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

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Abstract

Aqueous and methanol extracts of *Urtica urens*, *Capparis tomentosa*, *Dicoma anomala*, *Leonotis leonorus*, *Xysmalobium undulatum*, *Helichrysum foetidum*, *Pterocarpus 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 luciginin enhanced chemiluminescence at concentrations of 100 μg/ml and higher. However, the extracts had no effect on the growth of primary human fibroblasts.

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; Grierson and Afolayan, 1999; Kelmanson 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 *Pterocarpus 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) *Dicoma anomala* Sond. (Asteraceae) root is applied as a paste to sores and

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wounds (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996), (vii) powdered tuber of *Xysmalobium 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 wounds (Hutchings et al., 1996) and (ix) infusions of *Urtica urens* L. (Urticaceae) is applied externally to burns (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 Soutpansbergensis herbarium, Loius Trichardt. *Gunnera perpensa* was collected and identified at the Research Centre for Growth and Development (Pietermaritzburg, KwaZulu-Natal). *Pterocarpus angolensis* and *Leonotis leonorus* were collected at the National Botanical Institute (NBI) in Pretoria. *Dicoma anomala* (Hankey 1653) and *Xysmalobium 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 pyogenes* (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*-iodonitrotetrazolium 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% CO_2 in minimal essential medium supplemented with 10% heat inactivated foetal calf serum.

Cells were seeded into 96-well plates at a density of 2×10^5 cells per well. Plates were incubated for a further 24 h, after which the medium was replaced with 200 μl of Dulbecco'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).

Table 1
Scientific and trivial names of the plants investigated, plant part used, extraction solvent and w/w yields in terms of dry starting material

Scientific name	Plant part	Vernacular name		Solvent and % (w/w) yield	
		Zulu	English	MeOH	H_2O
Capparis tomentosa Lam.	Root	Umqoqolo	Woolly caper-bush	0.55	2.72
Dicoma anomala Sond.	Root	Umuna	Wormbos	0.53	2.95
Gunnera perpensa L.	Root	Uxobo	River pumpkin	1.05	1.99
Helichrysum foetidum (L.) Moench	Leaves	Isicwe	Stinking starflower	0.71	0.84
Leonotis leonurus (L.) R. Br.	Stems/leaves	Imunyamunya	Wild dagga	0.42	1.60
Pterocarpus angolensis DC.	Seeds	Umbilo	African teak	0.06	0.17
Terminalia sericea Burch. ex DC.	Bark	Amangwe	Silver cluster-leaf	3.30	2.41
Urtica urens L.	Whole plant	Imbati	Small stinging nettle	0.38	0.77
Xysmalobium undulatum (L.) Ait. f.	Tuber	Ishongwe	Uzara/Milkwort	1.46	2.31

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. Loius, MO). Briefly, 30 ml whole blood was layered into 15 ml Histopaque-1077 and centrifuged at 400 × *g* and 21 °C for 25 min. The neutrophils separated as a band just above the erythrocytes and were carefully removed by aspiration. Any contaminating erythrocytes were selectively lysed by exposure to cold 0.84% ammonium chloride for 10 min before washing the separated neutrophils with HEPES buffered (pH 7.4) Hanks Balanced Salt Solution (HBSS) and adjusting the cell concentration to 1×10^7 cells/ml. Cell purity was generally greater than 90% and cell viability better than 95%.

Oxidant generation by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated neutrophils was measured using lucigenin-dependant chemiluminescence as described by Allen (1986). Briefly, neutrophils (5×10^6/ml) were incubated at 4 °C in luciginin (1 mM) in phenol red free HEPES buffered HBSS for 30 min. Of this suspension, 200 μl was added to 100 μl of varying concentrations of plant extract in 600 μl HBSS and incubated for a further 15 min at 37 °C in a chemiluminometer equipped with an automatic dispensing system. Activation of the neutrophils was initiated by introduction of 100 μl FMLP (1 μM). The rate of oxidant production was monitored for 125 s.

3. Results

The methanol and water extracts of *Dicoma anomala*, *Helichrysum foetidum*, *Leonotis leonurus*, *Urtica urens*, *Pterocarpus angolensis* and *Xysmalobium undulatum* presented MICs >4 mg/ml against all the selected bacteria. The water extract of *Capparis tomentosa* showed activity of 4 mg/ml against *Staphylococcus aureus* and 1 mg/ml against *Streptococcus pyogenes*, whereas the methanol extract was active against *Streptococcus pyogenes* at 4 mg/ml. The methanol extract of *Gunnera perpensa* presented MICs of 1 mg/ml, 2 mg/ml and 4 mg/ml against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*, respectively. In the case of *Pseudomonas aeruginosa*, the MIC was >4 mg/ml. The water extract of this plant showed activity only against the Gram-positive bacteria tested: 4 mg/ml for *Staphylococcus aureus* and 1 mg/ml for *Streptococcus pyogenes*. Both the water and methanol extracts of *Terminalia sericea* had MIC values of 1 mg/ml against *Staphylococcus aureus* and *Streptococcus pyogenes*. No activity was present against the Gram-negative bacteria up to concentrations of 4 mg/ml for this plant extract. Tests could not be carried out using the water extracts of *Pterocarpus angolensis* due to the low yield. Phenol, which forms the base of many topically applied antiseptics, was included as positive control. Phenol presented MICs of 1 mg/ml, 1 mg/ml and 2 mg/ml against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Since *Terminalia sericea* and *Gunnera perpensa* were the most active plants, further tests were carried out using extracts of these two specimens only.

Both plant extracts 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 luciginin 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 (Burapadaja and Bunchoo, 1995; Sato et al., 1997; Silva et al., 1997; Fyhrquist et al., 2002; Gupta et al., 2002; Khan et al., 2002; Pawar and Pal, 2002; Suguna 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

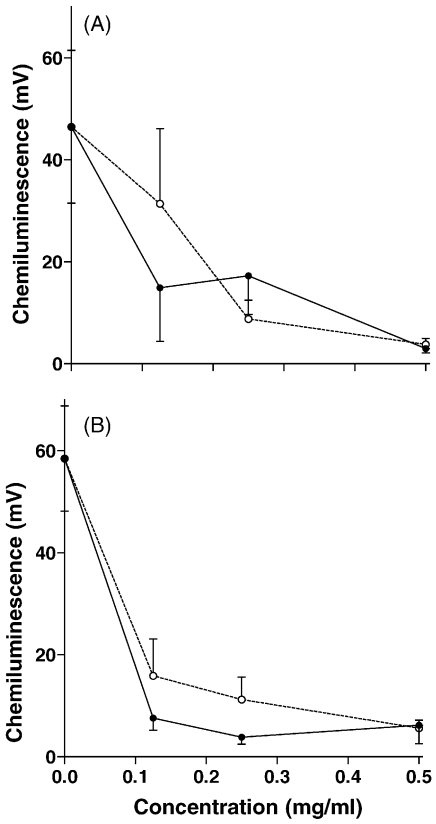

Fig. 1. Effects obtained for (A) methanol and (B) water extracts of *Terminalia sericea* (●) and *Gunnera perpensa* (◯) on luciginin enhanced chemiluminescence response of FMLP stimulated neutrophils.

effect on *Staphylococcus aureus* (Rabe and van Staden, 1997). In our study, both methanol and water extracts of the bark inhibited growth of *Staphylococcus aureus* and *Streptococcus pyogenes*. These results are in contradiction to that of Rabe and van Staden (1997) who also used bark as well as the same solvents but, however, they reported no inhibitory activity. Furthermore, our MIC was 1 mg/ml against *Staphylococcus aureus*, which is lower than the 3.0 mg/ml reported by Eloff (1999). Similar to our findings, Fyhrquist et al. (2002) reported no activity against *Escherichia coli* whereas Eloff (1999) reported activity with a MIC of 1.2 mg/ml. The MIC of 6.0 mg/ml reported by Eloff (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 *Xysmalobium undulatum* possess no antibacterial activity (Hutchings et al., 1996; Rabe and van Staden, 1997; Kelmanson et al., 2000). Various species or constituents of the genus *Helichrysum* have been reported to contain antimicrobial activities (Rios et al., 1991; Meyer and Afolayan, 1995; Salie et al., 1996; Afolayan and Meyer, 1997; Mathekga 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*, *Dicoma anomala* or *Pterocarpus angolensis* could be obtained from the literature.

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

Pentacyclic triterpenoids 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 *Staphylococcus 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.

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References

Afolayan, A.J., Meyer, J.J., 1997. The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. Journal of Ethnopharmacology 57, 177–181.

Allen, R.C., 1986. Phagocytic leukocyte oxygenation activities and chemiluminescence: a kinetic approach to analysis. Methods in Enzymology 133, 449–493.

Baird-Parker, A.S., 1972. Classification and Identification of Staphylococci and Their Resistance to Physical Agents. Wiley, New York.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Intercept, Hampshire.

Burapadaja, S., Bunchoo, A., 1995. Antimicrobial activity of tannins from *Terminalia citrina*. Planta Medica 61, 365–366.

Eloff, J.N., 1998a. A sensitive and quick method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–713.

Eloff, J.N., 1998b. Which extract should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.

Eloff, J.N., 1999. The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95, 148–152.

Fyhrquist, P., Mwasumbi, L., Haeggstrom, C.A., Vuorela, H., Hiltunen, R., Vuorela, P., 2002. Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. Journal of Ethnopharmacology 79, 169–177.

Grierson, D.S., Afolayan, A.J., 1999. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. Journal of Ethnopharmacology 66, 103–106.

Grinnell, F., Zhu, M.F., 1994. Identification of neutrophil elastase as the proteinase in burn wound fluid responsible for degradation of fibronectin. Journal of Investigative Dermatology 103, 155–161.

Gupta, M., Mazumder, U.K., Manikandan, L., Bhattacharya, S., Haldar, P.K., Roy, S., 2002. Antibacterial activity of *Terminalia pallida*. Fitoterapia 73, 165–167.

Hugo, W.B., Russell, A.D., 1995. Pharmaceutical Microbiology. Blackwell Science 132, 391–402.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.

Kang, J.S., Moon, K.H., 1990. Antibiotic resistance in *Staphylococcus aureus* isolated in Pusan. Yakhak-Hoeji 34, 122–125.

Karaman, I., Sahin, F., Gulluce, M., Oguutcu, H., Sengul, M., Adiguzel, A., 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. Journal of Ethnopharmacology 85, 231–235.

Kelmanson, J.E., Jager, A.K., Staden, J., 2000. Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacology 69, 241–246.

Khan, M.R., Kihara, M., Omoloso, A.D., 2002. Antimicrobial activity of *Terminalia complanata* and *Flacourtia zippelii*. Fitoterapia 73, 737–740.

Khan, F., Peter, X.K., Mackenzie, R.M., Katsoulis, L., Gehring, R., Munro, O.Q., van Heerden, F.R., Drewes, S.E., 2004. Venusol from *Gunnera perpensa*: structural and activity studies. Phytochemistry 65, 1117–1121.

Lee, C., Hiefets, L., 1987. Determination of minimal inhibitory concentrations of anti-tuberculous drugs by radiometric and conventional methods. American Review of Respiratory Disease 136, 349–352.

Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager, A.K., van Staden, J., 1999. Preliminary screening of some Zulu medicinal plants for anti-inflammatory and anti-microbial activities. Journal of Ethnopharmacology 68, 267–274.

Mabogo, D.E.N., 1990. The ethnobotany of the Vhavenda. M.Sc. thesis, University of Pretoria.

Mathekga, A.D., Meyer, J.J., Horn, M.M., Drewes, S.E., 2000. An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. Phytochemistry 53, 93–96.

McGaw, L.J., Jager, A.K., van Staden, J., 2000. Antibacterial anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72, 247–263.

Mensah, A.Y., Sampson, J., Houghton, P.J., Hylands, P.J., Westbrook, J., Dunn, M., Hughes, M.A., Cherry, G.W., 2001. Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. Journal of Ethnopharmacology 77, 219–226.

Meyer, J.J.M., Afolayan, A.J., 1995. Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). Journal of Ethnopharmacology 47, 109–111.

Mossmann, T., 1983. Rapid colourimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65, 55–63.

Pawar, S.P., Pal, S.C., 2002. Antimicrobial activity of extracts of *Terminalia catappa* root. Indian Journal of Medical Science 56, 276–278.

Phan, T.T., Hughes, M.A., Cherry, G.W., 1998. Enhanced proliferation of fibroblasts and endothelial cells treated with an extract of the leaves of *Chromolaena odorata* (Eupolin), a herbal remedy for treating wounds. Plastic and Reconstructive Surgery 101, 756–765.

Phan, T.T., See, P., Lee, S.T., Chan, S.Y., 2001a. Protective effects of curcumin against oxidative damage on skin cells in vitro: its implication for wound healing. The Journal of Trauma 51, 927–931.

Phan, T.T., Wang, L., See, P., Grayer, R.J., Chan, S.Y., Lee, S.T., 2001b. Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biology and Pharmacy Bulletin 24, 1373–1379.

Priya, K.S., Gnanamani, A., Radhakrishnan, N., Babu, M., 2002. Healing potential of *Datura alba* on burn wounds in albino rats. Journal of Ethnopharmacology 83, 193–199.

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56, 81–87.

Rios, J.L., Recio, M.C., Villar, A., 1991. Isolation and identification of the antibacterial compounds from *Helichrysum stoechas*. Journal of Ethnopharmacology 33, 51–55.

Salie, F., Eagles, P.F., Leng, H.M., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27–33.

Sato, Y., Oketani, H., Singyouchi, K., Ohtsubo, T., Kihara, M., Shibata, H., Higuti, T., 1997. Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* RETS against methicillin-resistant *Staphylococcus aureus*. Biological and Pharmaceutical Bulletin 20, 401–404.

Shirwaikar, A., Somashekar, A.P., Udupa, A.L., Udupa, S.L., Somashekar, S., 2003. Wound healing studies of *Aristolochia bracteolate* Lam. with supportive action of antioxidant enzymes. Phytomedicine 10, 558–562.

Silva, O., Duarte, A., Pimentel, M., Viegas, S., Barroso, H., Machado, J., Pires, I., Cabrita, J., Gomes, E., 1997. Antimicrobial activity of *Terminalia macroptera* root. Journal of Ethnopharmacology 57, 203–207.

Stevenson, P.C., Simmonds, M.S., Sampson, J., Houghton, P.J., Grice, P., 2002. Wound healing activity of acylated iridoid glycosides from *Scrophularia nodosa*. Phytotherapy Research 16, 33–35.

Suguna, L., Singh, S., Sivakumar, P., Sampath, P., Chandrakasan, G., 2002. Influence of Terminalia chebula on dermal wound healing in rats. Phytotherapy Research 16, 227–231.

Thang, P.T., Patrick, S., Teik, L.S., Yung, C.S., 2001. Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. Burns 27, 319–327.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, second ed. Livingstone, London.