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Antibacterial Activities of Some Commonly Used Medicinal Plants against Bacteria Isolates

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Abstract Despite the wide availability of clinically useful antibiotics and semi-synthetic analogues, a continuing search for new anti-infective agents remains indispensable, therefore this study was carried out to determine the antibacterial activities of some medicinal plants against bacterial isolates. The seeds, leaves and stem bark of some plants with medicinal claims were purchased from Itoku market, Abeokuta. The plant parts were grinded using an electric miller. The crude extract of the plants were prepared by cold maceration and were tested for antimicrobial activity using agar diffusion method. Those plants with activities were further extracted with N-hexane, Chloroform, Ethyl acetate, Butanol, Ethanol, Methanol and Water using continuous cold extraction technique with aid of separating funnel. Susceptibility testing of the extract obtained was performed using agar well diffusion and broth macro dilution techniques. Bactericidal effect and mode of action of the extracts were performed using Minimum Bactericidal Concentration (MBC) and time kill test technique. Phytochemistry was performed according to standard chemical techniques. Out of the twelve different medicinal plants screened, *Terminalia avicenniodes* and *Magnifera indica* possess antibacterial potentials. Methanol extract of *Terminalia avicenniodes bark* had the highest percentage yield of 17.8% while the ethanol extract of *Magnifera indica* leaves has the least percentage yield of 3.6 %. Susceptibility test revealed that the ethanol extract of the *Magnifera indica* leave against *Escherichia coli* ATCC 29929 has the widest Diameter Zone of Inhibition (DZI) (23.333±2.887), while the methanol extract of *Magnifera indica* leaves have no antibacterial effect on *Klebsiella pneumoniae* ATCC 4252. The methanol extract of the leaves of *Magnifera indica* against *Escherichia coli* ATCC 29929 and the methanol extract of the leaves of *Terminalia avicennoides* against *Klebsiella pneumoniae* ATCC 4252 and *Shigella dysentariae* have the highest MIC (12.500±0.000) while the ethanol extract of the leave and methanol extract of the leaves and stem bark of *Terminalia avicennoides* against *Staphylococcus aureus* ATCC 29293(3.125±0.000) has the lowest MIC. There was no significant difference in the MBC of all the extracts. The stem bark and leaves of the plants contains tannin, phytosterol, phenol, diterpenes, proteins and amino acid, reducing sugar and non reducing carbohydrate. The time kill test revealed that the methanol extract of the *Magnifera indica* leaves and the ethanol extract of *Terminalia avicenniodes* killed *Escherichia coli* ATCC 29929 within 60 mins, while most of the plant extracts killed *klebsiella pneumoniae* within 20hrs. In conclusion, *Terminalia avicennoides* and *Magnifera indica* have strong inhibitory activity against the bacteria isolates, and can be studied further for the Chemotherapeutic drug production.

Keywords: *Terminalia avicennoides, Magnifera indica, Antibacterial, Phytochemistry*

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1. Introduction

Gastrointestinal tract infections are very common. Diarrhea is the most common cause of death in developing countries (2.5 million deaths/year). There are an enormous number of microbes that cause disease in the intestines. Bacterial infections caused by *Salmonella enteritidis, Staphylococcus aureus, Escherichia coli* and newer food borne pathogens have become increasingly resistant to empirical antimicrobial agents [1]. Inspite of the fact that there is a wide range of current antibiotics available for treatment of bacterial infections, there are still some challenges to be met in microbial chemotherapy, some of which are the development of resistance by the microbes to chemotherapeutic agents due to abuse of use, high cost, limited effective lifespan and undesirable side effects of certain antibiotics [2,3,4]. Plants provide a variety of resources that contribute to the fundamental needs of

human such as food, clothing and shelter. Among plants of economic importance are medicinal plants. Plants have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms [5]. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs [6,7]. Natural products such as plant extracts, either as pure compounds or as standardized extracts provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [8]. Plants with potential medicinal activity have recently come to the attention of Western scientists, and studies have reported that some are bioactive [9]. Certain phytochemical components such as alkaloids, saponins, polyphenols, anthraqinones, cardiac glycosides which are present in the plants are the bioactive bases for the medicinal properties. These substances have been reported to be responsible for the antimicrobial activity exhibited by the medicinal characteristics in plant [10].

2. Materials and Methods

2.1. Plant Collection

Twelve different medicinal plants were purchased from the Itoku market of Abeokuta, Ogun State, Nigeria. The plant barks and seeds were identified by Mr. Owonikoko at the National Cereal Research Institute, Ibadan, Nigeria, while the leaves were identified by Mr. Olugbade at the Federal College of Animal Health and Production Technology, Ibadan, Nigeria as *Hunteria umbellata* (Abeere seed), *Magnifera indica* (Mango leaves), *Mandia whitei* (Ishirigun root), *Calliandra haematocephala* (Tude root), *Aristolochia ringens* (Akogun root), *Morinda lucida* (Oruwo bark), *Terminalia avicenniodes* (Idin bark), *Terminalia avicenniodes* (Idin leaves), *Enantia chlorantha* (Awopa bark), *Piper gineense* (Iyere seed), *Tetrapleura tetraplera* (Onigun merin), *Magnifera indica* (Mango bark). All plants were dried under cool environment condition for one week, after which they were grinded into powdered form using an electric miller. The powders were sieved to remove shaft in order to get a finer texture.

2.2. Crude Extraction Process

A pilot study was conducted on all the twelve plants in which three different extracting agents were used. Ten (10)g of each powder was dispensed in 100ml of each extracting solvents (ethanol, methanol or water) in beaker and was allowed to stand for 72 hours. The extracts were filtered using Whattman filter paper No 1. and the filtrates were used for antibacterial susceptibility test against six different American Type Culture Collection (ATCC) strains _ viz *Escherichia coli, Staphyloccus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosae and klebsiella pneumoniae.* Plants that showed clearer and wider zones of inhibition were extracted in large quantities of solvent to plant powder. The plants powder (200g) were soaked into 1000ml of solvent for seven (7) days (ratio 5:1). The extracts were filtered using Whattman filter paper No 1, After which the filtrates were evaporated to dryness at reduced pressure using a rotary evaporator. The extracts were further concentrated in a waterbath.

2.3. Antibacterial Susceptibility Testing Using Agar Well Diffusion Technique

Mueller Hilton agar was prepared according to the manufacturer's instruction and sterilized, It was dispersed into sterile petri dishes asceptically. The agar plates were allowed to solidify and were dried in the oven at 37°C for 15 mins. The Mueller Hilton agar plates were inoculated with 0.5 macfarland standardized bacterial broth culture by flooding and the excess was decanted. With the aid of 6mm diameter sterile core borer, wells were bored on the inoculated agar plate at equidistance to each other. Using a standard micropipette, 100ul of the extracts were dispersed into the wells with corresponding extract code and label. The plates were allowed to stay on the bench for 15 minutes for pre-diffusion of the extract and were incubated at 37°C for 24hours. The diameter zone of inhibition was measured in millimeters using a meter rule. The procedure was repeated in triplicates. Antibiotics susceptibility test using standard antibiotics disc was set up simultaneously. Controls were set up using only the extracting agents without the plant extract. The diameter zone of inhibition of the controls were noted, these were subtracted from the diameter zone of inhibition of the extract plus the extracting agent.

2.4. Percentage Yield

The residue was dried to powdered form to calculate the percentage yield as follows:

$$\% \; yield = \frac{W_2}{W_1} \times 100$$

W_1 = Weight of powdered plant before extraction
W_2 = Weight of powdered plant after extraction

2.5. Determination of Minimum Inhibitory Concentration (Mic) and Minimum Bactericidal Concentration (Mbc)

The MIC of the extracts were determined by diluting to 100g of the extract various concentrations. Serial dilution of plant extracts were prepared to achieve the following concentrations as follows: 100mg/ml to 50mg/ml to 25mg/ml to 12.5mg/ml to 6.25mg/ml to 3.125mg/ml to 1.56mg/ml to 0.78mg/ml to 0.39mg/ml to 0.195mg/ml. Twelve sterile tubes were and labeled accordingly corresponding to the decreased concentration order. 1ml of sterile nutrient broth was added into all test tubes except test tube one and test tube twelve. 1ml of the extract was added into test tube one and two. 1ml of the mixture was transferred from test tube two to three, three to four and so on, the process was continued till the last tube. Specifically 0.1ml of standardized inoculums of 1 to 2 X 10^7 cfu/ml was added to each tube. The tubes were incubated aerobically at 37°C for 18-24hrs. Two control

tubes was maintained for each test batch. This is as follows: tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculums (organism control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes. The MBC was determined by sub-culturing the test dilution on fresh solid medium and further incubated at 37°C for 18-24 hrs. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

2.6. Phytochemical Screening Methods

The screening procedures used was adapted from the work of Prashani Tiwari, et.,al [11].

The test for **alkaloids** was carried out by subjecting 0.5g aqueous extract in 5ml 1% HCl, boiled, filtered and treated with Mayer's reagent (Potassium Mecuric Iodide), Wagner's reagent and Dragendroff's reagent (Potasssium Bismuth Iodide). Formation of yellow precipitate, brown precipitate and red precipitate indicated presence of alkaloids.

Glycosides was identified by subjecting 0.5g extract in 5ml 1% HCl and treated with Ferric Chloride solution to carry out Modified Borntrager's test. It was then immersed in boiling water for about 5 mins, the mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution, formation of rose pink colour in the ammoniacal layer indicates the presence of anthranol glycosides. Legal's test was carried out by treating extract with sodium nitropruside in pyridine and sodium hydroxide, formation of pink to blood red colour indicates the presence of cardiac glycosides.

The presence of **flavonoid** was determined using alkaline reagent test, extracts were treated with few drops of sodium hydroxide solution, formation of intense-yellow color which becomes colorless on addition of dilute acid, indicates presence of flavonoids. The extract was also tested for **carbohydrates** using Molisch's test, benedict's test and fehling's test, Extract were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. For Molisch's test, Filtrates were treated with 2 drops of alcoholic α-naphtol solution in a testtube. For Benedict's test, Filtrates were treated with Benedict's reagent and heated gently. For fehling's test, filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions, formation of red precipitate indicates the presence of reducing sugars.

The extract was subjected to frothing test and foaming test for the identification of saponin. Extracts were diluted with distilled water to 20ml and was shaken in a graduated cylinder for 15 mins for froth test, formation of 1cm layer of foam indicates saponin and the persistence of the foam for 10mins indicated positive foam test.

For the detection of **phytosterols**, Salkowski test was carried out by treating extracts with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes. Libermann Burchard's test was also carried out, extracts were treated with chloroform and filtered. The filtered were treated with few drops of acetic anhydride, boiled and cooled. Conc Sulphuric acid was added, formation of brown ring at the junction indicated the presence of phytosterols.

For **phenols**, Ferric Chloride test was carried out by treating 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols. The test for **tannins** was carried out by subjecting 3g of each plant extract in 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicates the presence of tannins. Detection of **Protein and Amino acid** was carried out by Xanthoproteic test, the extract were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins. For the presence of **Anthraquinone**, 1g of powdered plant was boiled with 2ml of 10% HCL for 5 mins, it was filtered while hot and the filterate was allowed to cool. The cooled filtrates was partitioned against equal volumes of chloroform, vigorous shaking was avoided. A clean pipette was used to transfer the chloroform layer to a clean testtube and 10% ammonium solution, it was shaked and the layer was allowed to separate. Rose pink color indicated the presence of anthraquinone. The presence of was detected by dissolving extracts in water and treated with drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

2.7. Time Kill Test

Bactericidal kinetic assay were performed in sterile petri dishes containing 20ml of Mueller Hinton agar. The extracts were used at the MBC concentrations. An inoculum containing approximately 5x10cfu/ml was introduced into the Mueller Hinton broth containing various extracts. were inoculated on the sterile Mueller Hinton agar plate. One loopful was streaked on the plate at 0 mins, 5 mins, 30 mins, 1hrs, 2hrs, 4hrs, 8hrs, 12hrs, 16hrs, 20hrs and 24hrs and incubated at 37°C for 24hrs. After 24hrs, growth was observed and recorded, and kill charts were plotted with time against extract concentrations.

3. Results

The profile of some medicinal plants collected from Itoku market, Abeokuta and their uses is presented in Table 1, there are different claims for the plants which ranges from antimicrobial activities to anti-inflammatory activities.

Six ATCC strains of bacteria isolates used in this study includes *Salmonella typhi* (ATCC 14028), *Shigella dysentariae* (ATCC 23354), *Escherichia coli* (ATCC 29929), *Klebsiella pneumoniae* (ATCC 4252), *Pseudomonas aeruginosa* (ATCC 27953) and *Staphlococcus aureus* (ATCC 29293) (Table 2).

Table 3 shows the percentage yield of crude extract of the medicinal plants, the methanol extract of *Terminalia avicenniodes bark* possess the highest percentage yield of 17.8% while the ethanol extract *Magnifera indica* has the least percentage yield of 3.6 %.

Table 1. Profile of some medicinal plants collected from Itoku market, Abeokuta and their uses.

Plant botanical name	Family name	Local name	Part	Medicinal uses
Hunteria umbellata	Apocynaceae	Abeere	Seed	Treatment of Ascaris
Magnifera indica	Anacardiaceae	Mango	Leaves	Anti inflammatory action
Mandia whitei	Apocynaceae	Ishirigun	Root	Aphrodisiac & Antidepressant action
Calliandra haematocephala	Fabaceae	Tude root	Root	Anti ulcerogenic action Anti inflammatory
Aristolochia ringens	Aristolochiaceae	Akogun	Root	Anti cancer activity
Morinda lucida	Rubiaceae	Oruwo	Stem bark	Anti malaria activity
Terminalia avicenniodes	Combretaceae	Idin	Stem bark	Treatment of skin infection, GIT disorder
Terminalia avicenniodes	Combretaceae	Idin	Leaves	Dental care and ulcer treatment
Enantia chlorantha	Annonaceae	Awopa	Stem bark	Anti malarial action
Piper gineense	piperaceae	Iyere	Seed	Aseptic activity(flatulence)
Tetrapleura tetraplera	mimosaceae	Onigun merin ,Aidan,uhio	Seed	Anti inflammatory activity
Magnifera indica	Anacardiaceae	Mango	Stem bark	Anti inflammatory action

Table 2. ATCC Strains of Bacteria isolate used to determine the antibacterial efficacy of some plants in Itoku market, Abeokuta.

Bacteria isolates	Strains
Salmonella typhi	ATCC 14028
Shigella dysentariae	ATCC 23354
Escherichia coli	ATCC 29929
Klebsiella pneumoniae	ATCC 4252
Pseudomonas aeruginosa	ATCC 27953
Staphilococcus aureus	ATCC 29293

ATCC: American Type Culture Collection

Table 3. Percentage yield of crude extracts of some medicinal plants purchased at Itoku market, Abeokuta.

Plant botanical name	Local name	Part	Ethanol extract			Methanol extract		
			W1(g)	W2(g)	(%)YIELD	W1(g)	W2(g)	(%) YIELD
Magnifera indica	Mango	Stem bark	200	30.9	(15.45)	200	14.2	(7.1)
Magnifera indica	Mango	leaves	150	5.4	(3.6)	150	21.2	(14.13)
Terminalia avicenniodes	Idin	Stem bark	200	31.3	(15.65)	200	35.6	(17.8)
Terminalia avicenniodes	Idin	Leaves	100	12.2	(12.2)	100	12.2	(12.2)

Table 4. Antibacterial susceptibility test of some medicinal plant extract against ATCC strains of bacteria isolates.

Plant botanical name	*Salmonella typhi* ATCC 14028			*Shigella dysentariae* ATCC 23354			*Escherichia coli* ATCC 29929			*Pseudomonas aeruginosae* ATCC 27953			*Klebsiella Pneumoniae* ATCC 4252			*Staphilococcus aureus* ATCC 29293		
	W	M	E	W	M	E	W	M	E	W	M	E	W	M	E	W	M	E
Hunteria umbellata	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Magnifera indica	+	+	+	-	+	+	-	+	++	-	++	+	-	+	+	+	+	++
Mandia whitei	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Calliandra haematocephala	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
Aristolochia ringens	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Morinda lucida	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Terminalia avicenniodes	+	++	+	+	+	+	-	+	+	-	+	+	-	++	+	+	++	+
Terminalia avicenniodes	+	++	+	-	++	+	+	+	+	-	+	+	-	+	+	-	++	+
Enantia chlorantha	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
Piper gineense	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+	-	+	+
Tetrapleura tetraplera	-	+	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-
Magnifera indica	+	+	++	+	+	++	+	+	+	+	-	+	-	+	+	+	+	++

Key: (+) Sensitive, (-) Resistant, (W) Water, (E) Ethanol, (M) Methanol。

Antibacterial susceptibility of some medicinal plants extract against some ATCC strain bacteria isolates was shown in Table 4. None of the aqueous extracts of all the medicinal plants used was sensitive to *Klebsiella pneumoniae* (ATCC 4252), the methanol and ethanol extracts of some of the medicinal plants were sensitive to most of the organism used. *Mandai whitei* is not sensitive to all the bacteria isolates except *Shigella dysentariae* (ATCC 23354) the bark and leaf of *Magnifera indica* and *Terminalia avicenniodes* were strongly sensitive to all the ATCC bacteria strains used except in rare cases.

Table 5. Comparative Mean Diameter Zone of Inhibition among some Antibiotics against ATCC Strains of Bacteria using agar well diffusion method

Some ATCC Strains of Bacteria	N	Diameter Zones of Inhibition (mm)			F-ratio	P-value
		Gentamycin	Ofloxacillin	Ciprofloxacin		
		Mean±SD	Mean±SD	Mean±SD		
Salmonella typhi ATCC 14028	3	0.000 ± 0.000	0.000± 0.000	25.000± 1.000	1875.0	<0.05
Shigella dysentariae ATCC 23354	3	0.000 ± 0.000	17.333±4.933	12.000± 0.000	29.148	<0.05
Escherichia coli ATCC 29929	3	19.000±1.732	24.333± 2.517	22.667± 1.155	6.279	<0.05
Pseudomonas aeruginosae ATCC 27953	3	10.000± 0.000	9.000 ± 0.000	18.666± 1.155	190.62	<0.05
Staphilococcus aureus ATCC 29293	3	11.333±0.5774	27.000± 0.000	0.000 ± 0.000	4962.2	<0.05

Table 6. Comparative Mean Diameter Zone of Inhibition of extract of *Magnifera indica* against ATCC Strains of Bacteria

Bacteria strains	Diameter Zone of Inhibition of extract of *Magnifera indica* (mm)				F-ratio	P-value
	Leave		Bark			
	Methanol extract (mg/mL)	Ethanol extract (mg/mL)	Methanol extract (mg/mL)	Ethanol extract (mg/mL)		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Salmonella typhi ATCC 14028	14.667±2.309	20.333±4.509	18.333±5.774	21.000±5.568	1.080	>0.05
Shigella dysentariae ATCC 23354	9.000 ± 7.810	18.333±2.082	20.000±3.000	19.333±1.155	4.217	<0.05
Escherichia coli ATCC 29929	17.333±2.309	23.333±2.887	20.000±5.000	19.333±1.155	1.867	>0.05
Klebsiella Pneumoniae ATCC 4252	0.000 ± 0.000	6.667±11.547	6.667±11.547	6.667±11.547	0.3334	>0.05
Pseudomonas aeruginosae ATCC 27953	4.667 ± 8.083	10.000±8.660	11.667±10.116	16.667±5.774	1.064	>0.05
Staphilococcus aureus ATCC 29293	14.667±2.309	21.667±2.887	17.333 ±4.041	21.000±3.606	2.997	>0.05

Table 7. Comparative Mean Diameter Zone of Inhibition of *Terminalia avicenniodes* against ATCC Strains of Bacteria using agar well diffusion method

Bacteria strains	Diameter Zone of Inhibition of extract of *Terminalia avicenniodes* (mm)				F-ratio	P-value
	Leave		Bark			
	Methanol extract (mg/mL)	Ethanol extract (mg/mL)	Methanol extract (mg/mL)	Ethanol extract (mg/mL)		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
*Salmonella typhi*ATCC 14028	19.000±5.196	21.000±6.557	21.667±7.638	22.000±7.211	0.03539	>0.05
Shigella ATCC 23354	17.000±2.646	19.000±3.606	19.333±1.155	15.667±5.132	0.7526	>0.05
Escherichia coli ATCC 29929	17.667±3.786	20.667±1.155	18.333±2.887	17.333±2.309	0.9245	>0.05
Klebsiella Pneumoniae ATCC 4252	5.000±8.660	13.333±11.547	11.667±10.408	13.333±11.547	0.4197	>0.05
Pseudomonas aeruginosae ATCC 27953	11.667±10.408	11.667±10.408	11.667±10.408	10.408±11.269	0.01052	>0.05
Staphilococcus aureus ATCC 29293	15.333± 2.517	17.667± 6.429	16.667± 2.887	20.000± 5.000	0.5748	>0.05

The comparative mean diameter zone of inhibition of some standard antibiotics against ATCC strains of bacteria is shown in Table 5. Ofloxacillin has the significant increased diameter zone of inhibition against *Staphlococcus aureus* (ATCC 29293), p value <0.05. *Salmonella typi* ATCC 14028 and *Shigella dysentariae* (ATCC 23354) were significant to Gentamycin, F ratio 361.02, p value < 0.05, there is a significant increase in the mean diameter zone of inhibition of Gentamicin (19.00±1.73), Ofloxacillin (27.00±0.00) and Ciprofloxacin (25.00±1.00) against *Escherichia coli* (ATCC 29929), *Staphlococcus aureus* (ATCC 29293), and *Salmonella typhi(*ATCC 14028) respectively p< 0.05.

Table 6 shows the comparative mean diameter zone of inhibition of ethanol and methanol extracts of the leaves and stem bark of *Magnifera indica* against ATCC strains of bacteria, there is no significant difference in the diameter zone of inhibition of ethanol and methanol extracts of the leave and stem bark of *Magnifera indica* against all the ATCC strains of bacteria p>0.05 except in *Shigella dysentariae* (ATTC 23354) where there is statistical decrease in the diameter zone of inhibition of methanol extract, F ratio 4.217, p<0.05. However, The ethanol and methanol extract of the leaves of *Magnifera indica* has an increase in the mean diameter zone of inhibition (23.33±2.887) and (17.33±2.09) respectively against *Escherichia coli* ATCC 29929 when compared with other bacteria while the ethanol and methanol extracts of the bark of *Magnifera indica* has a significant increase in the diameter zone of inhibition among *Staphlococcus aureus* ATCC 29293, (21.00±3.606), *Salmonella typhi* ATCC 14028, (21.00±5.5680) and *Shigella dysentariae* ATCC,(20.00±3.00), *Escherichia coli* ATCC 29929, (20.00±5.00) respectively p value >0.05.Also it is statistically shown that there is no significant difference in the methanol and ethanol extract of *Magnifera indica* against all the ATCC strains of bacteria isolates used for this study.

Table 7 shows the comparative mean diameter zone of inhibition of methanol and ethanol extracts of *Terminalia avicenniodes* against ATCC strains of bacteria, there is no significant difference in the mean diameter zone of inhibition exhibited by the methanol and ethanol leave and bark extracts of *Terminalia avicenniodes* against all the ATTC strain of bacteria, p>0.05. However, there is an increase in the mean diameter zone of inhibition of methanol and ethanol extracts of the leave and stem bark of *Terminalia avicenniodes* against *Salmonella typhi* ATTC 14028 (19.0+-5.196, 21.667+-7.638, 21.00+-6.557, and 22.00+-7.211) while there is decrease in the mean diameter zone of inhibition of the leave of *Terminalia avicenniodes* against *Klebsiella pneumoniae* (ATTC4252) (5.0+-8.66).

Table 8. Comparative Mean Minimum inhibitory concentration between methanol and ethanol extracts of *Magnifera indica* against Some ATCC Strains of Bacteria using broth dilution method

Bacteria strains	Minimum inhibitory concentration (MIC) of *Magnifera indica* (mm)				F-ratio	P-value
	Leave		Bark			
	Methanol extract (mg/mL)	Ethanol extract (mg/mL)	Methanol extract (mg/mL)	Ethanol extract (mg/mL)		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Klebsiella Pneumoniae ATCC 4252	9.375±4.419	3.516± 3.867	6.250±0.000	4.688±2.210	1.310	>0.05
Pseudomonas aeruginosae ATCC 27953	7.813 ±6.629	4.688± 2.210	9.375±4.419	4.688 ± 2.210	0.5999	>0.05
Staphilococcus aureus ATCC 29293	6.250 ±0.000	4.688±2.210	3.125 ±0.000	3.125 ±0.000	3.666	>0.05
Salmonella typhi ATCC 14028	9.375 ±4.419	4.688±2.210	9.375±4.419	4.688 ±2.210	1.889	>0.05
Escherichia coli ATCC 29929	12.500±0.000	6.250±0.000	6.250 ± 0.000	7.813± 6.629	1.103	>0.05
Shigella dysentariae ATCC 23354	9.375 ± 4.419	6.250 ± 0.000	6.250 ± 0.000	6.250 ± 0.000	1.000	>0.05

Table 9. Comparative Mean Minimum inhibitory concentration between methanol and ethanol extracts of *Terminalia avicenniodes* against ATCC Strains of Bacteria using broth dilution method

Bacteria strains	Minimum inhibitory concentration (MIC) of *Terminalia avicenniodes* (mm)				F-ratio	P-value
	Leave		Bark			
	Methanol extract (mg/mL)	Ethanol extract (mg/mL)	Methanol extract (mg/mL)	Ethanol extract (mg/mL)		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Klebsiella Pneumoniae ATCC 4252	12.500 ±0.000	6.250 ± 0.000	9.375 ±4.419	3.125 ±0.000	6.668	<0.05
Pseudomonas aeruginosae ATCC 27953	6.250 ±0.000	9.375 ±4.419	6.250 ±0.000	9.375 ±4.419	0.6668	>0.05
Staphilococcus aureus ATCC 29293	1.563 ± 0.000	1.563 ±0.000	1.563 ± 0.000	3.125 ±0.000	8.021	<0.05
Salmonella typhi ATCC 14028	6.250 ± 0.000	6.250 ± 0.000	4.688 ±2.210	6.250 ± 0.000	0.9991	>0.05
Escherichia coli ATCC 29929	9.375 ± 4.419	9.375 ±4.419	7.813 ±6.629	4.688 ±2.210	0.4444	>0.05
Shigella dysentariae ATCC 23354	12.500± 0.000	9.375 ±4.419	6.250 ± 0.000	6.250 ± 0.000	3.667	>0.05

Table 10. Comparative Mean Minimum bactericidal concentration between methanol and ethanol extracts of *Magnifera indica* against ATCC Strains of Bacteria Isolates.

Bacteria strains	Minimum bactericidal concentration (MBC) of *Magnifera indica* (mm)				F-ratio	P-value
	Leave		Bark			
	Methanol extract (mg/mL)	Ethanol extract (mg/mL)	Methanol extract (mg/mL)	Ethanol extract (mg/mL)		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Salmonella typhi ATCC 14028	14.583 ± 9.547	14.583 ± 9.547	10.417 ± 3.608	16.667 ± 7.217	0.3333	>0.05
Shigella dysentariae ATCC 23354	16.667 ± 7.217	25.000 ± 0.000	12.500 ± 0.000	16.667 ± 7.217	3.167	>0.05
Escherichia coli ATCC 29929	29.167 ± 19.094	16.667 ± 7.217	8.594 ± 6.766	16.667 ± 7.217	1.683	>0.05
Klebsiella Pneumoniae ATCC 4252	10.417 ± 3.608	10.417 ± 3.608	10.417 ± 3.608	9.375 ± 5.413	0.04765	>0.05
Pseudomonas aeruginosae ATCC 27953	13.542 ± 10.975	18.750 ± 10.825	14.583 ± 9.547	13.542 ± 10.975	0.1642	>0.05
Staphilococcus aureus ATCC 29293	12.500 ± 0.000	5.208 ± 1.804	7.292 ± 4.774	8.333 ± 3.608	2.889	>0.05

Table 8 shows the comparative mean minimum inhibitory concentration of methanol and ethanol extract of the leaves and stem bark *Magnifera indica*. There is no significant difference in the mean minimum inhibitory concentration of methanol and ethanol extracts of the leaf and stem bark of *Magnifera indica* when compared with one another, p>0.05. However, the methanol extract of the leave of *Magnifera indica* against *Escherichia coli* ATCC 29929 (12.500±0.000) has the highest mean minimum inhibitory concentration while the ethanol and methanol of the stem bark of *Magnifera indica* against *Staphilococcus aureus* ATCC 29293 (3.125±0.000) has the lowest mean minimum inhibitory concentration.

Table 9 shows the comparative mean minimum inhibitory concentration of methanol and ethanol extract of the leaves and stem bark *Terminalia avicenniodes*. There is no significant difference in the mean minimum inhibitory concentration of methanol and ethanol extracts of the leaf and bark of *Terminalia avicenniodes* except against *Klebsiella pneumoniae* ATCC 4252 and *Staphilococcus aureus* ATCC 29293. Ethanol extract of the bark of *Terminalia avicenniodes* has the highest inhibitory activity against *Klebsiella pneumoniae* ATCC 4252 (3.125±0.000) while it has the least inhibitory activity against *Staphilococcus aureus* ATCC 29293 (3.125±0.000).

Table 10 shows the comparative mean minimum bactericidal concentration of methanol and ethanol extract of the leaves and stem bark of *Magnifera indica*. There is no significant difference in the mean minimum bactericidal concentration of methanol and ethanol extracts of the leaf and bark of *Magnifera indica*, p>0.05. There is an increase mean minimum bactericidal concentration of methanol extract of the leave of *Magnifera indica* which has the highest inhibitory activity against *Escherichia coli* ATCC 29929(29.167±19.094) while the ethanol extract of the *Magnifera indica* leave has the least inhibitory activity against *Staphilococcus aureus* ATCC 29293 (5.208±1.804).

Table 11. Comparative Mean Minimum bactericidal concentration between methanol and ethanol extracts of *Terminalia avicenniodes* against ATCC Strains of Bacteria Isolates.

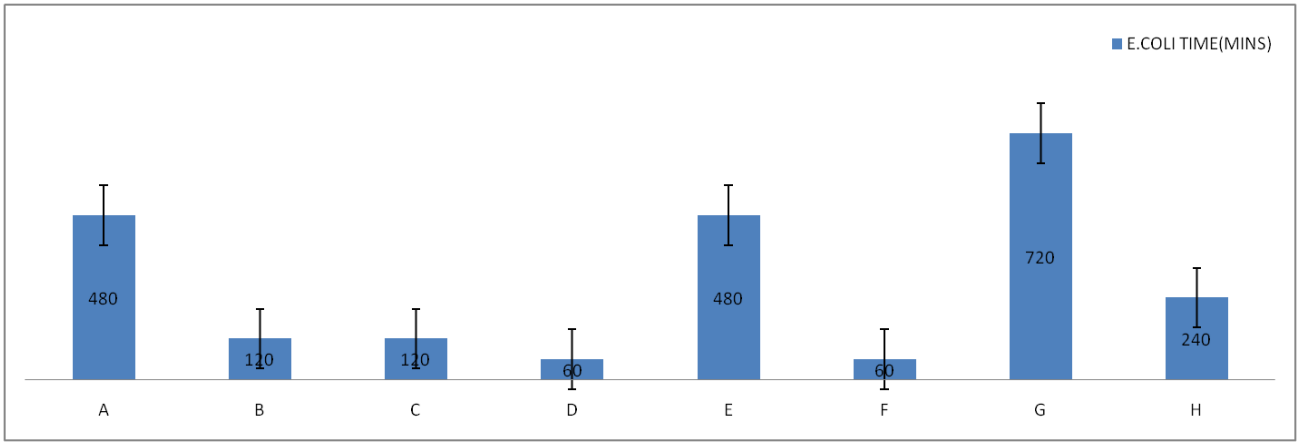
Bacteria strains	Minimum bactericidal concentration (MBC) of *Terminalia avicenniodes* (mm)				F-ratio	P-value
	Leave		Bark			
	Methanol extract (mg/mL)	Ethanol extract (mg/mL)	Methanol extract (mg/mL)	Ethanol extract (mg/mL)		
	Mean±SD Mean±SD	Mean±SD Mean±SD	Mean±SD Mean±SD	Mean±SD Mean±SD		
Salmonella typhi ATCC 14028	16.667 ± 7.217	10.417 ± 3.608	8.333 ± 3.608	12.500 ± 10.825	0.7779	>0.05
Shigella dysentariae ATCC 23354	16.667 ± 7.217	12.500 ± 0.000	16.667 ± 7.217	16.667 ± 7.217	0.3334	>0.05
Escherichia coli ATCC 29929	16.667 ± 7.217	50.000 ± 43.301	14.583 ± 9.547	41.667 ± 50.518	0.8310	>0.05
Klebsiella Pneumoniae ATCC 4252	9.375 ± 5.413	45.833 ± 47.324	12.500 ± 0.000	12.500 ± 0.000	1.574	>0.05
Pseudomonas aeruginosae ATCC 27953	14.583 ± 9.547	18.750 ± 10.825	14.583 ± 9.547	14.583 ± 9.547	0.1334	>0.05
Staphilococcus aureus ATCC 29293	14.583 ± 9.547	10.417 ± 3.608	12.500 ± 10.825	22.917 ± 23.662	0.4611	>0.05

Table 12. Phytochemical analysis of crude extract of the screened plants.

Constituents	Screened plants			
	Magnifera indica Stem Bark	*Magnifera indica* Leave	*Terminalia avicenniodes* Stem Bark	*Terminalia avicenniodes* Leave
Alkaloids	-	+	-	-
Tannins	+	+	+	+
Saponins	-	-	+	+
Anthraquinone	-	-	-	-
Flavonoids	-	-	-	-
Cardiac glycosides	+	+	-	+
Cyanogenic glycosides	+	+	-	+
Phytosterols	+	+	+	+
Phenol	+	+	+	+
Diterpenes	+	+	+	+
Proein and Amino acid	+	+	+	+
Reducing and non-reducing carbohydrates	+	+	+	+

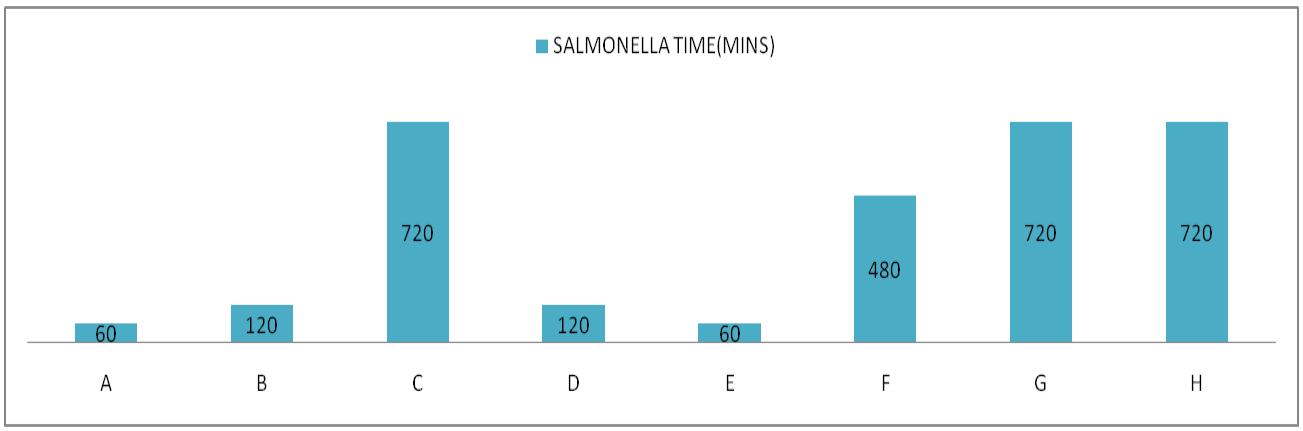
Table 11 shows the comparative mean minimum bactericidal concentration of methanol and ethanol extract of the leaves and stem bark of *Terminalia avicenniodes*. There is no significant difference in the mean minimum bactericidal concentration of methanol and ethanol extracts of the leaf and bark of *Terminalia avicenniodes*, p>0.05. There is an increase mean minimum bactericidal concentration of ethanol extract of the leave of *Terminalia avicenniodes* which has the highest inhibitory activity against *Escherichia coli* ATCC 29929 (50.000±43.301) while it has the least inhibitory activity against *Salmonella typhi* ATCC 14028 (8.333±3.608).

Table 12. *Magnifera indica* and *Terminalia avicennoides* stem bark and leaves contains tannin, phytosterol, phenol, diterpenes, proteins and amino acid, reducing sugar and non reducing carbohydrate except saponin and cardiac glycosides & cynogenic glycosides which is not present in *Magnifera indica* and *Terminalia avicennoides* stembark respectively. Meanwhile, both bark and leaves and *Terminalia avicennoides* and *Magnifera indica* contains no anthraquinonne and flavonoids.



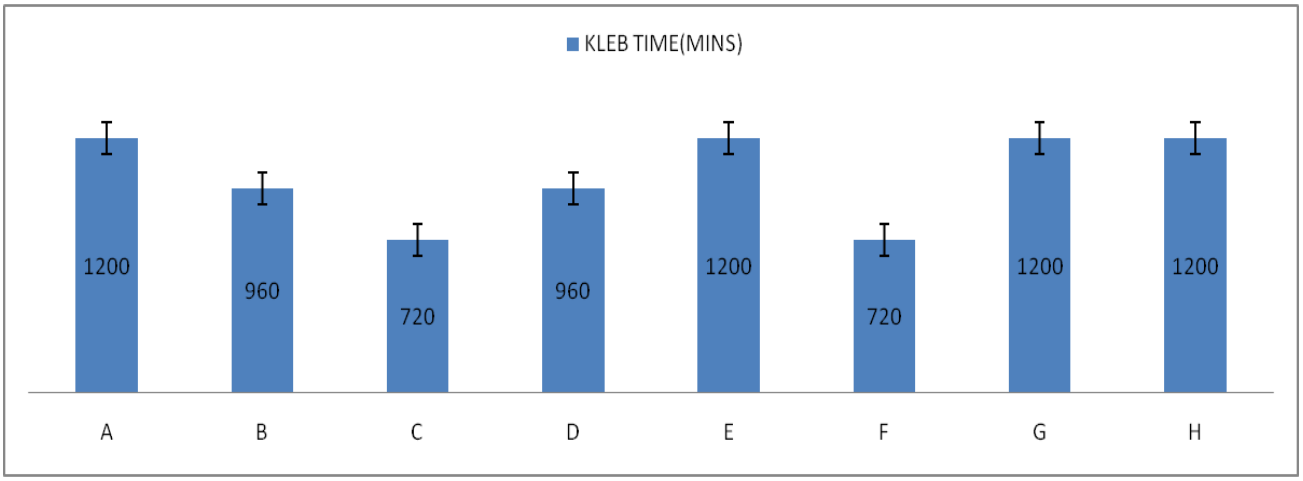
Key: A: Ethanol Extract of *Magnifera Indica* Stem Bark, B: Ethanol Extract of *Magnifera Indica* Leave, C: Methanol Extract of *Magnifera Indica* Stem Bark, D: Methanol Extract of *Magnifera Indica* Leave, E: Ethanol Extract of *Terminalia Avicenniodes* Stem Bark, F: Ethanol Extract of *Terminalia Avicenniodes* Leave, G: Methanol Extract of *Terminalia Avicenniodes* Stem Bark, H: Methanol Extract of *Terminalia Avicenniodes* Leave

Figure 1. Comparison of mode of action of various plant extract at 12.5mg/mL against *Escherichia coli* ATCC 29929 using time kill test in minutes



Key: A: Ethanol Extract of *Magnifera Indica* Stem Bark, B: Ethanol Extract of *Magnifera Indica* Leave, C: Methanol Extract of *Magnifera Indica* Stem Bark, D: Methanol Extract of *Magnifera Indica* Leave, E: Ethanol Extract of *Terminalia Avicenniodes* Stem Bark, F: Ethanol Extract of *Terminalia Avicenniodes* Leave, G: Methanol Extract of *Terminalia Avicenniodes* Stem Bark, H: Methanol Extract of *Terminalia Avicenniodes* Leave

Figure 2. Comparison of mode of action of various plant extract at 12.5mg/mL against *Salmonella typhi* ATCC 14028using time kill test in minutes



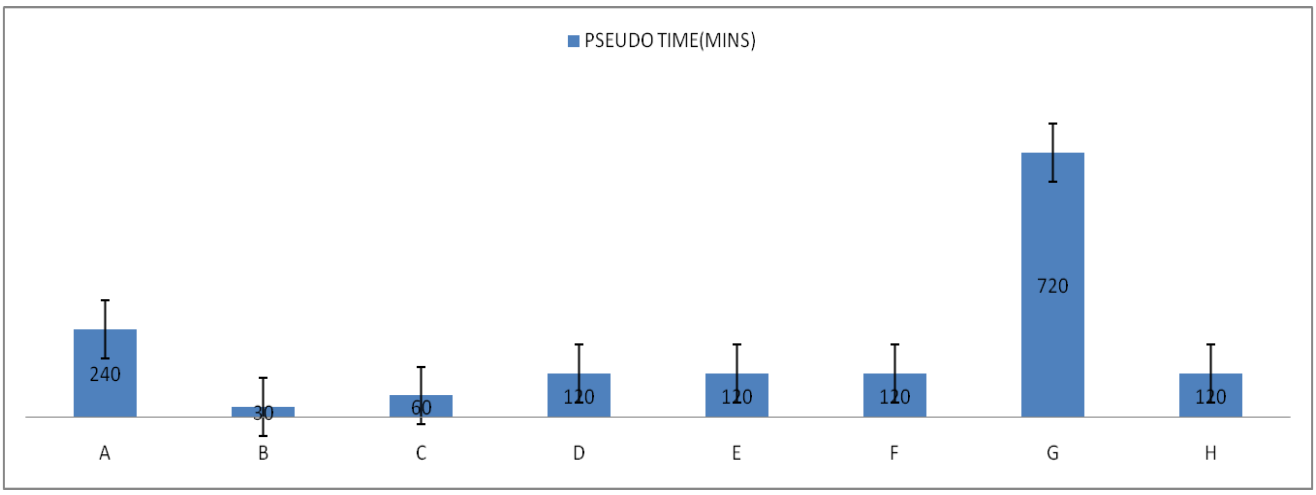
Key: A: Ethanol Extract of *Magnifera Indica* Stem Bark, B: Ethanol Extract of *Magnifera Indica* Leave, C: Methanol Extract of *Magnifera Indica* Stem Bark, D: Methanol Extract of *Magnifera Indica* Leave, E: Ethanol Extract of *Terminalia Avicenniodes* Stem Bark, F: Ethanol Extract of *Terminalia Avicenniodes* Leave, G: Methanol Extract of *Terminalia Avicenniodes* Stem Bark, H: Methanol Extract of *Terminalia Avicenniodes* Leave

Figure 3. Comparison of mode of action of various plant extract at 12.5mg/mL against *Klebsiella pneumoniae* ATCC 4252using time kill test in minutes

Figure 1- Comparison of mode of action of various plant extracts against *Escherichia coli*, using time kill test. The figure showed that at 12.5mg/ml of various plant extracts against *Escherichia coli*, methanol extract of the stembark of *Terminalia avicenniodes* killed *Escherichia coli* at 720mins which has the highest time of killing *Escherichia coli*, indicated that methanol extract of the stembark of *Terminalia avicenniodes* was least effective at the same concentration of 12.5mg/ml against *Escherichia coli*. Also, methanol extract of the leaves of *Magnifera indica* and ethanol extract of the leaves of *Terminalia avicenniodes* were able to kill *Escherichia coli* within 60mins of contact at 12.5mg/ml.

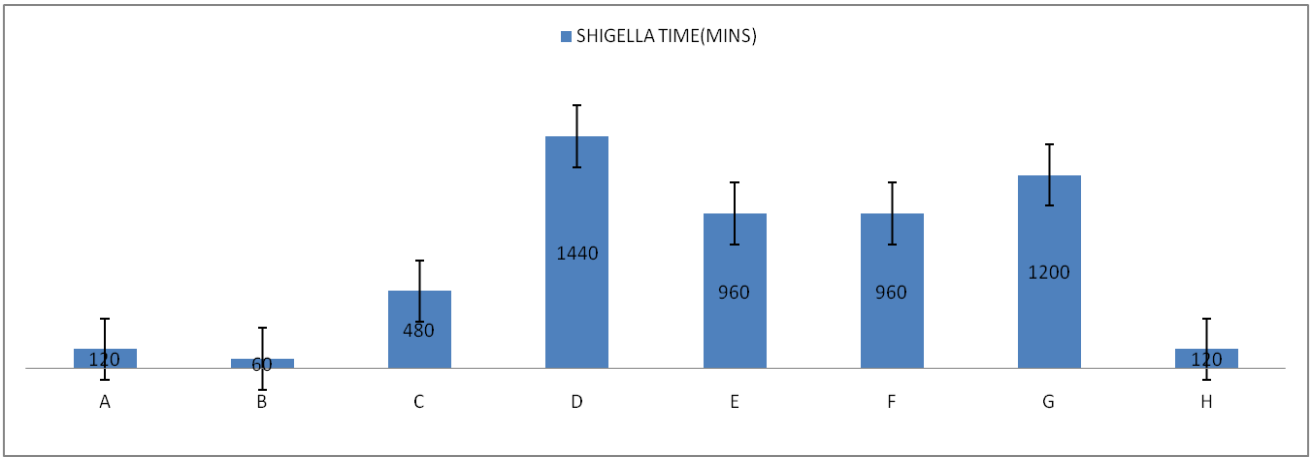
Figure 2- Comparison of mode of action of various plant extracts against *Salmonella typhi*, using time kill test. The figure showed that at 12.5mg/ml of various plant extracts against *Salmonella typhi*, methanol extract of the stembark of *Magnifera indica*, methanol extract of the stem bark of *Terminalia avicenniodes* and methanol extract of the leaves of *Terminalia avicenniodes* has the highest time of killing *Salmonella typhi* at 720mins, indicating the three plant were least effective at the same concentration of 12.5mg/ml against *Salmonella typhi*. Also, ethanol extract of the stembark of *Magnifera indica* and ethanol extract of the leaves of *Terminalia avicenniodes* were able to kill *Salmonella typhi* within 60mins of contact at 12.5mg/ml.

Figure 3- Comparison of mode of action of various plant extracts against *Klebsiella pneumoniae*, using time kill test. The figure showed that at 12.5mg/ml of various plant extracts against *Klebsiella pneumoniae*, ethanol extract of the stembark of *Magnifera indica*, ethanol extract of the leaves of *Terminalia avicenniodes*, methanol extract of the stembark of *Terminalia avicenniodes* and methanol extract of the leaves of *Terminalia avicenniodes* has the highest time of killing *Klebsiella pneumonia* at 1200mins, indicating the four plants were least effective at the same concentration of 12.5mg/ml against *Klebsiella pneumoniae*. Also, methanol extract of the stembark of *Magnifera indica* and ethanol extract of the leaves of *Terminalia avicenniodes* were able to kill *Klebsiella pneumoniae* within 720mins of contact at 12.5mg/m.



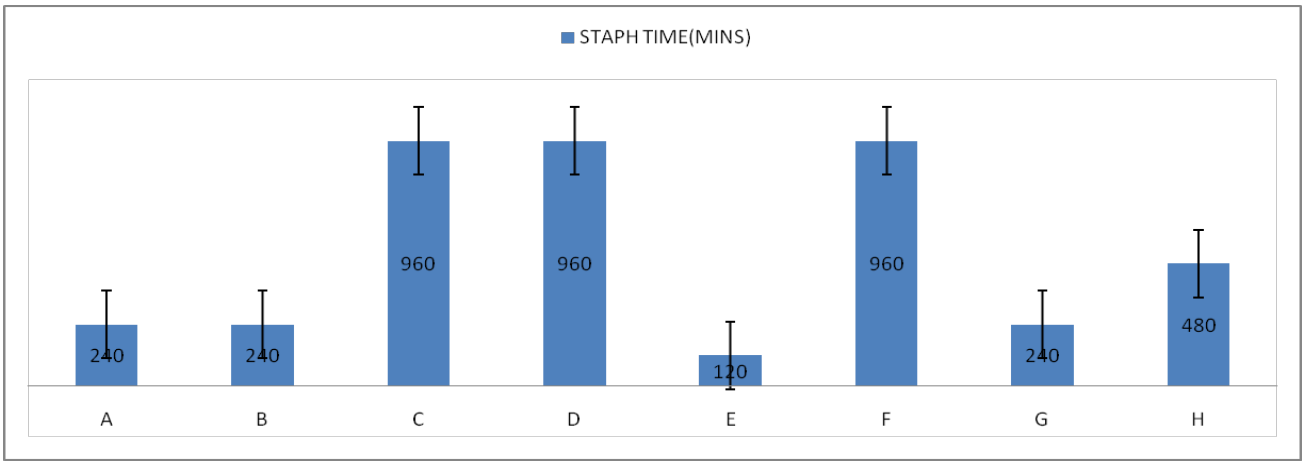
Key: A: Ethanol Extract of Magnifera Indica Stem Bark, B: Ethanol Extract of Magnifera Indica Leave, C: Methanol Extract of Magnifera Indica Stem Bark, D: Methanol Extract of Magnifera Indica Leave, E: Ethanol Extract of Terminalia Avicenniodes Stem Bark, F: Ethanol Extract of Terminalia Avicenniodes Leave, G: Methanol Extract of Terminalia Avicenniodes Stem Bark, H: Methanol Extract of Terminalia Avicenniodes Leave

Figure 4. Comparison of mode of action of various plant extract at 25mg/mL against *Pseudomonas aeruginosae* ATCC 27953 using time kill test in minutes



Key: A: Ethanol Extract of *Magnifera Indica* Stem Bark, B: Ethanol Extract of *Magnifera Indica* Leave, C: Methanol Extract of *Magnifera Indica* Stem Bark, D: Methanol Extract of *Magnifera Indica* Leave, E: Ethanol Extract of *Terminalia Avicenniodes* Stem Bark, F: Ethanol Extract of *Terminalia Avicenniodes* Leave, G: Methanol Extract of *Terminalia Avicenniodes* Stem Bark, H: Methanol Extract of *Terminalia Avicenniodes* Leave

Figure 5. Comparison of mode of action of various plant extract at 25mg/mL against Shigella dysentariae using time kill test in minutes.



Key: A: Ethanol Extract of *Magnifera Indica* Stem Bark, B: Ethanol Extract of *Magnifera Indica* Leave, C: Methanol Extract of *Magnifera Indica* Stem Bark, D: Methanol Extract of *Magnifera Indica* Leave, E: Ethanol Extract of *Terminalia Avicenniodes* Stem Bark, F: Ethanol Extract of *Terminalia Avicenniodes* Leave, G: Methanol Extract of *Terminalia Avicenniodes* Stem Bark, H: Methanol Extract of *Terminalia Avicenniodes* Leave

Figure 6. Comparison of mode of action of various plant extract at 12.5mg/mL against *Staphilococcus aureus* ATCC 29293using time kill test in minutes

Figure 4- Comparison of mode of action of various plant extracts against *Pseudomonas aureginosa*, using time kill test. The table showed that at 25mg/ml of various plant extracts against *Pseudomonas aureginosa*, methanol extract of the stembark of *Terminalia avicenniodes* has the highest time of killing *Pseudomonas aureginosa* at 720mins, indicated that methanol extract of the stembark of *Terminalia avicenniodes* was least effective at the same concentration of 25mg/ml against *Pseudomonas aureginosa*. Also, ethanol extract of the leaves *Magnifera indica* was able to kill *Pseudomonas aureginosa* within 30mins of contact at 25mg/ml.

Figure 5- Comparison of mode of action of various plant extracts against *Shigella dysentariae*, using time kill test. The figure showed that at 25mg/ml of various plant extracts against *Shigella dysentariae*, methanol extract of the leaves of *Magnifera indica* has the highest time of killing *Shigella dysentaria* at 1440mins, indicating that methanol extract of the leaves of *Magnifera indica* was least effective at the same concentration of 25mg/ml against *Shigella dysentariae*. Also, ethanol extract of the leaves of *Magnifera indica* was able to kill *Shigella dysentariae* within 30mins of contact at 25mg/ml.

Figure 6- Comparison of mode of action of various plant extracts against *Staphylococcus aureus* using time kill test. The figure showed that at 12.5mg/ml of various plant extracts against *Staphylococcus aureus*, methanol extract of the stembark of *Magnifera indica*, methanol extract of the leaves of *Magnifera indica* and ethanol extract of the leaves of *Terminalia avicenniodes* has the highest time of killing *Staphylococcus aureus* at 960mins, indicated that the three plant extract were least effective at the same concentration of 12.5mg/ml against *Staphylococcus aureus*. Also, ethanol extract of the leaves of *Terminalia avicenniodes* was able to kill *Staphylococcus aureus* within 120mins of contact at 12.5mg/ml.

4. Discussion Recommendation and Conclusion

4.1. Discussion

Despite tremendous progress in human medicines, infectious diseases caused by bacteria is still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance. From ancient times, different parts of medicinal plants have been used to cure specific ailments [12].

The percentage yield of the methanol and ethanol extract of the stem bark and leaves of *Terminalia avicenniodes* and *Magnifera indica* investigated in this study varied from one to another. The percentage yield of stem bark extract of both plants was found to be higher than those of the leaves extract. This was reported otherwise in the work of [13], which reported the leaves extract of *Terminalia avicenniodes* to have a higher yield than the bark extract. This difference could be as a result of the variation in the solvent used during extraction, the method of extraction could also be a reason for the variation. Also in this study, the ethanol extract of the leaves of *Magnifera indica* at a concentration of 23.333±2.887 strongly inhibited the growth of *Escherichia coli* ATCC 29929, this is comparable with the mean diameter zone of inhibition of Ofloxacillin and Ciprofloxacin against *Escherichia coli* ATCC 29929. Based on standard conversion, as little as 30µg of antibiotics is equivalent to 10mg of plant extract, it is important to note that the extract used in this study are in the crude form. The *Staphyloccus aureus* ATCC14028 used in this study is resistant to Gentamycin and Offloxacillin, whereas the ethanol and methanol extracts of the leaves and bark of *Terminalia avicenniodes* and *Magnifera indica* inhibited the growth of *Staphyloccus aureus* ATCC14028. The search for new antimicrobial natural products from plant materials is essential in order to curb the menace of multiple antibiotics resistant pathogens since plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines [14]. Though the antibacterial activity of the plant extracts in this study varied from one another, both stem bark and leaves of extracts of *Magnifera indica* possesses antibacterial activity against most of the bacteria isolates. This corresponds with the work of [15,16,17,18]. *Terminalia avicenniodes* is a member of the family combretaceae and most of the species from Combretaceae family have been shown to contain antibacterial activities [19]. Some studies also showed that the stem bark extract of *Terminalia avicennioides* exhibited both vibrocidal and typhoidal activities against *Vibrio cholera* and *Salmonella spp* (both *Salmonella typhi and Salmonella paratyphi*) respectively [20,21,22], while significant antimicrobial activities against Staphylococcus aureus was also reported [22]. However, there are differences in the diameter zone of inhibition of the plant extracts which could be due to the differences in the chemical composition of these extracts as revealed by phytochemical analysis. In this study, phytochemical analysis revealed that the leaves and stem bark of *Terminalia avicenniodes* contains tannins, saponin, steroid and phenol but do not contain anthraquinone and alkaloids which is in accordance with the work of [23]. Also in this study, *Magnifera indica* also does not contain anthraquinone and flavonoids. The presence of alkaloids in *Mangifera indica* leaves supported the use of this plant parts in the treatment of malaria and fever in Nigerian folk medicine. Furthermore, this study revealed that the ethanol extract of the *Magnifera indica* leaves killed *Pseudomonas aeruginosae* ATCC 27953 within 30 mins, while it took 24hrs for the methanol extract of *Magnifera indica* leaves to kill shigella dysentariae ATCC 23354, which is the longest time recorded. However, *klesiella pneumoniae* is the most resistance of all the ATCC strains of bacteria isolates. It took 20hrs for the and *Terminalia avicenniodes* stem bark and the methanol extract of *Terminalia avicenniodes* stem bark and leaves to kill *klesiella pneumonia*, which was the longest length of time recorded in this research work.

4.2. Conclusion

The following empirical conclusion emanciated from the study.

1. The methanol and ethanol extract of the stembark and leaves of *Magnifera indica* and *Terminalia avicenniodes* possesses bioactive compounds which is the bases of the antibacterial activity , so can be used in drug development.

2. The methanol and ethanol extract of the stembark and leaves of *Magnifera indica* and *Terminalia avicenniodes* were more active against *Escherichia coli, Staphyloccus aureus, Salmonella typhi, Shigella dysenteria* and *Pseudomonas aeruginosae* than *klesiella pneumonia.*

3. The nature of the solvent system used for extraction has an affect on the phytochemical components exhibited by the *Magnifera indica* and *Terminalia avicenniodes* extracts, thereby will affect the efficacy of the plant extracts against the bacterial isolates.

4. This study provides scientific basis for use of this plant in treatment of infections.

4.3. Recommendation

1. It is highly recommended that further investigation on purification and structural determination of the most promising constituents for in vivo evaluation of toxicity of these plants in animal and human studies should be encouraged.

2. *Magnifera indica* and *Terminalia avicenniodes* can be studied further for the chemotherapeutic drug production.

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