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ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF CALOTROPIS PROCERA AGAINST FOOD SPOILAGE ORGANISMS

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ABSTRACT

In a programme to source for local alternatives to chemical preservatives of food, a preliminary in-vitro antimicrobial activities of crude aqueous and ethanolic extracts of *Calotropis procera* leaves against some food spoilage organisms were determined. The test organisms were *Staphylococcus aureus, Escherichia coli, Pseudomonas, Klebsiella, Salmonella* and *Proteus* species. The in-vitro antimicrobial assay showed that at 5mg/ml, the test extracts inhibited some of the test bacteria on agar producing growth inhibitory zones ranging from 12.0mm (*Proteus, Salmonella, Klebsiella* species) for both aqueous and ethanolic extract to 20mm (*Pseudomonas* spp) for hot ethanolic extract. The minimum inhibitory concentrations (MIC) ranged from 1.09mg/ml (*Salmonella* spp) for the cold ethanolic extract to 66.3mg/ml (*Klebsiella* spp) for the hot ethanolic extract. The lowest minimum bacteriocidal concentration (MBC) was 6.25mg/ml (*E.coli*) for the cold aqueous extract and the highest was 50.0mg/ml (*Salmonella* spp) for the hot aqueous extract. The results indicated the efficacy of crude extracts of *Calotropis procera* in controlling the growth of food spoilage organisms.

Keywords; *Antimicrobial activity*, *Calotropis procera*, minimum inhibitory concentration (MIC) minimum bactericidal concentration (MBC), Spoilage organisms.

INTRODUCTION

In many African countries the diets comprise staple food such as milk, cassava, fish, cereals and there numerous products which are mainly preserved by fermentation (Odunfa, 1985).

Mostly, fermented food production in Africa, remains a traditional art, with the use of chance inoculation, or back slopping and are often associated with poor hygiene, quality variation and short shelf life (Onyekwere *et al*, 1989; Olasupo *et al,* 1999). This situations expose the food to microbial spoilage and apart from the economic loss to the manufacturer, more important is the health hazards to the unsuspecting consumers (Yamamura *et al,* 2000). Various measures, including pasteurization and use of additives have been adopted to prevent food spoilage. To further prevent spoilage and ensure food safety, antimicrobial agents called preservatives are added to eliminate or inhibit microbial growth.

The growing consumer apathy to chemical preservatives, however, has necessitated the search for alternatives means of food preservation with minimal side effect on the health of the consumers. Currently, there is growing interest on the use of the indigenous plant as veritable source of biopreservatives due to their promising anti microbial activities (Akpan and Fadeyi, 1990; Egwari, 1999; Hammer *et al,* 1999; Okemo *et al* 2001; Olasupo *et al,*_2003).

Calotropis procera (family; *Asdepiadaceae*) locally known as "*bomu-bomu*" in Yoruba land, Nigeria, because of its balloon-like flower, is a shrub found in both the savannah and tropical forest of West Africa, growing to height of 3m, with alternating, large, stiff oblivate leaves (Dingemans *et al.,* 1990). The plant is also known as "Sodom apple". The stem and leaves of the plant produce a whitish sap which traditionally is applied to fresh wound as dressing (Dingemans *et al.*, 1990). Decoctions from the leaves is used as a coagulant in the production of "*Wara*" and "*Waogachi*", cheese products in Nigeria and Benin Republic respectively.

Though the juice of the plant is used traditionally as wound dressing and coagulant in local cheese production, reports on its antibacterial spectrum are scanty. Our present study investigated and reports on the antibacterial activity of crude extracts of *C procera* on food spoilage organisms.

MATERIALS AND METHODS

Plant Materials: *Calotropis procera* (fresh leaves) was collected from the premises of the Federal Institute of Industrial Research, Oshodi (FIIRO) and other areas of Lagos in Nigeria. The taxonomic identification of the plant was established by Prof. Olowokudejo of the Department of Botany and Microbiology, University of Lagos. The leaves were dried at room temperature away from sunlight for one day after which they were further dried in the oven at 60%. The dried leaves were ground into fine powder using an electric blender. The powdered sample was aseptically kept in clean, sterile containers until the time of extraction.

Extraction: For the cold extraction, 200g of powdered plant material were infused separately in 250 ml sterile water and 2L of absolute ethanol for 5 days. Each preparation was sieved with fine wire mesh, centrifuged and then filtered using watmman no.1 filter paper. Both extracts were freeze-dried, weighed and stored in the refrigerator at 8°C prior to use. For the hot extraction, the powdered leaves (100g) were soxhlet extracted with sterile water and absolute ethanol respectively for about 10 h (*Chhabra et al,* 1982) and concentrated in a rotary evaporator at 40°C. The concentrated extracts were weighed and stored in the refrigerator at 8°C prior to use.

The test bacteria: The spoilage organisms isolated from food samples were obtained from the culture collection of Genetics Division of the National Institute for Medical Research (NIMR), Yaba/Lagos in Nigeria. The indicator strains were *Staphylococcus aureus, Escherichia coli, Salmonella, Klebsiella, Pseudomonas* and *Proteus* species.

Antimicrobial Assays

Disc diffusion assay: Each of the test organisms of inoculum size 10^8 cfu/ml (*Bauer et al,* 1966) was inoculated onto Mueller Hinton agar plates with sterile cotton swabs (sterilin) soaked in the inoculum. Disc of the extract (5mg/ml) was placed firmly on the surface of each of the inoculated agar plates and incubated at 37°C for 18h under aerobic conditions. Zones of inhibition of growth were measured and recorded in millimeters. Inoculated plates with disc impregnated with sterile water served as the positive control.

Broth dilution assay: Extract powder; 1g was dissolved in 10 ml of Mueller Hinton broth (MHB) to form the stock solution (100mg/ml). Minimum inhibitory concentrations were determined as the lowest concentration of extract that produced no turbidity in MHB.

With sterile cotton swab stick, broth was transfered from each negative tube in the MIC assay and inoculated on fresh Mueller Hinton agar (MHA) by spread plate technique. Control was set up by inoculating broth from the MIC assay control tube onto fresh MHA. Plates were incubated at 37°C for 24 hours. MBCs were determined as the lowest concentrations of extract that produced no visible growth on MHA.

RESULTS

The zones of growth inhibition (in mm) of the indicator organisms by cold and hot extracts of *Calotropis procera* at 5 mg/ml is present in Table 1. The cold and hot aqueous extract did not inhibit the growth of *S. aureus, Klebsiella* and *Pseudomonas* species. The cold aqueous extract inhibited the growth of *E. coli* and *Proteus* sp with zone diameter of 15.0 mm and 12.0mm respectively but did not inhibit *Salmonella* sp. The hot aqueous extract inhibited *Salmonella* and *Proteus* species producing zone diameters of 12.0mm but did not inhibit *E coli.* The cold ethanolic extract inhibited the growth of *S. aureus, Klebsiella, Pseudomonas, Salmonella* and *Proteus* species with zone diameters of 17.0, 12.0, 15.0, 17.0 and 17.0 mm respectively but did not inhibit the growth of *E. coli.* The hot ethanolic extract inhibited the growth of *E. coli, S. aureus, Klebsiella, Pseudomonas* and *Salmonella* species with zone diameters of 17.0, 15.0, 15.0, 20.0 and 14.0 mm respectively, but did not inhibit *Proteus* sp.

Table 2 shows the minimum inhibitory concentrations (MICs) of extracts of *C procera* against the test bacteria by the broth dilution method. The cold and hot aqueous extracts did not inhibit the growth of *S. aureus, Pseudomonas* and *Klebsiella* species at all the test concentrations. Also, the cold aqueous extract failed to inhibit the growth of *Salmonella* sp and the hot aqueous extract failed to inhibit the growth of *E. coli* and *Proteus* sp at all the test concentrations. The cold aqueous extract however, had MIC of 3.13 and 12.50 mg/ml, against *E. coli* and *Proteus* sp respectively. The hot aqueous extract had MIC of 25.0mg/ml against Salmonella sp. The cold ethanolic extract had MIC of 4.38 mg/ml against *S. aureus* and *Proteus* sp, 17.50 mg/ml against *E. coli* and *Klebsiella* sp, 8.75 mg/ml against *Pseudomonas* sp and 1.09 mg/ml against *Salmonella* sp. The hot ethanolic extract did not inhibit the growth of *E. coli* at all test concentrations but had MIC of 17.0 mg/ml against *S. aureus* and *Pseudomonas* sp, 66.3 mg/ml against *Klebsiella* sp, 13.30 mg/ml against *Salmonella* sp, 33.0 mg/ml against *Proteus* sp.

Table 3 shows the minimum bactericidal concentrations (MBCs) of extracts of *C. procera* against the test bacteria. The cold and hot aqueous extracts did not inhibit the growth of *S. aureus, Pseudomonas* and *Klebsiella* species at all the test concentrations during the MIC assay, therefore, the MBC of the aqueous extracts were not determined for these organisms. The cold aqueous extract had MBC of 6.25 mg/ml and 25.0 mg/ml against *E. coli* and *Proteus* sp respectively. The MBC of the hot aqueous extract was 50.0 mg/ml against *Salmonella* sp. The cold ethanolic extract had MBC of 35.0 mg/ml against *E. coli, Pseudomonas, Klebsiella* and *Proteus* species and 17.5 mg/ml against *S. aureus* and *Salmonella* sp. The hot ethanolic extract had MBC of 66.3 mg/ml against *S. aureus*, 33.0 and 26.5 mg/ml against Pseudomonas and *Salmonella* species and 13.3 mg/ml against *Klebsiella* and *Proteus* species respectively.

Table 1; Antimicrobial activity of leaf extracts of *C. procera* by disc diffusion method. Zone of inhibition at 5mg/ml (mm diameter) Organism

	Cold aqeous	Hot aqeous	Cold ethanolic	Hot ethanolic
S. aureus	0.0	0.0	17.0	15.0
E. coli	15.0	0.0	0.0	17.0
Pseudomonas sp.	0.0	0.0	15.0	20.0
Klebsiella sp.	0.0	0.0	12.0	15.0
Salmonella sp.	0.0	12.0	17.0	14.0
Proteus sp.	12.0	12.0	17.0	0.0

Table 2. Minimum Inhibitory Concentrations of extracts of *C procera* (against test bacteria) by the broth dilution technique.

Organism	Minimum Inhibitory Concentration (mg/ml)			
	Cold aqeous	Hot aqeous	Cold ethanolic	Hot ethanolic
S. aureus	NI	NI	4.38	17.0
E. coli	3.13	NI	17.50	NI
Pseudomonas sp	NI	NI	8.75	17.0
Klebsiella sp.	NI	NI	17.50	66.3
Salmonella sp.	NI	25.0	1.09	13.3
Proteus sp.	12.50	NI	4.38	33.0

NI = No inhibition.

Table 3. Minimum Bactericidal Concentrations of extracts of *C. procera* against test bacteria.

Organism	Minimum Bactericidal Concentrations (mg/ml)			
	Cold aqeous	Hot aqeous	Cold ethanolic	Hot ethanolic
S. aureus	ND	ND	17.5	66.3
E. coli	6.25	ND	35.0	ND
Pseudomonas sp	ND	ND	35.0	33.0
Klebsiella sp.	ND	ND	35.0	NC
Salmonella sp	ND	50.0	17.5	26.5
Proteus sp.	25.0	ND	35.0	NC

ND = Not determined. NC = Not cidal at all tested concentration.

DISCUSSION

There appears to be a revival in the use of traditional approaches to protecting livestock and food from disease, pest and spoilage in industrialized countries. This is especially true in regard to plant volatile oils and their antimicrobial evaluation (Dorman and Deans, 2002). Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and could serve as source of antimicrobial agents against food pathogens (Kim *et al.*, 1995).

This study shows that both aqueous and ethanolic leaf extracts of *Calotropis procera* possess antimicrobial activity against the indicator organisms. This finding gives credence to the traditional use of the juice from vegetative parts of the plant as wound dressing (Dingemans *et al,* 1990). It also implies that, the use of its leaf decoctions in local cheese might go beyond the purpose of coagulation (Dingemans *et al,* 1990) to include its possible effect on preservation.

Dada *et al.*(2002) reported a correlation between the antimicrobial activities of some plant extract and their traditional uses. The ethanolic extracts were greatly more active against the spoilage bacteria than the aqueous extracts of the same concentrations. Olukoya (1995) and Egwari (1999) proved organic extracts to be more effective against microorganisms, than the corresponding aqueous extracts of same concentrations. This may be attributed to the extraction of more bioactive principles of the plants by the organic solvents than the aqueous solvents and/or the greater diffusibility of the organic extract in the media than the aqueous extracts (Egwari, 1999)

E. coli was the only indicator organism that was not inhibited by the hot ethanolic extract at all the concentrations tested. Okemo *et al* (2001) reported resistance of *E. coli* to sub-inhibitory concentrations (2mg/ml and 4 mg/ml) of *Azadirachta india* and its susceptibility at higher concentration of 8mg/ml. From this report, it may be that at higher concentrations than tested, *E. coli* might be susceptible to the extracts of *C. procera*. The extracts were most active against *Salmonella* sp. This is of particular interest in view of increasing threat of antibiotic resistant strains of *Salmonella* sp (Giraud *et al.*, 2003). The MIC values of the extracts were lower than the MBCs, indicating that the extracts are bacteriostatic at lower concentrations but bactericidal at higher concentrations. This is important in considering effective preservative concentration of *C. procera*. Since strains of the indicator organisms are potential pathogens, concentrated decoctions of *C. procera* may be effective therapy against food poisoning due to *S. aureus, E. coli, Pseudomonas, Klebsiella, Salmonella* and Proteus species. The purification and subsequent concentration of the bioactive principles in *C. procera* would enhance their effectiveness against spoilage and food-borne pathogens.

For years, indigenous people in Nigeria and Benin Republic have been consuming wara and woagachi cheese containing leaf decoctions of *Calotropis procera* as coagulant without any evidence to suggest danger to their health, the safety concern of using leaf extracts of this plant to control food spoilage organism is minimal.

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