

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF THE METHANOL LEAF EXTRACT OF *LUFFA CYLINDRICA* ON CERTAIN PATHOGENIC MICROBES.

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ABSTRACT

The attention of the pharmaceutical scientists has been drawn to studies on the potential antimicrobial activities of plant derived substances, an untapped source of antimicrobials which are used in traditional medicine in different settings. This led to the study of the phytochemical analysis and antimicrobial activity of the methanol leaf extract of *Luffa cylindrica* on certain pathogenic microbes: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger*, which were isolated from the laboratory benches and toilet handles of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka. The percentage Ash value was determined to be 2.3%. Microscopic evaluation of the plant showed specific cellular structures like the parenchyma, oxalate crystals, epidermal hairs and stomata. *S. aureus*,

S. typhi, *P. aeruginosa* and *C. albicans* were susceptible to the methanol leaf extract of *L. cylindrica*, with the exception of *E. coli* and *A. niger* which were resistant to the methanol extract. The phytochemical compounds present are Flavonoids, Alkaloids, Saponins,

Anthraquinone, Terpenoids, Steroids. Overall, among the pathogenic microbes used, *E. coli* and *A. niger* were resistant to the effect of the leaf extract of *Luffa cylindrica*.

KEYWORDS: *Luffa cylindrica*; Phytochemical constituents; Pathogenic microbes.

INTRODUCTION

Nature has been the first resort in the past in the discovery of interesting compounds to fight diseases. The traditional medicine sector has become an important source of health care, especially in rural and tribal areas of the country. About 80% of the people in Africa use traditional medicine in maintaining health and wellness (WHO, 2015). The use of herbs & nutrition (diet, vitamins and minerals) offers many advantages over conventional medicine. Although taking a little longer to act, it is far gentler on the body and the overall result is more holistic because the whole body is treated instead of just the symptoms. If disease represents an imbalance in the body, then natural medicine is about correcting that imbalance in a sustainable way (Tam, 2010).

Luffa cylindrica Mill (*Cucurbitaceae*) is a plant from the cucumber family, grown for its multipurpose fruit in many tropical countries. It is an annual climbing or trailing herbaceous species that can be 15 m long (Grin, 2000). It is an annual climbing or trailing herbaceous species that can be 15 m long. The luffa fruit is a cylindrical, fusiform, smooth, and dehiscent capsule, 20-50 cm long x 6-10 cm broad, with has a characteristic fibrous mesocarp. The leaves are alternate, large (6-25 cm x 6-27 cm) ovate and dark green. The seeds are numerous, dull black, elliptic-ovoid, 10-12 mm long x 6-8 mm broad (Achigan-Dako *et al.*, 2011; Floras, 2014).

When the fruit becomes old and dry the endocarp becomes a persistent fibrous vascular network which is used in various ways. A major use is as a sponge for washing and scrubbing utensils as well as the human body. It is also used for the manufacture of hats, insoles of shoes, car-wipers, pot-holders, table-mats, door and bath-mats, sandals and gloves. The fibre has also been used for its shock and sound absorbing properties, for instance in helmets and armoured vehicles, and as a filter in engines. In Ghana the dry fibre is used to filter water and palm wine. In Central Africa it is used to brush clothes. Fungal biosorbents immobilized on *Luffa cylindrica* sponges have been used for the biosorption of heavy metals from olive oil mill wastewater and other wastewaters. The young fruit is eaten fresh or cooked as a vegetable, but it has to be picked before the fibrous vascular bundles harden and before the

purging compounds develop. In Guinea and Côte d'Ivoire edible cultivars are grown. Edible forms have also been developed in India and the Philippines where the plant is commonly cultivated. In India and China a type of curry is prepared with the fruit which is peeled, sliced and fried. In Japan the fruits are eaten fresh or sliced and dried to be eaten later. The leaves are also eaten as a vegetable. The roasted seeds are edible and contain an edible oil. The oil has been used in the United States in soap manufacture. The bitter and toxic seedcake is unsuitable as feed for cattle, but can be used as fertilizer given that it is rich in Nitrogen and Phosphorus. The fruits and leaves are browsed by goats. Bees feed on the flowers. (Bal, *et al.*, 2004).

In traditional African medicine pulp of the whole plant is used as a suppository against constipation. Root preparations are taken for the treatment of constipation and as a diuretic. In Gabon a root preparation is used as remedy of nose cancer. In DR Congo a decoction of the roots and leaves is recorded to be drunk or used in an enema as an abortifacient, but in Tanzania a root decoction and leaf sap are recorded to be drunk to reduce the danger of abortion. The leaves are used to promote wound healing and to maturate abscesses. In Togo leaf preparations are applied on oedemas and taken for the treatment of malaria. In the Central African Republic ground leaves are introduced rectally for the treatment of enterobiasis. In Congo the leaf juice is considered to be effective against filaria and an aqueous maceration of fresh leaves is taken for the treatment of whooping cough. In Rwanda the leaves are pounded with water and the sap is taken for the treatment of stomach-ache. In Uganda leaf preparations are used to facilitate childbirth. Zulu people in South Africa take a leaf decoction to treat stomach-ache. The fruit is used in Guinea on tumours and swellings, and the fruit pulp is used in Guinea and Nigeria as an emollient. The fruit sap is taken as a strong purgative. The seeds are credited with emollient and anthelmintic properties. They are also emetic and cathartic. The seed oil is used for the treatment of skin problems. Unripe fruits have been used as fish poison. (Neuwinger, 2000). *Luffa cylindrica* has been reported to possess both medicinal and nutritional properties. Its seeds have been used in the treatment of asthma, sinusitis and fever (Nagao *et al.*, 1991). It also reported that abortifacient proteins (Ng *et al.*, 1991) such as luffaculin which possess ribosome-inhibiting properties on the replication of HIV infected lymphocyte and phagocyte cells explain its potential as a therapeutic agent for AIDS (McGrath *et al.*, 1989). It has been reported that juice extracted from the stem has been used in the treatment of respiratory disorders and the seed has emetic action (Bailey, 1989). As part of our objective investigation of this on the antimicrobial

activity of the methanol leaf extract of *Luffa cylindrica* on certain pathogenic microbes isolated from the laboratory benches and toilet handles of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka.

MATERIALS AND METHODS

Materials

Plant materials

The plant sample *Luffa cylindrica* (leaves) was collected at Agulu, Anambra state of Nigeria and was authenticated by Mr. A.O Ozioko of International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. Voucher samples were prepared and deposited in the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, with voucher no. PCG/474/A/049, for reference. The plant was then washed and dried under the shade after which it was milled into fine powder with a mechanical grinder. The powdered plant was stored for future use.

Culture media and other reagents

Muller Hinton Agar (Titan Biotech Ltd; Rajasthan India), Sabouraud's dextrose agar, Nutrient Broth (LabM Ltd; United Kingdom), MacConkey Agar (Biotech Laboratories Pvt Ltd; Mumbai India), Mannitol Salt Agar, Cetrinide Agar, Salmonella Shigella Agar (Titan Biotech Ltd; Rajasthan India), Ethanol Absolute (Gunagdong Guandgus Chemical Factory; China), Buffered peptone water (HiMedia Laboratories Pvt Ltd ; Mumbai India), Swab stick, Inoculating loop, Bijou bottles, Disinfectant, Sodium hypochlorite, Distilled water, Normal saline

Reagents and equipment

Freshly prepared plasma, methyl red, marker, slides, crystal violet, tap water, sterile distilled water, iodine solution, ethanol, safranin, light microscope, hydrogen peroxide, ethanol, petri-dishes, test tubes, DMSO, oxidase reagent, Freezer, incubator, Bunsen burner, autoclave, hot air oven, mixer, syringes

Phytochemicals: 1% HCl, Mayer's reagent, Wagner's reagent, Ferric chloride solution (FeCl₃), 10% lead acetate solution, distilled water, concentrated sulphuric acid (H₂SO₄), chloroform, distilled water, aqueous ammonia, acetic acid, Fehling reagent.

Ash determination: Fume cupboard, crucible, weighing balance (Adventurer Ohaus USA).

Microscopy: Chloral hydrate solution, camera lucida microscope, glass slides, immersion oil

Method

Preparation of methanol extract

One kilogram (1kg) of the leave powder was cold macerated in one liter of methanol in two maceration containers for 48 hours. At the end of the extraction, the suspension was filtered with No. 1 filter paper (Whatman, Maidstone, England) which had a white muslin cloth over the funnel that contained the filter paper. The filterate was concentrated over a hot water bath regulated at 50°C and the dried filterate was weighed and stored at 4°C for further use.

Preparation of microorganisms for experiment

For the culturing of the organisms, 10 samples were collected from laboratory working benches and toilet handles of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka by using sterile swabsticks. The organisms were cultured at 37°C for 24 hrs in a nutrient broth which was prepared according to the manufacturers directions. After the 24hrs, the microbial broth was used in subculturing for the required organisms. For use in experiments, the organisms were sub-cultured in nutrient broth and nutrient agar (Oxoid Ltd.) while diagnostic sensitivity test agar (DST) (Oxoid Ltd.) was used in antibiotic sensitivity testing.

Phytochemical analysis

The following phytochemical analysis were conducted according to the description on Trease and Evans (2009) and Akinjogunla, *et al.*, (2010).

Sample culturing

For the culturing of the organisms, 10 samples were collected from laboratory working benches and toilet handles of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka by using sterile swabsticks. The organisms were cultured at 37°C for 24 hrs in a nutrient broth which was prepared according to the manufacturers directions. After the 24hrs, the microbial broth was used in subculturing for the required organisms.

CULTIVATION AND ISOLATION OF DESIRED MICROORGANISMS

Cultivation of *Salmonella typhi*

The glass wares used were sterilized using the hot oven at 150°C for 1 hr; the working slab was also disinfected with sodium hypochlorite. Then the required amount of *Salmonella*

Shigella Agar (SSA) was prepared according to the manufacturer's instructions and transferred to the sterile petri dishes under aseptic conditions after which it was allowed to cool. The agar plates were streaked with the cultured broth using a sterile swab stick and incubated upside down in an incubator at 37°C for 24hours.

Cultivation of *Escherichia coli*

The glass wares used were sterilized using the hot oven at 150°C for 1 hr; the working slab was also disinfected with sodium hypochlorite. Then the required amount of MacConkey Agar was prepared according to the manufacturer's instructions and transferred to the sterile petri dishes under aseptic conditions after which it was allowed to cool. The agar plates were streaked with the cultured broth using a sterile swab stick and incubated upside down in an incubator at 37°C for 24hours.

Cultivation of *Staphylococcus aureus*

The glass wares used were sterilized using the hot oven at 150°C for 1 hr; the working slab was also disinfected with sodium hypochlorite. Then the required amount of Mannitol salt Agar was prepared according to the manufacturer's instructions and transferred to the sterile petri dishes under aseptic conditions after which it was allowed to cool. The agar plates were streaked with the cultured broth using a sterile swab stick and incubated upside down in an incubator at 37°C for 24hours.

Cultivation of *Pseudomonas aeruginosa*

The glass wares used were sterilized using the hot oven at 150°C for 1 hr; the working slab was also disinfected with sodium hypochlorite. Then the required amount of Cetrimide Agar was prepared according to the manufacturer's instructions and transferred to the sterile petri dishes under aseptic conditions after which it was allowed to cool. The agar plates were streaked with the cultured broth using a sterile swab stick and incubated upside down in an incubator at 37°C for 24hours.

Isolation and Identification of *Salmonella typhi*

Presumptive test

The presence of black colonies on the SSA is indicative of *salmonella typhi* and this was used for further analysis.

Gram stain

The loop was sterilized by flaming to red hot and cooling. From the liquid culture, a loopful of culture was collected and fairly heavy smears were made in a circular manner on the slide. The slide was fixed by passing over Bunsen flame two or three times. The slide was flooded for 30 seconds with crystal violet and washed in tap water and excess water was drained off. The slide was flooded with iodine solution (mordant) for 1 minute. The slide was decolorized by adding ethanol drop wise on the tilted slide until all free colour (purple) has been removed, the slide was washed with water. Safranin was applied for 3 minutes. The slide was washed with water and air dried and immersion oil was dropped on the slide and covered with a slide cover and viewed under the microscope.

Isolation and identification of *Escherichia coli***Presumptive test**

The presence of reddish pink colonies on the MacConkey agar was presumptive for *E.coli*

Catalase test

The working slab was disinfected with sodium hypochlorite solution. A 3ml volume of hydrogen peroxide was poured into sterile test tubes, using a glass rod, a pink colony from the cultured plate was picked and transferred into the solution of hydrogen peroxide and the solution was checked for the presence of immediate bubbling.

Isolation and Identification of *Staphylococcus aureus***Presumptive test**

The presence of yellow colonies on Mannitol Salt Agar (MSA) was presumptive for *Staphylococcus aureus*.

Gram stain

The procedure for the gram stain that was used for *Salmonella typhi* was also used for the *Staphylococcus aureus*. The gram positive yellow colonies were further subjected to the following test for *Staphylococcus aureus*.

Coagulase test

The working slab was made aseptic by cleaning with disinfectant. Few drops of physiological saline were dropped on a clean slide and a colony of the organism was emulsified on the slide

using a sterile wire loop. A drop of plasma was dropped on the smear and mixed using an applicator stick, then clumping was checked for within 10 seconds.

Catalase test

The procedure for the catalase test that was used for *E. coli* was also used for *Staphylococcus aureus*.

Isolation and identification of *Pseudomonas aeruginosa*

Presumptive test

The presence of green colonies on Cetrimide agar was presumptive for *Pseudomonas aeruginosa*.

Oxidase test

Two drops of oxidase reagent was placed on a piece of filter paper on a slide. With the edge of another slide, the green colonies were collected and smeared across the emulsified filter paper. The filter paper was observed and the results were recorded.

Isolates purification and storage

The isolated microorganisms were purified by preparing the required volume of their respective selective nutrient media according to the manufacturer's description and allowed to solidify. Then a single colony was picked from the plate and was emulsified in sterile water. This was streaked on the agar plates from side to side in a systematic fashion without digging into the surface. It was then incubated at 37°C for 24hours.

Storage of isolates: Double strength Muller Hinghton agar was prepared into a bijou bottle to form an agar slant. Then the isolates were streaked on the agar in the properly labeled bottles and then incubated at 37°C for 24hours after which they were stored at 4°C for further use.

Fungi

Two fungi were used for the studies; *Candida albicans* and *Aspergillus niger*. The fungi were obtained from already isolated micro organisms in the department of pharmaceutical microbiology, Nnamdi Azikiwe University, Awka.

Antimicrobial activity of the plant extract

Determination of the minimum inhibitory concentration (MIC)

MIC against bacteria

The concentrated plant extracts were reconstituted in 10% DMSO in concentration of 200 mg/mL. A two fold serial dilution was made to obtain concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL and 1.563 mg/mL. The MIC determination was done as described below; 19 mL of the molten Mueller hington agar was mixed with 1mL of the plant extract dilutions in sterile petri dishes. It was allowed to solidify and a sterile wire loop was used to streak a loopful of the inoculum on the surface of the dried agar. The plates were then incubated in an inverted position at 37°C for 24 hours, after which the plates were observed for presence of colonies. These were done in duplicates of the plant extract against *E.coli*, *S. aureus*, *P. aeruginosa*, *S.typhi*.

Antibacterial positive Control

Ciprofloxacin was used as an antibacterial positive control against the four (4) bacterial. The IZD of ciprofloxacin was determined and the MIC of ciprofloxacin was extrapolated from a graph of IZD² against concentration C.

MIC against fungi

The concentrated plant extracts were reconstituted in 10% DMSO in concentration of 200 mg/mL. A two fold serial dilution was made to obtain concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL and 1.563 mg/mL The MIC determination was done as described below; 19 mL of the molten Sabouraud's Dextrose Agar (SDA) was mixed with 1mL of the plant extract dilutions in sterile petri dishes. It was allowed to solidify and a sterile wire loop was used to streak a loopful of the inoculum on the surface of the dried agar. The plates were then incubated in an inverted position at 25° C for 48 hours, after which the plates were observed for presence of colonies.

Antifungal Control

Ketoconazole was used as an antifungal positive control against the two (2) fungi. The IZD of ketoconazole was determined with 3 concentrations and the MIC was extrapolated from the graph of IZD² against concentration

Determination of Minimum Biocidal Concentration (MBC)

The MIC plates showing no growth of visible colonies were used to determine the MBC. Disc were cut out from each plate with no visible growth and transferred into a test tube containing fresh medium of double strength nutrient broth. The tubes were incubated at 37°C for 48 hours after which the test tubes will be observed for the presence of turbidity.

Ash value determination

A crucible was heated, cooled and weighed and 2g of the powdered plant leaves was added to the crucible in an even layer. This was heated gently in a fume compound until all the moisture has been driven out and the material was completely charred. The flame was gradually increased until (450°C) until the residue became white indicating that it is free from carbon. The crucible was cooled and weighed. The process of heating and cooling was continued until a constant weight was achieved. The final weight of the crucible containing the ash was taken and the ash value for the plant was calculated.

Evaluation of epidermal characters

0.5 g of the powdered plant material was added to a beaker and a sufficient quantity chloral hydrate of added to the plant to cover the material. This was left for 24 hrs to enable the total clearing of the of the plant material. After 24hrs, with the help of a paintbrush, the resulting solution was placed on a clean slide. 2 drops of glycerol were added on the slide and covered with a coverslip. The slide was mounted on the microscope and viewed accordingly with a magnification of 100x.

RESULT AND DISCUSSION**Percentage yield of extract**

$$\% \text{ yield} = \frac{\text{Mass of extract}}{\text{Mass of crude plant}} \times 100$$

$$\% \text{ yield} = \frac{23}{1000} \times 100 = 2.3\%$$

Ash value determination (Total ash value)

Weight of crucible = 35.22 g

Weight of crucible + burnt extract = 35.43 g

Weight of air dried sample = 2 g

Weight of ash = 35.43 - 35.22 = 0.21

$$\text{Percentage ash value} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

$$\text{Percentage ash value} = \frac{0.2}{2} \times 100 = 2.3 \%$$

Phytochemical analysis

The result of the phytochemical evaluation showed that the plant was abundantly rich in Alkaloids, Flavonoids and Saponin while Anthraquinones, cardiac glycosides, terpenoids and steroids were moderately present. Tannins, phlobatanins and reducing sugars were absent.

Table 1: Phytochemical screening of the plant extract.

Phytochemical test	Result
Alkaloid	+++
Flavonoid	+++
Tannin	-
Anthraquinone	++
Saponin	+++
Glycoside	++
Phlobatanin	-
Terpenoids	++
Steroids	++
Reducing sugar	-

Key : +++= abundantly present, ++= moderately present, - = absent.

Cultivation and isolation of desired organisms

Cultivation and isolation of *Staphylococcus aureus*

Yellow colonies of *Staphylococcus aureus* were observed on the Mannitol salt agar (MSA). The colonies were gram positive and catalase and coagulase positive. The results are seen in the table below.

Table 2: Identification and isolation of *Staphylococcus aureus*.

IDENTIFICATION TEST	RESULT
Colour of colony on MSA	Yellow colonies
Gram staining	Positive
Catalase test	Positive
Coagulase test	Positive

Cultivation and isolation of *Escherichia coli*

Reddish pink colonies of *Escherichia coli* were observed on the MacConkey agar. The colonies were gram negative and catalase positive. The results of the isolation and identification of *Escherichia coli* can be seen in the table below:

Table 3: Identification and isolation of *Escherichia coli*.

IDENTIFICATION	RESULT
Colour of colony on MacConkey agar	Reddish pink colonies
Gram staining	Negative
Catalase test	Positive

Cultivation and isolation of *Salmonella typhi*

Black colonies of *Salmonella typhi* were seen on the Salmonella Shigella Agar (SSA). The gram staining was negative. The results of the isolation and identification can be seen in the table below.

Table 4: Identification and Isolation of *Salmonella typhi*.

Identification Tests	Results
Colour of colony of SSA	Black
Gram staining	Negative

Cultivation and Isolation of *Pseudomonas aeruginosa*

Green colonies of *pseudomonas aeruginosa* were observed on the cetrimide agar. The gram staining was negative and the colonies were oxidase positive. The results are shown in the table below.

Table 5: Identification and isolation of *Pseudomonas aeruginosa*.

Identification Tests	Result
Colour of colonies on cetrimide agar	Green colonies
Gram staining	Negative
Oxidase test	Positive

Antimicrobial Studies

Minimum Inhibitory Concentration (MIC) --Bacteria

The result of the MIC determination of the plant extract against the selected organisms were as follows. For *Bacillus subtilis*, the MIC was 25 mg/ml. The MIC of *Pseudomonas aeruginosa* was 1.5625 mg/ml. The MIC of *Staphylococcus aureus* was 12.5 mg/ml. The plant extract was not active against *Escherichia coli* in the concentrations used.

Antibacterial Positive Control

For the positive, the IZD of ciprofloxacin against the selected organisms were determined with three concentrations. A graph of IZD^2 was plotted against concentration. The intercept on the concentration axis represents the MIC.

Table 6: Inhibition Zone Diameter of the Antibiotic Control Against the Bacteria.

Conc ug/ml	Inhibition Zone Diameter (IZD) (mm)			
	S. aureus	E. coli	S. typhi	P. aeruginosa
50	8	6	8	3
25	6	4	5	1
12.5	3	2	3	0.5

The graphs of the different IZD^2 against concentration were plotted accordingly.

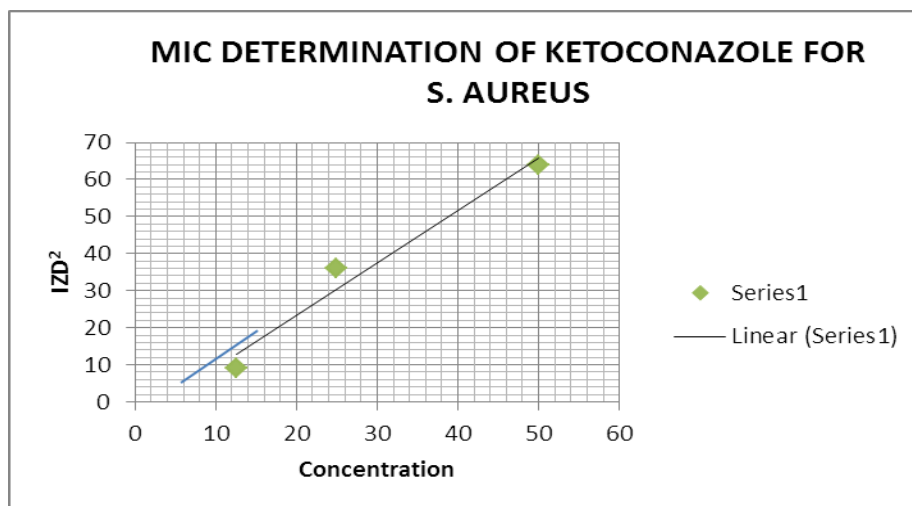


Figure 1: MIC DETERMINATION OF KETOCONAZOLE FOR S. AUREUS.

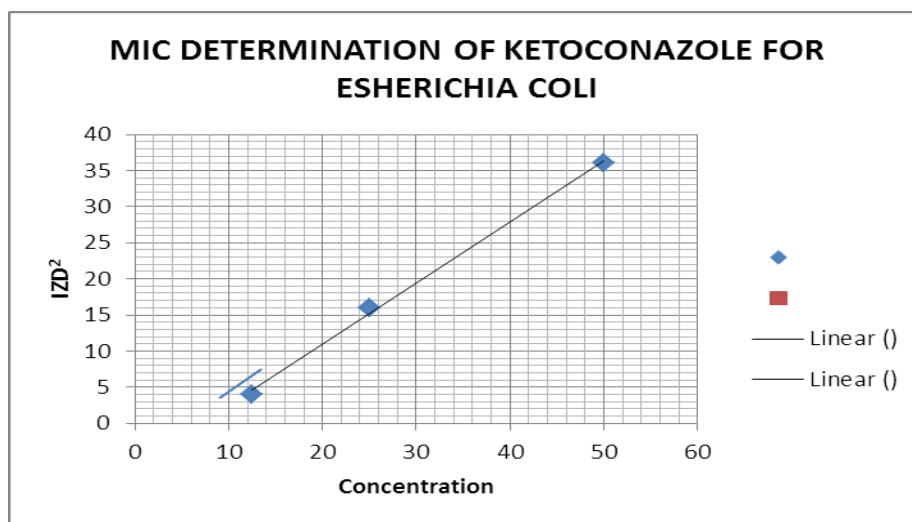


Figure 2: MIC DETERMINATION OF KETOCONAZOLE FOR ESHERICHIA COLI.

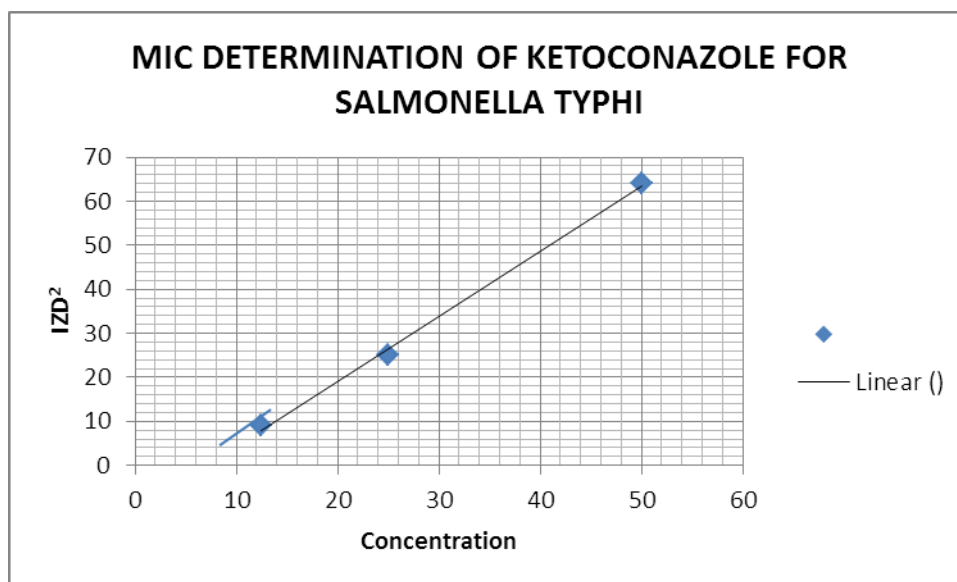


Figure 3: MIC DETERMINATION OF KETOCONAZOLE FOR SALMONELLA TYPHI.

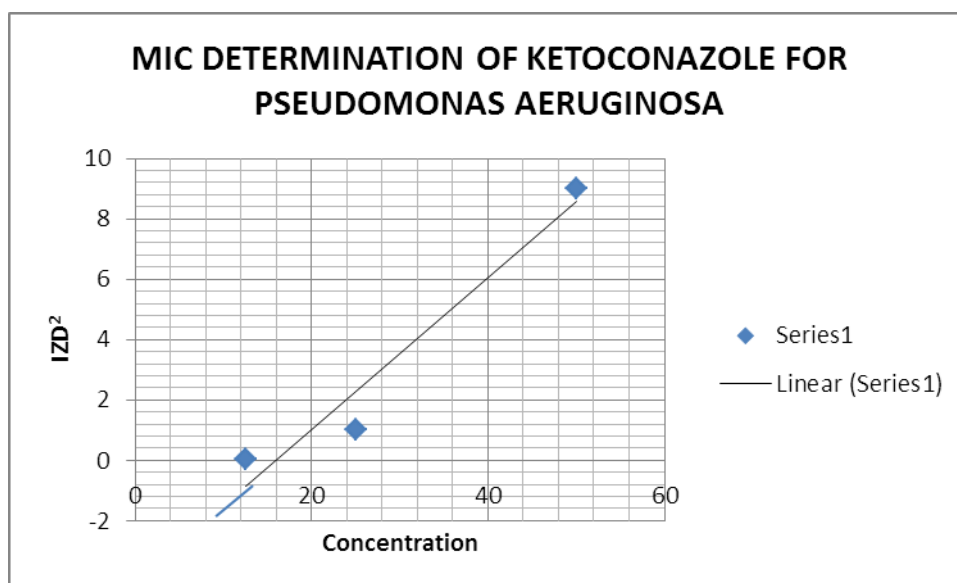


Figure 4: MIC DETERMINATION OF KETOCONAZOLE FOR PSEUDOMONAS AERUGINOSA.

The MIC of the control of the selected bacteria were extrapolated from the graph and tabulated

Organisms	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
MIC (ug/mL)	4.0	8.0	8.0	8.0

Minimum inhibitory concentration (MIC) -- Fungi

The MIC of the plant extract on *Candida albicans* was 50 mg/mL. The plant extract was not active against *Aspergillus niger*.

Antifungal positive Control

For the positive control, the IZD of ketoconazole against the selected organisms were determined with three concentrations. A graph of IZD^2 was plotted against concentration. The intercept on the concentration axis represents the MIC.

Table 7: Inhibition zone diameter of the antifungal control against the fungi.

Conc ug/ml	Inhibition Zone Diameter (IZD) (mm)	
	Candida albicans	Aspergillus niger
2.0	6	4
1.0	4	3
0.5	3	1

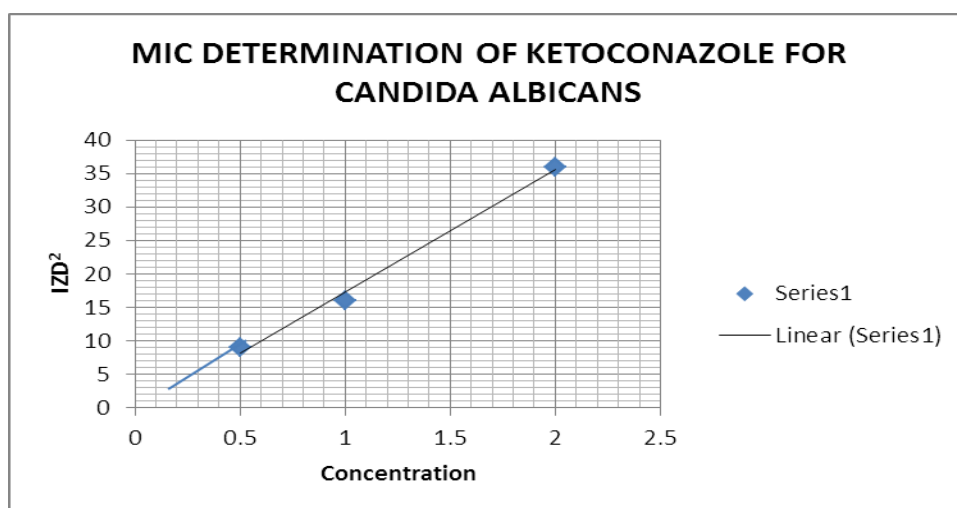


Figure 5: MIC DETERMINATION OF KETOCONAZOLE FOR CANDIDA ALBICANS.

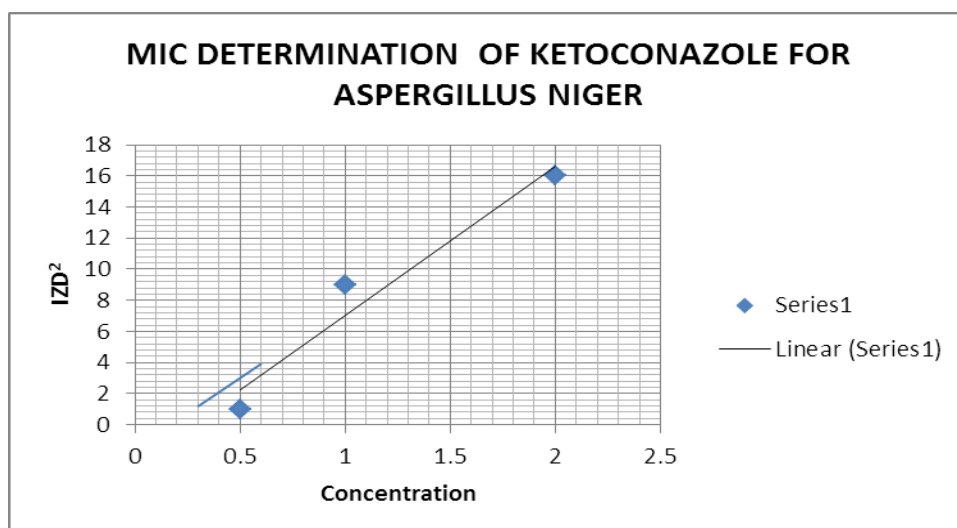


Figure 6: MIC DETERMINATION OF KETOCONAZOLE FOR ASPERGILLUS NIGER.

MIC of the control on the selected fungi were extrapolated from the graph and tabulated

Organisms	<i>C. albicans</i>	<i>A. niger</i>
MIC (ug/mL)	0.1	0.25

Minimum Biocidal Concentration--Bacteria

The Minimum Biocidal Concentration(MBC) of the extract against the selected organisms were determined and the result is as follows.

For *Bacillus subtilis*, the minmum MBC was 50 mg/mL. The MBC of *Pseudomonas aureginosa* was 6.25 mg/mL. The MBC of *Staphylococcus aureus* was 25 mg/ml.

Minimum Biocidal Concentration Fungi

The MBC of *Candida albicans* was 50 mg/mL, same with the Minimum Inhibitory Concentration (MIC).

The MBC was not determined for the organisms that did not show activity in the MIC.

The following results are represented into the table below.

Table 8: MIC of the Plant Extract Against the Organisms.

Conc (mg/ml)	<i>B. subtilis</i>	<i>E.coli</i>	<i>P. aureginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>A. niger</i>
200	–	+	–	–	–	+
100	–	+	–	–	–	+
50	–	+	–	–	+	+
25	+	+	–	–	+	+
12.5	+	+	–	+	+	+
6.25	+	+	–	+	+	+
3.125	+	+	–	+	+	+
1.5625	+	+	+	+	+	+

Key + = Growth; - = No growth

Table 9: MIC and MBC of the Selected Organisms.

Organisms	MIC (mg/ ml)	MBC (mg/ml)
<i>Salmonella typhi</i>	25	50
<i>Escherichia coli</i>	*	*
<i>Pseudomonas aureginosa</i>	1.5625	6.25
<i>Staphylococcus aureus</i>	12.5	25
<i>Candida albicans</i>	50	50
<i>Aspergillus niger</i>	*	*

Evaluation of epidermal characters

Evaluation of the epidermal characters of the whole leaf of *Luffa cylindrica*, with camera lucida and a Pharmacognostic atlas, the following features were revealed: Glandular trichomes, lamina surfaces, epidermal surface, palisade cells, calcium oxalate crystals, parenchyma and Sclerenchymatous layer, sieve tubes, endodermis with underlying pericyclic fibers as represented in Figures 7 – 12.

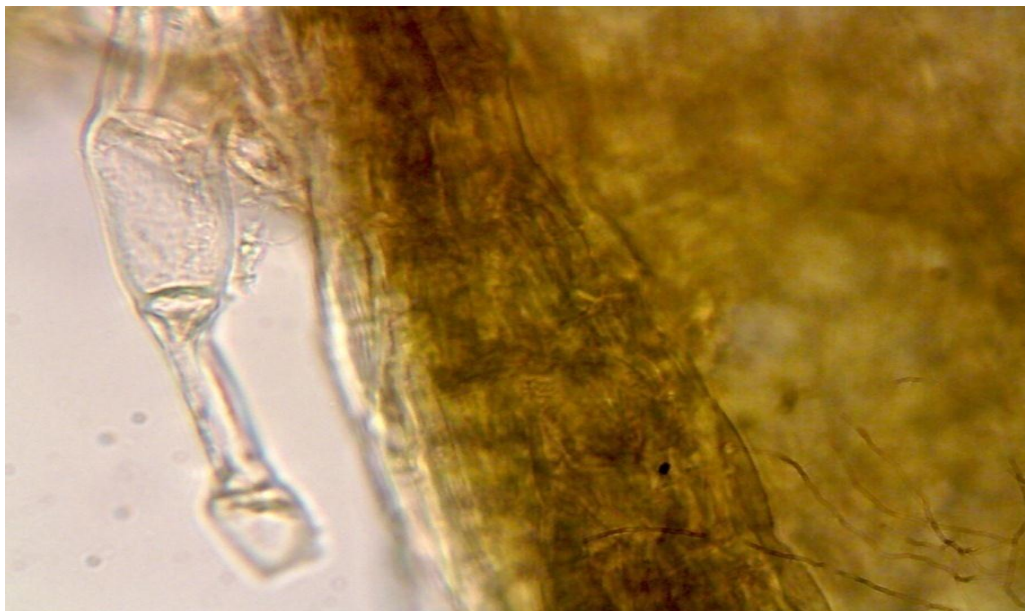


Figure 7: Microscopic view Glandular trichomes.

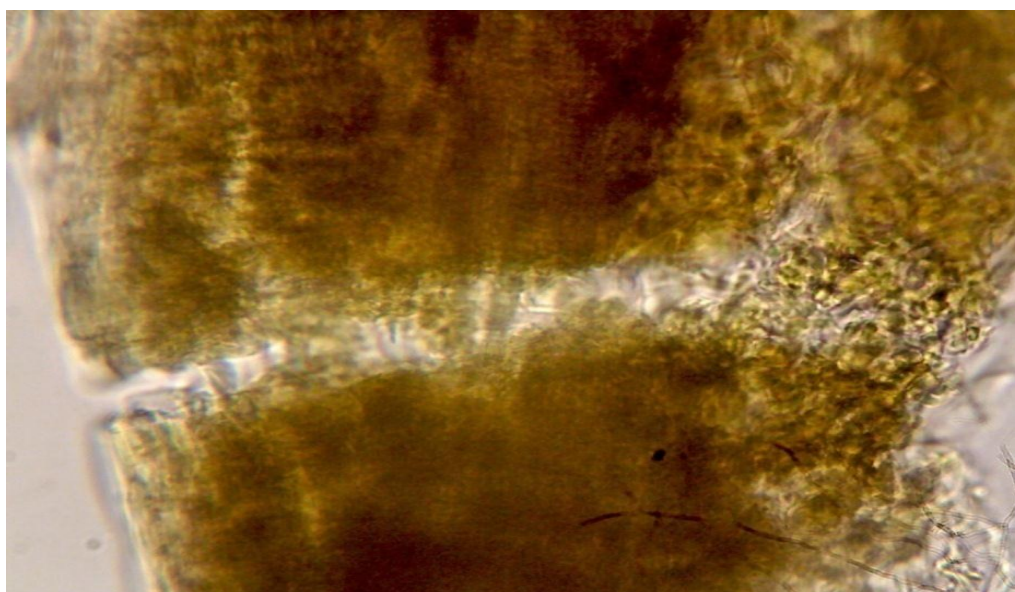


Figure 8: Microscopic view of part of the lamina surface in sectional view.

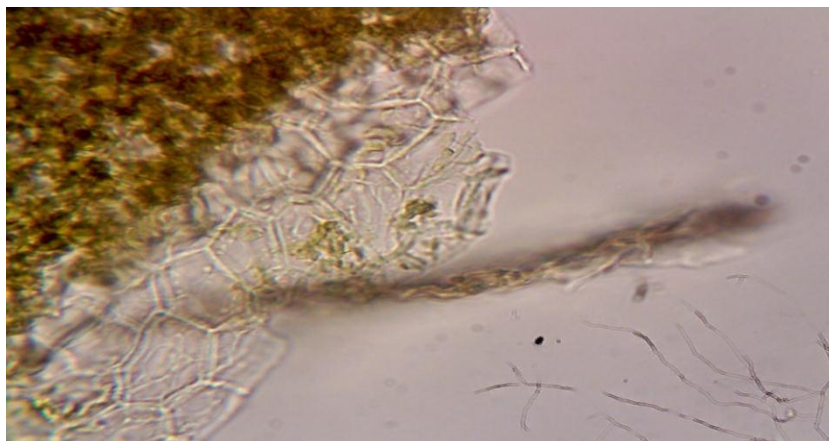


Figure 9: Microscopic view of upper epidermis in surface view with crystals of calcium oxalate and stomata.



Figure 10: Microscopic view of lacticiferous tissue with associated vessels and parenchyma.

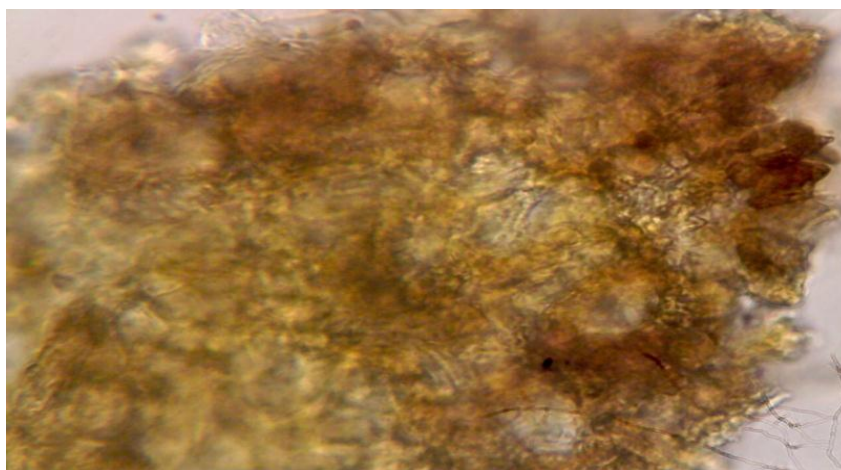


Figure 11: Microscopic view of sclerenchymatous layer of the pericarp in surface from above.



Figure 12: Microscopic view of parts of a vein in longitudinal section showing palisade cells.

DISCUSSION

The phytochemical active compounds of methanol extracts of *Luffa cylindrica* were quantitatively analyzed and the results are presented in a table 1. The phytochemicals; alkaloid, saponin and flavonoid were found to be abundant in the plant extract. Anthraquinone, glycoside, terpenoids and steroids were moderately present while tannin, phlobatanin and reducing sugar were absent.

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases (Mir *et al.*, 2013). Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compound with antimicrobial activity, with possibly new modes of action (Dhiman *et al.*, 2012).

The percentage ash value of the leave powder was determined and found to be 2.3%, which is low. This showed that the plant has a high percentage of volatile constituents and phytochemicals which evaporated on heating. As burning destroys all but the mineral components, the mass of the residue provides an indication of the contribution that minerals made to the original material (Evers *et al.*, 1999). This means that the plant sample contains a low percentage by mass of mineral components.

The antimicrobial activity of methanol extracts of the leaves of *Luffa cylindrical* against the environmentally isolated organisms are shown in a table 8. The results which were in Minimum Inhibitory Concentration (mg/mL) of the the methanol leaf extract against indicator bacteria and fungi. *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Candida albicans* were susceptible to the methanol leaf extract of *L. cylindrical*, with the exception of *Escherichia coli* and *Aspergillus niger* which were resistant to the methanol extract. *Pseudomonas aeruginosa* was the most sensitive bacterium to the plant extract with MIC of 1.56 mg/mL while *Candida albicans* was the most sensitive fungus to the extract with MIC of 50 mg/mL.

The methanol leaf extract of *Luffa cylindrical* had activity against the environmentally isolated microorganisms. The MIC of the controls used (ciprofloxacin and ketoconazole) were extrapolated from a graph of IZD² against concentrations.

For the antibacterial control, it was observed that the organisms were sensitive to the control (Ciprofloxacin). The control was active against *S. aureus* which was resistant to the plant test extract. The MIC of ciprofloxacin against *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa* were 4.0 mcg, 8.0 mcg, 8.0 mcg and 8.0 mcg respectively. For the antifungal control, it was also observed that the organisms were sensitive to the control (Ketoconazole). The control was active against *A. niger* which was resistant to the plant test extract. The MIC of ketoconazole against *A. niger* and *C. albicans* were 0.25 mcg and 0.1 mcg respectively.

Epidermal characters of the leaf showed the presence of the specific cellular structures like the parenchyma, crystals of calcium oxalates, epidermal hairs (glandular trichomes) and stomata.

CONCLUSION

The methanol leaf extract of *luffa cylindrical* demonstrated anti microbial activities against *S. aureus*, *S. typhi*, *P. aeruginosa* and *C. albicans* which were environmentally isolated. Some organisms like *E. coli* and *A. niger* were resistant to the methanol extract of *luffa cylindrical*. Therefore, from the results obtained, *luffa cylindrical* possesses antimicrobial activity against some environmentally isolated microorganisms.

Recommendation

I recommend that the plant *Luffa cylindrical* should be standardized, validated and used to treat infections caused by *Pseudomonas aeruginosa*. This is because it had profound activity against the organism even at the lowest concentration.

I also recommend that the plant *Luffa cylindrical* should be formulated into antimicrobial hand sanitizers as it was active against most of the environmentally isolated microorganisms which predisposes one to infections.

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