

Full Length Research Paper

Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections

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***Euphorbia hirta* powdered plant material was extracted using 3 solvents methanol, hexane and distilled water. The water extracts provided the higher yield and also more antibacterial effectiveness than when organic solvents were used. Phytochemical screening of the crude extracts revealed the presence of tannins, saponins, phenolics, flavonoids, cardiac glycosides, anthroquinones and alkaloids. This presence of these bioactive constituents have been linked to the antimicrobial activity of the plant material, the agar well diffusion method was used to determine the antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi* and *Proteus mirabilis*, a group of gram-negative bacteria that frequently cause enteric infections in humans. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranged from 25 to 100 mg/ml. The growth of all the bacteria were inhibited though to varying degrees, thus justifying the use of the herb in traditional medicine in treating enteric infections, with *E. coli* and *S. typhi* being more susceptible, the antibacterial activity of the plant material is enhanced under acidic conditions and at elevated temperatures. The herb *E. hirta* can be used as source of oral drugs to fight infections caused by susceptible bacteria.**

Key words: *Euphorbia hirta*, phytochemical, methanolic extract, hexane extract, aqueous extract, enteric infections, antimicrobial activity.

INTRODUCTION

Infectious diseases are major causes of morbidity and mortality in the developing world and accounts for about 50% of all deaths. In Bangladesh, about 17% of all children admitted to the paediatric wards die of diarrhea (Alam et al., 2001). Some 5.8 million deaths each year in infants and children below 5 years are caused by enteric diseases world wide (Amita et al., 2003). Records of morbidity and mortality occurring as a result of enteric infections are scanty in Nigeria. Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics. Bacterial resistance to antibiotics increases mortality, likelihood of hospitalization and the length of stay in the hospital (Winstanley et al., 1997). For most bacteria, there is evidence that increased usage of a particularly antimicrobial correlates with increased levels of bacterial resistance to that agent (Mordi and Erah, 2006). Spread of resistance, which is transferable amongst members of the enterobacteriaceae

has been attributed to the mobilization of drug resistance markers by a variety of agents encoded on plasmids, transposons and integrons (Amita et al., 2003). Isolation of bacteria less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is now a global problem (Muhammad and Muhammad, 2005). In the developing world, the situation is even worst because of poor sanitation and ignorance of good hygienic practices thus exposing a large number of people to infectious agents. Some of the bacteria implicated in causing enteric infections include but not limited to *E. coli*, *salmonella spp.*, *Proteus spp.*, *shigella spp.*, *pseudomonas spp.* and the *staphylococci*. These bacteria which are usually present as commensals, have several virulent factors and colonize in a biofilm fashion, causing a variety of intestinal and extra intestinal diseases (Lino and Deogracious, 2006). There is therefore the need to develop some newer, safer, effective and above all,

cheaper antimicrobial agents to tackle this problem. The success story of modern medicine lies in the continuous search for new drugs to counter the challenges posed by resistant strains of bacteria. There are several reports in the scientific literature describing the antimicrobial properties of crude extracts prepared from plants (Muhammad and Muhammad, 2005; Falodun et al., 2006; El-Mahmood and Amey, 2007) and such reports had attracted the attention of scientists worldwide (Falodun et al., 2006; Lai et al., 2008). Herbs have been used as sources of food and medicinal purposes for centuries and these knowledge have been passed on from generation to generation (Adedapo et al., 2005). This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far between and where the people nurse their ailments back to health using local herbs.

Euphorbia hirta Linn. is one of such herbs belonging to the family *Euphorbiaceae* which is frequently seen occupying open waste spaces and grasslands, road sides, and pathways. Though a native of central America, the herb is widely cultivated throughout the tropics, especially in west, central and east Africa (Adedapo et al., 2005). It is usually erect, slender-stemmed, spreading up to 45 cm tall, though sometimes can be seen lying down (Burkill, 1994). The plant is an annual broad-leaved herb that has a hairy stem with many branches from the base to the top. The stem and leaves produce white or milky juice when cut. The medicinal usefulness of the herb has been the subject of numerous chemical and microbiological studies. Some of the reported phytoconstituents of the herb included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid, while some of the reported scientific uses include its use as an antispasmodic, antiasthmatic, expectorant, anti-catarrhal and antisiphilitic (Burkill, 1994; Adedapo et al., 2005; Falodun et al., 2006). Most of the activities of the plant are believed to be due to the presence of choline, shikimic acid and the quercetin (Bala, 2006; Falodun et al., 2006). *E. hirta* is a very popular herb amongst practitioners of traditional medicine and some of its local names include *nonon furchiya* in Hausa, *tepel* in fulfulde, *Harvom* in Kaka and Hammock sand mat (Florida). Commonly called asthma weed in Asia and Australia, the herb is widely used in traditional medicine to treat a variety of diseased conditions including asthma, coughs, diarrhea and dysentery (Stuart, 1979; Ogbolie et al., 2007). In east, central and west Africa, a decoction of the herb is used to treat asthma, oral thrush, boils, sores, skin and wound infections, in addition to its been used as an antispasmodic, antipruritic, carminative, depurative, diuretic, febrifuge, galactagogue, purgative and vermifuge (Alabashi et al., 1999; Palombo and Semple, 2001; Darwish et al., 2002; Ogbolie et al., 2007). In Mauritius, a decoction of the plant is used to treat respiratory tract infections, vomiting, fever, bronchitis and pulmonary disorders (Darwish et al., 2002; Bala, 2006). In Nigeria, exudates of the stem is used to treat eye and ear infections

(Igoli et al., 2005), while a decoction of the plant is used to treat enteric infections including diarrhea and dysentery, constipations and other stomach problems, asthma, bronchitis, eczema, athletes foot and scorpion bite pains (Stuart, 1979; Bala, 2006; Ogbolie et al., 2007). It is usually taken in dosages of 0.1 - 0.3 g dried powdered herb 3 times a day and 0.12 - 2 ml 3 times daily and repeated over 2 - 3 weeks (Duke and Ayensu, 1985).

Because of its wide usage and easy availability, this study was undertaken to investigate the phytochemical properties and antibacterial activities of the plant against some economically important bacteria that cause a variety of intestinal and extra intestinal diseases. The effect of pH and temperature on the efficacy of the crude extracts were also investigated.

MATERIALS AND METHODS

Collection of plant material

The fresh plant *E. hirta* was collected around the surroundings of main campus of the Federal University of Technology, Yola, Adamawa State, Nigeria. The taxonomical identification of the plant was confirmed by Mr. Bristones Bariri of the Department of Biological Sciences, School of Pure and Applied Science, Federal University of Technology Yola, Nigeria.

Preparation of plant material

The fresh plant was harvested, rinsed with tap water and air dried under shade for 14 days and reduced to coarse powder using pestle and mortar and then grinded to fine powder using the kenwood electric blender (Kenwood Limited, Harvart, United Kingdom). The powder was stored in an airtight bottle until needed for use.

Preparation of the extracts

100 g of the powdered sample (whole plant) was soaked in 100 ml of solvent contained in a 500 ml sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminium foil and shaken vigorously. The mixture was left to stand over night (24 h) in a shaking water bath maintained at 40 °C. The mixture was then filtered using a clean muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using a rotary evaporator attached to a vacuum pump (Model type 349/2, Corning Limited). The percentage yield of the crude extract was determined for each solvent and was for water 39%, methanol 18% and hexane 13%. The percentage extract yield was estimated as dry weight/dry material weight x 100 (Parekh and Chanda, 2007). For the preparation of dilutions of crude extracts for antibacterial assay, the extracts was reconstituted by dissolving in the respective extracting solvents and further diluted to obtain 400, 200, 100, 50, 25, 12.5, 6.25, 3.085 and 1.03 mg/ml. The reconstituted extracts were maintained at a temperature between 2 - 8 °C.

Microorganisms

The bacteria were clinical isolates obtained from patients previously diagnosed with gastro-intestinal infections. The organisms were collected in peptone water from the Microbiology Department of the 750-bed, Specialist Hospital Yola, Nigeria, with the help of the labo-

Table 1. Percentage Yield of the Crude Extracts of *E. hirta*.

Extraction solvent	Raw plants powder (g)	Extracted plant powder (g)	Percentage yield (%)
Distilled water (aqueous)	100	3.9	3.9
Methanol	100	1.8	1.8
Hexane	100	1.3	1.3

ratory staff and immediately transported to the Microbiology Department of Federal University of Technology, Yola. The bacteria were isolated and identified as *E. coli*, *Proteus mirabilis*, *Shigella dysenteriae*, *Salmonella typhi* and *Klebsiella pneumoniae* following standard procedures as described by Cowan and Steel (1974) and Cheesbrough (2002). The bacteria were maintained at a temperature between 2 - 8°C. Standardization of culture was carried out according to Baker and Thomsberg (1983) and the National Committee for Clinical Laboratory Standards (NCCLS, 1990) by suspending an 18 h culture of bacteria into sterile universal bottles containing nutrient broth. Normal saline was gradually added so as to compare its turbidity to Mcfarland Standard of 0.5 which corresponds to approximately 1.0×10^8 cfu/ml.

Photochemical screening of the plant material

Phytochemical screening was carried out on the powdered plant material for the presence of bioactive components such as tannins, phenols, alkaloids, cardiac glycosides, anthroquinones, saponins and flavonoids (Emeruwa, 1982; Trease and Evans, 1996).

Determination of the antimicrobial activity

The method described by Emeruwa (1982) was used. Briefly, 1.0 ml of 18 h culture of bacteria adjusted to 1.0×10^8 cfu/ml was spread into a sterile plate so as to achieve a confluent growth. 3 petri dishes containing a particular bacteria was used. Then 19.0 ml of Mueller Hinton agar at 45°C was added to each plate and the plates were rocked for even spread and proper mixing of bacteria and agar. The content of the plates were allowed to solidify and wells approximately 6 mm in diameter and 2.5 mm deep were bored on the surfaces of the agar medium using a sterile cork borer. Then 0.5 ml of the reconstituted extract at a concentration of 100 mg/ml was pipetted in to one of the holes. 0.5 ml of pure solvent was pipetted into another hole as negative control while an aqueous solution of 12.5 ug amoxicillin was used as positive control. The plates were allowed to stand for 1 h for prediffusion of the extract to occur and then incubated at 37°C for 24 h and the zones of inhibition were measured to the nearest mm. The mean of triplicate results were taken.

Determination of MIC and MBC

Determination of the minimum inhibitory concentration (MIC) was carried out using the Broth dilution method (Sahm and Washington, 1990; Adesokan et al., 2007; Oyeleke et al., 2008). Briefly, 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100 mg/ml. 1 ml of this dilution was transferred to another test tube till the 7th test tube was reached. The 8th test tube did not contain any extract, but a solution of pure solvent and served as negative control. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 10^8 cfu/ml was put into each tube and thoroughly mixed on a vortex mixer. The

tubes were incubated at 37°C for 24 h and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

The MBC values were determined by removing 0.10 ml of bacterial suspension from the MIC tubes that did not show any growth and subcultured into Mueller Hinton agar plates and incubated at 37°C for 24 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC.

Effects of pH

This was carried out as previously described (El-Mahmood et al., 2008). Briefly, the extracts were reconstituted in to 3 separate test tubes each containing 100 mg/ml of extract in 4 ml test tubes. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 10^8 cfu/ml was put into each tube and thoroughly mixed on a vortex mixer. The first tube was treated with 1N hydrochloric acid by adding it drop wise until a pH of 2 was obtained. The second tube was treated with 1M sodium hydroxide by adding it drop wise until a pH of 10 was reached. The test tubes were left to stand for 1 h and then neutralized by acid or alkali treatment as the case might be. The third test tube was not treated and served as control. The test tubes were incubated at 37°C for 24 h. Antibacterial activity was determined as previously described.

Effect of temperature

The effects of temperature on the efficacy of the crude extracts was determined by reconstituting the powdered extracts to obtain a concentration of 100 mg/ml in 3 separate test tubes of 4 ml each. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 10^8 cfu/ml was put into each tube and thoroughly mixed on a vortex mixer. The first test tube was treated at a temperature of 10 °C in a refrigerator for 1 h and the second test tube was treated at a temperature of 100°C in a water bath, also for 1 h, after which both test tubes were removed and left to acclimatize at room temperature. The third test tube was not subjected to either cold or heat treatment and served as control.

RESULTS AND DISCUSSIONS

Percentage yield of the powdered plant *E. hirta* crude extracts obtained using various solvents is shown Table 1. Out of the 100 g of powdered plant material, the percentage yield obtained was for water 3.9%, methanol 1.8% and hexane 1.3%. This yield was far lower than that obtained by Doughari et al. (2008) for *Senna obtusifolia* who reported a yield of 52% for water extract, 50% for hexane extract and 28% for dichlormethane and that of Owolabi et al., (2007) who reported a yield of 10.74% for water extract and 3.78% of their ethanolic extracts. Ogbolie et al. (2007) also reported a yield of 9.1% in wa-

Table 2. Photochemical constituents of *Euphorbia hirta*.

Organism	Solvent (mg/ml)		
	Aqueous	Methanol	Hexane
Saponins	+	+	+
Alkaloids	+	+	+
Phenolics	+	+	+
Tannins	+	+	+
Cardiac glycosides	+	+	+
Anthroquinones	+	+	+
Flavonioids	+	+	+

Key: + = positive, - = negative.

Table 3. Antibacterial activity of *Euphorbia hirta*.

Organism	Zone of inhibition diameter (mm)			
	Aqueous	Methanol	Hexane	Amoxicillin
<i>E. coli</i>	18.0	13.0	11.0	26.0
<i>K. pneumoniae</i>	18.0	16.0	11.0	25.0
<i>S. typhi</i>	17.0	11.0	11.0	25.0
<i>P. mirabilis</i>	19.0	16.0	10.0	24.0
<i>S. dysenteriae</i>	21.0	10.0	9.0	22.0

ter extracts of *E. hirta*. Factors like the age of the plant and the polarity of the solvent used affect the yield. Thus in this studies, water seems to be the best solvent for this plant material, thus supporting the use of water as solvent of choice in traditional practice. Phytochemical screening of the crude extracts of *E. hirta* revealed the presence of some bioactive components as shown in Table 2. It contains tannins, phenolics, cardiac glycosides, anthraquinones, saponins, flavonoids and alkaloids. These compounds have potentially significant application against human pathogens, including those that cause enteric infections (El-Mahmood et al., 2008). Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Sahm and Washington, 1990; Adesokan et al., 2007; Ogbolie et al., 2007; Owolabi et al., 2007; Oyeleke et al., 2008). The presence of alkaloids is interesting, as significant quantities are used as antimalarials, analgesics and stumilants (Duke and Ayensu, 1985). The presence of glycosides moieties like saponins, anthraquinones, cardiac glycosides and flavonoids which are known to inhibit tumor growth and serve also to protect against gastrointestinal infections are of pharmacognostic importance and give credence to the use of the plant in ethnomedicine. Herbs that have tannins as their components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery thus exhibiting antibacterial activity (Akinpelu and Onakoya, 2006). Tannins are widely used in traditional medicine in treating wounds and to arrest bleeding (Nguyi, 1988). Some of these bioactive compounds which are synthesized as secondary metabolites as the plant grows, also serve to protect the plant against microbial

attacks and predation by animals (El-Mahmood et al., 2008). The increasing reliance on the use of medicinal plants by a sizeable proportion of the people in the so-called industrial world has been traced to the extraction and development of several drugs and chemotherapeutic agents from these plants as well as from traditionally used rural herbal remedies (El-Mahmood et al., 2008).

Antibacterial activity of crude extracts of *E. hirta* were evaluated by measuring the diameters of zones of growth inhibition on some members of the enterobacteriaceae and the results are presented as shown in Table 3. All the test organisms were susceptible to *E. hirta* extracts though to varying degrees. Karou et al. (2006) reported that the susceptibility of bacteria to plant extracts, on the basis of inhibition zone diameters varied according to strains and species, similar to the data obtained in this study. The highest zone of growth inhibition was shown by aqueous extract against *E. coli* (18.0 mm), *K. pneumoniae* (18 mm), *P. mirabilis* (19 mm), *S. dysenteriae* (21 mm) and *S. typhi* (17 mm). For hexane extract, the measured zone diameter was *E. coli*, (11.mm), *P. mirabilis* (10 mm), *S. typhi* (11 mm), *S. dysenteriae* (9 mm) and *K. pneumoniae* (11 mm). Methanol extract gave inhibitory zones of 13 mm for *E. coli*, 16 mm for *P. mirabilis*, 11 mm for *S. typhi*, 10 mm *S. dysenteriae* and 18 mm for *K. pneumoniae*. All the bacteria used in this study are gram-negative bacteria which are known to be resistant to the action of most antimicrobial agents including plant based extracts and these have been reported by several scholars (Geyid, 2002; Kambezi and Afolayan, 2008). Gram-negative bacteria have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents

Table 4. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extracts of *Euphorbia hirta*.

Organism	Solvents (mg/ml)							
	Aqueous		Methanol		Hexane		Amoxicillin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Escherichia coli</i>	50	100	100	100	100	200	12.5	25
<i>K. pneumoniae</i>	25	50	50	100	100	200	6.25	12.5
<i>S. typhi</i>	50	100	100	100	100	200	12.5	12.50
<i>P. mirabilis</i>	25	50	50	100	100	200	12.5	12.5
<i>S. dysenteriae</i>	25	50	50	50	100	100	6.25	6.25

(Nikaido and Vaara, 1985). However, Afolayan et al. (2002) have reported the inactivity of the shoots of *Helichrysum aureonitens* against some *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. marcescens*. The water extracts are more effective than methanol and hexane extracts. The large zone sizes produced by the plant extract against the test bacteria, especially the aqueous extracts is an indication of the potency of the bioactive components of the plant against all the test bacteria. Amoxicillin which served as positive control and at lower concentrations produced larger zone diameters for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. dysenteriae*, *P. mirabilis* and *S. typhi* than the crude extracts.

Aqueous extract showed minimum inhibitory concentration (MIC) for *E. coli* (50 mg/ml), *P. mirabilis* (25 mg/ml), *S. typhi* (50 mg/ml), *S. dysenteriae* (25 mg/ml) and for *K. pneumoniae* (50 mg/ml) as detailed in Table 4. For the hexane extract, the MIC for all the test bacteria were 100 mg/ml. The MIC values produced by the methanol extract for *E. coli* and *S. typhi* were 100 mg/ml, while those for *S. dysenteriae*, *P. mirabilis* and *K. pneumoniae* were 50 mg/ml respectively. The MIC values for hexane followed similar pattern. The aqueous extract gave an MBC of 100 mg/ml for *E. coli* and *S. typhi* respectively, while for *S. dysenteriae*, *K. pneumoniae*, *P. mirabilis*, the MBC values were 50 mg/ml, respectively. In the case of methanolic extract, the MBC recorded was 100 mg/ml for *E. coli*, *P. mirabilis*, *K. pneumoniae* and *S. typhi* respectively and for *S. dysenteriae* the MBC value was 50 mg/ml. For hexane extract, the MBC values were 200 mg/ml for all the pathogen, similar pattern to MIC values though higher. The MIC and MIB values for the control antibiotic, amoxicillin were far lower than the crude extracts. The effects of the crude extracts correlate with reports that microorganisms varied widely in their degree of susceptibility (Emeruwa, 1982; Banso and Mann, 2006). Agents with low activity against a particular organism usually gives high MIC and MBC values, while a highly reactive agent gives low MIC and MBC values. The MIC and MBC techniques are used to evaluate the efficacies of antimicrobial agents and in this study, the MIC and MBC values tend to support the results obtained in the antibacterial screening above showing clearly that the aqueous extracts were more potent than either methanol or hexane extracts. The MIC values obtained for the entire test

bacteria are high ranging from 25 to 100 mg/ml, when compared to the MIC values of 0.01-10 ug/ml frequently recorded for conventional antibiotics. The results obtained here are similar to those presented by Ade-sokan et al. (2007). George et al. (2002) explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities. Hugo and Russell, (1984) have reported that the MBC values can either be the same or higher than the MIC values. In this study, the MIC values were either the same or slightly lower than the MBC values, similar to the results of Karou et al. (2006). The MIC and MBC values are predictive of the efficacy of agents *in-vivo*. However, the MBC values which are obtained after plating various dilutions of the extracts, is more reliable than the MIC values, obtained using turbidity as an index of growth (Junaid et al., 2006)

The effects of pH on the activity of the extracts are given in Table 5. The antimicrobial activities of the crude extracts of *E. hirta* were evaluated at 2 different pH values (pH 2 and pH 10). For aqueous extracts, the zone diameters are: 28 mm at pH 2 and 24 mm at pH 10 for *E. coli*, 20 mm at pH 2 and 18 mm at pH 10 for *P. mirabilis*, 27 mm at pH 2 and 23 mm at pH 10 for *S. typhi*, 21 mm at pH 2 and 17 mm at pH 10 for *S. dysenteriae* and 22 mm at pH 2 and 20 mm at pH 10 for *K. pneumoniae*. A similar growth inhibition was exhibited by a methanol extract: 30 mm pH 2 and 26 mm at pH 10 for *E. coli*, 22 mm at pH 2 and 18 mm at pH 10 for *P. mirabilis*, 25 mm at pH 2 and 23 mm at pH 10 for *S. typhi*, 23 mm at pH 2 and 21 mm at pH 10 for *S. dysenteriae* and 25 mm at pH 2 and 23 mm at pH 10 for *K. pneumoniae*. Growth inhibition shown by hexane followed the same manner: 18 mm at pH 2 and 15 mm at pH 10 for *E. coli*, 16 mm at pH 2 and 16 mm at pH 10 for *P. mirabilis*, 20 mm at pH 2 and 16 mm at pH 10 for *S. typhi*, 15 mm at pH 2 and 14 mm at pH 10 for *S. dysenteriae* and 16 mm at pH 2 and 14 mm at pH 10 for *K. pneumoniae*. For all the extracts, activity was more under acidic than alkaline conditions. Similar observations were made by Doughari et al. (2008) and El-Mahmood et al. (2008). Acid stability is an important property of drugs, because it means that the plant components can be formulated to be taken orally and will not be inactivated

Table 5. Effect of pH on the antibacterial activity of *E. hirta*.

Organism	Aqueous		Methanol		Hexane	
	pH 2	pH 10	pH 2	pH 10	pH 2	pH 10
<i>E. coli</i>	28	24	30	26	18	15
<i>K. pneumoniae</i>	22	20	25	23	16	14
<i>S. typhi</i>	27	23	25	23	20	16
<i>P. mirabilis</i>	20	18	22	18	16	16
<i>S. dysenteriae</i>	21	17	23	21	15	15

Table 6. Effect of temperature on the antimicrobial activity of *E. hirta*.

Organism	Aqueous		Methanol		Hexane	
	100°C	10 °C	100°C	10 °C	100°C	10 °C
<i>E. coli</i>	18.0	14.0	17.0	13.0	14.0	10.0
<i>K. pneumoniae</i>	17.0	13.0	16.0	14.0	13.0	9.0
<i>S. typhi</i>	18.0	15.0	15.0	12.0	14.0	12.0
<i>P. mirabilis</i>	15.0	13.0	15.0	13.0	11.0	10.0
<i>S. dysenteriae</i>	14.0	12.0	14.0	11.0	12.0	11.0

under the acidic conditions of the stomach and the gastrointestinal tract.

The effects of temperature on the efficacy of the extracts are given in Table 6. The temperature was adjusted between 10 and 100°C for all extraction solvents. For methanolic extract, *E. coli* was inhibited by a zone diameter of 17 mm at 100 °C and 13 mm at 10°C, for *P. mirabilis*, the zone diameter were 15 mm at 100 °C and 13 mm at 10 °C, *S. typhi* gave a zone of growth inhibition diameter of 15 mm at 100°C and 12 mm at 10 °C, *S. dysenteriae* gave a zone of growth inhibition diameter of 14 mm at 100°C and 11 mm at 10°C and *K. pneumoniae* inhibited the growth of the bacteria with zone diameters of 16 mm at 100°C and 13 mm at 10°C. For aqueous extract, *E. coli* gave a zone diameter of 18 mm at 100 °C and 14 mm at 10 °C, *P. mirabilis* gave a zone diameter of 15 mm at 100°C and 13 mm, *S. typhi* gave a zone diameter of 18 mm at 100°C and 15 mm at 10°C, the diameter of zone of growth inhibition for *S. dysenteriae* was 14 mm at 100°C and 12 mm at 10°C while *K. pneumoniae* gave a zone diameter of 17 mm at 100°C and 13 mm at 10°C. The diameters of zones of inhibition of hexane extract for the test organisms were as follows: *E. coli*, 14 mm at 100°C and 10 mm at 10 °C, *P. mirabilis*, 11 mm at 100°C and 10 mm at 10°C, *S. typhi*, 14 mm at 100°C and 12 mm at 10°C, *S. dysenteriae*, 12 mm at 100°C and 11 mm at 10°C and *K. pneumoniae*, 13 mm at 100°C and 9 mm at 10°C. As the temperature was increased, the antibacterial activity also increased, similar to the data presented by Doughari et al. (2008) and El-Mahmood et al. (2008). The traditional practitioners usually boil the plants before dispensing out to patients. The results obtained in this study support the methods used by the traditional healers. It is evident from the

results that water extract has some significantly high antibacterial activity, suggesting that the active principles are more soluble in water and that water is the appropriate solvent for the extraction of the bioactive principles present in *E. hirta*, similar to the reports of Falodun et al. (2006) and El-Mahmood and Amey (2007), but contrary to that of Bansa and Mann (2006). This is a clear indication that the solvent system plays a significant role in the solubility of the active principles in the plant and influences the antibacterial activities. This can be explained in terms of the polarity of the compound being extracted by each solvent and in addition to their intrinsic bioactivity, their ability to dissolve or diffuse in the media used in the assay. The pathogenic bacteria varied in their susceptibility to the crude plant extracts, similar to the observations of Ergene et al. (2006). There are several other reports stating that other *Euphorbia* species extracts exhibit antibacterial activities. The methanolic, acetone and chloroform extracts of *Euphorbia fuciformis* exhibited significant antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris* and *S. typhi* and that the methanolic extracts were more potent (Natarajan et al., 2005). In their own studies, Alabashi et al. (1999) reported that the acetone and water extracts of *Euphorbia fruticososa* showed significant antibacterial activity as well as the methanol extracts of *Euphorbia macroclada* studied by Darwish et al. (2002). Also, Palambo and Semple (2001) reported that crude extracts of *Euphorbia australia* inhibited the growth of *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. typhi*. In this study, the crude extracts *E. hirta* herb inhibited the growth of such recalcitrant gram-negative bacteria that cause majority of diarrhoeal diseases and which usually display above average resistance to most

antibiotics and non-antibiotics antibacterial agents. These bacteria which have several virulence factors, also have intrinsic resistance from a restrictive outer membrane barrier and trans-envelope multidrug resistance pumps (Winstanley et al., 1997). The efficacy of the extracts, probably due to the identified secondary metabolites, further confirm its use as an antibacterial agent in folkloric medicine and may thus be useful in the treatment of enteric infections. The plant can be used to source for oral antibacterial drugs that can treat infections caused by susceptible gram-negative bacteria.

Although *E. hirta* was found to contain some bioactive compounds with pronounced antibacterial activities, further phytochemical and Pharmacological studies will be needed to isolate the active constituents and evaluate the antimicrobial activities against a wide range of microbial pathogens.

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