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16 α Hydroxy-(-)-kauran-19-oic Acid: An Antibacterial Diterpene from Sweet Apple (*Annona squamosa* L., Annonaceae)

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Abstract. The methanolic extract of sweet apple (*Annona squamosa* L.) showed a good antibacterial activity against both ATCC and clinical strains of *Staphylococcus aureus* and *Streptococcus pneumoniae*. 16 α Hydroxy-(-)-kauran-19-oic acid, was identified as the compound responsible for the antibacterial activity of this plant.

Key words. Antibacterial, kaurane, diterpenes

INTRODUCTION

Annona squamosa L. (Annonaceae) is a tropical tree which grows to a height of 6 m. The leaves are simple, alternate, exstipulate and thick in texture. The plant is cultivated throughout the tropical world for its fruit: the sweet apple^[1]. Sweet apple is known to contain acetogenins, alkaloids and kaurane diterpenes^[2-4].

MATERIALS AND METHODS

Plant material: The fruits were collected from Kuala Kangsar, Perak State, Malaysia in June 2004. The plant material was identified by comparison with specimens available at the Herbarium of the Forest Research Institute, Kepong, Malaysia. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacy, University of Malaya for future reference.

Extraction: Finely powdered air-dried sweet apples (1 kg) were extracted with methanol using a soxhlet apparatus. The liquid extract obtained was concentrated with a rotary evaporator and brought to complete dryness over water bath to yield the crude extracts (yield 4.58). The extract was screened for antibacterial activity using the method described under the section-antibacterial assay. Bioassay-guided chromatography of the methanol extract yielded a colorless crystal, the spectral data of which correspond to those reported for 16 α hydroxy-(-)-kauran-19-oic acid^[5].

16 α hydroxy-(-)-kauran-19-oic acid^[1] was obtained as colorless needles [CHCl₃]; mp 1H NMR [CDCl₃, 400 MHz]

δ 11.0 [1H, s, COOH], 1.75 [2H, m, H-3], 1.72 [1H, m, H-5], 1.60 [2H, s, H-15], 1.52 [2H, m, H-6, H-11, H-12,], 1.50 [1H, m, H-13] 1.49 [2H, m, H-1, H-2, H-7], 1.43 [2H, d, H14], 1.39 [1H, m, H-5a], 1.31 [3H, s, H-17], 1.26 [3H, s, H-18]. 13C NMR [CDCl₃, 100 MHz] δ 181.79 [C, C-19], 67.1 [C, C-16], 58.6 [CH₂, C-15], 49.8 [CH, C-13], 46.7 [C, C-4], 43.5 [C, C-8], 39.3 [CH₂, C-1], 37.7 [CH₂, C-14], 37.4 [CH₂, C-3, C-7], 36.9 [C, C-10], 25.5 [CH₂, C-17], 23.3 [CH₂, C-12], 22.3 [CH₂, C-11], 21.3 [CH₂, C-18], 20.8 [CH₂, C-20], 19.8 [CH₂, C-2], 19.2 [CH₂, C-6].

Antibacterial assay: Both gram-positive and gram-negative bacteria (Table 1) were obtained from the stock cultures of the Department of Medical Microbiology in University Malaya. The crude methanol extract of sweet apple and 16 α hydroxy-(-)-kauran-19-oic acid were subjected to antimicrobial assay using the disc diffusion method of Bauer *et al.*^[6]. The organisms used were of the American Typed Culture Collections (ATCC) and some isolates. The organisms included *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619 and clinical strains obtained from the University Hospital. Mueller-Hinton agar was prepared according to the manufacturer's instruction. It was dispensed into sterile plates in 20 mL aliquots. After gelling and drying, the plates were seeded with appropriate organisms by streaking evenly in 3 planes onto the surface of the medium with cotton swab. The inoculum was allowed 5 min to dry. Sterile filter paper disks (6 mm diameter) soaked with 50 μ L of extract (100 mg mL⁻¹) were placed

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onto the agar with flamed forceps and gently pressed down to ensure contact. The plates were incubated at 37°C for 24 h. The zones of inhibition were measured with a ruler. The experiment was carried out in triplicates.

The broth microdilution method described by the National Committee for Clinical Laboratory Standards Control was used to determine the minimum inhibitory concentrations^[7]. Material to be tested was dissolved in 25% dimethylsulfoxide, 25% Tween 80 and 50% sterile distilled water. Two fold serial dilutions from 64 to 0.0156 mg mL⁻¹ were prepared in 96-wells plates.

RESULTS AND DISCUSSION

Results obtained for antibacterial activity of the crude methanol extract of sweet apple and 16 α hydroxy-(-)-kauran-19-oic are reported in Table 1 and 2. Analysis of the data revealed that 16 α hydroxy-(-)-kauran-19-oic exhibited a good antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* (Fig. 1 and 2). This could be a source of new antibacterial compound which could be used as food preservative and as an antibiotic.

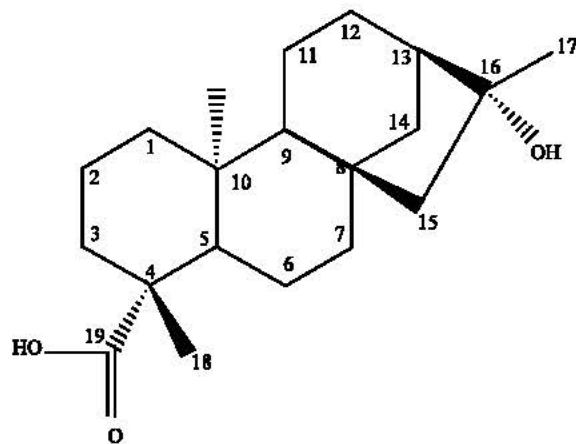


Fig. 1: 16 α hydroxy-(-)-kauran-19-oic acid

Table 1: Qualitative antibacterial activity of the methanolic extract and 16 α hydroxy-(-)-kauran-19-oic acid^a

Bacteria	M	[1]	V
<i>Staphylococcus aureus</i> ATCC 25923	14	21	15
<i>Staphylococcus aureus</i> ATCC 29213	13	20	14
<i>Staphylococcus aureus</i> (Clinical strain MSSA)	14	21	15
<i>Staphylococcus aureus</i> (Clinical strain MRSA)	13	21	14
<i>Streptococcus pneumoniae</i> ATCC 49619	13	20	13

Zone of inhibition, including the diameter of the filter paper disc [6 mm] and an average of triplicate. M = methanolic extract (5 mg/disc); (1)= 16 α hydroxy-(-)-kauran-19-oic acid (5 mg/disc); V= Vancomycin (5 μ g/disc)

Table 2: Quantitative antibacterial activity of the methanolic extract and 16 α hydroxy-(-)-kauran-19-oic acid^a

	M	[1]	V
<i>Staphylococcus aureus</i> ATCC 25923	0.25	15.6	1.2
<i>Staphylococcus aureus</i> Clinical strain MSSA [n = 10]	0.25	15.6	1.2
<i>Staphylococcus aureus</i> Clinical strain MRSA [n = 10]	0.25	15.6	1.2
<i>Streptococcus pneumoniae</i> Clinical strain [n = 10]	0.5	31.2	0.04

^a Minimum inhibitory concentration. M= methanolic extract (mg/mL); (1)= 16 α hydroxy-(-)-kauran-19-oic acid (mg/mL); V= Vancomycin (μ g/mL), n= number of strains tested

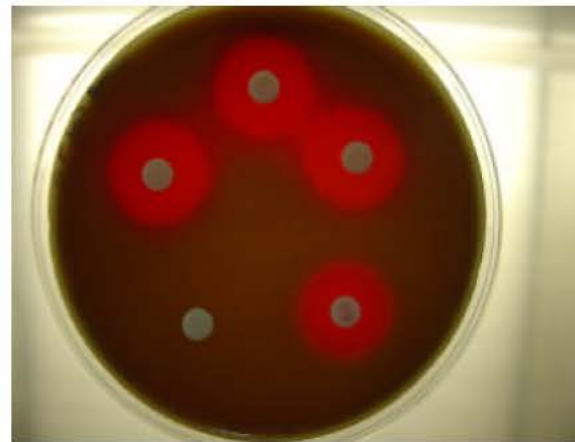


Fig. 2: Antibacterial activity of 16 α -hydroxy-(-)-kauran-19-oic acid against *Streptococcus pneumoniae* [ATCC49619]

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