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# Antimicrobial activity of South African *Podocarpus* species

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ABSTRACT

*Ethnopharmacological relevance:* Several species of *Podocarpus* (Podocarpaceae) are utilized in treating ailments across the world. In Africa, four species are used traditionally in both animal and human health.
*Aim of the study:* To investigate the antimicrobial activity of *Podocarpus* species against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*.
*Materials and methods:* Six solvents of varying polarity were used for extraction. Antibacterial activity was assessed using the microdilution bioassay and for antifungal activity, the microdilution bioassay and (M27-P) broth dilution were used.
*Results:* All species exhibited antimicrobial activity with MIC values of less than 1 mg/ml. Inhibition against Gram-positive bacteria was stronger with an MIC value of 98 μg/ml while for Gram-negative bacteria, the highest inhibition was against *Klebsiella pneumoniae* with an MIC value of 0.33 mg/ml. All species exhibited strong antifungal activity with the best MIC being 30 μg/ml after 48 h.
*Conclusions:* All four species exhibited strong inhibition against all tested microbials, based on Aligiannis et al. [Aligiannis, N., Kalpotzakis, E., Mitaku, S., Chinou, I.B., 2001. Composition and antimicrobial activity of the essential oils of two *Origanum* species. Journal of Agricultural and Food Chemistry 40, 4168–4170] classification they can be classified as strong inhibitors.



## 1. Plant

Leaves and young stems of *Podocarpus latifolius* (Thunb.) R. Br. ex Mirb. (syn.: *Podocarpus milanjianus* Rendle), *Podocarpus falcatus* (Thunb.) R. Br. ex Mirb. (syn.: *Podocarpus gracilior* in sensu of Burtt Davy), *Podocarpus henkelii* Stapf. ex Dallim. & Jacks. and *Podocarpus elongatus* (Ait.) L' Herit. ex Pers. (Podocarpaceae) were obtained from the University of KwaZulu-Natal and the National Botanical Gardens, Pietermaritzburg between the months of May–June 2007. Propagules of *Podocarpus henkelii* were also collected. Voucher specimens (HA 001NU, HA 002NU, HA 003NU and HA 004NU) were deposited at the University of KwaZulu-Natal Herbarium.

## 2. Uses in traditional medicine

In South Africa, the bark and sap of these species are used by the Zulu and woodmen working in southern African forests to treat chest complaints and also as a herbal remedy to treat a variety of livestock diseases including gallsickness (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). The Maasai of East Africa use the bark and stem of *Podocarpus falcatus* and *Podocarpus latifolius* as remedies against stomachache and cattle diseases (Beentje, 1994). The oil extracted from the reproductive propagules is used to treat gonorrhea and stem bark is used in deworming. A decoction of the fruit serves as a tonic for cleansing the kidneys, lungs and stomach. In Ethiopia *Podocarpus falcatus* oils are used to cure gonorrhoea and powder from the bark is used for headaches (Pankhurst, 2000).

## 3. Previously isolated classes of constituent

Taxol, a tubulin binding diterpene isolated from *Podocarpus falcatus*, has been shown to inhibit the growth of HeLa cells and is a promising new treatment for several forms of cancer (Stahlhut et al., 1998).

## 4. Materials and methods

### 4.1. Extraction

Six solvents, such as petroleum ether (PE), hexane, dichloromethane (DCM), acetone, 80% ethanol and water, were used in the extraction. Five grams of dried plant material was extracted in 100 ml of each solvent, then sonicated for 1 h in an

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**Table 1**
Antibacterial activity of *Podocarpus* species against Gram-positive and Gram-negative bacteria (MIC mg/ml)

| Plant species | Parts | Solvent | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Petroleum ether | | | | Dichloromethane | | | | Ethanol | | | | Water | | | |
| | | *B. s* | *S. a* | *E. c* | *K. p* | *B. s* | *S. a* | *E. c* | *K. p* | *B. s* | *S. a* | *E. c* | *K. p* | *B. s* | *S. a* | *E. c* | *K. p* |
| *Podocarpus elongatus* | Leaves | 0.65 | 0.65 | 5.21 | 3.13 | 0.65 | 0.52 | **0.39** | 1.04 | **0.098** | 0.52 | 2.08 | 2.08 | 5.20 | 10.47 | 3.13 | 6.25 |
| | Stem | 0.65 | 0.65 | 3.13 | 2.08 | 0.39 | 0.52 | 3.13 | 3.13 | 0.39 | 0.26 | 2.08 | **0.33** | 5.12 | 3.13 | 4.17 | 3.13 |
| *Podocarpus falcatus* | Leaves | 0.39 | 0.65 | 3.65 | 3.13 | 0.39 | 0.78 | 0.78 | 0.65 | 0.33 | 0.33 | 1.56 | 1.56 | 6.25 | 3.64 | 6.25 | 6.25 |
| | Stem | 0.52 | 0.65 | 2.08 | 1.56 | 0.52 | 0.65 | 1.56 | 0.78 | 0.33 | 0.26 | 1.56 | 1.56 | 2.08 | 1.56 | 3.13 | 3.13 |
| *Podocarpus henkelii* | Leaves | **0.098** | 0.65 | 3.13 | 2.08 | 0.26 | 0.52 | **0.39** | 0.65 | 0.26 | 0.39 | 1.56 | 1.56 | 3.13 | 3.13 | 4.17 | 2.60 |
| | Stem | **0.098** | 0.52 | 2.60 | 2.08 | 0.13 | 0.65 | 0.78 | 0.39 | 0.33 | 0.78 | 2.60 | 0.65 | 6.25 | 6.25 | 6.25 | 6.25 |
| | Epimatium | **0.098** | 0.65 | 0.81 | 2.08 | 0.23 | 0.65 | 2.60 | 0.91 | 0.65 | 0.39 | 0.78 | 0.65 | 6.25 | 6.25 | 6.25 | 6.25 |
| | Integument | 0.65 | 1.56 | 5.20 | 4.69 | 2.08 | 1.56 | 2.60 | 2.60 | 2.08 | 2.08 | 4.17 | 2.08 | >12.5 | >12.5 | >12.5 | >12.5 |
| *Podocarpus latifolius* | Leaves | 0.39 | 0.65 | 2.34 | 1.30 | 0.65 | 0.65 | 0.78 | 2.08 | 0.33 | **0.098** | 4.17 | 0.65 | 6.25 | 8.33 | 12.5 | 8.33 |
| | Stem | 0.195 | 0.65 | 2.08 | 0.65 | 0.163 | 0.78 | 2.08 | 0.65 | 0.65 | 0.33 | 5.20 | 0.78 | 8.33 | 8.33 | 12.5 | 8.33 |

Neomycin (μg/ml): *B. s* = 0.07; *S. a* = 0.26; *E. c* = 0.26; *K. p* = 0.26.
Bacteria: *B. s* = *Bacillus subtilis*; *E. c* = *Escherichia coli*; *K. p* = *Klebsiella pneumoniae*; *S. a* = *Staphylococcus aureus*.

ultra sound bath, kept overnight and then filtered under a vacuum using Whatman No. 1 filter paper. The clear filtrates were dried either under vacuum using a rotary evaporator or freeze dried.

### 4.2. Antibacterial bioassay

The microdilution bioassay by Eloff (1998) was used. The dried residues were redissolved to a concentration of 50 mg/ml in ethanol for non-aqueous extracts and water for aqueous extracts. Each extract was tested against *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775) and *Klebsiella pneumoniae* (ATCC 13883). One hundred microlitres of the extracts and neomycin (positive control) were serially diluted twofold with 100 μl distilled water. Solvents and bacteria were included as negative controls. To each well, 100 μl of the bacterial cultures was added. The plates were covered and incubated for 24 h at 37 °C. To indicate bacterial growth, 50 μl of 0.2 mg/ml ρ-iodonitrotetrazolum chloride (Sigma), was added to each well, and the plates were incubated for an hour. The wells which displayed no colour change represented antibacterial activity. The minimum inhibitory concentration (MIC) was taken as the lowest concentration of plant extract to inhibit growth of the tested bacterium. Each extract was tested in triplicates.

### 4.3. Antifungal bioassay

The (M27-P) broth dilution (Espinel-Ingroff and Pfaller, 1995) and microdilution bioassays (Eloff, 1998) were used. Each extract was bioassayed with a strain of *Candida albicans* (ATCC 10231). For M27-P method, aqueous extracts were resuspended in water and non-aqueous extracts in dimethyl sulfoxide (DMSO) to a concentration of 100 mg/ml. Twenty-five microlitres of non-aqueous and 100 μl of aqueous extracts were serially diluted twofold with 175 μl and 100 μl of YM broth, respectively. For microdilution bioassay, extracts were redissolved in DMSO and acetone to a concentration of 10 mg/ml. One hundred microlitres of the extracts were serially diluted in 100 μl of water. Amphotericin B was used as a positive control. Broth, fungus, water, acetone and DMSO were included as negative controls. To each well, 100 μl of the yeast cultures were added and incubated for 48 h at 37 °C. Fifty microlitres of 0.2 mg/ml ρ-iodonitrotetrazolum chloride (Sigma) was used as indicator of fungal growth. The wells which displayed no change in colour represented antifungal activity. The MIC was taken as the lowest concentration of plant extract to inhibit growth of the fungus after 24 h and 48 h. After 72 h YM broth was added to determine whether the inhibition was fungicidal or fungistatic. The assay was repeated three times for each extract.

### 4.4. Minimum inhibitory concentration

Although there is no validated criteria for the MIC end points for *in vitro* testing of plant extracts, Aligiannis et al. (2001) proposed a classification for plant materials based on MIC results as follows; MIC up to 0.5 mg/ml as strong inhibitors; between 0.6 mg/ml and 1.5 mg/ml as moderate inhibitors and above 1.6 mg/ml as weak inhibitors. For this study an MIC value of less than 1 mg/ml was considered to show good antimicrobial activity.

## 5. Results and discussion

The ranges of percentage dry weights isolated were between 3% and 5% for petroleum ether (PE), 0.9% and 3% for dichloromethane (DCM), 8% and 26% for ethanol, 0.1% and 2% for hexane, 2%, and 6% for acetone and 3% and 15% for water.

**Table 2**
Antifungal activity of *Podocarpus* species against *Candida albicans* (MIC mg/ml)

| Plant species | Plant parts | Solvent | (M27-P) broth dilution | Microdilution (DMSO) | | Microdilution (acetone) | |
|---|---|---|---|---|---|---|---|
| Time (hours) | | | 48[c] | 24[b] | 48[c] | 24[b] | 48[c] |
| *Podocarpus elongates* | Leaves | Petroleum ether | 6.25 | 0.39 | 0.78 | 0.16 | 0.42 |
| | | Hexane | ND[a] | ND[a] | | 0.23 | 0.47 |
| | | Dichloromethane | 0.16 | 0.07 | 0.15 | 0.02 | 0.06 |
| | | Acetone | ND[a] | ND[a] | | 0.04 | 0.08 |
| | | 80% ethanol | 6.25 | 1.25 | 1.25 | 1.04 | 1.67 |
| | Stem | Petroleum ether | 6.25 | 0.78 | 1.56 | 0.68 | 0.94 |
| | | Hexane | ND[a] | ND[a] | | 0.31 | 0.63 |
| | | Dichloromethane | 0.26 | 0.39 | 0.78 | 0.31 | 0.63 |
| | | Acetone | ND[a] | ND[a] | | 0.78 | 0.78 |
| | | 80% ethanol | 6.25 | 1.25 | 1.25 | 0.83 | 1.46 |
| *Podocarpus falcatus* | Leaves | Petroleum ether | 1.56 | 0.2 | 0.39 | 0.17 | 0.66 |
| | | Hexane | ND[a] | ND[a] | | 0.47 | 0.63 |
| | | Dichloromethane | 0.13 | 0.03 | 0.05 | 0.02 | 0.06 |
| | | Acetone | ND[a] | ND[a] | | 0.02 | 0.06 |
| | | 80% ethanol | 6.25 | 1.25 | 1.25 | 0.73 | 1.25 |
| | Stem | Petroleum ether | 3.13 | 0.78 | 1.56 | 0.73 | 1.04 |
| | | Hexane | ND[a] | ND[a] | | 0.47 | 0.63 |
| | | Dichloromethane | 0.26 | 0.2 | 0.39 | 0.31 | 0.31 |
| | | Acetone | ND[a] | ND[a] | | 0.63 | 0.93 |
| | | 80% ethanol | 6.25 | 1.25 | 1.25 | 0.73 | 1.67 |
| *Podocarpus henkelii* | Leaves | Petroleum ether | 3.13 | 0.39 | 0.78 | 0.16 | 0.42 |
| | | Hexane | ND[a] | ND[a] | | 0.23 | 0.47 |
| | | Dichloromethane | 0.163 | 0.03 | 0.05 | 0.02 | 0.06 |
| | | Acetone | ND[a] | ND[a] | | **0.02** | **0.04** |
| | | 80% ethanol | 6.25 | 1.25 | 1.25 | 0.94 | 2.08 |
| | Stem | Petroleum ether | 5.20 | 3.13 | 3.13 | 0.62 | 0.93 |
| | | Hexane | ND[a] | ND[a] | | 0.31 | 0.63 |
| | | Dichloromethane | 0.13 | 0.39 | 0.59 | 0.47 | 0.63 |
| | | Acetone | ND[a] | ND[a] | | 0.63 | 0.94 |
| | | 80% ethanol | 6.25 | 1.04 | 1.25 | 0.94 | 2.08 |
| | Epimatium | Petroleum ether | 0.78 | 0.39 | 0.78 | 0.16 | 0.31 |
| | | Hexane | ND[a] | ND[a] | | 0.94 | 0.94 |
| | | Dichloromethane | 0.65 | 0.39 | 0.65 | 0.63 | 0.63 |
| | | Acetone | ND[a] | ND[a] | | 0.63 | 0.94 |
| | | 80% ethanol | 3.13 | 1.25 | 1.56 | 1.04 | 1.25 |
| | Integument | Petroleum ether | 1.56 | 6.25 | 6.25 | 6.25 | 6.25 |
| | | Hexane | ND[a] | ND[a] | | 6.25 | 6.25 |
| | | Dichloromethane | 1.56 | 1.56 | 3.13 | 1.56 | 3.13 |
| | | Acetone | ND[a] | ND[a] | | 1.56 | 3.13 |
| | | 80% ethanol | 3.13 | 6.25 | 6.25 | 6.25 | 6.25 |
| *Podocarpus latifolius* | Leaves | Petroleum ether | 2.08 | 0.195 | 0.39 | 0.07 | 0.13 |
| | | Hexane | ND[a] | ND[a] | | 0.06 | 0.23 |
| | | Dichloromethane | 0.13 | **0.02** | **0.03** | 0.03 | 0.05 |
| | | Acetone | ND[a] | ND[a] | | **0.02** | **0.04** |
| | | 80% ethanol | 6.25 | 1.25 | 1.25 | 0.94 | 1.25 |
| | Stem | Petroleum ether | 3.13 | 0.78 | 2.34 | 0.52 | 0.83 |
| | | Hexane | ND[a] | ND[a] | | 0.94 | 0.94 |
| | | Dichloromethane | 0.16 | 0.39 | 0.59 | 0.31 | 0.47 |
| | | Acetone | ND[a] | ND[a] | | 0.63 | 0.94 |
| | | 80% ethanol | 6.25 | 0.89 | 1.25 | 0.23 | 1.04 |

[a] Not done; amphotericin B.
[b] $6.13 \times 10^{-3}$ μg/ml.
[c] $2.2 \times 10^{-2}$ μg/ml.

The four *Podocarpus* species exhibited broad-spectrum antibacterial activity against all the four tested microbials. Weak inhibitory activity against the test bacteria was detected with the aqueous extracts; this was previously reported in *Podocarpus latifolius*, which gave negative antibiotic tests (Watt and Breyer-Brandwijk, 1962). PE extracts of *Podocarpus henkelii* and ethanol extract of *Podocarpus elongatus* showed the strongest inhibition against *Bacillus subtilis* with an MIC value of 0.098 mg/ml. Ethanol extract of *Podocarpus latifolius* exhibited strong antibacterial activity against *Staphylococcus aureus* with an MIC value of 0.098 mg/ml. DCM leaf extracts of *Podocarpus henkelii* and *Podocarpus elongatus* gave an MIC value of 0.39 mg/ml against *Escherichia coli* and an ethanol stem extract of *Podocarpus elongatus* a MIC value of 0.33 mg/ml against *Klebsiella pneumoniae* (Table 1).

The two methods exhibited different antifungal activity against *Candida albicans*. For the M27-broth dilution method, extracts showed MIC values between 0.13 mg/ml and 6.25 mg/ml while in the microdilution assay, extracts showed lower MIC values between 0.03 mg/ml and 2.34 mg/ml after 48 h. DCM and acetone extracts of *Podocarpus henkelii* and *Podocarpus latifolius* inhibited growth of *Candida albicans* at the lowest concentration of 0.03 mg/ml and 0.04 mg/ml after 48 h, respectively (Table 2). After addition of broth, fungal growth resumed in most of the wells, except at 1.25 mg/ml and higher for DCM and acetone extracts, indicating fungicidal activity at higher concentrations and fungistatic activity at lower concentrations.

Several biological active compounds in these species may account for the antimicrobial activities observed. Compounds with

antifungal and antibacterial activities have been isolated from other species of *Podocarpus*. For example totarol isolated from *Podocarpus nagi*, *Podocarpus macrophyllus* and *Podocarpus totara* has been reported to be active against β-lactam resistant strains of bacteria (Moorhead and Bigwood, 2003). Diterpenes including ferrunigol, hinikiol and hinokione isolated from *Podocarpus nubigena* and *Podocarpus saligna* have showed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas* species and antifungal activity against *Aspergillus* sp. *Fusarium fujikuroi*, *Fusarium ciliatum*, *Mucor meihei*, *Nematospora coryli*, *Penicillium notatum* and *Paecilomyces variotii* (Becerra et al., 2002). Nagilactone E, the most abundant norditerpene dilactone from *Podocarpus nagi*, showed moderate to weak activity against *Candida albicans*, *Saccharomyces cerevisiae* and *Pityrosporum ovale* (Kubo et al., 1993).

The four *Podocarpus* species exhibited strong inhibition against all the tested microbials, based on Aligiannis et al. (2001) classification they can be classified as strong inhibitors. These species have displayed promising biological activity, hence they can be used to treat human and livestock diseases especially in terms of topical application where bioavailability and biotransformation are less important. The results obtained from our screening confirm the therapeutic potency of these four species and thus provide a rationale for their use in traditional medicine.

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