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Research Article

Health Implications of Using *Euphorbia hirta* Linn. (Euphorbiaceae) Extracts in the Treatment of 'Jedi Jedi' Infection in Newborns (Age one to six months) in Nigeria

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ABSTRACT: **Objective:** To determine the effects of the crude extracts of Euphorbia hirta in treating 'Jedi Jedi' infection in newborns, in Nigeria. **Materials and Methods:** The powdered plant material of *Euphorbia hirta* was extracted in three solvents ethanol, benzene and water using the Soxhlet extraction apparatus 241 (PSAWINDIA). Phytochemical screening was conducted to determine the plant constituents, while acute and chronic toxicity studies were carried out *in vivo* using nine albino mice. **Results:** The percentage yields of the extracts were 59 (water), 33 (ethanol), and 16 (benzene). Phytochemical screening of the crude extracts revealed the presence of saponins, flavonoids, cardiac glycosides, cyanogenic glycosides, anthraquinones, and alkaloids. The presence of these constituents was linked to the antibacterial activity of the plant, using the agar well-diffusion method against bacteria associated with 'Jedi Jedi' infection, namely, *Escherichia coli, Bacillus subtilis, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) of the extracts of *E. coli, B. subtilis, P. mirabilis, S. typhi*, and *P. aeruginosa* were 50 mg/ml, 25 mg/ml, 50 mg/ml, 25 mg/ml, and 25 mg/ml respectively. Toxicity studies revealed that most of the animals were adversely affected on long-term usage of the plant extracts, leading to the death of some of the animals while short-term usage produced organ inflammation, hypertrophy, ulcers and necrosis of some organs. **Conclusion:** Although these bacteria were susceptible to the extracts in various solvents that were significantly different from the control drug Cefuroxime Axetil® USP 100 ml (Bharat Parenterals Ltd., India) ($p \leq 0.05$), the results suggested that the use of the extracts of E. hirta as an oral medication, both on short -term and long-term was not safe, due to the presence of some toxic constituents.

KEYWORDS: Benzene extract, Ethanol extract, *Euphorbia hirta*, Infection, phytochemical, toxicity, water extract

INTRODUCTION

Infectious diseases are major causes of morbidity and mortality in newborns (infants) in the developing world and account for about 67% of all deaths.[1] In Bangladesh, about 17% of all children admitted to the paediatric wards die of diarrhoea.[1] Some 5.8 million deaths each year in infants and children below five years are caused by enteric diseases world wide.[2] Records of morbidity and mortality occurring as a result of enteric infections are scanty in Nigeria. Most of the pathogens causing infections have developed resistance to the commonly prescribed antibiotics.[3] Bacterial resistance to antibiotics increases mortality, the likelihood of hospitalization and the length of stay in the hospital.[3] For most bacteria, there is evidence that increased usage of a particularly antimicrobial correlates with increased levels of bacterial resistance to that agent.[4] Spread of resistance, which is transmitted among members of the enterobacteriaceae, has been attributed to the mobilization of drug resistance

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markers by a variety of agents encoded on plasmids, transposons and introns.[2] Isolation of bacteria less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is now a global problem.[5]

In the developing world, the situation is even worse 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 infections include, but are 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 bio film fashion, causing a variety of intestinal and extra intestinal diseases.[6] 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 scientific literature describing the antimicrobial properties of crude extracts prepared from plants[5,7,8] and such reports have attracted the attention of scientists worldwide.[7,9] Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation.[10] 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 such herb belonging to the family *Euphorbiaceae,* which is frequently seen occupying open waste spaces and grasslands, road-sides, and pathways. Although a native of Central America, the herb is widely cultivated throughout the tropics, especially in west, central and east Africa.[10] It is usually erect, slender-stemmed; spreading up to 45 cm tall, although sometimes can be seen lying down.[11] 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 phyto-constituents of the herb include 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, anticatarrhal, and antisyphilitic[7, 10, 11] Most of the activities of the plant are believed to be due to the presence of choline, shikimic acid, and the quercetin.[7, 12]

E. hirta is a very popular herb among practitioners of traditional medicine and some of its local names include '*Nonon furchiya*' in Hausa, '*Tepel*' in Fulfulde, '*Harvom*' in Kaka, 'Tawa tawa' in Chinese, and Hammock sand mat (Florida). Commonly called the asthma weed in Asia and Australia, the herb is widely used in traditional medicine to treat a variety of disease conditions including asthma, cough, diarrhoea and dysentery.[13,14] In east, central, and west Africa, a decoction of the herb is used to treat asthma, oral thrush, boils, sores, and skin and wound infections, in addition to its been used as an antispasmodic, antipruritic, carminative, depurative, diuretic, febrifuge, galactagogue, purgative, and vermifuge.[1,17] On account of the folkloric use of the plant in almost all parts of Nigeria to treat 'Jedi Jedi' infection; a disease that infects newborns (age one to six months) on their skin, like pimples. It is reddish in color when it appears, and causes severe pain to the newborns. There is a need to investigate this plant for its acclaimed potency as well as its safety.

MATERIALS AND METHODS

Collection of Plant Material

The fresh plant of *E. hirta* was collected from the premises of the Federal polytechnic Idah, Kogi State, Community School garden in Ogurugu, Enugu State, Ijanikin forest Lagos, and a forest in Jaji Kaduna State. It was identified by Mr. U.S Galla of the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria; where a voucher number is deposited for the plant.

Preparation of Plant Material

The fresh plant was rinsed with tap water and air-dried under shade for a week, and then reduced to coarse powder using a pestle and mortar. Subsequently, it was ground into fine powder using the Kenwood electric blender (Kenwood Limited, Havant, United Kingdom). The powder was then stored in an airtight bottle for further use.

Preparation of the Extracts

Two hundred grams of the powdered sample (whole plant) was soaked in 100 ml of solvent in a Soxhlet extractor 241(PSAWINDIA) and left to stand for 24 hours, to extract. 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; for water it was 59%, for methanol 33%, and for benzene 16%. The percentage extract yield was estimated as dry weight/dry material weight × 100.[18] For the preparation

of the dilutions of crude extracts for antibacterial assay, the extracts was reconstituted by dissolving in the respective extracting solvents and further diluted with distilled water 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 and 8°C.

Phytochemical 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, anthraquinones, cyanogenic glycosides, saponins, and flavonoids.[19]

Biological Evaluation of the Extracts of *E. hirta*

The following were carried out in order to assess the potency of the crude extracts of the plant on bacteria associated with 'Jedi Jedi' infection in the newborns. The bacteria tested were both Gram-positive and Gram-negative. These are *E. coli, Bacillus subtilis, Proteus mirabilis, Salmonella typhi, and Pseudomonas aeruginosa*. All the bacteria were obtained from the Department of Microbiology ABUTH. The Isolates were checked for purity and maintained in the slant of the nutrient agar.

Antibiotics Susceptibility Testing of Extracts

The method described by Emeruwa[18] was used. Briefly, 1.0 ml of an 18- hour culture of bacteria adjusted to 1×10^8 cfu/ml was spread onto a sterile plate so as to achieve a confluent growth. Three Petri dishes containing a particular bacterium 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. Pure solvent 0.5 ml was pipetted into another hole as the negative control while an aqueous solution of 12.5 ml Cefuroxime Axetil was used as the positive control. The plates were allowed to stand for one hour for pre-diffusion of the extracts to occur and then incubated at 37°C ± 2°C for 24 hours and the zones of inhibition were measured to the nearest millimetre. The means of triplicate results were taken.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was carried out using the Broth dilution method.[19,20]

Acute and Chronic Toxicity Investigations of Extracts

Using Lork's method of 1983,[24] 0.5 g of the crude extracts were weighed and prepared into appropriate millilitres in order to determined the lethal dose (LD_{50}) (acute toxicity) of the extracts in Swiss white male albino mice numbering thirty-nine(39), that is, 13 for each extract .The animals were weighed using an electronic scale balance. The experiment was divided into two phases. In phase one, nine animals, three animals per group were used and doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg b.w aqueous, ethanol and benzene extracts administration i.p were given to the animals. The animals were fed with ordinary water on the first day and later with maize feeds, and then monitored for any deaths or changes in behaviour for one week.

In the second phase, the remaining four animals were divided into three groups of one animal per group and administered doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg b.w extract (i.p) and observed for changes or death for 24 hours. The LD_{50} was calculated as the geometric mean of the dosage that resulted in 100% mortality and that dosage which caused no mortality at all.[24] Recovery and body weight gain after each investigation was taken as a sign of surviving the acute intoxication. The experiment was terminated after 3 months and the animals were observed for pathological damage as a result of prolonged intake (chronic) of the extracts.[24]

RESULTS

Percentage yield of the powdered plant *Euphorbia hirta* crude extracts, obtained using various solvents are shown Table 1. Out of the 200 g of powdered plant material, the percentage yield obtained for water was 59%, for ethanol 33%, and for benzene 16%.

Phytochemical screening of the crude extracts of *E. hirta* revealed the presence of some bioactive components as shown in Table 2. It contained tannins, terpenes, cardiac glycosides, anthraquinones, saponins, cyanogenic glycosides, flavonoids, and alkaloids.

Table 1: Percentage yields of extracts of *E. hirta*

Extraction Solvent	Yields (%)
Water	59.0
Ethanol	33.0
Benzene	16.0

Table 2: Phytochemical screening and chromatographic resolution of crude extracts

Class of compounds	R_F values of spots		
	Aqueous	Ethanol	Benzene
Alkaloids	0.53	0.81	–
Flavonoids	0.74	0.84	0.83
Saponins	0.81	0.65	0.78
Tannins	0.85	0.90	0.86
Anthraquinones	0.91	0.63	–
Cyanogenic gly	0.72	0.67	0.65
Terpenes	0.90	0.64	0.68
Cardiac gly	0.82	–	–

– (absent), solvent system (methanol-chloroform; 4:1) gly (glycosides), R_F values were obtained by spraying detecting reagents and then calculated.

Table 3: Zones of inhibition of extracts of *E. hirta* against the microbes

Test Organism	Mean zone of inhibition ± SE (mm)		
	Aqueous	Ethanol	Benzene Cefx®
Escherichia coli	39 + 0.60	37 ± 0.60	30 + 0.17 43 ± 0.7
Bacillus subtilis	42 ± 0.65	38 ± 0.60	37 + 0.60 47 ± 0.8
Salmonella typhi	*19 + 0.11	*23 ± 0.14	19 + 0.50 48 ± 0.8
Proteus mirabilis	38 + 0.60	36 ± 0.58	24 + 0.37 39 ± 0.6
Pseudomonas aeruginosa	36 ± 0.58	35 ± 0.58	32 ± 0.18 39 ± 0.7

Cefuroxime Axetil® is the standard drug used, *A significant difference exists between the control and extracts ($p \geq 0.05$); t-test, n = 3.

These compounds had a potentially significant application against human pathogens, including those that caused infections [Table 3].

The antibacterial activity of the crude extracts of *E. hirta* was evaluated by measuring the diameters of the zones of growth inhibition on some members of enterobacteriaceae, and the results are presented in Table 3. All the test organisms were susceptible to *E. hirta* extracts, although in varying degrees. The highest zone of growth inhibition was shown by the aqueous extract against *B. subtilis* (42 ± 0.65 mm), followed by *E. coli* (39 ± 0.60 mm), *P. mirabilis* (38 ± 0.60 mm), *P. aeruginosa* (36 ± 0.58 mm), and *S. typhi* (19 ± 0.11 mm); others showed significant diameter zone of inhibition against the microbes, but comparably, the aqueous extract presented the best activity on the microbes (Table 3). The large zone sizes produced by the plant extract against the test bacteria, especially the aqueous extracts, was an indication of the potency of the bioactive components of the plant against all the test bacteria.

The aqueous extract showed MIC for *E. coli* (50 mg/ml), *P. mirabilis* (25 mg/ml), *S. typhi* (50 mg/ml), *B. subtilis* (25 mg/ml), and *P. aeruginosa* (25 mg/ml) (Table 4). For the benzene extract, the MIC for all the test bacteria was

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of extract against the microbes

Test organism	MIC (mg/ml)			MBC (mg/ml)		
	Aq	Et	Be	Aq	Et	Be
Escherichia coli	50	100	100	100	200	200
Bacillus subtilis	25	50	100	50	100	200
Salmonella typhi	50	100	100	100	200	200
Proteus mirabilis	25	50	100	50	100	200
Pseudomonas aeruginosa	25	50	50	50	100	100

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), ?Aq (Aqueous extract), Et (Ethanol extract), Be (Benzene extract), results are means of original values, n = 3.

Table 5: LD_{50} determination of crude extracts of *E. hirta*.

Doses (mg/kg) 1.p	Animals died/Animals survived		
	Aqueous	Ethanol	Benzene
10	0/3	0/3	0/3
100	0/3	0/3	0/3
1000	0/3	0/3	0/3
1600	0/1	0/1	0/1
2900	1/1	0/1	0/1
5000	1/1	1/1	0/1

LD_{50} > 5000 mg/kg, $P \leq 0.05$ (t - test).

100 mg/ml. The MIC values produced by the ethanol extract for *E. coli* and *S. typhi* were 100 mg/ml, while the others had 50 mg/ml.

In the toxicity studies *in vivo*, no mortality was recorded at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg b.w (i.p) of the ethanol extracts. Although, there were reduction in activities and deaths of the animals when the dosage was increased to 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg (in the aqueous extract) (Table 5). The LD_{50} was found to be greater than 5000 mg/kg as deaths were recorded at this dosage in the aqueous extract.

DISCUSSION

The percentage yield obtained for water was 59%, for ethanol 33%, and for benzene 16% (Table 1). This yield was not far lower than that obtained by Doughari et al.[22] for *Senna angustifolia* as they reported a yield of 52% for water extract, 50% for benzene extract, and 28% for dichloromethane, and also that of Owolabi et al.[23] who reported a yield of 10.74% for water extract and 3.78% for their ethanol extracts. Ogbolie et al.[14] also reported a yield of 9.1% for water extracts of *E. hirta*. Factors like the age of the plant and the polarity of the solvent used affected the yield. Thus, in this study, water seemed to be the best solvent for this plant material, thus supporting

the use of water as a solvent of choice in traditional practice.

Agents with low activity against a particular organism usually give 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 mg/ml frequently recorded for conventional antibiotics. The results obtained here are similar to those presented by Adesokan et al.[24] George et al.[25] explains that the observed differences may be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contain some impure substances that may be inert and do not have any antibacterial activities. This is the case with ethanol and benzene extracts, as they present high MIC and MBC values, while the aqueous extract displays low values on the bacteria (Table 4). In this study, the MIC values have been either the same or slightly lower than the MBC values, similar to the results of Karou et al.[26] 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, are more reliable than the MIC values, obtained using turbidity as an index of growth.[27]

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 low 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 constituents present in *E. hirta,* similar to the reports of Falodun et al.[7] and El-Mahmood and Amey.[8] In this study, the crude extracts the *E. hirta* herb have inhibited the growth of such recalcitrant Gram-negative bacteria that cause a majority of infectious diseases and which usually display above-average resistance to most antibiotic and non-antibiotic 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.[3] The efficacy of the extracts is probably due to the identified secondary metabolites, and this is harmful to animals in long-term use, as seen in Table 5, which further confirms its use as an antibacterial agent in folkloric medicine and may thus be useful in the treatment of infections.

CONCLUSION

The study showed that the extracts of *E. hirta* displayed antibacterial activities against the various bacteria that caused 'Jedi Jedi' infection in newborns. However, long-term use of the extract was slightly toxic in experimental animals, even though it was potent on the bacteria, which points to its toxicity in the newborns vital organs such as liver, lungs, kidneys, spleen, and heart. However, in this present study, only the pathology done to the liver was shown. It is therefore suggested that detoxification of the constituents responsible for its toxicity be researched upon for effective ethno- medicinal prescriptions of the plant.

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