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In vitro anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine

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Abstract

Many plants are used for ethnoveterinary purposes in South Africa, particularly in rural areas. Extracts of 17 plant species employed to treat infectious diseases were prepared using three solvents and the antibacterial activity of the extracts was determined against two Gram-positive and two Gram-negative bacteria. Anthelmintic activity was evaluated against the free-living nematode *Caenorhabditis elegans* and toxicity was determined using the brine shrimp larval mortality test. Most of the plant extracts demonstrated antibacterial activity, with the best minimum inhibitory concentration (MIC) being 0.1 mg mL^{-1}. More than a third of the extracts displayed anthelmintic activity. Toxic effects against brine shrimp larvae were shown by 30% of extracts, with the lowest LC_{50} recorded as 0.6 mg mL^{-1}. The promising biological activity displayed by a number of plant extracts supports the ethnoveterinary use of these plants but in vivo tests are required to ascertain fully their medicinal properties and potential toxicity.

Keywords: Antibacterial; Anthelmintic; Brine shrimp; Cytotoxicity; Ethnoveterinary medicine

1. Introduction

Ethnoveterinary medicine is a broad field covering people's beliefs, skills, knowledge and practices relating to the care of their animals (McCorkle, 1986). The recent revival of scientific interest in traditional veterinary medicine has followed the well-documented interest in traditional practices in human medicine (Schillhorn van Veen, 1997). In animal health, as in human health, the market in traditional medicines is expanding, and traditional practices are increasingly becoming mainstream (Schillhorn van Veen, 1997).

Ethnoveterinary medicine is important in areas of developing countries that lack access to conventional medicines for animal health care, which are often unaffordable to poor rural farmers. A key objective of the scientific study of ethnoveterinary practices is the development and promotion of effective veterinary medicines based on inexpensive locally available plants. While the value of conventional medicines in combating infectious diseases is irrefutable, for common diseases such as mild diarrhoea, skin diseases, intestinal worms, wounds and reproductive disorders, ethnoveterinary medicine may have much to offer (Martin et al., 2001). Drawbacks of traditional medicinal plant remedies include seasonal unavailability of plants, the possibility of ineffective or harmful treatments, uncertain dosages and lack of standardisation (Martin et al., 2001).

A survey of the use of medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the

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North West Province of South Africa recorded the use of 45 plant species representing 24 families (Van der Merwe et al., 2001). Ethnoveterinary plant use was widespread in this rural part of the country, and many different plants were used for various ailments. The most commonly treated disorders include diarrhoea, eye inflammation, general gastrointestinal problems, retained placenta, heartwater, internal parasites, coughing, redwater and tick infestation (Van der Merwe et al., 2001). The plant material was traditionally prepared in various ways including infusion, decoction, ground fresh material and sap expressed from fresh material, and in most cases a liquid was orally administered using a bottle.

Based predominantly on the ethnoveterinary use of plants reported by Van der Merwe et al. (2001), as well as on knowledge gathered in unreported studies, plants used to treat infectious disorders were collected and assayed for biological activity in an endeavour to discover those that were highly active and also to validate their traditional use. The plant parts employed to prepare the extracts were those used in traditional ethnoveterinary medicine.

Antibacterial effects against two Gram-positive and two Gram-negative species were investigated using a microdilution assay. Anthelmintic activity was examined using a free-living nematode as a model. Additionally, the extracts were submitted to the brine shrimp lethality assay as activity in this assay has been correlated to pharmacological activity (McLaughlin, 1991).

2. Materials and methods

2.1. Collection of plant material and extract preparation

Plant material was collected in the northern and eastern parts of South Africa in the summer of 2001/2002. Voucher specimens [2] were prepared and deposited in the Herbarium of the Onderstepoort Veterinary Institute (Pretoria). The place of collection as well as the known ethnoveterinary use of the plants were recorded.

After drying at room temperature in a well-ventilated room, the plant material was ground to a powder using a Janke and Kunkel mill. Three separate aliquots of 2 g of parts of each plant were extracted by shaking vigorously for 20 min on a Labotec Model 20.2 shaker with 20 mL of hexane, methanol or water. The extract was allowed to settle, centrifuged at 2000*g* for 10 min and the supernatant filtered through Whatman No. 1 filter paper. The extracts were dried in a stream of cold air before resuspending in acetone in the case of the organic extracts, and water for aqueous extracts, to a concentration of 100 mg mL^{-1}. In total, 70 extracts were prepared from 17 species (24 plants) for bioassay screening against bacteria, nematodes and brine shrimp larvae.

[2] A voucher specimen is a pressed sample of plant material deposited in a herbarium for future reference as it may be examined to verify the identity of the specific plant used in a study.

2.2. Antibacterial assay

The serial microplate dilution method of Eloff (1998) was used to screen the plant extracts for antibacterial activity. This method allows the determination of the minimal inhibitory concentration (MIC) of each plant extract against each bacterial species by measuring reduction of tetrazolium violet.

The bacteria used in the present study included two Gram-positive bacteria, *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 29213), and two Gram-negative species, *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35219). The bacterial cultures were incubated in Müller-Hinton (MH) broth overnight at 37 °C and a 1% dilution of each culture in fresh MH broth was prepared prior to use in the microdilution assay. Twofold serial dilutions of plant extract (100 μL) were prepared in 96-well microtitre plates, and 100 μL of bacterial culture were added to each well. The plates were incubated overnight at 37 °C and bacterial growth was detected by adding 40 μL *p*-iodonitrotetrazolium violet (INT) (Sigma) to each well. After incubation at 37 °C for 1 h, INT is reduced to a red formazan by biologically active organisms, in this case, the dividing bacteria. Bacterial growth was shown to be inhibited when the solution in the well remained clear. This concentration was taken to be the minimal inhibitory concentration (MIC). Solvent controls and the standard antibiotic neomycin (Sigma) were included in each experiment.

2.3. Anthelmintic assay

Anthelmintic activity of plant extracts was assayed using the free-living nematode *Caenorhabditis elegans* var. Bristol (N2) following the method of Rasoanaivo and Ratsimamanga-Urverg (1993), modified by McGaw et al. (2000). The nematodes were cultured on nematode growth (NG) agar seeded with *E. coli* (Brenner, 1974). Approximately 500 nematodes (7–10 day old cultures) in M9 buffer (Brenner, 1974) were incubated with 0.5, 1 and 2 mg mL^{-1} of plant extract for 2 h at 25 °C in the dark. The anthelmintic levamisole (Sigma) was used as a positive control, and solvent blanks were included. Using a stereomicroscope, the percentage of living nematodes was estimated.

2.4. Brine shrimp lethality assay

The plant extracts were tested against larvae of *Artemia salina* (brine shrimp) using the method of Solís