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ANTIBACTERIAL ACTIVITY OF *JATROPHA CURCAS* AND *VIGNA UNGUICULATA* LEAF EXTRACTS AGAINST *STAPHYLOCUCCUS AUREUS* ISOLATED FROM CHILDREN WITH OTITIS MEDIA ATTENDING MURTALA MUHAMMAD SPECIALIST HOSPITAL

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Abstract— **The study was conducted to determine the antibacterial activity of *Jatropha curcas* and *Vigna unguiculata* leaf extracts against *Staphylococcus aureus,* with the aim to determine the phytochemical properties and antibacterial activity of *Jatropha curcas* and *Vigna unguiculata* leaf extracts against *Staphylococcus aureus* isolated from children with otitis media attending Murtala Muhammad Specialist Hospital with the objectives as follows, To determine the qualitative and quantitative phytochemical constituents of *Jatropha curcas* and *Vigna unguiculata* leaf extracts, using quantitative procedure of alkaline precipitation gravimetric method described by Harbome (1998). To identify and characterize *Staphylococcus aureus* from children with otitis media using biochemical tests and serological test of dryspot staphytect plus latex agglutination test, to determine the antibacterial activity of the *Jatropha curcas* and *Vigna unguiculata* leaf extracts against *Staphylococcus aureus* of otitis media origin and finally to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal Concentration (MBC) of *Jatropha curcas* and *Vigna unguiculata* leaf extracts against *Staphylococcus aureus.* In which result of the study indicated the presence of alkaloids, saponins, terpenoids, and free anthraquinone in both *Jatropha curcas* and *Vigna unguiculata* leaf extracts respectively. However, Steroids is present in *Vigna unguiculata* leaf while absent in *Jatropha curcas* meanwhile, flavonoid is present in *Jatropha curcas*, which is absent in *Vigna unguiculata.* Quantitatively, the content of alkaloids in *Jatropha curcas* and *Vigna unguiculata* leaves is 2 and 10%, saponin is 6.2 and 3.56%, and terpenoid is 94.3 and 78.57% respectively. Meanwhile, the flavonoids content in *Jatropha curcas* is 2% and also that of Steroidss in *Vigna unguiculata* is 2.0%. Out of 176 samples collected, from ENT unit of Murtala Muhammad Specialist Hospital, 33(18.75%) appeared to be Gram positive, cocci, golden yellow, slightly raised colonies on Mannitol salt agar and clustered under the microscope. Moreover, based on gender, out of these 176 sample collected, 112(63.64%) out of which 24(72.73%) are positive in male patients, while 64(36.36%) are females, with 9(27.27%) positive samples. Biochemically, 33 samples are catalase positive, coagulase positive, DNase positive and fermentannitol. Out of 33 biochemically positive samples, 31(93.94%) are positive while 2(6.06%) are negative for Dryspot Staphytect Plus latex agglutination test.**

Keywords- **antibacterial activity, *Jatropha curcas,* leaf extracts, MBC, MIC, Otitis media, phytochemical, *Vigna unguiculata* and *Staphylococcus aureus***

I. INTRODUCTION

Plants are being used as valuables sources of food and medicine for the prevention of illness and maintenance of human health. Medicinal plants are cheap and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to bacteria

(Basile *et al*., 2009). The uses of medicinal plants as possible therapeutic measures have become a subject of scientific investigation (Patwardhan *et al.,* 2004). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. The most important bioactive compounds plants are alkaloids, Flavonoids, Tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agent from higher plants (Duraipandiya *et al*., 2006). Historically, plants have provided a source of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being.

Jatropha curcas

Jatropha curcas is called physic nut, poison nut or Purging nut in English, Fula in fulfulde, Binidazugu in hausa, Lapalapa, Yalode or Ologbotuje in Yoruba, etookpa in Efik and oru-ebo in Edo (Burkill, 1994). *Jatropha* is a large genus comprising over 170 species. Commonly occurring species in India are*: J. curcas, J. giandulifera, J. gossypifolia, J. multifida, J. nana and J. podagrica. J. curcas,* the commonest species in Nigeria, mainly grown for bio-diesel because of its high oil content (48%). It is a shrub or small tree with smooth grey bark, which exudes whitish coloured watery latex when cut. *Jatropha curcas* is a medicinal crop that belongs to the family *Euphorbiaceae* and has a long history of cultivation in tropical America, Africa, and Asia (Ravindrath *et al.,* 2004). The seed kernels contain a high amount of oil between 58 and 60% (v/w) (Aderibigbe *et al*., 2007), and serve as a source of biodiesel currently being used inIndia, Thailand, and other Soth East Asian countries. The seeds are reported to contain high protein, lectin, saponin, phytic acid, and toxic compounds (Martinez *et al*., 2006), as well as a wide of phytochemicals to which its antimicrobial effect is possibly attributable (Namuli *et al*., 2011). It has also been reported that all parts of *Jatropha curcas* can be used for a wide range of purposes. Extracts from various parts of *Jatropha curcas*, such as seeds and leaves, have been shown to have molluscicidal, insecticidal, and fungicidal properties (Rug and Ruppel 2000). *Jatropha curcas* seed extracts were found to inhibit the growth of Colletotrichummusaemycelia that cause anthracnose disease in bananas (Thangavelu *et al.,* 2004).The chemicals responsible for those effects were suggested to be phorbol esters in the extract (Goel *et al*., 2007), also stated that some derivatives of phorbol esters are known to have antimicrobial and antitumourproperties, as well as molluscicidal and insecticidal effects. *Jatropha curcas* is becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (Mkoma and Mabiki, 2012). *Jatropha curcas* grows well particularly in the Northern part of Nigeria and can be used to provide cheap source of antibiotic and disinfectant due to its antimicrobial activities that have been reported (Igbinosa, *et al*., 2009). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world‘ s population, especially in developing countries (WHO, 2002). Previous studies have reported that *J. Curcas* exhibits antimicrobial activity (Aiyela, *et al*., 2007; Igbinosa *et al*., 2009; Ekundayo *et al*., 2011; Namuli *et al*., 2011). The search for new antibacterial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics (Iwu *et al*., 2009; Wurochekker *et al*., 2008; Krishnaiah *et al*., 2009).

Vigna unguiculata

Cowpea (*Vigna unguiculata* (L) Walp.) is legume, belongs to the family *Leguminosae*. It is known as black eyed bean in English and *Lobia or Chawli* in Indian name (Marathe *et al*., 2011). Cowpea seed weighs between 80 to 320 mg and ranges in shape from round to kidney-shaped. Seed coat varies in texture (*e.g.*, smooth, rough, or wrinkled), color (*e.g.*, white, cream, green, buff, red, brown, black) and uniformity (*e.g.*, solid, speckled, or patterned) (Timko and Singh, 2008). It is one of the most ancient food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al*., 2010). Like for many other legumes, its seeds are the most economically valuable plant part of cowpea and are well-known due to their ascribed nutritional and medicinal properties. Known to be an excellent source of protein, cowpea is also rich in important vitamins, minerals, and soluble and insoluble dietary fiber. All parts of cowpea plants are used for food or fodder. The tender shoot tips and leaves are consumed when they reach the seeding stage while immature pods and seeds are consumed during the fruiting stage. Harvested dry seeds can be ground into slurry to make cowpea cake, or deep fried into bean balls, or the seeds could be boiled, mixed with sauce or stew and consumed directly. Plant residues are used as fodder for farm animals (Ferry *et al*., 2000).

Otitis Media

Otitis Media is inflammation of the middle ear. Otitis media occurs in the area between the ear drum and the inner ear, including a duct known as the eustachian tube (Richard and Robert, 1996). Otitis media is very common in childhood, with the average toddlers having two to three episodes a year and this is always accompanied by a viral upper respiratory infection (URI), mostly common cold caused by influenza virus (Richard and Robert, 2006). Children below the age of seven years are much more susceptible to otitis media (OM) since the eustachian tube is shorter and at more of a horizontal angle than in the adult and this is also because they have not developed the same resistance to bacteria, fungi and viruses as found in adults (Weiner and Collison, 2003). Breast feeding for the first twelve months of life is associated with a reduction in the number and duration of all otitis media infections (Owen *et al.,* 2003; Dewey *et al.,* 2005). Poor Eustachian tube function can overwhelmingly increase the

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likelihood of more frequent and severe episodes of otitis media. Progression to chronic otitis media is much more common in the group of people, who often have a family history of middle ear diseases. Over the past two decades there has been a dramatic increase in the number of consultations to the paediatricians for otitis, this probably reflects a combination of factors ranging from a change in the pattern of disease with more children in child care (Dewey *et al.*, 2005).

II. MATERIALS AND METHODS

A. Study Site

The study area for this research is Murtala Muhammad Specialist Hospital, Kano. Murtala Muhammad Specialist Hospital is located within Kano metropolis. The state is located between Latitude 12.2^0North and Longitude9.4^0 East Kano city as the capital of the state (Barau, 2007). Presently, the state is the most populous in Nigeria, with over nine million (9,000,000) people (National Population Commission, 2006). People from within and outside the metropolis and also some other neighbouring states are rapidly attending this health facility to seek healthcare. Infact, Murtala Muhammad Specialist Hospital is the state hospital with high number of attendees within the state, most especially by middle class.

B. Determination of Sample Size

One hundred and seventy-Six (176) samples were collected from children with Otitis Media Attending Murtala Muhammad Specialist Hospital, Kano.
This sample size was determined using formula below

$$n = z^2p\frac{(1 - p)}{d^2}$$

(Lwanga and Lemeshow, 1991;Kuta *et al*., 2014a).

Where n= sample size,

z= confidence level at 95%

p= prevalence 9% (Akesola and Fasina, 2012)

d= margin of error at 5% (0.05)

$$n=\frac{1.96^2 \times 0.09 \times (1 - 0.91)}{0.05^2}$$

$$=125.8$$

$$n\approx126 +50=176$$

C. Sample Collection

Fresh leaves of *Jatropha curcas* were collected from a very healthy and fully growing plants from BurumBurum, Tudunwada Local Government while *Vigna unguicula* samples were collected from Yakasai Town, along Zaria road, Kura Local Government, Kano State, Nigeria. The leaves of the two plants were collected in the morning hours and washed with water to ensure proper sanitation (Mukhtar and Tukur, 1999).
Also, an ear swap from one hundred and seventy six children was collected from Murtala Muhammad Specialist Hospital, Kano. The ear swap was collected using swap stick, recapped and transported to the Microbiology laboratory, Kano University of Science and Technology, Wudil for analysis in an icebox to avoid further contamination during handling and to ensure the maintenance of appropriate temperature (Cheesbrough, 2010).

D. Identification of Plant Materials

The leaves of the two plants, *J. curcas* and *Vigna unguiculata* were transported to the Department of Plant Science, Bayero University, Kano for identification and obtaining a voucher number of the two plants. They were identified and authenticated at Herbarium in Department of Plant Science as *Jatropha curcas* and *Vigna unguiculata* BUKHAN 60 and 99 respectively. After identification, two plants were transported to the Microbiology Laboratory in the Department of Microbiology, Kano University of Science and Technology, Wudil, Kano State Nigeria for further processing. The leaves were air-dried by spreading them on the table in the laboratory for a period of three (3) weeks, after which they were seperately grinded to powder and then sieved by 35mm sized mesh using pestle and mortar (Mukhtar and Tukur 1999).

E. Preparation of Plant Extract

One hundred grams (100g) each of the powdered leaves of *Jatropha curcas* and *Vigna unguiculata* were added to 300ml each of ethanol, methanol and distilled water respectively. Each was allowed to stand for 3days at room temperature (28 ±2ºC), with agitations at intervals. Each extract was sieved through a muslin cloth, filtered through a Whatman (no.1) filter paper, poured unto a clean evaporating dish and placed on a rotary evaporator and then water bath at 50ºC until all the solvent evaporated. The dried extracts were stored in a sterile beaker and kept in the refrigerator at 4ºC until required for use (Suresh *et al*., 2008).

F. Qualitative Phytochemical Screening

The extracts of *Jatropha curcas* and *Vigna unguiculata* were subjected to various phytochemical analysis which identified the chemical constituents present using standard method described by Sofowora (1993). One gram (1g) of each powdered extract was weighed and dissolved in 10ml of sterile distilled water and filtered using WhatmanNo. 1 filter paper. One millilitre each of the filtrate of each dissolved extract, was dispensed into various test tubes and used for the following tests:saponins, tannins, alkaloids, flavonoids, cardiac glycosides, free antrhaquinone, steroids and terpenoids.

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G. Quantitative Phytochemical Determination

This analysis was carried to determine the amount or concentration of the phytochemical constituents present in the plant leaves screened above.

Determination of alkaloids

The determination of the concentration of alkaloids in the each of the two samples (*Jatropha curcas* and *Vigna unguiculata* leaf extracts) was carried out using the alkaline precipitation gravimetric method described by Harborne, (1990). A 5g of the powdered sample was soaked in 20 ml of 10% ethanol acetic acid. The mixture was stored for four hours at room temperature and thereafter was filtered through Whatman filter paper (No. 42). The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60ºC for 30 min, cooled in a dessicator and reweighed. The weight of alkaloid was determined by the weight differences and expressed as a percentage of weight of sample analyzed (Harbome, 1990).

Determination of flavonoids

The Flavonoids content of the samples was determined by the gravimetric method described by Harborne (1990). A 5g of each powdered sample was placed into a conical flask and 50 ml of water and 2 ml of ethyl acetate solution were added. The solution was allowed to boil for 30 min. The boiled mixture was allowed to cool and filtered through Whatman filter paper (No. 42). About 10 ml of ethyl acetate extract which contained flavonoids was recovered, while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filter the second (ethyl acetate) layer. The residue was then placed in an oven to dry at 60ºC. It was cooled in a dessicator and weighed (Harbome, 1999).

Determination of saponins

The saponins content of the samples was determined by double extraction gravimetric method.

A 5g of each powdered sample was mixed with 50 ml of 20 % aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 min at 55ºC. It was then filtered through Whatman filter paper (No. 42). The residue was extracted with 50 ml of 20% ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90^0C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer became clear in colour. The saponins were extracted with 60 ml of n-butanol; the combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60ºC in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. Saponins content was determined by difference and calculated as a percentage of the original sample (Harbome, 1990).

Determination of steroids

The extract (1 g) was marcarated with 20 ml of ethanol and filterd. To the filterate (2 ml), 2ml of chromagen solution was added and the solution left to stand for 30 min. The absorbance was read at 550 nm (Ekwueme *et al*., 2015)

Determination of terpenoids

Dried powdered of *Jatropha curcas*and *Vigna unguiculata* 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hours (Indumathi *et al*., 2014). The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100) Ekwueme *et al*., (2015).

H. Culture and Identification

The samples were inoculated aseptically with a wire- loop on the prepared Nutrient Agar (Titan Biotech Ltd) plates and incubated at 37^OC between 18hours and 24hours. Then the result was observed for the growth of microorganisms. Discrete colonies were purified by sub-culturing into Mannitol Salt agar (Bichanan and Gibbons, 1994) plates and was subsequently identified using standard methods. Golden yellow colonies grow on Mannitol Salt Agar due to its high salt concentration. The isolates were characterized and identified based on their cultural characteristics and biochemical reaction according to Buchanan *et al.*, (1994).

I. Gram staining and microscopy

To differentiate gram positive and gram negative organisms, a gram reaction test was carried out. Smear of the isolates was fixed on clean grease free slides and stained with crystal violet solution (primary dye) for 60 seconds, rinsed with tap water and drained to avoid diluting with the mordant. It was further flooded with lugol' s iodine solution (mordant) for 30 seconds and rinsed. Then the decolourizer was then applied drop wise on the tilted slide until all free colours had been removed and subsequently be rinsed with tap water, the slides were then flooded with Safranin (secondary dye). The slides were then examined under the microscope at ×100 oil immersion objective (Cheesbrough, 2010).

J.Biochemical tests

Catalase test

Catalase test helps to differentiates staphylococci from streptococci. Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide into oxygen and water.

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This was done by addition of a drop of the bacterial suspension to a drop of hydrogen peroxide on a clean microscope slide. The appearance of effervescence and bubbling is an indication of a positive reaction. This test was done to identify members of the genus Staphylococci (Ochai and Kolhatkar, 2007).

Coagulase test

The test was used to identify *Staphylococcus aureus* which produce the coagulase enzymes that cause plasma to clot by converting fibrinogen to fibrin. Slide method wasused. The slide required dropping of sterile distilled water and placing on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspension and mixed gently. The slide was examined for clumping or clotting of the organisms within 10 seconds. Plasma was not added to the second suspension which serves as control (Nwakanwa *et al.,* 2015).

Deoxyribonuclease (DNase) test

This test is used to helping the identification of *S. aureus* which produces deoxyribonuclease (DNase) enzyme. The DNase test is particularly useful when plasma is not available to perform coagulase test or when the results of a coagulase test are difficult to interpret. Deoxyribonuclease hydrolyses deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitate unhydrolysed DNA. DNase producing colonies are therefore surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2010).

Fermentation of mannitol

Staphylococcus aureus ferments mannitol and is able to grow well on mannitol salt agar. A selective medium containing peptone, lab-lemco powder, mannitol, 70-100g/l sodium chloride, phenol red, pH 7.3 at room temperature and agar would be prepared according to manufacturer ‘ s instruction by suspending 111 g of the agar in 1000ml distilled water, boiled and sterilized by autoclaving at 121ºC for 15minutes and cooled to 45ºCbefore dispensing in Petridishes 0.1 ml of the bacterial suspension would be placed on the plates and spreaded with sterile swab stick (Cheesbrough, 2010).

Serological analysis
Dryspot Stapyhtect Plus was used for serology. This latex agglutination test, available from Oxoid, uses reagent that has been dried on a reaction card. It detects up to 97% of *S. aureus* strains, including most MRSA. Colonies of *S. aureus* are emulsified in saline and mixed with dry reagent. Agglutination of the blue latex particles indicates a positive test. Test cards can be stored at room temperature up 25ºC (Ochai and Kolhatkar, 2007).

Preparation of turbidity standard

McFarland standard are used as a reference to adjust the turbidity of microbial suspension so that the number of bacteria will be within a given range. Firstly, BaC_{l2}(1%w/v) and H_2S0_4(1% v/v) would be prepared by dissolving 1g of BaC_{l2} in 100ml of sterile distilled water and 1ml of concentrated H_2S0_4 in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5 McFarland scale was prepared by adding 9.95ml of (1%v/v) H_2S0_4 to 0.05ml of (1% w/v) Ba_2SO_4 whose density is equivalent to 1.5×10^8 CFU/ml approximate cell density of bacteria. The barium sulphate suspension in 6ml aliquots was transferred in to screw-cap tubes, tightly sealed, and stored at room temperature in order to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial inoculum (Cheesbrough, 2010).

Preparation of bacterial inoculastandard

For inocula standardization, the density of *S. aureus* isolated culture was adjusted equal to that of 0.5 McFarland standards (1.5×10^8 CFU/ml) by suspending 2 or 3 colonies of each bacterial culture into 2ml of sterile physiological saline as a suspending medium. The physiological saline was prepared by dissolving 8.5g of $NaCl_2$ in 1L of distilled water before sterilizing. To aid comparison, the test organisms and standard were compared against a white background with contrasting black lines (Cheesbrough, 2010).

Preparation of extract concentration
This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solution of the plant extracts were prepared by adding 1g of each crude plant extract in 10ml of 10% dimethylsulphuroxide (DMSO). From each of the stock solutions, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentrations were prepared using Two-fold serial dilution method. These concentrations were labelled and kept in bijou bottles for subsequent use.

Solvent fractionation of crude plant extracts
The active crude extracts of *Jatropha curcas* and *Vigna unguiculata* leaves was fractionated in accordance with the procedures of Andrews, (2001). The extraction solvents were hexane (non-polar), ethyl-acetate, n-butanol and water. The procedure was carried out in a separating funnel in which fractions obtained were evaporated to dryness on a water bath to remove the solvent.

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K. Sensitivity test (In-vitro Demonstration of Antimicrobial Activity)

The antimicrobial activity of *Jatropha* curcas and *Vigna unguiculata* leave extracts against the *Staphylococcus aureus* was evaluated using agar well diffusion method of susceptibility test (Srinivasan *et al*., 2009). Mueller-Hinton agar plates (Titan Biotech Ltd. Bhiwadi- 301 019, Rajasthan, India) was inoculated with 0.1ml of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Wells of 8mm size were made with sterile cork borer into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml each of the extracts was dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for10minutes, allowing the diffusion of the extracts into the incubation at 37 ºC for 24 hours. The diameter of inhibition zones (DIZ) was measured and expressed in millimetres after incubation. The mean values of the diameter of inhibition zones were calculated to the nearest whole number. DMSO was used as negative control. Commercially available standard antibiotic, Ciprofloxacin (10mg) was used as positive control parallel with the extracts. For the antibiotic inhibition zone was interpreted in accordance with Clinical Laboratory Standards Institute (CLSI, 2011) interpretation guidelines.

Determination of minimum inhibitory concentration (MIC)

Extracts that exhibited activity against the test organisms were further assayed for their minimum inhibitory concentrations (MIC). The broth dilution method was employed using Mueller Hinton broth as described by Andrews (2001). The dilutions of each reconstituted extract was made to obtain the following concentrations; 25, 20, 15, and 10mg/ml. Each extract concentration was then inoculated into tubes containing 100µl of active inoculum of standardized bacterial isolates and incubated for 24h at 37ºC.The MIC was determined as the lowest concentration of the extract that inhibited the organism and results were observed in the form of turbidity (Andrews, 2001).

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined from broth dilution test resulting from the MIC tube by sub-culturing to antimicrobial free agar as described for the MIC earlier. The lowest concentration of the extract which shows no growth or turbidity was regarded as the minimum bactericidal concentration (Usman *et al.*, 2007).

L. Data Analysis

The data generated are presented in tables, charts, percentage and were analysed statistically using the Statistical Package and Service Solution (SPSS) version 20. The details are attached in the appendices. T test was used to compare means of the two plant extracts and that of individual plans at different concentrations, the standard strain, and the positive control antibiotics if there is any statistically significant difference in the diameter of zones of inhibition.

III. RESULTS AND DISCUSSION

Table 1: Qualitative Phytochemical Screening of *Jatropha curcas* and *Vigna unguiculata* leaves

Phytoconstituents	*Jatropha curcas*	*Vigna unguiculata*
Tannins	-	-
Steroids	-	+
Flavonoid	+	-
Saponins	+	+
Alkaloids	+	+
Terpenoids	+	+
Cardiac glycoside	-	-
Free anthraquinone	+	+

Key; + = Positive; - = Negative

Table 2: Quantitative Analysis of *Jatropha curcas* and *Vigna unguiculata* leaves extracts

Phytoconstituents	*J. curcas*	*V. unguiculata*
Steroids	-	2.0%
Flavonoids	2.0%	-
Terpenoids	94.3%	78.6%
Saponins	6.2%	3.6%
Alkaloids	2.0%	10.0%
Mean±STD Deviation	29.9±41.1	18.84±33.6

t (4) = .528, p = .62. Key; % = Percentage; - = Negative;

Table 3: Biochemical and Cultural Characterization of *Staphylococcus aureus*

Col	Grn	Cat	Coa	DNAse	Fma
+	+	+	+	+	+
33	33	33	33	33	33

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Key; Col=Colonial Appearance, Grn=Gram Reactions Cat= *Catalase*, Coa=*Coagulase* DNAse=Deoxyribonuclease, Fma=Fermentation of Mannitol,

Table 4: Gender grouping of children with otitis media attending MurtalaMuhammadSpecialist Hospital

Gender	No.tested (%)	Positive (%)	Negative (%)
Male	112(63.64)	24(72.73)	88(61.54)
Female	64(36.36)	9(27.27)	55(38.46)
Total	**176(100)**	**33(100)**	**143(100)**

Table 5: Age grouping of children with otitis media attending Murtala Muhammad Specialist Hospital

Age Group(years)	No tested (%)	Positive (%)	Negative (%)
0-2	51(28.98)	12(36.3)	39(27.27)
3-4	39(22.16)	8(24.24)	31(21.67)
5-6	27(15.34)	4(12.12)	23(16.08)
7-8	34(19.33)	5(15.15)	29(20.28)
9-10	21(11.93)	2(6.06)	19(13.29)
11-12	4(2.27)	2(6.06)	2(1.40)
Total	**176(100)**	**33(100)**	**143(100)**

Table 6: Dryspot Staphytect Plus latex agglutination test result of the 33 biochemically positive samples.

Positivity/Negativity	Frequency (%)
Positive	31(93.94)
Negative	2(6.06)
Total	**33(100)**

Table 7: Mean Zone of Inhibition of Ethanolic and Methanolic *Jatropha curcas* Leave Extracts Against *Staphylococcus aureus*

Concentration (mg/m)	Ethanolic Extract (mm)	Methanolic Extract (mm)
100	24	17
50	18	13
25	12	8
12.5	9	6
Control	42	42
Mean±STD Deviation	15.74±6.65	11±4.97

t (3) = 5.563, p = .011, α = .05

Table 8: Mean Zone of Inhibition of Ethanolic and Methanolic Leave *Vigna unguiculata* Extracts Against *Staphylococcus aureus*

Concentration (mg/m)	Ethanolic Extract (mm)	Methanolic Extract (mm)
100	25	21
50	18	18
25	14	10
12.5	11	6
Control	41	41
Mean±STD Deviation	17±6.01	13.75±6.95

t (3) = 2.93, p = .061, α = .05

Table 9: Mean Zone of Inhibition of various fractions of Ethanolic *Jatropha curcas* and *Vigna unguiculata* Extracts Against *Staphylococcus aureus*

Solvents	Ethanolic *J. curcus* (mm)	Ethanolic *V. unguiculata* (mm)
Hexane	30.8	34.7
Ethyl acetate	27.1	29.0
n-butanol	16.0	18.0
Water	1.6	2.3
Mean±STD Deviation	18.87±13.12	21±14.26

t (3) = -3.12, p = .049, α = .05

Table 10: Mean Zone of Inhibition of various fractions of Methanolic *Jatropha curcas* and *Vigna unguiculata* Extracts Against *Staphylococcus aureus*

Solvents	Methanolic	Methanolic *V.*

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	J. curcus (mm)	*unguiculata* (mm)
Hexane	32.0	33.2
Ethyl acetate	27.0	28.8
n-butanol	17.0	19.0
Water	1.8	3.5
Mean±Standard Deviation	19.7±13.6	21.1±13.2

t(3) = -3.45, p = 0.041, α = 0.05

Table 11: The Minimum Inhibitory Concentration (MIC) and Minimum Batericidal Concentrations (MBC) of Ethanolic and Methanolic *Vigna unguiculata* Leaf Extracts against *Staphylococcus aureus.*

	Ethanolic leaf extract	Methanolic leaf extract
MIC	6	6
MBC	12.5	12.5

Table 12: The Minimum Inhibitory Concentration (MIC) Minimum Batericidal Concentrations (MBC) Of Ethanolic and Methanolic Leaf Extracts of *Jatropha curcas* Against *Staphylococcus aureus.*

	Ethanolic leaf extract	Methanolic leaf extract
MIC	20	20
MBC	25	25

The result of this study indicated the presence of alkaloids, saponins, terpenoids, and free anthraquinone in both *Jatropha curcas* and *Vigna unguiculata* leave extracts respectively. However, Steroids is present in *Vigna unguiculata* leaf while absent in *Jatropha curcas* meanwhile, Flavonoid is present in *Jatropha curcas*, which is absent in *Vigna unguiculata* as shown in Table 4.1 of qualitative analysis. Quantitatively, the content of alkaloids in *Jatropha curcas* and *Vigna unguiculata* leaves is 2 and 10%, saponin is 6.2 and 3.56%, and terpenoid is 94.3 and 78.57% respectively. Meanwhile, the flavonoids content in *Jatropha curcas*is 2% and also that of Steroidss in *Vigna unguiculata* is 2.0%. as shown in Table 4.2 of quantitative analysis. A paired t test failed to reveal a statiscally significance difference between mean in phytochemical content of *Jatropha curcas* (M = 29.9, s = 41.1) and *Vigna unguiculata* (M = 18.84, S = 33.6), t (4) = .528, p = .625, α = .05. The cultural and physiologic properties of clinical isolate (*Staphylococcus aureus),* is presented in Table 4.3. Out of 176 samples collected, from ear noe and throat (ENT) unit of Murtala Muhammad Specialist Hospital, 33(18.75%) appeared to be Gram positive, cocci, golden yellow, slightly raised colonies on Mannitol salt agar and clustered under the microscope. Biochemically, 33 samples are catalase positive, coagulase positive, DNAse positive and fermentannitol. Table 7 shows the result of antibacterial activity using inhibitory zone of each of the ethanolic and methanolic leave extracts of *Jatropha curcas* while the result of inhibitory zone of each of the of ethanolic and methanolic leave extracts of *Vigna unguiculata* was shown in Table 8.Theresult of this study shows that, the ethanolic extract of *Jatropha curcas* shows considerable zone of inhibition of 9mm for the least concentration of 12.5mg/ml on *Staphylococcus aureus*, 13mm for 25mg/ml, 18mm and 24mm for 50 and 100mg/ml respectively, with 42mm for ciprofloxacin as control. The methanolic leaf extract of *J. curcas*on the other hand shows a considerable inhibitory zone of 6mm for the least concentration of 12.5mg/ml, 8mm for 25mg/ml, 13 and 17mm for 50 and 100mg/ml respectively, with a control of 41mm. A paired t test revealed a statiscally significance difference in mean anti-bacterial activity of ethanolic leave extract of *Jatropha curcas*(M = 15.74, s = 6.65) and methanolic leave extract of *Jatropha curcas* (M = 11, s = 4.97), t (3) = 5. 563, p = .011, α = .05. However, the ethanolic extract of *Vigna unguiculata* displays a significant zone of inhibition of 11mm for the least concentration 12.5mg/ml against *Staphylococcus aureus* 14mm for 25mg/ml, 18 and 25mm for 50 and 100mg/ml respectively. While ciprofloxacin as control, shows an inhibitory zone 41mm. Also, a methanolic leave extract of *Vigna unguiculata* shows considerable zone of inhibition of 6.mm for the least concentration of 12.5mg/ml against *Staphylococcus aureus*, 10mm for 25mg/ml 18mm and 21mm for 50 and 100mg/ml respectively, with 41mm for ciprofloxacin as control. A paired t test failed to revealed a statiscally significance difference in mean zone of inhibition of various fractions of ethanolic*Jatropha curcas* (M =17, s = 6.01) and methanolic leave extract of *Vignaunguiculata* (M = 13.75, s = 6.95), t (3) = 2.93, p = .061, α = .05. However, the result of antibacterial susceptibility test of various fractions of ethanolic leave extracts of *J. curcas*and *V. unguiculata* against *Staphylococcus aureus* was presented Table 9. The result for the fraction of ethanolic *J. curcas* and *V. unguiculata* leaves hexane had the highest activity on *S. aureus* with mean inhibition zone of 30.8 and 34.7mm±0.1 followed by fraction of ethanolic *J. curcas* and *V. unguiculata* leaves ethyl acetate with mean

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inhibition zone of 27.1 and 29mm±0.1 then fraction of ethanolic leave n-butanol with 16.0 and 18.0mm±0.1, while the lowest mean inhibition zone is with fraction of ethanolic *J. curcas* and *V. unguiculata* leaves aqueous with 1.6 and 2.3mm±0.1 at a concentration of 100mg/ml respectively. A paired t test revealed a statiscally significance difference in mean anti-bacterial activity of ethanolic leave extract of *Jatropha curcas*(M = 18.87, s = 13.12) and *V. unguiculata*(M = 21, s = 14.26), t (3) = -3.21, p = .049, α = .05.For methanolic *J. Curcas* and *V. unguiculata* leaf extracts, the result for the fraction of methanolic leaves, hexane had the highest activity on *S. aureus* with mean inhibition zone of 32.0 and 33.2mm±0.1 followed by fraction of methanolic leaves ethyl acetate with mean inhibition zone of 27.0 and 28.8mm±0.1 then fraction of methanolic leave n-butanol with 17.0 and 19.0mm±0.1 while the lowest mean inhibition zone is with fraction of methanolic leaves aqueous with 1.8 and 3.5mm±0.1 at a concentration of 100mg/ml respectively as shown in Table 4.10 of various fractions of methanolic *J. curcas* and *V. unguiculata* leave extracts.

Table 12 represents the MIC and MBC of ethanolic extracts of *J. curcas* and *V. unguiculata* on *S. aureus* where ethanolic *J. Curcas* and *V. unguiculata* leaf exracts had the highest value ranges from 20 and 25mg/ml, 6 and 12.5mg/ml for MIC and MBC respectively.

IV. DISCUSSION

The result of phytochemical analysis on *Jatropha curcas* and *Vignaunguiculata*, revealed the presence of alkaloids, terpenoids, saponins and free anthraquinones in both the leave extracts. In addition, *Jatropha curcas* in particular, has flavonoids content, while *Vigna unguiculata*has a content of steroids. Statistically, there is no significant difference between the two plants based on quantitative analysis. The major components of the phytochemical detected in two plant samples as shown on table 4.1. is in agreement with the report of Ulubelen, (2003) who reported that all medicinal leaves have phytochemical compounds. These phytochemical compounds are one of the largest and most ubiquitous group of plant metabolite (Cowan, 1999). For example, flavonoidss, which are present in *Jatropha curcas* are phenol in nature, which is known to cause denaturation and coagulation and are known to be produced by plants in response to microbial infections to which this aspect has been extensively studied and found to have antimicrobial activity against an array of microorganisms in vitro (Cowan, 1999). Their ability has been attributed to their ability to form complexes with extracellular and sable protein and bacterial cell wall (Trease and Evans, 1999). terpenoids, present in both *Jatropha curcas* and *Vignaunguiculata* although mainly used for their aromatic qualities, but have also been found to be potential agents in inhibiting bacterial growth (Tsuciya*et al.*, 1996). Saponinss, present in both *Jatropha curcas* and *Vignaunguiculata* leave exracts which are glycosides have been found to have inhibitory effect against microbes Cowan, 1999).

In another study conducted by Agarwal *et al.*, (2012) also states that the presence of metabolic toxins or broad spectrum antimicrobial compounds in *J. curcas*acts against most Gram positive and some Gram negative bacteria especially in solvents ethanol, ethyl acetate and cold aqueous. In the same vein, Igbinosa *et al.*, (2011) reported that the activities of *J. curcas* to both Gram positive and negative microorganisms can be attributed to the presence of phenolic compounds which showed to be powerful antioxidants and free radical scavengers, and those compounds are able to induce reactions of electron transfer which reacts with nitrogen compound in microbial cell like nucleic acid and proteins, this helps as a strong barrier against bacterial infection. Result reported by Kalimathu*et al.*, in (2010) on *Pseudomonas aeruginosa* and *Staphylococcus aureus* with leaf extract of *Jatropha curcas*, this study recorded high antibacterial activity on Gram positive of *S. aureus.* While slight or no activity on Gram negative, *E. coli* and *Pseudomonas aeruginosa* which might be attributed to their difference in cell wall composition.

The study also shows that, *Vigna unguiculata* leave extract, recorded the highest activity in almost all concentrations and also in various fractions than the extract of *Jatropha curcas*, this may be attributed to the presence of Steroidss, which is absent in *Jatropha curcas* leaf, high concentration of Saponis (Saponinss content in *Vigna unguiculata* multiplies that of *Jatropha curcas),* and the Alkaloids content, which its concentration in *Vignaunguiculata* is five times higher than that of *Jatropha curcas*, as shown in the result of quantitative analysis.

The result of this study also reveals the efficacy of ethanolic and methanolic extracts of leaves of *Jatropha curcas* and *Vigna unguiculata* based on *in vitro* evaluation of antibacterial activity of the extracts on *Staphylococcus aureus* isolates. The ethanolic extract in both the plant samples, in most of the concentrations, recorded the highest antibacterial activity on the clinical isolates than the methanolic extract and the significant difference exist beweenethanolic and methanolic leaf extracts of *Jatropha curcas* while there is no that statistical difference between the ethanolic and methanolic leaf extracts of *Vigna unguiculata.* Although both ethanol and methanol are polar, but they also have the ability to attract non-polar molecules due to ethyl group of ethanol being non-polar. This contributes to their ability to extract highly polar and non-polar components from the plant materials. They characterized by very low toxicity, completely miscible in water, volatile and easily removed from plant material at low temperature, which significantly contribute to the retaining the viability of heat-labile bioactive compounds presents in the extracts (Roy *et al.*, 2014).

Moreover, the susceptibility pattern of the ethanolic and methanolic *Jatropha curcas* and *Vigna unguiculata* fractions in this study shows Hexane, n-butanol and ethyl acetate fractions of leaves are very effective against *S. aureus* with aqueous fractions not active against *S. aureus* with mean inhibition zone as high as 30.8mm and 32.0mm, 34.7 and 33.2

for ethanolic and methanolic *Jatropha curcas* and *Vigna unguiculata* respectively, and the mean weight between the two plants is signicantly different statistically.

The MIC and MBC assay procedures are frequently used to evaluate some diverse agents such as antibiotics, antiseptics, disinfectants and chemotherapeutic agents (Andrews, 2001). In this study, the MIC and MBC values of both ethanolic and methanolic *Jatropha curcas* and *Vigna unguiculata* on *S. aureus*, indicates significant bacteriostatic and bactericidal activities. This implies the strong efficacy of the extracts as stated by Arekemase (2011) that the constituents of leaves of *J. curcas* contain phenols, flavonoidss and some secondary metabolites that are very useful in antimicrobial activity. The MIC and MBC effects observed with different concentrations of various extracts against susceptible *S. aureus* in this study, it could be attributed to the presence of organic bioactive compounds detected in the extracts as shown on Table 1 and 2 above for qualitative and quantitative analysis respectively, the interactions of these hydrocarbons with the hydrophobic structures of bacteria had been reported to result in antimicrobial activity (Sikkema *et al*., 1995; Cowan, 1999; Zulfiker *et al*., 2011).

This study is also in agreement with statement of David, (2009), which states that Plants are one of the most important sources of medicine. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. Extracts of plants used for the treatment of various diseases, forms the basis for all systems of medicine, and that of Glombitza*et al*., (1993) which stated that, the importance of plants in medicine remain of greats relevance with current global shift to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value remedies for safety, efficacy and economy.

The result also shows that, out of 176 ear swap samples collected, 33 samples, which is equivalent to 18.75% *S. aureus* are biochemically positive, while 31 are serologically positive. This result is in agreement with that of Rajani *et al*., (2017), with 19.7% MRSA, Maan *et al*., (2014), with 17.7% *S. aureus* and Chirwa, (2014), with 20.1% *S. aureus* positive. The result of this study also disagrees with that of Mwaniki, (2009; Ibekwe and Okafor, 2014) and Harrison, (2016) with 39%, 29% and 10.7% respectively. This is normal because, according to Bluestone, (1998), there are three types of otitis media, namely acute otitis media, chronic otitis media and chronic supporative otitis media. Each one above is characterized witha peculiar organism that are most frequently isolated, for example, the major pathogens causing acute otitis media *Streptococcus pneumonia* and *Haemophilus influenza*, with *Maraxellacatarrhalis*, *Streptococcus pyogenes*, and *Staphylococcus aureus* less frequently isolated. The same organisms and *Staphylococcus epidermidis* are found in chronic otitis media with effusion. In chronic supporative otitis media, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are most frequently found Bluestone, (1998).

V. CONCLUSION

In extraction using ethanol and methanol, shows that ethanolic leaf etract had high antibacterial effect against *Staphylococcus aureus* isolated from otitis media samples from Murtala Muhammad Specialist Hospital Kano, Kano State.

VI. RECOMMENDATIONS

1. Both *Jatropha curcas* and *Vignaunguiculata* ethanolic leaf extracts are recommended to be used for the control of *Staphylococcus aureus* infections, though *Vigna unguiculata* is most recommended in the treatment of infections caused by *Staphylococcus aureus*, then *Jatropha curcas*, as it gives wider inhibitory zone subject to in vivo test.
2. It is also recommended that, extracton using aqueous water, is not suitable, as it soaks the available water content in the container at regular intervals.
3. The *Jatropha curcas*and*Vignaunguiculata*leaves should be tested on*Streptococcuspneumonia, Streptococcuspyogenes, Haemophilus influenza, Moraxella catarrhalis, Pseudomonas aeruginosa Staphylococcus epidermidis* and also on fungi to establish their inhibitory activities as they all associated with otitis media.
4. Further investigation should be made to identify molecular targets in the bacterial cell like spore formation, active sites of enzymes, inhibition of cell wall and DNA synthesis etc in order to develop compounds of plants origin, to serve as their specific inhibitors.
5. Government should also help in providing standard research laboratory centers at local, state and federal levels for screening and optimization of quality and safety of traditional medicine.

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