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Antimicrobial activity of southern African medicinal plants with dermatological relevance: From an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound

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ABSTRACT

Ethnopharmacological relevance: Ethnobotanical reports on more than 100 southern African medicinal plants with dermatological relevance have been highlighted, yet there is still limited scientific data to support claims for their antimicrobial effectiveness against skin pathogens. Guided by ethnobotanical data, this paper explores the antimicrobial efficacies of southern African medicinal plants used to treat skin ailments.

Aim of the study: To investigate the antimicrobial properties of southern African medicinal plants against dermatologically relevant pathogens. The study also aimed at providing a scientific rationale for the traditional use of plant combinations to treat skin diseases and the isolation of the bioactive compound from the most active species, *Aristea ecklonii* (Iridaceae).

Materials and methods: Organic and aqueous extracts (132) were prepared from 47 plant species and screened for antimicrobial properties against dermatologically relevant pathogens using the micro-titre plate dilution method. Four different plant combinations were investigated for interactive properties and the sum of the fractional inhibitory concentration (ΣFIC) calculated. Isobolograms were used to further investigate the antimicrobial interactive properties of *Pentanisia prunelloides* combined with *Elephantorrhiza elephantina* at varied ratios. A bioactivity-guided fractionation process was adopted to fractionate the organic leaf extract of *Aristea ecklonii*.

Results: Plants demonstrating notable broad-spectrum activities (MIC values ≤1.00 mg/ml) against the tested pathogens included extracts from *Aristea ecklonii, Chenopodium ambrosioides*, *Diospyros mespiliformis, Elephantorrhiza elephantina, Eucalyptus camaldulensis, Gunnera perpensa*, *Harpephyllum caffrum, Hypericum perforatum, Melianthus comosus, Terminalia sericea* and *Warburgia salutaris*. The organic extract of *Elephantorrhiza elephantina*, a plant reportedly used to treat acne vulgaris, demonstrated noteworthy antimicrobial activity (MIC value of 0.05 mg/ml) against *Propionibacterium acnes*. Similarly, *Diospyros mespiliformis* reported for its traditional use to treat ringworm, also displayed noteworthy antimicrobial activity against *Trichophyton mentagrophytes* (MIC 0.10 mg/ml) and *Microsporum canis* (MIC 0.50 mg/ml). The aqueous root extracts of *Pentanisia prunelloides* combined (1:1) with *Elephantorrhiza elephantina* displayed synergistic interactions (ΣFIC values 0.31–0.38) against *Staphylococcus aureus*, gentamycin–methicillin resistant *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans*. Fractionation of *Aristea ecklonii* resulted in the isolation of the known bioactive compound, plumbagin, displaying noteworthy antimicrobial activity (MIC range between 2.00 μg/ml and 16.00 μg/ml).

Conclusion: Most of the plant extracts demonstrated pathogen specific antimicrobial effects with a few exhibiting broad-spectrum activities. Positive antimicrobial effects noted for plants such as *Elephantorrhiza elephantina* and *Diospyros mespiliformis* used for acne vulgaris and ringworm infections, respectively, give some validation to their reported traditiona l uses. Synergistic interactions noted for *Pentanisia prunelloides* combined with *Elephantorrhiza elephantina* validate an enhanced antimicrobial

Abbreviations: Aq, Aqueous extract; CC, column chromatography; CFU/ml, colony forming units/ml; D:M, 1:1 mixture of dichloromethane and methanol; DMSO, dimethyl sulfoxide; HSCCC, high speed counter-current chromatography; MIC, minimum inhibitory concentration; NMR, nuclear magnetic resonance; ΣFIC, the sum of the fractional inhibitory concentration; INT, iodonitrotetrazolium chloride; TSB, Tryptone Soya broth; UHPLC, ultra-high performance liquid chromatography..

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effect when used in combination. Noteworthy antimicrobial activities (MIC range between 2.00 μg/ml and 16.00 μg/ml) were observed for plumbagin isolated from *Aristea ecklonii*.

1. Introduction

The readily-available ethnobotanical literature has reported over 100 medicinal plants that are used in southern Africa for treating dermatological disorders (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Von Koenen, 1996; Felhaber, 1997; Rabe and Van Staden, 1997; Van Wyk et al., 2000, 2009). A review by Van Vuuren (2008), on South African medicinal plants, highlights numerous studies which have focused on evaluating the antimicrobial efficacies of plant species used for a variety of ailments, including skin inflictions. While most studies have focused on antimicrobial screening against common pathogens such as, *Staphylococci* species, *Pseudomonas aeruginosa* and *Candida albicans*, it has been noted that skin dermatophytes such as *Trichophyton mentagrophytes* and *Microsporum canis* have been neglected in most screening assays. This is possibly due to difficulties encountered with culturing mould dermatophytes, which is a time-consuming process. However, to validate the antimicrobial efficacies of medicinal plants used traditionally to treat common skin diseases such as ringworm infections, it is necessary to include all the relevant, including fastidious pathogens.

Propionibacterium acnes is an important skin pathogen responsible for the chronic inflammatory disease of the sebaceous glands and hair follicles of the skin. The infection usually results in acne vulgaris, a skin condition common but not exclusive to teenagers, which has considerable psychological impact (Magin et al., 2006). The antibacterial effects of South African medicinal plants against acne causing bacteria have been rarely addressed, even though attention has been given to this pathogen in other geographical ethnobotanical- relevant studies (Chomnawang et al., 2005, Kim et al., 2007, 2008; Tsai et al., 2010; Balakrishnan et al., 2011).

While many studies have focused on either antimicrobial screening or the phytochemistry, very little is reported on plant–plant interactions when used in combination, in spite of the traditional use (Smith, 1895; Hutchings, 1996; Felhaber, 1997). Although some studies have been conducted to evaluate medicinal plant interactions from southern African species (Kamatou et al., 2006; Van Vuuren, 2008; Suliman et al., 2010; Ncube et al., 2012), the use of plant combinations to treat specific skin ailments has been sorely neglected.

The identification of bioactive compounds is another important factor to be examined to gain insight into the antimicrobial properties of medicinal plants of dermatological relevance. Previously, a number of antimicrobial related studies have highlighted the value of identifying the antimicrobial active compound/s (Rabe and Van Staden, 1997; De Paiva et al., 2003; Van Vuuren et al., 2006; Shai et al., 2008; Van Vuuren, 2008). Hence, this comprehensive study of southern African dermatologically relevant plants aims to present a detailed account of the antimicrobial properties, plant–plant interactive efficacies and isolation of a bioactive naphthoquinone from one of the most active plant species.

2. Materials and methods

2.1. Plant collection and identification

Various plant parts (related to traditional use) of 47 different plant species (representing 38 families) were harvested from designated botanical gardens. Voucher specimens were prepared for each species and are housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand. Table 1 details the plant species collected, reported traditional use, parts of the plants used, voucher numbers and the collection sites.

2.2. Preparation of plant extracts

Plant samples were left to dry at room temperature. They were then ground to a fine powder using the high speed Fritsch Pulverisette grinder (Labotec). Organic extracts were prepared by submerging (± 20 g) of the dried, crushed plant material in a 1:1 mixture of dichloromethane and methanol (D:M) and left on the platform shaker incubator (Labcon) at 37 °C for 24 h.

Aqueous extracts (Aq) were prepared by submerging the macerated plant material in sterile distilled water, and were then left on the platform shaker incubator and kept at ambient temperature overnight. Thereafter, the liquid extracts were filtered and stored at −80 °C before lyophilisation (Virtis). All extract samples were stored at room temperature until further use.

2.3. Antimicrobial activity assays

2.3.1. Culture preparation

Bacterial and fungal test organisms were selected based on conditions that the plants are reported to treat and on their prevalence to cause skin infections. These include aerobic Gram-positive bacteria; *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, gentamycin–methicillin-resistant *Staphylococcus aureus* (GMRSA) ATCC 33592, *Staphylococcus epidermidis* ATCC 2223, *Brevibacillus agri* ATCC 51663 and anaerobic *Propionibacterium acnes* ATCC 11827. The Gram-negative bacterium selected for the study was *Pseudomonas aeruginosa* ATCC 27858. Dermatophytes such as *Trichophyton mentagrophytes* ATCC 9533, *Microsporum canis* ATCC 36299 and the yeast *Candida albicans* ATCC 10231 were also included.

Each bacterial culture was grown in Tryptone Soya broth (TSB) (Oxoid, Ltd), for 18–24 h at 37 °C. *Propionibacterium acnes*, however, was grown in Thioglycolate broth (Oxoid, Ltd) and incubated under anaerobic conditions using a candle gas jar for seven days at 37 °C.

Dermatophytes, *Trichophyton mentagrophytes* and *Microsporum canis* were grown and maintained on Sabouraud's Dextrose agar (Oxoid, Ltd), incubated at 35 °C for up to seven days in a humidified environment (Masoko et al., 2007). *Candida albicans* was grown in TSB and incubated at 37 °C for 48 h.

2.3.2. Micro-titre plate dilution technique: Minimum inhibitory concentration (MIC)

A serial micro-dilution assay was used to quantify the minimum inhibitory concentration (MIC) values for plant extracts using tetrazolium violet reduction as an indicator of growth (Eloff, 1998; NCCLS, 2003). Using aseptic manipulation, 100 μl of distilled sterile water was instilled in each well of a 96 well micro-titre plate. The plant extracts at starting concentrations of 64 mg/ml in acetone or dimethyl sulfoxide (DMSO) (Table 2) were transferred to the first row of the micro-titre plate. The solvent DMSO was used when select extracts were insoluble in acetone. Serial dilutions were performed on each plate, and thereafter the cultures (sub-cultured 1:100 in suitable broth) with an approximate inoculum size of 1×10^6 colony forming units/ml (CFU/ml)

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