Journal of Bangladesh Academy of Sciences, Vol. 37, No. 1, 109-113, 2013

- Short communication

STEROIDS FROM THE STEM OF *NYMPHAEA STELLATA*

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ABSTRACT

Three steroids, namely 24-ethyl-5α-cholestan-3-one **(1)**, 5α-stigmast-22-en-3-one **(2)**, stigmast-5, 22-dien-3-one **(3)** have been isolated from *N. stellata.* The phytochemical and antimicrobial as well as cytotoxic activities of *Nymphaea stellata* were investigated in this study. Crude extracts of *N. stellata* and various column fractions exhibited poor antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria and fungi. The crude extract and the fractions showed significant cytotoxic effect when subjected to brine shrimp lethality bioassay.

Key words: Nymphaeaceae, *Nymphaea stellata*, Steroids, Antimicrobial activity, Cytotoxicity

The Nymphaeaceae family comprises of many medicinally and economically important plants. Some species of this family are being medicinally used by the indigenous people of South Africa, America, India and Bangladesh. Number of coumarins, terpenoids, flavonoids and steroids were reported from species of the genus *Nymphaea* (Marquina *et al*. 2005, Elegami *et al*. 2003, Zhang *et al*. 2003). Consequently, some biological investigations were carried out on the genus *Nymphaea* (Benson 1985, Elakovich 1989). From the literature survey it appears that no phytochemical work has been done on *Nymphaea stellata.*

Nymphaea stellata Willd. (Syn. *Nymphaea nouchali* Burm) locally known as Lal Shapla is an aquatic plant under the family of Nymphaeaceae. A large aquatic herb with floating orbicular stem bark, white or red showy flowers and spongy berries, grows abundantly in water all over the country specially in beels and haors of Kishorgonj and Sylhet. *Nymphaea stellata* is distributed from Africa to India and throughout South-East

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Asia to Australia, both wild and cultivated (UNESCO 1998) and it has been used in folk medicine to treat blennorrhea, diarrhoea, diuretic, dyspepsia, fever, piles and tumor (Dassanayake and Fosberg 1980, Fu *et al*. 2001) and also the plant extracts has antihyperglycemic (Rajagopal *et al*. 2008) and antihepatotoxic effect (Manoj and Khan 2004).

In the present study, the authors report the isolation of 24-ethyl-5α-cholestan-3-one **(1)** (Takatsuto *et al*. 1999, Noguchi *et al*. 1999), 5α-stigmast-22-en-3-one **(2)** (Noguchi *et al*. 1999, Shu *et al*. 2002) and stigmast-5, 22-dien-3-one **(3)** (Ikhan 1991) from *N. stellata*.

NMR spectra were obtained on a Bruker AMX-400 (400 MHz for ^{1}H NMR) spectrometer. Column chromatography was carried out using Merck kiesel gel 60 (mesh 70 - 230). The PTLC fractions were monitored by TLC on silica gel 60 PF_{254} (Merck, Germany).

The stem bark of *N. stellata* were collected from Mymensingh and was identified at the Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB Accession No. 32563). The air-dried and powdered plant material (80 g) was extracted with petroleum ether, dichloromethane and methanol sequentially using a Soxhlet apparatus. The crude petroleum ether extract (3.5 g) was subjected to column chromatography on silica gel (Merck, 70 - 230 mesh) by using gradients of petroleum ether/dichloromethane, then dichloromethane, followed by a gradient of dichloromethane/ methanol, and finally with methanol to afford a total of 30 fractions (each 100 mL). Fractions 11, 14 and 15 were found to yield crystals. The crystals were washed with petroleum ether to give pure compounds **1** (15 mg), **2** (12 mg) and **3** (10 mg), respectively. The structures of the isolated compounds were deduced by analysis of ^{1}H NMR spectroscopic data as well as by comparison with previously reported values.

24-ethyl-5α-cholestan-3-one (**1**) : Amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ : 0.95 (3H, *s*, H-19), 0.92 (3H, *d*, *J* = 6.4 Hz, H-21), 0.84 (3H, *t*, *J* = 6.8 Hz, H-29), 0.81 (3H, *d*, *J* = 7.2 Hz, H-26), 0.78 (3H, *d*, *J* = 7.2 Hz, H-27), 0.70 (3H, *s*, H-18).

5α-stigmast-22-en-3-one (**2**) : Amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ : 5.14 (1H, *dd*, *J* = 8.4; 14.8 Hz, H-22), 5.02 (1H, *dd*, *J* = 8.4; 14.8 Hz, H-23), 1.02 (3H, *d*, *J* = 6.4 Hz, H-21), 1.18 (3H, *s*, H-19), 0.84 (3H, *t*, *J* = 6.8 Hz, H-29), 0.81 (3H, *d*, *J* = 7.2 Hz, H-26), 0.78 (3H, *d*, *J* = 6.4 Hz, H-27), 0.68 (3H, *s*, H-18).

Stigmast-5, 22-dien-3-one (**3**) : Amorphous solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ : 5.33 (1H, *m*, H-6), 5.13 (1H, *dd*, *J* = 8.4; 15.2 Hz, H-22), 5.05 (1H, *dd*, *J* = 8.4; 15.2 Hz, H-23), 1.02 (3H, *d*, *J* = 6.4 Hz, H-21), 0.95 (3H, *s*, H-19), 0.92 (3H, *d*, *J* = 6.4 Hz, H-26), 0.87 (3H, *t*, *J* = 6 .8 Hz, H-29), 0.79 (3H, *d*, *J* = 6.4 Hz, H-27), 0.70 (3H, *s*, H-18).

The crude petroleum ether extract (at a concentration of 500 µg/disc), the column fractions F-8, F-9, F-13, F-17 and F-18 of crude petroleum ether extract (at a concentration of 400 µg/disc) were tested for antimicrobial activity against five Gram-positive bacteria, seven Gram-negative bacteria and two fungi by disc diffusion method (Bauer *et al*. 1966, Barry 1980). Standard disc of kanamycin was used for comparison purpose. All the fractions and the crude petroleum ether extract exhibited poor antimicrobial activity.

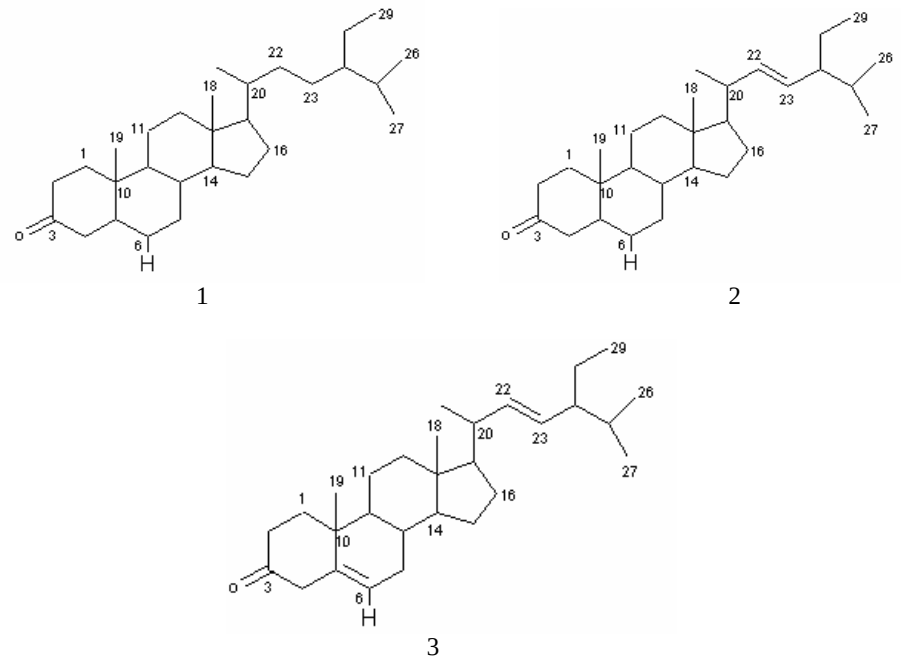

Fig. 1. Structure of compounds 1 - 3.

Following the procedure of Meyer (Meyer *et al*. 1982), the lethality of crude methanol (ME), petroleum ether (PE) and dichloromethane (DCM) extracts to brine shrimp was evaluated on *A. salina*. Table 1 shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS). The LC_{50} values of ME, PE and DCM were found to be 25.12, 6.68, 17.78 µg/mL, respectively. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by petroleum ether (PE) was significant. This result suggests that the petroleum ether (PE) extract may contain antitumor or pesticidal compounds (Meyer *et al*. 1982).

Table 1. LC_{50} data of test samples of *N. stellata*.

Samples	LC_{50} (µg/mL)
VS	0.33
ME	25.12
PE	6.68
DCM	17.78

The values of LC_{50} are expressed in µg/mL. VS: Vincristine sulphate; ME: Methanol extract; PE: Petroleum ether extract and DCM: Dichloromethane extract.

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(*Received revised manuscript on 28 March, 2013*)