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EVALUATION OF ANTIBACTERIAL AND TOXICOLOGICAL PROPERTIES OF STEM EXTRACTS OF *Eurporbia heterophylla* ON SOME ENTERIC BACTERIA

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ABSTRACT

Enteric pathogenic organisms overtime, have been a menace to the general public, causing numerous morbidities and mortalities. However, in recent times, they have progressively developed resistance to existing antibiotics. A study was carried out to determine the antibacterial activity of *Euphorbia heterophylla* crude extracts on four enteric organisms namely: *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli* and *Proteus vulgaris.* The clinical isolates of *these organisms* were subjected to antimicrobial susceptibility test using agar diffusion technique. Two thousand milligram per kilogram body weight of the crude extracts was also administered to the mice orally and each group administered with methanolic crude extract of the stem, experienced a single death. Methanolic and aqueous crude extracts of *Euphorbia heterophylla* produced clear zones of inhibition at concentrations ranging from 100 to 200 mg/ml at 24 hours of incubation with the test organisms. However, the efficacy and antibacterial activity of the extracts, decreased at 48 and 72 hours of incubation with the enteric organisms respectively. From the result of this study, it may be concluded that *Euphorbia heterophylla* crude extract has some potential therapeutic properties for the treatment of diseases associated with enteric organisms such as *Salmonella typhi*, *Shigella flexneri, E. coli* and *Proteus vulgaris,* if adequate doses are administered at the appropriate time.

Keywords: Phytochemicals; *In vitro* activity; *In vivo* activity; *Euphorbia heterophylla*; Enteric bacteria; Acute oral toxicity

INTRODUCTION

Enteric bacteria are Gram negative bacteria that are associated with the gastrointestinal flora or disease (Murray, 1994; AL-Ouqaili, 2013). Enterics can be found in various natural habitats, and not only in the intestinal tract. However, these organisms are said to be chemoorganotrophs that exhibit both respiratory and fermentative metabolism (AL-Ouqaili, 2013). Most enterics are motile by peritrichous flagella; however, two major exceptions that lack peritrichous flagella, are *Klebsiella* and *Shigella* (Kolling *et al.,* 2012; McMahon, 2014). Enteric organisms are anaerobic in nature, a trait which allows them to thrive in the environment of the gut, producing energy by

feeding on sugars and converting them into lactic acid (AL-Ouqaili, 2013). Some of the enterics can live in the gut without causing health problems in individuals of good health, where as others are opportunistic, causing signs of infection, including vomiting, diarrhoea, and related symptoms (Murray, 1994).

Plants have served as sources of drugs and pharmaceuticals for man and other animals since medieval period. There are about half a million plants now growing on earth, many of which possess therapeutic and pharmaceutical properties for the treatment of various diseases (Muller, 1973; Okeniyi *et al.*, 2012). The ability of plants to produce many phytochemicals that are used to perform important biological functions is one of the characteristics they possess. According to an earlier survey, about 25 % of therapeutic drugs and medicinal products are derived from plant secondary metabolites (Hamburger and Hostettmann, 1991). Many of these phytochemicals have beneficial effects on long-term health of human and animals when consumed, and can be used to effectively treat human diseases (Ehrlich, 2013). Additionally, such antimicrobial compounds produced by plants are generally used for defence against predators that could induce harmful effects on living things such as humans, animals and plants (Hasegawa *et al.*, 1995). These substances also inhibit the growth of pathogens or destroy them, having little or no toxic effects on the host cells and based on this, they are considered as potential candidates for developing new antimicrobial drugs (Kunle *et al.*, 2012; Oyedum *et al*., 2015).

Euphorbia heterophylla is one of the numerous plants found especially in the fields of most tropical regions. It grows as weeds in cultivated and waste land, in gardens and along roadsides from sea-level up to 3000 m altitude (Mosango, 2008). It is a toxic plant which belongs to the family of *Euphorbiaceae*. It is referred to as Mexican fire plant, milk weed and Spurge weed in English. In Nigeria it is commonly called *Nono-kunchiya* in Hausa, *Egele* in Ibo and *Adimeru* in Yoruba (Okeniyi *et al.*, 2012). All parts of *Euphorbia heterophylla* contain latex: 0.42 % of leaves, 0.11 % of stems, 0.06 % of roots and the whole plant comprises up to 0.77 % (Mosango, 2008). The presence of latex in the plant is one of the main reasons it is considered to be toxic. In-spite of its toxicity, it is also known to possess numerous medicinal properties, hence it is

widely used as traditional African medicine in most tropical countries (Falodun and Agbakwuru, 2004; Falodun *et al.,* 2006). Generally, this plant is regarded as a purgative, antiasthmatic, anti-inflammatory and an arbotifacient (Erden *et al.*, 1999; Falodun *et al.,* 2006). It has also been reported to be oxytocic (Unekwe *et al.*, 2006). Records show that the plant is used for the treatment of gonorrheal disease, respiratory tract infection, malaria, eczema, and wart cure in traditional medicine (Falodun *et al.,* 2006; Unekwe *et al.,* 2006).

In African traditional medicine a decoction or infusion of the stems are taken as a purgative and laxative to treat body pain, stomach-ache and constipation, and to expel intestinal worms (Saimo *et al.,* 2003). The methanol stem extract, showed moderate antiplasmodial activity, whereas, the leaf extract showed significant nematicidal activity against *Meloidogyne graminicola* (Mosango, 2008). An extract of the aerial parts given orally to goats revealed moderate activity against several intestinal nematodes, such as *Haemonchus*, *Trichostrongylus*, *Bunostomum* and *Oesophagostomum* (Saimo *et al.,* 2003). However, despite the antibacterial reports of the stem of this plant against various bacteria, it is also observed that pharmacological studies of other parts of this plant are few. It is therefore imperative to determine the antibacterial properties and toxicological effects of stem extracts of *Euphorbia heterophylla* on some enteric bacteria and subsequently determine the antibacterial potentials of each fraction from the crude extracts on same test organisms.

MATERIALS AND METHODS

Collection and Identification of the Plant Materials

Fresh samples of the plants were uprooted from Garatu in Bosso Local Government Area of Niger State, Nigeria. The geographic location of Garatu lies on Longitude 6.44^{o}N, and Latitude 9.4^{o}E. The plant materials were placed in sterile sample bags and taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification. The leaves and roots were aseptically removed with sterile knife and the stems of the plant were taken to Microbiology Laboratory for further analyses.

Procedure for Drying Sampled Stems

The stems were thoroughly washed, air dried at room temperature (28 ^{o}C) and ground into coarse powder using a clean mortar and pestle. The dried plant parts were further ground into a fine powder using an electric blender (ES-242, Eurosonic, China), according to published protocols and procedures of (Iyamabo, 1991).

Extraction of Bioactive Components from Sampled Stems

One thousand milliliters (1000 mL) of four different extracting solvents namely; distilled water, methanol, chloroform and petroleum ether were dispensed into four different sterile conical flasks containing one hundred grammes (100 g) of the ground stem part. The four different mixtures were macerated successively for three days using cold maceration technique (Elmahmood *et al.,* 2008). Each mixture was occasionally rotated for one hour (1 hr) using an electronic rotator in the Microbiology Laboratory. The macerated samples were sieved with muslin cloth and evaporated to dryness using a steam bath. The dried extracts were weighed and stored in sterile sample bottles and kept in the refrigerator for further studies (Iyamabo, 1991).

Culture Media for the Isolation of Enteric Organisms

MacConkey agar (6.3 g) and *Salmonella- Shigella* agar (6.5g) were added to individual conical flask containing one hundred milliliters (100 mL) of distilled water. Each mixture (used either as differential or selective media for the confirmation of the test organisms) was heated and MacConkey agar was further sterilized using an autoclave, before use. Nutrient agar (2.8 g) was added to one hundred milliliters (100 mL) of water and was sterilized before use for susceptibility testing (Idu and Igekele, 2012).

Identification of the Test Organisms

The test organisms used (*Salmonella typhi, Shigella flexneri, Escherichia coli,* and *Proteus vulgaris*) were obtained from the stock culture in Microbiology Laboratory, General Hospital, Minna, Nigeria. The isolates were identified as described by Cheesbrough (2006) via various biochemical tests such as; indole test, triple sugar iron test, catalase test or citrate test.

Antibacterial Assay of the Stem Extracts

The antibacterial assay of the crude extracts was carried out according to the method described by Idu and Igekele (2012). Plates were prepared briefly, by dispensing 20 mL of nutrient agar into sterile Petri plates and allowed to set. A 4 mm cork borer was used to punch holes in the medium. Four holes (with a distance of 3cm from each other) were made on each Petri plate, after inoculation. About 0.2 mL of the different concentrations (of Chloroform stem extract of *Euphorbia heterophylla*; Methanolic stem extract of *Euphorbia heterophylla* ; Aqueous stem extract of *Euphorbia heterophylla* ; Petroleum ether stem extract of *Euphorbia heterophylla*) was introduced into each well. The Petri plates were incubated at a temperature of 37 ^{o}C for 24 hours, after which observation for the zones of inhibition were made. Measurement of the zones of inhibition was carried out using metre rule and the results recorded, in comparison to that of the standard antibiotic (Ciprofloxacin) as the control (Idu and Igekele, 2012). Only extracts that showed high zone of inhibition were used for the acute oral toxicity studies.

Thin–Layer Chromatography

Thin layer chromatography was performed according to already published protocols of Abalaka *et al.* (2011). The sample was applied at one end of the sheet of glass, coated with a thin layer of silica gel. The sheet of glass was then placed in a TLC (Thin Layer Chromatography) tank containing 100 mL of the mixture of solvents (called the mobile phase which consisted of ethyl acetate and n-hexane, in the ratio of 1: 4). After the sample had been applied on the plate and placed in the TLC tank, the solvent was drawn up the plate via capillary action. The different analytes ascended the TLC plate at different rates, and so separation was achieved (Abalaka *et al.*, 2011).

Acute Oral Toxicity Studies

Acute toxicity study was performed on 30 animals using a single dose of 2000 mg/kg body weight (based on the fact that the LD_{50} > 2000 mg/kg). The animals were divided in to 6 groups, each containing 5 animals. The animals were starved overnight before they were administered with a crude extract orally (Mukinda and Syce, 2007). After drug administration the animals

were provided with food and water immediately and were under observation for any mortality/ adverse signs (Mukinda and Syce, 2007).

Statistical Analysis

The data obtained will be analyzed using package for Statistical Analysis System (SAS) version 9.4 to determine the significant differences between the data obtained**.**

RESULTS

Tables 1 – 4 reveal that stem extracts of *Euphorbia heterophylla* had significant antibacterial activity on all the test organisms at 50 mg / mL or 24 hours and as the time increased to 48 hours and 72 hours, microbial population increased, and there was decrease in the potency and antibacterial activity of the stem extracts.

Table 1: Zone of inhibition of the stem of *Euphorbia heterophylla* at 50mg/ml

	24hr				48hr				72hr			
Extracts	S. typhi	S. flexneri	E. coli	P. vulgaris	S. typhi	S. flexneri	E. coli	P. vulgaris	S. typhi	S. flexneri	E. coli	P. vulgaris
EHCS	4.00 ± 0.58^{cde}	3.67 ± 0.33^{cd}	4.33 ± 0.67^{de}	4.33 ± 0.33^{cd}	2.00 ± 0.58^{a}	1.33 ± 0.33^{abc}	2.33 ± 0.68^{cde}	$1.33\pm\pm0.33^{bc}$	0.33 ± 0.03^{ab}	0.00^{a}	0.67 ± 0.33^{ab}	0.33 ± 0.03^{a}
EHMS	8.33 ± 0.67^{g}	6.33 ± 0.88^{f}	7.00 ± 1.15^{fg}	7.67 ± 0.33^{e}	4.67 ± 0.33^{c}	3.33 ± 0.88^{cd}	3.67 ± 0.88^{e}	4.00 ± 0.58^{e}	2.33 ± 0.67^{c}	1.33 ± 0.88^{a}	1.00 ± 0.58^{b}	2.00 ± 0.58^{b}
EHAS	5.67 ± 1.20^{ef}	5.33 ± 0.67^{def}	6.00 ± 0.58^{ef}	5.33 ± 0.33^{e}	3.33 ± 1.33^{bc}	2.67 ± 0.33^{bcd}	3.00 ± 0.00^{de}	2.33 ± 0.33^{cd}	1.67 ± 1.20^{bc}	0.67 ± 0.03^{a}	1.00 ± 0.00^{b}	0.33 ± 0.03^{a}
EHPS	3.33 ± 0.88^{bc}	$4.00\pm0..56^{cde}$	3.33 ± 0.33^{bc}	3.67 ± 0.33^{c}	1.33 ± 0.88^{ab}	2.00 ± 0.577^{abcd}	1.00 ± 0.5^{abc}	1.67 ± 0.33^{cd}	0.00^{a}	0.33 ± 0.03^{a}	0.00^{a}	0.33 ± 0.03^{a}
CONTROL	9.00 ± 0.58^{g}	8.00 ± 0.57^{g}	$8.67\pm$O.68^{g}	8.67 ± 0.33^{e}	7.00 ± 0.58^{d}	6.00 ± 0.58^{e}	6.67 ± 0.68	6.67 ± 0.33^{f}	5.00 ± 0.58^{d}	3.33 ± 0.33^{b}	3.00 ± 0.58^{c}	3.33 ± 0.33^{c}

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet is significantly ($p < 0.05$)

Key: EHCS---Chloroform stem extract of *Euphorbia heterophylla*; EHMS---Methanolic stem extract of *Euphorbia heterophylla* ; EHAS---Aqueous stem extract of *Euphorbia heterophylla* ; EHPS---- Petroleum ether stem extract of *Euphorbia heterophylla*

Table 2: Zone of inhibition of the stem of *Euphorbia heterophylla* at 100mg /ml

	24hr				48hr				72hr			
Exracts	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCS	10.00 ± 0.58^{ef}	9.33 ± 1.20^{gh}	9.33 ± 0.90^{def}	9.33 ± 0.33^{def}	7.00 ± 0.58^{de}	7.67 ± 0.88^{e}	7.00 ± 1.00^{ef}	6.67 ± 0.70^{f}	5.00 ± 0.60^{ef}	5.33 ± 1.20^{d}	4.67 ± 0.70^{de}	3.33 ± 0.33^{de}
EHMS	10.67 ± 1.21^{g}	10.33 ± 0.90^{h}	4.67 ± 1.40^{bc}	9.67 ± 1.67^{efg}	8.33 ± 0.90^{e}	8.00 ± 0.60^{e}	7.33 ± 0.66^{f}	7.00 ± 1.00^{f}	6.33 ± 0.90^{e}	5.33 ± 0.70^{d}	6.33 ± 0.67^{e}	4.67 ± 0.88^{e}
EHAS	9.67 ± 1.22^{efg}	8.67 ± 0.86^{fgh}	9.67 ± 1.20^{ef}	10.33 ± 0.33^{fg}	7.67 ± 1.17^{de}	6.33 ± 0.85^{de}	6.67 ± 1.76^{def}	6.33 ± 0.88^{f}	5.00 ± 1.53^{ef}	3.00 ± 0.58^{c}	2.33 ± 0.88^{bc}	3.00 ± 0.60^{cde}
EHPS	7.33 ± 0.67^{cd}	6.00 ± 0.60^{bcd}	7.00 ± 0.58^{cde}	7.33 ± 0.33^{cde}	3.33 ± 0.70^{b}	2.33 ± 0.33^{b}	3.00 ± 0.60^{bc}	3.33 ± 0.33^{bcd}	1.67 ± 0.31^{abc}	0.33 ± 0.03^{ab}	1.33 ± 0.33^{abc}	1.33 ± 0.33^{abc}
CONTROL	15.00 ± 0.60^{h}	13.33 ± 0.90^{i}	13.33 ± 1.45^{f}	12.33 ± 1.45^{g}	13.33 ± 0.33^{e}	10.33 ± 0.90^{e}	11.00 ± 1.15^{g}	10.00 ± 1.20^{g}	10.00 ± 0.6^{g}	8.00 ± 0.58^{e}	8.67 ± 1.20^{f}	7.00 ± 0.60^{f}

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet is significantly ($p < 0.05$)

Key: EHCS---Chloroform stem extract of *Euphorbia heterophylla*; EHMS---Methanolic stem extract of *Euphorbia heterophylla* ; EHAS---Aqueous stem extract of *Euphorbia heterophylla* ; EHPS---- Petroleum ether stem extract of *Euphorbia heterophylla*

Table 3: Zone of inhibition of the stem of *Euphorbia heterophylla* at 150mg/ml

	24hr				48hr				72hr			
Extracts	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCS	12.00±0.57^{de}	10.67±0.70^{ef}	11.33±0.90^{def}	9.67±0.31^{cd}	10.33±0.88^{ef}	9.00±1.15^{fg}	10.00±1.20^{e}	8.00±0.60^{de}	7.67±1.20^{ef}	6.33±0.33^{de}	6.67±0.31^{fgh}	5.33±0.33^{d}
EHMS	14.00±0.60^{e}	12.00±1.15^{f}	12.33±1.45^{f}	12.00±1.00^{e}	12.00±0.60^{f}	10.33±0.90^{g}	10.67±1.76^{e}	10.00±0.58^{f}	10.00±0.60^{g}	8.67±1.20^{f}	8.67±1.76^{h}	8.00±0.58^{e}
EHAS	12.33±0.90^{de}	11.33±0.33^{e}	11.67±1.20^{ef}	11.00±1.00^{de}	11.33±0.88^{ef}	9.33±0.33^{fg}	10.33±0.90^{e}	9.67±0.70^{ef}	9.33±0.33^{fg}	7.00±0.60^{ef}	8.00±0.58^{fg}	7.67±0.70^{e}
EHPS	8.33±0.88^{c}	8.67±0.30^{cd}	8.67±0.70^{cd}	7.67±0.90^{c}	6.33±0.88^{c}	6.33±0.33^{cde}	6.33±0.90^{cd}	5.67±0.88^{bc}	4.00±1.00^{cd}	3.00±0.58^{bc}	4.00±1.00^{de}	2.67±0.31^{bc}
CONTROL	20.00±0.60^{f}	18.67±0.70^{g}	19.33±0.33^{g}	19.00±0.58^{f}	18.00±0.60^{g}	16.33±0.88^{h}	16.333±0.90^{f}	16.67±0.67^{g}	15.33±0.33^{h}	13.33±1.23^{g}	15.33±0.90^{i}	15.33±1.15^{f}

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet is significantly ($p < 0.05$)

Key: EHCS---Chloroform stem extract of *Euphorbia heterophylla*; EHMS---Methanolic stem extract of *Euphorbia heterophylla* ; EHAS---Aqueous stem extract of *Euphorbia heterophylla* ; EHPS---- Petroleum ether stem extract of *Euphorbia heterophylla*

Table 4: Zone of inhibition of the stem of *Euphorbia heterophylla* at 200mg/ml

	24hr				48hr				72hr			
Extracts	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*	***S. typhi***	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCS	11.33±0.67^{de}	10.33±0.33^{de}	10.67±0.33^{cd}	10.00±0.60^{de}	10.00±0.60^{d}	8.67±0.30^{c}	9.33±0.33^{e}	9.00±0.60^{d}	9.00±0.57^{e}	7.33±0.67^{f}	7.33±0.33^{e}	7.33±0.67^{d}
EHMS	16.33±0.33^{f}	15.33±0.33^{f}	15.67±0.90^{e}	15.33±1.20^{f}	15.00±0.60^{e}	14.00±0.60^{d}	14.33±0.33^{f}	13.67±0.90^{e}	13.33±0.88^{f}	12.00±0.58^{g}	12.33±0.33^{f}	11.67±0.90^{e}
EHAS	14.67±0.33^{e}	14.00±0.60^{f}	14.33±0.33^{e}	14.00±0.00^{f}	13.67±0.30e	13.00±0.57^{d}	13.33±0.33^{f}	12.67±0.33^{e}	12.00±0.00^{f}	11.00±0.57^{g}	11.67±0.33^{f}	10.67±0.30^{e}
EHPS	10.33±0.33^{cd}	9.00±0.60^{cd}	9.67±0.30^{bc}	8.67±0.31^{cde}	9.33±0.33^{cd}	8.00±0.58^{c}	8.67±0.33^{de}	7.33±0.33^{cd}	8.33±0.33^{de}	6.67±032^{def}	7.00±0.58^{de}	6.33±0.33^{cd}
CONTROL	26.00±0.60^{h}	25.00±0.70^{g}	25.33±0.33^{f}	24.33±0.33^{g}	25.00±0.60^{f}	24.00±0.60^{e}	23.33±0.33^{g}	22.33±0.33^{f}	24.00±0.57^{g}	23.00±0.60^{h}	22.33±0.33^{g}	21.33±0.33^{f}

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet is significantly ($p < 0.05$)

Key: EHCS---Chloroform stem extract of *Euphorbia heterophylla*; EHMS---Methanolic stem extract of *Euphorbia heterophylla* ; EHAS---Aqueous stem extract of *Euphorbia heterophylla* ; EHPS---- Petroleum ether stem extract of *Euphorbia heterophylla*

Table 5 reveals the different number of bioactive components found in each stem extract. Samples EHCS and EHPS posses two bioactive components each while samples EHMS and EHAS possess to three bioactive components each.

Table 5: Fractionated crude extracts of the stem and its corresponding antibacterial effect on each test organism

Extracts	Bioactive components	*Salmonella typhi*	*Shigella flexneri*	*E.coli*	*P.vulgaris*
EHMS	A1	+	+	+	+
	A2	+	+	+	+
	A3	+	+	+	+
EHAS	B1	+	+	+	+
	B2	+	_	+	+
	B3	+	+	+	_
EHCS	C1	_	_	_	_
	C2	_	+	+	_
EHPS	D1	_	_	+	+
	D2	_	_	_	_

Key: + = antibacterial activity ; - =no antibacterial activity; EHCL---Chloroform stem extract of *Euphorbia heterophylla*; EHML---Methanolic stem extract of *Euphorbia heterophylla* ; EHAL--- Aqueous stem extract of *Euphorbia heterophylla* ; EHPL---- Petroleum ether stem extract of *Euphorbia heterophylla.*

Table 6: Acute oral toxicity of the crude extracts on the mice

Extract	No of mice per extract	Dose (mg/kgbw)	Number of mice that died
EHMS-I	5	2000	1/5
EHAS-II	5	2000	0/5
EHMS-III	5	2000	1/5
EHAS-IV	5	2000	0/5
EHMS-V	5	2000	1/5
EHAS-VI	5,	2000	0/5
EHMS-VII	5	2000	1/5
EHAS-VIII	5	2000	0/5

Key: EHMS-I: Methanolic stem extract of *Euphorbia heterophylla* against *S.typhi*; EHAS-II: Aqueous stem extract of *Euphorbia heterophylla* against *S.typhi*; EHMS-III: Methanolic stem extract of *Euphorbia heterophylla* against *S.flexneri*; EHAS-IV: Aqueous stem extract of *Euphorbia heterophylla* against *S.flexneri*; EHMS-V: Methanolic stem extract of *Euphorbia heterophylla* against *E.coli*; EHAS-VI: Aqueous stem extract of *Euphorbia heterophylla* against *E.coli*; EHMS-VII: Methanolic stem extract of *Euphorbia heterophylla* against *Proteus vulgaris*; EHAS-VIII: Aqueous stem extract of *Euphorbia heterophylla* against *Proteus vulgaris*.

DISCUSSION

All the stem extracts of *Euphorbia heterophylla* showed significant antibacterial activity on the test organisms at 50 mg/ mL concentrations with greater antibacterial activity recorded for aqueous and methanol crude extracts, after 24 and 48 hours which declined after 72hours of incubation, on all the test organisms, when compared to the petroleum ether extracts, which recorded no antibacterial activity after 24, 48 or 72 hours of incubation. This could be attributed to the different variations in polarity of the solvents and solubility of the bioactive compounds in the plant as reported by Elmahmood *et al*. (2008). In addition, extracting solvents which are alcohol based such as the methanol are usually known to have greater extracting abilities compared to their counterparts. This therefore enables them extract and retain the potency of large quantities of bioactive phytochemicals from little concentration of a plant. Similarly, the study also revealed that 48 hours after the antibiogram was carried out, the zones of inhibition gradually reduced. This could be due to the fact that the potency of the extracts after 48hours reduced, and this in turn encouraged the growth of more microorganisms in the plate, thereby subjecting the microorganisms to develop resistance to the stem extract in the plate. This agrees with the findings of Mbata and Salkia (2008).

Furthermore, the antibacterial activities of methanolic and aqueous crude extracts of *Euphorbia heterophylla* at 150 mg/ mL and 200 mg/ mL were significant on the test organisms after 24, 48 and 72 hours as compared to the antibacterial activities of methanolic crude extracts of *Euphorbia heterophylla* at 100 mg/ mL. The high antibacterial activities of methanolic and aqueous crude extract observed at 150 mg/ mL and 200 mg/ mL could be due to the enhanced effect of the plant extracts based on the increased concentration of the individual extract, which are said to contain more phytochemical constituents. Similarly, the high antibacterial activities observed, could also be based on the fact that, the content of latex in the stem extracts, which is said to show enhanced antimicrobial properties (Okeniyi *et al.*, 2012), could be high . This therefore, conforms to the result obtained in a study by Ahmed *et al.* (2012).

The presence of different bioactive components in the crude extracts of the stem of *Euphorbia heterophylla* indicates that the stem contains diverse potent active ingredients .This result agrees with the findings of Jayashree (2013); Falodun and Agbakwuru (2004). All bioactive

components obtained in the methanoic crude extracts of the stem showed antibacterial activity against the test organisms in this study compared to all bioactive components of the aqueous, chloroform and petroleum ether extracts. This suggests that the individual components or bioactive compounds that were not able to exhibit antibacterial activity on the test organisms, may be due to their inactive nature or requires a synergistic relationship with other bioactive components, as reported by Harborne (1984); Oyeleke *et al.* (2008). The thin layer chromatography (TLC) analysis also revealed that retardation factor (RF) values for the stem are 0.7 and 0.9 respectively which agrees with the result of Adamu *et al.* (2013).

Acute oral toxicity screening of these extracts revealed that the food intake and water consumption were not affected by the intake of both the methanoic and aqueous extracts of the stem of *Euphorbia heterophylla* and as such, induced appetite suppression and deleterious effect were not experienced. This indicates that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen, 2001). Although within the first 24 hours in which the mice were administered with the extracts at a single dose of 2000 mg/kg, the mice revealed minor abnormalities such as, weakness, slight decrease in locomotion, and aggression, after two to four hours of administering the extracts to them. Such abnormalities are said to be triggered by the presence of these foreign extracts, when they enter the body system as reported by Pillai *et al*. (2011). In addition to this, the intake of plant extracts or chemicals by animals might also show slight changes in behavior as a consequence of the metabolism of the plant extracts or chemicals, and such behavioral changes or abnormalities are said to be quickly reversible (Eaton and Klaassen, 1996). In addition, after the administration of 2000 mg/kg bw, the weight of the mice at the end of the toxicity test as compared with the control showed no significant change. This therefore implies that the stem extract of *Euphorbia heterophylla* are non-toxic. This therefore agrees with the report by Sangetha *et al.* (2008).

Similarly, the acute oral toxicity study on the mice also revealed that the oral administration of *Euphorbia heterophylla*, at a single dose of 2000 mg/kg bw, caused single deaths in each group of mice administered with methanolic crude extract, when compared with the group of mice administered with aqueous crude extracts. The death of the mice administered with 2000 mg/kg bw of methanolic extracts could be attributed to the fact that this high single dose of *Euphorbia*

heterophylla, was toxic in relation to their body weights. However, this result disagrees with the findings of Arsad *et al.* (2013), who reported that treatment at any dose of *Rhaphidophoria decursiva* irrespective of the weights of the mice was non-toxic. In addition, the deaths of the mice could also be based on the fact that the mice could not exhibit normal physiological adaptation responses to the plant extracts or compounds as compared to their counterparts. This in turn, led to the low appetite and low calorie intake which therefore resulted to the reduction in the body weight and death of the mice and this agrees with the findings of Rhiouani *et al.* (2008).

CONCLUSION

The methanolic, chloroform and aqueous extracts of the stem of *E. heterophylla*, contained efficient phytochemicals that were active against all test organisms at a concentration as low as 50 milligram, indicating that the stem extract of *Euphorbia heterophylla* is potent and contains numerous bioactive components that could be potential sources of synthetic drugs. However, the activity of the crude extracts at concentrations ranging from 50-200 mg/ml showed antibacterial activity, particularly after 24 to 48 hours but the activity declined after 72 hours of incubation. In addition, the crude extracts of the plant was found to be safe at higher dose of 2000 mg/kg bw. But since the administration of methanolic crude extracts of the stem caused single death, it is therefore recommended that before an extract is administered, side effects of the extract and appropriate dose in relation to the weight of the mouse should be ascertained.

REFERENCES

Abalaka, M.E., Onaolapo, J.A, Inabo, H.I. and Olonitola, O.S (2011). Comparative Evaluation of the Bioefficacy of crude extracts and chromatographically separated fractions of *Momordica charantial. Advance Agricultural Biotechnology*, 1(1), 53-59.

Adamu, H.M.,Ushie, O.A., and Nansel, E. (2013). Antimicrobial Activity of Oil from *Butyrospermum parkii* (Shea Butter)Seed. *International Journal of Modern Biology and Medicine,* 3(2), 50-59.

Ahmed, R.N., Abdulrahaman,A.A., and Sani, A.(2012). *Invitro* Evaluation of Antifungal Potentials of Methanolic Extracts of Three Organs Of *Vitellaria Paradoxa* (Shea plant). *Journal of Science, Technology,Mathematics and Education*, 8(2), 8-16.

AL- Ouqaili M.T.S. (2013). The Enteric Bacteria. A Ph.D thesis presented at Cairo University. Pp 1-10.

Arsad, S. S., Esa, N. M., Hamzah, H. and Othman, F. (2013). Evaluation of acute, subacute and subchronic oraltoxicity of *Rhaphidophora decursiva* (Roxb.) Schott in male Sprague Dawley rats. *Journal of Medicinal Plant Research*, 7(41), 3030-3040.

Cheesbrough**,** M. (2006). District Laboratory Practice in Tropical Countries. 2nd edition.Cambridge: Cambridge University Press; Pp. 100-103.

Eaton, D.L. and Klaassen, C.D. (1996). Principles of toxicology. In: Klaassen, C.D. (Ed.),Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th ed. McGrawHill, pp. 13.

Ehrlich, S. D. (2013). Herbal Medicine. Review provided by VeriMed Herbal Network. Pp1-5.

El-Mahmood, A.. M,., Doughari, J. H. and Ladan, N. (2008). Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*, 2(5), 089-094.

Erden, Y S., Ekrem, H., Gisho, T. and Yoshiohiro, T. (1999) Traditional medicine in Turkey IX, folk medicine in NorthWest Anatolia. *Journal of Ethnopharmacology*, 64, 201.

Falodun, A. and Agbakwuru, E.O.P. (2004). Phytochemical analysis and laxative activity of the leaf extracts of *Euphorbia heterophylla* L. (Euphorbiaceae). *Pakistan Journal of Scientific and Industrial Research*, 47(5),345–348.

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.

Hamburger, M. and Hostettmann, K. (1991). Bioactivity in plants: the link between phytochemistry and medicine, *Phytochemistry*, 30,3864-3874.

Harborne, J.B. (1984). Phytochemical methods; A guide to modern techniques of plant analysis. 2nd edition, London ,Chapman and Hall, London, pp.1-19, 37-168.

Hasegawa, H., Matsumya, S. and Yamasak, K. (1995). Reversal of efflux mediated tetracycline resistance in *Staphylococcus aureus* clinical isolates by Ginseng prosaponenins. *Phytotherapy. Resource*, 9, 260-263.

Idu, M. and Igekele, C.L. (2012). Antimicrobial activity and phytochemistry of *Khaya senegalensis* root. *International Journal of Ayurvedic and Herb Medicine*, 2(3), 416 - 422.

Iyamabo, P. A. (1991). Thesis on comparative antimicrobial activity of crude extract of Terminalia Macroptae with phenol chlorhexidine and gentamycin. *Pharmacognosy Journal*,1(1),12.

Jayashree, D. (2013). Phytochemicals analysis and TLC fingerprinting of methanolic extracts of three medicinal plants. *International Research Journal Pharmacy*, 4(6), 123-126.

Klaassen, C.D. (2001). Principles of Toxicology. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed.; McGraw-Hill: New York, NY, USA, p. 13.

Kolling, G., Wu, M., & Guerrant, R..L. (2012). Enteric pathogens through life stages.*Front. Cell Infectious Microbiology*, 2(1):114.doi; 10.3389/fcimb.2012.00114 PMCID: PMC3427492.

Kunle, O.F., Egharevba, H.O. and Ahmadu, P. O. (2012). Standardization of herbal medicines – A review.*International Journal of Biodiversity and Conservation*, 4(3), 101-112.

Mbata, T.I. and Salkia, A. (2008). Antibacterial acivity and phytochemical screening of crude ethanolic extract of leaves of *Ocimumgratissimum* L on *Listeria monocytogenes*. *The Internet Journal of Microbiology*,4, 2.

McMahon, M. (2014). What is Enteric Bacteria. Retrieved March 1,2014. From http://www.wisegeek.com/ Microbiology Laboratories (2014). "Microbes in our world and what they do". Retrieved January 31st 2014. From http://inst.bac.wisc.edu/inst/index.php.

Mosango, D.M. (2008). Euphorbia heterophylla L. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1),1-2.

Mukinda, J.T. and Syce, J.A. (2007). Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. *Journal of ethnopharmacology*,112 (1),138-44.

Muller, L.P. (1973). Importance of secondary metabolites constituents as drugs, *Phytotherapy*, 3: 354.

Murray, P.R. (1994). Enterobacteriaceae, In:*Medical Microbiology*, R. Farrell, (Ed.), 227-240, Mosby Year Book Inc., ISBN 0723420106, London, UK.

Okeniyi, S.O., Adedoyin, B.J. and Garba, S. (2012). Phytochemical Screening, Cytotoxicity,Antioxidant and Antimicrobial Activities of stem and leave extracts of

Euphorbia heterophylla . *Bulletin of Environmental Pharmacology of Life Science*, 1(8),87-91.

Oyedum, M.U. (2015). Phytochemical Screening, *In vitro* and *In* vivo activity of extracts of parts of *Vitellaria paradoxa* against *S.typhi* and *S.flexneri.* An unpublished M.tech thesis, Federal University of Technology, Minna, Niger State, pp. 56-59**.**

Oyeleke, S.B., Dauda, B.E.N. and Boye, O.A.(2008). Antimicrobial activity of *Ficus cupensis. African Journal of Biotechnology*, 7 (10),1414 -1417.

Pillai, P. G., Suresh, P., Mishra, G. and Annapurna, M. (2011). Evaluation of the acute and sub acute toxicity of the methanolic leaf extract of *Plectranthus amboinicus* (Lour) Spreng in Balb C mice. *European Journal of Experimental Biology*, 1 (3), 236-245.

Rhiouani, H.R., Nazari, P., Kamli-Nejad, M. and Lyoussi, B. (2008). Acute and subchronic oral toxicity of an aqueous extract of leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology*, 118,378 -386.

Saimo, M.K., Bizimenyera, E.S., Bwanika, A., Ssebuguzi, F., Weny, G. and Lubega, G.W. (2003). Ethnoveterinary practices in Uganda: use of medicinal plants in treating helminthosis and coccidiosis in rural poultry and goats in Uganda. Bulletin of Animal Health and Production in Africa 51(3): 133–138.

Sangetha, S., Zuraini, Z., Sasidharan, S., and Suryani, S. (2008). Fungicidal effect and oral acute toxicity of *Cassia spectabilis* leaf extract. *Japan Journal of Medical Mycology*,49, 299-304.

Unekwe, P .C., Ughachukwu, P. O. and Ogamba, J .O .(2006). Some pharmacological Studies of Aqueous extract of leaves of *Euphorbia heterophylla*. *Tropical Journal of Medical Resources*, 10 (2),1-5.