Asian Journal of Fisheries and Aquatic Research

Volume 27, Issue 1, Page 57-66, 2025; Article no.AJFAR.128481
ISSN: 2582-3760

In vitro Study of Antibacterial Potential of *Moringa oleifera* Ethanolic Leaf Extract on Bacteria Isolated from *Clarias gariepinus*

Kanu, Ezinne Maryann [a], Nwiyi, Paul Okechukwu [a*] and Owunna, Cyprain Onyeama [a]

[a] Department of Veterinary Microbiology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajfar/2025/v27i1863

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, are available here: https://www.sdiarticle5.com/review-history/128481

Original Research Article

Received: 25/10/2024
Accepted: 30/12/2024
Published: 09/01/2025

ABSTRACT

The aim of this study was to determine the in vitro antibacterial potential of ethanolic leaf extract of *Moringa oleifera* on bacteria organisms isolated from at 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml concentration. The antibacterial potential of ethanolic leaf extracts of *Moringa oleifera* were determined against bacteria isolated from the body of *Clarias gariepinus*. The leaf of *Moringa oleifera* was extracted with 99.9% ethanol and distilled deionized water and reconstituted at 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml concentrations. The phytochemical analysis carried out on the leaf extract of Moringa oleifera reveals the presence of alkaloids, tannins, flavonoids,

**Corresponding author: Email: afodiokechukwu@yahoo.com;*

Cite as: *Maryann, Kanu, Ezinne, Nwiyi, Paul Okechukwu, and Owunna, Cyprain Onyeama. 2025. "In Vitro Study of Antibacterial Potential of Moringa Oleifera Ethanolic Leaf Extract on Bacteria Isolated from Clarias Gariepinus". Asian Journal of Fisheries and Aquatic Research 27 (1):57-66. https://doi.org/10.9734/ajfar/2025/v27i1863.*

saponins, oxalates and phytate. The micro-organisms isolated include; *Escherichia coli, Staphylococcus aureus and Salmonella spp*. Antibacterial testing was by agar well diffusion method. The highest zone of inhibition mm achieved with ethanolic extract of *Moringa oleifera* at concentration of 200 mg/ml against *Staphylococcus aureus* while the lowest zone of inhibition was mm at 50mg/ml concentration. The highest and lowest zone of inhibition and mm at concentrations of 200 mg/ml and 50 mg/ml was achieved with ethanolic leaf extract of *Moringa oleifera* against *Escherichia coli*, the highest and lowest zone of inhibition was and mm at concentrations of 200 mg/ml and 100mg/ml, respectively. There was no susceptibility to the organisms at concentration 25 mg/ml and 50 mg/ml for Salmonella spp with *Moringa oleifera* leaf extract. The most susceptible organism was *Staphylococcus aureus* and the least susceptible was *Salmonella spp*. The presence of the phytochemicals in *Moringa oleifera* supports the use of this leaf extract as an antibacterial agent at higher concentration which might justify its ethno-medicinal use.

Keywords: Antibacterial potential; ethanolic leaf extract; Moringa oleifera; Clarias gariepinus.

1. INTRODUCTION

Moringa oleifera is a fast growing, drought-resistant tree of the kingdom Plantae (Vascular plant), Division Magnoliophyta (Flowering plants), Family Moringaceae Martionov (Horse-radish tree family), Genus Moringa Adans and Specie *Moringa oleifera*.

"Common names: Ben oil tree of benzo live tree, Moringa drumstick tree (from the long, slender, triangular seed pods), Horse-radish tree (from the taste of the root, which resembles horse-radish). In local Nigerian languages, Moringa is known as 'Barambo' in Hausa, 'Odudu Oyibo' in Igbo and 'Ewele' in Yoruba. It is widely cultivated for its young seed pods and leaves used as vegetables and for traditional herbal medicine. Moringa is economically important in the production of several commodities, such as oils, foods, condiments and medicine" (Makkar & Becker 1997).

> *"Moringa oleifera have been used in the treatment of hard water, and proved that hardness removal efficiency of M. oleifera increases with increasing dosage. Moringa seed powder is a natural alternative to imported alum" (Jahn et al. 1990).*

"Bacteria disease and its effect on all species of fish have become a concern in fish industries and its effect on the consumers. Fish living in natural environments are known to harbor pathogenic Enterobacteriaceae" (Pillay 1990). Water of poor physico-chemical quality has adverse effects on fish and fish consumers thereby resulting in serious economic and human loss (Amadi 2009) although there are vaccines that have been developed and marketed but these vaccines cannot be used as a universal control measure in aquaculture.

"During the last decades, antibiotics were not only used as a traditional strategy for fish disease management but also for the improvement of growth and efficiency of feed conversion" (Kim et al. 2004). "On the other hand, antibiotics inhibit or kill beneficial microbiota in the gastrointestinal ecosystem but it also makes antibiotic residue accumulated in fish products to be harmful on human consumption" (WHO, 2006). Bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body (Douglas 2007). Animal scientist and Veterinarians are now turning attention towards alternative sources of natural ingredients like plants or herbs to replace antibiotics (Ogbe et al. 2009).

"Plant oil from seeds and leaves such as *Moringa oleifera* are in high demand for their medicinal value. Apart from the medicinal uses *M. oleifera* was reported to be a good source of vitamin and amino acids" (Olugbemi et al. 2010). "Its extract was reported to have antibacterial properties and a conclusion was made to investigate it as a Phyto-therapeutic agent to combat infectious agents" (Patel 2018). The views by Cowman (1999, Parekh & Sumitra 2007) that medicines produced from raw plants are safe is in agreement with this study. The growing interest in plant's usage for medicinal purposes due to the presence of several antibacterial compounds present in them, the safety of plant extracts and the economically friendly cost necessitates this study. The findings by Van den Berg and Saskia Kuipers demonstrate minimal inhibition of bacterial growth of *Moringa oleifera.*

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The fresh leaves of *Moringa oleifera* used in this study was collected from in and around Umudike and its environment on 16th June 2023.

2.2 Processing of the Plant Material

The leaves of *M. oleifera* were thoroughly cleaned and dried at room temperature for three weeks at the Veterinary Biochemistry Laboratory bench in the College of Veterinary Medicine, MOUAU. The dried plant material was then pulverized to fine powder using an electric blender and stored in a sterile container until the time of extraction and analysis.

2.3 Preparation of The Plant Sample

The leaves were carefully separated from the stalk, air dried for three weeks. The dried leaves were grounded with an electric blender to a fine powder form for extraction and was stored in an airtight container.

2.4 Extraction Procedure

Two solvents; ethanol and distilled water were used for the extraction of the grounded plant leaves. These solvents were chosen to yield the most active extracts (Ugochukwu & Babady 2003) with documented advantageous properties (Fasakin et al. 2021, Zygler et al. 2012).

The ethanoic extraction was done using Soxhlet extractor and reflux method in the report of Harborne (1998). "Using electronic weighing balance, 200.54 g of the stored dried powder of *M. oleifera* were collected into the Soxhlet apparatus with 250ml of ethanol. External heat source was attached to the extractor with temperatures between 30^0C to 60^0C. The extraction ran for some days with anti-pumping silicone beads added each day before the apparatus was turned on. The extracts were collected into a clean beaker and filtered with a Whatman No.1 filter. The ethanol solvent was then evaporated in *vacuum* and later in a water bath at 4^0C until completely dry crude extract was obtained. The whole extract was refrigerated at 4^0C until further use. The store extract was reconstituted using distilled water to obtain extracts of several concentrations 25, 50, 100, and 200 mg|ml" [16].

2.5 The Qualitative Phytochemical Screening of leaf extract of *M. oleifera*

Qualitative phytochemical analysis of aqueous and ethanolic extracts of *M. oleifera* leaf was carried out as per method (Negi & Jayaprakasha 2003).

2.5.1 Detection of flavonoids

To 2.0 mL of aqueous and ethanolic extracts, few drops of sodium hydroxide solution were added. Formation of intense yellow color, which became colorless in addition to dilute HCL indicated the presence of flavonoids.

2.5.2 Detection of tannins

To 2.0 mL of aqueous and ethanolic extracts, three drops of 1% ferric chloride was added. Appearance of blue-green color indicated the presence of tannins.

2.5.3 Detection of phytate

The method reported (Day & Underwood 1986) was adapted for phytate quantification. "Powdered samples (4 g) were soaked in 100 cm^3 of 2% HCl (w/v) and allowed to stand for over three hours before filtration. From the filtrate, 25 cm^3 was taken and placed in a conical flask, 5 cm^3 of 0.3% NH_4SCN (aq) and 53.5 cm^3 of distilled water were mixed together and titrated against standard $FeCl_3$ (aq) having 0.00195 g Fe/cm^3 and observed the formation of brown-yellow color which may persist for 5 minutes. Blank was treated in a similar manner".

2.5.4 Detection of saponins

Two mL of aqueous and ethanolic extracts were diluted with 10 mL of distilled water and mixed for 15 min. formation of layers of foam which remain for 10 min indicated the presence of saponins.

2.5.5 Detection for alkaloids

To 2.0 mL of aqueous and ethanolic extracts, 2 mL of picric acid (Hager's reagent) was added. Formation of orange or yellow color precipitate indicated the presence of alkaloids.

2.5.6 Detection for oxalate

Detection of oxalate according to Allen et al. (1986) proposed "a method for determination of oxalate. The leaves sample should be (1 g) and put inside a 100 cm^3 flask. 75cm^3 of $3MnH_2SO_4$ was measured, placed into the same conical

flask and stirred for about 1 hour. The filtration of the solution was carried out using a Whattman No 1 filter paper. 25 cm^3 of the filtrate was measured and titrated over 0.05N potassium permanganate (KMnO4) solution till the appearance of pale-pink color. 1ml of 0.05 m KMnO4 was used to calculate the oxalate content".

2.6 Quantitative Determination of Phytochemical Constituents of Leaf Extract of *M. oleifera*

2.6.1 Flavonoids

"Flavonoids in plant extracts were determined by aluminum chloride colorimetric method (Chang 2002). About 0.25 ml of extract (10mg|ml) was mixed with 0.75 ml of ethanol. 0.005 ml of 10% aluminum chloride, 0.02 ml of 1M potassium acetate and 1.4 ml of distilled water. The reaction mixture was incubated at 37^0C for 30 minutes. The absorbance of the mixture was measured at 415 nm using UV- VIS spectrophotometer" (Negi & Jayaprakasha 2003).

2.6.2 Tannins

"Tannin content was estimated using the Person method, about 1.0 g of the sample was dispersed on 10 ml distilled water and agitated. This was left to stand for 30 minutes at room temperature and shaken every 5 minutes. After 30 minutes, it was centrifuged and the extract was obtained. About 2.5 ml of the supernatant extract was dispensed into a separate 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid was dispensed into a separate 50 ml flask. The absorbance was measured at 250 nm" (Negi & Jayaprakasha 2003).

2.6.3 Saponin

"Saponin content was estimated as per the method of Harborne (1973) About 2 g of dried plant sample was dissolved in 50 ml of petroleum ether. The suspension was heated over a hot water bath at 55^0C for 1 hour with continuous stirring. The mixture was filtered and the residue was re-extracted in 50 ml of methanol. The combined filtrates were reduced to10 ml by placing a water bath at about 90℃. The concentrate was transferred into a 250 ml separating funnel and acetone was added slowly and shaken well. The aqueous layer was recovered and the purification process was repeated. The remaining solution which contains saponin was heated in a water bath and the sample was dried in the oven and weighed. The saponin content was calculated in percentage" (Negi & Jayaprakasha 2003).

> % saponin = (weight of saponin) \ (weight of sample) *100

2.6.4 Alkaloids

"The quantity of alkaloids was estimated as per the method of Lammert & John (2007). About 1 g of powdered sample was mixed with 40 ml of 10% acetic acid and allowed to stand for 4 hours. It was filtered and concentrated on a water bath to one fourth of its original volume then concentrated ammonium hydroxide was added drop by drop to the extracts until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered" (Negi & Jayaprakasha 2003). The residue is the alkaloid, which was dried and weighed. The percentage of alkaloid is expressed mathematically as;

> % Alkaloid = (weight if alkaloid)/ (weight of sample) * 100

2.6.5 Phytate

The method reported by Negi & Jayaprakasha (2003) was adapted for phytate quantification. "Powdered samples (4g) were soaked in 100 cm3 of 2% HCl (w/v) and allowed to stand for over 3 hours before filtration. From the filtrate, 25 cm3 was taken and placed in a conical flask, 5 cm3 of 0.3% NH4SCN (aq) and 53.5 cm3 of distilled water were mixed together and titrated against standard FeCl3 (aq) having 0.00195g Fe/cm3 and observed the formation of brown-yellow color which may persist for 5 minutes. Blank was treated in a similar manner".

2.7 Preparation of Crude Plant Extract

0.5 g of extract was weighed out using an electronic weighing balance and then dissolved in 20 ml, 10 ml, 5 ml and 2.5 ml of distilled water to obtain 25, 50, 100, and 200 mg/ml concentrations.

2.8 Media Preparation

All media used were prepared according to the manufacturer's directives.

2.8.1 Nutrient agar (fluka)

It was prepared according to Amin et al. (2006), by suspending 28 grams of the medium in one

liter of distilled water and sterilized by autoclaving at 121^{0}C for 15 mins and checked for sterility at 37^{0}C for 24 hours. The nutrient agar was used for plating out the organisms as well as storing them in slants and also was used for sensitivity tests.

2.8.2 Mac-conkey's agar

It was prepared as described by Amin et al. (2006), "Mac-Conkey agar is a differential medium used to distinguish lactose-fermenting from non-lactose fermenting bacteria. 51.53grams of Mac-Conkey agar was suspended in the demineralized water. The powder dissolved completely by heating in a water bath sterilized by autoclaving for 15 minutes at 121^{0}C. It was then poured into petri-dishes, about 16-18 ml in each and allowed to solidify at room temperature. The shelf life is about one month. It is stored at 2-8^{0}C".

2.8.3 Blood agar

It was prepared according to Amin et al. (2006), "Blood agar is an enriched medium. It can also be made selective by adding some antibiotics like Kanamycin. When blood agar is heated, the red blood cells are lysed and the media becomes brown, called Chocolate agar. Dissolve 40.0 g in 1 liter of purified water, heat in boiling water and agitate frequently until completely dissolved. Autoclave for 15 minutes at 121^{0}C, it was then poured into petri-dishes, about 16-18 ml in each and allowed to solidify at room temperature. The shelf life is about one month. It was stored at 2-8^{0}C".

2.8.4 Eosin methylene blue agar (EMB)

It was prepared according to Amin et al. (2006), "to suspend 36 grams of EMB agar in 1000 ml of distilled water. Heat to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 Ibs, pressure (121^{0}C) for 15 minutes. Avoid overheating. Cool to 50^{0}C and shake the medium in order to oxidize the methylene blue (i.e to restore its blue color) and to suspend the flocculent precipitate and store the dehydrating medium at 24^{0}C and the prepared medium at 2-8^{0}C".

2.8.5 Salmonella shigella agar (Ss-Agar)

It was prepared according to Amin et al. (2006), suspending 63 grams in 1 liter of distilled water. Bring to boiling with frequent agitation and allow to simmer gently to dissolve the agar. Do not autoclave. Cool to about 50^{0}C, mix and pour into petri-dishes and store at 8^{0}C.

2.8.6 Gram- staining technique

The technique was introduced by Karen (2010) "Make a smear and fixed, by passing the slide rapidly over a flame. Cover the slide with crystal violet and allow it to act for 30 seconds. Pour off and wash freshly with iodine solution. Cover with fresh iodine solution and allow it to act for 30 seconds. Pour off the iodine solution and wash freely with acetone iodine. Cover with acetone iodine and allow it to act for about 30 seconds (until the stain stops carrying out). Wash thoroughly with water, counterstain with safranin for 30 seconds and wash with water, blot and dry. See under the microscope with an oil immersion lens" (Negi & Jayaprakasha 2003).

2.8.7 Catalase test

Test was carried out as described by Patricia et al. (2010). Pour 2-3 drops of fresh hydrogen peroxide solution on a glass slide. Using a sterile wooden or glass rod or wire loop, remove a good piece of growth of the test organism and immerse it in 3% hydrogen peroxide solution. Look for immediate bubbling, which indicates the production of oxygen. Active bubbling produced is a positive result.

2.8.8 Oxidase test

"The test was performed as described Lino et al. (2006) by the indirect paper strip procedure in which a few drops of the reagent were added to a filter paper strip. A loop full of suspected colonies was smeared into the reagent's zones of the filter paper. Bacterial colonies having cytochrome oxidase activity developed a deep purple color at the site of inoculation within 10 seconds. Precautionary measures were taken so as not to use stainless steel inoculating loops or wires so as to avoid surface oxidation products formed by metals when flamed for sterilization which gives a false positive reaction" (Negi & Jayaprakasha 2003).

2.9 Isolation of Sample Organism

"Samples were taken from the dorsal and ventral parts of the body of live fishes using sterile swab sticks and inoculated into nutrient agar plates, incubated for 48 hours at 37^{0}C. The samples were later subcultured into different medias like

eosin methylene blue, *Salmonella shigella* agar, blood agar and Mac-Conkey agar and incubated at 37^0C for 48 hours. The presence of green metallic sheen was confirmatory of *Escherichia coli*, the presence of incomplete zones of hemolysis confirms *Staphylococcus aureus* on blood agar, discrete raised colonies with black centers in SSA is suggestive of *Salmonella spp"* (Negi & Jayaprakasha 2003).

2.9.1 Preparation of test organisms and test for potency of bacteria pathogen

Microorganisms were obtained from the prepared and stored stock cultures of test organisms in the Department of Veterinary Microbiology, MOUAU. The organisms are as follows; *Salmonella spp. Staphylococcus aureus and Escherichia coli.*

"Bacteria organisms were sub-cultured in nutrient agar. Overnight cultures were prepared by inoculating approximately 2 ml nutrient broth with colonies of the appropriate organism taken from the agar slant. Broth was incubated overnight at 37^0C, bacteria inoculum was prepared by diluting overnight cultures in saline to approximately 10 raise to power 8 CFU/ml for each of the organisms" (Negi & Jayaprakasha 2003).

2.9.2 Determination of antimicrobial activity of the extract

The antimicrobial screening of the aqueous and ethanolic extract was carried out using the agar well diffusion method as described by Duke (2008). "Nutrient agar was poured into sterile petri-dishes and allowed to solidify. About 1.0 ml of the test culture was dropped on the appropriate solidified agar and spread over the surface of the medium using sterile swab stick. Wells of approximately 6 mm in diameter were made in the agar medium using sterile cork borer. Each well was filled with 0.2 ml of the appropriate concentration of extract. The dishes were allowed to stand for 40 minutes at room temperature to allow proper diffusion of the extract to occur. Control experiments were set up with 0.2 ml of 99% ethanol and 0.2 ml distilled water in separate wells. The plates were incubated for 24 hours. All tests were performed in 5 replicate and antimicrobial activity was expressed as the mean diameter of the clear zone (mm) produced by the plant extract. Zones of clearance around each well means inhibition and the diameter of each zone are measured in millimeters (mm)" (Negi & Jayaprakasha 2003).

Fig. 1. Ethanolic extract of M. oleifera at concentration 25, 50, 100 and 200 mg/ml against *E. coli*

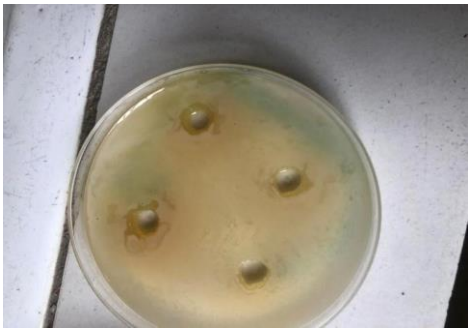

Fig. 2. Ethanolic extract of M. oleifera at concentration 25, 50, 100, and 200

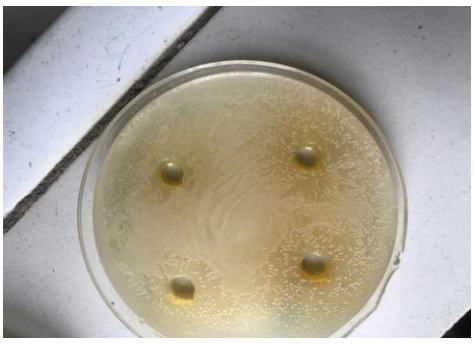

Fig. 3. Ethanolic extract of M. oleifera at concentration 25, 50, 100 and 200 mg/ml

3. RESULTS

3.1 Yield from Aqueous and Ethanolic Extractions

The leaves were extracted with 99.9% ethanol and distilled water. The highest yield was achieved with ethanol *M. oleifera* 6.63%, while the highest yield achieved with distilled deionized water was 6.56%.

Table 1. The qualitative analysis of *Moringa oleifera*

Sample	Alkaloid	Saponins	Tannins	Flavonoids	Oxalate	Phytate
Moringa oliefera	+	+	+	++	+	+

Key: ++ = highly present, + = present

Table 2. The aqueous phytochemical analysis of *Moringa oleifera.*

Sample	Alkaloid	Saponins	Tannins	Flavonoids	Oxalate	Phylate
Moringa oliefera	0.59 ± 0.62	1.68 ± 1.72	3.51±3.66	6.60 ± 6.63	0.35 ± 0.28	2.69 ± 1.25

Measurement are in mg/100 g

Table 3. The ethanolic phytochemical analysis of *Moringa oleifera*

Sample	Alkaloid	Saponins	Tannins	Flavonoids	Oxalate	Phytate
Moringa oliefera	0.66 ± 0.45	1.70 ± 0.20	3.58 ± 0.75	6.59 ± 0.35	0.37 ± 0.20	2.71 ± 0.28

Measurement are in mg/100g

Table 4. Anti-microbial activity of ethanolic leaf extract of *Moringa oleifera* with zones of inhibition measured in (mm) at different concentrations. Zone of inhibition (mm)

Test Organisms	Ethanol	25 mg/ ml	50 mg/ml	100 mg/ml	200 mg/ml
Staphylococcus aureus	0	0	1.66 ± 1.52	1.66 ± 1.54	2.14 ± 2.10
Escherichia coli	0	0	1.58 + 1.51	1.60 ± 1.47	1.68 ± 1.70
Salmonella spp	0	0	0	0.72 ± 1.02	0.98 ± 1.36

Moringa oleifera used in this research contained alkaloid, saponins, tannins, flavonoids, oxalates and phytate in different proportions in ethanolic extraction with higher levels of flavonoids followed by tannins.

The inhibition diameter of the aqueous leaf extract of *Moringa oleifera* against *Staphylococcus aureus* ranges from to , *Escherichia coli* ranges from to *, and Salmonella spp* ranges from to. Higher values suggest a greater zone of inhibition.

4. DISCUSSION

The susceptibility of the test microorganisms is related to the inhibition zone size in millimeters via agar well diffusion assay. The phytochemical screening conducted on the leaf extract *of Moringa oleifera* revealed the presence of phytochemicals constituents such as alkaloids, saponins, tannins, flavonoids, oxalates and phytate which have been shown to possess some pharmacological activities. According to Duke (2008) (Evans 1996 Lawal et al., (2015) Wasagu et al., (2005), Ibrahim & Oluwosuland (2006) Magaji & Yaro (2006) as well as Magaji and Yaro (2006), Kumar et al. (2010) phytochemical components are responsible for both pharmacological and toxic activities in plants. The ethanolic quantitative analysis composition of the phytochemical in *Moringa oleifera* is similar to the findings of previous studies. Several functions and roles are attributed to flavonoids in humans and animals; this includes protection and fight against inflammatory disorders, allergies, diarrhea, microbes' invasion, platelet aggregation, ulcers, hepatotoxins, viruses, and tumors Kumar *et al.*, (2010), Makkar et al., (1997). Flavonoids were able to achieved the aforementioned properties because of their antipyretic (fever-reducing), antioxidant, analgesic (pain-relieving), and spasmolytic (spasm-inhibiting) activities Kokoska et al*,* (2009) Akpuaka (2009) also known to possess some pharmacological activities. "Saponins are used medically for the treatment of increased blood cholesterol and are beneficial to patients with arteriosclerosis and hypertension and in the control of post-menopausal syndrome Akpuaka" (2009), Brain & Turner 1975).

The ethanolic extract of *Moringa oleifera* gave the yield of 0.00 at the concentration of 25 mg/ml. At concentrations 50 mg/ml, 100 mg/ml and 200 mg/ml there were little zones of inhibition (reactions, as shown in the pictures later) against *Escherichia coli,* same reaction

(zone of inhibition) was also achieved against *Staphylococcus aureus.* There was insignificant antimicrobial potential of *Moringa oleifera* to the tested bacterial organisms. Little reaction or zone of inhibition was observed in *Salmonella spp* at the four different concentrations. Ethanol has been shown to be a stronger extractant than water Brain and Turner (1975) Akinyemi (2000), Pandit & Langfield (2004). The presence of ethanol in addition to achieving better extraction may also enhance the efficacy of the active principle or ingredients. This may justify the traditional use of alcohol in extracting the leaf component of the medicinal plants from application against pathogens Panditt and Langfield (2004) Pandit & Langfield (2004). Earlier, Eloff in 1998 reported that due to the inability of water to extract nonpolar compounds, the water preparation is usually not suitable for antibacterial discovery. Another reason for organic extract to be more active than water extract is due to the better solubility of the active components in organic solvents which is in agreement with the work done by Doughari *et al*., (2007) The presence of phytochemicals supports the use of this plant as antibacterial agent and therefore can be used as antibacterial agent in higher concentrations; this is in agreement with the work done by Gupta *et al*. (2010).

Salmonella spp was found to show no inhibitory activity and this disagrees with the study of Siddhuraju & Becker (2003), that indicated significant inhibitory effects of *Salmonella spp* on the plants extract. *Salmonella spp* was the least test organism with zone of inhibition while *Staphylococcus aureus* had the highest zone of inhibition in this study which is not up to the standard inhibition diameter. The statistical analysis was insignificant since none of the three organisms used in this study exhibited a clear zone of inhibition by the extract at different concentrations.

5. CONCLUSION AND RECOMMENDATION

From the study, it has been revealed *that Moringa oleifera* leaf extracts may possess antibacterial properties when used at a higher concentration. The extracts showed little antibacterial effect on pathogens that affect *Clarias gariepinus*.

Further work should be carried out and there should be an increase in the concentration of the leaf extract in order to verify the ethnomedicinal claim.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Akinyemi, K. A. (2000). Antibacterial screening of five Nigerian medicinal plants against *Salmonella typhi* and *Salmonella paratyphi*. *Journal of the Nigerian Infection Control Association*, 3, 1–13.

Akpuaka, M. U. (2009). *Essentials of Natural Product Chemistry*. Mason Publishers.

Allen, A. L., Fernández, B., & Rubia, E. (1986). A simple, rapid, and reliable spectrophotometric method for the determination of oxalate in aqueous solution. *Microbiology Journal*, 34, 51–55.

Amadi, A. N. (2009). Physico-chemical and bacteriological evaluation of groundwater in parts of Aba, Abia State, Southeastern Nigeria. *International Journal of Applied Biological Research*, 1(1), 63–71.

Amin, A. L., Thomson, Z. M., Al-Qattan, M., Peltonen-Shalaby, K. K., & Ali, M. (2006). Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *Brazilian Journal of Nutrition*, 96(4), 660–666.

Brain, K. R., & Turner, T. D. (1975). *Practical Evaluation of Phytopharmaceuticals* (1st ed.). Wright-Scientechnica.

Chang, C. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, 178–182. https://doi.org/10.38212/2224-6614.2748.

Cowman, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12, 564–582.

Day, R. A., & Underwood, A. L. (1986). *Quantitative Analysis* (5th ed.). Prentice Hall Publication.

Doughari, J. H., El-mahmood, A. M., & Tyoyina, I. (2007). Studies on the antibacterial activity of *Balanites aegyptiaca* and *Moringa oleifera* on *Salmonella typhi*. *African Journal of Biotechnology*, 6(19), 2212–2215.

Douglas, D. (2007). Identifying freshwater aquarium fish disease. Retrieved from http://fishsuite101.com/article.cfm/identifying_fish_diseases

Duke, J. A. (2008). *Handbook of Medicinal Herbs*. CRC Press. Sofowara, E. A. (2008). *Medicinal Plants and Traditional Medicine in Africa* (3rd ed.). Spectrum Books.

Evans, W. C. (1996). *Trease and Evans' Pharmacognosy* (14th ed.). WB Saunders.

Fasakin, O. W., Oboh, G., & Ademosun, A. O. (2021). Neuromodulatory evaluation of commonly abused plants ex vivo: A comparative study. *Comparative Clinical Pathology*, 30, 671–680.

Gupta, R. N., Kartik, V., Manoj, P., & Sing, P. S. (2010). Antibacterial activities of ethanolic extracts of plants used in folk medicine. *International Journal of Resources in Ayurveda Pharmaceutical*, 1(2), 529–535.

Harborne, J. B. (1973). *Phytochemical Methods*. Chapman and Hall Ltd.

Harborne, J. B. (1998). *Textbook of Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (5th ed.). Chapman and Hall Ltd.

Ibrahim, Y. K. E., & Oluwosuland, A. K. (2006). Studies on the antimicrobial screening of aqueous extracts of five plants used in folk medicine in Nigeria. *West African Journal of Biological Science*, 3, 21–26.

Jahn, S. A. A., Jahn, S. A. L., Aria, A. Z., & Azharia, J. S. (1997). Nutrients and anti-quality factors in different morphological parts of the Moringa oleifera tree. *Journal of Agricultural Science*, 128(5), 311–322.

Kim, S., Nonaka, L., & Suzuki, S. (2004). Occurrence of tetracycline resistance genes tet(M) and tet(S) in bacteria from marine aquaculture sites. *FEMS Microbiology Letters*, 237, 147–156.

Kokoska, L., & Janovska, D. (2009). Chemistry and pharmacology of Rhaponticum carthamoides: a review. Phytochemistry, 70(7), 842-855.

Kumar, P. S., Mishra, D., Ghosh, G., & Panda, C. S. (2010). Medicinal uses and pharmacological properties of *Moringa oleifera*. *International Journal of Phytomedicine*, 2, 210–216.

Lammert, A., & John, M. (2007). *Techniques in Microbiology: A Student Handbook*. Pearson Education Inc.

Lawal, B., Shittu, O. K., Abubakar, A. N., Umar, M. B., Ibrahim, A. M., & Haruna, G. M. (2015). Biochemical evaluation in Wistar rats (*Rattus norvegicus*) following chronic exposure to methanol leaf extract of *Telfairia occidentalis*. *Journal of Pharmaceutical and Biomedical Sciences*, 5(9), 740–744.

Lino, A., Apak, D., & Deogracious, O. (2006). The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata*, and *Steganotaenia araliacea*—Ugandan medicinal plants. *African Health Sciences*, 6, 31–35.

Magaji, A., Rabiu, A., & Yaro, A. (2006). Acute toxicity evaluation of the cold aqueous extract of *Syzygium aromaticum* in mice. *Biological and Environmental Journal for the Tropics*, 3, 4–6.

Makkar, H. P. S., & Becker, K. (1997). Nutrients and anti-quality factors in different morphological parts of the *Moringa oleifera*. *Journal of Agricultural Science*, 128(3), 311–322.

Makkar, H. P. S., & Becker, K. (1997). Water treatment with traditional plant coagulant and clarifying clay. *Record 10 and 26 CAB Abstract*, 87-89.

Negi, P. S., & Jayaprakasha, G. K. (2003). Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *Journal of Food Science*, 68, 1473–1477.

Ogbe, A. O., Atawodi, S. E., Abdu, P. A., Sannusi, A., & Itodo, E. A. (2009). Changes in weight, faecal oocyst count and packed cell volume of *Eimeria tenella*-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract. *Journal of South African Veterinary Association*, 80, 97–102.

Olugbemi, T. S., Mutayoba, S. K., & Lekule, F. P. (2010). Effect of Moringa (*Moringa oleifera*) inclusion in cassava-based diets on broiler chickens. *International Journal of Poultry Science*, 9(4), 363–367.

Pandit, K. and Langfield, R.D. (2004). Antibacterial Activity of Some Italian Medicinal Plant.Journal of Ethnopharmacology, 82: 135-142.

Parekh, J., & Sumitra, C. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Resources*, 10, 175–181.

Patel, A. S. (2018). Bacterial diseases of fish: A review. *Veterinary Science Resources*, 3(3), 000164.

Patricia Shields, Laura Cathcart. (2010), Oxidase test protocol. American Society for Microbiology. https://asm.org/getattachment/00ce8639-8e76-4acb-8591-0f7b22a347c6/oxidase-test-protocol-3229.pdf

Patricia, M., Lucas, S., Caio, G., Ademar, A., Marcio, L., Wilson, R., & Ana, H. (2010). Halogenated indole alkaloids from marine invertebrates. *Marine Drugs*, 8, 1526–1549.

Pillay, T. V. R. (1990). Fish and public health and disease. In *Aquaculture, Principles and Practices* (eds), Fishing New Book. Farrihan, New York, 174–215.

Reiner, K. (2010). Catalase test protocol. *American Society for Microbiology*.

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agriculture and Food Chemistry*, 51, 2144–2155.

Ugochukwu, N. H., & Babady, N. E. (2003). Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sciences Journal*, 3, 13–21.

Wasagu, R. S. U., Hassan, S. W., & Lawal, M. (2005). Evaluation of phytochemical and antioxidant levels and antimicrobial screening of aqueous leaf extract of *Aloe vera* (*Aloe barbadensis Miller*). *Biological and Environmental Sciences Journal for the Tropics*, 2(2), 21–25.

World Health Organization. (2006). Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance: Seoul, Republic of Korea.

Zygler, A., Slomińska, M., & Namiesnik, J. (2012). Soxhlet extraction and new developments such as Soxtec. In J. Pawliszyn (Ed.), *Comprehensive Sampling and Sample Preparation: Analytical Techniques for Scientists* (Vol. 2, pp. 65–82). Theory of Extraction Techniques. Elsevier.

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