

**IN VITRO EVALUATION OF THE PRELIMINARY ANTIFUNGAL ACTIVITY OF CERTAIN EPIPHYTIC ORCHIDS FOUND IN SACRED GROVES OF PENCHALIKONA, TIRUMALA, TALAKONA AND SRISAILAM OF ANDHRA PRADESH**

N. V. Ramana\*

Department of Botany, Government Degree College, Darsi, Prakasam (Dist.).



\*Corresponding Author: N. V. Ramana

Department of Botany, Government Degree College, Darsi, Prakasam (Dist.).

DOI: <https://doi.org/10.5281/zenodo.17746556>

**How to cite this Article:** N.V. Ramana\*. (2025). *In Vitro* Evaluation Of The Preliminary Antifungal Activity Of Certain Epiphytic Orchids Found In Sacred Groves Of PENCHALIKONA, Tirumala, Talakona And Srisailam Of Andhra Pradesh. European Journal of Biomedical and Pharmaceutical Sciences, 12(12), 283-289.

This work is licensed under Creative Commons Attribution 4.0 International license.



Article Received on 31/10/2025

Article Revised on 21/11/2025

Article Published on 01/12/2025

**ABSTRACT**

*In vitro* evaluation of antifungal activity by disc diffusion method carried out on epiphytic roots and leaves of *Vanda tessellate* Hook. Ex G. Don (NV01) from PENCHALIKONA, and its ecotypes *Vanda tessellate* Hook. Ex G. Don (NV14) from Talakona and *Vanda tessellata* Hook. Ex G. Don (NV16) from Srisailam, *Acampe praemorsa* (Roxb.) Blatt. & McCann (NV06) and its ecotype *Acampe praemorsa* (Roxb.) (N V11) Blatt. & McCann from Tirumala and *Vanda testacea* Rchb.f. from Srisailam with three solvents methanol, n-hexane and ethyl acetate. None of the n-hexane extracts of both leaves and roots of the tested species were effective inhibitors against the three fungal strains tested. Overall, of all tested specimens, ethyl acetate extracts of both roots and leaves were shown to be more efficient growth retarders compared to methanolic counterparts.

**KEYWORDS:** Epiphytes, Orchidaceae, Antifungal activity, Methanol, N-Hexane, Ethyl acetate, Disc diffusion method.

**INTRODUCTION**

Nature's plants are all beneficial. Every species contributes chemical compounds and benefits people in a variety of ways, particularly in the field of medicine. According to World Health Organization (W.H.O) estimates, the majority of the world's population uses plant extracts or their active components as medicine in traditional therapies. A thorough literature review revealed that there are few studies on epiphytes, which are unique plant families. Epiphytes are a significant and fascinating plant group that has adapted to thrive in the harsh climatic and ecological conditions of the canopy. According to a study by Haroon Khan et al. (2019)<sup>[1]</sup>, there are 73 species in the Genus *Vanda* (family: Orchidaceae) that are found across Southeast Asia. This genus includes plants that have medicinal significance and are utilized in indigenous medical systems in Asian nations, notably India, Nepal, China, and Bangladesh. Various species of *Vanda* have a variety of traditional applications, primarily for the treatment of indigestion, earaches, rheumatism, and broken bones. Scientific research has been done on a small number of plants for

their therapeutic effects extracts of various types have been evaluated for a variety of pharmacological effects, including neuroprotective, anti-aging, antibacterial, anti-inflammatory, antioxidant, membrane stabilizing, wound healing, and hepato-protective effects. *Vanda tessellata* (Roxb.) Hook. Ex.G. is a threatened epiphytic orchid. According to Chopra, 1956<sup>[2]</sup>, indigenous medical sources, such as Ayurveda and local traditional medical practices, have used the various components of this plant. *A. praemorsa* is a commonly used epiphytic orchid for medicinal purposes in many regions of India and other nations. According to Reddy et al., 2005<sup>[4]</sup>, the entire plant is used to treat fractures in Andhra Pradesh, India. The seed and leaf juice are used to treat stomachaches, earaches, lower body temperature, and wounds in Kerala, India, as reported by Shanavaskhan et al., 2012.<sup>[3]</sup> Kaur et al., 2010<sup>[5]</sup>, reported that *Vanda testacea* (Lindl.) Reichb. f., also known as Banda or Rasna, is a well-known epiphytic orchid species with a high concentration of alkaloids that has therapeutic effects. In herbal treatments, almost all plant components, including the roots, leaves, and flowers, are utilized in powder form

or as an extract to address rheumatism, bronchitis, nervous disorders, piles, inflammations, and possibly even cancer. With about 880 species, Orchidaceae is the second-largest family in India. The Botanical Survey of India (BSI) has recorded around 1256 orchid species in India, Which Belongs to 155 genera Out of which 388 species are endemic to India.<sup>[6]</sup> Rajendran *et al.*, 1997<sup>[7]</sup> reported medicinal uses of nine species of orchids of southern India.

**MATERIALS AND METHODS**

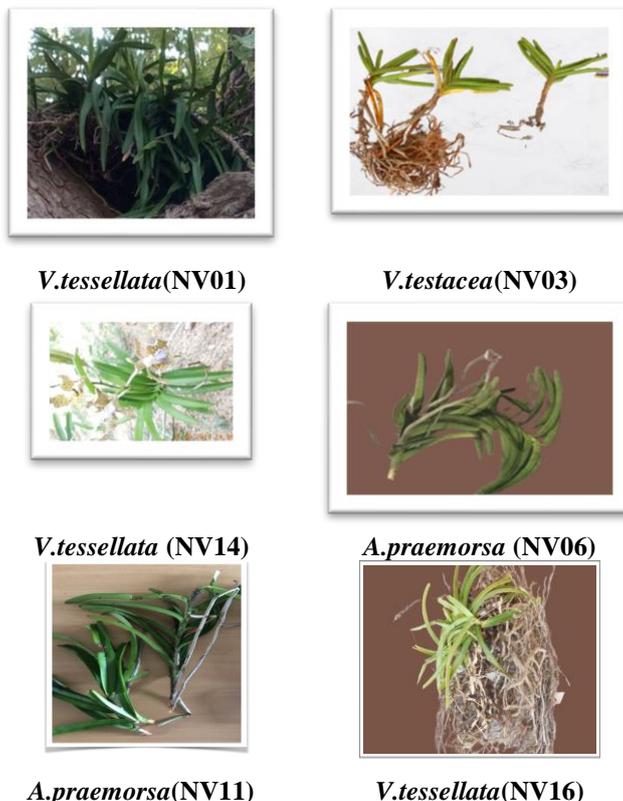
In the present study, four species of genus *Vanda* and two species of genus *Acampe* were collected from different forest regions of Andhra Pradesh. All the plant species selected for the present study are epiphytes belong to the Orchidaceae family. The tested species are collected from different regions are duly authenticated by Botanical Survey of India (B.S.I), Deccan regional

center, Hyderabad. Herbarium specimens of each of the species have been maintained separately in the lab. The list of the species tested is presented in Table.1. The three different species tested in the present study are viz 1. *Vanda tessellata* (NV01) (Penchalikona forest region) 2. *Vanda testacea* (NV03) (Srisailam forest region) and 3. *Acampe praemorsa* (NV06) (Talakona region). Besides, two *Vanda tessellata* (NV14 and NV16) species have also been collected from two different regions (Talakona forest region and Srisailam forest regions respectively), and one *Acampe praemorsa* (NV11) collected from Tirumala forest region, which are morphological variants to 1 and 3 respective species referred to as `ecotypes`. (Fig-1) Ecotypes are morphological variants of a species with little phenotypic differences which are too small for consideration as subspecies.<sup>[8]</sup>

**Table 1: The list of tested plant species and ecotypes.**

S. NO	Plant code*	Scientific name	Place of collection	Type
1	NV01	<i>Vanda tessellata</i> (Roxb.) Hook. Ex G. Don	Penchalikona	Species
2	NV03	<i>Vanda testacea</i> Rchb.f.	Srisailam	Species
3	NV06	<i>Acampe praemorsa</i> (Roxb.) Blatt. & McCann	Talakona	Species
4	NV11	<i>Acampe praemorsa</i> (Roxb.) Blatt. & McCann	Tirumala	Ecotype
5	NV14	<i>Vanda tessellata</i> (Roxb.) Hook. Ex G. Don	Talakona	Ecotype
6	NV16	<i>Vanda tessellata</i> (Roxb.) Hook. Ex G. Don	Srisailam	Ecotype

\*Authentication code denoted for each plant by B.S.I, Hyderabad.



**Fig. 1: Plant species and ecotypes.**

**Preparation of plant extracts**

The leaves and roots were separated, and surface

sterilized with 0.1% Hg Cl 2 for 5 minutes washed thrice with sterilized distilled water 5 minute each time. They

were shade dried for forty days and powdered. Powders of the test material were dissolved in three different solvents viz methanol, ethyl acetate, and n-hexane for *in vitro* antimicrobial studies. Inoculums of three fungal strains were selected in the present study viz., *Fusarium oxysporium*, *Phytophthora infestans*, *Sclerotium rolfisii* were obtained from Department of biotechnology, Mahatma Gandhi University, Nalgonda, Telangana, India. Antifungal activity was tested employing a disc diffusion method.

#### Disc diffusion method<sup>[9,10]</sup>

##### Media preparation

Dissolve 24 gm of PDB in 1000 ml water to obtain PDB-Potato Dextrose Broth for fungal growth. The broth was sterilized by autoclaving at 121°C and 15 lb. pressure for fifteen minutes. The sterilized medium (20 ml) was poured in sterilized Petri dishes under aseptic conditions, allowing them to solidify on a plane table.

##### Procedure

Inoculation of fungal strains in autoclaved PDB media and Incubate 3-4 days at 30° C in a shaker for fungal growth. From that 20 µl of fungal culture was taken and inoculated by inoculation loop on freshly prepared autoclaved agar plates. Filter paper discs(Whatman N0.1 filter paper) of about 6 mm in diameter impregnated with the test compound at the desired concentration, are placed on the agar surface on the Fungal plate. The incubation of the plates was done for 2 to 4 days at 30°C in the BOD incubator. Inhibition diameter around each disc was measured by measuring scale and recorded. Negative control was prepared with only methanol extract used for extraction.

The inhibition percentage (I %) was calculated using the formula.  $I\% = \frac{(C-T)}{C} \times 100$

Where I = Inhibition % of mycelial growth (growth reduction over control), C = radial growth of fungus in the control plate (mm), T = radial growth of fungus on the inoculated plate.

## RESULTS

### Antifungal activity of plant extracts

Leaf and root extracts of *Vanda tessellata* (NV01), and its two ecotypes (NV14 & NV16), *Acampe praemorsa* (NV06), and its ecotype (NV11), and *Vanda testaceae* (NV03) were tested for their antifungal activity against three fungal strains Viz *Fusarium oxysporium*, *Sclerotium rolfisii* and *Phytophthora infestans* by disk diffusion method using three different solvents viz., methanol, n- hexane and ethyl acetate.

Among the six species tested, methanolic leaf extracts of *V. tessellata* (NV01) and its ecotype (NV16) exerted an inhibitory effect on *F. oxysporium* growth while none of the remaining had any effect on the strain. (Table:2, Fig:2 & Plates:1-6)

Ethyl acetate leaf extracts of all the tested species exhibited inhibitory activity on *P. infestans* excepting *V. tessellata* (NV14). Notably, *A. praemorsa* (NV06) and its ecotype (NV11) exerted an inhibitory effect on the growth of *S. rolfisii*. Comparatively, ethyl acetate leaf extracts were better performers compared to methanolic leaf extracts. (Table:2 Fig:2&Plates: 1-6)

Of all the extracts methanolic root extracts *V. tessellata* (NV01) was the only one that exerted inhibition on the growth against *F. oxysporium* while none of the other specimens shown any activity on the three tested fungal strains. (Table:3 Fig:3 & Plates: 1-6)

Similarly, ethyl acetate root extracts of *V. tessellata* (NV01) and its two ecotypes (NV14 & NV16) exerted an effect on the growth of *P. infestans*. Whereas, root extracts of *V. tessellata* (NV01) and its ecotype (NV14) impaired growth against *F. oxysporium*. None of the root extract samples tested had any impact on *S. rolfisii* (Table:3 Fig:3 & Plates: 1-6).

None of the n-hexane extracts of both leaves and roots of the tested species were effective inhibitors against the three fungal strains tested (Table:2-3, Fig:2-3 & Plates: 1-6). Overall, of all tested specimens, ethyl acetate extracts of both roots and leaves were shown to be more efficient growth retarders compared to methanolic counterparts.

**Table 2: Antifungal activity of different leaf extracts of six epiphytes.**

Fungal species	<i>Vanda tessellata</i> (NV01)			<i>Vanda tessellata</i> (NV14) ecotype			<i>Vanda tessellata</i> (NV16) ecotype			<i>Acampe praemorsa</i> (NV06)			<i>Acampe praemorsa</i> (NV11) ecotype			<i>Vanda testacea</i> (NV03)		
	Zone of Inhibition (mm)			Zone of inhibition (mm)			Zone of Inhibition (mm)			Zone of Inhibition (mm)			Zone of Inhibition (mm)			Zone of Inhibition (mm)		
	ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA
<i>S. rolfisii</i>	**	**	**	**	**	**	**	**	**	**	**	25	**	**	25	**	**	**
<i>F.oxysporum</i>	22	**	**	**	**	**	22	**	**	**	**	**	**	**	**	**	**	**
<i>P. infestans</i>	**	**	20	**	**	**	**	**	20	**	**	25	**	**	25	**	**	25

Key: ME- Methanol extract, NH- N-hexane extract, EA-Ethyl acetate extract; \*\* - No activity

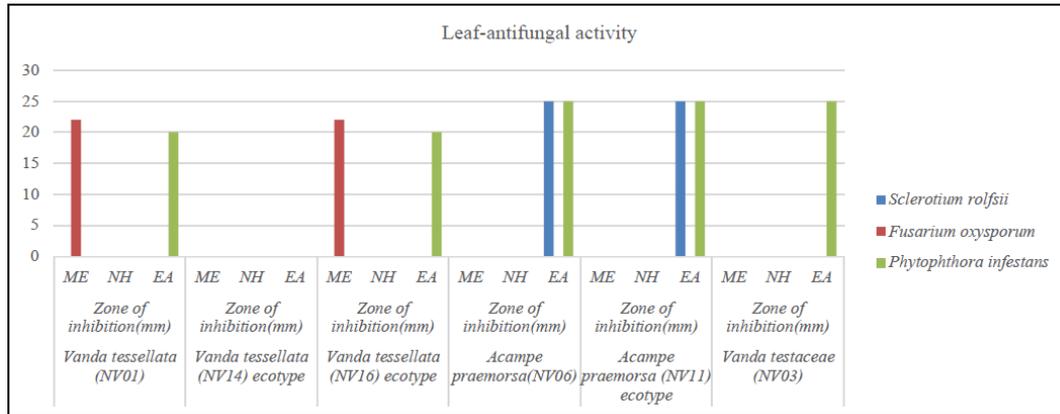


Fig. 2: Antifungal activity of different leaf extracts of six epiphytes.

Table 3: Antifungal activity of different root extracts of six epiphytes.

Fungal species	<i>Vanda tessellata</i> (NV01)			<i>Vanda tessellata</i> (NV14) ecotype			<i>Vanda tessellata</i> (NV16) ecotype			<i>Acampe praemorsa</i> (NV06)			<i>Acampe praemorsa</i> (NV11) ecotype			<i>Vanda testacea</i> (NV03)		
	Zone of inhibition (mm)			Zone of inhibition (mm)			Zone of inhibition (mm)			Zone of inhibition (mm)			Zone of inhibition (mm)			Zone of inhibition (mm)		
	ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA
<i>S. rolfsii</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
<i>F. oxysporum</i>	20	**	20	**	**	20	**	**	**	**	**	**	**	**	**	**	**	**
<i>P. infestans</i>	**	**	20	**	**	20	**	**	20	**	**	**	**	**	**	**	**	**

Key: ME-Methanol extract, NH-N-hexane extract, EA-Ethyl acetate extract; \*\* - No activity

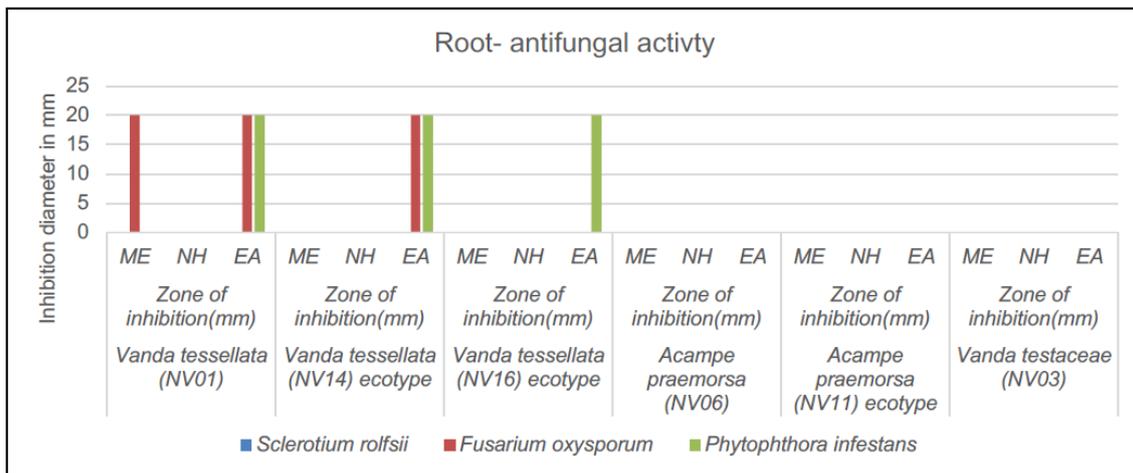
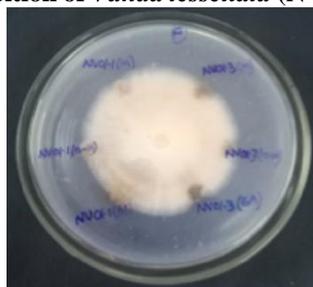


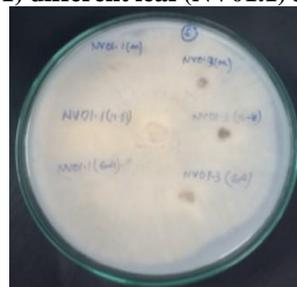
Fig. 3: Antifungal activity of different root extracts of six epiphytes.

Plate-1

Zone of inhibition of *Vanda tessellata* (NV01) different leaf (NV01.1) and root (NV01.3) extracts



Fusarium oxysporum



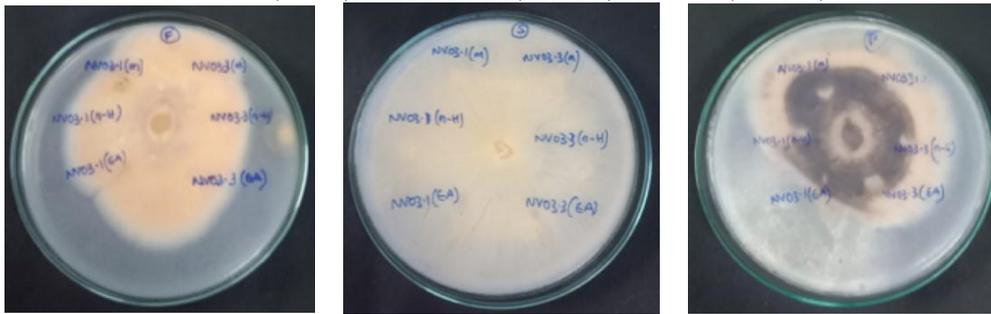
Sclerotium rolfsii



Phytophthora infestans

**Plate-2**

Zone of inhibition of *Vanda testacea* (NV03) different leaf (NV03.1) and root (NV03.3) extracts



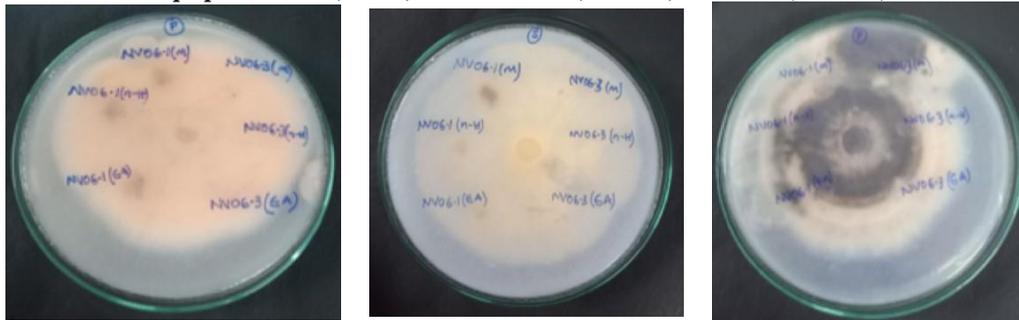
**Fusarium oxysporum**

**Sclerotium rolfsii**

**Phytophthora infestans**

**Plate-3**

Zone of inhibition of *Acampe praemorsa* (NV06) different leaf (NV06.1) and root (NV06.3) extracts.



**Fusarium oxysporum**

**Sclerotium rolfsii**

**Phytophthora infestans**

**Plate-4**

Zone of inhibition of *Acampe praemorsa* ecotype (NV11) different leaf extracts (NV11.1).



**Fusarium oxysporum**

**Sclerotium rolfsii**

**Phytophthora infestans**

**Plate-5**

Zone of inhibition of *Vanda tessellata* ecotype (NV14) different leaf (NV14.1) and root (NV14.3) extracts.



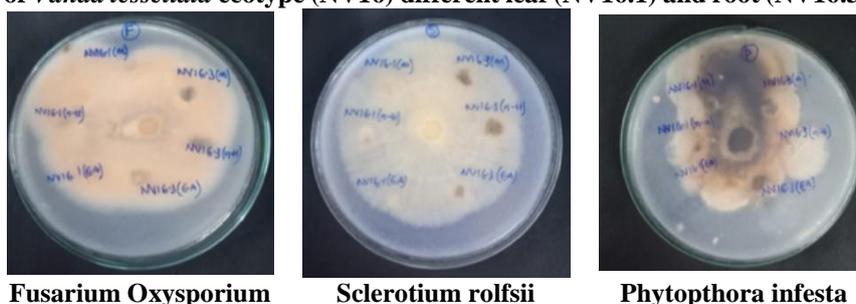
**Fusarium Oxysporum**

**Sclerotium rolfsii**

**Phytophthora infestans**

## Plate-6

Zone of inhibition of *Vanda tessellata* ecotype (NV16) different leaf (NV16.1) and root (NV16.3) extracts.



Fusarium Oxysporium

Sclerotium rolfsii

Phytophthora infesta

## DISCUSSION

One of the significant reasons influencing the extraction efficiency of bioactive compounds from plant extracts is an extraction solvent. Besides, the concentration of the crude drug, temperature, plant parts used for the extraction of secondary metabolites and rate of diffusion are the other factors that influence the efficacy of the extract.<sup>[11]</sup> None of the hexane extracts of the tested species had any inhibitory activity against the fungal strains tested. Reduced ability of hexane to extract polar solutes could be the reason for non-performance of hexane extracts.<sup>[12]</sup> These species and ecotypes demonstrated a more or less similar degree of antifungal activity against *S. rolfsii*. Antifungal activity of *A. praemorsa* (NV06) against *S. rolfsii* and *F. oxysporum* was in accordance with the studies of Akarsh S *et al.* (2016).<sup>[13]</sup> Results noted with ethyl acetate root and leaf extracts of the three test species against *F. oxysporum* and *P. infestans* are in agreement with the earlier reports.<sup>[14,15]</sup> Orchids are known to contain a host of phytochemicals, including alkaloids, triterpenoids and phenolic compounds.<sup>[16]</sup> Phytochemical analysis of *Vanda tessellata* revealed several phytochemicals such as flavonoids, tannins, saponins, terpenoids, steroids and alkaloids.<sup>[17,18]</sup> Several reports confirm the presence of similar secondary metabolites in *A. praemorsa*.<sup>[19,20,21,22]</sup> Antifungal activity of the test species observed in the present study could be attributable to these metabolites like terpenoids, alkaloids phenolics in general and flavonoids in particular.<sup>[23]</sup> As could be seen from published literature and to the best of the knowledge of the author, the present study on antifungal activity of *Vanda testacea* is the first report.

## CONCLUSION

Despite advancements in medicinal plant research, epiphytic plants are still underutilized for their medicinal uses. Preliminary research on antifungal activity conducted at present suggests that ethyl acetate root extracts of *V. tessellata* and its two ecotypes exerted growth inhibition against *P. infestans*, while *V. tessellata* (NV01) and its ecotype (NV16) were effective against *F. oxysporum*. Conversely, leaf extracts of ethyl acetate of *A. praemorsa* and its ecotype were effective against *S. rolfsii*. Ethyl acetate leaf extracts of

*V. testaceae* were effective on *P. infestans*. The current

study in Andhra Pradesh is preliminary but essential for future work on isolating the elements present in the above species.

## REFERENCES

1. Haroon Khan, Marya, Tarun Belwal, Mohd Tariq, Atanas G. Atanasov, Hari Prasad Devkota, Genus *Vanda*: A review on traditional uses, bioactive chemical constituents and pharmacological activities, Journal of Ethnopharmacology, 2019; 229: 4653, ISSN03788741, <https://doi.org/10.1016/j.jep.2018.09.031> (<http://www.sciencedirect.com/science/article/pii/S037887411831540X>) Hoque MM, Khaleda L, Al-Forkan M. Evaluation of pharmaceutical properties on microbial activities of some important medicinal orchids of Bangladesh. J Pharmacogn Phytochem, 2015; 4(4): 265-269.
2. Chopra R.N. (1956) Glossary of Indian medicinal plants. New Delhi. CSIR.
3. Shanavaskhan, AESivadasan, MAlfarhan, Ahmed H, Thomas, Jacob. (2012) Indian Journal of Traditional Knowledge, April. 2012; 11(2): 250-258. Ethnomedicinal aspects of angiospermic epiphytes and parasites of Kerala, India <http://nopr.niscair.res.in/handle/123456789/13853>
4. K.N. Reddy, C. Sudhakar Reddy & S.N. Jadhav (2005) Ethnobotany of certain orchids of eastern ghats of Andhra Pradesh.
5. Kaur, S., & Bhutani, K.K. In vitro Propagation of *Vanda testacea* (Lindl.) Reichb. f. - A Rare Orchid of High Medicinal Value. *Plant Tissue Culture and Biotechnology*, 2010; 19: 1-7.
6. Singh S.K., Agrawala D.K., Jalal J.S., Dash S.S., Mao. A and SinghP. Orchids of India-A Pictorial guide, 2019.
7. Rajendran A, Rao RN, Kumar RK, Henry AN. Some medicinal orchids of Southern India. *Ancient Science of Life.*, 1997; 17(1): 10-14.
8. Turesson, Turesson G. *The genotypical response of the plant species to the habitat.* Hereditas, 1922; 3: 211-350.
9. Mounyr Balouiri, Moulay Sadiki, Saad Koraihi Ibsouda, Methods for in vitro evaluating antimicrobial activity: A review, Journal of Pharmaceutical Analysis, 2016; 6(2): 71-79, ISSN 2095-1779, <https://doi.org/10.1016/j.jpha.2015.11.005>

10. J. F. Hindler and J. K. Jorgensen, "Antimicrobial susceptibility testing: procedures in antimicrobial susceptibility testing," in *Textbook of Diagnostic Microbiology*, C. R. Mahon, D. C. Lehman, and G. Manuselis, Eds., 2007; 319–353, Saunders Elsevier, Beijing, China.
11. Prescott LM, Harley PJ, Klein AD. Microbiology. 7th ed. McGraw Hill Publisher, Singapore, 2008; 94-122.
12. T J Siek. Effective use of organic solvents to remove drugs from biological specimens. *Clin Toxicol.*, 1978; 13(2): 205-30. doi: 0.3109/15563657808988234
13. Akarsh S, Kekuda PTR, Ranitha MC, Vidya P, Monica, Firdos G. Inhibitory activity of some plants against *Colletotrichum capsici* and *Fusarium oxysporum* f. sp. zingiberi. *Journal of Medicinal Plants Studies*, 2016; 4(4): 165-168: 95.
14. Bhattacharjee B., Islam T., Rahman Z., and Islam S. M. S. Antimicrobial activity and phytochemical screening of whole plant extracts of *Vanda tessellata* (Roxb.) Hook. Ex. G. Don. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2015; 4(1): 72-83.
15. Gupta, C., & Katewa, S. S. In vitro Evaluation of the antimicrobial activity of different solvent extracts of Stem of *Vanda tessellata* (Roxb.) Hook. Ex G. Don. *Indo. Am.J.Pharm. Res.*, 2014; 4: 2386-2391.
16. Gutierrez RMP. Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. *J Med Plants Res.*, 2010; 4(8): 592-638.
17. Ghani A. Medicinal plants of Bangladesh with Chemical Constituents and Uses. 2nd ed. Dhaka, Asiatic Society of Bangladesh, 2003; 424.
18. Sirisha J.V., Sailakshmi K. and Vijayal K. Human red blood cell (HRBC) membrane stabilizing activity of leaves of a pet-ether extract of *Vanda tessellata* Roxb. *International Ayurvedic Medicinal Journal*, 2013; 1: 5-8.
19. Akter M, Huda MK, Hoque MM. Investigation of secondary metabolites of nine medicinally important orchids of Bangladesh. *J Pharmacogn Phytochem*, 2018; 7(5): 602-606.
20. Maridass M, Hussain ZMI, Raju G. Phytochemical survey of orchids in the Tirunelveli hills of South India. *Ethnobotanical Leaflets*, 2008; 12: 705-712.
21. Marjoka A, Alam O, Huda MK. Phytochemical screening of three medicinally important epiphytic orchids of Bangladesh. *Jahangirnagar University J Biol Sci.*, 2016; 5(1): 95-99.
22. Suja MR, Williams CB. Micropropagation, phytochemical screening and antioxidant potential of a wild epiphytic orchid *Acampe praemorsa* (Roxb) of Kanyakumari district, India. *European Journal of Pharmaceutical and Medical Research*, 2016; 3(5): 572-576.