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Full Length Research Paper

Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*

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Leaves of *Euphorbia hirta* used in traditional medicine for the treatments of boils, wounds and control of diarrhoea and dysentery were extracted by maceration in ethanol. The agar diffusion method was used to determine the antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis* at different concentrations while toxicological studies was carried out through intraperitoneal injection of albino rat with varying concentrations of the extract. Antibacterial sensitivity test indicated that the extract inhibited the growth of *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa* to varying degrees while S*. typhi* was not affected. Minimum inhibitory concentration (MIC), of the extract against *E. coli*, *S aureus*, *P. aeruginosa* and *B. subtilis* were 58.09, 22.55, 57.64 and 74.61 mg/ml respectively. Hematological analyses revealed that there was no significant difference (p = 0.05) between the total red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC) values of the rats used as control and those treated with the different concentrations of the extract. Also, the erythrocyte sedimentation rate (ESR) and mean corpuscular volume (MCV) values were significantly different at certain concentrations of the extract administered which indicates that the plant extract is heamatologically not toxic to rats. The plant extract was found to contain tannins, alkaloids and flavonoids which may be responsible for its antimicrobial properties. The results justify the traditional use of the plant in the treatment of sores, boils, wounds and control of dysentery and diarrhoea.

Key words: *Euphorbia hirta*, ethanolic extract, agar diffusion, inhibition, toxicity.

INTRODUCTION

Euphorbia hirta belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries (Soforowa, 1982). It can grow to a height of 40 cm. The stem is slender and often reddish in colour, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate and are usually greenish or reddish underneath measuring about 5 cm long. In the axils appear very small dense round clusters of flowers. The small green flowers constitute the inflorescence characteristic of the euphorbias. The stem and leaves produce white or milky juice when cut (Lind and Tallantire, 1971; Anononymous, 2005).

In East and West Africa, extracts of the plant are used in treatment of asthma and respiratory tract inflammations (Kokwaro, 1993). It is also used for coughs, chronic bronchitis and other pulmonary disorders in Mauritius (Wong-Ting-Fook, 1980). The plant is also widely used in Angola against diarrhea and dysentery, especially amoebic dysentery. In Nigeria, extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Igoli et al., 2005, Annon, 2005). Interaction with some traditional medical practitioners revealed that the plant is very popular amongst them, thus there is need to determine its antibacterial potentials. This work was therefore undertaken to authenticate the antibacterial potentials of *Euphorbia hirta*.

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MATERIALS AND METHODS

Sample preparation and extraction procedure

Fresh leaves of *E.* hirta were collected from Umuguma, Owerri West Local Government Area of Imo State, Nigeria. The plant was identified by Dr. I. I. Ibeawuchi of the Department of Crop Science Technology, Federal University of Technology, Owerri. Voucher specimens were also kept with number E.h.cco 002.

The fresh leaves were air dried for about one week and ground into fine powder using a mechanical grinder. 20 g of the fine powder were weighed into 250 ml of ethanol (95%) in a conical flask. This was covered, shaken every 30 min for 6 h and then allowed to stand for about 48 h. The solution was subsequently shaken and filtered using Whatman filter paper. The filtrate was evaporated to dryness using a rotary evaporator (Model type 349/2, Corning Limited.). A yield of 9.1% was obtained. The extract was then stored below ambient temperature.

The methods of Akujobi et al. (2004) and Esimone et al. (1998) were adopted For the preparations of dilutions of crude extract for antibacterial assay. The crude extracts were dissolved in 30% dimethylsulphoxide (DMSO) and further diluted to obtain 250, 200, 150, 100 and 50 mg/ml concentrations. These were stored at 15°C until required.

Evaluation of antimicrobial activity

Escherichia coli, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* were obtained from the Federal Medical Centre, Owerri while *Bacillus subtilis* was isolated from fermented African Oil bean seeds. They were re-isolated and the pure cultures subcultured on Nutrient agar slants. They were stored at 4°C until required.

The agar diffusion method as described by Esimone et al. (1998) was adopted for the study. About 15 ml of sterile molten nutrient agar in a Petri dish was seeded with 1.0 ml of standardized broth cultures of the bacteria (1.0×10^7 cfu/ml) and swirled gently to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. Three holes were made in the plates (about 5.0 mm diameter) using a sterile cork borer and equal volumes of the extracts were transferred into the holes using a Pasteur's pipette. Two Petri dishes containing a particular microorganism were used for each concentration of the extract. The plates were allowed to stand for one hour for prediffusion of the extract to occur (Esimone et al., 1998) and were incubated at 37°C for 24 h.

At the end of incubation, zones of inhibition that developed were measured and the average of zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values (Esimone et al., 1998; Osadebe and Ukwueze, 2004).

Administration to rats

Initial LD_{50} studies carried out were used to determine the maximum dose that did not produce any death in rats. Four groups of albino rats each comprising three rats, randomly selected having an average weight of 132.5 g were used. They were placed in different cages. Based on the LD_{50} studies, doses of 60.4 body weight, 120.8 body weight, 241.5 body weight and 483.0 mg/kg body weight were intraperitoneally injected into each group of the rats (Iyaniwura et al., 1991, EFPIA/ECVAM, 2001). The administration of the injection was carried out on daily basis for 14 days (EFPIA/ECVAM, 2001). The control group was injected with the diluents (30% DMSO). Food and water were provided *ad libitum*.

On the 15th day, blood samples drawn through the sublingual vein according to the method described by Zeller et al. (1998). This method has been found to be suitable for laboratory animal's well being as stated in EFPIA/ECVAM (2001). Two milliliter of blood sample was immediately transferred to ethylene diamine tetracetic acid (EDTA) treated bottles for hematological assay.

Blood samples were analyzed within 3 h of collection for total red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), hemoglobin (Hb) content, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamics (2003). Erythrocyte sedimentation rate (ESR) was determined according to the method described by Orji et al. (1986). Various hematological indices were calculated from the results obtained. These included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Preliminary phytochemical analysis of extract

This was carried out according to the methods described by Trease and Evans (1989).

Statistical analysis

The data obtained from the study were analyzed statistically using the analysis of variance (ANOVA). Fisher's Least Significant Difference (LSD) was used to separate the means (Sanders, 1990).

RESULTS

Results of the antibacterial screening of the different concentrations of the extract on the test isolates are shown in Table 1. The results show that increase in concentration of extract increased the zone of growth inhibition of some of the microorganisms. The extract did not inhibit the growth of *S. typhi* at any of the concentrations administered. The highest zone of growth inhibition of 13.5 mm diameter was exhibited by 250 mg/ml concentration of the extract against *S. aureus.* Only the 200 and 250 mg/ml concentrations had effects against *B. subtilis* while at 50 mg/ml the extract had no effect against *E. coli* and *P. aeruginosa*. The lowest zone of growth inhibition was observed with 200 mg/ml concentration of the extract against *B. subtilis* which gave a zone of inhibition measuring 5.6 mm.

The minimum inhibitory concentrations of the extract on the test isolates are shown in Table 2. The lowest minimum inhibitory concentration (MIC) was produced against *S. aureus* with a concentration of 22.55 mg/ml while the highest MIC was against *B. subtilis* with a concentration of 74.61 mg/ml. The extract had MIC of 58.09 and 57.64 mg/ml, respectively, against *E. coli* and *P. aeruginosa*.

Table 3 shows the results of the hematological analyses of the blood samples of rats injected with different concentrations of the extracts. In general although the values obtained for RBC counts, total WBC counts, Hb

Table 1. Antibacterial screening of the different concentrations of crude ethanolic extract of *Euphorbia hirta*.

Concentrations of extract (mg/ml)	Zones of inhibition (mm)				
	E. coli	***S. aureus***	***P. aeruginosa***	***B. subtilis***	***S. typh*i**
250	11.9[a]	13.5[b]	12.1[a]	8.4[c]	NI
200	9.8[a]	12.9[b]	11.3[c]	5.6[d]	NI
150	8.0[a]	11.5[b]	8.2[a]	NI	NI
100	5.8[a]	10.6[b]	6.1[a]	NI	NI
50	NI	7.8[a]	NI	NI	NI

*Values are means of triplicate readings. NI = No inhibition.
[a,b,c]Values with different superscripts on the same row are significantly different (p=0.05).

Table 2. Minimum inhibitory concentrations of the ethanolic extract of *Euphorbia hirta* against test isolates.

Plant	E. coli	S. aureus	P. aeruginosa	B. subtilis	Sa. typhi
E. hirta	59.09[a]	22.55[b]	57.64[a]	74.61[a]	NIL

[a,b,c]Values with different superscripts on the same row are significantly different (p=0.05).

Table 3. Hematological analysis of blood samples of rats injected with different concentrations of crude ethanolic extract of *Euphorbia hirta*.

Parameters	Concentrations of extract injected (mg/kg body weight)						
	Control	**60.4**	**120.8**	**241.5**	**483.0**	**SEM**	**LSD**
RBC (x10^6 cells/mm^3)	5.32[a]	5.28[a]	4.96[a]	4.84[a]	4.68[a]	0.43	0.96
PCV (%)	36.3[a]	32.0[b]	32.0[b]	33.0[b]	33.0[b]	0.49	1.09
ESR (mm/h)	3.50[a]	4.0[a,b]	4.0[a,b]	4.0[a,b]	5.0[b]	0.639	1.42
MCV (cubic microns)	68.23[a]	60.61[b]	64.52[c]	68.18[a]	70.51[a]	1.076	2.40
Hb content (g/100 ml)	9.8[a]	9.1[a]	9.1[a]	9.2[a]	9.3[a]	0.341	0.76
MCHC (%)	27.0[a]	28.44[a]	28.44[a]	27.88[a]	28.18[a]	0.847	1.89
WBC (x 10^3 cells/mm^3)	4.77[a]	3.72[a]	3.96[a]	4.38[a]	4.98[a]	0.568	1.27

level and MCHC differed. They were not significantly different ($p \geq 0.05$) from the values obtained from the control for these parameters. For ESR, the value obtained from the rats treated with 483.0 mg/kg body weight of extract was significantly different from the value obtained from control but not different from those rats treated with the other concentrations. Also MCV values from rats treated with 60.4 body weight and 120.8 mg/kg body weight concentrations of the extract were significantly different ($p = 0.05$) from the values of control and others.

The results of the preliminary phytochemical screening are shown in Table 4. The extract was found to contain tannins, flavonoids, alkaloids and cardiac glycosides. No saponins and cyanogenic glycosides were identified.

DISCUSSION

In this study, the results obtained indicated that the ethanolic extract of the *E. hirta* inhibited the growth of the test isolates except *S. typhi*. This, therefore, shows that the extract contains substance(s) that can inhibit the growth of some microorganisms. Other workers have also shown that extracts of some plants inhibited the growth of various microorganisms at different concentrations (Akujobi et al., 2004; Esimone et al., 1998; Nweze et al., 2004; Ntiejumokwu and Alemika, 1991; Osadebe and Ukwueze, 2004). The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to posses antibacterial properties (Cowan, 1999; Draughon, 2004). Some workers have also attributed their observed antimicrobial effects of plant extracts to the presence of these secondary metabolites (Nweze et al., 2004). Some workers have also identified tannins, flavonoids and alkaloids in the extracts of some medicinal plant (Yoshida et al., 1990; Blanc and Sacqui-Sannes, 1972; Abo, 1990; Baslas and Agarwal, 1980).

The observed antibacterial properties corroborate its use in traditional medicine. Traditionally, extracts of the plant are used in sore and wound healing, as ear drop for boils in the ear and treatment of boils. They are also used

Table 4. Preliminary phytochemical screening of ethanolic extract of *Euphorbia hirta*.

Plant	Saponins	Tannins	Flavonoids	Alkaloids	Cardiac glycosides	Cyanogenic glycosides
E. hirta	-	+	+	+	+	-

+ = Present; - = absent.

in the control of diarrhoea and dysentery (Kokwaro, 1993; Igoli et al., 2005). The large zones of inhibition exhibited by the extract against *S. aureus* and *P. aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. *S. aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds (Braude, 1982). Also the moderate growth inhibition against *E. coli* justifies its use in the control of diarrhoea and dysentery. *E. coli* is the common cause of traveler's diarrhoea and other diarrheagenic infections in humans (Adams and Moss, 1999). The low MIC exhibited by the extract against *S. aureus* is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections caused by these microbes, especially as they frequently develop resistance to known antibiotics (Singleton, 1999). Their use also will reduce the cost of obtaining health care. The relatively high zone of inhibition exhibited by the extract against *E. coli* is also of significance, since *E. coli* is a common cause of diarrhea in developing countries.

The inability of the extract to inhibit *Salmonella typhi* may be that it possesses a mechanism for detoxifying the active principles in the extract. Some bacteria are known to posses mechanisms by which they convert substances that inhibit their growth to non-toxic compounds. For examples *S. aureus* produces the enzyme penicillinase which converts the antibiotic penicillin to penicillinoic acid which is no longer inhibitory to its growth (Singleton, 1999).

Statistical analysis revealed that for RBC there was no significant ($p \geq 0.05$) between the values obtained for the different concentrations of the extract injected and the control. This shows that the extract did not affect either the circulating red blood cells or the erythropoetic centres of the animals. Some workers (Aniagu et al., 2005) have also shown that some extracts of plants do not have deliterious effects on RBC even up to 400 mg/kg body weight after 28 days of administration. This is also true for the WBC counts. Thus, the extract did not induce production or destruction of the WBC. The same trend was also observed for the Hb content which indicates that the extract did not affect synthesis of hemoglobin by the animals. Some plants have been suggested to interfere with the synthesis of Hb by inhibition of the uptake and utilization of iron (Sokunbi and Egbunike, 2000, Iheukwumere et al., 2002).

These results indicate that the extract is less toxic heamatologically, at least to the rats, at the concentrations administered. *E. hirta* is commonly used traditionally in the treatment of wounds and boils as well as in the control of diarrhoea and dysentery in Nigeria (Igoli et al., 2005). However, more work needs to be carried out to determine the chemistry of the particular active principle and the effect on the organs at these concentrations

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