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Phytochemical Screening of Ayer Hitam Forest Reserve : Isolation of Ariskanin-A from *Thottea corymbosa* (Griff.) Ding Hou (Aristolochiaceae)

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ABSTRAK

Semasa penyaringan fitokimia ke atas tumbuhan tinggi dari Hutan Simpan Ayer Hitam, Puchong, Selangor, suatu sampel akar Thottea corymbosa telah dikutip dengan banyaknya untuk kajian lanjutan ke atas juzuk bioaktifnya. Tumbuhan ini yang telah diguna sebagai ubat tradisi ialah suatu syrab yang senang dijumpai di rintis terbuka dalam hutan. Akar telah diekstrakkan secara berjujukan menggunakan petroleum eter, kloroform dan metanol dan diikuti oleh pemisahan pecahan kloroform dengan kromatografi turus dan kromatografi lapisan nipis. Dalam kajian ini kita ingin melaporkan pemencilan suatu aristolaktam, Ariskanin-A dengan nilai ketoksikan LC50 <200 ppm dalam Bioasai Kemautan Anak Udang Air Masin, berdasarkan data spektroskopik dan perbandingan kepustakaan.

ABSTRACT

During a phytochemical screening of higher plants at Ayer Hitam Forest Reserve, Puchong, Selangor, we collected a large amount of sample of Thottea corymbosa roots for further investigation of its bioactive constituents. The plant is a shrub commonly used in herbal medicine and could easily be found along the open forest trails. The roots of the collected sample were consecutively extracted with petroleum-ether, chloroform and methanol followed by separation of the chloroform fraction by column chromatography and preparative thin-layer chromatography. In this study we reported the isolation of an aristolactam, Ariskanin-A with a toxicity value of LC50 <200 ppm in Brine-Shrimp Lethality Bioassay based on spectroscopic data and comparison with literature.

INTRODUCTION

As a continuation of our previous preliminary work (Mat So'ad and Nik Idris Yusoff 2001) on the phytochemistry of *Thottea tomentosa* (Blume) Ding Hou to isolate bioactive components of some pharmacological interests (or possibly toxic) especially Aristolochic acids and aristolactam, we chose to investigate the chemical constituents of its next-of-kin, *T. corymbosa*. The plant is called 'hempedu beruang' in Malay, a shrub found growing commonly along the open trails of lowland forests throughout Malaysia. In herbal medicine, the pounded leaves are applied to sore gums or tooth cavity for toothache. Also it is claimed that the shrub can be used even in drug form as an analgesic, antiasthmatic, antifertility and for treating impotence and snake-bite. Although the plant is widely used among the locals, to date no study on the phytochemistry and biological activity has ever been reported.

In Peninsular Malaysia the family Aristolochiaceae ia represented by two genera, namely *Aristolochia* and *Thottea*. The former consists of five species, namely *A. curitisii*, *A. foveolata*, *A. jackii*, *A. minutiflora* and *A. tagala* and the latter contains a total of seven species, namely, *T. corymbosa*, *T. dependens*, *T. grandiflora*, *T. parviflora*, *T. sumatrana*, *T. tomentosa* and *T. tricornis*. The species within *Aristolochia* have a very narrow range of distribution except *A. tagala* which is widespread. Turner (1995) stated that *A. curtisii* is only found in Penang, *A. foveolata* is found in Terengganu, *A. jackii* is found in Pahang

and *A. minutiflora* is known to occur only in Perak and Johore. However, all species of *Thottea* are widespread and *T. dependens* is endemic to Peninsular Malaysia. Several species of *Aristolochia* from India and China were reported to contain bioactive compounds in their respective pharmacological studies.The roots of *Aristolochia indica* revealed phenanthrene derivatives and a sesquiterpene which posseses antifertility activity (Pakrashi *et al.* 1977) besides being a source of aristolochic acid, a tumour-inhibitory principle, rarely found in nature as it contains a NO_2 group. In China, *Aristolochia debilis* decoction known as 'qing mu xiang' has a direct constrictive action on blood vessels and shows an inhibitory action on the heart (Wang 1983). Recently, it was found that Aristolochic acids I and II were reported to cause interstitial nephritis (Kazunori *et al.* 1999).

MATERIALS AND METHODS

The clean air-dried roots of *T. corymbosa* (Voucher No. AZ 6681) were collected from Ayer Hitam Forest Reserve during the expedition and were cut into small pieces and milled. A specimen voucher was deposited in the Herbarium, Universiti Kebangsaan Malaysia, Bangi (UKMB).

UV spectrum was recorded on a Shimadzu UV Spectrophotometer in ethanol solution. Mass spectrum was obtained on Hewlett Packard 5989A Spectrometer operating at 70 ev and attached to a VG-Display Digispec data acquisition system computer. The ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ solvent on a JEOL FX400 (400 Mhz) Spectrometer and the chemical shifts are expressed as δ values (ppm) downfield from tetramethylsilane (TMS) as an internal reference.

About 1.35 kg sample was then consecutively extracted with petroleum-ether, chloroform and methanol at room temperature, filtered and solvents dried off under reduced pressure on a rotary evaporator.

The slightly toxic chloroform extract (9.18 g) with a value of LC_{50} <200 ppm on Brine Shrimp Lethality Bioassay was then subjected to dry-flash column chromatography, silica gel 60 (230-400 mesh) as adsorbent and hexane, ethylacetate and finally methanol as elueting solvents. The methanol fractions were combined and further fractionated by silica gel column chromatography, using gradient hexane-ethylacetate as solvent system. Then the hexane-ethylacetate (4:6) fraction was concentrated and checked on a thin-layer chromatography resulting in at least four spots. Finally, the crude was subjected to a further micro-column chromatography separation affording two major components labelled as AC3 (61.3 mg) and AC9 (30.7 mg) after a final preparative thin-layer chromatography purification. AC3 was then analysed by spectroscopic methods of ultra-violet spectrophotometry, mass spectroscopy and ^1H and ^{13}C nuclear magnetic resonance measurements to elucidate its chemical structure (Fig.1).

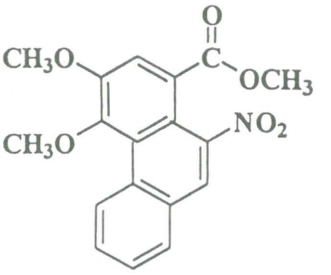

Fig. 1. Compound AC3

Yellow needles , 61.3 mg. TLC (silica gel) with solvent system hexane-ethylacetate (8:2), R_f 0.9. UV λ_{max} (MeOH, nm) : 256,272, 295,372. IR ν_{max} (cm^{-1}): 1714 (C=O ester), 1584, 1513 (NO_2), 1448, 1342, 1235. MS m/z: 341[M+, $C_{18}H_{15}NO_6$], 295 [M-NO_2]$^+$, 280 [M - NO_2- Me]$^+$, 264 [M - NO_2- OMe]$^+$, 252, 237, 222, 194, 166. ^1H-NMR, δ (ppm): 9.66 (1H,d,H-5), 8.33 (1H, s, H-9), 7.97 (1H, dd, H-8), 7.89 (1H,s,H-2), 7.80 (1H,td, H-6), 7.71 (^1H, td, H-7), 4.10(3H,s,3-OMe), 3.98 (3H,s, 4-OMe), 3.88 (3H, s, OCOMe). ^{13}C-NMR δ (ppm): 167.6 (C=O), 151.6(C-5), 149.6(C9), 146.1(C8), 131.1(C4), 130.6, 130.5(C10),129.8, 128.4, 128.3, 127.4, 126.3, 124.9, 116.9, 116.4, 60.4(C3-OMe), 56.7 (C4-OMe) and 52.2 (OCOMe).

RESULTS AND DISCUSSION

Compound AC3 was isolated from the hexane-ethylacetate (4:6) fraction by column chromatography from the slightly toxic chloroform extract with LC50 < 200 ppm on Brine-Shrimp Lethality Bioassay. The UV spectrum of AC3 showed bathochromic shifts at 256, 272, 295 and 372 nm. The IR spectrum clearly showed the absorption peaks at 1714 cm^{-1} for carbonyl ester group and at 1513 cm^{-1} for NO_2. Its mass spectrum gave a molecular weight of 341 which corresponds to a molecular formula of $C_{18}H_{15}NO_6$.The fragmentation peak at m/z 295

TABLE 1
The ^{1}H NMR spectral data of compound AC3 as compared to Ariskanin-A

δ 1H (ppm)	Compound AC3	Ariskanin-A (Wu *et al.* 1994)
1-COOMe	3.88 (3H, s)	3.86 (3H, s)
H-2	7.89 (1H, s)	7.89 (1H, s)
3-OMe	4.10 (3H, s)	4.10 (3H, s)
4-OMe	3.98 (3H, s)	3.97 (3H, s)
H-5	9.66 (1H, d, 8.6 Hz)	9.66 (1H, dd, 8.0, 1.8 Hz)
H-6	7.80 (1H, m, 1.0, 0.9 Hz)	7.81 (1H, td, 8.0, 1.6 Hz)
H-7	7.71 (1H, dd, 1.4, 1.4 Hz)	7.71 (1H, td, 8.0, 1.8 Hz)
H-8	7.97 (1H, dd, 1.4, 1.4 Hz)	7.99 (1H, dd, 8.0, 1.6 Hz)
H-9	8.33 (1H, s)	8.34 (1H, s)

shows an elimination of NO_2 which supports the existence of NO_2 suggesting a typical aristolochic acid for the structure of AC3, fragment 280 a further loss of Me and 264 that shows a loss of OMe.

Integration of 1H-NMR spectrum showed the presence of 15 protons altogether consisting of six aromatic protons, and three each for 3-OMe, 4-OMe and OCOMe belonging to a methylester moeity. When comparing these proton signals with that of a known compound, Ariskanin-A from a published report (Wu *et al.* 1994), they are without any doubt very consistent and agreeable (Table 1).

In addition, the ^{13}C-NMR spectral data clearly showed the presence of 18 carbon nuclei and this tally well with the molecular formula, $C_{18}H_{15}NO_6$ for Ariskanin-A. The signals at δ 52.2, 56.7 and 60.4 ppm are typical of a methylester aristolochic acid derivative.

CONCLUSION

Therefore, it can be concluded that the compound isolated from the chloroform root extract of *T. corymbosa* collected from Ayer Hitam Forest Reserve can be identified beyond doubt as a methylester of aristolochic acid derivative i.e. Ariskanin-A based on the spectroscopic data which are comparable and agreeable with that of previous work done on a different species, *A. kankauensis* (Wu *et al.* 1994). Hence, this report constitutes the first report on the isolation of Ariskanin-A from *Thottea corymbosa* in Malaysia. It is worth mentioning that Ariskanin-A also showed some significant cytotoxity activity against certain cancer cell-lines. Finally, since species of Aristolochiaceae are the only source of aristolochic acids which cannot be synthesized, it is not far-fetched to say that future new drugs discovery begins here.

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