



Antibacterial screening of the leaf extracts of *Vanda coerulea* Griff. ex Lindl

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ABSTRACT

The various leaves extracts such as ethanol, chloroform and petroleum ether: ethanol (1:1) of *Vanda coerulea* Griff. ex Lindl. were investigated for an *in vitro* antibacterial activity by disc diffusion method. Ethanolic leaf extracts produced definite antibacterial activity against gram positive *Bacillus cereus* than *Streptococcus faecalis*, *Streptococcus pneumonia* and *Staphylococcus aureus* and very less inhibition by gram negative bacteria such as *E. coli* and *Salmonella typhi*. The present study reveals that the ethanolic extracts of *Vanda coerulea* leaf had more antibacterial activity against all bacteria tested followed by chloroform and petroleum ether: ethanol (1:1) extracts. From the present study, it reveals that the selected plant material has potential antibacterial activity against gram positive bacteria *Bacillus cereus*.

Keywords: *Vanda coerulea*; antibacterial activity; herbal medicines; active compounds.

INTRODUCTION

According to WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (WHO, 2001). Traditions of collecting, processing and applying plants and plant based medications have been handed down from generations to generations (Von Maydell, 1996). Plants contain numerous biologically active compounds many of which have been shown to have antimicrobial properties (Cowan, 1999). Since the advent of synthetic chemicals the use of natural products in medicinal and pesticidal applications has reduced. However, the scenario is once again changing in the light of environmental and other secondary effects of synthetic products. There are several reports in the literature regarding the antimicrobial activity of plant extracts and the bioassay guided fractionation of them to yield active principles (Rabe and Vanstaden, 1997; Zgodapols et al, 2002; Rojas et al., 2003).

Orchids are one of the largest groups of Angiosperms belonging to the family Orchidaceae. They are known for their diversity of habitats, and they occur in diverse habitat conditions of our country. India is one of the richest orchid habitats with about 2500 species in 167 genera represented in six sub-families, 17 tribes and 30 sub-tribes. (Hedge, 1997). One of the unscreened medi-

cal plant is *Vanda coerulea*. It is a rare orchid species available in the high altitudes of North eastern Region of Himalayas, India and has been used as a traditional medicine in India. Different parts of the plant have been claimed to possess medicinal properties in the traditional medical system. The medicinal value of the present study plant was also discussed in 'Charaka samhita' – a classic ancient Indian medicinal treatise in Sanskrit. The ancient Indian people were also well aware of the medicinal values of orchids (Manilal and Sathishkumar, 1986) and they are rich in alkaloids, flavonoids, glycosides and other phytochemical contents besides they also cure diseases viz., eye diseases, amoebic dysentery, high fever, scabies and other skin disease (Nagrare, 2001). Thus the present study was undertaken to determine the antibacterial effect of the *Vanda coerulea* leaf extracts.

MATERIALS AND METHODS

Plant material: The plant material used for the study was collected from Shillong, North Eastern region of India. The collected plant material was identified at Rapinat Herbarium St. Joseph's College (Autonomous), Tiruchirappalli, India.

Preparation of extracts: The method of Alade and Irobi (1993) was adopted for preparation of plant extract with little modification. Three 20 gm portions of the powdered plant materials were soaked separately in 100 ml of ethanol, chloroform and petroleum ether: ethanol (1:1) for 72 hr. Each mixture was stirred every 24 hr using a sterile glass rod. At the end of fraction each extract was passed through Whatman No.1 filter paper. The filtrate obtained was concentrated in vacuum using rotary evaporator.

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Table 1: Antibacterial activity of Leaf Extracts of *Vanda coerulea* Griff. Ex. Lindl

Test Bacteria	Inhibition Zone Diameter in mm			
	Ethanol Extracts	Chloroform Extracts	Petroleum Ether : Ethanol (1:1)	Negative Control for Ethanol
Gram-positive				
<i>Bacillus cereus</i>	9.0	8.2	7.2	1.5
<i>Streptococcus faecalis</i>	6.0	6.1	5.2	1.0
<i>Streptococcus pneumoniae</i>	1.6	0.9	0.6	0.3
<i>Staphylococcus aureus</i>	2.0	2.6	2.0	1.0
Gram-negative				
<i>Escherichia coli</i>	5.3	4.4	3.2	1.5
<i>Salmonella typhi</i>	4.2	3.0	2.0	1.1
<i>Proteus vulgaris</i>	5.1	5.0	4.1	1.2
<i>Enterobacter aerogenes</i>	4.1	4.0	3.0	1.0

Culture Media: The media used for antibacterial test was Nutrient agar/ Broth of Himedia Pvt . Ltd., Mumbai, India.

Inoculum Preparation: Cultures of bacteria was inoculated into Nutrient broth (Liquid medium) and incubated at 37°C for 4 hr and suspension was checked to provide approximately 10 CFU/ ml.

Bacteria Tested: Gram positive bacteria Viz., *Bacillus cereus*, *Streptococcus faecalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and Gram negative bacteria Viz., *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris* and *Enterobacter aerogenes*. (Microbial Type culture collection, Chandigarh, India) were used as test organisms. The bacteria maintained on nutrient agar slants (Hi Media) at 4°C.

Antibacterial Testing: The disc diffusion assay of Lennette (1985) as described by Rosoanaivo and Ratsimamanga (1993) and Rabe and Vanstadan (1997) with modifications was used to determine the growth inhibition of bacteria by the plant extracts. Diluted bacterial cultures (0.2 ml) were spread over sterile nutrient agar plates by using sterile glass rod spreader and the plates allowed to dry at room temperature. Subsequently, 6mm diameter filter paper discs (Whatman) were impregnated with the plant extract were placed and gently tapped to remove excess liquid, positioned on seeded plates. After incubation for 24 hr at 37°C all plates were observed for zones of growth inhibition and these zones were measured in centimeters.

RESULTS AND DISCUSSION

The antibacterial activity of ethanol, chloroform and petroleum ether: ethanol (1:1) leaf extracts of *Vanda coerulea* on both gram positive and gram negative bacteria are presented in the Table -1. The ethanolic leaf extract exhibited significant inhibition against gram positive *Bacillus cereus*, whereas the same extract showed less inhibition on *Streptococcus faecalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*. In case of gram negative bacteria *Escherichia coli* showed significant zone of inhibition in ethanolic extracts when

compared to *Salmonella typhi*, *Proteus vulgaris* and *Enterobacter aerogenes*. However, the leaf extracts with chloroform and petroleum ether: ethanol (1:1) showed moderate and very low inhibition against both gram positive and gram negative bacteria taken for the study.

In this study, it was observed that the ethanolic leaf extract had a significantly higher antibacterial activity than the other two extracts. This difference is attributed to the solubility of the active component in different solvents. This report has also revealed that the solvents used for extraction plays a very important role in its level of activity. Our results hence indicated that the organic extracts exhibited better antibacterial activity because of the antibacterial active principles which are either polar or non-polar are effectively extracted only through the organic solvent medium. (Santayana et al., 2003).

A number of phytochemicals have been studied for their antibacterial activity which is potentially useful against infectious diseases. It is clear that the chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plant secondary metabolites. Examples include alkaloids (Vanbeek et al., 1985; Chakraborty and Brantner, 1999), naphthoquinones (Cai et al., 2000), Coumarins (Hamburger et al., 1985), flavonoids (Watcher et al., 1999), phenolic acids (Fernandez et al., 1996), terpenes (Coveney et al., 1985) and terpenoids (Osawa et al., 1990; Habibi et al., 2000).

CONCLUSION

It has been concluded that the ethanolic leaf extract of *Vanda coerulea* possess antibacterial activity. Its potential application in the treatment of bacterial infection would therefore be promising. Further work is required in order to isolate the active constituents of the plant responsible for the antibacterial activity.

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