Journal of Natural and Applied Sciences, Vol. 7 No. 1 December, 2018

PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES OF ROOT EXTRACTS OF *EUPHORBIA HETEROPHYLLA* ON SOME ENTERIC BACTERIA

Oyedum, U.M., Kuta, F.A. and Adabara, N.U.
Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna
Corresponding author: hemdi41@gmail.com

Abstract

Phytochemical components and antibacterial activity of *Euphorbia heterophylla* crude extracts on four enteric organisms namely: *Salmonella typhi, Shigella flexneri, E.coli* and *Proteus vulgaris* were determined. The clinical isolates of *Salmonella typhi, Shigella flexneri, E.coli* and *Proteus vulgaris* were subjected to antimicrobial susceptibility test, using agar diffusion technique. Phytochemistry of the *Euphorbia heterophylla*, crude extracts revealed the presence of flavonoids, alkaloids, saponins, tannins and cardiac glycosides. Methanolic and aqueous crude extract produced clear zones of inhibition at concentration ranging from 50 to 200 mg/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 minute pathological effects. On the other hand, untreated mice (control) died after 48hours of inoculation with *Salmonella typhi, Shigella flexneri, E.coli* and *Proteus vulgaris. Euphorbia heterophylla* crude extracts, most especially its methanolic and aqueous fractions, 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 toward isolation and characterization of the active compounds in the crude extracts.

Keywords: Phytochemicals, *in vitro* activity, *in vivo* activity, *Euphorbia heterophylla*, Enteric bacteria

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, apart from 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, while others cause signs of infection such as vomiting, diarrhoea, and related symptoms (Murray, 1994).

Plants have served as sources of drugs and pharmaceuticals for man and other animals from time immemorial. 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). The ability of plants to produce many phytochemicals that are used to perform important biological functions is one of the many characteristics they possess. According to an earlier survey, about 25% of modern drugs and medicinal products are derived from plant secondary metabolites (Hamburger & Hostettmann, 1991). Many of these phytochemicals have beneficial effects on long-term health of humans and animals when consumed, and can be used to effectively treat human diseases (Ehrlich, 2013). In the same vein 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). Also, these substances can either inhibit the growth of pathogens or kill them, and have little or no toxicity to host the cells; and in most cases they are considered as potential candidates for developing new antimicrobial drugs (Kunle *et al.*, 2012; Oyedum, 2015).

Euphorbia heterophylla is one of the numerous plants found in the field. *Euphorbia heterophylla* grows in disturbed localities as a weed of cultivation and wasteland, 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). The presence of latex in this plant is one of the main reasons, it is considered to be a toxic plant. In spite of its toxicity, it is also known to posses numerous medicinal properties. *Euphorbia heterophylla* is widely used in traditional African medicine and elsewhere in tropical countries.

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

In East Africa, the roots are used in the treatment of gonorrhoea or to increase milk production in breast-feeding women (Sundaram *et al.*, 2010). In Nigeria, the latex and preparations of the roots are applied to treat skin tumours (Falodun & Agbakwuru, 2004). A methanol extract of the aerial parts showed moderate antiplasmodial activity. A leaf extract showed significant nematicidal activity against *Meloidogyne graminicola* (Mosango, 2008). An extract of the aerial parts given orally to goats showed moderate activity against several intestinal nematodes such as *Haemonchus*, *Trichostrongylus*, *Bunostomum* and *Oesophagostomum*.

However, despite the antibacterial activity of the leaves of this plant against various bacteria, it is also observed that pharmacological studies of other parts of the plant are few. It is therefore imperative to further evaluate the chloroform, aqueous, methanolic and petroleum ether extracts of the root 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 root were collected from Garatu in Bosso Local Government Area of Niger State, Nigeria. The geographic location of 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 roots were thoroughly washed, air-dried at room temperature (25°C) for 3-5 days, and ground into coarse powder using a sterile mortar and pestle. The dried plant parts were 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. The macerated samples were sieved with muslin cloth and the filtrate 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 (1983), were employed.

Culture Media

A measure of 6.3 g of MacConkey and *Salmonella-Shigella* agars were dissolved in one hundred milliliters (100 ml) of water, through heating and were used as both differential and selective media for the confirmation of the test organisms. Then, 2.8 g of nutrient agar dissolved in one hundred milliliters (100 ml) of water was sterilized and used for susceptibility testing (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. The isolates were identified using the method recommended by Cheesbrough (2006).

Antibacterial Assay of the Extracts

The antibacterial assays of the crude extracts were done, using punch hole method, described by Idu & Igekele (2012). The plates were prepared by dispensing 20 ml of nutrient agar into sterile Petri dishes and allowed to set. A 4mm cork borer was used to punch holes in the medium. Four holes were made on each Petri dish, adequately spaced out after inoculation. About 0.2 ml, of the different concentrations of extracts (namely 50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml) was introduced into each well. The Petri dishes were incubated at a temperature of 37°C for 24 hours, after which observation for the zones of inhibition were made; measurement of the zones of inhibition were carried out and the results recorded, in comparison with the effect of the standard antibiotic (namely ciprofloxacin) as the control (Idu & Igekele, 2012). Only extracts that showed high antibacterial activity (such as 5.0 mm and above at 200 mg/ml) 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

Albino 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 grouped and kept in standard cages with adequate food (a combination of both cereal and soya grain products), 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 each 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 1.0×10^5 cfu/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, each of 5 replicates (n=5). In each particular 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 & Hoda. (2008). After the inoculation of the mice, administration of each extract and antibiotics was done orally, according to the procedure of Itelima and Agina (2014).

Determination of Mortality Rate, Survival Rate and Observation of Pathological Manifestations

The mortality rate and survival rate of the mice in the sub- groups were calculated, as numbers of the mice that died or survived during the course of the experiment, in relation to all the mice that were used (Eman & Hoda, 2008). The animals were observed for the consistency, frequency and colour of their faecal matter (and any faecal matter that was loosely formed, consistent and frequent was recorded as watery diarrhoea). The mice were also observed for any abnormalities and pathological manifestations (such as loss of appeptite, loss of weight and body weakness) using a laboratory weighing balance, during the period of the experiment (Itelima & Agina, 2014). At the end of the experiment, which was after the seventh (7^{th}) day, the infected mice were killed, using chloroform to prevent the spread of the infection associated with enteric pathogens in the environment (Itelima & Agina, 2014).

Results

The common phytochemical components of *Euphorbia heterophylla* include: cardiac glycosides, saponins, alkaloids, flavonoids and tannins in all the crude extracts. Other compounds such as carbohydrates, starch and steroids were present in only the methanolic and aqueous leaf extracts, while phenolics and phlobatannins were detected in only methanolic and petroleum ether extracts of the root (Table 1).

Table 1: Phytochemical constituents of *Euphorbia heterophylla*

PHYTOCHEMICAL COMPOUNDS	Root			
	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 reveal that EHMR and EHAR had significant antibacterial activity on all the organisms from 50 mg to 200 mg. EHCR on the other hand, showed antibacterial activity from 100 mg to 200 mg, while EHPR revealed significant activity on all the organisms at 200 mg.

Table 2 reveals that EHMR and EHAR had significant antibacterial activity on all the organisms from 50 mg to 200mg, while EHPR revealed significant activity on all the organisms at 200mg.

Table 2: Zones of Inhibition of the root of *E.heterophylla* at 50mg

Extracts	*S.typhi*	*S.flexneri*	*E.coli*	*P. vulgaris*
EHCR	2.00 ± 0.58^{b}	1.67 ± 0.33^{b}	1.67 ± 0.33^{ab}	2.00 ± 0.58^{b}
EHMR	4.33 ± 0.33^{cdef}	4.00 ± 0.58^{cde}	4.67 ± 0.33^{de}	3.67 ± 0.33^{c}
EHAR	3.67 ± 0.33^{bcd}	3.67 ± 0.67^{cd}	5.00 ± 0.58^{de}	3.67 ± 0.66^{c}
EHPR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
CONTROL	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 root of *E.heterophylla* at 100mg

Exracts	*S. typhi*	*S.flexneri*	*E.coli*	*P. vulgaris*
EHCR	4.00 ± 0.60^{b}	4.33 ± 0.33^{b}	5.00 ± 0.00^{bcd}	3.67 ± 0.33^{b}
EHMR	7.67 ± 0.30^{cde}	7.33 ± 0.33^{defg}	6.33 ± 1.76^{cde}	6.67 ± 1.20^{cd}
EHAR	6.67 ± 0.70^{cd}	6.67 ± 0.30^{cdef}	7.00 ± 00^{cde}	6.33 ± 0.90^{bc}
EHPR	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.00 ± 0.00^{ab}	0.00 ± 0.00^{a}
CONTROL	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 root *E.heterophylla* at 150mg

Extracts	*S. typhi*	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCR	5.33±0.70^{b}	4.67±0.28^{b}	5.00±0.60^{b}	4.33±0.90^{b}
EHMR	8.33±0.90^{c}	8.00±0.60^{c}	8.33±0.88^{c}	7.67±0.90^{c}
EHAR	8.67±0.30^{c}	7.67±0.90^{c}	8.33±0.33^{c}	8.33±0.30^{c}
EHPR	2.67±0.30^{a}	1.67±0.90^{a}	2.00±0.30^{a}	1.33±0.33^{a}
CONTROL	20.00±0.60^{f}	18.67±0.70^{g}	19.33±0.33^{g}	19.00±0.58^{f}

Key: EHCR---Chloroform root extract of *Euphorbia heterophylla*; EHMR---Methanolic root extract of *Euphorbia heterophylla* ; EHAR--- Aqueous root extract of *Euphorbia heterophylla* ; EHPR---- Petroleum ether root extract of *Euphorbia heterophylla* ; Control---

Table 5: Zones of Inhibition of the root *E.heterophylla* at 200mg

Extracts	*S. typhi*	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCR	8.33±0.33^{b}	7.00±0.60^{b}	8.33±0.33^{b}	7.33±0.67^{bc}
EHMR	11.00±00de	10.33±0.33^{de}	10.67±0.70^{cd}	9.67±0.30^{de}
EHAR	11.00±0.00^{de}	10.33±0.33^{de}	10.67±0.30^{cd}	10.00±0.60^{de}
EHPR	3.67±0.30^{a}	2.67±0.70^{a}	3.33±0.67^{a}	3.00±0.60^{a}
CONTRO	26.00±0.60^{h}	25.00±0.70^{g}	25.33±0.33^{f}	24.33±0.33^{g}

Values are presented as Mean±Standard Error of Mean of triplicate determinations. Values in a column with different alphabet are significantly different ($p < 0.05$)

Table 6 reveals the mortality rate and pathological effects of the crude extracts on mice at 2000mg/kgbw after infection with *S.typhi, S.flexneri, E.coli, P.vulgaris.*

At the end of the 7days treatment with the various extracts; only 12 mice had diarrhoea, 17 experienced weight loss and 17 experienced loss of appetite.

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

Sub-group	No of Mice	Dilution Factor	Mortality rate (%) 1-7days	W.D (%) 1-3 Days	W.D (%) 4-6 Days	W.D (%) at 7days	Loss of appetite (%) at 7days	Loss of weight (%) at 7days	Body weakness/ Slow movement (%) at 7days
S. typhi-I	5	10^{-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	10^{-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	10^{-5}	0/5(0)	5/5(100)	3/5(60)	1/5(20)	2/5(40)	2/5(40)	2/5(40)
S.flexneri-IV	5	10^{-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	10^{-5}	0/5(0)	5/5(100)	2/5 (40)	1/5(20)	1/5(20)	1/5(20)	1/5(20)
S.flexneri-VI	5	10^{-5}	0/5(0)	5/5(100)	3/5 (60)	1/5(20)	2/5(40)	2/5(40)	2/5(40)
E.coli-VII	5	10^{-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	10^{-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	10^{-5}	0/5(0)	5/5(100)	2/5 (40)	2/5(40)	2/5(40)	2/5(40)	2/5(40)
P.vulgaris-X	5	10^{-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	10^{-5}	0/5(0)	5/5(100)	2/5 (40)	1/5(20)	1/5(20)	1/5(20)	1/5(20)
P.vulgaris-XII	5	10^{-5}	0/5(0)	5/5(100)	2/5 (40)	1/5(20)	2/5(40)	2/5(40)	2/5(40)
XIII	5	10^{-5}	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)
XIV	5	10^{-5}	5/5(0)	5/5(100)	5/5 (100)	5/5(100)	5/5(100)	5/5(100)	5/5(100)
XV	5	10^{-5}	0/5(0)	0/5 (0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)

Key: Sub-group I= Infected with *S.typhi* and treated with EHMR; II= Infected with *S.typhi* and treated with EHAR: III= Infected with *S.typhi* and treated with EHCR; IV = Infected with *S.flexneri* and treated with EHMR ; V = Infected with *S.flexneri* and treated with EHAR; VI= Infected with *S.flexneri* and treated with EHCR; VII= Infected with *E.coli* and treated with EHMR; VIII= Infected with *E.coli* and treated with EHAR; IX= Infected with *E.coli* and treated with EHCR; X= Infected with *P.vulgaris* and treated with EHMR; XI= Infected with *P.vulgaris* and treated with EHAR ; XII= Infected with *E.coli* and treated with EHCR; XIII=Treated with Ciprofloxacin; XIV = Infected but not treated; XV= Not infected not treated; W.D= Watery diarrhoea.

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. Table 1 shows that the chloroform, methanolic, aqueous and petroleum ether root 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 (Oyedum, 2015; Yakubu *et al.*, 2005). This study therefore reveals that the root extract of *Euphorbia heterophylla* contains cardiac glycosides, saponins, alkaloids, flavonoids and tannins. Phytochemical components such as carbohydrates, starch and steroids were also detected in the crude root extracts. The findings agree with the previous reports by Sundaram *et al.* (2010) and Okeniyi et *al.* (2012). Some of these chemical compounds, like the flavonoids and tannins, have been shown to have inhibitory effect on some Gram-negative bacteria, most especially inhibiting the synthesis of bacterial cell wall (Jimoh & Oladigi, 2005; Daniyan *et al.*, 2011). The flavonoids are also reported to have strong antioxidant effects (Nakayama *et al.*, 1993; Pari & Latha, 2004) while the saponins are known to have immune modulation activities (Plohmann *et al.*, 1997). Based on these chemical compounds present in the root, *E. heterophylla* is said to possess both pharmacological and antibacterial activities.

The antibacterial activity of the root revealed that the root shows significant antibacterial activity on the various test organisms which are mainly Gram-negative bacteria, indicating that the root extract is highly potent (Table 2). This result, however, is contrary to an earlier report indicating that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria (Jigna and Sumitra, 2006). There may be several factors that will predispose bacteria to antibacterial agents, such as previous encounters with the agents or the nature of the medium used, which may affect the diffusability of the agent (Doughari *et al.*, 2007). In addition to this, the root may contain fewer pigments and other phenolics which have been reported to interfere with the antimicrobial activities of most extracts (Doughari, 2006).

Among all the four solvents used, the methanol extract showed the highest antibacterial activity at a concentration as low as 50 mg/ml (Table 2). This could be attributed to the fact that methanol is an alcohol; thus, it is said to extract more bioactive components than other solvents (El-Mahmood *et al.*, 2005). Most of all the identified components from plants that are 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 differences in polarity of the solvents and solubility of the bioactive compounds in plants, as reported by El-Mahood *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 extracts of the root on all the test organisms (Table 3). This could be due to the fact that the active components in 100 mg/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' development of resistance to the extract (Oyedum, 2015).

However, the antibacterial activities of petroleum ether crude extract of the root of *E. heterophylla* showed some antibacterial activity at 150 mg/ml and 200 mg/ml on all the test organisms, as was also observed in the case of the other crude extracts of the root of *E. heterophylla* from other solvents at same concentrations of 150mg/ml and 200mg/ml, which also revealed higher antibacterial activities compared to the antibacterial activities at 50 mg/ml and100 mg/ml (Tables 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 root 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 and aqueous root extracts of *E.hetrophylla* in mice infected with all the test organisms, as seen in Table 6, revealed that the extract showed significant antibacterial effect 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 signs 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 root of *E. heterophylla*, 20% of the mice showed signs of watery diarrhea. 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, and thus rendering the mode of action and potency of the aqueous extract slow. Similarly, the observation that 20% of the mice treated with aqueous extracts of the root of *E.heterophylla* showed loss of appetite, loss of weight and body weakness/slow movement after 7 days, could be

based on the fact that the mice were experiencing some side effects due to the presence of some foreign chemical component in them (Arsad *et al.*, 2013).

Conclusion

The methanolic, chloroform and aqueous extracts of the root of *E. heterophylla* contained phytochemicals that were active against all test organisms at a concentration as low as 50 milligram, indicating that the root is potent and contains therapeutic properties. However, the *in vivo* studies revealed that only the methanolic, chloroform and aqueous extract of the stem 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. & Sani, A. (2012). *In vitro* 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. & Othman, F. (2013). Evaluation of acute, subacute and subchronic oral toxicity 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.

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

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

Daniyan, S.Y., Abalaka, M.E., Garba, S.A & Adeyemo, S. (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., Elmahmood, A. M. and Manzara, S. (2007) Studies on the antibacterial activity of root extracts of Carica papaya L. *African Journal of Microbiology Research*, 1(1),037-041.

Doughari, J.H. (2006). Antimicrobial activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research*, 5 (2), 597-603

Ehrlich, S. D. (2013). Herbal Medicine. Review provided by VeriMed Herbal Network. pp1-5.

El-Mahmood, A. M., Doughari, J. H. & Ladan, N. (2005). Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some

enteric pathogenic microorganisms..*African Journal of Pharmacy and Pharmacology*, 2(5),089-094.

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.

Eman, M.A. & Hoda, M.Z. (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. & Yoshiohiro, T. (1999) Traditional medicine in Turkey IX, folk medicine in NorthWest Anatolia. *Journal of Ethnopharmacology*, 64, 201.

Falodun, A., Okunrobo, L. O. & 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.

Falodun, A. & 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.

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

Idu, M. & Igekele, C.L. (2012).Antimicrobial activity and phytochemistry of *Khaya senegalensis* root. *International Journal of Ayurvedic and Herb Medicine*, 2(3), 416-422. *Internet Journal of Microbiology*, 4, 2.

Itelima, J. U. and Agina, S. E. (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). Thesis on comparative antimicrobial activity of crude extract of Terminalia Macroptae with phenol chlorhexidine and gentamycin. *Pharmacognosy Journal*,1(1),12.

Jigna, P. & Sumitra, C. (2006).In-vitro antimicrobial activities of extracts of *Launaea procumbns* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L.(Cyperaceae). *Afr. J. Biomed,Res.*, 9(2),89-93.

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

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

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

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.), pp.227-240, Mosby Year Book Inc., ISBN 0723420106, London, UK.

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

Okeniyi, S.O., Adedoyin, B.J. & 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. M.tech thesis, Federal University of Technology, Minna, Niger State, pp. 56-59.

Pari, L. & Latha, M (2004). Effect of Scoparia dulcis (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. & Franz, G. (1997). Immunomodulatory and antitumoral effects of triterpenoid saponins. *Pharmazie*, 52, 955-957.

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

Trease,G.E. & Evans,W.C.(1983).A Textbook of Pharmacognosy. ELSB/ Bailliere Tindal, Oxoford, UK, pp. 1055.

Unekwe, P .C., Ughachukwu, P. O. & Ogamba, J.O. (2006). Some