Vol. 20 (2017) 13-16

Antimicrobial activity of extracts and phytosterols from the root bark of *Lonchocarpus eriocalyx*

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The root bark of *Lonchocarpus eriocalyx* was dried, powdered and extracted using chloroform, methanol and hot water. The extracts exhibited antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Saccharomyces cerevisiae*. The decoction (100mg/ml) was more active than the chloroform and methanol extracts against the four microorganisms. Chromatographic fractionation of the chloroform extract using normal phase silica yielded the phytosterols lupeol and lupenone. At 100 mg/ml, the compounds were active against all the four microorganisms, with lupeol being more active than lupenone. This is the first report of the isolation of lupenone from *Lonchocarpus eriocalyx*.

Key words: *Lonchocarpus eriocalyx*, lupeol, lupenone, antimicrobial activity.

INTRODUCTION

Despite the current surge in drug discovery, there is increased preference for alternative medicine [1]. In Kenya, for example, over 70% of the population uses traditional medicine in addition to conventional medicine [2]. The WHO recommends that individual countries develop standard procedures to validate medicinal products for incorporation into mainstream health care [1].

Lochocarpus eriocalyx (*Fabaceae)* is a slender, deciduous tree, growing up to 15m in height, with a pale greyish bark. It is native of South America, but grows naturally worldwide at altitudes of 500m-1680m. In Kenya, *L. eriocalx* is commonly found in the lower eastern parts of Machakos, Tharaka and Mbeere [3].

A decoction of the root bark of *L. eriocalyx* has been used to manage pimples among the Haya community of North-western Tanzania [4] and for the treatment of eye infections among the Tharaka people of Eastern Kenya [5].

Previous studies on this plant are limited. For instance, Kiplagat isolated lupeol from a dichloromethane root extract [6]. This study sought to carry out further phytochemical and antimicrobial investigation on the root bark of *L. eriocalyx*.

MATERIALS AND METHODS

Plant material

The *Lonchocarpus eriocalyx* root bark was collected from Makanyanga sub-location, Igamba- Ng'ombe division, Tharaka-Nithi County (Kenya) on September 10, 2015. Taxonomic specimen authentication was done at the University of Nairobi (Department of Botany) by Mr. Anthony Mutiso and voucher specimens deposited at the University Herbarium (voucher number MJK/1/2015).

Solvents and reagents

Solvents used for extraction and fractionation were general purpose grade and were distilled before use. Chloroform and methanol were from Alpha Chemicals Ltd (Nairobi, Kenya) while hexane and ethyl acetate were from Synerchemie Chemicals (Nairobi, Kenya). Reagents used were prepared as per pharmacopoeial procedures.

Equipment

A Soxhlet extractor equipped with a Graham condenser and a thermostatic heating mantle (Quickfit, Birmingham, U.K.) were used for solvent extractions. A Mini UV/Vis box (Desaga GmbH, Heidelberg, Germany) was used for

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visualizing TLC plates. Infrared spectra were obtained by running KBr discs of the samples on a Prestige-21 (A21000501-21) FT-IR spectrometer (Shimadzu Corporation, Kyoto, Japan). Nuclear magnetic resonance spectra of the isolated compounds were acquired on a Varian Unity 400 MHz NMR spectrometer (Varian Inc., Palo Alto, California, USA).

Extraction

The root bark was chopped into small pieces, air-dried at room temperature, ground to powder and stored in closed containers at room temperature before use. To obtain the organic extracts, 1 kg of the powder was placed in a Soxhlet extractor and extracted sequentially with chloroform and methanol, each solvent extraction lasting 48 hrs. The decoction was obtained by extracting 1 kg of root powder in hot water for one hour. The three extracts were filtered, reduced to dryness *in vacuo* and stored at 2-8°C.

Chromatography

About 12.5 g of chloroform extract was loaded onto a column packed with 320 g of normal phase silica gel (60-120 mesh) and eluted with a hexane/ethyl acetate mobile phase starting with 95/5 and reducing the hexane content by 5% after every 100 fractions (about 500 ml). The fractions were monitored by TLC on pre-coated silica gel F_{254} plates.

Isolation of compounds

Fractions with a similar TLC profile were pooled to give super-fractions A - E, covered with aluminium foil and left to stand on the laboratory bench. Super-fractions B and D formed crystals after three to five days.

The crystals from super-fraction B were recrystallized in dichloromethane to form white shiny crystals (96.4 mg, 0.017% yield). Similarly, the crystals from super-fraction D were recrystallized in acetone to give white feathery crystals (5.6 g, 1.871% yield).

Antimicrobial activity testing

The extracts and isolated compounds (all at 100 mg/ml in DMSO) were tested for antifungal activity against *Saccharomyces cerevisiae* (local strain), and for antibacterial activity against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The agar well diffusion method was employed [7]. Antibacterial and antifungal assays were carried out in Nutrient Agar (NA) and Sabouraud's Dextrose Agar (SDA), respectively. Gentamcin and nystatin each at a concentration of 0.3 mg/ml in DMSO served as the standards, respectively, for the antibacterial and antifungal assays.

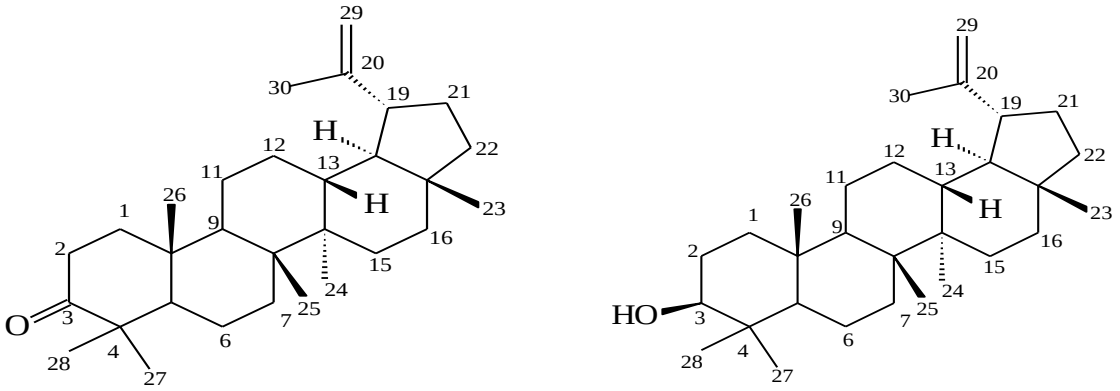
RESULTS AND DISCUSSION

Structural elucidation

Physical and spectroscopic methods (melting point, UV, IR, MS and NMR analysis) were used to identify 2 compounds (lupenone, and lupeol) from the fractions. The data obtained was consistent with literature values for the two compounds [8-12].

Lupenone: white shiny crystals; m.p. 165-167 °C; IR ν_{max} (KBr) cm^{-1}: 2926 (CH_3, C-H str), 2856 (CH_2, str), 1705 (C=O str); MS (*m/z*): 424 (M^+); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.82 (3H, s, H-27), 0.96 (3H, s, H-25), 0.98 (3H, s, H-28), 1.05 (3H, s, H-23), 1.10 (3H, s, H-26), 1.28 (3H, s, H-24), 1.71 (3H, s, H-30), 1.90-1.95 (1H, m, H-19), 2.40-2.51 (1H, m, H-19), 4.60 (1H, d, *J*=1.9 Hz, H-29b) and 4.71 (1H, d, *J*=1.9 Hz, H-29a); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 39.63 (C-1), 34.15 (C-2), 218.10 (C-3), 47.33 (C-4), 54.96 (C-5), 19.70 (C-6), 33.60 (C-7), 40.81 (C-8), 49.82 (C-9), 36.90 (C-10), 21.50 (C-11), 25.19 (C-12), 38.21 (C-13), 42.91 (C-14), 27.45 (C-15), 35.54 (C-16), 43.00 (C-17), 48.28 (C-18), 47.97 (C-19), 150.87 (C-20), 29.86 (C-21), 39.99 (C-22), 26.67 (C-23), 21.04 (C-24), 15.96 (C-25), 15.80 (C-26), 14.49 (C-27), 18.02 (C-28), 109.39 (C-29) and 19.32 (C-30).

Lupeol: white feathery crystals; m.p: 211-213 °C; IR (KBr) cm^{-1}: 3356 (O-H str), 2939 (CH_3, C-H str), 2872 (CH_2, str), 1643 (C=C str), 1458 (CH_3, C-H bend), 1377 (CH_2, bend), 1055 (cycloalkane); MS (m/z): 426 (M^+); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.69 (1H, m, H-5), 0.78 (3H, s, H-24), 0.81 (3H, s, H-28), 0.86 (3H, s, H-25), 0.97 (3H, s, H-27), 0.99 (3H, s, H-23), 1.06 (3H, s, H-26), 1.62 (1H, m, H-2), 1.71 (3H, s, H-30), 1.91-1.95 (2H, m, H-21), 2.36-2.43 (1H, m, H-19), 3.20 (1H, dd, *J*=3.4, 8.4 Hz, 8.4 Hz, H-3), 4.60 (1H, d, *J*=1.9 Hz, H-29b) , 4.71 (2H, d, *J*=1.9 Hz, H-29a); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 38.75 (C-1), 27.45 (C-2), 79.01 (C-3), 38.88 (C-4), 55.35 (C-5), 18.35 (C-6), 34.33 (C-7), 40.87 (C-8), 50.49 (C-9), 37.21 (C-10), 20.97 (C-11), 25.19 (C-12), 38.10 (C-13), 42.86 (C-14), 27.49 (C-15), 35.61 (C-16), 43.02 (C-17), 48.35 (C-18), 48.00 (C-19), 150.95 (C-20), 29.89 (C-21), 40.03 (C-22), 28.01 (C-23), 15.38 (C-24), 16.13 (C-25), 16.01 (C-26), 14.57 (C-27), 18.03 (C-28), 109.33 (C-29), 19.33 (C-30).



Lupenone **Lupeol**

Figure 1: Chemical structures of compounds isolated from *L. eriocalyx* root bark.

Antimicrobial activity

The decoction was active against all the antibacterial and antifungal strains tested. The three extracts showed notable activity against *Pseudomonas aeruginosa* with zones of inhibition of between 24-32% that of gentamicin. Against the three bacterial strains, the zone of inhibition of the decoction ranged from 20-35% that of gentamicin. The extracts and isolated compounds exhibited good antifungal activity. The antifungal activity of the decoction was 86.8% that of nystatin. Lupeol and lupenone both had >30% the activity of gentamicin against the three bacterial strains. Lupeol showed greater activity than lupenone in all cases. The zones of inhibition of extracts and isolated compounds are shown in table 1.

Table 1: Zones of inhibition of extracts and isolated compounds

Test substances	Diameters of zones of inhibition (cm)			
	S. aureus	*P. aeruginosa*	*E. coli*	*S. cerevisiae*
	D (% of std)	D (% of std)	D (% of std)	D (% of std)
Decoction	0.8 (34.8)	0.8 (32.0)	0.8 (20.4)	1.3 (86.8)
Methanol extract	0 (-)	0.6 (24.0)	0 (-)	1.1 (73.3)
Chloroform extract	0.7 (30.4)	0.7 (28.0)	0 (-)	1.2 (80.0)
Lupenone	0.7 (30.4)	0.9 (36.0)	0.7 (33.3)	0.9 (60.0)
Lupeol	0.9 (39.1)	1.2 (48.0)	1.0 (47.6)	1.0 (66.7)
Gentamicin	2.3 (100)	2.5 (100)	2.1 (100)	-
Nystatin	-	-	-	1.5 (100)

D = diameter of the zone of inhibition less the 0.3 cm diameter of the well; % of std= D of test/D of std × 100%; a zone of inhibition of 0 cm implies no activity; - = Test not done.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support received from the Department of Pharmaceutical Chemistry of the University of Nairobi. In particular, the support of Mr. Hannington Mugo during the antimicrobial screening is appreciated. For the NMR and MS analysis, we thank Prof Kelly Chibale of the Department of Chemistry at the University of Cape Town.

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