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Research Paper

ANTIMICROBIAL ACTIVITY OF EXTRACTS OF EUPHORBIA *KAMERUNICA PAX*

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Crude ethyl acetate, methanol and aqueous extracts of *Euphorbia kamerunica* plant were investigated for the antimicrobial properties using agar well diffusion technique against pathogenic bacteria and yeast (*Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Candida albicans).* The extract exhibited greater activity on Gram positive bacteria and methanol extract showed greater activity than ethyl acetate and aqueous extracts.

Keywords: *Euphorbia kamerunica*, Crude extracts, Pathogenic bacteria, Yeast, Antimicrobial activities

INTRODUCTION

Euphorbia kamerunica Pax belong to the family Euphorbiaceace comprising trees, shrubs and herds and found in the rain forest Guinea and xerophytic habitats with most of them being lactiferous. Many species of this family are used in folk medicine as drugs or as raw materials for medicinal preparations. (Wiriyachitra *et al.*, 1985), as alternative industrial resources (Calvin, 1980) and used for agriculture and horticulture processes (Hecker *et al*., 1979). Toxic diterpene esters found in *Euphorbia* species result in skin inflammation, and reddening and formation of edematous swellings. There has been cases of poisoning with severe gastroenteritis, vomiting and colicky diarrhoea when taken internally (Frohne and Pfander, 1984).

Fai and Fagade (2005) on assaying acute toxicity of acute toxicity of *Euphorbia kamerunica* on *Oreochrmis niloticus* fingerlings, reported that poisoning of streams with this plant to capture fish may have ecological consequences due to destruction not initially targeted at aquatic life.

Many higher plants are known to produce antimicrobial agents and indeed extracts of plants from different parts of the world have been shown to possess antimicrobial properties (Malcolm and Sofowora, 1969). Plants that are used in traditional medicines contain substances that can

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be employed in the treatment of chronic and infectious diseases (Kumara *et al*., 2011). Discovery of new drugs from the screening of plants that can be effective in treatment of diseases is of interest to scientists (Dimayuga and Garcia, 1991). Medicines derived from plant extracts have been used in parts of the world as traditional health care for a very long time with interest being focused now on how to use these plant extracts as antimicrobial agents (Chariandy *et al.*, 1999). Production of antimicrobial agents from plants using different solvents to extract them has well been documented and these have been shown to have antimicrobial properties.

The objective of this present study was to evaluate the antimicrobial activity using ethyl acetate, water and methanol extracts of *Euphorbia kamerunica* plant against pathogenic bacteria and yeast.

MATERIALS AND METHODS

Plant Material and Extraction

The whole plant of *Euphorbia kamerunica* was collected from the Botanical gardens of the University of Ibadan, Oyo state, Nigeria. The plant was authenticated by Prof Abiodun Ayodele of the Department of Botany and Microbiology, University of Ibadan. A voucher specimen with number 22278 was deposited at the herbarium. The plant was washed, cut and pounded using a mortar and pestle.

Extraction with sterile water, ethyl acetate and methanol was carried out. The extracts were then filtered and evaporated to dryness under reduced pressure and kept until needed.

Microorganisms and Medium

The microorganisms used were isolates obtained from the Department of Medical Microbiology and Parasitology, University College Hospital (UCH) Ibadan.

Antimicrobial Sensitivity Test

0.1 mL of the bacteria (10^6 CFU/mL) was introduced into the Petri dishes and 15 mL of Mueller Hinton Agar (Lab M) distributed into Petri dishes. Also, 0.1 mL of yeast was introduced into Petri dish and 15 mL of Sabouraud Dextrose Agar (Lab M) was distributed. Nine millimeter diameter wells were cut into the agar using sterile cork borer and 0.2 mL of the different plant extracts of different concentrations of 10-100 mg/mL were introduced into the wells. The plates were incubated at 37^{o}C for the bacteria and 25^{o}C for the yeast for 24 h after which the plates were examined for any zone of inhibition around the wells. Gentamycin (10 ug/mL) and Nystatin (10 ug/mL) served as control antibiotics for the bacteria and yeast respectively.

STATISTICAL ANALYSIS

Results were expressed as mean$\pm$S.E of two separate experiments. Statistical significance was determined using SPSS 10 software after one way variance analysis

RESULTS AND DISCUSSION

Table 1 shows the activity of ethyl acetate extract on microorganisms. The extract was not effective on all the microorganisms at 10 mg/mL. The highest zone of inhibition of 30.5$\pm$2.5 mm was observed for *Staphylococcus aureus* at 100 mg/mL and was significantly different statistically from other inhibition zones obtained for this organism using different concentrations. The organism with the least zone of inhibition at 100 mg/mL was *Klebsiella pnuemoniae* at 12.0±2.0a. At other concentrations for this organism, the extract was not effective.

Table 1: Antimicrobial Activity of Different Concentrations of Crude Ethyl Acetate Extract of *Euphorbia kamerunica* Plant Using Agar Well Diffusion Technique

Zones of Inhibition (mm) ± S.D with Different Concentration (mg/ml)									
Organisms	**100**	**80**	**50**	**30**	**10**	**Control**	**Ethyl Acetate**	**NYST**	**GENT**
Bacillus cereus	18.5 ± 2.5a*	17.0 ± 1.0ab†	15.0 ± 0.0b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	15.0 ± 0.0b
Bacillus licheniformis	15.5 ± 2.5a	14.5 ± 1.5a	10.5 ± 0.5b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	NT	18.0 ± 0.0a
Bacillus subtilis	12.5 ± 1.5b	11.0 ± 1.0bc	9.0 ± 0.0c	9.0± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	17.0 ± 0.0c
Escherichia coli	18.5 ± 2.5a	13.5 ± 0.5bc	13.0 ± 3.0b	9.5 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	15.0 ± 0.0a
Klebsiella pneumoniae	12.0± 2.0a	9.0± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	NT	NT
Proteus mirabilis	23.5 ± 1.5a	21.5 ± 2.5ab	18.5 ± 0.5b	130 ± 1.0c	9.0 ± 0.0d	9.0 ± 0.0d	9.0 ± 0.0d	NT	18.0 ± 0.0b
Pseudomonas aeruginosa	21.5 ± 0.5a	19.5 ± 2.5ab	19.0 ± 1.0ab	13.5 ± 2.5bc	9.0 ± 0.0c	9.0 ± 0.0c	9 0± 0.0c	NT	23.0 ± 0.0a
Salmonella typhi	24.0± 0.0a	22.0 ± 1.0b	17.5 ± 0.5c	11.0 ± 1.0d	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	21.0 ± 0.0b
Staphylococcus aureus	30.5 ± 2.5a	22.0 ± 2.0b	19.0 ± 1.0bc	14.0 ± 2.0cd	9.0 ± 0.0d	9.0 ± 0.0d	9.0 ± 0.0d	NT	14.0 ± 0.0c
Candida albicans	23.0 ± 2.0a	22.5 ± 1.5a	17.0 ± 0.0b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0. ± 0.0c	23± 0.0a	NT

Note: * Means of two readings ± standard deviation; † Values in the same row followed by the same letter are not significantly different ($p>0.05$) from each other; Diameter of cork borer = 9.0 mm; NT = Not Tested, GENT = Gentamycin, NYST =Nystatin

Table 2 shows the activity of aqueous extract. The extract had no activity on *P. aeruginosa, S. typhi*, and *Candida albicans*. At 100 mg/mL, zones of inhibition for *B cereus* was 35.5±2.5a, *B. Licheniformis*; 34.5±2.5a and *S. aureus* 27.0±0.0a.

Table 3 shows the activity of methanol extract. Methanol had the highest activity on *S. aureus* at 36.5± 2.5a out of all the extracts used. Activity was recorded at only 100 mg/mL for *K. pneumoniae* with zone of inhibition at 13.0±1.0a

In this study, greater resistance to the extracts was observed by more Gram negative bacteria for example *P aeruginosa* and *S. typhi* for aqueous extract while for the methanol extract, lower activities were observed for *K. pneumoniae* and *S. typhi*.

Kelmanson *et al.* (2000) reported on traditional medicinal plants used by the Zulus having antibacterial activity with aqueous, methanol and ethyl acetate extracts on *Staphylococcus aureus, Bacillus subtilis, Esherichia coli, Klebsiella pnuemoniae,* and *Pseudomonas aeruginosa*. From this study, ethyl acetate was effective against *S. aureus*. Antimicrobial activity was found in the methanol extracts of the leaves and stems of *Cheilanthes viridis* and *Dioscorea dregeana* tubers, both methanol and ethyl acetate extracts of *D. sylvatica* tuber bark, water and methanol extracts of leaves and stems of *Melianthus comosus* and methanol and ethyl acetate extracts

Table 2: Antimicrobial Activity of Crude Aqueous Extract of *Euphorbia kamerunica* Plant at Different Concentrations Using Agar Well Diffusion Technique

Zones of Inhibition (mm) ± S.D with Different Concentration (mg/ml)									
Organisms	**100**	**80**	**50**	**30**	**10**	**Control**	**Water**	**NYST**	**GENT**
Bacillus cereus	35.5 ±2.5a*	29.0±3.0b†	24.5 ± 0.5b	18.0 ± 2.0c	9.0 ±0.0d	9.0 ±0.0d	9.0± 0.0d	NT	15.0 ± 0.0c
Bacillus licheniformis	34.5 ± 2.5a	23.5 ± 1.5b	20.0 ± 1.0bc	15.0 ± 1.0d	9.0 ±0.0e	9.0 ±0.0e	9.0 ± 0.0e	NT	18.0 ± 0.0cd
Bacillus subtilis	20.0 ± 2.0a	17.0 ± 1.0a	13.5 ± 1.5b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	17.0 ± 0.0a
Escherichia coli	17.0 ± 0.0a	14 ± 2.0ab	12.0 ± 0.2bc	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	15.0 ± 0.0ab
Klebsiella pneumoniae	23.5 ± 2.5a	20 ± 2.0a	14.0 ± 2.0b	9.0 ± 0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ± 0.0b	NT	NT
Proteus mirabilis	15.0 ± 1.0b	11.5 ± 0.5c	9.0 ± 0.0d	9.0 ± 0.0d	9.0 ±0.0d	9.0 ±0.0d	9.0 ± 0.0d	NT	18.0 ± 0.0a
Pseudomonas aeruginosa	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ± 0.0b	NT	23.0 ± 0.0a
Salmonella typhi	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ± 0.0b	NT	21.0 ± 0.0a
Staphylococcus aureus	27.0 ± 0.0a	18.0 ± 0.0b	12.5 ± 0.5d	10.0 ± 0.5e	9.0 ± 0.0f	9.0 ± 0.0f	9.0 ± 0.0f	NT	14.0 ± 0.0c
Candida albicans	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ± 0.0b	23.0 ± 0.0a	NT

Note: * Means of two readings ± standard deviation; † Values in the same row followed by the same letter are not significantly different (p>0.05) from each other; Diameter of cork borer = 9.0 mm; NT = Not Tested, GENT = Gentamycin, NYST =Nystatin.

Table 3: Antimicrobial Activity of Crude Methanol Extract of *Euphorbia kamerunica* Plant at Different Concentrations Using Agar Well Diffusion Technique

Zones of Inhibition (mm) ± S.D with Different Concentration (mg/ml)									
Organisms	**100**	**80**	**50**	**30**	**10**	**Control**	**Methanol**	**NYST**	**GENT**
Bacillus cereus	30.0 ± 1.0a*	15.5 ± 0.5b†	14.5 ± 0.5b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	15.0 ± 0.0b
Bacillus licheniformis	16.0 ± 1.0a	11.5 ± 0.5b	10.0 ± 0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	NT	18.0 ± 0.0a
Bacillus subtilis	16.0 ± 2.0a	12.0 ± 2.0ab	9.5 ± 0.5b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	NT	17.0 ± 0.0a
Escherichia coli	19.0 ± 2.0a	18.0 ± 2.0a	15.5±2.5ab	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	NT	15.0 ± 0.0ab
Klebsiella pneumoniae	13.0 ± 1.0a	9.0 ± 0.0b	9.0 ± 0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	9.0 ±0.0b	NT	NT
Proteus mirabilis	23.5 ± 1.5a	23.0 ± 0.0a	16.5 ± 0.5b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	18.0 ± 0.0a
Pseudomonas aeruginosa	21.0 ± 1.0ab	21.5 ± 1.5ab	19.5 ± 0.5b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	NT	23.0 ± 0.0a
Salmonella typhi	15.0 ± 2.0b	13.0 ± 1.0bc	11.5 ±2.5bc	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0± 0.0c	NT	21.0 ± 0.0a
Staphylococcus aureus	36.5 ± 2.5a	27.5 ± 0.5b	25.5 ± 0.5b	9.0 ±0.0d	9.0 ±0.0d	9.0 ±0.0d	9.0 ±0.0d	NT	14.0 ± 0.0c
Candida albicans	15.5 ± 0.5b	14.5 ± 0.5b	14.5 ± 0.5b	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	9.0 ± 0.0c	23.0 ± 0.0a	NT

Note: * Means of two readings ± standard deviation; † Values in the same row followed by the same letter are not significantly different (p>0.05) from each other; Diameter of cork borer = 9.0 mm; NT = Not Tested, GENT = Gentamycin, NYST =Nystatin.

of leaves, stems and roots of *Vernonia colorata.* In general, these extracts were most active against Gram positive bacteria and from this study also, the extracts had more activity against Gram positive than Gram negative organisms. These results were in line with those from the previous screenings of medicinal plants for the antimicrobial activity, where most of the active plants showed activity against Gram-positive strains only. (Vlietinck *et al.,* 1995; Rabe and van Staden, 1997).

Bisht *et al.* (2006) reported on the antimicrobial activity of *Hedychium spicatum* using ethyl acetate, acetone, ethanol and aqueous extracts on *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pnuemoniae, Pseudomonas aeruginosa, Salmonella typhi, Aspergillus flavus, Aspergillus niger* and *Candida albicans*. All the extracts were active on *Bacillus cereus, Salmonella typhi* and *Escherichia coli*. The extracts were active on *Staphylococcus aureus* except for ethyl acetate extract. No activities were recorded on *Klebsiella pnuemoniae* and *Pseudomonas aeruginosa* for all the extracts.

E. hirta exhibited activity against both Gram positive and Gram negative organisms which are *E. coli, P. vulgaris, P. aeruginosa* and *S. aureus* (Sudhakar *et al.*, 2006) and antibacterial activity having also been reported for different *Euphorbia* spps (Annapuma *et al*., 2004) and this s in agreement with this study.

From the results of aqueous extracts of *Euphorbia kamerunica*, *Bacillus cereus* had inhibition zone of 35.5±2.5 mm, *Bacillus licheniformis* 34.5±2.5 mm and *Bacillus subtilis* 20.0±2.0 mm. All Gram-positive organisms gave greater inhibition zones than the Gram-negative ones. The aqueous extract had no activities on *Pseudomonas aeruginosa* and *Salmonella typhi* and the inhibition zones for *Escherichia coli* was 17.0±0.0 mm, *Klebsiella pneumoniae* 23.5±2.5 mm and *Proteus mirabilis* 15.0±1.0 mm at 100.0 mg/mL.

The greater resistance of Gram- negative bacteria to plant extracts has been reported previously (Paz *et al.,* 1995; Vlietinck *et al.,* 1995; Kudi *et al.,* 1999) and it is supported by this study. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a barrier to the many environmental substances including antibiotics (Tortora *et al.,* 2001).

CONCLUSION

From this study, extracts of *Euphorbia kamerunica* had antimicrobial properties on both Gram positive and Gram negative organisms at varying degrees and this can be further studied and the compounds used in the treatment of some infections associated with these organisms.

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