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BIOACTIVITY OF ENDEMIC ORCHIDS OF WESTERN GHATS; *PHOLIDOTA PALLID* LINDL AND *ARUNDINA GRAMINIFOLIA* (D. DON) HOCHR.

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ABSTRACT

Pholidota pallida And *Arundina graminifolia* is an Endemic orchid species belongs to orchidaceae family. It is mainly found in Western Ghats of India. It is used to treat various diseases such as liver affection, wound healing, rheumatism, abdominal pain, ear pain, diabetes etc. The present study is to identify the phytochemicals, Anticancer, Antioxidant, Antimicrobial and nanoparticle synthesis from leaves extract of *Pholidota pallid* and *Arundina graminifolia*. The phytochemical analysis revealed the presence of Alkaloids, Terpenoids, Phenols, Sugar, Saponins, Flavonoids, Quinin's and Steroids. While comparing *Pholidota pallida* possess more bioactivity than *Arundina graminifolia*.

Keywords: *Pholidota pallida, Arundina graminifolia*, Endemic, Nanoparticle synthesis

INTRODUCTION

Phytochemically some orchids have been reported to contain alkaloids, terpenoids, flavonoids and stilbenoids (Singh *et al*., 2009) and more than 44 orchid species of 34 genera have medicinal value (Ghanaksha *et al*., 1993). Orchids are also commercially important for its glycosidal value where four kinds of glycosides have been reported to be present in some orchids (Bose *et al*., 1989). Loroglosin from Loroglossum, coumarin from Angraceum fragrance and saponin from *Paphiopedilum javanium* (Bose *et al.,* 1989) are commercially important glycosides. *Vanilla planifolia* is the main source of commercial vanilla flavor (A. Marjoka *et al.,* 2016)

Pholidota pallida belongs to family Orchidaceae, one of the native epiphytic orchids from the Western Ghats forests of Karnataka. The pseudo bulb of *P. pallida* is used in controlling intestinal worms and abdominal pain and root is used in. Based on its importance the present work was designed to evaluate its phytochemical constituents and free radical scavenging activity of pseudo bulb cold and hot successive extracts. The phytochemical secondary metabolite screening of extracts revealed the presence of alkaloids, flavonoid, phenols, phytosterols and total antioxidant components. Based on the quantitative estimation studies it revealed that extracts have a good amount of secondary metabolites. The cold and hot successive extracts were subjected to free radical scavenging activity on DPPH and ABTS radical cation decolorization assay the result revealed that the highest DPPH scavenging activity was seen in the hot methanolic extract and highest ABTS scavenging activity was seen in the cold methanolic extract. The present investigations prove that the *Pholidota pallida* plant is a reservoir of the phytochemicals that can be utilized for the development of Phyto-therapeutics (Nagananda G S, *et al.,* 2014).

A. graminifolia a reedly terrestrial tropical orchid species generally grows in clumps. It is available in newly developed habitats of anthropogenic origin, such as road cuts and abandoned farm fields and mostly occurs in limited areas, its natural habitat being steep, rocky sites or open grassy areas. The rhizome of the plant is used as antibacterial agent and its root decoction is commonly used for the ailments of diabetes, tumour, hyperliposis and hepatitis. The phenolic compound of this orchid has antihepatitic and anti HIV activity (Bimal Debnath *et al.,* 2016).

MATERIALS AND METHODS

Pholidota pallida and *Arundina graminifolia* leaves were collected from TBGRI, Palode, Trivandrum. Leaves were washed thoroughly using distilled water and shade dried. Then it was pulverized through mechanical grinder. The powdered plant material was extracted using solvents petroleum ether, chloroform, ethanol, methanol, Aqueous solution respectively. Qualitative analysis of phytochemicals mainly alkaloids, terpenoids, phenols, sugar, saponins, flavonoids, quinines, steroids were screened out on *Pholidota pallid* and *Arundina graminifolia*. Antioxidant, Anti-cancerous, Antimicrobial analysis of leaves of both *Pholidota* and *Arundina* were screened out. Nanoparticle synthesis was also analyzed in aqueous extract. Based on the results got it is concluded that *Pholidota* has the highest bioactivity than *Arundina graminifolia*. Current study gives innovative and new findings on the capacity of these orchids and supports the continued research of medicinal orchids of Western Ghats.

Phytochemical screening

Preliminary phytochemical screening of secondary metabolites such as alkaloids, flavonoid, terpenoids, phenols, sugar, saponins steroids was carried out according to standard phytochemical method.

Antioxidant activity

Total Antioxidant activity

Using phospho molybdenum method total antioxidant activity was confirmed by the protocol of Jesteena *et al.,* (2018) .0.5ml of the sample was mixed with the 0.5ml of reaction mixture of 0.6 M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate reagent solution and the tube was incubated at 45^0C for 90minutes with blank solution. After incubation the tubes were normalised to room temperature and the absorbance was read at 695nm. Ascorbic acid was used a standard to calculate the mg/gm of the total antioxidant activity. Triplicate value was used to finalise the total antioxidant activity.

FRAP (ferric reducing antioxidant power assay)

To the 1 ml of the plant extract added 1 ml of phosphate buffer solution and 1 ml of potassium ferric cyanide (0.1 %), these were incubated at 50 ˚c for 20 minutes. After incubation with the addition of 1 ml of 10% trichloro acetic acid, 1 ml of distilled water and 0.5 ml of 0.1% ferric chloride solution the sample was again and incubated for 5 to 10 minutes and the reducing power activity was measured by taking OD value at 700 nm using spectrophotometer (Labtronics LT 291). The results were reported in mg/g by using ascorbic acid as a standard.

SOD (superoxide assay)

Superoxide dismutase (SOD)**,** which catalyses the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, is one of the most important antioxidant_enzymes. To the 1ml of reaction mixture 1 (1 ml of 50 millimolar phosphate buffer solution, 0.075 ml of 20 millimolar l-methionine, 0.04 ml of 10 millimolar hydroxyl amide hydrochloride and 0.1 ml of 50 millimolar ethylene diamine tetra acetic acid (EDTA), added 0.1 ml of the plant extract and incubated at 30˚c for 5 to 10 minutes. 80 µl of 50 millimolar riboflavin was added and the mixture was exposed under 200 v of fluorescent light for 1 to 2 minutes. 1 ml of the reaction mixture 2 (reaction mixture 2 - 1 % sulphonyl amide in 5 % phosphoric acid) added and od value at 543 nm was measured by using spectrophotometer (Labtronics LT 291).

Antimicrobial activity

Antimicrobial activity of the sample was done by using standard agar well diffusion method. For the antibacterial activity mueller Hinton agar was prepared by dissolving 39 gm in 1000ml of distilled water and sterilised under autoclave at 121^0c and the media were poured to Petri plate and allowed for solidification. After solidification bacterial suspension of *E. coli* and *S.aureus* were swabbed. cork borer was used to make well and each well samples was poured. Cefotaxime (30mcg) was used as a positive control, after placing the samples plate were incubated at 37^0 Cfor 24 hrs and the zone of inhibition was measured. potato dextrose agar was prepared by dissolving 39gm in 1000ml of distilled water and sterilised this was used for antifungal activity, *Aspergillus flavus* and *Fusarium Spp*. were swabbed and using above method sample added and incubated at 30^0C for 5 -7 days and zone of inhibition was measured, fluconazole were used as a positive control.

Anticancer activity

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm.(Alley, M. C *et al.,* 1986, Mossman *et al.,* 1983).

Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet). Incubate the plate for 24 hrs at 37°C in a 5% CO2 atmosphere. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume. Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.) Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength. The IC_{50} value was determined by using linear regression equation i.e., Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph.
In this study, 2 test compounds labelled as A and B are used to check the Cytotoxicity Study on the 1 cell line namely, MCF7.The results suggest that the test compound, B have significant cytotoxicity potential against human breast cancer cells (MCF7). Further studies have to be

Table 1 Total antioxidant activity

Sample	Mg /gm of total antioxidant activity
Sample Achloroform	64 68 64
Sample A water	74 76 80
Sample Bchloroform	101 108 107
Sample B water	117 115 120

Nanoparticle synthesis result

Sample (Pholidota)	Mg/gm of total antioxidant activity
Nanoparticle	503 508 505

Table 2 FRAP results

Sample used	Mg/g of FRAP
Sample A (arundina) water	152 124 142
Sample B (Pholidota)water	54 52 53

Nanoparticle result

Sample (Pholidota)	Mg /gm of total antioxidant activity
Nanoparticle	166 163 169

Table 3 SOD Results

Samples used	Percentage of inhibition
SampleA(arundina) water	11.6 % 12.6% 12.6 %
SampleB(Pholidota) water	60.5 61.8 60.8

Nanoparticle result

Sample	Mg /gm of total antioxidant activity
Nanoparticle	68.28 % 71.19 % 70.55 %

Antimicrobial Results

Table 4 sample A (Arundina)

Extracts used	Bacteria		Fungus	
	E. coli	*S. aureus*	*A. flavus*	*Fusarium Spp*
Water	3mm	5 mm	Nil	3mm
Chloroform	Nil	3mm	Nil	1mm
Acetone	Nil	Nil	Nil	1mm
Petroleum ether	Nil	Nil	Nil	1mm
Methanol	4mm	Nil	1mm	1mm
Cefotaxime	10 mm	9mm	NA	NA
Fluconazole (STD)	N A	N A	3mm	4mm

Table 5 sample B (Pholidota)

Extracts used	Bacteria		Fungus	
	E. coli	*S.aureus*	*A.flavus*	*Fusarium Spp*
Water	4mm	4mm	Nil	2mm
Chloroform	Nil	Nil	Nil	1mm
Acetone	3mm	5mm	Nil	1mm
Petroleum ether	4 mm	2mm	Nil	1mm
Methanol	Nil	Nil	1mm	1mm
Cefotaxime	10 mm	9 mm	NA	NA
Fluconazole (STD)	N A	N A	Nil	3mm

Nanoparticle results (Pholidota)

Sample used	Bacteria		Fungus	
	E. coli	*S. aureus*	*A. flavus*	*Fusarium Spp*
Nanoparticle	7	5	7mm	3mm
AgNO3	2	2	Nil	Nil
Cefotaxime	10	9	NA	NA
Fluconazole (STD)	NA	NA	4mm	4mm

Anticancer results

Table 7

Sl. No	Test Compounds	Cell line	Concentrations treated to cells
1	Cell Control	MCF7	No treatment
2	Standard (Camptothecin)	MCF7	25uM
3	Blank	-	Only Media without cells
4	Test Compounds (A & B)	MCF7	25,50,100,200and 400ug/ml

(A & B: Arundina and Pholidota respectively)

conducted to determine the molecular mechanism behind the anti-cancer properties of the test compound.

RESULTS

Phytochemical screening

The preliminary screening of crude extracts of leaves of *Pholidota pallida* and *Arundina graminifolia* revealed the presence of some constituents such as Alkaloid, Terpenoid, Phenol, sugar, saponin, flavonoid, Quinoine, Steroid.

Antioxidant analysis

The best results got from phytochemical analysis was in chloroform and in aqueous solution so it is selected for further tests. Total antioxidant activity, frap analysis and Sod analysis have been done on both Pholidota and arundina leaf extracts. In the results got it shows the highest antioxidant activity is in aqueous solution. Synthesis of nanoparticles were also noted in antioxidant analysis.

Antimicrobial analysis

Antibacterial analysis done using E. coli and S. aureus, In *Arundina graminifolia* 3mm and 5mm (Aqueous extract) inhibition zones were measured and in the case of *Pholidota pallida* too mm and 9mm (aqueous extract) inhibition zones were measured

For Antifungal analysis, *Aspergillus flavus* and *Fusarium spp* were used. In *Arundina graminifolia* highest inhibition zone is measured in Aqueous extract; 3mm and 4mm. In *Pholidota pallida* highest inhibition zone is measured in *Fusarium spp* 3mm and no inhibition zone were detected in *Aspergillus flavus*.

Anticancer analysis

The results suggest that the test compound, B have significant cytotoxicity potential against human breast cancer cells (MCF7). Further studies have to be conducted to determine the molecular mechanism behind the anti-cancer properties of the test compound.

Nanoparticle synthesis

Synthesis of Silver nanoparticles were studied in Antioxidant and antimicrobial analysis using UV Spectroscopy, FTIR Analysis, SEM and XRD.

Preparation of nanoparticle

The aqueous extract was used for the synthesising of silver nanoparticles. 10ml of the aqueous extract was mixed with equal amount of the 1mM AgNO3 solution (prepared by standard formula, MW of AgNO3- 169.87) and incubated for 24 to 48hrs in dark room temperature. After incubation the sample changes were observed and characterised to confirm the nanoparticle.

Characterisation of nanoparticles

UV-Visible spectroscopy

The synthesised nanoparticle was primarily characterised by UV-Visible spectroscopy. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical surroundings, UV Visible analysis was done from the nanometer of 300 to 600nm after setting the baseline in UV-Visible spectrophotometer of Labtronics LT 291.

FTIR

The synthesised sample was further characterised by Fourier-transform infrared spectroscopy. FTIR analysis of the sample was done from the range of 4000cm-1 to 500 cm-1 using Shimadzu instrument to identify the functional group which is in the synthesised nanoparticle.

SEM

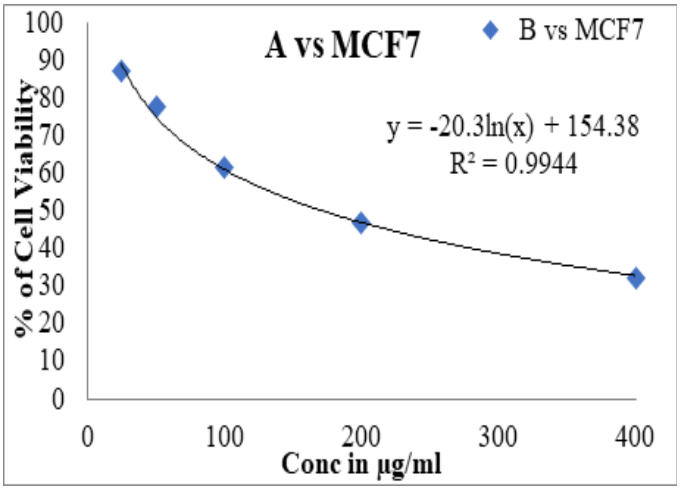
SEM is a surface imaging method, totally capable of determining diverse particle sizes, nanomaterial shapes, size distributions and the surface morphology of the synthesized silver particles at the micro and nanoscales. The synthesised sample was powdered and the fine powder was used for the SEM analysis to characterise the morphological analysis.

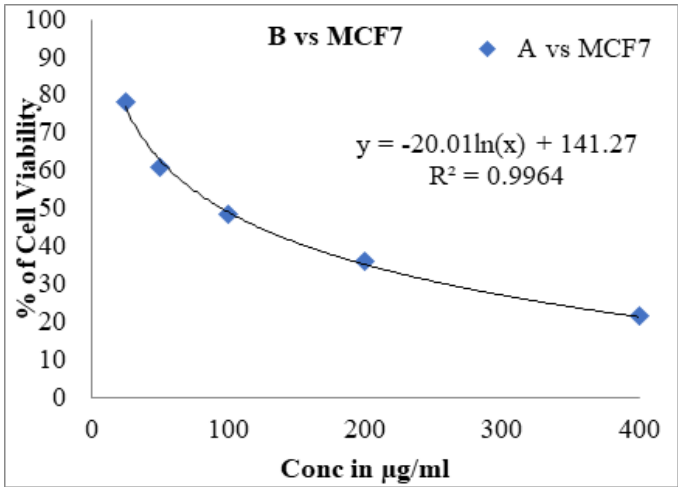
XRD

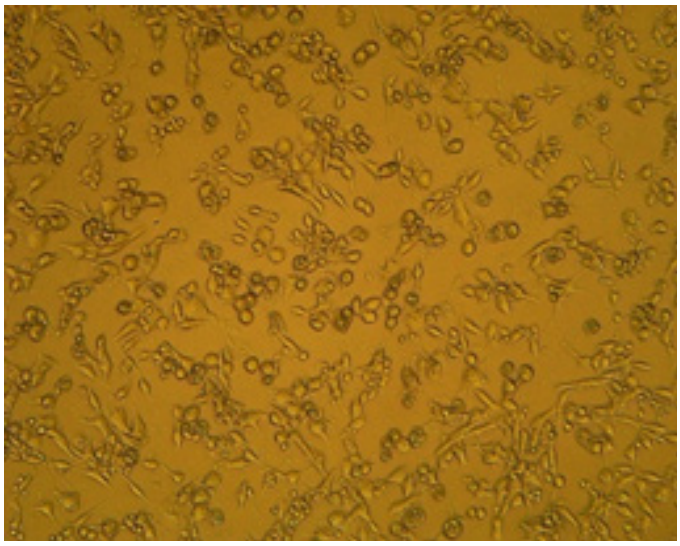
The crystalline nature of AgNPs was further confirmed by Xray Diffraction (XRD) analysis. Operated at 35 kV and 28 mA in flat plane geometry mode with each scan taking 2 seconds. The respective diffraction patterns were collected over a 2θ range of 20° to 90°.

DISCUSSION

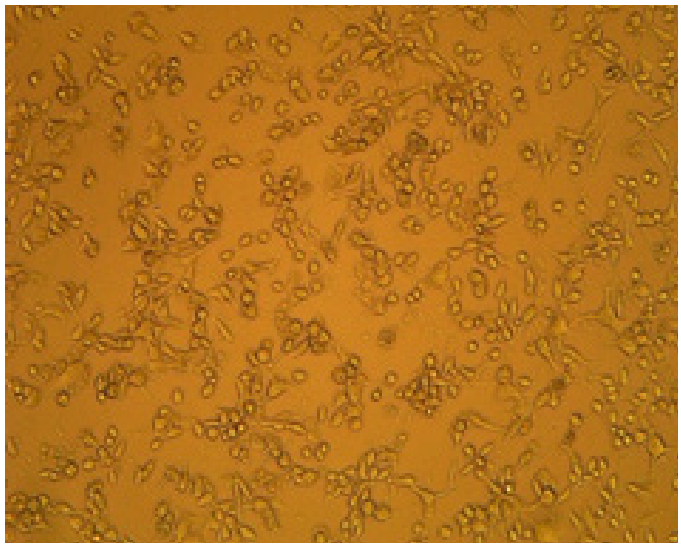
In this present study about phytochemical activity on *Pholidota* and *Arundina*, we haveobtained Alkaloid, terpenoid, phenol, sugar, saponin, flavonoid, quinine, steroids. Presence of Alkaloids, flavonoids, terpenoids, tannins, steroids, quinines, coumarins, phytosterols were reported on *Pholidota pallida* by *Seema akter et al., 2019*; *Nagananda GS et al., 2014*. In the case of Total Antioxidant activity, we have got highest activity on *Pholidota pallida* while comparing to *Arundina graminifolia*. In the case of SOD and FRAP analysis Pholidota possess highest antioxidant activity. But in the Antioxidant study in Pholidota articulata species it is showed that Methanolic extract have significant antioxidant activity by Darshan





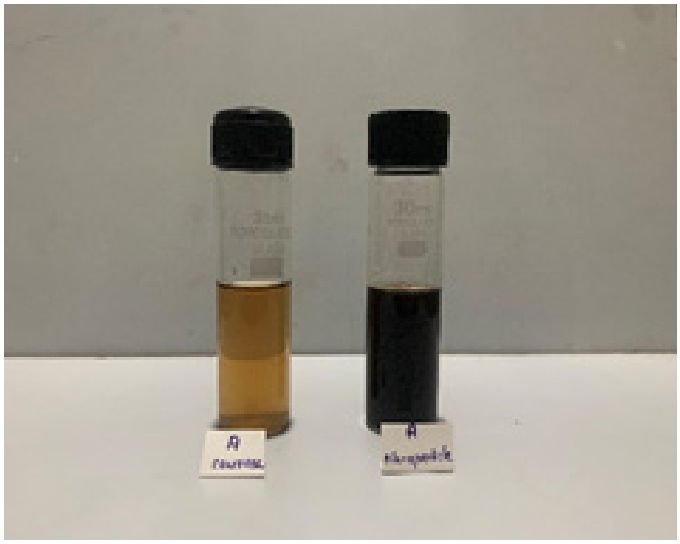


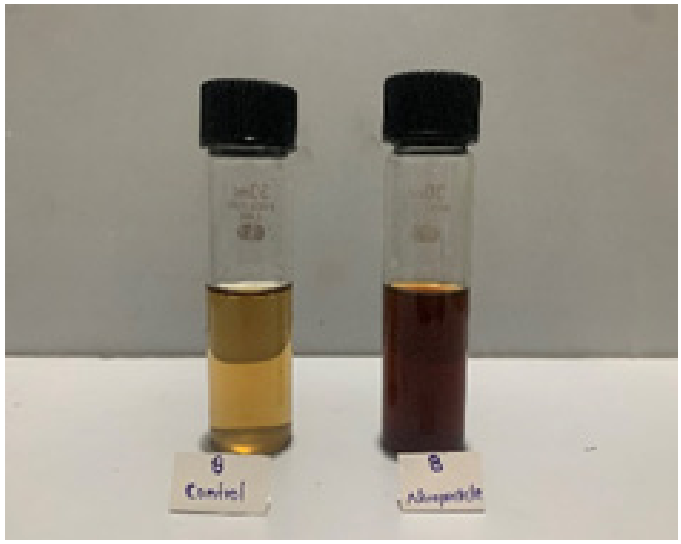
(MCF7 Arundina 100µg)



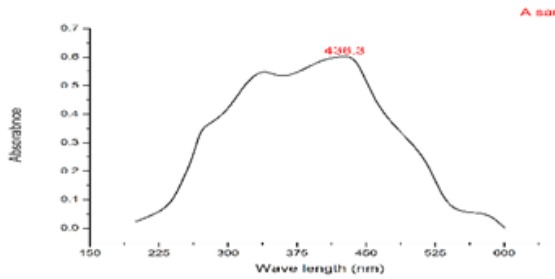
(MCF7 Pholidota 100µg)

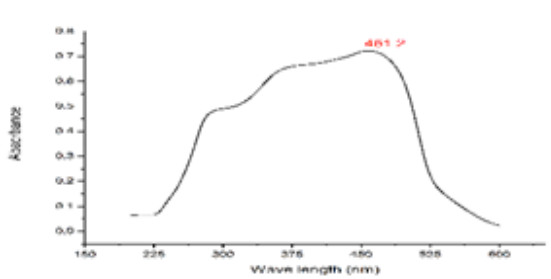
Nanoparticle synthesis result



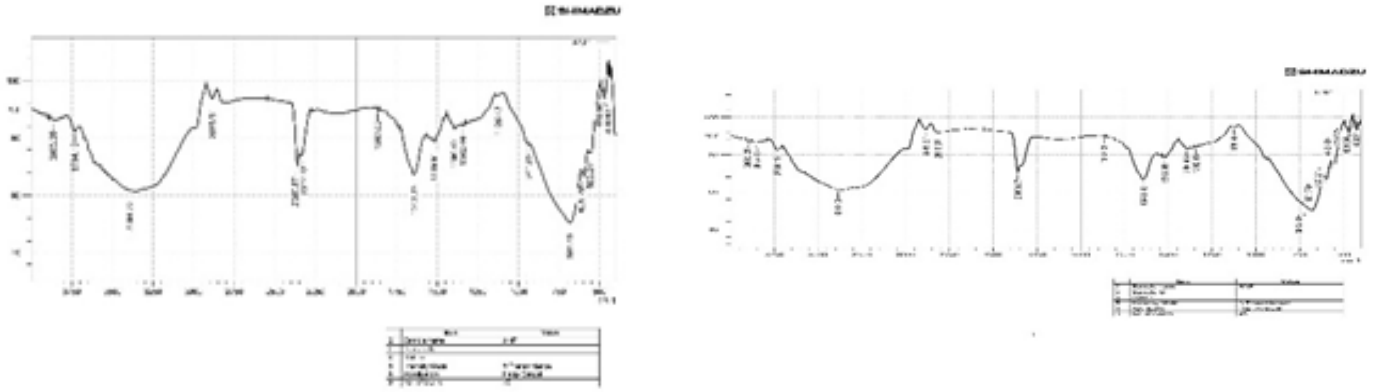


(preparation of nanoparticles)

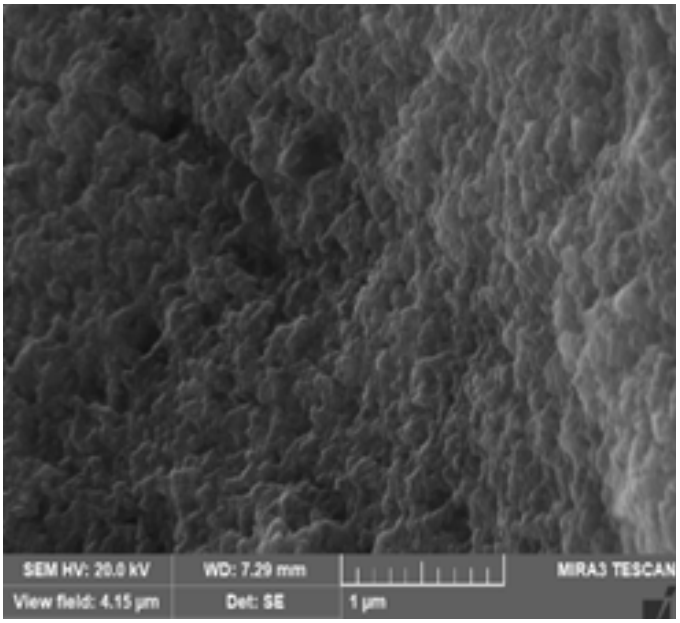


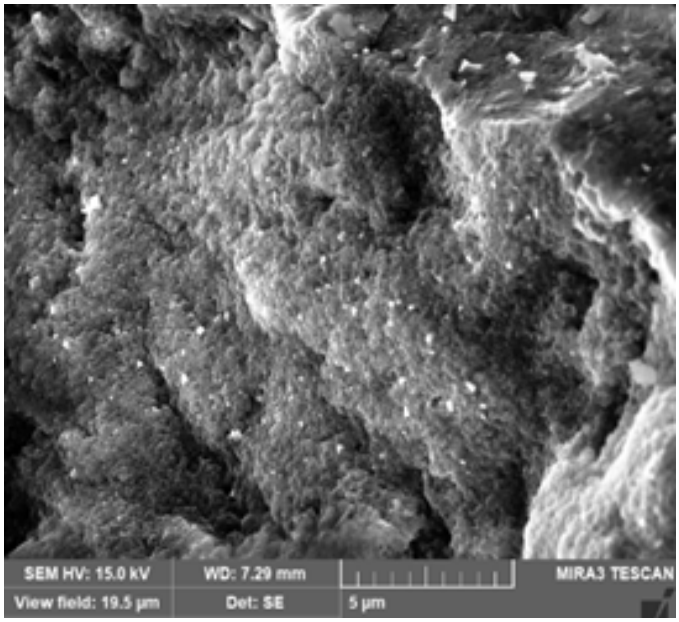


(UV Spectroscopy)



(FTIR Analysis)





Singh *et al.,* 2016.In Antimicrobial activity aqueous extract showed significant inhibiting activity in both *Pholidota* (4mm, 4mm in *E. coli*& *S. aureus* respectively and no activity in *Aspergillus flavus*,2mm in *Fusarium spp*) and *Arundina* (3mm, 5mm in *E. coli & S. aureus* respectively and no activity in *Aspergillus flavus*, 3mm in *Fusarium spp*). In the antimicrobial study of *Pholidota articulata*, Ethyl acetate extract shows significant activity with 18mm, 14mm by Darshan Singh *et al.,* 2016. Anticancerous activity showed *Pholidota pallida* possess significant cytotoxicity potential against Human breast cancer cells (MCF7) rather than *Arundina*.

CONCLUSION

The present study revealed that the various phytoconstituents and bioactive compounds present in the leaf extract of *Pholidota pallida* and *Arundina graminifolia*. This study confirms the use of leaves of this orchids *Pholidota pallida* and *Arundina graminifolia* for various disease by traditional practitioners. These beneficial compounds have antioxidant, antimicrobial, and anticancer activity. While comparing, Pholidota possess more bioactive compounds than *Arundina.*

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