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Phytochemical and Biological Investigation of *Ravenia spectabilis* Leaves

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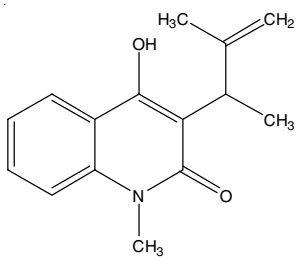
The methanolic extract of the leaves of *Ravenia spectabilis* was investigated for isolation of secondary metabolites and evaluation of bioactivities. Two alkaloids ravenoline (**1**) and arborinine (**2**) were obtained by using vacuum liquid chromatography, gel permeation chromatography and TLC. The compounds were identified by NMR spectroscopy and by comparing the NMR data with those published in the literature. The pure compounds and the petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions of the crude methanolic extract of the plant were evaluated for thrombolytic, antioxidant and antimicrobial properties. In the study for thrombolytic property, the pure compounds and different fractions showed thrombolytic activity ranging from 30.43 to 48.85 % as compared to standard streptokinase (74.34 %). All the fractions and the pure compounds showed mild to moderate antibacterial activity. Carbon tetrachloride fraction showed moderate antioxidant activity (97.53 µg/mL) as compared to BHT (27.50 µg/mL), whereas other fractions showed mild antioxidant activity.

Keywords: ***Ravenia spectabilis,*** **Antioxidant, Thrombolytic, Antimicrobial, Ravenoline, Arborinine.**

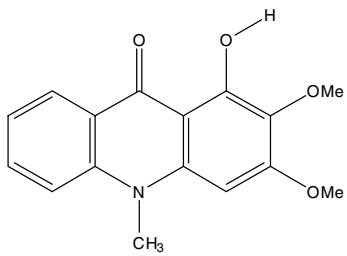
INTRODUCTION

Ravenia spectabilis is a medium sized shrub [1] under the family Rutaceae and the subfamily Rutoideae and tribe Cusparieae [2]. The plant has been cultivated as ornamental in many countries of the world and found widespread in South America [1]. It is also found in India, Pakistan, Bangladesh *etc*. [3]. Many important Rutaceous plants (*Ruta, Zanthoxylum, Murraya, Glycosmis* species) have been used in medicine [4]. The plants of this family also produce a number of anticancer [5] and antitumor agents [6]. 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 [7].

Chemical investigations of *Ravenia spectabilis* has reported various types of compounds such as quinolone alkaloids like arborinine [8,9], atanine [9], ravenine [9,10], ravenoline [9,10], ravesilone, paraensin, acridone alkaloids [11,12], the steroids stigmasterol and spinasterone, alkaloids γ-fagarine, geranyl indole and 3-methoxy-4-hydroxy cinnamic acid [1] and among them γ-fagarine, arborinine and atanine were found to be bioactive [11]. In 2018, dichloromethane fractions of the leaves was analyzed by GC-MS and led to the identification of the compounds isatin, lichexanthone and the terpenes α-spinasterone, lupeol, sitostenone and α-cadinol in its composition [3]. Previous biological studies of the plant extract reported antimicrobial, cytotoxicity [13,14] and antiacetylcholinesterase activity [3]. The present study has been undertaken to isolate and identify biologically active secondary metabolites. We, herein, report the isolation of two alkaloids ravenoline and arborinine from this plant and the evaluation of antioxidant, thrombolytic and antimicrobial activities of different fractions of the extracts and as well as the isolated pure compounds.



Ravenoline



Arborinine

EXPERIMENTAL

General experimental procedures: Preparative TLC was conducted over silica gel 60 PF254 (0.5 mm thickness, Merck) coated glass plates and vanillin H_2SO_4 spray reagent were used to detect compounds. Sephadex LH-20 was used to perform gel permeation chromatography. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Avance100 and 400 MHz Ultrashield NMR spectrophotometer equipped with broadband and selective (^{1}H and ^{13}C) inverse probes.

Leaves of *Ravenia spectabilis* was collected at its fully from the campus of University of Dhaka in the month of February 2015 and was identified by the taxonomist of Bangladesh National Herberium, Dhaka, Bangladesh. A voucher specimen (Accession No. 46423) of the plant has been deposited in National Herbarium, Mirpur, Dhaka, Bangladesh for future reference.

Extraction and isolation: The air dried powdered plant material (leaf) 1 Kg was soaked in methanol (3 L) for 20 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated under reduced pressure using a Buchii rotary evaporator. An aliquot of the crude methanolic extract (34.5 g) was fractionated by vacuum liquid chromatographic (VLC) technique using silica gel 60H and petroleum ether, petroleum ether-dichloromethane, dichloromethane-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol in increasing order of polarity. This provided 45 VLC fractions. Following TLC screening of fractions of VLC, fraction 15 was subjected to column chromatography over lipophilic Sephadex (LH-20) and petroleum ether-chloroform combination as mobile phase. VLC fraction 15 and column fraction 19 yielded the first compound namely ravenoline, which was purified by PTLC and from VLC fraction 21 arborinine was obtained by recrystallization technique.

Preparation of sample for biological investigation: By using the protocol designed by Kupchan [15] and modified by Van Wagenen *et al*. [16], the crude methanoic extract of *Ravenia spectabilis* was subjected to solvent-solvent partitioning. 5 g crude leaf extract was dissolved in10 % aqueous methanol to make the mother solution which was successively partitioned by petroleum ether, carbon tetrachloride, chloroform and water in order of increasing polarity by using separating funnel.

Thrombolytic property: *In vitro* clot lysis activity of the leaves of *R. spectabilis* was carried out according to the method of Prasad *et al*. [17]. The thrombolytic property was assessed by standard streptokinase (100 µL) as positive control and water as negative control. Whole blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy.

Antioxidant activity: For the current investigation of evaluation of antioxidant activity, DPPH assay, designed by Brand-Williams *et al*. [18] was selected. In this investigation, evaluation of scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as the basis for assessing the antioxidant activities of the test samples and butylated hydroxyl toluene (BHT) was taken as the reference standard.

***In vitro* antimicrobial activity:** The method described by Bauer *et al*. [19] was selected for the current study for screening antimicrobial activity against two Gram-positive bacteria and six Gram-negative bacteria. Sterile blank discs and standard antibiotic discs (kenamycin 30 µg/disc) were as negative and positive controls respectively. The test samples were loaded on the discs at a concentration of 400 µg/disc and for the pure compounds 100 µg/disc. The antimicrobial activity of the test samples were than evaluated by measuring the zone of inhibition.

RESULTS AND DISCUSSION

Chemical investigation of *Ravenia spectabilis*: The methanolic extract of the leaves of *R. spectabilis* (Family: Rutaceae) was investigated for isolation of the potent secondary metabolites from this plant. Successive chromatographic separation and purification yielded two compounds and the structures of two compounds were solved as ravenoline and arborinine.

Ravenoline: Compound **1** was isolated as yellow non-crystalline mass from VLC fraction 15 of the crude methanol extract. The ^{1}H NMR spectral data (400 MHz, $CDCl_3$) of compound **1** demonstrated the presence of four aromatic protons at δ_H 7.95 dd (J = 8.0, 1.6 Hz), 7.24 ddd (J = 8.0,7.2, 0.8 Hz), 7.57 ddd (J = 8.0, 7.2, 1.6 Hz) and 7.34 d (J = 8.0 Hz) assignable to H-5, H-6, H-7 and H-8 respectively. The *N*-methyl group (3H s), at position 1 and hydroxyl proton at position 4 appeared as singlets at δ_H 3.75 and 7.33. In addition the spectrum revealed the presence of a methyl doublet at δ_H 1.42, 3H d (J = 7.2 Hz) indicating an adjacent methine proton at δ_H 4.18 q (J = 7.2 Hz), an exomethylene group at δ_H 5.36 s, 5.28 s and a deshielded tertiary methyl group at δ_H 1.85 (3H s). All these NMR data suggested the presence of a 1,2-dimethyl-2-propenyl chain at C-3 of the quinolone molecule. Thus, compound **1** was identified as ravenoline whose structure was further confirmed by comparing its ^{1}H NMR data with those published in the literature [1].

Arborinine: Compound **2** was isolated from VLC fraction 21 as yellow gum. The ^{1}H NMR spectrum showed five aromatic protons, two methoxls, an *N*-methyl and a hydrogen bonded hydroxyl group. The later was attributed to OH-1 of the benzene ring A. The four aromatic protons resonating at δ 7.53 (J = 8.0, 1.6 Hz), 7.74 (J = 8.0, 6.8, 1.6 Hz) 7.33 (J = 8.0, 6.88 Hz) and 8.5 (J = 8.0, 1.6 Hz) suggested an *ortho* disubstituted benzene ring and could be assign to H-5, H-6, H-7 and H-8 respectively. The appearance of H-8 proton at a much lower field (δ 8.50) is due to the deshielding effect of the carbonyl oxygen at C-9 of the acridone molecule. The remaining aromatic protons which was appeared as a singlet at δ 6.32 and two methoxy resonating at δ 3.94 and δ 4.03 must be placed in ring A. The ^{1}H NMR data of compound **2** was found to be in close agreement with those reported for 1-hydroxy-2,3-dimethoxy-10-methyl-9(10*H*)-acridinone or arborinine [1].

Thrombolytic activity: The extractives and the pure compounds of leaf of *Ravenia spectabilis* showed moderate thrombolytic activity. Among all the fractions and pure compounds, the petroleum ether fraction showed highest clot lysis activity (48.85 %), whereas standard streptokinase at 37 °C showed 73.34 % lysis of the clot as compared to distilled water showing a negligible lysis of clot (3.87 %) (Table-1).

Antioxidant activity: Different organic fractions of *Ravenia spectabilis* and isolated pure compounds were subjected to

TABLE-1
THROMBOLYTIC ACTIVITY OF DIFFERENT FRACTIONS AND PURE COMPOUNDS OF *R. spectabilis*

Sample	Clot lysis (%)
Negative control (water)	3.93
Petroleum ether fraction	48.85
Carbon tetrachloride fraction	30.43
Chloroform fraction	41.96
Aqueous fraction	33.37
Arborinine	31.74
Positive control (streptokinase)	74.34

evaluation of antioxidant activity by the method suggested by Brand-Willium *et al.* The IC_{50} value of BHT was used as reference for which was 27.50 µg/mL. In the evaluation, carbon tetrachloride and aqueous fraction of the plant showed the moderate inhibitory concentration of all the fractions with IC_{50} value of 97.53 µg/mL and 110.89 µg/mL, respectively (Table-2).

TABLE-2
ANTIOXIDANT ACTIVITY OF DIFFERENT FRACTIONS AN PURE COMPOUNDS OF *R. spectabilis*

Sample	Free radical scavenging activity (IC_{50}, µg/mL)
BHT	27.50
Petroleum ether fraction	282.91
Carbon tetrachloride fraction	97.53
Chloroform fraction	351.77
Aqueous fraction	110.89
Ravenoline	206.68
Arborinine	301.76

Antibacterial activity: Among eight Gram-positive and Gram-negative bacteria, antimicrobial activity of all fractions of *R. spectabilis* and the isolated pure compounds ravenoline and arborinine showed highest antibacterial activity against *Vibrio cholerae* and in case of *Salmonella typhi*, all of the fractions and pure compounds showed no activities. In case of petroleum-ether fraction of the plant it showed the highest activity against *Bacillus subtilis*. This fraction showed very small zone of inhibition against all other bacteria. Carbon tetrachloride fraction of the plant showed very little activity against all the bacteria and against *Staphylococcus aureus,* it has no effect. The chloroform fraction of the plant *Staphylococcus aureus*, *Shigella dysenteriae* and *Vibrio cholera* showed pretty good activity, whereas *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia* showed very small zone of inhibition. The aqueous fraction and the pure compound ravenoline have shown moderate activity against *Bacillus subtilis*, high activity against *Vibrio cholera* and in case of other bacteria they showed very little activity or no activity at all. The pure compound arborinine showed very little activity or no activity against all bacteria except *Vibrio cholera* (Table-3).

Conclusion

From the above results it may be concluded that various fraction of the leaves of *Ravenia spectabilis* have mild to moderate antibacterial, thrombolytic activities and mild antioxidant activities. The isolated pure compounds showed mild antibacterial, thrombolytic and antioxidant activities. Therefore, considering the potential bioactivity, the plant material can further be studied extensively to find out their unexplored efficacy and many potentially active drug candidate could provide from this plant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. C.M. Hasan, N. Ahmed, R. Haque, M.M. Haque, S. Begum, M.H. Sohrab and M. Ahsan, *Pharmacogn. Mag.*, **9**, 76 (2013); https://doi.org/10.4103/0973-1296.108147.
2. V.H. Heywood, Flowering Plants of The World, Oxford University Press, New York, 202 (1993).
3. M.M. Haque, S. Begum, M.H. Sohrab, M. Ahsan, C.M. Hasan, N. Ahmed and R. Haque, *Pharmacogn. Mag*., **9**, 76 (2013); https://doi.org/10.4103/0973-1296.108147.
4. G.A. Usher, Dictionary of Plants used by Man. Constable: London (1974).
5. J.L. Hartwell, *Lloydia*, **34**, 153 (1971).
6. G.H. Svoboda, G.A. Poore, P.J. Simpson and G.B. Boder, *J. Pharm. Sci.*, **55**, 758 (1966); https://doi.org/10.1002/jps.2600550803.
7. P.G. Waterman, eds.: P.G. Waterman and M.F. Grundon, Chemistry and Chemical Taxonomy of the Rutales, Academic Press: London, p. 337 (1983).

TABLE-3
SCREENING OF ANTIMICROBIAL ACTIVITY OF DIFFERENT FRACTIONS AND PURE COMPOUNDS OF *R. spectabilis*

Sample	Diameter of zone of inhibition (mm)							
	Gram-positive bacteria		Gram-negative bacteria					
	B. subtilis	*S. aureus*	*P. aeruginosa*	*S. typhi*	*S. dysenteriae*	*E. coli*	*V. cholerae*	*K. pneumonia*
Petroleum ether fraction (400 µg/disc)	20	8	7	Absent	7	6	17	6
Carbon tetracholoride (400 µg/disc)	10	Absent	6	Absent	7	9	18	Absent
Chloroform fraction (400 µg/disc)	16	11	6	Absent	15	8	18	7
Aquaous fraction (400 µg/disc)	11	6	Absent	Absent	6	8	16	Absent
Arborinine (100 µg/disc)	6	7	Absent	Absent	6	7	16	Absent
Ravenoline (100 µg/disc)	10	8	6	Absent	Absent	6	17	Absent
Kanamycin (30 µg/disc)	34	30	43	35	27	37	30	40

8. K.C. Das and P.K. Bose, *Trans. Bose Res. Inst. (Calcutta)*, **26**, 129 (1963).
9. B.D. Paul and P.K. Bose, *Indian J. Chem.*, **7**, 678 (1969).
10. M. Kupinic, M. Medic-Šaric, M. Movrin and D. Maysinger, *J. Pharm. Sci.*, **68**, 459 (1979); https://doi.org/10.1002/jps.2600680417.
11. P. Bhattacharyya and B.K. Chowdhury, *Phytochemistry*, **23**, 1825 (1984); https://doi.org/10.1016/S0031-9422(00)83512-8.
12. M.A. Khan and P.G. Waterman, *Fitoterapia*, **61**, 282 (1990).
13. A. Alam, M. Asaduzzaman and N. Nasrin, *Int. J. Pharmacog. Pharm. Res.*, **3**, 80 (2011)
14. M.H. Sohrab, R. Chowdhury, K.M. Rahman, C.M. Hasan and M.A. Rashid, *Fitoterapia*, **75**, 510 (2004); https://doi.org/10.1016/j.fitote.2004.03.008.
15. S. Morris Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970); https://doi.org/10.1351/pac197021020227.
16. B.C. VanWagenen, R. Larsen, J.H. Cardellina, D. Randazzo, Z.C. Lidert and C. Swithenbank, *J. Org. Chem.*, **58**, 335 (1993); https://doi.org/10.1021/jo00054a013.
17. S. Prasad, R.S. Kashyap, J.Y. Deopujari, H.J. Purohit, G.M. Taori and H.F. Daginawala, *BMC Complement. Altern. Med.*, **7**, 36 (2007); https://doi.org/10.1186/1472-6882-7-36.
18. W. Brand-Williams, M.E. Cuvelier and C. Berset, *Lebensm.-Wiss. Technol.*, **28**, 25 (1995); https://doi.org/10.1016/S0023-6438(95)80008-5.
19. A.W. Bauer, W.M. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45(4_ts)**, 493 (1966); https://doi.org/10.1093/ajcp/45.4_ts.493.