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Antimicrobial activity of cowpea (*Vigna unguiculata*) leaf extracts

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Cowpea (*Vigna unguiculata* (L.) Walp), an indigenous African legume crop, is used to treat epilepsy, bilharzia, chest pains and constipation. Acetone and ethanol extracts of the leaves of Bechwana White (BW) and Kpodjiguégué (Kpod) cultivars were investigated for their antimicrobial properties against bacterial and fungal pathogens. With the exception of *Fusarium equiseti*, all the extracts significantly inhibited growth of the fungal pathogens at 5.0mg ml^{-1}. *Alternaria alternata* was significantly reduced by both BW extracts at 2.5mg ml^{-1} whereas only the ethanol extract showed antifungal activity against *Fusarium proliferatum* at the same concentration. The acetone extract from Kpod significantly inhibited the growth of *A. alternata* at 2.5mg ml^{-1}. The acetone and ethanol extracts showed no inhibitory activity at 1.0mg ml^{-1}. BW acetone extracts inhibited growth of the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, at 2.5mg ml^{-1} and *Bacillus cereus*, *B. subtilis* and *Enterobacter cloacae* at 5.0mg ml^{-1}. Ethanol extracts of the same cultivar only showed antibacterial activity against *Enterococcus faecalis* and *Enterobacter cloacae* at 5.0mg ml^{-1}. The Kpod extracts exhibited no inhibitory effect on the bacteria. This is the first report on the inhibitory effect of cowpea leaf extracts on the growth of bacterial and fungal pathogens.

Introduction

Bacterial and fungal pathogens play a negative role with regard to the nutritional and economical value of various important crop plants. These pathogens cause severe damage to the roots and aerial parts of the plants. Many pesticides as well as various other chemical formulations used to control plant pathogens are inaccessible to small-scale farmers due to financial constraints. The use of plant extracts provides a less expensive means of controlling these pathogens (Poswal *et al.* 1993). Furthermore, since chemicals pose a danger to the environment and non-targetted organisms, the use of plant extracts as an alternative means of controlling plant fungal and bacterial pathogens has been widely exploited (Poswal *et al.* 1993). There have been numerous investigations into the use of plants extracts in controlling plant pathogens (NRC 1992, Eksteen *et al.* 2001).

Moreover, bacterial and fungal pathogens are also capable of causing serious diseases in humans and animals (Van Burik and Magee 2001, Worthington and Bigalke 2001). Some fungal pathogens infest the seed during storage and produce toxic secondary metabolites — mycotoxins — which, when ingested, can cause acute and chronic toxicities in humans and animals (Barrett 2000). Due to problems with the toxicity of existing antimicrobial agents as well as the emergence of drug-resistant strains, the use of plant extracts can be exploited as an alternative way to control these pathogens. Many of these plant extracts contain secondary compounds that have an inhibitory effect on harmful bacterial and fungal human pathogens (Afolayan and Meyer 1997, Lall and Meyer 2000, Mathekga *et al.* 2000).

Cowpea (*Vigna unguiculata* (L.) Walp) is an important legume crop that is widely grown in many countries of sub-Saharan Africa and Latin America (Lattanzio *et al.* 2000). This versatile crop has various uses, which include a good source of nutritious food, animal fodder and a source of cash through trade of the seed (Singh *et al.* 1997). It also increases soil nitrogen levels and prevents soil erosion (Singh *et al.* 1997). Furthermore, an infusion of the seed can be taken orally to treat amenorrhoea whilst powdered roots eaten with porridge are believed to treat painful menstruation, epilepsy and chest pain by the indigenous people of South Africa (Van Wyk and Gericke 2000). Leaves are applied on burns and can be used as a snuff to treat headaches (Hutchings *et al.* 1996). The Zulus (a South African tribe) make emetics from the plant that are taken to relieve fever (Gerstner 1939, as cited by Hutchings *et al.* 1996). Cowpea has also been identified as a plant that

traditional healers use to treat urinary shistomiasis (bilharzia) in Zimbabwe (Ndamba *et al.* 1994). Cowpea seeds cooked with the roots of *Lannea edulis* (Sond.) Engl. (Van Wyk and Gericke 2000), *Euclea divinorum* Hiern or *Terminalia sericea* Burch ex DC. (Nyazema 1987) are used to treat blood in the urine and bilharzias by South Africans.

However, as far as the literature is concerned, no report on the antimicrobial activity of cowpea has been found thus far. This is the first report on the inhibitory effect of extracts made from cowpea leaves on the growth of various bacterial and fungal pathogens.

Materials and Methods

Plant material

Seeds of two cowpea cultivars, 'Bechwana White' (BW), obtained from the Grain Crops Institute, Agricultural Research Council, Potchefstroom (South Africa), and 'Kpodjiguégué' (Kpod), collected from a market in Cotonou, Benin (West Africa), were planted under greenhouse conditions. The plants were harvested after ± two months' growth.

Preparation of extracts

Two solvents, namely acetone and ethanol, were used for the extractions of both the cultivars. Air-dried plant material (100g) was homogenised with 250ml of the solvent for 1min and then filtered. This process was repeated three times. The filtrate was concentrated to dryness at reduced pressure with a rotary evaporator (Büchi Laboratoriums, Technik AG, Germany). The resultant residues were later dissolved with the respective solvent to 100mg ml^{-1}. In the case of the antibacterial tests, the ethanol extract was re-dissolved using dimethyl sulphoxide, since prior investigations showed ethanol to be toxic to the bacteria.

Micro-organisms

The fungal pathogens used in this investigation were *Alternaria alternata* (Fr: Fr.), *Aspergillus flavus* Link ex. Fries, *Fusarium equiseti* (Corda) Sacc., *F. proliferatum* (Matsushima) Nirenberg and *Penicillium chrysogenum* Thom. The fungal cultures were maintained on potato dextrose agar (PDA) at ±25°C. The bacteria used in this study to determine antibacterial activity of the extracts included five Gram-positive bacteria: *Bacillus cereus* Frankland and Frankland, *B. pumilus* Meyer and Gottheil, *B. subtilis* (Ehrenberg) Cohn, *Staphylococcus aureus* Rosenbach, *Enterococcus faecalis* (Andrews and Horder) Schleifer and Kilpper-Balz and five Gram-negative bacteria: *Enterobacter cloacae* (Jordan) Hormaeche and Edwards, *Escherichia coli* (Migula) Castellani and Chalmers, *Klebsiella pneumoniae* (Schroeter) Trevisan, *Pseudomonas aeruginosa* (Schroeter) Migula and *Serratia marcescence* Bizio. All the bacteria were obtained from the bacterial collection at the Department of Microbiology and Plant Pathology, University of Pretoria. Bacterial cultures were recovered for testing by culturing in nutrient broth for 24h at 37°C.

Antimicrobial tests

For the antifungal assay, the required amount of extract was added to sterile PDA in 65mm Petri-dishes before congealing to yield final concentrations of 0.5, 1.0, 2.5 and 5.0mg ml^{-1}. Unamended PDA plates served as controls. Once the agar had solidified, a 5mm plug of a seven-day old fungal culture was placed in the centre of the Petri-dish containing the extract-amended and unamended PDA plates. The plates were sealed with Parafilm and placed in an incubator at 25°C. Fungal growth was measured on two preset diametral lines after three, six and nine days of growth. Each treatment was analysed in triplicate. The results of the six-day growth was statistically analysed using two-way analysis of variance (ANOVA) and least significant differences ($P = 0.05$) were determined according to the Student's t-test.

Prior to streaking, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth. The minimum inhibitory concentration (MIC) of the extracts was determined by incorporating various amounts (0.5–5.0mg ml^{-1}) of the extract into 65mm Petri-dishes containing sterile nutrient agar (NA). Petri-dishes containing only the culture medium served as controls. The bacteria were streaked out in radial patterns onto the extract-amended NA and unamended NA plates. The Petri-dishes were sealed with Parafilm and incubated for 24h at ±37°C. The MIC was regarded as the lowest concentration of an extract where no growth of a bacterium was visible. Each treatment was replicated three times.

Results and Discussion

The results pertaining to the antifungal investigations revealed that both the acetone and ethanolic extracts of the leaves of BW and Kpod cultivars, with the exception of *F. equiseti*, significantly inhibited growth of the fungal pathogens at 5.0mg ml^{-1} (Figures 1a–d). Only the BW ethanolic extract inhibited the growth of *F. equiseti* at the same concentration when compared to the control (Figure 1b). *Alternaria alternata* was significantly reduced by both BW extracts at 2.5mg ml^{-1} whereas only the ethanolic extract exhibited antifungal activity against *F. proliferatum* at the same concentration. The acetone extract from Kpod also inhibited the growth of *A. alternata* at 2.5mg ml^{-1} when compared to the control. The acetone and ethanolic extracts of both cultivars showed no inhibitory activity at 1.0mg ml^{-1}.

The Gram-positive bacteria were found to be more susceptible than the Gram-negative bacteria (Table 1), as previously reported by earlier researchers (Kuhnt *et al.* 1994, Meyer and Afolayan 1995). The weak activity shown by the extracts against the Gram-negative bacteria could be due to lipophilic characteristics displayed by certain compounds in the extracts (Werner *et al.* 1979). However, a minimum inhibition concentration of 5.0mg ml^{-1} was observed when the acetone and ethanol extracts of BW were tested against *E. cloacae* (Table 1).

The results from this study have shown that cowpea extracts do have the potential to inhibit the growth of certain bacterial and fungal pathogens. This is likely to occur since

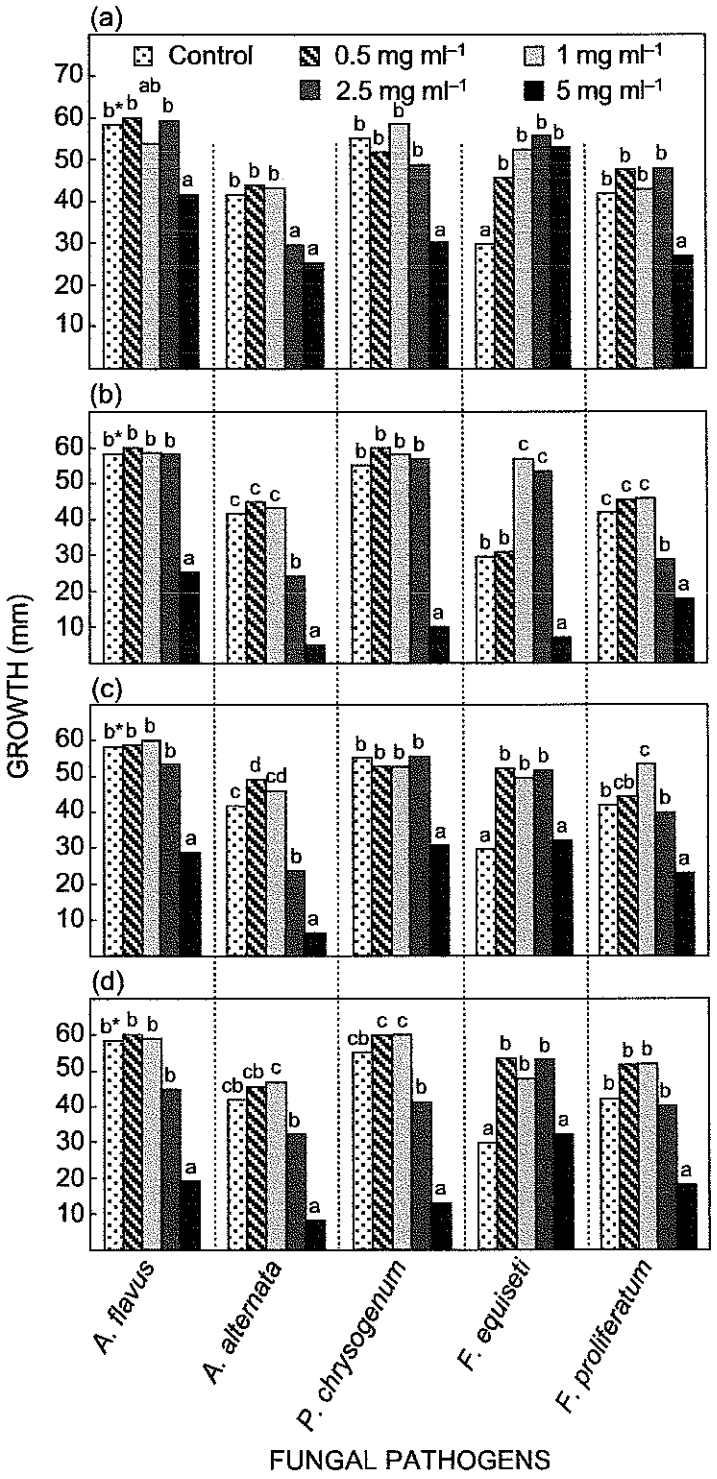

Figure 1: Antifungal activity of (a) Bechwana White acetone leaf extracts, (b) Bechwana White ethanol leaf extracts, (c) Kpodjiguégué acetone leaf extracts and (d) Kpodjiguégué ethanol leaf extracts — on selected fungal pathogens. * Each value of a bar is a mean of three replicates. Values of the bars within each fungal species not followed by the same letter are significantly different (P = 0.05) according to the Student's t-test

it is known that cowpea leaves do contain flavonoids and these same flavonoids, isolated from other plant species, have shown antimicrobial activity. Lattanzio *et al.* (1997) found three flavonoid aglycones, namely quercetin, kaempferol and isorhamnetin, always to be present in the

Table 1: Antibacterial activity of acetone and ethanol leaf extracts of two cowpea cultivars

Bacterial species	Gram +/–	MIC[a] (mg ml⁻¹)			
		Acetone		Ethanol	
		BW[b]	Kpod[c]	BW	Kpod
Bacillus cereus	+	5.0	na	na	na
Bacillus pumilus	+	na[d]	na	na	na
Bacillus subtilis	+	5.0	na	na	na
Staphylococcus aureus	+	2.5	na	na	na
Enterococcus faecalis	+	2.5	na	5.0	na
Enterobacter cloacae	–	5.0	na	5.0	na
Escherichia coli	–	na	na	na	na
Klebsiella pneumoniae	–	na	na	na	na
Pseudomonas aeruginosa	–	na	na	na	na
Serratia marcescence	–	na	na	na	na

[a] Minimum inhibitory concentration
[b] Bechwana White cultivar
[c] Kpodjiguégué cultivar
[d] Not active

leaves of cultivated cowpea lines. Quercetin, a naturally occurring bioflavonoid, is known to inhibit the growth of various fungi and bacteria (El-Gammal and Mansour 1986, Aziz *et al.* 1998). Further phenolic aglycons including p-coumaric acid and caffeic acid have also been isolated from cowpea leaves (Lattanzio *et al.* 2000) and it has been shown that p-coumaric acid, caffeic acid and kaempferol do exhibit antimicrobial activity against various bacterial and fungal pathogens (El-Gammal and Mansour 1986, Aziz *et al.* 1998).

It was noted in this study that the growth of *F. equiseti* was actually stimulated by the cowpea leaf extracts. Morris and Ward (1992) noted that an isoflavone, daidzein, acted as a germination stimulant for the zoospores of *Phytophthora sojae* (Kaufman and Gerdemann) and *Pythium irregulare* Buisman on soybean (*Glycine max* (L.) Merrill). However, Dakora (1995) reported that daidzein did not have the same effect on *Phytophthora vignae* Purss. on cowpea.

This study shows the potential of using cowpea extracts to control fungal pathogens that cause problems to various agricultural crops by causing disease and those that are capable of producing mycotoxins. The isolation of these active compounds from cowpea and other legumes, due to their rich source of flavonoid compounds (Dakora 1995), should be explored further for their use in disease control which can lead to the increase of the yield of agricultural commodities. Further exploitation of this activity could also increase the use of these extracts in the medicinal field.

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