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PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF *MORINGA oleifera*.

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ABSTRACT

The leaves of Moringa oleifera were subjected to ethanol extraction. The extract was fractioned and patitioned using different organic solvents in order of increasing polarity. Standard methods were used for both extraction and phytochemical screening. Modified agar well diffusion method was adopted for screening the antimicrobial activities of the leaf extracts. The test was carried out in triplicates. The extract was phytochemically screened and revealed the presence of Flavonoid, Alkaloid, Tannins, Steroid, Saponins, Cardic glycosides, Terpenoids, Anthraquinone. The chloroform extract at 100 mg / ml concentration showed highest antimicrobial activity of 18mm on both gram positive and gram negative organisms (S. aureus, P. mirabilis and P. vulgaris) and 17mm on P. auriginosa, E. coli and K. pneumoniae. However it had 16mm on S. epidermidis and 14mm on C.albicans. The aqueous extract had strong antimicrobial activity (17mm, 14mm, 14mm, 14mm, 13mm, 13mm, 13mm and 12mm, on S.aureus, P.auriginosa, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, S.epidermidis, and C.albicans respectively). Ethyl acetate extract showed moderate antimicrobial activity of 13mm, 12mm, 15mm, 13mm, 14mm, 13mm, 13mm and 12mm on S. aureus, S.epidermidis, P.auriginosa, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris and C.albicans respectively. n-hexane extract had the least antimicrobial activity of 14mm, 12mm, 14mm, 14mm, 13mm, 12mm, 12mm and 12.5mm on S.aureus, S.epidermidis, P.auriginosa, E. coli, K.pneumoniae, P. mirabilis, P. vulgaris and C.albicans respectively. The 50mg/ml and 25mg/ml extract's concentrations had lesser diameter zones of inhibitions in decreasing order of the decreasing concentrations.The extracts were subjected to MIC evaluation and the findings of the chloroform extract showed that S. aureus was the most sensitive; followed by E. coli, K. pneumoniae, followed by P. auriginosa, P. mirabilis, P. vulgaris, S. epidermidis and C.albicans. The MBC showed no value. This study proved that the leaves of M. oleifera contain some active principles with antimicrobial activity and could enhance its use in preventing and curing diseases, when purified to the appropriate pharmacological level.

Key words: M. oleifera, Phytochemical, Antimicrobial, Infectious isolates.

INTRODUCTION

Moringa oleifera Lam. (Moringaceae) is an evergreen tree, and is the most broadly cultivated species of the monogeneric family, the Moringaceae, that is native to India, Pakistan, Bangladesh and Afghanistan and is widespread all through the tropical and subtropical areas. This fast-

growing tree (also identified as the horseradish tree, drumstick tree, miracle tree), was used by the ancient Romans, Greeks and Egyptians. It is now extensively planted and has grown naturally in many locations in the tropics. *Moringa oleifera* is a medicinal plant. Sofowora, (2008) in his book upheld that WHO (2008) described medicinal plant as any plant which in one or more of its organs harbors substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs . Finally, what is the view of WHO concerning medicinal plant? Sofowora, (2008) stated that WHO consultative group that formulated the above definition reiterated that such a assertion enables differentiation to be made between medicinal plants whose therapeutic properties and constituents have been established scientifically, and plants that are regarded as medicinal, though have not yet been subjected to thorough scientific study.. Such a plant is commonly used in treating or preventing specific ailments or diseases and plays valuable role in health care. *Moringa oleifera* is an economically essential tree and vegetable, and initial proof suggests that it has reasonable antioxidant and anti-inflammatory properties. It contains compounds structurally comparable to Sulforaphane and seems to be protective when orally ingested (Fahey, 2005). It is a perennial supple wood tree with timber of low quality, yet for centuries, it has been promoted for traditional medicinal and industrial uses. It is an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands and it's importance is becoming appreciated globally. All parts of the *Moringa* tree are edible and have been eaten by humans for a long time. Fuglie, (1999) stated that all parts of *Moringa* are used for therapeutic purposes and some of these parts are also used for domestic and industrial purposes. Tsaknis *et al.* (1999) and Oliveira *et al.* (1999) added that the plant has been considered a multipurpose plant that could be used as a medicinal plant, vegetable, animal fodder, and a source of vegetable oil, which is used in condiments and the manufacture of perfumes, cosmetics, and hair care products. In the West, one of the best known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water. (Fahey, 2005). Nutritionally, *Moringa* trees have been used to combat malnutrition, especially among infants and nursing mothers. Trees for Life, Church World Service and Educational Concerns for Hunger Organization are three non-governmental organizations in particular, that have advocated *Moringa* as "natural nutrition for the tropics." The Trees for Life organization characterized *Moringa* many years ago thus: "that "ounce-for-ounce, *Moringa* leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and that the protein quality of *Moringa* leaves rivals that of milk and eggs". *Moringa* leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly maintain its nutritional value. All the organs of this plant have been traditionally used for medicinal purposes as an antioxidant, anti-inflammatory and anti-cancer agent (Il Lae, 2014). The leaves are used as antibacterial, anti-diabetic and cardiotonic agents, and

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also for the treatments of stomachaches, sprains, fever and various infections but not much control experiment ts has been provided by researchers to con vin ce the orthodox doctors (Fahey, 2005). Infection is the diseased state caused by infectious agents, which is a multi-stage process that leads to the establishment of infectious agents in a part or parts of the body of the living being. This establishment leads to varying degrees of tissue damage and undermining known as pathologies. The organisms responsible for tissue damage (infections) are the targets of laboratory investigations and when isolated are known as the isolates. For this study, 900 isolates were obtained from these pathological samples(infectious sites)and from these specimens (ear swab, throat swab, wound swab, pus aspirates, urethral swab, high vaginal swab, intra cervical swab, and catheter). These isolates (test organisms) were challenged with the leaf extracts of *M. oleifera.*The emergence of resistant strains of microorganisms has undermined the effectiveness of existing antimicrobial agents and hence renewed the interests in the discovery of new plant derived antimicrobial compounds. Therefore this study targets the phytochemical and antimicrobial potential of the leaves of *M. oleifera.*

MATERIALS AND METHODS

Collection and Preparation of the Plant Extracts

The leaves of *Moringa oleifera* were collected within the University of Port Harcourt premises. The identity of the plant was authenticated by the office of Herbarium Plant Science and Biotechnology, University of Port Harcourt. The fresh leaves were collected and washed, hot-air dried and ground to powder form.

Extraction of Plant Leaves

The plant leaves were subjected to ethanol extraction using maceration technique. The leaf powder (1kg) was suspended in 80% ethanol(10 litres) at room temperature. After 48 hours, the crude extract was then decanted and filtered using Whatman filter paper No. 1. The filterate was evaporated under vacuum, using rotary evaporator at 45^0C. The concentrated ethanol extract was dried further in a water bath, before the extract was suspended in 20% ethanol and partitioned with different organic solvents in order of increasing polarity. The percentage yield of the extract was 20%. The solid residue was stored in glass vials in a refrigerator (4^0C) from which portions were taken for further experiments.

Source of Organisms

The Medical Microbiology Department of the University of Port Harcourt Teaching Hospital provided the microbial cultures. Specimen collection, transport, and processing were carried out using conventional methods. The test organisms (microbial isolates) were identified by their cultural, microscopic and biochemical characteristics using standard methods (Willey *et al.* 2014, Forbes *et al.* 2007, Cheesbrough, 2002.).

Antimicrobial Assays

Preparation of Microbial Inocula

About 4 – 5 colonies of 16 to 24 hours of age of the test organisms from agar plate were suspended in Mueller Hinton broth solution and allowed to achieve good active growth – indicated by a turbid suspension (Willey *et al.* 2014, Forbes *et al.* 2007, Cheesbrough, 2002.). Standard inoculum size is as important asculture purity which was achieved by comparison of the turbidity of the organism suspension with a turbidity

standard. Commercially prepared 0.5 Mcfarland was used, which provided an optical density comparable to the density of a bacterial suspension of 1.5 x 10^8 colony forming units (CFU) / ml. further hundred fold dilution was made to achieve a x 10^6 colony forming units (CFU) / ml.

Screening for Antimicrobial Activity

Modified Agar well diffusion method was adopted for antimicrobial screening (Akerele *et al.* 2011, Anibijuwon, *et al.* 2010). The extracts were reconstituted to obtain 100mg / ml stock solution using DMSO (Dimethyl Sulfoxide). The Mueller Hinton agar was autoclaved and cooled to about 45^0C and aseptically seeded with 100 µl of the test organism at a turbidity of approximately 10^6 CFU / ml and poured into sterile Petri dishes of 8.5cm (85mm) in diameter. The poured plates were allowed to set at room temperature before using sterile cork borer to punch four uniform wells of 7mm in diameter in each of the plates. Each of the wells was filled with 0.1ml of a working solution of the different extracts to give a final strength of 10mg/ml, 5mg/ml and 2.5mg/ml of the different extracts (100mg/ml, 50mg/ml and 25mg/ml concentrations) respectively. Gentamicin (0.1 ml) solution was added to the fourth well to give a final concentration of 10µg/ml which serves for positive control. A one hour pre-diffusion period was allowed before the plates were incubated aerobically at 37^0C for 18 – 24 hours. With a transparent millimeter rule, the diameters of the zones of inhibitions were measured to the nearest millimeter.

Determinaton of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal (Microbicidal) Concentration (MBC)

The MIC was determined using micro broth dilution method (Rakholiya *et al.*, 2015) with slight modification. Sterile flat bottom 96 well micro test plates were used to perform this study. One hundred and fifty microliters of Mueller – Hinton broth was placed into each of the 96 wells and 20 µl of varying concentrations (concentration range of 3µg - 6250µg/ml) of the extracts were introduced in increasing order. Then followed with 30µl (the desired organisms at a turbidity approximately 10^6 CFU / ml) inoculation of test organism suspension to achieve a final volume of 200µl. Three control wells were incubated along with the test. The organism control (Mueller - Hinton broth, test organism and DMSO), the test control (Mueller - Hinton broth, test organism and the standard antimicrobial agent), and the sterility control (DSMO and Mueller – Hinton broth). Plates were incubated at 37^0C for 24 hours. Forty microliters of phenol red was added to each well after incubation, that is, the growth of the organisms was determined by adding 40µl of phenol red (0.2%) indicator and observing a change in color from red to yellow when there was growth of the organism and after further incubation for 30 minutes, the plates were examined for any colour change from red to pink which was an indication of bacterial growth. The lowest concentration (highest dilution) that showed no growth (no change in color) was recorded as MIC. The set up were performed in triplicate.

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Phytochemical Tests

Standard methods were used for the phytochemical analysis (Sofowora, (2008), Akerele et *al*., (2011), Tease and Evans, 1989, Harbone and Baxter, 1993).

RESULTS

Distribution of Test Organisms

The total number of microbial isolates was nine hundred (900) isolates. Out of these, seven hundred and fifty (750) microbial isolates were used as test organisms representing 83.3%. These test organisms were isolated from the following specimens: ear swab, throat swab, wound swab, pus aspirates, urethral smear, high vaginal swab, intra cervical swab, and catheter. One hundred and fifty isolates representing 16.7% were discarded for lack of preservation facilities.*S. aureus*(36%) was the most prevalent isolate, followed by *E. coli*(20%)and then *K.pneumoniae*(16%). This was followed by *P. vulgaris*(8%)and *P.auriginosa*(8%). The least prevalentwere*P .mirabilis* (4%), *S.epidermidis*(4%)and *C.albicans*(4%). *S.aureus*and *S.epidermidis* were the gram positive bacterial isolates and these represent forty percent (40%); and the gram negative bacterial isolates were *P. auriginosa*, *E. coli*, *K.pneumoniae*, *P. mirabilis*, *P. vulgaris* which represents fifty-six percent (56%); while the frequency distribution of fungal isolates was thirty isolates of *C.albicans*, representing four percent (4%).The frequency distribution of the microbial isolates is shown in Figure 1.

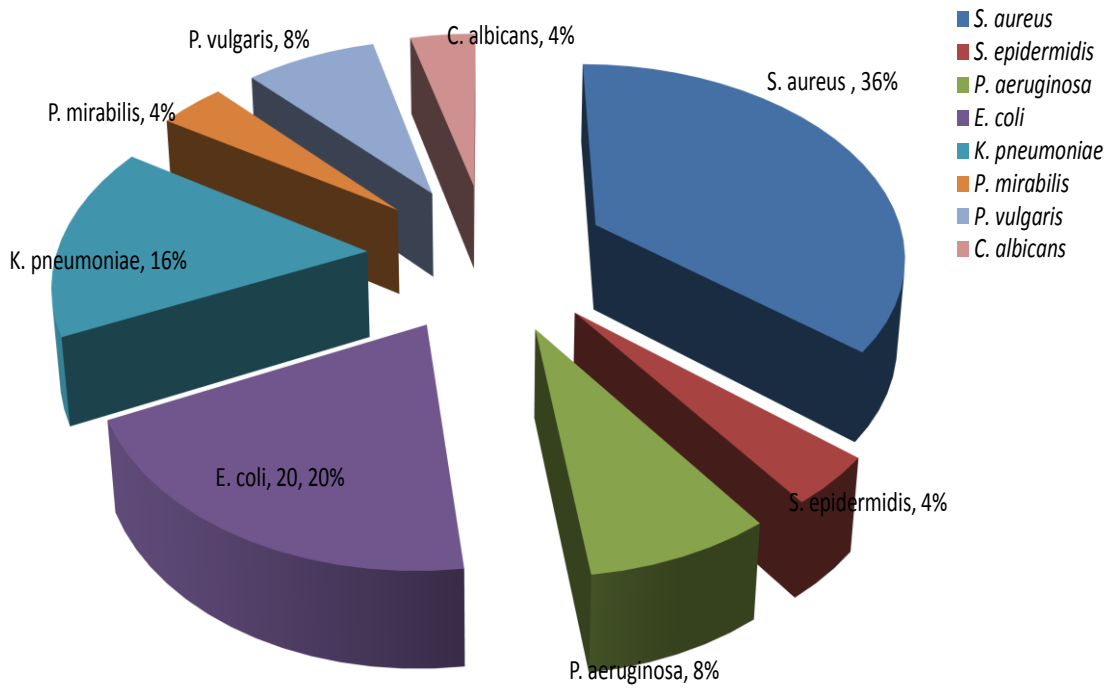

Figure.1. Frequency Distribution of the Microbial Isolates used as Test

Result of Antimicrbial activity of *Moringaoleifera* Leaves' Extracts of Different Concentrations (100mg/ml, 50mg/ml, 25mg/ml) of the Solvents on the Test Organsms.

The chloroform extract exerted higher activity than the other solvents (see figures 3 to 10). And the higher concentration (100mg/ml) of the different solvents had higher activity than the lower concentrations (50mg/ml and 25mg/ml), while that of 50mg/ml was higher than that of 25mg/ml as shown in Figures 2 to 9.

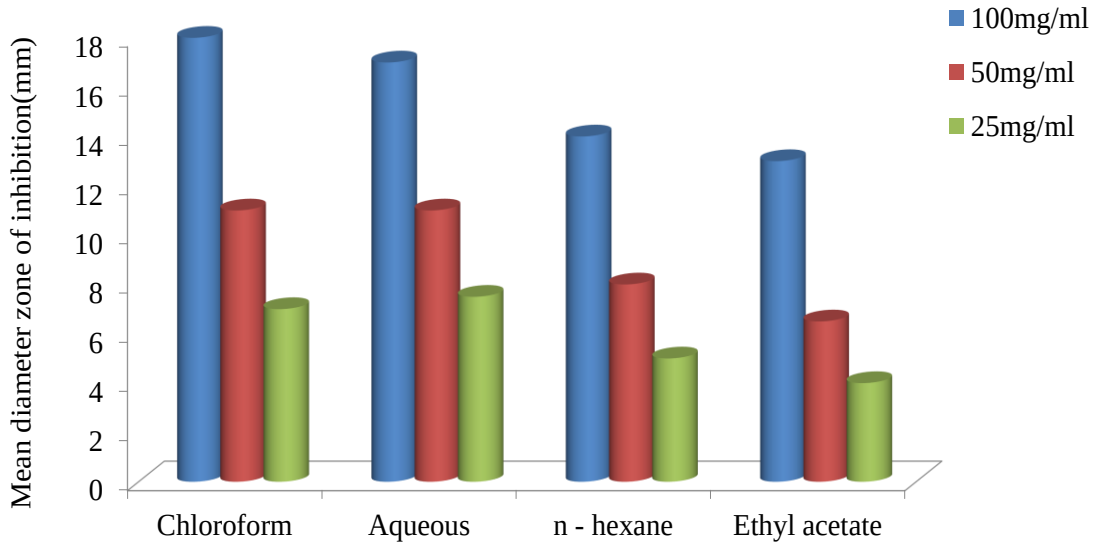

Figure .2. Antimicrobial activity of the different concentrations(100, 50, 25mg/ml) of the leaf extracts of *M. oleifera* against *S. aureus*

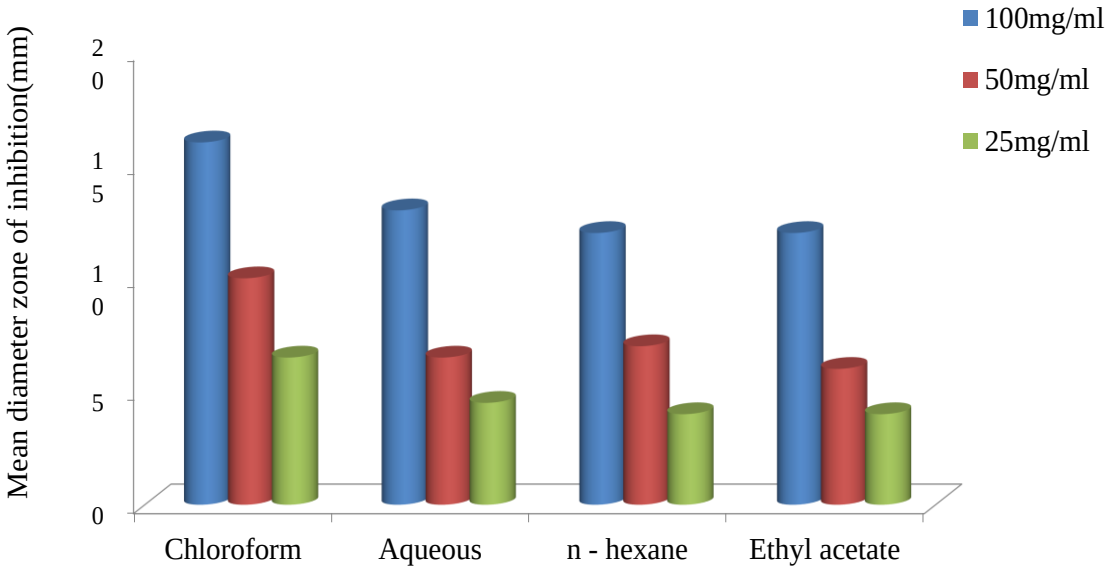

Fig.ure 3. Antimicrobial activity of the different concentrations(100, 50, 25mg/ml) of the leaf extracts of of *M. oleifera* against *S. epidermidis.*

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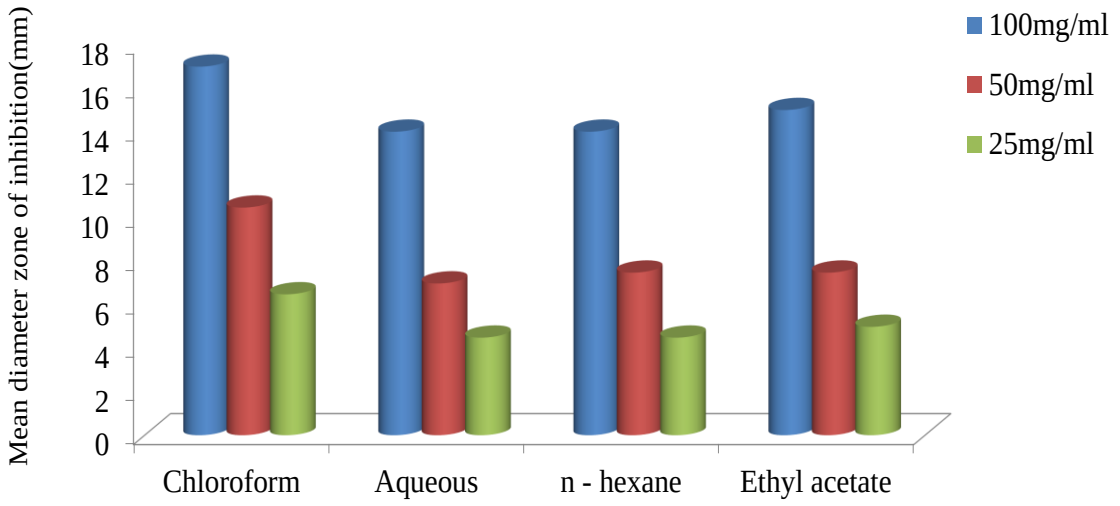

Figure.4. Antimicrobial activity of the different concentrations (100, 50, 25mg/ml) of the leaf extracts of of *M. oleifera* against *P. aeruginosa*

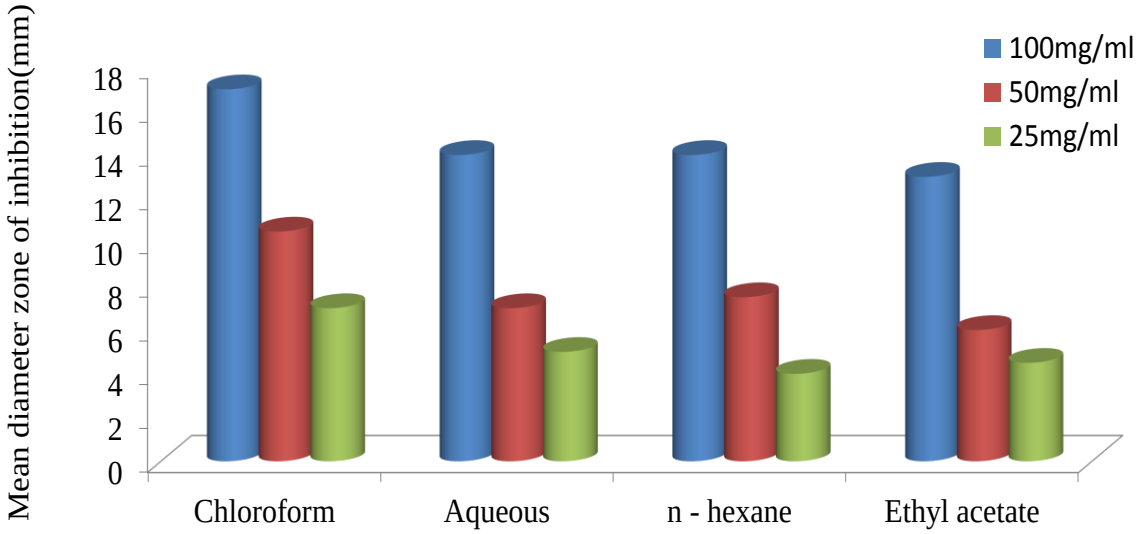

Figure.5. Antimicrobial activity of the different concentrations (100, 50, 25mg/ml) of the leaf extracts of *M. oleifera* against *E. coli.*

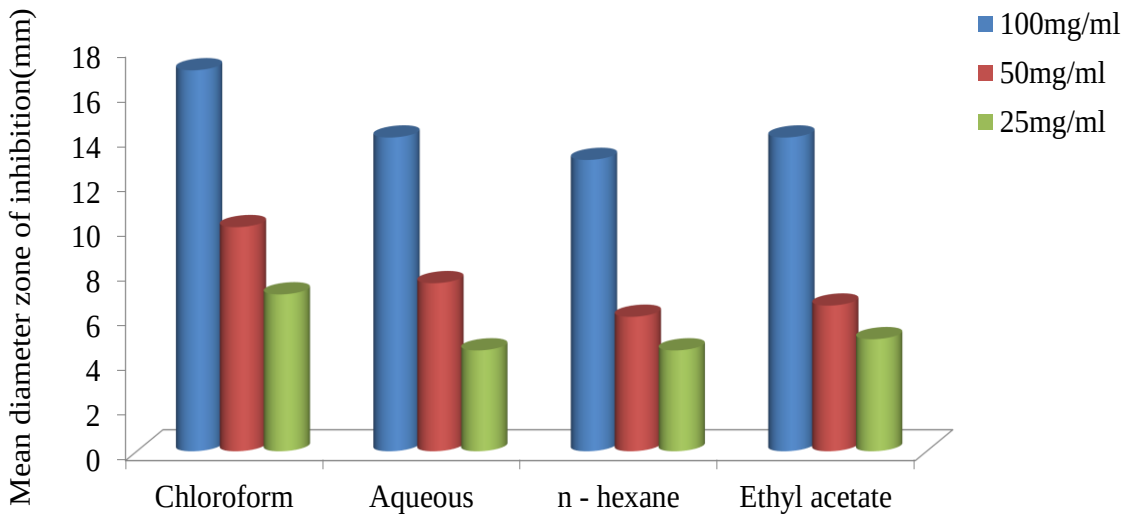

Figure.6. Antimicrobial activity of the different concentrations (100, 50, 25mg/ml) of the leaf extracts of *M. oleifera on K. pneumoniae*

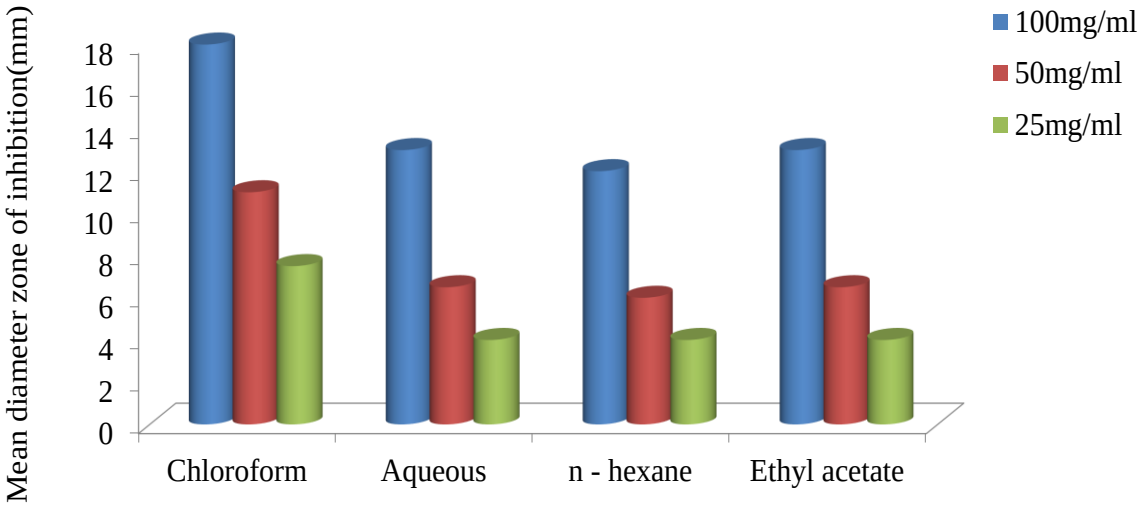

Figure.7. Antimicrobial activity of the different concentrations (100, 50, 25mg/ml) of the leaf extracts of *M. oleifera* against *P. mirabilis*

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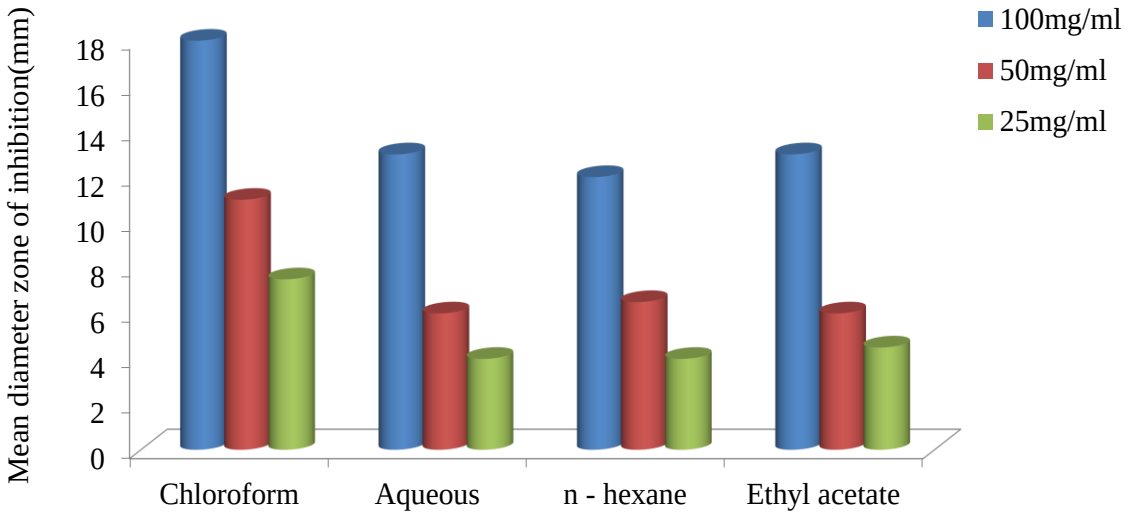

Figure.8. Antimicrobial activity of the different concentrations (100, 50, 25mg/ml) of the leaf extracts of *M. oleifera* against *P. vulgaris*

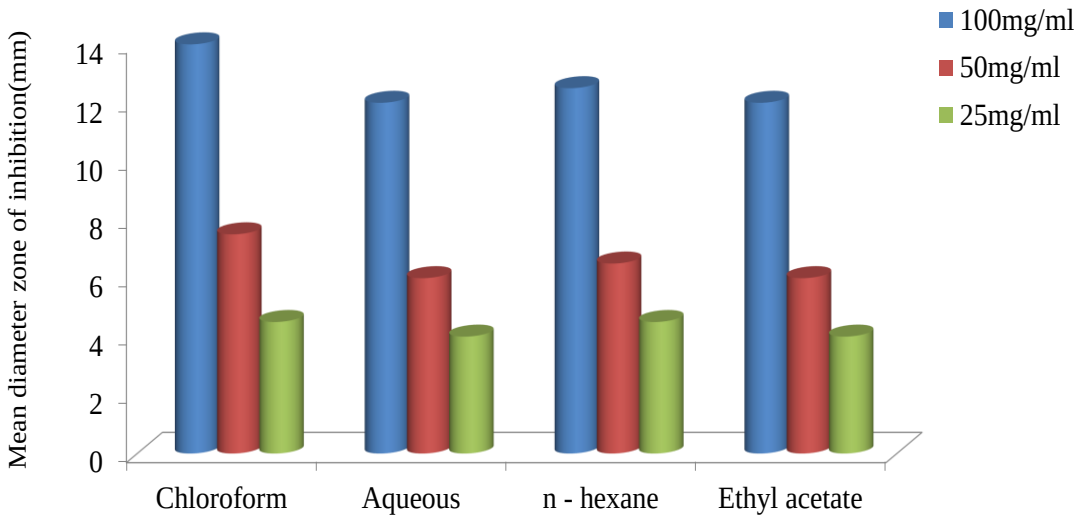

Figure.9. Antimicrobial activity of the different concentrations (100, 50, 25mg/ml) of the leaf extracts of *M. oleifera* against *C. albicans.*

Result of MIC

The findings of the MIC evaluation of the chlorofoam extract showed that *Staphylococcus aureus* was the most sensitive with MIC vaue of 391µg/ml; followed by *E. coli* and *K. pneumoniae,* followed by *P. auriginosa, P. mirabilis, P.vulgaris, S.epidermidis and C.albicans.* The MBC showed no value.

DISCUSSION

The chloroform extract had higher activity than the other solvents' extracts tested (n-hexane, ethyl acetate and aqueous fraction). The higher antimicrobial activity of

chloroform extract might be due the fact that more active chemical constituents were dissolved and recovered in it than others. Similar result was reported by some workers in which the chloroform extract was able to inhibit microbial growth including *S. aureus* and *P. aeruginosa* (Devendra et *al.*, 2011). Where as some reporters had the opposite view (Patil and Jane, 2013). Such variation might be due to climatic conditions, geographical distribution,verity of plant and source of pathogen used. However, Unaeze et *al.*, (1986) stated that the yield and activity of plants' extracts depends on extraction methods. The aqueous fraction exerted good activity, contrary to popular belief that active principles are not readily soluble in water; this strong activity of the aqueous fraction could be attributed to the method of our extraction (Unaeze et *al.*, 1986), in which 80% ethanol was used for maceration and 20% ethanol used in suspending the extract in fractionation. It could also be reasoned that more active ingredients could have yielded if more fractionation was done, thereby reducing the active constituents retained in the aqueous fraction. The solubility of the chemical components in solvents also depend on the nature and type of the plant; as some researchers have reported of certain plants not being soluble in cold water, like the leaves of *Aspilaafricana* (Anibijuwon et *al.*, 2010); and some that are soluble in cold water like the leaves of *Moringaoleifera* (Il Lae, 2014). Generally, most researchers use organic solvents for extraction of active principles. And this justify thepreference for use of organic solvents as extractants in preparation of crude drugs by herbalists. This is also provenand supported by the present study, as all the organic solvents used as extractants in this work, exerted antimicrobial activity. Most researchers supported this(Khalafalla et *al.*, 2010., Sultana et *al.*, 2009., Kumar et *al.*, 2012., Buddaetal .2011. Guevara et *al.*, 1999). In this study, the leaves' extracts of *M. oleifera*at different concentrations (100mg/ml, 50mg/ml, 25mg/ml) showed growth inhibition on all the test organisms with higher concentrations exerting more activity, and this is true with the works of other researchers (Fahey, 2005., Anibijuwon et *al.*, 2010.,Sofowora, 2008). However, the level of diameter of zone of inhibition obtained vary from one researcher to another because they might have used different strains and their methods of purification might differ and might yield different amounts of active ingredients. Other things that may lead to varied results are the ageof the plant when harvested, the season and time of samples' collection, the nature of the materials – fresh or dry (Harbone, 1998.,Sofowora, 2008). It may also be due to the consequence of different plantation if obtained from different regions. And it may be due to the genetic diversity of the plants. The standard antimicrobial agent exerted higher antimicrobial activity than most solvents' extracts used in this study (n-hexane, ethyl acetate and the aqueous fraction) except the chloroform extract. This is also true with some other reports that constitutional (conventional) antibiotics are more active than some plants' extracts (Akerele et *al.*, 2011., Anibijuwon et *al.*, 2010). However, the chloroform extract in this present study, exerted more antimicrobial activity than the constitutional (standard) antimicrobial agent used; this is similar to the works of other researchers who had greaterdiameter of zones of inhibition with plants' extracts (Usman and Osuji, 2007). Our results obtained in the present study is similar to other reports that both plants' extracts and conventional (standard) antimicrobial agent exert higher antimicrobial activity on bacteria than on the fungal organisms. Our report in MIC, that *S. aureus* is the most sensitive, is

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similar to the work of Patil and Jane, 2013. The highest sensitivity of this gram positive organism may be due to its cell wall structure and outer membrane (Zaika, 1988). This study proves that *M. oleifera's*leaves contain some active principles with antimicrobial activity and could enhance its use in preventing and curing diseases, when purified to the appropriate pharmacological level and it means that *M. oleifera* holds a promise as antimicrobial agent for treating infectious diseases.

REFERENCES

Akerele, J. O., Ayinde, B. A. and Ngiagah, J. (2011). Phytochemical and Antimicrobial Evaluations of the Stem Bark of *Newbouldialaevis* against Isolates from Infectd Wounds and Eyes. *Tropical Journal Pharmaceutical Research; 10 (2): 211 – 218.*

Anibijuwon, I. I., Duyilemi, O. P. and Onifade, A. K. (2010). Antimicrobial activity of leaf of *Aspilaafricana* on some pathogenic organism of clinical origin. *Nigerian Journal of Microbiology, 24: 2048 – 2055.*

Budda S, Butryee C, Tuntipopipat S, Rungsipipat A, Wangnaithum S, et al. (2011) Suppressive effects of *Moringaoleifera* Lam pod against mouse colon carcinogenesis induced by azoxymethane and dextran sodium sulfate. *Asian Pac J Cancer Prev12: 3221–3228*[PubMed]

Chanda, S. and K. Rakholiya.(2011). Combination Therapy: Synergism between Natural Plant extracts and Antibiotics against Infectious Diseases. In: Science against Microbial Pathogens: Communicating Current Research and Technological Advances, Volume 1, Mendez-Vilas, A. (Ed.). Formatex Research Spain, ISBN-13: 978-84-939843-1-1, pp: 520-529.

Cheesbrough, M. (2002). District Laboratoy Practice in Tropical Countries, Part 2 Microbiology. Cambridge University Press. Pp 132 – 143.

Devendra BN, Shrinivas N, Prasad Talluri VSSL and SwarnaLatha P (2011) Antimicrobial activity of Moringaoleifera lam., leaf extract, against selected bacterial and fungal strains. *Int. J. Pharma and Biosciences, 2(3): 13-18.*

Fahey, J. W. (2005). Moringaoleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Lewis B. and Dorothy Cullman Cancer Chemoprotection Center, 725 N. Wolfe Street, 406. WBSB, Baltimore, Maryland, USA 21205-2185. http://www.tfljournal.org/article.php/20051201124931586)

Forbes, B. A., Sahm, D. F., Weissfeld, A. S. (2007): Overview of Bacterial Identification Methods and Strategies.In:Diagnostic Microbiology.Pp.216 – 247. 12 edition.

Fuglie LJ (1999) The Miracle Tree: Moringaoleifera: Natural Nutrition for the Tropics. Church World Service, Dakar. 68 pp.; revised in 2001 and published as The Miracle Tree: The Multiple Attributes of Moringa, 172 pp.http://www.echotech.org/bookstore/advanced_search_result.php?keywords=Miracle+Tree. ANT AST CIR DET DIG INF NER NUT REP SKI).

Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, et al. (1999) An antitumor promoter from *Moringaoleifera* Lam. *Mutat Res440: 181–188 [PubMed]*

Harborne, J. B. (1994). Methods of Plants Analysis. *DOI.10.1007/978-94-009-5570-7-1.* Print ISBN 978-94-010-8956-2.

IlLae, (2014). Soluble Extract from *Moringaoleifera* Leaves with a New Anticancer Activity. *Doi: 10.1371/journal.pone.0095492*

Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah AA, Aboul-Enein KM, et al. (2010) Active principle from *Moringaoleifera* lam leaves effective against two leukemias and a hepatocarcinoma. *Afr J Biotech9: 8467–8471*

Kumar V, Pandey N, Mohan V, Singh RP (2012) Antibacterial and antioxidant activity of extract of *Moringaoleifera* leaves-An in vitro study, S. *Int J Pharm Scis Rev Res12: 89–94*

NCCLS (2003) Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard 6th edition, NCCLS document M7-A6, National Committee for Clinical Laboratory Standards, Pennsylvania, USA. Oliveira JTA, Silveira SB, Vasconcelos IM, Cavada BS, Moriera RA (1999) Compositional and nutritional attributes of seeds from the multiple purpose tree *Moringaoleifera*Lamark. J Sci Food Agric79: 815–820.

Patil, S.D. and Jane, R. (2013). Antimicrobial activity of Moringaoleifera and its synergism with Cleome viscosa*Int. J. of Life Sciences, Vol. 1(3): 182-189. © 2013|IJLSCI. www.ijlsci.in*

Rakholiya, K. D., Kaneria, M. J. and Chanda, S. V. (2015). *In vitro* Assessment of Novel Antimicrobial from Methanol Extracts of Matured Seed Kernel and Leaf of *Mangiferaindica* L. (Kesar Mango) for Inhibition of *Pseudomonas* spp. And their Synergistic Potential. *American Journal of Drug Discovery and Development, 5: 13-23*

Sofowora, E. A. (2008). Definitions and Terminology; Standardisaion of Herbal Medicine; Screening plants for bioactive agents. In: Medicinal plants and traditional Medicine in Africa. Pp. 1 – 9; 70 – 88; 181 – 207. Spectrum books Ltd, Ibadan.

Sultana B, Anwar F, Ashraf M (2009) Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules14: 2167–2180 [PubMed]*

Trease, G. E. and Evans, M. C. (1989). Text book of Pharmacognosy. 13th Edition. BailiereTindall, London, Toronto, Tokyo. Pp. 200-201, 340-348, 419-423,626-630, 765-775.

Tsaknis J, Lalas V, Gergis V, Douroglou V, Spiliotis V (1999) Characterization of *Moringaoleifera* variety Mbololo seed oil of Kenya. *J Agric Food Chem47: 4495–4499 [PubMed]*

Unaeze, M. C. and Abarikwa, P. O. (1986). Antimicrobial activity of certain medicinal plants used in traditional medicine in Nigeria. A preliminary study. *Journal of Microbiology, 6(1 – 2), 32 – 40.*

Usman, H. and Osuji, J. C. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of *Newbouldialaevis. African Journal of Traditional, Complementary and Alternative Medicines; 4(4) :476 – 480.*

Willey, J., Sherwood, L., Woolverton, C. J. (2014): Clinical Microbology and Immunology. In Prescot'sMcobiology. McGraw Hill Inc. New York. U. S. A. Pp. 808 – 829.

Zaika LL (1988) Spices and herbs- their antimicrobial activity and determination, *J. food safety. 9,97-118.*