EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 9
324-333
Year: 2020

IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF CERTAIN EPIPHYTIC ORCHIDS

N. V. Ramana[1], Ratna Kumar P. K.*[2] and B. V. Rao*[3]

[1]Department of Botany, K.R.K Government Degree College, Addanki, Prakasam.
[2]Department of Botany, Andhra University, Vishakhapatnam.
[3]Department of Botany, Andhra University, Vishakhapatnam.

***Corresponding Author: B. V. Rao**
Department of Botany, Andhra University, Vishakhapatnam.

Article Received on 09/07/2020 **Article Revised on 29/07/2020** **Article Accepted on 19/08/2020**

ABSTRACT
In vitro evaluation of **antibacterial activity** by **disc diffusion method** carried out on epiphytic roots and leaves of ***Vanda tessllata*** Hook. Ex G. Don **(NV01)** from Penchalikona, ***Vanda tessellata*** Hook. Ex G. Don (**NV16**) and ***Vanda testacea*** Rchb.f**.(NV03**) from Srisailam, ***Acampe praemorsa*** (Roxb.) Blatt. & McCann **(NV06**) and ***Vanda tessellate*** Hook. Ex G. Don **(NV14)** from Talakona and ***Acampe praemorsa*** (Roxb**.) (N V11)** Blatt. & McCann from Tirumala with three solvents **Methanol, N-Hexane and Ethyl acetate**. Except N-Hexane roots and Leaves of these plants shown substantial anti - bacterial activity.

KEYWORDS: Epiphytes, Orchidaceae, Antibacterial activity, Methanol, N-Hexane, Ethyl Acetate, Disc diffusion method

INTRODUCTION
An exhaustive survey of literature revealed that studies on the epiphytes which are special groups of plants are comparatively limited. Epiphytes are extreme specialists adapted to climatically and ecologically harsh conditions in the canopy; they represent an important and interesting plant group. A global assessment of the uses and misuses of orchids including epiphytic species in medicine was made and summarised some important uses of orchids in controlling fevers, curing eye diseases, treating fatigue, headaches and their function as anticancer agents. Even though there are several scattered works on the ethnobotanical and ethnomedicinal aspects related to the epiphytes of several other parts of India and neighbouring areas, only a few are available on those of Andhra Pradesh.

Orchidaceae is the second largest family in India, consisting of about 990 Genera. Rajendran *et al*., (1997) 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*) ecotypes 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)

Table -1. The list of tested plant species and ecotype.

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

*Authentication code by B.S.I, Hyderabad.

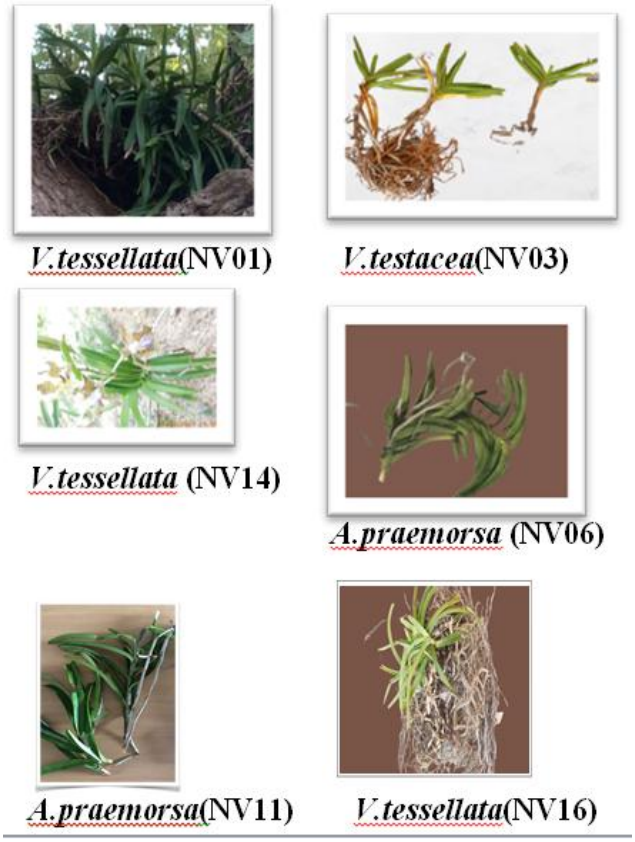


Fig-1 Plant species and ecotypes.

Selection of microorganisms

The organisms employed in this study were two gram-positive bacteria such as *Staphylococcus aureus* (MTCC – 96), *Bacillus subtilis* (MTCC – 121) and two gram-negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC–424). The bacterial cultures were obtained from the Microbial Type Culture Collection, Chandigarh, India

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.

Disc diffusion method

Principle

Agar disk-diffusion testing method provides a simple and effective test in antimicrobial studies to measure the effect and sensitivity of a particular substance on a specific bacterium. In this method agar plates are inoculated with standardized inoculums of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at the desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. The substance diffuses into the agar and inhibits the growth of the test microorganism. The degree of susceptibility is determined by measuring the diameters of inhibition zone due to diffusion of the test compound from the disc into the surrounding medium.

Preparation of the antibiotic disc with Plant extracts

A stock solution of extract was prepared with the dried powdered plant materials by hot extraction process by using a Soxhlet extraction device with respective solvents viz. methanol, ethyl acetate and n-hexane (1:1). The stock solution was then diluted with different concentrations. 0.2 ml of each dilution was impregnated into sterile, blank discs (Whatman N0.1 filter paper) 6 mm in diameter. All discs were fully dried.

Procedure

Agar plates with Mueller Hinton Agar (MHA) are seeded with the test bacterium strain and labelled. Leave culture Plate for 5-10 min at room temperature by closing the culture plate. Place the impregnated disc at the centre of plate using disc dispensers. Gently press antibiotic paper disc by forceps so that there is no gap left between the disc and bacterial culture. Incubate plates at 37^0C for 24 hours. After 24 hours, the inhibition diameter around each disc was measured and recorded. Each extract was tested in triplicate. Negative control was prepared with only methanol extract used for extraction.

RESULTS

Antibacterial activity of plant extracts

Leaf and root extracts of *Vanda tessellate* (NV01), and its two ecotypes (NV14&NV16), *Acampe praemorsa* (NV06), and its ecotype (NV11), and *Vanda testacea* (NV03), were evaluated in three different solvents viz.,

methanol, n-hexane and ethyl acetate against two gram-positive bacteria *Staphylococcus aureus* (MTCC – 96), *Bacillus subtilis* and two gram-negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC – 424) by disk diffusion method.

Methanolic leaf extracts of the *V. tessellata* (NV 01) and its ecotypes (NV 14 &NV 16) exerted inhibitory effect against both the gram-negative bacteria. Among the three, *V. tessellata* (NV01) was most effective. Interestingly, *A. praemorsa* (NV06), and its ecotype (NV11) induced a higher zone of inhibition on all the four bacterial strains, while *V. testacea* (NV03) was effective only on *P. aeruginosa.* (Table: 2, Fig: 2 & Plates: I-VI).

Ethyl acetate leaf extracts of *V. tessellata* (NV01), *A. praemorsa* (NV06), and its ecotype (NV11) exerted inhibition on all the four bacterial isolates. While the two ecotypes of *V. tesellata* (NV14 & NV16) and *V. testacea* (NV03) were more effective on three of the four bacterial isolates barring *E. coli.* (Table: 2, Fig: 2 & Plates: I-VI)
Methanolic root extracts of *V. testacea* (NV03) was the most effective in suppression against gram-positive isolate *S. aureus* while *A. praemorsa* (NV06) exerted inhibition on the other gram-positive isolate *B.subtilis. V. tessellata* (NV01) and its ecotype (NV14) were effective against gram-negative bacteria *E. coli* and *P. aeruginosa* whereas *V. tessellate* ecotype (NV16) exerted inhibition on *S. aureus.* (Table: 3, Fig: 3 & Plates: I-VI).

Ethyl acetate root extracts of *V. tessellata* and its two ecotypes (NV14*)* (NV16) were most effective on all the four bacterial strains tested among which *V. tessellata* (NV01) was more efficacious. *V. testacea* (NV03) followed a similar trend. (Table: 3, Fig: 3 & Plates: I-VI)

None of the n-hexane extracts of both leaves and roots of the tested species has shown any inhibitory activity on the bacterial strains tested. (Tables: 2-3, Fig: 2-3 & Plates: I-VI)

Overall, of all the tested species, ethyl acetate extracts of both roots and leaves were shown to be more efficient inhibitory activity compared to its methanolic extract counterparts. However, among the six specimens tested, methanolic leaf extracts of *A. praemorsa* (NV06), and its ecotype (NV11) exerted growth inhibition on all the bacterial strains tested.

Table-2. Anti-bacterial activity comparative analysis of different leaf extracts of six epiphytes

Species and ecotypes	***Vanda tessellata* (NV01)**			***Vanda tessellata* (NV14) Ecotype**			***Vanda tessellata* (NV16) Ecotype**			***Acampe praemorsa* (NV06)**			***Acampe praemorsa* (NV11) Ecotype**			***Vanda testacea* (NV03)**		
Bacterial Species	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**
B. subtilis	**	**	1	**	**	1	**	**	1.5	**8**	**	4	5	**	3	**	**	3
S. aureus	**	**	2	**	**	1	**	**	1.8	3	**	4	**7**	**	3	**	**	6
P.aerungiosa	2	**	2	1	**	1	**	**	2	**4**	**	3	3	**	2	3	**	2
E. coli	2	**	2	1	**	**	1	**	**	3	**	2	**6**	**	4	**	**	**

ME=Methanolic extract, NH=N-Hexane extract, EA= Ethyl acetate extract ** = No activity

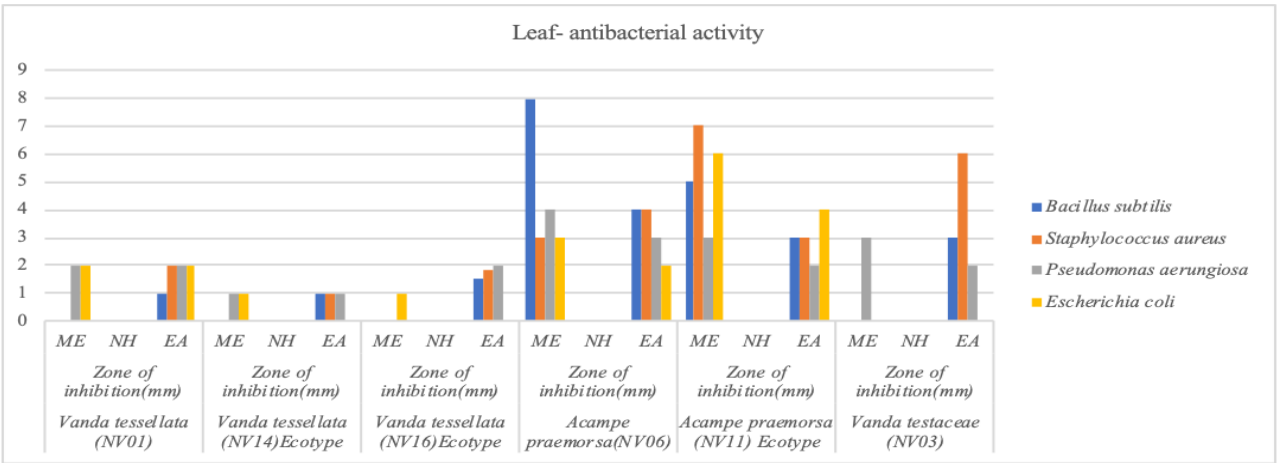


Fig-2. Anti-bacterial activity comparative analysis of different leaf extracts of six epiphytes.

Table-3. Anti-bacterial activity comparative analysis of different root extracts of six epiphytes.

Plant species and ecotypes	***Vanda tessellata* (NV01)**			***Vanda tessellata* (NV14) ecotype**			***Vanda tessellata* (NV16) ecotype**			***Acampe praemorsa* (NV06)**			***Acampe praemorsa* (NV11) ecotype**			***Vanda testacea* (NV03)**		
Bacterial species	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
B. subtilis	**	**	5	**	**	3	**	**	2	6.5	**	**	**	**	**	**	**	2
S. aureus	**	**	5	**	**	4	3	**	2	6	**	**	**	**	**	7	**	4
P.aerungiosa	5	**	5	4	**	4	**	**	2	2	**	3	**	**	**	**	**	2
E. coli	6	**	7	5	**	5	**	**	3	**	**	**	**	**	**	**	**	6

ME=Methanolic extract, NH=N-Hexane extract, EA= Ethyl acetate extract ** = No activity

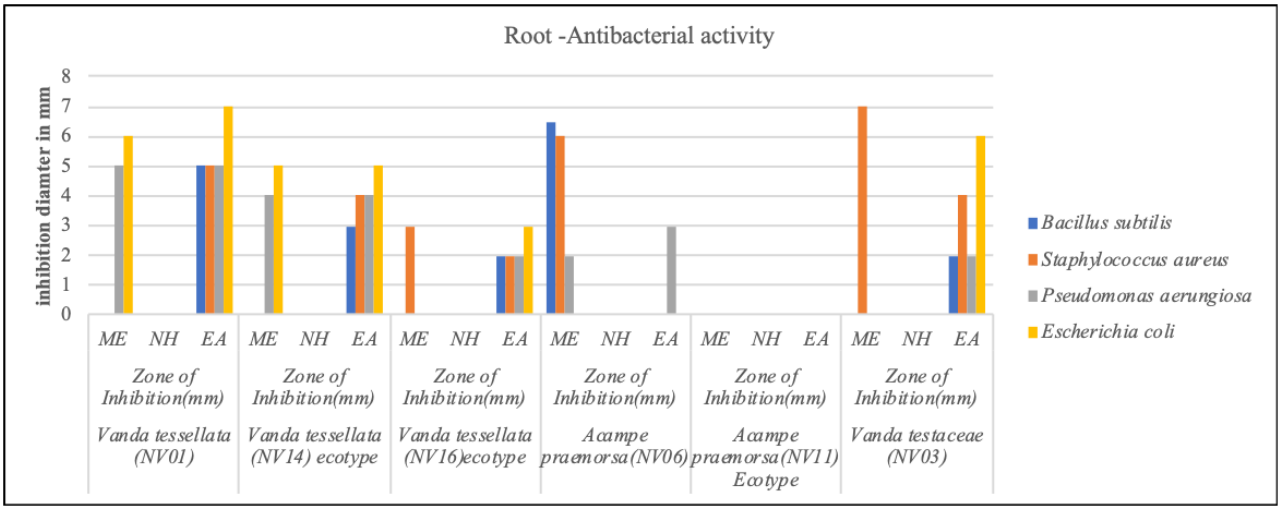
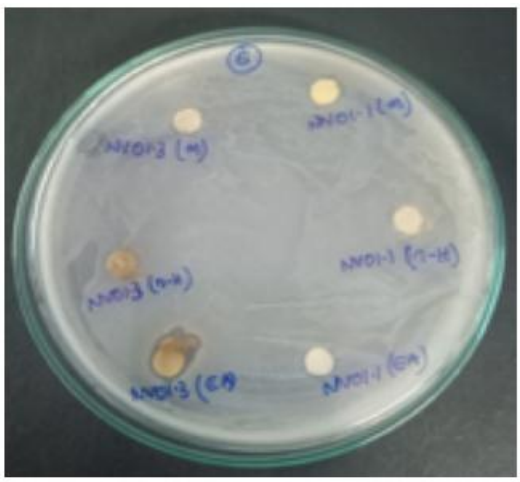


Fig-3. Anti-bacterial activity comparative analysis of different root extracts of six epiphytes.

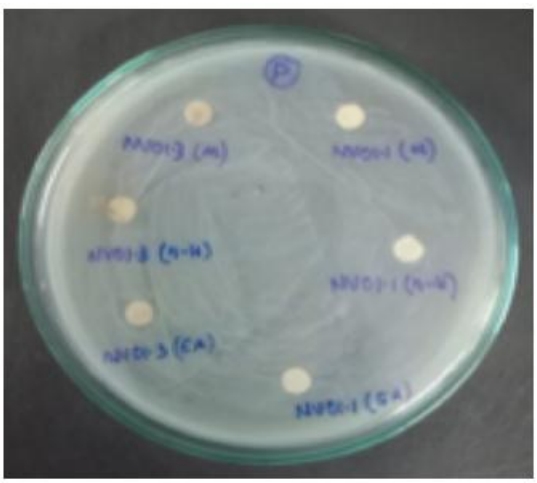
Plate-I

Anti-bacterial activity

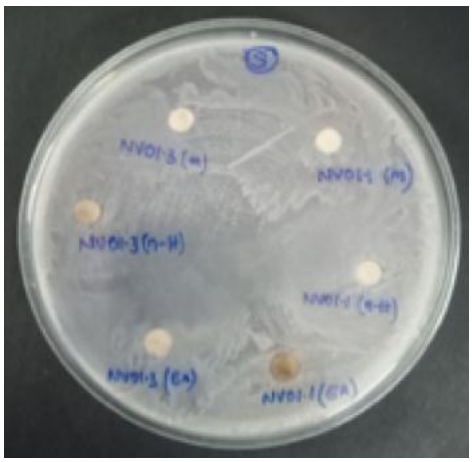
Zone of inhibition of *Vanda tessellata* (NV01) Methanol Hexane and Ethyl acetate Leaf (NV01.1) and root (NV01.3) extracts.



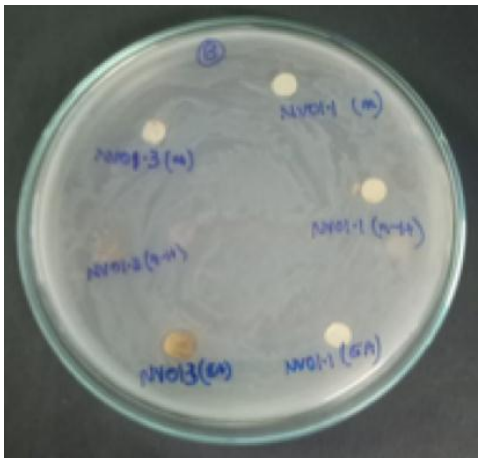
E. coli



Pseudomonas aeruginosa

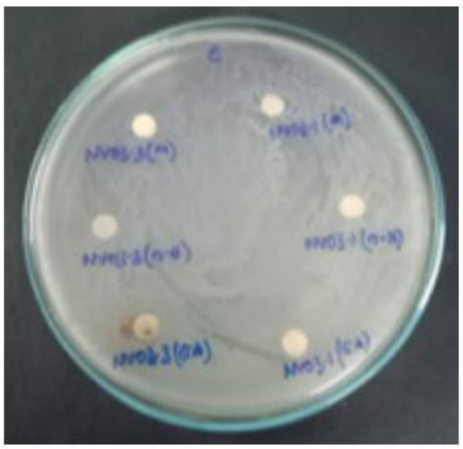


Staphylococcus aureus

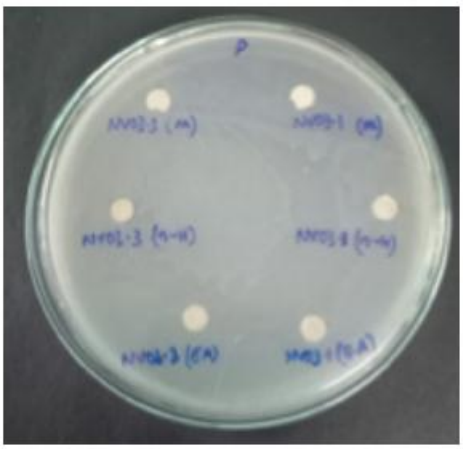


Bacillus subtilis

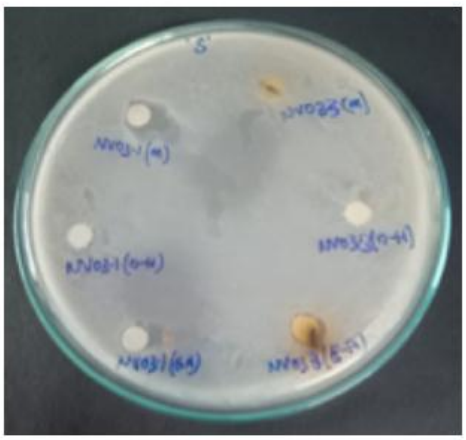
Plate- II
Anti-bacterial activity
Zone of inhibition of *Vanda testacea* (NV03) **Methanol,N Hexane and Ethyl acetate Leaf(NV03.1) and root(NV03.3) extracts.**



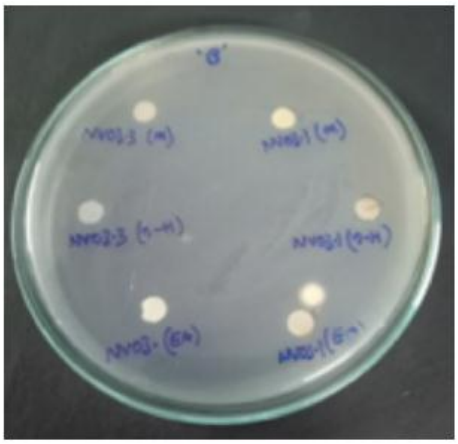
E.coli



Pseudomonas aeruginosa

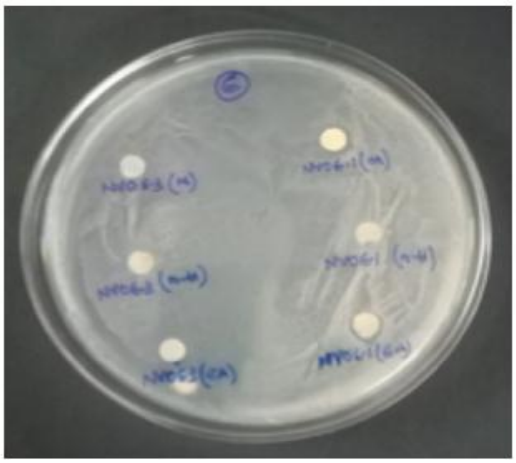


Staphylococcus aureus

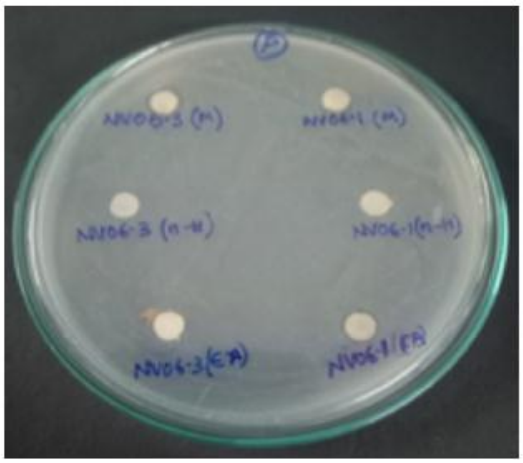


Bacillus subtilis

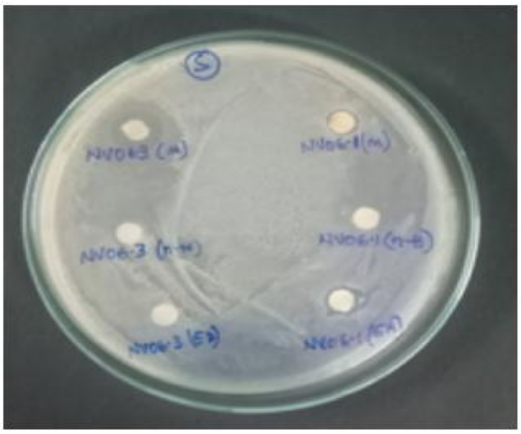
Plate-III
Antibacterial activity
Zone of inhibition of *Acampe praemorsa* (NV06) **Methanol,N Hexane and Ethyl acetate Leaf (**NV06.1) **and root**(NV06.3) **extracts.**



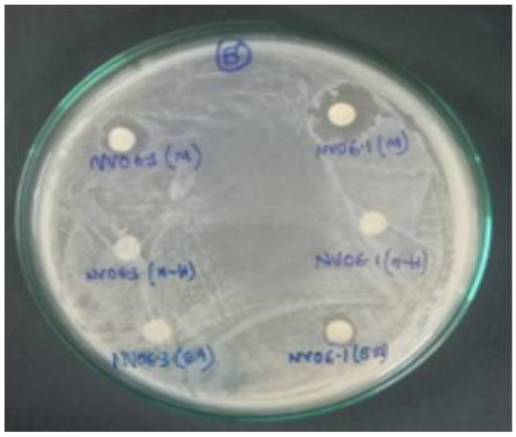
E. coli



Pseudomonas aeruginosa

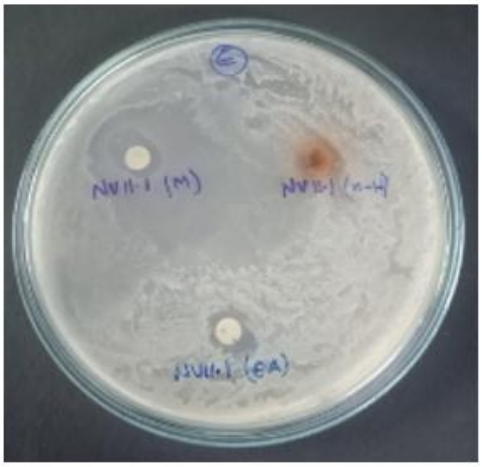


Staphylococcus aureus

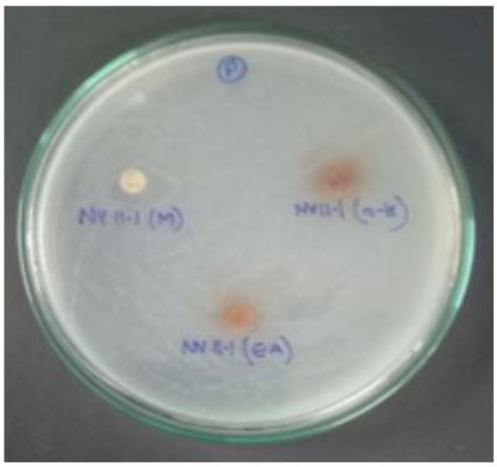


Bacillus subtilis

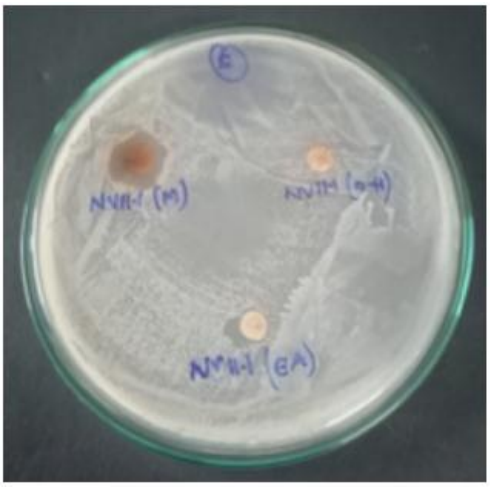
Plate-IV
Antibacterial activity
Zone of inhibition of *Acampe praemorsa Ecotype (*NV11) **Methanol, N Hexane and Ethyl acetate Leaf (**NV11.1) **extracts.**



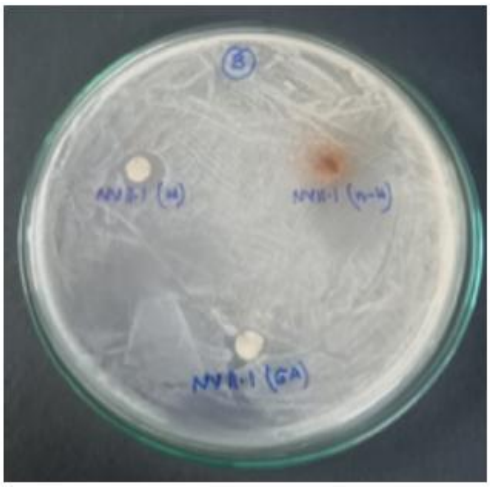
E.coli



Pseudomonas aeruginosa

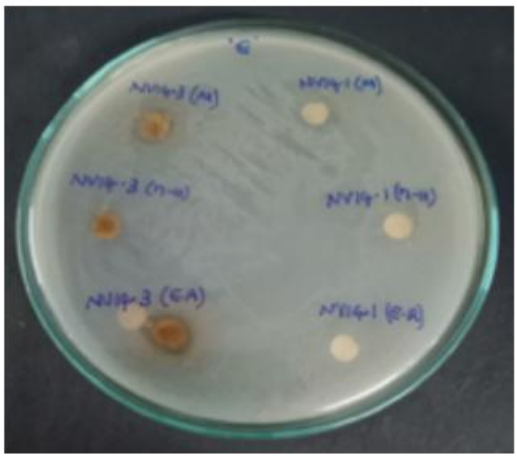


Staphylococcus aureus

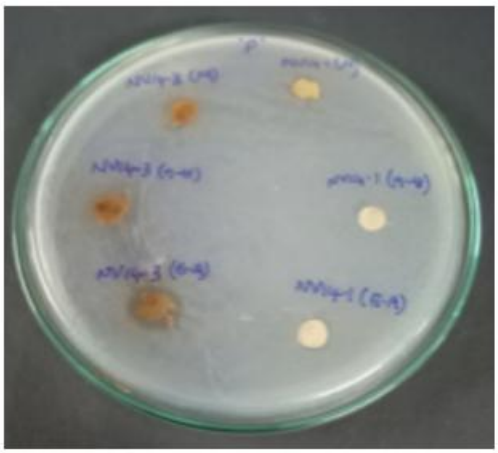


Bacillus subtilis

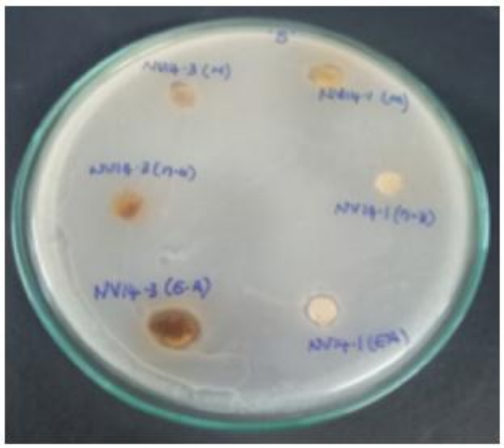
Plate-V
Antibacterial activity
Zone of inhibition of *Vanda tessellata ecotype* (NV14) Methanol, N Hexane and Ethyl acetate Leaf (NV14.1) and root (NV14.3) Extracts.



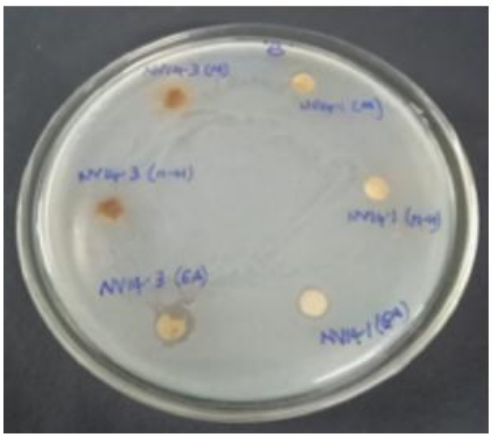
E.coli



Pseudomonas aeruginosa

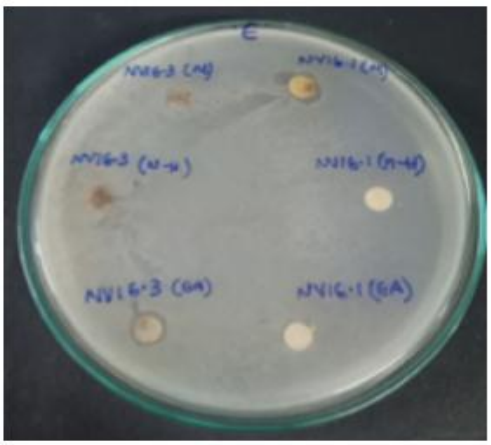


Staphylococcus aureus

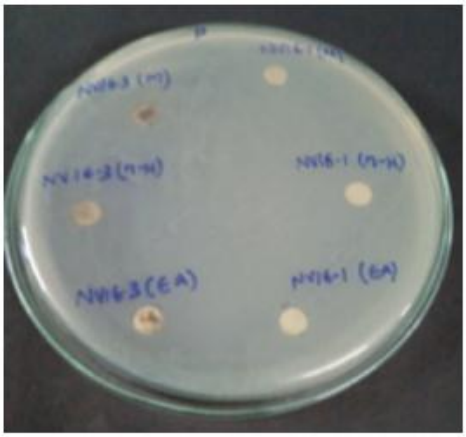


Bacillus subtilis

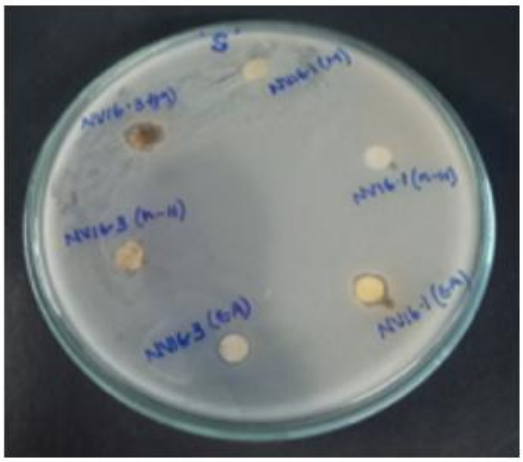
Plate-VI
Antibacterial activity
Zone of inhibition of *Vanda tessellata ecotype* (NV16) Methanol, N Hexane and Ethyl acetate Leaf (NV16.1) and root (NV16.3) Extracts.



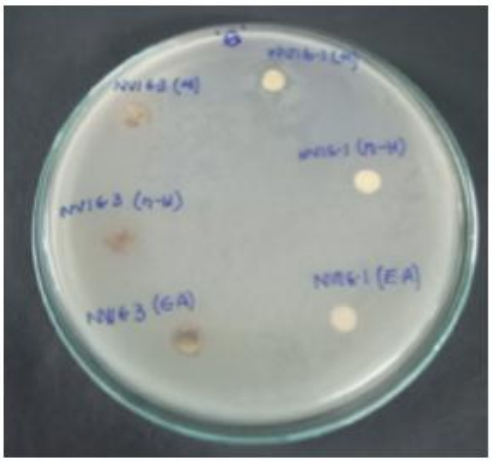
E. coli



Pseudomonas aeruginosa



Staphylococcus aureus



Bacillus subtilis

DISCUSSION

One of the key factors 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. (Prescott LM *et al.*, 2008). In the overall preliminary screening, ethyl acetate extracts showed more promising activity against tested bacteria compared to its methanolic counterparts. (Gupta &Katewa, 2014) The better performance noted with methanol leaf extracts of *A.praemorsa* (NV06), and its ecotype (NV11) against all bacteria were in line with the studies of (Behera *et al.*,

2013). None of the hexane extracts of the tested species had any inhibitory activity against the bacterial strains tested. Reduced ability of hexane to extract polar solutes could be the reason for non-performance of hexane extracts. (Siek, 1978; Thanh *et al.*, 2017).

CONCLUSION

Even though there is a good progress in medicinal plant research, but epiphytic species are not exploited fully for their medicinal application. The present finding indicated the high potential of these species for acting as anti-microbial agents. The present study in Andhra Pradesh is preliminary but necessary for further work of isolation of the elements present in the above species.

REFERENCES

1. C, Sudhakar Reddy & Kn, Reddy & SN, Jadhav. Ethnobotany of certain orchids of Eastern Ghats of Andhra Pradesh. Indian Forester, 2005; 135: 90-94.
2. Vibha, S & S Hebbar, Sushmita & Mahalakshmi, S.N. & T.R, Prashith. A comprehensive review on ethnobotanical applications and pharmacological activities of Acampa praemorsa (Roxb.) Blatt. & McCann (Orchidaceae). Journal of Drug Delivery and Therapeutics, 2019; 9: 331-336. 10.22270/jddt. v9i1.2224.
3. Literature: H.O. Saxena & M. Brahmam, Flora of Orissa, 1995; 3.

4. R. S. Rao et al., Flora of West Godavari District, 1986.
5. R. S. Rao and S. H. Sreeramulu, Flora of Srikakulam District, 1986.
6. H. O. Saxena and M. Brahmam, The Flora of Similipahar (Similipal), Orissa, 1989.
7. Prasad, K et al Conservation of Wild Orchids in Sri Krishnadevaraya University botanical garden, Ananthapur, Andhra Pradesh, India. Journal of Threatened Taxa, 2012; 4(7): 2705-2708.
8. Matthew, K.M., The Flora of Tamil Nadu Carnatic, Diocesan Press, Madras.; 9. Sasidharan, N. Flowering plants of Kerala. DVD, V 2, KFRI; Rao & Razi, Mysore; Gopalakrishnan Bhat, Udupi; Gopalakrishnan Bhat, Flora of South Kanara, 1983; 3(2).
9. Literature: H.O. Saxena & M. Brahmam, Flora of Orissa, 1995; 3.
10. R. S. Rao et al., Flora of West Godavari District, 1986.
11. R. S. Rao and S. H. Sreeramulu, Flora of Srikakulam District, 1986.
12. H. O. Saxena and M. Brahmam, The Flora of Similipahar (Similipal), Orissa, 1989.
13. Prasad, K et al Conservation of Wild Orchids in Sri Krishnadevaraya University botanical garden, Anantapur, Andhra Pradesh, India. Journal of Threatened Taxa, 2012; 4(7): 2705-2708.
14. Sasidharan, N. Flowering plants of Kerala. DVD, V 2, KFRI, 2011.
15. Literature: J.L.Ellis, Flora of Nallamalais, 1990; 2.
16. H.O. Saxena & M. Brahmam, Flora of Orissa, 1995; 3.
17. R. S. Rao et al., Flora of West Godavari District, 1986.
18. R. S. Rao and S. H. Sreeramulu, Flora of Srikakulam District, 1986.
19. H. O. Saxena and M. Brahmam, The Flora of Similipahar (Similipal), B. D. Sharma, S. Karthikeyan & N. P. Singh Flora of Maharashtra state – Monocotyledons, 1996.
20. Matthew, K.M., The Flora of Tamil Nadu Carnatic, Diocesan Press, Madras. Sasidharan, N. Flowering plants of Kerala. DVD, V 2, KFRI, 1983; 22.
21. Literature: Matthew, K.M., The Flora of Tamil Nadu Carnatic, Diocesan Press, Madras. 1983.; IUCN Red List of Threatened Species. Version, 2014.1.
22. Sasidharan, N. Flowering plants of Kerala. DVD, V 2, KFRI, 2011.
23. Literature: Sasidharan, N. Flowering plants of Kerala. DVD, V 2, KFRI, 2011.
24. Gamble, Fl. Pres. Madra, 1925; Vol 1.pp: 1252.
25. Literature: H.O. Saxena & M. Brahmam, Flora of Orissa, 1995; 3.
26. R. S. Rao et al., Flora of West Godavari District, 1986.
27. R. S. Rao and S. H. Sreeramulu, Flora of Srikakulam District, 1986.
28. H. O. Saxena and M. Brahmam, The Flora of Similipahar (Similipal), Orissa; 31.N.P.Singh, 1988. Flora of Eastern Karnataka, 1989.
29. Matthew, K.M., The Flora of Tamil Nadu Carnatic, Diocesan Press, Madras, 1983; 3(2).
30. Literature: Pullaiah et al., Flora of Eastern Ghats, 2011; 4.
31. Matthew, K.M., The Flora of Tamil Nadu Carnatic, Diocesan Press, Madras. Sasidharan, Flowering plants of Kerala. DVD, V 2, KFRI; Saldanha, 1996; 3(2).
32. Ramaswamy & Razi, Bangalore; Keshava Murthy & Yoganarasimhan, - Coorg; Bhaskar & Kushalappa, Flora of Tumkur District, 2013.
33. Literature: J.L.Ellis, Flora of Nallamalais, 1990.
34. H.O. Saxena & M. Brahmam, Flora of Orissa, 1995.
35. R. S. Rao et al., Flora of West Godavari District; R. S. Rao and S. H. Sreeramulu, Flora of Srikakulam District, 1986.
36. H. O. Saxena and M. Brahmam, The Flora of Similipahar (Similipal), 1989.
37. B. D. Sharma, S. Karthikeyan & N. P. Singh Flora of Maharashtra state – Monocotyledons, 1996.
38. Matthew, K.M., The Flora of Tamil Nadu Carnatic, Diocesan Press, Madras. 1983.
39. Sasidharan, N. Flowering plants of Kerala. DVD, V 2, KFRI.Saldanha & Nicolson, – Flora of Hassan District, 2011.
40. Saldanha, Rao & Razi, Flora of Mysore; Keshava Murthy & Yoganarasimhan, Flora of Coorg district, 1990.
41. Gopalakrishnan Bhat, Flora of Udupi; Manjunath et al., Flora of Devanagari, 2003.
42. Sankara Rao, K., Raja K Swamy, Deepak Kumar, Arun Singh R. and K. Gopalakrishna Bhat Flora of Peninsular India. http://peninsula.ces.iisc.ac.in/plants.php?name=Acampe praemorsa. Downloaded on, 2019; 6.
43. Sankara Rao, K., Raja K Swamy, Deepak Kumar, Arun Singh R. and K. Gopalakrishna Bhat Flora of Peninsular India. http://peninsula.ces.iisc.ac.in/plants.php?name=Vanda testacea. Downloaded on, 2019; 19.
44. Sankara Rao, K., Raja K Swamy, Deepak Kumar, Arun Singh R. and K. Gopalakrishna Bhat Flora of Peninsular India. http://peninsula.ces.iisc.ac.in/plants.php?name=Vanda tessellata. Downloaded on, 2019; 11.
45. Rajendra Yonzone. Taxonomic Notes and Medicinal Uses of Two Taxa of Orchids Cymbidium devonianum Lindl. ex Paxt., and Vanda testacea (Lindl.) Rchb. f., in Darjeeling Himalaya of West Bengal, India. J Complement Med Alt Healthcare, 2018; 8(2): 555733. DOI: 10.19080/JCMAH.2018.08.555733.
46. Miria, A & Khan, Anisa & Boyina, Ravi & Chatla, Prasada. Orchids of Talakona Sacred Grove, Andhra Pradesh, India, 2019;
47. Ethnobotanical Studies on Japali Hanuman Theertham- A.

48. Sacred Grove of Tirumala hills, Andhra Pradesh, India. N. Savithramma, P. Yugandhar* and M. Linga Rao Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.
49. Traditional Medicinal Plant Diversity of Penusilanarasimha Wildlife Sanctuary, Eastern Ghats, Andhra Pradesh.
50. Siva Kumar Reddy P1, Basha SKM2, and Indira Priyadarsini A1
51. Gupta, C., & Katewa, S. S. In vitro Evaluation of the antimicrobial activity of different solvent extracts of Stem of *Vanda tessellata* (Roxb.) Hook, 2014. ex G. Don. Indo.Am.J.Pharm. Res. 4. 2386-2391)
52. Prescott LM, Harley PJ, Klein AD. Microbiology. 7th ed. McGraw Hill Publisher, Singapore; 2008, p.94-122.
53. Behera D., Rath C.C., Tayung K. and Mohapatra U.B. Ethnomedicinal uses and antibacterial activity of two orchid species collected from Similipal Biosphere Reserve Odisha, India. Journal of Agricultural Technology, 2013; 9(5): 1269-1283.