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Isolation of an anthelmintic compound from *Leucosidea sericea*

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The leaves of *Leucosidea sericea* (Rosaceae) are used medicinally by some indigenous South African people as a vermifuge and astringent. No information on the biological activity and phytochemistry of the plant could be found in the literature. Bioassay-guided fractionation was used to identify the active compound. Partial purification of the petroleum ether extract of the leaves and flowers was followed by fractionation using chromatographic methods. This process yielded the phloroglucinol derivatives, aspidinol and desaspidinol, which were previously reported to be present in a *Dryopteris* species. Aspidinol was further isolated and structurally elucidated. This is the first report of the presence of these compounds in *Leucosidea sericea.* The disk diffusion method used in our study, indicated that the plant has antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*.

Introduction

The Rosaceae family consists of 107 genera which occur worldwide and encompass approximately 300 species (Jordaan 2000). Nine of these genera, including approximately 140 species, are indigenous to South Africa. The genus *Leucosidea* consists of only one species, *L. sericea*, also known as 'Ouhout' or 'Oubaas' in Afrikaans and 'umTshitshi' in Zulu (Killick 1969). Pooley (1997) describes the plant as a straggling silvery grey shrub or small tree (2–9m high), which occurs at high altitude, often near water, in grasslands or on mountain slopes. It is found in parts of Zimbabwe and Lesotho, as well as in KwaZulu-Natal, the Eastern Cape, Gauteng, Mpumulanga and the Free State (Pooley 1997). As far as could be established, no previous investigations have been conducted on the chemistry and biological activity of the plant.

Leucosidea sericea Eckl. & Zeyh (Rosaceae) is often regarded as an aggressive invader of overgrazed and disturbed areas (Killick 1969). According to a note on a specimen of *L. sericea* in the National Herbarium, Pretoria, collected by Thomas Cooper in 1861 (PRE number 0440498), the leaves of the plant were used as a vermifuge by Basuto tribes. There are also references to its use as an astringent medicine (Harvey and Sonder 1894, Watt and Breyer-Brandwijk 1962). In this paper we report on the isolation of a bioactive compound found in the petroleum ether extract of the aerial parts of *L. sericea*.

Materials and Methods

Plant material

Aerial parts of *L. sericea* were collected from the Sabie region (Mpumulanga Province) of South Africa. A voucher specimen (PRE 576807) of the plant was prepared and deposited at the National Botanical Institute, Pretoria, South Africa.

Extraction and isolation of antimicrobial compounds

Five solvents of decreasing polarities, i.e. methanol, acetone, ethyl acetate, dichloromethane and petroleum ether (distillation range 40–60EC; Merck, AR grade), were compared as to their abilities to extract antimicrobial compounds from the plant extracts (Gailliot 1998: 53). The bioactive fraction, petroleum ether, was further purified.

Air-dried, ground leaves (100g) were extracted with petroleum ether (3 x 400ml) using an orbital shaker. Each extract was filtered (Whatman No. 4), the filtrates combined and concentrated (to 100ml) under reduced pressure at 40°C using a rotary evaporator. The petroleum ether extract was partitioned with aqueous NaOH solution (200ml, pH 12) in a separating funnel. The layers were separated and the petroleum ether layer was re extracted with a second portion of aqueous NaOH (200ml). The petroleum ether layer was discarded. The aqueous NaOH layers were combined, acidified to pH 3 using 0.5M aqueous HCl solution, and extracted with petroleum ether (2 x 300ml). The aqueous layer was discarded, while the combined petroleum ether layers were evaporated under reduced pressure to yield a residue

(1.4g). This extract was re dissolved in petroleum ether and fractionated by silica gel column chromatography (Merck Kieselgel 60, 80–230 mesh) using four different gradients ranging from 7% to 30% chloroform/petroleum ether as solvent systems. The main active fraction (12% chloroform/petroleum ether), was further separated by TLC on silica gel plates (G 60 Macherey-Nagel, 0.20mm thick, without a fluorescent indicator) using chloroform:petroleum ether (1:4). A fluorescent band, clearly visible under UV light, was scraped off and the silica gel eluted with chloroform to yield the active compound (18mg).

Antimicrobial assay

Seven bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Shigella sonnei*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and one yeast (*Candida albicans*) were initially selected as test organisms. The antimicrobial activity of sample fractions was tested at each purification stage using the disk diffusion method as described by Bauer *et al.* (1965). An aliquot (20µl) of the test solution was applied at a concentration of 1mg ml^{-1}, using chloroform as solvent, to each disk. A positive control (2µg, Ampicillin) and a negative control (chloroform) were subjected to the same assay (Table 1).

Identification of the pure compound

Data obtained from GC-MS analysis (Agilent 6890GC-5973-MSD; DB5 column with length 30m) was used for the preliminary identification of the compounds. The obtained mass spectra were compared to the NIST mass spectral library. The structure of the purified compound was further elucidated using ^{1}H and ^{13}C NMR (Varian Gemini 2000, broadband, 300 MHz; $CDCl_3$) with TMS as internal standard and 85% phosphoric acid (^{31}P) as external standard. The values obtained were compared to those reported in the literature.

Results

The antimicrobial activity-guided fractionation by disk diffusion of the petroleum ether extract from *L. sericea* led to the isolation of the bioactive compound, aspidinol (2,6-Dihydroxy-4-methoxy-3-methyl-l-butyrophenone). Aspidinol (Figure 1) and desaspidinol were detected and identified in a partially purified extract using GC-MS. The spectra obtained for both these components correlated with those in the NIST library. The ^{1}H and ^{13}C NMR values obtained for the isolated compound were consistent with those previously reported for aspidinol (Wollenweber *et al.* 1998). Partially purified extracts and the isolated compound caused growth inhibition of *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* (Table 1). However, no extracts were significantly active against the other test organisms.

Discussion and Conclusions

Aspidinol, a phloroglucinol, was first reported to be present in extracts of the male fern, *Aspidium felix-mas* in 1899 (Hausmann 1899). Aspidinol and its derivatives were isolated in 1957 from *Dryopteris austriaca*, a member of the fern family (Aebi *et al.* 1957). These phloroglucinols are present in *Agrimonia pilosa* (Kasai *et al*. 1992) and *Hagenia abyssinica* (Woldemariam *et al*. 1992) which both belong to the Rosaceae family. This is the first time that the presence of aspidinol and desaspidinol have been reported in *L. sericea*. Aspidinol and its derivatives are known anthelmintic agents (Aebi *et al.* 1957) and the presence of these compounds in *L. sericea* could therefore explain the use of the plant as a vermifuge in tribal medicine.

The antimicrobial potential of *L. sericea* extracts was found to be limited. *B. subtilis* is common in soil and is not usually considered pathogenic although it can be responsible for opportunistic infections. *S. aureus* is, however, a common cause of wound infections and extracts of this plant

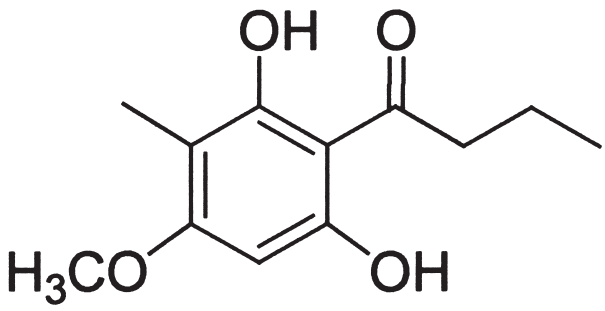

Figure 1: Chemical structure of Aspidinol

Table 1: Growth inhibition of several organisms by different extracts and the isolated compound from the leaves of *L. sericea*

	Methanol	Acetone	Ethyl acetate	Dichloromethane	Petroleum ether	Isolated compound	Ampicillin
S. aureus	x		x		xx	xxx	xxx
S. sonnei							
S. typhimurium							
C. albicans			x				xxx
B. subtilis		x	x	x	xx	xxx	xxx
P. aeruginosa							
E. coli							

x indicates inhibition zones with diameter <5mm
xx indicates inhibition zones with diameter 5–10mm
xxx indicates inhibition zones with diameter >10mm

could possibly be used topically for minor wounds. The use of a paste made from the plant and used on the eyes by African tribes could possible relate to the antibacterial action of the plant material (Watt and Breyer-Brandwijk 1962). Of more significance are the anthelmintic and anti-protozoal potential of aspidinol. Helminth infestation is common in both humans and animals in Africa. In humans, worm infestations may contribute to the effect of malnutrition, while in cattle and sheep the control of internal worms increases production. Resistance by worm parasites of animals to anthelmintics has escalated in South Africa to such an extent that it has become a major problem in sheep and goat farming (Van Wyk *et al.* 1999). Investigation of chemicals for use as anthelmintics in animal husbandry is therefore important. This is even more critical for small scale farmers in rural areas where the cost of commercial anthelmintics and their availability is a problem. Aspidinol derivatives have been shown to be effective against *Plasmodium falciparum* (US patent 5807898), one of the protozoan parasites responsible for malaria. Although no ethnopharmacological or phytochemical studies have previously been conducted on *L. sericea*, it can be concluded that the plant has medicinal potential and should be further investigated for application as a natural ethnomedicinal product. *L. sericea* can be utilised as a medicinal plant, without endangering the species, because it occurs commonly and has a tendency to invade.

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