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# Free Radical Scavenging and Antibacterial Activity of Crude Extracts from Selected Plants of Medicinal Value Used in Zululand

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**Abstract:** *Ledebouria revoluta*, *Berkheya setifera* and *Carissa bispinosa* are some of the medicinal plants used in South African traditional medicine. An investigation to evaluate antimicrobial, anti-inflammatory and free radical scavenging activities of the root crude extracts and fractions from the three plants was carried out. Reduction by 2, 2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS^{\cdot+}$) and 2, 2-di (4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH$^{\cdot}$) radicals were used to assess antioxidant capacities by change in absorbance in a UV-VIS spectrophotometer. Anti-inflammatory activity of the plant extracts against 15-soybean derived lipoxygenase (15-LOX) enzyme was evaluated as increase in absorbance at 234 nm after addition of soya bean derived 15-LOX, using linoleic acid (134 μM) as substrate. Anti-microbial activities were determined by assessing the plant samples' Minimum Inhibitory Concentrations (MICs) by a micro dilution method. *Carissa bispinosa* and *Berkheya setifera* have shown good to moderate antimicrobial activity. *Ledebouria revoluta* displayed good activities (20 μg/mL) for both acetone and methanol fractions against *E. coli* and good-moderate activity (160-320 μg/mL) against the rest of the bacterial strains used. Anti-fungal activity for *Ledebouria revoluta* methanol fraction (20 μg/mL) is high against *Candida albicans*. Percentage inhibition of 15-soybean lipoxygenase enzyme by the crude extracts at concentration of 25 μg/mL was for *Berkheya setifera*, 80%, *Carissa bispinosa*, 65% and *Ledebouria revoluta*, 40%.

**Key words:** 2, 2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid, $ABTS^{\cdot+}$, 2, 2-di (4-*tert*-octylphenyl)-1-picrylhydrazyl, DPPH$^{\cdot}$, Minimum Inhibitory Concentration, MIC, reactive oxygen species, ROS and reactive nitrogen species RNS, 15-soybean lipoxygenase (15-LOX)

## INTRODUCTION

Identification and characterization of physiologically active constituents from plant extracts leads to the development of new drugs and the information produces a valuable database for creating new formulas. It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use of the plants (Ncube, 2008). Traditionally used medicinal plants have recently attracted the attention of the pharmaceutical and scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor and Van Staden, 2001). Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Osbourne, 1996).

Natural Products contribute significantly as a source for derivation of lead compounds and development of drugs that are introduced into the market. Traditional knowledge applications and its use of plant extracts in medicinal practices provide an excellent database for potential identification of sources that may yield lead compounds with bioactive properties. New pathogens are fast emerging and with some of them being resistant to existent drugs hence the focus to research in medicinal plant extracts. Reserves of herbs and stocks of medicinal plants in developing countries are diminishing and in danger of extinction as a result of growing trade demands for cheaper health care products and new plant based therapeutic markets in preference to more expensive target specific drugs and biopharmaceuticals (Hoareau, 1999).

Although 3-benzyl-4-chromanones(homoisoflavanones) have been isolated from *Ledebouria revoluta* (Moodley *et al*., 2006), more research has to be done on further isolation and identification of the plant's secondary metabolites to validate its use in traditional medicine (Table 1). Reports on the biological activities, isolation and identification of secondary metabolites in *Berkheya setifera* and *Carissa bispinosa* have not yet been made. All the three plants are claimed to be used in treating inflammation and other complications resulting from it. The aim of this study is to scientifically validate the traditional use of these plants in treating infections and

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Table 1: Summary of South African plants used in this study and their traditional medicinal use

| Botanical name and specimen and South African name | Voucher No. | Traditional medicinal use part used and reference |
|---|---|---|
| *Berkheya setifera* (B.s.) D.C. (Asteraceae) (ulwimi lwenkomo isiZulu) | 1342-0 | Leaf and root extracts are used to treat ailments like colds and diarrhea in children. (Traditional healer, Mabandla Village, Umzimkulu, KwaZulu-Natal, South Africa 2009) |
| *Ledebouria revoluta (L.r.)* L.f. Jessop (Hyacinthaceae) Bookhoe(Sesotho) Inqwebebana(Xhosa) | 758-3 | Root and bulb extracts are used to treat sores, wound and skin eruptions. Causes mortality in sheep and cattle. Also used as an expectorant, diuretic and irritant to gastro-intestinal tract (Watt and Breyer-Brandwijk, 1962; Dold and Cocks, 2001; Abegaz, 2002; Moodley, 2006). |
| *Carissa Bispinosa (C.b.)* (L.) Desf. Ex Brenan Umvhusankunzi(isiZulu) big num-num (English) grootnoemnoem (Africans), | 984-2 | Roots are used to treat toothache. Stem and root extract used to stimulate male sex hormone (Traditional healer, Mabandla Village, Umzimkulu, KwaZulu-Natal, South Africa, 2009) |

oxidative stress relating to inflammation. This work reported further biological activities of *Staphylococcus aureus*, ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli*, ATCC25922 and clinically isolated fungal pathogens *Candida albicans* and *Aspergillus fumigatus* and anti-oxidative properties of the plant extracts.

## MATERIALS AND METHODS

**Plant selection and collection:** Ethno botanical survey was conducted in Mabandla village of UMzimkhulu Local Municipality, Kwa-Zulu Natal, South Africa for plants used traditionally for treating infectious and inflammatory diseases. The Plants listed in Table 1 were identified and collected with the aid of the head of traditional healers from the village (Mr. Sanoyi Paulos Dlamini, traditional healer). Authentication of plants was done at South Africa National Biodiversity Institute, Pretoria and their voucher specimens are maintained at Pretoria Botanical Gardens, South Africa.

**Plant treatment:** Plant root and bulb materials were washed, air dried at room temperature for three weeks and ground to powder using a Lasec Polymix PX-MFC 90D crusher. Dried plant pulverized material was stored in glass containers in a cool dry place. Crude extracts were made by shaking plant powder (200 g) in a ratio of 1 g to 10 mL solvent for 6 h (Elloff, 1998). Excess solvent was recovered on a rotary vapor until the extract was concentrated. The concentrated slurry was dried at room temperature in the fume hood. The crude plant extract was stored in the fridge at 4°C until it is required for biological assays.

**Liquid-liquid extraction (Fractionation):** Dried crude extracts were dissolved in 70% acetone in a separating funnel and extracted with hexane, dichloromethane and ethyl acetate respectively (Fig. 1). The residual water fraction was dried in a conventional oven at 50°C for 96 h. Absolute methanol was added every 24 h to prevent the growth of fungi. The dried water fractions were extracted with acetone and methanol successively to produce fractions of different polarities. Fractions were concentrated on a rotary evaporator and then air dried at room temperature under a fan. The dried fractions were weighed and results are recorded in Table 2. The fractionation protocol is presented in Fig. 1.

**Quantitative evaluation of the biological activities of the plant extracts**

**Minimum inhibitory concentrations (MIC):** Minimum inhibitory concentrations (MIC) of the crude extracts and fractions were determined by twofold serial dilution using 96-well microtitre plate against *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 25922) (Eloff, 1998). In brief, 100 µL distilled water was placed in each of the wells using a multichannel micropipette. Thereafter, 100 µL of extract (10 mg/mL) was added to the first well of column and serially diluted to prepare a concentration range between 5.0 and 0.04 mg/mL in the first well and last well, respectively. The solvent blank (negative control) and antibiotic (gentamicin for bacteria and amphotericin B for fungi) were included as positive control. From an overnight culture of bacteria grown in diluted Mueller Hinton broth, 100 µL of relevant bacteria dispensed into each well and incubated at 37°C for 18-24 h before adding 40 µL (0.2 mg/mL) of INT (iodonitrotetrazolium violet, Sigma) solution. Color change was noted after 30, 60 and 120 min to obtain the lowest concentration where growth was inhibited (MIC). Minimum inhibitory concentrations (MIC) of the crude extracts and fractions against *Candida albicans* and *Aspergillus fumigatus* were determined using the above-mentioned serial dilution assay with some modifications and the fungal stock was prepared using Sabouraud dextrose broth instead of Mueller Hinton broth used for bacteria culture.

**DPPH anti-oxidant assay:** For DPPH anti-oxidant assay, using 96 well plates and a VERSAmax™ tunable microplate reader (Labotech), 40 µL of 0,5 mg/mL plant extract and fraction was determined by a twofold serial dilution in 160 µL of 0.0025% of DPPH (total volume 200 µL), methanol (negative control) or trolox (positive control). After 30 min the absorbance was measured at

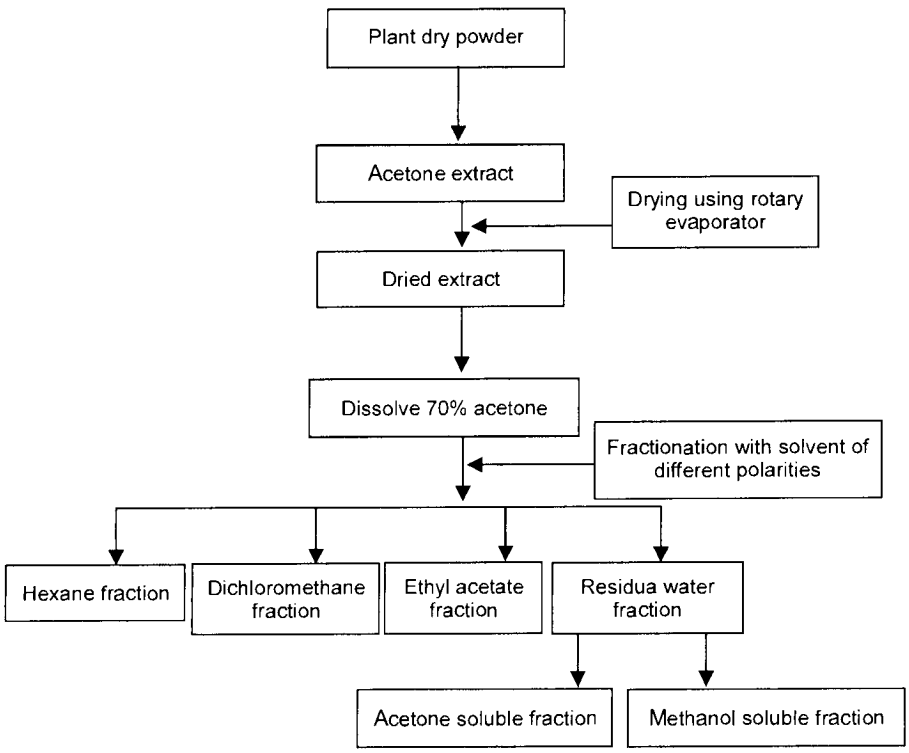


Fig. 1: Flow chart of the fractionation protocol

Table 2: Percentage yield of the crude and fractions of the extracts

| Plant sp. | I crude | Hexane | DCM | Et | Acetone | Methanol |
|---|---|---|---|---|---|---|
| *B. setifera* | 5.00 | 46.00 | 18.00 | 4.00 | 4.00 | 6.20 |
| *L. revoluta* | 2.50 | 40.00 | 4.00 | 26.00 | 12.00 | 0.03 |
| *C. Bispinosa* | 2.50 | 3.80 | 28.40 | 1.20 | 2.06 | 0.02 |

Table 3: Soya bean based 15 LOX inhibitory activity expressed as $EC_{50}$ values for the crude and fractions in mg/L

| Plant sp. | $EC_{50}$ | 95%CL |
|---|---|---|
| *L. revoluta* | 1030 | 0.4237 to 2.504 |
| *C. Bispinosa* | 12.24 | 5.647 to 26.54 |
| *B. setifera* | 11.91 | 5.482 to 25.89 |

516 nm wavelength. Methanol was used as negative control and trolox (25-0.5 g/mL) was used as positive control. The free radical DPPH˙ scavenging (reduction) was calculated from the equation:

$$\text{Activity [\% of DPPH reduction]} = \{(A-A_x)/A)X100\%$$

where, A is absorbance of DPPH solution with methanol, $A_x$ is absorbance of a DPPH solution with a tested fraction solution or trolox (positive control).

**ABTS$^{\cdot+}$ Scavenging assay:** The ABTS$^{\cdot+}$ radical cation was prepared by mixing 7 mM ABTS stock solution and incubating for 12-16 h in the dark at room temperature until a steady absorbance was obtained to indicating that the reaction was complete. Plant extracts and fractions (40 µL of 0.5 mg/mL) were mixed with 160 µL of the ABTS$^{\cdot+}$ radical cation (total volume 200 µL) in a 96-well microtitre plate. Absorbance of the mixture was read at 734 after six minutes using VERSAmax$^{TM}$ tunable microplate reader (Labotech). Methanol was used as negative control and trolox (25-0.5 g/mL) was used as positive control.

**Lipoxygenase inhibition:** Inhibitory activity of the plant extracts against 15-soybean lipoxygenase (15-LOX) enzyme was evaluated as described by Malterud and Rydland (2000) in borate buffer (0.2 M, pH 9.00). Increase in absorbance at 234 nm was read 5 min at interval of 30 s after addition of 15-LOX, using linoleic acid (134 µM) as substrate. The final enzyme concentration was 167 µg/mL. Test substances were added as DMSO solutions (final DMSO concentration of 1.6%) while DMSO alone was added in control experiments. The enzyme solution was kept on ice and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant. All measurements were carried out three times.

Table 4: Antimicrobial activity of the crude extract and fractions of varied polarities (µg/mL)

| | *Ledebouria revoluta* | | | | | | *Carissa bispinosa* | | | | | | *Berkheya setifera* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S.a | E.f | E.c | P.a | C.a | A.f | S.a | E.f | E.c | P.a | C.a | A.f | S.a | E.f | E.c | P.a | C.a | A.f |
| Cr | 320 | 160 | 160 | 160 | 625 | 160 | 320 | 320 | 20< | 320 | 625 | 160 | 320 | 160 | 320 | 160 | 625 | 320 |
| H | 160 | 320 | 160 | 360 | 625 | 160 | 160 | 320 | 20< | 160 | 625 | 160 | 80 | 160 | 320 | 80 | 625 | 320 |
| DCM | 320 | 160 | 160 | 320 | 625 | 160 | 160 | 320 | 320 | 160 | 625 | 160 | 160 | 160 | 320 | 160 | 625 | 320 |
| ET | 320 | 160 | 160 | 320 | 625 | 160 | 160 | 160 | 160 | 320 | 625 | 160 | 160 | 625 | 160 | 160 | 625 | 320 |
| Ac | 160 | 320 | 20< | 320 | 1250 | 160 | 160 | 160 | 320 | 160 | 1250 | 320 | 320 | 320 | 320 | 320 | 20 | 320 |
| Met | 160 | 160 | 20< | 320 | 1250 | 320 | 320 | 320 | 320 | 160 | 1250 | 320 | 320 | 160 | 160 | 320 | 1250 | 1250 |
| Gent | 4 | 4 | 4 | 4 | - | - | - | 4 | 4 | 4 | - | - | 4 | 4 | 4 | 4 | - | - |
| Amp B | - | - | - | - | 13 | 0.6 | - | - | - | - | 13 | 0.6 | - | - | - | - | 13 | 0.6 |

Cr: Crude extract; H: Hexane fraction; DCM: Dichloromethane fraction; ET: Ethyl acetate fraction
Ac: Acetone fraction; Met: methanol fraction; Gent: Gentamicin; Amp B: Amphotericin B
Sa: *Staphylococcus aureus*; E.f: Enterococcus faecalis; E.c: *Escherichia coli*; P.a: *Pseudomonas aeruginosa*
C.a: *Candida albicans*; A.f: *Aspergillus fumigatus*

## RESULTS

Investigations for different biological assays revealed that crude extracts and fractions of differing polarities had varied activities depending on the assays carried out. Results from the DPPH and $ABTS^{2+}$ scavenging assay investigations with *Ledebouria revoluta*, *Berkheya setifera* and *Carissa bispinosa* root extracts and fractions are diplayed in Table 5. Results for the 15-LOX anti-inflammatory assay displayed in Table 3 indicate that the crude extracts had some anti-inflammatory activities. The minimum inhibitory concentrations of the methanol crude extracts and fractions were presented in Table 4 display high to moderate antimicrobial activities.

**Yield:** *Berkheya setifera* had the highest percentage yield of 10% aetone crude extract while lower yieds of 2.5% were obtained with *Ledebouria revoluta* and *Carissa bispinosa.*The highest non polar fraction was from *Berkheya setifera* hexane nfraction of 46%. The lowest yield was from *Carissa bispinosa* of 0.02%. *Berkheya setifera* had then highest amount of the methanol fraction of 6.2% (Table 2).

**Antimicrobial activities:** Most samples from the three plants had high to moderate antimicrobial activities (20-320 µg/mL) as displayed in Table 4. However some low antifungal activities (1250 µg/mL) were exhibited by fewer samples of methanol fractions of *Berkheya setifera* against *Candida albicans* and *Aspergillus fumigatus*. The acetone fraction of *Berkheya setifera* had high activity against *Candida albicans of* 20 µg/mL.

**Antioxidant and lipoxygenase inhibitory activity:** Plant samples displayed differing antioxidant and lipoxygenase inhibitory activity depending on the polarity of sample. In general, most activity was displayed by the polar acetone and methanol fractions. *Berkheya setifera* crude sample displayed the highest activity of $EC_{50}$ 2.471 µg/mL against DPPH and 1.967 µg/mL with ABTS radical ion comparing with very well with trolox (0.04 µg/mL). The other two plant crude extracts displayed moderate activates of compared to the activities of their fractions.

## DISCUSSION

*Ledebouria revoluta* displayed good activities (20 µg/mL) for both acetone and methanol fractions against *E. coli* and good-moderate activity (160-320 µg/mL) against the rest of the bacterial strains used. Anti-fungal activity for *Ledebouria revoluta* methanol fraction (20 µg/mL) is high against *Candida albicans*. The bulb infusion of *Ledebouria revoluta* are used diarrhea in goats and leaf decoction for gall sickness (Dold and Cocks (2002) quoted in McGaw *et al.* (2008). *Carissa bispinosa* and *Berkheya setifera* have shown good to moderate antimicrobial activity of 160 to 320 µg/mL although their methanol fractions displayed low activities of 1250 µg/mL against clinically isolated fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. The fact that acetone fraction of *Berkheya setifera* had high activity against *Candida albicans of* 20 µg/mL indicate that the antifungal compounds are polar. The activities which were demonstrated by the plant acetone crude extracts and fractions of different polarities indicate presence of active compounds in them. These activities are being reported here for the first time.

*Carissa bispinosa* acetone fraction displayed high activity $EC_{50}$ of 1.108 µg/mL against ABTS radical ion when compared to the rest of the fractions. Although the *Carissa bispinosa* acetone fraction corresponding activity against DPPH free radical $EC_{50}$ of 11.18 µg/mL, the activity is the highest when compared to other fractions and crude of the same plant and acetone fractions of the other two plants. Most antioxidants are extracted by methanol as displayed by the fractions of *Berkheya setifera* ($EC_{50}$ 1.008 µg/mL) and *Ledebouria revoluta* ($EC_{50}$ 1.867 µg/mL) against DPPH radical. This antioxidant activity is linked to the antimicrobial activity of the same acetone methanol fractions which whose activities were between 20 and 320 µg/mL excluding the activity against the fungal strains *Candida albicans* and *Aspergillus fumigatus* ($EC_{50}$ 1250 µg/mL) which were low.

Soya bean derived 15 LOX inhibitory activity of crude extracts of *Carissa bispinosa* ($EC_{50}$ 12.24 µg/mL) and *Berkheya setifera* ($EC_{50}$ 11.91 µg/mL) were high and

also have and radical and antimicrobial activities. These observations were significant because LOXs are the family of the key enzymes in the biosynthesis of leukotrienes that are postulated to play an important role in the pathophysiology of several inflammatory diseases (Rackova *et al*., 2007). Plants have been reported before to be used to manage inflammation related conditions (Iwalewa *et al*., 2007). Results obtained during this study illustrate that *Ledebouria revoluta, Berkheya setifera* and *Carissa bispinosa* root extracts and fractions can be used in the treatment of inflammation related diseases.

**Conclusion:** A relationship between the 15 LOX inhibitory activity, antimicrobial and anti-radical activities illustrate that the crude fractions have medicinal value and explain why traditional healers use them to manage inflammation and related diseases.

Although the *in vitro* investigations illustrate that the extracts and fractions of root samples of *Ledebouria revoluta, Berkheya setifera* and *Carissa bispinosa* have medicinal properties, there is need for more *in vivo* investigationstions for recommendations of such plants to be used in medicine in managing inflammation.

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## REFERENCES

Abegaz, B.M., 2002. Novel phenylanthraquinoine, isofuranonaphthoquinones, homoisoflavonoids and biflavonoids from African Plants in the genera Bulbine, Scilla, Ledrebouria and Rhus Phytochemical Reviews, 1: 299-310.

Dold T. and M. Cocks, 2001. A succulent herbal-the medicinal and cultural use of some succulent plants traded in the Eastern Cape Province of South Africa. Cactus and Succulent J., 73: 141-145.

Dold, A.P. and M.L. Cocks, 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. S. Afr. J. Sci., 98: 589-597.

Elloff, J.N., 1998. A sensitive and quick micro-plate method to determine the minimal Inhibitory concentration of plant extracts for bacteria. Planta Med., 64: 711-713.

Hoareau, L. and E.J. DaSilva, 1999. Medicinal Plants: a re-emerging health aid. Elec. J. Biotechnol., 2: 56-70.

Iwalewa, E.O., J. McGaw, V. Naidoo and J.N. Eloff, 2007 Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. Afr. J. Biotechnol., 6: 2868-2885.

Malterud, K.E. and K.M. Rydland, 2000. Inhibitors of 15-lipoxygenase from orange peel. J. Agri. Food Chem., 48: 5576-5580.

McGaw, L.J., N. Lall, J.M. Meyer and J.N. Eloff, 2008. The Potential of South African plants against Mycobacterium infections. J. Ethnopharmacol., 119: 482-500.

Moodley, N., N.R. Crouch, D.A. Mulholland, D. Slade and D. Ferreira, 2006. 3-Benzyl-4-chromanones (homoisoflavanones) from bulbs of the ethno-medicinal geophyte *Ledebouria revoluta* (Hyacinthaceae). Afr. J. Bot., 72: 517-520.

Ncube, N.S., A.J. Afolayan and A.I. Okoh, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotechnol., 7: 1797-1806.

Osbourne, A., 1996. Saponins and plant defense-a soap story. Trends in Plant Science: Rev., 1: 4-9.

Rackova, L., M. Oblozinsky, D. Kostalova, Viktor V. Kettmann and L. Bezakova, 2007. Free radical scavenging activity and lipoxygenase inhibition of Mahonia aquifolium extract and isoquinoline alkaloids. J. Inflammation, 4: 15.

Taylor, J.L.S. and Van J. Staden, 2001. The effect of age, season and growth conditions on anti-inflammatory activity in Eucomis autumnalis (Mill.) Chitt. Plant extracts Plant Growth Regulation, 34: 39-47.

Watt, J.M. and M.G. Breyer-Brandwijk, 1962. The medicinal and poisonous plants of southern Africa. 2nd Edition Livingstone. London.