Pharmacological and phytochemical properties of *Dombeya rotundifolia*

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Dombeya rotundifolia (Hochst.) Planch, is used in traditional medicine to treat heart problems, nausea in pregnant women, intestinal ulcers, headaches, stomach complaints, haemorroids, diarrhoea, dyspepsia and to hasten the onset of labour. *D. rotundifolia* material was collected from two sites and screened pharmacologically for anti-inflammatory and anti-bacterial activity using the COX-1 and disc-diffusion assays. High anti-inflammatory activity was detected in the ethanolic and dichloromethane leaf and young shoot extracts. Anti-bacterial activity was highest in the ethanolic leaf and young shoot extracts. Material was phytochemically screened for alkaloids, saponins, tannins, cardiac glycosides and cyanogenic glycosides. The results obtained for material from both collection sites was very similar. No alkaloids and no cyanogenic glycosides were detected. Tannins were present in the leaf and young shoot material. Saponins were detected in the bark material using the haemolysis test. Cardiac glycosides were detected in the leaf, young shoot and bark material.

Introduction

The prime advantage of traditional medicine is that it is an immediate, existing source of health care for people where they live. It may not be as 'good' as what may be considered ideal, but, in the absence of better alternatives use should be made of what is available (Iwu 1993). Evaluation and screening of routinely used medicinal plants is becoming a necessity to ensure the acceptance of the plants by the medical doctors and health care authorities as potential aids or cures for a number of ailments.

Dombeya rotundifolia (Hochst.) Planch belongs to the *Sterculiaceae* which is found throughout the world (Palmer and Pitman 1961). This family is often referred to as the chestnut family (Van Wyk 1974). It comprises approximately 1 200 species (50 genera), mainly trees and shrubs found in tropical and subtropical regions. Many of the African members of the family serve as sources of medicine, fibre, firewood, timber for furniture, and as decorative plants (Van Wyk 1974).

D. rotundifolia, commonly known as the wild pear, is a single-stemmed deciduous tree that grows to about 5–6m tall, with a moderate, irregular-shaped canopy (Immelman *et al.* 1973). It occurs in woodlands over a wide range of altitudes in Mpumalanga and KwaZulu-Natal (Coates-Palgrave *et al.* 1985) and is more abundant in the warmer, drier habitats (Immelman *et al.* 1973).

The inner bark is used to treat heart problems and nausea in pregnant women. Decoctions of the bark are sometimes used in delayed labour, to hasten the onset of the process. The preparation is thought also to be used for procuring abortion (Watt and Breyer-Brandwijk 1962, Hutchings *et al.* 1996, Van Wyk *et al.* 1997, Thomas and Grant 1998). Aqueous infusions of the bark or wood are used as enemas or are taken orally for treatment of intestinal ulcers, headaches, stomach complaints, haemorroids and diarrhoea (Watt and Breyer-Brandwijk 1962, Coates-Palgrave *et al.* 1985, Hutchings *et al.* 1996, Van Wyk *et al.* 1997, Thomas and Grant 1998).

The roots are made into a tonic and administered as enemas for dyspepsia and sharp pains in the stomach. In Tanzania the Zigula use the roots as a remedy for abdominal pains. It is also used as a colic remedy (Watt and Breyer-Brandwijk 1962, Coates-Palgrave *et al.* 1985, Hutchings *et al.* 1996, Venter and Venter 1996). In Zambia, the leaves are rubbed on abscesses as a counter irritant (Watt and Breyer-Brandwijk 1962).

No phytochemical and pharmacological studies of this species have previously been undertaken, except for the screening for alkaloids (Raffauf 1996). This study involved the screening of *D. rotundifolia* for biologically active compounds. Plant material was obtained from two different sites to determine whether there is a difference in the chemical composition of the plant from widely different localities.

Materials and Methods

Plant material

Plant material of *D. rotundifolia* was obtained from two sites: 1) Umgeni Valley Nature Reserve, Howick, KwaZulu-Natal, and 2) Monato, a private nature reserve in the Droogekloof area, approximately 30km from Warmbaths in the Northern Province. Voucher specimens were deposited in the University of Natal Herbarium, Pietermaritzburg, under the numbers: REID1UN and REID2UN. The habitats varied with respect to climate, altitude and soil type (Table 1). Leaf, shoot and outer bark material was collected and dried at 50°C for 72h. The plant material was homogenously ground and stored in the dark at room temperature in airtight containers until further processing.

Extraction of plant material

Ground, dried plant material (leaves, shoot and bark) was extracted with water, ethyl acetate and ethanol respectively (100mg ml⁻¹) in an ultrasonic bath for 30min. The extracts were filtered and air-dried overnight. They were re-suspended in the same solvents at 50mg residue ml⁻¹ (anti-bacterial activity) and 2.5mg residue ml⁻¹ (anti-inflammatory activity) respectively.

Anti-bacterial activity

The plant extracts were tested for anti-bacterial activity using the disc-diffusion assay (Rasoanaivo and Ratsimamanga-Urverg 1993). Activity was tested against six strains of bacteria: *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 11775), *Klebsiella pneumoniae* (ATCC 13883), *Micrococcus luteus* (ATCC 4698), *Staphylococcus aureus* (ATCC 12600) and *Staphylococcus epidermidis*. Stock cultures were stored at 4°C on nutrient agar plates. Anti-bacterial activity was expressed as the ratio of the inhibition zone (mm) produced by the plant extract to the inhibition zone caused by the neomycin control (Rabe and Van Staden 1997).

The Minimal Inhibition Concentration (MIC) technique, developed by Eloff (1998), using 96-well microplates, was used to determine the MIC's of extracts. Activity was tested against 4 strains of bacteria. *K. pneumoniae* and *E. coli* (Gram-negative) and *B. subtilis* and *S. aureus* (Gram-positive). Bacterial growth in the wells was indicated by a red colour. Clear wells indicated inhibition by the test substance.

Anti-inflammatory activity

Anti-inflammatory activity of leaf, shoot and bark extracts of *D. rotundifolia* was determined using the prostaglandin-synthesis inhibitor assay (COX-1) (Jäger *et al.* 1996). Inhibition of the test solutions was obtained by analysing the amount of radioactivity present relative to the radioactivity in the solvent blank (McGaw *et al.* 1997).

Cardiac glycosides

Extracts were screened for 2-deoxy-sugars using the Keller-Killiani test (Evans 1989, Jäger and Van Staden 1995a). If a reddish-brown-purple ring appears at the interphase, and the upper layer slowly becomes bluish-green, it is an indication that 2-deoxy-sugars are present, suggesting the presence of cardiac glycosides.

The extracts were screened for unsaturated lactone rings (Jäger and Van Staden 1995a). A TLC plate was sprayed with Kedde's reagent (Wagner *et al.* 1984) to test for the presence of cardenolides. A second and third TLC plate were sprayed with chloramine T-trichloroacetic acid reagent and antimony (III) chloride reagent respectively (Wagner *et al.* 1984) to test for the presence of bufadienolides.

Cyanogenic glycosides

Plant material was screened for cyanogenic glycosides using the Grignard test (Jäger *et al.* 1995b). A positive control was prepared containing 0.1mg amygdalin.

Saponins

Plant material was tested for saponins using the haemolysis test (Fong *et al.* 1974). Columbia Blood Agar Base (Oxoid CM 331) was prepared. Fifteen ml of sterile 20% sodium citrate was added to 500ml of whole blood. Aseptically, 50–70ml sterile citrated cattle blood was added to the Columbia Blood Agar solution. Distilled water was used as a negative control and *Saponaria officinalis* extract as a positive control.

Tannins

Tannins were detected using the Gelatin-Salt Block test (Duncan *et al.* 1999).

Alkaloids

pH partitioning for alkaloids was performed according to Brimer *et al.* (1989). Dragendorff's reagent (Wagner *et al.* 1984) was added to one part of the prepared extracts, and Mayer's reagent (Wagner *et al.* 1984) to the other. Observations were made for the development of a red-orange precipitate on the addition of Dragendorff's reagent, and a white precipitate on the addition of Mayer's reagent.

Results and Discussion

The ethyl acetate, ethanol and water extracts, from plant material collected at both collection sites in March 1999, showed varying degrees of anti-bacterial activity (Table 2). Highest activity was recorded for the ethanolic leaf extracts from both collection sites, they were bacteriostatic against *M. luteus* (0.83 and 0.71 respectively). Bacteriostatic extracts are those which prevent the multiplying of bacteria without destroying them. A zone of inhibition forms which is not clear. Ethanolic shoot extracts were also bacteriostatic against *S. aureus* (1.22).The ethanol extract from Monato showed anti-bacterial activity against *M. luteus* (0.89). Bark material showed low anti-bacterial activity. The extracts from

Table 1: Comparison of the two sites where material of *D. rotundifolia* were collected

Umgeni Valley Nature Reserve	Monato Farm(Warmbaths)
• Mean annual rainfall 950–1 100mm	• Mean annual rainfall: 500–650mm
• Vegetation type: lowveld bushveld	• Vegetation type: mixed bushveld
• Altitude: 1 000–1 500m	• Altitude: 1 500–1 700m
• The trees were about 3m tall	• Due to better spacing, the trees grew to about 4–6m
• Leaves were dark green in colour	• The leaves were a lighter green, some with signs of yellowing. This is probably due to the more acidic nature of the soil
• Trees grew in a cluster	•Trees grew scattered over a hillside

Table 2: Anti-bacterial[a] activity of extracts of *D. rotundifolia* detected using the disc-diffusion assay

Collection site	Plant part analysed	Extract	Bacteria tested[b]					
			Gram-positive			Gram-negative		
			B.s.	M.l.	S.a.	S.e.	E.c.	K.p.
UVNR	Leaf	H₂O	0	0	0	0.23	0	0
		Ethyl acetate	0	0	0	0	0	0.19
		Ethanol	0	0.83[c]	0	0	0	0
	Shoot	H₂O	0.12	0	0	0.23	0	0
		Ethyl acetate	0	0	0	0	0	0.14
		Ethanol	0	0	1.22[c]	0	0	0
	Bark	H₂O	0	0	0	0	0	0.13
		Ethyl acetate	0	0	0.07	0.14	0	0
		Ethanol	0.10	0	0	0	0	0.25
Monato	Leaf	H₂O	0	0	0	0.08	0	0
		Ethyl acetate	0	0	0	0	0	0.13
		Ethanol	0	0.71[c]	0	0	0	0
	Shoot	H₂O	0	0.21	0	0.23	0	0
		Ethyl acetate	0.07	0	0	0	0.07	0.07
		Ethanol	0	0.89	1.22[c]	0	0	0
	Bark	H₂O	0	0	0	0	0.08	0.13
		Ethyl acetate	0	0	0	0	0	0
		Ethanol	0	0	0	0	0	0.08

a — Anti-bacterial activity is expressed as a ratio of the inhibition zone of the extract (100mg ml⁻¹) to the inhibition zone of the reference (neomycin 500µg ml⁻¹).
b — Bacteria: B.s., *Bacillus subtilis*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; M.l., *Micrococcus luteus*; S.a., *Staphylococcus aureus* and S.e.,*Staphylococcus epidermidis*.
c — Bacteriostatic activity

both collection sites showed similar results in their inhibition of the bacterial strains. *K. pneumoniae* (Gram-negative) was inhibited by four of the nine extracts from each of the two collection sites. Gram-negative bacteria are more resistant strains. Anti-bacterial activity was previously detected in leaf material from *D. rotundifolia* (McGaw 2000). Ethanol and water extracts had anti-bacterial activity against *B. subtilis* (0.40 and 0.40 respectively) and *S. aureus* (0.33 and 0.36 respectively). This study could not confirm these previous results.

The results obtained from the disc-diffusion assay and the MIC values obtained for extracts obtained from *D. rotundifolia* plant material, concurred. These results did not agree with the results obtained by McGaw (2000). Here, ethanol, water and hexane extracts of the leaf material were screened. Ethanol and water extracts were shown to have anti-bacterial activity against *B. subtilis* (0.40 and 0.40 respectively) and *S. aureus* (0.33 and 0.36 respectively).

The MIC values obtained in this study were modest. None of the water extracts showed any anti-bacterial activity

(Table 3). Water extracts in the disc-diffusion assay (Table 2) were shown to have anti-bacterial activity. This activity must therefore have insignificant MIC values. It is important for traditional healers to know that the aqueous extracts are ineffective in treatment against bacterial infections at low concentrations. Organic solvents are more efficient in the extraction process for anti-bacterials. *D. rotundifolia* extracts are taken as decoctions, infusions, enemas or are rubbed on the skin (Watt and Breyer-Brandwijk 1962, Coates-Palgrave *et al.* 1985, Hutchings *et al.* 1996, Van Wyk *et al.* 1997, Thomas and Grant 1998).

The ethanol and dichloromethane extracts exhibited high and the water extract low anti-inflammatory activity (Table 4). The results were very similar for the two collection sites. Prostaglandins are involved in the complex processes of inflammation and are responsible for the sensation of pain (Jäger *et al.* 1996). The presence of anti-inflammatory activity in the *D. rotundifolia* extracts is in line with its use in traditional medicine to treat intestinal ulcers and headaches. Tannins inactivate cyclooxygenase. The leaf and shoot

Table 3: The minimum inhibitory concentration (MIC) of plant extracts against selected bacteria

Plant collection site	Plant part analysed	Extract	Bacteria used (MIC (mg ml⁻¹))			
			B.s.	E.c.	K.p.	S.a.
UVNR	Leaf	H₂O	–	–	–	–
		Ethyl acetate	–	–	–	6.25
		Ethanol	–	–	12.5	–
	Shoot	H₂O	–	–	–	–
		Ethyl acetate	12.5	–	6.25	12.5
		Ethanol	–	–	–	12.5
	Bark	H₂O	–	–	–	–
		Ethyl acetate	6.25	–	–	–
		Ethanol	–	–	–	–
Monata	Leaf	H₂O	–	–	–	–
		Ethyl acetate	–	–	–	12.5
		Ethanol	–	–	–	–
	Shoot	H₂O	–	–	–	–
		Ethyl acetate	12.5	–	6.25	12.5
		Ethanol	–	–	–	–
	Bark	H₂O	–	–	–	–
		Ethyl acetate	–	–	–	–
		Ethanol	12.5	–	–	12.5
Neomycin			56×10^{2}	6.25×10^{2}	1.56×10^{2}	3.1×10^{2}

Bacteria: B.s., *Bacillus subtilis*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; and S.a., *Staphylococcus aureus*

Table 4: The percentage inhibition of cyclo-oxygenase exhibited by *D. rotundifolia* extracts

Collection site	Plant part analysed	Inhibition (%)		
		H₂O	EtOH	CH₂Cl₂
UVNR	Leaf	79	97	95
	Shoot	65	87	94
	Bark	5	87	93
Monata	Leaf	55	90	89
	Shoot	76	88	79
	Bark	5	87	80

results in the COX-1 assay (Table 4) may be false positives due to the presence of tannins, but results obtained for the bark are correct as no tannins were present.

Leaf, bark and shoot material, from both the Umgeni Valley Nature Reserve (UVNR) and Monato, were tested for cardiac glycosides. Visible brown rings formed at the interphase of all the extracts, suggesting the presence of 2-deoxy-sugars in the plant material. The brown rings were more evident in the leaf extracts from both collection sites. They began to form with the addition of the glacial acetic acid (containing 2.2mM FeCl₃) and extract onto the H₂SO₄. Kedde's reagent indicates the presence of the y-lactone ring of cardenolides appearing as pink/blue violet spots. It does not indicate the presence of bufadienolides. Pink zones appeared on the TLC plate with the leaf, twig and bark extracts from UVNR. Twig and bark extracts from Monato also showed pink zones, with identical Rf values to those from UVNR, suggesting the presence of similar cardenolides. Unexpectedly, no pink zones appeared in the leaf extract from Monato. Chloramine T-trichloroacetic acid reagent was viewed under ultraviolet light at 365nm for yellow-orange zones. Similar positive spots were extracted

from corresponding plant material from the two localities, however, there was a higher concentration from the Monato extracts. Yellow bands appeared in the shoot and bark extracts collected at UVNR. Several yellow-orange bands appeared in the leaf and shoot extracts from Monato, and yellow bands in the bark extracts, suggesting the presence of bufadienolides. Blue-green bands appeared in the UVNR leaf extract. Antimony (III) chloride reagent–sprayed TLC plates were observed under ultraviolet light at 365nm for yellow to yellow-brown spots. Identical compounds were extracted from corresponding plant material from the two localities, again, there was a higher concentration from the Monato extracts. Yellow bands appeared in the shoot and bark extracts from Monato.

Saponins are often detected in plant extracts through their haemolytic activity (Harborne and Baxter 1993). No clear zones were visible around the cups containing water, the negative control. After 3h clear zones appeared around the positive control. The leaf and shoot extracts from material obtained from both the collection sites showed no clear zones. The bark extracts, from both collection sites, developed clear zones 16h after their addition to the blood agar plates. Some anti-fungal activity is attributed to saponins, and some saponins may aid plants to resist microbial infection (Harborne and Baxter 1993).

Fresh leaf, shoot and bark material was used in the preparation of the extracts to test for cyanogenic glycosides. None were detected.

The extracts were tested for tannins. Colour changes and the formation of a precipitate occurred in the leaf and shoot extracts from both collection sites. An off-white precipitation formed with the addition of 1 drop of 1% gelatin and 1 drop of 1% gelatin with 1 drop 10% NaCl to the leaf and shoot extracts. A dark green solution with brown precipitate in the leaf extracts and a light green solution with brown precipitate

formed in the shoot extracts. There was no colour change or precipitate formation in the bark extracts. It can therefore be concluded that tannins are present in the leaves and shoots of *D. rotundifolia*, but not in the bark. Most tannins that have been purified and studied are biologically active. Chemically, there are two main types of tannin: condensed tannins and hydrolysable tannins. Condensed tannins have been used in medicine to aid the healing of wounds and burns. When applied to the skin, they produce an impervious layer under which the healing process can take place (Harborne and Baxter 1993). They are also thought to have some protective value against toxins when taken internally. Hydrolysable tannins are of pharmacological interest because of their anti-viral and anti-tumour properties (Harborne and Baxter 1993). The extracts from both collection sites showed similar results with respect to the presence of tannins.

Leaf, bark and shoot material from both collection sites were tested for alkaloids. In all the extracts, no orange-red precipitate developed with the addition of Dragendorff's reagent and no white precipitate developed with the addition of Mayer's reagent indicating the absence of alkaloids. It has previously been reported that alkaloids are not present in *D. rotundifolia* (Raffauf 1996).

D. rotundifolia extracts from both collection sites showed similar results despite the differences in their habitats. *D. rotundifolia* has notable anti-bacterial and anti-inflammatory activity, and was found to contain cardiac glycosides, saponins and tannins. There was no cyanogenic glycosides or alkaloids present in the plant material tested.

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