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Original Article

PHYSICOCHEMICAL, PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY AGAINST CLINICAL PATHOGENS OF MEDICINALLY IMPORTANT ORCHID *GEODORUM DENSIFLORUM* (LAM) SCHLTR

M. KEERTHIGA[1], S. P. ANAND[2]

[1]Department of Biotechnology, National College (Autonomous), Tiruchirappalli–01, [2]Department of Botany, National College (Autonomous), Tiruchirappalli-01
Email: keerthigamanohar@gmail.com

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ABSTRACT

Objective: To evaluate the Physicochemical, Preliminary Phytochemical analysis and Antibacterial activity of *Geodorum densiflorum* against clinical pathogens and to prove the orchid will target to meet the therapeutic demands.

Methods: The physicochemical parameters and Preliminary Phytochemical analysis of the orchid were studied. The antibacterial properties using the following bacterial strains, *ie*., *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Klebseilla pneumoniea, Pseudomonas aeruginosa* were studied. Streptomycin were used as a control and study was conducted using disc diffusion method and the MIC value also be evaluated.

Results: The Physicochemical parameters show the presence of bioactive compounds. The phytochemical screening revealed the presence of Alkaloids, Tannins, Flavonoids, Sopanins, Steroid and Terpenoids. The ethanol extract showed significant total activity against *Staphylococcus aureus* than other extracts.

Conclusion: The ethanol extract of *Geodorum densiflorum* can successfully used as a pharmaceutical agents as antibacterial drug.

Keywords: *G. densiflorum,* antibacterial, Phytochemical analysis, Physicochemical analysis.

INTRODUCTION

Infectious diseases are the number one among all cautions of death, accounting approximately one-half all death throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases [1]. Scientists from divergent fields is investigating plants with a new eye for their antimicrobial usefulness and as an alternative source to chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity [2]. Plant materials remain an important resource to combat serious diseases in the world. Still they play a vital role to cover the basic health needs in the developing countries. Plants are also rich in compounds which have pain relieving and healing abilities [3]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [4].

Orchidaceae family is one of the largest, most diverse and most important categories of botanically and commercially significant flowering plants with 20,000-30,000 species [5]. Orchids are well known for their beauty and its medicinal use [6].

Geodorum densiflorum (Lam.) Schltr. (Family: *Orchidaceae*) is an endangered terrestrial orchid [7], commonly known as ground gem orchid appearing above the ground only during the rainy season and is widely distributed in humid tropical forests of south India especially in the Western Ghats, Eastern Ghats (all districts of Orissa, Andhra pradesh, Tamil Nadu) [8] and in Bangladesh. Literature review indicates no studies of *G. densiflorum* root has so far been undertaken to provide enough scientific data in favour of reported traditional uses.

Traditional use varies among the local practitioners though traditionally the orchid is used for a wide range of indications including menstrual cycle regulation [9], joint pain and arthritis [10], diabetic [11], applied externally to cure Carbuncles [12], etc. As part of the endeavour for search of medicinal properties in local floristic resources we herein report a study of preliminary phytochemical, physiochemical and antibacterial activity of the whole plant of *G. densiflorum*.

MATERIALS AND METHODS

Collection of plant

The healthy plant materials of *Geodorum densiflorum* were collected from the Periyakombai Hills in Tamil Nadu, South India. The specimen was identified with the help of regional floras and the voucher specimen was deposited at St. Joseph's college (Autonomous), Trichy, Tamil Nadu. The plant material was shade dried, powdered and stored in air tight container for further use.

Solvent extraction of plant drug

The fine powdered plant material was subjected to extraction in soxhlet apparatus. The powdered plant drug was successively extracted with chloroform, and ethanol. The extract was collected and concentrated to get dried plant extract.

Aqueous extraction

100 gm of dried powder were extracted in distilled water for 6 hrs at slow heat. Every 2 hrs the extract was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min.

The supernatant was collected and procedure was repeated twice and after 6 hrs the supernatant was concentrated to make the dried plant extract. Percent extractive values were calculated by the following formula.

$$Percent\ Extract = Weight\ of\ dried\ extract / Weight\ of\ dried\ plant\ material$$

Preliminary phytochemicals screening

For preliminary phytochemical screening, plant extract of the whole plant was subjected to various qualitative chemical tests to determine the presence of various phyto-constituents like glycosides, tannins, phytosterols, proteins, amino acids, flavonoids, saponins [13, 14].

Physicochemical evaluation

The whole plant powder of *Geodorum densiflorum* was subjected to evaluate total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content [15, 16].

Ash values

Total ash value was obtained by incinerating plant powder at temperature 650-700º C in Silica crucible until free from carbon, weight of ash was taken and the percentage of it was calculated.

Acid insoluble ash

The total ash obtained was boiled with 2N HCl, filtered and the insoluble matter was collected on ashless filter paper. It was washed with hot water, ignited in silica crucible. Cooled in desiccators and the residue obtained was weighed and the percentage of acid insoluble ash was calculated.

Water soluble ash

The total ash obtained was boiled with water and the insoluble matter was collected on ash less filter paper, washed with hot water and ignited. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated.

Water soluble extractive value

Plant powder was macerated with chloroform water (2.5 ml chloroform in 1000 ml water) in a closed flask. It was filtered and 25 ml filtrate was evaporated to dryness at 105º C and weight. The percentage of water soluble extract value was calculated.

Alcohol soluble extractive value

Plant powder was macerated with alcohol in a closed flask. It was filtered and 25 ml filtrate was evaporated to dryness at 105º C and weight. The percentage of water soluble extract value was calculated.

Loss on drying

1.0 g of plant powder weight was taken. The sample was heated in oven maintained at 105-110°C, for 3h, after which the sample was allowed to cool to room temperature in desiccators. The percentage of moisture contain was calculated.

Microbiological screening

Antimicrobial activities of different extracts were evaluated by the Disc diffusion method [17] modified by [18]and Minimum Inhibitory Concentration (MIC) [19].

Disc diffusion method assay

Disc diffusion method was used to test the antibacterial activity of the extracts against several bacteria. The essential leaf extracts were used for studying their antibacterial activity. A loopful of bacterial strains were inoculated into 5 ml of nutrient broth and incubated for 24 hrs at 37º C to get active strain.

Nutrient agar plates were prepared by pouring 20 ml of molten media into sterile petriplates. After solidification of media, inoculums of MTCC strains such as *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumonia* (MTCC 3384) and *Streptococcus pyogenes* (MTCC 442) were swabbed uniformly and the inoculums was allowed to dry for 5 minutes. The different concentrations of extracts (25mg, 50mg, 75mg and 100mg) were loaded on 10 mm sterile disc which was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37º C for 24 hrs.

At the end of incubation, zones formed around the disc were measured with transparent ruler in millimetre. Based on the diameter of the zone of inhibition, antibacterial susceptibility was ranked [20]. Inhibition zones were measured and compared with the standard reference antibiotics. Activity index for each extracts was calculated.

$$Activity\ Index = Inhibtion\ zone\ of\ the\ sample / Inhibition\ zone\ of\ the\ standard$$

Minimum inhibitory concentration (MIC)

Minimum Inhibitory Concentration (MIC) was determined by Micro dilution method using serially diluted plant extracts. The extracts were diluted into different concentrations of 0.125 - 8 mg/ml respectively with DMSO. Then each tubes was filled with 1 ml of sterile nutrient broth and inoculated with 0.1 ml of broth culture of the test organism (inoculums contains $1\text{-}2\times10^{-7}$CFU/ml).

The tubes were incubated aerobically at 37ºC for 18-24 hrs. The control tubes were maintained for each test tube. Inhibition of growth observed in those test tubes (No turbidity) which has lowest or minimum concentration of extract. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC) [21].

Total activity (TA) determination

Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 gm plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [22].

$$Total\ Activity\ (TA) = Extract\ per\ gram\ dried\ plant\ part / MIC\ of\ extract$$

Statistical analysis

Mean value and standard deviation were calculated for each test bacteria. Data were analysed by one –way ANOVA and p values were considered significant at $p > 0.005$ [23].

RESULTS AND DISCUSSION

Phytochemical evaluation

The Whole plant powder of *Geodorum densiflorum* was subjected to successive solvents and aqueous extraction. Percentage yield of the selected successive extracts were recorded in Fig 1.

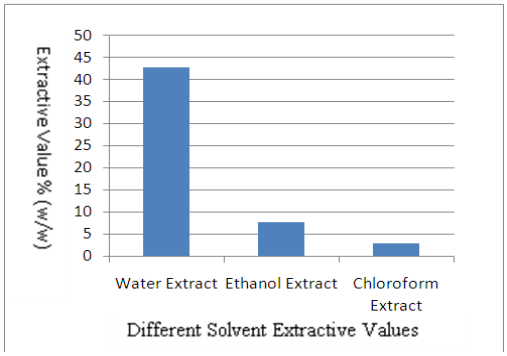

Fig. 1: Successive extraction of the plant of *Geodorum densiflorum* (Lam.) Schltr.

Preliminary phytochemical screening of *Geodorum densiflorum* shows presence of alkaloids, steroids, carbohydrates, flavonoids, tannin and saponins in different extract were reported in Table 1. From this analysis it was clear that ethanol has higher extractive value, then water and chloroform respectively. In whole plant many chemicals were detected that helps for treating different diseases. Now day, tribal peoples were used pseudobulb and their roots to cure many diseases, so it has potential to provide valuable drugs for human beings.

Physicochemical evaluation

Physicochemical evaluation of whole plant powder in silica crucible by using different parameters. The results of physicochemical characterization including ash value, extractive value and moisture contain were measured and mentioned in Table 2.

Table 1: Preliminary phytochemical screening of *Geodorum densiflorum* (Lam.) Schltr

Phytochemical Test	Plant Extracts		
	Chloroform Extract	Ethanol Extract	Aqueous Extract
Alkaloids	+++	+++	++
Flavonoids	+++	+++	+++
Steroids	+++	++	+
Cardiac Glycosides	-	-	-
Terpenoids	++	+	-
Triterpenoids and Steroids	-	-	-
Phenol	-	+	-
Tannins	+	+++	++
Sopanins	-	++	++
Phlobatannins	-	-	-
Reducing Sugar	++	+++	+
Anthroquinones	-	-	-
Gum and Mucilages	-	-	-

Physicochemical evaluation was carried out by using parameters like total ash, acid insoluble ash, water soluble ash, loss on drying, alcohol extractive value and aqueous extractive value are 5.47, 8.89, 2.55, 9.87, 7.643 and 42.89 respectively. Physicochemical evaluation is important in identification of distinctive features of the drug and also helps to avoid adulteration. The ash values were used to examine quality and purity of drugs. Moisture contain was also important at the time of storage to avoid microbial contamination of drugs [24].

Table 2: Physicochemical parameters of *Geodorum densiflorum* (Lam.) Schltr

Parameter	Values % (w/w)
Total Ash	**5.47**
Acid Insoluble Ash	**8.89**
Water Soluble Ash	**2.55**
Loss on Drying	**9.87**
Alcohol Soluble Extractive Value	**7.643**
Aqueous extractive value	**42.89**

Antibacterial activity

The results of *in vitro* antibacterial activity of the different extracts of *Geodorum densiflorum,* determined by inhibition zones are presented in Table - 3. Streptomycin used as a standard antibiotic at the concentration of 30µg/disc exhibited higher diameters of inhibition than other extracts. These results revealed that the diameter of inhibition zones increased with the concentration of extract.

Table 3: Antibacterial activity of various extracts of *Geodorum densiflorum* (Lam.) Schltr. against clinical pathogens

Test microorganisms	Values	Solvents											
		Water				Ethanol				Chloroform			
		25mg	50mg	75mg	100mg	25mg	50mg	75mg	100mg	25mg	50mg	75mg	100mg
Bacillus subtilis	IZ±S.D	- -	- -	12±0.1 3	14±0.2 6	11±0.3 3	13±0.2 3	15±0.5 6	19±0.3 4	- -	- -	- -	- -
	AI			1.090	1.272	1.003	1.181	1.363	1.727				
Staphylococcus aureus	IZ±S.D	- -	- -	- -	- -	14±0.2 4	17±0.2 4	22±0.2 7	29±0.2 4	14±0.2 7	16±0.0 6	17±0.4 8	22±0.2 4
	AI					0.933	1.133	1.466	1.933	0.933	1.066	1.133	1.466
Streptococcus pyogenes	IZ±S.D	- -	- -	- -	- -	10±0.2 9	12±0.2 3	14±0.4 2	21±0.2 5	14±0.2 9	17±0.0 7	19±0.3 6	22±0.1 8
	AI					0.476	0.571	0.666	1.005	0.666	0.809	0.904	1.047
Escherichia coli	IZ±S.D	- -	- -	- -	13±0.2 4	12±0.3 3	14±0.2 7	18±0.1 0	20±0.2 4	18±0.0 9	19±0.2 3	21±0.2 5	25±0.2 3
	AI				0.812	0.75	0.875	1.125	1.25	1.125	1.187	1.312	1.562
Pseudomonas aeruginosa	IZ±S.D	- -	12±0.1 2	15±0.2 3	18±0.2 7	10±0.1 3	15±0.1 9	17±0.0 8	22±0.2 3	11±0.1 2	14±0.2 5	16±0.3 4	18±0.1 4
	AI		0.5	0.625	0.75	0.416	0.625	0.708	0.916	0.458	0.583	0.666	0.75
Klebseilla pneumoniae	IZ±S.D	- -	- -	- -	- -	10±0.2 3	16±0.2 5	20±0.2 8	25±0.3 4	12±0.2 5	13±0.2 4	16±0.6 5	17±0.0 9
	AI					0.714	1.142	1.428	1.785	0.857	0.928	1.142	1.214

IZ – Inhibition zones in mm, S.D – Standard Deviation, AI – Activity Index, - - No zone formation

In the present study total three extracts of different concentrations of selected plant were tested for their bioactivity. Alcoholic extracts showed significant antimicrobial potential against test microbes. Most susceptible organism in the investigation was *Staphylococcus aureus* against which the plant extracts showed inhibition zone. Maximum antimicrobial activity were recorded for ethanolic extract (IZ – 29.00 ± 0.24 mm, AI – 1.933) against *Staphylococcus aureus* followed by *Klebseilla pneumoniae* showing (IZ – 25.00 ± 0.34 mm, AI – 1.785). Water extracts of the plant didn't show any significant antibacterial activity.

Table 4: Screening MIC (mg/ml) performance of different extracts of *Geodorum densiflorum* (Lam.) Schltr. against pathogenic organisms.

Test microorganisms	Aqueous Extract	Ethanol Extract	Chloroform Extract
Bacillus subtilis	5±0.013	0.500±0.027	-
Staphylococcus aureus	-	0.125±0.032	0.250±0.007
Streptococcus pyogenes	-	0.500±0.018	0.250±0.014
Escherichia coli	-	0.250±0.024	0.125±0.005
Pseudomonas aeruginosa	3	0.500±0.017	0.500±0.024
Klebseilla pneumoniae	-	0.500±0.043	0.500±0.031

Minimal inhibitory concentration

MIC values (Table 4 & Fig 2) were recorded for those plant extracts only which shows activity in disc diffusion assay.

The range of MIC of extract recorded was 0.125 to 5 mg/ml. In the present investigation MIC values were recorded for water extracts (5

and 3 mg/ml) against *Bacillus subtilis* and *Pseudomonas aeruginosa* as well as for extract in ethanol solvent against *Staphylococcus aureus* indicating significant antimicrobial potential of test extracts. The chloroform extracts shows efficient activity on *E. coli* and followed by *Staphylococcus aureus.*

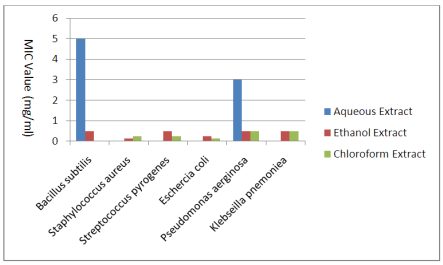

Fig. 2: Comparative MIC (mg/ml) performance of different extracts of *Geodorum densiflorum* (Lam.) Schltr. against pathogenic organisms.

Total activity

Total activity indicates the volume at which extracts can be diluted with still having ability to kill microorganism (Table 5 & Fig 3). Mostly ethanolic extracts showed high value of total activity (61.14 & 30.57) against *Staphylococcus aureus* and *Escherichia coli* respectively, which proves the potential of extracts to inhibit growth of the test microorganisms, even at low concentration. Total activity values were calculated in Ethanolic solvent extracts (15.28) followed by Water extract (14.29) & Chloroform extract (5.77) against *P. aeruginosa*. The findings revealed that ethanolic extracts from *Geodorum densiflorum* contain phytochemicals which offer an enormous potential as bio control of these pathogens and source of antimicrobial agents of therapeutic importance.

Table 5: Screening Total Activity performance of different extracts of *Geodorum densiflorum* (Lam.) Schltr. against pathogenic organisms

Test microorganisms	Aqueous Extract	Ethanol Extract	Chloroform Extract
Bacillus subtilis	8.57	15.28	-
Staphylococcus aureus	-	61.14	11.55
Streptococcus pyogenes	-	15.28	11.55
Escherichia coli	-	30.57	23.11
Pseudomonas aeruginosa	14.29	15.28	5.77
Klebseilla pneumoniae	-	15.28	5.77

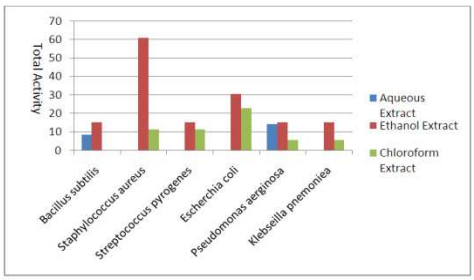

Fig. 3: Comparative Total Activity performance of different extracts of *Geodorum densiflorum* (Lam.) Schltr. against pathogenic organisms

CONCLUSION

From the above studies, it is concluded that the traditional plants may represent new sources of anti microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedicinal preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

CONFLICT OF INTERESTS

Declared None

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