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RESEARCH ARTICLE

GC-MS PROFILE, ANTIBACTERIAL, ANTIFUNGAL, AND ANTICANCER ACTIVITY OF ROOT OF *VANDA TESSELLATA* AN EPIPHYTIC ORCHID

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Abstract

Background:The presence of phytochemical constituents has been reported from species of the Orchidaceae. Hitherto no reports exist on the GC-MS Profile of *Vanda tessellata* Hook. Ex G. Don which was an epiphyte from the sacred groove of Penchalikona of Andhra Pradesh.

Objective:The current study was to determine the antibacterial, antifungal, anticancer activities, and GC-MS profiles of the root of *Vanda tessellata* Hook. Ex G. Don.

Materials and Methods:The root of the *Vanda tessellata* Hook. Ex G. Don which was an epiphytic orchid from the sacred groove of Penchalikona of Andhra Pradesh.

An *in-vitro* evaluation of antibacterial and antifungal activity was performed by disc diffusion method and dilution technique, and anticancer activity was performed by SAB assay.

Gas chromatography-mass spectrometry (GC-MS) analysis of the root extracts of *Vanda tessellata* Hook. Ex G. Don was performed on GC-MS equipment (GCMSQP2010, SHIMADZU).

Results:The GC-MS profile has shown the presence of different phytochemical compounds in the epiphytic root of *Vanda tessellata* Hook. Ex G. Don. A total of 33 compounds were identified. Furthermore, the results of antibacterial and antifungal tests (both primary and secondary) shown that substantial antibiotic activity, and the anticancer activity results are shown inactivity.

Conclusion:From the results, it is evident that epiphytic root extracts of *Vanda tessellata* Hook. Ex G. Don has shown substantial antibacterial, and antifungal activity and GC-MS profile revealed the 33 analytes confirming the root was a source of therapeutic compounds useful for the pharmaceutical applications.

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Introduction:-

Epiphytes are extreme specialists adapted various morphological and physiological characters for their survival. Scientists summarised some important applications of orchids in controlling fevers, curing eye diseases, treating fatigue, headaches and their function as anticancer agents [15]. Orchidaceae is the second-largest angiosperm family in India, consisting of about 990 Genera. Nine species of orchids used for medicine in south India [25].

Materials and Methods:-

This species was collected from the Penchalikona area. Penchalikona is located in Sri Potti Sri Ramulu district of Andhra Pradesh (Latitude 14° 18' N, Longitude 70°28') 3000 ft (900m) above sea level. It is an epiphytic herb, belongs to the family Orchidaceae. (Fig-1)

Table-1:- Plant species and medicinal uses

S.NO	Place of Collection	Scientific name	Part	Uses
1	Penchalikona	<i>Vanda tessellata</i>	Roots	Treat bronchitis, inflammation, hiccup, piles and boils on the scalp

Description of plant species:**Habit:**

Herb

Habitat:

Dry and moist deciduous forests and also in the plains and on foothills to about 750 m, on slopes, sometimes in dense masses on decaying trees. Flower, Fruit: March-October

Morphology:

An epiphytic orchid, with stem 30-60 cm in height, thick, scandent with branching aerial roots. Its leaves 15-20 cm thick, fleshy, long, linear, recurved and complicate. Flowers in 6-10 flowered racemes, reaching with the peduncle 15-25 cm long. Its sepals are yellow, tessellated with brown lines and with white margins and petals are yellow with brown lines and white margins, shorter than the sepals. Lip is 16 mm long, bluish, with purple dots. Capsules are 7.5-9 cm long, narrowly clavate- elongated with acute ribs.

**Fig-1:-** *Vanda tessellate*.**Plant Identification:**

Herbarium specimens are prepared and identified by B.S.I of Hyderabad. Herbarium of the plant was preserved in the Department of Botany, Andhra University, Vishakhapatnam.

Preparation of plant extracts:

The roots were separated, and surface sterilized with 0.1% Hg Cl₂ for 5 minutes washed thrice with sterilized distilled water 5 minute each time. They were shade dried for forty days and powdered. Powder of the test material was dissolved in three different solvents viz methanol, ethyl acetate, and n-hexane for *in vitro* antimicrobial studies.

Antibacterial activity:

Antibacterial activity of different extracts of the test species was conducted in two phases, i.e., i) Antibacterial activity (disc-diffusion method) ii) Antibacterial activity (MIC) (dilution method).

Disc diffusion method [19]**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 root 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°C 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.

Minimal inhibition concentration (MIC):**Dilution technique**[19]:**Principle:**

Dilution method is the primary method of determination of MIC values. Agar dilution method used to quantitatively measure the *In vitro* antimicrobial activity against bacteria and fungi. MIC value recorded is the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the tested microorganism and expresses in µg/ml [20].

Procedure:

The secondary antibacterial activity of the root extracts was carried out using the dilution technique, and minimum inhibitory concentration (MIC) was determined [19]. Root extracts were dissolved in different solvents with different concentrations was tested for antibacterial activity.

Actively growing selected bacterial strains were respectively spread on nutrient agar plates and wells were made using sterile agar borer. Different aliquots (100 µg/ml, 75 µg/ml, 50 µg/ml, 25 µg/ml) of root extracts were added in separate wells and incubated at 28°C-30°C and incubated for 24h. After incubation measure the radius of the zone of inhibition.

Antifungal activity:

Antifungal activity of different extracts of the test species was conducted in two phases. i) Antifungal activity (disc-diffusion method) ii) Antifungal activity (MIC) (dilution method).

Antifungal activity:

Inoculums of three fungal strains were selected in the present study viz., *Fusarium oxysporium*, *Phytophthora infestans*, *Sclerotium rolfsii* were obtained from Department of Biotechnology, Mahatma Gandhi University, Nalgonda, Telangana, India. Antifungal activity was tested employing a disc diffusion method [20].

Disc diffusion method:**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. Zone of inhibition 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.

$$I\% = \frac{(C-T) * 100}{C}$$

Where I = Inhibition % of mycelial growth (growth reduction over control),

C = Radial growth of the fungus in the control plate (mm),

T = Radial growth of fungus on the inoculated plate.

Minimal Inhibition concentration (MIC):

An agar plugs of actively growing fungal culture was placed in the center of the PDA plates, respectively. Plates were incubated for 24h then, wells were made using sterile agar borer 2 cm away from the center where fungal was placed, and different aliquots (100 µg/ml, 75 µg/ml, 50 µg/ml, 25 µg/ml) of root extracts were added in separate wells and incubated at 28°C-30°C and incubated for 24h-96h. The inhibition percentage (I %) was calculated using the formula $I\% = \frac{(C-T) * 100}{C}$

Where I = Inhibition % of mycelial growth (growth reduction over control),

C = Radial growth of the fungus in the control plate (mm),

T = Radial growth of fungus on the inoculated plate.

Anticancer activity:**SRB assay:****Principle:**

SRB assay is developed in the year 1990. After its development, the SRB assay used to conduct various screening assays to investigate cytotoxicity in cell-based studies[34]. This method mainly relies on the property of SRB, which binds stoichiometrically to proteins under mildly acidic conditions and it can be extracted using alkaline conditions, the amount of bound dye considered for proxy for cell mass which can be extrapolated to measure cell proliferation.

Experimental Procedure:**Preparation of treatment:**

In the present study, the cell lines (Human Hepatoma Cell Line Hep-G2, Murine Skin Melanoma Cell Line B16-F10) were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mm L-glutamine. Depending on the doubling time of individual cell lines cells were inoculated into 96 well microtiter plates in 100 µL at plating densities depending on the doubling time of individual cell lines for the present screening experiments.

Incubation of cells:

After the cell inoculation completed, microtiter plates were incubated at conditions 37°C, 5 per cent CO₂, 95 per cent air and 100 per cent relative humidity for 24 h before the addition of experimental drugs.

Cell fixation and SRB staining:

roots powder was initially solubilized in dimethyl sulfoxide (DMSO) at 100 mg/ml and using water diluted to 1mg/ml and stored frozen before use. An aliquote of frozen concentrate (1mg/ml) was melted and a dilute solution of 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml prepared with complete medium containing test article before the time of drug addition. Already appropriate microtiter plates containing 90 µl of the medium are present. For

these aliquots of 10 μ l of this different drug dilutions were added resulting in the required final drug concentrations, i.e., 10 μ g/ml, 20 μ g/ml, 40 μ g/ml, 80 μ g/ml. After that compound is added to the plates and plates were incubated at standard conditions for 48 hours, after that by the addition of cold TCA and the assay was terminated. After that gentle addition of 50 μ l of cold 30 % (w/v) TCA (final concentration, 10 % TCA) the in-situ fixation of the cells took place and incubated for sixty minutes at 4°C. Discarded the supernatant after the experiment, the plates were washed five times with tap water and air-dried. For each of the wells Sulforhodamine B (SRB) solution (50 μ l) at 0.4 % (w/v) in 1 % acetic acid was added and plates were incubated for 20 minutes at room temperature conditions. The unbound dye was recovered after staining, and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air-dried. With a 10 mm trizma base, the bound stain was subsequently eluted. By using a plate reader and the absorbance was read at a 540 nm wavelength using a reference wavelength of 690 nm. On a plate-by-plate basis for test wells, per cent growth was calculated by comparing with control wells. The ratio of average absorbance of the test well to the average absorbance of the control well*100 is called growth percentage.

Absorbance measurement:

Mainly six absorbance measurements (time zero (Tz), control growth (C) and test growth in the presence of drug at four concentration levels (Ti) are taken into account for the calculation of percentage growth at each concentration level. Growth inhibition percentage was calculated as $\frac{Ti}{C} * 100$.

GC-MS Analytical technique:

Principle:

GC-MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), which analyse complex biochemical and organic mixtures [28]. The two main components of the GC-MS instrument are the gas chromatography portion and a mobile phase. The gas chromatography portion which splits dissimilar compounds in the sample into pulses of pure substances based on their instability [22] by flowing inert gas (mobile phase), which moves the sample, across a stationary phase fixed in the column [28]. Spectra of compounds are gathered as they are leaving a chromatographic column by the mass spectrometer, which recognizes and counts the chemicals according to the mass-to-charge ratio (m/z). These generated spectra can then be stored on the computer and analysed [22].

Methodology:-

Gas Chromatography-Mass Spectrum Analysis (GC-MS) GC-MS technique was used in this study to identify the phyto components present in the root extracts. The GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced with a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length: 30.0m, Diameter: 0.25 mm, Film thickness: 0.25 μ m Composed of 100% Dimethylpolysiloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. For each sample, the lyophilized compounds were dissolved in 50 μ l pyridine (Sigma-Aldrich, Steinheim, Germany), sonicated, and then derivatized in 60 μ l bis (trimethylsilyl)-trifluoroacetamide (Sigma-Aldrich) at 75 °C for 45 min.

GC-MS analysis was accomplished on a Shimadzu QP 2010 GC-MS system using a 30 m \times 0.25 mm (i.d.) \times 0.25 μ m DB5-MS fused-silica capillary column (J & W Scientific, Folsom, CA). The temperature injector was 280°C, and the split ratio was 10: 1. The carrier gas (Helium) is with a linear velocity of 35.0 cm s⁻¹ and an equilibration time of 3 minutes. The temperature of the column keeps at 70°C at the beginning for 5 min and then increased to 280°C at 5°C min⁻¹, held for 5 min. The MS scan parameters included a mass scan range of m/z 40–600, a scan-interval of 0.5s, a scan speed of 1000 u s⁻¹, and a detector voltage of 0.9 kV. The ion source temperature was fixed to 200°C, and the interface temperature was 280°C. The solvent cut time was 6 min.

The carrier gas such as Helium was used at a constant flow rate of 1.51ml/minutes, and a volume of injection 2 μ l was used. Injector temperature was 200°C, and Ion-source temperature was 200°C. The programming of oven temperature was at 70°C (isothermal for two min.), with an increase of 300°C for 10 min. Mass spectra were noted at 70eV; a scan-interval of 0.5 seconds with a scan range of 40 –1000 m/z. Total GC running time was 35 min. After the comparison of its average peak area to the total areas, the relative percentage amount of each component was calculated Software adapted to handle mass spectra, and chromatograms was a GC MS solution ver. 2.5.

Results:-

Antibacterial activity:

Antibacterial activity of different root extracts was conducted in two phases. Preliminary studies were carried out by disk diffusion method to find out the susceptibility of selected bacterial strains to each of the tested root extracts. Based on the primary activity of the tested extracts on the bacterial strains, selected root extracts were tested on the bacterial isolates to determine the minimum inhibitory concentration (MIC) to find out the effectiveness. The lowest concentration of the tested root extracts that inhibits the visible growth of the microorganism tested, recorded as MIC value and is expressed as µg/ml or mg/L.

Root extracts of *Vanda tessellata*, 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 root extracts of *V. tessellata* were effective against gram-negative bacteria *E. coli* and *P. aeruginosa*. (Table-2 & Fig-2)

Table 2:- Analysis of the preliminary antibacterial activity of different root extracts.

Plant species	<i>Vanda tessellata</i>		
	Zone of Inhibition(mm)		
	ME	NH	EA
<i>Bacillus subtilis</i>	**	**	5
<i>Staphylococcus aureus</i>	**	**	5
<i>Pseudomonas aeruginosa</i>	5	**	5
<i>Escherichia coli</i>	6	**	7

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

Ethyl acetate root extracts of *V. tessellata* were most effective on all the four bacterial strains tested. (Table-2 & Fig-2)

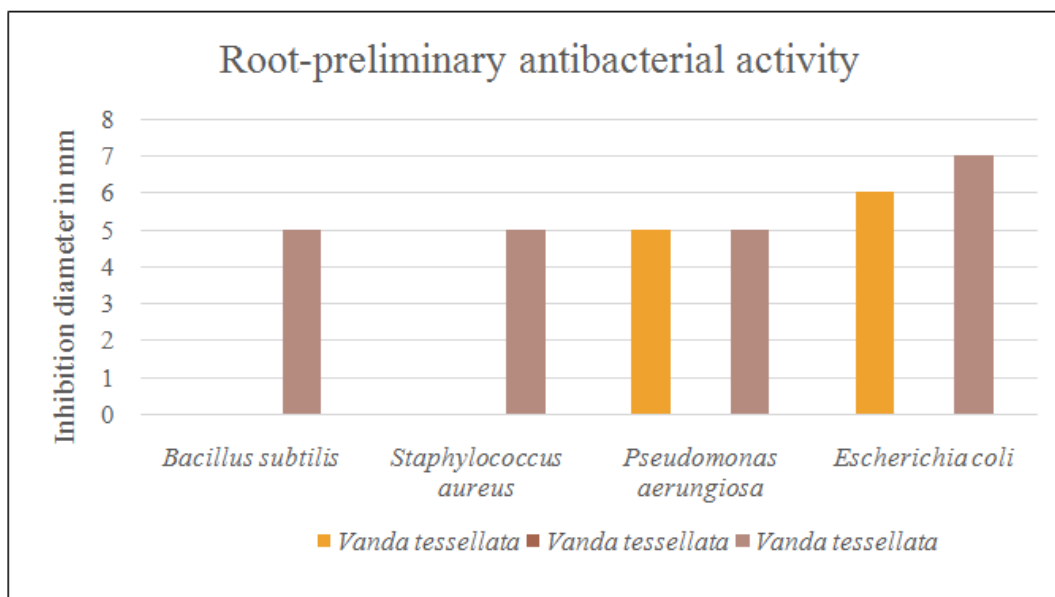


Fig 2:- Analysis of preliminary antibacterial activity of different root extracts.

None of the n-hexane root extracts of *V. tessellata* has shown any inhibitory activity on the bacterial strains tested.

Minimum inhibitory concentrations (MIC's) of the effective root's extracts:

Basing on the results obtained minimum inhibitory concentrations (MIC's) of the effective root extracts were employed to study their bacteriostatic properties.

Methanolic root extracts of *V. tessellata* induced inhibitory activity against *B. subtilis*, *E. coli* and *S. aureus*, and exerted the highest inhibition on the gram-negative *P. aeruginosa* at three lower doses. (Table:3 & Fig:3&Plate-1)

Table3:-Antibacterial activity (MIC) comparative analysis of root extracts

Plant	Solvent	<i>B. subtilis</i>				<i>S. aureus</i>				<i>P. aeruginosa</i>				<i>E. coli</i>			
		Zone of inhibition(mm)				Zone of inhibition(mm)				Zone of inhibition(mm)				Zone of inhibition(mm)			
		25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
<i>V.tessellata</i>	ME	2 ±0.05	2 ±0.05	2.5 ±0.02	**	2 ±0.05	4 ±0.05	3 ±0.05	3 ±0.05	5 ±0.05	4 ±0.05	4 ±0.05	**	2 ±0.05	3.5 ±0.02	3 ±0.05	3 ±0.05
	EA	2 ±0.05	2.5 ±0.02	3 ±0.05	5 ±0.05	3 ±0.05	3.5 ±0.02	3 ±0.05	3.5 ±0.02	2.5 ±0.02	2.5 ±0.02	2.5 ±0.02	3 ±0.05	**	2 ±0.05	2 ±0.05	2 ±0.05

Concentrations(µg/ml) 25,50,75,100 ME=Methanolic extract; EA= Ethyl acetate extract; ± standard mean error; ** =No activity

Ethyl acetate root extracts of *V. tessellata* exerted inhibitory activity on both gram + and gram- bacterial isolates at all concentrations. (Table-3&Fig-3&Plate-1)

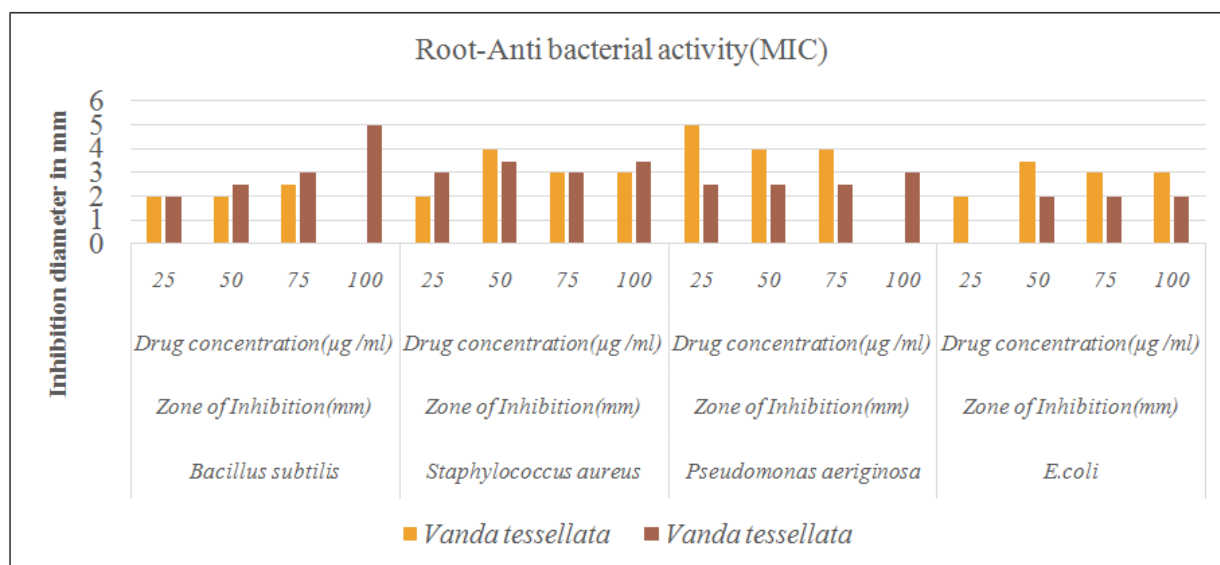
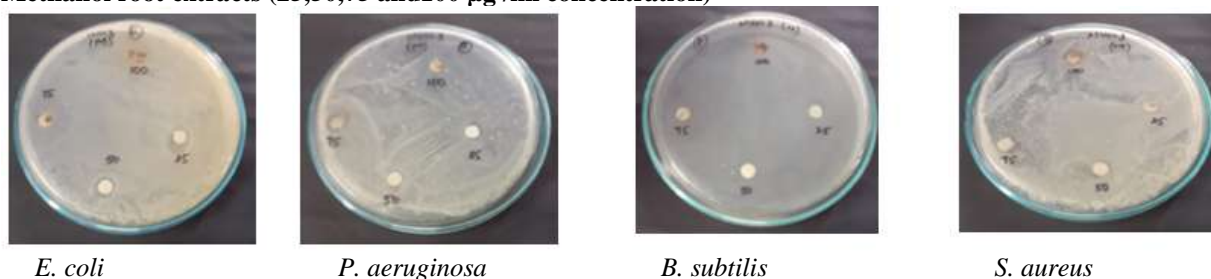


Fig 3:-Antibacterial activity (MIC) comparative analysis of root extracts.

Plate 1: Methanol root extracts (25,50,75 and100 µg/ml concentration)



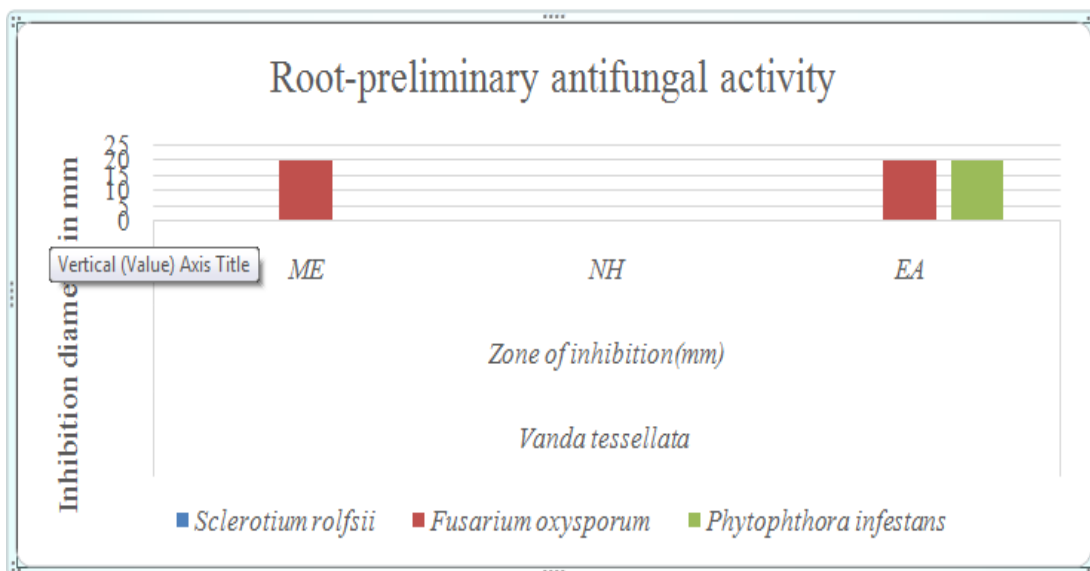
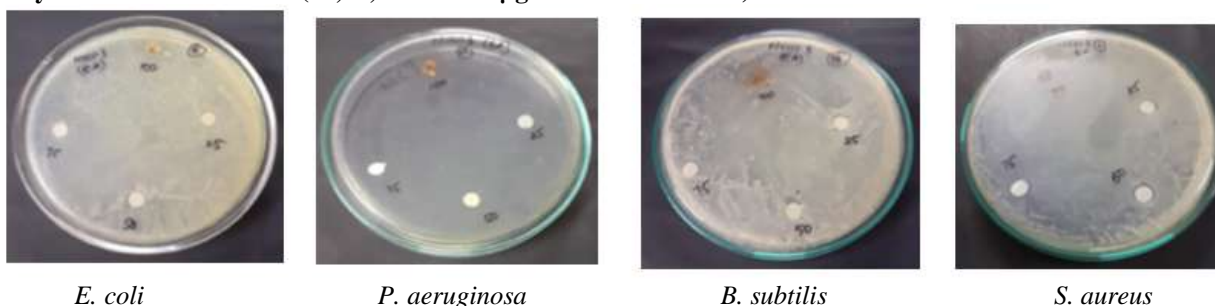


Fig 4:- Analysis of preliminary antifungal activity of different root extracts.

Ethyl acetate root extracts (25,50,75 and 100 µg/ml Concentration):



Antifungal activity:

Antifungal activity of different root extracts was conducted in two phases. Preliminary studies were carried out by disk diffusion method to find out the susceptibility of the selected fungal strains to each of the tested plant extracts. Based on the primary activity of the tested extracts on the fungal strains, minimum inhibitory concentration (MIC) of effective root extracts were employed to evaluate antifungal properties.

Antifungal activity of root extracts:

Root extracts of *Vanda tessellata* were tested for their antifungal activity against three fungal strains viz *Fusarium oxysporum*, *Sclerotium rolfsii* and *Phytophthora infestans* by disk diffusion method using three different solvents viz., methanol, n-hexane and ethyl acetate.

Of all the extracts methanolic root extracts *V. tessellata* was the only one that exerted inhibition on the growth against *F. oxysporum*. (Table:4, Fig:4&Plate-2)

Similarly, ethyl acetate root extracts of *V. tessellata* exerted an effect on the growth of *P. infestans* and *F. oxysporum*. None of the n-hexane extracts of roots of *V. tessellata* was effective inhibitors against the three fungal strains tested. (Table:4, Fig:4&Plate-2)

Table 4:- Analysis of preliminary antifungal activity of different root extracts

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

Fungal species	<i>Vanda tessellata</i>
----------------	-------------------------

	Zone of inhibition (mm)		
	ME	NH	EA
<i>Sclerotium rolfsii</i>	**	**	**
<i>Fusarium oxysporum</i>	20	**	20
<i>Phytophthora infestans</i>	**	**	20

Plant Name	Solvent	<i>S.rolfsii</i>				<i>F. oxysporum</i>				<i>P. infestans</i>			
		Zone of inhibition(mm)				Zone of inhibition(mm)				Zone of inhibition(mm)			
		25	50	75	100	25	50	75	100	25	50	75	100
<i>V.tessellata</i>	ME	**	**	**	20±0.028	**	24±0.028	22±0.028	20±0.028	**	**	18±0.57	16±0.57
	EA	**	**	**	23±0.028	**	20±0.028	19±0.57	16±0.57	**	**	24±0.028	22±0.028

Table 5:- Antifungal activity (MIC) comparative account of root extracts.

Minimum inhibitory concentrations (MIC's) of the effective root's extracts:

Minimum inhibitory concentrations (MIC's) of two effective root extracts were employed to evaluate for their antifungal activity

Concentrations (µg/ml) 25,50,75,100 ME=Methanolic extract; EA= Ethyl acetate extract; ± standard mean error; ** =No activity.

Methanol root extracts of *V. tessellata* was most effective on *S. rolfsii* fungal growth retardation and *F. oxysporum*. (Table:5, Fig:5 &Plate-2)

Results noted with ethyl acetate root extracts of *V. tessellata* against *F. oxysporium* and *P. infestans* were more or less to similar to their methanolic counterparts. (Table:5, Fig:5 &Plate-2)

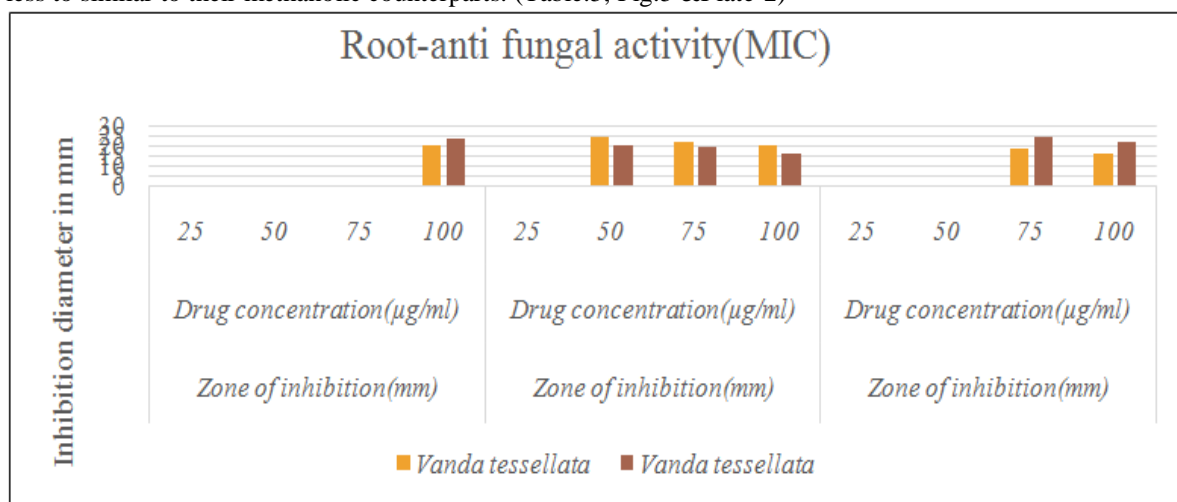


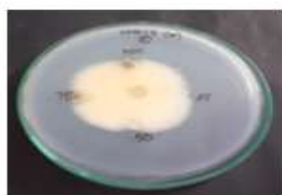
Fig 5:-Antifungal activity (MIC) comparative account of root extract.

Plate 2:

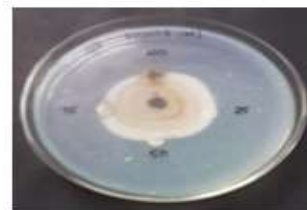
Methanol root extracts (25,50,75 ,100µg /ml concentration):



S. rolfsii



F. oxysporum



P. infestans

Ethyl acetate root extracts (25,50,75 ,100µg /ml concentration):*S. rolfsii**F. oxysporum**P. infestans***Anticancer activity:**

In the present study, the anti-proliferative activity of root extracts of *V. tessellata* was carried out on two cell lines Viz Human hepatoma cell line Hep G2 and Murine skin Melanoma Cell line- B16-F10 by SRB assay (Sulforhodamine B colourimetric assay). SRB assay is a useful and highly sensitive technique for qualitative analysis of anticancer activity. As per the SRB assay protocol, GI₅₀, TGI, LC₅₀ values are calculated as stated by the National Cancer Institute. If percentage growth inhibition is ≥ 50 at any of above concentration, then it is indicative of the effectiveness of the study drug. Adriamycin is used as a positive control. Triplicate experiments were performed, and the average values are calculated and tabulated in Table-6.

An *in-vitro* study of root extracts of *V. tessellata* exhibited little anti-proliferative activity on the Hep G2 and B16-F10 cell lines. Based on the calculations from graphs, using GraphPad prism software, it is extrapolated that above 80 µg/ml concentration, of any of the extracts, is necessary to exert 50% growth inhibition of the two cell lines. (Table:6-7, Fig:6 & Plate-3)

Table 6:-Anticancer activity comparative analysis of the root on two cancer cell lines

Plant name	HEP-G2				B16-F10			
	Drug Concentration(µg/ml)				Drug Concentration(µg/ml)			
	10	20	40	80	10	20	40	80
<i>Vanda tessellata</i>	95.3 ±1.08	95.4±1.06	111.6±1.7	145.9±8.3	111.3±2.01	121.6±1.65	132.1±4.29	125±3.11
Positive control compound (ADR)	-15.3±0.01	-25.2± 0.02	-32.3±0.04	-21.9±0.05	-71.0±0.04	-71.8±0.05	-71.0±0.06	-56.1±0.07

HEP G2: Human hepatoma cell line, B16-F10: Murine skin Melanoma cell line ADR=Adriamycin. ± =S.M. E

Table 7:- GI 50 values of root.

Hep-G2&B16-F10	Drug concentrations (µg/ml) calculated from the graph
	GI50*
<i>Vanda tessellata</i>	>80
Positive control compound (ADR)	<10

GI50 = concentration of drug causing 50% inhibition of cell growth.

ADR=Adriamycin.

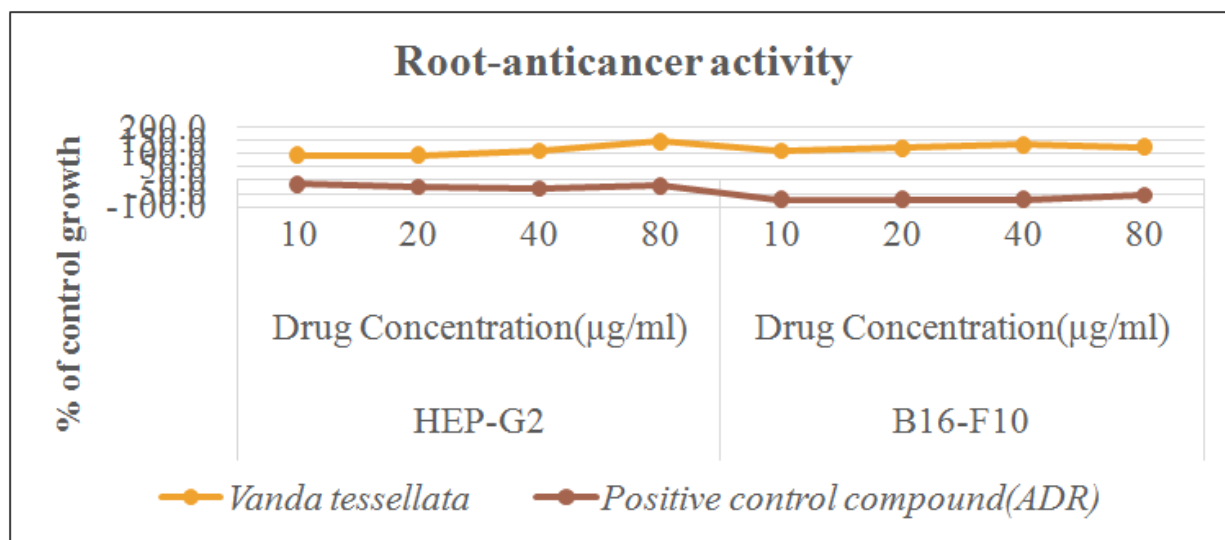
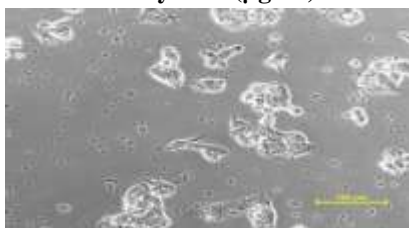


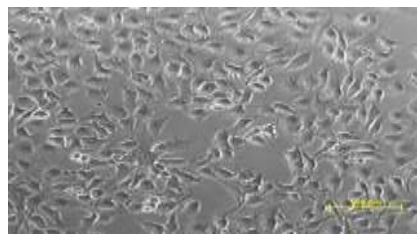
Fig 6:-Anticancer activity comparative analysis of the root on two cancer cell lines

Plate 3:

Anticancer activity at 80(µg/ml) concentration:



Hep-G2



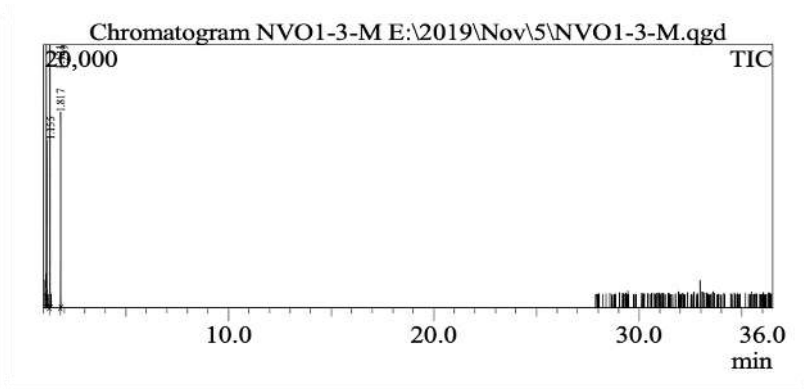
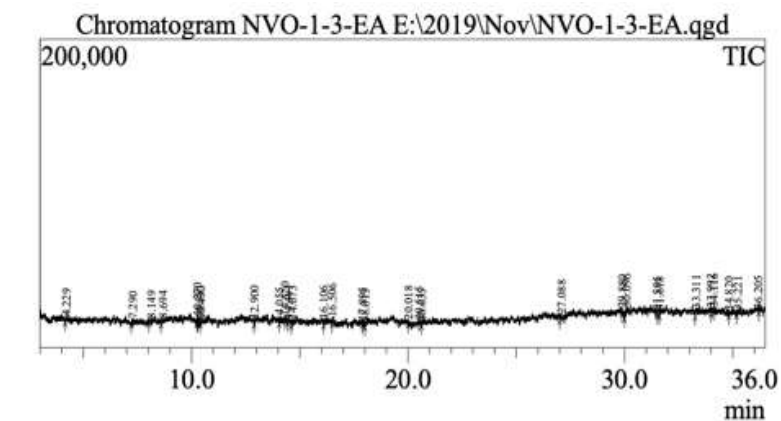
B16-F10

GC-MS (Gas Chromatography and Mass Spectroscopy analysis):

Gas chromatography-mass spectrometry (GC-MS), a key technological platform for secondary metabolite profiling in plant species, was carried out on root extracts of *Vanda tessellata*, using two solvents methanol and ethyl acetate. The total analysis of the extracts of samples revealed about 33 active compounds. Therapeutic values of the compounds are gathered from the PubChem, which is the database of chemical molecules which maintains three types of information namely, substance, compound and bioassays and the web link is <https://pubchem.ncbi.nlm.nih.gov/>. The list of compounds with their chemical names and therapeutic values are given in Table 8.

GC-MS analysis of plant extracts:

A maximum number of compounds with antimicrobial property were found in ethyl acetate root extracts of *V. tessellata*. The methanolic root extract of *V. tessellata*, revealed four active compounds among which dimethyl ether is used in wart treatment. (Table:8, Fig:7) Whereas ethyl acetate root extract revealed thirty compounds in which 3-Carboxymefenamic acid was the most noted compound with anti-inflammatory, analgesic, and antipyretic activities and joint disorders. Another compound, Norzimeldine, was known for antidepressant activity and the compound, 2-(Trimethylsilyl) benzothiazole is an anti-diabetic compound, Ferrocene, 1,2,3,4-tetrachloro was antimicrobial. The compound 5-Imidazolic acid, 2-[4-bromophenylazo] was known for antifungal activity and 4,6,8-Tetrathiatricyclo [3.3.1.1^{3,7}] decane with anticancer activity. The roots are popularly used in the treatment of cancer by tribals of the region. (Table:8, Fig:7).

Methanolic root extract:**Ethylacetate root extract:****Fig 7:- Chromatograms****Table 8:- GC-MS profile:
Methanolic root extract:**

S.NO	Name of the compound	Therapeutic activity**
1	Ethane, 1-chloro-1-fluoro-	Manufacture of ethylene
2	Dimethyl ether	Wart treatment
3	4-Penten-2-one	Component of the fruit of <i>Tamarindus indicus</i>

Ethyl acetate root extract:

S.NO	Name of the compound	Therapeutic activity**
1	2,4(1H,3H)-Pyrimidinedione, 6-chloro-5-nit	Anti-viral, antineoplastic
2	Spiro [5. alpha. -androstane-3,2'-thiazolidine],	Anticancer
3	1-Pyrrolidinecarboxylic acid, 2-[[[5-[[[3-am	Unknown
4	5.alpha. -Androstan-12-one, cyclic ethylene	Antioxidant
5	Norzimeldine	Anti-depressant
6	3-Carboxymefenamic acid	Anti-inflammatory, analgesic, and antipyretic activities.
7	2,7:3,6-Dimethanonaphth[2,3-b] oxirene, 3,	Anti-cancer

8	Cyclohexanone, 2-[(4-bromophenyl)-1-pipe	Anti-tubercular
9	Acetic acid, 8-acetoxy-6-benzenesulfonyl-2	Anti-microbial
10	Ethyl 2-(2-chloroacetamido)-2-[4-chloro-2-	Anti-inflammatory
11	5-(6-Butoxy-1-butylbenz[cd]indol-2(1H)-yl	Antihypertensive
12	Thionaphthene, 2-[1-[1-piperidyl] cyclohexy	Anti-Parasitic
13	2,2-Dimethylpropane, 1,3-dioxybis (N, N-dii	Anti-microbial
14	Ferrocene, 1,2,3,4-tetrachloro-	Antimicrobial,
15	3',4',5',6'-Tetrabromobenzo[1',2'-b]-1,4-diaz	Enzyme inhibitor
16	Dispiro[1,3-dioxolane-2,1'(4H)-cyclobutadi	Anti-microbial
17	D(15)-Norpregnan-20-ol, 20-methyl-, (5.alpha	Unknown
18	2,4,6,8-Tetrathiatricyclo [3.3.1.1.3,7] decane,	Anti-cancer
19	5-Imidazolic acid, 2-[4-bromophenylazo]-	Anti-fungal
20	Pregnan-20-one, 3,11,21-tris[(trimethylsilyl	Anti-bacterial
21	3-Methylindole-2-carboxylic acid, 4,5,6,7-t	Anti-depressant
22	3-Allyl-5-(3-ethyl-2(3H)-benzothiazolylide	Anti-cancer
23	2-[3-Chloro-4-methylphenyl]-2-[4-pyridinyl	Anti-hypersensitive
24	Silane, trimethyl [5-methyl-2-(1-methylethyl	Antioxidant
25	N-(3-Chlorophenyl)-2-hydroxyimino-aceta	Unknown
26	1-Methoxy-6-trimethylsilyloxyhexane	Antioxidant
27	4-Hydroxy-3-phenyl-4,6,6-trimethylhexahy	Anti-depressant
28	2-(Trimethylsilyl)benzothiazole	Antidiabetic
29	. alpha. -D-Galactopyranose, 6-O-(trimethyls	Inhibitors of phospholipase C isozymes anti-cancer
30	Arabinitol, pentaacetate	Cell immobilization

PubChem- Database of chemical molecules which maintains three types of information namely, substance, compound and bioassays.

Discussion and Conclusions:-

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 [23].

In the overall preliminary screening, ethyl acetate extracts showed more promising activity against tested bacteria compared to its methanolic counterparts [12].

None of the hexane extracts of the root 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 [30,31].

Bacteriostatic efficacy of *V. tessellata* against all the four bacterial strains as can be inferred from minimum inhibitory concentrations (MIC), was in accordance with the studies of earlier workers[1,4,5,10,12]. Better performance of *V. tessellata* against the two gram-negative bacteria amply supports the earlier studies[4,5,26].

Orchids are known to contain a host of phytochemicals, including alkaloids, triterpenoids and phenolic compounds [13]. Phytochemical analysis of *Vanda tessellata* revealed several phytochemicals such as flavonoids, tannins, saponins, terpenoids, steroids and alkaloids [14,30].

Antibacterial and the antifungal activity of the root extracts was observed in the present study could be attributable to these metabolites like terpenoids, alkaloids phenolics in general and flavonoids in particular [18].

Absence of anticancer activity by the root extracts, in the present study, could be attributable to the solvent, DMSO, which might have failed to extract the bioactive chemicals that could mediate respective biological activity. Ascertaining the pharmacokinetics of an extract was difficult, if a compound, necessary for anti-proliferative activity is insoluble in the solvent [7,31].

The absence of anti-proliferative activity by the tested doses of *V. tessellata* was in line with studies of Chowdary *et al.*, 2014 [9] where in LC 50 value with methanol extracts of *V. tessellata* was observed at 574.32µg/ml. Similarly,

Shamsul Islam *et al.*, 2016 [27] reported that the methanolic root extract of the *V. tessellata* induced LC50 value at a concentration of 25.19 mg/ml on Brine Shrimp nauplii.

Gas chromatography-mass spectrometry (GC-MS), analyses were done to find out the active principles in root extracts of *V. tessellata* in two different solvents. GC-MS analyses revealed a battery of different compounds, many of which are with multi-therapeutic properties.

Both methanolic and ethyl acetate root extract a total of 33 analytes are revealed. The data indicate that the ethyl acetate extracts performed better and are a more promising therapeutic agent. The extracts can be used in various pharmaceutical applications.

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