Isolation of two antibacterial compounds from the bark of *Salix capensis*

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Livestock owners in the Eastern Cape Province of South Africa continue to use medicinal plants to treat their livestock. The bark of *S. capensis*, one of the plants commonly used, was extracted in ethanol. The resultant extract was partitioned between n-hexane and water. The n-hexane extract was subjected to a series of column chromatography using various solvent systems, yielding two known compounds: catechol and 2-hydroxybenzyl alcohol. When tested on several bacterial strains, the compounds exhibited significant antibacterial activity with minimum inhibitory concentrations ranging from 62.5–250µg ml⁻¹.

The use of plant remedies to treat both human and livestock ailments is still widely practised worldwide. One important goal of ethnobotanical research is the understanding of the relative importance of medicinal plants to a society and their perceptions of plants and plant products. Medicinal plants have become the focus of intense study, to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. Where they are effective, such plant-derived drugs serve as prototypes for the development of more effective and less toxic medicines (Samy and Ignacimuthu 2000).

In the Eastern Cape, livestock farmers continue to use a number of plants for the treatment of livestock disease (Dold and Cocks 2001, Masika and Afolayan 2003). An example of plants used is *Salix capensis* (Salicaceae). It occurs along stream- and riverbanks in a wide range of places (Palgrave 1990). Its leaves and bark are traditionally used in many parts of South Africa to treat rheumatism and fever (Watt and Breyer-Brandwijk 1962). The decoction from the bark is used in the treatment of redwater and gall-sickness in cattle (Masika and Afolayan 2003). Some farmers also use alcoholic beverages to extract the plant. Decoctions are administered in large volumes (750ml and 375ml), and extracts of alcoholic beverages are given in smaller volumes (20ml and 5–10ml) for cattle and small stock (goats and sheep), respectively.

Previous phytochemical work on this genus revealed that most of the species contain numerous phenolic compounds, of which salicortin and salicin are of special medicinal interest (Van Wyk et al. 1997). We now report on the phytochemical analysis of *Salix capensis*, which led to the isolation of two compounds, catechol and 2-hydroxybenzyl alcohol, together with their antibacterial activity.

¹H NMR and ¹³C NMR were recorded on a Bruker AMX400 spectrometer at 400 MHz and 100.60 MHz, respectively. 2D NMR spectra were recorded on the same instrument, using a field gradient BBI (inverse) probe. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 H (15µm) and silica gel 60 (0.063–0.2mm) respectively. Silica gel 60 F₂₅₄-coated aluminum plates were used for thin layer chromatography (TLC), all supplied by Merck.

The bark of *S. capensis* was collected from the natural population at the University of Fort Hare Research Farm, Alice campus, allowed to air-dry and pulverised. The pulverised plant material (1kg) was extracted by shaking in ethanol at room temperature for two days. The extracts were filtered using Whatman No. 1 filter paper under suction and concentrated to dryness at 40°C under reduced pressure, yielding a mass of 63g. The extract was partitioned between n-hexane and distilled water. The aqueous part was further portioned between ethyl acetate (EtOAc) and water. The aqueous part was further portioned between ethyl acetate (EtOAc) and water. The aqueous part was further portioned between ethyl acetate (EtOAc) and water.

The n-hexane extract (16.4g) was fractionated by VLC on silica gel, eluted with solvents of increasing polarity (n-hexane-EtOAc) and then EtOAc-MeOH (0–15%). The fractions eluted with 50–60% EtOAc in n-hexane were combined (550mg) and purified by CC using EtOAc in CHCl₃ (30:70 v/v) isocratic system. Fractions 16–17 were combined (261mg), based on similar bioactivity, and subjected to GFC (Sephadex LH-20) eluting with CHCl₃. Fractions 33–39 were combined to yield catechol 1 (29.2mg). Fractions 18–19 (182mg) were treated by CC using EtOAc.
in n-hexane (80:20). Fractions 20–25 (84.6mg) were treated by silica gel using MeOH in CHCl₃ (5:95) and further Fractions 11–17 (34.5mg) were combined and treated by silica gel using MeOH in CHCl₃ (10:90), from which Fractions 3–6 were combined, to yield 2-hydroxybenzyl alcohol 2 (15.2mg).

Laboratory strains of Bacillus subtilis (ATCC11774), Staphylococcus aureus (ATCC29213), Escherichia coli (ATCC25922) and Pseudomonas aeruginosa (ATCC27853) were used for the antibacterial assay. During the extraction and purification procedure, a bioautographic assay (Slusarenko et al. 1989) was performed on TLC plates using B. subtilis. An inoculated layer of agar was sprayed with fresh culture bacteria over a developed TLC plate and incubated for 24h at 37°C. Bacterial growth was detected by spraying the plates with 0.2mg ml⁻¹ p-iodonitrotetrazolium (INT) solution and incubating at 37°C for 30min. The inhibition of bacterial growth by the compounds on the TLC plate was observed as white spots against the deep red background.

The minimum inhibitory concentration (MIC) values of the pure compounds were further determined using the microplate dilution method against two Gram-positive (B. subtilis, S. aureus) and two Gram-negative (E. coli and P. aeruginosa) bacteria. Microtitre plates of 96 wells were used. Test organisms were prepared by diluting 24h-old broth culture with sterile nutrient broth. The culture was then diluted 100-fold to give approximately 10⁶ bacteria ml⁻¹. The microtitre plates were prepared using serial dilution (Elloff 1998) and incubated for 24–48h at 37°C. As an indicator of bacterial growth, 40µl of 0.2mg ml⁻¹ INT solution was added to each well and incubated at 37°C for 30min. The colourless tetrazolium salt was reduced to a red-coloured product by biological activity of the organisms, thereby making the inhibition of bacterial growth visible as clear wells. MIC values were recorded as the lowest concentration resulting in complete inhibition of bacterial growth. Each treatment was replicated thrice. Streptomycin, chloramphenicol, dimethyl sulfoxide (DMSO) and sample-free solutions were used as positive and negative controls.

Compounds 1 (catechol 1,2-dihydroxybenzene) and 2 (2-hydroxybenzyl alcohol) shown in Figure 1, were identified based on their spectral data (¹H and ¹³C NMR), which were in agreement with the literature: Breitmaier and Volter (1987), and Huang and Wan (1991), respectively.

Catechols are a large class of substances of natural or synthetic origin that contain a 1,2-dihydroxybenzene group. The term generally applies to different classes of compounds, including small catechols, which are biosynthesised in plants containing a carboxylic function, neurotransmitters and catechol drugs (Antonio et al. 2003).

Catechol and 2-hydroxybenzyl alcohol both exhibited similar antibacterial activity (Table 1). They showed more activity on P. aeruginosa, a Gram-negative bacterium. The activity of the isolated compounds was rather low, compared to that of the standard antibiotics. This is probably because plant extracts used by the farmers are administered in large quantities (750ml for cattle, and 250ml for sheep and goats), hence meeting the required physiological levels in the animals. On the other hand, the compounds in the plant extracts used by the farmers may not be having direct effect on the causative organisms, but act more as antioxidants (Rice-Evans et al. 1996). These compounds are toxico-logically potent, depending on their concentration (Van Zyl et al. 1991).

Both catechol and 2-hydroxybenzyl alcohol have been isolated from various plant sources (Zenk 1967, Pearl and Darling 1968). However, this is their first isolation from S. capensis.

Catechol has been associated with various biological and medicinal activities (Gigante et al. 2002, Roginsky 2003). For example, it possesses antioxidant properties, by virtue of the structural arrangements and hydrogen-donating potential of their phenolic groups (Begona and Nicholas 2002), that may afford some protection against oxidative stress.

Despite its biological and medicinal activities, catechol also exhibits a wide variety of toxicological properties. It is a well-known carcinogen in long-term exposure (Hirose et al. 1998) and is also reported to be a genotoxicant, which is able to induce chromosomal aberrations in V79 cells (do Céu Silva et al. 2003). It is also a potent inhibitor of T cell proliferation (Li et al. 1997), by quenching the essential tyrosyl radical of ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis; it can also induce oxidative DNA damage (Oikawa et al. 2001). Inhibitory effects of these compounds on pro-oxidant enzymes such as xanthine

![Figure 1: Structures of compounds isolated from S. capensis](image_url)

| Bacteria          | Catechol | 2-Hydroxybenzyl alcohol | Streptomycin | Chloramphenicol | DMSO |
|-------------------|----------|-------------------------|--------------|----------------|------|
| B. subtilis       | 250      | 250                     | 4            | 4              | >500 |
| S. aureus         | 250      | 250                     | 4            | 4              | >500 |
| E. coli           | 250      | 250                     | 4            | 4              | >500 |
| P. aeruginosa     | 62.5     | 62.5                    | 4            | 4              | >500 |

Table 1: Antibacterial activity of catechol and 2-hydroxybenzyl alcohol isolated from S. capensis.
oxidase, myeloperoxidase and lipoxygenases have also been reported (Middleton et al. 2000).

Little information is available on the biological and physiological activities of 2-hydroxybenzyl alcohol. Evidence of various biological and medicinal activities of compounds from *S. capensis* provides better understanding for the reason why farmers have been using these remedies for time immemorial. However, caution has to be exercised, since these plants possess compounds which are toxic, despite their biological and medicinal activities.

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