Oyedum et al. (2018).Phytochemical and antibacterial study of stem extracts of Euphorbia heterophylla on some enteric bacteria. 13(1): 97-104.

Phytochemical and antibacterial study of stem extracts of *Euphorbia heterophylla* on some enteric bacteria

Oyedum, U.M., Kuta, F.A. and N. U. Adabara. Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna, Niger State, Nigeria.

Abstract

A study was carried out to determine the phytochemical components and antibacterial activity of Euphorbia heterophylla crude extracts on four enteric bacteria namely; Salmonella typhi, Shigella flexneri, Escherichia coli and Proteus vulgaris. The isolates of Salmonella typhi, Shigella flexneri, E.coli and Proteus vulgaris were subjected to antimicrobial susceptibility test using agar diffusion technique. Phytochemical tests of the Euphorbia heterophylla crude extracts revealed the presence of starch, flavonoids, alkaloids, saponins and tannins. Methanolic and aqueous crude extracts produced clear zones of inhibition such as, 8.33 ± 0.67^{g} 6.33 ± 0.88^{f} 7.00 ± 1.15^{fg} 7.67 ± 0.33^{e} and 5.67 ± 1.20^{ef} 5.33 ± 0.67^{def} 6.00 ± 0.58^{ef} 5.33 ± 0.33^{e}; 10.67 ± 1.21^{g} 10.33 ± 0.90^{h} 4.67 ± 1.40^{bc} 9.67 ± 1.67^{efg} and 9.67 ± 1.22^{efg} 8.67 ± 0.86^{fgh} 9.67 ± 1.20^{ef} 10.33 ± 0.33^{fg}; 14.00 ± 0.60^{e} 12.00 ± 1.15^{f} 12.33 ± 1.45^{f} 12.00 ± 1.00^{e} and 12.33 ± 0.90^{de} 11.33 ± 0.33^{e} 11.67 ± 1.20^{ef} 11.00 ± 1.00^{de}; 16.33 ± 0.33^{f} 15.33 ± 0.33^{f} 15.67 ± 0.90^{e} 15.33 ± 1.20^{f} and 14.67 ± 0.33^{e} 14.00 ± 0.60^{f} 14.33 ± 0.33^{e} 14.00 ± 0.00^{f}, at concentrations ranging from 50 to 200mg/ml. In vivo antimicrobial assay revealed that the mice treated with the crude methanolic and aqueous extracts, after being infected with the various test organisms, survived and showed mild pathological effects. Similarly, untreated mice (control) died after 48hours of inoculation with Salmonella typhi, Shigella flexneri, E.coli and Proteus vulgaris. Euphorbia heterophylla based on the methanolic and aqueous extracts activity, could be a potential source for the treatment of diseases associated with enteric organisms such as Salmonella typhi, Shigella flexneri, E.coli and Proteus vulgaris. Further studies should be directed towards isolation and characterization of the active compound in the crude extracts.

Keywords: Bioactive components; Antibacterial activity; *Euphorbia heterophylla*; Enteric bacteria; Toxicity
Email: hemdi41@gmail.com
Received: 2017/03/10.
Accepted: 2017/12/17.
DOI: https://dx.doi.org/10.4314/njtr.v13i1.10

Introduction

Enteric bacteria are Gram negative bacteria that are associated with gastrointestinal flora or disease (Murray, 1994). Enterics can be found in various natural habitats, not just in the intestinal tract. However, these organisms are said to be chemoorganotrophs and they 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*.

Many enteric organisms are anaerobic in nature, a trait which allows them to thrive in the environment of the gut, and most produce energy by feeding on sugars and converting them into lactic acid. Some of the enterics can live in the gut without causing health problems in individuals of good health, while others cause signs of infection, such as vomiting, diarrhoea, and related symptoms (Murray, 1994).

There are about half a million plants now growing on earth, many of which possess therapeutic and pharmaceutical properties which are used in all major systems of medicine for the treatment of various diseases (Muller, 1973; Okeniyi *et al.*, 2012). According to an earlier survey, about 25% of modern drugs and medicinal products are derived from plant secondary metabolites also regarded as phytochemicals (Hamburger *et al.*, 1991;Ehrlich, 2013), and such antimicrobial compounds produced by plants are usually active against plants and human pathogenic microorganisms (Kunle *et al.*, 2012; Oyedum, 2015). Such substances can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells and in most cases are considered as potential candidates for developing new antimicrobial drugs.

Euphorbia heterophylla is one of the numerous plants found in the field. *Euphorbia heterophylla* grows in disturbed localities as a weed of cultivation and waste land, in gardens and along roadsides from sea-level up to 3000 m altitude (Mosango, 2008). *Euphorbia heterophylla* 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: leaves 0.42%, stems 0.11%, roots 0.06% and whole plant up to 0.77% (Mosango, 2008). 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). It has also been recorded that this plant is used for the treatment of gonorrheal disease, respiratory tract infection, malaria, Eczema, and wart cure in traditional medicine.

In Africa 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). A methanol extract of the aerial parts showed moderate antiplasmodial activity. 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 further evaluate the chloroform, aqueous, methanolic and petroleum ether extract of the stem of *E.heterophylla* against some enteric organisms namely: *Salmonella typhi*, *Shigella flexneri, E. coli* and *Proteus vulgaris.*

Materials and methods

Collection and Identification of the Plant Materials

Fresh samples of the stem were collected from Garatu, in a village called Anguwan noma. Anguwan noma in Garatu lies on Longitude 6.44°N, and Latitude 9.4°E. The plant materials were taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification.

Drying Procedure

The stem was thoroughly washed, air dried at room temperature (28°C) and ground into coarse powder using a sterile mortar and pestle. The dried plant part was further ground into a fine powder using an electric blender. This was done to enhance the penetration of the extracting solvent, thus facilitating the release of active components (Iyamabo, 1991).

Extraction

One hundred grammes (100 g) of the ground part was macerated successively for three days (with occasional shaking) using cold maceration technique. One thousand milliliters (1000 ml) each of distilled water, methanol, chloroform and petroleum ether were used as extraction solvents respectively. 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).

Phytochemical Screening

The phytochemical screening of the crude extracts was carried out to detect the presence or absence of some secondary metabolites. The methods by Harbone (1984) and Trease and Evans, (1987) were employed.

Culture Media

MacConkey agar, *Salmonella - Shigella* agar and nutrient agar were used as differential medium, selective medium and for susceptibility testing of the test organisms respectively, as described by Idu and Igekele, 2012.

Identification of the Test Organisms

The test organisms (*Salmonella typhi, Shigella flexneri, E.coli,* and *Proteus vulgaris*) were obtained from the stock culture in the Microbiology Laboratory, General Hospital, Minna, Niger State.

Antibacterial Assayof the Extracts

Well grown activated cultures were serially diluted in test tubes with normal saline until a cell concentration of 1.0 $x10^5$ cfu/ml was obtained. The antibacterial assay of the crude extracts was done using punch hole method described by Idu *et al.* (2012). The plates were prepared by dispensing 20ml of sterile molten nutrient agar into sterile Petri plates and allowed to set. A 4mm cork borer was used to punch holes in the medium. Four holes were made in each agar, adequately spaced out after inoculation (which was carried out by aseptically streaking the inoculums on the surface of the media with a wire loop). About zero point two milliliter (0.2ml) of the different concentrations was introduced into each well. The Petri plates were incubated at a temperature of 37°C for 24 hours, after which observed zones of inhibition were measured and the results recorded in comparison with the effect of the standard antibiotic (known as Ciprofloxacin) which was used as the control (Idu *et al.*, 2012).Only extracts that showed high antibacterial activity and served as potential source of drug development were used for the *in vivo* studies.

In vivo Antibacterial Activity of the Crude Extracts

Experimental Animals

Mice within the age of 8-12 weeks with body weight from 18-22 g were acquired from Ibrahim Badamosi Babangida University Lapai. The mice were kept in standard cages with adequate food, water and under hygienic conditions for 2 weeks before inoculation (Canadian Council on Animal Care, 1997).

Challenge Culture Preparation (Preparation of Inoculum)

A loopful of the organisms was inoculated on Salmonella-Shigella agar to activate the test organisms. The test organisms were further transferred into test tubes containing ten milliliters (10 ml) of sterilised nutrient broth and incubated at 37□ C for 18-24 hours. The activated culture was serially diluted in test tubes with normal saline until a cell concentration of 1.0×10^5cfu/ml was obtained (Eman and Hoda, 2008).

Inoculation of Test organisms and Administration of Plant Extracts and Antibiotic to Albino Mice

The mice were divided into 15 sub- groups, 5 each. In each sub-group, a specific volume of an inoculum (approximately 1ml of the infective dose of the inoculum) was introduced into each mouse intraperitoneally as prescribed by Eman *et al.* (2008). After the inoculation of the mice, administration of each extract (namely chloroform stem extract of *Euphorbia heterophylla*, aqueous stem extract of *Euphorbia heterophylla* and methanolic stem extract of *Euphorbia heterophylla*) and antibiotics (namely Ciprofloxacin) were done orally for seven (7) days (Itelima and Agina,2014). The mice were closely observed daily and the mortality rate and other physical manifestations were recorded.

Observation of Mortality Rate, Survival Rate and other Physical Manifestations

The mortality rate and survival rate of the mice in the sub groups were calculated as numbers of the mice that died and survived during the course of the experiment in relation to all the mice that were used (Eman *et al.*, 2008). The animals were observed to note the consistency, frequency and colour of their faecal waste. The mice were also observed for any abnormalities and physical manifestations (such as loss of appetite, loss of weight and body weakness) during the period of the experiment (Itelima *et al.*, 2014). At the end of the study, the infected mice were killed using chloroform and were buried, to prevent the spread of the infection associated with enteric pathogens in the environment (Itelima *et al.*, 2014).

Results

The phytochemical components of *Euphorbia heterophylla* were: starch, saponins, alkaloids, flavonoids and tannins in all the crude extracts. Other compounds such as steroids, phenolics and phlobatannins were present in only the methanolic and aqueous stem extract (Table 1).

Table 1: Results of phytochemical screening of *Euphorbia heterophylla*

Phytochemicals compounds	Stem			
	Chloroform	Methanol	Aqueous	Petroleum ether
Carbohydrates	+	+	+	–
Starch	+	+	+	+
Cardiac glycosides	+	+	–	–
Saponins	+	+	+	+
Steroids	–	+	+	–
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Phenolics	–	+	+	–
Tannins	+	+	+	+
Phlobatannins	–	+	+	–

Key: + = Presence of the phytochemical compound ; - = Absence of the phytochemical compound

Tables 2 - 5 revealed that EHMS (Methanolic stem extract of *Euphorbia heterophylla*) and EHAS (Aqueous stem extract of *Euphorbia heterophylla*) had significant antibacterial activity on all the organisms from 50 mg/ml to 200 mg/ml. EHCS (Chloroform stem extract of *Euphorbia heterophylla*) on the other hand, showed antibacterial activity from 100 mg/ml

to 200 mg/ml while EHPS (Petroleum ether stem extract of *Euphorbia heterophylla*) revealed significant activity on all the organisms at 200 mg/ml.

Table 2: Zones of inhibition of the stem extract of *E.heterophylla*at 50mg on test organisms

Extracts	*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}
EHMS	8.33 ± 0.67^{g}	6.33 ± 0.88^{f}	7.00 ± 1.15^{fg}	7.67 ± 0.33^{e}
EHAS	5.67 ± 1.20^{ef}	5.33 ± 0.67^{def}	6.00 ± 0.58^{ef}	5.33 ± 0.33^{e}
EHPS	3.33 ± 0.88^{bc}	$4.00\pm0..56^{cde}$	3.33 ± 0.33^{bc}	3.67 ± 0.33^{c}
Ciprofloxacin	9.00 ± 0.58^{g}	8.00 ± 0.57^{g}	8.67 ± 0.68^{g}	8.67 ± 0.33^{e}

Table 3: Zones of inhibition of the stem extract of *E.heterophylla* at 100mg on test organisms

Extracts	*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}
EHMS	10.67 ± 1.21^{g}	10.33 ± 0.90^{h}	4.67 ± 1.40^{bc}	9.67 ± 1.67^{efg}
EHAS	9.67 ± 1.22^{efg}	8.67 ± 0.86^{fgh}	9.67 ± 1.20^{ef}	10.33 ± 0.33^{fg}
EHPS	7.33 ± 0.67^{cd}	6.00 ± 0.60^{bcd}	7.00 ± 0.58^{cde}	7.33 ± 0.33^{cde}
Ciprofloxacin	15.00 ± 0.60^{h}	13.33 ± 0.90^{i}	13.33 ± 1.45^{f}	12.33 ± 1.45^{g}

Table 4: Zones of inhibition of the stem extract of *E.heterophylla* at 150mg on test organisms

Extracts	*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}
EHMS	14.00 ± 0.60^{e}	12.00 ± 1.15^{f}	12.33 ± 1.45^{f}	12.00 ± 1.00^{e}
EHAS	12.33 ± 0.90^{dc}	11.33 ± 0.33^{e}	11.67 ± 1.20^{ef}	11.00 ± 1.00^{de}
EHPS	8.33 ± 0.88^{c}	8.67 ± 0.30^{cd}	8.67 ± 0.70^{cd}	7.67 ± 0.90^{c}
Ciprofloxacin	20.00 ± 0.60^{f}	18.67 ± 0.70^{g}	19.33 ± 0.33^{g}	19.00 ± 0.58^{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: Zones of Inhibition of the stem extract of *E.heterophylla*at 200mg on test organisms

Extracts	*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}
EHMS	16.33 ± 0.33^{f}	15.33 ± 0.33^{f}	15.67 ± 0.90^{e}	15.33 ± 1.20^{f}
EHAS	14.67 ± 0.33^{e}	14.00 ± 0.60^{f}	14.33 ± 0.33^{e}	14.00 ± 0.00^{f}
EHPS	10.33 ± 0.33^{cd}	9.00 ± 0.60^{cd}	9.67 ± 0.30^{bc}	8.67 ± 0.31^{cde}
Ciprofloxacin	26.00 ± 0.60^{h}	25.00 ± 0.70^{g}	25.33 ± 0.33^{f}	24.33 ± 0.33^{g}

Table 6 reveals the mortality rate and physical changes due to the crude extracts on mice at 2000 mg/kgbw after infection with *S.typhi*, *S.flexneri, E.coli* and *P.vulgaris*. At the end of the 7days treatment with the various extracts only 9 mice had diarrhoea, 13 experienced weight loss and 13 experienced loss of appetite.

Table 6: Effects of the methanolic, aqueous and chloroform stem extracts on the mice infected with *S.typhi*, *S.flexneri*, *E.coli* and *P.vulgaris*

Group	No of Mice	Mortality rate (%)	W.D (%) 1-3 Days	W.D (%) 4-6 Days	W.D (%) >7days	Loss of appetite (%)>7days	Loss of weight (%) >7days	Body weakness/ Slow movement (%) >7days
S. typhi-I	5	0/5 (0%)	2/5 (40%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
S. typhi-II	5	0/5 (0%)	3/5 (60%)	1/5 (20%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
S. typhi-III	5	0/5 (0%)	3/5 (60%)	2/5 (40%)	1/5 (20%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
S.flexneri-IV	5	0/5(0%)	2/5 (40%)	1/5(20%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
S.flexneri-V	5	0/5 (0%)	5/5 (100%)	2/5 (40%)	0/5 (0%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
S.flexneri-VI	5	0/5 (0%)	5/5 (100%)	3/5 (60%)	1/5 (20%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
E.coli-VII	5	0/5(0%)	3/5 (60%)	1/5(20%)	0/5(0%)	0/5(0%)	0/5 (0%)	0/5(0%)
E.coli-VIII	5	0/5 (0%)	5/5 (100%)	2/5 (40%)	0/5 (0%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
E.coli-IX	5	0/5 (0%)	5/5 (100%)	2/5 (40%)	1/5 (20%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
P. vulgaris-X	5	0/5(0%)	3/5 (60%)	1/5(20%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
P. vulgaris-XI	5	0/5 (0%)	5/5 (100%)	2/5 (40%)	0/5 (0%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
P. vulgaris-XII	5	0/5 (0%)	5/5 (100%)	2/5 (40%)	1/5 (20%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
XIII	5	0/5 (0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
XIV	5	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
XV	5	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)

Key: Group I= Mice infected with *S.typhi*and treated with EHMS; II= Mice infected with *S.typhi* and treated with EHAS: III= Mice infected with *S.typhi*andtreated with EHCS; IV = Mice infected with *S.flexneri*and treated with EHMS ; V = Mice infected with *S.flexneri*and treated with EHAS; VI= Mice infected with *S.flexneri*andtreated with EHCS; VII= Mice infected with *E.coli* and treated with EHMS; VIII= Mice infected with *E.coli* andtreated with EHAS; IX= Mice infected with *E.coli* andtreated with EHCS; X= Mice infected with *P.vulgaris*andtreated with EHMS; XI= Mice infected with *P.vulgaris*andtreated with EHAS ; XII= Mice infected with *E.coli* andtreated with EHCS; XIII=Mice infected and treated with Ciprofloxacin; XIV = Mice infected and not treated; XV= Mice not infected and not treated; W.D= Watery diarrheoa.

Discussion.

The bioactive substances in plants are produced as secondary metabolites, which may not only be developmental stage specific but also organ and tissue specific. The findings of this study revealed that the chloroform, methanolic, aqueous and petroleum ether stem extracts of *Euphorbia heterophylla* had various bioactive compounds which are well known for their therapeutic abilities and could be used in the synthesis of very useful drugs (Yakubu *et al.*, 2005: Oyedum, 2015). This study therefore reveals that the stem extract of *Euphorbia heterophylla* contains starch, saponins, alkaloids, flavonoids and tannins (Table 1). Other phytochemical components such as steroids, phenolics and phlobatannins were also detected in the crude stem extracts (Table.1). The findings agree with the previous reports by Sundaram *et al.* (2010) and Okeniyi *et al.* (2012). Phytochemical compounds such as flavonoids and tannins observed in the stem of this plant are said to have inhibitory effects on some gram-negative bacteria, most especially inhibiting the synthesis of bacterial cell wall (Jimoh *et al.*, 2005; Daniyan *et al.*, 2011). In addition to this, the flavonoids are also reported to have strong antioxidant effects (Nakayama *et al.*, 1993; Pari *et al.*, 2004). In the same vein, other observed phytochemical compounds in this study, such as saponins and alkaloids are known to have immune modulation activities (Plohmann *et al.*,1997) and antibacterial activities (Itelima *et al.*, 2014). The diverse active compounds present in the stem of *E. heterophylla*, are therefore said to enhance both the pharmacological and antibacterial activities of this plant on various bacteria.

The antibacterial activity of the stem on the various test organisms was observed to be significant, indicating that the stem is highly potent (Table 2). This may be due to the fact that the stem of *E. heterophylla* is adequately developed and mature and may contain fewer pigments or other phenolics, which have been reported to interfere with the antimicrobial activities of most stem or leaf extracts of other plants (Doughari, 2006).

In addition to this, among all the four solvents used, the methanol extract of the stem, showed the highest antibacterial activity at a concentration as low as 50mg/ml (Table 2). This could be attributed to the fact that methanol, as a solvent is regarded as an alcoholic derivative; thus, it is said to extract more bioactive components than other solvents (El-Mahmood, Doughari and Ladan, 2005). In the same vein, all the identified components from plants active against microorganisms are aromatic or saturated organic compounds, and they are most often obtained through initial ethanol or methanol extraction (Eloff, 1998; Cowan, 1999). Similarly, the different results observed among the various chemical solvents used in this study (Table 2) could be attributed to the different variations in the polarity of the solvents and solubility of the bioactive compounds of the stem of *E. heterophylla* in different solvents as reported by Elmahood *et al.* (2005).

The antibacterial activities of chloroform, methanolic and aqueous crude extracts of *E. heterophylla* at 100 mg/ml were significant on all the test organisms, compared to the effects of petroleum ether extract of the stem on all the test organisms(Table 3). This result obtained could be due to the fact that the active components in 100mg/ml of petroleum ether crude extract are insufficient to exhibit significant antibacterial activities as reported by Mbata and Salkia,(2008). Thus, enhancing the various test organisms to develop resistance to the extract (Oyedum, 2015).

However the antibacterial activities of petroleum ether crude extract of the stemof *E. heterophylla* was significant at 150 mg/ml and 200 mg/ml on all the test organisms as it was also observed in the case of the other crude extracts of the stem of *E. heterophylla* from other solvents at same concentrations of 150mg/ml and 200 mg/ml, which also revealed higher antibacterial activities compared to the antibacterial activities at 50 mg/ml and100 mg/ml (Table 4 and 5). The high antibacterial activities observed at 150 mg/ml and 200 mg/ml could be due to the enhanced effect of the stem extracts based on the increased concentration of the individual extract, which are said to contain more phytochemical constituents. The outcome of this agrees with the result obtained in a study by Ahmed *et al.* (2012).

The *In vivo* determination of the efficacy of methanolic, aqueous and chloroform stem extracts of *E.hetrophylla* in mice infected with all the test organismsas seen in Table 4.6 revealed that the extracts showed significant antibacterial effects upon administration for the treatment of the infected mice. The extent of the extract's activities on the treated mice was dose and time- dependent; and daily observation of the mice also revealed that after seven days most clinical and physical symptoms observed in the mice few days after inoculation disappeared. However, with respect to mice infected with the different test organisms and treated with aqueous extracts of the stem of *E. heterophylla*, the result revealed that 40% of the mice showed pathological symptoms of watery diarrhoea as compared to its methanolic counterpart between the 4^{th} to 6^{th} days of treatment with the stem extract of *E. heterophylla*. This could be as a result of low concentration of the bioactive components present in the aqueous dose administered for the treatment of the infected mice which is insufficient compared to the level of infection in the mice, thus, rendering the mode of action and potency of the aqueous extract slow. Similarly, the observation that 20% of the mice treated with aqueous and chloroform extracts of the stem of *E.heterophylla,* showed loss of appetite, loss of weight and body weakness/slow movementafter 7 days, could be based on the fact that the mice are experiencing some side effects due to the presence of some foreign chemical component in them, as reported by Arsad *et al.*(2013) .

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 is potent and contains

therapeutic properties. However, the *In vivo* studies revealed that only the methanolic and aqueous extract of the leaf had significant therapeutic effect on the infected mice. It is therefore recommended that adequate administration of extracts should be time and dose- dependent to ensure complete eradication of the pathogenic organisms and prevent development of resistant genes.

References

Ahmed, R.N., Abdulrahaman, A.A. and A.Sani (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 F. Othman (2013). Evaluation of acute, subacute and subchronicoraltoxicity of *Rhaphidophora decursiva* (Roxb.)Schott in male Sprague Dawley rats.*Journal of Medicinal Plant Research*, 7(41): 3030-3040.

Canadian Council on Animal Care (CCAC) (1997). Guidelines on animal use protocol review.Pp.1-5.

Cowan, M.M. (`1999). Plant products as antimicrobial agents. *Clin Microbiol Rev.*, 12: 564-582.

Daniyan, S. Y., Abalaka, M. E., Garba, S. A and S. Adeyemo (2011). Investigation into the antimicrobial properties of *Euphorbia heterophylla* on typhoid disease causative agents.*International Journal of Research in Ayurveda and Pharmacy,* 2(4): 1214-1217.

Doughari, J. H. (2006). Antimicrobial Activity of *Tamarindusindica*Linn.*Tropical Journal of Pharmaceutical Research*, 5 (2): 597-603

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

Eloff, J N.(1998). Which extractant should be used for the screening and isolation of antimicrobial compounds from plants? *Journal of Ethnopharmacology*, 60: 1-8.

EL- Faraley, Y. S., Cheatham, S. F. and R. L.Breelove, (1983). Antimicrobial neolignan of *Sassafras ranaiense*root.*Lloydia*, 46 (4): 493-497.

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

Eman, M.A. and M. Z. Hoda (2008). Studies on the effect of garlic preparation on *Escherichia coli O157:H7* causing enteritis in lambs. *Egyptian Journal of Clinical Pathology,* 21(4): 102-129.

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

Falodun, A. and E. O. P.Agbakwuru (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 N. Uzoamaka (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 K. Hostettmann (1991). Bioactivity in plants: the link between phytochemistryand 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 K. Yamasak (1995). Reversal of efflux mediated tetracycline resistance in *Staphylococcus aureus* clinical isolates by Ginseng prosaponenins. *Phytotherapy. Resource*,9: 260-263.

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

Itelima, J. U. and S. E. Agina (2014). In vivo antimicrobial activity of plant species on *Escherichia coli O157:H7* inoculated into albino rats. *World Journal of Microbiology*, 1(1): 002-009.

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

Jimoh, F. O. and A. T. Oladigi (2005). Preliminary studies on Pilostigmathonninggii seeds: Proximate analysis, mineral composition and phytochemical screening. *African J. Biotech.* 4(12):1439-1442.

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

Mbata, T.I., and A. Salkia (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.

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

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.

Nakayama, N. G., Lindsey, M. L. and L. H. Michael, (1993).Inhibition of infectivity of influenza virus by tea polyphenoids.*Antiviral Resource*, 21: 289-299.

Okeniyi, S.O., Adedoyin, B.J. and S. Garba (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

Vitellariaparadoxa against*S.typhi*and *S.flexneri*.. An unpublished M.tech thesis, Federal University of Technology, Minna, Niger State, Nigeria, pp. 56-59.

Pari, L. and M. Latha (2004). Effect of Scopariadulcis (Sweet Broomweed) plant extract on plasma antioxidants in STZ-induced experimental diabetis in male albino rats. Die Pharmazie, 59: 557-560.

Plohmann, B., Bader, G., Hiller, K. and G. Franz (1997). Immunomodulatory and antitumoral effects of triterpenoid saponins.Pharmazie, 52: 955-957.

Saimo, M.K., Bizimenyera, E.S., Bwanika, A., Ssebuguzi, F., Weny, G. and G. W. Lubega (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.

Sundaram, M.M., Karthikeyan, K., Sudarsanam, D. and P. Brindha(2010). Antimicrobial and anticancer studies on *Euphorbia heterophylla*, *Journal of Pharmacy Research*, 3(9): 2332-2333.

Trease,G.E. and W. C. Evans (1984). A Text Book of Pharmacognosy.ELSB/ Bailliere Tindal, Oxoford, UK. 1055.

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

Yakubu, M.T., Akanji, M.A. and A. T. Oladiji (2005). Aphrodisiac potentials of the aqueous extract of Fadogiaagrestis (Schweinf.Ex Hiern) stem in male albino rats.*Asian J. Androl.* 7(4): 399-404.