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Research Article

Phytochemical and Antimicrobial screening of the polar and Non-polar solvent Root extracts of *Vanda tessellate* (Roxb.)Hook.ex G.Don

P. Jyothi Chaitanya[2], M.Lalagoud[1], R.Chandrashekar [2], N.Lakshmi Bhavani[2]*, Karunakar Rao Kudle[3]

[1]*Department of Botany, Government Degree College for Women, Begumpet,*
[2]*Department of Botany, University College of Science, Saifabad, Osmania University*
[3]*Department of Biochemistry, University College of Science, Osmania University, Hyderabad, Andhra Pradesh-500007, India.*

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ABSTRACT

The present study deals with the polar and non polar root extracts of *Vanda tessellate*. In this study polar (Methanol) and non-polar (Chloroform) solvent extracts of *Vanda tessellate* were investigated for their phytochemical and antimicrobial activity. Phytochemical analysis revealed the presence of the Tannins, phenols, alkaloids, flavonoids, anthocyanins, terpenoids, cyogenic glycosides and steroids. Polar extracts showed more phytochemicals than the non polar extracts. The cyogenic glycosides are found to be present in non polar extracts that are absent in the polar solvent. The microorganisms employed were *E.coli, Psuedomonas putida, Staphylococci, Klebsiella pneumonia, Bacillus subtilis and* certain fungal species such as *Aspergillus niger, Fusarium, Colletotrichum, Rhizopus and Mucor*. Among the two aqueous and methanol extracts used methanolic extracts were found to more active towards the organisms tested than the non polar extracts. The analysis revealed maximum activity of polar solvent against bacteria in order of the *Klebsiella pneumonia, E.coli, Staphylococcus aureus, Pseudomonas putida, and Bacillus subtilis.* Whereas non polar solvent extracts showed their maximum activity on bacteria in order *Pseudomonas putida, E.coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis.* Both Polar and non polar solvents showed broad spectrum inhibition zone against the fungal species *Aspergillus niger, Aspergillus flavus, Fusarium, Colletotrichum, Rhizopus and Mucor*. Due to the presence of various active phytochemicals present in *Vanda tessellate* may be attribute the broad spectrum inhibition zone against microorganisms, which may be their individual or combined action. Methanolic extracts of almost all samples dominated aqueous extracts in inhibiting the growth of the pathogenic bacteria and fungi under study, but were less potent when compared to those of Ampicillin and ketocnogole used as positive controls.

Key Words: *Vanda tessellate,* Polar, non polar, Root extracts, Antibacterial, Antifungal.

INTRODUCTION

World's population (80%) needs herbal medicines to their health need to cure diseases, especially for millions of people in the vast rural areas of developing countries (WHO, 2001). Collection of plants, applying and processing of the plants and plant based medications has becoming down from generations to generations (Von Maydell, 1996). Medicinal properties of medicinal plant ranges from the administration of the leaves, bark, roots, wood and seeds used as an extract and decoction to be prepared from the plants (Ogbulie *et al.*, 2007). In different parts of the world a very large number of researchers have been studied the effects of plant extracts on bacteria (Reddy *et al*., 2001; Ateb & ErdoUrul, 2003). Most of the Plants can synthesize aromatic substance like phenolic, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites. In Angiosperms Orchids is one of the largest groups belonging to the family Orchidaceae. They are known for their diversity of habitats, and they occur in our country in diverse habitat. India is one of the richest orchid habitats with about 2500 species and 167 genera represented in six sub-families, 17 tribes and 30 sub-tribes. (Hedge, 1997). In traditional medical system different parts of the plant have been claimed to possess medicinal properties. The roots of vanda possess significant anti-inflammatory activity by their novel aphrodisiac compound has been reported by (A Subramanian, *et al* 2013).The present investigation evaluates the phytochemical analysis and antimicrobial effect of the *Vanda tessellate* root extracts. In the system of Yunani, the roots of Vanda is used as a tonic for the liver and brain, which is effective against bronchitis, piles, lumbago, toothache, and boils of the scalp. It is also used to cure inflammation and heal fractures. The roots are fragrant and bitter in nature and used to cure rheumatism and allied disorders. It is also used in the composition of

*Author for correspondence: E-mail: mlalagoud@yahoo.com

Table1: Photochemical screening test of root extracts

S.No	phytochemicals	Polar solvent (Methanol)	Non-polar solvent (Chloroform)
1	Tannins	+	+
2	Phenols	+	_
3	Saponins	_	_
4	Alkaloids	+	+
5	Flavonoids	+	_
6	Anthocyanins	+	+
7	Amino acids	-	_
8	Carbohydrates	_	_
9	Terpenoids	+	+
10	Cyanogenic glycosides	+	+
11	Steroids	+	+

(+) = Present, (-) = Absent

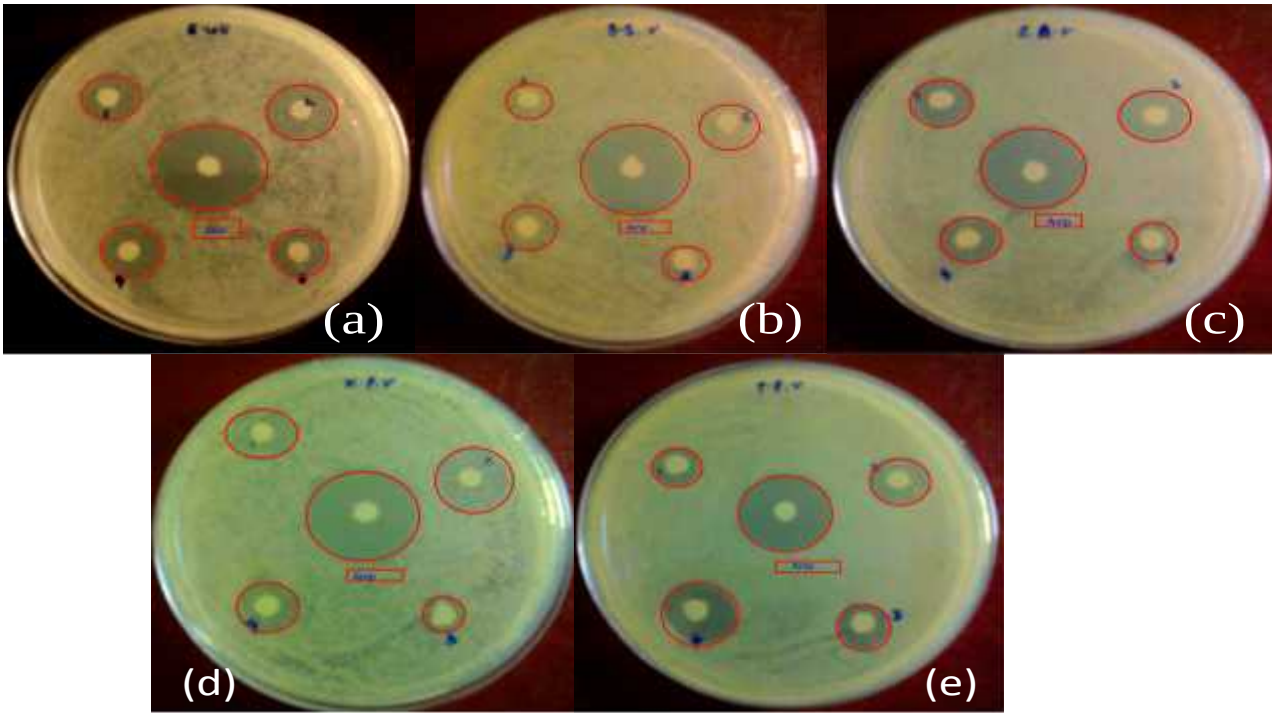

Fig-1 : Antibacterial Activities Of Root Extract Polar solvent (Methanol)1,2 and Non-polar solvent (Chloroform) 3,4 different (µl) effected by (a) Escherichia Coli,(b) Bacillus Subtilis,(c) Staphylococcus aureus,(d) Klebsiella Pneumonia,(e) Pseudomonas putida.

Table 2: Antibacterial activities of root extract Inhibition Zone Diameter in (mm)

Test Bacteria	Polar solvent (Methanol)		Non-polar solvent (Chloroform)		Control (Ampicillin)
	10µl	20µl	10µl	20µl	10 µl
Escherichia Coli	6.49	8.85	6.2	8.19	16.6
Bacillus Subtilis	5.2	7.8	5.51	5.62	17.06
Klebsiella Pneumonia	8.85	9.44	3.76	6.84	14.26
Pseudomonas putida	5.3	7.21	5.21	10.24	13.42
Staphylococcus aureus	6.05	8.5	4.12	6.79	12.97

several medicated oils for external application in rheumatism and diseases of the nervous system.

MATERIALS AND METHODS

Plant material: The plant material used for the study was collected from Matloodhi village Neradigonda mandal, Adilabad District, Andhra Pradesh, India. The collected plant material was identified at University College of science,Saifabad and Antimicrobial activity Studied in Department Biochemistry, Osmania University, Hyderabad, Andhra Pradesh India.

Preliminary phytochemical analysis: The healthy and disease free Vanda root material was collected from the Matloodhi village Neradigonda mandal, Adilabad District,

Andhra Pradesh, India. The root was washed thoroughly in tap water, shade dried in open air. Powder of the root obtained by grinding them mechanically. About100 gram of dried powder of the root powder is soaked separately in 100 ml of different polar and non-polar solvents in a conical flask. It is then subjected to agitation on a rotary magnetic shaker for about 72 hours. After three days the root extracts were subjected to filtration, filtered with No 42 what man filter paper separately. The extract was filtered under reduced pressure using rotary flash evaporator and subjected for further preliminary phytochemical tests. For the phytochemical identification different tests are adopted by using the methods described by Edeogal *et al* (2005) and R. Chandrashekar *et al* (2013), B.Thamilmarai Selvi (2011).

- To the 5ml of extract 5ml of 2N HCL is added and boiled and then the mixture is filtered. To the filtrate a few drops of Mayer's reagent is added. A cream colour precipitate was produced immediately indicating the presence of alkaloids (Dragendorff's test).
- Saponins are tested by boiling 5ml of extract in 10ml of distilled water in a test tube and are shaken vigorously
- for about 30 seconds. The test tube is allowed to settle for half an hour. Formation of froth indicates the presence of saponins.
- Tannins are tested by adding a few drops of 1% lead acetate to 5 ml of plant extract. Appearance of yellow precipitate indicates the presence of tannins.
- Phenols are tested by adding 2ml of ferric chloride solution to 2ml of plant extract. Appearance of bluish green colour solution indicates the presence of phenols. (R. Chandrashekar *et al., 2013*).
- For testing the presence of steroids 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added from the walls of the test tube. Appearance of red colour in the upper layer and yellow with green fluorescence indicates the presence of steroids.
- To 1ml of extract glacial acetic acid, few drops of ferric chloride and then finally concentrated sulphuric acid were added from the walls of the test tube. Appearance of the reddish brown at the junction of two layers and the bluish green colour in the upper layer indicates the presence of cardiac glycosides.
- To one ml of the extract, a few drops of dilute sodium hydroxide are added. An intense yellow colour was produced in the plant extract, which became colorless on addition of few drops of dilute acid. This indicates the presence of flavonoids.
- 1ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid. Formation of reddish colour indicates the presence of terpenoids.
- 1ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour indicates the presence of amino acids.
- 1ml of extract was added 5 to 10 drops of Fehling's solution. Mixture was then subjected to boiling for 15 minutes. Appearance of brick red precipitate indicates the presence of reducing sugars.
- To the 1ml of extract, 1ml of Barfoed´s reagent was added and heated on water bath. Formation of brown precipitate indicates the presence of monosaccharides.
- To the 1ml of extract, 1ml of water was added and swirl the test tube then to the test tube add NaOH by drop wise colour will change to blue green that eventually fades that indicates the presence of anthocyanins.
- To the 1ml of extract add 6 drops of chloroform and then the tube was stoppered with a cork containing a strip of picrate impregnated paper hanging down from the stopper. Incubated the tube for 2 hrs. A colour change of the paper, from yellow to brown red, indicated the presence of Cyanogenic glycosides.

Antimicrobial activities:

Test organisms: Microbial cultures are obtained from the Department of Biochemistry, University College of science, Osmania University, Hyderabad. Among seven bacterial species investigated four gram negative bacteria (*E.coli*,*Psuedomonas, Salmonella typhi, Klebsiella pneumonia*), gram positive bacteria (*Sterptococci, Bacillus subtilis, Bacillus cereus*) were carefully identified using standard microbiological methods. All the bacterial were maintained at 4°C nutrient agar slants respectively.

Preparation of concentrations: Methanol, ethanol, acetone extracts of root of *Vanda tessellate* (Roxb.)Hook.ex G.Don were prepared as a different concentrations (25µg/ml, 50mµg/ml, and 100µg/ml) to get the final drug concentration 5µg, 10mg, and 15µg respectively, control (DMSO) and standard (Ampicillin10µg/ml for bacteria and ketoconazole 10µg/ml for fungi). Concentrations of extracts were prepared by filter paper disc method; discs with 5mm diameter were prepare by using No1 whatman filter paper and sterilized by autoclaving. Then, the discs were impregnated with different concentrations of extracts.

Antibacterial activity: Twenty four hours old cultures of the organisms to be tested were used. The nutrient agar medium plates were prepared by pouring 15ml of nutrient agar media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum was inoculated onto the solidified plates by pour plate method allowed to dry for 5 minutes. In agar disc diffusion method by (Bauer *et al.*, in 1966) in this paper disc method, discs with 5mm diameter were prepared using No1 whatman filter paper and sterilized by autoclaving. Then, the discs had been impregnated with various concentrations of the plant extract and introduced onto upper layer of the seeded agar plates. The plates were then incubated at 37^0C for 24hours. Triplicates were maintained and the averages of the zones of inhibition were calculated.

Antifungal activity: The extracts of leaf and bark were screened for antifungal activity by agar disc diffusion method (P. Jyothi Chaitanya *et al*., 2013). Discs with 5mm diameter were prepared by using No1 whatman filter paper and sterilized by autoclaving. The cultures of 48 hours old fungal culture grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An

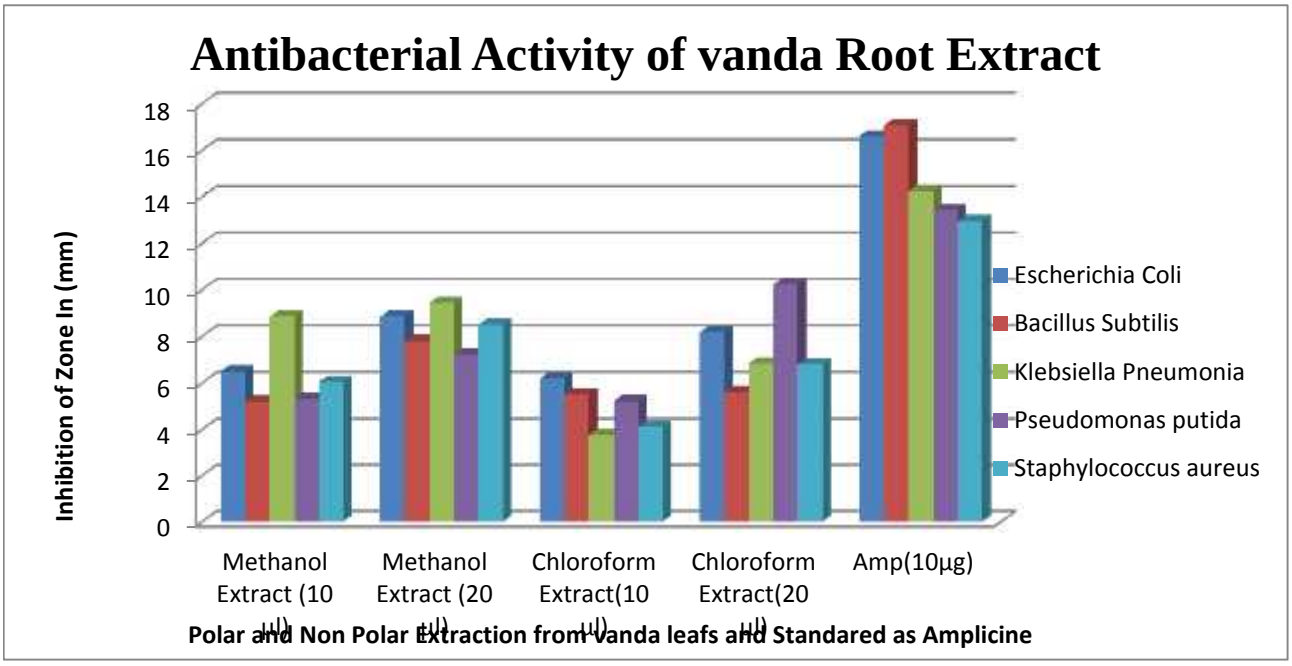

Fig-2: Graphical picture of Antibacterial activities of root extract in Diameter in (mm)

Table 3: Antifungal activities of root extract

Test fungal organism	Inhibition Zone Diameter in mm	
	Polar solvent (Methanol) 10µl	Non-polar solvent (Chloroform) 10µl
Aspergillus flavus	5mm	2mm
Aspergillus niger	6.9mm	3mm
Fusarium sp.,	10mm	3.8mm
Colletotrichum sp.,	15mm	8mm
Rhizopus sp.,	6mm	4mm
Mucor sp.,	8mm	6mm
Control	9.7mm	7.5mm

aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a Petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method different concentrations of extracts were introduced medium. Incubation period of 24-48hours at was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

RESULTS

The phytochemicals that are present in the root screened by different screening tests. The root extracts revealed the presence of the Tannins, phenols, alkaloids, flavonoids, anthocyanins, terpenoids, cyogenic glycosides and steroids. Table 1 showed the presence of tannins, phenols, alkaloids flavonoids, anthocyanins, terpenoids, cyogenic glycosides and steroids both in polar and non polar solvents but during the phytochemical analysis polar solvents when reacted with the phytochemical tests more rapidly than the non polar solvents. (Figure.1and 2) shows antibacterial activities of Vanda tessellate root extracts was assayed and revealed the data on effect of plant extracts on the growth of series of bacterial strains *E.coli, Psuedomonas putida, Staphylococci, Klebsiella pneumonia, Bacillus subtilis* . Among the two polar and nonpolar solvent extracts tested methanol extracts of root showed broad inhibition zone on the bacteria *Klebsiella pneumonia, E.coli, Staphylococcus aureus, Pseudomonas putida and Bacillus subtilis.* But when compared with the zones of inhibition of methanolic and chloroform extracts, chloroform extracts are also showed their maximum activity on bacteria in order *Pseudomonas putida, E.coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis.* The methanol extracts of Vanda showed maximum activity even at lower concentrations. Both the polar and non polar extracts showed the anti fungal activity on the fungi *Aspergillus niger, Aspergillus flavus, Fusarium, Colletotrichum, Rhizopus and Mucor* (Table 3).Polar solvent extracts showed maximum activity on fungi than the non polar solvent extracts.

CONCLUSION

The result shows that most of the phytochemicals which are present in the root extracts solubulised abundantly in

water and polar solvents. Most of the extracts showed that the similar properties to the screening tests. 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). Due to the presence of some different phytochemicals the roots of the Vanda tessellate has the ability to cure eye diseases, amoebic dysentery, high fever, scabies, other skin disease and antimicrobial activities. The broad spectrum inhibition zones exhibited by *Vanda* may be attributed to the various active constituents present in it, which either due to their individual or combined action, exhibit antibacterial and antifungal activity. This study suggests that methanol extracts of screened root extracts would be helpful in treating diseases caused by bacteria *Klebsiella pneumonia, E.coli, Staphylococcus aureus, Pseudomonas putida and Bacillus subtilis.* In particular that the methanol extracts of bark to be used as potent biocide to treat diseases caused by bacteria *Klebsiella pneumonia, E.coli, Staphylococcus aureus, Pseudomonas putida and Bacillus subtilis.* It also supports the earlier investigation (Banso&Adeyemo, 2007) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance. Extensive bioprocess parameter studies should be undertaken for the methanol extract of Vanda as a strong antibacterial agent against curing diseases.

The secondary metabolites of various chemical types present in the plant species are known to possess antimicrobial activities. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall; more lipophilic flavonoids may also disrupt microbial membrane (Tsuchiya, H., Sato, 1996 *et al*). Phenolics and polyphenols present in the plants are known to be toxic to micro-organisms (Mason, T.L. and Wasserman, B.P, 1987). Antibacterial activity of tannins may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins, they also complex with polysaccharides (Ya, C., Gaffney *et al* 1998). Many plant genetic resources have been analyzed for their active constituents possessing antibacterial activities. For example, broad spectrum antibacterial activity of leaf extract of Bolusanthus specis is due to flavonoids (Bojase, G., *et al* 2002). Landolphiaowrience is known to possess glycosides, flavonoids, tannins, saponins, which either individually or in combination, exert antibacterial activity (Ebi, G.C. 1997). The broad spectrum antibacterial activity exhibited by Vanda may be attributed to the various active constituents present in it, which either due to their individual or combined action, exhibit antibacterial activity. The results of the study have justified the traditional use of the plants to curing the diseases.

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