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Antimicrobial Activity of Extracts and a Glycoside from *Vanda roxburghii* Br.

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Abstract: The petroleum ether, ethyl acetate and methanol extracts of *Vanda roxburghii* as well as glycoside, melianin (VR-1) isolated from it were screened for antimicrobial activity against a wide variety of bacteria and fungi. The ethyl acetate and methanol extracts showed moderate antibacterial activity against almost all the tested organisms. The compound melianin (VR-1) exhibited strong activity against all the tested organisms and produced zone of inhibition between 17 and 27 mm. The petroleum ether extract was found comparatively less active against the organisms. All the tested materials showed antifungal activity against *Aspergillus fumigatus*, *Candida albicans*, *Hensinela californica* and *Rhizopus arijae*. The minimum inhibitory concentrations (MIC) of melianin against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Shigella dysenteriae* were 32, 64, 64 & 128 μg ml^{-1} respectively. The findings may provide the basis for traditional use of this plant in the treatment of infectious diseases.

Key words: *Vanda roxburghii*, antimicrobial activity, glycoside

Introduction

Vanda roxburghii Br., an epiphytic herb belonging to the family Orchidaceae is widely distributed in tropical Asiatic & Australian Zones (Kirtikar and Basu, 1994). It is found in Bengal, Chosta, Nagpur, Bihar, Central Provinces, W. Penisula, Travancore, Ceylon (Hooker, 1985). In Bangladesh it grows as an epiphyte in Mango, Black berry and Guava trees. The plant has a folkloric reputation as a cure of various infectious diseases (Biswas, 1973). The juice of leaves is used by the native physicians of Bangladesh as a wonderful painkiller of ears. The root is used in the treatment of diarrhoea, dysentery, dyspepsia, bronchitis, rheumatic pains, diseases of the abdomen, hiccough and tremor. The root of this plant is also used as laxative and tonic to the liver & brain. It is also effective against piles, lumbago toothache, boils on the scalp, inflammation, fracture (Kirtikar and Basu, 1994; Biswas, 1973).

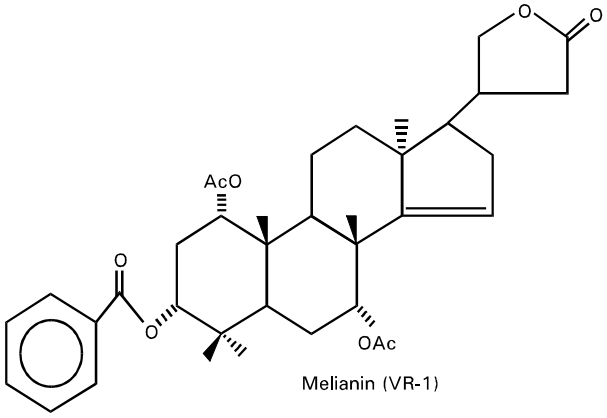
Although *V. roxburghii* is locally credited with medicinal properties, no antimicrobial study of this plant has been reported. As a part of our continuing search for novel antimicrobial principles from the medicinal plants of Bangladesh, we studied *V. roxburghii* and herein reported the results of *in vitro* antimicrobial investigation.

Materials and Methods

Collection of the plant: The roots of *V. roxburghii* Br. were collected from Rajshahi University campus and were identified by Bangladesh National Herbarium, Dhaka.

Extraction, isolation and characterization: The roots were dried in an oven at 45°C, crushed and then extracted successively with petroleum ether (C_2H_5–O-C_2H_5) (40-60°C), ethyl acetate (CH_3COO C_2H_5) and methanol in a soxhlet apparatus (Morrison and Boyd, 1994). The extracts were concentrated by a vacuum rotary evaporator under reduced pressure and then were used for antimicrobial screening.

Compound VR-1 was isolated from the ethyl acetate extract by column chromatography (Beckett and Stenlake, 1986) followed by TLC and PTLC (Stahl, 1969). The compound was identified as melanin on the basis of their ^1H and ^{13}C-NMR, and mass spectral data (Ahmed, 1996) and comparison with previously reported data for melianin from *Melia volkensii* (Lingling *et al.*, 1998). It was then subjected to antimicrobial screening.



Melianin (VR-1)

Antibacterial screening: The *in vitro* antibacterial activity of the extracts and isolated compound VR-1 were studied against 5 gram-positive and 9 gram-negative bacterial strains by the standardized disc-diffusion method (Barry, 1980; Berghe and Vlietnck, 1991) against selected test organisms. Nutrient agar media was used as the bacteriological media. The petroleum ether,

ethyl acetate and methanol extracts were dissolved separately in sufficient amount of methanol to get a concentration of 400µg 10µl^{-1}. Compound VR-1 was dissolved in sufficient volume of methanol to get a concentration of 200µg 10µl^{-1}. The diameters of zone of inhibition produced by the agent were compared with those produced by the standard antibiotic (Kanamycin, 30µg disc^{-1}). The experiment was performed in duplicate to minimize the errors.

Minimum inhibitory concentration (MIC): The MIC value of the compound VR-1 was determined against one gram-positive (*Bacillus cereus*) and one gram-negative (*Escherichia coli*) bacteria. The test was carried out by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Antifungal screening: Seven pathogenic fungi were used for the test. Potato Dextrose Agar (PDA) was used as fungicidal media. The *in vitro* antifungal activities of the samples were performed by disc diffusion method. Clotrimazole was used as a standard one.

Results and Discussion

In antibacterial screening, it was found that the ethyl acetate and methanol extracts showed moderate activity against almost all gram-positive and gram-negative bacteria (Table 1). Whereas the petroleum ether extract exhibited mild antibacterial activity against the test organisms. The zone of inhibition of ethyl acetate and methanol extracts was found in between 11-17 mm and 9-14 mm respectively. On the other hand, compound melianin (VR-1), isolated from the ethyl acetate extract showed strong activity against all the tested organisms and produced zone of inhibition in between 17 and 29 mm. Compound VR-1 showed intense activity against *Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Shigella dysenteriae.*

Antifungal screening of the extracts and compound revealed that all the tested materials were active against *Aspergillus fumigatus, Candida albicans, Hensinela californica* and *Rhizopus arijae*. The compound did not show any activity against *Saccharomyces cerevisae, Pigmented yeast* and *Rhizopus digasporum* (Table 2).

The minimum inhibitory concentration (MIC) of the compound VR-1 against *Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Shigella dysenteriae* was determined and the values were 32, 64, 64 & 128µg ml^{-1} respectively (Table 3).

Table 1: Antibacterial activity of extracts and compound VR-1 isolated form *Vanda roxburghii* Br.

	Diameter of zone of inhibition in mm				
Test organism	A	B	C	D	E
Gram positive					
1. Bacillus cereus	11	16	14	27	27
2. Bacillus subtilis	09	15	12	25	26
3. Bacillus megaterium	-	12	09	20	21
4. Staphylococcus aureus	10	14	12	21	24
5.Streptococcous β-haemolyticus	08	11	13	20	23
Gram negative					
1. Escherichia coli	09	17	13	29	31
2. Shigella dysenteriae	13	15	14	24	29
3. Shigella shiga	-	15	12	21	25
4. Shigella flexneriae	09	14	11	20	23
5. Shigella sonnei	11	13	12	22	27
6. Shigella boydii	-	11	10	18	21
7. Salmonella typhi-A 10	12	11	19	24	
8. Salmonella typhi-B 09	11	12	17	25	
9. Pseudomonas aeruginosa	08	13	09	21	19

A= Petroleum ether extract, 400µg disc^{-1}
B= Ethyl acetate extract, 400µg disc^{-1}
C= Methanol extract, 400µg disc^{-1}
D= VR-1, 200µg disc^{-1}
E= Standard Kanamycin, 30µg disc^{-1}
'-' = No sensitivity

Table 2: Antifungal activity of extracts and compound VR-1 isolated form *Vanda roxburghii* Br.

	Diameter of zone of inhibition in mm				
Test organism	A	B	C	D	E
1. Aspergillus fumigatus	08	10	07	12	24
2. Hensinela californica	-	10	06	07	22
3. Pigmented Yeast	-	-	-	-	23
4. Saccharomyces cerevisae	-	-	-	-	24
5. Rhizopus arizae	06	07	09	13	26
6. Candida albicans	06	07	-	08	25
7. Rhizopus digasporum	-	-	-	-	22

A= Petroleum ether extract, 400µg disc^{-1}
B= Ethyl acetate extract, 400µg disc^{-1}
C= Methanol extract, 400µg disc^{-1}
D= VR-1, 200µg disc^{-1}
E= Standard Clotrimazole, 30µg disc^{-1}
'-' = No sensitivity

Table 3: The minimum inhibitory concentration (MIC) values of the compound VR-1 against test organisms.

	Minimum inhibitory concentration in µg ml^{-1}									
	512	256	128	64	32	16	8	4	2	1
Bacillus cereus	-	-	-	-	-	+	+	+	+	+
Bacillus subtilis	-	-	-	-	+	+	+	+	+	+
Escherichia coli	-	-	-	-	+	+	+	+	+	+
Shigella dysenteriae	-	-	-	+	+	+	+	+	+	+

'+' = Growth; '-' = No Growth,

In conclusion, the present study reports for the first time the antibacterial and antifungal activity of the compound and extracts of *V. roxburghii* Br. The findings may provide the basis for traditional use of this plant in the treatment of infectious diseases.

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References

Ahmed, F., 1996. Chemical and antimicrobial investigation of *Vanda roxburghii* Br. M.Sc. Thesis, Department of Applied Chemistry and Chemical Technology, University of Rajshahi, Bangladesh.

Barry, A. L., 1980. Procedures for testing antimicrobial agents in agar media. In : Antibiotic in laboratory medicine. (V. Lorian Ed.) Williams and Wilkins Co. Baltimore, USA. pp: 1-23.

Beckett, A.H. & J.B. Stenlake, 1986. Chromatography. In: Practical Pharmaceutical Chemistry., Delhi, India, pp: 75-76.

Berghe D.A.V. and A. J. Vlietnck, 1991. Screening methods for antibacterial and antiviral agents from higher plants. In : Assay for Bioactivity (Hostettmann K. Ed) Vol. 6 in the series, Methods in plant Biochemistry (Dey P. M. and J. B. Harborne, series Eds). Academic press, London, pp: 47-56.

Biswas, K., 1973. Indian Medicinal plants, India: Dera Dun Publisher Ltd., vol. 4, p: 1140-1141.

Hooker J.D., 1985. Flora of British India, L. Reeve & Co. Ltd., London, vol. 4, pp: 131-132.

Kirtikar R.K. and B.D. Basu, 1994. Indian Medicinal Plants. 2nd Edn., Vol. 4, Lalit Mohan Basu, Allahabad, India, pp: 1668.

Lingling, L.R, L. Zeng, J.F. Kozlowski, H. Shimada, F.Q. Alali, H.A. Johnson and J.L. Mclaughlin, 1998. New bioactive triterpenoids from *Melia volkensii*. Heterocycles, 41: 64-70.

Morrison, R.T. and R.N. Boyd, 1994. Organic Chemistry, 6th ed., Prentice-Hall of India Pvt. Ltd., pp: 96.

Reiner, R., 1982. Detection of antibiotics activity. In: Antibiotics an introduction. Roche Scientific Service, Switzerland, pp: 21-25.

Stahl, E., 1969. Thin Layer Chromatography-A Laboratory Hand Book Revised and Expanded. 2nd Edn., Springer Verlag, New York, USA.