Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa

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Received 9 March 2004; received in revised form 27 June 2004; accepted 9 August 2004

Abstract

Aqueous and methanol extracts of Urtica urens, Capparis tomentosa, Dicomia anomala, Leonotis leonorus, Xysemalobium undulatum, Helichrysum fordiem, Piericarpus angolensis, Terminalia sericea and Gunnera perpensa, plants documented as being used for topical wound healing in the literature, were tested for antibacterial activity against Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Pseudomonas aeruginosa. Methanol and water extracts of two of these plants, Terminalia sericea and Gunnera perpensa, were more active compared to the other extracts against Streptococcus pyogenes and Staphylococcus aureus. The effects of the latter plants on fibroblast growth as well as oxidant production by N-formyl-methionyl-leucyl-phenylalanine were also studied. The water and methanol extracts of Terminalia sericea and Gunnera perpensa significantly decreased lucigenin enhanced chemiluminescence at concentrations of 100μg/ml and higher. However, the extracts had no effect on the growth of primary human fibroblasts.

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Keywords: Wounds; Antioxidant; Antibacterial; Herbal remedies

1. Introduction

More than 80% of the world’s population still depends upon traditional medicines for various skin diseases (Priya et al., 2002). Wound healing is a complex process characterized by homeostasis, re-epithelialisation, granulation tissue formulation and remodelling of the extracellular matrix (Priya et al., 2002). Though the healing process takes place by itself, an infection can seriously delay this healing process (Priya et al., 2002). Although there are many published reports on the effectiveness of South African traditional remedies against Gram-positive and Gram-negative microorganisms, only three publications could be found where the antibacterial activity of remedies used specifically for the treatment of wounds has been determined (Rabe and van Staden, 1997; Grieson and Afolayan, 1999; Kelmanon et al., 2000).

The following nine plants, based on their ethnomedical use for topical wound healing, were selected for testing: (i) the bark of Terminalia sericea Burch ex DC. (Combretaceae) (Watt and Breyer-Brandwijk, 1962; Mabogo, 1990; Van Wyk et al., 1997), (ii) Capparis tomentosa Lam. (Capparaceae) roots, which are applied to snake-bites, wounds and leprosy (Hutchings et al., 1996), (iii) decoctions of the roots of Gunnera perpensa L. (Haloragaceae) are applied as a dressing for wounds and psoriasis (Watt and Breyer-Brandwijk, 1962), (iv) seed ashes of Piericarpus angolensis DC. (Fabaceae) are used as a dressing on wounds and psoriasis whereas the fruits are used to treat head wounds (Hutchings et al., 1996), (v) decoctions of the leaves and stems of Leonotis leonurus (L.) R. Br. (Lamiaceae) commonly known as the wild dagga, are applied to boils, snake-bites and sores on the leg and head as well as for the treatment of eczema and other skin diseases (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Van Wyk et al., 1997), (vi) Dicomia anomala Sond. (Asteraceae) root is applied as a paste to sores and...
wounds (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996), (vii) powdered tuber of Xysema undulatum (L.) Ait. f. (Asclepiadaceae) is applied to sores, wounds and abscesses (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Van Wyk et al., 1997), (viii) Helichrysum foetidum (L.) Moench (Asteraceae) leaves are used as a dressing of septic sores (Watt and Breyer-Brandwijk, 1962) and circumcision sores (Watt and Breyer-Brandwijk, 1962).

In African communities, few people visit health services and thus rely on ethnomedicines. In addition, the cost of antibiotics is high enough being not affordable to these people. Although the above plants are widely used as remedies, most of them have not yet been evaluated for antibacterial activity. In this study, we investigated the above-mentioned South African plants, used for topical wound healing, for their potential antibacterial activity since microbial infections can hamper the healing process. The two most promising candidates were selected for further testing on the growth of fibroblasts and effects on oxidant production by stimulated neutrophils. Antioxidant activity was determined since the release of oxygen radicals kills invading foreign organisms and clears the wound of fibrin matrix, thus enhancing the healing process (Grinnell and Zhu, 1994), whereas fibroblasts play a crucial role in wound healing by initiating the proliferative phase of repair (Mensah et al., 2001).

Results from our study found both Terminalia sericea and Gunnera perpensa to have no effect on fibroblast growth. Various researchers investigated the ability of aqueous extracts of herbal remedies, used for wound healing, to stimulate the growth of fibroblasts in vitro (Phan et al., 1998; Mensah et al., 2001; Stevenson et al., 2002), however, these two species have not previously been investigated. Numerous publications reported the antioxidant activity of plants traditionally used for wound healing (Mensah et al., 2001; Thang et al., 2001, Phan et al., 2001a,b; Shirwaikar et al., 2003). This study is the first to determine the antioxidant activity of Terminalia sericea and Gunnera perpensa.

2. Materials and methods

2.1. Plant material

Terminalia sericea (N.H. 1878) and Capparis tomentosa (N.H. 1882) are lodged at the Southpanberrgenis herbarium, Loui Trichardt. Gunnera perpensa was collected and identified at the Research Centre for Growth and Development (Pietermaritzburg, Kwazulu-Natal). Pterocarpus angolensis and Leontis leonorus were collected at the National Botanical Institute (NBI) in Pretoria. Discma unonala (Hankey 1653) and Xysema undulatum (Hankey 1654) were collected at the NBI in Johannesburg. Helichrysum foetidum was collected at the NBI in Bettiesbaai and Urtica urens was purchased from a healthshop. Voucher materials from the plants collected in the botanical institutes are deposited in the respective herbariums.

2.2. Preparation of extracts

Different parts of the plants were dried and ground to a fine powder (Table 1). One gram of plant material was added to 10 ml of deionised water and boiled for 15 min. The extracts were allowed to cool, centrifuged and the supernatants filtered through 0.22 µm filters. Methanol extracts of the remedies were prepared by adding 1 g plant material to 10 ml methanol and macerated for 24 h at room temperature. Yields (w/w) were determined by weighing sterile glass Petri-dishes, placing 2 ml of the filtered supernatant into the dish, drying at 100 °C overnight and re-weighing the dishes (Table 1).

2.3. Antibacterial assay

Antibacterial activity tests were carried out against Staphylococcus aureus (ATCC 25923), Streptococcus pneumoniae (clinical isolate; identified and obtained from the Department of Microbiology, Faculty of Health Sciences, University of Pretoria), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27893).

Inocula of the microorganisms were prepared from 24 h Mueller–Hinton broth (Davies Diagnostics) cultures and suspensions were adjusted to a 0.4 reading using a standard radiometric method (Lee and Hiefets, 1987) and a colorimeter (Sherwood). Methanol extracts were dried and reconstituted in distilled water. Minimal inhibition concentration (MIC) values of the extracts were determined based on a micro-well dilution method (Eloff, 1998a).

The 96-well sterile plates were prepared by dispensing 180 µl of the inoculated broth plus a 20-µl aliquot of the plant extract made up in broth or 20 µl broth in the case of the negative control into each well. Phenol (Merck) was included as positive control. Plates were covered and incubated for 24 h at 37 °C. Bacterial growth was determined after addition of 50 µl p-sodium tetrazolium violet (0.2 mg/ml, Sigma).

2.4. In vitro test for fibroblast growth stimulation

Semi-continuous human fibroblasts (MRC5, ATCC CCL-171) were grown as a mono-layer culture at 37 °C in 5% CO2 in minimal essential medium supplemented with 10% heat inactivated foetal calf serum.

Cells were seeded into 96-well plates at a density of 2 × 103 cells per well. Plates were incubated for a further 24 h, after which the medium was replaced with 200 µl of Dalbceco’s modified Eagle’s medium/10% fetal calf serum in the presence or absence of different concentrations of the plant extract. The plates were incubated and cell growth determined on after treatment using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) test (Mossmann, 1983).
2.5. Antioxidant activity

Neutrophils were isolated from heparinized venous blood obtained from normal healthy volunteers by density gradient centrifugation on Histopaque-1077 (Sigma diagnostics, St. Louis, MO). Briefly, neutrophils (5 × 10⁶/ml) were incubated at 4°C in lucigenin (1 mM) in phenol red free HBSS and incubated for a further 15 min at 37°C. The use of decoctions of several Terminalia species is widespread in Africa and many species are known to contain antimicrobial constituents and activity (Bhuradajala and Bunchoo, 1995; Sato et al., 1997; Silva et al., 1997; Fyhrquist et al., 2002). Three studies on the antimicrobial activity of Terminalia sericea have been carried out. In one of them, methanol, ethanol, acetone and water extracts of the roots were shown to inhibit Staphylococcus aureus (Fyhrquist et al., 2002). In a second work, acetone leaf extracts inhibited growth of Staphylococcus aureus (MIC 3.0 mg/ml), Escherichia coli (MIC 1.2 mg/ml) and Pseudomonas aeruginosa (MIC 6.0 mg/ml) (Eloff, 1999). In a third report, both the water and methanol extracts of bark had no significant effect on the growth of the fibroblasts up to concentrations of 1 mg/ml (results not shown). However, the water extracts of both plants as well as the methanol extract of Terminalia sericea significantly decreased the lucigenin enhanced chemiluminescence response of FMLP stimulated neutrophils at a concentration of 100 μg/ml and higher (Fig. 1).

4. Discussion and conclusion

The use of decoctions of several Terminalia species is widespread in Africa and many species are known to contain antimicrobial constituents and activity (Bhuradajala and Bunchoo, 1995; Sato et al., 1997; Silva et al., 1997; Fyhrquist et al., 2002). Three studies on the antimicrobial activity of Terminalia sericea have been carried out. In one of them, methanol, ethanol, acetone and water extracts of the roots were shown to inhibit Staphylococcus aureus (Fyhrquist et al., 2002). In a second work, acetone leaf extracts inhibited growth of Staphylococcus aureus (MIC 3.0 mg/ml), Escherichia coli (MIC 1.2 mg/ml) and Pseudomonas aeruginosa (MIC 6.0 mg/ml) (Eloff, 1999). In a third report, both the water and methanol extracts of bark had no significant effect on the growth of the fibroblasts up to concentrations of 1 mg/ml (results not shown). However, the water extracts of both plants as well as the methanol extract of Terminalia sericea significantly decreased the lucigenin enhanced chemiluminescence response of FMLP stimulated neutrophils at a concentration of 100 μg/ml and higher (Fig. 1).
and 0.78 mg/ml against Staphylococcus aureus of the root of disc diffusion assay and found ethanol and water extracts of aureus. Other authors tested antibacterial activity using the cherichia coli, the disc diffusion assay against Escherichia coli, which is lower than the 3.0 mg/ml reported by Eloff (1999) against coccus aureus. These results are in contradiction to that of Eloff (1999). Similar to our findings, Fyhrquist et al. (2002) reported activity with a MIC of 1.2 mg/ml. The MIC of 6.0 mg/ml reported by Eloff (1999) against Staphylococcus aureus, which is lower than the 3.0 mg/ml reported by Elof (1999). Similar to our findings, Fyhrquist et al. (2002) reported no activity against Escherichia coli whereas Elof (1999) reported activity with a MIC of 1.2 mg/ml. The MIC of 6.0 mg/ml reported by Elof (1999) against Pseudomonas aeruginosa was not witnessed by us, since the highest concentration at which the extracts were tested was 4 mg/ml.

McGaw et al. (2000) determined the antibacterial activity of Gunnera perpensa against Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus. Other authors tested antibacterial activity using the disc diffusion assay and found ethanol and water extracts of the root of Gunnera perpensa to have a MIC of 3.13 and 0.78 mg/ml against Staphylococcus aureus, respectively (McGaw et al., 2000). No inhibitory activity was found in the disc diffusion assay against Escherichia coli by the same authors (McGaw et al., 2000). We found the methanol extract of Gunnera perpensa to have a MIC of 2.0 mg/ml, 1 mg/ml and 4 mg/ml against Streptococcus pyogenes, Staphylococcus aureus and Escherichia coli, respectively. The water extracts were less active with a MIC of 4 mg/ml and 2 mg/ml against Streptococcus pyogenes and Staphylococcus aureus, respectively.

Various studies support our results that Urtica urens, Leonotis leonurus and Xymalobium undulatum possess no antibacterial activity (Hutchings et al., 1996; Rabe and van Staden, 1997; Kelmanon et al., 2000). Various species or constituents of the genus Helichrysum have been reported to contain antimicrobial activities (Rin et al., 1991; Meyer and Afolayan, 1995; Sale, 1996; Afolayan and Meyer, 1997; Matleka et al., 2000). However, no antibacterial information is available on Helichrysum foetidum. We detected no activity with both the water and methanol extracts against the microorganisms tested. No reports of antibacterial studies carried out using Capparis tomentosa, Dicomum amonu or Plectusangolens could be obtained from the literature.

Extracts of both Terminalia sericea and Gunnera perpensa showed possible scavenging activity in a concentration dependent manner. Water extracts demonstrated higher activity than the methanol extracts.

Pentacyclic terpenoids have been isolated from Terminalia species (Van Wyk et al., 1997), which are known for their antimicrobial activity (Bruneton, 1995). Various acids as well as venusol has been isolated from the rhizome of Gunnera perpensa (Khan et al., 2004). Although, venusol has been reported to stimulate a contractile response of the ileum and uterus muscles, no other activity has been reported thus far (Khan et al., 2004).

The antibacterial effect of the crude methanol extracts and to a lesser extent, the water extracts of Terminalia sericea and Gunnera perpensa against the mentioned pathogens confirms the evidence of previous studies reporting that methanol is a more consistent extraction solvent of antimicrobial substances from medicinal plants compared to ethanol, hexane and water (Eloff, 1998b; Lin et al., 1999; Karaman et al., 2003). As Staphyllococcus aureus is one of the most persistent infectious microorganisms (Baird-Parker, 1972; Kang and Moon, 1990) and commonly found in nosocomial infections (Hugo and Russell, 1995), the methanol extracts of Terminalia sericea and Gunnera perpensa could be advantageous for the treatment of such wound infections.

Acknowledgements

We wish to thank the Department of Microbiology, University of Pretoria for providing us with the bacteria. This work was supported by a Research and Development grant of the University of Pretoria.

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