Bioresearch Communications
Volume 01, Issue 02. July 2015.
Journal Homepage: www.bioresearchcommunications.com

Short Communication

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

Md. Enamul Haque[1*], Zimam Mahmud[1], A. K. M. Mahbub Hasan[1], Roman Sardar[1], Md. Lutful Kabir[1] and Sheikh Tanzina Haque[2]

[1]Department of Biochemistry and Molecular Biology, University of Dhaka. [2]Department of Applied Chemistry and Chemical Engineering, University of Dhaka.

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, antimalerial, antidiarrehal, antiprotozoal, antidyesntry stimulating properties. The coarsely powdered stem bark of *B. acutangula* was extracted with $CHCl_3$. The concentrated extract was then fractioned 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 amyrin. The structures of these compounds were determined by ^{1}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, amyrin, ^{1}H NMR.

CITATION: Haque, M. E., Mahmud, Z., Hasan, A. K. M. M., Sardar, R. and Haque, S. T. 2015. Betulin-3-caffeate and Amyrin from the Stem Bark of *Barringtonia acutangula* (L). *Biores Comm.* **1**(2), 121-123.

CORRESPONDENCE: enamulmd@yahoo.com

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 canels 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.[1, 2]

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.[2] From the bark, a study yielded nine triterpene saponins, acutangulosides A-F, and acutanguloside D-F methyl esters and a single triterpene aglycone.[3] 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 ß-sitosterol.[4-7] 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 dicarboxilic acid, barrigtonic acid and other triterpenoids, barrigenic acid and hexa-hydorxytriterpene, tanginol. Branch wood cotains barringtogenol E and triterpenic acid, barrinic acid.[8-11]

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 speciman 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 temparature 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 50^0C 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 (Kisel 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 (Kisel 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 propoprtions 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_2SO_4 followed by heating at 110^0C. 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 amyrin respectively. The structures of the compounds were determined by ^{1}H NMR spectroscopy and by comparing with reported values.[1,2]

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 110^0 C for 5-10 minutes. It was found to be soluble at petroleum ether, methanol and chloroform.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of BA-1 (Table - 1) displayed signal for an isopropenyl group [δ_H 4.66 and 4.50 (1H each , br s) and (1.64, 3H , s)], five tertiary methyl groups [δ_H 0.99, 0.96, 0.89, 0.86, 0.84], a secondary ester function (δ_H 4.56, 1H, m, H-3), a primary alcohol [δ_H 3.80 and 3.36 (ABq, J 11.0 Hz, 2H-28)] and an allylic proton [δ_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 δ_H 6.24 (1H, d, J 15.8Hz, 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 [10, 11] this compound was confirmed as betulin-3-caffeate.

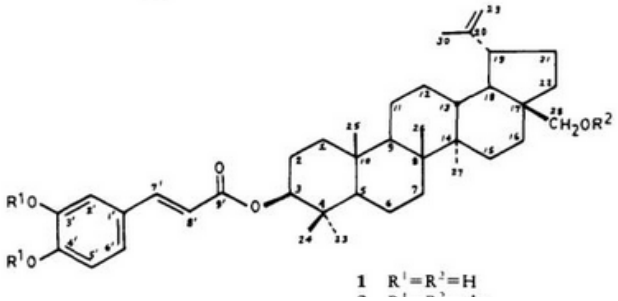

Figure 1. Structure of compound of fraction BA-1 (Betulin 3-caffeate).

Table 1. ^{1}H -NMR spectral data (400 MHz) of isolated compound BA-1 and betulin 3- caffeate in $CDCl_3$.

No. of protons	Chemical shift value (δ)	
	Value for compound BA-1 (δ_H)	Reported value (δ_H)[10,11]
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 amyrin
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 110^0 C for 5-10 minutes produced purple spot. It appeared as a single spot on TLC plate.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of BA-2 (Table-2) showed signal for eight three protons singlets at δ_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 δ_H 3.10 demonstrated a hydroxyl group. The spectrum also

showed a singlet at δ_H 5.27 demonstrated an olefenic proton at H-12 of a triterpene nucleus. On this basis and comparing with published data [12, 13] this compound, BA-2 isolated from the fraction 2 was identified as amyrin [2].

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.

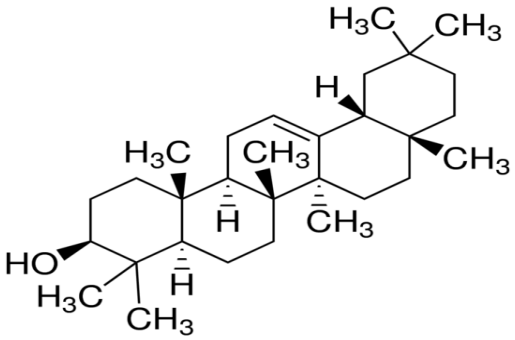

Figure 2. Structure of compound of fraction BA-2 (Amyrin).

Table 2. ^{1}H -NMR spectral data of isolated compound of BA-2 along with the published data.

No. of proton	Chemical shift value (δ_H) in ppm	
	Value for compound (δ_H)	Reported value (δ_H)[12,13]
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 (1H, t, *J* = 3.5 Hz)	5.27	5.17

ACKNOWLEDGEMENTS

The authors wish to give thanks to the BCSIR NMR laboratory for providing NMR facilities.

REFERENCES

1. Ghani, A. 2003. Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edition, Asiatic Society of Bangladesh.
2. Rastogi, R.P. and Mehrotra B.N. 1993. Compendium of Indian Medicinal Plants. Vol. 2, Central Drug Research Institute, Lucknow Publications and Information Directorate, New Delhi, India.
3. Mills, C. 2000, Suppressors from *B. acutangula* and inhibition of Protein phosphatase activity. *B. actangula*, External PhD thesis. Faculty of Science and technology, Griffith University, Brisbane.
4. Nguyen, V.D., Nguyen, T.L., Tran, H.T., Ha, T.A., Bui, V.H., Nguyen, H.N. and Nguyen, T.D. 2014. Flavan-3-ols from the barks of *Barringtonia acutangula*, *Biochemical Systematics and Ecology* **55**, 219-221.
5. Padmavathi, D., Sushula, D. and Bherathi, R.V. 2011. Pharmacognostical evaluation of *Barringtonia acutangula* leaf. *Int J of Agricultural Research* **2(1)**, 39-41.
6. Daniel, M. and Robin, E.M. 2011. Phytochemical and pharmacognostic studies on the bark of leaves of *B. acutangula Gaertrn*. *Int J of Pharmaceutical and Biological Sciences* **2**, 128-134.
7. Mohan, A. and San Kun, R. 2014. Antioxidant, antimicrobial studies and investigation of secondary metabolites from stem bark of *B. acutangula* (L). *Int J of Pharmacognosy and Phytochemical Research*. **6**, 969-972.
8. Kathirval, A and Sujatha, V., 2012. Phytochemical analysis and antioxidant activity of *Barringtonia acutangula (L) Gaertn* leaves. *I J Pharmacognosy and Pharmaceutical Sciences* **4**, 277-281.
9. Flored, M and Seha, T. 2012. Phytochemical investigation of tropical medicinal plants, *Stereospermum colias* and *B. acutangula* (L). *I J of Research in Plant Sciences* **1**, 109-115.
10. Jeong, W., Hong, S.S., Leins, N., Yong, Y.S., Shein, Y.S., Lee, C., Hway, B.Y. and Lee, D. 2005. Bioactive triterpinoids from *Callislemon lanceolatus*. *Archives of Pharmaceutical Research* **32**, 845-849.
11. Guo, H., Li, B.G., Wu, Z-J. and Zhang, G-L. 2006. Leupene triterpinoids and a decyl heptanoid from *Clemaloclethra actividades*. *Plant Med* **72**, 180-183.
12. Vazquez, L.H., Palazan, J. and Ocana, A.N. 2005. The pentacyclic triterpines, α and β amyrin: A review of sources and biological activities. www.intechopen.com. 487-502.
13. Ragosa, C. Y., Ng, V.A.S. and Shen, G. C., 2013. Triterpenes from *Ficus nervosa*. *J of Chemical and Pharmaceutical research* **5**, 1070-1073.