

SHORT COMMUNICATION

Antimicrobial and brine shrimp activity of *Acanthus pubescens* root extracts

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Abstract: The root dichloromethane and ethyl acetate extracts of *Acanthus pubescens* (Oliv.) Engl (ACANTHACEAE) exhibited weak antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus anthracis*, *Salmonella typhi*, *Streptococcus faecalis*, *Streptococcus agalactiae* and *Pseudomonas aeruginosa*, with MIC values ranging from 1.6-6.25 mg/ml. The two extracts also exhibited weak antifungal activity against *Candida albicans* (MIC 6.25 mg/ml). Using the brine shrimps lethality test ethanol, ethyl acetate and aqueous extracts were virtually non-toxic to brine shrimp larvae, but the dichloromethane extract (LC₅₀ 38.9 µg/ml) was mildly toxic. These results support the use of the plant in traditional medicine to treat gonorrhoea, syphilis, gastroenteritis and pneumonia. Since the plant is used in combination with other plants it is difficult to make any final conclusions regarding safety and efficacy. Further work is needed to evaluate the activity of an extract made from a combination of the six plants.

Keywords: *Acanthus pubescens*, traditional medicine, antimicrobial activity

Acanthus pubescens (Oliv.) Engl (ACANTHACEAE) also known among the Haya people of Bukoba in Tanzania as "Amatoju" is used in traditional medicine for treatment of syphilis and gonorrhoea. The roots are cut into small pieces and boiled with water or meat broth and two bowls are administered per day, for up to a week for the treatment of gonorrhoea and syphilis. The roots are boiled in a mixture with the roots of *Sapium ellipticum*, *Trichilia emetica*, *Zehneria scabra*, *Emilia javanica* and *Oxygonum sinuatum*. Reports from the literature indicate that a decoction of the leaves is used in Rwanda for the treatment of gastroenteritis, pneumonia and anthrax (Boily & van Puyvelde, 1986). It is also reported that, in Rwanda, a preparation of the dried leaves is used externally as a remedy for scabies (Heyndrickx *et al.*, 1992).

An 80% ethanol extract of the leaves exhibited antifungal activity against *Microsporum canis* and *Trichophyton mentagrophytes*, a weak antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and antiviral activity against the poliovirus and measles viruses (Vlietinck *et al.*, 1995).

The present study is part of an ongoing initiative to evaluate ethnobotanical resources in the Kagera Region of Tanzania and ways in which these resources could contribute to poverty alleviation strategies. Plants used as anti-infectives can be developed into topical preparations, evaluated for skin hypersensitivity reactions, and put into clinical

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use in a short time. The need to provide data to support commercial exploitation of these plants is significant, given the amount of poverty that exists among these remotely located communities. The current study, therefore, aims to demonstrate presence of antimicrobial activity in root extracts which has not been reported before and in addition through the brine shrimp test to obtain preliminary indication of safety of the plant extracts.

Ethanol, dichloromethane, and ethyl acetate were purchased from Fisher Scientific UK Ltd (Leicestershire, LE 11 5RG, UK). Saboraud's dextrose agar (SDA) and Tryptone soya agar and broth were purchased from Oxoid Ltd (Basingstoke, Hampshire, England), while Iodonitro tetrazolium chloride and dimethyl sulphoxide (DMSO) were purchased from Sigma (Poole, Dorset, UK). Brine shrimp eggs were bought from Aquaculture Innovations (Grahamstown 6140, South Africa). Sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam coast in Tanzania.

Acanthus pubescens roots were collected from Buzi village, Bugabo Ward in Bukoba Rural District in north-west Tanzania. The plant (Voucher No. MJM 3028) was identified at the Department of Botany, University of Dar es Salaam, Tanzania. Duplicate vouchers are kept at the Herbarium of the same Department and the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences in Dar es Salaam.

The roots of the plant were ground into powder and 460 g of the powder was sequentially soaked for 48 h, with dichloromethane, ethyl acetate, ethanol and distilled water. The extracts were dried using a rotary evaporator, at 40°C, followed by freeze drying to remove remaining water. The powders were then stored at -20°C until the time of testing. The respective yields were 2.6 g (0.56%), 0.9 g (0.20%), 2.3 g (0.50%) and 4.4 g (0.96%) for dichloromethane, ethyl acetate, ethanol and distilled water, respectively.

Plant extracts were all dissolved in dimethyl sulphoxide. Antibacterial and antifungal activities were tested by the disc-diffusion method (Singh *et al.*, 2002). Eight standard bacteria, *Staphylococcus aureus* (NCTC 6571), *Streptococcus faecalis* (clinical isolate), *Sterptococcus agalactiae* (NCTC 8181), *Pseudomonas aeruginosa* (NCTC 10662), *Salmonella typhi* (NCTC 8385), *Bacillus anthracis* (NCTC), *Bacillus subtilis* (clinical isolate), and one fungus, *Candida albicans* (Strain HG 392) were used. Filter paper discs (Whatman No. 1; 5 mm diameter) were impregnated with crude extracts (5 mg/disc) while standard drugs (10 µg/disc gentamicin; for bacteria) and clotrimazole (20 µg /disc for fungi) were purchased from Sigma (Poole, Dorset, UK). Discs containing DMSO alone were used as a negative control. The discs were overlaid on pre-inoculated tryptone soya agar plates (for bacteria) and Saboraud's dextrose agar plates (for fungi) and incubated at 37 °C, for 24 h. The discs were tested in triplicate, including one with a solvent blank (DMSO) and one with a standard drug. The results of the disc diffusion method were only used to detect active extracts for MIC determination using the microdilution method. This was necessary to cut down on wastage of the microtitre plates.

MICs were determined using the microdilution method (Ellof, 1998). The 96 well microtitre plates were used and each plant extract was tested in duplicate at serial dilutions of 10, 5, 2.5, 1.25, 0.625, 0.31, 0.16 and 0.08mg/ml. Columns 1 and 2 were used for solvent controls and Columns 11 and 12 for positive controls. Each well was first filled with 100 µl broth followed by addition of 100 µl test drug, DMSO or standard drugs in the first well of each column and serially diluted. The bacteria suspension (100µl) was then added in each well and the plates were incubated at 37°C for 24h. After 24h, 40 µl of 2 mg/ml (0.02%) iodonitrotetrazolium chloride was added in each well and the plates were incubated for 1h

at 37°C for detection of bacterial and fungal growth. MICs for fungi and bacteria were determined by visual inspection.

The brine shrimp lethality test (BST) was used to predict the presence, in the extracts, of cytotoxic activity (Meyer *et al.*, 1982). Solutions of the extracts were made in DMSO or distilled water, at varying concentrations and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the duplicate vials. Control brine shrimp larvae were placed in a mixture of artificial sea water (3.8g of sea salt per litre) and DMSO only. After 24 h the nauplii were examined against a lighted background, and the average number of survived larvae in each triplicate was determined. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC₅₀) was determined from the graph (Meyer *et al.*, 1982).

The mean percentage brine shrimp mortalities were plotted against the logarithms of concentrations using the Fig P computer program (Biosoft Inc, USA), which also gives the regression equations. The regression equations were used to calculate LC₁₆, LC₅₀ and LC₈₄ values. Confidence intervals (95% CI) were then calculated using the three results (Litchfield & Wilcoxon, 1949; Meyer *et al.*, 1982). An LC₅₀ value greater than 100 µg/ml was considered to represent an inactive compound or extract

Using the disc diffusion method dichloromethane and ethyl acetate extracts of *Acanthus pubescens* root extracts exhibited antibacterial activity against both gram positive and gram negative bacteria. They exhibited weak antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus anthracis*, *Salmonella typhi*, *Streptococcus faecalis*, *Streptococcus agalacticus* and *Pseudomonas aeruginosa*. They also showed weak antifungal activity against *Candida albicans*. The MICs results ranged from 1.6-6.25 mg/ml (Table 1).

Table 1: MICs results for *Acanthus pubescens* root extracts.

Organism	MIC (mg/ml)	
	DCM extract	Ethyl acetate extract
<i>Staphylococcus aureus</i>	6.25	1.6
<i>Salmonella typhi</i>	-	3.12
<i>Streptococcus faecalis</i>	6.25	-
<i>Bacillus subtilis</i>	3.12	-
<i>Bacillus anthracis</i>	6.25	1.6
<i>Pseudomonas aeruginosa</i>	-	6.25
<i>Streptococcus agalactiae</i>	6.25	25.0
<i>Candida albicans</i>	6.25	6.25

The results for brine shrimp toxicity are reported in Table 2. The aqueous and ethanolic extracts were least toxic, while the dichloromethane extract was the most toxic.

Table 2: Results for brine shrimp toxicity of the extracts

Extracts	LC ₅₀ (µg/ml)	95% CI (µg/ml)
Dichloromethane	38.9	27.4 -55.2
Ethylacetate	136.2	92.0 – 201.6
Ethanol	>1000	-
Aqueous	>1000	-
Cyclophosphamide	16.3	10.6-25.1

Preparations of *Acanthus pubescens* are used in traditional medicine for the treatment of syphilis, gonorrhoea, gastroenteritis, anthrax and scabies. This suggests that preparations of the plant have both antibacterial and antifungal activity. The results have shown that ethyl acetate and dichloromethane root extracts of *Acanthus pubescens* have weak activity against both Gram positive and Gram negative bacteria. They also have weak antifungal activity against *Candida albicans*. According to Aligiannis *et al.* (2001) MICs above 1.6 mg/ml indicate weak activity. Since all the MICs of the *Acanthus pubescens* extracts were 1.6 mg/ml and above then they all exhibited weak antibacterial and antifungal activity.

Traditionally the plant roots are not used alone; the roots are boiled together with the roots of *Sapium ellipticum*, *Trichilia emetica*, *Zehmeria scabra*, *Emilia javanica* and *Oxygonum sinuatum*. The long duration of use (1 week) and the fact that the plant is not used alone shows how this plant may be effective for treatment of infections even though it does not have very good *in vitro* activity. However, what one can not say is on the possible metabolism of the active compounds *in vivo* to more active compounds, which at this point remains a speculation. It will also be interesting to test the activity of the mixture as compared to the single plants.

The brine shrimp lethality test (BST) was used to predict trends of toxicity and possible presence of potential anticancer compounds (Moshi *et al.*, 2004). The results are interpreted as follows: $LC_{50} < 1.0 \mu\text{g/ml}$ – highly toxic; $LC_{50} 1.0\text{-}10.0 \mu\text{g/ml}$ – toxic; $LC_{50} 10.0\text{-}30.0 \mu\text{g/ml}$ – moderately toxic; $LC_{50} > 30 < 100 \mu\text{g/ml}$ – mildly toxic, and $> 100 \mu\text{g/ml}$ as non-toxic. Cyclophosphamide ($LC_{50} 16.3 \mu\text{g/ml}$) was used as a standard so that it can allow inference to be made for potential to yield anticancer compounds.

The results show that the aqueous and aqueous ethanolic extracts have LC_{50} values above $1000 \mu\text{g/ml}$, indicating that they are virtuously non-toxic. The dichloromethane and ethyl acetate extracts showed LC_{50} values of 38.9 and $136.2 \mu\text{g/ml}$, respectively. This indicates that the ethyl acetate extract is also virtually non-toxic. However, the dichloromethane extract showed mild toxicity.

One way of preparing the plant for use is by boiling the roots with meat broth, which would effectively provide a fatty base for extraction of compounds picked up in the dichloromethane and ethyl acetate extracts. This suggests that the potential for toxicity is higher when the preparation is made with meat broth. However, in the absence of information on the mixture that is used not much can be emphasized regarding either efficacy or safety.

In conclusion extracts of *Acanthus pubescens* exhibited weak antibacterial activity against Gram positive and Gram negative bacteria and antifungal activity against *Candida albicans*. This supports the traditional medicine use and shows potential for isolation of active compounds.

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