



## Is the use of *Gunnera perpensa* extracts in endometritis related to antibacterial activity?

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### ABSTRACT

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Rhizome extracts of *Gunnera perpensa* are used in traditional remedies in South Africa to treat endometritis both in humans and animals. An investigation was undertaken to determine whether this plant possesses antibacterial activity, which may explain its efficacy. *Gunnera perpensa* rhizome extracts were prepared serially with solvents of increasing polarity and tested for antibacterial activity. Test bacteria included the Gram-positive *Enterococcus faecalis* and *Staphylococcus aureus* and the Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. A moderate to weak level of antibacterial activity in most of the extracts resulted, with the best minimal inhibitory concentration (MIC) value of 2.61 mg mL<sup>-1</sup> shown by the acetone extract against *S. aureus*. The extracts were also submitted to the brine shrimp assay to detect possible toxic or pharmacological effects. All the extracts were lethal to the brine shrimp larvae at a concentration of 5 mg mL<sup>-1</sup>. The acetone extract was extremely toxic at 1 mg mL<sup>-1</sup>, with some toxicity evident at 0.1 mg mL<sup>-1</sup>. The remainder of the extracts generally displayed little activity at concentrations lower than 5 mg mL<sup>-1</sup>. In summary, the results indicate that although the extracts demonstrated a level of pharmacological activity, the relatively weak antibacterial activity is unlikely to justify the use of *G. perpensa* rhizomes in the traditional treatment of endometritis. Rather, the slightly antibacterial nature of the rhizomes may contribute to an additive effect, along with their known uterotonic activity, to the overall efficacy of the preparation.

**Keywords:** Antibacterial, brine shrimp, endometritis, ethnoveterinary, *Gunnera perpensa*, traditional medicine

### INTRODUCTION

*Gunnera* is the only genus of the family Gunneraceae, which includes 30–40 herbaceous species, mostly distributed in the southern hemisphere (Wanntorp, Wanntorp, Oxelman & Källersjö 2001).

The greatest diversity is found in South and Central America. A few species are also found in Hawaii, New Zealand, Tasmania, Africa and the Malay Archipelago (Wanntorp *et al.* 2001). *Gunnera perpensa* L. is widely distributed in the southern, eastern and northern parts of South Africa and northwards into tropical Africa (Van Wyk, Van Oudtshoorn & Gericke 1997). It is a vigorous perennial herb that is restricted to moist habitats and grows in marshy areas and along river banks. It has large, round, pumpkin-like leaves and fleshy, pink tuberous rhizomes (Van Wyk & Gericke 2000). The plant is known in South Africa by various names including ugobho (Zulu), qobo (Sotho), rambola-vhadzimu (Venda), river pumpkin (English) and rivierpampoep (Afrikaans) (Van Wyk *et al.* 1997).

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Infusions or decoctions of the rhizome of *G. perpensa* are used by black South African women to induce or augment labour and as a postnatal medication to expedite the expulsion of the after-birth (Hutchings, Scott, Lewis & Cunningham 1996; Kaido, Veale, Havlik & Rama 1997; Van Wyk *et al.* 1997; Van Wyk & Gericke 2000). Decoctions are also administered to animals by the Zulu and southern Sotho to assist the expulsion of the placenta (Watt & Breyer-Brandwijk 1962). According to anecdotal reports, this herbal remedy is used to treat cattle by subsistence farmers and also by commercial farmers of the Underberg region of KwaZulu-Natal, South Africa to assist in the expulsion of fetal membranes, and in the treatment and prevention of endometritis (T. Collins, unpublished data 2002).

Conventional treatment of retained foetal membranes and endometritis in cattle includes manual removal of the membranes, stimulation of uterine contraction and stimulation of uterine defences as well as topical and systemic antibacterial therapy (Kudlác 1991). The use of *G. perpensa* for treating humans and animals may therefore be due to antibacterial effects. In a preliminary screening, McGaw, Jäger & Van Staden (2000) reported antibacterial activity of the aqueous rhizome extract against one bacterial pathogen, the Gram-positive *Staphylococcus aureus*. However, no activity was demonstrated against *Bacillus subtilis* (Gram-positive) or the Gram-negative bacteria *E. coli* and *Klebsiella pneumoniae*.

The chemistry of *Gunnera* species appears not to have been thoroughly studied, although Watt & Breyer-Brandwijk (1962) mention the presence of a bitter principle, celastrin, in *G. perpensa*. Doyle & Scogin (1988) detected caffeic acid, ellagic acid, ellagitannins and quercetin in *Gunnera densiflora* and *Gunnera manicata*. Alkaloids, iridoids, proanthocyanidins, saponins and sedoheptulose were not present in these species (Doyle & Scogin 1988). The compounds present in these two *Gunnera* species that have antibacterial activity, are caffeic acid (Thiem & Goślińska 2004), ellagic acid (Ohemeng, Schwender, Fu & Barrett 1993), ellagitannins (Nishizawa, Nakata, Kishida, Ayer & Browne 1990) and quercetin (Basile, Sorbo, Giordano, Ricciardi, Ferrara, Montesano, Castaldo Cobianchi, Vuotto & Ferrara 2000).

Regarding pharmacological activity, Kaido *et al.* (1997) reported on the uterotonic activity of aqueous root decoctions of *G. perpensa*. Joseph & Mafatle (1994) considered the effects of intraperitoneal administration of the aqueous extracts of rhizomes of the plant on the maintenance of pregnancy and

parturition in rats, and discovered that the extract had a significant deleterious effect on foetuses. The authors postulated that the presence of celastrin or an unknown chemical in the extract was sufficient to kill the unborn rats, but was not enough to kill the adults (Joseph & Mafatle 1994). Antithrombin activity has been detailed in a related species, *Gunnera tinctoria* (De Medeiros, Macedo, Contancia, Nguyen, Cunningham & Miles 2000). Recently, Khan, Peter, MacKenzie, Katsoulis, Gehring, Munro, Van Heerden & Drewes (2004) isolated a major constituent, Z-venusol, along with the minor components pyrogallol, succinic acid, lactic acid, and the trimethyl ether of ellagic acid glucoside from the aqueous extract of *G. perpensa* rhizomes. These authors demonstrated the stimulation of a direct contractile response of isolated rat uterus by the aqueous rhizome extract, which also induced continuous uterine contractility after removal of all additives from the organ bath (Khan *et al.* 2004). Interestingly, venusol did not trigger the contractile response but induced the state of continuous contractility after flushing of the organ bath (Khan *et al.* 2004).

Because endometritis is an important economic disease in stock farming, and resource-poor farmers frequently do not have access to orthodox veterinary medicine, we examined the biological activity of *G. perpensa* in more depth as part of an ethnoveterinary medicine project.

## MATERIALS AND METHODS

### Collection of plant material

*Gunnera perpensa* L. rhizomes were collected in May 2002 from the Underberg region of KwaZulu-Natal. They were washed, air-dried and cut into small pieces of c. 1 cm<sup>3</sup> using an electric band saw. The material was then milled in a rotomill (Macslab) and passed through a 1 mm mesh.

### Preparation of extracts

The dried powder (35.7 g) was extracted by vigorous shaking for 15 min with 150 ml hexane. The extract was removed by vacuum filtration through Whatman No. 1 filter paper using a Buchner funnel. The extraction process was repeated on the marc until the extract had little colour. Extraction was continued on the marc in a serial manner using dichloromethane, acetone, methanol and 20 % ethanol in water (ethanol/water). The extracts were subsequently dried using a rotary vacuum evaporator (Büchi) at 40 °C.

## Antibacterial screening

The dried plant extracts were dissolved to a known concentration. The acetone and ethanol/water extracts were dissolved in acetone and water respectively, while the remainder of the extracts were suspended in a small volume of dimethyl sulfoxide (DMSO) and made up to the required concentration with water.

The test organisms in the investigation of antibacterial activity 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 specific strains used are recommended for antibacterial activity testing by the National Committee for Clinical Laboratory Standards (NCCLS 1990). These bacteria are responsible for most nosocomial diseases in hospitals (Sacho & Schoub 1993). The tetrazolium violet serial dilution microplate method of Eloff (1998) was used to evaluate the antibacterial activity of the extracts. This method allows the calculation of minimal inhibitory concentration (MIC) values for active plant extracts against bacterial species.

Freshly inoculated bacterial cultures in Müller-Hinton (MH) broth were incubated overnight at 37 °C. The cultures were diluted (1:100) with MH broth before use in the assay. A twofold serial dilution of plant extract (100 µl) was prepared in 96-well microtitre plates, and 100 µl bacterial culture was added to each well. The presence of bacterial growth was detected by adding to each well 40 µl *p*-iodonitro-tetrazolium violet (INT, 0.2 mg ml<sup>-1</sup>) that is reduced to a red-coloured formazan by respiring bacteria. When there was a substantial decrease in colour 1 hour after addition of the INT, the concentration in that well was taken to be the MIC of the plant extract against the particular bacterial strain. The antibiotic neomycin was included as a standard in each assay, and appropriate negative solvent controls were included.

The direct thin-layer chromatography (TLC) bioautography assay (Hamburger & Cordell 1987) was employed to determine the number of antibacterial compounds and their R<sub>f</sub> values in the *Gunnera* extracts. In this assay, bacterial cultures were incubated in MH broth overnight at 37 °C, centrifuged, and the resulting pellet resuspended in fresh MH broth. The bacteria were sprayed onto a thoroughly dried TLC chromatogram and incubated overnight at 37 °C. A solution of INT (2 mg ml<sup>-1</sup>) was sprayed

on the plate and incubated again at 37 °C for approximately an hour. White spots appearing on a pink background of bacterial growth indicated the presence of antibacterial compounds on the chromatogram.

## Screening for toxicity

The possible toxicity of the extracts was investigated using the brine shrimp (*Artemia salina*) microwell cytotoxicity bioassay (Solís, Wright, Anderson, Gupta & Phillipson 1993). Brine shrimp eggs were obtained from a local pet shop and hatched in artificial seawater (3.8 g NaCl per 100 ml distilled H<sub>2</sub>O). After 48 h, the phototropic nauplii (first stage larvae) were collected using a Pasteur pipette. The plant extracts were diluted to concentrations of 0.1, 1, 5 and 10 mg ml<sup>-1</sup> with the artificial seawater. For each concentration, the plant extract solution was placed in two replicate wells of a 96-well microtitre plate. A suspension of nauplii (100 µl, containing approximately 10–15 nauplii) was added to each well. The microplate was covered and incubated for 24 h at room temperature. The number of dead and live nauplii in each well was counted using a dissecting microscope. If control deaths occurred, the percent death values were corrected using Abbott's formula:

$$\text{Corrected mortality percentage} = \frac{m - M \times 100}{S}$$

m = mean percentage of dead larvae in treated tubes

M = mean percentage of dead larvae in controls

S = mean percentage of living organisms in controls

Podophyllotoxin (Sigma) at a concentration of 5 µg ml<sup>-1</sup> was used as a positive control and solvent blanks were included in each assay.

## RESULTS AND DISCUSSION

### Antibacterial activity

The MIC values for the different bacterial species varied from 2.6–12.5 mg ml<sup>-1</sup> (Table 1). The MIC value was higher than 12.5 mg ml<sup>-1</sup> for all the extracts on *P. aeruginosa* and for the hexane extract on *E. coli*.

McGaw *et al.* (2000) reported that ethanol and water extracts of *G. perpensa* displayed antibacterial activity against *S. aureus*, with MIC values of 3.13 and 0.78 mg ml<sup>-1</sup>, respectively. These extracts

TABLE 1 Average MIC values for *G. perpensa* extracts (serial extraction)

Extracting solvent	mg extracted from 1 g	<i>Escherichia coli</i>		<i>Enterococcus faecalis</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>	
		MIC <sup>a</sup> mg ml <sup>-1</sup>	Total activity ml g <sup>-1</sup>	MIC mg ml <sup>-1</sup>	Total activity ml g <sup>-1</sup>	MIC mg ml <sup>-1</sup>	Total activity ml g <sup>-1</sup>	MIC mg ml <sup>-1</sup>	Total activity ml g <sup>-1</sup>
Hexane	1.46	– <sup>b</sup>	–	12.5	0.12	–	–	6.25	0.23
Dichloromethane	3.78	12.5	0.30	5.21	0.73	–	–	6.25	0.60
Acetone	69.38	12.5	5.55	4.17	16.64	–	–	2.61	26.58
Methanol	54.63	6.25	8.74	3.13	17.45	–	–	3.13	17.45
Ethanol/water (20 %)	21.50	–	–	–	–	–	–	–	–
Neomycin (x 10 <sup>-3</sup> )		1.56		6.25		25		0.78	

<sup>a</sup> MIC is taken as last concentration of plant extract to completely inhibit bacterial growth (= MLC, minimum lethal concentration)

<sup>b</sup> MIC > 12.5 mg ml<sup>-1</sup>

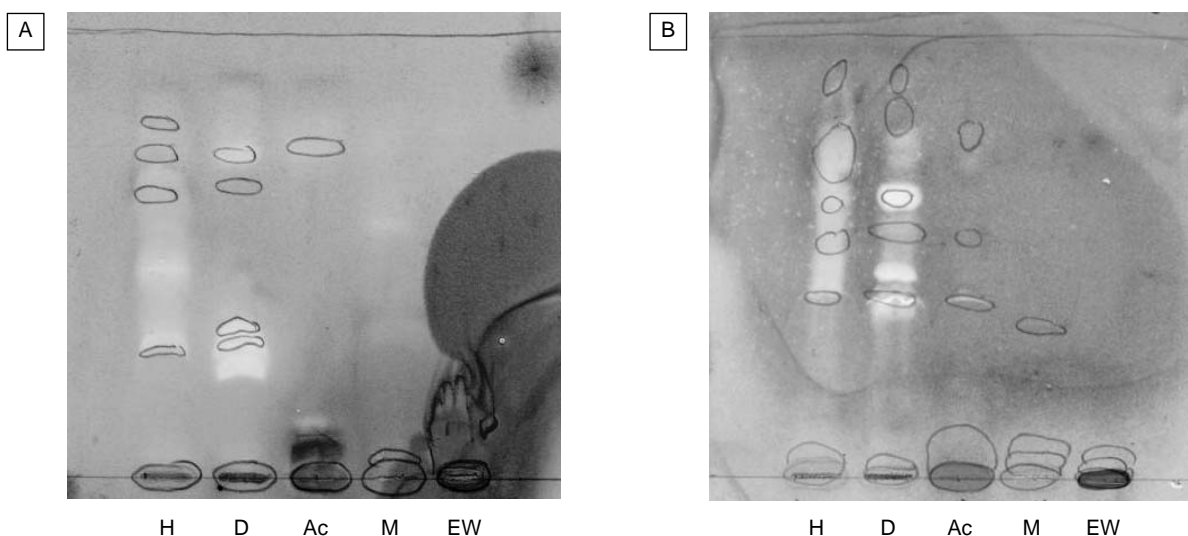


FIG. 1 *Gunnera perpensa* extracts separated on a TLC plate

A: solvent system BEA (benzene:ethyl acetate:ammonia = 18:2:0.2) and sprayed with *E. coli*. B: solvent system CEF (chloroform:ethyl acetate:formic acid = 10:8:2) and sprayed with *S. aureus*. Extracts: H = hexane, D = dichloromethane, Ac = acetone, M = methanol, EW = ethanol/water (20 %)

were prepared using separate aliquots of plant material, in contrast to the present investigation where the extracts were prepared serially with the same dried, ground plant material. The first extracting solvent used was hexane followed by the other solvents with increasing polarity on the same plant material. This may partially explain the absence of activity in the 20 % ethanol extract as the preceding solvents possibly removed some of the antibacterial compounds. The extract prepared using acetone had the highest antibacterial activity against the Gram-positive bacteria. Gram-positive species are generally more susceptible than Gram-negative species to plant extracts (Vlietinck, Van Hoof, Totté, Lasure, Van den Berghe, Rwangabo & Mvukiyu-

mwami 1995), and this was also true in our experiments. According to the MIC level of 8 mg ml<sup>-1</sup> to denote antibacterial activity of plant extracts suggested by Fabry, Okemo & Ansorg (1998) most extracts were inactive or had borderline antibacterial activity.

The magnitude of the antibacterial activity depends not only on the MIC value but also on the total quantity of the specific extract obtained from the plant. The total activity can be calculated from the quantity in mg extracted from 1 g divided by the MIC in mg ml<sup>-1</sup> (Eloff 2000). The resultant value reflects the volume in ml to which the antibacterial compound can be diluted and still inhibit the growth of

TABLE 2 Percentage mortality of *Gunnera perperensa* extracts against *Artemia salina* (brine shrimp) larvae

Extract	Percentage mortality at various concentrations of extract			
	0.1 mg mL <sup>-1</sup>	1 mg mL <sup>-1</sup>	5 mg mL <sup>-1</sup>	10 mg mL <sup>-1</sup>
Hexane	0	0	14	24
Dichloromethane	0	0	92	100
Acetone	24	100	100	100
Methanol	12	69	100	100
Ethanol/water	17	36	100	100

the test organism. The highest total activity value of 26.6 mL g<sup>-1</sup> for the acetone extract on *S. aureus* compares poorly with values of 182 found for *Combrethum erythrophyllum* extracts on *S. aureus* and *E. coli* (Eloff 1999) and of values higher than 258 mL g<sup>-1</sup> for *Cassine peragua* var. *peragua* (Eloff 2000).

The bioautography plates are depicted in Fig. 1.

The serial extraction led to some fractionation of the antibacterial compounds in *G. perperensa* (Fig. 1). The presence of antibacterial compounds on the bioautogram with activity against *E. coli* and *S. aureus* supports the results obtained in the microtitre plate assay, with the hexane, dichloromethane and acetone extracts showing antibacterial activity. One compound with major activity (dichloromethane extract: R<sub>f</sub> = 0.28) and at least three compounds with minor activity (dichloromethane extract: R<sub>f</sub> = 0.65; hexane extract: R<sub>f</sub> = 0.31, R<sub>f</sub> = 0.48) against *E. coli* are present. The growth of *S. aureus* was inhibited strongly by at least four compounds (dichloromethane extract: R<sub>f</sub> = 0.32, R<sub>f</sub> = 0.46, R<sub>f</sub> = 0.63, R<sub>f</sub> = 0.77). In all cases the active compounds were relatively non-polar. It has been shown that *G. densiflora* and *G. manicata* contain caffeic acid, ellagic acid, ellagitannins and quercetin (Doyle & Scogin 1988), all of which are known to have antibacterial activity. If a chemotaxonomic relationship exists between *G. perperensa* and these two related species, it is possible for similar compounds to be responsible for the antibacterial activity of *G. perperensa*.

### Brine shrimp assay

The bioassay against brine shrimp larvae (nauplii) has been employed to test for the presence of toxic substances, and also as a means of facilitating the isolation of biologically active substances (MacLaughlin 1991, cited by Solís *et al.* 1993). This assay can be used to evaluate plants for pharmacological activity, taking into account the principle that pharmacology is merely toxicology at a lower dose (Vlie-

tinck & Apers 2001). All of the *Gunnera* extracts studied were toxic at a concentration of 10 mg mL<sup>-1</sup> (Table 2). At 1 and 0.1 mg mL<sup>-1</sup>, the hexane and dichloromethane extracts had no effect on the nauplii. This indicates that highly non-polar compounds from *G. perperensa* rhizomes are not toxic to brine shrimp nauplii. The acetone extract appeared to be the most toxic, as at a concentration of 1 mg mL<sup>-1</sup>, no nauplii survived. In relation to results obtained for other plant extracts, the *G. perperensa* extracts are not regarded as being highly toxic. For instance, Wanyoike, Chhabra, Lang'at-Thoruwa & Omar (2004) reported toxic effects against brine shrimp larvae by a selection of Kenyan medicinal plant extracts at concentrations of less than 0.03 mg mL<sup>-1</sup>.

Further tests should be performed to evaluate the potential mammalian toxicity of *G. perperensa* extracts, since toxicity against brine shrimp larvae is merely a preliminary indication of the likely presence of toxic compounds in an extract. Nevertheless, the toxic level for brine shrimp larvae is probably considerably higher than the therapeutic dose used on animals.

### CONCLUSIONS

*Gunnera perperensa* contains several compounds with antibacterial activity against *E. coli* and *S. aureus*. Based on the MIC values and total activity, it appears unlikely that the purported efficacy of *G. perperensa* extracts could be due solely to an antibacterial effect. The total antibacterial activity of the extracts was substantially lower than values found for many other plants. If one considers that aqueous extracts are used by subsistence level farmers and that the bioactive compounds according to the bioautography results were all relatively non-polar compounds this also indicates that efficacy can probably not be ascribed to antibacterial effects of the plant extract. On the other hand McGaw *et al.* (2000) found that water extracts had a higher activity than ethanol extracts and thus in this study it may be that first extracting plant material with non-

polar extractants may have inhibited the solubility or activity of antibacterial compounds present in the *G. perpensa* rhizomes. In the serial extraction procedure, acetone and methanol may have already extracted the more polar active compounds in the rhizomes before the ethanol/water extract was prepared.

The application of *Gunnera perpensa* in treating endometritis and related ailments may therefore largely be due to its previously reported uterotonic activity (Kaido *et al.* 1997), rather than its antibacterial activity. This is supported by the brine shrimp assay results, which indicate a level of pharmacological activity present in all the extracts. The mild effect against various bacteria demonstrated in this investigation may, however, contribute to the overall efficacy of the traditional treatment.

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## REFERENCES

- BASILE, A., SORBO, S., GIORDANO, S., RICCIARDI, L., FERRARA, S., MONTESANO, D., CASTALDO COBIANCHI, R., VUOTTO, M.L. & FERRARA, L. 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. *Fitoterapia*, 71 (Supplement 1): S110–S116.
- DE MEDEIROS, J.M.R., MACEDO, M., CONTANCIA, J.P., NGUYEN, C., CUNNINGHAM, G. & MILES, D.H. 2000. Antithrombin activity of medicinal plants of the Azores. *Journal of Ethnopharmacology*, 72:157–165.
- DOYLE, M.F. & SCOGIN, R. 1988. A comparative phytochemical profile of the Gunneraceae. *New Zealand Journal of Botany*, 26:493–496.
- ELOFF, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64:711–713.
- ELOFF, J.N. 1999. The antibacterial activity of 27 southern African members of the Combretaceae. *South African Journal of Science*, 95:148–152.
- ELOFF, J.N. 2000. A proposal on expressing the antibacterial activity of plant extracts—a small first step in applying scientific knowledge to rural primary health care in South Africa. *South African Journal of Science*, 96:116–118.
- FABRY, W., OKEMO, P.O. & ANSORG, R. 1998. Antibacterial activity of East African medicinal plants. *Journal of Ethnopharmacology*, 60:79–84.
- JOSEPH, M.M. & MAFATLE, T.J.P. 1994. Abortifacient effect of *Gunnera perpensa* L. extract in pregnant rats. *South African Journal of Science*, 90:574.
- HAMBURGER, M.O. & CORDELL, G.A. 1987. A direct bioautographic TLC assay for compounds possessing antibacterial activity. *Journal of Natural Products*, 50:19–22.
- HUTCHINGS, A., SCOTT, A.H., LEWIS, G. & CUNNINGHAM, A.B. 1996. *Zulu medicinal plants: an inventory*. Pietermaritzburg: University of Natal Press.
- KAIDO, T.L., VEALE, D.J.H., HAVLIK, I. & RAMA, D.B.K. 1997. Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. *Journal of Ethnopharmacology*, 55:185–191.
- KHAN, F., PETER, X.K., MACKENZIE, R.M., KATSOUKLIS, 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.
- KUDLÁC, E. 1991. Einige Fragen der Therapie und Prophylaxe der Nachgeburtsverhaltung bei Kühen und ihre weitere Fruchtbarkeit. *Tierärztliche Umschau*, 46:407–411.
- MCGAW, L.J., JÄGER, A.K. & VAN STADEN, J. 2000. Antibacterial, anthelmintic and antiamebic activity in South African medicinal plants. *Journal of Ethnopharmacology*, 72:247–263.
- NCCLS. 1990. The National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility tests*, 4th ed. Approved Standard. NCCLS Document M2-A4. Villanova, Pa. USA.
- NISHIZAWA, K., NAKATA, I., KISHIDA, A., AYER, W.A. & BROWNE, L.M. 1990. Some biologically active tannins of *Nuphar variegatum*. *Phytochemistry*, 29:2491–2494.
- OHMENG, K.A., SCHWENDER, C.F., FU, K.P. & BARRETT, J.F. 1993. DNA gyrase inhibitory and antibacterial activity of some flavones (1). *Bioorganic and Medicinal Chemistry Letters*, 3:225–230.
- SACHO, H. & SCHOUB, D.B. 1993. *Current perspectives on nosocomial infections*. Pietermaritzburg: Natal Witness Printing and Publishing.
- SOLÍS, P.N., WRIGHT, C.W., ANDERSON, M.M., GUPTA, M.P. & PHILLIPSON, J.D. 1993. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica*, 59:250–252.
- THIEM, B. & GOŚLIŃSKA, O. 2004. Antimicrobial activity of *Rubus chamaemorus* leaves. *Fitoterapia*, 75:93–95.
- VAN WYK, B.E., VAN OUDTSHOORN, B. & GERICKE, N. 1997. *Medicinal plants of South Africa*. Pretoria: Briza.
- VAN WYK, B.E. & GERICKE, N. 2000. *People's plants: a guide to useful plants of southern Africa*. Pretoria: Briza.
- VLIETINCK, A.J. & APERS, S. 2001. Biological screening methods in the search for pharmacologically active natural products, in *Bioactive compounds from natural sources: isolation, characterization and biological properties*, edited by C. Tringali. London: Taylor & Francis.
- VLIETINCK, A.J., VAN HOOF, L., TOTTÉ, J., LASURE, A., VAN DEN BERGHE, D., RWANGABO, P.C. & MVUKIYUMWAMI, J. 1995. Screening of a hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of Ethnopharmacology*, 46:31–47.
- WANNTORP, L., WANNTORP, H-E., OXELMAN, B. & KÄLLER-SJÖ, M. 2001. Phylogeny of *Gunnera*. *Plant systematics and evolution*, 226:85–107.
- WANYOIKE, G.N., CHHABRA, S.C., LANG'AT-THORUWA, C.C. & OMAR, S.A. 2004. Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants. *Journal of Ethnopharmacology*, 90:129–133.
- WATT, J.M. & BREYER-BRANDWIJK, M.G. 1962. *The medicinal and poisonous plants of southern and eastern Africa*. London: E. S. Livingstone.