ORIGINAL RESEARCH ARTICLE

Current Research on Biosciences and Biotechnology **2** (2) 2021 145-148

Current Research on Biosciences and Biotechnology

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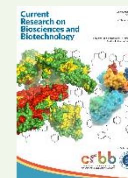

Antibacterial activity of lupeol from the bark of *Dehaasia cuneate* (Lauraceae)

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ABSTRACT

Dehaasia is a member Lauraceae. It is locally known as 'gajus hutan' or 'pekan'. A triterpenoid, lupeol was isolated from the bark of *Dehaasia cuneate.* The structure of the isolated compound was determined using spectroscopic methods, such as UV–vis, FT-IR, 1D and 2D NMR, and mass spectrometer. The isolated compound was tested against Gram-negative and positive bacteria using agar disc diffusion technique. The results showed that lupeol had a moderate inhibition zone value of 10.0±0.00 mm against Gram-negative *Serratia marcescens* ATCC 14756 whereas low inhibition which is 7.0±0.00 mm against *Escherichia coli* ATCC 25922, *Vibrio fluvialis* ATCC 33809, *Bacillus subtilis* ATCC 6633, and Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300.

Article history:
Received 21 Jan 2021
Revised 19 Feb 2021
Accepted 22 Feb 2021
Available online 28 Feb 2021

Keywords:
Dehaasia cuneate
lupeol
antibacterial activity
plausible pathway

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DOI: 10.5614/crbb.2021.2.2/BOFY6724

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1. Introduction

Dehaasia is a member of the Lauraceae family (Burkill, 1935). Blume (1837) reported that *Dehaasia* consists of four species namely *D. cuneata* Blume, *D. elongata* Blume, *D. media* Blume and *D. microcarpa* Blume. The plant is locally known as 'gajus hutan' or 'pekan', and its timber is durable and used for building houses (Hsuen,1969).

The chemical constituents of Lauraceae include neolignans, alkaloids, sesquiterpenes, flavonoids lignans, butanolides, benzopyrans, steroids, alkylphenols, arylpropene, coumarins, ester, and saponin (Salleh and Ahmad, 2017). Previous studies reported some species of the Lauraceae exhibiting useful biological activities, such as antisplasmodic, antipyretic, antitumor, anticonvulsant, antibacterial, fungicidal, cytotoxic, cruzain inhibitory and antiviral (Zahari et al, 2014; Oliveira et al, 2015).

In our recent study, we have successfully isolated a triterpenoid from the bark of D. *cuneate* and performed an antibacterial test against four Gram–negative and four Gram–positive bacteria. A plausible biogenetic pathway for the formation of isolated compound leading to Ruzicka's biogenetic terpene rule is also described in this study.

2. Materials and methods

2.1. General procedures

Several spectroscopic methods were used to elucidate the structures of lupeol (1). Ultraviolet (UV) and infrared (IR) spectra were recorded in an absolute methanol solvent using Shidmadzu UV-160 (FST, UKM) and FT-IR (ATR) Perkin-Elmer Spectrum 400 (CRIM, UKM), respectively. 1H and 13C NMR spectra were recorded at 600 MHz and 150 MHz, respectively, using a Bruker NMR 700 MHz cryo-probe (CRIM, UKM). The chemical shifts (δ) were determined from residual solvent peaks. ESIMS was determined using a Bruker (Micro ToF-Q) mass spectrometer. Melting points was determined using a hot stage melting point apparatus equipped with a microscope, XSP-12 model 500X, and was uncorrected. Column and gel permeation chromatography were performed using silica gel (Merck 7734, Merck 9385, and Merck 7749, Germany). Thin-layer chromatography, aluminium sheets 20 × 20 cm of the silica gel 60 F254 of 0.25 mm thickness (art. no. 5554, Merck, Germany) with detection accomplished by visualizing with a UV lamp at 254 and 366 nm.

2.2. Plant Material

The plant material was collected from Fraser's Hill (elevation 1000 M from sea level) in Peninsular Malaysia. The voucher

specimen (UKMB 40311) was deposited at the Herbarium of Universiti Kebangsaan Malaysia, Bangi (UKM).

2.3. Extraction and isolation

The bark was washed and cut into small pieces and air dried. The dried samples were weighed (1.1 kg), grounded to powder, and then extracted (three times each) with methanol (MeOH). The crude extracts were then filtered and evaporated under reduced pressure at 45 °C using a rotary evaporator. Eighty grams of the extract was subjected to vacuum liquid chromatography on a column (80 mm id x 500 mm) of silica gel (7734). The sample was eluted using n-hexane/ethyl acetate (EtOAc) (in ascending polarity manner) and finally with MeOH to afford 10 fractions. Purification of fraction 6 was carried out using a radial chromatography (RC) with silica gel plate of 1 mm thickness eluted with 95:5 n-hexane-EtOAc in 5% polarity increment to yield compounds 1 (25 mg) (Rosandy et al. 2013; 2018).

2.4. Bioassay

The pure compound was assayed for antibacterial activity using disc diffusion methods which is the concentration of lupeol (1) 30 µg/disc as described by Sabri et al. (2017).

3. Results and discussion

3.1. NMR data of isolated compounds

Lupeol (1) was isolated as white crystal from fraction 3 with melting point of 215-216 ºC, lit. 215-216 ºC (Reynolds et al., 1986). The mass spectrum of this compound showed molecular ion peak at m/z 426 and the molecular formula for this compound is $C_{30}H_{50}O$. The FTIR spectrum shows a broad absorbance peak at vmax of 3367 cm^{-1} indicating the stretching vibration of O-H bond. The C_{sp3}-H stretching bands appear at 2943 and 2870 cm^{-1}. The 1H NMR spectrum of this compound indicated that the compound is a triterpene in nature, due to its characteristic of methyl and methylene 1H chemical shifts. The 30 carbon signals observed in the 13C NMR spectrum were characterized by a DEPT experiment, which indicated that compound was a triterpene having seven methyls, eleven methylenes, six methines and six quaternary carbons. The chemical shifts of one methine carbon signal δH 3.21 (1H, *dd*, *J* = 4.5, 11.5 Hz, δC 79.0 ppm) and one quaternary carbon signal at δC 151.0 ppm suggested the presence of one hydroxyl group and an unsaturated carbon system In the HMBC spectrum revealed the correlation between the (H-23) and (H-24) to δC 38.8 (C-4) and δC 79.0 (C-3), and the correlation between the (H-30) and δC 151.0 (C-20), δC 109.3 (C-29) and δC 47.9 (C-19). In the correlations of H-H COSY explain that the carbons and the protons are in the correct positions (Fig. 1). The 3J coupling appears between (H-2) and (H-3) in the COSY spectrum, as well as between the (H-5) and (H-6), confirming the position of the protons in the molecule, in addition to comparison with literature data (Reynolds et al., 1986). Lupeol was isolated from many plants in the Lauraceae such as *Neolitsea sericea* (Cao et al., 2015), *Beilschmiedia glabra* (Lenta et al., 2015) and *B. erythrophloia* (Salleh and Ahmad, 2017). In addition, it was isolated from other families such as *Ficus pseudopalma* Blanco (Santiago and Mayor, 2014) and *Scapium macropodum* (Al Muqarrabun et al., 2014). It was first isolated in 1989 from *Lupinus luteus* (Barcelóñ and Muñoz, 1889).

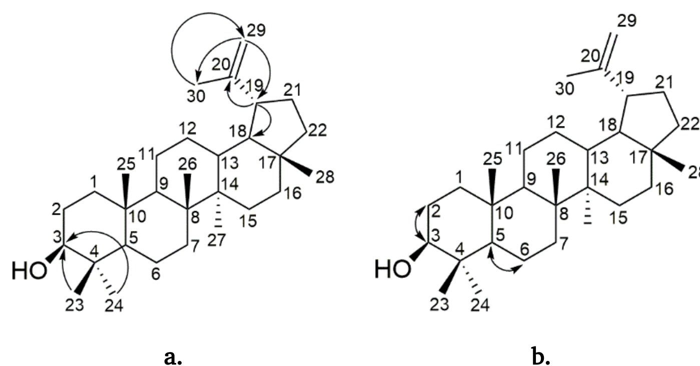

Fig. 1. Correlations of lupeol (1), (a) HMBC and (b) COSY

3.2. Antibacterial activity

Lupeol was tested for antibacterial activity. Using disc methods, lupeol showed low inhibition which is 7.0±0.00 mm against *E. coli* ATCC 25922, *V. fluvialis* ATCC, *B. subtilis* and MRSA whereas this compound exhibited moderate inhibition (4+) against *S. marcescens*. Based on the results, it was observed that lupeol has inhibition against one Gram-negative and two Gram-positive bacteria. This is probably due to the morphological differences between Gram-negative and positive bacteria in terms of their cell wall composition. The Gram-positive bacteria only have an outer peptidoglycan which is not effective permeability barrier compared to Gram-negative bacteria that have both outer membrane and peptidoglycan. This makes the Gram-negative bacteria cell wall impermeable to most of the drugs (Sabri et al., 2017). The inhibitory zone values for all compounds are shown in Table 1.

Table 1. Antibacterial activity of the Lupeol (1) (30 µg/disc) in disc diffusion method

Bacteria	Inhibitory zone (mm) of lupeol (1)	K* and V**
Escherichia coli ATCC 25922 *	7.0 ± 0.0	20 ± 1.00 (K)
Serratia marcescens ATCC 14756*	10.0 ± 0.0	23 ± 1.52 (K)
Vibrio fluvialis ATCC 33809*	7.0 ± 0.0	35 ± 1.04 (K)
Vibrio cholera ATCC 39315*	-	10 ± 0.00 (K)
Bacillus subtilis ATCC 6633 **	7.0 ± 0.0	28 ± 0.00 (V)
Staphylococcus aureus ATCC 25923 **	-	25 ± 0.00 (V)
Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300 **	7.0 ± 0.0	20 ± 0.00 (V)
Bacillus thruriengiensis ATCC 10792**	-	22 ± 0.00 (V)
DMSO	6.0 ± 0.0	-

*Gram negative, **Gram positive, Diameter of disc = 6 mm, DMSO (negative control), K* (Kanamycin) 30 µg/disc (positive control of Gram-negative) and V** (Vancomycin) 30 µg/disc (positive control of Gram-positive)

3.3. Plausible biogenetic pathway

A triterpene, lupeol (1) is built from a simple molecule of isopentenylpyrophosphate (IPP) reacting with its allyl isomer, dimethylallylpyrophosphate (DMAPP), to form geranylpyrophosphate (GPP) catalysed by prenyltranferase. The GPP then reacts with another IPP to give farnesyl pyrophosphate (FPP). The reaction was catalyzed by farnesylpyrophosphate synthase (FPS). The squalene synthase mediates the tail-to-tail dimerization of two molecules of FPP to form squalene, which is then oxidized into 2,3-oxidosqualene by squalene epoxidase (SQE). Intramolecule cyclization occurs within the molecule, driven by enzyme oxidosqualene cyclase (OSC) forming dammarenyl cation, which then undergoes rearrangement reaction to form bacharenyl cation. A further electrophilic addition of bacharenyl cation results in lupanyl cation, which is converted to lupeol (1) by deprotonation of the methyl group (C-29) by lupeol synthase (LUS) (Gallo and Sarachine, 2009; Phillips et al., 2006). A plausible biogenetic pathway from lupeol is shown in Fig. 2.

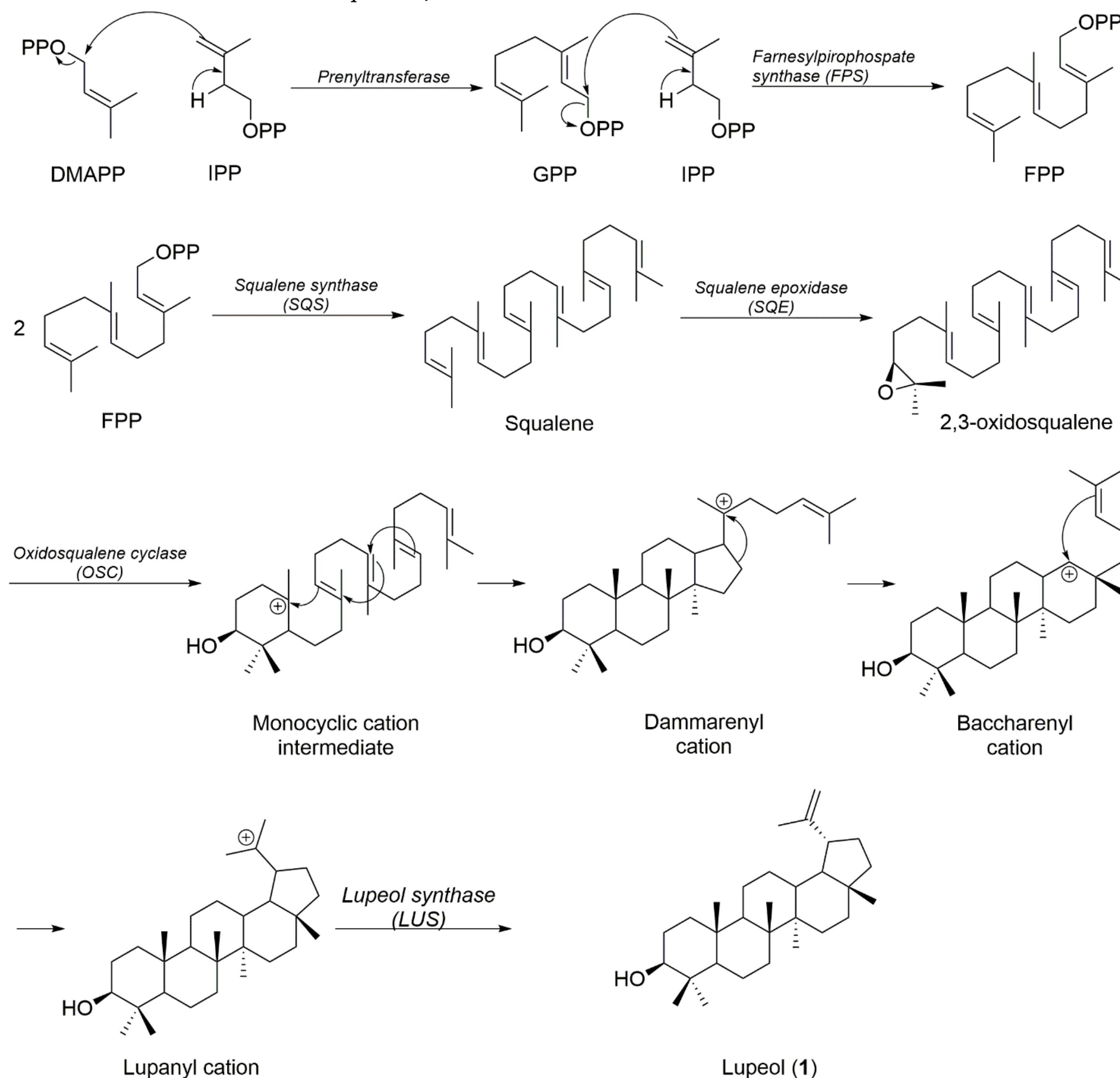

Fig. 2. A plausible biogenetic pathway of lupeol

4. Conclusion

The phytochemical investigation of the bark of *Dehaasia cuneate* resulted in the isolation of a known triterpenoid named lupeol and reported for the first time from this plant. The results of the antibacterial activity test showed that lupeol had a low inhibition zone value of 7.0±0.00 mm against *Escherichia coli, Vibrio fluvialis, Bacillus subtilis,* and methicillin-resistant *Staphylococcus aureus* (MRSA).

Acknowledgement

Authors would like to thank the Ministry of Education, Malaysia and Universiti Kebangsaan Malaysia (UKM) for the financial support under DLP-2013-018 grant and "Program Pelajar Cemerlang (PPC)" scholarship under Public Service Department, Malaysia. Special thanks to School of Chemical Sciences and Food Technology, Faculty of Science and Technology, UKM and Centre for Research and Innovation Management (CRIM) UKM for technical support on NMR, LC-MSToF, UV and FTIR instrumentation.

Conflict of interest

No potential conflict of interest was reported by the author.

NMR Data

Lupeol (1)

Form: White crystal

MP: 215-216 ^{o}C.

IR ν max (cm-1): 3367 (stretching vibration of O-H bond), 1040 vibration band of C-O bending), 761 (out-of-plane bending vibration of O-H), 2943 and 2870 (Csp3-H). 1457 (bending vibrations of Csp3-H, asymmetrical) and 1382 (symmetrical), 720 (methylene rocking bending), 1641 (olefinic, symmetrical C=C), 3072 (vibration of Csp2-H) and 882 (stretching of of Csp2-H).

UV λ max (MeOH) nm (log ε): 203.53 nm.

ESIMS [M+Na]+ m/ z 449.2842

1H NMR (Chloroform-d, 500 MHz) δ H: 1.70 (3H, s, H-30), 4.59 (1H, s, H-29a) and 4.71 (1H, s, H-29b), 0.81 (3H, s, H-28), 0.96 (3H, s, H-27), 1.05 (3H, s, H-26), 0.85 (3H, s, H-25), 0.78 (3H, s, H-24), 0.99 (3H, s, H-23), 1.41 (1H, m, H-22), 1.33 (1H, m, H-21a) and 1.94 (1H, m, H-21b), 1.36 (1H, m, H-18), 1.38 (1H, m, H-16a) and 1.49 (1H, m, H-16b), 1.68 (1H, m, H-15), 1.68 (1H, m, H-13), 1.68 (1H, m, H-12), 1.40 (2H, m, H-11), 1.26 (1H, m, H-9), 1.38 (2H, m, H-7), 1.39 (1H, m, H-6a) and 1.54 (1H, m, H-6b), 0.69 (1H, d, H-5), 3.21 (1H, dd, J = 4.5, 11.5 Hz, H-3), 1.54 (1H, m, H-2), 0.90 (1H, m, H-1a) and 1.68 (1H, m, H-1b)

13C NMR (Chloroform-d, 125 MHz) δ H: 19.3 (C-30), 109.3 (C-29), 18.0 (C-28), 14.5 (C-27), 15.9 (C-26), 16.1 (C-25), 15.4 (C-24), 27.9 (C-23), 39.9 (C-22), 29.8 (C-21), 151.0 (C-20), 47.9 (C-19), 48.2 (C-18), 43.0 (C-17), 35.5 (C-16), 27.4 (C-15), 42.8 (C-14), 38.0 (C-13), 25.1 (C-12), 20.9 (C-11), 37.1 (C-10), 50.4 (C-9), 40.8 (C-8), 34.2 (C-7), 18.3 (C-6), 55.2 (C-5), 38.8 (C-4), 79.0 (C-3), 27.4 (C-2), 38.6 (C-1)

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