Ethnobotanical Leaflets 14: 654-64. 2010.

Antimicrobial Activities of Some Euphorbiaceae Plants Used in the Traditional Medicine of Akwa Ibom State of Nigeria

Uduak, A. Essiett[1], Kola', K. Ajibesin* [2]

[1]Department of Botany and Ecological studies, University of Uyo, Uyo, Akwa Ibom State, Nigeria

[2]Department of Pharmacognosy, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Ogun State, Nigeria
*E-mail: kay_ajib@yahoo.com

Issued: June 1, 2010

Abstract

Nine plant species belonging to the Euphorbiaceae family and used in traditional medicine in Akwa Ibom State of Nigeria were evaluated for *in vitro* antimicrobial activity using agar diffusion method. The stem bark of *Maesobotrya dusenii* gave the most significant effect followed by its root bark. The inhibitory effect of *M. dusenii* stem bark extract (37 mm) on *Pseudomonas aeruginosa* was higher than that of Chloramphenicol (35 mm). However, *Alchornea laxiflora* leaf extract showed the weakest activity. The minimum inhibitory concentration of the extracts ranged between 12.5 and 250 µg/mL. The results of the antimicrobial effects validated the use of the plants to treat infections caused by these microorganisms.

Key words: Euphorbiaceae; antimicrobial activities; traditional medicine; Akwa Ibom State; Nigeria.

Introduction

The Euphorbiaceae is the 4th largest family of the angiosperms comprising over 300 genera and about 7500 species distributed widely in tropical Africa (Gill, 1988). The euphorbiaceae plants are shrubs, trees, herbs or rarely lianas (Pandey, 2006). Many of them are xerophytes and cactoid and most often with milky latex. The family provides food (Pandey, 2006; Etukudo, 2003) and varied medicinal properties used in ethnobotany (Gill, 1988; Vasishta, 1974; Agbovie *et al*., 2002; Betti, 2004; Kubmarawa, 2007). They are useful in the treatment of ailments such as respiratory infections, venereal diseases, toothache, rheumatism, cough, ulcer and wounds (Oliver, 1960). However, some are also found as toxic. For instance, ricin contained in *Ricinus communis* is a well-known poisonous compound that elicits violent purgative action in man (Trease and Evans, 2002)), while the leaves of *Euphorbia kamerunica* are toxic to rats (Ajibesin, 2002). The plant is also a known irritant having *in vitro* cytotoxic activities (Abo and Evans, 1981).

In the traditional medicine of Akwa Ibom State, the under listed euphorbiaceae plants (Table 1) are used to treat various microbial diseases such as diarrhea, dysentery, skin infections and gonorrhea (Ajibesin *et al*., 2008). The antimicrobial uses of the plants in Akwa Ibom State, their ethnomedical uses in other parts of Nigeria, Africa and the world, as well as their chemical constituents are given.

Thus, this study aims at determining the antimicrobial effects of these euphorbiaceae plants, thereby validating their use in the traditional medicine of Akwa Ibom State, Nigeria.

Methods

Plant collection and authentication

Each fresh part (1 kg) of the plants (Table 1) was collected in 2006 and authenticated by Dr. U. Essiett of the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State, Nigeria. Plant specimens were deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The various plant parts were dried in the oven (30-65 ^{o}C), powdered by electric mill and stored.

Extraction

500 g of the respective plant part powder was macerated with 50% aqueous ethanol (2 x 5mL) for 72h at room temperature and filtered. The pooled liquid extract was concentrated to dryness *in vacuo* at 40^{o}C to give dry ethanol extract.

Phytochemical screening

The respective dry ethanol extracts were subjected to phytochemical screening using standard methods (Harborne, 1984; Sofowora, 1993) to show the classes of bioactive compounds in the plants.

Test organisms

The bacteria used in this study were typed cultures obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, while the fungi were clinical isolates collected from the same source. The bacteria: *Staphylococcus aureus* NCIB 8588, *Bacillus subtilis* NCIB 3610, *Escherichia coli* NCIB 86, *Proteus vulgaris* NCIB 67, *Pseudomonas aeruginosa* NCIB 950 and *Klebsiella pneumoniae* NCIB 418 were sustained on nutrient agar (Oxoid) slant at 4^{o}C prior to use. However, the fungi *Candida albicans* and *Aspergilus flavus* were sustained on Sabouraud's Dextrose Agar (Oxoid) slants at 4^{o}C before use.

Antimicrobial susceptibility test

The dry ethanol extracts were evaluated against the test microorganisms using agar-gel diffusion method described by Alade and Irobi (1993). The ethanol extracts were redissolved in distilled water and tested at concentration level of 20 mg/ml. Fixed volumes (150µl) of the extracts and distilled water were separately introduced into equidistant wells (6 mm) bored on the surface of the agar and Sabouraud's plates, which had been previously, inoculated with one of the test organisms. A well containing a standard drug, Chloramphenicol was made in the bacteria plates, while the fungal plates had a hole containing Nystatin as standard drug.

The bacteria were incubated at 37^{o}C for 24h, while the fungi were incubated at 25^{o}C for seven days. The presence of zones of inhibition surrounding the wells was taken as an evidence of antimicrobial activity.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by incorporating various amounts (250 – 6.25 µg/ mL) of the solution of extracts and fractions into sets of test tubes containing the culture media.50 µl of the standard test bacterial and fungal broth cultures were added into each of the test tubes. The set of tubes containing a mixture of bacteria and the sample (extracts and fractions) were incubated at 37 ^{O}C for 24 h, while those

containing the fungi were incubated at 25 ^{O}C for 7 days.

A positive control tube containing only the growth medium of each of the organisms was also set up. The MIC was regarded as the lowest concentration of the extract or fraction that did not permit any visible growth when compared with that of the control tubes.

Results and Discussion

The different parts of the nine plants studied gave various classes of bioactive constituents (Table 2). All the plants contained cardiac glycosides, flavonoids, terpenes and saponins at varying concentrations, while tannins and phlobatannins were lacking only in *M. dusenii* root bark. Alkaloids were absent in *M. fulvum* leaves and root bark, *M. barteri* leaves, *M. oppositifolius* leaves and stem bark and *M. dusenii* root bark, while anthraquinones were absent in all the plants.

The extracts of the different parts of the plants gave varying degrees of antimicrobial effects (Table 3). The stem bark extract of *M. dusenii* elicited the most significant activity, while *A. laxiflora* leaf extract gave the least. Moreover, the *M. dusenii* stem bark extract showed the highest inhibitory effect against *P. aeruginosa* with a zone of inhibition of 37 mm. This inhibitory effect was higher than the Chloramphenicol effect against the same organism. Only the extracts of *R. heudelotii* stem bark, *T. conophora* leaves, *A. laxiflora* leaves and *M. dusenii* stem bark and root bark were active against *A. flavus*.

The result of this study showed that the different parts of all the plants elicited different levels of antimicrobial activities against all the test organisms. The extract of *M. dusenii* stem bark and root bark exhibited significant inhibitory effects against the entire test organisms ($p < 0.01$), while *A. laxiflora* leaf extract and *M. fulvum* leaf and root bark extracts gave weak activities. The other plants elicited moderate activities. However Ogundipe (2001) reported good antimicrobial activity for *A. laxiflora* leaf extract and identified quercetin, rutin and quercitrin as the flavonoids responsible for its activity. Similarly, the good antimicrobial effects recorded for *E. heterophylla* and *R. heudelotii* in this study are consistent with previous reports on the plants (Falodun *et al.*, 2003; Momeni *et al.*, 2005).

The results of the antimicrobial effects of all extracts showed that some of the plant species might be weak antimicrobial plants, while others may be potent. Similar observation has been reported for the antimicrobial activities of some Euphorbiaceae plants (de Lima et al., 2006; Parekh and Chanda, 2007). Also, antimicrobial screening of some plants with ethnobotanical antimicrobial uses was reported to show similar result (Rajakaruna *et al*., 2002).

Minimum inhibitory concentration values of 12.5 - 250 µg/mL were recorded for the extracts of some of the Euphorbiaceae plants against the test bacteria, while a range of 25 – 250 µg /mL was obtained against *Candida albicans and Aspergillus flavus* (Table 4). The result of minimum inhibitory concentration of extracts showing 12.5 and 25 µg /mL suggests that the extracts may act as bactericidal and fungicidal agents to these microorganisms.

The bioactive compounds responsible for the inhibitory effects of these plants were detected in their phytochemical screening, some of which were reported in literature as antimicrobial constituents. Flavonoids are known to be antimicrobial in nature (Joseph *et al.*, 2002). Flavonoids isolated from the leaves of *Euclea crispa*

sub sp. *crispa* were reported to give antimicrobial activity (Pretorius *et al*., 2003). Tannins identified from *Vaccinium vitis-idaea*, terpenes from *Vernonia amygdalina* and saponins from *Allium minutifolium* and were all established as antimicrobial constituents of the plants (Ho *et al*., 2001; Barile *et al*., 2007).

The antimicrobial effects of the plants, established in the results, have lent credence to the ethnobotanical use of these euphorbiaceae plants in treating infections caused by these organisms.

In conclusion, this study showed the antimicrobial activity of the extracts of various plants of the euphorbiaceae family used in ethnomedicine of Akwa Ibom State of Nigeria. The inhibitory effect of the plant extracts validated the medicinal use of the plants. Further work is required for identifying the active constituents of the plants.

Acknowledgments

The authors acknowledge the contributions of Mr. Patrick Iberi, a Technologist in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria, and Mr. Etefia Okon, a Laboratory staff in the same Department.

References

Abo, K., and Evans, F.J. 1981. The composition of a mixture of Ingol Esters from *Euphorbia kamerunica*, *Planta Medica* 43(12): 392-395.

Agbovie, T., Amponsah, K., Crentsil, O.R., Dennis, F., Odamtten, G.T. and Ofusohene-Djan, W. 2002. *Conservation and sustainable use of medicinal plants in Ghana*, Ethnobotanical Survey, UNEP-WCMC, Cambridge, UK.

Ajibesin, K.K., Bala, D.N., Ekpo, B.A.J. and Adesanya, S.A. 2002. Toxicity of some plants implicated poisons in Nigerian ethnomedicine to rats, *Nig. J Nat Prod Med* 6: 7-9.

Ajibesin, K.K., Ekpo, B.A., Bala, D.N., Essien, E.E. and Adesanya, S.A. 2008. Ethnobotanical survey of Akwa Ibom State of Nigeria, *J Ethnopharmacology* 115(3): 387-408.

Alade, P.I. and Irobi, O.N. 1993.Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*, *J. Ethnopharmacology* 39: 171-174.

Barile, E., Bonanomi, G., Antignam, V., Zolfaghari, B., Sajjadi, S.E., Scala, F. and Lanzotti, V. 2007. Saponins from *Allium minutiflorum* with antifungal activity, *Phytochemistry* 68(5): 596-603.

Betti, J.L. 2004. An ethnobotanical study of medicinal plants among the Baka Pygmies in the Dja Biosphere Reserve, Cameroon, *African Study Monographs* 25(1): 1-27.

de Lima, M.R.F., Luna J de, S., dos Santos, A.F., de Omena, M.C. and de Erasto, P. and Grierson, D.S. Afolayan A. J. 2006. Bioactive sesquiterpene lactones from the leaves of *V. amygdalina*, *J. Ethnopharmacol*ogy. 106(1): 117-120.

Etukudo, I. 2003. *Ethnobotany: conventional and traditional uses of plants*, Verdict Press, Uyo, Akwa Ibom, Nigeria.

Falodun, A., Agbakwuru, E.O.P. and Ukoh, G.C. 2003. Antibacterial activity of *Euphorbia heterophylla* L (Euphorbiaceae), *Pak. J. Sci Res* 46(6): 471-472.

Gill, L.S. 1988. *Taxonomy of flowering plants*, Africana-Fep Publishers Ltd., Nigeria.

Harborne, J.B. 1984. *Phytochemical Methods*, 2nd ed, Champion and Hall Publishers, London. Ho, K.Y., Tsai, C. C., Huang, J.S., Chen, C.P., Lin, T.C. and Lin, C.C. 2001. Antimicrobial activity of tannin components from *Vaccinium vitis-idaea* L, *J Pharmacy Pharmacology* 53(2): 187-191. Joseph, J. and Nadeau D. 2002. *Underwood A. The Colour Code: A Revolution Eating Plan For Optimal Health*, Hyperion, New York. Kubmarawa, D., Ajoku, G.A., Enwerem, N.M. and Okorie, D.A. 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria, *African J Biotechnology* 6(14): 1690-1696. Momeni, J., Djoulde, R.D., Akam, M.T. and Kimbu, S.F. 2005. Chemical constituents and antibacterial activities of the stem bark of *Ricinodendron heudelotii* (Euphorbiaceae), *Indian J Pharm Sci* 67(3): 386-389. Ogundipe, O.O., Moody, J.O., Houghton, P.J. and Odelola, H.A. 2001. Bioactive chemical constituents from *Alchornea laxiflora* (benth) Pax and Hoffman, *J Ethnopharmacology* 74: 275-280. Oliver, B. (1960). *Medicinal plants in Nigeria*, Nigerian College of Arts, Science and Technology, Lagos. Pandey, B.P. 2006. A textbook of Botany: Angiosperms, Taxonomy, Anatomy, Embryology (including tissue culture) and Economic Botany, S Chand & Co., Ltd., Ram Nagar, New Delhi. Parekh, J. and Chanda, S. 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal plants, *Turk J Biol* 31: 53-58. Pretorius, J.C, Magama, S. and Zietsman, P.C. 2003. Purification and identification of antibacterial compounds from *Euclea crispa* subsp. *crispa* (Ebenaceae) leaves, *South African J Bot* 69(4): 579-586. Rajakaruna, N., Harris, C.S. and Towers, G.H.N. 2002. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka, *Pharmaceutical Biology* 40(3): 235-244. Sofowora, A. 1993. *Medicinal plants and traditional medicine in Africa*, Spectrum books Ltd., Ibadan, Nigeria. Trease, G. E. and Evans, W.C. 2002. *Pharmacognosy*, 15th Ed, Saunders. Vasishta, P.C. 1974. *Taxonomy of Angiosperms* 2nd ed., R. Chand & Co., New Delhi.

Table 1. Traditional uses of some Euphorbiaceae plants.

Plant species	Source Plant part Used	Traditional uses	Chemical constituents

Euphorbia heterophylla L. Leaves	Ab	Purgative, malaria, rashes, respiratory tract infection (26).	Alkaloids, phenolics, saponins, (27), resins, triterpenes (28).
Mallotus Oppositifolius (Geisel.) Mull. Arg. Leaves, stem bark.	Es	Lumbago (29), dysentery, wounds, measles, whitlow (5), worms (30), laxative, vermifuge, aphrodisiac headache (31, 28, 32).	Rottelerin, flavonols (28, 34).
Macaranga barteri Mull. Arg Leaves	In	Ulcer, stomatitis (34), amnesia (35).	Phenolics (36)
Maesobotrya dusenii (Pax) Hutch. Stem bark,	Ek	Diarrhoea, dysentery(3).	root bark.
Phyllanthus amarus Schum. & Thonn. Whole plant	Ik	Gall stone, kidney stone (37), boil, sores, skin disease, pile (30).	Phyllanthin, hypophyllanthin, (38), inulin, saponins (39), flavones (28).
Ricinodendron heudelotii (Baill.) Pierre ex Pax Stem bark	Ur	Constipation, miscarriage, painful menstruation, (32), female infertility (5), diarrhoea, dysentery (40), asthma (41).	Aleuritolic acid, phenolic (17).
Tetracarpidium conophorum (Mull. Arg.) Hutch & Dalz. Leaves	It	Hiccups (42), fibroid, female infertility, irregular menstrual Flow (30), skin rash, dysentery	
Alchornea laxiflora (Benth.) Pax K. Hoffm. Leaves	Uy	Oral hygiene, malaria (42), infectious disease, inflammation (43,44).	Terpenoids (44), quercetin, rutin, quercitrin (38).
Manniophyton fulvum Mull. Arg. Leaves,	Or	Insanity (35), skin infection	Terpenoids (45) root bark.

Ab = Abak, Es = Eastern Obolo, In = Ini, Ek = Eket, Ik = Ikono, Ur = Uruan, It = Itu, Uy = Uyo, Or = Oron, 26 = Falodun et al., 2006; 27 = Falodun and Agbakwuru, 2007; 28 = Iwu, 1986; 29 = Abiww, 1990; 30 = NNMDA, 2006 a; 31 = Lewis and Elvin-Lewis, 1977; 32 = Burkill, 1994; 34 = Adjanohoun et al., 1981; 35 = Bouquet, 1969; 36 = Adesegun et al., 2007; 37 = Anonymous, 2003; 38 = Sharma et al., 1993; 39 = Gill, 1992; 40 = Cunningham, 1993; 41 = Barnish and Samai, 1992; 42 = Walker and Sillans, 1995; 43 = NNMDA, 2006 b;

44 = Farombi et al., 2003; 45 = Nia et al., 2005; 3 = Etukudo Etukudo, 2003; 5 = Agbovie et al., 2002; 17 = Momeni et al., 2005.

Table 2 Phytochemical screening of some Euphorbiaceae plants.

Metabolites	E.h	M.o		M.d		M.b	P.a	R.h	T.c	A.l	M.f	
	L	L	Sb	Sb	Rb	L	Wp	Sb	L	L	Rb	L
Saponins	++	+++	++	++	++	+++	+	+	+++	+	++	+
Tannins	+++	+++	++	-	-	++	+++	+++	+++	+++	+++	+++
Flavonoids	+	++	++	++	+++	+	+++	++	++	+++	++	+++
Alkaloids	++	-	-	-	-	-	+	++	+++	++	-	-
Phlobatannins	++	+++	++	-	-	++	+++	+	++	+	+++	++
Anthraquinones (Borntrager's test)	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides												
(i) Lieberman	+	++	++	+	++	++	+	+	++	++	++	+++
(ii) Salkowski	++	++	+++	++	++	++	+++	++	++	+	++	+++
(iii) Keller Kiliani	++	+++	++	++	+++	++	+++	++	+++	+++	+++	++
Terpenes	++	++	+++	++	++	++	+	++	++	+++	++	+++

E.h = *Euphorbia heterophylla*; M.o = *Mallotus oppositifolius*; M.b = *Maesobotrya dusenii*; P.a = *Phyllanthus amarus*; R.h = *Ricinodendron heudelotii*; T.c = *Tetracarpidium conophora*; A.l = *Alchornea laxiflora*; M.f = *Maniophyton fulvum.*
L = Leaves; Sb = Stem bark; Rb = Root bark; Wp = Whole plant.

Table 3 Antimicrobial effects of the extracts of some Euphorbiaceae plants.

Plant Species	Plant part	Microorganisms (Inhibition zone in mm)[a]							
		B.s	E.c	S.a	P.a	K.p	P.v	C.a	A.f
E. heterophylla	Leaves	21±1.4*	15±1.7	28±2.0*	10±1.0	25±2.6*	30±2.0*	20±1.4*	-
M. oppositifolius	Leaves	-	23±3.6*	33±1.0*	31±2.0*	-	24±1.4*	28±2.0*	-
	Stem bark	31±1.7*	16±1.2	14±0.0	22±2.4	30±2.0*	16±0.0	25±1.7*	-
M. dusenii	Stem bark	25±3.6*	17±1.0	23±1.7*	37±1.0*	21±2.0	13±0.0	31±2.0*	8±0.0*
	Root bark	25±1.0*	21±1.2*	24±2.0*	23±1.4*	20±2.4*	17±1.2	23±2.0*	7±1.0
M. barteri	Leaves	30±1.7*	13±0.0	-	25±1.6*	26±2.6*	28±2.0*	30±1.2*	-
P. amarus	Whole plant	14±1.4	18±1.6*	7±0.0	15±1.9	-	10±1.0	9±0.0	-
R. heudelotii	Stem bark	2±0.0	6±0.0	5±1.0	6±2.0	21±1.7*	10±1.4	10±1.2	25±1.0*

T. conophora	Leaves	-	20±2.0*	9±0.0	-	-	20±2.4*	4±1.0	9±1.2
A. laxiflora	Leaves	-	-	-	15±3.6	-	11±0.0	-	9±0.0
M. fulvum	Leaves	-	3±1.0	11±2.0	-	-	6±2.0	16±2.6*	-
	Root bark	-	-	15±1.7	8±2.4	-	13±1.2	16±2.0*	-
Chloramphenicol (4 µg/mL)		35±0.0*	28±0.0*	37±0.0*	35±0.0*	32±0.0*	33±0.0*	NA	NA
Nystatin (4 µg/mL)		NA	NA	NA	NA	NA	NA	28±0.0*	34±0.0*

B.s = *B. subtilis* NCIB 3610; E.c = *E. coli* NCIB 86; S.a = *S. aureus* NCIB 8588; P.a = *Ps. aeruginosa* NCIB 950; K.p = *K. pneumoniae* NCIB 418; P.v = *P. vulgaris* NCIB 67; C.a = *C. albicans*; A.f = *A. flavus*.
a = Values are the mean of quadruplicate readings; P<0.01.
Saline solution (150 µL) = - ; - = No inhibition zone

Table 4 Minimum inhibitory concentration of the extracts of some Euphorbiaceae plants.

Plant Species	Plant part	Microorganisms (Inhibition zone in mm)[a]							
		B.s	E.c	S.a	P.a	K.p	P.v	C.a	A.f
E. heterophylla	Leaves	50	200	25	250	50	25	100	-
M. oppositifolius	Leaves	-	50	12.5	25	-	50	50	-
	Stem bark	25	250	>250	100	25	250	50	-
M. dusenii	Stem bark	50	250	100	12.5	100	>250	25	>250
	Root bark	50	100	50	50	100	250	50	>250
M. barteri	Leaves	25	>250	-	50	25	25	25	-
P. amarus	Whole plant	250	200	>250	200	-	>250	>250	-
R. heudelotii	Stem bark	>250	>250	>250	>250	50	>250	>250	25
T. conophora	Leaves	-	100	>250	-	-	100	>250	>250
A. laxiflora	Leaves	-	-	-	250	-	>250	-	250
M. fulvum	Leaves	-	>250	>250	-	-	>250	250	-
	Root bark	-	-	250	>250	-	>250	250	-
Chloramphenicol (4 µg/mL)		12.5	12.5	6.25	12.5	25	12.5	NA	NA
Nystatin (4 µg/mL)		NA	NA	NA	NA	NA	NA	12.5	6.25

B.s = *B. subtilis* NCIB 3610; E.c = *E. coli* NCIB 86; S.a = *S. aureus* NCIB 8588; P.a = *Ps. aeruginosa* NCIB 950; K.p = *K. pneumoniae* NCIB 418; P.v = *P. vulgaris* NCIB 67; C.a = *C. albicans*; A.f = *A. flavus*.
a = Values are the mean of quadruplicate readings
- = No inhibition