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Pharmacological screening of six Amaryllidaceae species

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Abstract

Dichloromethane and 90% methanol extracts of different parts of 6 Amaryllidaceae species were investigated for anti-inflammatory (COX-1 and COX-2), antibacterial and mutagenic (*Salmonella*/microsome test strain TA98) activities. Antibacterial activity was carried out using disc diffusion assay against three Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and two Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria. With the exception of the leaf of *Cyrtanthus mackenii*, all CH_2Cl_2 extracts of different parts of *Cyrtanthus* species inhibited the growth of at least one bacterium, with the CH_2Cl_2 bulb/root extracts of *Cyrtanthus suaveolens* showing broad-spectrum antibacterial activity. In the anti-inflammatory assay, CH_2Cl_2 extracts, resuspended at 500 μg/ml, from different parts of all species under investigation, inhibited activity of both COX-1 and COX-2 by at least 70%. The most active, 90% methanolic extracts (resuspended at 500 μg/ml) were from the leaves (78%) and roots (76%) of *Cyrtanthus falcatus* and the leaves of *Gethyllis ciliaris* (70%). Only CH_2Cl_2 extracts of *Cyrtanthus falcatus* (leaf and root) and *Cyrtanthus suaveolens* (bulb/root and leaf) had a mutagenic effect in the *Salmonella*/microsome assay strain TA98.
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1. Introduction

Multiple drug resistance has become a very real issue in pharmacotherapeutics as there is an increasing number of diseases which are exhibiting various levels of drug resistance, including bacterial infections (Henry, 2000). The search for new drugs to combat this problem is receiving much attention (Coates et al., 2002). Plants used in traditional medicine have the potential to provide pharmacologically active natural products which can be used to treat various ailments. This could be achieved by taking advantage of information available from traditional medicine and/or ethnobotanical knowledge.

The species belonging to the genus *Cyrtanthus* (Amaryllidaceae) are amongst the plants used in South African traditional medicine. *Cyrtanthus mackenii* is used as a protective charm against storms and evil (Hutchings et al., 1996). Other related species are used to treat diseases like scrofula, chronic coughs, headache, cystitis, leprosy and during pregnancy and childbirth (Watt and Breyer-Brandwijk, 1962; Herrera et al., 2001). The distribution of the genus *Cyrtanthus*, comprising 51 species in southern Africa range into tropical Africa. In South Africa, *Cyrtanthus* species are mainly distributed in the Western and Eastern Cape provinces with a smaller distribution in Transvaal and KwaZulu-Natal (Reid and Dyer, 1984; Du Plessis and Duncan, 1989).

Amaryllidaceae species are an exclusive source of Amaryllidaceae alkaloids (Viladomat et al., 1997). Phytochemical investigations within the genus *Cyrtanthus* led to the isolation of Amaryllidaceae alkaloids from *Cyrtanthus elatus* (Herrera et al., 2001), *Cyrtanthus obliquus* (Brine et al., 2002) and *Cyrtanthus falcatus* (Elgorashi and van Staden, 2003).

The genus *Gethyllis* is a genus of the Amaryllidaceae. It comprises about 32 species found mainly in southern Africa (Namibia and South Africa). They are widely distributed in the Western and Northern Cape. The genus is endangered and little is known about its chemistry and biological activity (Du Plessis and Duncan, 1989; Van Wyk et al., 1997). *Gethyllis* species are used traditionally in the South African folk medicine. *Gethyllis ciliaris* is used in the Cape as a remedy for colic, flatulence and indigestion (Watt and Breyer-Brandwijk, 1962). The fruit pods of many *Gethyllis* species are boiled or administered as an alcohol infusion of brandy for stomach disorders while flower decoctions

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are used for toothache (Van Wyk et al., 1997; Louw et al., 2002).

The objective of this study was to investigate the anti-inflammatory and antibacterial activities of *Cyrtanthus falcatus*, *Cyrtanthus mackenii*, *Cyrtanthus suaveolens*, *Gethyllis ciliaris*, *Gethyllis multifolia* and *Gethyllis villosa*. The risk associated with the use of these plants in traditional medicine was also evaluated using the *Salmonella*/microsome mutagenicity test.

2. Materials and methods

2.1. Plant material

Cyrtanthus plants were obtained from Green Goblin Nursery, Durban while those of the *Gethyllis* species came from Summerfield's Indigenous Bulbs and Seeds, Cape Town. Prof. T.J. Edwards, Curator of the Herbarium, University of Natal Pietermaritzburg, confirmed the identity of the species investigated. Voucher specimens were deposited in this herbarium (Table 1).

2.2. Preparation of plant extracts

Dried and powdered plant parts were extracted sequentially with dichloromethane and then 90% methanol (10 ml/g) using a sonication bath for 1 h. The crude extracts were filtered and dried under reduced pressure at 40 °C.

2.3. Cyclooxygenase assay

COX-1 and COX-2 assays were performed as described by Jäger et al. (1996) and Noreen et al. (1998). Inhibition refers to reduction of prostaglandin formation in comparison to an untreated sample. Enzyme (60 μl) and sample solution (500 μg/ml in 12.5% ethanol) were incubated at room temperature for 5 min. The reaction was started by adding 20 μl [l-^{14}C] arachidonic acid (30 μM). Samples were incubated at 37 °C (8 min for COX-1 and 10 min for COX-2) and the reaction terminated by adding 10 μl 2 M HCl. Results given are the mean of two experiments. Indomethacin was used as a positive control at a concentration of 20 μM for COX-1 and 200 μM for COX-2.

2.4. Antibacterial assay

The bacteria used were *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 13883, *Micrococcus luteus* ATCC 4698 and *Staphylococcus aureus* ATCC 12600. A colony of each bacterial strain was suspended in 1 ml of Mueller–Hinton broth and incubated for 18 h at 37 °C. A subculture was made after 6 h. The subculture was diluted 1/50 in the same broth before use.

A disc diffusion assay was used to determine the inhibition of bacterial growth by the plant extracts (Rasoanaivo and Ratsimamanga-Urverg, 1993). Plant extracts were dissolved in the extracting solvents at a concentration of 100 mg/ml and 10 μl were dispensed on a 6 mm sterile paper disc (Whatman No. 3). Neomycin was used as a positive control (500 g/ml). The diluted cultures were spread on sterile Muller–Hinton agar plates. Antibacterial activity was measured as a ratio of the inhibition zone of bacterial growth (mm) produced by the plant extract and the inhibition zone around the neomycin standard (Vlietinck, 1997).

2.5. Mutagenicity assay

A mutagenicity test was carried out using the *Salmonella*/microsome assay based on the plate—incorporation

Table 1
Percentage prostaglandin synthesis inhibition detected in CH_2Cl_2 and 90% CH_3OH plant extracts using the COX-1 and COX-2 assays

Plant species	Specimen number	Plant part	COX-1		COX-2	
			CH_2Cl_2 extract	90% CH_3OH extract	CH_2Cl_2 extract	90% CH_3OH extract
Cyrtanthus falcatus R.A. Dyer	Elgorashi NU 5	Bulbs	nt	nt	91.7 ± 4.1	29.8 ± 7.9
		Leaves	98.1 ± 2.5	78.0 ± 0.8	86.4 ± 4.6	30.1 ± 7.6
		Roots	98.5 ± 0.2	76.4 ± 1.8	96.3 ± 2.3	57.3 ± 2.6
Cyrtanthus mackenii Hook.f	Elgorashi NU 5a	Bulbs/roots	99.7 ± 0.4	36.1 ± 5.8	85.9 ± 2.3	37.6 ± 1.0
		Leaves	93.7 ± 4.7	65.3 ± 3.3	93.7 ± 2.2	37.7 ± 7.9
Cyrtanthus suaveolens Schonland	Elgorashi NU 6	Bulbs/roots	nt	nt	81.6 ± 5.4	16.6 ± 7.7
		Leaves	91.7 ± 5.8	45.8 ± 2.3	85.5 ± 3.5	53.4 ± 0.9
Gethyllis ciliaris linn.f	Elgorashi NU 7	Bulbs	93.9 ± 8.4	29.1 ± 5.7	83.5 ± 4.2	48.0 ± 4.7
		Leaves	97.1 ± 3.6	70.4 ± 0.3	70.5 ± 2.5	22.2 ± 7.7
		Roots	100.1 ± 8.6	13.2 ± 6.3	73.1 ± 3.8	39.5 ± 7.4
Gethyllis multifolia L. Bolus	Elgorashi NU 9	Whole plants	103.6 ± 0.7	30.0 ± 1.1	95.8 ± 1.4	33.1 ± 4.8
Gethyllis villosa linn.f	Elgorashi NU 8	Whole plants	97.32 ± 2.4	6.8 ± 0.2	91.3 ± 0.3	25.2 ± 8.4

Percentage prostaglandin synthesis inhibition by indomethacin in COX-1 was 71.8–79.8%, COX-2 was 54–70%. nt: not tested.

Table 2
Determination of antibacterial activity of plant extracts using the disc diffusion assay

Plant species	Plant part	CH_2Cl_2 extracts (μg/ml)					90% CH_3OH extracts (μg/ml)				
		BS	EC	KP	ML	SA	BS	EC	KP	ML	SA
Cyrtanthus falcatus	Bulbs	0	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Leaves	0.30	Bacteriostatic	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
	Roots	0.20	Bacteriostatic	Bacteriostatic	0.30	0.20	Bacteriostatic	0.00	0.00	0.00	Bacteriostatic
Cyrtanthus mackenii	Bulbs/roots	0.01	0.00	Bacteriostatic	0.17	0.39	0.00	0.00	Bacteriostatic	0.00	0.00
	Leaves	Bacteriostatic	0.00	0.00	0.00	0.00	0.00	0.00	Bacteriostatic	0.00	0.00
Cyrtanthus suaveolens	Bulbs/roots	0.28	0.37	0.13	0.57	0.91	0.00	0.00	Bacteriostatic	0.00	0.00
	Leaves	0.15	0.00	0.00	Bacteriostatic	Bacteriostatic	0.00	Bacteriostatic	Bacteriostatic	0.00	0.00
Gethyllis ciliaris	Bulbs	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Leaves	Bacteriostatic	Bacteriostatic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Roots	Bacteriostatic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gethyllis multifolia	Whole plants	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gethyllis villosa	Whole plants	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

BS: *Bacillus subtilis*, EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, ML: *Microccus luteus*, SA, *Staphylococcus aureus*.

procedure with *Salmonella typhimurium* tester strain TA98. The assay was performed according to Maron and Ames (1983). Stock (100 μl) bacteria in 20 ml Oxoid nutrient broth No. 2 were incubated for 16 h at 37 °C. The bacterial cultures (100 μl) were added to 100 μl of the plant extract in 500 μl phosphate buffer and 2 ml of agar containing biotin-histidine (0.5 mM). The mixture was poured on a minimal agar plate and incubated at 37 °C for 48 h. 4-Nitroquinoline-*N*-oxide (4 NQO) was used as a positive control at a concentration of 2 μg/ml. Samples were tested in triplicate with two replicates. Three dilutions (5000, 500, 50 μg/ml) were prepared per sample.

2.6. Test for histidine in the plant extracts

All plant extracts were tested for the presence of histidine according to Schimmer et al. (1994). Experiments were performed without histidine in the agar plates and the number of bacterial colonies and background lawn were evaluated. The presence of more than a few colonies and of a normal background lawn is indicative of an internal histidine source in the test sample.

3. Results

3.1. Anti-inflammatory test

The effects of the dichloromethane and 90% methanol extracts made from different parts of the plant species on the inhibition of prostaglandin synthesis by COX-1 and COX-2 are presented in Table 1. All dichloromethane extracts tested showed strong inhibitory activity against COX-1 (>90%) and COX-2 (>70%). Dichloromethane extracts of the bulbs and roots of *Cyrtanthus falcatus*, leaves of *Cyrtanthus mackenii*, whole plants of *Gethyllis multifolia* and whole plants of *Gethyllis villosa* showed 90% or more inhibitory activity in COX-2. All the 90% methanol extracts showed lower inhibitory activity of both the COX-1 and COX-2 assays compared to the dichloromethane extracts. Only 90% methanol extracts of the leaf and root extracts of *Cyrtanthus falcatus* and leaves of *Gethyllis ciliaris* had activity of 70% or more.

3.2. Antibacterial assay

Antibacterial activity results for dichloromethane and 90% methanol extracts of the different plant parts of the species are given in Table 2. Seven of the 12 dichloromethane extracts tested showed antibacterial activity against at least one bacterial strain. Three dichloromethane extracts (bulbs of *Cyrtanthus falcatus*, bulbs/roots of *Cyrtanthus suaveolens* and bulbs of *Gethyllis ciliaris*) exhibited activity against Gram-negative bacterial strains. The extracts that showed activity against Gram-positive bacteria were: *Cyrtanthus falcatus* (leaves and roots), *Cyrtanthus mackenii* (bulbs/roots) and *Cyrtanthus suaveolens* (bulbs/roots and leaves). The dichloromethane extract of bulbs/roots of *Cyrtanthus suaveolens* showed broad-spectrum activity. Apart from bacteriostatic effects, none of the 90% methanol extracts exhibited antibacterial activity.

3.3. Mutagenicity test

The mutagenic effects of different plant part extracts obtained with a *Salmonella* strain TA98 without metabolic activation are shown in Table 3. Plant extracts inducing revertant colonies numbering at least twice the revertant control number were considered positive for mutagenic

Table 3
Number of his+ revertants in *Salmonella typhimurium* strain TA98 produced by crude plant extracts

Plant species	Plant part	No. of colonies							
		CH_2Cl_2 extracts (μg/ml)			90% CH_3OH extracts (μg/ml)			Positive control	Negative control
		5000	500	50	5000	500	50		
Cyrtanthus falcatus	Bulbs/leaves	143 ± 24.2[a]	37.5 ± 2.9	39.6 ± 3.8	36.3 ± 3.8	38.6 ± 0.6	40.0 ± 3.6	245 ± 40.8	36.6 ± 3.1
	Roots	927 ± 48[a]	290.0 ± 30.9[a]	75.5 ± 9[a]	30.6 ± 5.6	37.3 ± 5.0	32.0 ± 3.0		
Cyrtanthus mackenii	Bulbs/roots	32.0 ± 8.0	37 ± 4.3	30.3 ± 3.7	32.6 ± 3.5	32.0 ± 7.0	31.0 ± 7.9	271 ± 42.2	23 ± 1.7
	Leaves	21.6 ± 2.5	20.6 ± 1.1	18 ± 2.6	25.3 ± 10.4	25.6 ± 6.4	22.0 ± 2.0		
Cyrtanthus suaveolens	Bulbs/roots	904.0 ± 80.0[a]	438.6 ± 29.0[a]	38.6 ± 6.8	20.0 ± 4.0	19.3 ± 0.6	22.3 ± 1.5	315 ± 23.7	26 ± 3.4
	Leaves	128.3 ± 7.0[a]	44.6 ± 9.8	34.0 ± 7.0	17.3 ± 2.3	20.0 ± 2.6	18.3 ± 1.5		
Gethyllis ciliaris	Bulbs	23.3 ± 7.3	27.0 ± 4.3	24.0 ± 7.0	24.3 ± 3.5	25.6 ± 2.1	27.0 ± 2.6		
	Leaves	29.6 ± 5.0	25.2 ± 6.4	27.5 ± 0.7	23.0 ± 0.7	30.0 ± 6.2	24.0 ± 2.8		
	Roots	23.6 ± 4.7	24.0 ± 3.0	24.0 ± 1.0	20.3 ± 5.6	19.6 ± 3.2	28.3 ± 7.2		
Gethyllis multifolia	Whole plants	27.5 ± 3.5	31.6 ± 7.7	30.0 ± 2.1	26.0 ± 1.4	19.6 ± 2.8	20.6 ± 4.2	242 ± 7.9	34.3 ± 7
Gethyllis villosa	Whole plants	25.2 ± 3.5	28.5 ± 0.7	33.0 ± 5.6	18.6 ± 9.1	19.6 ± 0.7	22 ± 4.3		

[a] Plant extracts induced revertant colonies numbering at least twice the revertant control number.

activity. Dichloromethane extracts of leaves and roots of *Cyrtanthus falcatus*, and bulbs/roots and leaves of *Cyrtanthus suaveolens* induced mutation in TA98. The mutagenic effects of the dichloromethane extracts of the roots of *Cyrtanthus falcatus* and bulbs/roots of *Cyrtanthus suaveolens* were higher compared to the dichloromethane extracts of the leaves of these species. None of the 90% methanol extracts of the different plant parts of the different species induced mutagenicity in *Salmonella* strain TA98.

4. Discussion and conclusions

Dichloromethane extracts showed high anti-inflammatory activity in both the COX-1 and COX-2 assays and relatively low antibacterial activity, while that of 90% methanol extracts were not highly active in both assays. The two extraction procedures were used in a sequential manner in order to extract apolar (dichloromethane) and polar (methanol–water soluble) compounds. The detected anti-inflammatory and antibacterial activities are in line with the uses of some the plants investigated and used in traditional medicine. However, as traditional healers use water as a solvent for preparation of plant extracts, mostly as decoctions and/or infusions, the dichloromethane procedure may be irrelevant, compared to the methanol–water extracts, at least in terms of validation of the use of plants in traditional medicine.

The Amaryllidaceae in general, including the genus *Cyrtanthus*, had been extensively studied for their alkaloid content and little is known about their non-alkaloidal constituents. Methanol, ethanol, water or aqueous alcoholic mixtures (polar solvents) are the solvents of choice in classical alkaloid extraction protocols while the apolar solvents like petroleum ether and dichloromethane are normally used for the removal of acidic fractions, mostly fatty acids, prior to alkaloid isolation (Cordell, 1981). Antibacterial and anti-inflammatory properties of fatty acids are well known. It was reported that both saturated and unsaturated fatty acids are implicated in the control of bacterial growth and inflammation in vivo and in vitro (Henry et al., 2002; McGaw et al., 2002). Thus, the possibility that the antibacterial and anti-inflammatory activity of the plants studied in this work is due to the presence of fatty acids cannot be ruled out.

The presence of Amaryllidaceae alkaloids is considered to provide scientific validation for the traditional medicinal use of Amaryllidaceae plants (Viladomat et al., 1997). This needs to be investigated further as alkaloids were only detected, using Dragendorff's reagent, in the 90% methanol extracts. Moreover, work in our laboratory on the activity of 15 Amaryllidaceae alkaloids indicated that these alkaloids had no or low antibacterial and anti-inflammatory activity (Elgorashi et al., 2003).

Plants used in traditional medicine are assumed to be safe due to the long-term use by traditional healers. Information about the safety and effective use of medicinal plants is difficult to find due to the lack of rigorous clinical studies and limited toxicological data available (Melo et al., 2001). The detection of mutagenic effects for dichloromethane extracts of *Cyrtanthus falcatus* and *Cyrtanthus suaveolens* revealed that plants used in traditional medicine should be administered with caution and after rigorous toxicological investigations.

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