

Indole Alkaloids of *Leuconotis eugenifolius*

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ABSTRACT In this study the alkaloid content of Malaysian *Leuconotis eugenifolius* has been carried out. From the bark of *L. eugenifolius*, two indole alkaloids were isolated and identified as leuconolam and E-akuammidine. The crude extract also showed positive anti-plasmodial activity. The isolation of the alkaloids is achieved by chromatographic techniques and the structural elucidations were performed via spectral methods; namely NMR, MS, IR and UV.

ABSTRAK Kajian terhadap kandungan alkaloid pada pokok *Leuconotis eugenifolius* telah dijalankan. Pengasingan dan pengenalpastian dua alkaloid indol iaitu leuconolam dan E-akuammidin. Ekstrak mentah juga menunjukkan aktiviti antiplasmodial yang positif. Pengasingan alkaloid dicapai menggunakan teknik kromatografi dan pengenalpastian struktur menggunakan kaedah spektroskopi iaitu RMN, SJ, IR dan UV.

(*Leuconotis eugenifolius*, Apocynaceae, indole alkaloid)

INTRODUCTION

Leuconotis is a small genus of climbing shrubs of the family Apocynaceae. *Leuconotis eugenifolius*, D.C. is indigenous to Malaysia and Indonesia. In Peninsula Malaysia, it is restricted to the north-west region. Medicinally, its latex was once used for the treatment of yaws by applying it on the infected skin. It was also used to cure worm infection [1]. The study on *Leuconotis eugenifolius* was repeated because firstly, the plant sample was collected from a different site in Malaysia and secondly, the alkaloids were extracted from the bark with dichloromethane instead of methanol as previously reported [2, 3]. Thirdly, most importantly, this extract is found to exhibit antiplasmodial activity. Previous work on Apocynaceae plants of this region has shown wide diversity in their alkaloidal content including those with medicinal values. We identified the alkaloids from the bark of *L. eugenifolius* collected from a different site in Malaysia in order to compare with the previously reported results [3, 4].

EXPERIMENTAL METHODS

General Methods

All solvents, except those used for bulk extractions are AR grade. Glass and aluminium supported silica gel 60 F₂₅₄ plates were used for TLC and preparative TLC respectively. The plates were activated at 100°C for one hour and stored in a dessicator until needed. TLC spots were visualized under ultra-violet light (254 nm and 365 nm) followed by spraying with the Dragendorff's reagent for alkaloidal screening. Silica gel 60, 70-230 mesh ASTM (Merck 7734) and silica gel 60, 230-400 Mesh ASTM (Merck 9385) were used for column and flash chromatography, respectively. Mayer's reagent was used for alkaloid screening.

Spectroscopic methods

The optical rotations were recorded on Jasco (Japan) P1010 with tungsten lamp. HRMS was obtained on Automass Multi Thermofinnigan. The ultraviolet spectra were obtained in MeOH on Shimadzu UV-160A ultraviolet-visible spectrometer. IR spectra were taken on a Perkin Elmer 2000 Double-Beam recording spectrometer. The ¹H NMR spectra were

recorded in deuterated chloroform and/or methanol on a JEOL 400 MHz (unless stated otherwise); chemical shifts are reported in ppm on δ scale, and the coupling constants are given in Hz.

Plant material

The bark of *Leuconotis eugenifolius*, D.C. (Apocynaceae) were collected at Caruk Puyoh Forest Reserve, Sik, Kedah, Malaysia (May 12, 1993). Voucher specimen (KL 4240) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and isolation of the alkaloids

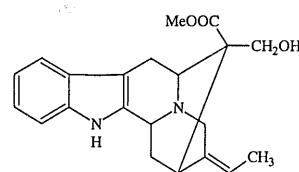
1 kg of the dried and milled bark of the plant was first defatted by soaking in petroleum ether for 24 hours. The petroleum extracts were evaporated to dryness. The residue (plant material) was dried and left overnight after moistening with 10% ammonia. They were then re-extracted with dichloromethane exhaustively by Soxhlet extractor for 17 hours. The CH_2Cl_2 extract were concentrated under reduced pressure to 500 ml and examined for its alkaloid content.

The dichloromethane extract was repeatedly extracted with 5% HCl until Mayer's test becomes negative. The aqueous solution obtained was basified with concentrated ammonia solution to ca. pH 11 and re-extracted with CH_2Cl_2 until Mayer's test was negative. This was followed by washing with distilled H_2O and dried over anhydrous sodium sulfate. Finally, the extract was concentrated to give crude alkaloids (10.1 g).

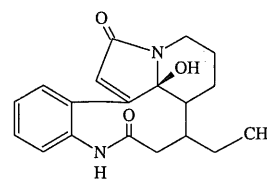
The crude alkaloid mixture was subjected to exhaustive column chromatography over silica gel using dichloromethane with increasing proportions of methanol and finally with pure methanol as eluants. At present, two alkaloids were isolated; *E*-akuammidine **1** (CH_2Cl_2 :MeOH 97:3) and leuconolam **2** (CH_2Cl_2 :MeOH 96:4). The structural elucidation was carried out by spectroscopic methods: 1D and 2D NMR, IR, UV and MS. The crude extract was screened for anti-malarial activity towards the chloroquine resistant isolate, Gombak A and the sensitive strain, D10 of *plasmodial falciparum* in vitro using the lactate dehydrogenase (LDH) assay [5].

RESULTS AND DISCUSSION

Further purification by a small column and preparative TLC (Silica gel 60 F₂₅₄) yielded *E*-akuammidine **1** (CH_2Cl_2 : MeOH 97:3) and 10 mg leuconolam **2** (CH_2Cl_2 : MeOH 96:4). The identify of both alkaloids were confirmed through comparison of their spectral data (1D and 2D NMR, IR, UV and MS) [6,7].



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The crude dichloromethane extract was also found to possess antiplasmodial activity: effective against the chloroquine resistant isolate, Gombak A (IC₅₀ of 1.1853 $\mu\text{g}/\text{ml}$) and the sensitive strain D10 (IC₅₀ of 1.6105 $\mu\text{g}/\text{ml}$).

CONCLUSION

Both compounds isolated *E*-akuammidine and leuconolam are known. However, the presence of akuammidine in *L. eugenifolius* has not been reported before. The method of extraction, location of the plant collected and part of plant studied led to slight differences in the alkaloids obtained. The crude dichloromethane extract of *L. eugenifolius* was screened for anti-malarial activity using the lactate dehydrogenase assay. It was found that the crude dichloromethane extract exhibited strong activity against both strains of parasites used: effective against the chloroquine resistant isolate, Gombak A (IC₅₀ of 1.1853 $\mu\text{g}/\text{ml}$) and the sensitive strain D10 (IC₅₀ of 1.6105 $\mu\text{g}/\text{ml}$).

Acknowledgements We gratefully acknowledge the financial support provided by University of Malaya (Vote F 0160/2002B).

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