STUDIES ON PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF THE FRUIT PEELS OF *LANSIUM DOMESTICUM* CORR.

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Abstract

Phytochemical investigation on the fruit peels of *Lansium domesticum* Corr. had furnished one pure compound identified as lupeol (**1**) together with mixtures of zierone (**2**), cadinol (**3**), stigmasterol (**4**) and β-sitosterol (**5**). Structure of the pure compound was determined on the basis of spectroscopic data (^{1}H NMR, IR, GC and GCMS) and also by comparison with literature while structures of (**2**) – (**5**) were determined based on the GC and GCMS analysis. Antioxidant test was carried out to all crude extracts (*n*-hexane, dichloromethane and ethyl acetate extracts) using DPPH free radical scavenging assay. The percentage values of scavenging activities showed that all crude extracts have weak antioxidant properties.

Keywords: Lansium domesticum, Meliaceae, triterpernoid, antioxidant, DPPH assay

INTRODUCTION

Meliaceae or Mahogany family is one of the members in flowering plants with medium-sized family. This family consists of about 50 genera and 575 species of trees and shrubs. About 15 genera and 91 species can be found in Peninsular Malaysia [1]. Genus *Lansium* has only one unique species which is *Lansium domesticum*. This genus of small to medium trees was widely cultivated in Malaysia, Thailand and other countries in Southern Asia [2].

Lansium. domesticum Corr. is a popular tropical plant producing economic edible fruits found mainly in Southeast Asia, especially in the south of Thailand [2-3]. It is a highly variable species, with different forms which has been classified by some taxonomist as distinct species. There are five basic varieties which are known as langsat, duku, dokong, rambai and kokosan but there may be intermediate forms with overlapping characteristics [4]. Duku are spheroid in shape with skin ripens to a greyish buff or pale muddy yellow colour with brown blemishes. It has thick peel, little to no latex, and a sweet, aromatic pulp. Duku pulp is white in colour and no translucent. Within the scope of continuation search for bioactive compounds from natural plants, the peels of *L. domesticum* were investigated. The peels of *L. domesticum* were reported to contain triterpenoids and phenolic compounds such as flavonoids. From the previous study, large constituents of triterpenoids have been found in the *L. domesticum* peels and seeds [5]. In this paper, we report the isolation and structural elucidation of one pure compound (**1**) and mixtures of four known compounds (**2**) – (**5**). In addition, the crude extracts were evaluated for their antioxidant activities by DPPH free radical scavenging assay.

EXPERIMENTAL

Vacuum liquid chromatography (VLC) and gravity column chromatography 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 F254). Spots on TLC were visualized by UV light (254 nm and 365 nm) and sprayed with vanillin sulphuric acid reagent. Melting points were measured using melting point apparatus equipped with microscope, Leica Gallen III and were uncorrected. The 400 MHz ^{1}H NMR 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) for liquid samples. Gas chromatography (GC) analysis was carried out on Hewlett Packard HP6890. GC-MS analysis was equipped with Wiley Library Software.

Plant Material

Fruits of *L. domesticum* (12 kg) were bought at Pasar Malam, Taman Seri Pulai, Johor (September 2013). The fruit peels were air-dried at room temperature and ground to form powdered samples.

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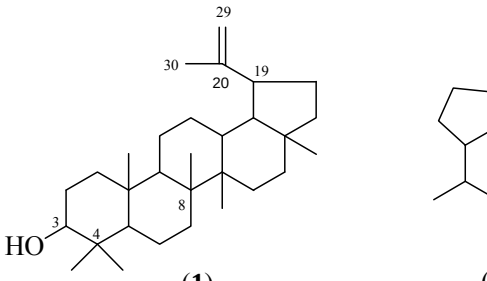
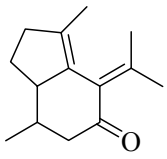
Extraction and Isolation

The powdered peels of *L. domesticum* (200 g) were extracted using cold extraction method with *n*-hexane, CH_2Cl_2 and EtOAc as the solvents at room temperature for 3 days each. Then the sample was filtered and the solvent was concentrated under reduced pressure using rotary evaporator to give *n*-hexane crude extract (18.58 g, 9.29%), CH_2Cl_2 crude extract (13.67 g, 6.84%) and EtOAc crude extract (1.89 g, 0.95%) as viscous dark brown gums.

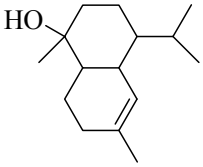
The CH_2Cl_2 crude extract (10.0 g) was fractionated by VLC on a sintered funnel (10 cm × 9 cm) packed with Merck silica gel of 230-400 mesh (250 g). The solvent system used was combination of *n*-hexane and EtOAc. The polarity was increased by 10%. A total of sixteen fractions were collected and each fraction was analyzed by TLC. Fractions which showed similar pattern of TLC profile were combined to yield six major fractions (LDPD1 - LDPD6). LDPD 5 (3.2 g) was subjected to Si gel column chromatography and eluted with *n*-hexane-EtOAc (3:2) to afford compound (**1**) (0.013 g, 0.13%) as white flakes. The *n*-hexane crude extract (5.0 g) was subjected to silica gel column chromatography and eluted with combination of *n*-hexane-EtOAc (4:1) to give 160 fractions. Fractions with similar TLC profiles were combined to give 13 major fractions labeled as LDPH 1- LDPH 13. Analysis of fractions LDPH 6 (3.3 mg), LDPH 7 (6.1 mg) and LDPH 9 (6.7 mg) by GC and GCMS showed that all fractions contained mixtures of compound (**2**) and (**3**). Analysis by GC and GCMS on LDPH 11 (10.7 mg) showed the presence of compound (**4**) and (**5**).



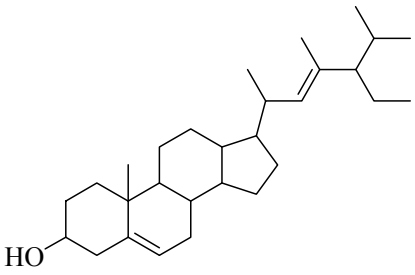
(**1**)



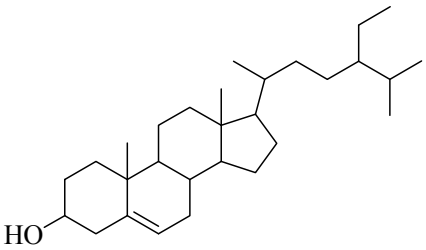
(**2**)



(**3**)



(**4**)



(**5**)

RESULTS AND DISCUSSION

Cold extraction of dried peels of *Lansium domesticum* (200 g) at room temperature using *n*-hexane, CH_2Cl_2 and EtOAc for 3 days each had afforded *n*-hexane crude extract (18.58 g, 9.29%), CH_2Cl_2 crude extract (13.67 g, 6.84%) and EtOAc crude extract (1.89 g, 0.95%).

Compound (**1**) was obtained from the CH_2Cl_2 crude extract as white flakes (0.013 g, 0.13%) with m.p. 215-216°C ([6] lit. 213-215°C). The TLC analysis with vanillin sulphuric acid spraying reagent revealed a purple spot suggesting a triterpenoid. The IR spectrum of compound (**1**) showed a broad absorption band at 3389 cm^{-1} which corresponded to the O-H bond. Absorption band at 2941 cm^{-1} showed the presence of sp^3 C-H stretching. Absorption band at 1639 cm^{-1} was attributed to the olefinic (-C=C-) stretching while absorption band at 1032cm^{-1} was due to C-O stretching. The ^{1}H NMR spectrum of (**1**) showed the presence of singlet signals attributable for seven tertiary methyl protons (H-23, H-24, H-25, H-26, H-27, H-28 and H-30) at chemical shifts between δ 0.76 - δ 1.69. A pair of broad singlets at δ 4.70 and δ 4.57 was due to the exomethylene protons, H-29. A multiplet signals at δ 2.38 was attributable to H-19. A doublet of doublet signal was observed at δ 3.19 (*J*

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= 11.2 Hz, 4.8 Hz) for H-3. The spectroscopic data of (**1**) were compared to literature [6] and hence, (**1**) was identified as lupeol.

Purification of the *n*-hexane crude extract (5.0 g, 2.5%) by gravity CC using Merck silica gel 70-230 (150.0 g) as stationary phase gave 160 fractions which were combined to give 16 fractions labeled as LDPH 1 to LDPH 16. Analysis of fractions LDPH 6 (3.33 mg), LDPH 7 (6.14 mg) and LDPH 9 (6.71 mg) by GC and GCMS showed that all fractions contained mixture of zierone (**2**) and cadinol (**3**). Analysis by GC and GCMS on LDPH 11 (10.73 mg) showed the presence of stigmasterol (**4**) and β-sitosterol (**5**). Antioxidant evaluation on the *n*-hexane, dichloromethane and ethyl acetate crude extracts using DPPH assay showed that all crude extracts have weak scavenging activity towards DPPH radicals.

CONCLUSION

Cold extraction of the fruits peels of *Lansium domesticum* yielded *n*-hexane, CH_2Cl_2 and EtOAc crude extracts. Purification of the CH_2Cl_2 extracts afforded lupeol (**1**) and mixtures of zierone (**2**), cadinol **(3**), stigmasterol (**4**) and β-sitosterol (**5**). All crude extracts showed weak antioxidant activity.

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