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EVALUATION OF *MANGIFERA indica* FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The leaves of Mangifera indica were subjected to ethanol extraction and the extracts fractioned by suspending the extract in 20% ethanol and partitioned with different organic solvents (n-hexane, chloroform, ethyl acetate) 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. The test was carried out in triplicates. The test organisms were S. aureus, S. epidermidis, P. aeruginosa, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris and C. albicans. The leaf extract was phytochemically screened and revealed the presence of Flavonoid, Alkaloid, Tannins, Steroid, Saponins, Cardiac glycosides, Anthraquinone, Triterpenoids. The chloroform leaf extract showed strong antimicrobial activity on both gram positive and gram negative organisms; followed by the aqueous extract. The ethyl acetate leaf extract ranked third. n-hexane leaf extract had the least antimicrobial activity. The 100 mg/ml concentration exerted a broad spectrum antimicrobial activity with the highest diameter zone of inhibition of 18mm against Staphylococcus aureus and Klebsiella pneumoniae. This activity was followed by the 50 mg/ml and 25 mg/ml concentrations in that order. Fifty (50) mg/ml exerted the diameter zones of inhibitions of 10.5 mm while 25 mg/ml concentrations exerted the diameter zones of inhibitions of 7.5 mm. Also the least antimicrobial activity was exerted by the 25 mg/ml concentration, followed by the 50 mg/ml concentration with diameter zones of inhibitions of 4 mm and 7.5mm respectively, against S. epidermidis and C. albicans and some Gram negative organisms. The data obtained showed broad spectrum activities with increasing diameter zone of inhibitions at increasing concentrations, which showed that the inhibitory effects of the extracts were not only solvent dependent but also concentration dependent (Figures 1 – 8). The extracts were subjected to MIC evaluation and the findings showed that E. coli and K. pneumoniae were the most sensitive. The MBC showed no value. This study shows that the leaves of M. indica when purified to the appropriate pharmacological level, contain some active principles with antimicrobial potential that will be useful in preventing and curing diseases.

Key Words: *M. oleifera*, Antimicrobial, Phytochemical, Infectious isolates.

INTRODUCTION

Mangifera indica is known as mango and regarded as the king of herbs. Mangoes belong to genus *Mangifera* that have about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae. The

leaves contain 43-46 percent euxanthin acid and some euxanthon. Leaves and flowers yield an essential oil containing humulene, elemene, ocimene, linalool and nerol. In India most plants are used as bases for drugs' manufacturing in the Indian systems of natural medicine such as Ayurveda, Unani, Siddha and also in Homeopathy (Chukwuma and Nwachukwu. 2016). Phytochemical screening of leaves yielded total phenols, flavonoids, tannins, and saponins, alkaloids, cardiac glycoside, terpenoids. Mango is one of the most popular of all tropical fruits. The extensive survey of literature revealed that *M. indica* is an important source of many pharmacologically and medicinally essential chemicals such as mangiferin, mangiferonic acid, hydroxymangiferin, polyphenols and carotenes. Mangiferin has been reported to have many different pharmacological activities. Bbosa *et al*., (2007), in their study, showed that leaf extracts of *M. indica* possess some antibacterial activity against *S. aureus, E. coli, P. aeruginosa,* which has provided a basis for its medical use in Uganda and some fractions of leaf extracts (like ethereal and ethanolic) showed anti-clostridium tetani activity. Aderibigbe *et al*., (1999) showed that leaf extract of *M. indica* possess hypoglycemic activity, which could be attributable to reduction in intestinal absorption of glucose. Some studies revealed that *M. indica* contained some active ingredients like flavonoids which effectively reduce lipid levels in serum and tissues of rats with induced-excessive quantity of lipid in the blood (hyperlipidemia) and degradation and expelling of cholesterol were enhanced and mouth intake of flavonoids showed significant antioxidant action in cholesterol-fed experimental rats. The activities of free radical-scavenging enzymes were extensively elevated and lipid peroxide content was considerably reduced in flavonoid-treated hypercholesterolemic rats (Anila, 2002). Bharti, (2013) studied antimicrobial and phytochemical profile of *M. indica* leaf extract and reported that Hexane – ethyl acetate extract had significant activities against all the tested organisms.

Awad El – Gied, (2012) stated that the seed extract was able to inhibit the activities of the isolates studied and it has some anti-syphilitic activities. Most parts of the *Mangifera indica* have wide medical applications and the bark also contains tannins, which are used in dyeing industry. Since 1861 – 1880, that Koch described bacteria as the causative agents of diseases, the laboratory has positioned itself through technological advancement to identification of microorganisms as aid to doctors to facilitate and speed up cure of patients. This pathological state known as infection, can be acute or chronic and identification of the causative agent and as in Microbiology, obtaining a sensitivity (susceptibility) pattern of these microorganisms offer a high level of cure. Unfortunately, bacterial resistance to currently available antibiotics has rapidly emerged to a global problem and posing a growing public health risk. This increase incidence of drug-resistant pathogens has drawn the attention of the scientific communities towards studies on the potential antimicrobial activity of plant-derived substances, as potential source of novel drug. More – so, the huge success recorded by quinine and quinidine isolated from *Cinchona* tree bark and recently artemisinin from *Artemisia Annua* in the treatment of malaria has now created much interest on scientific workers in the search

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on medicinal plants like *M. indica* for novel drugs (Di Flumeri et al. 2000). Hence this study aims at the phytochemical and antimicrobial potential of the leaves of *M. indica.*

MATERIALS AND METHODS

Collection and Preparation of the Plant Extracts

The leaves of *M. indica* were collected within the University of Port Harcourt's premises. The identity of the plant was authenticated by the office of Herbarium Plant Science and Biotechnology, University of Port Harcourt. The fresh leaves, after collection, were washed, hot-air dried and ground to powder.

Extraction of Plant Leaves

The plant's material was 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 under vacuum through 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 fractioned by suspension in 20% ethanol and partitioned with different organic solvents in order of increasing polarity. The percentage yield of the extract was 18%. The solid residue was stored in glass vials in a refrigerator (4^0C) from which portions were taken for further experiments.

Source of the 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.).

Collection of Specimens

Nine hundred samples were collected from various patients suspected of having infections. The different types of specimens encountered were wounds, reproductive, surgical, ear, nose and throat specimens. The exudates, smears, puses, etc, were collected using sterile cotton tipped swab before treatments were given to the patients. Seven hundred and fifty (750) positive cultures were used as test isolates.

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 inoculums size is as important as culture 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, Sofowora, E. A. 2008).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.

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

Seven hundred and fifty (750) microbial isolates out of nine hundred (900) 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 swab, high vaginal swab, intra cervical swab, and catheter. One hundred

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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 followed by *K. pneumoniae* (16%). And followed by *P. vulgaris* (8%) and *P. auriginosa* (8%). While the least prevalent were *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%).

Table 1: Distribution of the Test Isolates Among Different Pathological Samples.

Pathological Sites	**Frequency of Isolates (%)**								
	A	b	c	d	e	f	g	h	Total
Wound	80(11)	0(0)	20(3)	40(5)	30(4)	10(1)	15(2)	5(1)	200(27)
Reproductive organs	100(13)	10(1)	20(3)	70(9)	60(8)	5(1)	30(4)	5(1)	300(40)
Surgical	40(5)	20(3)	5(1)	5(1)	10(1)	5(1)	5(1)	10(1)	100(13)
Ear and Nose	30(4)	0(0)	10(1)	20(3)	10(1)	5(1)	5(1)	0(0)	80(11)
Respiratory	20(3)	0(0)	5(1)	15(2)	10(1)	5(1)	5(1)	10(1)	70(9)
Total	270	30	60	150	120	30	60	30	750

Key:a - *Staphylococcus aereus, b - Staphylococcus epidermidis, c - Pseudomonas aeruginosa , d - E. coli, e - K. pneumoniae, f -Proteus mirabilis*, g -*P. vulgaris*,h - *Candida albicans.* Figures in bracketsare percentages of the microorganisms in different pathological types (approximated to whole numbers).

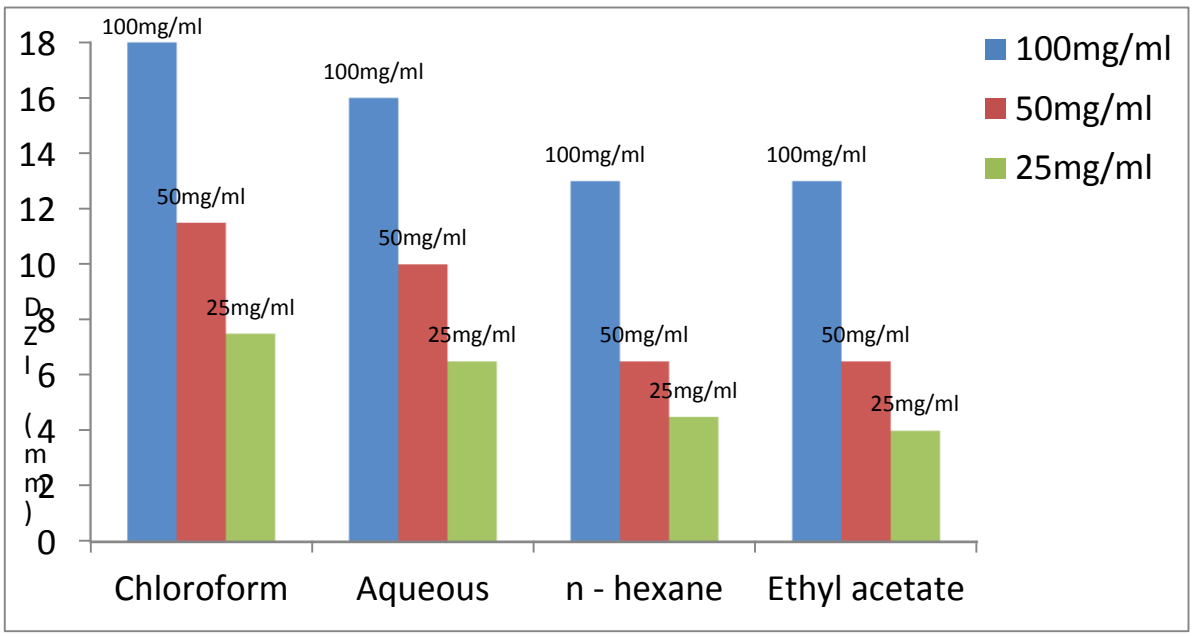

Figure 1. Antimicrobial activity of the leaf' extracts of different concentrations (100, 50, 25mg/ml) of the solvents of *M. indica* against *S.aureus.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

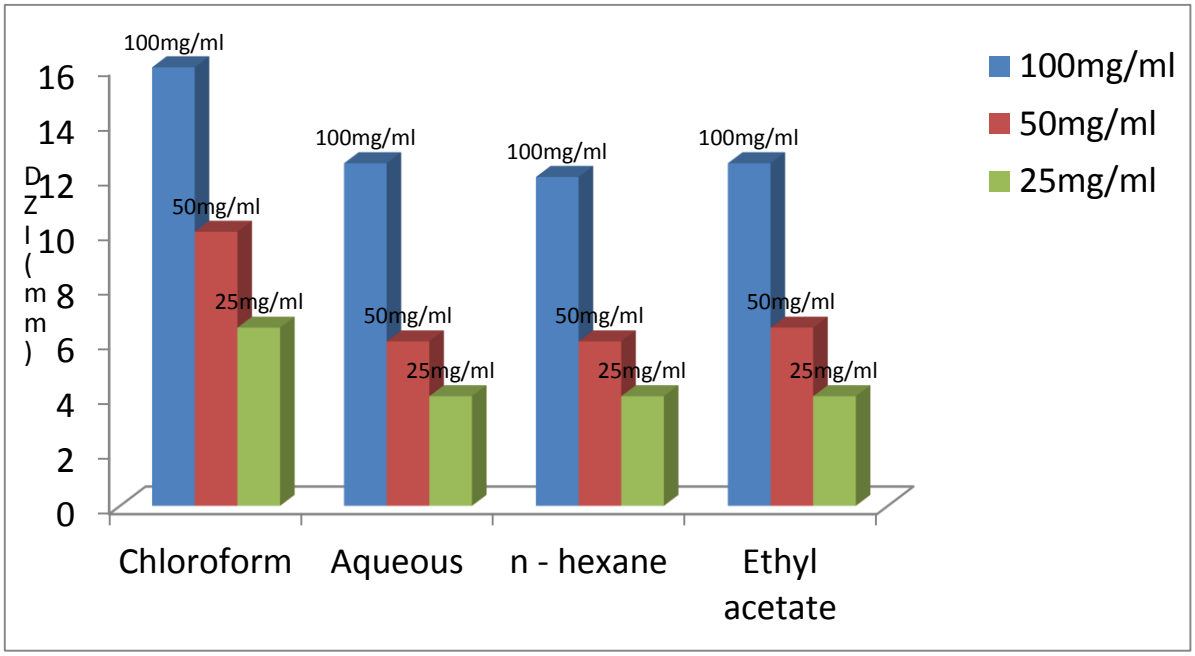

Figure 2. Antimicrobial activity of the leaf' extracts of different concentrations(100, 50, 25mg/ml) of the solvents of *M. indica* against *S.epidermidis.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

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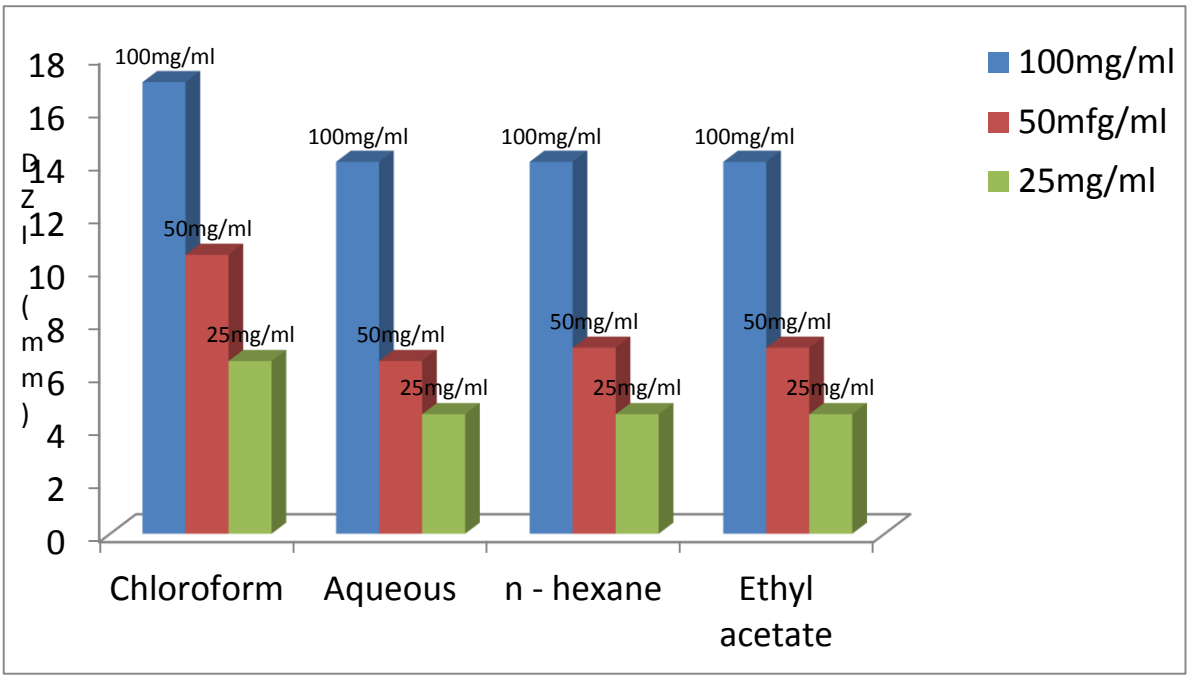

Figure 3. Antimicrobial activity of the leaf' extracts of different concentrations (100, 50, 25mg/ml) of the solvents of *M. indica* against *P.aeruginosa.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

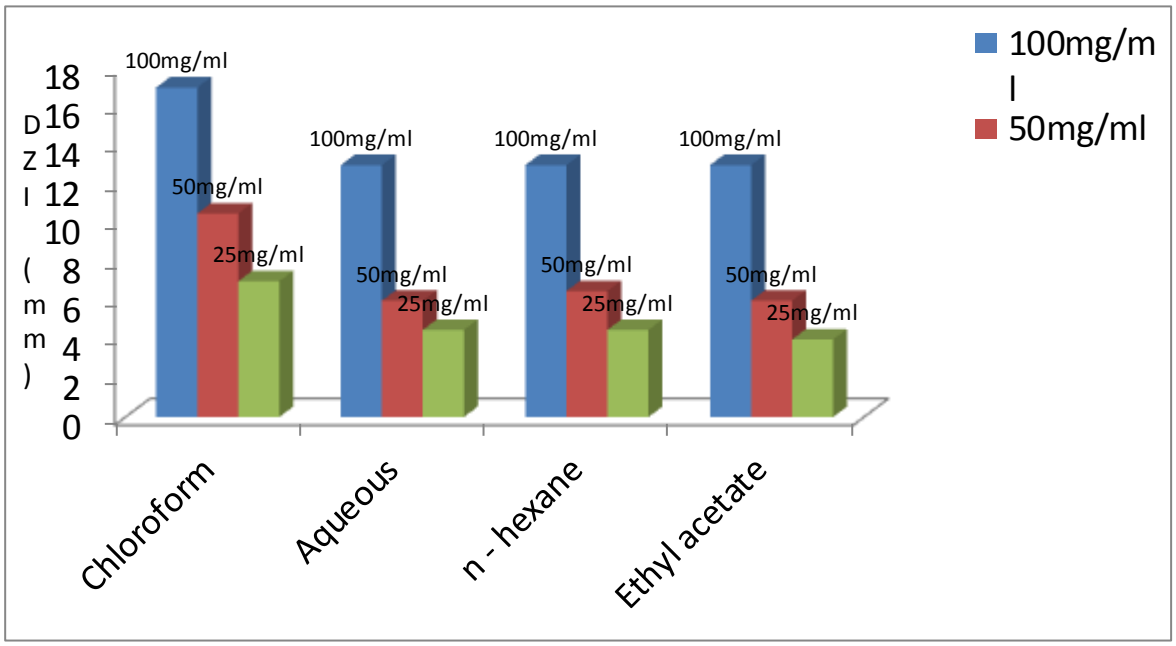

Figure 4. Antimicrobial activity of the leaf' extracts of different concentrations (100, 50, 25mg/ml) of the solvents of *M. indica* against *E. coli.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

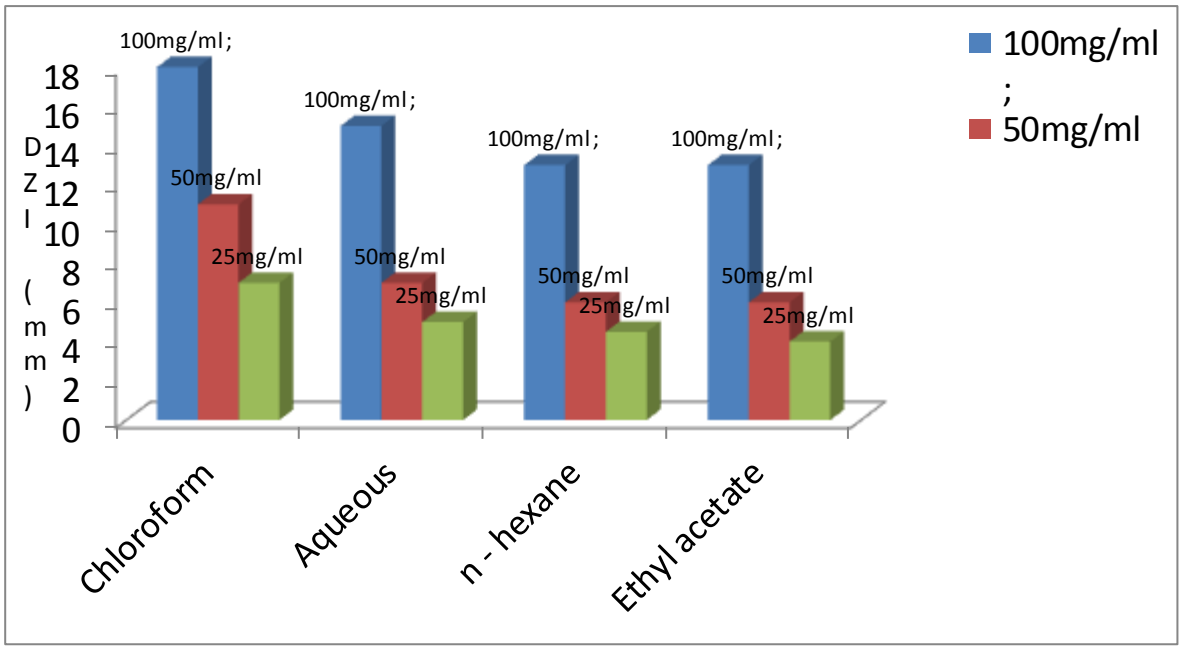

Figure 5. Antimicrobial activity of the leaf' extracts of different concentrations (100, 50, 25mg/ml) of the solvents of *M. indica* against *K. pneumoniae.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

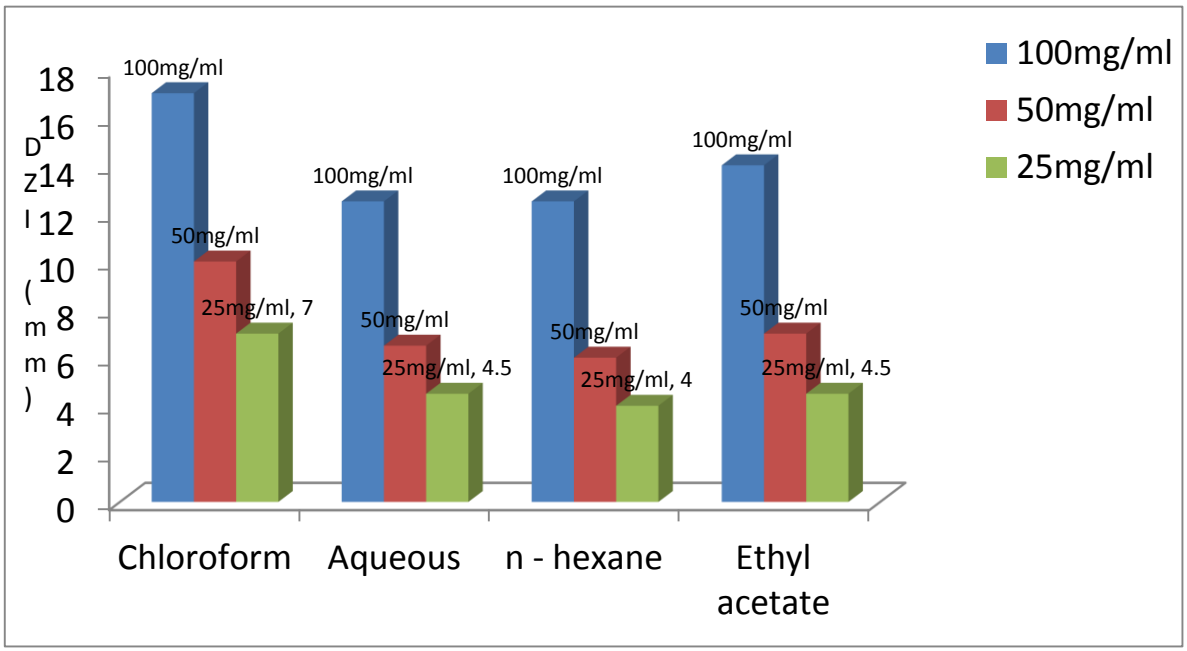

Figure 6. Antimicrobial activity of the leaf' extracts of different concentrations (100, 50, 25mg/ml) of the solvents of *M. indica* against *P.mirabilis.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

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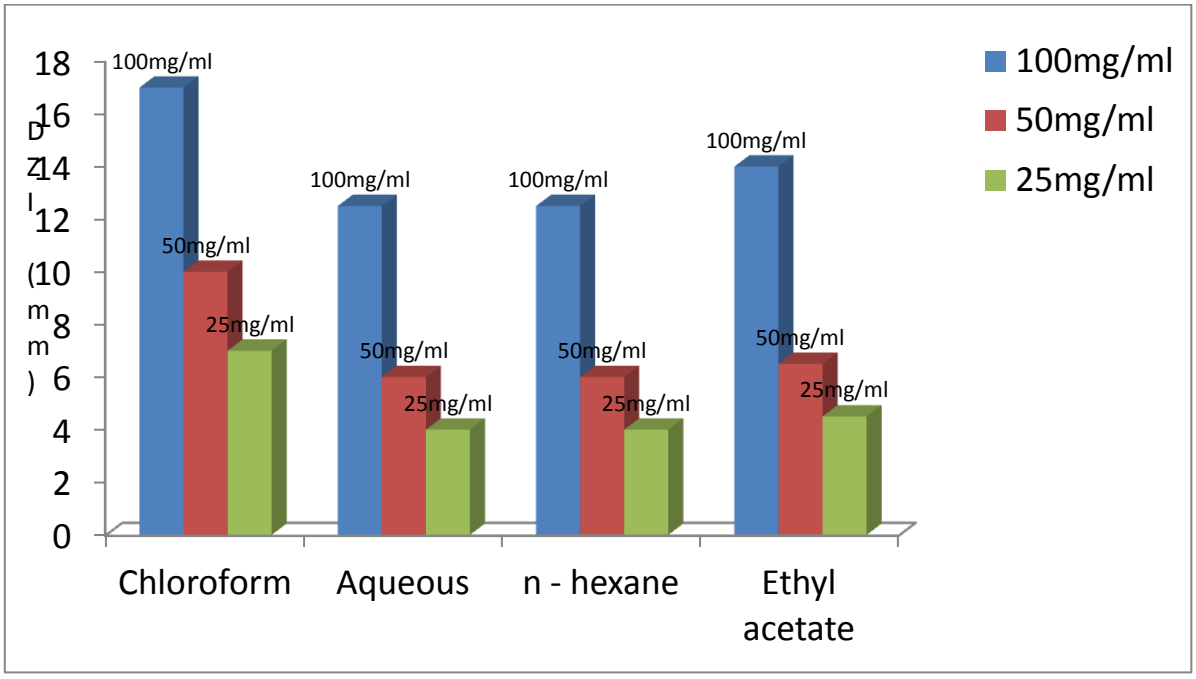

Figure 7. Antimicrobial activity of the leaf' extracts of different concentrations(100, 50, 25mg/ml) of the solvents of *M. indica* against *P.vulgaris.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

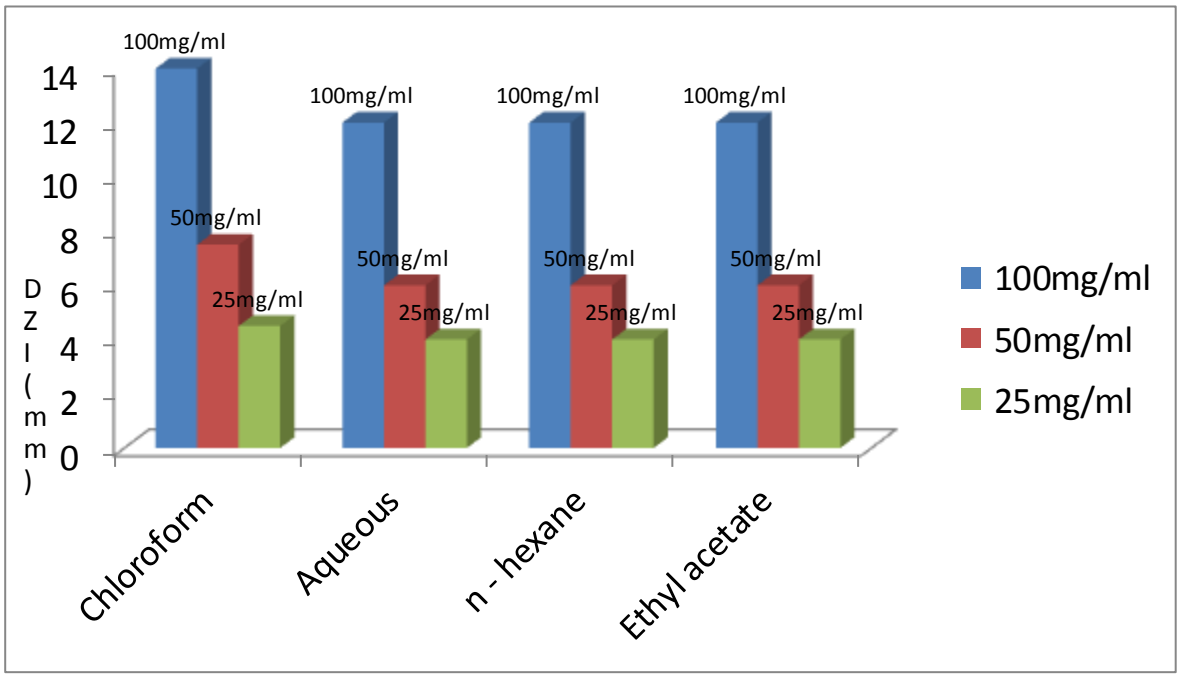

Figure 8. Antimicrobial activity of the leaf extracts of different concentrations (100, 50, 25mg/ml) of the solvents of *M. indica* against *C.albicans.*

Key: DZI (mm) = Diameter zone of inhibition (mm)

Result of MIC and MBC

The findings of the MIC evaluation of the chlorofoam extract showed that *E. coli, K.pneumoniae* were the most sensitive with MIC value of 1563 µg/ml; *C. albicans* and *S. epidermidis* were the least affected The MBC showed no value.

Result of Phytochemical Screening

Table 1: Phytochemical Screening of Leaf Extract of *Mangifera indica*

S/N	Active Principle	Test conducted	Result
1	Flavonoid	Alkali test	+++
2	Alkaloid	Drangendroff's test	++
3	Tannins	Ferric chloride test	++
4	Steroid	Salkowski test	++
5	Saponins	Froth test	+
6	Cardiac glycosides	General test	++
7	Anthraquinone	Borntrager's test	+
8	Triterpenoids	Salkowski test	++

Key: - = Negative, + = weak positive, ++ = moderate positive, +++ = strong positive

DISCUSSION

The leaf extracts of *M. indica* exerted various degrees of antimicrobial activities against the test organisms (tables 2 to 9). This is due to the fact that these leaves contain active ingredients that can prevent, inhibit and destroy microorganisms. The level of activity of these natural plants against a given disease depends on the amount of chemical components derivable from such plant. These phytochemicals (table 2) are essential in medicine and commerce. For example, in medicine, phenol is used to embalm bodies, which indicates a bacteriostatic and bactericidal activity; it is also used as an antiseptic for surgical instruments, this also confers a bacteriostatic and bactericidal effect. It is used in other things like in the production of drugs, in cosmetics and as an oral analgesics. Our research showed that *M. indica* possess other active principles like the alkaloids, saponins, etc. The great importance of alkaloids and saponins, etc, in medicine was reported by Akpuaka, 2009, in which alkaloids are prescribed for treatment of cough, cold, diabetes, hypertension, cancer and malaria. And saponins are beneficial for sufferers of hypertension and arteriosclerosis and in the control of post menopausal syndrome and in the treatment of high blood cholesterol. Our study also had result of phytochemicals such as flavonoids and tannins, which are believed to have played an antimicrobial role observed in the present study. Other researchers have similar findings and reported that flavonoids act as an antioxidant and enhance the effects of vitamin C and are biologically known to be active against liver toxins, tumors, viruses and other microorganisms (Korkina and Afanas'ev, 1997). On the other hand, tannins are used to treat dysentery, diarrohea and other intestinal disorders, which indicates an antimicrobial activity.

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The antimicrobial activity of the leaf's extracts of *M. indica* has been proven and reported by many researchers (Morsi et al. 2010, Bbosa et al., 2007). This is also true in the present study, in which all the leaf's extracts had various degrees of antimicrobial activities against the test organisms. Our study had results similar to those of most researchers. For example, in a study carried out by Bbosa et al. 2007, they showed that leaf's extracts of *M. indica* possess some antimicrobial activities against *S. aureus, E. coli, P.aeruginosa* and this is also true in the present case in which *M. indica* leaf's extract exerted antimicrobial activity against those microbes also. The present study is similar to the work reported by Morsi et al. 2010, in which they evaluated various leaf's extracts for antimicrobial activity against human and plant pathogenic bacteria and their study revealed a significant inhibition of almost all tested pathogenic organisms. The importance of *M. indica* commonly known as mango in treating various diseases can not be over emphasized and awareness to its medicinal value should be created as many people don't know that mango has other benefits other than its edible fruits, especially now that antibiotic resistance microorganisms are major public health problems.

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