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Full Length Research Paper

Antimicrobial activities of four plant species from the Southern Overberg region of South Africa

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Four plant species used for medicinal purposes in the Bredasdorp/Elim (Southern Overberg) region of the Western Cape Province in South Africa, were screened for their antimicrobial activity. The antimicrobial activity of aqueous, methanol, ethanol and ethyl-acetate leaf extracts of *Bulbine lagopus* (Asphodelaceae), *Chironia baccifera* (Gentianaceae), *Conyza scabrida* (Asteraceae) and *Dodonaea viscosa* var. *angustifolia* (Sapindaceae), were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Mycobacterium smegmatis.* In the disc-diffusion assay, 20 out of the 80 extracts showed activity. Better activity was observed in the liquid dilution assay with all extracts showing a degree of activity. The best activity was observed in the ethanol extract of *B. lagopus* and the methanol extract of *C. scabrida* both having an MIC value of 0.3125 mg/ml. In the bioautography, *M. smegmatis* was chosen as the test organism along with the ethanol, ethyl-acetate and methanol extracts of *C. scabrida* and *D. viscosa* var. *angustifolia*. All six extracts showed at least two zones of inhibition on the TLC plates overlaid with *M. smegmatis*.

Key words: Southern Overberg, South Africa, antimicrobial activity, ethnobotanical approach, scientific validation.

INTRODUCTION

Many individuals still use plants as a source of medicine, using their own personal recipes which have been passed down from generation to generation. This is true for many people in the Bredasdorp/Elim region of the Western Cape Province in South Africa. From the results of the survey performed in this area (Thring and Weitz, in press), four plant species were chosen to undergo preliminary antimicrobial tests (Thring, 2004). In this survey, individuals who use plants in self care were interviewed to find out which plants were in use and the methods regarding their use and preparation. Thirty-six plant species out of 19 families were found to be in general use in the area. Many of these species in use are those which are commonly used in South Africa, such as *Artemisia afra*, *Ruta graveolens*, *Elytropappus rhinocerotis* and *Sutherlandia frutescens*. However, some plant species were not found to be scientifically known for their antimicrobial activity and so it was decided to test some of these species based on both their use in the survey and reported uses in the literature. The species chosen were:

Bulbine lagopus (Thunb.) N.E. Br. (Asphodelaceae) (TSAT 011), common name "geel katstert", is used in the study area to treat wounds, sores and skin conditions. *Bulbine species* are reported to be used to treat burns, wounds and skin conditions (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997).
Chironia baccifera L. (Gentianaceae) (TSAT012), common name "aambeibossie", is used in the

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survey for stiff muscles and sore legs. Reported to be used to treat acne, sores and diarrhea (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997). Some active compounds have been isolated from *Chironia* species (Wolfender et al., 1993), and a recent publication by Springfield et al. (2005) tested positive for the presence of tannins and saponins but negative for alkaloids and cadiac-, cyanogenic- and anthraquinone glycoside, in *Chironia baccifera*.

Conyza scabrida DC. (Asteraceae) (TSAT 0013), common name "paddabossie", is used in the study area to treat chest, heart, fever, diabetes, rheumatism, colds, influenza and inflammation. This herb is reported to be used to treat influenza, chest and stomach afflictions, fever, diarrhea, sores, and inflammation in the literature (Watt and Breyer-Brandwijk, 1962; Smith, 1966, Scott et al, 2004). Scott et al (2004) gave a pharmacognostical overview on *Conyza scabrida* which covers correct identification of this herb by means of thin-layer chromatography, high-performance liquid chromatography and classical microscopy.

Dodonaea viscosa Jacq. var. *angustifolia* (L.f.) J.G.West (Sapindaceae) (TSAT 014), common name "ysterhouttoppe", is used in the study area to treat arthritis, bladder and kidney disorders, colds, influenza, convulsions, fever, inflammation, rheumatism and stomach complaints. This herb is reported to contain diterpenoids and used in the treatment of fever, colds, influenza, stomach ailments and sore throats (van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962). Amabeoku et al. (2001) found *D. viscosa* var. *angustifolia* to have both analgesic and antipyretic activities, and tested positive for the presence of flavonoids, reducing sugars, alkaloids, saponins and tannins.

The aim of this study was to investigate these plant species to determine their antimicrobial activity and to determine whether the ethnobotanical approach (Thring and Weitz, in press) in finding anti-infectives is a useful approach.

METHODOLOGY

Plant material

The four plant species were collected in the Bredasdorp area (3420CA) in September 2002 as recommended by the individuals interviewed in the survey. It was said that the plants should be collected after the rain but before flowering and that was when the plants were at their best. Voucher specimens (TSAT 011 for *Bulbine lagopus*; TSAT 012 for *Chironia baccifera*; TSAT 0013 for *Conyza scabrida*, and TSAT 014 for *Dodonaea viscosa* var. *angustifolia*) were authenticated by the curator of the University of the Western Cape Herbarium, prepared and deposited in the UWC Herbarium. The plant material was washed with distilled water prior to being dried in an oven at 40ºC for approximately 72 hours with the exception of the *Bulbine lagopus* material, which was kept fresh and extracted immediately. The dried plant material consisting of leaves and stems from the other three species was coarsely ground in a pestle and mortar in a similar way to how the plant material is used by the people in the survey.

Solvent extraction

Five different extracts for each plant were prepared using four solvents; methanol, ethanol, ethyl-acetate and distilled water. In each case 10 g of dried material in 100 ml of solvent or 20 g fresh material in 200 ml of solvent was extracted. Two aqueous extracts were prepared for each plant. The one aqueous extract being a "tea" (infusion) where the dried material was placed in a flask and boiling water poured over it and left to steep (this is a very common way of preparing plant material by the people interviewed). The other aqueous extract was obtained by placing the material in water, and boil it on a hotplate for 5-10 min as was suggested by some people in the survey. These aqueous extracts were left to cool overnight before being filtered under vacuum. The filtrate was then frozen at -70ºC for 48 h before being freeze-dried. For the ethanol, methanol and ethyl-acetate extracts, the plant material was placed in flasks with the solvent and then sonicated in an ultrasound bath for 30 min. These extracts were left overnight then sonicated the next day for 30 min before being filtered under vacuum. The filtrate was then evaporated to dryness in a rotary evaporator before being frozen at -70ºC for 48 h prior to being freeze dried. The yields of all the extracts were determined as follows: *Bulbine*: aqueous infusion (3.01%); aqueous boiled (2.70%); ethanol (2.14%); ethyl acetate (2.6%) and methanol (1.01%). *Chironia*: aqueous infusion (24.10%); aqueous boiled (23.8%); ethanol (14.5%), ethyl acetate (6.70%) and methanol (17.43%). *Conyza*: aqueous infusion (17.62%); aqueous boiled (17.54%); ethanol (14.32%); ethyl acetate (13.83%) and methanol (18.29%). *Dodonaea*: aqueous infusion (12.36%); aqueous boiled (11.78%); ethanol (4.11%); ethyl acetate (3.07%) and methanol (9.32%).

The extracts were stored at 4ºC in glass vials for use in the antimicrobial bioassays.

Microorganisms and growth media

The microorganisms used were *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231) and *Mycobacterium smegmatis*. *M. smegmatis* was a gift from Professor Paul van Helden of the Department of Biochemistry and Physiology at the University of Stellenbosch Medical School. The *S. aureus*, *P. aeruginosa* and *C. albicans* were obtained from the Medical Biosciences Department at the University of the Western Cape.

The disc-diffusion method

The dried plant extracts were resuspended to 20 mg/ml in their respective solvents and sonicated to dissolve and sterilize the extracts. Sterile 9 mm discs were impregnated with 50 µl of extract and placed on the surface of agar plates inoculated with a microbial culture. Each extract was tested in triplicate. Negative controls were prepared in the same way but using 50 µl of pure solvent on sterile discs. Ciprofloxacin (40 µg/disc) served as positive control for *S.*

Table 1. Results from the disc diffusion assay showing the antibacterial activity of the 20 extracts tested against the four microorganisms.

Plant	Extract	Growth inhibition activity			
		S. a[a]	P. a	C.a	M.s
Bulbine lagopus	Aqueous boiled	-	-	-	-
	Aqueous infusion	-	-	-	-
	Ethanol	-	-	-	1+
	Ethyl-acetate	1+	2+	-	1+
	Methanol	-	-	-	-
Chironia baccifera	Aqueous boiled	-	-	-	-
	Aqueous infusion	-	-	-	-
	Ethanol	-	-	-	1+
	Ethyl-acetate	-	-	-	1+
	Methanol	-	-	-	-
Conyza scabrida	Aqueous boiled	1+	-	-	-
	Aqueous infusion	1+	-	-	1+
	Ethanol	2+	-	-	3+
	Ethyl-acetate	1+	-	-	3+
	Methanol	1+	-	-	3+
Dodonaea viscosa var. *ngustifolia*	Aqueous boiled	1+	-	-	-
	Aqueous infusion	1+	-	-	-
	Ethanol	1+	-	-	-
	Ethyl-acetate	-	-	-	-
	Methanol	1+	-	-	1+
	Ciprofloxacin	3+	3+	-	3+
	Amphotericin	-	-	3+	-

[a]S.a – *Staphylococcus aureus;* P.a – *Pseudomonas aeruginosa;* C.a – *Candida albicans;* M.s – *Mycobacterium smegmatis*.
- Indicates no inhibition, while 1+ = 0.5 – 1 mm; 2+ = 1-2 mm; 3+ = 2-4 mm represents the size of the zone.

aureus, P. aeruginosa and *M. smegmatis*, whereas Amphotericin B (25 µg/disc) was the control for *C. albicans*. Agar plates containing the fungi, bacteria and mycobacteria were incubated at 37°C for 24 and 48 h, respectively. After incubation, inhibition zones were recorded as the diameter of the growth free zones around the disc.

Minimum inhibitory concentrations (MIC)

This was based on the liquid dilution method of Rios et al. (1988) and the microplate method by Eloff (1998). Each extract was resuspended to 50 mg/ml in sterile distilled water and sonicated. Minimum inhibitory concentration (MIC) was determined by two-fold serial dilution of extracts beyond the concentration where growth of the relevant cultures was observed. Controls of distilled water inoculated with the test organism were used to ensure the viability of the test organism. The wells in the dilution series were inoculated with the relevant cultures, incubated overnight at 37ºC for *S. aureus*, *P. aeruginosa*, *C. albicans*, and 48 h for *M. smegmatis.*

After the incubation period, 40 µl of 0.2 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT or thiazolyl blue) was added to each well. The extracts were then incubated for a further 30 min, and bacterial growth was indicated by the purple-blue colour of the MTT formazan produced.

Bioautography

Ethanol, methanol and ethyl-acetate extracts of *C. scabrida* and *D. viscosa* var. *angustifolia* were tested in this bioassay due to them having the best overall activity in the above assays. Extracts were resuspended to a concentration of 50 mg/ml in methanol and 30 µl applied to 5 x 20 cm silica F254 (Merck) glass TLC plates. The plates were developed in the organic phase of 1.75 M acetic acid : ethyl-acetate : toluene (1:1:1) which was the solvent system that gave the best separation out of the various solvent systems attempted. The plates were dried overnight, and agar, which was inoculated with *M. smegmatis*, was poured over the plates. Blank plates were also inoculated to ensure that the test organism was viable and not sensitive to the solvent system. The plates were incubated at 37ºC for 48 h and then sprayed with 0.2 mg/ml MTT. Clear zones on the chromatogram indicated inhibition of growth (Beugue et al., 1972).

RESULTS AND DISCUSSIONS

All the plant extracts tested showed a degree of antimicrobial activity. The results from the bioassays are tabulated in Tables 1, 2, and 3.

The disc-diffusion bioassay

The most activity was found against *S. aureus* and *M. smegmatis* with no activity against *C. albicans*. *P.*

Table 2. Minimum inhibitory concentration (MIC) results of the 20 extracts tested against the four microorganisms.

Plant	Extract	MIC (mg/ml)			
		S. a	P. a	C.a	M.s
Bulbine lagopus	Aqueous boiled	5	-	5	5
	Aqueous infusion	5	5	5	5
	Ethanol	2.5	-	-	0.3125
	Ethyl-acetate	5	5	-	5
	Methanol	5	-	5	2.5
Chironia baccifera	Aqueous boiled	-	-	5	-
	Aqueous infusion	-	-	-	-
	Ethanol	-	-	-	2.5
	Ethyl-acetate	5	-	5	5
	Methanol	1.25	1.25	1.25	1.25
Conyza scabrida	Aqueous boiled [b]	2.5	-	1.25	1.25
	Aqueous infusion [b]	1.25	-	0.625	0.625
	Ethanol	2.5	-	5	5
	Ethyl-acetate	0.625	-	2.5	5
	Methanol	5	-	5	0.3125
Dodonaea viscosa var. *angustifolia*	Aqueous boiled [b]	5	-	-	5
	Aqueous infusion [b]	5	-	-	5
	Ethanol	2.5	-	2.5	1.25
	Ethyl-acetate	5	-	-	5
	Methanol	2.5	-	1.25	1.25

[a]S.a – *Staphylococcus aureus;* P.a – *Pseudomonas aeruginosa;* C.a – *Candida albicans;* M.s – *Mycobacterium smegmatis*
[b]Students "t" test was performed between the two water extracts. There was no significant difference found between the activities of the two types of extracts:
Pooled $s^2 = 5.5436$
$t_{30} = 0.8212 \pm 8.3\%$ (ns) ; $P > 0.05$

aeruginosa is among the microbes which are harder to inhibit (Martìnez-Vásquez et al, 1999), and in our study only the ethyl-acetate extract of *B. lagopus* showed activity against *P. aeruginosa*. The plant which showed the most activity was *C. scabrida*. This plant showed particularly good activity against *M. smegmatis* with the biggest zones of inhibition being observed (Table 1).

The aqueous extracts did not perform well in the disc-diffusion assay, but due to most of the plants being used in the form of "teas" or infusions, it was valuable to test aqueous extracts for these plants.

The minimum inhibitory concentration (MIC) bioassay

Our MIC results confirm that this bioassay is more sensitive as more activity was recorded (Table 2). In this case, the methanol extracts showed the most activity followed by the ethyl-acetate extracts. The ethanol and aqueous extracts each showed a degree of activity in nine extracts. All but two extracts of *C. baccifera* aqueous extracts, showed activity against *M. smegmatis*. The lowest MIC value was found in the *B. lagopus* ethanol extract and the *C. scabrida* methanol extracts, both inhibiting microbial growth against *M. smegmatis* at 0.3125 mg/ml. It is recommended that both these extracts be tested against *M. tuberculosis* and further investigated as possible anti-TB agents. *S. aureus* was observed to be the second best micro-organism to be inhibited, with all but three extracts demonstrating activity. In this bioassay, *C. albicans* was inhibited by 13 out of the 20 extracts. The aqueous infusion of *C. scabrida* showed good inhibition against *C. albicans* with a MIC value of 0.625 mg/ml. *P. aeruginosa* proved to be the most difficult microbe to inhibit with only three extracts showing activity in this bioassay.

Placing the plant material either in alcohol (such as brandy) or sprinkling the material with alcohol before making a poultice are other ways in which the people in the survey prepare the plants for use (Thring and Weitz, article in press). Due to this, it was decided to extract the plant material in ethanol. In the disc-diffusion assay, none of the ethanolic extracts was able to inhibit *C. albicans* whereas in the MIC assay, two out of four extracts (*C. scabrida* and *D. viscosa* var. *angustifolia*) showed activity.

A comparison (using the "Student T test") between the MIC results (Table 2) of the "boiled" aqueous extracts

Table 3a. Bioautography results for *C. scabrida* ethanol extract.

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.46	10x30	Red	N/v	Brown
		Purple	N/v	Grey
		Green	Yellow	N/v
2. 0.68	25x34	Purple	N/v	N/v
		Orange	Pale yellow	Purple

N/v: not visible

Table 3b. Bioautography results from *C. scabrida* methanol extract.

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.05	10x20	N/v	N/v	Grey
2. 0.38	20x30	Grey	Cream	Dark grey
		Orange	Cream	N/v
		Purple	Yellow	Purple/red
3. 0.60	12x20	N/v	N/v	Light grey

N/v: not visible

Table 3c. Bioautography results from *C. scabrida* ethyl-acetate extract.

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.05	12x21	N/v	Brown	N/v
2. 0.39	25x30	Brown	Pale yellow	Purple
		Orange	N/v	Red/purple
		Brown	Yellow	Brown
		N/v	N/v	
3. 0.60	7x26	N/v	N/v	Grey

N/v: not visible

Table 3d. Bioautography results from *D. viscosa* var. *angustifolia* ethanol extract.

Rf value of Zones of inhibition	Sizes of zones (LXB, mm)	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.05	12x15	N/v	N/v	Light brown
2. 0.40	19x30	Light brown	Yellow	Brown
		Brown	Yellow	Red
3. 0.57	28x29	Purple/brown	N/v	N/v
		Cream	N/v	N/v
		N/v	N/v	Grey

N/v: not visible

and the “infusion” aqueous extracts show no significant difference found between the activity of the extracts thus indicating that active secondary compounds are being extracted whether these medicinal plants are “boiled” or “infused”.

Bioautography

By separating bioactive extracts on thin layer chromatography, it is possible to get information on the compounds present in the mixture, and in our study, six plant extracts which was tested show chemical compounds responsible for the antimicrobial activity (Tables 3a-f) (Bioautograms not shown). The ethanol, methanol and ethyl-acetate extracts of *C. scabrida*, and the ethanol extract of *D. viscosa* var. *angustifolia* all showed three zones of inhibition against *M. smegmatis* (Tables 3a, 3b, 3c, 3d). The remaining two *D. viscosa* var. *angustifolia* extracts showed two zones of inhibition

Table 3e. Bioautography results from *D. viscosa* var. *angustifolia* methanol extract.

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.38	5x25	Light blue	N/v	Violet
2. 0.42	8x25	Red	Green	N/v
		Light blue	Cream	Violet
3. 0.61	13x30	Black	Yellow	Purple

N/v: not visible

Table 3f. Bioautography results from *D. viscosa* var. *angustifolia* ethyl-acetate extract.

Rf value of Zones of inhibition	Sizes of zones (LXBmm)	Band colours under UV 366nm	Visible band colours	Band colours under UV 254nm
1. 0.45	18x20	Pink	N/v	N/v
		Blue	Yellow	N/v
		Green	Yellow	Brown
2. 0.66	19x20	N/v	Yellow	Purple
		Purple	Yellow	Red
		Red	N/v	Brown

N/v: not visible

(Tables 3e, 3f). Compounds 2 and 3 (Table 3a), compound 2 (Table 3b), compound 2 (Table 3c), and compound 2 (Table 3e) show particularly good activity with respect to zone size, and from the bioautograms it appear to be respective overlapping bands of several compounds. From the above results it is evident that bioautography is a method that facilitates the localization of antimicrobial activity on a chromatogram, and proves to eliminate the diffusion step (Hamburger and Cordell, 1987).

Conclusion

The preliminary findings of these plants all showed activity against the tested microbes and further studies on the isolation and characterization of the active compounds in these medicinal herbs may well provide us with novel antimicrobial agents. This scientific study validated the medicinal claims of *Bulbine lagopus*, *Chironia baccifera*, *Conyza scabrida* and *Dodonaea viscosa* var. *angustifolia*, used in the Southern Overberg region of the Western Cape Province in South Africa.

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