



Short Communication

## Betulin-3-caffeate and Amyrin from the Stem Bark of *Barringtonia acutangula* (L)

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**ABSTRACT:** *Barringtonia acutangula* (Indian Oak) is an important medicinal plant of Bangladesh and India for its phytochemical and prognostic biomarkers in stem. This plant was selected based on its medicinal properties including antiasthmatic, anticold, antimalarial, antidiarrheal, antiprotozoal, antidystry stimulating properties. The coarsely powdered stem bark of *B. acutangula* was extracted with CHCl<sub>3</sub>. The concentrated extract was then fractionated with flash column chromatography using petroleum ether, ethyl acetate and methanol. The column fractions were further fractionated with gel permeating chromatography. Extensive preparative thin layer chromatography resulted in the isolation of two compounds which were identified as betulin-3-caffeate and amyryn. The structures of these compounds were determined by <sup>1</sup>H NMR spectroscopy. This is the first report of the isolation of these compounds from this plant stem bark.

**KEYWORDS:** *Barringtonia acutangula*, chromatography, betulin-3-caffeate, amyryn, <sup>1</sup>H NMR.

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### INTRODUCTION

*Barringtonia acutangula* (Lecythidaceae) is a shrub or small bushy, multi-stemmed tree that can grow up to 12 m tall. It is a small to medium evergreen tree, with spreading crown. It is occur in the fresh water swamp forests of Sylhet ("Haor"). Also occur along the canals and roadside ditches throughout the country. This is a widely distributed species found from India to Myanmar and Thailand, Laos, Vietnam, Philippines, Malaysia to New Guinea.<sup>1,2</sup>

Principal constituents of the plant are starch, protein, cellulose, fat, caoutchouc, alkaline sales, and an active principal similar to saponin which forms into a stable froth when shaken on a watery solution.<sup>2</sup> From the bark, a study yielded nine triterpene saponins, acutangulosides A-F, and acutanguloside D-F methyl esters and a single triterpene aglycone.<sup>3</sup> Leaves contain a trihydroxy triterpene monocarboxylic acid, acutangulic acid, and other organic acids, barrigtogenic, tangulic and oleanolic acids; saponins and sapogenins, acutagenol A and acutagenol B, three triterpenoid sapogenols, barringtogenols, B, C and D, E, two triterpenoid acid sapogenins, stigmasterol glucoside and  $\beta$ -sitosterol.<sup>4-7</sup> Fruits contain barringtogenol D, C and B, saponins and barrigenic acid. Seeds contain triterpenoid glycosides, barringtogenin. Bark contains tannins and a small amount

of sapogenin. Wood contains a triterpenoid dicarboxylic acid, barrigtonic acid and other triterpenoids, barrigenic acid and hexa-hydorxytriterpene, tanginol. Branch wood cotains barringtogenol E and triterpenic acid, barrinic acid.<sup>8-11</sup>

Therefore, with the above background, the present study was carried out to identify the compounds present in the stem bark of *B. acutangula*.

### MATERIALS AND METHODS

#### *Collection and preparation of the plant sample*

At first with the help of a comprehensive literature review a plant was selected for investigation and then the plant sample of *B. acutangula* was collected from the district Shariatpur in the month of March 2013. A voucher specimen for these collection has been deposited in Department of Botany, University of Dhaka for proper identifiacation. After washing and cutting into small pieces, the stem barks were sun dried separately for several days. The plant materials were then over dried for 24 hours at considerably low temperature for better grinding. The dried plant was then ground in course powder using high capacity grinding machine. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use.

### Collection and preparation of the plant material

About 600 g of the powder plant material was taken in a clean, round bottomed flask (5 liters) and soaked in 2.5 liters of chloroform. The container with its content was sealed with foil and kept for a period for 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through filter paper and the filtrate thus obtained was concentrated at 50°C with a rotatory evaporator and got 12 g of crude extract.

### Investigation of the crude chloroform soluble extract

The chloroform crude extract was subjected to TLC screening to see the type of compounds present in the extract. A portion of the crude chloroform extract (8 g) was subjected to column chromatography (CC) for rapid fractionation.

### Column chromatography of crude chloroform extract

The column, packed with fine TLC grade silica gel (Kiesel gel 60 g) was used as the packing material. A column having 50.0 cm in length and 5.0 cm in diameter was packed with the silica gel up to a height of 43 cm under reduced pressure. The column was washed with chloroform to facilitate compact packing. The sample was prepared by adsorbing 8 g of chloroform soluble extract onto silica gel (Kiesel gel 60, mesh 70-230), allowed to dry and subsequently applied on top of the adsorbent layer. The column was then eluted with petroleum ether followed by mixtures of petroleum ether and ethyl acetate and then ethyl acetate and methanol. The polarity was gradually increased by adding increasing proportions of ethyl acetate and methanol. A total of 27 fractions were collected each in 100 mL beakers.

### Isolation of compounds

All the fractions were analyzed by analytical thin layer chromatography (TLC). The TLC plates were run in different percentage of solvent system, such as 1%, 2%, 4%, 6% and 10% of ethyl-acetate and petroleum-ether.

### Analysis of column chromatography fractions by TLC

All the column chromatographic fractions were screened by TLC under UV light and by spraying with vanillin H<sub>2</sub>SO<sub>4</sub> followed by heating at 110°C. Depending on the TLC behavior fraction F-1 and F-2 were selected for further investigations.

## RESULTS AND DISCUSSION

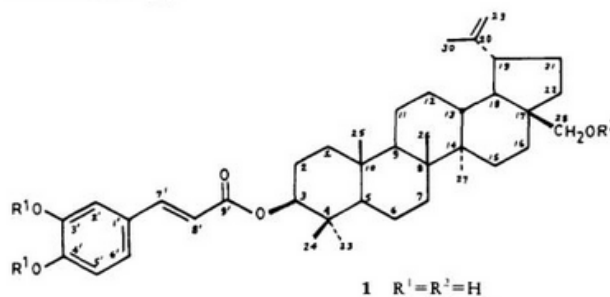
### Characterization of isolated compounds from *B. acutangula*

Repeated chromatographic separation and purification of the chloroform extract of *B. acutangula* resulted in the isolation of BA-1 (1) and BA-2 (2) which was identified as betulin 3-caffeate and amyryn respectively. The structures of the compounds were determined by <sup>1</sup>H NMR spectroscopy and by comparing with reported values.<sup>1,2</sup>

### Characterization of BA-1 as betulin 3-caffeate

Compound BA-1 was obtained as needle shaped crystals. It was evident as a purple spot on TLC (Silica gel PF254) when the developed plate was sprayed with vanillin-sulfuric acid followed by heating at 110°C for 5-10 minutes. It was found to be soluble at petroleum ether, methanol and chloroform.

The <sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>) of BA-1 (Table - 1) displayed signal for an isopropenyl group [ $\delta_H$  4.66 and 4.50 (1H each, br s) and (1.64, 3H, s)], five tertiary methyl groups [ $\delta_H$  0.99, 0.96, 0.89, 0.86, 0.84], a secondary ester function ( $\delta_H$  4.56, 1H, m, H-3), a primary alcohol [ $\delta_H$  3.80 and 3.36 (ABq, J 11.0 Hz, 2H-28)] and an allylic proton [ $\delta_H$  2.46 (1H, m, H-19)]. These data are consistent with the presence of a lupeol type triterpenoid skeleton with a hydroxyl at C-28. The appearance of signal at  $\delta_H$  6.24 (1H, d, J 15.8 Hz, H-8'), 6.85 (1H, d, J 7.8, H-5'), 6.96 (1H, br d, J 8.3 Hz, H-6'), 7.15 (1H, br s, H-2') and 7.90 (1H, d, J 15.8 Hz, H-7') indicates the presence of a 3',4'-dihydroxycinnamoyloxy moiety. Thus BA-1 is most likely to be betulin 3-caffeate. By comparing with published data<sup>10, 11</sup> this compound was confirmed as betulin-3-caffeate.



**Figure 1.** Structure of compound of fraction BA-1 (Betulin 3-caffeate).

**Table 1.** <sup>1</sup>H-NMR spectral data (400 MHz) of isolated compound BA-1 and betulin 3-caffeate in CDCl<sub>3</sub>.

No. of protons	Chemical shift value ( $\delta$ )	
	Value for compound BA-1 ( $\delta_H$ )	Reported value ( $\delta_H$ ) <sup>10,11</sup>
5H(1-3)	4.66,4.50,1.64	4.66,4.56,1.66
3H-23	0.99	0.99
3H-24	0.96	0.96
3H-25	0.89	0.90
3H-26	0.86	0.88
3H-27	0.84	0.84
2H-28	3.80 and 3.36 (ABq, J 11.0 Hz)	3.85 and 3.36 (ABq, J 11.0 Hz)
1H-19	2.46	2.36
1H-8'	6.24	6.25
1H-5'	6.85	6.85
1H-6'	6.96	6.96
1H-2'	7.15	7.08
1H-7'	7.90	7.53

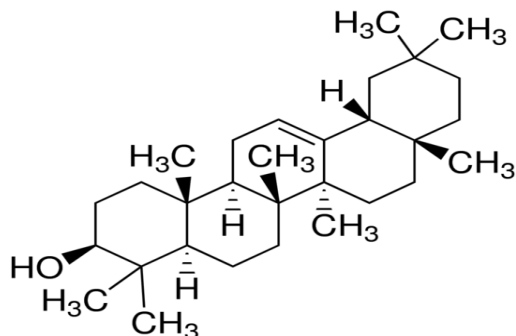
### Characterization of BA-2 as amyryn

BA-2 was isolated from FCC fraction no.2 that was eluted with (95% PE+5% EA). BA-2 was obtained as colorless liquid. Spraying the developed TLC plate of BA-2 with vanillin sulphuric acid and subsequent heating at 110°C for 5-10 minutes produced purple spot. It appeared as a single spot on TLC plate.

The <sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>) of BA-2 (Table-2) showed signal for eight three protons singlets at  $\delta_H$  0.80, 0.87, 0.87, 0.88, 0.93, 1.12, 1.24, 1.37 for eight methyl groups. The spectrum showed a double singlet at  $\delta_H$  3.10 demonstrated a hydroxyl group. The spectrum also

showed a singlet at  $\delta_H$  5.27 demonstrated an olefinic proton at H-12 of a triterpene nucleus. On this basis and comparing with published data<sup>12, 13</sup> this compound, BA-2 isolated from the fraction 2 was identified as amyrin<sup>2</sup>.

This is the first report of the isolation of these compounds from this plant stem bark. Further studies are necessary to isolate, purify and for identification of more bioactive compounds.



**Figure 2.** Structure of compound of fraction BA-2 (Amyrin).

**Table 2.** <sup>1</sup>H-NMR spectral data of isolated compound of BA-2 along with the published data.

No. of proton	Chemical shift value ( $\delta_H$ ) in ppm	
	Value for compound ( $\delta_H$ )	Reported value ( $\delta_H$ ) <sup>12,13</sup>
3H-22	0.80	0.82
3H-23	0.87	0.87
3H-24	0.87	0.87
3H-25	0.88	0.88
3H-26	0.93	0.93
3H-27	1.12	1.13
3H-28	1.24	1.25
3H-29	1.37	1.35
1H-3	3.10	3.20
1H-12	5.27	5.17

(1H, t,  $J = 3.5$  Hz)

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