

BIOACTIVITY AND PHYTOCHEMICAL SCREENING OF NIGERIAN MEDICINAL PLANTS GROWING IN ONDO AND EKITI STATE AGAINST BACTERIAL ISOLATES FROM PEDIATRICS HOSPITAL.

ABSTRACT

The Aqueous, ethanol, hexane and ethyl acetate extracts of the leaves, bark and roots of *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta* and *Moringa oleifera* were tested for possible source of antibacterial activities against selected clinical organisms found in the pediatrics hospital in Ondo and Ekiti State, Nigeria. The selected clinical organisms used for this research work are *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. It was observed that all plant were potent against the clinical test organism. *Anchomanes difformis*, *Moringa oleifera* and *Indigofera arrecta* extracts were the most active medicinal plants during the course of this research work. *Boscia senegalensis*, *Acacia albida*, *Bridelia ferruginea* and *Ficus ingens* extract were not as active against the test organism. All the medicinal plants extract shows evidence of antibacterial properties. Phytochemical screening of the medicinal plants reveals, very important and vital phytochemicals like saponin, tanins, alkaloids, flavonoids, cardiac glucosides and anthraquinones, at a very appreciable quantity. It also reveals presence of minerals like sodium, potassium, calcium, iron, zinc, copper and phosphorus at mg/100g which are essential to biological and physiological activities. The main objectives of this research work is to investigate the antimicrobial potency and phytochemical activities of seven Nigerian Medicinal plants on Selected Clinical Organisms.

Keywords: Antibacterial assay, agar well diffusion method, phytochemical screening; minerals composition; nutrient and anti-nutrient composition, proximate composition.

INTRODUCTION

The discovery of new antibiotics is a very important aspect of medicinal plants, which is dated as far back to 500 to 700 BC. Pharmacist, Pharmacologist, Biochemist and Pharmaceutical microbiologist are taking the necessary clue from the advent of medicinal plants in the discovery and production of new antibiotic for the cure of different diseases (Iwu 1999).

Medicinal Plants are good source of antimicrobial agents, which can be used as a form of chemotherapy in the cure of different illness. Some are used as chewing sticks, some are used for soup condiment and some Medicinal plants are use even for spiritual purpose in the South Western part of Nigeria (Osuntokun 2014). Major health hazard may not be cure by the conventional medicine, but medicinal plants are found to be useful in such areas. Poor people may not even have enough money to buy the conventional drug, but nature has blessed us with the medicinal plants for food and also for the cure of major health hazard.(Osuntokun2014, Sofowora(2008).

The use of medicinal plants for therapy predates the introduction of synthetic antibiotics. It also predates the religious, social and economic barriers (Akinyemi *et al.*,2000).Antibiotics are naturally occurring or synthetic organic compounds which inhibit or destroy bacteria, generally at low concentration(Brooks *et al.*,2007).Many lives have been saved by the use of synthetic antibiotics but in recent times, these disease causing microbes have developed resistance to some of these antibiotics and have become difficult to treat in our various health care homes,an example of our pediatric hospital. This situation may lead to increased cost of medication and in the long run leads to increased morbidity and mortality(Osuntokun 2015).

The medicinal plants is taking a place of pride in the world health economy, knowing fully well that, developing countries can make earn a lot foreign exchange in this aspect of drug production, drug discovery, development of new drugs and antibiotic through medicinal plants, especially in Nigeria, where God has blessed us abundantly with various degree of medicinal plants.(Nmorsi & Osuntokun 2014),(Okwu 2005).

Traditional medicine like orthodox medicine has its own methods and techniques of applications, which however aims at finding a cure to various health hazards (Wurochekke *et al.*,2008). The treatment and control of diseases by the use of the available medicinal plants in a locality will continue to play a significant roles in medical health care. Nearly all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. Our empirical belief in Nigeria and Africa on herbal drugs gives us an edge on the use of medicinal plants which is on the high increase in our daily activities, due to ineffectiveness of most conventional drugs as a result of microbial resistance in some health challenge in the developing countries, more patients in Nigerian are seen in herbal medical centers than ever. The intractable problem of antimicrobial resistance

has led to the resurgence of interest in herbal products as sources of novel compounds to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases (Udekwe 2010).

Phytochemicals are plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into two, this are the primary metabolites such as sugars and fats found in all plants; and secondary metabolites which are compounds found in a smaller range of plants, serving a more specific function. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs examples are inulin from the roots of dahlias, quinine from the cinchona, morphine, codeine and digoxin from the foxglove.(Carrubba, & Scalenghe 2012).

Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the anti-hypertension agent reserpine; the choline-mimetic galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arrhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. (Springbob *et al.*,2009),(Edgar *et al.*,2002, Cravotto *et al.*,2010).

In view of this, it is therefore very important to search for effective, efficient, low cost and reliable traditional therapeutic agents ,for the development of locally made drugs(antibiotics) , to reduce abuse of drugs and drug resistant organisms which is a common factors in some conventional drugs (Trease 1989). The main objectives of this research work is to investigate the antimicrobial potency and phytochemical activities of seven Nigerian Medicinal plants on selected clinical organisms

MATERIALS AND METHOD

The experiment involved seven types of Nigerian medicinal plants, with four different extracting solvent, four clinical organisms, and three concentrations of medicinal plants extracts, the four clinical isolates are Bacteria.

Source of plant materials

The Nigerian Medicinal plants selected were obtained from the roots and the branch of *Acacia albida*, *Anchomaens difformis*, *Boscia senegalenss*, *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta* and *Moringa oleifera*. The plant were collected in Ikare Akoko, a tropical rainforest, Ondo State, Nigeria with latitude (7.21692 North) and longitude (5.21561 East) The plant parts were authenticated by a Botanist at the Department of Botany, Ekiti State University Ado Ekiti, and Adekunle Ajasin University, Akungba Akoko, Ondo State ,Nigeria. A voucher specimen number: 1784 was assigned to it for future reference.

Table 1;Profile of the Medicinal Plants under study

Botanical Name	Family	Local Names	Part used	Medicinal uses
1. <i>Acacia albid.a</i>	Leguminosae	Gawo	Root	Skin infections
2. <i>Anchomanes difformis</i> .	Araceae	Chakara	Roots	1.Cough. 2.Respiratory diseases.
3. <i>Boscia senegalensis</i> .	Capparidaceae	Anza	Roots	1.Anticancer. 2.Ulcer swellings.
4. <i>Bridelia ferruginea</i> .	Euphorbiaceae	Kirni	Leaf	1Antibiotic. 2.Skin infection, 3.Diuretic, 4.Febrifuge urethral discharge.
5. <i>Ficus ingens</i> .	Moraceae	Kawuri	Root	1.Piles. 2.diarrhea. 3.laxative. 4.diuretic.
6. <i>Indigofera arrecta</i> .	Papilionoideae	Ba-ba	Leaf	1.Infections stomach. 2.Pain.

				3.Snakebite. 4.Epilepsy.
7. <i>Moringa oleifera</i> .	Moringaceae	Zogale	Leaf	1Asthma. 2.Eye infection. 3.Migraine. 4.Headache. 5.Febrifuge. 6.Abortifacient.

96 Sofowora(2008), Osuntokun (2014).

97 Plant profile, local and botanical names and its uses, are elucidate in the table 1, to show that this
98 plant are been in use for centuries. Sofowora(2008),Osuntokun (2014).

99 Source of microorganisms

100 Pure cultures of *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pnumonniae*,
101 *Escherichia coli* isolated from children stools attending the Pediatrics from the Federal
102 Medical Center (Pediatrics) (FMC), Owo, Ondo State, and Ekiti State Teaching Hospital
103 (Pediatrics), Ekiti State, Nigeria Bacterial cultures were maintained on Blood agar slant.
104 Authenticity of the isolates were preform through the use of API kits 20.(Osuntokun 2015)

105 Extract preparation

106 The leaf and root of the tested plant were well dried and then grinded to powder. About 200g
107 of the powder were separately soaked in 400ml of 95% Ethanol, Distilled water, Ethyl
108 acetate and Hexane in a 500ml reagent bottle and stoppered. This was allowed to stand for 14
109 days to permit full extraction of the active ingredients. The fluids were then filtered using
110 Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was
111 then kept in refrigerator at -4⁰c prior to use. (Appelbaum 1992), (Osuntokun 2015).

112 Extract preparation

113 The root and branch of the test plant were well dried and then grinded to powder. About 200g
114 of the powder were separately soaked in 400ml of **95% ethanol, distilled water and ethyl**
115 **acetate** in a 500ml reagent bottle and stoppered. This was allowed to stand for 14 days to
116 permit full extraction of the active ingredients. The fluids were then filtered using Whatman

No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge prior to use. A 2.0g/l solution of each extract was prepared and fractionated into 0.4g/l, 0.2g/l and 0.1g/l concentrations needed for the bioassay(Osuntokun *et al.*,2014).

Antimicrobial Assay (Determination of Zones of Inhibition) using Agar Well Diffusion method.

Twenty five milliliters of Mueller-Hinton agar were poured into sterile petri dishes and allowed to set. Standardized test bacterial cultures were inoculated into the sterile Kliger iron agar plate using sterile cotton swab. A sterile cork borer of 6mm diameter were used to punch wells on the agar on each of the petri dishes. Three holes were made on the surface of the plate with one in the center to serve as the control. Each hole was labeled (A,B,C, D) representing a particular concentration of 20mg/ml, 40mg/ml and 60mg/ml.The dishes were then filled into the wells with three drop(2ml) of their respective type of extract according to the labeled format. The central well containing the diluent reagent Streptomycin was used as control. The process was carried out for each extract and the inoculated petri dishes were left for few minutes for extract of diffuse into agar. The plates were incubated at 37°C for 18-24 hours after which the zones of inhibition (if any) were measured(Osuntokun & Olajubu 2014).

PHYTOCHEMICAL SCREENING

The extract were analysed for the presence of Alkaloid, Glycosides, Tannins, Saponins, Anthraquinones, Anthocyanosides, Flavonoids, Reducing sugars, Cyanogenic (Edeoga *et al.*,2005, Osuntokun & Ajayi 2014, Harbone 1998,Sofowora 2008).

Qualitative Method of Analyses

Plant filtrates were prepared by boiling 20g of the fresh plant in distilled water. The solution was filtered through a vacuum pump.The filtrate were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides.

(i).Test for Alkaloids

143 About 0.2g was warmed with 2% of H_2SO_4 for two minutes, it was filtered and few drops of
144 Dragendoff's reagent were added. Orange red precipitates, indicate the present of Alkaloids (Trease
145 and Evans 1989).

146 **(ii) Test for Tannins**

147 One milliliter of the filtrate was mixed with 2ml of FeCl_3 , A dark green colour indicated a positive
148 test for the tannins.

149 **(iii) Test for Saponins**

150 One milliliter of the plant filtrate were diluted with 2 ml of distilled water; the mixture were
151 vigorously shaken and left to stand for 10min during which time, the development of foam on the
152 surface of the mixture lasting for more than 10mm, indicates the presence of saponins.

153 **(iv) Test for Anthraquinones**

154 One milliliter of the plant filtrate were shaken with 10ml of benzene; the mixture was filtered and 5
155 ml of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a
156 positive test

157 **(v).Test for Anthocyanosides**

158 One milliliter of the plant filtrate was mixed with 5 ml of dilute HCl ; a pale pink colour indicates
159 the positive test.

160 **(vi)Test for Flavonoids**

161 One milliliter of plant filtrate were mixed with 2 ml of 10% lead acetate; a brownish precipitate
162 indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 ml of the plant filtrate
163 were mixed with 2ml of dilute NaOH ; a golden yellow colour indicated the presence of flavonoids

164 **(vii)Test for Reducing Sugars**

165 One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown
166 colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars.

167 **(viii) Test for Cyanogenic glucosides.**

168 This was carried out subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium
169 picrate to the filtrate and heated to boil (Trease and Evans 1989).

170 **(ix) Test for Cardiac glucosides.**

171 Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic
172 anhydrate plus H_2SO_4 (Trease and Evans 1989)

173 **Quantitative Method of Analyses**

174 **(i) Saponins**

175 About 20g each of dried plant samples were ground and, put into a conical flask after which 100 ml
176 of 20 % aqueous ethanol were added. The mixture was heated using a hot water bath. At about $55^{\circ}C$,
177 for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted
178 with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water
179 bath at about $90^{\circ}C$. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of
180 diethyl ether were added and then shaken vigorously. The aqueous layer were recovered while the
181 ether layer was discarded. The purification process was repeated three times. 60 ml of n-butanol
182 were added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium
183 chloride. The remaining solution was heated in a water bath. After evaporation, the samples were
184 dried in the oven to a constant weight, the saponin content was calculated as percentage of the
185 starting material (Osuntokun 2015).

186 **(ii) Flavonoids**

187 About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at
188 room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate
189 were later transferred into a crucible and evaporated into dryness over a water bath; the dry content
190 were weighed to a constant weight (Edeoga *et al.*, 2005).

191 **(iii) Cardiac glucosides**

192 Legal test and the killer-kilianiw was adopted, 0.5g of the extract were added to 2ml of acetic
193 anhydrate plus H_2SO_4 (Trease and Evans 1989).

(iv) Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1M Hydrochloride and 0.008 M potassium ferrocyanide. The absorbance were measured at 120 nm within 10 minutes. The tannins content was calculated.

(v) Alkaloids

5g of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hour. This were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass (Trease and Evans 1989).

(vi) Phlobatannins

About 0.5g of each plant extracts were dissolved in distilled water and filtered. The filtrates were boiled in 2% HCl, red precipitate show the present of phlobatannins.

Total Phenol (Spectrophotometric methods): 2g each of the samples were defatted with 1ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free samples were boiled with 50ml of ether for the extraction of the phenolic components for 15 minutes. 5ml of the extracts were pipetted into 5ml flask and then 10ml distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min. for colour development. This was measured at 505 nm. (Osuntokun 2015), (Obadoni *et al.*, 2001), (Boham, &Kocipai-Abyazam, 1974).

Determination of Proximate analysis of medicinal plants

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods.

222 **1.Determination of moisture content** was done by drying samples in oven (WiseVen, WON-50,
 223 Korea) at 110 °C until constant weight was attained (Horwitz 2003).

224 **2.Nitrogen estimation** was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360,
 225 Switzerland) method with some modification_Hussain *et al.*,2011.

226 **3.The crude proteins** were subsequently calculated by multiplying the nitrogen content by a factor
 227 of 6.25, Hussain *et al.*,2011 The energy value estimation was done by summing the multiplied
 228 values for crude protein,

229 **4.Crude fat and carbohydrate** respectively at Water Factors (4, 9 and 4). Crude fats were
 230 determined by Soxhlet apparatus using *n*-hexane as a solvent.

231 **5.The ash values** were obtained by heating samples at 550 °C in a muffle furnace (Wise Therm,
 232 FHP-03, Korea) for 3 h. Hussain *et al.*,2011

233 **6.The carbohydrate content** was determined by subtracting the total crude protein, crude fiber, ash
 234 content and crude fat from the total dry matter(Horwitz 2003)..

235 **7. Crude fiber was estimated** by acid-base digestion with 1.25% H₂SO₄ (v/v) and 1.25% NaOH
 236 (w/v) solutions Al-Harrasi *et al* ,.2012.

237 RESULTS-

238 **Table 2 shows the Antibacterial Activity of Aqueous Extract of Medicinal Plants at**
 239 **20mg/mL,40mg/mL, and 60mg/mL Concentration.** The medicinal plants used for this bioassay
 240 are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*,
 241 *Indigofera arrecta*, *Moringa olefera* and the clinical organism are *Salmonella typhi* *Staphylococcus*
 242 *aureus*, *Klebsiella pneumonniae*, *Escherichia coli*. The control for the experiment is Streptomycin at
 243 20mg/mL concentration.

244 Table 2

Selected Clinical organisms	<i>Salmonella typhi</i>			<i>Staphylococcus aureus</i>			<i>Klebsiella pneumonniae</i>			<i>Escherichia coli</i>			Streptomycin (control)
Extracts	20 mg/mL	40 mg/mL	60 mg/mL	20 mg/mL	40 mg/mL	60 mg/mL	20 mg/mL	40 mg/mL	60 mg/mL	20 mg/mL	40 mg/mL	60 mg/mL	20 mg/mL

						L	L			L			
<i>Acacia albida</i>	10.0	12.0	13.0	10.0	12.0	14.0	10.0	12.0	13.0	4.0	7.0	10.0	2.50
<i>Anchomanes difformis</i>	10.0	11.0	12.0	12.0	15.0	16.0	10.0	11.0	12.0	3.0	5.0	7.0	2.40
<i>Boscia senegalensis</i>	7.0	8.0	10.0	10.0	12.0	13.0	8.0	10.0	11.0	7.0	8.0	10.0	2.0
<i>Bridelia ferruginea</i>	9.0	12.0	13.0	13.0	14.0	15.0	5.0	7.0	8.0	3.0	6.0	8.0	3.0
<i>Ficus ingens</i>	7.0	14.0	15.0	10.0	15.0	19.0	5.0	8.0	10.0	6.0	7.0	8.0	2.7
<i>Indigofera arrecta</i>	6.0	10.0	13.0	10.0	11.0	12.0	7.0	9.0	9.5	2.0	6.0	7.0	3.5
<i>Moringa olefera.</i>	6.0	7.0	10.0	7.0	10.0	12.0	4.0	5.0	7.0	5.0	8.0	10.0	4.0

245 Unit of zone of inhibition- mm

246

247 Table 3 shows the Antibacterial Activity of Ethanol Extract of Medicinal Plants at
248 20mg/mL,40mg/mL, and 60mg/mL Concentration.The medicinal plants used for this bioassay
249 are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*,
250 *Indigofera arrecta*, *Moringa olefera* and the clinical organism are *Salmonella typhi* *Staphylococcus*
251 *aureus*, *Klebsiella pneumonniae*, *Escherichia coli*. The control for the experiment is Streptomycin at
252 20mg/mL concentration.

253 Table 3

Selected Clinical organisms	<i>Salmonella typhi</i>	<i>Staphylococcusa aureus</i>	<i>Klebsiella pneumonniae</i>	<i>Escherichia coli</i>	Strepto mycin (control)
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Extracts	20 mg/ mL	40 mg/ mL	60 mg/ mL	20 mg/ mL	40 mg/ mL	60 mg/ mL	20 mg/ mL	40 mg/ mL	60 mg/ mL	20 mg/mL	40 mg/mL	60 mg/mL	20 mg/mL
<i>Acacia albida</i>	2.0	5.0	6.0	1.0	2.0	4.0	3.0	5.0	7.0	2.0	3.0	4.0	3.0
<i>Anchomanes difformis</i>	3.0	5.0	7.0	1.0	4.0	6.0	2.0	5.0	8.0	1.0	3.0	8.0	2.9
<i>Boscia senegalensis</i>	2.0	2.0	5.0	1.0	3.0	5.0	2.0	4.0	5.0	2.0	3.0	6.0	4.5
<i>Bridelia ferrug inea</i>	5.0	6.0	6.0	2.0	4.0	6.0	3.0	3.5	5.0	2.0	4.0	7.0	4.0
<i>Ficus Ingens</i>	8.0	10. 0	11. 0	10.	12. 0	13.	5.0	6.0	9.0	8.0	11.0	10	5.0
<i>Indigofera arrecta</i>	10.0	11. 0	13. 0	10. 0	11. 0	12. 0	7.0	8.0	10	2.0	7.0	8.0	4.0
<i>Moringa oleifera.</i>	8.0	1.0	12. 0	10. 0	11. 0	13. 0	4.0	5.0	8.0	1.0	5.0	7.0	3.5

254

Unit of zones of inhibition-mm

255 **Table 4 shows the Antibacterial Activity of Ethyl Acetate Extract of Medicinal Plants at**
256 **20mg/mL,40mg/mL, and 60mg/mL Concentration.**The medicinal plants used for this bioassay
257 are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*,
258 *Indigofera arrecta*, *Moringa oleifera* and the clinical organism are *Salmonella typhi* *Staphylococcus*
259 *aureus*, *Klebsiella pneumonniae*, *Escherichia coli*. The control for the experiment is Streptomycin at
260 20mg/mL concentration.

261

Table 4

Selected Clinical organisms	<i>Salmonella typhi</i>			<i>Staphylococcus aureus</i>			<i>Klebsiella pneumonniae</i>			<i>Escherichia coli</i>			Streptomy cin (control)
Extracts	20	40	60	20	40	60m	20	40mg	60m	20mg/	40mg	60mg/	20mg/mL

	mg /m L	mg/ mL	mg /m L	m g/ m L	mg /m L	g/mL	mg/ mL	/mL	g/m L	mL	/mL	mL	
<i>Acacia albida</i>	4.0	7.0	9.0	1.0	2.0	3.0	4.0	7.0	9.0	4.0	7.0	9.0	1.0
<i>Anchomanes difformis</i>	1.0	1.0	8.0	1.0	3.0	5.0	6.0	3.0	6.0	1.0	1.0	8.0	5.0
<i>Boscia senegalensis</i>	1.0	1.0	4.0	1.0	4.0	8.0	2.0	3.0	4.0	1.0	3.0	4.0	8.0
<i>Bridelia ferruginea</i>	3.0	3.0	5.0	2.0	3.0	5.0	1.0	2.0	7.0	3.0	4.0	6.0	7.0
<i>Ficus ingens</i>	1.0	1.0	2.0	1.0	5.0	8.0	1.0	4.0	8.0	1.0	4.0	5.0	5.0
<i>Indigofera arrecta</i>	1.0	4.0	8.0	1.0	4.0	7.0	1.0	3.0	6.0	1.0	4.0	8.0	5.0
<i>Moringa oleifera.</i>	1.0	7.0	9.0	1.0	4.0	8.0	1.0	4.0	8.0	1.0	7.0	9.0	8.0

262

Unit of zones of inhibition-mm

263 **Table 5 shows the Antibacterial Activity of Hexane Extract of Medicinal Plants at**
264 **20mg/mL,40mg/mL, and 60mg/mL Concentration.**The medicinal plants used for this bioassay
265 are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*,
266 *Indigofera arrecta*, *Moringa oleifera* and the clinical organism are *Salmonella typhi* *Staphylococcus*
267 *aureus*, *Klebsiella pneumoniae*, *Escherichia coli*. The control for the experiment is Streptomycin at
268 20mg/mL concentration.

269

Table 5

Selected Clinical organisms	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	Strept omycin

													(contr ol)
Extracts	20 mg /m L	40 mg /m L	60 mg /m L	20 mg/ mL	40 mg/ mL	60m g/m L	20 mg/ mL	40m g/m L	60m g/m L	20 mg/ mL	40m g/m L	60m g/mL	20mg/ mL
<i>Acacia albida</i>	1.0	3.0	4.0	0.0	1.0	6.0	2.0	5.0	8.0	3.0	5.0	10.0	5.0
<i>Anchomanes difformis</i>	5.0	6.0	7.0	4.0	5.0	10.0	5.0	6.0	7.0	4.0	8.0	10.0	4.0
<i>Boscia senegalensis</i>	4.0	6.0	8.0	6.0	8.0	10.0	5.0	6.0	7.0	4.0	8.0	10.0	5.0
<i>Bridelia ferruginea</i>	2.0	4.0	6.0	1.0	7.0	9.0	8.0	12.0	5.0	10. 0	12.0	16.0	6.0
<i>Ficus ingens</i>	2.0	5.0	8.0	3.0	5.0	10.0	2.0	6.0	6.0	7.0	10.0	11.0	3.5
<i>Indigofera arrecta</i>	2.0	6.0	6.0	7.0	5.0	11.0	5.0	6.0	7.0	4.0	8.0	10.0	4.0
<i>Moringa oleifera.</i>	8.0	12. 0	5.0	10. 0	8.0	16.0	2.0	5.0	8.0	3.0	5.0	10.0	4.2

Unit of zones of inhibition -mm

Table 6 shows the Qualitative Analysis Of Phytochemicals Screening of The Medicinal Plants. The medicinal plants are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta*, *Moringa oleifera* and the qualitative parameter are Alkaloid, Cardiac Glycoside, Steroid, Anthraquinone, Phenol, Tannins, Saponin and Flavonoids.

Medicinal plants used	Alkaloid	Cardiac Glycoside	Steroid	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids
<i>Acacia albida</i>	+	+	+	-	+	+	+	-
<i>Anchomanes difformis</i>	+	+	+	-	+	+	+	+
<i>Bosciasenegalensis</i>	+	+	+	+	+	+	+	-
<i>Bridelia ferruginea</i>	-	+	+	+	+	+	-	+
<i>Ficus ingens</i>	+	+	-	+	+	+	+	-
<i>Indigofera arrecta</i>	+	+	+	+	+	+	+	-
<i>Moringa oleifera</i>	-	+	-	+	+	+	+	+

278 **Key- Positive (+), Negative (-)**

279 **Table 7 shows Quantitative Analysis Of Minerals Present In Medicinal Plants Extract**
 280 **(mg/100g).**The medicinal plants are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*,
 281 *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta*, *Moringa oleifera*. The minerals that were are
 282 Sodium(Na^{2+}),Potassium(K^{+}),Calcium(Ca^{2+}),Magnesium(Mg^{+}),Zinc(Zn^{2+}),Iron(Fe^{2+}),Lead(Pb^{2+}),Co
 283 pper(Cu^{+}),Manganese(Mn^{+}) and Phosphorus (P^{+})

284

285

Table 7

Medicinal Plants sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn	P
<i>Acacia albida</i>	22.73	29.11	30.45	26.05	28.04	7.88	ND	0.03	6.00	24.51
<i>Anchomanes difformis</i>	20.56	22,34	21.89	22.10	20.89	5.92	ND	0.04	6.34	25,91
<i>Boscia senegalensis</i>	14.59	14.20	18.77	22.10	21.59	22.10	2.24	1.20	6.23	17.90
<i>Bridelia ferruginea</i>	19.00	23.98	22.12	23.34	16.89	22.12	3.56	2.16	5.79	18.78
<i>Ficus ingens</i>	20.92	23.12	29.34	24.78	17.34	20.34	2.78	3.00	4.92	27.64
<i>Indigofera arrecta</i>	21.33	43.21	16.50	26.37	17.75	4.36	ND	ND	14.33	88.43
<i>Moringa oleifera.</i>	20.00	26.14	31.46	22.05	25.03	5.77	ND	0.01	6.20	25.12

287

288 **Table 8 shows the Quantitative Analysis Of Anti- Nutrient Present In Medicinal Plants Extract**
 289 **In Percentage (%).**).The medicinal plants are *Acacia albida*, *Anchomanes difformis*, *Boscia*
 290 *senegalensis*, *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta*, *Moringa oleifera*..the anti-
 291 nutrient detected are Tannin, Phenol, Phylate, Oxalate, Saponin, Flavonoid, Alkaloids,Tannin and
 292 Phenol

293

Table 8

PARAMETERS	<i>Acacia albida</i>	<i>Anchomanes difformis</i>	<i>Boscia senegalensis</i>	<i>Bridelia ferruginea</i>	<i>Ficus ingens</i>	<i>Indigofera arrecta</i>	<i>Moringa oleifera</i>
Tannin	2.20	2.10	2.32	2.37	2.30	2.25	ND
Phenol	2.50	3.57	2.56	2.49	3.49	3.45	ND
Phylate	16.30	15.27	15.68	15.79	12.33	12.45	2.25
Oxalate	3.66	3.74	6.57	6.51	8.51	8.55	1.50
Saponin	13.87	14.04	9.78	9.70	7.52	7.61	1.67
Flavonoid	8.59	8.52	6.43	6.56	10.38	10.41	ND
Alkaloids	3.23	1.25	4.25	4.31	4.36	4.37	
Tannin	2.20	2.10	2.32	2.37	2.30	2.25	ND
Phenol	2.50	3.57	2.56	2.49	3.49	3.45	ND

294

295 **Table 9 shows the Quantitative Analysis Of Proximate Composition of Medicinal Plants**
296 **Extract(%).**The medicinal plants are *Acacia albida*, *Anchomanes difformis*, *Boscia senegalensis*,
297 *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta*, *Moringa oleifera* The detectable parameter are
298 percentage composition of Ash,Moisture content, Crude protein, Fat, Fibre and Carbohydrate.

Table 9

Medicinal plants used	% Ash	% MC	% CP	% Fat	% Fibre	%CHO
<i>Acacia albida</i>	10.67	9.31	13.33	6.43	11.49	46.00
<i>Anchomanes difformis</i>	11.69	9.46	12.87	6.46	11.41	47.12
<i>Boscia senegalensis</i>	9.37	3.74	14.68	7.22	4.33	60.55
<i>Bridelia ferruginea</i>	9.39	3.79	14.72	6.82	4.46	60.90
<i>Ficus ingens</i>	8.72	7.32	16.25	5.34	8.53	53.72
<i>Indigofera arrecta</i>	8.78	7.33	16.19	5.40	8.50	53.79
<i>Moringa oleifera.</i>	10.53	9.00	14.45	6.50	10.37	42.00

300 **Keys: MC=Moisture content CP=Crude protein. CHO=Carbohydrate.**

301 **DISCUSSION**

302 The pediatrics hospital, little or nothing is known about the presence of microorganism in this health
303 care facility, but with this present study, it is a fact that microbes care also be present and isolated in
304 this health care facility, putting into consideration that children are prone to microbe attack even if
305 ,they are still in the hospital. Adequate care must be taken to reduce the presence of pathogen in the
306 pediatrics hospital to avoid cross contamination and reemergence of infectious diseases.

307 The Aqueous, Ethanol, Hexane and **Ethyl acetate** extracts of the leaves, and roots of *Acacia albida*,
308 *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Ficus ingens*, *Indigofera arrecta*
309 and *Moringa oleifera* were examined for possible properties of antimicrobial and phytochemical

activities against the selected clinical organisms. The clinical organisms used are *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*.

It was observed that in Aqueous extract of *Anchomanes difformis*, *Ficus ingens*, *Moringa oleifera* and *Boscia senegalensis* shows appreciable activity of antibacterial potency against the test organism with the zone of inhibition from 10 mg/mL and 19 mg/mL and *Acacia albida*, *Bridelia ferruginea* and *Indigofera arrecta* shows lesser activity of antibacterial potency between the ranges of 6mg/mL to 12 mg/mL. This result conform with the finding of Indrayan *et al.*, 2002, Osuntokun & Olajubu 2014, the action of plant against microorganism.

Ethanol extracts shows appreciable high antibacterial activity in the plant extract of *Anchomanes difformis*, *Ficus ingens*, *Indigofera arrecta* and *Moringa oleifera* while in *Acacia albida*, *Boscia senegalensis*, *Bridelia ferruginea* shows appreciable low zones of inhibition between the range of 6mL/mg to 12 mg/mL. this conform to the fact that plant are probable source of antimicrobial and therapeutic agent against some nosocomial organisms especially the organism found in pediatrics hospital and other hospital related organism, if this is possible, Medicinal plants serves as a source good antibiotics, then it can be consumed in are fined form in our day to day activity, like in our coffee tea, beverages, soup ingredient, food seasons and etc. this is a proof evident by De and James 2002.

Ethyl acetate plant extracts shows high zones of inhibition between 7mg/mL and 10mg/mL in the plant extract of *Anchomanes difformis*, *Indigofera arrecta* while *Acacia albida*, *Boscia senegalensis*, *Bridelia ferruginea* and *Ficus ingens* shows low zone of inhibition between 1mg/mL and 5mg/mL. *Anchomanes difformis* and *Indigofera arrecta* are good source of inane booster in the case of HIV patient and some researchers are in the advent of making a good HIV drugs from the duo of *Anchomanes difformis*, and *Indigofera arrecta*. This another proof that medicinal plant is a good source of therapitic agent. (Lambert, 2002; Russell, 2002).

Hexane plant extracts shows appreciable antibacterial activity. *Anchomanes difformis*, *Boscia senegalensis*, *Bridelia ferruginea*, *Indigofera arrecta* and *Moringa oleifera* shows higher zones of inhibition compare with low zones of inhibition in exhibited by *Acacia albida* and *Ficus ingens*.

The activities of all the medicinal plants extracts, predicts the presence of antimicrobial evidence in the medicinal plants under study, it also brighten the hope for the advents of new antibiotics which

can be derived from medicinal plants source but not only new antibiotics but evidence for new drugs for the cure of different infection associated with pathogen microorganism, under which gram positive and gram negative bacteria has been adequately taken into consideration (Osuntokun 2015).

Ogundare *et al.*, (2006) shows that plant species of *Anchomanes difformis*, *Indigofera arrecta* and probable *V. tenoreana* containing saponins, flavonoids, tannins and anthraquinones which were found to have very potent antibacterial as well as antifungal activities. These phytochemical constituents were further reported to be responsible for many antimicrobial activities of different plant species (Ghoshal *et al.*, 1996; Iwu *et al.*, 1999). Flavonoids have been reported to be synthesized by plants in response to microbial infections and are good antibacterial agents; tannins have been demonstrated to have antibacterial activities (Akiyama *et al.*, 2001), (Osuntokun 2015).

Proximate composition of the medicinal plant under study, shows that, carbohydrate, moisture, fat, fibres, ash and crude protein are all present inclusive in plant analysis. It was observed that carbohydrate has the highest proportion followed by crude protein. Scientifically, two proximate quantities are the most important element in life. Plants are the medicinal intrinsic worth providing essential nutrients. Proteins and carbohydrates are important nutrients to be assessed in medicinal plants Ekanayake *et al.*, 1999. All fractions (cellulose, lignin, hemicellulose, pectin, gums and mucilage) of the fiber play a significant role treating various diseases such as diabetes, constipation, diverticulosis, cardio-vascular diseases, and fatness, Ekanayake *et al.*, 1999, Ramulu and Rao 2003. In recent years, much work has been performed for producing and evaluating various sources of proteins and fibers, Ramulu and Rao 2003. Proteins enclose essential amino acids and have the nutritional values for human health.

The importance of phytochemicals must be clearly emphasized in this present study. Phenols is another phytochemical that were found in the medicinal plant, they are weakly acidic, Hydroxyl group attached directly to an aromatic ring, it serves as Antiseptic, anti-inflammatory, antimicrobial, anti-tumor and also a good disinfectant in its activity which is found in table 6 Ogundare *et al.*, (2006).

Flavonoids is water soluble, super antioxidant and free radical scavenger as antioxidant, anticarcinogens, antimicrobial, antitumor. Saponins were observed to have bitter taste, foaming

property, haemolytic effect on red blood cells and emulsifying agent. it were also observed that saponin containing plant are good expectorant, cough suppressant, hemolytic activity (Osuntokun & Oluwafoise 2015).

Tannins were observable in medicinal plant under study. they have unpleasant taste, tans leather, used in the production of leather and ink, in treating wounds, varicose ulcers, hemorrhoids, frostbite and burns, and it has a soothing relief, regenerates skin, anti-inflammatory and diuretics in its activity (Osuntokun & Oluwafoise 2015). In other word phytochemical and medicinal plants plays unquantifiable role in our day to day activities, this important were x-rayed in this research work.

CONCLUSION

Nigerian populace are fed-up with the foreign conventional drugs which can be, and always been adulterated by some disgruntle elements in the society, but, there is need for the herbal drugs from our natural environment must be developed. This herbal drugs cannot be adulterated, but can be improved upon, by the standardizing the production and herbal practice, due to the fact that medicinal plants are all over the four geo-political zone of the country, in other word the production and the use of the medicinal plant must be standardized and encouraged which can also be a source of revenue to the Nigerian. The medicinal plants has an antibacterial and phytochemical effectiveness on the tested bacterial isolates resulted within 24 hours of incubation of the organism to all the plant extract. The aqueous, ethanol and ethyl acetate extract display a high level of antibacterial activity compared to the extract of hexane, but all the plant tested against the clinical organism have a certain degree of antibacterial activity, the best activity were found in *Anchomanes difformis* in aqueous extract, *Moringa oleifera* in ethanol and ethyl acetate extract and *Indigofera arrecta* in hexane extract have little or less activity against the organism, knowing fully well, that the hexane extract do not really do well in this research work, but in all, all plant were proven active because of their zones of inhibition. this result shows that medicinal plants have a great effect on clinical organism found on pediatric hospital in Ekiti and Ondo state.

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