

ANTIBACTERIAL AND TOXICOLOGICAL EFFECTS OF LEAF EXTRACTS OF *Euphorbia heterophylla* ON SOME ENTERIC BACTERIA

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ABSTRACT

Aim: A study was carried out to determine the antibacterial activity of *Euphorbia heterophylla* crude extracts on four enteric bacterial organisms namely; *Salmonella typhi*, *Shigella flexneri*, *E.coli* and *Proteus vulgaris*.

Method: The clinical isolates of *Salmonella typhi*, *Shigella flexneri*, *E.coli* and *Proteus vulgaris* were subjected to antimicrobial susceptibility test using agar diffusion technique.

Result: Methanolic and aqueous crude extract produced clear zones of inhibition at concentration ranging from 100 to 200mg/ml at 24 hours and as the time increased to 48hours and 72hours, the antibacterial activity decreased. Two thousand milligram per kilogram body weight of the crude extracts was administered to the mice orally, and single death accompanied each group of mice that were administered with methanolic crude extract of the leaf.

Conclusion: *Euphorbia heterophylla* crude extract could be a potential source of antimicrobial agent 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

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; 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 almost always cause signs of infection, including vomiting, diarrhoea, and related symptoms (Murray, 1994).

Fermentation and decarboxylation are anaerobic processes and will result in acid and alkaline reactions respectively. Another anaerobic process - production of hydrogen sulfide from thiosulfate – is also possessed by some of these microorganisms that predominate the intestinal tract (Pitout and Church, 2004).

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 *et al.*, 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).

The antimicrobial compounds produced by plants are active against plant 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 in waste land, in gardens and along roadsides, from sea-level up to 3000m 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 and commonly called Non-kunchiya in Hausa, Egele in Ibo and Adimeru in Yoruba, Nigeria (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 possess 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 abortifacient (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 gonorrhoea, respiratory tract infection, malaria, Eczema, and wart cure by traditional medicine.

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 nematocidal 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 noticeable 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*.

MATERIALS AND METHODS

Collection and Identification of the Plant Materials

Fresh samples of the leaves were collected from Garatu in Bosso local government area of Niger State. 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 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 principles (Iyamabo, 1991).

Extraction

One hundred grammes (100g) of the ground part was macerated successively for three days (with occasional shaking) using cold maceration technique. One thousand milliliters (1000ml) 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).

Culture Media

MacConkey and *Salmonella - Shigella* agar plates were used for differential and selective media for the isolation of enteric bacterial flora. Susceptibility testing of isolated organisms was performed in nutrient agar plates (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 cultures from the Microbiology Laboratory, General Hospital, Minna, Niger State. The isolates were identified using the schemes of Cheesbrough (2006).

Bacterial Assay of the Extracts

The antibacterial assay of the crude extracts was done using the agar diffusion plate method described by Idu *et al.* (2012). Briefly, a sterile 4mm cork borer was used to punch four holes in the medium after spread inoculation of the plated medium. About 0.2ml of the different concentrations of the extracts was introduced into each well and the plates were allowed to stand for 20mins. The petri plates were incubated at a temperature of 37°C for 24 hours. Observations for the zones of inhibition were conducted and measurements of the zones of inhibition in diameters were carried out and the results recorded. Comparisons were made with zone diameters of standard antibiotic 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 oral acute toxicity.

Thin-Layer Chromatography

Thin layer chromatography was performed on a sheet of glass coated with a thin layer of silica gel. The sample was then applied at one end of the plate and placed in a TLC tank containing a shallow amount of the mixture of solvents (mobile phase) to be used. After the sample had been applied on the plate and placed in the beaker, the solvent was drawn up the plate via capillary action. The different analytes ascended the TLC plate at different rates, and so separation was achieved (Abalaka *et al.*, 2011).

Acute Oral Toxicity Studies

Acute toxicity study was performed on 30 animals using a single dose of 2000mg/kg body weight. The animals were

divided in to 6 groups, each containing 5 animals. The animals were starved overnight before they were administered with a crude extract orally. After drug administration the animals were provided with food and water immediately and were under observation for any mortality/ adverse signs. (Mukinda and Syce, 2007).

Statistical Analysis

The result would be analysed statistically using ANOVA

RESULTS PRESENTATION

Table 4.1 reveals that among all the leaf extracts of *Euphorbia heterophylla*, only the methanolic extract of the leaf had significant antibacterial activity on all the test

organisms at 50mg in 24hours and there was decrease in the antibacterial activity after 48hours.

Table 4.2 reveals that all the leaf extracts of *Euphorbia heterophylla*, except petroleum extract of the leaf had significant antibacterial activity on all the test organisms at 100mg in 24hours and there was decrease in the antibacterial activity after 48hours.

Table 4.3 and 4.4 reveal that all the leaf extracts of *Euphorbia heterophylla*, including petroleum extract of the leaf had significant antibacterial activity on all the test organisms at 150mg and 200mg in 24hours and 48hours, after which a decrease in the antibacterial activity was observed after 72hours.

Table 4.1: Plant extracts of *Euphorbia heterophylla* at 50mg

Extracts	24hr				48hr				72hr			
	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>
EHCL	3.33±0.30 ^{bc}	3.00±0.58 ^{bc}	2.33±0.33 ^{bc}	2.00±0.58 ^b	1.00±0.58 ^a	1.00±0.58 ^{ab}	0.33±0.03 ^{ab}	0.33±0.03 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
EHML	6.00±0.58 ^f	5.67±0.67 ^{ef}	3.33±0.88 ^{bc}	5.67±0.88	3.33±0.83 ^{bc}	3.67±1.20 ^d	1.67±1.20 ^{abcc}	2.67±0.33 ^d	0.67±0.07 ^{ab}	1.33±0.88 ^a	0.33±0.003 ^{ab}	0.67±0.33 ^a
EHAL	5.33±0.33 ^{def}	5.67±0.33 ^{ef}	4.00±0.58 ^{cd}	5.00±0.58	3.33±0.67 ^{bc}	3.33±0.33 ^{cd}	2.00±0.57	2.33±0.33 ^{cd}	0.00 ^a	0.67±0.07 ^a	0.33±0.03 ^{ab}	0.33±0.03 ^a
EHPL	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	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	7.00±0.58 ^d	6.00±0.58 ^e	6.67±0.68	6.67±0.33 ^f	5.00±0.58 ^d	3.33±0.33 ^b	3.00±0.58 ^c	3.33±0.33 ^c

Inhibition zone diameters (mm) values are represented as Mean± Standard Error of Mean of triplicate determinations. Values along each column with different alphabets are significantly different ($p < 0.05$)

Table 4.2: Plant extracts of *Euphorbia heterophylla* at 100mg

Extracts	24hr				48hr				72hr			
	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>
EHCL	5.67±0.67 ^{bc}	5.00±0.58 ^{bc}	6.67±0.33 ^{cde}	6.33±0.33 ^{bc}	4.67±0.70 ^{bc}	3.33±0.88 ^{bc}	5.33±0.33 ^{cdef}	4.67±0.30 ^{cdef}	2.67±0.67 ^{bcd}	2.00±0.57 ^{bc}	1.67±0.70 ^{abc}	2.33±0.33 ^{bcd}
EHML	8.67±0.88 ^{de}	8.33±0.33 ^{efgh}	8.00±0.58 ^{cde}	7.67±1.20 ^{cdef}	6.67±0.90 ^{cde}	6.33±0.33 ^{de}	5.67±0.31 ^{cdef}	6.00±1.16 ^{ef}	4.67±0.90 ^{def}	3.67±0.33 ^{cd}	3.33±0.33 ^{cd}	4.33±0.88 ^e
EHAL	8.33±0.33 ^{def}	6.33±0.90 ^{bcd}	7.00±0.60 ^{cde}	7.33±1.20 ^{cde}	6.00±0.58 ^{cde}	4.33±0.88 ^{bcd}	4.33±0.90 ^{bcd}	5.67±0.88 ^{def}	3.33±0.33 ^{cde}	2.00±0.60 ^{bc}	3.33±0.33 ^{cd}	2.33±0.70 ^{bcd}
EHPL	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	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	13.33±0.33 ^e	10.33±0.90 ^e	11.00±1.15 ^g	10.00±1.20 ^g	10.00±0.6 ^g	8.00±0.58 ^e	8.67±1.20 ^f	7.00±0.60 ^f

Inhibition zone diameters (mm) values are represented as Mean± Standard Error of Mean of triplicate determinations. Values along each column with different alphabets are significantly different ($p < 0.05$)

Key: EHCL---Chloroform leaf extract of *Euphorbia heterophylla*; EHML---Methanolic leaf extract of *Euphorbia heterophylla*; EHAL--- Aqueous leaf extract of *Euphorbia heterophylla*; EHPL---- Petroleum ether leaf extract of *Euphorbia heterophylla*

Table 4.3: Plant extracts of *Euphorbia heterophylla* at 150mg

Extracts	24hr				48hr				72hr			
	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>
EHCL	8.33±0.33 ^c	7.67±0.30 ^c	7.67±0.88 ^{bc}	7.33±0.90 ^c	6.33±0.88 ^c	5.67±0.33 ^{cd}	5.33±0.70 ^c	4.67±1.20 ^b	4.33±0.90 ^{cd}	2.67±0.30 ^b	2.67±0.28 ^{bcd}	2.00±0.60 ^a
EHML	11.33±0.60 ^d	10.33±0.33 ^{def}	10.33±0.88 ^{cdef}	9.67±0.90 ^{cd}	9.33±0.70 ^{de}	8.33±0.33 ^{efg}	8.33±0.67 ^{de}	7.00±0.60 ^{cd}	6.67±0.88 ^e	6.00±0.60 ^{de}	5.67±0.88 ^{efg}	4.00±0.57 ^{cd}
EHAL	10.33±1.33 ^{cd}	9.00±0.60 ^{cde}	9.33±1.45 ^{cde}	8.67±0.30 ^c	7.33±0.80 ^{cd}	7.33±0.88 ^{def}	7.33±0.80 ^{cd}	7.67±0.31 ^d	6.00±0.58 ^{de}	4.67±1.17 ^{cd}	4.67±1.20 ^{def}	3.67±0.30 ^c
EHPL	4.00±0.58 ^{ab}	3.67±0.70 ^b	5.33±0.90 ^b	3.67±0.31 ^b	2.67±0.88 ^{ab}	1.67±0.70 ^a	2.33±0.33 ^{ab}	1.67±0.27 ^a	1.00±0.15 ^{ab}	0.33±0.03 ^a	0.67±0.30 ^a	0.33±0.03 ^a
CONTROL	20.00±0.60 ^f	18.67±0.70 ^g	19.33±0.33 ^g	19.00±0.58 ^f	18.00±0.60 ^g	16.33±0.88 ^h	16.33±0.90 ^f	16.67±0.67 ^g	15.33±0.33 ^h	13.33±1.23 ^g	15.33±0.90 ⁱ	15.33±1.15 ^f

Inhibition zone diameters (mm) values are represented as Mean± Standard Error of Mean of triplicate determinations. Values along each column with different alphabets are significantly different (p < 0.05)

Table 4.4: Plant extracts of *Euphorbia heterophylla* at 200mg

Extracts	24hr				48hr				72hr			
	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. flexneri</i>	<i>E. coli</i>	<i>P. vulgaris</i>
EHCL	9.33±0.66 ^{bc}	8.00±0.58 ^{bc}	8.00±1.16 ^b	8.00±0.60 ^{bcd}	8.00±0.60 ^{bc}	6.33±0.33 ^b	6.67±1.45 ^{bc}	6.67±0.70 ^{bc}	7.00±0.58 ^{cd}	5.33±0.33 ^{cde}	5.33±1.20 ^{bcd}	6.33±1.33 ^{cd}
EHML	12.00±0.60 ^e	11.33±0.90 ^e	11.67±0.88 ^d	10.33±0.33 ^e	10.00±0.60 ^d	9.00±0.58 ^c	9.00±0.58 ^e	8.33±0.33 ^{cd}	8.00±0.58 ^{cde}	7.00±0.60 ^{ef}	6.67±0.67 ^{cde}	6.00±0.60 ^{cd}
EHAL	10.67±0.70 ^{cde}	10.00±0.60 ^{de}	10.67±0.33 ^{cd}	9.67±1.20 ^{de}	8.67±1.20 ^{bcd}	8.00±0.60 ^c	8.33±0.67 ^{cde}	7.67±1.21 ^{cd}	6.33±0.90 ^{bc}	5.00±1.00 ^{bcd}	6.33±0.33 ^{cde}	4.33±0.88 ^{bc}
EHPL	8.33±0.33 ^b	7.33±0.33 ^b	8.00±0.00 ^b	6.33±0.88 ^b	7.00±0.58 ^b	5.66±0.33 ^b	6.33±0.33 ^b	5.00±0.60 ^b	4.67±0.70 ^b	3.33±0.33 ^b	4.33±0.33 ^b	2.33±0.33 ^{ab}
CONTROL	26.00±0.60 ^h	25.00±0.70 ^g	25.33±0.33 ^f	24.33±0.33 ^g	25.00±0.60 ^f	24.00±0.60 ^e	23.33±0.33 ^g	22.33±0.33 ^f	24.00±0.57 ^g	23.00±0.60 ^h	22.33±0.33 ^g	21.33±0.33 ^f

Inhibition zone diameters (mm) values are represented as Mean± Standard Error of Mean of triplicate determinations. Values along each column with different alphabets are significantly different (p < 0.05)

Key: EHCL---Chloroform leaf extract of *Euphorbia heterophylla*; EHML---Methanolic leaf extract of *Euphorbia heterophylla*; EHAL--- Aqueous leaf extract of *Euphorbia heterophylla*; EHPL---- Petroleum ether leaf extract of *Euphorbia heterophylla*

Table 4.5 reveals the different number of bioactive components found in each extract. Samples EHCL and EHPL gave rise to two bioactive components each while samples EHML and EHAL gave rise to three bioactive components each.

Table 4.5: Fractionated crude extracts of the leaf and its corresponding antibacterial effect on each test organism

Extracts	Bioactive components	<i>Salmonella typhi</i>	<i>Shigella flexneri</i>	<i>E.coli</i>	<i>P.vulgaris</i>
EHML	A1	+	+	+	+
	A2	+	+	+	+
	A3	+	+	+	+
EHAL	B1	-	-	-	-
	B2	+	-	+	+
	B3	+	+	+	-
EHCL	C1	-	-	-	-
	C2	-	+	+	-
EHPL	D1	-	-	-	-
	D2	-	-	-	-

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

Table 4.6 reveals the oral acute toxicity (LD50) of the crude methanolic and aqueous extracts of the leaf of *Euphorbia heterophylla* at a dose of 2000mg/kgw. Single death was recorded in all groups administered with methanolic extracts.

Table 4.6: Acute oral toxicity of the crude extracts on the mice

Extract	No of mice per extract	Dose (mg/kgbw)	No of mice that died
Methanolic leaf extract of <i>Euphorbia heterophylla</i> -I	5	2000	1/5
Aqueous leaf extract of <i>Euphorbia heterophylla</i> -II	5	2000	0/5
Methanolic leaf extract of <i>Euphorbia heterophylla</i> -III	5	2000	1/5
Aqueous leaf extract of <i>Euphorbia heterophylla</i> -IV	5	2000	0/5
Methanolic leaf extract of <i>Euphorbia heterophylla</i> -V	5	2000	1/5
Aqueous leaf extract of <i>Euphorbia heterophylla</i> -VI	5	2000	0/5
Methanolic leaf extract of <i>Euphorbia heterophylla</i> -VII	5	2000	1/5
Aqueous leaf extract of <i>Euphorbia heterophylla</i> -VIII	5	2000	0/5

Key;

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

DISCUSSION

The methanol, aqueous and chloroform crude extracts showed significant activity at 100mg/ml concentrations, after 24 and 48 hours on all organisms, when compared to the petroleum ether extracts (Table 4.2) based on the potency they possessed. This 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). However, the study also revealed that 48 hours after the antibiogram was carried out, the zones of inhibition gradually reduced (Table 4.1 to Table 4.4). This could be due to the fact that the potency of the extracts after 48 hours reduced, and thus bringing about the development of resistance towards the extracts. This is findings is said to agree with the findings of Mbata and Salkia, (2008).

Furthermore, the antibacterial activities of methanolic and aqueous crude extracts of *Euphorbia heterophylla* at 150mg/ml and 200mg/ml were significant on the test organisms after 24 hours, 48 hours and 72 hours as compared to the antibacterial activities of methanolic crude extracts of *Euphorbia heterophylla* at 100mg/ml (Table 4.3 and 4.4). The high antibacterial activities of methanolic and aqueous crude extract observed at 150mg/ml and 200mg/ml could be due to the enhanced effect of the plant extracts based on the increased concentration of the individual extract, which are said to contain more phytochemical constituents. The outcome of this, conform with the result obtained in a study by Ahmed, Abdulrahman, and Sani, (2012).

The presence of different bioactive components in the crude extracts of the leaf of *Euphorbia heterophylla* (Table 4.6) indicates that the leaf contains diverse potent active ingredients. This result agrees with the findings of Jayashree (2013); Falodun *et al.* (2004). All bioactive components obtained in the methanolic crude extracts of the leaf showed antibacterial activity against the test organisms in this study compared to the aqueous, chloroform and petroleum ether extracts (Table 4.6). This suggests that the individual components or bioactive compounds that were not able to exhibit antibacterial activity on the test organisms, may be due to their inactive nature or requires a synergistic relationship with other bioactive components, as reported by Harborne (1984); Oyeleke, Dauda, and Boye, (2008).

Toxicity screening of these extracts revealed that the food intake and water consumption was not affected by the intake of both the methanolic and aqueous extracts of the leaf of *Euphorbia heterophylla* and as such, induced appetite suppression and deleterious effect were not experienced. This indicates that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen, 2001). Although within the first 24 hours in which the mice were administered with the extracts at a single dose of 2000mg/kg, the mice revealed minor abnormalities such as, weakness, slight decrease in locomotion, and aggression, after two to four hours of administering the extracts to them. Such abnormalities are said to be triggered by the presence of these foreign extracts, when they enter the body system as reported by Pillai *et al.* 2011.

Similarly, the acute oral toxicity study on the mice also revealed that the oral administration of *Euphorbia heterophylla*, at a single dose of 2000mg/kg bw, caused single deaths in each group of mice administered with methanolic crude extract, when compared with the group of mice administered with aqueous crude extracts (Table 4.5). The death of the mice administered with 2000mg/kg bw of methanolic extracts could be attributed to the fact that this high single dose of *Euphorbia heterophylla*, was toxic in relation to their body weights. However, this result disagrees with the findings of Arsad *et al.* (2013), who reported that treatment at any dose of *Rhaphidophora decursiva* irrespective of the weights of the mice was non-toxic.

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 activity of the crude extracts at concentrations ranging from 50-200mg/ml showed antibacterial activity, particularly after 24 to 48 hours but the activity declined after 72 hours. In addition, the crude extracts of the plant was found to be safe at higher dose of 2000mg/kgbw. But since the administration of methanolic crude extracts of the leaf caused single death, it is therefore recommended that before an extract is administered, side effects of the extract and appropriate dose in relation to the weight of the mouse should be ascertained.

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