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Preliminary Phytochemical Investigations and Evaluation of Anti-Microbial Activity of Methanol Extract of the leaves of *Morinda Lucida* Benth (*Rubiaceae*)

Chinedu Fredrick Anowi[1*], TU Onyekaba[2], Favour Chidimma Ezekwibe[1], Emmanuel Ezeokafor[3]
[1]*Dept of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.*
[2]*Dept of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University, Abraka, Nigeria.*
[3]*Dept of Physiology, Faculty of Medical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.*

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ABSTRACT

Purpose: *the leaves of Morinda Lucida Benth Family Rubiaceae were claimed to have antimicrobial properties. The leaves were used to treat wounds, running stomach, as well as fever in Ogidi, Idemili North Local Government area of Anambra State, Nigeria. This investigation was carried out to ascertain the veracity of the claim.*

Methodology: *The leaves were collected and dried at ambient temperature and pulverized. 500gm of the powdered drug was extracted with 1000ml of methanol using the cold maceration technique for 24hours with occasional shaking. This was filtered and the process repeated using the marc. The combined filtrates were concentrated using rotary evaporator under reduced pressure. The preliminary phytochemical tests were carried out using standard methods. The antimicrobial activity was evaluated using agar diffusion method.*

Result: *The methanol extract of the leaves of Morinda Lucida exhibited antimicrobial property. Alkaloid, flavonoids, saponins, tannins, phenols, proteins, starch, essential oil and glycosides were found.*

Conclusion: *The claim on the use of Morinda Lucida appears to be obvious in line with the results of the investigation.*

Key words: *Morinda Lucida, agar diffusion, marc.*

INTRODUCTION

Over the past decade herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use (Inamul Haq 2004). Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations (Srinivas *et al*, 2007). Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use (in some case) and the cultural and spiritual point of view of the people of these countries (Srinivas *et al, 2007)*.

In developed countries however, after a downturn in the pace of herbal use in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited (Satyejji and lutfun, *2007)*. Worldwide spending on finding new anti-infective agents (including vaccines) was expected to increase to 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Secondly, the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. All these makes the knowledge of chemical, biological and therapeutic activities of medicinal plants used become necessary. (Fagbohun *et al*, 2010).

Before the era of Louis Pasteur (1822-1895), world renowned chemist and biologist who proved the germ theory of disease, the notion that tiny organisms could kill vastly larger ones (including human) seemed ridiculous to many people (Karanayil et al 2011; Sheo Singh and John Barrett 2006). Nowadays, it has been accepted that infectious diseases are the number one causes of death worldwide, accounting for approximately one half of all deaths in tropical countries (Iwu *et al*., 1999). In fact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents.

The use of plant parts as a source of medicine to treat infectious diseases predates history (Sunil Mishra and Singh PN 2011). Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Erdemeier et al. 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control (Majorie Murphy Cowan 1999). Herbal medicine practice plays an important role in the primary healthcare delivery system in most developing countries including Nigeria. Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. The WHO estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health needs (WHO Traditional Medicine Strategy, 2002). Much of the exploration and utilization of natural products as antimicrobials arise from microbial sources (Ajaiyeoba et al 1998). It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin. Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have also been a source of antibiotics. Examples of these are the bacteriostatic and antifugicidal properties of *Lichens,* the antibiotic action of allinine in *Allium sativum* (garlic), or the antimicrobial action berberines in goldenseal (*Hydrastis canadensis*). Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur. Plants based antimicrobials have enormous therapeutic potential (Ajaiyeoba EO. 2000). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Awe S and Omojasola PF 2008). They are effective, yet gentle. Many plants have tropisms to specific organs or systems in the body (Tomas-Barberan et al 1988). Phytomedicines usually have multiple effects on the body.Their actions often act beyond the symptomatic treatment of disease (Ajaiyeoba EO and Okogun JI 1996). An example of this is *Hydrastis canadensis*. *Hydrastis* not only has antimicrobial activity, but also increases blood supply to the spleen promoting optimal activity of the

***Corresponding author:**
Chinedu Fredrick Anowi
Dept of Pharmacognosy and Traditional Medicine,
Faculty of Pharmaceutical Sciences,
Nnamdi Azikiwe University, Awka, Nigeria.
**E-Mail: cromwell_pharm@yahoo.com*

spleen to release mediating compounds (Murray 1995; Abdullahi et al 2008).

However, in spite of the obvious and important contribution the herbal medicine makes to primary health care, it continues to be antagonized by majority of allopathic medical practitioners as it is considered to have no scientific basis. This work is therefore a preliminary work to prove that there is scientific evidence to the use of the root of Morinda Lucida in the treatment of diseases.

Taxonomy of *Morinda Lucida* Benth:

Morinda comprises approximately 80 species, distributed in all tropical regions of the world.

Taxon	*: Morinda Lucida Benth.*
Genus	*: Morinda*
Family	*: Rubiaceae .*
Subfamily	*: Rubioideae.*
Tribe	*: Morindeae.*
Nomenclature number	*:* 417533
Common names	*:* Brimstonetree.
Igbo name	*:* Akpakwulu nniewu

Economic importance:

Materials	*:* wood
Medicines	*:* Folklore

Description of the Plant:

Morinda Lucida is an Evergreen shrub or small to medium-sized tree up to 18-25m tall, with bole and branches often crooked, the bark is smooth to roughly scaly, grey to brown, often with some district purple layers. Leaves are opposite, simple and entire; stipules ovate or triangular,1-7mm long, petiole up to 1.5 cm long, blade elliptical,6-18 cm ×2-9 cm, base rounded to cuneate, apex is acute to acuminate, shiny above, sometimes finely pubescent when young. The flowers are bisexual, regular and fragrant. The bear aggregate or multiple fruits that can be fleshy or dry. The fruit are drupe, several together arranged into an almost succulent syncarp 1-2.5cm in diameter, soft and black when matured (Smith et al 1995).

Distributional Range:

Native	*:* Africa
Northeast Tropical Africa	**:** Sudan
East Tropical Africa	**:** Tanzania; Uganda
West-Central Tropical Africa	**:** Cameroon; Congo; Equatorial Guinea –Bioko; Zaire
West Tropical Africa	**:** Benin; Cote D'Ivoire; Ghana; Liberia; Nigeria; Senegal; Sierra Leone; Togo
South Tropical Africa	**:** Angola; Zambia

Most species of this genus originate in the area of Borneo, New Guinea, Northern Australia, and New Caledonia.

Ecology:

Molinda Lucida grows in grassland, exposed hillsides, thickets, forests, often on termite mounds, sometimes in areas which are regularly flooded, from sea-level up to 1300 m altitude.

Management:

The useful parts of Morinda Lucida are mostly collected from wild plants. Only occasionally are plants grown in home gardens. Propagation is possible by seed and cuttings, but no details are known.

Ethnobotanical uses of *Morinda Lucida* Benth*:*

According to the revised literature, in West Africa *Morinda Lucida* Benth *is an important plant in Traditional* Medicine. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during child birth (Thompson PE and Werbel LM 1972). The plant is also employed in cases of diabetes, hypertension, cerebral congestion, dysentery, stomach ulcers, leprosy, and gonorrhea.

In Cote d'Ivoire a bark or leaf decoction is applied against jaundice itch and ringworm.

In Nigeria, (particularly the root and leaves) are used in the treatment of various illness. The root is chewed to relieve pain, fever and it promotes gastric emptying and intestinal motility. The fresh leaves are grinded and the juice obtained is applied to an inflamed area. The local palm wine extract is used for the treatment of Malaria, Diabetics and it have inhibiting effect on tumours.

Safety of *Morinda Lucida:*

From review, experts have indicated that the decoction of this multipurpose herb could be relatively safe as oral remedy if not taken beyond twenty one days.

Scientists investigated the toxicological effect of the ethanolic root extract of the plant at 50,100,200 and 300mg/kg body weight on the blood, kidney, liver function in wistar rats for twenty one days and found that the extract did not exhibit any significant effect on red blood cells, volume of whole blood (hematocrits), hemoglobin (Hb) as well as platelet distribution width.

It also caused a significant reduction in the level of blood cells, platelets, and cholesterol. Similarly, the extract at all doses led to significant increase in the body and absolute organ weights of the animals but no effect on the liver, kidney, heart, and lungs body-weight ratios.

The reduction in the levels of lipid profile (cholesterol, especially the bad cholesterol) suggest that the extract at 50, 100, 200 and 300mg/kg body weight may not predispose the animal to heart related problems but protective to the heart.

Aim and Objectives:

The aims and scopes of this study are;

1. To investigate antibacterial and antifungal activities of *Morinda Lucida Benth leaves against* human pathogens.
2. To verify the local use of the plant.

MATERIALS AND METHODS

Materials:

Chemicals and solvent:

The chemicals used for extraction processes include methanol, Dimethyl sulphoxide (DMSO), Nutrient agar and Sarbouraud dextrose agar.

The following reagents were used for the phytochemical analysis –Fehling solution A and B, Wagner's reagents(mixture of iodine and potassium iodide), Hager's reagent (saturated solution of picric acid),concentrated Sulphuric acid, naphthol solution in ethanol(Molisch reagents), picric acid, Ammonium solution, nitric acid Aluminum chloride solution.

Microorganisms:

The microorganisms used includes bacteria (both gram negative and gram positive) and fungi obtained from laboratory stock of the department of pharmaceutical Microbiology and Biotechnology, Faculty of pharmaceutical sciences, Nnamdi Azikiwe University, Awka. The organisms include bacteria (*Pseudomonas aeruginosae, staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Salmonella typhi*) and fungi (*Candida albicans and Aspergillus niger*).

Equipments and Glassware:

Autoclave, test tubes, test tube rack, syringe and needle, fliter paper, Pasteurs pipette, conical flask, weighing balance(scout prou401), incubator (Gentlab UK),Beakers, measuring cylinder, glass rod, inoculation loop, Tripod stand, water bath, reagent bottles, permanent marker and Bunsen burner.

Methods:

Collection and Identification:

The fresh leaves of *Morinda Lucida* were obtained from Ogidi in idemili North local Government Area of Anambra state and was identified by Mr. A.O Ozioko, a taxonomist with the Biosource Development and conservation program (BDCP). The plant leaves were washed thoroughly, cut into small parts and then air-dried for 2 weeks in the Pharmacognosy laboratory. They were then milled and 500g of powdered plant material was obtained.

Extraction Processes;

Cold marceration was done by placing 500g of the powdered plant- material in a closed vessel with 1000ml of Methanol and it was allowed to stand for twenty four hours with occasional agitation after which it was strained off and the marc was pressed to remove as much solution as possible and the marc was placed back into the vessel and the process repeated. The liquid so obtained was clarified by filtration using whatman filter paper No.1. The filtrates were concentrated using rotary evaporator under reduced pressure.

Phytochemical Test:

Standard screening tests was carried out on the powered Plant material. The procedure used was obtained from Trease and Evans (2002) and Harborne JB (1983)

Test for Protein:

Xanthoproteic reaction test: In 5 ml volume of the filtrate is heated with few drops of concentrated nitric acid, yellow colour that changes to orange on addition of alkali indicates the presence of protein.

Test for Carbohydrates:

0.1g of the powered leave was boiled with 2ml of distilled water and was filtered. To the filtrate, few drops of naphthol solution in ethanol (Molish reagent) were added. Concentrated sulphuric acid was then poured gently down the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrate (starch).

Test for Alkaloids

About 5g of powered leave placed in the test tube and 200ml methanol added to the tube, the mixture was heated in water bath and allowed to boil for two minutes. It was cooled and filtered. 5ml of the filtrate was tested with two drops of Wagner's reagent (solution of iodine and potassium iodide).

To another 5ml portion of the extract 2 drops of Hager's reagent (saturated picric acid solution) was added. The presence of precipitate indicates alkaloid.

Tests for Saponins:

About 20ml of water was added to 0.25g of crude extract and boiled gently in a hot water bath for 20minutes. The mixture was filtered hot and allowed to cool and the filtrate was used for the following tests:

a) Frothing test: 5ml of filtrate was diluted with 20ml of water and vigorously shaken. The test tube was observed for the presence of stable foam upon standing.
b) Emulsion test: To the frothing solution, 2 drops of olive oil was added and the content shaken vigorously and observed for the formation of emulsion.
c) Fehling's test: To 5ml of the filtrate was added 5ml of Fehlings solutions (equal parts of A and B) and the content was heated in a water bath and reddish participate which turns brick red on further heating with sulphuric acid indicates the presence of saponins.

Test for Flavonoids:

About 10ml of ethylacetate was added to 0.2g of the (crude entract) extracts and heated on a water bath for 3 minutes. The mixture was cooled, filtered and used for the following test.

a) Ammonium test: 4ml of filtrate was shaken with 1ml of dilute ammonium solution. The yellow color in the ammonical layer indicates the presence of the flavonoids.
b) Ammonium chloride solution (1% test) another 4ml portion of the filtrate was shaken with 1ml of 1% of ammonium chloride solution. They layers were allowed to separate; a yellow colour in the ammonium chloride indicates the presence of flavanoids.

Essential oil:

Whole extract solution (0.5ml) with two drops of 1M alcoholic K_2CrO_7 and 3 drops of phenolphthalein were added in a clean test tube. Soap formation shown by frothing indicated the presence of essential oil.

Phenolic group:

Alcoholic plant extract (0.5ml) was taken in a test tube. Two drops of 1M ferric chloride was added. Appearance of intense color indicated the presence of phenolic groups.

Cyanogenetic glycosides:

About 1g of powered sample was boiled with distilled water and moist sodium picture paper held inside the tube with a cork. A color change from yellow to Brick - red of the picrate paper is positive for Cyanogenetic glycosides.

Tannin:

One gram of powdered plant material was boiled with 50ml of water filtered and used for the test.

Ferric chloride test: 3ml of the filtrate and few drops of ferric chloride added.A greenish black precipitate indicates the presence of tannins.Gallitannins and Ellagitannins gives blue black colour while Condensed tannins gives brownish green precipitates.

Antimicrobial Assay:

Microorganisms: 24hours culture of five human pathogenic bacteria made up of both gram positive and gram negative bacteria were used for the in-vitro antibacterial assay. For the antifungal assay, two fungi were utilized for the studies and these were made up of *Aspergillus nigger and Candida albican*. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

Preparation of the Media: The glassware used here were thoroughly washed and then sterilized using hot air oven at 160 Oc for one hour(oven made by Gallenkamp).The test Nutrient agar, sabouraud dextrose agar (SDA) were used in the assay. The media were prepared by dispersing the weighed amount in distilled water and then were sterilized with autoclave.

Antimicrobial Agents: Ampicilin (Juhel industrial Ltd, Awka); Clotrimazole cream, 1mg/ml (Drug field, Nigeria) were included in the study as standard reference drugs(positive control).

Antimicrobial activity determination: An overnight broth culture used to obtain 0.5 Marcfarland standard of bacterium was used to seed sterile molten nutrient agar medium maintained at 45^{0}C. Sabouraud dextrose agar plate was similarly seeded with fungi. Nine holes (6mm) respectively, were bored in each of the plates (9cm, diameter) with an aseptic cork borer, when seeded plates had solidified. 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml 6.25mg/ml,3.125mg/ml and 1.5625mg/ml of extract were prepared in dimethly sulphoxide (DMSO) by double fold dilutions and with the aid of a Syringe, the wells were filled with 0.1ml (3drops) of different dilutions of the extract while the centre wells were filled with 20µg/ml and 1mg/ml of ampicilin and clotrimazole cream for bacteria and fungi respectively. The tests were carried out in triplicate for each of the concentrations. Diameters of zones of inhibition were determined after incubating plates at 37^{0}C for 24 for bacteria and at 25^{0}C for 72 hours for fungi. Dimethlsulphoxide was used as negative control (Hassan et al 2006).

RESULTS AND ANALYSIS

Table No. 1: The Result of Phytochemical Screening of *Morinda Lucida* Benth

Secondary metabolites	It presence or absent
Alkaloids	++
Tanins	+
Phenols	+++
Proteins	++
Starch	+
Flavonoids	+++
Saponin	+++
Glycosides	++
Essential oil	++

Legend: +++=High, ++=Moderate, +=Low.

Table No. 2: Antibacterial Activity of Methanol extract

Methanol Extract	Extract concentration/mean inhibitory zone diameter (mg/ml/mm)								
Bacteria	200mg/ml	100	50	25	12.5	6.25	3.125	1.562	Ampicilin 20ug/ml
S. aureus	10	8	6	4	2	+	+	+	6
E. coil	+	+	+	+	+	+	+	+	16
B. subtilis	6	4	2	1	+	+	+	+	10
P.aeruginosa	4	2	+	+	+	+	+	+	6
S.typhi	6	5	4	2	+	+	+	+	6

Table No. 3: Antifungal properties of methanol extract

Fungi	200	100	50	25	12.5	6.25	3.125	1.562	CLT 1mg/ml
A. niger	6	4	2	1	+	+	+	+	1
C.albican	6	4	2	1	+	+	+	+	16

key: + means absences of antimicrobial activity.

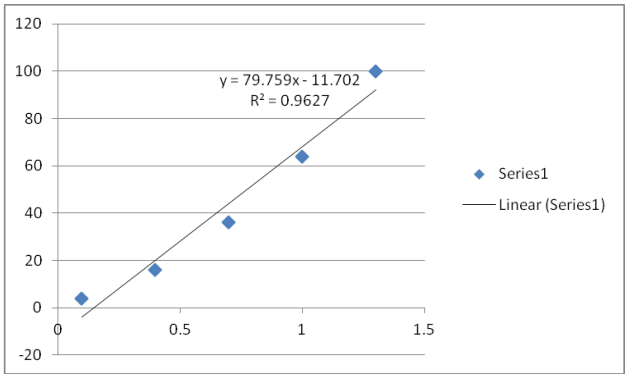
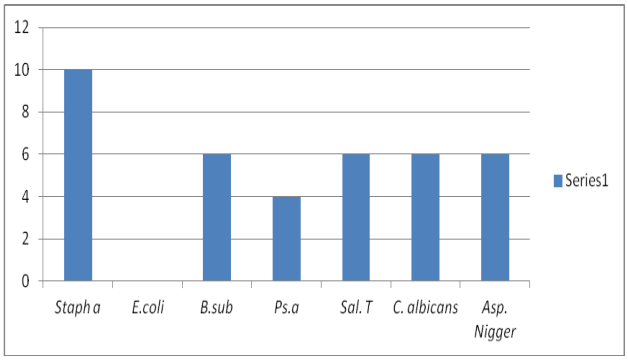


Fig. 1: MIC of the extract was determined by agar diffusion method by plotting IZD² against log concentration

Table No. 4: Minimum Inhibitory Concentration Of Themethanol Extract

Bacteria	Methanol extract
S.aureus	12.5
E.coil	-
B.subtilis	25
P.aeruginosa	100
S.typhi	25

Key: Means No Inhibition



Y axis = IZDmm ,X AXIS= organisms.

Fig. 2: Proportion indices of methanol at 200mg/ml

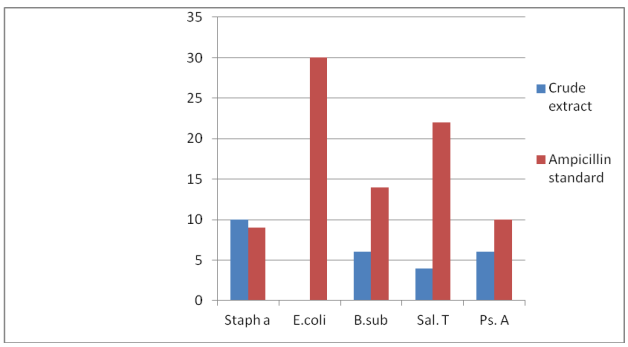


Fig. 3: Antibacterial Activity of Methanol Extract at 200mg/ml Compared with the Standard at 20ug/ml.

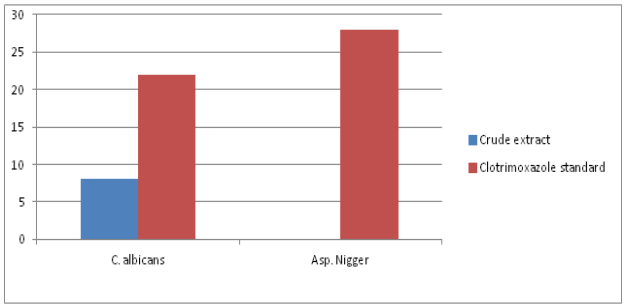


Fig. 4: Antifungal Activity of Methanol Extract at 200mg/ml Compared with Standard at 1mg/ml

DISCUSSION/CONCLUSION

The result of phytochemical screening showed abundant of saponin, flavonoid and phenols and moderate availability of Alkaloids, proteins, glycosides and essential oil; low availability of Tannins and starch. Some of this secondary metabolite has been reported to have activity against microorganisms. Flavonoid, phenolics, Alkaloids, and essential oil have been shown to have activity (Majorie, 1999). Flavonoids have been shown to have the following biological activities, antioxidants, antimutagenic antiturmour and free radical scavengers. The presence of alkaloid, saponin, Glycosides, and essentials oil are normally present in the plant of these family. Alkaloid have antidiarrheal effects and also it may be useful against HIV infections associated with AIDS while phenols have antipyretic , fungistatic and bacteriostatic .Its activity increases as the number of hydroxylation increases (e.g: Eugenol).

As shown above ,the activity of the crude extract was well *demonstrated against S.aureus were all the dilutions except* 1.5625mg/ml exerted some effect ranging from 8 to 1 mm for concentration 200 to 3.125.It also exerted some effect against *S.typhi.*

The activity of the Methanol extract was well demonstrated against S. aureus with 1ZD of 10mm to 2mm for concentration 200mg/ml to 12.5mg/ml. It also exerted some effect against certain other organism like B. subtilis and S. typhi with 1ZD of 6mm to 1mm and 6mm to 2mm for concentration ranging from 200mg/ml and 25mg/ml. It has very little effect against P. aeruginosa for only concentration of 200mg/ml and 100mg/ml with 1ZD of 4mm and 2mm. The extract has no effect against E. coli for all the concentrations.

For the fungi, it demonstrated some effect against C. albicans and A.nigger with the same 1ZD of 6mm to 1mm for concentrations of 200mg/ml to 25mg.

CONCLUSION AND RECOMMENDATIONS

The extract demonstrated activities against certain bacteria and fungi. From the MIC Methanol extract exhibited activity. Since the root extract is more often used locally in Nigeria, it is yet to be confirmed it has activity. The active principle in the plant is very suggestive of good antibacterial and antifungal drugs, therefore this plant can served as a lead in the development of a potent antibiotic since its safety is assured from revised literature.

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