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ANTIBACTERIAL ACTIVITY OF *Vitis trifolia* Linn.

K. M. Rahman, M. M. Rahman and F. Ahmed*

Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

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Abstract: The plant *Vitis trifolia* (local name Amallata, Bundal, Roktomurmur) is traditionally used for the treatment of various diseases including diarrhoea and dysentery. The scientific basis of the traditional use of this plant was tested in this investigation. The collected plant parts were cut into small pieces, dried and grinded into fine powder with the help of a suitable grinder. About 400 gm of powdered material was extracted with 90% methanol and was tested for its antibacterial activity by disk diffusion method using amikacin as a standard. The extract showed a remarkable antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermis*, and a mild activity against *Escherichia coli and Shigella dysenteriae.* The results of the present investigation support the notion that this traditional plant could be used for partial remedy of diarrhoea and dysentery.

Key words: *Vitis trifolia* Linn, antibacterial activity, traditional medicine

Introduction

Vitis trifolia Linn. (Vitaceae) commonly known as 'Amallata' is a 'climber' and distributed through out India, Ceylon, Malay and Java. In Bangladesh it is known as 'Bundal' and 'Roktomurmur' as well. The plant is widely used for the treatment of diarrhoea and dysentery (Rahman, 2003). Roots are used for the treatment of inflammation of the spleen, liver and heart diseases, blood purification and biliousness (Kirtikar *et al.*, 1987). The whole plant is used as tonic, stomachic and expectorant (Ambasta, 1986). It is evident from the existing information that this plant may possess some important biological activity. Kundu *et al.* (2000) reported its anti-tumor activity. The primary objective of this study was to evaluate the antibacterial activity of the methanolic extract of *Vitis trifolia*.

Materials and Method

Collection of plant: *Vitis trifolia* Linn. (Vitaceae) was collected from Batiaghata, Khulna during the month of January, 2003 and was identified by the National Herbarium of Bangladesh (accession no. 29753).

Extraction: The collected plants were dried for one week after cutting into small pieces and grinded into fine powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until use.

About 400 gm of powdered material was taken in a clean, flat-bottomed glass container (4 lit) and soaked in 1300 ml of 90% methanol (prepared by adding water). The container was sealed and kept on standing for a period of 7 days with occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a filtration through whatmann filter paper, and the filtrate thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to obtain a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to obtain a dried extract. The crude extract obtained was used for its antibacterial activity (Rahman, 1994).

Test of antibacterial activity: Antimicrobial activity of the crude extract was determined by disk diffusion method (Bauer *et al.*, 1966; Ahmed *et al.*, 2001).

Preparation of disks: Three types of disks were used for antibacterial activity test.

Sample disks: Sterile filter paper disks (5 mm in diameter) were taken in a petridish. Six micro litter of the sample solution (prepared by dissolving 1 g of the crude extract in 10 ml of methanol) with desired concentration (100 µg/µl) was applied on the disks by using a micropipette in an aseptic condition. These disks were left for few minutes in an aseptic condition for the complete removal of the solvent.

Standard disks: These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antibiotics with that produced by test samples. In this investigation standard amikacin disks (30 µg/disk, Oxoid, U.K.) were used as the reference.

Blank disks: These disks were used as negative control to ensure that the residual solvents (that left over the disks even after air drying) and the filter paper were not active themselves. Six micro litter of methanol was applied on the sterile filter paper disk with the help of a micropipette and left for few minutes.

*Corresponding author: E-mail: Tel: +880-41-720171, Ext. 253; Fax: 880-41-731244; firoj72@yahoo.com
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Preparation of media: 14 g of dried Nutrient Agar Media (Oxoid, UK) was dissolved in 500 ml of distilled water and a clear medium was obtained by thorough shaking and heating in a water bath. The media was then sterilized in an autoclave at temperature of 121°C and pressure of 15 lbs/sq-inch for 20 min.

Selection of the test organisms: Following bacteria were used as test organisms for the antibacterial activity test.

Table 1. List of bacteria used for the antibacterial screening

Gram Positive	Gram Negative
Staphylococcus aureus	*Escherichia coli*
Staphylococcus epidermis	*Shigella boydii*
	Shigella dysenteriae
	Shigella flexneri
	Shigella sonnei
	Vibrio cholerae

Preparation of the seeded test plates: Sixteen ml of the sterilized medium was poured on to each (sterilized) test tube aseptically under laminar air hood. Each of the test organisms was then transferred from the subculture to the test tube with the help of the sterilized inoculating loop at 45°C. The test tubes were shaken by rotation to get a uniform suspension of organisms. The bacterial suspensions were immediately transferred to the sterile petridishes and then were rotated several times, first clockwise and then anticlockwise, to ensure a homogeneous distribution of the test organisms to give a uniform layer of depth of approximately 4 mm. After the medium returned to room temperature, it was stored in a refrigerator (4°C) for 2 h. All of the three disks (sample, standard and blank) were then placed in the seeded test plates using sterile transfer loop for antibacterial activity test. The plates were kept at 4-8^{0}C facilitating maximum diffusion. Afterwards the plates were kept in an incubator at 37°C for 12-18 h to allow the growth of bacteria. The experiments were carried out thrice and the mean of the readings were recorded.

Results and Discussion

Table 2 showed the antibacterial activity of the methanolic extract of *V. trifolia* relative to that of the standard amikacin. It showed a strong antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermis* where the zone of inhibition was 21 and 17 mm, respectively. The extract showed a mild activity against *E. Coli* and *S. dysenteriae* where the zone of inhibition was 9 mm and 10 mm, respectively. It was, however, ineffective against *S. boydii*, *S. flexneri*, *S. sonnei* and *V. cholerae.*

Table 2. Antibacterial activity of methanolic extract of *V. trifolia*

Bacteria	Zone of inhibition (mm)	
	Methanol Extract 600 µg/disk	Amikacin (30 µg/disk)
Gram Positive		
Staphylococcus aureus	21	27
Staphylococcus epidermis	17	30
Gram Negative		
Escherichia coli	09	31
Shigella boydii	-	30
Shigella dysenteriae	10	28
Shigella flexneri	-	29
Shigella sonnei	-	25
Vibrio cholerae	-	27

(-): No inhibition

Since the crude extract showed a mild activity against *E. coli* and *S. dysenteriae*, the results, thus, at least partially support the basis of the traditional use of this plant as a remedy of diarrhoea, dysentery and systemic shigellosis that are caused by these bacteria.

Conclusion

The plant is widely and effectively used by the traditional practitioner for the treatment of various microbial diseases including diarrhoea and dysentery. It partially supports the basis of traditional use. In case of *E. coli* and *S. dysenteriae*, the crude extract had about one third of the antibacterial activity as compared to that

obtained for amikacin. This may relate either to the low concentration and/or thermal/chemical destruction of the active ingredients of the crude extract. In conclusion, it is suggested that Bundal crude extract (*Vitis trifolia*) though could not be fully supported to use as remedy of diarrhoea and dysentery, its purified fractions might demand so. Therefore further researches are essential particularly using its various fractions.

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