World Journal of Microbiology
Vol 4(1), pp 072-078, April, 2017. © www.premierpublishers.org ISSN: 2141-5032

WJM

Research Article

Invitro and *Invivo* antibacterial study of leaf extracts of *Euphorbia heterophylla* on some enteric bacteria

Oyedum Uche Mary

Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna.
Email of corresponding author: hemdi41@gmail.com

A study was carried out to determine the phytochemical components and 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 *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 and cardiac glycosides. Methanolic and aqueous crude extract produced clear zones of inhibition at concentration 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 minute pathological effects. Similarly, untreated mice (control) died after 48hours of inoculation with *Salmonella typhi*, *Shigella flexneri*, *E.coli* and *Proteus vulgaris*. *Euphorbia heterophylla* crude extract, most especially its methanolic and aqueous counterpart could be a potential source for the treatment of diseases associated with enteric organisms. Further studies should be directed towards isolation and characterization of the active compound in the crude extracts.

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

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).

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 (Sudipta *et al.*, 2012, Swamy *et al.*, 2015a and, 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 (Arumugam *et al.*, 2016). 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). 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 (Kunle *et al.*, 2012; Oyedum *et al.*, 2015).

Euphorbia heterophylla is one of the numerous plants usually found in the field is said to grow 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). 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 properties, it is also known to posses numerous medicinal properties too. *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 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 fresh or dried leaves 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 leaf infusion is used to treat skin problems, including fungal diseases, and abscesses. In Nigeria the latex and preparations of the leaves are applied to treat skin tumours (Falodun *et al.*, 2004 ; Sundaram *et al.*, 2010).

The butanol extract of the dried leaves exhibited marked inhibitory action on the growth of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* at 100 mg/ml (Mosango, 2008). 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 reports of the leaves of this plant against various bacteria, it is also observed that pharmacological studies of this plant are few. It is therefore imperative to further evaluate the chloroform, aqueous, methanolic and petroleum ether extract of the leaves of *E.heterophylla* against some enteric organisms namely: *Salmonella typhi*, *Shigella, flexneri*, *E.coli* and *Proteus vulgaris*, based on the fact that most of these organisms have become resistant to available synthetic drugs, and this in turn, have led to a global threat (Rudramurthy *et al.*, 2016).

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh samples of the leaves were collected from Garatu in Bosso Local Government Area of Niger State, Nigeria. Garatu is geographically located 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 leaves were thoroughly washed, air dried at room temperature (28°C) 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

100 g of the ground part was macerated successively for three days (with occasional shaking) using cold maceration technique. 1000 ml of distilled water, methanol, and 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

6.3 g of MacConkey and *Salmonella Shigella* agar were dissolved in 100 ml of water through heating and were used as both differential and selective media for the confirmation of the test organisms. 2.8g of nutrient agar dissolved in 100 ml of water was sterilized and used for susceptibility testing (Idu *et al.*, 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 district laboratory practice for tropical countries by Cheesbrough (2006) as a guide.

Bacterial Assay of the Extracts

Well grown activated cultures were serially diluted in test tubes with normal saline until a cell concentration of 1.0×10^5 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 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 on each Petri plate, adequately spaced out after inoculation. About 0.2 ml 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 observation for the zones of inhibition were conducted, measurement of the zones of inhibition were carried out and the results recorded in comparison with the effect of the standard antibiotic used as a control in this study (which was Ciprofloxacin) (Idu *et al.*,2012).

***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 1.0×10^5 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 11 sub- groups, each of 5 replicates (n=5). In each particular sub-group, a specific volume of an inoculum (approximately 1ml of the infective doses of the inoculum) was introduced into each mouse intraperitoneally as prescribed by Eman *et al.* (2008). After the innoculation of the mice, administration of each extract and antibiotics were done orally according to the procedure of Itelima and Agina, (2014).

Observation of Mortality Rate, Survival Rate and other Pathological 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 pathological 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 to prevent the spread of the infection associated with enteric pathogens in the environment (Itelima *et al.*, 2014).

Statistical Analysis

The result would be analysed statistically using ANOVA

RESULTS

The common phytochemical components of *Euphorbia heterophylla* include: saponins, steroids, phenolics and flavonoids in all the crude extracts. Other compounds such as alkaloids and tannins were present in only the methanolic and aqueous leaf extract while phlobatannins were not detected in any extract except the aqueous extracts of the leaf (Table 1).

Table 2-5 revealed that EHML and EHAL had significant antibacterial activity on all the organisms from 50 mg to 200 mg. EHCL on the other hand, showed antibacterial activity from 100 mg to 200 mg while EHPL revealed significant activity on all the organisms at 200mg.

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 8 mice had diarrhoea, 11 experienced weight loss and 11 experienced loss of appetite.

DISCUSSION

The findings of this study revealed that the chloroform, methanolic, aqueous and petroleum ether leaf 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 leaf extract of *Euphorbia heterophylla* contain saponins, steroids, phenolics and flavonoids (Table 1). Other phytochemical components such as alkaloids and tannins were also detected in the crude leaf extracts (Table 1). The findings agree with the previous reports by Sundaram *et al.*, 2010; Okeniyi *et al.*, 2012; Falodun *et al.*, 2004. 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

Table 1. Phytochemical constituents of *Euphorbia heterophylla*

PHYTOCHEMICALS COMPOUNDS	Leaf			
	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

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

Extracts	*S. typhi*	*S.flexneri*	*E. coli*	*P. vulgaris*
EHCL	3.33 ± 0.30^{bc}	3.00 ± 0.58^{bc}	2.33 ± 0.33^{bc}	2.00 ± 0.58^{b}
EHML	6.00 ± 0.58^{f}	5.67 ± 0.67^{ef}	3.33 ± 0.88^{bc}	5.67 ± 0.88^{b}
EHAL	5.33 ± 0.33^{def}	5.67 ± 0.33^{ef}	4.00 ± 0.58^{cd}	5.00 ± 0.58^{b}
EHPL	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 leaves of *E.heterophylla* at 100mg

Exracts	*S. typhi*	*S. flexneri*	*E.coli*	*P. vulgaris*
EHCL	5.67 ± 0.67^{bc}	5.00 ± 0.58^{bc}	6.67 ± 0.33^{cde}	6.33 ± 0.33^{bc}
EHML	8.67 ± 0.88^{defg}	8.33 ± 0.33^{efgh}	8.00 ± 0.58^{cde}	7.67 ± 1.20^{cdef}
EHAL	8.33 ± 0.33^{def}	6.33 ± 0.90^{bcde}	7.00 ± 0.60^{cde}	7.33 ± 1.20^{cde}
EHPL	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	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}

bacterial cell wall (Jimoh *et al.*, 2005; Daniyan *et al.*, 2011). The flavonoids and terpenoids have been reported to have strong antioxidant effects (Nakayama *et al.*, 1993; Pari *et al.*, 2004) while the saponins are known to have immune modulation activities (Plohmann *et al.*,1997). Based on these chemical compounds the leaf of *E. heterophylla* is said to possess both pharmacological and antibacterial activities.

The methanolic leaf of *E.heterophylla* at 50mg/ml concentration revealed the highest significant activity (6.00 ± 0.58^{f} , 5.67 ± 0.67^{ef} , 3.33 ± 0.88^{bc} and 5.67 ± 0.88^{b}) for all the test organisms as compared to the its aqueous counterpart and other solvents used in this study at the same concentration (Table 2). The inability of the aqueous leaf extract to show significant activity is due to the fact that the use of aqueous as an extraction solvent enhances the release of some enzymes such as phenolases and hydrolases that might have affected the activity of the active compounds. In addition to this, methanol is regarded as alcoholic derivatives, and thus, it is said to extract more bioactive components than aqueous and in most cases, incomplete extraction of the active components is said to occur when aqueous is used as a solvent,.as earlier reported by El-Mahmood, Doughari and Ladan, (2005). Similarly, the different results observed among the various chemical solvents used in this study (Table 2) could be attributed to the different variations in polarity of the solvents and solubility of the bioactive compounds in the leaf of this plant as reported by Elmahood *et al.*, (2005). In addition to this, previous studies have revealed that phytochemical composition of medicinal plants exhibit varied chemical composition that mainly differs on the different solvents

Table 4. Zones of Inhibition of the leaves E.heterophylla at 150mg

	S. typhi	*S. flexneri*	*E.coli*	*P. vulgaris*
Extracts				
EHCL	8.33 ± 0.33^{e}	7.67 ± 0.30^{c}	7.67 ± 0.88^{bc}	7.33 ± 0.90^{c}
EHML	11.33 ± 0.60^{d}	10.33 ± 0.33^{def}	10.33 ± 0.88^{cdef}	9.67 ± 0.90^{cd}
EHAL	10.33 ± 1.33^{cd}	9.00 ± 0.60^{cde}	9.33 ± 1.45^{cde}	8.67 ± 0.30^{c}
EHPL	4.00 ± 0.58^{ab}	3.67 ± 0.70^{b}	5.33 ± 0.90^{b}	3.67 ± 0.31^{b}
CONTROL	20.00 ± 0.60^{f}	18.67 ± 0.70^{g}	19.33 ± 0.33^{g}	19.00 ± 0.58^{f}

Table 5. Zones of Inhibition of the leaves E.heterophylla at 200mg

	S. typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
Extracts				
EHCl	9.33 ± 0.66^{bc}	8.00 ± 0.58^{bc}	8.00 ± 1.16^{bc}	8.00 ± 0.60^{bcd}
EHML	12.00 ± 0.60^{e}	11.33 ± 0.90^{e}	11.67 ± 0.88^{d}	10.33 ± 0.33^{e}
EHAL	10.67 ± 0.70^{cde}	10.00 ± 0.60^{de}	10.67 ± 0.33^{cd}	9.67 ± 1.20^{de}
EHPL	8.33 ± 0.33^{b}	7.33 ± 0.33^{b}	8.00 ± 0.00^{b}	6.33 ± 0.88^{b}
CONTROL	26.00 ± 0.60^{h}	25.00 ± 0.70^{g}	25.33 ± 0.33^{f}	24.33 ± 0.33^{g}

Table 6. Effects of the methanoic and aqueous leaf extracts on the mice infected with *S.typhi*, *S.flexneri*, *E.coli* and *P.vulgaris*

Sub-group	Mortality rate (%)	W.D (%) 1-3 days	W.D (%) 4-6 days	W.D (0%) >7days	Loss of appetite (%)>7days	Loss of weight (%) >7days	Body weakness/ slow movement (%) >7days
S. typhi-I	0/5(0%)	2/5 (40%)	1/5 (20%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
S. typhi-II	0/5(0%)	3/5 (60%)	2/5 (40%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
S.flexneri-III	0/5(0%)	5/5 (100%)	2/5 (40%)	0/5(20%)	1/5(20%)	1/5(20%)	1/5(20%)
S.flexneri-IV	0/5(0%)	5/5 (100%)	3/5 (60%)	1/5(20%)	1/5(20%)	1/5(20%)	1/5(20%)
E.coli-V	0/5(0%)	5/5 (100%)	2/5(40%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
E.coli-VI	0/5(0%)	5/5 (100%)	2/5 (40%)	1/5(20%)	1/5(20%)	1/5(20%)	1/5(20%)
P.vulgaris-VII	0/5(0%)	5/5 (100%)	1/5 (20%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
P. vulgaris-VIII	0/5(0%)	5/5 (100%)	3/5 (60%)	1/5(20%)	1/5(20%)	1/5(20%)	1/5(20%)
IX	0/5(0%)	0/5 (0%)	0/5 (0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
X	5/5(100%)	5/5 (100%)	5/5 (100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
XI	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 EHML; II= Infected with *S.typhi* and treated with EHAL. III = Infected with *S.flexneri* and treated with EHML ; IV = Infected with *S.flexneri* and treated with EHAL; V= Infected with *E.coli* and treated with EHML; VI= Infected with *E.coli* and treated with EHAL; VII= Infected with *P.vulgaris* and treated with EHML; VIII= Infected with *P.vulgaris* and treated with EHAL ; IX=Treated with Ciprofloxacin; X = Infected not treated; XI= Not infected not treated; W.D= Watery diarrhoea n= 5, Dilution factor=10^{-5}.
used in the process of extraction (Armugam *et al.*, 2016; Swamy *et al.*, 2015b).

The antibacterial activity of methanolic and aqueous crude extracts of *E. heterophylla* at 100mg/ml was significant on all the test organisms, compared to the effects of petroleum ether extracts of the leaf 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). In addition to this, petroleum ether compared to other extracting solvents used in this

study, is regarded as a non-polar solvent, based on this fact, its ability to extract bioactive compounds from the leaf was impossible.

However the antibacterial activities of petroleum ether crude extract of the leaf of *E. heterophylla* was significant at 150mg/ml and 200mg/ml on all the test organisms as it was also observed, in the case of the other crude extracts of the leaf of *E. heterophylla* from other solvents at concentrations of 150mg/ml and 200mg/ml, which also revealed higher antibacterial activities compared to the antibacterial activities at 50mg/ml and100mg/ml (Table 4 and 4.5). The high antibacterial activities observed at 150mg/ml and 200mg/ml could be due to the enhanced effect of the leaf extracts based on the increased concentration of the individual extract, which are said to contain more phytochemical constituents. The outcome of this, conform with the result obtained in a study by Ahmed *et al.* (2012).

The *Invivo* determination of the efficacy of methanolic and aqueous leaf 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 symptoms observed in the mice few days after inoculation disappeared. However, mice infected with the different test organisms treated with aqueous extracts of the leaf of *E. heterophylla* revealed that 20% of the mice showed pathological symptoms of watery diarrhoea, this could be as a result that the concentration of the bioactive components present in the aqueous dose administered for the treatment of the infected mice 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 20% of loss of appetite, loss of weight and body weakness/slow movement, observed after 7 days in which aqueous extracts of the leaf of *E. heterophylla* were administered 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 leaf of *E. heterophylla*, contained efficient phytochemicals that were active against all test organisms at a concentration as low as 100 milligram, indicating that the leaf 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 RN, Abdulrahaman AA, 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 MTS (2013). The Enteric Bacteria. A Ph.D thesis presented at Cairo University. Pp 1-10.

Arumugam G, Swamy MK, Sinniah UR (2016). Plectranthus amboinicus (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. *Molecules*, *21*(4), p.369.

Arsad SS, Esa NM, Hamzah H, 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.

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.

Daniyan SY, Abalaka ME, Garba SA, Adeyemo S (2011). Investigation into the antimicrobial properties of *Euphorbia heterophylla* on typhoid disease causative agents. *International Journal of Research in Ayurveda & Pharmacy*, 2(4), 1214-1217.

Ehrlich SD (2013). Herbal Medicine. Review provided by VeriMed Herbal Network. Pp1-5.

EL- Faraley YS, Cheatham SF, Breelove RL (1983). Antimicrobial neolignan of *Sassafras ranaiense* root.*Lloydia*, 46 (4), 493-497.

El-Mahmood AM, Doughari, J H 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.

Eman MA, Hoda MZ (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 YS, Ekrem H, Gisho T, Yoshiohiro T (1999) Traditional medicine in Turkey IX, folk medicine in NorthWest Anatolia. *Journal of Ethnopharmacology*, 64, 201.

Falodun A, Agbakwuru EOP (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 LO, 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, Hostettmann K (1991).Bioactivity in plants: the link between phytochemistry and medicine, *Phytochemistry*, 30,: 3864-3874.

Harborne JB (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 CL (2012).Antimicrobial activity and phytochemistry of *Khaya senegalensis* root. *International Journal of Ayurvedic and Herb Medicine*, 2(3), 416-422.

Itelima, JU Agina SE (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 PA (1991). Comparative antimicrobial activity of crude extract of Terminalia Macroptae with phenol chlorhexidine and gentamycin. *Pharmacognosy Journal*,1(1), pp.12.

.Jimoh FO, Oladigi AT (2005). Preliminary studies on Pilostigma thonninggii seeds: Proximate analysis, mineral composition and phytochemical screening. African J. Biotech. 4(12):1439-1442.

Kunle OF, Egharevba HO, Ahmadu PO (2012). Standardization of herbal medicines – A review. *International Journal of Biodiversity and Conservation*, 4(3), 101-112.

Mbata TI, 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.

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

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

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

Nakayama NG, Lindsey ML, Michael LH (1993). Inhibition of infectivity of influenza virus by tea polyphenoids. *Antiviral Resource*, 21, 289-299.

Okeniyi SO, Adedoyin BJ, 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 MU (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, Nigeria, 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.

Rudramurthy GR, Swamy MK, Sinniah UR Ghasemzadeh A (2016). Nanoparticles: alternatives against drug-resistant pathogenic microbes. *Molecules*, *21*(7), p.836.

Saimo MK, Bizimenyera ES, Bwanika A, Ssebuguzi F, Weny G, Lubega GW (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 MM, Karthikeyan K, Sudarsanam D, Brindha P (2010). Antimicrobial and anticancer studies on *Euphorbia heterophylla*, *Journal of Pharmacy Research*, 3(9), 2332-2333.

Sudipta KM, Lokesh P, Rashmi W, Vijay R, Ssn K (2012). Phytochemical screening and in vitro antimicrobial activity of Bougainvillea spectabilis flower extracts. *International journal of phytomedicine*, *4*(3), p.375.

Swamy MK, Sinniah UR (2015a). A comprehensive review on the phytochemical constituents and pharmacological activities of Pogostemon cablin Benth.: an aromatic medicinal plant of industrial importance. *Molecules*, *20*(5), pp.8521-8547.

Swamy MK, Sinniah UR, Akhtar MS (2015b). In vitro pharmacological activities and GC-MS analysis of different solvent extracts of Lantana camara leaves collected from tropical region of Malaysia. *Evidence-Based Complementary and Alternative Medicine*, *2015*.

Trease GE, Evans WC (1984). A Text Book of Pharmacognosy. EL SB/ Bailliere Tindal, Oxoford, UK. 1055.

Unekwe PC, Ughachukwu PO, Ogamba JO (2006). Some pharmacological Studies of Aqueous extract of leaves of *Euphorbia heterophylla*. *Tropical Journal of Medical Resources*, 10 (2),1-5.

Yakubu MT, Akanji MA, Oladiji AT (2005). Aphrodisiac potentials of the aqueous extract of

Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. *Asian J. Androl.* 7(4): 399-404.

Accepted 18 February, 2017.

Citation: Oyedum UM (2017). Invitro and *Invivo* antibacterial study of leaf extracts of *Euphorbia heterophylla* on some enteric bacteria. World Journal of Microbiology, 4(1): 072-078.