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Original Research Article

Evaluation of Antibacterial and Phytochemical Analysis of *Mangifera indica* Bark Extracts

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A B S T R A C T

Mangifera indica extract is used as a natural medicine. It is a cheaper and safe alternative source of drugs. Antibacterial activity of *Mangifera indica* bark extracts was determined using agar well diffusion method. It had significant antibacterial potency against the tested pathogens. In the present study the plant active components of *M. indica* bark were extracted using four different extraction solvents namely distilled water, ethanol, methanol and ethyl acetate. The phytochemical compounds such as steroids, cardiac glycosides, saponins and resins were revealed. It was demonstrated that more percentage of yields was observed for distilled water extract (27.34%) followed by ethanol (8.86%), methanol (2.28%) and least for ethyl acetate (1.15%). In the present study bark powder extract of *M. indica* was studied by using 100%, 50%, 25%, 12.5% and 6.25% dilution of four different solvents against the tested pathogens such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The ethyl acetate extract (100%) was found to be most effective against *Klebsiella pneumoniae* (28 mm) followed by *Bacillus subtilis* (25 mm), *Pseudomonas aeruginosa* (22mm), *Staphylococcus aureus* and *Escherichia coli* (20 mm each). Distilled water extract of *Mangifera indica* bark was not effective for the entire tested pathogens.

Keywords

Mangifera indica, Bark Extract, Antibacterial Activity, Phyto-chemicals

Introduction

Plant derived products like gums, oils and extracts have been used for therapeutic purpose before the introduction of modern drugs (Haslam, 1989; Lima et al., 2006) and continues to provide health coverage for over eighty percent of the world's population (Ndip et al., 2006). Serious attention is being given to medicinal plants as evidenced by the recommendation given by the World Health Organization (WHO) in 1970.WHO given emphasis on the need to include traditional remedies within national drug policies as these plants serve as the best sources of a variety of drugs. An estimated 74% of pharmacologically

active plant derived components were discovered after following up on ethnomedicinal use (Ncube et al., 2007). Thus, medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine and chemical entities for synthetic drugs.

The emergence of multidrug resistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agent. The new compound may actually be more effective than the parent compound. Man has resorted to plants for treatment due to high prices of synthetic drugs. Plants are regarded as cheaper and safe alternative source of drugs (Emeruwa, 1991; Pretorious and Watt, 2001). Easy accessibility and affordability of plants compared to commercial drugs tends to self-medication with these substances. Contrary to the belief that natural medicine has no ill effects, several people have been hospitalized by consuming plants of unknown properties. To address such challenges, plants must be investigated to validate and standardize their dosages.

Mangifera indica belongs to the family Anacardiaceae which consists of about sixty genera and six hundred species, which are mainly tropical trees and shrubs. It is widely used as a source of food, medicines and timber. Mango bark is the major by product of any mango processing industry. These bark, as a waste product, cause tremendous investment of capital to decompose it safely and to prevent any environmental pollution. If these waste products can be converted as a raw material for the production of any bioactive compounds, then it will keep the food processing industries free from investing its capital in decomposing these wastes. The bark contains Mangiferine and is astringent and employed against rheumatism and diphtheria in India. Among the compounds isolated from *M. indica* extract are terpenoidal saponins, polygalacturonase, fructose-1-6-diphosophatase, triterpenoid, 2-hyroxymangiferonic acid tetracyclic triterpenoid and pentacyclic triterpenoid. The bark infusion has been used as gargle to treat mouth infections in children (Doughari and Manzara, 2008; Abubakar, 2009). Therefore aim of the present study was to evaluate the antibacterial and phytochemical analysis of *Mangifera indica* bark extracts by using distilled water, ethanol, methanol and ethyl acetate.

Materials and Methods

Preparation of *Mangifera indica* Bark Powder

Fresh bark pieces were collected from a *Mangifera indica* tree. The bark pieces were washed thoroughly with water and then air dried at room temperature for five days. After drying, the pieces were ground into powder form and then sieved using a sieve. Two kilograms of powdered plant extracts were transferred into airtight containers and stored at room temperature.

Extraction of Crude Extracts from Bark Powder

Plant active components were extracted using the cold extraction method. The 25 gm bark powder was mixed with 250ml of four different extraction solvents each namely distilled water, ethanol, methanol and ethyl acetate in the conical flasks and allowed to soak at room temperature for

48 hours. A rotary shaker set at 120 rpm was used to improve the extraction of phytochemicals. The filtrate was obtained by means of filter funnel aided by a Whatman filter paper. Filtering was repeated three times with same plant material until the solution was clear. The filtrate was evaporated in a weighed flask, with a water bath set at 40^{0}C. Drying was done to allow the calculation of the yield of the extraction process.

Calculation of % yield obtained

After drying, the remaining four different powdered extracts were quantified by determining the weight of each of the extracts and the percentage yield was calculated as (Weight of dry extracts in grams /Initial dry plant extracts) × 100.

Preparation of Dilutions

The procedure was done separately for the four solvents used. Five different types of dilutions such as 100%, 50%, 25%, 12.5% and 6.25% were prepared. For the preparation of dilutions of dry extracts, the dry extracts in powdered form were reconstituted by re-dissolving in their respective extracting solvents and above mentioned dilutions were prepared. The filtrate was obtained by means of filter funnel aided by a Whatman filter paper. Sterile extracts obtained were stored separately in labeled, sterile capped bottles, in a refrigerator before using for the antibacterial sensitivity tests.

Test organisms

The test organisms used were *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2064, *Bacillus subtilis* NCIM 2010, *Klebsiella pneumoniae* NCIM 2098 and *Pseudomonas aeruginosa* NCIM 2036.

Sterility Proofing of Extracts

After membrane filtration, extracts were tested for sterility, by streaking on freshly prepared sterile nutrient agar plate which was incubated for 24 hours at 37^{0}C.

Assay for Antibacterial Activity

Mueller Hinton Agar plates were prepared, on which the lawn of test organisms were made separately by using sterile cotton swab. Well puncture machine (cork borer) having 6mm diameter was used for making wells in the inoculated plates. A 10ul of each solvent extract was added in different wells for each organism tested. Plates were incubated at 37^{0}C for 24hours. Diameter of zone of inhibition was observed in mm by using the zone size interpretative chart (Hi Media Laboratories Pvt. Limited) (Lino and Deogracious, 2006).

Phytochemical Tests

The powdered bark extracts of *M.indica* were evaluated for the presence of phytochemical compounds using standard methods (Fowler, 2006). Phytochemical examination was carried out separately for all the extracts and the procedure was done three times for confirmatory purpose. For performing phytochemical tests the extracts were allowed to convert in the powdered form (Joshua and Takudzwa, 2013).

Detection of Steroids (Salwoski's Test)

The 100mg of dry extracts were dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were added to form a lower layer. A reddish brown

colour at the interface was indicative of the presence of steroidal ring.

Detection of Cardiac Glycosides (Keller Killian's Test)

The 100mg of dry extracts were dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolides.

Detection of Saponins (Froth Test)

The 100mg extracts were diluted with distilled water to 20ml which was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

Detection of Resins

The 100mg of dry extracts were dissolved in ethanol then 5 ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 ml of concentrated sulphuric acid was added. Bright purple colour produced indicated the presence of resins.

Results and Discussion

In the present study the plant active components of *M. indica* bark were extracted using four different extraction solvents namely methanol, ethyl acetate, ethanol and distilled water. The filtrate obtained was evaporated and dried. Drying was done for calculating the yield of the extraction process. The colour and state of the final extract was observed after 72 hrs of soaking the plant material in different solvents. Ethyl acetate extract showed brown colour, after vaporization of solvent brown solid extract was obtained. Methanol extract had a brownish blackish colour; the extract was solid after vaporization of solvent. Ethanol extract was yellowish in colour and gummy extract was obtained. Distilled water extract was black in colour; a solid extract was obtained after vaporization. The bark powder obtained after evaporation of the solvents was then measured for calculating the yield. In the bark powder extraction of *M. indica,* more percentage of yields was observed for distilled water extract i.e. 27.34%, and least for ethyl acetate i.e. 1.15% only. For methanol the yield was 2.28% and for ethanol it was 8. 86 % (Table 1).

In the present study bark powder extract of *M. indica* was studied by using 100%, 50%, 25%, 12.5% and 6.25% dilution of four different solvents such as Distilled water, Methanol, Ethanol and Ethyl acetate against the tested pathogens. The test pathogens include *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* It was observed that methanol and ethyl acetate extract have shown antibacterial activity against *S. aureus* at 100% and 50% dilution respectively. Methanol extract was efficient (12mm zone) while 100% dilution of ethyl acetate extract has given the highest zone of inhibition (20mm) (Figure 1) followed by 50% (15mm), 25% (12mm) and 12.5% (12mm). This was the lowest dilution of this extract for showing antibacterial activity because in 6.25% of dilution was found to be non effective against *S. aureus*. Distilled water does not show any antibacterial activity against *S. aureus* for all the dilutions tested (Table 2) (Chart 1).

The 100% of ethanol solvent extract was more effective against *E. coli* (24mm)

followed by 50% (13mm), 25% (12mm), 12.5% and 6.25% (10mm each) (Figure 2) while for 100% extract in methanol (12mm) followed by 50% and 25% (10 mm each). The 100% ethyl acetate (20mm) zone was observed against *E.coli* (Figure 3) followed by 50% and 25% (16mm each), 12.5% (12mm) and 6.25% (10mm). However, Distilled water did not show any antibacterial activity against *E.coli* for all the dilutions prepared (Table 3) (Chart 2).

Except distilled water extract, all other extract were found to be antibacterial against *Pseudomonas aeruginosa*. At 100% and 50% dilutions of ethanol 18mm and 16mm zone of inhibition was observed respectively (Figure 4) followed by 25% (15mm), 12.5% (11mm) and 6.25% (10mm). At 100% dilution in methanol (13mm) followed by 50% and 25% (12mm each), 12.5% (11mm) and 6.25% (10mm) while 100% and 50% dilution of ethyl acetate showed (22mm) zone of inhibition each followed by 25% (15mm), 12.5% (12mm) and 6.25% (10mm) against *Pseudomonas aeruginosa* (Table 4) (Chart 3).

For *Bacillus subtilis*, 100% dilution of ethyl acetate extract have shown highest antibacterial activity (25mm) (Figure 5), at 50% (18mm) followed by 25% (14mm), 12.5% and 6.25% (10mm each) as compared to 100% dilution of ethanol (12mm) followed by 50%, 25% and 12.5% (10mm each). For 100% methanol (10mm) zone was observed. Distilled water extract, was not effective on *Bacillus subtilis* and some dilutions of methanol also not effective against *Bacillus subtilis* (Table 5) (Chart 4).

At 100% ethanol extract (13mm) (Figure 6) followed by 12 mm for remaining dilutions was observed. Methanol extract of 100%, 50%, 25%, 12.5% dilution showed 10mm zone of inhibition each. Ethyl acetate extract was more effective against *Klebsiella pneumoniae* as compared to ethanol and methanol extract. Ethyl acetate extract (100%) have showed 28mm zone of inhibition (Figure 7), 25mm for 50%, 21mm each for 25% and 12.5% dilution while 6.25% (12mm) (Table 6) (Chart 5).

The phytochemical test for all four extracts such as distilled water, methanol, ethanol, ethyl acetate was performed for the presence of steroids (Salwoski's test) cardiac glycosides (Keller Killian's test), saponins (Froth Test) and resins (Detection of Resin). In the Salwoski's test reddish brown colour was observed at the interface indicated the presence of steroidal ring. In Keller Killian's test brown ring obtained at the interface indicated the presence of de-oxy sugar characteristics of the cardenolides (cardiac glycosides). In the Froth test formation of 1cm layer of foam indicated the presence of saponins. The phytochemical test for Resins showed bright purple colour production indicated the presence of resins (Table 7).

Antimicrobial activity of bark extract of *Mangifera indica* on different test pathogens was carried out in the study. In the present study bark powder extract of *M. indica* was studied by using 100%, 50%, 25%, 12.5% and 6.25% dilution of four different solvents such as Distilled water, Methanol, Ethanol and Ethyl acetate against the tested pathogens. Hence the findings on extraction potential of the different solvents demonstrated the highest percentage yield (27.34%) of distilled water extract than the other solvent used.

Table.1 Percentages obtained after using different solvents in extraction of compounds from the bark of *Mangifera indica*

Solvents	Weight of Starting Material	% of yield extract [(w/w)x100]
Distilled Water	25g	27.34%
Ethanol	25g	8.86%
Methanol	25g	2.28%
Ethyl acetate	25g	1.48%

Table.2 Antibacterial activity of *Mangifera indica* bark solvent extracts against *Staphylococcus aureus*

Solvent Dilutions	Distilled Water	Ethanol	Methanol	Ethyl acetate
100%	R	R	12mm	20mm
50%	R	R	12mm	15mm
25%	R	R	R	12mm
12.5%	R	R	R	12mm
6.25%	R	R	R	R

Table.3 Antibacterial activity of *Mangifera indica* bark solvent extracts against *Escherichia coli*

Solvent Dilutions	Distilled Water	Ethanol	Methanol	Ethyl acetate
100%	R	24mm	12mm	20mm
50%	R	13mm	10mm	16mm
25%	R	12mm	10mm	16mm
12.5%	R	10mm	R	12mm
6.25%	R	10mm	R	10mm

Table.4 Antibacterial activity of *Mangifera indica* bark solvent extracts against *Pseudomonas aeruginosa*

Solvent Dilutions	Distilled Water	Ethanol	Methanol	Ethyl acetate
100%	R	18mm	13mm	22mm
50%	R	16mm	12mm	22mm
25%	R	15mm	12mm	15mm
12.5%	R	11mm	11mm	12mm
6.25%	R	10mm	10mm	10mm

Table.5 Antibacterial activity of *Mangifera indica* bark solvent extracts against *Bacillus subtilis*

Solvent Dilutions	Distilled Water	Ethanol	Methanol	Ethyl acetate
100%	R	12mm	10mm	25mm
50%	R	10mm	R	18mm
25%	R	10mm	R	14mm
12.5%	R	10mm	R	10mm
6.25%	R	R	R	10mm

Table.6 Antibacterial activity of *Mangifera indica* bark solvent extracts against *Klebsiella pneumoniae*

Solvent Dilutions	Distilled Water	Ethanol	Methanol	Ethyl Acetate
100%	R	13mm	10mm	28mm
50%	R	12mm	10mm	25mm
25%	R	12mm	10mm	21mm
12.5%	R	12mm	10mm	21mm
6.25%	R	12mm	R	12mm

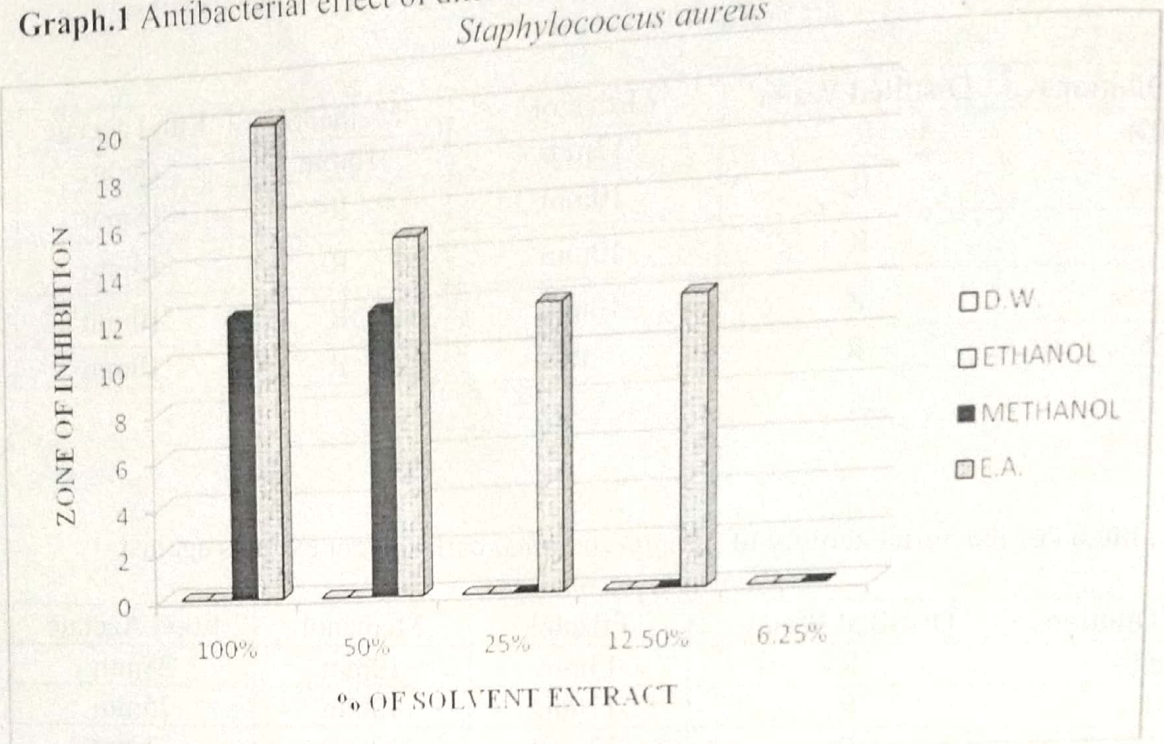
Where, R = Resistant

Table.7 Phytochemical analysis of *Mangifera indica* bark extracts

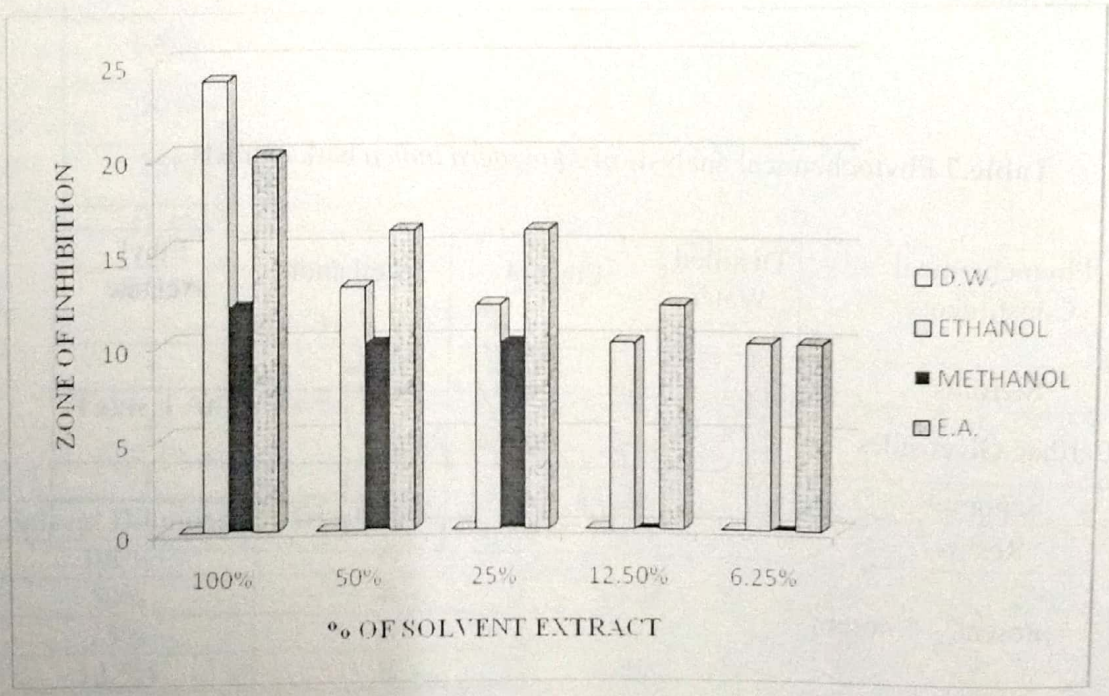
Phytochemical Constituents	Distilled Water	Ethanol	Methanol	Ethyl Acetate
Steroids	+	+	+	+
Cardiac Glycosides	+	+	+	+
Saponins	+	+	+	+
Resins	+	+	+	+

Where: + = present, - = absent

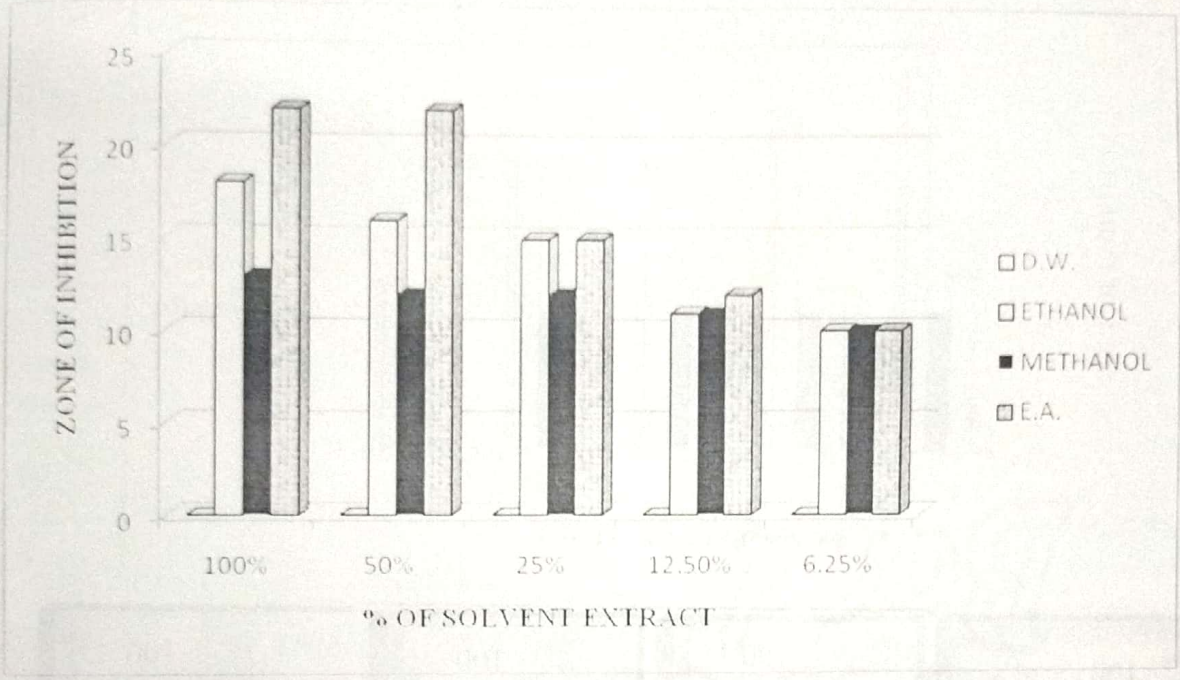
Graph.1 Antibacterial effect of different solvent extracts of *Mangifera indica* bark against *Staphylococcus aureus*



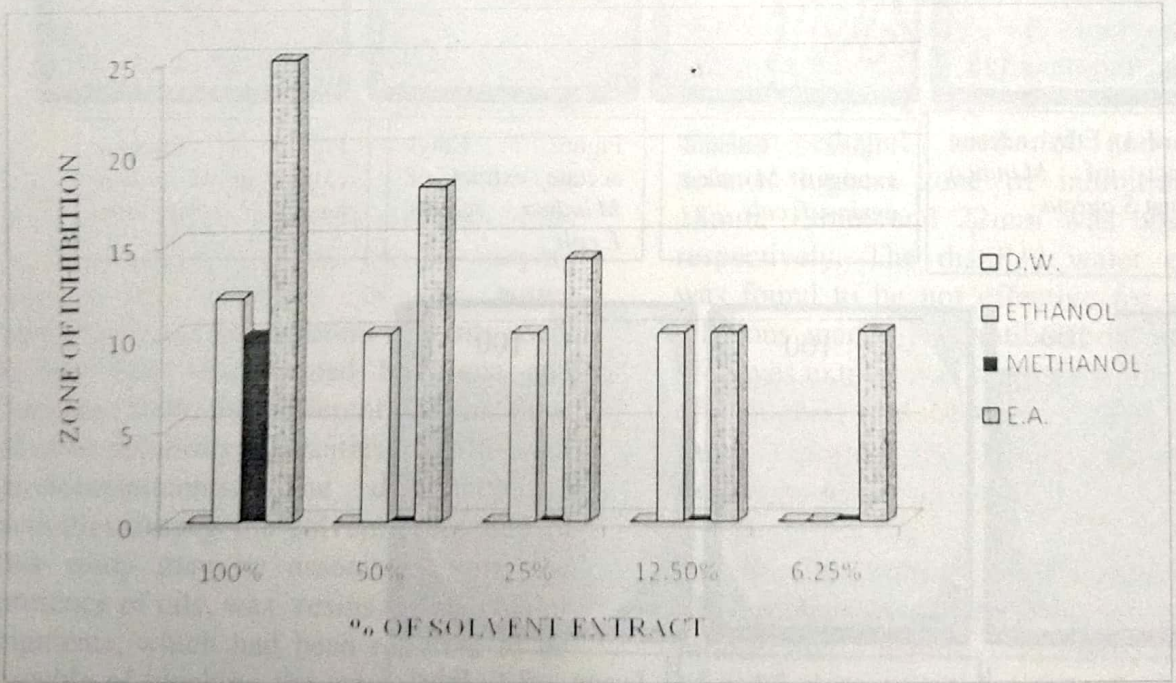
Graph.2 Antibacterial effect of different solvent extracts of *Mangifera indica* bark against *Escherichia coli*



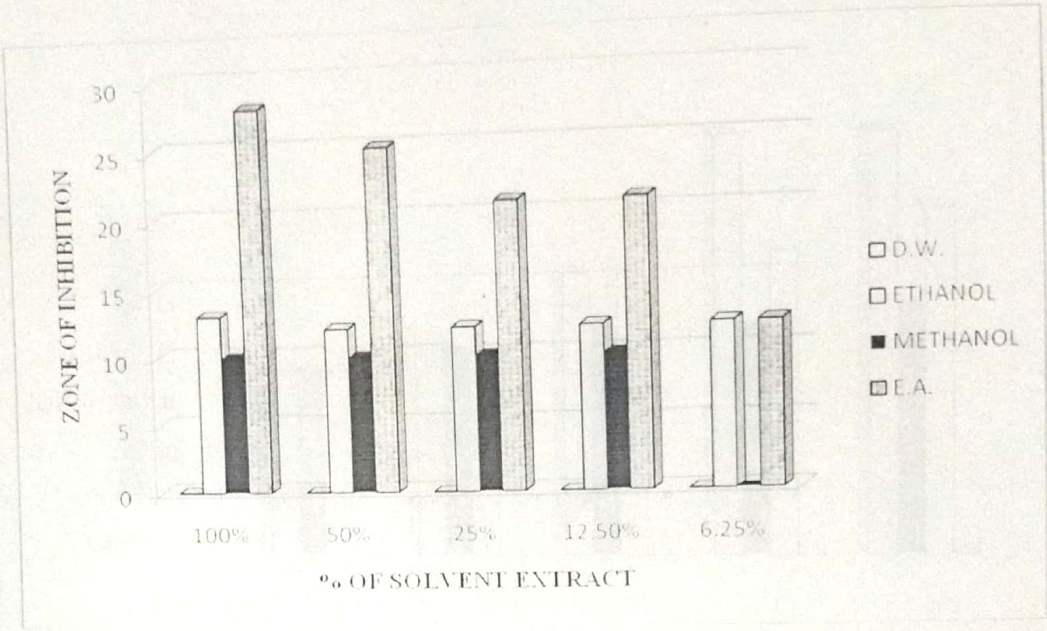
Graph.3 Antibacterial effect of different solvent extracts of *Mangifera indica* bark against *Pseudomonas aeruginosa*



Graph.4 Antibacterial effect of different solvent extracts of *Mangifera indica* bark against *Bacillus subtilis*



Graph.5 Antibacterial effect of different solvent extracts of *Mangifera indica* bark against *Klebsiella pneumoniae*



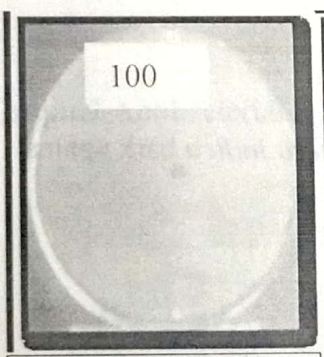


Figure 1: Ethyl acetate extract of *M.indica* against *S.aureus*

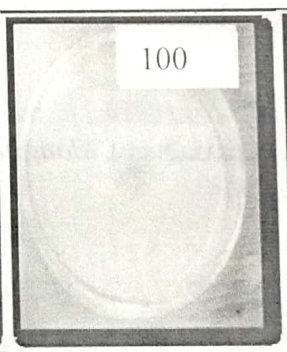


Figure 2: Ethanol extract of *M.indica* against *E.coli*

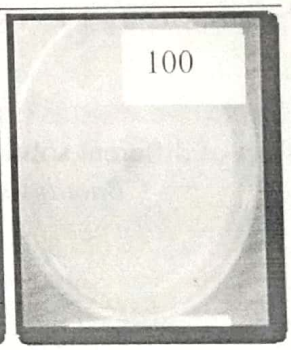


Figure 3: Ethyl acetate extract of *M.indica* against *E.coli*

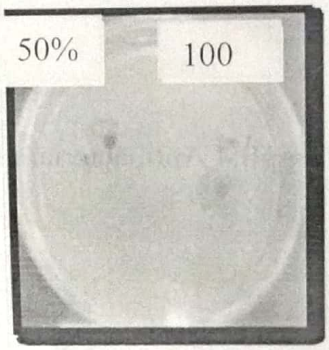


Figure 4: Ethanol extract of *M. indica* against *P. aeruginosa*

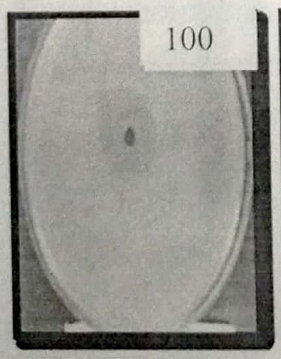


Figure 5: Ethyl acetate extract of *M. indica* against *B.subtilis*

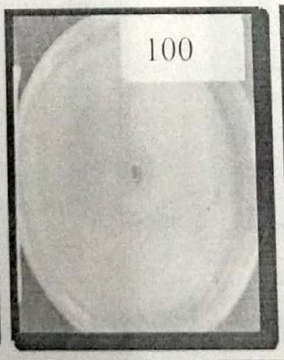


Figure 6: Ethanol extract of *M. indica* against *K.pneumoniae*

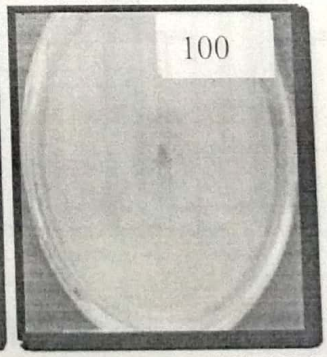


Figure 7: Ethyl acetate extract of *M. indica* against *K.pneumoniae*

The observed difference in the extract yields of different solvents might be due to the fact that the extracts have different solubility or due to the polarity of the solvents and was suggested by Lino and Deogracious (2006). The basic parameters influencing the quality of an extract are the plant part used as a starting material, the solvent used for extraction, extraction technology and sterilization method (Ncube et al., 2007). Another fact is that different extractable components were present in different quantities within the extracts (Joshua and Takudzwa, 2013).

The phytochemical test for all four extracts such as distilled water, methanol, ethanol, ethyl acetate was performed for the presence of steroids (Salwoski's test) cardiac glycosides (Keller Killian's test), saponins (Froth Test) and resins (Detection of Resins). The phytochemical analysis conducted on *M. indica* bark extracts revealed the presence of steroids, cardiac glycosides, saponins and resins which are known to be useful in the treatment of stress-related ailments and as dressings for wounds. These results were correlated with that of the Hausteen, 1983; Nobori et al., 1994; Li et al., 2003. The differences in the observed activities of the various extracts may be due to varying degrees of solubility of the active constituents in the different solvents used. It has been documented by Jigna and Sumitra (2006) that different solvents have diverse solubility capacities for different phytoconstituents. The difference in activities among the solvents recorded in this study may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported to be capable of blocking the active ingredients in the extract, thus preventing the bark extract from accessing the bacterial cell wall.

The methanol and ethyl acetate extract have shown antibacterial activity against *S. aureus*. Ethyl acetate extract has given the highest zone of inhibition (20mm) at 100% dilution. Distilled water did not show any antibacterial activity against *S. aureus* for all the dilutions tested. But there was some contradiction in the study conducted by Joshua and Takudzwa (2013), in that they obtained the antibacterial activity against *S. aureus* for all the dilutions of the extracts including that of distilled water extracts. According to their study distilled water extract was less potent. This can be attributed to the presence of water soluble compounds such as polysaccharide and polypeptides and have no real impact as antibacterial agent.

The 100% of ethanol solvent extract was more effective (24mm) against *E. coli* than ethyl acetate (20mm) and methanol (12mm). However, distilled water did not show any antibacterial activity against *E .coli* for all the dilutions tested. Except distilled water extract, all other extracts were found to be antibacterial against *Pseudomonas aeruginosa*. At 100% dilution ethanol, methanol and ethyl acetate, highest zone of inhibition i.e. 18mm, 13mm and 22mm was observed respectively. The distilled water extract was found to be not effective for all the dilutions made. The antibacterial activity of leaves extract was reported in the study of Doughari and Manzara (2008). Their study reported the similar results on *Pseudomonas aeruginosa*.

For *Bacillus subtilis*, 100% dilution of ethyl acetate extract have shown highest antibacterial activity (25mm), 100% dilution of ethanol (12mm) and methanol (10mm) zone was observed. Distilled water extract was not effective against *Bacillus subtilis* and some dilutions of

methanol i.e. 50%-6.25% also not effective against *Bacillus subtilis*.

Ethyl acetate extract was more effective against *Klebsiella pneumoniae* as compared to ethanol and methanol extract. Ethyl acetate extract have showed 36mm zone of inhibition at 12.5% dilution, 28mm for 50% and 21mm for 100% dilution. Methanol extract of 100%, 50%, 25%, 12.5% dilution showed 10mm zone of inhibition each. At 100% ethanol extract (13mm) while for the remaining dilutions 12mm zone of inhibition was observed.

Methanol and Ethyl acetate have showed good antibacterial activity comparatively than distilled water and ethanol extract. This is because they are the polar solvents. It can be suggested that some principle antibacterial components of this plant were polar compound. Most of the identified components with antibacterial activity extracted from plants might be the aromatic or saturated organic compounds which are more soluble in polar solvents such as distilled water and methanol reported by Eisenberg et al (1993). The present study demonstrated variation in antibacterial activity of different solvent extracts which suggested that the active components in the crude extract may be acting synergistically to produce antimicrobial effects (Elloff, 1998), the disparity between the activities of the extracts and the standard antimicrobial drug, may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotic (Olajuyigbe and Afolayan, 2012).

The results suggested that all the extracts except distilled water extract posses the compound with antibacterial properties which can be used as antibacterial agent in new drug for therapy of infectious diseases in human. According to Ncube et al (2007), the distilled water extracts were less potent. This can be attributed to the presence of water-soluble compounds such as polysaccharides and polypeptides, which are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents. On the other hand the antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases. Thus besides choice of a good solvent for extraction of active compounds, antibacterial activity also depends on phyto-constituents present in the plant. The contents of active ingredients in plant materials have been shown to fluctuate constantly with the genetic heterogeneity of plant species, differences in soil condition, variation in seasonal cycle, climatic influences, age of plant, alteration in weather, sun and shade fluctuations.

The bark extracts of *Mangifera indica* were found to have antibacterial activity against all the tested bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae*. The ethyl acetate extract was found to be most effective among all other solvent extracts used in the study. Distilled water extract of *Mangifera indica* bark was not effective of the entire tested organisms. Therefore it was suggested that the plant could be a source of oral drugs to be used in the treatment of infections and diseases and may be act as a source for industrial drug production. The bark extracts of *Mangifera indica* revealed the phytochemical compounds such as

steroids, cardiac glycosides, saponins and resins. The demonstration of activity against both gram positive and gram negative bacteria is an indication of broad spectrum of activity and thus can be used as a source of antibiotic substances for drug development that can be used in the control of the bacterial infections. Further investigations of its activity against a wider range of bacteria and fungi, identification and purification of its chemical constituents, and toxicological investigations of *Mangifera indica* plant extracts should be carried out with a view to developing novel drugs for human consumption.

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