CYCLOARTANE TYPE TRITERPENOID ISOLATED FROM THE LEAVES *OF BRUCEA JAVANICA* (L.) MERR

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Abstract

Phytochemical investigation on the leaves of *Brucea javanica* (L.) Merr had been carried out. The powdered dried leaves were extracted with methanol using Soxhlet extraction method to get methanol crude extract. The methanol crude extract had been partitioned to get *n*-hexane, chloroform and ethyl acetate crude extracts. The *n*-hexane crude extract was fractionated and purified to afford 9,19-cyclolanost-24-en-3-acetate (**1**). The structure of this compound was elucidated on the basis of spectroscopic data and also comparison with data reported in the literature. Antioxidant test was carried out on the *n*-hexane crude extract and the pure compound using DPPH method. The result showed that both crude extract and pure compound were inactive as antioxidant agents.

Keywords: *Brucea javanica, Soxhlet extraction, antioxidant, DPPH*

INTRODUCTION

Brucea is a genus of Simaroubaceae that widely distributed in tropical Africa and Asia. The genus *Brucea* comprises of ten species including with very bitter monoecius or dioecious shrubs or small trees ranging from 0.3-1.0 m in height [1]. *Brucea* species is noted presence of various secondary metabolites such as terpenes, quassinoids and flavonoids which have chemistry and biological activity properties including antimicrobial, anti-inflammatory, insecticidal and cyctotoxic activitities [1-2].

Brucea javanica is the only one *Brucea* species found in Malaysia. This plant is locally known as 'lada pahit'and is an evergreen shrub with 1 to 3 m in height [1,3]. Within the scope of continuation search of the bioactive compound from natural plant, the leaves of *B. javanica* were investigated. In this paper, we report the isolation and structural elucidation of a compound **(1)**. In addition, the compound was evaluated for the antioxidant activity by DPPH free radical scavenging method [4].

EXPERIMENT

Vacuum liquid chromatography (VLC) and gravity column chromatography (CC) were carried out by using Merck silica gel 60 (230-400 mesh) and Merck silica gel 60 (70-230 mesh) respectively. Thin layer chromatography (TLC) was performed on 0.20 mm precoated silica gel aluminium sheets (Merck Kieselgel 60 F_{254}). TLC spots were visualized by UV light (254 nm and 365 nm) and sprayed with vanillin sulphuric acid reagent. The NMR (^{1}H and ^{13}C) spectra were recorded on Bruker Avance 400 spectrometer. Residual solvents were used as an internal standard. Infrared spectra were recorded on Perkin-Elmer series 1600 spectrophotometer as thin film (NaCl windows). Gas chromatography (GC) analysis was carried out on Hewlett Packard HP6890. GC-MS analysis was equipped with Wiley Library Software.

Plant Material

Brucea javanica was collected from Kuantan, Pahang in 2009. The leaves of *Brucea javanica* were separated from the plant and dried at room temperature. After dried, the leaves were ground using mill to form fine powdered samples.

Extraction of the Leaves of *Brucea javanica*

The powdered leaves of *Brucea javanica* (500 g) were extracted using soxhlet extractor with MeOH (3 L) as solvent for 18 hours. The MeOH crude extract was concentrated by rotary evaporator under reduced pressure to give methanol crude extract (100 g, 20%) as thick brown syrup. The MeOH crude extract (100 g) was suspended in mixture of methanol-water (300 ml, 9:1). Then it was partitioned with *n*-hexane (3 × 500 ml). The *n*-hexane layer was separated and evaporated to yield dark green *n*-hexane crude extract (23 g, 4.6%). The process was continued by adding distilled water (300 mL) to the defatted residue and partitioned again using $CHCl_3$ (3 × 1 L). The $CHCl_3$ crude extract (5.52 g, 5.5%) was then added with water containing 1% NaCl (3×1

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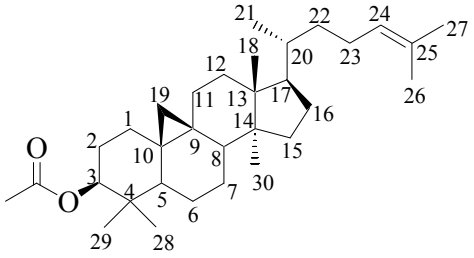
L) to give chloroform-soluble extract (4.7 g, 85.1 %). Finally, the chloroform-soluble extract was partitioned with EtOAc (3 × 1 L) to yield ethyl acetate crude extract (0.76 g, 16.2%).

Purification of the *n*-hexane crude extract

The *n*-hexane crude extract (23 g) was fractionated using VLC on a sintered funnel (10 cm × 9 cm) packed with Merck silica gel of 230-400 mesh. Combination of *n*-hexane – ethyl acetate with increasing polarity was used as the solvent system to afford 11 fractions. Fractions with similar TLC profile were combined to yield 7 major fractions, BJH1-BJH7. BJH4 (4.7 g) was subjected to silica gel CC to yield compound **(1)** (0.96 g, 22.2%) as pale yellow liquid with R_f value of 0.65 in *n*-hexane: EtOAc (4:1). IR ν_{max} (film) cm^{-1} : 2931 (sp^3 C-H), 1733 (C=O, ester), 1643 (olefinic C=C), 1465 and 1377 (CH_3 and CH_2 bending); ^1H NMR δ_H ($CDCl_3$): δ 5.11 (1H, *t*, *J*=7.2 Hz, H-24), 2.18 (6H, s, 2 × CH_3), 1.69 (1H, s, H-5), 1.62 (3H, s, H-27), 1.26 (8H ,m, 4 × CH_2), 0.97 (3H, s, H-18), 0.90 (12H, m, 6 × CH_2), 0.86 (3H, s, H-30), 0.58 (1H, *d*, *J*=4.0 Hz, H-19a) and 0.35 (1H, *d*, *J*=4.0 Hz, H-19b); ^{13}C NMR δ_C ($CDCl_3$): δ 171.1(C-31), 157.0 (C-4), 151.2 (C-25), 130.9 (C-13), 125.0 (C-3), 110.0 (C-2), 106.1(C-1), 100.1 (C-14), 81.0 (C-24), 55.3 (C-17), 52.2 (C-8), 50.3 (C-5), 45.2 (C-9), 39.5 (C-10), 36.5 (C-24) 36.1 (C-20) , 32.8 (C-6), 31.5 (C-7), 29.8 (C-16), 28.0 (C-15), 24.7 (C-11), 21.1 (C-12) and eight methyl groups at δ 22.0 (CH_3C=O), 18.7 (C-26, C-27), 17.6 (C-18, C-30), 14.9 (C-21) and 14.0 (C-29,C-28). GCMS: $[M]^+$ *m/z* 468, $C_{32}H_{52}O_2$.



(1)

DPPH Free Radical Scavenging Assay

A sample stock solution was prepared by diluting each sample (1.2 mg) in MeOH (1.2 mL). A DPPH methanolic solution which was purple in colour was prepared by diluting DPPH (8.3 mg) in methanol (8.3 mL).The sample solution (0.5 mL) was added to the solution of DPPH (0.5 mL) in a test tube and allowed to react at room temperature for 30 minutes. The sample was considered active if the colour of DPPH solution changes to yellow.

RESULTS AND DISCUSSION

Soxhlet extraction of dried leaves of *Brucea javanica* (500 g) using MeOH had afforded thick brown syrup of MeOH crude extract (100 g, 20%). Extraction of the MeOH crude extract by *n*-hexane, $CHCl_3$ and EtOAc had afforded the *n*-hexane, $CHCl_3$-soluble and EtOAc crude extracts with percentage of yield 4.6%, 5.5%, 85.1%, and 16.2% respectively. Fractionation of the *n*-hexane crude extract (23 g, 4.6%) using VLC yielded 7 major fractions (BJH1 – BJH7). Purification of BJH4 (4.7 g) by CC gave compound (**1**) with R_f value of 0.65 in *n*-hexane: EtOAc (4:1). The IR spectrum of **(1)** exhibited a strong absorption band at 2931 cm^{-1} associated to sp^3 C-H stretching. A strong intensity absorption band was observed at frequency of 1733 cm^{-1} for C=O of an ester. Absorption band for olefinic C=C stretching was observed at 1643 cm^{-1}. Two absorption bands at 1465 cm^{-1} and 1377 cm^{-1} was corresponding to CH_2 and CH_3 bending. The EIMS of **(1)** showed the molecular ion peak at *m/z* 468 which corresponding to molecular formula $C_{32}H_{52}O_2$.

The ^1H NMR spectrum of **(1)** showed a triplet signal resonated at δ 5.11 (1H, t, *J*=7.2 Hz) which was corresponded to the olefinic proton H-24. Two doublets appeared at δ 0.58 (1H, d, *J*=4.0 Hz) and 0.35 (1H, d, *J*=4.0 Hz) were assigned to the nonequivalent protons H-19a and H-19b respectively. These two doublets were the characteristic for cycloartane type triterpenoid. A singlet at δ 2.18 was attributable for the acetoxyl proton (CH_3C=O). Multiplets between δ 0.86- δ 2.18 were assigned to methyl protons H-11, H-12, H-18, H-21, H-26, H-27, H-28, H-29 and H-30. Multiplets between δ 0.90-δ 1.26 were attributable to H-1, H-2, H-6, H-7, H-11, H-12, H-15, H-16, H-22 and H-23 The chemical shift at Signals at δ 1.69 and 1.62 were attributable to H-17

and H-5 respectively. This is because they are located at the environment which does not have anisotropic effect. Based on the spectroscopic data and comparison with reported data [5], compound **(1)** was identified as 9,19-cyclolanost-24-en-3-acetate. Antioxidant test on the *n*-hexane crude extract and compound **(1)** revealed that both samples were very weak antioxidant agent.

CONCLUSION

Chemical investigation on the chemical constituent of the leaves of *B. javanica* had been carried out using soxhlet extraction with methanol as a solvent. Purification of the n-hexane crude extract had afforded a triterpenoid 9,19-cyclolanost-24-en-3-acetate (**1**).

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