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Evaluation of the Potency of Some Medicinal Plants and Some Common Antibiotics on Bacteria Isolated from Otitis Media Effusion

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ABSTRACT: *Otitis media is a common childhood disease and it is associated with microbial pathogens within the middle ear. This aim of this study was to identify bacterial isolates associated with Otitis media effusions, determine their susceptibility to antibiotics and plants extracts of Ficus exasperata, Securinega virosa, and Tamarindus indica, as well as determine the MIC and MBC of the plants extracts on the bacteria isolated. A total of 48 samples of Otitis media effusion were collected from 28 males (58.33%) and 20 females (41.67%) at the out-patient department of Sacred Heart hospital, Abeokuta, Nigeria from February 2017 to September 2017 for bacteriological analysis. Bacteria isolated were identified according to Bergey's Manual of Determinative Bacteriology, (2006) while their antibiotic susceptibility testing was carried out using Disc Diffusion method. Children below the age of 5 years had the highest occurrence rate (83.33%) with the disease. Bacteria isolated include Staphylococcus aureus 19 (39.58%), Klebsiella pneumoniae 15 (31.25%), Pseudomonas aeruginosa 10 (20.83%), Proteus mirabilis 3 (6.25%) and Streptococcus pneumoniae 1 (2.08%). Antibiotic sensitivity test against gram positive isolates showed that Staphylococcus aureus was most resistant to Gentamicin (10mcg), Ofloxacin (5mcg), Ciprofloxacin (10mcg), Chloramphenicol (30mcg) and Streptomycin (25mcg), unlike Streptococcus pneumoniae which was highly susceptible. Gram negative isolates all showed significant ($P < 0.05$) sensitivity to Tetracycline (30mcg), Ciprofloxacin (10mcg), Amoxycillin (25mcg), Augmentin (30mcg), and Streptomycin (25mcg). At different concentrations (0.5mg - 1.0mg) of the plant extracts (Ficus exasperata, Securinega virosa and Tamarindus indica) all had significant effect ($P < 0.05$) on Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae but least effect on Proteus mirabilis. Conclusively, the outcome of this study indicates the need for proper bacteriological screening of patient Otitis media effusion/pus before treatment with conventional antibiotics or herbal remedies to ensure adequate treatment and prevent antibiotic resistance.*

KEYWORDS: Otitis media, MIC, MBC, Antibiotic sensitivity, antibiotic resistance, plants extracts

INTRODUCTION

Otitis media (OM) effusion or ear discharge is a relatively serious and unpleasant bacterial infection of the ear. It is one of the most common diseases encountered by an otolaryngologist. OM is defined as redness or swelling of the external auditory canal or debris within the canal, accompanied by itchiness, discharge (otorrhoea), loss of hearing or painful stuffy feeling (Wasihun and Zemene, 2015, Monasta *et al*., 2012). Chronic Otitis media (COM), common among infants and children of lower socioeconomic status, causes hearing loss and impacts the language and speech development of such children, thereby affecting their school performance and social interactions (Afolabi *et al.,* 2012). The incidence is 1.2 – 6.0 in 100,000 and usually occurs in children under 2 years of age (Chesney *et al.,* 2013).

Antibiotics sensitivity testing (AST) before the treatment of infections, is very important in making accurate prescription of the drug that will be most effective against a pathogen. One of the significant roles of a clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of various bacterial isolates. The main objectives of the testing are to find out possible drugs that will be sensitive or resistant to bacterial growth (Uddhav and Sivagurunathan, 2016).

Nowadays, a greater emphasis has been given towards researches on traditional medicine, particularly medicinal plants that deals with microbial diseases management. Based on ethno-botanical data, considerable number of studies have been conducted on the antimicrobial activity of medicinal plants, of which many showed promising potency against multi-drug resistant microorganisms after the current antibiotics failed to eradicate them (Emad, 2011). Plant-derived compounds are mostly secondary metabolites, most of which are phenols or their oxygen-substituted derivatives. These secondary metabolites possess various benefits including antimicrobial properties against pathogenic and spoilage microbes (Baliga *et al*., 2012).

Although a significant improvement has been achieved in terms of antibiotic care in the treatment of Otitis media, but OM continues to be a worldwide health problem that may develop serious complications resulting from antibiotics resistant pathogens. Antimicrobials derived from plants and spices are expected to be non-toxic and effective in humans since they are organic, and could serve as good alternatives to conventional antibiotics.

Ficus exasperata also called the sandpaper tree or "ewe ipin" in Yoruba, is widely used as a source of sandpaper and as a valuable medicinal plant. The extracts from the tree are locally used as anti-ulcer, anti-inflammatory, antipyretic, antihypotensive, lipid-lowering and analgesic potions (Ahmed *et al.,* 2012). Tamarind (*Tamarindus indica*) called "ewe ajagbon" in Yoruba language is a leguminous tree in the family Fabaceae, and it is indigenous to tropical Africa. The genus *Tamarindus* has only a single species and it produces pod-like fruits that contain an edible pulp, used in cuisines around the world. Its pulp is also used in traditional medicine and metal polish. *Securinega virosa* is a plant in the family Phyllanthaceae. It's a shrub that grows up to 5m

in most parts of East Africa and it is used by traditional healers in Tanzania to heal different kinds of diseases, and in the management of inflammatory conditions and tumor in African traditional medicine (Magaji *et al.*, 2015). These three plants which have been reported to possess some medicinal properties need to be explored for drug discovery as there in urgent need to develop new alternative therapies of organic origin with less harmful propensities in the treatment of diseases. This research is aimed at evaluating the antimicrobial effects of commonly used antibiotics as well as the antimicrobial efficacy of some medicinal plants (*Ficus exasperata, Securinega virosa and Tamarindus indica*) that have been suggested to have antimicrobial activity on bacterial isolates of Otitis media effusion.

MATERIALS AND METHODS

Study Area and Design

The area of study was Sacred Heart Hospital, Lantoro, Abeokuta, Ogun State. Ear swab samples of 48 patients were aseptically taken to increase the representativeness of the sample, minimize sampling errors, increase generalizability of the results and cater for attrition. An *in-vitro* experimental study of antimicrobial activity of plant extracts on bacterial isolates and disc diffusion method for antibiotics sensitivity was carried out. Experiments were performed in triplicates, while positive and negative controls were used to monitor the antimicrobial activities of the extracts. The media used includes MacConkey agar, Manitol Salt agar, Nutrient agar and Peptone water. Media were prepared according to the manufacturers' instructions.

Ethical Approval

Approval for the study was obtained (Reference number- SHH/EC/07/07/18) from the Ethics Committee of the Sacred Heart Hospital, Lantoro, Abeokuta, Ogun State (Appendix III).

Plant materials

Fresh leaves of *Ficus exasperata*, *Securinega virosa* and *Tamarindus indica* without any signs of disease were obtained in the month of February 2018 from the localities of Federal University of Agriculture, Abeokuta, Nigeria. They were identified taxonomically and authenticated by Dr. Oyelakin of the department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria. These plant leaves samples were deposited for future reference and were given voucher numbers FUNAABH-00101, FUNAABH-00102 and FUNAABH-00103 respectively.

Preparation of Extracts

Fresh leaf samples of *Ficus exasperata, Securinega virosa* and *Tamarindus indica* were washed with tap water to remove dust and other inpurities. The washed leaves were dried under shade for 3 to 7 days approximately. The air-dried whole leaves were pulverized into powdered form by using heavy duty blander. The powdered samples (up to 50g) were extracted with aqueous methanol (500 mL) for 72 h. The soaked extracts were filtered and filtrate concentrated using rotary evaporator under reduced pressure to obtain the methanolic crude extracts. The residues left

in the separation funnel were re-extracted twice following the same procedure, then filtered and stored in the refrigerator.

Macroscopic and Microscopic Identification of Isolates

Ear swab specimens collected were aseptically inoculated on sterile plates of Nutrient agar, MacConkey agar and Manitol salt agar respectively and was placed in the incubator at 37^0C for 18 hours, and afterwards examined for growth. Distinct colonies were subcultured to obtain pure cultures of bacteria.Bacterial isolates were identified morphologically via: size, form, colour, consistency, edges, elevation, and opacity. They were further identified morphologically using Gram staining and viewed under the microscope using oil lens.

Biochemical Identification of Isolates

Biochemical characterization was carried out according to Bergey's Manual of Determinative Bacteriology, (2006) using tests such as; catalase, oxidase, indole, motility, coagulase, urease and citrate tests were carried out appropriately on each of the isolated bacteria.

Antibacterial Activity Test

Antibacterial activity tests basically measure the ability of an antibiotic or other antimicrobial agent to inhibit the *in vitro* growth of microorganisms.

Procedure for Antibiotics Sensitivity Test

The Kirby-Bauer Disc Method also called the agar diffusion method or the disc diffusion method was followed. Filter discs impregnated with selected antibiotics; Amoxycillin (AMX) 25mcg, Ofloxacin (OFL) 5mcg, Streptomycin (STR)10mcg, Chloramphenicol (CHL) 30mcg, Cotriazone (CEF) 30mcg, Gentamicin (GEN) 10mcg, Pefloxacin (PEF) 5mcg, Cotrimoxazole (COT) 25mcg, Ciprofloxacin (CPX) 10mcg, Erythromycin (ERY) 5mcg (for gram positive organism) and; Augmentin (AUG) 30mcg, Chloramphenicol (CHL) 30mcg, Nitrofurantone (NIT) 20mcg, Gentamicin (GEN) 10mcg, Streptomycin (STR) 25mcg, Ofloxacin (OFL) 5mcg, Amoxycillin (AMX) 25mcg, Tetracycline (TET) 30mcg, Ciprofloxacin (CPX) 10mcg, Pefloxacin (PEF) 5mcg (for gram negative bacteria) were applied to the surface of Nutrient agar plate inoculated with the bacteria organism to be tested. The plates were incubated at 37°C for 24 – 48 hours. The diameter of the zones of inhibition were measured with a ruler and interpreted according to Clinical Laboratory Standard Institute (CLSI).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Plant Extracts

Determination of Minimum Inhibitory Concentration (MIC) of the extracts on the bacterial isolates

The MIC of the plant extracts was determined respectively using the method described by Akinpelu and Kolawole, (2004). The MIC helps to measure exactly the concentration of an antibiotic assay necessary to inhibit growth of a standardized inoculum (0.5 McFarland standard) under defined conditions. Reducing concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39

and 0.19mg/ml) of the extracts (*Ficus exasperata, Securinega virosa and Tamarindus indica*) in liquid medium were prepared respectively. The medium was poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 hours old standardized bacterial cultures. The plates were incubated at 37˚C for 24 to 72 hours and afterwards examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the bacteria.

Determination of Minimum Bactericidal Concentration (MBC) of the extracts on bacterial isolates.

The MBC of the plant extracts was determined respectively in accordance with the method of Akinpelu and Onakoya (2006). Samples were taken from plates with no visible growth in the MIC assay and sub-cultured on to freshly prepared Nutrient Agar medium and later incubated at 37˚C for 48hours. The MBC was taken as the lowest concentration of the extract that does not allow any bacterial growth on the surface of the agar plates.

Data Analysis

All the experiments were carried out in triplicates and data obtained from the study were subjected to analysis of variance. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of significance using Statistical Analysis System (SAS).

RESULTS

Demography of Subjects

A total of 48 samples were obtained in all, with majority of the patients (83.33%) within the age group 0 to 5 years, while only 10.42% were within the age group 6 to 10 years. There was no patient within the age group 11 to 40 years, 4.17% of the patients were within 41 to 45 years and 2.08% were within 51 years and above. Twenty eight of the patients (58.33%) were males while 20 (41.67%) were females.

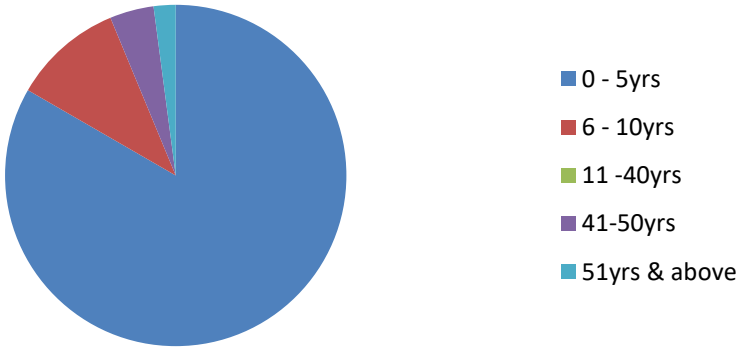

Figure 1: Demography of Patients with Otitis media from Sacred Heart Hospital, Lantoro, Abeokuta

Percentage Occurrence of Bacteria Species Isolated from Otitis Media Effusion

The percentage occurrence of bacteria species isolated were *Staphylococcus aureus* (39.58%) with the highest percentage occurrence, followed by *Klebsiella pneumoniae* (31.25%), *Pseudomonas aeruginosa* (20.83%), *Proteus mirabilis* (6.25%) and *Streptococcus pneumoniae* (2.08%) with the lowest percentage occurrence (Table 1).

Table 1. Percentage occurrence of bacterial species isolated from Otitis media effusion

S/N	Isolate	Frequency	Percentage Occurrence (%)
1	*Proteus mirabilis*	3	6.25
2	*Staphylococcus aureus*	19	39.58
3	*Klebsiella pneumoniae*	15	31.25
4	*Pseudomonas aeruginosa*	10	20.83
5	*Streptococcus pneumoniae*	1	2.08
	Total	**48**	**100**

Occurrence Rate of Bacterial Species Isolated from Otitis Media Effusion with Respect to Patients Age

The occurrence rate of each bacterial species isolated across the age groups shows that *Staphylococcus aureus* occurred at 29.52% and 10.42% within age groups 0 to 5 years and 6 to 10 years respectively. *Klebsiella pneumoniae* occurred at 21.17% and 2.08% within age intervals 0 to 5years and 41 to 50 years respectively. *Pseudomonas aeruginosa* occurred at 18.75% and 2.08% within age intervals 0 to 5years and 51 above respectively. *Proteus mirabilis* occurred at 4.17% and 2.08% within age intervals 0-5years and 41-50 years respectively. *Streptococcus pneumoniae* occurred at 2.08% within the age group 0-5years (Table 2).

Table 2: Occurrence rate of bacterial species isolated from Otitis media pus with respect to patients age

Age group	SA (%)	PM (%)	KP (%)	PA (%)	SP (%)
0 – 5	14 (29.17)	2 (4.17)	14 (21.17)	9 (18.75)	1 (2.08)
6 – 10	5 (10.42)	ND	ND	ND	ND
11 - 15	ND	ND	ND	ND	ND
16 - 20	ND	ND	ND	ND	ND
21 – 25	ND	ND	ND	ND	ND
26 – 30	ND	ND	ND	ND	ND
31 - 35	ND	ND	ND	ND	ND
36 - 40	ND	ND	ND	ND	ND
41 – 50	ND	1 (2.08)	1 (2.08)	ND	ND
51 and Above	ND	ND	ND	1 (2.08)	ND
Total	**19 (39.58)**	**3 (6.25)**	**15 (31.25)**	**10 (20.83)**	**1 (2.08)**

KEY: ND = Not Detected, SA = *Staphylococcus aureus*; PM = *Proteus mirabilis*, KP = *Klebsiella pneumoniae*, PA = *Pseudomonas aeruginosa*, SP = *Streptococcus pneumoniae*

Morphological and Biochemical Identification of Isolates

Table 3 shows the morphological and biochemical characteristics of each bacterium isolated from the Otitis media effusion samples. The bacteria identified fell into the following species as follows: *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Streptococcus pneumoniae.*

Table 3: Morphological and biochemical identification of bacteria isolated from Otitis media effusion samples

S/No	Gram	Shape	Smell	Size	Shape	Colour	Consistency	Edges	Elevation	Opacity	CO	CA	OX	IN	UR	CI	Suspected Organism
1	-	B	Fishy	Swarmer	R	Cream	Wet	Rough	Flat	Translucent	-	+	-	-	+	+	*Proteus mirabilis*
2	+	C	None	1-2mm	R	Cream	Wet	Rough	Flat	Opaque	+	+	-	-	-	-	*Staphylococcus aureus*
3	-	B	None	2-3mm	R	Cream	Slimy	Smooth	Raised	Opaque	-	+	-	-	+	+	*Klebsiella pneumoniae*
4	-	B	Sweet	2-3mm	R	Green	Wet	Smooth	Flat	Transparent	-	+	+	-	-	-	*Pseudomonas aeruginosa*
5	+	C	None	1-2mm	R	Cream	Dry	Rough	Flat	Opaque	-	-	-	-	-	-	*Streptococcus pneumoniae*

Key: ORG= Organism; B= Bacilli; R= Round; C= Cocci; CA= Catalase; CO= Coagulase; IN= Indole; OX= Oxidase; CI= Citrate utilization; UR= Urease; + = Positive; - = Negative

Antimicrobial Sensitivity of Common Antibiotics on Bacterial Isolates

Gram Positive Isolates

The commonly used antibiotics against Gram positive bacteria had significant effects ($P < 0.05$) on the growth of *Staphylococcus aureus* and *Streptococcus pneumoniae* in this study (Table not shown). The zones of inhibition (18.33mm), (20.00mm), (20.66mm), (14.00mm) and (20.00mm) were observed for *Streptococcus pneumoniae* treated with Gentamicin, Ofloxacin, Ciprofloxacin, Chloramphenicol and Streptomycin respectively These were significantly higher ($P > 0.05$) than the zones of inhibition (14.08mm), (13.87mm), (15.07mm), (12.29mm) and (15.29mm) observed in *Staphylococcus aureus.* This indicates that *S. aureus* was less susceptible to the antibiotics. Significant susceptibility were observed in Pefloxacin, Cotrimoxazole, Amoxycillin, Cotriazone and Erythromycin giving the zones of inhibition 20.33mm, 15.00mm, 20.00mm, 20.00mm, 18.33mm and 16.28mm, 16.04mm, 15.36mm, 16.83mm, 15.68mm against *Streptococcus pneumoniae* and *Staphylococcus aureus* respectively.

Gram Negative Isolates

The commonly used antibiotics against Gram negative bacteria had significant effects ($P < 0.05$) on the growth of *Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* (Table not shown). The zones of inhibition (11.69mm, 16.20mm, 14.51mm, 16.53mm, 16.98mm) observed against *Klebsiella pneumoniae* treated with Nitrofurantone, Chloramphenicol, Gentamicin, Pefloxacin and Ofloxacin respectively were significantly higher ($P > 0.05$) than the zones of inhibition (10.83mm, 7.50mm, 17.25mm, 13.38mm, 12.13) observed in *Pseudomonas aeruginosa. Proteus mirabilis* showed resistance to Nitrofurantone and Ofloxacin. Significant sensitivity was observed in Tetracycline, Ciprofloxacin, Amoxycillin, Augmentin, and Streptomycin for all the gram negative isolates.

Antimicrobial Effect of Plant Extracts (*Ficus exasperata, Securinega virosa and Tamarindus indica*) on Bacterial Isolates Using Agar Well Diffusion Method

Antibiotic effect of Plant Extract at Low Concentration (0.5mg)

Table 4 reveals that the zones of inhibition (11.00mm) observed in *Streptococcus pneumoniae* treated with 0.5 mg *Ficus exasperata* leaf extract and 11.08mm observed in *Pseudomonas aeruginosa* were significantly ($P < 0.05$) high showing susceptibility, as compared to the zone of inhibition (3.82mm), (3.93mm) and (0.00mm) observed in *Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis* respectively. This indicated that *Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis* were resistant to 0.5mg of *Ficus exasperata*. The zones of inhibition (3.88mm) and (2.50mm) were significantly higher ($P < 0.05$) in *Pseudomonas aeruginosa* treated with 0.5 mg *Securinega virosa* and *Tamarindus indica* leaf extract indicating susceptibility.

Antibiotic effect of Plant Extract at Low Concentration (1.0mg)

An increase in the concentration of the plant extracts to 1.0mg showed better susceptibility against some of the bacterial isolates. Zones of inhibition (13.00mm) observed against *Streptococcus pneumoniae* treated with 1.0mg *Ficus exasperata* leaf extract and (14.17mm) observed against *Pseudomonas aeruginosa* were significantly higher ($P < 0.05$) compared to the zones of inhibition (8.11mm, 4.91mm) observed against *Klebsiella pneumoniae and Staphylococcus aureus* respectively. *Proteus mirabilis* was resistant to 1.0mg of *Ficus exasperata* and *Securinega virosa.* The zones of inhibition (5.71mm, 4.82mm and 3.67mm) were significantly higher ($P < 0.05$) in *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* treated with 1.0 mg *Securinega virosa* leaf extract respectively. The zones of inhibition (5.17mm) and (10.00mm) shows significant susceptibility ($P < 0.05$) in *Proteus mirabilis and Streptococcus pneumoniae* treated with 1.0 mg *Tamarindus indica* leaf extract respectively (Table 4).

Table 9: Inhibitory effect (mm) of F*icus exasperata, Securinega virosa* and *Tamarindus indica* leaf extracts on isolated microorganisms.

Organisms	Conc. of Extract	Zones of Inhibition (mm)		
		Ficus exasperata	***Securinega virosa***	***Tamarindus indica***
Klebsiella pneumoniae	0.5mg	3.82 ± 0.84^{b}	0.67 ± 0.38^{ab}	0.00 ± 0.00
Pseudomonas aeruginosa	0.5mg	11.08 ± 0.22^{a}	3.88 ± 1.04^{a}	2.50 ± 0.90^{a}
Staphylococcus aureus	0.5mg	3.93 ± 0.78^{b}	1.667 ± 0.51^{b}	1.11 ± 0.43^{b}
Streptococcus pneumoniae	0.5mg	11.00 ± 0.01^{a}	0.00 ± 0.00	0.00 ± 0.00
Proteus mirabilis	0.5mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Klebsiella pneumoniae	1.0mg	8.11 ± 0.92^{b}	4.82 ± 0.82^{a}	4.31 ± 0.78^{b}
Pseudomonas aeruginosa	1.00mg	14.17 ± 0.37^{a}	5.71 ± 1.21^{a}	3.79 ± 1.20^{b}
Staphylococcus aureus	1.00mg	4.91 ± 0.97^{cd}	3.67 ± 0.71^{a}	4.24 ± 0.73^{b}
Streptococcus pneumoniae	1.0mg	13.00 ± 0.03^{a}	0.00 ± 0.00	10.00 ± 0.01^{a}
Proteus mirabilis	1.0mg	0.00 ± 0.00	0.00 ± 0.00	5.17 ± 0.29^{a}

Means followed by the same superscripts(s) on the same column are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Antimicrobial Effect of Plant Extracts (*Ficus exasperata, Securinega virosa and Tamarindus indica*) on Bacterial Isolates Using Agar Mixed Diffusion method

The sensitivity of the plant extracts (*Ficus exasperata, Securinega virosa* and *Tamarindus indica*) against the bacterial isolates at different concentrations (1mg, 5mg and 10mg) was indicated by the inhibition of bacterial growth. The growth of *Staphylococcus aureus* was inhibited when treated with 10mg *Ficus* exasperata, 5mg and 10mg *Securinega virosa* and 10mg *Tamarindus indica.* The growth of *Streptococcus pneumoniae* was inhibited when treated with 5mg and 10mg *Ficus* exasperata, 10mg *Securinega virosa* and 1mg, 5mg and 10mg *Tamarindus indica.* The

growth *Klebsiella pneumoniae* was inhibited when treated with 10mg *Ficus* exasperata, 5mg and 10mg *Securinega virosa* and 10mg *Tamarindus indica.* The growth of *Proteus mirabilis* was inhibited when treated with 10mg *Tamarindus indica.* The growth of *Pseudomonas aeruginosa* was inhibited when treated with 1mg, 5mg and 10mg *Ficus* exasperata, 5mg and 10mg *Securinega virosa* and 10mg *Tamarindus indica.* (Table 5).

Table 5: Growth Inhibition of isolated bacteria to different concentrations of *Ficus exasperata*, *Securinega virosa* and *Tamarindus indica* leaf extracts using Agar mixed diffusion method

S/No	Organism	*Ficus exasperata* (mg)			*Securinega virosa (mg)*			*Tamarindus indica* (mg)		
		1.0	**5.0**	**10**	**1.0**	**5.0**	**10**	**1.0**	**5.0**	**10**
1	*Staphylococcus aureus*	G	G	NG	G	NG	G	NG	NG	G
2	*Streptococcus pneumoniae*	G	NG	NG	G	G	NG	G	NG	NG
3	*Klebsiella pneumoniae*	G	G	NG	G	NG	NG	G	G	NG
4	*Proteus mirabilis*	G	G	G	G	G	G	G	G	NG
5	*Pseudomonas aeruginosa*	NG	NG	NG		NG	NG	G	G	NG

KEY: NG = No growth observed; G = Growth observed

Minimum Inhibitory Concentration (MIC) of plant extracts on the bacterial isolates

Minimum Inhibitory Concentration of *Ficus exasperata* on the bacterial isolates.
The MIC of the *Ficus exasperata* extract against the bacterial isolates showed that out of the ten concentrations of the double fold dilution, 0.39mg/ml was the MIC for *Pseudomonas aeruginosa*, 0.78mg/ml for *Streptococcus pneumoniae* while 1.56mg/ml for both *Staphylococcus aureus* and *Klebsiella pneumoniae*. The MIC against *Proteus mirabilis* was 50mg/ml (Table 6)**.**

Minimum Inhibitory Concentration of *Securinega virosa* on bacterial isolates.
The MIC of the *Securinega virosa* extract against the bacterial isolates showed that out of the ten concentrations of the double fold dilution, 0.78mg/ml was MIC for *Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae*, 1.56mg/ml for *Streptococcus pneumoniae* while *Proteus mirabilis* had 50mg/ml as the MIC (Table 7).

Minimum Inhibitory Concentration of *Tamarindus indica* on bacterial isolates.
The MIC of the *Tamarindus indica* extract against the bacterial isolates showed that out of the ten concentrations of the double fold dilution, 1.56mg/ml was the MIC for *Pseudomonas aeruginosa,* 6.25mg/ml for *Staphylococcus aureus* and 3.12mg/ml for *Klebsiella pneumoniae,* 0.39mg/ml for *Streptococcus pneumoniae* while *Proteus mirabilis* had 25mg/ml as the MIC (Table 8).

Table 6: Minimum Inhibitory Concentration of *Ficus exasperata* on the bacterial isolates

Concentration of Plants Extract (mg/ml)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*
100	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG
25	NG	NG	NG	NG	G
12.50	NG	NG	NG	NG	G
6.25	NG	NG	NG	NG	G
3.12	NG	NG	NG	NG	G
1.56	NG	NG	NG	NG	G
0.78	G	NG	NG	G	G
0.39	G	G	NG	G	G
0.19	G	G	G	G	G

KEY: NG= No growth observed; G = Growth observed

Table 7: Minimum Inhibitory Concentration of *Securinega virosa* on the bacterial isolates.

Concentration of Plant Extract (mg/ml)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*
100	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG
25	NG	NG	NG	NG	G
12.50	NG	NG	NG	NG	G
6.25	NG	NG	NG	NG	G
3.12	NG	NG	NG	NG	G
1.56	NG	NG	NG	NG	G
0.78	NG	G	NG	NG	G
0.39	G	G	G	G	G
0.19	G	G	G	G	G

KEY: NG= No growth observed; G = Growth observed

Table 8: Minimum Inhibitory Concentration of *Tamarindus indica* on the bacterial isolates.

Concentration of Plant Extract (mg/ml)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*
100	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG
25	NG	NG	NG	NG	NG
12.50	NG	NG	NG	NG	G
6.25	NG	NG	NG	NG	G
3.12	G	NG	NG	NG	G
1.56	G	NG	NG	G	G
0.78	G	NG	G	G	G
0.39	G	NG	G	G	G
0.19	G	G	G	G	G

KEY: NG = No growth observed; G = Growth observed

Minimum Bactericidal Concentration (MBC) of plant extracts (*Ficus exasperata, Securinega virosa and Tamarindus indica*) on the bacterial isolates

Minimum Bactericidal Concentration of *Ficus exasperata* on the bacterial isolates

The MBC for *Staphylococcus aureus* and *Klebsiella pneumoniae* was 3.12mg/ml, 1.56mg/ml for *Streptococcus pneumoniae*, 0.78mg/ml for *Pseudomonas aeruginosa* and no MBC value was gotten for *Proteus mirabilis* (Table 9).

Minimum Bactericidal Concentration of *Securinega virosa* on the bacterial isolates

The MBC for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* is 1.56mg/ml, *Streptococcus pneumoniae* is 3.12mg/ml and no MBC value was gotten for *Proteus mirabilis* (Table 10).

Minimum Bactericidal Concentration of *Tamarindus indica* on the bacterial isolates

The MBC for *Staphylococcus aureus* is 12.50mg/ml, *Streptococcus pneumoniae* is 1.56mg/ml, *Pseudomonas aeruginosa* is 3.12mg/ml, *Klebsiella pneumoniae* is 6.25mg/ml and 50mg/ml was gotten for *Proteus mirabilis* (Table 11).

Table 9: Minimum Bactericidal Concentration of *Ficus exasperata* on bacterial isolates

Concentration of Plant Extracts (mg/ml)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*
100	NG	NG	NG	NG	G
50	NG	NG	NG	NG	G
25	NG	NG	NG	NG	G
12.50	NG	NG	NG	NG	G
6.25	NG	NG	NG	NG	G
3.12	NG	NG	NG	NG	G
1.56	G	NG	NG	G	G
0.78	G	G	NG	G	G
0.39	G	G	G	G	G
0.19	G	G	G	G	G

KEY: NG = No growth observed; G = Growth observed

Table 10: Minimum Bactericidal Concentration of *Securinega virosa* on the bacterial isolates

Concentration of Plant Extracts (mg/ml)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*
100	NG	NG	NG	NG	G
50	NG	NG	NG	NG	G
25	NG	NG	NG	NG	G
12.50	NG	NG	NG	NG	G
6.25	NG	NG	NG	NG	G
3.12	NG	NG	NG	NG	G
1.56	NG	G	NG	NG	G
0.78	G	G	G	G	G
0.39	G	G	G	G	G
0.19	G	G	G	G	G

KEY: NG = No growth observed; G = Growth observed

Table 11: Minimum Bactericidal Concentration of *Tamarindus indica* on the bacterial isolates

Concentration of Plant Extracts (mg/ml)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*
100	NG	NG	NG	NG	NG
50	NG	NG	NG	NG	NG
25	NG	NG	NG	NG	G
12.50	NG	NG	NG	NG	G
6.25	G	NG	NG	NG	G
3.12	G	NG	NG	G	G
1.56	G	NG	G	G	G
0.78	G	G	G	G	G
0.39	G	G	G	G	G
0.19	G	G	G	+	G

KEY: NG = No growth observed; G = Growth observed

DISCUSSION

This study entails the analysis of ear swab cultures and antibiotic sensitivity of the bacteria isolated from patients who presented with Otitis media within a period of 7months at Sacred Heart Hospital, Abeokuta, Ogun state, Nigeria. A total of 48 samples of Otitis media effusions were collected from the patients presenting with symptoms of Otitis media. Twenty eight of the patients were males **(**58.33%) while 20 were Females (41.67%). Occurrence rate of Otitis media was high in children below 5 years (83.33%). This study agrees with the study conducted by Garba *et al.*, (2017) where laboratory test results of 53 patients were retrieved from the laboratory record book of which Males were 33 (62.3%) while Females were 20 (37.74%) and 71.7% of the patients were below the age of 5 years.

Results from this present study revealed that children within ages 5 years and below are at high risk of the infection which may be due to their anatomical predisposition. At that age, the Eustachian tube of children is usually more flexible, shorter and horizontal than that of adults, which allows nasopharyngeal pathogens to enter the middle ear with relative ease as described by

Renko *et al*., (2007). Otitis media was less common within the age ranges 41-45 years and 51 years above which are 4.17% and 2.08% respectively, thus supporting the research of Farhan *et al.,* (2011) that Otitis media infection also affects adults but with a lower rate.

In this study, the bacteria isolated from Otitis media effusion were *Staphylococcus aureus* (39.58%), *Klebsiella pneumoniae* (31.25%), *Pseudomonas aeruginosa* (20.83%), *Proteus mirabilis* (6.25%) and *Streptococcus pneumoniae* (2.08%). It is noteworthy that all the species of bacteria identified in this study were mainly cultured from the samples of patients within age 5 years and below. This may be due to host risk factors including age (5 years old and below), male sex, premature birth (37 weeks gestation), ethnicity, low birth weight (2.5 kg), as well as environmental factors including season of birth, lack of breastfeeding, parental education/employment (lower socioeconomic groups), household income (below poverty level), day care attendance, number of siblings, personal and family history of ear infections (Hoffman *et al.,* 2013).

This finding partly agrees with the study of Nwogwugwu *et al*., (2014) who also reported the presence of *Pseudomonas aeruginosa.* However they reported *Pseudomonas aeruginosa* as the most prevalent causative bacteria to be isolated from Otitis media effusion. This is in disagreement with this present study which found *Staph. aureus* as the most prevalent bacteria, a finding which is in agreement with the study by Nwankwo and Okeke, (2014) who worked in Abia State, Nigeria and reported *Staph. aureus* as the most predominant isolate. This might be due to environmental factors and geographical location of the patients (Ettehad *et al*., 2006).

Antibiotics sensitivity of gram positive isolates in this study showed that *Staphylococcus aureus* was less susceptible to Gentamicin, Ofloxacin, Ciprofloxacin, Chloramphenicol and Streptomycin, unlike *Streptococcus pneumoniae* which was highly susceptible. Both *Staphylococcus aureus* and *Streptococcus pneumoniae* were significantly susceptible to Pefloxacin, Cotrimoxazole, Amoxycillin, Cotriazone, and Erythromycin. Gram negative isolates on the other hand, for example *Klebsiella pneumoniae* was susceptible to Nitrofurantone, Chloramphenicol, Gentamicin, Pefloxacin and Ofloxacin unlike *Proteus mirabilis and Pseudomonas aeruginosa* which were less susceptible. The entire gram negative bacteria cultured in this study showed significant susceptibility to Tetracycline, Ciprofloxacin, Amoxycillin, Augmentin, and Streptomycin. This corresponds with the report of Paradise (2004) and the study of Qureishi *et al*., (2014) on treatment and prevention of Otitis media. This buttresses the fact that there is always a need for microbiological screening of patients' Otitis media pus before treatment. The patients' consumption of antibiotics should be in accordance with physician's prescription to ensure proper treatment and prevent drug resistance.

The evaluation of the antibacterial activities of the methanol leaf extracts of *Ficus exasperata*, *Securinega virosa* and *Tamarindus indica* reveals that increasing concentrations of the extracts have increasing significant effects on *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Staphylococcus aureus, Streptococcus pneumoniae* and less effect on *Proteus mirabilis*. It was

observed that zone of inhibition showed significant increase with increase in concentration (1.0mg > 0.5mg). At 1.0mg, *Ficus exasperata* had highest zone of inhibitions 14.17mm and 13.00mm against *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* respectively. Also at 1.0mg concentration for Agar well diffusion method and 10mg concentration for Agar mixed diffusion method, *Tamarindus indica* was able to inhibit all the isolates. The extracts exerted inhibition against both the Gram negative and Gram positive organisms hence the extracts can be referred to as having a broad spectrum activity.

The minimum inhibitory concentration and minimum bactericidal concentration values obtained for the plants extract indicates a good bacteriostatic and bactericidal potency of the extracts against the isolates except *Proteus mirabilis* which showed resistance to the *Ficus exasperata* and *Securinega virosa* but was inhibited at higher concentrations (50mg/ml) of *Tamarindus indica.* This corresponds with the study of Afolayan *et al.,* (2017) where *Proteus mirabilis* isolated from Otitis media was only inhibited by *Emilia pratermissa* leaf extracts at 50mg/ml. This might be as a result of the swarming characteristics of *Proteus mirabilis* and/or the presence of substantial concentration of the extract may be required for minimum inhibition.

Agar well diffusion method and Agar mixed diffusion method used for the plant extracts' sensitivity test yielded corresponding results showing effectiveness of the methods. Doughari *et al*., (2007) reported that the state of administration of an antimicrobial agent affects the effectiveness of such agent, and that antibiotics being in a refined state, as compared to plant extracts in crude state, may record higher antimicrobial activity. Also, the small molecular size possessed by antibiotics as reported by Mailard, (2002) aids their solubility in diluents as this could enhance their penetration through the cell wall into the cytoplasm of the organism. However, the antimicrobial activities of the crude leaf extracts, if purified, may exhibit wider zones of inhibition on the test organisms and may serve as a substitute but not complete replacement or eradication of the available conventional antibiotics.

CONCLUSION

Conclusively, the outcome of this study indicates the need for a proper microbiological screening of patient Otitis media effusion/pus before treatment with conventional antibiotic to ensure proper treatment and prevent drug resistance as the bacterial isolates reacts differently to the antibiotics. The antimicrobial activity of the methanolic leaf extracts of *Ficus exasperata, Securinega virosa* and *Tamarindus indica* exhibited considerable antimicrobial effects on the test isolates except *Proteus mirabilis.* The results of antimicrobial activity indicate the bacteriostatic and bactericidal potential of the methanolic leaf extracts showing a promising development that will help in the discovery of new classes of antibiotics which might be used in the treatment of Otitis media infections. Hence further purification of the extracts and identification of the active components is necessary to improve antimicrobial potency.

RECOMMENDATION

The authors recommend further research in the following directions:

- Phytochemical screening of the different plant parts of *Ficus exasperata, Securinega virosa* and *Tamarindus indica.*
- Purification of the plant extracts for isolation and identification of the active ingredients in these
- In-vivo evaluation of these extracts on animal models (preferably mammals) that will elicit the physiological effects of these plants extracts on human usage.

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APPENDIX I

Gram-staining procedure: A smear of the organism was made on a glass slide, and crystal violet was added as a primary stain for 1minute**.** It was washed and Lugol's iodine was added as a mordant for 1min, Iodine was drained and alcohol was added as decolourizer for 15seconds**.** It was washed off with water and safranin was added as secondary staining for 1min**.** It was washed, dried and the glass slide was observed using oil immersion lens**.** Purple colour indicated Gram positive while the pink/red colour indicated Gram negative and the shape/forms of the organisms were also observed.

Procedures for Biochemical Tests

1. Catalase test: The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive *Micrococcaceae* from catalase-negative *Streptococcaceae*. Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; Hydrogen peroxide.

The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme.

Procedure: A sterile inoculating loop was used to pick a small amount of organism from a well-isolated 18 to 24 hour colony and placed on the microscope slide. A dropper or Pasteur pipette was used to pick a drop of 3% H_2O_2 (Hydrogen peroxide) on the organism on the microscope slide. Without mixing, it was observed for immediate bubble formation (O2+water= bubbles).

Catalase positive bacteria: *Staphylococcus sp.*

Catalase negative bacteria: *Streptococcus sp.*

2. Coagulase test: The coagulase test differentiates strains of *Staphylococcus aureus* from other coagulase-negative species. *S. aureus* strains are capable of coagulating plasma in the tube test and will produce clumps of cells in the slide test. The coagulase test can be performed using two different procedures - Slide test and tube test. The slide test is simple, giving results within 10 seconds. The tube test is definitive, however it can take up to 24 hours to complete. For both tests, clumping or clots of any size indicate a positive response.

Procedure: The slide test was performed by preparing a suspension of bacterial cells mixed into a drop of plasma on a microscope slide. If bound coagulase is present on the bacterial cells, then the presence of plasma will cause the bacterial cells to clump. The clumping will occur because the clumping factor is an adhesin, which causes the cells to bind to fibrinogen in the plasma. This resulted in visible clumping of bacterial cells on the microscope slide as a positive response.

Coagulase positive bacteria*: Staphylococcus aureus*

Coagulase negative bacteria: *Staphylococcus epidermis, Staphylococcus saprophyticus*

3. Oxidase test: A small piece of filter paper was soaked in 1% Kovács oxidase reagent and let dry and a loop was used to pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto the treated filter paper. It was observed for colour changes. Microorganisms that are oxidase positive gave colour change to dark purple within 5 to 10 seconds, delayed oxidase positive gave colour change to purple within 60 to 90 seconds and oxidase negative does not change or it takes longer than 2minutes.

Oxidase positive bacteria: *Pseudomonas, Vibrio cholera*

Oxidase negative bacteria*: E. coli, Klebsiella, Salmonella.*

4. Indole test: It is used as part of the IMViC (indole, Methyl Red-Voges Proskaurer, Citrate) procedures, a battery of tests designed to distinguish among members of the family Enterobacteriaceae. A test tube of tryptone broth (peptone water) was inoculated with a small amount of a pure culture and incubated at 37°C for 24 to 48 hours. To test for indole production, 5 drops of Kovác's reagent was added directly to the tube. A positive indole test was indicated by the formation of a pink to red colour ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent and for indole negative the reagent layer remained yellow or slightly cloudy.

Indole positive bacteria: *E. coli, Vibrio cholera*

Indole negative bacteria: *Klebsiella, Salmonella, Shigella sp.*

5. Urease test using Christensen's (modified) urea broth: Testing for urease enzyme activity is important in differentiating enterobacteria. *Proteus* strains are strong urease producers. The test organism was heavily inoculated in a bijou bottle containing 3 ml sterile Christensen's

modified urea broth and was Incubated at 35–37^{0}C for 3–12 h. After wish the medium was checked for a pink colour. A pink colour indicates a positive urease test.

Urease positive bacteria: *Proteus sp.*

Urease negative bacteria: *Salmonellae sp.* and *shigellae sp.*

6. Citrate test: This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon. This was performed using Simmon's citrate agar. Slant of the medium was prepared in test tubes as recommended by the manufacturer and stored at 2–8^{0}C. A sterile straight wire loop was used to pick the test organism, stab the butt and then streak the slope out. It was incubated at 35^{0}C for 24hours. It was observed for colour change in medium to a bright blue colour. Bright blue colour indicates a positive citrate test.

Citrate positive bacteria: *Klebsiella pneumoniae*

Citrate negative bacteria: *Escherichia coli.*

APPENDIX II

Procedures for Antibacterial Sensitivity Test

1. **Agar well diffusion method**: A suitable agar medium was prepared (Nutrient Agar), and once the agar has solidified the medium was inoculated using a sterile wire loop to spread throughout the plate. The wells were prepared by punching with a eight millimeters (8mm) diameter standard sterile cork borer (heat flamed), these wells were filled with the extracts (at different measurement of 0.5mg, 1mg and 2mg) respectively. The plates were kept in the refrigerator for 15 to 20minutes before they were incubated at 35 ± 2°C for 18 – 24h. The antimicrobial activity (i.e. zones of inhibition) was measured with ruler and calculated in millimeter. The size of the cork borer remained constant throughout the experiment.

2. **Agar mixed Method** was carried out, a suitable agar medium was prepared (Nutrient Agar), and once the agar cooled to 40^{0}C the plant extract was added and gently swirled for thorough mixing. It was dispensed into the sterile Petri dishes and was allowed to solidify. The medium was inoculated using a sterile wire loop to spread throughout the plate. The plates were incubated at 35 ± 2°C for 18 – 24h. The antimicrobial activity was determined by observing the plates to see if the organism inoculated was inhibited by the presence of the plant extract or there was no inhibition which meant the plant extract added to the medium has not affected the growth of the organism.