- Short communication

2,5-Dihydroxy-3-heptadecyl-1,4-benzoquinone and Stigmasterol from *Heliotropium indicum* L.

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From the crude methanol extract of whole plant of *Heliotropium indicum* L., two compounds 2,5-dihydroxy-3-heptadecyl-1,4-benzoquinone (**1**) and stigmasterol (**2**) were isolated by a combination of column chromatography and preparative thin layer chromatography over silica gel. The structure of these compounds were determined by ^{1}H NMR spectroscopic method and by comparing with published data.

H. indicum (Bengali name Hatisur; Fam-Boraginaceae) is a small to medium-sized, much-branched, evergreen plant of the family native to Asia. It is also widely available throughout Bangladesh in fallow lands.[1] Different parts of the plant are used as astringent, emollient, diuretic, ulcers, sores and wounds, stings of insects, eye disease, fever and rheumatism. Leaves are used for ringworm; juice is used in fever. Roots are used aphrodisiac, for the cure of night blindness. The flowers are considered emmenagogue in small doses and abortifacient in large doses.[2] Aqueous and alcoholic extract of roots are oxytocic.[3] Previous studies also led to the isolation of heliotrine, indicine-*N*-oxide, indicine, supinine, heleurine, lasiocarpine, rapanone and estradiol.[4] Few aldehydes like phenyl-acetaldehyde (22.2%), (*E*)-2-nonenal (8.3%) and (*E, Z*)-2-nonadienal (6.1%), with a notable quantity of hexahydrofarnesylacetone (8.4%) and another pyrrolizidine alkaloid named as helindicine were identified with moderate antioxidant activity.[5,6] Most of these alkaloids showed hepatotoxic activities and therefore, the internal use of this plant is not recommended.[7] The aqueous extract of leaves of *H. indicum* was used in ulceration where dose dependent histo-gastroprotective effects were observed.[8] As part of our ongoing investigations on medicinal plants of Bangladesh,[9-11] the present work has been undertaken to isolate and identify biologically active secondary metabolites of the methanol extract of whole plant of *H. indicum.* We, herein, first report the isolation of 2,5-dihydroxy-3-heptadecyl-1,4-benzoquinone (**1**) and the re-isolation of stigmasterol (**2**) from the extractive of *H. indicum*.

The ^{1}HNMR spectra were recorded using a Bruker AMX400 (400 MHz) instrument and the spectra were referenced to the residual nondeuterated solvent signal. PTLC (20 × 20 cm) and TLC (20 × 5 cm) were carried out using Merck Silica gel 60 PF_{254} on glass plates at a thickness of 0.5 mm. Spots on TLC and PTLC plates were visualized by spraying with vanillin-sulfuric acid followed by heating for 5 minutes at 110 ºC. All solvents used in this study were of reagent grade.

Aerial parts of the plant, *H. indicum* were collected from Comilla, Bangladesh in the month of June 2014. A voucher specimen (DACB-43063) for this collection has been deposited in Bangladesh

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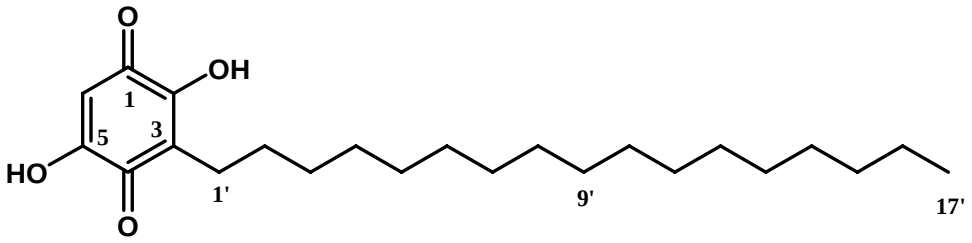
National Herbarium, Mirpur, Dhaka for future reference.

The dried powdered aerial parts (980 g) of *H. indicum* was soaked in 2.5 L methanol for 10 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a Buchii Rotary Evaporator. A portion (5.0 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method[12] into pet-ether (PF, 1.5 g), dichloromethane (DCMF, 1.0 g), chloroform (CF, 1.5 g) and aqueous soluble fractions (AF, 0.8 g). The petroleum ether soluble partitionate was fractionated by column chromatography (CC) over silica gel (Kieselgel 60, mesh 100-200) using petroleum ether-ethyl acetate and ethyl acetate-methanol mixtures of increasing polarities to give 60 fractions (each 25 ml). Preparative thin layer chromatography of fractions eluted with 25% ethyl acetate in petroleum ether afforded compound **1** namely 2,5-dihydroxy-3-heptadecyl-1,4-benzoquinone. On the other hand, compound **2** namely stigmasterol was separated using 15% ethyl acetate in petroleum ether.

2,5-Dihydroxy-3-heptadecyl-1,4-benzoquinone (1). Colorless powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.34 (1H, br. s, H-6), 2.34 (2H, t, *J*=7.5 Hz, H-1'), 2.00-1.25 (30H, m, H-2'-H-16'), 0.87 (3H, t, *J*=6.5 Hz, H-17').

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of compound **1** showed a broad singlet (*J* = 10.0Hz, 5.0 Hz) at δ 5.34 which could be assigned for H-6 of benzoquinone. The spectrum further showed a terminal methyl group (C-17') at δ 0.87 (3H, t, *J*=6.5 Hz). The methylene groups at δ 2.36 (2H, m, *J*= 7.50 Hz), δ 2.00 (2H, m, *J*=6.5 Hz) and δ 1.62 (2H, m, *J*=26 Hz) could be assigned to H-1' to H-3' of the heptadecyl chain. The methylene groups of C-4' to C-16' were observed at δ 1.30 that integrated for 26 protons. Although ^{1}HNMR data of this compound was not available for comparison but the ^{1}H NMR resonance was found to be in close agreement to those of embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone), rapanone (2,5-dihydroxy-3-tridecyl-1,4-benzoquinone) and their alkyl derivatives.[13-16]



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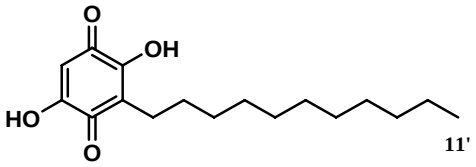
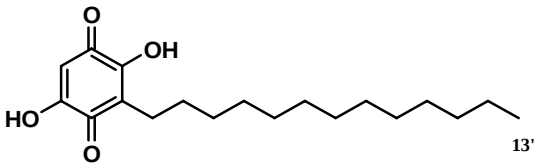


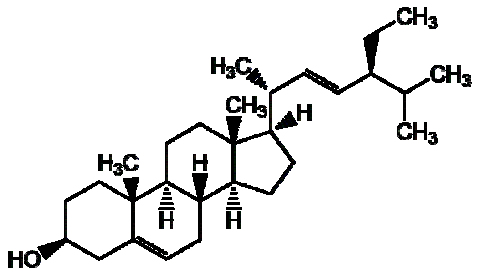
Embelin



Rapanone

Stigmasterol 2. ^{1}H NMR (500 MHz, $CDCl_3$): Colorless gum; δ 5.2 (1H br. s, *J*=7 Hz, H-6 of a steroidal nucleus), 3.49 (m, H-3), multiplet signals at δ 1.14 to 2.21(18H, 9×CH_2 and 8H, CH proton) and δ 0.62 to 1.09 (multiplet, 18H, 6 × CH_3). Based on the above data, the compound was confirmed as

stigmasterol.[17-19] The identity was further substantiated by co-TLC with authentic sample.



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