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

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ABSTRACT

Dehaasia cuneata, locally known in Malaysia as 'gajus hutan or pekan', belongs to family Lauraceae. From its bark, A triterpenoid, lupeol was isolated and characterized. The chemical structure of the isolated compound was determined using spectroscopic methods, such as UV–vis, FT-IR, 1D & 2D-NMR, and ESIMS 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.

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1. Introduction

Dehaasia cuneata belongs to Lauraceae family (Burkill, 1935). This plant is also known as 'gajus hutan' or 'pekan' in Malaysia (Hsuen,1969).

The chemical constituents of Lauraceae include neolignans, alkaloids, lignans, sesquiterpenes, flavonoids, butanolides, benzopyrans, saponin, arylpropene, alkylphenols, coumarins, ester, and steroids (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

Determination of lupeol (**1**) structure were done using several spectroscopic methods. Ultraviolet (UV) spectrum recorded in absolute methanol using Shidmadzu UV-160 (FST, UKM). Infrared (IR) experiment was conducted at CRIM, UKM using ATR method on FTIR Perkin-Elmer Spectrum 400. ^{1}H and ^{13}C NMR were reordered using Bruker NMR 700 MHz cryo-probe (CRIM, UKM) at 700 and 175 MHz respectively. Mass spectrometry data (ESIMS) was obtained using Bruker (Micro ToF-Q). Melting points was measured using XSP-12 model 500X and was uncorrected. were performed using silica gels (Merck 7734, Merck 9385, and Merck 7749, Germany) were utilized in Column and gel permeation chromatography. 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

Dehaasia cuneata bark 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 cleaned with water and cut into small pieces. The sample was then air-dried to give 1.1 kg of dry sample which was then turn into powder powder using grinder. Methanol (MeOH) was used for extraction. After filtration, the extract was dried in *vacuo* at 45 °C to give crude extract. Fractionation of the extract was carried out with vacuum liquid chromatography (VLC) using a column (80 mm id x 500 mm) of silica gel (7734), to which 80 grams of the crude extract was subjected. The mobile phase used was n-hexane/ethyl acetate (EtOAc) with increasing polarity. Radial chromatography (RC) was utilized for purification process, using 95:5 n-hexane-EtOAc in 5% polarity increment to yield compounds 1 (25 mg) (Rosandy et al. 2013; 2018).

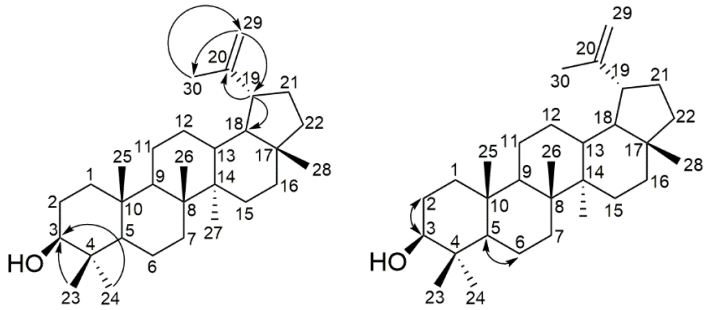
2.4. Bioassay

The pure compound was assayed for antibacterial activity using disc diffusion methods in which the concentration of lupeol (**1**) was 30 µg/disc (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 °C, lit. 215-216 °C (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 νmax 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 ^{1}H chemical shifts. The ^{13}C-NMR spectrum showed that 30 carbon signals, while from the DEPT-135 experiment, it was revealed that the compound comprised seven methyls, eleven methylenes, six methines. A methine carbon signal at δ_H 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, respectively. The HMBC spectrum revealed the correlation between H-23 and H-24 to C-4 (38.8) and C-3 (79.0), and the correlation between the (H-30) and C-20 (151.0), C-29 (109.3), and C-19 (47.9). H-H COSY experiment indicated that the carbons and the protons are in the correct positions (Fig. 1). The ^{3}J 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. These results were in accordance to literature (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).



a. **b.**

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 a poor 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.

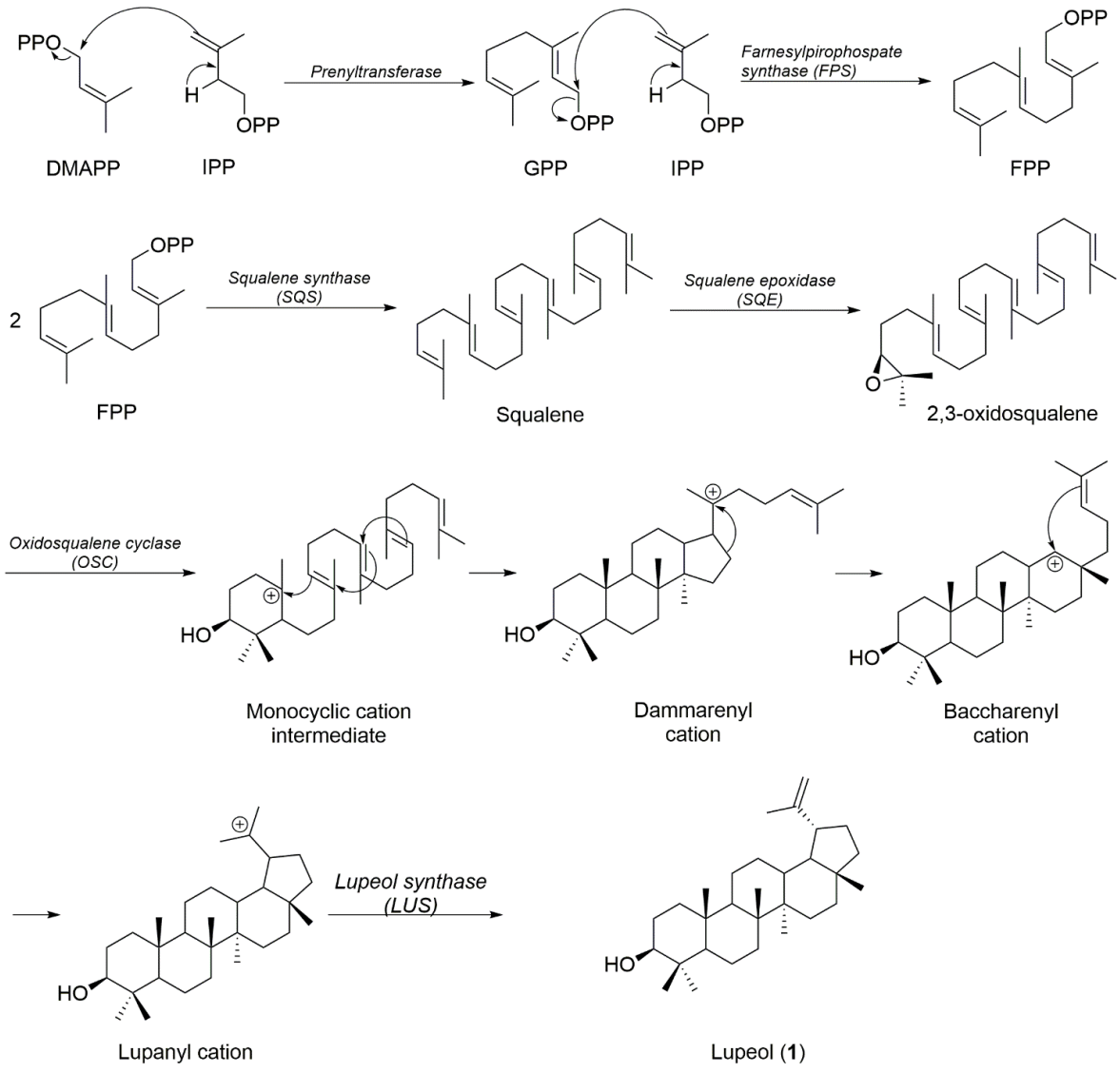


Fig. 2. A plausible biogenetic pathway of lupeol

4. Conclusion

The phytochemical investigation of the bark of *Dehaasia cuneata* resulted a known triterpenoid named lupeol. 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). To the best our knowledge, this compound was reported for the first time from this plant.

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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 (O-H bond), 1040 (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

^{1}H 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)

^{13}C 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)

References

Al Muqarrabun LMR, Ahmat N, Aris SRS, Shamsulrijal N, Baharum SN, Ahmad R, Rosandy AR, Suratman MN, Takayama H. 2014. A new sesquiterpenoid from *Scaphium macropodum* (Miq.) Beumee. *Nat Prod Res* 28(9): 597–605. doi: 10.1080/14786419.2014.886211

Barcelóñ AR, Muñoz R. 1989. Epigenetic control of a cell wall scopoletin peroxidase by lupisoflavone in *Lupinus*. *Phytochemistry* 28(5), 1331–3. doi: 10.1016/S0031-9422(00)97740-9

Blume CL. 1837. Rumphia 1. Sulpke, Leiden, Amsterdam, etc.

Burkill IH. 1935. A dictionary of the economic products of the Malay peninsula (1st Ed.). London: Crown Agents for the Colonies.

Cao Y, Gao X-L, Su G-Z, Yu X-L, Tu P-F, Cha X-Y. 2015. The genus *Neolitsea* of Lauraceae: a phytochemical and biological progress. *Chem Biodivers* 12(10): 1443-65. doi: 10.1002/cbdv.201400084

Gallo MB, Sarachine MJ. 2009. Biological activities of lupeol. *Int J Biomed Pharm Sci – Global Science Books* 3(1): 46-66.

Hsuen K. 1969. Orders and families of Malayan seed plants. Kuala Lumpur, Malaysia: University of Malaya.

Lenta BN, Chouna JR, Nkeng-Efouet PA, Sewald N. 2015. Endiandric acid derivatives and other constituents of plants from the genera *Beilschmiedia* and *Endiandra* (Lauraceae). *Biomolecules* 5(2): 910-42. doi: 10.3390/biom5020910

Oliveira Filho AA, Fernandes HMB, Assis TJCF. 2015. Lauraceae's family: a brief review of cardiovascular effects. *IJPPR* 7(1): 22-6.

Phillips DR, Rasbery JM, Bartel B, Matsuda SP. 2006. Biosynthetic diversity in plant triterpene cyclization. *Curr Opin Plant Biol* 9(3): 305–14. doi: 10.1016/j.pbi.2006.03.004

Reynolds WF, McLean S, Poplawaki J, Enriquez RG, Escobar LI, Leon I. 1986. Total assignment of 13C and 1H spectra of three isomeric triterpenol derivatives by 2D NMR: an investigation of the potential utility of ^{1}H chemical shifts in structural investigation of complex natural products. *Tetrahedron* 42(13): 3419-28. doi: 10.1016/S0040-4020(01)87309-9

Rosandy AR, Din LB, Yaacob WA, Yusoff NI, Sahidin I, Latip J, Baharum SN, Noor NM. 2013. Isolation and characterization of compounds from the stem bark of *Uvaria rufa* (Annonaceae). *Malaysian Journal of Analytical Sciences* 17(1): 50-8.

Rosandy AR, Ying YC, Kqueen CY, Lim SJ, Latip J, Murad AMA, Bakar MA, Khalid RM. 2018. (-)-Glaciantarcin, a new dipeptide and some secondary metabolites from the psychrophilic yeast *Glaciozyma antarctica* PI12. Sains Malaysiana 47(11): 2693–8. doi: 10.17576/jsm-2018-4711-12

Sabri NA, Ahmad WYW, Abdullah NS, Ibrahim N. 2017. The chemical constituents from the fruits of *Johannesteijsmannia altifrons* and antibacterial activity. *Malaysian Journal of Analytical Sciences* 21(2): 304-11. doi: 10.17576/mjas-2017-2102-05

Salleh WMNHW, Ahmad F. 2017. Phytochemistry and biological activities of the genus *Ocotea* (Lauraceae): a review on recent research results (2000-2016). *JAPS* 7(5): 204-18. doi: 10.7324/JAPS.2017.70534

Santiago LA, Mayor ABR. 2014. Lupeol: an antioxidant triterpene in *Ficus pseudopalma* Blanco (Moraceae). Asian Pac J Trop Biomed. 4(2): 109-18. doi: 10.1016/S2221-1691(14)60218-5

Zahari A, Cheah FK, Mohamad J, Sulaiman SN, Litaudon M, Leong KH, and Awang K. 2014. Antiplasmodial and antioxidant isoquinoline alkaloids from *Dehaasia longipedicellata*. *Planta Med* 80(7): 599-603. doi: 10.1055/s-0034-1368349