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# Comparative study of the phytochemical and antibacterial activity of leaf extracts of *Euphorbia heterophylla* and *Vitellaria paradoxa*

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A comparative study was carried out to determine the phytochemical components and *Euphorbia* heterophylla antibacterial activity as well as *Vitellaria paradoxa*leaf crude extracts on four enteric organisms, namely: *Proteus vulgaris, Salmonella* Typhi, *Shigella flexneri*, and *Escherichia coli*. The clinical isolates of the enteric organisms were subjected to test of antimicrobial susceptibility using technique of agar diffusion. Phytochemistry of the *E. heterophylla* crude extracts exposed the presence of more phenolics, phlobatannins, tannins and cardiac glycosides than *V. paradoxa*, which revealed the presence of more steroids. All crude *E. heterophylla* extracts produced high clear inhibition zones, compared to the *V. paradoxa* counterpart at concentration ranging from 50 to 200 mg/ml. *In vivo* antimicrobial assay discovered that the mice treated with the crude methanolic *E. heterophylla* extracts, after being infected with the test organisms, survived and showed no pathological effects as compared to the *V. paradoxa* counterpart, which showed 20% pathological effects. *E. heterophylla* crude extract could be a possible source for the diseases treatment associated with enteric organisms such as *P. vulgaris, S.* Typhi, *S. flexneri*, as well as *E. coli*. Additional studies should be directed towards isolation as well as characterisation of the active compound in the crude extracts.

Key words: In vitro activity, in vivo activity, Euphorbia heterophylla, Vitellaria paradoxa, Enteric bacteria.

### INTRODUCTION

Plants, which are not only regarded as integral parts of the earth, have served as drugs sources as well as pharmaceuticals for man and other animals from immemorial time. They consist of 32% of the surface of the earth (Ghose, 2016). There are about half a million plants now growing on earth, which possess many therapeutic as well as pharmaceutical properties (Muller, 1973). According to a previous survey, about 25% of medicinal products as well as modern drugs are derived from plant secondary metabolites (Hamburger and Hostettmann, 1991). Essentially, most of these plants are said to be helpful in achieving stable health as well as most diseases treatment associated with the human race, and as such they are termed medicinal plants. Medicinal plants can be defined as natures' pharmacy for nearly 80% of people living in Africa (World Health Organisation,

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> 2002). A medicinal plant has been defined as any plant in which, one or more of its parts contain substances that can be used for therapeutic purpose or which acts as precursors for the useful drugs synthesis (Sofowora, 1982). Based on the cultural acceptability as well as fewer side effects, herbal medicine still remains the mainstay of 75 to 80% of the whole population for primary health care in Africa (Ghasi et al., 2000). In Nigeria, thousands of plant species are known to have medicinal values (Sofowora, 1982). Generally, the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Rios and Recios, 2005); but the current emergence scenario of multiple drug resistance to human pathogenic organisms, has required a search for new antimicrobial substances from plants.

Though, the plants ability to produce many phytochemicals that are used to perform significant biological functions is one of the many characteristics they possess as well as the medicinal values of these plants lie in the phytochemicals present in the plants and these phytochemical in turn produce certain physiological actions on the body of human (Lee et al., 1998; Afolabi et al., 2001; Doughari and Manzara, 2008). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well known that plants produce these chemicals to protect themselves and recent researches have demonstrated that they can also protect humans against diseases. These chemicals possess varied actions such as antioxidants, hormonal activity, stimulatory activities, antimicrobial effects, antidiarrhoreal effects, anti histamic effects, anti diabetic effects, anti malarial effects and anti carcinogenic effect. In most cases because of the these bioactive components presence or phytochemicals in plants, plants may be considered as possible candidates for developing new antimicrobial drugs or alternative treatments of numerous ailments caused by microorganisms resistant to most available synthetic drugs. Two effective medicinal plants that are gradually gaining grounds in the developing countries because of their medicinal benefits are Vitelleria paradoxa as well as Euphorbia heterophylla.

*E. heterophylla* is one of the several plants found in the field. *E. heterophylla* grows in distressed localities, as a cultivation weed and waste land, in gardens and along roadsides, from sea-level up to 3000 m altitude (Mosango, 2008). *E. heterophylla* belongs to the family of Euphorbiaceae. It is referred to as Mexican fire plant, milk weed as well as spurge weed in English, and commonly called *Nono-kunchiya* in Hausa, *Egele* in Ibo and *Adimeru* in Yoruba, Nigeria (Okeniyi et al., 2012). All parts of *E. heterophylla* comprised latex (Mosango, 2008). *E. heterophylla* is used widely in traditional African medicine and elsewhere in tropical countries. In general, this plant is regarded as a purgative, antiasthmatic, anti-inflammatory as well as an arbotifacient (Erden et al., 1999; Falodun et al., 2006). It has also been reported to

be oxytocic (Unekwe et al., 2006). The dried leaves butanol extract exhibited marked inhibitory action on the *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus subtilis* growth at 100 mg/ml (Mosango, 2008). An extract of the aerial parts showed antiplasmodial activity. A leaf extract showed significant nematicidal activity against *Meloidogyne graminicola* (Mosango, 2008).

*V. paradoxa*, on the other hand, is generally regarded as a multipurpose as well as deciduous plant, found and used in Africa. The size of the mature tree of *V. paradoxa* differs from 7 to 25 m. This plant belongs to the family Sapotaceae and is said to produce Shea butter as its main product (Djekota et al., 2014). *V. paradoxa* formerly called *Butyrospermum parkii* (which means butter seed) could also be called *Butyrospermum paradoxa*. In Nigeria, it is locally known as '*emi-emi*' among the Yorubas, '*ka'danya*' among the Hausas and '*okwuma*' among the Igbos (Orwa et al., 2009). The plant is said to possess white latex. The shea tree grows naturally in the wild in the dry savannah belt of West Africa from Senegal in the west to Sudan in the east, and onto the Ethiopian highlands foothills.

The leaves of this plant are mostly used as medicine to treat stomach ache in children (Fobil et al., 2002). A decoction of young leaves is used as a vapour bath to treat headaches in some Africa countries such as Ghana, Nigeria. The leaves usually contain saponins, which produces lathers in water and such water can be used for washing and cleansing various organs of the body such as the eyes. Generally, these plants are used for the diseases treatment caused by enteric bacteria which are known to be associated with high morbidity and mortality cases among the populace.

Enteric bacteria are Gram negative bacteria that are associated with gastrointestinal flora or disease (Murray, 1994). Enterics can be found in numerous natural habitats, not just in the intestinal tract. Most enterics are motile by peritrichous flagella; two major exceptions that lack peritrichous flagella, are Shigella as well as Klebsiella (AL-Ougaili, 2013). Numerous 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 as well as changing them into lactic acid. Some of the enterics can live in the gut without causing health problems in individuals of good health, whereas others almost always cause infection signs, including diarrhoea, vomiting, and related symptoms (Murray, 1994). Based on this health related threats posed by the enteric organisms, many individuals tend to abuse the use of available synthetic drugs, thereby encouraging these enteric organisms to develop resistance to these drugs. It is consequently imperative to seek other alternative remedy sources to numerous enteric diseases as well as determine the differences between them in this perspective, which is the main aim of this study.

#### METHODOLOGY

#### Collection and identification of the plant materials

Both plants leaves of fresh samples (namely *E. heterophylla* and *V. paradoxa*) 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. These plants' materials were taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification and processing. The voucher specimens of these plants' material have been deposited in the herbarium with the deposition numbers such as: NIPRD/P/001/961 and NIPRD/H/6865 for *V. paradoxa* and *E. heterophylla*, respectively.

#### **Drying procedure**

Both plants leaves were washed thoroughly, air dried at room temperature (28°C) and ground into coarse powder using a sterile pestle as well as mortar. The dried leaves were further ground into a fine powder using an electric blender. This was done to improve the extracting solvent penetration, therefore facilitating the active components release (Iyamabo, 1991).

#### Extraction

One hundred grams of each ground leaves, were softened successively for three days (with occasional shaking) using cold maceration technique. 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 as well as 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 (lyamabo, 1991).

#### Phytochemical screening

The crude extracts phytochemical screenings of both leaves were carried out to detect the presence or absence of some secondary metabolites. The methods by Harborne (1984) and Trease and Evans (1987) were employed.

#### Culture media

MacConkey, *Salmonella-Shigella* and nutrient agars were, respectively 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 Multi drug resistant (MDR) test organisms (*Proteus vulgaris, Salmonella* Typhi, *Shigella flexneri* and *E. coli*) were gotten from the stock culture in the Microbiology Laboratory, General Hospital, Minna. The test organisms were identified via Gram staining and other conventional biochemical tests such as: Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar tests as described by Cheesbrough (2010).

#### Antibacterial assay of the extracts

Well grown activated cultures were diluted serially in test tubes with

normal saline until a cell concentration of 1.0 ×10<sup>5</sup> cfu/ml was gotten. The antibacterial assay of the crude extracts of both plants leaves was done using punch hole method defined by Idu et al. (2012). The plates were prepared by dispensing 20 ml of sterile molten nutrient agar into sterile Petri plates and allowed to set. A 4 mm cork borer was used to punch holes in the medium. Four holes 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 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 h, after which observed inhibition zones were measured as well as the results recorded in comparison with the effect of the standard antibiotic, known as Ciprofloxacin (5 µg) which was used as the control (Idu and Igekele, 2012). Only leaf extracts of each plant which showed high antibacterial activity and served as possible drug development source 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 picked up from Ibrahim Badamasi Babangida University Lapai. The mice were kept in standard cages with suitable food, water as well as 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 stimulate the test organisms. The test organisms were transferred further into test tubes containing 10 ml of sterilised nutrient broth and incubated at 37°C for 18-24 h. The activated culture was diluted serially in test tubes with normal saline until a cell concentration of  $1.0 \times 10^5$  cfu/ml was gotten (Eman and Hoda, 2008).

## Inoculation of test organisms and administration of plant extracts and antibiotic to albino mice

Division of the mice was into 15 sub-groups of 5 each. In each subgroup, a precise volume of an inoculum (approximately 1 ml of the infective dose of the inoculum) was introduced into each mouse intraperitoneally as approved by Eman et al. (2008). After the innoculation of the mice, each extract administration (namely *E. heterophylla* chloroform leaf, *E. heterophylla* aqueous leaf, *E. heterophylla* methanolic leaf, *V. paradoxa* chloroform leaf, *V. paradoxa* aqueous leaf) and antibiotics namely Ciprofloxacin (5  $\mu$ g) were done orally for 7 days (Itelima and Agina, 2014). The mice were closely observed daily and the mortality rate as well as other physical manifestations was recorded.

## Observation of mortality rate, survival rate and other physical manifestations

The survival as well as mortality rate of the mice in the sub groups was calculated as numbers of the mice that died as well as survived during the experiment course in relation to all the mice that were used (Eman and Hoda, 2008). The animals were perceived to note the consistency, frequency as well as colour of their faecal waste. The mice were also perceived for any abnormalities and physical manifestations (for example loss of appetite, loss of weight

Phytochemical compound	Lea	f ( <i>Euphorbi</i>	a heterophy	rlla)	Leaf (Vitellaria paradoxa)			a)
	Chloroform	Methanol	Aqueous	Petroleum ether	Chloroform	Methanol	Aqueous	Petroleum ether
Carbohydrates	+	+	-	-	+	+	+	-
Starch	+	+	-	-	+	+	+	-
Cardiac glycosides	-	+	+	+	-	-	-	+
Saponins	+	+	+	+	+	+	+	+
Steroids	-	-	+	+	-	+	+	+
Alkaloids	-	+	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+	+	+
Phenolics	+	+	+	+	+	-	-	+
Tannins	-	+	+	-	+	-	-	-
Phlobatannins	-	-	+	-	-	-	-	-

Table 1. Phytochemical constituents of the leaves of Euphorbia heterophylla and Vitellaria paradoxa.

+= Presence of the phytochemical compound; - = Absence of the phytochemical compound.

and body weakness) during the experiment period (Itelima and Agina, 2014). The infected mice were killed using chloroform and were buried, to stop the spread of the infection associated with enteric pathogens in the environment at the end of the study (Itelima et al., 2014).

### **RESULTS AND DISCUSSION**

The phytochemical components of the leaf extract of *E. heterophylla* were: starch, saponins, alkaloids, flavonoids and more of phenolics, phlobatannins, tannins and cardiac glycosides than its *V. paradoxa*, which revealed the presence of more steroids (Table 1).

The basis of the therapeutic activities of plants lies on the phytochemical components contained in these plants (Oyedum, 2015). This therefore implies that variations observed among the phytochemicals of two different plants will result to difference in their therapeutic abilities. This in turn is observed in this study, where the phytochemicals were found present at varying proportions among solvent extracts of the leaves of the various plants. From this study, more than 50% of E. heterophylla extracts (namely chloroform, methanol, petroleum aqueous and ether) possessed phytochemicals such as cardiac glycosides, phenolics, tannins and phlobatannins more than its V. paradoxa counterpart which revealed high contents of steroids, carbohydrates and starch among its extracts (Table 1). Onwuliri (2004) have also observed the presence of such constituents as phlobatannins, alkaloids, saponins, phenolics, tannins, cardiac glycosides among others in tropical plants growing in Nigeria, and some have been shown to exhibit varying biological activities. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowora, 1993). Mensah et al., (2008) reported the importance of alkaloids, saponins and tannins in manufacturing various antibiotics used in treating common pathogenic strains and these phytochemicals have also been known to produce a definite physiological action on human body (Edeoga et al., 2005). The presence of cardiac glycosides indicates that they may act as good sedatives and have antispasmodic properties (Egunyomi et al., 2009). Schneider and Wolfhing (2004) have reported the therapeutic effects of some phytochemical constituents such as tannins and cardiac alvcoside against cardiovascular disease and digestive problems. Therefore, the observation of these phytochemical constituents in different proportions, together with other phytochemicals found in these plants of study may be responsible for some of the observed antibacterial activity observed in this study.

Furthermore, this study showed great disparity in the results obtained from the antibacterial activities of the leaves of both E. heterophylla and V. paradoxa. The results revealed that the leaves of E. heterophylla showed high antibacterial activities than its V. paradoxa counterparts (Tables 2 to 9). This result could be attributable to the fact that high proportion of phytochemicals was observed in the leaves of E. heterophylla. This is based on the fact that the age and nature of the harvested leaves of E. heterophylla which is a shrub, as compared to its V. paradoxa counterpart which is a full grown tree, is said to affect the yield and quality of the bioactive components obtained, thereby giving rise to well concentrated and effective bioactive components. This observation conforms to the study of Calixto (2000), who reported that difference in the antibacterial activity could be due to differences in geographical location, season of plant, age of the plant, and method of extraction, all of which affect the yield and the active constituents of medicinal plants. Similarly, the difference observed in the antibacterial activities of both leaves that were studied, could be attributed to the

Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
VPCL	1.33±0.33 <sup>b</sup>	0.33±0.03 <sup>a</sup>	0.67±0.033 <sup>a</sup>	0.00±0.00 <sup>a</sup>
VPML	5.67±0.33 <sup>b</sup>	4.67±0.33 <sup>c</sup>	5.67±0.33 <sup>b</sup>	5.00±0.58 <sup>bc</sup>
VPAL	0.67±0.33 <sup>a</sup>	0.00±0.00 <sup>a</sup>	3.33±0.33 <sup>a</sup>	2.33±0.33 <sup>a</sup>
VPPL	$0.00 \pm 0.00^{a}$	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Control	18.00±0.58 <sup>d</sup>	17.33±0.67 <sup>c</sup>	17.00±0.58 <sup>d</sup>	14.33±0.67 <sup>d</sup>

Table 2. Zones of inhibition of the leaf of V. paradoxa at 50 mg.

Table 3. Zones of Inhibition of the leaf of E. heterophylla at 50 mg.

Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
EHCL	3.33±0.30 <sup>bc</sup>	3.00±0.58 <sup>bc</sup>	2.33±0.33 <sup>bc</sup>	2.00±0.58 <sup>b</sup>
EHML	6.00±0.58 <sup>f</sup>	5.67±0.67 <sup>ef</sup>	3.33±0.88 <sup>bc</sup>	5.67±0.88 <sup>b</sup>
EHAL	5.33±0.33 <sup>def</sup>	5.67±0.33 <sup>ef</sup>	4.00±0.58 <sup>cd</sup>	5.00±0.58 <sup>b</sup>
EHPL	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Control	9.00±0.58 <sup>g</sup>	8.00±0.57 <sup>g</sup>	8.67±0.68 <sup>9</sup>	8.67±0.33 <sup>e</sup>

Table 4. Zones of inhibition of the lesf of V.paradoxaat 100 mg.

Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
VPCL	4.67±0.33 <sup>b</sup>	3.33±0.90 <sup>ab</sup>	4.00±0.58 <sup>b</sup>	3.00±1.00 <sup>ab</sup>
VPML	7.00±0.58 <sup>a</sup>	5.33±0.88 <sup>a</sup>	6.67±0.33 <sup>b</sup>	6.00±0.58 <sup>a</sup>
VPAL	6.00±0.58 <sup>b</sup>	4.00±0.58 <sup>a</sup>	5.00±0.57 <sup>a</sup>	4.67±0.33 <sup>a</sup>
VPPL	2.67±0.33 <sup>a</sup>	2.00±0.58 <sup>a</sup>	2.33±0.33 <sup>a</sup>	1.67±0.67 <sup>a</sup>
CONTROL	24.67±0.33 <sup>d</sup>	23.00±0.00 <sup>c</sup>	24.00±0.58 <sup>d</sup>	20.00±0.58 <sup>c</sup>

Table 5. Zones of Inhibition of the leaf of E. heterophylla at 100 mg.

Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
EHCL	5.67±0.67 <sup>bc</sup>	5.00±0.58 <sup>bc</sup>	6.67±0.33 <sup>cde</sup>	6.33±0.33 <sup>bc</sup>
EHML	8.67±0.88 <sup>defg</sup>	8.33±0.33 <sup>efgh</sup>	8.00±0.58 <sup>cde</sup>	7.67±1.20 <sup>cdef</sup>
EHAL	8.33±0.33 <sup>def</sup>	6.33±0.90 <sup>bcde</sup>	7.000.60 <sup>cde</sup>	7.33±1.20 <sup>cde</sup>
EHPL	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Control	15.00±0.60 <sup>h</sup>	13.33±0.90 <sup>i</sup>	13.33±1.45 <sup>f</sup>	12.33±1.45 <sup>9</sup>

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet are significantly (p < 0.05). 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*; EHMS-Methanolic stem extract of *Euphorbia heterophylla*; EHCR-Chloroform root extract of *Euphorbia heterophylla*; EHMR-Methanolic root extract of *Euphorbia heterophylla*; EHAR-Aqueous root extract of *Euphorbia heterophylla*; EHRP-Petroleum ether root extract of *Euphorbia heterophylla*; EHRP-Petroleum ether root extract of *Euphorbia heterophylla*; EHRP-Petroleum ether stem extract of *Euphorbia heterophylla*; EHPR-Petroleum ether root extract of *Euphorbia heterophylla* 

different content of latex present in each plant; which is said to enhance the antibacterial potentials of various plants that contain it (Okeniyi et al., 2012; Oyedum, 2015). However, other studies have shown that *E. heterophylla* as a plant contains 0.77% of latex, of which the leaf contains 0.42% of the latex (Mosango, 2008). This

Table 6. Zones of inhibition of the leaf of V. paradoxa at 150 mg.

Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
EHCL	8.33±0.33 <sup>c</sup>	7.67±0.30 <sup>c</sup>	7.67±0.88 <sup>bc</sup>	7.33±0.90 <sup>c</sup>
EHML	11.33±0.60 <sup>d</sup>	10.33±0.33 <sup>def</sup>	10.33±0.88 <sup>cdef</sup>	9.67±0.90 <sup>cd</sup>
EHAL	10.33±1.33 <sup>cd</sup>	9.00±0.60 <sup>cde</sup>	9.33±1.45 <sup>cde</sup>	8.67±0.30 <sup>c</sup>
EHPL	4.00±0.58 <sup>ab</sup>	3.67±0.70 <sup>b</sup>	5.33±0.90 <sup>b</sup>	3.67±0.31 <sup>b</sup>
Control	20.00±0.60 <sup>f</sup>	18.67±0.70 <sup>9</sup>	19.33±0.33 <sup>9</sup>	19.00±0.58 <sup>f</sup>

Table 7. Zones of Inhibition of the leaf of E. heterophylla at 150 mg.

Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
VPCL	9.00±0.60 <sup>cd</sup>	8.33±0.33 <sup>bcd</sup>	8.67±0.30 <sup>cd</sup>	8.00±0.57 <sup>b</sup>
VPML	11.00±0.58 <sup>a</sup>	10.00±0.58 <sup>a</sup>	10.00±0.58 <sup>ab</sup>	9.33±0.30 <sup>ab</sup>
VPAL	10.33±0.33 <sup>a</sup>	9.33±0.33 <sup>a</sup>	9.33±0.65 <sup>a</sup>	8.33±0.32 <sup>a</sup>
VPPL	4.33±0.33 <sup>a</sup>	3.33±0.30 <sup>a</sup>	3.33±0.33 <sup>a</sup>	3.00±0.60 <sup>a</sup>
Control	27.00±0.58 <sup>c</sup>	26.00±0.58 <sup>d</sup>	26.33±0.33 <sup>d</sup>	25.67±0.33 <sup>d</sup>

Table 8. Zones of Inhibition of theleaf of V. paradoxa at 200 mg.

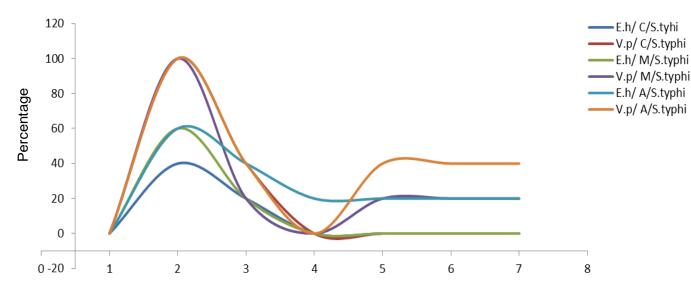
Extract	S. Typhi	S. flexneri	E. coli	P. vulgaris
EHCL	9.33±0.66 <sup>bc</sup>	8.00±0.58 <sup>bc</sup>	8.00±1.16 <sup>bc</sup>	8.00±0.60 <sup>bcd</sup>
EHML	12.00±0.60 <sup>e</sup>	11.33±0.90 <sup>e</sup>	11.67±0.88 <sup>d</sup>	10.33±0.33 <sup>e</sup>
EHAL	10.67±0.70 <sup>cde</sup>	10.00±0.60 <sup>de</sup>	10.67±0.33 <sup>cd</sup>	9.67±1.20 <sup>de</sup>
EHPL	8.33±0.33 <sup>b</sup>	7.33±0.33 <sup>b</sup>	8.00±0.00 <sup>b</sup>	6.33±0.88 <sup>b</sup>
Control	26.00±0.60 <sup>h</sup>	25.00±0.70 <sup>g</sup>	25.33±0.33 <sup>f</sup>	24.33±0.33 <sup>9</sup>

Table 9. Zones of Inhibition of the leaf of E. Heterophylla at 200 mg.

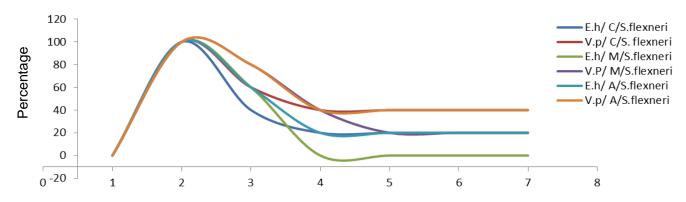
Extracts	S. typhi	S. flexneri	E. coli	P. vulgaris
VPCL	10.33±0.33 <sup>c</sup>	7.67±1.20 <sup>b</sup>	9.67±0.33 <sup>cd</sup>	9.00±0.60 <sup>bcd</sup>
VPML	12.00±0.58 <sup>a</sup>	11.33±0.33 <sup>a</sup>	11.00±0.58 <sup>a</sup>	10.68±0.30 <sup>a</sup>
VPAL	11.67±0.33 <sup>a</sup>	11.00±0.58 <sup>a</sup>	9.67±0.65 <sup>a</sup>	10.00±0.56 <sup>a</sup>
VPPL	5.33±0.30 <sup>a</sup>	4.33±0.33 <sup>a</sup>	5.00±0.00 <sup>a</sup>	4.00±0.60 <sup>a</sup>
Control	30.00±0.58 <sup>c</sup>	29.67±0.33 <sup>c</sup>	29.33±0.67 <sup>c</sup>	28.00±0.58 <sup>c</sup>

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet are significantly (p < 0.05). EHCL-Chloroform leaf extract of *Euphorbia heterophylla*; EHML-Methanolic leaf extract of *Euphorbia heterophylla*; EHAL-Aqueous stem extract of *Euphorbia heterophylla*; EHAL-Aqueous stem extract of *Euphorbia heterophylla*; EHAL-Aqueous ether stem extract of *Euphorbia heterophylla*; EHAL-Aqueous ether stem extract of *Euphorbia heterophylla*; EHAL-Aqueous root extract of *Euphorbia heterophylla*; EHAL-Aqueous ether stem extract of *Euphorbia heterophylla*; EHAL-Aqueous root extract of *Euphorbia heter* 

therefore reveals that the percentage of latex in the leaf of *E. heterophylla* is far more than its *V. paradoxa* counterpart, which in turn enhanced its antibacterial activities on the various test organisms. Figures 1 to 4 reveal that among the various groups of mice treated with the leaves of *E. heterophylla* and *V. paradoxa* which were infected with *S. typhi, S. flexneri, E. coli* and *P. vulgaris*, all the infected mice groups



**Figure 1.** Pathological signs and symptoms of *S*. Typhi infected mice. 1= Mortality; 2=Watery diarrhorea(1-3days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa*.



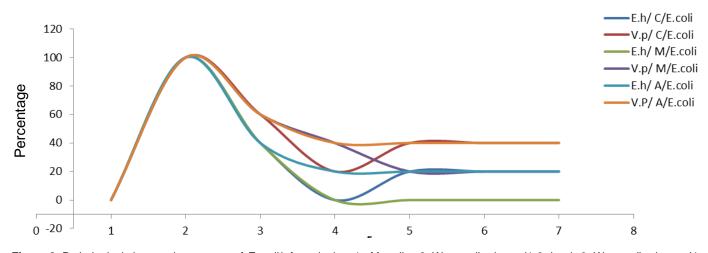
**Figure 2.** Pathological signs and symptoms of *S. flexneri* infected mice treated with various extract. 1= Mortality; 2=Watery diarrhorea(1-3days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/M=Methanolic leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa*.

treated with methanolic extracts of *E. heterophylla* showed 0% of pathological signs and symptoms (such as watery diarrhorea> 7 days, weight loss, loss of appetite and body weakness, while infected mice treated with chloroform and aqueous extracts of *E. heterophylla* showed 20% pathological signs and symptoms (such as watery diarrhorea> 7 days, weight loss, loss of appetite and body weakness) against the groups of mice treated with its *V. paradox*a counterpart, which showed 40% pathological signs and symptoms after the extracts were administered orally. This result could be attributed to the fact that bioactive components contained in the leaves of *E. heterophylla* are diverse and highly potent than those

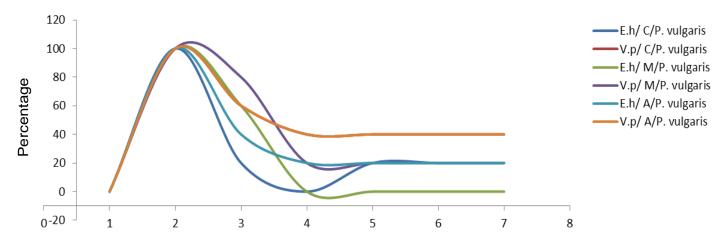
contained in the leaves of *V. paradox*a. This agrees with the result of Jayashree (2013).

#### Conclusion

The methanolic, chloroform and aqueous extracts of *E. heterophylla* contained diverse and efficient phytochemicals that were active against all test organisms at all the applied concentrations, namely: 50, 100, 150 and 200 mg/ml, indicating that *E. heterophylla* is potent and contains high yield of therapeutic properties against its *V. paradoxa* counterpart. However, the *in vivo* 



**Figure 3.** Pathological signs and symptoms of *E. coli* infected mice. 1= Mortality; 2=Watery diarrhorea(1-3 days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa*.



**Figure 4.** Pathological signs and symptoms of *Proteus vulgaris* infected mice. 1= Mortality; 2=Watery diarrhorea (1-3 days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Vitellaria paradoxa*.

studies revealed that only the methanolic leaf extract of *E. heterophylla* showed 0% pathological signs and symptoms against all the infected groups of mice treated. It is therefore recommended that in order to obtain goodyield of bioactive components from plants with effective eradication, preventive and therapeutic potentials, most especially against the emerging resistant antibiotic genes associated with most third world countries, alcohol based solvents should be utilized for extraction.

#### **CONFLICT OF INTERESTS**

The author has not declared any conflict of interest.

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