Antibacterial Activities And Toxicological Potentials Of Crude Ethanolic Extracts Of *Euphorbia hirta*

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ABSTRACT: Leaves of *Euphorbia hirta* used in traditional medicine for the treatment of boils, wounds and control of diarrhoea and dysentery was extracted by maceration in ethanol. The agar diffusion method was used to determine the antibacterial activity on *Staphylococcus aureus, E coli, Pseudomonas aeruginosa, Salmonella typhi and Bacillus subtilis* at different concentrations while it was tested for toxicity on albino rats by injecting varying concentrations of the extracts through the intraperitoneal route. The results indicated that the extract inhibited the growth of *Staph aureus*, *E. coli* and *P. aeruginosa* to varying degrees. The extract did not inhibit the growth of *S. typhi*. The minimum inhibitory concentration (MIC) of the extract for *E. coli*, *Staph aureus*, *P. aeruginosa* and *B. subtilis* were 58.09mg/ml, 22.55 mg/ml, 57.64 mg/ml and 74.61 mg/ml respectively. Hematological analyses revealed that there was no significant difference (p=0.05) between the values obtained from rats used as control and those treated with the different concentrations of the extract for RBC, WBC, Hb and MCHC . However, ESR and MCV values were significantly different at some concentrations of the extract administered. Thus the plant extract is hematologically not toxic to rats. The observed antibacterial activities were believed to be due to the presence of tannins, alkaloids and flavonoids which were identified in the extract. The results are of significance in the health care delivery system and apparently justifies the use of the plant in the treatment of sores, boils, wounds and control of dysentery and diarrhoea. [The Journal of American Science. 2007;3(3):11-16]. (ISSN: 1545-1003).

Keywords: ***Euphorbia hirta***; Ethanolic extract; Agar diffusion; MIC; Inhibition; Hematological.

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 color, 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 euphobias. The stem and leaves produce white or milky juice when cut (Lind and Tallantire, 1971).

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 Malagasy (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). Personal communications with some traditional medical practitioners revealed that the plant is very popular amongst them, thus there is used to determine it's antibacterial potentials. This work was therefore undertaken to authenticate the plant's antibacterial potentials.

MATERIALS AND METHODS

Plant collection and identification:

Fresh leaves of Euphorbia 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. Specimen vouchers were also kept with number E.h.cco; 002

Sample preparation and extraction procedure:

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 was weighed into 250 ml of ethanol (95%) in a conical flask. This was covered, shaken every 30 min. for 6 hrs. and then allowed to stand for about 48 hrs. 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 Ltd.). A yield of 9.1% was obtained. The extract was then stored below ambient temperature.

Preparations of dilutions of crude extract for antibacterial assay:

The methods of Akujobi et al., (2004) and Esimone et al., (1998) were adopted. The crude extracts were dissolved in 30% dimethylsulphoxide (DMSO) and further diluted to obtain 250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml concentrations. These were stored at 15^0C until required.

Test microorganisms:

The organisms *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 Oilbean seeds. They were re-isolated and the pure cultures subcultured on Nutrient agar slants. They were stored at 40C until required for the study.

Evaluation of antimicrobial activity:

The agar diffusion method as described by Esimone et al. (1998) was adopted for the study. 15 ml of molten nutrient agar was seeded with 1.0 ml of standardized broth cultures of the bacteria (1.0×10^7 cfu/ml) by introducing the broth cultures into sterile Petri dishes, incorporating the molten agar, rotating slowly 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 37^0C for 24 hrs.

At the end of incubation the plates were collected and zones of inhibition that developed were measured. The average of the 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 a*l., 1998, Osadebe ad Ukwueze, 2004).

Administration of extract for 14 days:

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

On the 15^{th} day, the animals were collected and 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 wellbeing as stated in EFPIA/ECVAM (2001). 2.0 ml of blood sample was immediately transferred to ethylene diamine tetracetic acid (EDTA) treated bottles for hematological assay.

Hematological analysis:

Blood samples were analysed within 3 hr. of collection for total erythrocyte (RBC) and leukocyte (WBC) counts, packed cell volume (PCV), hemoglobin (Hb) content, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) according to the methods described by Okeudo *et al.* (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 *Salmonella typhi* at any of the concentrations administered. The highest zone of growth inhibition was exhibited by the extract on *Staph aureus* giving a zone diameter of 13.5 mm when administered at 250 mg/ml concentration. Only the 200 mg/ml and 250 mg/ml concentrations had effects on *Bacillus subtilis* while at 50 mg/ml the extract had no effect on E. coli and *Pseudomonas aeruginosa*. The lowest zone of growth inhibition was observed with 200 mg/ml concentration of the extract on *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 on *Staph aureus* with a concentration of 22.55 mg/ml while the highest MIC was on *B. subtilis* with a concentration of 74.61 mg/ml. The extract had MIC of 58.09 mg/ml and 57.64 mg/ml respectively on *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 content and MCHC differed, they were not significantly different (p= 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 from control but not different from those rats treated with the other concentrations. Also MCV values from rats treated with 60.4 mg/kg body weight and 120.8 mg/kg body weight concentrations of the extract were significantly different (p= 0.05) from the values from the 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.

Table 1: * Results of 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	Sa. typhi
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: The minimum inhibitory concentrations of the ethanolic extract of *Euphorbia hirta* on 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 are significantly different (p = 0.05)

Table 3: Preliminary phytochemical screening of ethanolic extract of Euphorbia hirta

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

\+ Present
\- Absent

Table 4: Results of the 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/hr)	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

a,b,…. Values with same superscript on the same row are not significantly different ($\rho \leq 0.05$)

DISCUSSION

The use of plants and their extracts in treatment of diseases dates back to 460 – 370 BC when Hippocrates practiced the art of healing by the use of plant-based drugs (Soforowa, 1982). In this study the results obtained indicated that the ethanolic extract of the plant inhibited the growth of the test isolates except *Salmonella 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 plants inhibit 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 the plant (Yoshida et al., 1990, Blanc and Sacqui-Sannes, 1972, Abo, 1990, Baslas and Agarwal, 1980).

The observed antibacterial properties corroborates 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 in the control of diarrhoea and dysentery. (Kokwaro, 1993, Igoli et al., 2005). The large zones of inhibition exhibited by the extract on *Staph aureus* and *P. aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. *Staph aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds (Braude, 1982). Also the moderate

growth inhibition on E. coli justifies its use in the control of diarrhoea and dysentery. *E. coli* is the common cause of travelers diarrhoea and other diarrhoeagenic infections in humans (Adams and Moss, 1999). The low MIC exhibited by the extract on *Staph 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 due to this microorganism, 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 on *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 *Staph aureus* produces the enzyme penicillinase which converts the antibiotic penicillin to penicillinic acid which is no longer inhibitory to its growth (Singleton, 1999).

Statistical analysis revealed that for RBC there was no significant ($p = 0.05$) 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 delirious effects on RBC even up to 400mg/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 hematologically, at least to the rats, at the concentrations administered. *E.hirta* is commonly used 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 effect of the extract on organs of at least albino rats at these concentrations.

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REFERENCES

1. Abo, K. A. (1990). Isolation of ingenol from the lattices of *Euphorbia* and *Elaeophorbia* species. Fitoterapia. (61 (5); 462 – 463.
2. Akujobi, C., Anyanwu, B. N., Onyeze, C. and Ibekwe, V. I. (2004). Antibacterial Activities and Preliminary Phytochemical Screening of Four Medicinal Plants. Journal of Applied Sciences. 7(3); 4328 – 4338.
3. Aniagu, S.O., Nwinyi, F.C., Akumka, D.D. and Ajoku, G.A. (2005). Toxicity studies in rats fed nature cure bitters. African Journal of Biotechnology.4 (1); 72-78.
4. Anon. (2005). The use of *Euphorbia hirta* in the treatment of sores, boils and wounds.
5. Baslas, R. K. and Agarwal, R. (1980). Chemical Examination of *E. hirta.* In International Research Congress on National Products as Medicinal Agents, Strasbourg, France. Book of Abstracts II (eds. Michler, E. and Reinhard, E.). p25.
6. Blanc, P. and Sacqui-Sonnes, G. de (1972). Flavonoids of E. hirta. Plant Med. Phytother. 6; 106 – 109.
7. Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. Clinical Microbiology Review. 12; 564 – 583.
8. Draughon, F. A. (2004). Use of Botanicals as Biopreservatives in Foods. Food Technology. 58(2); 20-28.
9. EFPIA/ECVAM (2001). Paper on good practice in administration of substances and removal of blood. Journal of Applied Toxicology .21; 15-23.

10. Esimone, C. O., Adiukwu, M. U. and Okonta, J. M. (1998). Preliminary Antimicrobial Screening of the Ethanolic Extract from the Lichen *Usnea subfloridans* (L). Journal of Pharmaceutical Research and Development. 3(2); 99 – 102.
11. Igoli, J. O., Ogaji, T. A., Tor-Anyiin and Igoli, N. P. (2005). Traditional Medicine Practice Amongst the Igede People of Nigeria. Part II. Afri. J. Traditional, Complementary and alternative Medicines. 2(2); 134 – 152.
12. Iheukwumere, F.C., Okoli, I.C. and Okeudo, N.J. (2002). Preliminary studies on raw *Napoleona imperialis* seed as feed ingredient; II. Effect on performance, hematology, serum biochemistry, carcass and organ weight of weaner rabbits. Tropical Animal Production Investigation. 5; 219-227.
13. Khan, M. and Olusheye, R. (1980). Studies in African Medicinal Plants. Part 1: Preliminary Screening of Plants for Antibacterial Activity. Planta Medica Supplement.
14. Kokwaro, J. O. (1993). Medicinal Plants in East Africa. 2nd edn. East African Literature Bureau, Nairobi, Kenya.
15. Lind, E. M. and Tallantire, A. C. (1971). Some Common Flowering Plants of Uganda. Oxford University Press, Nairobi. p182.
16. Ntiejumokwu, S. and Alemika, T. O. E. (1991). Antimicrobial and Phytochemical Investigation of the Stem bark of *Boswellia dalziella*. West African Journal of Pharmacology and Drug Research. 10; 100 – 104.
17. Nweze, E. I., Okafor, J. I. and Njoku, O, (2004). Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* Benth used in Nigerian Herbal Medicinal Practice. Journal of Biological Research and Biotechnology. 2(1); 39 – 46.
18. Osadebe, P. O. and Ukwueze, S. E. (2004). A Comparative Study of the Phytochemical and Antimicrobial Properties of the Eastern Nigerian Species of African Mistletoe (*Loranthus micranthus*) sourced from different host trees. Journal of Biological Research and Biotechnology. 2(1); 18 – 23.
19. Sanders, D. H. (1990). Statistics; A fresh Approach. 4th edition. McGraw-Hill Inc., Singapore.
20. Singleton, P. (1999). Bacteria in Biology, Biotechnology and Medicine. 4th edn. John Wiley and Sons Ltd, New York.
21. Soforowa, E. A. (1982). Medicinal plants and traditional medicine in Africa. John Wiley and Sons, Chichester. p198.
22. Trease, G. E. and Evans, W. C. (1989). A textbook of Pharcognosy. 13th edn. Bacilliere Tinall Ltd., London.
23. Wong-Ting-Fook, W. T. H. (1980). The medicinal plants of Mauritius. ENDA publication No. 10, Dakar.
24. Yoshida, T., Namba, O., Chen, L. and Okuda, T. (1990). Euphorbin E, a hydrolysable tannin dimmer of highly oxidized structure from *Euphorbia hirta*. Chem. Pharm. Bull. 38(4); 1113 – 1115.
25. Braude, A. I. (1982). Microbiology. W. B. Sauders Company, London.
26. Zeller, W., Weber, H and Panoussis, B. (1998). Refinement of blood sampling from the sublingual vein of rats. Laboratory Animals. 32; 369-376.