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Characterization and *in vitro* Antimicrobial Activity of 17-β-hydroxy-14, 20-epoxy-1-oxo-[22R]-3β-[O-β-d-glucopyranosyl]-5, 24-withadienolide from *Vanda Roxburghii* Br

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The ethyl acetate ($C_2H_5COOCH_3$) extract of the roots of *Vanda roxburghii* Br. is known to have a significant antimicrobial activity. The investigation was carried out on the $C_2H_5COOCH_3$ extract for the isolation and characterization of the active principle (s). The extract afforded a glycoside, 17-β-hydroxy-14, 20-epoxy-1-oxo- [22R]-3β-[O-β-D-glucopyranosyl]-5,24-withadienolide (VR-2), the structure of which was elucidated on the basis of spectral evidences. Compound VR-2 was screened against a wide variety of pathogenic bacteria and fungus for its antimicrobial activity by disc diffusion method. The compound showed significant antibacterial activity against most of the tested strain of bacteria and produced zone of inhibition between 14 to 28mm. The compound also showed significant antifungal activity and the minimum inhibitory concentration (MIC) of VR-2 against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Shigella dysenteriae* and *pseudomonus aeruginosa* was determined and the values were 8, 32, 16, 8 and 16μg ml^{-1}, respectively.

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

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Introduction

Vanda roxburghii Br. locally known as Rasna, is one of the important medicinal plants of Orchidiaceae family available in Bangladesh (Kirtikar and Basu, 1987). The plant has been used in Bengal folk medicine as a cure for various diseases. The juice of the plant is used in the treatment of dyspepsia, bronchitis, inflammations, rheumatic pains, disease of the abdomen, hiccough and tumors (Biswas, 1973). It is also used against piles, lumbago, toothache, boils on the scalp and as a remedy for otitis media. The paste of leaves is applied to the body during fever (Kirtikar and Basu, 1987). As a part of our continuing research, (Ahmed, 1996) reported that the petroleum ether (C_2H_5-O-C_2H_5) ethyl acetate ($CH_3COOC_2H_5$) and methanol (CH_3OH) extracts of the roots of *V. roxburghii* possess mild to prominent antimicrobial activity. Among the extracts tested, the $CH_3COOC_2H_5$ fraction showed significant antimicrobial activity. Therefore, we have studied further for the isolation of bioactive principle the plant.

In this paper we report the isolation, characterization and antimicrobial activity of a compound isolated from the ethyl acetate extract of *V. roxburghi* Br.

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 powdered plant material (950gm) was successively extracted with petroleum ether (C_2H_5-O-C_2H_5) (40-60°C) and ethyl acetate ($CH_3COOC_2H_5$) in a soxhlet apparatus (Morrison and Boyd, 1994). The extract was concentrated by a vacuum rotary evaporator under reduced pressure and was subjected to column chromatography over silica gel (Beckett and Stenlake, 1986). The column was successively eluted with C_2H_5-O-C_2H_5, increasing amounts of $CH_3COOC_2H_5$ and finally with CH_3OH which gave fractions from 1 to 29. PTLC (Egon and Stahl, 1969) of the fractions 14-19 using silica gel GF_{254} and the solvent system, C_2H_5-O-C_2H_5: $CH_3COOC_2H_5$ (4:1) afforded compound VR-2 (12 mg). VR-2 was characterized by spectroscopic methods of analysis i.e. EIMS, ^{1}H-NMR and ^{13}C BNMR.

Antibacterial screening: The *in vitro* antibacterial activity of the isolated compound VR-2 were studied against five gram positive and nine gram negative bacterial strains by the standardized disc-diffusion method (Barry 1980; Berghe and Vlietnck, 1991) against selected test organisms. Nutrient agar was used as the bacteriological media. Compound VR-2 was dissolved in sufficient volume of CH_3OH to get a concentration of 200μg 10 μl^{-1}. The diameters of zone of inhibition produced by the agent were compared with those produced by the standard antibiotic (Kanamycin, 30 μg disc^{-1}).

Minimum Inhibitory Concentration (MIC): The MIC value of the VR-2 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 a bacteriological media.

Antifungal screening: Seven pathogenic fungi were used for the test. Potato Dextrose Agar (PDA) was used as fungicidal media. Compound VR-2 was dissolved in sufficient volume of CH_3OH to get a concentration of 200μg 10 μl^{-1}. The *in vitro* antifungal activities of the compound VR-2, was performed by disc diffusion method (Bauer, 1966). Clotrimazole was used as a standard one.

Result and Discussion

The $C_2H_5COOCH_3$ extract of *V. roxburghii* Br. after chromatography over silica gel yielded a pure compound VR-2 which was obtained as white, amorphous powder having melting point 210-211°C. It produced a single spot on the TLC plate after spraying with vanillin-sulphuric acid and heating. The compound was characterized by EIMS ^{1}H-NMR and ^{13}C BNMR.

EIMS m/z (ret.int.): 633.78 [M+], 569.75, 434.54, 379.63, 338.12, 283.21, 243.34, 152.45 and 125.31. ^{1}H-NMR: δ 2.52 (H-α), δ 2.62 (H-β), δ 3.88 (*m*), δ 2.63 (H-α), δ 2.58 (H-β), δ 5.65 (*m*), δ 1.71 (H-α), δ 2.09 (H-β), δ 1.22 (H-á), δ 1.53 (H-β), δ 0.98 (s), δ 1.11 (s), δ 1.25 (s), δ 4.65 (dd), δ 2.33 (H-á), δ 2.41 (H-β), δ 1.73 (s), δ 1.85 (s), δ 4.22 (d), δ 3.98 (t), δ 4.21 (m), δ 4.18 (m), δ 3.88 (m), δ 4.56 (dd), δ 4.32 (s); ^{13}C BNMR: 210.10 (C-1), 45.52 (C-2), 74.11 (C-3), 37.45 (C-4), 134.21 (C-5), 125.59 (C-6), 36.00 (C-7), 35.10 (C-8), 35.41 (C-9), 52.18 (C-10), 21.60 (C-11), 25.31 (C-12), 54.00 (C-13), 87.29 (C-14), 29.92 (C-15), 31.89 (C-16), 78.17 (C-17), 19.09 (C-18), 18.21 (C-19), 81.32 (C-20), 20.21 (C-21), 80.80 (C-22), 34.51 (C-23),150.80 (C-24),120.01 (C-25), 166.00 (C-26), 12.12 (C-27), 20.13 (C-28), 101.21 (C-1′)73.32 (C-2′),76.52 (C-3′), 70.33 (C-4′), 76.51 (C-5′), 61.22 (C-6′).

The mass spectrum of the compound displayed a highest ion peak (M+) at m/z 633.78 corresponding to $C_{34}H_{48}O_{11}$. Other peaks appeared at 569.75, 434.54, 379.63, 338.12, 283.21, 243.34, 152.45 and 125.31. The ^{1}H NMR and ^{13}C-NMR spectra of compound VR-2 were in complete agreement with 17-β-hydroxy-14,20-epoxy-1-oxo-[22R]-3β-[O-β-D-glucopyranosyl]-5,24-withadienolide, previously reported from *Physalis peruviana* (Ahmed *et al.*, 1999). This is the first report of isolation of the compound from *V. roxburghii* Br.

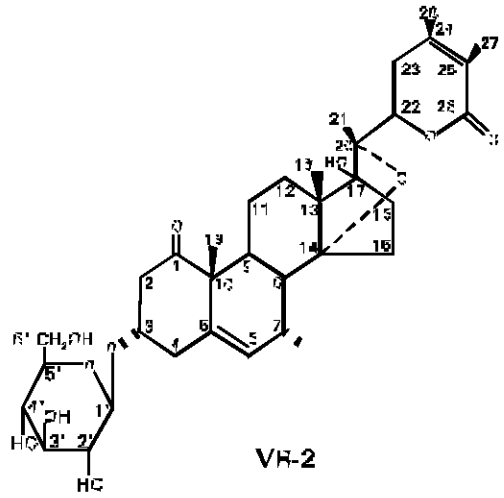

Table 1: Antibacterial activity of compound VR-2 isolated form *V. roxburghii* Br.

	Diameter of zone of inhibition in mm	
Test organism	A	B
Gram positive		
Bacillus cereus	24	27
Bacillus subtilis	23	26
Bacillus megaterium	18	21
Staphylococcus aureus	21	24
Streptococcus-β-haemolyticus	20	23
Gram negative		
Escherichia coli	28	31
Shigella dysenteriae	26	29
Shigella shiga	24	25
Shigella flexneriae	20	23
Shigella sonnei	22	27
Shigella boydii	18	21
Salmonella typhi-A	15	24
Salmonella typhi-B	14	25
Pseudomonus aeruginosa	16	19

A = VR-2, 200μg disc^{-1} B = Standard Kanamycin, 30μg disc^{-1}

Table 2: Antifungal activity of compound VR-2 isolated form *V. roxburghii* Br.

	Diameter of zone of inhibition in mm	
Test organism	A	B
1. Aspergillus fumigates	11	24
2. Hensinela californica	08	22
3. Pigmented Yeast	-	23
4. Saccharomyces cerevisae	-	24
5. Rhizopus arizae	12	26
6. Candida albicans	09	25
7. Rhizopus digasporum	-	22

A = VR-2, 200 μg disc^{-1} B = Standard Clotrimazole, 30 μg disc^{-1} > "-" No sensitivity

Table 3: The minimum inhibitory concentration (MIC) values in μg ml^{-1} of compound VR-2 against test organisms.

Test organism	VR-2
Bacillus cereus	8
Bacillus subtilis	32
Escherichia coli	16
Shigella dysenteriae	8
Pseudomonus aeruginosa	16

The results of antibacterial and antifungal activity of compound VR-2 are presented in Table 1 and Table 2. VR-2 showed significant antibacterial activity against all the gram-positive and gram-negative bacteria. The zone of inhibition produced by VR-2 was in between 14 to 28 mm.

Compound VR-2 showed antifungal activity against *Aspergillus fumigates*, *Candida albicans*, *Hensiela colifornica*, *Rhizopus arijae* and produced zone of inhibition between 8 to 12 mm. The compound did not show any activity against *Saccharomyces cerevisae*, *Pigmented yeast* and *Rhizopus digasporum*.

The minimum inhibitory concentration (MIC) of the compound against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Shigella dysenteriae* and *pseudomonus aeruginosa* was determined and the values were 8, 32, 16, 8 and 16μg ml^{-1} respectively (Table 3).

In conclusion, this investigation reports here the characterization, antibacterial and antifungal activity of the compound VR-2 isolated from *V. roxburghii* Br. Further work is required from a toxicological point of view and necessary to better evaluate the potential effectiveness of the compound as an antimicrobial agent.

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