Studies on the Antibacterial Properties of the Leaf Extracts of *Chromolaena odorata* (L.) King and Robinson (Asteraceae)

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Abstract

Ethanol and aqueous extracts of the leaf of Chromolaena odorata (L.) King and Robinson were tested against nine bacterial strains using the agar well diffusion method. The ethanol extract was active against 4 (44%) of the test organisms. The aqueous extract was also active against 4 (44%) of the test bacterial species. None of the extracts had any activity against Proteus mirabilis. Also none of the test bacterial strains was susceptible to the effect of both extracts. Ethanol extract was active against Escherichia coli (ATCC 11775), Salmonella typhi, Pseudomonas aeruginosa (ATCC 10145) and Staphylococcus aureus. The water extract had activity against Escherichia coli, Salmonella kintanbo (Human 1, 13, 32: mt:-), Pseudomonas aeurginosa and Staphylococcus aureus (ATCC 12600). The MIC values for the ethanol extracts range from 6.25 to 25mg/ml and correlates with the MIC values for the water extracts which was equally between 6.25 to 25mg/ml. Our result provides a justifiable platform on which the use of C. odorata in folk and traditional medicine for treatment of various ailments is based.

Keywords: *Chromolaena odorata*, Ethnomedicine, Phytochemical analysis

Introduction

Chromolaena odorata, family of Asteraceae, (common name: Siam weed, Jack in the Bush) has wide applications in African ethnomedicine. In Nigeria especially, herbal preparations made from the leaf extracts of C. odorata is dispensed by traditional health practitioners for the treatment of cough, stomach upset due to Samonellosis or dysentery and in dressing of wounds. When administered mixed with decoction of lemon grass and guava leaves, a very potent antiplasmodial regimen, which can be applied against malaria is produced (Iwu, 1993; Dalziel, 1961). The leaf extract have been reported to have anti fungal properties (Ngane et al., 2006) as well as other medicinal uses which include antidiarrhoea, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Igbo, 1981). In spite of the rich and promising potential of this plant, it is surprising that not much scientific knowledge about its antibacterial properties is available as basis for use in human pathology affordable. Also, due to the challenge costs and drug resistance poses to qualitative health care delivery, we felt prompted to search in the direction of traditional medicine for affordable alternative to orthodox medical treatment from common Nigerian plants. In this work, we determined qualitatively, the active phytochemical components of *Chromolaena odorata* as well as its antibacterial activity against nine bacterial strains comprising three strains from the American Type Culture Collection (ATCC), one stereotyped local *Salmonella* strain and five strains isolated from our clinical laboratory.

Materials and Methods

Plant material: The leaf of *C. odorata* used in this study was collected form a forest at Ajuona in Nsukka, Enugu state of Eastern Nigeria. The plant was identified taxonomically by Mr. Gabriel Ugwu of the Botany Department of the University of Nigeria, Nsukka. Thereafter, a voucher specimen was deposited in the department herbarium.

Extraction of plant material: The *C. odorata* leaves were first air dried at room temperature (27 ± 2°C) of the laboratory and thereafter pulverised to powder by a mechanical grinder. A 50g portion of the pulverised plant material was separately soaked in a 500ml flask containing 400ml of 98% reagent grade ethanol or distilled water and left stranding for 24hrs. Each preparation was filtered through a Whatman No 1 filter paper and the filtrate evaporated to dryness in a steady air current for about 24hrs in a previously weighed crucible (Okoli *et al.*, 2002). The dried extract was exposed to UV rays for 24hrs and checked for sterility by streaking on nutrient agar plates.

Phytochemical screening: The ethanol and distilled waterleaf extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical method for the presence of alkaloids, flavonoid, tannins, saponin, glycosides and protein (Harbone, 1984).

Test bacterial strains: Standard typed cultures of *Escherichia coli* (ATCC 11775), Pseudomonas aeruginosa (ATCC 16145). *Staphylococcus aureus* (ATCC 12600) were obtained from Bioresources Development and Conservation Project (BDCP), Nsukka. Salmonella kintanbo (human 1, 13, 23, mt:) was supplied by the Veterinary Microbiology an Pathology Laboratory of the University of Nigeria, Nsukka. Proteus mirabilis, *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the clinical laboratory of the Department of Microbiology, University of Nigeria, Nsukka. All test strains were reisolated successively on Nutrient Agar (Oxoid) and their identity confirmed by

standard bacteriological methods (Collins and Lyne, 1970).

Screening of extract for antimicrobial activity: The extracts were spot checked for antimicrobial activity using the agar well diffusion technique (Okeke et al. 2001). 1ml of each test bacterium standardised to an inoculum concentration equivalent to 1.0 McFarlands ($3.0x\ 10^8$ cfu/ml) was introduced by a Finn pipette and spread onto sterile nutrient agar plates so as to achieve a confluent growth. The excess was drained using a Finn pipette. The plates were allowed to dry and a sterile Cork borer of 8mm diameter was used to bore wells in the agar plates.

The extract was reconstituted in distilled water to a concentration of 50mg/ml. Subsequently, a 100µl volume of the extract was introduced in triplicate wells into the nutrient agar cultures. The plates were allowed to stand for 1hr or more for diffusion to take place and then incubated at 37°c for 24hrs. The zone of inhibition was recorded to the nearest millimetre (mm). Only extract exhibiting apparent zone of inhibition was chosen for further evaluation.

Determination of minimum inhibitory concentration (MIC): The MIC was determined for the ethanolic and aqueous leaf extracts by a modified agar well diffusion technique (Okeke *et al.*, 2001). A two fold serial dilution of the extract was prepared by first reconstituting and then diluting in sterile distilled water to achieve a decreasing concentration range of 50 to 6.25 mg/ml, a 100µl volume of each dilution was introduced in triplicate wells into Nutrient agar plates already seeded with the standardised inoculum ($3.0x10^8$) of the test bacterial cells. All test plates were incubated at 37°c for 24hrs. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

Results

The phytochemical analysis showed that the ethanol and aqueous extracts of *C. odorata* contained saponins, phenolic compounds and alkaloids (Table 1). While the ethanolic leaf extract contained terpenoids, the water extract showed presence of steroids and flavonoids. Both extracts lack tannins.

Table 1: Phytochemical Screening of Ethanolic and Aqueous Extracts of *C. odorata*

Plant constituent	Ethanolic Extract	Aqueous Extract
Terpenoids	+ + +	-
Steroids	-	+
Saponins	+	+ +
Phenols	+	+
Flavonoids	-	+
Alkaloids	+ + +	+ + +
Tannins	-	-

- Negative, + Low, ++ Moderate, +++ High

Of the nine bacterial strains screened, against the effect of the two extracts, the ethanolic extract showed activity against 4(44%) of the test strains namely *E. coli* (ATCC 11775), *P. aeruginosa* (ATCC 10145) and the clinical isolates S. typhi and S. aureus (Table 2).

Table 2: Inhibition Zone Diameter (IZD)mm of Extracts Against the Bacterial Strains

Test Organism	Extracts	
	Ethanol	Aqueous
E. coli (T)	+ + +	-
E. coli (C)	-	+
S. kintanbo (T)	-	+ +
S. typhi (C)	+ +	-
P. aeruginosa (T)	+ + + +	-
P. aeruginosa (C)	+	+ +
S. aureus (T)	+ +	+ +
S. aureus (C)	+	-
P. mirabilis (C)	-	-
% Showing Activity per Strain	4(44)	4(44)
Control Distilled Water	0.0	0.0

+ = Presence of Inhibition, - Absence of Inhibition, + = 10 – 12mm, T = Typed Strain (ATCC), ++ = 13 –15mm, C = Clinical Strain, +++ = 16 – 18mm, ++++ = >18mm

The water extract equally had activity against 4(44%) of the test bacterial strains which included *S. kintanbo* (1, 13, 23 mt) *S. aureus* (ATCC 12600) as well as the clinical isolates *E. coli and P. aeruginosa*. Both extracts had no activity against *P. mirabilis*. The highest mean zone of inhibition against test isolates was >18mm recorded against *S. aureus* (ATCC 12600) for the aqueous extract and *Pseudomonas aeruginosa* (ATCC 10145) for the ethanolic extracts.

The minimum inhibitory concentration (MIC) was determined for both extracts because of their wide spectrum of anti bacterial activity. The MIC values of the ethanolic extracts ranged from 6.25mg/ml for S. typhi and P. aeruginosa (ATCC 10145) to 25mg/ml for E. coli (ATCC 11775) and S. aureus. The aqueous extract MIC values ranged from 6.25mg/ml for *E. coli*, *S. kintambo* (human 1, 13, 23 mt) and P. aeruginosa (ATCC 10145) to 25mg/ml for *S. aureus* (ATCC 12600) (Table 3).None of the extracts had any activity against *P.mirabilis*.

Table 3: Minimum Inhibitory Concentration (MIC) of the Ethanolic and Aqueous Extracts against Test Bacterial Strains

Test Bacterial Strains	Ethanol	Aqueous
E. coli (T)	25(22)	ND -
E. coli (C)	ND -	6.25 (22)
S. kintanbo	ND -	6.25 (23)
S. typhi (C)	6.25 (22)	ND -
P. aeruginosa (T)	6.25 (25)	6.25 (20)
P. aeruginosa (C)	ND -	ND -
S. aureus (T)	ND -	25 (27)
S. aureus (C)	25 (21)	ND -
P. mirabilis (C)	ND -	ND –

ND = Not determined, T = Typed Strains (ie ATCC), C = Clinical, - = No activity

Discussion

The yield and anti bacterial activity of plant extracts vary extensively with the solvent used for extraction. Ethanol and water are known to be better extraction solvents than other compounds such as benzene and chloroform (Nkere and Iroegbu, 2005).

The leaf extract of *C. odorata* obtained with analytical grade ethanol gave exactly the same spectrum of activity (44.44%) against the test strains as compound with the aqueous extracts. This is significant because herbal medicines are traditionally administered mixed with water or locally distilled liquor to achieve the same effects.

The phytochemical components on the extracts e.g. Terpenoids, Saponins, Phenols, Alkanoids are known to have antimicrobial activity (Leven *et al.*, 1979). However due to the absence of Terpenoids in the aqueous extract of *C. odorata* probably due to the lipophilic nature of the compound, it seems most probable that the anti microbial properties associated with the extract could be due to the presence of alkaloids principally and some traces of saponins and phenols. Thus for both extracts to have comparable effects, the active principles were soluble to the same degree in both ethanol and water. The antibacterial activity displayed by both extracts against test bacterial strains is quite significant. Some of the test organisms are usually implicated in some diseases against which *C. odorata* is a preferred herbal remedy. Our results support folklore claims that herbal preparations of *C. odorata* whether extracted in alcohol or water is active sufficiently for use in curative therapy of such diseases.

Further research is probably required to extract and concentrate the active principles to fully establish their antibacterial profiles.

References

Collins, C. H. and Lyne, P. M. (1970). Microbiological Methods. Butherwort, London.

Dalziel, J. M. (1961). The Useful Plants Of West Tropical Africa. The Crown Agents, London

Horbone, J. B. (1984). Phytochemical Methods. Chapman and Hall, London. pp 166 – 226.

Igbo, T. N. (1981). B. Pharm Project Report. University of Nigeria, Nsukka.

Iwu, M. M. (1993). Handbook of African Medicinal Plants. CRC Press Inc, USA, pp 219 – 221.

Ngane, A. V., Etame, R. E., Ndifor, F., Biyiti, L. Amvanzollo, P. H. and Bouchet P. H. (2006). Antifungal Activity of Chromolaena odorata. L. King & Robinson (Asteraceae) of Cameroun. Chemotherapy 2006; 52; 103 – 106

Nkere, C. K. and Iroegbu, C. U (2005). Antibacterial Screening of the Root, Seed and Stem bark extracts of *Picralima nitida*. Afr. Jour. Biotech. 4(6); pp 522-526.

Okeke, M. I., Iroegbu, C. U., Eze, E. N., Okoli A.S. and Esimone, C. O. (2001). Evaluation of extracts of the Root of *Landolphia owerrience* for antibacterial activity. J. Ethnopharmacol. 78: (2001) pp 119-127.

Okoli, A. S., Okeke, M. I., Iroegbu, C. U., Ebo, P. U. (2002) Activity of *Harungana madagascariensis* leaf extracts. Res 16: 174 –179.