Secondary metabolites from the stem of *Ravenia spectabilis* Lindl

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ABSTRACT

Background: *Ravenia spectabilis* is a medium tall shrub found widespread in South America. It also found in India, Pakistan, Bangladesh etc. Few alkaloid and steroid compounds were reported from the plant previously. **Materials and Methods:** Methanol extract from the stems of *Ravenia spectabilis* were partitioned into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions, respectively. The crude methanol extract, carbon tetrachloride fraction and chloroform fraction were fractionated by column chromatography of Silica gel and Sephadex LH-20 for isolation and purification of compounds. The structures of the isolated compounds were determined by extensive NMR spectral analysis, including 2D NMR, mass spectroscopy etc. **Results:** Ten compounds, γ-fagarine (1), ravenoline (2), N-methyl atanine **(3),** 2,3,3,5-tetramethyl-2,3,4,5-tetrahydrofurano [3,2-c] quinolin-4-one **(4),** arborinine (5), 3-geranyl indole (6), atanine (7), steroids sitosta-4-en-3-one (8), stigmasterol (9) and 3-methoxy-4-hydroxy cinnamic acid (10) were isolated from the stems of *Ravenia spectabilis.* **Conclusion:** Compounds N-methyl atanine **(3),** 2,3,3,5-tetramethyl-2,3,4,5-tetrahydrofurano [3,2-c] quinolin-4-one **(4),** 3-geranyl indole (6), sitosta-4-en-3-one (8) and 3-methoxy-4-hydroxy cinnamic acid (10) were isolated from this plant for the first time. 3-geranyl indole (6) was also isolated second time from natural sources.

Keywords: 3-methoxy-4-hydroxy cinnamic acid, alkaloids, *Ravenia spectabilis*, rutaceae, steroids

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INTRODUCTION

Development of new drugs is essential due to the invasion of new threats or the formation of resistance against existing drugs. Medicinal plants are an ideal target for discovery of potential bioactive compounds or lead structures for new drugs. The main aim of this study was to identify and characterize the bioactive compounds from the stem of *Ravenia spectabilis. Ravenia spectabilis* is a medium tall shrub found widespread in South America. This species is cultivated in many districts of Bangladesh. It is included under the family Rutaceae. The Rutaceous plants contain a wide range of pharmacologically active compounds[1] including anti-inflammatory, anti-implantation, anti-neoplastic and anti-mutagenic activities. The family is well known for producing a wide range of secondary metabolites, such as phenanthridine, acridone and furo- and pyranoquinoline alkaloids, complex furo- and pyranocoumarins, flavonoids and various types of terpenoids, including limonoids.[2]

A literature survey indicated that *R. spectabilis* possesses antimicrobial and cytotoxic activities.[3,4] Previous phytochemical studies revealed the occurrences of a number of compounds such as paraensine,[5] ravesilone,[5] spectabiline,[6] ravenine,[7] ravenoline,[7] atanine,[7] γ-fagarine,[3] arborinine,[3,4] stigmasta-4,22-dien-3-one[3] and stigmasterol[3] from this plant and among them γ-fagarine, arborinine and atanine were found to be bioactive.[8-15]

As a continuation of our phytochemical studies on medicinal plants, this article reports the isolation of 7 alkaloids, namely γ-fagarine **(1),**[3,16,17] ravenoline **(2),**[7] N-methyl atanine **(3),**[18,19] 2,3,3,5-tetramethyl-2,3,4,5-tetrahydrofurano [3,2-c] quinolin-4-one **(4),**[20] arborinine **(5),**[3,4,21] 3-geranyl indole **(6),**[22,23] atanine **(7),**[7] two steroids, namely sitosta-4-en-3-one **(8),**[24] stigmasterol **(9)**[3,25,26] and

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also the 3-methoxy-4-hydroxy cinnamic acid **(10)**[27] from the stem of *R. spectabilis*.

MATERIALS AND METHODS

General experimental procedures

Accurate mass measurements were determined on JMS600H Mass Spectrometer. NMR spectra (both 1D and 2D) were obtained on a Bruker spectrometer (400 and 500 MHz for ^{1}H and 100 and 125 MHz for ^{13}C), using the residual solvent peaks as internal standard. *J*-modulated ^{13}C spectra were acquired with a relaxation time (d_1) of 4s. HMBC spectra were optimized for a long range J_{H-C} of 7 Hz (d_6 = 0.07s). Vacuum-liquid chromatography (VLC) was carried out using Merck Si gel 60 H. PTLC was carried out using Merck Si gel 60 PF_{254} on glass plates (20 cm X 20 cm) at a thickness of 0.5 mm. TLC was conducted on normal-phase Merck Si gel 60 PF_{254} plates. Spots on TLC and PTLC plates were visualised under UV light (254 and 366 nm) and by spraying with ceric sulphate and Vanillin-Sulfuric acid reagent.

Plant material

The stems of *R. spectabilis* were collected from Balda Garden (District-Dhaka) and the plant was identified by Professor Salar Khan of Bangladesh National Herbarium (BNH), Dhaka, where a voucher specimen has been deposited (DACB Accession no. 28090). The stems were collected again from the campus of Dhaka University (DACB Accession no. 34694) to undergo phytochemical work using a different method to isolate more compounds. The stems were first air dried and then ground into coarse powder using a grinding machine.

Extraction and isolation

About 1.0Kg of the powdered plant material of stem from the first collection was soaked in 2.5 litre of methanol for 7 days. The concentrated methanol extract (10.13g) was partitioned by the modified Kupchan partitioning procedure[28] into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions, respectively.

Column fractionation of the carbon tetrachloride soluble fraction (1.42g) on Silica gel was performed using a mobile phase of petroleum ether, chloroform and methanol in order of increasing polarities. The column fraction of 20-60% petroleum ether in chloroform was subjected to further column chromatography on Silica gel using a mobile phase of petroleum ether, chloroform and methanol in order of increasing polarities. Preparative TLC (toluene-EtOAc-AcOH = 85:5:1) of the column fraction eluted with 25-35% petroleum ether in chloroform afforded compound **1** (28mg), compound **2** (4.2mg), compound **3** (5mg) and compound **4** (3mg). The chloroform soluble fraction (2.98g) was fractionated by VLC over Silica gel 60H using petroleum ether, chloroform and methanol mixtures of increasing polarity. The VLC fraction eluted with 90% chloroform in methanol was subjected to preparative TLC (toluene-EtOAc = 90:10) to obtain compound **5** (10.5mg).

About 1.5Kg of the powdered plant material of stem from the second collection was soaked in 4.0 litre of methanol for 7 days. The concentrated methanol extract (15.0g) was fractionated by VLC over Silica gel 60H using petroleum ether, ethyl acetate and methanol mixtures of increasing polarity. The VLC fraction of 8–15% ethyl acetate in petroleum ether was subjected to Gel Permeation Chromatography on Sephadex LH-20 using a mobile phase of petroleum ether, chloroform and methanol in order of increasing polarities to induce adsorption and partition quality like a normal phase column chromatography for better separation of components. The sephadex column fraction eluted with 80–90% petroleum ether in chloroform was subjected to preparative TLC (toluene-EtOAc = 98:2) to obtain compound **6** (6mg), compound **7** (10mg) and compound **8** (9mg). Compound **9** (8mg) was obtained as a white crystal directly from the VLC fraction of 20% ethyl acetate in petroleum ether by treatment with different solvents. The VLC fraction of 25% ethyl acetate in petroleum ether was subjected to further Gel Permeation Chromatography on Sephadex LH-20 using a mobile phase of petroleum ether, chloroform and methanol in order of increasing polarities. The sephadex column fraction eluted with 80–100% petroleum ether in chloroform was subjected to preparative TLC (toluene-EtOAc = 95:5) to obtain compound **10** (6mg).

RESULTS

γ-fagarine (**1**): Pink gum. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data have been given in Table 1.

Ravenoline (**2**): Yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, *d*, *J* = 7.2 Hz, CH_3-1'), 1.82 (3H, *s*, CH_3-2'), 3.72 (3H, *s*, N-CH_3), 4.17 (1H, *m*, H-1'), 5.26 (1H, *s*, H-3'), 5.33 (1H, *s*, H-3'), 7.22 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-6), 7.31 (1H, *d*, *J* = 8.2 Hz, H-8), 7.31 (1H, *s*, O*H*-4), 7.54 (1H, *dd*, *J* = 7.2, 1.5 Hz, H-7), 7.92 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-5).

N-methyl atanine (3): Yellow gum, ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60 (3H, *s*, CH_3-3'), 1.81 (3H, *s*, CH_3-3'), 3.41 (2H, *d*, *J* = 6.8 Hz, -CH_2-1'), 3.72 (3H, *s*, N-CH_3), 3.92 (3H, *s*, OCH_3-4), 5.25 (1H, *m*, H-2'), 7.24 (1H, *tt*, *J* = 7.2, 0.4 Hz, H-6), 7.36 (1H, *d*, *J* = 8.0 Hz, H-8), 7.53 (1H, *ddd*, *J* = 7.2, 7.2, 1.6 Hz, H-7), 7.82 (1H, *dd*, *J* = 8.0, 1.6 Hz, H-5).

2,3,3,5-tetramethyl-2,3,4,5-tetrahydrofurano [3,2-c] quinolin-4-one (4): Yellow gum. EI-MS *m/z* (%): 243[M^+] (30), 228 (100), 214 (10), 200 (13), 186 (8), 167 (15), 149 (32), 57 (20). ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data have been given in Table 2.

Arborinine (**5**): Yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (3H, *s*, N-CH_3), 3.93 (3H, *s*, OCH_3-3), 4.02 (3H, *s*, OCH_3-2), 6.29 (1H, *s*, H-4), 7.30 (1H, *t*, *J* = 7.4 Hz, H-7), 7.51 (1H, *d*, *J* = 8.8 Hz, H-5), 7.72 (1H, *ddd*, *J* = 7.2 Hz, H-6), 8.47 (1H, *dd*, *J* = 6.4 Hz, H-8).

3-Geranyl indole (**6**): Yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data have been given in Table 3.

Atanine (**7**): Yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 (3H, *s*, CH_3-3'), 1.82 (3H, *s*, CH_3-3'), 3.56 (2H, *d*, *J* = 6.9 Hz, H-1'), 5.28 (1H, *br.t*, J = 6.9 Hz, H-2'), 7.20 (1H, *ddd*, *J* = 8.1, 7.2, 1.0 Hz, H-6), 7.27 (1H, *d*, *J* = 8.1 Hz, H-8), 7.45 (1H, *ddd*, *J* = 8.1, 7.2, 1.2 Hz, H-7), 7.76 (1H, *dd*, *J* = 8.1, 1.1 Hz, H-5), 10.82 (1H, *br.s*, N*H*).

Table 1: ^{1}H- and ^{13}C-NMR spectroscopic data for compound 1

C	DEPT	$\delta_{H,\,J\,Hz}$	δ_C	HMBC (H→C)
2	CH	7.62 d (2.4)	143.9	8b, 3a
3	CH	7.05 br.s	104.5	8b
3a	C		103.9	
4	C		156.9	
4a	C		119.7	
5	CH	7.82 d (8.4)	114.1	7, 8, 8a
6	CH	7.34 t (8.2)	123.4	4a , 8
7	CH	7.04 d (8.2)	107.5	5, 8
8	C		154.6	
8a	C		137.5	
8b	C		163.2	
4-OCH_3	CH_3	4.42 s	59.0	4
8-OCH_3	CH_3	4.06 s	56.0	8

Table 2: ^{1}H- and ^{13}C-NMR spectroscopic data for compound 4

C	DEPT	$\delta_{H,\,J\,Hz}$	δ_C
2	CH	4.59 q (6.5)	91.7
3	C		45.3
3a	C		114.0
4	C		164.0
5a	C		138.2
6	CH	7.34 bd (8.5)	114.8
7	CH	7.56 ddd (8.5, 8.0, 1.0)	131.4
8	CH	7.21 dd (8.0, 8.0)	121.9
9	CH	7.76 dd (8.0,1.0)	123.6
9a	C		115.8
9b	C		159.2
>N-CH_3	CH_3	3.67 s	29.1
2-CH_3	CH_3	1.45 d (6.6)	15.0
3-CH_3	CH_3	1.26 s	20.7
3-CH_3	CH_3	1.48 s	26.0

Sitosta-4-en-3-one (**8**): Needle like crystal. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, *s*, CH_3-18), 0.81 (3H, *d*, *J* = 6.6 Hz, CH_3-26), 0.83 (3H, *d*, *J* = 6.6 Hz, CH_3-27), 0.84 (3H, *t*, *J* = 6.8 Hz, CH_3-29), 0.91 (3H, *d*, *J* = 6.6 Hz, CH_3-21), 1.15 (3H, *s*, CH_3-19), 5.70 (1H, *s*, H-4).

Stigmasterol (**9**): White crystal. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.70 (3H, *s*, CH_3-13), 0.82 (3H, *t*, CH_3-28), 0.84 (3H, *d*, *J* = 6.6 Hz, CH_3-25), 0.86 (3H, *d*, *J* = 6.6 Hz, CH_3-25), 0.94 (3H, *d*, *J* = 6.6 Hz, CH_3-20), 1.02 (3H, *s*, CH_3-10), 3.53 (1H, *m*, H-4), 5.03 (1H, *dd*, *J* = 15.0, 9.0 Hz, H-23), 5.16 (1H, *dd*, *J* = 15.0, 6.5 Hz, H-22), 5.36 (1H, *m*, H-6).

3-Methoxy-4-hydroxy cinnamic acid (**10**): Yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (3H, *s*, OCH_3-3), 5.83 (1H, *br.s*, O*H*-4), 6.29 (1H, *d*, *J* = 15.9 Hz, H-8), 6.91 (1H, *d*, *J* = 8.1 Hz, H-5), 7.03 (1H, *d*, *J* = 1.8 Hz, H-2), 7.07 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6), 7.61 (1H, *d*, *J* = 15.9 Hz, H-7).

DISCUSSION

The air-dried and ground stem of the first collection of *R. spectabilis* were macerated with methanol and partitioned with *n*-hexane, carbon tetrachloride and chloroform. Column fractionation of the carbon tetrachloride soluble fraction followed by preparative TLC afforded compound **1** (28mg), compound **2** (4.2mg), compound **3** (5mg) and compound **4** (3mg), as well as VLC fractionation of the chloroform soluble fraction followed by preparative TLC afforded compound **5** (10.5mg). Again, VLC fractionation of the crude methanol extract obtained from the second collection followed by Gel Permeation Chromatography on Sephadex LH-20 and preparative TLC afforded compound **6** (6mg), compound **7** (10mg) and compound **8** (9mg). Compound **9** (8mg) was obtained as a white crystal directly from the VLC fraction of the crude methanolic extract. Another VLC fractionation of the crude methanolic extract and further Gel Permeation Chromatography on Sephadex LH-20 followed by preparative TLC afforded compound **10** (6mg). The structures of **1** - **10** were determined by extensive NMR spectral analysis, including 2D NMR, mass spectroscopy and comparison with published literatures [Figure 1].

Compound **1** was obtained as a Pink gum. The structure of compound **1** was identified as γ-fagarine by comparing its ^{1}H and ^{13}C-NMR data with previously published

Table 3: ^{1}H- and ^{13}C-NMR spectroscopic data for compound 6

C	DEPT	$\delta_{H, J Hz}$	δ_{C}	HMBC (H→C)
2	CH	6.94 br.s	121.2	1', 9
3	C		116.2	
4	CH	7.61 d (7.8)	119.1	3, 6, 8
5	CH	7.12 dd (7.8, 7.2)	119.2	7, 9
6	CH	7.20 dd (7.2, 7.2)	121.9	4, 8
7	CH	7.35 d (8.0)	111.0	5, 9
8	C		136.5	
9	C		127.5	
>NH		7.86 br.s		
1'	CH_2	3.48 d (7.2)	24.0	2, 9, 3'
2'	CH	5.47 t (7.2)	122.9	3, 4', 3'-CH_3
3'	C		135.6	
4'	CH_2	2.08 m	39.7	2'
5'	CH_2	2.14 m	26.7	7'
6'	CH	5.14 t (6.8)	124.4	4', 7'-CH_3, 7'-CH_3
7'	C		131.4	
3'- CH_3	CH_3	1.77 s	16.1	2', 3'
7'- CH_3	CH_3	1.70 s	25.8	6', 7'
7'- CH_3	CH_3	1.62 s	17.8	7', 7'

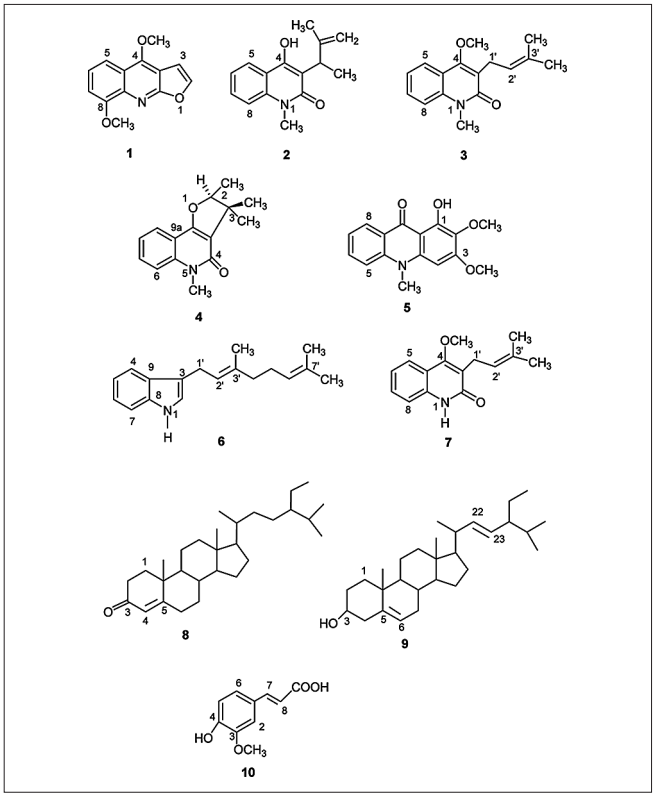

Figure 1: Structure of compounds 1-10 isolated from the stem of *R. spectabilis*

report.[3,16,17] γ-fagarine was reported to have antitubercular,[8] antileishmanial,[9] and anti-HIV[10] activities. Compound **2** was obtained as a yellow gum and identified as ravenoline by comparing its ^{1}H-NMR data with published value.[7]

Compound **3** was isolated as a yellow gum. The structure of compound **3** was established as N-methyl atanine by comparison with published paper.[18,19] Compound N-methyl atanine was isolated from this plant species for the first time.

The structure of compound **4** was confirmed as 2,3,3, 5-tetramethyl-2,3,4,5-tetrahydrofurano [3,2-c] quinolin-4-one by the mass spectrum with $[M^{+}]$ at $m/z = 243$, suggesting the molecular formula $C_{15}H_{17}NO_2$ in agreement with the NMR spectra and by comparison with published article.[20] 2,3,3,5-tetramethyl-2,3,4,5-tetrahydrofurano [3,2-c] quinolin-4-one was isolated from this plant for the first time.

Compound **5** was obtained as a yellow gum. Compound **5** was identified as arborinine by comparing its ^{1}H-NMR data with published value.[3,4,21] Arborinine showed anti-HRV-2,[11] antiplasmodial[12] and cytotoxic[13] activities.

Compound **6** was found as a yellow gum. The structure of compound **6** was confirmed as 3-geranyl indole by comparison with previous published literature.[22,23] 3-Geranyl indole was reported for the first time from this plant species and second time from natural sources. We, herein, report 2D NMR for compound **6** for the first time.

Compound **7** was obtained as a yellow gum. Compound **7** was identified as atanine by comparing its ^{1}H-NMR data with published value.[7] Atanine was found to be cytotoxic[14] and anthelmintic.[15]

The ^{1}H NMR spectra of compound **8** were found in close agreement with those reported for sitosta-4-en-3-one.[24] It was reported from this plant species for the first time.

Compound **9** was obtained as white crystal. The structure of compound **9** was identified as stigmasterol by comparing its ^{1}H-NMR data with published article.[3,25,26]

Compound **10** was isolated as yellow powder. According to its NMR data and a comparison with those given in the literature, the structure of **10** was identified as 3-methoxy-4-hydroxy cinnamic acid.[27] This compound was isolated from this plant for the first time.

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