PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL SUSCEPTIBILITY OF WHOLE PLANT OF *EUPHORBIA HETEROPHYLLA* CRUDE EXTRACTS AGAINST SELECTED BACTERIA PATHOGENS

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ABSTRACT
In this study, four solvents namely normal hexane, ethyl acetate, methanol and water were used for the reflux extraction of whole plant of E. heterophylla successively and exhaustively. The phytochemicals present in the crude extracts were determined. The bacterial pathogens used were subjected to biochemical tests and molecular characterization for proper identification and antibacterial susceptibility of the crude extracts against selected clinical isolates were determined at varying concentrations of 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml using agar well diffusion. The MIC and MBC of the crude extracts were also determined. The study revealed that phytochemicals such as saponins, flavonoids, terpenoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars were present in the crude extracts. The highest mean values of antibacterial susceptibility against S. typhimurium and E. coli were 23.33±0.33 and 17.33±0.33 at 100 mg/ml of the aqueous and methanol crude extracts respectively while there was no antibacterial activity with K. pneumoniae and P. fluorescens at varying concentrations. The MIC and MBC values of aqueous extract against S. typhimurium were 20 mg/ml and 40 mg/ml. The MIC values of aqueous and methanol extracts against E. coli were both 40 mg/ml while the MBC values of 160 mg/ml and 80 mg/ml were recorded respectively. This suggests that aqueous crude extract have good antibacterial activity than the other crude extracts.
Keywords:
Phytochemical screening, Antibacterial susceptibility, Euphorbia heterophylla, Bacteria, Pathogens

INTRODUCTION
Over the years, the therapeutic properties of various medicinal plants such as *Euphorbia heterophylla* have been used to treat human diseases. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002). Consumers are increasingly interested in complementary alternative medicines, including herbal medicine as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. In the US, a number of medicinal plants have been documented as important source of bioactive compounds (Balunas and Kinghorn, 2005).

As reported by Ogunnusi and Oso (2017) and Omoya *et al*. (2015), *Euphorbia heterophylla* is herbaceous, erect and 20-200 cm in height (depending on growing conditions). It belongs to the family of Euphorbiaceae and has been identified as plants widely used in traditional medicine in various parts of Africa and are used in the treatment of skin infections, wart, respiratory tract infection, tumors and diseases of viral origin. The most common size is 40-60 cm tall. Milky latex is present when most parts of the plant

are broken. The stem is branched and cylindrical, with nodes at regular intervals. The surface is smooth and reddish-green (Rowan and Onwukaeme, 2001). The plant is used in traditional medicine as laxative, in the treatment of gonorrhoea, migraine and viral warts while the latex is used as fish poison, insecticide, treatment of constipation, bronchitis, asthma and as a purgative (Falodun *et al*., 2003). *Euphorbia heterophylla* with the common name "spurge weed" grows in semi humid places especially in cassava, cowpea and soya beans plantations as reported by Ajibesin *et al*. (2008) and Falodun *et al*. (2006).

In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes *et al*., 2007). Phytochemicals which are plant-derived substances have recently become great interest owing to their versatile application. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids and chlorophyll's.

Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides. Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytoconstituents (Mamta *et al.*, 2013). There are thousands of species of medicinal plants used globally for the cure of different infections. These plants are used as antimicrobial agents and several works have been carried out by scientists to find out its scientific basis (Omotayo, 1998). The phytochemicals possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities (Bidlack *et al.*, 2000). The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients (Lepape *et al.*, 2009). Increasing bacterial resistance is prompting a resurgence in research of the antimicrobial role of herbs against resistant strains (Alviano and Alviano, 2009; Hemaiswarya *et al.*, 2008). This research work focused on the phytochemical screening and antibacterial susceptibility of whole plant of *E. heterophylla* crude extracts against bacterial pathogens.

MATERIALS AND METHODS
Collection and Identification of Plant Materials
Fresh *Euphorbia heterophylla* plants were collected inside the farms opposite Guiding Angel Secondary School, Sauka-Kahuta in Minna, Niger State. The plant materials were taken to the Department of Biological Sciences in Federal University of Technology, Minna for botanical identification. The identified plants were authenticated by a botanist from the Herbarium Department of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. Nigeria with the voucher number: NIPRD/H/6865 and the voucher specimen deposited in the same Herbarium Department. The identified/authenticated medicinal plant was thoroughly washed with water to remove any dirt and the whole plants dried at room temperature away from sunlight and dust for eight weeks until a constant weight was obtained.

Preparation of Extracts
The air dried whole plants were blended with Panasonic Blender model MX-JS10PN and the pulverized plant samples were sieved with a 150 µm pore size.. The extraction was done using reflux extraction method as described in the work of Ewansiha *et al.* (2016). The reflux extraction method was used to obtain the crude extract at a reduced temperature for 2 h. The extraction was carried out successively beginning from non-polar solvent to polar solvent. The solvents used were *n*-hexane, ethyl acetate, methanol and water. One hundred grams (100 g) of the well blended dried plant materials was each dissolved in 400 ml of the extracting solvents (1:4). At the end of 2 h of refluxing, the mixture was filtered firstly with muslin cloth and later with Whatman No. 1 filter paper with pore size 0.7 µm in

order to obtain a clear filtrate which was then concentrated to a semi-solid substance with the use of a Rotary Evaporator (model RE-6000) at a reduced temperature and then dried using water bath set at $60^{\circ}C$. The dried extract was then stored in an air-tight amber bottle and kept in the refrigerator for further analysis. The marc was air-dried for 1 hour and was extracted with the next solvent in increasing polarity. This procedure was repeated using other solvents.

Determination of the Phytochemical Properties in the Crude Extracts
The crude extracts were subjected to phytochemical analysis to screen for the presence of secondary metabolites such as alkaloids, reducing sugars, saponins, phenols, flavonoids, cardiac glycosides, terpenoids, steroids and tannins. The phytochemical screening was carried out using standard procedure (Evans, 2002).

Collection, Biochemical Tests, Molecular Characterization and Identification of Selected Bacterial Pathogens
The *Escherichia coli*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae* and *Salmonella typhimurium* used were collected from the Microbiology Department of General Hospital, Minna. They were Gram stained and their biochemical tests (triple sugar iron (TSI); methyl red; indole; catalase; citrate utilisation and urease) were carried out in order to identify them according to the methods of Cheesbrough (2010) and then sub cultured onto Nutrient Agar slants for molecular characterization for confirmation (Bioneer Incorporation, 2012; Beckman Coulter Incorporation, 2016; National Centre for Biotechnology Information, 1988).

Standardization of Selected Bacterial Pathogens
The population of the bacteria pathogens were determined from the McFarland Turbidity Standard (Murray *et al.*, 2007). The bacteria pathogens were cultured for 24 h in a Nutrient Broth and then ten-fold dilutions from 10^{-1} to 10^{-6} were made. The absorbance of each of the dilution was determined at 540 nm using Jenway 6305 UV/Visible Spectrophotometer and then compared with the absorbance of the 0.5 McFarland Turbidity Standard prepared.

Preparation of Extract Concentration
The extract concentration was prepared as described in the work of Ewansiha *et al.* (2016). Two hundred milligram (200 mg) each of the *n*-hexane, ethyl acetate, methanol and aqueous crude extract were weighed in 5 ml each of 20% Dimethyl sulfoxide (DMSO) (20 ml DMSO was made up to 100 ml with distilled water) to give 40 mg/ml concentrations respectively. The other concentrations of 60 mg/ml, 80 mg/ml and 100 mg/ml were prepared following similar procedure.

Determination of the Antibacterial Susceptibility of the Crude Extracts
The antibacterial susceptibility of the crude extract was carried out using Agar Well Diffusion method of NCCLS (1993) and CLSI (2015). Mueller Hinton Agar (MHA) was prepared and sterilized as instructed by the manufacturer.

Petri dishes containing about 20 ml MHA were streaked with standardized 24 h culture of the bacteria pathogens using sterile swab sticks. Wells were cut with a 6 mm sterile cork borer and then sealed at the bottom with a drop of molten agar so as to prevent the extract from sipping beneath the agar. Four holes were made on each plate and adequately spaced out. About 100 μl of the crude extracts (40, 60, 80 and 100 mg/ml) were delivered into each well and 40, 60, 80 and 100 mg/ml of the standard drug (Ciprofloxacin) were used for positive controls while dimethylsulfuroxide (DMSO) served as the negative control. One hour pre-diffusion time was allowed after which the plates were incubated at 37°C for 24 h. The zones of inhibition were measured by direct linear measurement using a meter scale rule. The above method was carried out in triplicates and the mean of the triplicate result was taken.

Determination of the Minimum Inhibitory Concentration (MIC) of the Crude Extracts
The MIC of the crude extracts was determined by broth dilution method (Ewansiha *et al.*, 2016). Nutrient Broths were prepared and labeled A-I. Two fold serial dilutions of the crude extracts were prepared to give a decrease in concentration ranging from 160, 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/2ml respectively. This concentration was achieved by weighing and dissolving 320 mg of the crude extract in a test tube labeled T containing 4 ml of 20% of DMSO. Two milliliter (2 ml) was transferred from test tube T to test tube A containing 2 ml of Nutrient Broth to give 160 mg/2ml. This procedure continued until a concentration of 0.625 mg/2 ml was obtained in the ninth test tube labeled I (0.625 mg/2ml). Homogenous mixture was obtained by vortexing each tube for at least 5 seconds. Zero point two millilitre (0.2 ml) of bacteria suspension (1.5 x 10^8) was inoculated in each of the test tubes containing 2 ml sterile Nutrient Broth. In the control tube, the test crude extract was not added. The test tube not inoculated was used to check the sterility of the medium and as negative control while the positive control tube was used to check the suitability of the medium for growth of the microorganisms and the viability of the inoculums. All the test tubes were properly shaken and then incubated at 37°C for 24 h and the change(s) in turbidity were observed. The MIC was determined by the lowest concentration of the crude extract that prevented visible growth (Andrews, 2005; Ewansiha *et al.*, 2016).

Determination of the Minimum Bactericidal Concentration (MBC) of the Crude Extracts
The MBC of the crude extracts was determined from the MIC tubes that showed no visible growth. Zero point one millilitre (0.1 ml) from these concentrations that showed no visible growth was inoculated into 9 ml recovery Nutrient Broth (Nutrient broth containing 3% v/v Tween 80). These were incubated at 37°C for another 24 h. The least concentration of the extracts that showed no bacterial growth in the recovery liquid medium was taken as the MBC (Bergen *et al.*, 2010).

RESULTS
Gram's Staining and Biochemical Characteristics of the Bacterial Pathogens
The result of the Gram stain reactions and biochemical tests conducted on the four bacteria pathogens is shown in Table 1. The four bacteria pathogens were Gram negative. The 4 isolates tested for TSI were all positive. *E. coli* and *S. typhimurium* were positive for methyl red while *K. pneumoniae* and *P. fluorescens* were negative. Table 1 also revealed that *E. coli* was indole positive while *P. fluorescens*, *S. typhimurium* and *K. pneumoniae* were indole negative. However, all the isolates were catalase positive as revealed in Table 1. The citrate utilisation test was positive in *P. fluorescens* and *K. pneumoniae* but *E. coli* and *S. typhimurium* were negative (Table 1). The urease test was positive in *E. coli*, *P. fluorescens* and *K. pneumoniae* but negative in *S. typhimurium* (Table 1).

Table 1: Gram's Reactions and Biochemical Characteristics of the Bacterial Pathogens

Tests	*E. coli*	*P. fluorescens*	*S. typhimurium*	*K. pneumoniae*
Gram Reactions	-	-	-	-
TSI	+	+	+	+
Methyl Red	+	-	+	-
Indole	+	-	-	-
Catalase	+	+	+	+
Citrate Utilisation	-	+	-	+
Urease	+	+	-	+

KEY:
+ = Positive; - = Negative; TSI= Triple Sugar Iron

Molecular Characterization of the Bacterial Isolates
The results of bacterial isolates characterized molecularly are shown in Table 2. The PCR sequencing and the BLAST results revealed the identity of the bacteria pathogens with their accession numbers as follows: *E. coli* strain MRE 600 (CP014197.1); *P. fluorescens* strain 2P24 (CP025542.1); *Salmonella* enteric subsp. enteric serovar *typhi* PMO 16/13 (CP12091.1) and *K. pneumoniae* strain HZW25 (CP025211.1) (*www.ncbi.nlm.nih.gov*) (Table 2).

Table 2: Molecular Characterization of Bacterial Isolates

Bacterial Isolates	Max Score	Total Score	Query Cover	Expected Value	Identity	Accession Number
E. coli strain MRE 600	2660	16064	100%	0.0	100%	CP014197.1
P. fluorescens strain 2P24	2689	2689	100%	0.0	100%	CP025542.1
Salmonella enteric subsp. enteric serovar *typhi* PMO 16/13	3546	3546	100%	0.0	100%	CP12091.1
K. pneumoniae strain HZW25	797	6348	100%	0.0	98%	CP025211.1

Phytochemical Properties of the Whole Plant of *E. heterophylla* Crude Extracts

Table 3 shows the phytochemical properties of the whole plant of *E. heterophylla* crude extracts. The *n*-hexane crude extract (NHE) contained flavonoids, terpenoids, tannins, phenols, alkaloids, steroids and reducing sugars while saponins and cardiac glycosides were absent. On the other hand, alkaloids, flavonoids, saponins, tannins, phenols, reducing sugars and terpenoids were present in both ethyl acetate (EAE) but cardiac glycosides and steroids were absent. The methanol (ME) crude extracts had saponins, flavonoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars while terpenoids was absent. The aqueous extract (AQE) had saponins, flavonoids, terpenoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars (Table 3).

Table 3: Phytochemical Properties of the Whole Plant of *E. heterophylla* Crude Extracts

	Phytochemicals								
Extracts	**Saponins**	**Flavonoids**	**Terpenoids**	**Cardiac Glycosides**	**Tannins**	**Phenols**	**Alkaloids**	**Steroids**	**Reducing Sugars**
n-hexane	-	+	+	-	+	+	+	+	+
Ethyl acetate	+	+	+	-	+	+	+	-	+
Methanol	+	+	-	+	+	+	+	+	+
Aqueous	+	+	+	+	+	+	+	+	+

KEY
+ Present
- Absent

Antibacterial Susceptibility of the Crude Extracts

The results of antibacterial activity of *n*-Hexane Extract (NHE), Ethyl Acetate Extract (EAE), Methanol Extract (ME) and Aqueous Extract (AQE) of whole plant crude extracts of *E. heterophylla* tested against standardized *S. typhimurium*, *E. coli*, *P. fluorescens* and *K. pneumoniae* using varying concentrations of 40 mg/ml; 60 mg/ml; 80 mg/ml and 100 mg/ml are shown in Tables 4 and 5. There was no antibacterial activity of all the crude extracts tested against standardized *K. pneumoniae* and *P. fluorescens* (Tables 4-5). *E. coli* did not show antibacterial activity with NHE and EAE but recorded 14.67-17.33 mm with ME and 15.00-16.33 mm with AQE (Tables 4-5). *S. typhimurium* had no antibacterial activity with EAE but recorded 16.67-18.67 mm with NHE; 10.67-17.33 mm with ME and 20.67-23.33 mm with AQE (Tables 4-5). The DMSO had no antibacterial activity while Ciprofloxacin showed antibacterial activity of 11.67-49.67 mm; 45.33-48.67 mm; 50.00-55.00 mm and 34.67-38.33 mm for *E. coli*, *S. typhimurium*, *K. pneumoniae* and *P. fluorescens* respectively (Tables 4-5).

Table 4: Antibacterial Susceptibility of Whole Plant Crude Extracts (40-100 mg/ml) of *Euphorbia heterophylla* (mm)

	Clinical Isolates							
	Escherichia coli				*Salmonella typhimurium*			
Extracts/Control	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**
n-Hexane	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	16.67±0.33[c]	17.67±0.33[c]	18.33±0.33[c]	18.67±0.33[c]
Ethyl Acetate	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Methanol	14.67±0.33[c]	15.33±0.33[b]	16.67±0.33[c]	17.33±0.33[c]	10.67±0.33[b]	12.33±0.33[b]	16.33±0.33[b]	17.33±0.33[b]
Aqueous	15.00±0.00[d]	15.33±0.33[b]	15.67±0.33[b]	16.33±0.33[b]	20.67±0.33[d]	21.33±0.33[d]	21.67±0.33[d]	23.33±0.33[d]
Ciprofloxacin	11.67±0.33[b]	44.33±0.67[c]	46.00±0.58[d]	49.67±0.33[d]	45.33±0.33[e]	46.00±0.58[e]	48.00±0.58[e]	48.67±0.33[e]
DMSO	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p < 0.05$

Table 5: Antibacterial Susceptibility of Whole Plant Crude Extracts (40-100 mg/ml) of *Euphorbia heterophylla* (mm)

	Clinical Isolates							
	Klebsiella pneumoniae				*Pseudomonas fluorescens*			
Extracts/Control	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**
n-Hexane	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Ethyl Acetate	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Methanol	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Aqueous	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Ciprofloxacin	50.00±0.00[b]	52.00±0.58[b]	53.00±0.33[b]	55.00±0.58[b]	34.67±0.33[b]	35.00±0.58[b]	37.00±0.58[b]	38.33±0.67[b]
DMSO	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at $p < 0.05$

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Whole Plant Crude Extracts of *E. heterophylla*

The results of MIC and MBC of normal hexane extract, methanol extract and aqueous extract of whole plant *E. heterophylla* showed that the MIC of whole plant aqueous extract of *E. heterophylla* recorded 20 mg/ml while the MBC was 40 mg/ml each (Figures 1 and 2).
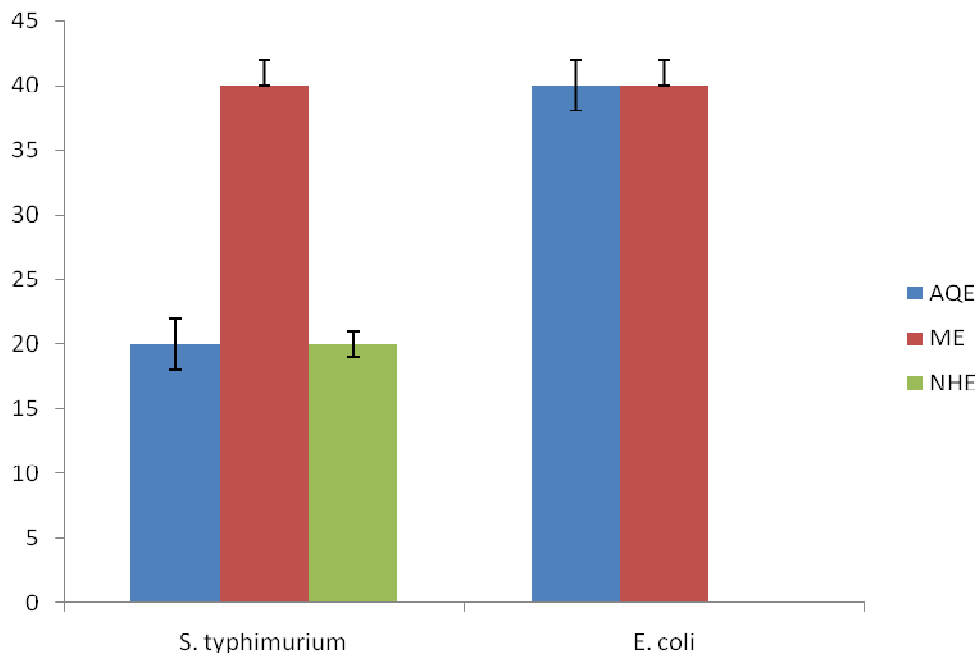

Figure 1: MIC of Whole Plant crude Extracts of *E. heterophylla*
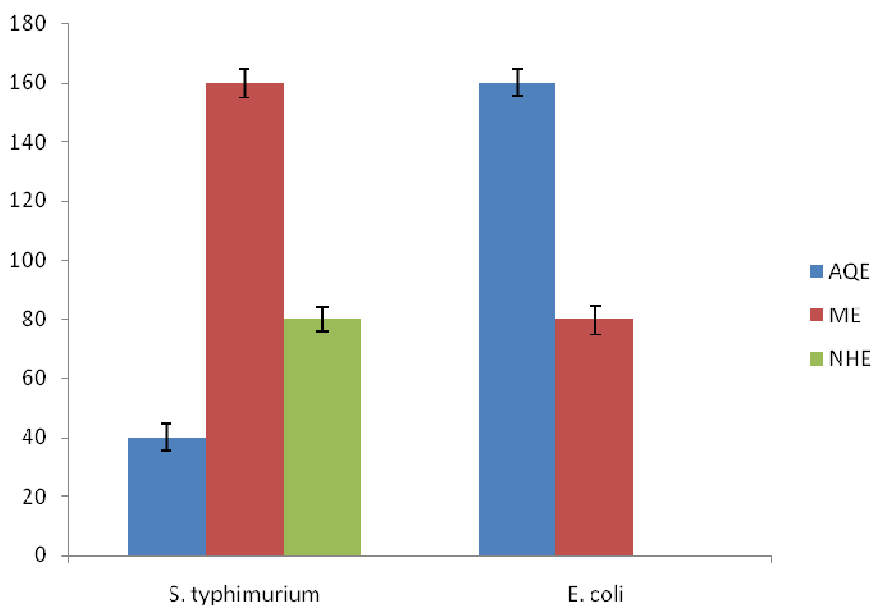**KEY:** AQE: Aqueous Extract; ME: Methanol Extract; NHE: *n*-Hexane Extract

Figure 2: MBC of Whole Plant crude Extracts of *E. heterophylla*
KEY: AQE: Aqueous Extract**;** ME: Methanol Extract**;** NHE: *n*-Hexane Extract

DISCUSSION

The results of biochemical and molecular characterization confirmed the bacteria pathogens to be *E. coli*; *S. typhimurium*; *P. fluorescens* and *K. pneumoniæ*. The use of biochemical and molecular approach in identifying bacteria is in agreement with the work of Ewansiha *et al*. (2016).

The presence of phytochemicals such as saponins, flavonoids, terpenoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars in the whole plant crude extracts of *E. heterophylla*. This result is in agreement with the work of Ughachukwu *et al*. (2014). The aqueous extract (AQE) contained all the phytochemical components compared to other crude extracts (Methanol Extract (ME)**,** *n*-Hexane Extract (NHE) and ethyl acetate extract (EAE)**)**. This possibly suggests that aqueous (water) might be a good solvent for the extraction of phytochemicals in *E. heterophylla*. The presence of these bioactive agents in the whole plant crude extracts of *E. heterophylla* is in line with the work of Ukoha *et al*. (2011) and Abalaka *et al*. (2016). These phytochemicals were known to exhibit medicinal physiological activities as reported by Sofowora (2013).

More so, flavonoids, tannins, phenols, alkaloids and reducing sugars are present in all the four extracts. Renu (2005) noted the potential of alkaloids as effective drugs and associated it to their sedative properties and powerful effect on the nervous system. Flavonoids are an integral phytochemical constituent of higher plants. They have antioxidant potentials hence could offer protection against heart disease and cancer probably by enhancing the body defence against pathology induced free radicals generation as opined by Al-Humaid *et al*. (2010). As reported by Mamta *et al*. (2013), the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and

haemostatic pharmaceuticals. Phenols are famous group of secondary metabolites with wide pharmacological activities. This includes antiulcer, anti- inflammatory, antioxidant, cytotoxic, antitumor, antispasmodic, and antidepressant activities (Ghasemzadeh *et al*., 2010; Silva *et al*., 2007).

The results of antibacterial activity of EAE of *E. heterophylla* revealed that there was no antibacterial activity with all the test organisms even with the increment in concentrations from 40 mg/ml to 100 mg/ml. These results might suggest that there are no sufficient bioactive components in the EAE to cause antibacterial activity against the test organisms. There was antibacterial activity with *n*-hexane extract (NHE), methanol extract (ME) and aqueous extract (AQE) at varying concentrations of the crude extracts. The zones of inhibition increased as the concentrations were increased. This is line with the work of Ughachukwu *et al*. (2014) and Mann *et al*. (2008). Prescott *et al*. (2010) reported that the activity of antimicrobial agent is concentration dependent. Hugo (1998) also indicated that the position of the zone edge (diameter of inhibition) is determined by the initial population density of the test organism, their growth rate and the rate of diffusion of the antimicrobial agent.

The AQE recorded the highest zone of inhibitions in this study with *S. typhimurium* at different concentrations. This result is in agreement with the work of Ogunnusi and Oso (2017). The highest antibacterial activity recorded with AQE in this work might also suggest that the extract contains more bioactive components than the other extracts subjected to antibacterial activity.

Furthermore, the results of the antibacterial activity of the Ciprofloxacin increases as the concentration used were increased from 40 mg/ml to 100 mg/ml. The wideness in the zones of inhibition recorded with Ciprofloxacin as compared to the crude extracts show its purity level.

The crude extracts might perform better if they are refined and purify further. Hence, the bioactive agents in this plant can be used for chemotherapy as reported by Mann *et al.* (2008). The results obtained for the DMSO were expected since it contains no antibacterial agent.

The values of MIC and MBC obtained for *E. heterophylla* did not agree with the works of Ogunnusi and Oso (2017) and Ugachuckwu *et al.* (2014). This might be due to the differences in the test organisms used, geographical location of the plant, season of plant, age of the plant and method of extraction, all of which affect the yield and the active constituents of medicinal plants as reported in the work of Calixto (2000) and it could also be due to differences in laboratory procedures and reagents used (Bonini *et al.*, 2002; Wallack, 2007).

CONCLUSION

The research work showed that different types of phytochemicals such as saponins, flavonoids, terpenoids, cardiac glycosides, tannins, phenols, alkaloids, steroids and reducing sugars are present in the crude extract of whole plant of *E. heterophylla* and the aqueous extract has a better antibacterial activity than other crude extracts when tested against

selected bacteria pathogens. The low values of MIC (20 mg/ml) and MBC (40 mg/ml) recorded for the aqueous extract of whole plant of *E. heterophylla* against *S. typhimurium* suggests that it has a good antibacterial activity.

Conflict of Interest

There is no conflict of interest among the authors.

REFERENCES

Abalaka, M. E., O. B. Akpor, O. B., Bello, A. B., Olonitola, O. S., Sani, A. and Osemwegi, O.O. (2016). Synthesis of Silver Nanoparticles (AgNPs) from Bark and Root of African Mahogany (*Khaya Senegalensis*) and the Comparative Studies of their Antimicrobial Properties. *Asian Journal of Microbiology and Biotechnology*, 1(1): 25-32.

Ajibesin, K. K., Ekpo, B. A., Bala, D. N., Essien, E. E. and Adesanya, S. A. (2008). Ethnobotanical survey of Akwa Ibom State of Nigeria. *Journal of Ethnopharmacology*,115: 387-408.

Al-Humaid, A.I., H.M. Mousa, R.A. El-Mergawi and A.M. Abdel-Salam, 2010. Chemical composition and antioxidant activity of dates and dates-camel-milk mixtures as a protective meal against lipid peroxidation in rats. *American Journal of Food Technology*, 5: 22-30.

Alviano, D. S. and Alviano, C. S. (2009). Plant Extracts: Search for New Alternatives to Treat Microbial Diseases. *Current Pharmaceutical Biotechnology, 10,* 106-121

Andrews, J. M. (2005). BSAC standardized disc susceptibility testing method (version 4). *Journal of Antimicrobial Chemotherapy,* 56(1): 60-76.

Balunas, M. J. and Kinghorn, A. D. (2005). "Drug Discovery from Medicinal Plants." *Life Sciences*, 78 (5): 431-441.

Barnes, J., Anderson, L. A. and Phillipson, J. D. (2007). Herbal Medicines, 3rd Edition. London: Pharmaceutical Press.

Beckman Coulter Incorporation (2016). GenomeLab™ Dye Terminator Cycle Sequencing and DNA Clean Up for Sequencing Reaction and Protocol. 608019 BA (pp.1-4). Kraemer Blvd, Brea, USA: 250 S, CA 92821. Available at *www.beckmancoulter.com*

Bergen, P. J., Bulitta, J. B., Forrest, A., Tsuji, B. T., Li, J. and Nation, R. L. (2010). Pharmacokinetic/pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an *in vitro* model. *Antimicrobial Agents Chemotherapy,* 54: 3783- 3789.

Bidlack, W. R., Omaye, S. T., Meskin, M. S, and Topham, D. K. W. (2000). Phytochemicals as Bioactive Agents. CRC press, Boca Raton, FL.

Bioneer Incorporation (2012). Accuprep Genomic DNA Extraction Protocol and Accupower Hotstart PCR Protocol. Revision 0 (pp. 1-10). Alameda, USA: 1000 Atlantic Avenue, CA 94501. Available at *www.bioneer.com*.

Bonini, P., Plebani, M., Cerrioti, F. & Rubboli, F. (2002). *Errors in laboratory medicine. Clinical Chemistry*, 48: 691-698.

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. *Brazilian Journal of Medical and Biological Research*, 33: 179- 189.

Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries in Laboratory Manual* (pp. 146-157). UK, Cambridge University Press.

Clinical Laboratory Standards Institute (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100- S25, Volume 35, No. 3.950 West Valley Road, Suite 2500 Wayne, Pennsylvania 19087, USA.

Evans, W. C. (2002). Trease and Evans Pharmacognosy15th Edition (pp. 42-44. 214-314, 221-229, 246-249, 304-306, 331-332, 391-393). London, U.K: Saunders Publishers.

Ewansiha, J. U., Garba, S. A., Musa, G., Daniyan, S. Y., Busari, M. B., Innalegwu, D. A. and J. H. Doughari, J. H. (2016). Preliminary Phytochemical and Antimicrobial Activity of Citrus x limon (L) Burm. f. (lemon) Leaf Extract against Some Pathogenic Microorganisms. *Journal of Applied Life Sciences International*, 6(4): 1-10.

Falodun, A., Agbakwuru, E. O. P. and Ukoh, G. C. (2003). Antibacterial activity of *Euphorbia heterophylla* Linn (Family Euphorbiaceae). *Pakistan Journal of Scientific and Industrial Research*, 46(6): 471-472.

Falodun, A., Okunrobo, L. O. and Uzoamaka, N. (2006). Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn (Euphorbiaceae). *African Journal of Biotechnology*, 5(6): 529-531.

Gardam, M. A. (2000). Is methicillin-resistant *Staphylococcus aureus* an emerging community pathogen? A review of the literature. *Canadian Journal of Infectious Diseases*, 11:202-211.

Ghasemzadeh, A., Jaafar, H. Z.E. and Rahmat, A. (2010). Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale Roscoe*). *Molecules*, 15: 4324-4333.

Hemaiswarya, S., Kruthiventi, A. K. and Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*, 15: 639-652.

Hugo, W. B. & Russell, A. D. (1998). Pharmaceutical Microbiology. 6th Edition (pp. 91-100). Oxford: Clarendon press.

Lepape, A. and Monnet, D. L. (2009). Experience of European intensive care physicians with infections due to antibiotic-resistant bacteria, 2009. *Eurosurveillance*, 12; 14(45). Pii: 19393.

Mamta, S., Jyoti, S., Rajeev, N., Dharmendra, S. and Abhishek, G. (2013). *Journal of Pharmacognosy and Phytochemistry*, Vol. 1 No. 6.

Mann, A., Yahaya, Y., A. Banso, A. and John, F. (2008). Phytochemical and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. *Journal of Medicinal Plants Research*, 2(5): 094-097.

Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. and Pfaller, M. A. (2007). Manual of Clinical Microbiology. Ninth Edition. Washington DC: ASM Press.

National Centre for Biotechnology and Information (NCBI) (1988). Sequence BLAST. Available at *www.ncbi.nlm.nih.gov*.

NCCLS (National Committee for Clinical Laboratory Standards) (1993) Performance Standard for Antimicrobial Disc Susceptibility Tests. Approved Standard. National Committee for Clinical Laboratory Standards, Villanova, P.A. Publication M2-A5. USA.

Ogunnusi, T. A. & Oso, B. A. (2017). Antibacterial Activity of Methanolic Extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* against Some Microorganisms. *European Journal of Medicinal Plants*, 20(3): 1-8.

Omotayo, A. E. (1998). Antibacterial Activity of Some Antimalarial Plants. *Proceeding of Nigerian Society for Microbiology*. 39: 69-72

Omoya, F. O., Momoh, A. O. & Olaifa, O. A. (2015). In vivo Effect of Cassava Flakes Mixed with *Euphorbia heterophylla* against *Salmonella typhi*. *European Journal of Medicinal Plants*, 7(1): 38-45.

Prescott, L. M., Harley, J. P. and Klein, D. A. (2002). Microbiology (International Edition). 5th Edition (pp. 811). New York: McGraw-Hill Companies, Incorporation.

Renu, S. (2005). Useful metabolites from plant tissue cultures. *Biotechnology*, 4(2): 79-93.

Rowan, N. G. and Onwukaeme, D. N. (2001). Deterpenoid esters of Euphorbiaceae in *Euphorbia hyles. Nigerian Journal of Pharmacolology*, 32: 60-64.

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F. and Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry*, 101: 1012-18.

Sofowora, A., Ogunbodede, E. and Onayade, A. (2013). The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *African Journal of Traditional Complement Alternative Medicine*, 10(5): 210-229.

Ughachukwu, P. O., Ezenyeaku, C. C. T., Ochiogu, B. C., Ezeagwuna, D. A. and Anahalu, I. C. (2014). Evaluation of antibacterial activities of *Euphorbia heterophylla. IOSR Journal of Dental and Medical Sciences*, 13 (11): 69-75.

Ukoha, P. O., Cemaluk, E. A. C., Nnamdi, O. L. and Madus, E. P. (2011). Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*, 5(8): 237-244.

Wallack, J. B. 2007). Interpretation of diagnostic tests. Philadelphia: Lippincott Williams & Wilkins.

WHO (World Health Organization) (2002). WHO Traditional medicine strategy 2002-2005. *WHO/EDM/TRM/2002,* 1: 1-74.