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Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species

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Extracts from 26 plant species representing 16 families, collected in the Free State Province of South Africa, were tested *in vitro* for their potential to inhibit the growth of eight plant pathogenic fungi and five plant pathogenic bacteria. None of the crude extracts showed any mycelial growth inhibition of the eight test fungi. All of the extracts inhibited the growth of one or more of the five plant pathogenic test bacteria, but to varying degrees. Crude extracts from *Acacia karroo* and *Elephantorrhiza elephantina* inhibited the growth of four bacteria, while that of *Euclea crispa*, *Acacia erioloba, Senna italica* and *Buddleja saligna* inhibited the growth of all five plant pathogenic bacteria. Of these, the crude extract of *Euclea crispa* was clearly superior to the rest as it compared more favourably to that of a commercial bactericide, Dimethyl Dodecyl Ammonium Chloride (DDAC). Subsequently, the *Euclea crispa* crude extract was fractionated by means of liquid-liquid extraction using four organic solvents, hexane, diethyl ether, chloroform and ethyl acetate, in order of increasing polarity. This was done in an attempt to assess the antimicrobial potential of the more concentrated fractions. Once again, none of the semi-purified fractions showed any antifungal activity. However, antibacterial activity was located in the more polar ethyl acetate fraction indicating that the substances involved were very similar in polarity and/or structure. From this it seems justified to further purify the ethyl acetate fraction of the *Euclea crispa* extract and attempt to identify the active substance(s) involved.

Introduction

Over the past decade there has been an elevated interest in searching for antimicrobial agents of plant origin, as well as in isolating and identifying active components with possible use in integrated crop protection and pest management programmes (Hostettman and Wolfender 1997, Shivpuri *et al*. 1997, Eksteen *et al*. 2001). This is mainly because synthetic pesticides, bactericides, fungicides and herbicides, and even mineral fertilisers as sources of nutrients, are known to contribute to ecological imbalances, particularly with their excessive and sometimes inappropriate application (Swaminathan 1993). Chemically induced ill health in humans as well as environmental pollution have reached unacceptable levels, threatening not only the people of today but also future generations (Jansma *et al.* 1993, Waibel 1993, Zadoks 1993).

As organised agriculture has a major impact on the environment in terms of habitat loss, degradation and pollution (Jones 1993), compounds of plant origin are preferred to synthetic compounds since they are environmentally safer, biodegradable and leave no harmful residues (Ganesan and Krishnaraju 1995). Compounds isolated from plants may be used directly as natural agrochemicals or they may constitute useful starting materials for the synthesis of analogues with improved biological and physical properties (Beautement *et al.* 1991). The combined use of plant derived and synthetic chemicals could also assist in reducing the amount of active synthetic chemicals applied to crops (Gorris and Smid 1995).

Application of synthetic chemicals often leads to increased resistance by bacteria, fungi, weeds and insect pests alike, as well as the appearance of other diseases formerly unknown (Beautement *et al.* 1991), necessitating the search for new environmentally friendly natural products. This has led to the commercialisation of plant derived preparations such as Milsana™, a dried extract of *Reynoutria sachalensis* (Polygonaceae) which inhibits powdery mildew on a variety of crops (Gorris and Smid 1995). Despite the development of transgenic cultivars resistant to certain pathogens and more efficient agricultural techniques, a number of plant diseases that remain problematic are bacterial wilt, downy mildew, ergot of rye, phytophtora root rot, potato late blight, cereal smuts and wheat rusts (Lucas *et al.* 1992).

Numerous research publications on the constituents and biological activity of medicinal plants from Africa are available in the literature and some of these medicinal plants are already exploited commercially. However, this information

has not been documented as fully as in other traditional societies, such as the Indian and the Chinese, while research on the potential of these plants to be applied as natural agrochemicals in organised agriculture has remained neglected. South Africa is no exception and the Free State Province, in comparison to the rest of the country, is probably the least documented.

Moreover, despite existing knowledge on the antimicrobial activity of South African plants towards human pathogenic bacteria and fungi (Hutchings *et al.* 1996, Lis-Balchin and Deans 1996, Meyer *et al.* 1996, Mathekga and Meyer 1998), virtually no reports on the potential of extracts from these plants to inhibit plant pathogens could be found in the literature. Subsequently, as part of a research programme directed towards the isolation of active compounds from South African plants with application potential in agriculture, this paper describes the preliminary screening of 26 plant species growing in the Free State Province against some important plant pathogenic fungi and bacteria.

The Free State Province, one of nine provinces in South Africa, is situated on the central plateau between latitudes 26°S and 31°S and longitudes 24°E and 30°E. The area consists mainly of open rolling plains interrupted at regular intervals by low rocky hills (Henderson 1991). The most abundant species were collected in the Dry Sandy Highveld Grassland area around the towns of Bloemfontein and Brandfort. The remainder were collected in the Sandveld Nature Reserve, situated in the Kimberley Thorn Bushveld Savanna (Low and Rebelo 1996).

It is believed that *in vitro* antimicrobial screening methods are sufficient to provide the required preliminary observations for selecting, among crude plant extracts, those with potentially useful properties for further chemical and other investigations (Mathekga and Meyer 1998). For the purpose of this study we concentrated on collecting common plants and not necessarily plants with known medicinal properties. We also aimed at only screening plant parts that could be harvested sustainably (e.g. leaves and twigs). Root material was therefore not collected except for *Bulbine asphodeloides* where the whole plant was collected.

Materials and Methods

Plant material

Twenty-six plant species, representing 16 families, were collected between January and March 1997 and are listed in Table 1. Voucher specimens of each species were processed according to standard procedures and deposited in the herbarium of the National Museum in Bloemfontein (NMB). Although it may not necessarily be true for the areas where the plants were collected, 15 of these species (Table 1) are listed as problem plants in agriculture (Wells *et al*. 1986). The reasons vary, but primarily concern competition for space, light, water and nutrients, that they smother crops and that their seeds or fruits are regarded as contaminants. The woody species are mainly listed as problem plants because of their ability to replace preferred vegetation (mainly grazing) and obstruct the movement of machinery.

Table 1: List of plant species used in the preliminary screening for antimicrobial properties

District	Family	Plant species	Voucher no. (National Museum Bloemfontein, RSA)
Bloemfontein	Anacardiaceae	*Rhus ciliata* Licht ex Schult.	20482
		Rhus erosa Thunb	20480
		* *Rhus lancea* L.f.	17047
	Asclepiadaceae	* *Asclepias fruticosa* L.	13202
	Asphodelaceae	* *Bulbine asphodeloides* (L.) Willd.	19490
	Asteraceae	*Senecio radicans* (L.f) Sch. Bip.	12965
		Vernonia oligocephala (DC.) Sch. Bip. Ex Wolp.	17523
	Boraginaceae	* *Ehretia rigida* (Thunb.) Druce	17526
	Ebenaceae	*Diospyros austro-africana* De Winter var. *microphylla* (Burch.) De Winter	20485
		Euclea crispa (Thunb. Guerke) subsp. *crispa*	20483
	Fabaceae	* *Elephantorrhiza elephantina* Burch. (Skeels)	20481
	Loganiaceae	* *Buddleja saligna* Willd.	20477
	Oleaceae	*Olea europaea* L. subsp. *africana* (Mill.) P.S. Green	12868
	Ranunculaceae	* *Ranunculus multifidus* Forssk.	17066
	Rhamnaceae	* *Ziziphus mucronata* Willd. subsp. *mucronata*	20475
	Santalaceae	*Osyris lanceolata* Hochst. and Steud.	20474
	Tiliaceae	* *Grewia occidentalis* L.	13207
Brandfort	Asparagaceae	*Asparagus laricinus* (Burch.) Oberm.	17652
	Polygonaceae	* *Rumex lanceolata* Thunb.	20202
Hoopstad	Cucurbitaceae	*Coccinia sessilifolia* (Sond.) Cong.	13040
	Fabaceae	* *Acacia erioloba* E.Mey.	20479
		* *Acacia hebeclada* DC. subsp. *hebeclada*	11455
		* *Acacia karroo* Hayne	20478
		Senna italica Mill. subsp. *arachoides*	12882
	Ranunculaceae	* *Clematis brachiata* Thunb.	16302
	Tiliaceae	* *Grewia flava* DC.	14456

* Species listed as problem plants (Henderson 2001)

Microorganisms

All microorganisms used in this study were provided by the Department of Plant Pathology, University of the Free State, Bloemfontein, South Africa. Five plant pathogenic test bacteria were used for screening purposes including *Agrobacterium tumefaciens* Smith and Townsend, *Clavibacter michiganense* Spieckermann pv. *michiganense* Smith, *Erwinia carotovora* pv. *carotovora* Jones, *Pseudomonas solanacearum* Smith and *Xanthomonas campestris* Pammel pv. *phaseoli* Smith.

Eight common South African plant fungal pathogens were chosen to test for the fungitoxic properties of the plant extracts. These pathogens included the mitosporic fungi *Aspergillus niger* Tiegh., *Botrytis cinerea* Pers.: Fr., *Colletotrichum acutatum* J.H. Simmonds, *Fusarium oxysporum* Schlechtend.: Fr., a *Phoma* species. Sacc., *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc. as well as *Phytophthora cinnamomi* Rands (Chromista: Oomycetes).

Preparation of crude extracts

Dried plant material was powdered, using a Retsch SM2000 cutting mill and soaked in 95% methanol (v/v) at a ratio of 2ml g^{-1} dry weight on a roller mill overnight and the supernatant subsequently decanted. This was repeated ten times. The combined suspensions were filtered twice, first under vacuum through a double layer of Whatman filter paper (No. 3 and No. 1) and then by gravity through a single sheet of Whatman No. 1 filter paper after which it was centrifuged at 12 000 x g for 10min. The methanol was removed from the clear supernatant by means of vacuum distillation at 30–35°C using a Büchi Rotary Evaporator. The remaining aqueous solution, referred to as the crude extract, was found to give higher yields of active compounds with subsequent liquid-liquid extraction than did crude extracts obtained with either 100% methanol or acetone. For this reason extraction with 95% methanol was adopted as standard procedure. However, in the event of a sediment forming in certain crude water extracts after a while, dimethyl sulphoxide (DMSO) was added to a maximum of 10% (v/v), in order to keep more non-polar substances in suspension (Villarreal *et al.* 1994). Three replicates of 1ml aliquots were dried at 80°C, until the weight remained constant, to determine the concentrations of crude extracts. From the dried crude material, a stock solution of 50mg ml^{-1} in water was prepared.

Fractionation of crude extracts

Semi-purified extracts were prepared for *Euclea crispa* only. Five hundred ml aqueous crude solution were fractionated by means of liquid-liquid extraction using four organic solvents, *n*-hexane (DC = 2.0), diethyl ether (DC = 4.3), chloroform (DC = 4.8) and ethyl acetate (DC = 6.0), in order of increasing polarity and at a ratio of 2ml solvent ml^{-1} aqueous crude extract. Fractionation was repeated ten times with fresh solvent for each step by shaking vigorously on a mechanical shaker for 10min. The four fractions were collected separately and evaporated to dryness under vacuum at 35°C by means of a Büchi rotavapor. The mass of recovered dry material was determined for each fraction. From the dried material of each fraction, 1mg ml^{-1} stock solutions in water were prepared. In order to establish the success of the fractionation process, a thin layer chromatography (TLC) profile was obtained for each fraction with a 0.5mm Silica Gel 60 plate using chloroform : methanol : water (80:20:10) as mobile phase.

Screening for antifungal properties

A modified agar dilution method (Rios *et al.* 1988) was used for determining the inhibition of mycelial radial growth of the test organisms by the plant extracts. All plant pathogenic test fungi were cultured on 2% (m/v) potato dextrose agar (PDA), prepared according to the specifications of the manufacturers, and autoclaved for 20min at 121°C. On cooling to 45°C in a waterbath, 300µl of a 33% (m/v) Streptomycin solution was added to the basal medium for controlling bacterial growth. Dried material of each plant extract was dissolved in 100ml sterile distilled water and amended in the agar to yield a final concentration of 1mg ml^{-1}. Working in a laminar flow cabinet, the medium was poured into 90mm sterile plastic petri dishes, to a thickness of 2–3mm, and allowed to set. The centre of each test plate was subsequently inoculated with a 5mm size plug of 7–10-day-old cultures, for each of the pathogens separately, and incubated for eight days at 25 ± 2°C (March *et al*. 1991) in a growth cabinet. Radial mycelial growth was determined after eight days by calculating the mean of two perpendicular colony diameters on each replicate. The measurement included the assay wells (March *et al.* 1991, Pfaller *et al*. 1992). A plate containing only the basal medium served as control. Additionally, a plate containing 1µg ml^{-1} carbendazim/difenoconazole (Eria® — 187.5g l^{-1} EC) was used as a standard fungicide against each test organism separately to determine the effectiveness of the extracts by comparison. Each assay was performed in triplicate.

Screening for antibacterial properties

A modified agar diffusion method (Caceres *et al.* 1993a) was used. Plate count agar (PCA, Biolab) was prepared in the same way as PDA used in the antifungal screening tests except that the plant extracts were not suspended in the agar. Mother cultures of all plant pathogenic bacteria were sustained on PCA (Caceres *et al*. 1991, Rasoanaivo and Ratsamimanga 1993).

Overnight soup cultures of the test bacteria were initially prepared separately in sterile 1% (w/v) nutrient broth (Biolab) solutions at 30°C (Meyer and Afolayan 1995). 100µl of each of these bacterial suspensions were subsequently separately transferred to 90mm petri dishes containing sterile PCA agar and evenly streaked on the surface with a glass spreader. Petri dishes were divided into four quarters and a hole, 6mm in diameter, plunged into the agar of each quarter by means of a sterile cork borer. 50µl of the 50mg ml^{-1} crude stock solution extract (2.5mg active substance), or 50µl of each of the 1mg ml^{-1} semi-purified stock solution extracts (50µg active substance), were transferred into the holes in the agar. The plates were equilibrated at 4°C for 1h

to allow the extracts to diffuse into the agar before incubation commenced. In this way the development of clear zones of inhibition was optimised. Plates were incubated at 30°C for 24h. Each assay was performed in triplicate.

Antibacterial controls were run together with the other tests under identical conditions. The negative controls were distilled water and a solution of 10% (v/v) DMSO in water. The positive control was a 1mg ml^{-1} solution of dimethyl dodecyl ammonium chloride (DDAC) in water. Antibacterial inhibition zones in the range of 6.0–6.5mm were recorded as zero (no inhibition) since 6mm holes were used (Heisey and Gorham 1992).

Results

Preliminary screening of crude extracts against seven plant pathogenic fungi showed that none of the 27 crude extracts (from 26 plant species) was toxic against any of the test fungi, even when the test concentration was elevated to 300mg ml^{-1} (results not shown). However, to differing degrees, most crude extracts exhibited antibacterial activity towards one or more of the test bacteria except the crude extract of *Vernonia oligocephala* which was totally inactive (Table 2).

Crude extracts from four plants (14.8% of plants tested) inhibited the growth of all five plant pathogenic test bacteria, namely *Euclea crispa*, *Acacia erioloba*, *Buddleja saligna* and *Senna italica* (Table 2). Of these, inhibition zones obtained with crude extracts of only *E. crispa* and *A. erioloba* were 10mm or more for all test bacteria and this compared favourably to that obtained with the standard bactericide, DDAC. Of the remaining two extracts, that of *S. italica* was the less active with all of the measured inhibition zones being <10m.

Extracts from two plants (7.4% of plants tested), *Acacia karroo* and *Elephantorrhiza elephantina*, inhibited the growth of four of the five test bacteria (Table 2). In both cases inhibition zones of >10mm were measured for all of the sensitive test organisms. Neither showed any inhibitory activity against the Gram-negative bacterium, *Erwinia carotovora*.

Three plant extracts (11.1% of plants tested) inhibited the growth of three of the five test bacteria (Table 2). Only in the case of *Diospyros austro-africana* subsp. *microphylla* were the measured inhibition zones >10mm for all three organisms. Inhibition zones measured with the *Rumex lanceolatus* crude extract exceeded 10mm only in the case of two test bacteria while inhibition zones of below 10mm were measured for all three organisms with the *Ziziphus mucronata* extract.

Nine plant extracts (33.3% of plants tested) inhibited the growth of two of the five test organisms. Of these, inhibition zones larger than 10mm were obtained only with the *Osyris lanceolata* crude extract. Only the root extract of *Bulbine asphodeloides* showed weak inhibitory activity against the Gram-negative bacterium, *Erwinia carotovora*. The remaining eight plant extracts (29.6% of plants tested) showed weak inhibitory activity (<10mm zones) against only one test organism.

The most resistant organism was the Gram-negative bacterium, *Erwinia carotovora* pv. *carotovora*. Inhibition zones of 10mm or more were obtained with crude extracts from only three plant species, *Euclea crispa, Acacia erioloba* and *Buddleja saligna,* against this test organism (Table 2). Moreover, only these three crude plant extracts compared favourably to the standard control, DDAC, in terms of inhibition range and zone size. As the crude extract of *Euclea crispa* outperformed the standard bactericide in terms of inhibition zone size for three of the five test organisms, and also outperformed the other three extracts on the basis of zone size (Table 2), it was fractionated by applying liquid-liquid extraction. The antibacterial activities of these fractions were compared to that of a standard bactericide, DDAC, at equal concentrations.

Only the ethyl acetate fraction of *Euclea crispa* showed antibacterial activity (Table 3) and, at equal concentrations, compared favourably to the standard bactericide in terms of both inhibition range and zone size. The qualitative TLC profile of the different liquid-liquid chromatography fractions, developed with 5% (v/v) ethanolic H_2SO_4, confirmed proper separation of different groups of chemicals with the four organic solvents used (results not shown). However, none of the liquid-liquid chromatography fractions showed any toxic activity against the eight plant pathogenic fungi tested (results not shown).

Discussion

Antimicrobial compounds in plants are usually present in small quantities (Hamburger and Hostettmann 1991, Zavala *et al.* 1997). Therefore, in this study the concentration used for screening each crude extract (50mg ml^{-1}, 2.5mg $hole^{-1}$) for possible antifungal activity was 50 times higher than that of the control (carbendazim/difenoconazole, 50µg $hole^{-1}$). Despite this, none of the crude extracts tested showed any activity against eight plant pathogenic fungi. Even at elevated concentrations of 100–300mg ml^{-1} (Caceres *et al.* 1993b, Zavala *et al.* 1997) the crude extracts failed to inhibit the lateral mycelial growth of the fungi. This could either have been due to the absence of biologically active antifungal compounds (Farnsworth and Bingel 1977, Gordon *et al.* 1980) or due to the presence of growth factors in the methanolic extract that stimulated the growth of fungi, negating the effect of possible inhibitory substances that might have been present (Ieven *et al.* 1979, Qasema and Abu-Blan 1996). However, as growth promoting substances are known to be very concentration specific, the lack of activity at these high concentrations probably implicates the absence of antifungal active substances rather than a screen effect by growth substances. A wider range of fungi will probably have to be tested to come to a definite conclusion regarding the antifungal status of these plant species.

Most of the plant extracts tested showed some level of antibacterial activity. This supported previous observations made by other investigators that plant pathogenic fungi are more resistant to natural extracts from plants than plant pathogenic bacteria (Ieven *et al.* 1979, Naqvi *et al.* 1991, Heisey and Gorham 1992). In a preliminary screen of 60 crude extracts, Heisey and Gorham (1992) observed that 13 extracts (21.67%) inhibited the growth of bacteria, while only five extracts (8.33%) inhibited fungal growth. Naqvi *et al*.

Table 2: Preliminary screening of crude extracts from South African plant species at a concentration of 50mg ml^{-1} (2.5mg active substance/hole) for antibacterial activity

Family and plant name	Mean inhibition zone diameter (mm)				
	A. tumefaciens (Gram-negative)	*C. michiganense* pv. *michiganense* (Gram-positive)	*E. carotovora* pv. *carotovora* (Gram-negative)	*P. solanacearum* (Gram-negative)	*X. campestris* pv. *phaseoli* (Gram-negative)
Anacardiaceae					
Rhus ciliata	0	7.0 ± 0.8	0	8.0 ± 0.0	0
Rhus erosa	9.0 ± 0.8	9.0 ± 0.0	0	0	0
Rhus lancea	0	7.0 ± 0.0	0	8.0 ± 1.0	0
Asclepiadaceae					
Asclepias fruticosa	0	0	7.0 ± 1.0	0	0
Asparagaceae					
Protasparagus laricinus	0	0	7.0 ± 0.8	0	0
Asphodelaceae					
Bulbine asphodeloides (inflorescence)	0	0	0	0	9.0 ± 0.0
Bulbine asphodeloides (roots)	0	0	9.0 ± 0.8	0	9.0 ± 0.8
Asteraceae					
Senecio radicans	0	0	7.0 ± 0.6	0	0
Vernonia oligocephala	0	0	0	0	0
Boraginaceae					
Ehretia rigida	0	0	7.0 ± 0.4	0	0
Cucurbitaceae					
Coccinia sessilifolia	0	7.0 ± 0.7	0	8.0 ± 0.6	0
Ebenaceae					
Diospyros austro-africana var. *microphylla*	15.0 ± 0.8	** 16.0 ± 0.0	0	** 15.0 ± 0.8	0
Euclea crispa subsp. *crispa*	16.0 ± 0.6	** 15.0 ± 0.7	10.0 ± 0.4	** 15.0 ± 0.4	** 15.0 ± 0.8
Fabaceae					
Acacia erioloba	13.0 ± 0.4	11.0 ± 0.8	** 13.0 ± 0.4	** 12.0 ± 0.8	** 15.0 ± 0.4
Acacia hebeclada subsp. *hebeclada*	0	0	7.0 ± 07	0	0
Acacia karroo	11.0 ± 0.4	11.0 ± 0.8	0	** 12.0 ± 0.8	11.0 ± 0.0
Elephantorrhiza elephantina	11.0 ± 0.4	11.0 ± 0.8	0	** 12.0 ± 0.9	11.0 ± 0.9
Senna italica subsp. *arachoides*	7.0 ± 0.6	7.0 ± 0.6	7.0 ± 04	8.0 ± 0.0	8.0 ± 0.6
Loganiaceae					
Buddleja saligna	10.0 ± 0.0	10.0 ± 0.7	11.0 ± 0.4	9.0 ± 0.8	9.0 ± 0.4
Oleaceae					
Olea europaea subsp. *africana*	0	0	0	8.0 ± 0.8	7.0 ± 0.8
Polygonaceae					
Rumex lanceolatus	0	** 15.0 ± 0.4	0	** 12.0 ± 0.4	8.0 ± 0.0
Ranunculaceae					
Clematis brachiata	0	7.0 ± 0.8	0	7.0 ± 0.6	0
Ranunculus multifidus	0	9.0 ± 0.8	0	0	0
Rhamnaceae					
Ziziphus mucronata subsp. *mucronata*	9.0 ± 0.8	9.0 ± 0.8	0	0	8.0 ± 0.7
Santalacea					
Osyris lanceolata	12.0 ± 0.8	12.0 ± 0.7	0	0	0
Tiliaceae					
Grewia flava	0	9.0 ± 0.8	0	8.0 ± 0.6	0
Grewia occidentalis	0	0	0	7.0 ± 0.0	0
Control*	20.0 ± 0.4	13.0 ± 0.7	13.0 ± 0.8	12.0 ± 0.8	13.0 ± 07

* Dimethyl Dodecyl Ammonium Chloride (DDAC, 1mg ml^{-1})
** Equal or more effective inhibition than control

(1991) concluded that many plants possess effective defense mechanisms against bacterial attacks but the same cannot be said for fungal attacks.

Subsequently, in order to assess the degree of effectiveness of the extracts as antibacterial agents by comparison, a wide spectrum systemic bactericide (DDAC) was used as a control. Among the 26 plant species tested and at a concentration 20 times higher than that of the DDAC control, crude extracts of *E. crispa*, *A. erioloba*, *B. saligna* and *S. italica* were most effective against the five plant pathogenic test bacteria as they inhibited the growth of all. However, when a >10mm diameter criterium for inhibition zone size was applied, only the crude extracts of *E. crispa* and *A. erioloba* compared favourably to inhibition by the standard broad spectrum bactericide. Applying the >10mm inhibition zone criterium, extracts from *A. karroo* and *E. elephantina*, inhibiting the growth of four test bacteria each, as well as *D. austro-africana*, inhibiting the growth of three test bacteria, also

Table 3: Antibacterial activity of semi-purified liquid-liquid chromatography fractions of an *Euclea crispa* subsp. *crispa* leaf extract against plant pathogenic bacteria as compared to that of a standard bactericide, DDAC, at equal concentrations of 1mg ml^{-1} (50µg active substance/hole)

		Mean inhibition zone diameter (mm)					
Bacterium	Gram -ve/ +ve-	Crude extract	Hexane	Diethyl ether	Chloroform	Ethyl acetate	* Control
A. *tumefaciens*	-	16.0 ± 0.6	0	0	0	13.0 ± 0.0	20.0 ± 0.4
B. michiganense pv. *michiganense*	+	15.0 ± 0.7	0	0	0	15.0 ± 0.4	16.0 ± 0.4
E. carotovora pv. *carotovora*	-	10.0 ± 0.4	0	0	0	14.0 ± 0.6	16.0 ± 0.8
R. solanacearum	-	15.0 ± 0.0	0	0	0	11.0 ± 0.7	16.0 ± 0.4
X. campestris pv. *phaseoli*	-	15.0 ± 0.8	0	0	0	20.0 ± 0.8	15.0 ± 0.0

* Dimethyl Dodecyl Ammonium Chloride (DDAC, 1mg ml^{-1})

compared favourably to inhibition by the DDAC control.

When both inhibition zone size and broad spectrum inhibition were considered as indicators of antibacterial effectiveness and applied as criteria, the *E. crispa* and *A. erioloba* crude extracts emerged as the most potent of all plant extracts tested. As the former crude extract produced the largest inhibition zones compared to the rest, and inhibited the growth of three of the bacteria more effectively than the DDAC control, it was fractionated by means of liquid-liquid extraction. Subsequently, growth inhibition of bacteria by the semi-purified fractions was compared to that of the DDAC control, but this time at equal concentrations of 1mg ml^{-1} or 50µg active substance per hole. Antibacterial activity was detected only in the ethyl acetate fraction.

The fact that this semi-purified ethyl acetate fraction of *Euclea crispa* leaves showed a high degree of bacterial growth inhibition, with the actual substances exhibiting the inhibitory effect probably being diluted by the presence of other non-active compounds, makes the comparative inhibitory performance to that of a pure synthetic bactericide remarkable. The latter is based on the assumption that a pure antibacterial compound, as in a pure synthetic product, will probably show higher antibacterial activity than a crude or semi-purified plant extract where not all the compounds in the extract will necessarily contribute to the measured *in vitro* activity.

Moreover, the five test bacteria controlled by the ethyl acetate fraction of *E. crispa* all cause diseases that are economically important in organised agriculture. *Agrobacterium tumefaciens* is a soil bacterium that causes lumpy tumors, followed by wilting, chlorosis and eventual death in a wide range of crops (Binns and Thomashow 1988). *Clavibacter michiganense* causes bacterial canker on tomatoes (Chang *et al*. 1992), ring rot of potato (Gudmestad and Secor 1993) and bacterial wilt of alfalfa (Stutevill and Erwin 1990). *Erwinia carotovora* causes bacterial rot in most species of the Brassicaceae, beans, onions, carrots, cucumber, sorghum and a variety of fruits (Snowdon 1992). Bacterial wilt or brown rot is a devastating disease of potatoes caused by *Pseudomonas solanacearum*, also known as *Ralstonia solanacearum* (Anguiz and Mendoza 1997). The bacterium *Xanthomonas campestris* pv. *phaseoli* infects many types of beans causing common blight and is very difficult to control (Hall 1991).

The results from this study warrant further purification of the ethyl acetate fraction from an *E. crispa* leaf extract, as well as an *A. erioloba* crude extract in order to isolate and identify the active substances responsible for bacterial growth inhibition with a view to their possible use as bactericides.

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