

**ISOLATION, STRUCTURAL
ELUCIDATION AND ANTIBACTERIAL
ACTIVITY OF THE CHEMICAL
CONSTITUENTS OF
*SCAEVOLA SPINESCENS***

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LIST OF ABBREVIATIONS

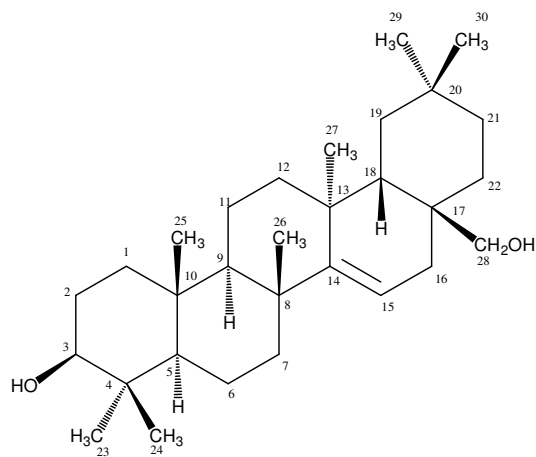
°C	degrees Celsius
v/v	volume per volume
ppm	parts per million
Hz	hertz
RNA	ribonucleic acid
CFU	colony forming units
TLC	Thin layer chromatography
UV	Ultraviolet
MP	melting point
FTIR	Fourier Transform InfraRed
NMR	Nuclear magnetic resonance
1D	one-dimensional
2D	two-dimensional
DEPT	Distortionless Enhancement by Polarization Transfer
COSY	Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
ROESY	Rotational nuclear Overhauser Effect Spectroscopy
m	multiplet
s	singlet
d	doublet
t	triplet
q	quadruplet
δ_C	chemical shift for ^{13}C
δ_H	chemical shift for ^1H
ATCC	American Type Culture Collection
IC ₅₀	50% inhibitory concentrations

ABSTRACT

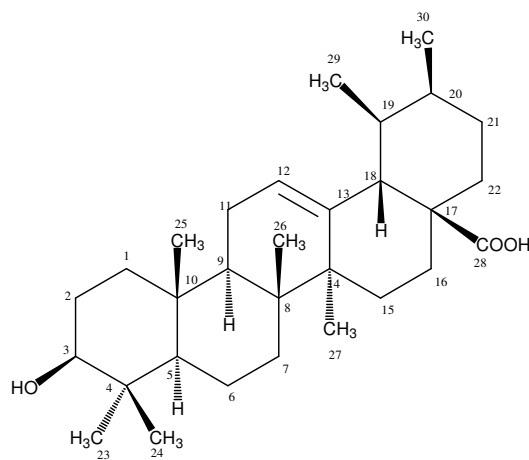
Scaevola spinescens, an Australian indigenous plant has been used by Australian Aboriginal people in their traditional medicines for treating colds, stomach ache, urinary problems and pain in the alimentary tract, skin rashes, boils and sores. An infusion of leaves and twigs of *Scaevola spinescens* and *Codonocarpus cotinifolius* has been reputed to cure cancer. Therefore, this plant has been deemed desirable for investigation to identify possible active compounds that contribute to these medicinal therapies used by the Aboriginal people.

Previous work has shown that coumarins, terpenoids, iridoids and flavonoids are the classes of compounds isolated from *Scaevola spinescens*. So far, chemical constituents of *Scaevola spinescens* have only been isolated from hexane and methanol fractions. One of the aims of this research was to isolate more of the chemical constituents of *Scaevola spinescens*. Therefore in this research, the ethyl acetate and aqueous fractions were also included to broaden the range of compounds being isolated.

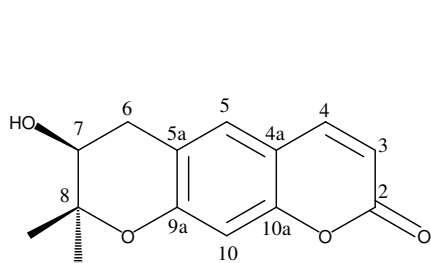
In this research, eleven compounds have been isolated from *Scaevola spinescens*. Five of the isolated compounds are known – myricadiol, 20-*epi*-ursolic acid, decursinol, luteolin-7-*O*-glucoside, and 2-*deoxy*-D-chiro-inositol. A novel compound identified as 18-*epi*-taraxerol has been isolated from *Scaevola spinescens* in this study. The structures of these compounds were determined using 1D and 2D NMR, UV-Visible spectroscopy, FTIR and high-resolution mass spectrometry. The structures of the five remaining compounds are yet to be determined.



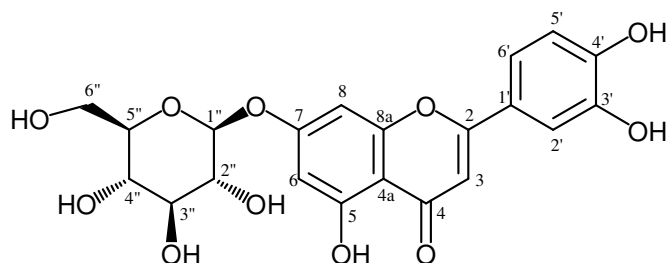
Myricadiol



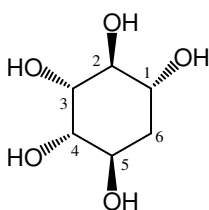
20-*epi*-ursolic acid



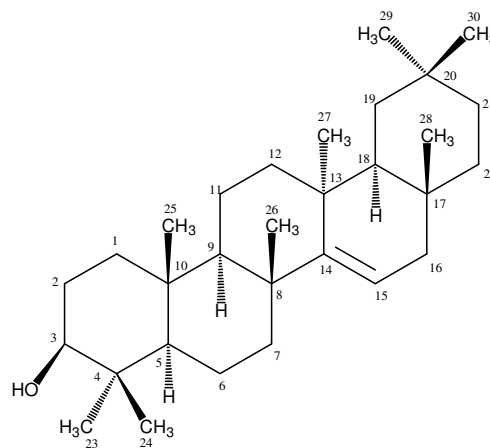
Decursinol



Luteolin-7-O-glucoside



2-deoxy-D-chiro-inositol



18-*epi*-taraxerol

The antiviral, antitumor and antibacterial activity of *Scaevola spinescens* extracts has been previously investigated. Previous work has shown that *Scaevola spinescens* was active against human cytomegalovirus (HCMV). However, previous work done on the antitumor activity of *Scaevola spinescens* was inconclusive. Previous work done on the antibacterial activity showed that some of the methanol fractions from *Scaevola spinescens* might contain antibacterial agents. However, these active compounds that contribute to the antibacterial activity were yet to be identified.

In this work, the organic (hexane, ethyl acetate and methanol) and aqueous crude extracts of *Scaevola spinescens* were screened for antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* ATCC 10389) and Gram-negative (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) bacteria using a broth micro-dilution assay to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

The hexane, ethyl acetate and methanol crude extracts were found to have antibacterial activity against Gram-positive bacteria specifically *Streptococcus pyogenes* while the aqueous fractions showed significant antibacterial activity against *Staphylococcus aureus*. No antibacterial activity was observed against Gram-negative bacteria.

A promising antibacterial activity was observed on one of the isolated compounds, 20-*epi*-ursolic acid. This compound showed good antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus* with MIC in the range 1.87 to 7.5 µg/ml.

The research presented within this thesis shows that 20-*epi*-ursolic acid isolated from *Scaevola spinescens* might be a potential candidate as an antibacterial agent. This is supported by the results obtained from preliminary antibacterial screening on the organic and aqueous crude extracts.

STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Michele Mejin

February, 2009

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