WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF VANDA EPIPHYT (ROOTS & STEM)

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ABSTRACT :

The different organic extracts of the roots and stem of Vanda Epiphyte was investigated for its possible antibacterial activity against four human pathogenic bacterial strains. The plant extracts were evaluated against some gram positive and gram negative bacterial strains like Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli was carried out by the disk diffusion technique. The pattern of inhibition varied with the solvent used for extraction and the microorganism tested. Among all the extracts the methanolic extracts showed significant antibacterial activity against most of the tested microbes. The most susceptible microorganism was Staphylococcus aureus (24 mm zone of inhibition in methanolic extract) followed by Bacillus subtilis (20 mm zone of inhibition in methanolic extract) again followed by Escherichia coli (15 mm zone of inhibition in methanolic extract) and Staphylococcus epidermidis (10 mm zone of inhibition in methanolic extract). Minimal inhibitory concentration (MIC) values of extracts and antibiotics were comparatively determined by agar dilution method. Preliminary phytochemical analysis of different extracts was carried out. The results obtained from the present study suggested that Vanda Epiphyte plant extracts possess significant antibacterial property. Vanda Epiphyte crude extracts exerted a strong antifungal activity against C.albicans are often implicated in the infections of genitourinarytract; consequently the reputed usefulness of extracts in treating venereal diseases might be due to their inhibitory effect against this group of fungal species.

Key words: Antibacterial activity, Antifungal activity, Vanda Epiphyte, Preliminary phytochemical analysis.

1.Introduction :

Vanda epiphyte **is** an epiphytic orchid, 30-60 cm high, with leafy stem. This plant has been used as indigenous medicine sources such as *Ayurveda* and local traditional medical practices. [1] In different countries plants are using for its medicinal value and an important source of many potent and powerful drugs. [2] Medicinal plants represent arch source of antimicrobial agents. So, a wide range of medicinal plant parts is used forest extract as raw drugs and they possess varied medicinal properties. [3] The roots of *Vanda epiphyte* are antipyretic; useful in dyspepsia, bronchitis, inflammations, piles and hiccup. Externally the root is used in rheumatism and allied disorders and diseases of the nervous system. It is also remedy for secondary syphilis and scorpion-sting. Juice of the leaves is given in otitis and the paste as febrifuge. The roots possess significant anti-inflammatory activity. The plant contains an alkaloid, a glucoside, tannins, β- sitosterol, γ sitosterol and a long chain aliphatic compound, fatty oils, resins and coloring matters. Roots contain tetrazolyl ferrulate and β-sitosterol-D-glycoside. [4] A novel aphrodisiac compound isolated from the flowers of *Vanda epiphyte* which activates neuronal and endothelial, but not inducible. [5] Antibiotics are powerful medicines that fight bacterial infections and have greatly benefited the health-related quality of human life since their introduction. Antibiotic resistance has become a global concern. [6] One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Thus, drug resistance in human pathogens developed the necessity to search antimicrobial compounds as an alternative. [7] Therefore, screening of medicinal plants is vital to overcome these emerging problems. [8] On the contrary, the plant-derived antimicrobial agents are not associated with side effects and they have a prospective therapeutic benefit to heal many infectious diseases. [9,10] The purpose of the present study is to investigate the antibacterial activity of three different extracts of *epiphyte* inner bark and flowers against four strains of antibiotic multi-resistant bacteria's.

Figure No.1: *Vanda epiphyte* plant

2.Material and Methods :

Plant material

Plant was selected for this study is based on its traditional medicinal use. Plant material collected from manchippa reserve forest nizamabad district.


Figure No.2: stem and bark of *Vanda epiphyte*

Preparation of the extracts

Figure No.3: Soxhlet apparatus

The roots and stems are cleaned thoroughly and shade dried material were cut into small pieces and powdered in a grinder separately. The plant material (500 gm) was sequentially extracted with different solvents (petroleum ether, chloroform and methanol according to their increasing polarity by using Soxhlet apparatus for 24 hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated under vacuum at 400 C by using a rotary evaporator and then lyophilized. The extractive value of the extract (percentage yield, water-soluble extractive and alcohol soluble extractive) was calculated. The residual extracts were stored in refrigerator at 40 C in small and sterile plastic bottles. The antibacterial activity was carried out by disc diffusion method. The required bacterial strains were obtained from college.

Figure No.4: *Vanda epiphyte* plant material

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of the extract was carried out to find an idea of the natural of compounds present in the various extracts of plant. Hence, the presence and absence of compound such as tannins, saponins, flavonoids, etc., are identified by carrying out the phytochemical investigation.

Preparation of inoculums

Stock cultures were maintained at 4° C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37° C. The cultures were diluted with fresh Mueller-Hinton to achieve optical densities corresponding to 2.0·106 colony forming units (CFU/ml) for bacteria.

Antibacterial Activity Assay

Antibacterial activity was determined by cup diffusion method on MHA medium The sterile medium(20ml) was poured into 9 cm Petri plates. The medium was allowed to cool in a sterile condition and plates were then inoculated with cultures of test bacteria. Agar cup of 5 mm diameter were made in the plates with the help of sterile borers. The desired different concentrations of the extracts were prepared by first reconstituting in methanol then diluting in sterile distilled water. A 100 µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculums of the test bacterial cells. All test plates were incubated at 37° C for 24 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC. Negative controls were prepared using the same solvent employed to dissolve the extracts. Gentamycin was used as positive reference to determine the sensitivity of each bacterial species tested [11-13].

Minimal inhibitory concentration (MIC) determination

Serial agar microdilution method was performed for MIC determination. The tests were performed in MHA medium. Serial two-fold dilutions of each extract were added to equal volume of medium. Control dishes containing the same volume of ethanol or distilled water were made. After cooling and drying, the plates were inoculated in spots of 2 µl with each bacterial cell suspension (1×104 cfu) and incubated aerobically for 16-20 hr at 350 C. A growth control of each tested strain was included [14].

Fungal medium preparation :

Sabouraud dextrose agar and Sabouraud dextrose broth

Peptone agar and dextrose were dissolved in water, boiled and stirred well. Choloramphenicol in 2ml ethanol [95%] was added to the hot medium. Cycloheximide was dissolved in 2ml acetone and added, while stirring to the hot medium. Streptopenicillin was mixed with the hot medium was yellow and the final PH of the medium was 5.6±0.2.

Minimum inhibitory concentration [MIC]

For MIC determination 0.5ml of various concentrations of extract [125 to1.95mg/ml] and

synthetic compounds [50 to 0.78ul] of bacterial strains inoculum was transferred on to each tube. The last tube of Muller-Hinton broth with 50 µl of inoculums served as positive control. The whole set up in triplicate was incubated at 370 c for 24 hrs. The MIC was the lowest concentration of the extract that did not permit any visible growth after 24 hrs incubation.

Minimum Fungiricidal concentration [MFC]

The MFC was determined by sub culturing the above [MIC] serial dilutions after 24 hrs, in Muller-Hintonagar plates using 0.01 µl loop and incubating at 370 cfor 24 hrs. MFC was regarded.

3. RESULTS & DISCUSSION :

TABLE NO. 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ROOTS EXTRACT OF VANDA EPIPHYT (REVE)

BIOACTIVE COMPOUND	OBSRVATION	RESULTS
Tannins	Deep Blue To Black Color	+
Saponins	Formation Of Foam	+
Flavanoids	Pink Color	+
Alkaloids	Yellow Precipitate	+

Cardiac Glycosides	Blue Colour	+
Steroids	Red Colour To Green Flouresence	+

TABLE NO. 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF STEM EXTRACT OF VANDA EPIPHYT (SEVE)

BIOACTIVE COMPOUND	OBSRVATION	RESULTS
Tannins	Deep Blue To Black Color	+
Saponins	Formation Of Foam	+
Flavanoids	Pink Color	+
Alkaloids	Yellow Precipitate	+
Cardiac Glycosides	Blue Colour	+
Steroids	Red Colour To Green Flouresence	+

ANTIBACTERIAL ACTIVITY OF MADHUCA INDICA

TABLE NO. 3: ANTIBACTERIAL ACTIVITY OF ROOTS EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE)

S.NO.	Organisms	Solvent extracts (mm)						Gentamycin
		Ether		Methanol		Chloroform		
		REVE	SEVE	REVE	SEVE	REVE	SEVE	
1	*Staphylococcus aureus*	12	11	26	25	18	18	28
2	*Bacillus subtilis*	12	11	22	21	16	15	26
3	*Staphylococcus epidermidis*	14	13	17	16	15	14	25
4	*Escherichia coli*	15	14	20	20	14	14	26

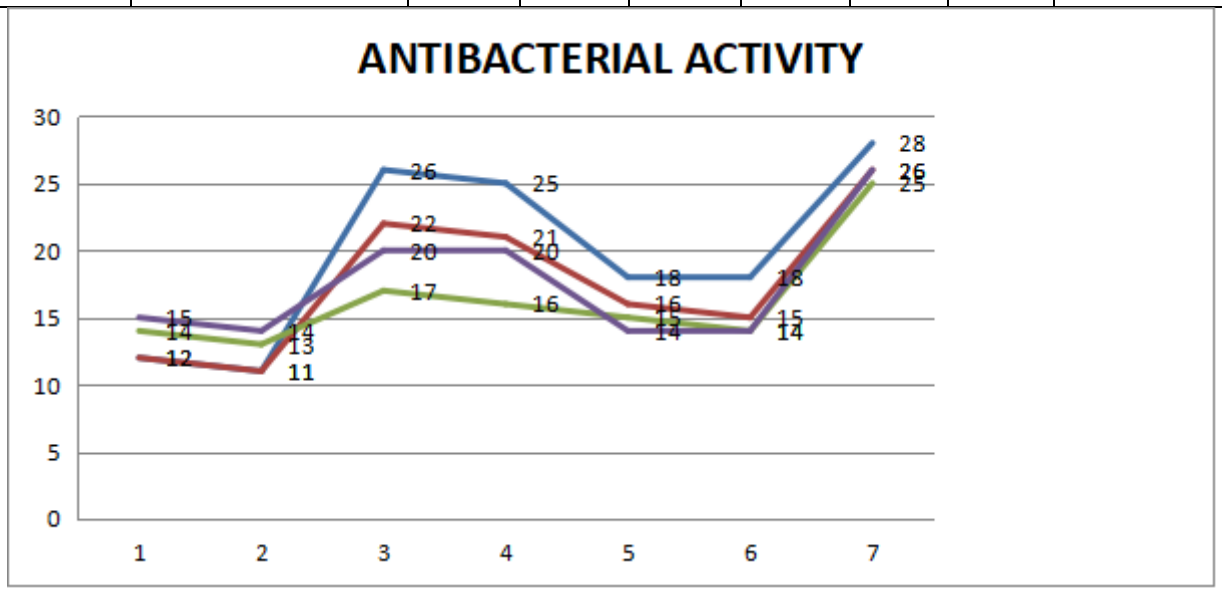

Figure No.6. ANTIBACTERIAL ACTIVITY OF ROOTS EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE)

TABLE NO. 4: MIC OF ROOTS EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE) AGAINST *STAPHYLOCOCCUS AUREUS*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE
1	Ether	-	-	+	+	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	+	+	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

TABLE NO. 5: MIC OF ROOT EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE) AGAINST *BACILLUS SUBTILIS*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE
1	Ether	-	-	+	+	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	+	+	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

TABLE NO. 6: MIC OF ROOT EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE) AGAINST *STAPHYLOCOCCUS EPIDERMIDIS*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE
1	Ether	-	-	+	+	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	-	-	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

TABLE NO. 7: MIC OF ROOT EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE) AGAINST *ESCHERICHIA COLI*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE	REVE	SEVE
1	Ether	-	-	-	-	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	+	+	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

In the initial stages the plant inner bark extracts in three different solvent viz. ether, chloroform and methanol, were evaluated for antibacterial activity of REVE & SEVE against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli*. Table no. 3 & 4 shows the zone of inhibition of different solvent extracts from these tables it is investigated that the methanolic extracts having the more potent activity against all the pathogenic bacteria as compared to other extracts. The bacterium growth inhibition produced by *Vanda Epiphyte* extracts varied in relation to the type of extract and to the bacterial strains used compared with standard Gentamycin. The lowest MIC value were found to be 6.25 mg/ml for methanolic extract against the *Staphylococcus aureus* compared to other solvent as shown in table no. 5&6.

FIGURE NO.11 :ANTIBACTERIAL ACTIVITY OF ROOT EXTRACT OF VANDA EPIPHYT & STEM EXTRACT OF VANDA EPIPHYT (REVE & SEVE)

ANTIFUNGAL ACTIVITY OF VANDA EPIPHYT :

Table – 8 ANTIFUNGAL ACTIVITY OF *VANDA EPIPHYT* ETHER EXTRACT

S.NO	NAME OF THE FUNGAL SPECIES	ZONE OF INHIBITIONS (MM)*	
		REVE	SEVE
1	*Candida albicans*	16	15
2	*Aspergillus Niger*	9	8
3	*Aspergillus flavus*	11	10
4	*Aspergillus fumigates*	13	12
5	*Mucor species*	No activity	No activity
6	*Rhizopus species*	13	12
7	*Penicillium species*	8	7

Table – 9 MIC & MFC OF *VANDA EPIPHYT* ETHER EXTRACT

S.NO	NAME OF THE FUNGAL SPECIES	ZONE OF INHIBITIONS (MM)*			
		REVE		SEVE	
		MIC (mg/ml)	MFC (mg/ml)	MIC (mg/ml)	MFC (mg/ml)
1	*Candida albicans*	16.2	31.1	16.4	32
2	*Aspergillus Niger*	62.1	121	61.9	124.1
3	*Aspergillus flavus*	31.3	62.3	31.4	62.1
4	*Aspergillus fumigates*	31.8	63.1	32.1	63.1
5	*Mucor species*	NA	NA	NA	NA
6	*Rhizopus species*	32.3	63.2	32.1	63.5
7	*Penicillium species*	61.4	125.2	61.9	125.4

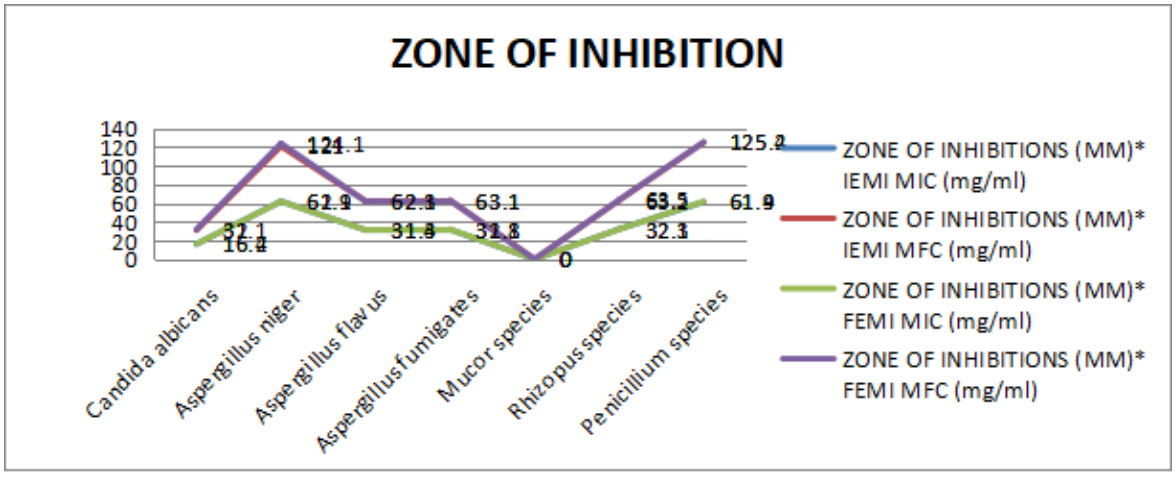

Figure No.12.MIC & MFC OF *VANDA EPIPHYT* ETHER EXTRACT

The results obtained from the present study suggested that *Vanda Epiphyte* plant extracts possess significant antibacterial property. *Vanda Epiphyte* crude extracts exerted a strong antifungal activity against *C. albicansare* often implicated in the infections of genitourinarytract; consequently, the reputed usefulness of extracts in treating venereal diseases might be due to their inhibitory effect against this group of fungal species. The *Vanda Epiphyte* crude extracts in our work also exhibited moderate antifungal activity against *Fumigates, A. Niger, A.flavus, Rhizopus* species and *Penicillium* species. The *Mucor* species showed no activity up to125 mg/ml. A previous investigation revealed that water extract from *Amorellos* leaves contained potential antifungal agent against *Candidia albicans* and antibacterial agent against *Escherichiacoli* for the treatment of opportunistic infections in patients afflicted with acquired immunodeficiency syndrome [aids]. These results were comparable to commercial antifungal drug Amphotericin B andantibiotic Chloramphenicol [15].

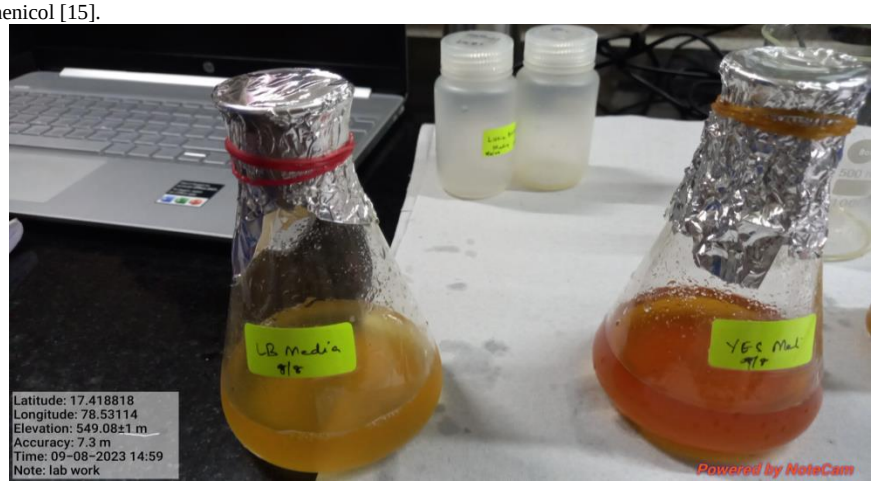

FIGURE NO.13: ANTIFUNGAL ACTIVITY OF *VANDA EPIPHYT* ETHER EXTRACT

4.CONCLUSION :

Overall, the results obtained by these extracts revealed better control of these pathogens used in study. Thus, it is concluded that the inner bark and flower of the plant *Vanda Epiphyte* is a potential source for antibacterial activity and antifungal activity provides some idea about phytochemical evaluation on *Vanda Epiphyte*. Minimal inhibitory concentration (MIC) and its activity against various clinical isolates may be sufficient to perform further studies for isolation and identification for active principles. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antibacterial effect and anti fungal effect and to determine the degree of toxicity of these extracts.

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