Bioassay-guided purification, GC-MS characterization and quantification of phyto-components in an antibacterial extract of *Searsea lancea* leaves

M. Vambe, D. Naidoo, A. O. Aremu, J. F. Finnie and J. Van Staden*

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa.

*Corresponding author (J. Van Staden), E-mail address: rcpgd@ukzn.ac.za.

Tel: +27 33 2605130.

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Abstract

Phytocompounds in an aqueous methanol (70% MeOH) leaf extract of *Searsia lancea* were separated using liquid-liquid partitioning techniques and gravity-assisted column chromatography. The resultant fractions were screened for antibacterial properties (minimum inhibitory concentration, MIC) against four bacterial strains (*Enterococcus faecalis*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae* and *Staphylococcus aureus*). Bioactive fractions were purified using preparative thin layer chromatography (TLC) and subjected to further antibacterial screening. Phytocompounds in antibacterial sub-fractions were characterized and quantified using Gas Chromatography-Mass Spectrometry (GC-MS). An ethyl acetate sub-fraction purified from the aqueous methanol extracts of the leaves demonstrated potent antibacterial properties (MIC range: 31-61 µg/ml against *E. faecalis* and *S. aureus*). Based on GC-MS analysis, 81.5% of the sub-fraction consisted of broad-spectrum antibacterial compounds namely tetracosanol (43.98%) and nonadecanol (37.5%). Current research findings support the traditional use of *S. lancea* leaves to manage gastro-intestinal disorders and gonorrhoea.

Key words: Anacardiaceae; Antibacterial; GC-MS; Ethnobotany; *Searsia lancea*.

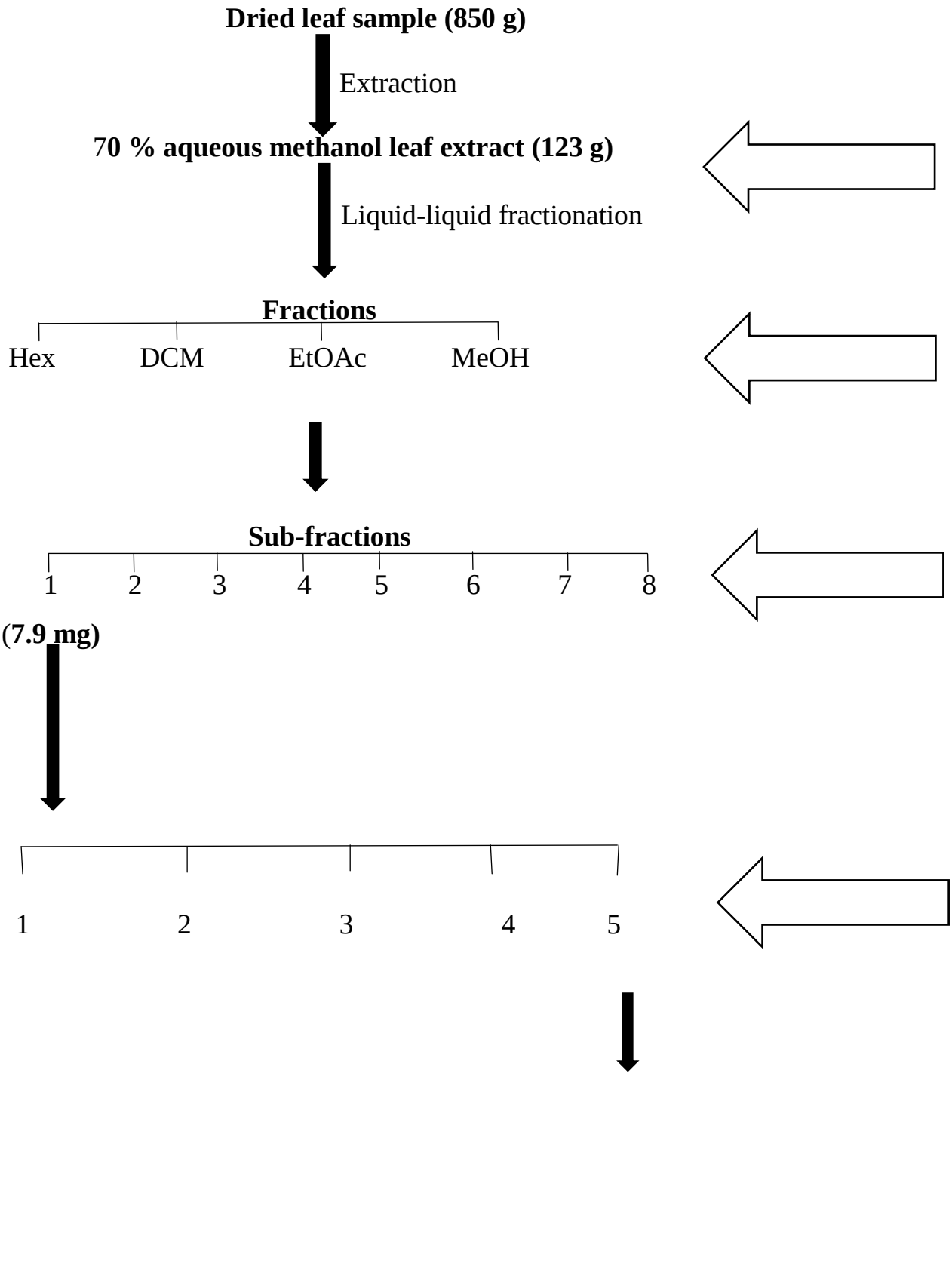
Supplementary materials

Experimental section

The plant (*Searsia lancea*) was positively identified by the Curator at the Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg, South Africa. A voucher specimen (NU0042514) was prepared and deposited at the same Herbarium. The scheme presented in Figure S1 was applied to a methanolic extract of *S. lancea* leaves which yielded several fractions. Briefly, a dry powdered leaf sample (850 g) of the plant was mixed with 1 L of 70% MeOH and stirred for 24 h at room temperature. The resultant extract was filtered *in vacuo* and then concentrated using a rotary evaporator (Heldolph vv 2000, Germany) at 30 ^{o}C. The concentrated extract was sequentially extracted with n-hexane (3x250 ml), dichloromethane (3x250 ml) and ethyl acetate (3x400 ml). Each fraction was concentrated to dryness *in vacuo* to give four solvent fractions: hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and MeOH. The four fractions were screened for antibacterial properties (Eloff, 1998) against four bacterial strains (*E. faecalis*, *K. pneumoniae*, *N. gonorrhoeae* and *S. aureus*). The ethyl acetate fraction demonstrated noteworthy antibacterial properties and was further purified on a Sephadex LH-20 column using a DCM/EtOAc (1:0-0:1) solvent system followed by MeOH (up to 40%) in ethyl acetate. The resultant fractions were spotted on TLC plates and developed in DCM/EtOAc (4:1). Fractions with similar chemical profiles were combined and subjected to antibacterial susceptibility tests (Eloff, 1998). Fraction 1 (7.9 mg) displayed the best antibacterial activity and was thus further purified through preparative TLC. The resultant bioactive sub-fraction was then analysed using Gas Chromatography-Mass Spectroscopy (GC-MS). The compounds were identified by direct comparison of the mass spectrum of the analyte at a particular retention time to that of reference standards found in the 2011 National Institute of Standards and Technology (NIST11) library. The area percentage of each component was calculated by comparing its average peak area to the total areas obtained.

Reference

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


Dried leaf sample (850 g)
Extraction
70 % aqueous methanol leaf extract (123 g)
Liquid-liquid fractionation
Fractions
Hex
DCM
EtOAc
MeOH
Sub-fractions
1
2
3
4
5
6
7
8
(7.9 mg)
1
2
3
4
5

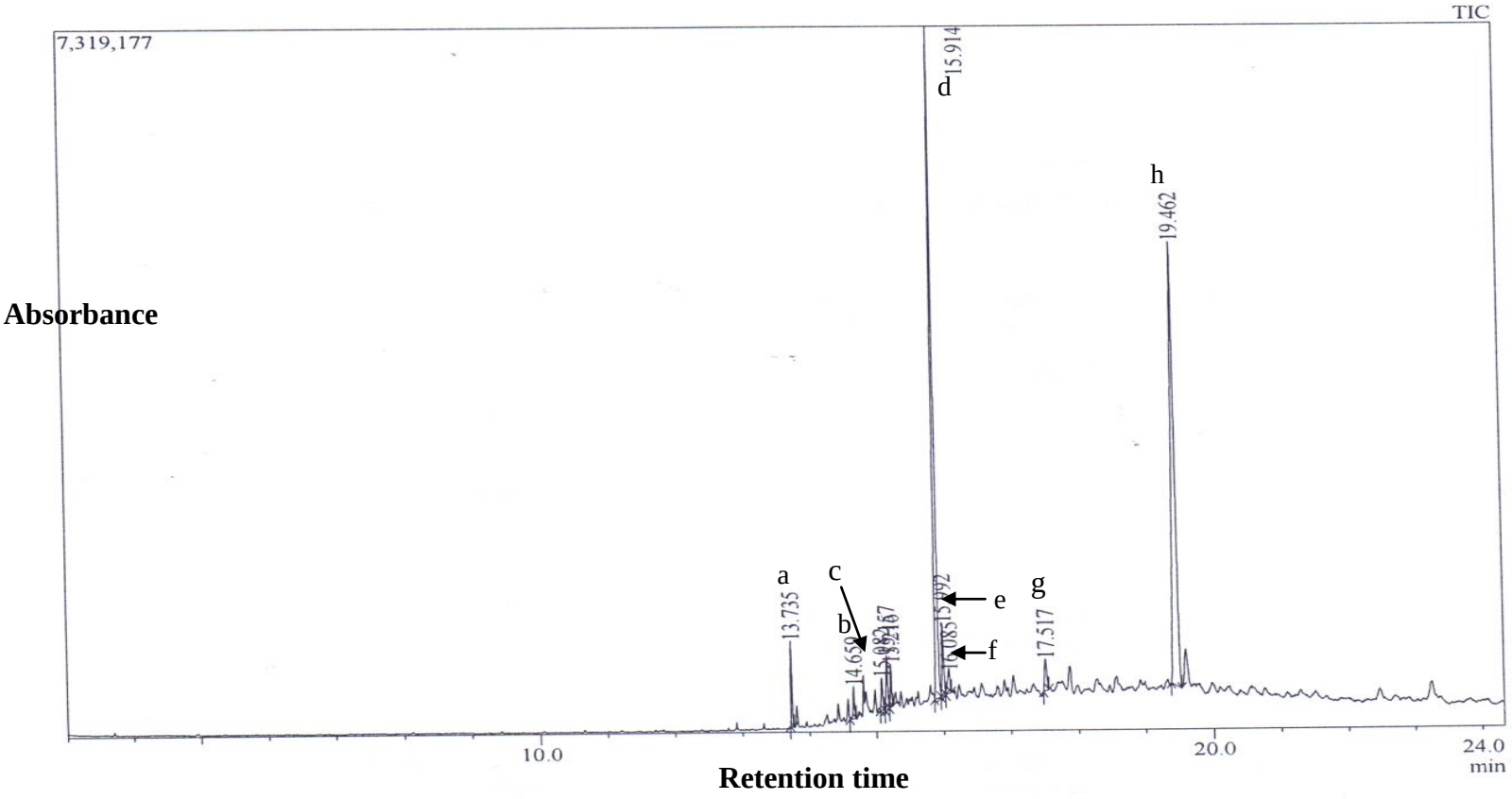
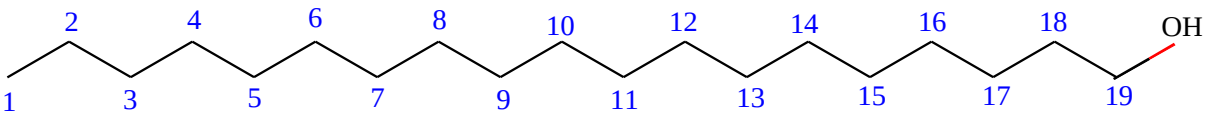
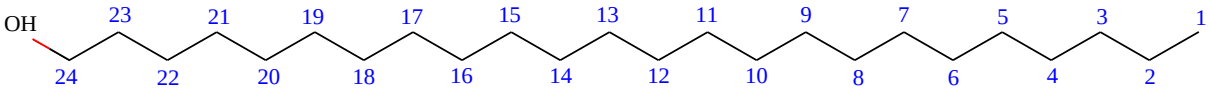

TIC
7,319,177
15.914
d
h
19.462
Absorbance
a
c
b
e
g
f
13.735
14.652
17.517
10.0
20.0
24.0
min
Retention time

Figure S2



A. Nonadecanol



B. Tetracosanol

Figure S3