for plant part extraction. The importance of the leaf extract of *Moringa oleifera* and *Gongronema latifolium* as an alternative to modern day antibacterial therapy should be considered as seen from this research work, it has the potential of been used as an antimicrobial agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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