

Indole Alkaloids from *Rauvolfia reflexa* (Apocynaceae)

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Abstract: From the methanolic crude extract of *Rauvolfia reflexa* six indole alkaloids were isolated namely; (1), akuammilan (2), vomilenine (3), isoreserpiline (4), rescinnamine (5), and cantleyine (6). The methanol crude extract showed moderate anticancer activity against MCF-7 and WRL-68 cell lines, although the isolated compounds and dichloromethane crude extract were also tested for anticancer activity but they didn't show significant activity against MCF-7 and WRL-86 cancer cell lines.

Keywords: Alkaloids, anti cancer activity, Apocynaceae, *Rauvolfia*, NMR.

INTRODUCTION

Bioactive indole alkaloids have been isolated from *Rauvolfia reflexa* (Apocynaceae). The chemistry of the *Rauvolfia* species has been comprehensively investigated for the presence of alkaloids over a long period of time [1]. First part of research program was on the occurrence of alkaloids in Malaysian species of *Rauvolfia*, a phytochemical analysis from bark of *Rauvolfia reflexa* is described. Six alkaloids were isolated; the structures of all alkaloids isolated were determined by a detailed analysis of the ¹H NMR, ¹³C NMR, HMBC, HSQC, NOESY, COSY spectral data and confirmed by UV, IR and MS. Second part of research was carried out In vitro studies of the crude extracts and pure compounds isolated from *Rauvolfia reflexa* which the methanolic crude extracts exhibited considerable anticancer Activity.

RESULTS AND DISCUSSION

Plant material was collected at Kelantan, and air-dried. The methanol crude extract, from the bark, were submitted to acid-base treatment and fractions corresponding to different pH ranges were purified by flash-chromatography, LH-20 sephadex and preparative TLC, leading to the isolation of six indole alkaloids (Fig. 1). Undulifoline (1) [2], akuammilan (2) [3], vomilenine (3) [4], isoreserpiline (4) [5], rescinnamine (5) [6], and cantleyine (6) [7] were confirmed by spectroscopic analysis.

GENERAL EXPERIMENTAL PROCEDURES

CC was run on silica gel 60 (40-63 μm). TLC was performed on aluminum and glass plates pre-coated with silica gel 60 F₂₅₄ (Merck). ¹H NMR and ¹³C NMR and 2D NMR spectra were determined in CDCl₃ (JEOL JNM-FX400), UV spectra were recorded on a Shimadzu UV-160A spectrophotometer using MeOH as solvent. MS was obtained

with Agilent 6530. The IR spectrum was measured by FT-IR: pelkin-elmer RX 1 (fourier transform infra-red) spectrometer for frequencies 4000-400 cm⁻¹.

COLLECTING OF PLANT MATERIAL

The plant materials (barks) of *Rauvolfia reflexa* (KL 4900) were collected from Kelantan The botanical identification was made by Mr.Teo Leong Eng, Faculty of Science, University of Malaya. Voucher specimens are deposited in the Herbarium of Chemistry Department, University of Malaya.

EXTRACTION AND ISOLATION

The extraction of the plant (2.0 kg) was carried out by extracted exhaustively with hexane for 48 hours to removed non-polar organic compound, waxes and fats. Then the extract was dried on the rotary evaporator. The plant material was dried and wetted with 10 % ammonia solution and left for overnight. They were then re-extracted successively with dichloromethane (CH₂Cl₂) and methanol (MeOH). After removal of the solvents, the hexane crude extract (1.2 g), dichloromethane (15 g) and methanol (15 g) were obtained.

MATERIALS AND METHODS

Cell Culture

In this study, all cells used were obtained from American Type Cell Collection (ATCC) and maintained in a 37°C incubator with 5% of CO₂ saturation. WRL-68 normal hepatic cells were maintained in Dulbecco's modified Eagle's medium (DMEM) whereas A549, non-small cell human lung adenocarcinoma epithelial cells; MCF-7, human breast adenocarcinoma cells and PC-3, prostate adenocarcinoma cells were maintained in Roswell Park Memorial Institution-1640 media (RPMI-1640). Both media were supplemented with 10% of fetal bovine serum (FBS) [8].

Cellular Viability

The above mentioned cell lines were used to determine the inhibitory effect of dichloromethane (CH₂Cl₂), methanol

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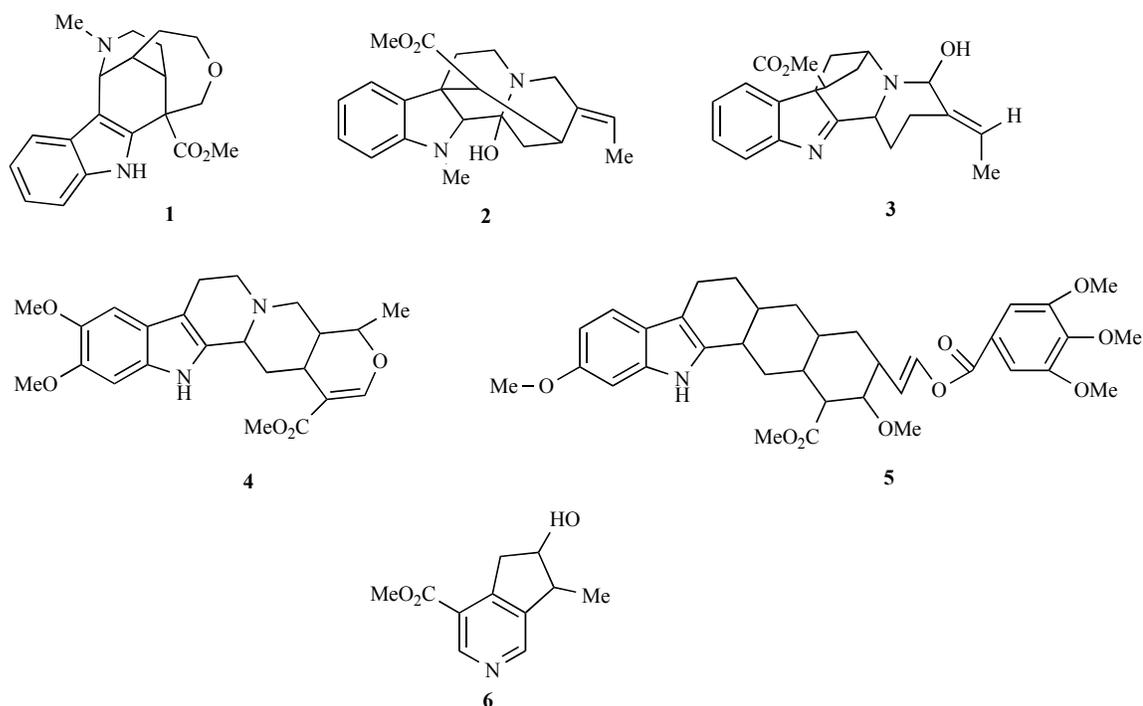


Fig. (1). Indole alkaloids isolated from the bark of *Rauvolfia reflexa*.

Table 1. Shows the Effect of Crude Extracts (MeOH, CH₂Cl₂, G25, Thymoquinone) on Two Cell Lines (MCF-7 and WRL68) Expressed as EC₅₀ Values in 24 Hours MTT Assay

EC ₅₀ ± S.D. (µg/ml)	Cell line	
	MCF-7	WRL-68
MeOH crude extract	> 200	12.51
CH ₂ Cl ₂ crude extract	20.10	11.95
G25	19.65	33.89
Thymoquinone	~ 5.106	~ 8.517

(MeOH) crude extracts compare with the standard compounds (G25 and Thymoquinone), on cell growth using MTT assay [9]. This calorimetric assay is based on the conversion of yellow tetrazolium bromide (MTT) to purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells. To measure cell viability, cells were seeded at a density of 1×10^5 cells/ml in a 96-well plate and incubated for 24 hours at 37°C with 5% of CO₂. Cells were then treated with the test agents in the next day and incubated for another 24 hours. After 24 hours, 10 µl of MTT solution at 5 mg/ml was added to each well and then the plates were incubated for another 4 hours at 37°C. then, water-insoluble formazan was dissolved by adding 100 µl dimethyl sulfoxide (DMSO) to each well. To finish, optical density (OD) was monitored at 570 nm as a reference wavelength using. Results were expressed as a percentage of control giving percentage cell viability after 24 hours exposure to test agents. The potency of cell growth inhibition for each test agent was expressed as an inhibitory concentration 50 value (IC₅₀). Viability was defined as the ratio (expressed in percentage) of absorbance of treated cells to untreated cells [10].

Statistical Analysis

Each experiment was performed at least three times. Results are expressed as the mean value ± standard deviation (SD).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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