

RESEARCH ARTICLE

Screening of Biological Activity of Selected Medicinal Orchids of Western Ghats, Karnataka, India

Hemanthkumar Madhavamurthy¹, Mahendra Chikkamadaiah², and Sharada M. Suryanarayana^{2*}

¹Plant Pathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India

²Plant Biotechnology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India

*Corresponding author: Sharada M. Suryanarayana, Email: mssharada.botany@gmail.com

Received: October 27, 2022; **Accepted:** June 21, 2023; **Published:** July 24, 2023; **DOI:** <https://doi.org/10.36922/itps.239>

Copyright: Author(s). This is an open-access article distributed under the terms of the Attribution Non-Commercial 4.0 International 4.0 (CC BY-NC 4.0), which permits all non-commercial use, distribution, and reproduction in any medium, which provided that the original work is properly cited.

Abstract

The present work was carried out to investigate the presence of bioactive compounds in selected medicinal orchids such as *Dendrobium jerdonianum*, *Dendrobium barbatulum*, *Eria mysorensis*, *Bulbophyllum neilgherrense*, and *Pholidota pallida*. The phytochemical, antibacterial, and antioxidant properties of the plants were evaluated using extracts obtained through chloroform, methanol, and aqueous extraction methods, respectively. The results of the phytochemical test showed the presence of diverse classes of secondary metabolites such as alkaloids, steroids, triterpenes, saponins, tannins, flavonoids, carbohydrates, resins, proteins, glycosides, and phenolics. The presence of these phytochemicals varied among different solvent extracts among the selected plants. Among the plant extracts tested, *D. jerdonianum* and *P. pallida* offered significant $p \geq 0.05$ antibacterial properties against *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Shigella flexneri*, and *Pseudomonas aeruginosa* with a minimum inhibitory concentration (MIC) ranging from 0.312 to 2.5 mg/mL. Among the plant extracts tested, methanol extract of *Erythrina mysorensis* (29.38%), *B. neilgherrense* (15.92%), *P. pallida* (13.84%), and *D. barbatulum* (13.10%) showed highest phenolic contents, followed by chloroform and aqueous extracts in all the plants. *E. mysorensis* and *D. jerdonianum* exhibited highest antioxidant properties at 85.41% and 81.28% (with IC_{50} values of 36.49 and 43.72 μ g/mL), respectively, while the positive control, gallic acid, displayed antioxidant properties of 94.61% (with an IC_{50} values of 30.49 μ g/mL). These results warrant further studies on using these selected orchids for therapeutic and pharmaceutical applications.

Keywords: Antibacterial, Antioxidants, Orchids, Phytochemistry

1. Introduction

Medicinal plants such as herbs, shrubs, and woody plants have played a crucial role in the development of new therapeutic drugs, contributing to the improvement of human life for thousands of years. These biologically active therapeutic agents have served as a valuable source of new natural products for humans [1]. Orchidaceae is one of the biggest and most well-known families of flowering plants in the plant kingdom [2]. With approximately 779 genera and 22,500 species, it stands as the

most ecologically and morphologically diverse member of angiosperms [3]. In India, the diversity of Orchidaceae members is represented by 1331 species across 186 genera, due to their adaptable nature. Orchids can be classified as epiphytic (growing on trees or shrubs), saprophytic (growing on dead and decaying matter), or terrestrials (growing on the ground). They are highly sensitive to habitat degradation and fragmentation [4,5].

The majority of orchid plants are highly medicinal and used to combat various ailments [6,7]. These plants are also used to extract various natural

products such as perfumes and medicines [8,9]. Approximately 60% of orchids are used every year for various medicinal purposes, and they are known to contain abundant chemical constituents such as alkaloids, flavonoids, and terpenoids [10,11]. One well-known example is vanillin, a flavoring agent obtained from *Vanillin planifolia* [12,13]. Vanillin is a phenolic aldehyde, commercially available, and extensively used in the food industry [14]. Purified compounds obtained from orchid extracts exhibit several bioactivities such as antimicrobial, antioxidant, anthelmintic, insecticidal, antiviral, analgesic, antipyretic, anti-allergic, wound healing, anti-aging, anti-cancer, and anti-inflammatory properties [15-17]. The Indian subcontinent, characterized by different climatic regimes, habitat conditions, and forest types, provides a favorable environment for a diverse group of orchid species [18,19]. In southern India, the Western Ghats is the main hub for many natural products, encompassing a wide range of forest areas, ranging from tropical wet evergreen forests to grasslands with rich flora, and enriched with several endangered flowering plant species [20]. The Western Ghats area of Karnataka, which includes Kodagu, Hassan, Chikmagalur, Shimoga, and Uttara Kannada, boasts a high diversity of orchids [20-22] (**Figure 1**). Thus, in light of the medicinal importance of the orchids, certain epiphytic orchids, such as *Dendrobium jerdonianum* Wight, *Dendrobium barbatulum* Lindl, *Eria mysoriensis* Lindl, *B. neilgherrense* Wight, and *Pholidota pallida* Lindl (**Figure S1**) obtained from the Western Ghats region, were selected for the present study. The objective of this study is to isolate bioactive secondary metabolites and evaluate the phytochemical, antibacterial, and antioxidant properties of these orchid plants using various organic solvent extraction methods.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals were purchased from Sigma Aldrich and Hi-Media Lab. Pvt. Ltd. Bangalore, including DPPH (2,2-diphenyl-1-picrylhydrazyl [99%]), hexane (98%), chloroform (98%), methanol (99%), sterile distilled water and streptomycin, gallic acid (99%), nutrient agar, nutrient broth, Lieberman–Burchard (LB) reagent (98%),

Folin–Ciocâlteu (FC) reagent (98%), phosphate buffer, and ferric chloride.

2.2. Plant materials collection

Fresh plant materials were collected from the Western Ghats of Karnataka, India (12° 25'37 N) and (75° 44'51 E) and identified using the Flora of the Presidency of Madras [23]. The specimens were submitted to the Department of Studies in Botany, University of Mysore (Voucher specimen no.: *D. jerdonianum* (UOMBOT20DJ05), *D. barbatulum* (UOMBOT20DB04), *E. mysoriensis* (UOMBOT20EM06), *B. neilgherrense* (UOMBOT20BN03), and *P. pallida* (UOMBOT20PP07).

2.3. Preparation of plant extracts

Collected plant materials (pseudobulbs/tubers) were thoroughly washed with running tap water, cut into small pieces, shade dried at room temperature, and made into a fine powder using a mixture grinder. Each powdered sample (100 g) was mixed in 250 mL of chloroform, methanol, and water, respectively, and left for 24 h in a rotary shaker at room temperature and then filtered. The filtrate was collected and subjected to flash evaporation to obtain the extracts. The extracts were stored at 4°C in an airtight glass bottle until further experiments [24].

2.4. Preliminary phytochemical screening

Each plant extract was subjected to qualitative preliminary phytochemical analysis to detect the presence or absence of various classes of secondary metabolites such as alkaloids, carbohydrates, flavonoids, glycosides, phenolics, proteins, resins, triterpenes, steroids, saponins, and tannins, using the method described by Harborne (1998) [25].

2.5. Antibacterial activity

2.5.1. Test pathogens

Bacterial strains, such as *Salmonella typhi* (MTCC733), *Shigella flexneri* (MTCC1457), *Pseudomonas aeruginosa* (MTCC2453), *Escherichia coli* (MTCC7410), and *Bacillus subtilis* (MTCC121), were procured from the Microbial Type of Culture Collection (MTCC) and Gene Bank, Chandigarh, India.

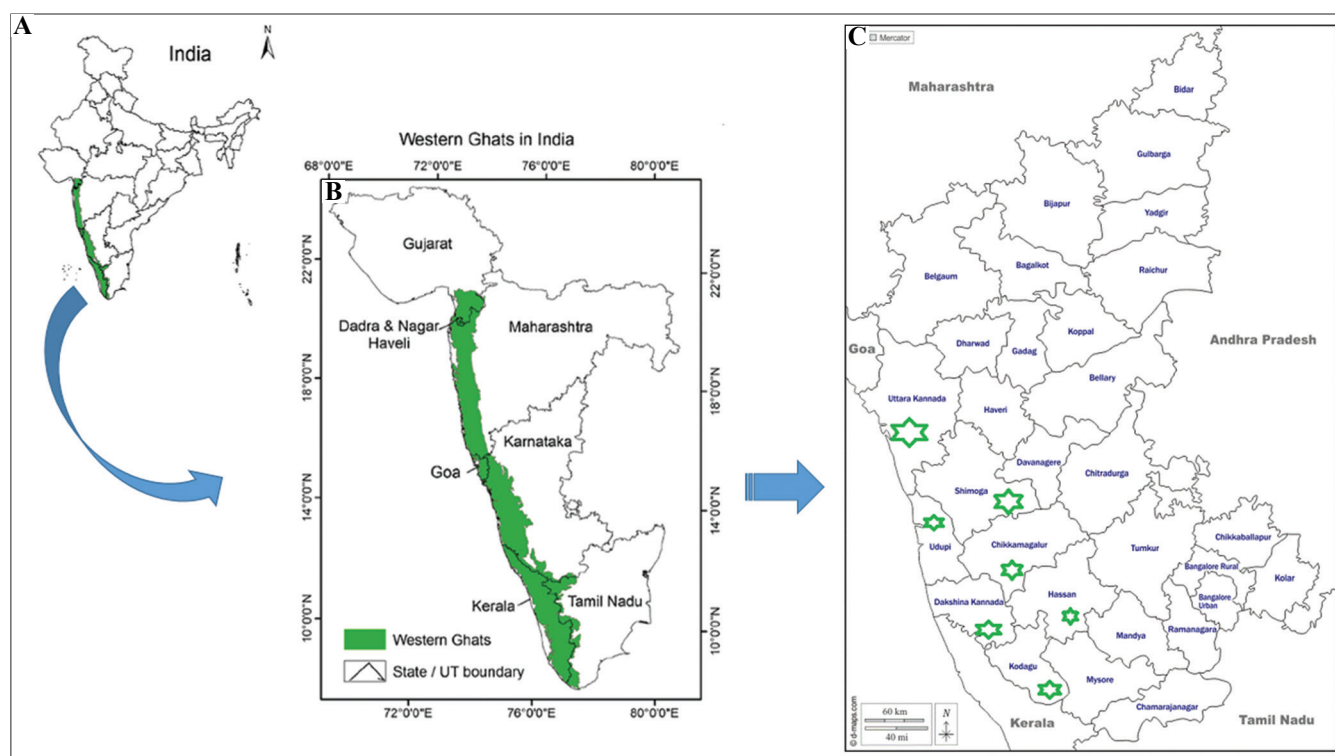


Figure 1. The map shows the diversity of orchids in the Western Ghats. (A and B) Western Ghats of India and their states. (C) Diversity of orchids in Karnataka districts. Note: Green stars – indicate the areas inhabited by orchids.

2.5.2. Disk diffusion method

The antibacterial activity of the extract was tested using the disk diffusion method [26]. Briefly, 100 μ L (1.5×10^8 CFU/mL) of the bacterial test sample (24-h-old) was spread uniformly on the surface of the Petri plates containing solidified nutrient agar media using a sterile glass spreader. The sterile disks (6 mm) were loaded with 50 μ L (3.5 mg) and 75 μ L (6.5 mg) of each plant extracts, respectively, from the stock (50 mg/mL) and placed equidistantly. The streptomycin (25 μ g/mL) and respective solvents (25 μ L) were used as positive and negative controls, respectively, and plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 h. After incubation, the zone of inhibition was measured, and the experiment was repeated in triplicates.

2.5.3. Minimum inhibitory concentration (MIC)

The MIC was determined using the micro-broth dilution method [27]. The extracts were prepared at a concentration of 50 mg/mL in respective solvents. In a 96-well plate, each well was dispensed with 100 μ L of nutrient broth, followed by the addition of 100 μ L stock solutions that were serially diluted

(0.002–5 mg/mL). Subsequently, a 10 μ L bacterial inoculum suspension was added to the mixture and incubated at $37 \pm 2^\circ\text{C}$ for 24 h. Absorbance was measured without agitation at 620 nm using an ELISA plate reader (LabTech 4000). The MIC was confirmed by adding 10 μ L of TTC (2, 3, 5-triphenyl tetrazolium chloride) per well at a concentration of 2 mg/mL and incubating the mixture for 30 min. The MIC was determined as the lowest concentration at which no color changes were observed. The experiments were performed in triplicates.

2.6. Antioxidant activity

The free radical scavenging activity (RSA) of each extract was evaluated using the DPPH assay, following the method described by Wahab *et al.* [28]. Each extract at varying concentrations (20, 40, 60, 80, and 100 μ g/mL) was prepared from the stock of 1mg/mL and tested for antioxidant activity. Four milliliters (4 mg 100/mL in methanol) of DPPH solution was added to each test tube containing extract, and methanol only was used as blank. The reaction mixture was incubated for 20 min at room temperature in the dark. The absorbance was measured

at 517 nm after incubation using a spectrophotometer. The experiment was performed in triplicates. The percentage of RSA and the inhibitory concentration (IC_{50}) were calculated using the following formula:

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100 \quad (I)$$

2.7. Total phenolic content

The presence of phenolic substances was measured using the FC reagent method, referring to gallic acid standard [29]. Each plant extract was tested at a concentration of 100 $\mu\text{g/mL}$. A combination of 0.5 mL of FC reagent and 1.0 mL of Na_2CO_3 (20%) was added to each test tube and incubated in the dark for 42 min. The absorbance was measured at 765 nm using a spectrophotometer (Thermo Fischer). The experiment was performed in triplicates. The absorbance of samples was plotted against the concentration and expressed in terms of gallic acid equivalence ($\mu\text{g GAE/g}$ of extract).

2.8. Statistical analysis

Data from three replicates were averaged and analyzed for each experiment. Analysis of variance was performed using SPSS Inc. 16.0. To determine the significant effects of treatments, Tukey's HSD test was conducted to compare, and separate treatment means based on F -values ($p \leq 0.05$).

3. Results and discussion

3.1. Preliminary phytochemical screening

Plants with a greater number of phytoconstituents generally exhibit higher medicinal properties. The phytochemical analysis of tested plant extracts revealed the presence of various classes of secondary metabolites in chloroform, methanol, and aqueous extracts, and the results of the biochemical test showed the presence of various classes of phytoconstituents such as alkaloids, steroids, triterpenes, saponins, tannins, flavonoids, carbohydrates, resins, proteins, glycosides, and phenolics in the plant. The results are presented in **Table 1**.

Among the tested plants, *D. barbatulum* showed no alkaloids, while *B. neilgherrense* and *D. Jerdonianum* were positive for the majority of the test in all extracts. These results are consistent with findings reported by Nagananda *et al.* [30], which found alkaloids, flavonoids, phenols, phytosterols, and other compounds in the methanol extracts of *P. pallida*. The majority of the phytochemical tests yielded the highest number of positive results in methanol extracts, followed by aqueous and chloroform extracts in all the plants. The findings align with previous reports by Kumari *et al.* [31] on *B. neilgherrensis* and Maridass *et al.* on *D. barbatulum*, where the majority of the phytochemicals were present in methanolic and aqueous extracts [32]. The study results indicate that methanol is the most effective solvent for extracting secondary metabolites from orchid plants. Methanol has been found to dissolve the highest number of secondary metabolites. These findings are consistent with the previous research conducted by Esmaeili *et al.*, who reported that methanol extracted the highest phytochemical content in *in vitro*-grown *Trifolium pratense* L. [33].

3.2. Antibacterial activity

The antibacterial activity of the crude extracts from selected orchids was evaluated at 50 mg/mL stock solution, and the results are presented in **Table 2**. The MIC values for each crude extract are shown in **Table S1**. Among the tested plant extracts, the methanolic extract of *P. pallida* effectively inhibited *E.coli* (18.64 mm), *P. aeruginosa* (15.36 mm), *S. typhi* (14.32 mm), *S. flexinari* (13.24 mm), and *B. subtilis* (13.34 mm) (**Figure 2**). The MIC values ranged 0.312–2.5 mg/mL. The methanol extract of *D. Jerdonianum* showed moderate antibacterial activity against all the tested bacteria, with inhibition zones of 9.54, 9.64, 10.24, 11.64, and 8.58 mm, respectively. The MIC values for *D. Jerdonianum* ranged from 0.625–2.5 mg/mL. No antibacterial activity was observed in the methanolic extracts of *D. barbatulum* and *Erythrina mysorensis*. The methanol extracts of *B. neilgherrense* only showed inhibition against *E.coli* (8.54 mm) and *S. flexinari* (8.12 mm), while chloroform and aqueous extracts of the other plants showed negative results. The results align with the previous reports by

Table 1. Preliminary phytochemical screening of selected orchids in various solvent extracts

Phytochemical tests	<i>D. jerdonianum</i>			<i>D. barbatulum</i>			<i>E. mysorensis</i>			<i>B. neilgherrense</i>			<i>P. pallida</i>		
	Chl	Meth	Aq	Cchl	Meth	Aq	Chl	Meth	Aq	Chl	Meth	Aq	Chl	Meth	Aq
Sterols															
Salkowski	-	-	-	-	+	-	-	-	-	+	+	-	-	+	-
LB test	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
Triterpenes															
Salkowski	-	-	-	-	+	-	-	-	-	+	+	-	-	+	-
LB test	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
Saponins															
Foam	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+
Alkaloids															
Mayer's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Dragondroff's	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
Wagner's	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-
Hager's	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Tannins															
FeCl ₃	-	+	+	-	+	+	-	+	+	+	+	+	-	+	+
Gelatine	-	+	-	-	-	-	+	+	-	+	+	-	+	+	-
Flavonoids															
Shinado	+	-	+	+	+	+	+	+	+	+	-	-	+	-	-
FeCl ₃	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+
Lead acetate	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+
Carbohydrates															
Molisch's	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+
Fehling's	+	+	+	-	-	-	+	-	-	+	-	-	+	+	-
Benedict's	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Resins															
Turbidity	+	-	+	+	-	-	+	-	-	+	-	-	+	-	-
Acetic anhydride	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-
Proteins															
Biuret	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Ninhydrin	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
Glycosides															
Killer-killiani	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-
Phenolics															
Ferric chloride	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+

Notes: “+”: presence; “-”: absence; Aq: Aqueous; Chl: Chloroform; Meth: Methanol; *D. jerdonianum*: *Dendrobium jerdonianum*; *D. barbatulum*: *Dendrobium barbatulum*; *E. mysorensis*: *Erythrina mysorensis*; *B. neilgherrense*: *Bulbophyllum neilgherrense*; *P. pallida*: *Pholidota pallida*

Rashmi et al. on *P. pallida*, which demonstrated effective inhibition *B. subtilis*, *B. coagulans*, *S. typhi*, and *E. coli* using methanol extracts [34]. The results indicated that among the tested plant extracts, methanolic extract was found to possess

significantly higher antimicrobial properties due to the presence of a higher number of secondary metabolites, as evidenced in the preliminary phytochemical screening test. Extracts with high potential secondary metabolites effectively

Table 2. Antibacterial activity of selected orchids in various solvent extracts

Plant and extract	Zone of inhibition measured in mm												Standard (25 µg)	Negative control		
	<i>E. coli</i>			<i>P. aeruginosa</i>			<i>S. typhi</i>			<i>B. subtilis</i>					<i>S. flexneri</i>	
	50µL	75 µL		50 µL	75 µL		50 µL	75 µL		50 µL	75 µL	50 µL			75 µL	
<i>D. jerdonianum</i>																
Chl	-	-	-	-	-	-	-	-	-	-	-	-	-	17.20±0.25	-	
Meth	8.12±0.25	9.54±0.54	-	8.64±0.54	9.64±0.98	10.24±0.87	9.22±1.24	11.64±0.54	8.58±0.98	8.24±0.26	15.12±0.25	-	-			
Aq	-	-	-	-	-	-	-	-	-	-	17.23±0.63	-	-			
<i>D. barbatulum</i>																
Chl	-	-	-	-	-	-	-	-	-	-	14.63±0.54	-	-			
Meth	-	-	-	-	-	-	-	-	-	-	17.25±0.56	-	-			
Aq	-	-	-	-	-	-	-	-	-	-	15.56±0.85	-	-			
<i>E. mysorensis</i>																
Chl	-	-	-	-	-	-	-	-	-	-	11.12±0.64	-	-			
Meth	-	-	-	-	-	-	-	-	-	-	12.54±0.32	-	-			
Aq	-	-	-	-	-	-	-	-	-	-	10.44±0.87	-	-			
<i>B. neilgherrense</i>																
Chl	-	-	-	-	-	-	-	-	-	-	13.12±0.25	-	-			
Meth	8.54±0.87	8.54±0.24	-	-	-	-	-	-	-	8.12±0.39	19.56±0.74	-	-			
Aq	-	-	-	-	-	-	-	-	-	-	13.26±0.26	-	-			
<i>P. pallida</i>																
Chl	-	-	-	-	-	-	-	-	-	-	17.64±0.54	-	-			
Meth	11.14±0.64	18.64±0.24	14.25±0.46	15.36±0.52	11.32±0.24	14.32±0.57	12.32±0.62	13.24±0.65	12.21±1.32	13.34±0.29	15.54±0.54	-	-			
Aq	-	-	-	-	-	-	-	-	-	-	18.36±0.58	-	-			

Note: Values are means of three independent replicates±SE. Notes: “-”: absence; Aq: Aqueous; Chl: Chloroform; Meth: Methanol; negative control: Respective solvents; standard: Streptomycin; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. typhi*: *Salmonella typhi*; *B. subtilis*: *Bacillus subtilis*; *S. flexneri*: *Shigella flexneri*; *D. jerdonianum*: *Dendrobium jerdonianum*; *D. barbatulum*: *Dendrobium barbatulum*; *E. mysorensis*: *Erythrina mysorensis*; *B. neilgherrense*: *Bulbophyllum neilgherrense*; *P. pallida*: *Pholidota pallida*

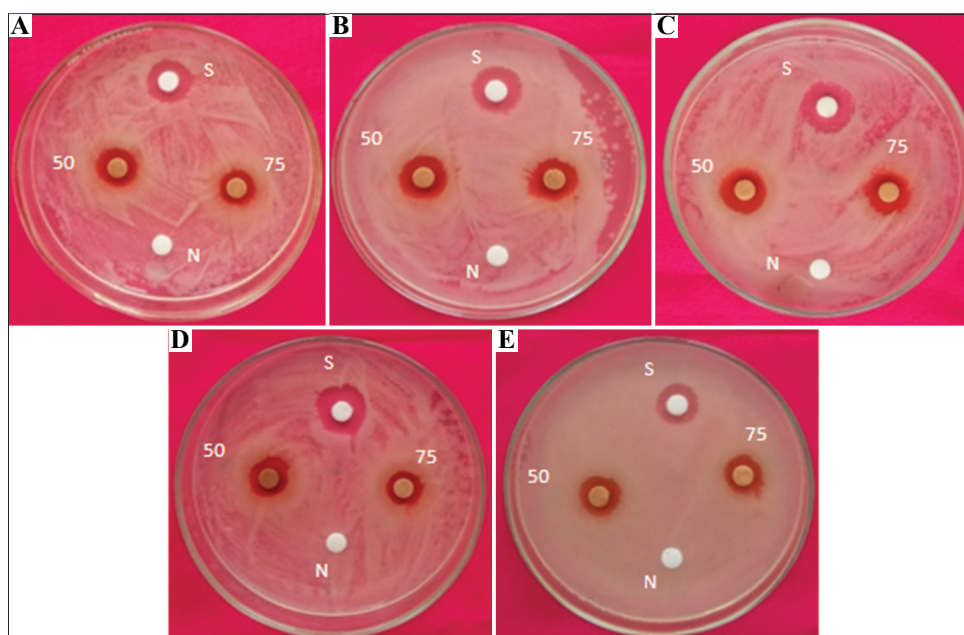


Figure 2. The antibacterial activity of the maximum zone of inhibition showed in methanolic extracts of *Dendrobium jerdonianum*. (A) *Salmonella typhi*, (B) *Shigella flexneri*, (C) *Pseudomonas aeruginosa*, (D) *Escherichia coli*, and (E) *Bacillus subtilis*.

Notes: N: Negative control; S: Standard.

inhibited the growth of bacteria, a finding in line with the report of Bhatnagar and Ghosal (2018), who demonstrated that methanolic extracts effectively inhibit *E. coli*, *S. aureus*, and *Acinetobacter* sp. It was also noticed that the zone of bacterial inhibition was also increased with plant extracts in a dose-dependent manner [35].

3.3. Antioxidant activity

The antioxidant activity of each plant extract was evaluated using the DPPH method. The percentage of RSA was measured, and the results are presented in **Table 3**. Among the tested plant extracts, aqueous extracts of *P. pallida*, *D. jerdonianum*, and *B. Neilgherrensis* exhibited antioxidants properties of 91%, 81%, and 74%, respectively, with inhibitory concentrations (IC_{50}) of 55.68, 43.72, and 54.84 $\mu\text{g/mL}$. The methanol and chloroform extracts of *D. barbatulum* and *E. mysorensis* showed significant RSA of 83% and 85% at higher concentrations, with IC_{50} values of 44.02 and 36.49 $\mu\text{g/mL}$, respectively. The standard gallic acid showed 94% inhibition at 100 $\mu\text{g/mL}$ ($IC_{50} = 30.49 \mu\text{g/mL}$), and it was dose-dependent. Similar results on medicinal orchids have been reported by Chand *et al.*, who screened various solvent extracts for antioxidant properties [36]. The

results revealed that the aqueous extract showed excellent antioxidant activity, followed by the methanol and chloroform extracts. The assay also revealed a dose-dependent with RSA. Furthermore, Paudel *et al.* reported the antioxidant activity of *D. moniliforme*, wherein the ethanol extracts showed antioxidant properties of 94%, significantly higher than acetone and chloroform extracts [37].

3.4. Total phenolic content (TPC)

The total phenolic content was measured and quantified in terms of gallic acid equivalent. These plants are often associated with polyphenolic substances and are considered a good source of antioxidants. Orchid plants, in particular, are well-known for their own medicinal properties (Moretti *et al.*, 2013) [38]. The phenolic content was measured according to the Folin–Ciocâlțu method at 100 $\mu\text{g/mL}$, and the results are presented graphically in **Figure 3**. The assay revealed that all the extracts contained a considerable amount of polyphenols. The percentage of TPC was determined by referencing the plotted standard gallic acid curve and applying a linear regression coefficient with an R^2 value of 0.9986 (**Figure S2**). Among the tested plant extracts, the methanol extracts of *E. mysorensis*, *B. neilgherrense*, *P. pallida*, and *D. barbatulum* showed the highest phenolic

Table 3. DPPH radical scavenging activity of selected orchids in various solvent extracts

Plants and extracts	Concentrations (µg/mL)					IC ₅₀ (µg/mL)
	20	40	60	80	100	
<i>D. jerdonianum</i>						
Chloroform	4.37±2.41 ⁿ	25.27±0.39 ⁿ	37.42±0.06 ^k	40.34±0.22 ^j	54.07±0.47 ^k	83.77
Methanol	23.93±0.82 ^l	39.85±0.19 ^h	47.75±2.13 ^h	56.86±0.13 ^h	62.21±1.42 ⁱ	68.30
Aqueous	38.91±0.81 ^c	47.26±1.61 ^e	57.22±0.65 ^c	68.40±0.26 ^d	81.28±0.97 ^e	43.72
<i>D. barbatulum</i>						
Chloroform	28.18±0.55 ^j	32.80±0.68 ^k	45.32±1.99 ⁱ	51.64±0.34 ⁱ	65.97±0.13 ^h	71.06
Methanol	32.07±0.76 ^g	51.76±1.85 ^c	62.21±0.42 ^b	67.92±2.24 ^e	83.83±0.61 ^d	44.02
Aqueous	31.59±1.74 ^h	51.76±0.85 ^c	62.21±0.42 ^b	70.83±0.61 ^c	59.65±0.78 ^j	46.14
<i>E. mysorensis</i>						
Chloroform	39.79±0.51 ^b	54.44±0.71 ^b	61.44±0.93 ^b	74.08±0.62 ^b	85.41±0.92 ^c	36.49
Methanol	33.90±0.36 ^d	50.18±2.26 ^d	56.74±0.62 ^d	68.28±0.75 ^d	75.69±0.66 ^f	42.47
Aqueous	29.76±0.14 ⁱ	34.38±0.39 ^j	39.85±0.19 ^j	40.94±0.75 ^j	61.60±0.89 ⁱ	84.76
<i>B. neilgherrense</i>						
Chloroform	23.57±0.23 ^l	27.46±0.51 ^m	29.16±0.16 ^l	33.29±0.83 ^k	38.88±2.14 ^m	47.79
Methanol	12.02±0.16 ^m	21.74±0.97 ^o	26.00±0.43 ^m	33.17±1.32 ^k	45.20±0.49 ^j	117.50
Aqueous	35.96±0.98 ^d	41.31±2.27 ^g	51.03±0.81 ^f	59.29±0.26 ^f	74.72±0.61 ^f	54.84
<i>P. pallida</i>						
Chloroform	33.04±0.82 ^f	38.03±1.59 ⁱ	44.10±0.93 ⁱ	57.71±0.67 ^g	68.40±0.26 ^g	63.87
Methanol	31.34±0.72 ^h	42.64±0.85 ^f	55.52±0.55 ^e	72.29±0.48 ^c	82.38±1.53 ^e	49.67
Aqueous	26.85±0.98 ^k	28.67±0.58 ^l	49.08±0.87 ^g	72.90±2.01 ^c	91.13±0.21 ^b	55.68
<i>Standard</i>						
Gallic acid	41.33±0.51 ^a	59.41±0.65 ^a	68.38±0.61 ^a	80.34±0.58 ^a	94.61±0.47 ^a	30.49

Values are the means of three independent replicates $\pm$ SE. Means followed by the same letter (s) within the same column are not significantly different, according to Tukey's HSD.

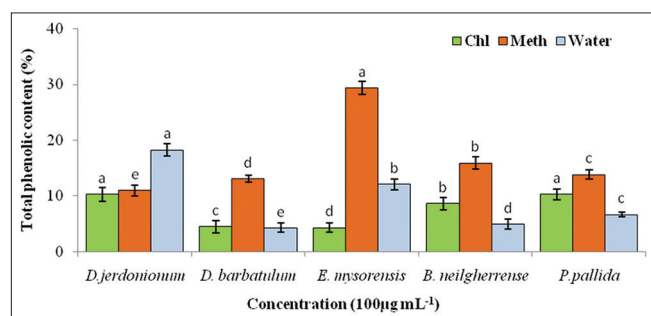


Figure 3. Total phenolic contents of selected orchid in various solvent extracts at the concentration of 100 $\mu\text{g/mL}$ and expressed in gallic acid equivalence per gram of dry weight. Values are the means of three independent replicates ($n = 3$), and bars sharing the same letters are not significantly different ($p \leq 0.05$) according to Tukey's HSD test results.

Notes: Chl: Chloroform; Meth: Methanol.

contents of 29.38%, 15.92%, 13.84%, and 13.10%, respectively. The aqueous extracts *D. Jerdonianum* showed a phenolic content of 18.26%, followed by the chloroform extracts. The findings are in agreement with the studies conducted by Poudel *et al.* [39] on *D. amoenum* and Minh *et al.* [40] on *Phalaenopsis* sp., which reported significant phenolic and flavonoid contents in these orchid species, making them good source of antioxidants [40].

4. Conclusion

This study highlights the bioefficacy of selected medicinal orchids from the Western Ghats. The phytochemical analysis in various solvent extracts revealed the presence of secondary metabolites such as alkaloids, steroids, triterpenes, saponins,

tannins, flavonoids, carbohydrates, resins, proteins, glycosides, and phenolics. The diverse range of phytochemicals present in the tested extracts contributes to their potential antioxidant properties and their status as good sources of phenolic substances. Furthermore, among the various extracts tested, methanolic extracts of *D. Jerdonianum* and *P. pallida* showed significant inhibition against tested pathogens, indicating the potential of isolating the bioactive metabolites for antibacterial purposes. The overall bioefficacy of the selected orchids can be attributed to the presence of numerous groups of secondary metabolites in various solvent extracts. Therefore, the study paves the way for their better utilization as therapeutic agents in the near future.

Acknowledgments

The first author is grateful to Dr. Sharif M. U., Joint Director, Acharya Jagadish Chandra Bose Institute, Indian Botanical Garden Kolkatta, West Bengal, for his kind support in the identification of plants. He is also thankful to the Department of Studies in Botany and its faculty members. The authors are also grateful to the Institute of Excellence (IOE), Vignana Bhavan, University of Mysore, for spectral analysis.

Funding

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

Conceptualization: Hemanthkumar Madevamurthy
Investigation: Hemanthkumar Madevamurthy,
Mahendra Chikkamadaiah

Writing – original draft: Sharada Suryanarayana
Writing – review & editing: Sharada Suryanarayana,
Mahendra Chikkamadaiah

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data generated and analyzed during this study are included in this article and provided some data as supplementary files. The data are available at the corresponding author's request.

References

- [1] Farnsworth, N.R. The Role of Ethnopharmacology in Drug Development. *Ciba Found Symp.*, **1990**, 154, 2–11.
- [2] Nongdam, P. Ethno-Medicinal Uses of Some Orchids of Nagaland, North-East India. *Res. J. Med. Plant*, **2014**, 8, 126–39.
- [3] Mabberley, D.J. *Mabberley's Plant-Book: A Portable Dictionary of Plants, their Classification, and Uses*. 3rd ed. Cambridge University Press, Cambridge, **2008**. p1021.
- [4] Puri, H.S. *American Orchid. Soc. Bull.*, **1970**, 39, 39–723.
- [5] Misra, S. *Orchids of India--A Glimpse*. Vol. 402. Bishen Singh, Mahendra Pal Singh, Dehradun, **2017**.
- [6] Singh, S.; Singh, A.K.; Kumar, S.; Kumar, M.; Pandey, P.K.; Singh, N.C.K. Medicinal Properties and Uses of Orchids: A Concise Review. *Eli. Appl. Bot.*, **2012**, 52, 11627–34.
- [7] Teoh, E.S. *Medicinal Orchids of Asia*. Springer, Berlin, **2016**.
- [8] Gutierrez, R.M.P. Orchids: A Review of Uses in Traditional Medicine, its Phytochemistry and Pharmacology. *J. Med. Plant Res.*, **2010**, 4(8), 592–638.
- [9] Ramesh, T.; Koperuncholan, M.; Praveena, R.; Ganeshkumari, K.; Vanithamani, J.; Muruganatham, P.; Renganathan, P. Medicinal Properties of Some *Dendrobium* Orchids--A Review. *J. Appl. Adv. Res.*, **2019**, 4(4), 119–28.
- [10] Attri, L.K. Therapeutic Potential of Orchids. *World J. Pharm. Pharm. Sci.*, **2016**, 5(2), 438–46.
- [11] Vaidya, B.N. Nepal: A Global hotspot for medicinal orchids. In: *Medicinal Plants: From Farm Pharmacy*. Springer Nature Switzerland, Cham, **2019**. p35–80.
- [12] Singh, A.; Duggal, S. Medicinal Orchids: An Overview. *Ethnobot. Leaf.*, **2009**, 13, 351–63.
- [13] Tsering, J.; Tam, N.; Tag, H.; Gogoi, B.J.; Apang, O. Medicinal Orchids of Arunachal Pradesh: A Review. *Bull. Arunachal For. Res.*, **2017**, 32(1–2), 1–16.
- [14] Menon, S.; Nayeem, N. *Vanilla Planifolia*: A Review of a Plant Commonly Used as Flavoring Agent. *Int. J. Pharm. Sci. Rev. Res.*, **2013**, 20(2), 225–8.
- [15] Kiran, R.; Kekuda, P.T.R.; Kumar, P.H.G.; Hosetti, B.B.; Krishswamy, K. Biological Activities of *Sarcanthus pauciflorus*. *J. Appl. Pharm. Sci.*, **2013**, 3(70), 105–10.
- [16] Keerthiga, M.; Anand, S.P. Physiochemical, Preliminary Phytochemical Analysis and Antibacterial Activity Against Clinical Pathogens of Medicinally Important Orchid *Geodorum densiflorum*. *Int. J. Pharm. Pharm. Sci.*, **2014**, 6(8), 558–61.
- [17] Keerthiga, M.; Anand, S.P. Study on Preliminary Phytochemical and Antibacterial Activity Against Human Pathogens of an Endangered Orchid *Habenaria plantaginea*. *Int. J. Pharm. Sci. Res.*, **2017**, 6(3), 1101–6.
- [18] Jalal, J.S.; Jayanthi, J. Endemic Orchids of Peninsular India: A Review. *J. Threatened Taxa*, **2012**, 4(15), 3415–25.
- [19] Asseleih, L.M.C.; Garcia, R.A.M.; Cruz, J.Y.S.R. Ethnobotany, Pharmacology, and Chemistry of Medicinal Orchids from Veracruz. *J. Agric. Sci. Technol. A*, **2015**, 5, 745–54.
- [20] Ramachandra, T.V.; Subash Chandran, M.D.; Bhat, S.P.; Aithal, B.H.; Rao, G.R.; Mukri, V. Status of Forest in Shimoga District, Karnataka. ENVIS Technical Report53. Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science Bangalore 560012, India, **2013**.
- [21] Nayar, M.P. Hot Spots of Endemic Plants of India, Nepal, and Bhutan. Tropical Botanical Garden and Research Institute, Thiruvananthapuram, **1996**. p252.

- [22] Nayar, M.P. Changing the Pattern of Distribution of Endemic Genera (Angiosperm). *J. For.*, **1997**, 7, 99–110.
- [23] Gamble, J.S. Flora of the Presidency of Madras. Vol. 1, 2, 3. Published under the Authority of the Secretary of State for India in Council, Dehra Dun, **2008**.
- [24] Maizura, M.; Aminah, A.; Wanaida, W.M. Total Phenolic Content and Antioxidant Activity of Kesum, Ginger, and Turmeric Extract. *Int. Food Res. J.*, **2011**, 18, 529–34.
- [25] Harborne, J.B. In: Chadwick, D.J.; Marsh, J. (eds). *Textbook of Phytochemical Methods. A Guide to Modern Techniques of the Compound from Plants*. John Wiley, Chichester, **1998**. p2-21.
- [26] Sen, A.; Batra, A. Evaluation of the Antimicrobial Activity of Different Solvent Extracts of Medicinal Plant: *Melia azedarach* L. *Int. J. Curr. Pharm. Res.*, **2012**, 4(2), 67–73.
- [27] CLSI. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Second Informational Supplement. CLSI Document, USA, **2012**. pM100–S22.
- [28] Abdul-Wahab, N.Z.; Shahar, S.; Abdullah-Sani, H.; Pihie, A.H.L.; Ibrahim, N. Antioxidant, Antibacterial, and Antiviral Properties of *Goniathalamus umbrosus* Leaves Methanolic Extract. *Afr. J. Microbiol. Res.*, **2011**, 5, 3138–43.
- [29] Chlopicka, J.; Pasko, P.; Gorinstein, S.; Jedryas, A.; Zagrodzki, P. Total Phenolic and Total Flavonoid Content, Antioxidant Activity and Sensory Evaluation of Pseudocereal Bread. *LWT Food Sci. Technol.*, **2012**, 46, 548–55.
- [30] Nagananda, G.S.; Patil, A.M.; Kambli, J.V.; Rajath, S. Phytochemical Evaluation and *In-Vitro* Free Radical Scavenging Activity of Cold and Hot Successive Pseudobulb Extracts of Medicinally Important Orchid *Pholidota pallida*. *Adv. Bio. Res.*, **2014**, 5(1), 100–5.
- [31] Kumari, H.; Nishteswar, K.; Shukla, V.J.; Harisha, C.R. Development of Pharmacognostic and Phytochemical Standards for Pseudobulb of *Bulbophyllum neilgherrense*. *Int. Ayur. Med. J.*, **2013**, 1(4), 1–8.
- [32] Maridass, M.; Zahir Hussain, M.I.; Raju, G. Phytochemical Survey of Orchids in the Tirunelveli Hills of South India. *Ethnobot. Leaf.*, **2008**, 12, 95.
- [33] Esmaili, A.K.; Taha, R.M.; Mohajer, S.; Banisalam, B. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from *In Vivo* and *In Vitro* Grown *Trifolium pratense* L. (Red Clover). *Biomed Res. Int.*, **2015**, 2015, 643285.
- [34] Rashmi, K.; Shweta, S.D.; Sudeshna, C.S.; Vrushala, P.S.; Kekuda, P.T.R.; Raghavendra, H.L. Antibacterial and Radical Scavenging Activity of Selected Orchids of Karnataka, India. *Sci. Technol. Arts Res. J.*, **2015**, 4(1), 160–4.
- [35] Bhatnagar, M.; Ghosal, S. Antibacterial and Antimycobacterial Activity of Medicinal Orchid of Arunachal Pradesh. *Int. J. Pharm. Sci. Res.*, **2018**, 9(2), 712–7.
- [36] Chand, M.B.; Paudel, M.R.; Pant, B. The Antioxidant Activity of Selected Wild Orchids of Nepal. *J. Coastal Life Med.*, **2016**, 4(9), 731–6.
- [37] Paudel, M.R.; Chand, M.B.; Pant, B.; Pant, B. Antioxidant and Cytotoxic Activities of *Dendrobium moniliforme* Extracts and the Detection of Related Compounds by GC-MS. *BMC Complement Altern. Med.*, **2018**, 18(1), 134.
- [38] Moretti, M.; Cossignani, L.; Messina, F.; Dominici, L.; Villarini, M.; Curini, M.; Marcotullio, M.C. Antigenotoxic Effect, Composition and Antioxidant Activity of *Dendrobium speciosum*. *Food Chem.*, **2013**, 140(4), 660–5.
- [39] Poudel, M.R.; Chand, M.B.; Karki, N.; Pant, B. Antioxidant Activity and Total Phenolic and Flavonoid Contents of *Dendrobium amoenum* Wall. Ex. Lindl. *Bot. Orientalis J. Plant Sci.*, **2015**, 9, 20–6.
- [40] Minh, T.N.; Khang, D.T.; Tuyen, P.T.; Minh, L.T.; Anh, L.H.; van Quan, N.; Xuan, T.D. Phenolic Compounds and Antioxidant Activity of *Phalaenopsis* Orchid Hybrids. *Antioxidants*, **2016**, 5(3), 31.

Publisher's note

AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.