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RESEARCH ARTICLE

Ethnobotanical survey and *in vitro* quality assessment of *Dendrobium thyrsiflorum* B. S. Williams from the Ultapani Forest Range, Assam, India

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Abstract

Dendrobium thyrsiflorum B. S. Williams (Orchidaceae), locally known as 'Garudi Baha/ Khejari Baha' by the Santhal community of the Ultapani Forest Range, Bodoland Territorial Region (BTR), Assam. The species occurs anthesis between March and May, with flowers reaching full bloom and attracting pollinators. This study investigated the ethnobotanical uses, macroscopic and microscopic pharmacognostic characteristics, qualitative and quantitative phytochemical composition, heavy metal content, antioxidant activity and volatile compound profile of *D. thyrsiflorum*. Ethnobotanical surveys conducted among the Santhal community revealed the traditional use of leaves and pseudobulbs as a medicine for the treatment of various gynecological and parasitic ailments. The examination of powdered plant material showed satisfactory organoleptic properties (colour, aroma/odour, flavour/taste and texture) for both leaves and pseudobulbs. Microscopic analysis identified fibers in both leaves and pseudobulbs, parenchyma cells and stomata in leaves and acicular crystals and spiral vessels in pseudobulbs. Aqueous extracts of leaves and pseudobulbs underwent qualitative phytochemical screening, revealing the presence of alkaloids, carbohydrates, glycosides, terpenoids and steroids in both tissues. The heavy metal analysis indicated the absence of bismuth, cadmium and lead in the samples. Quantitative phytochemical analysis determined the total polyphenol content (TPC) to be 27.62 ± 0.03 mg gallic acid equivalents (GAE)/g dry weight in leaves and 17.57 ± 0.02 mg GAE/g dry weight in pseudobulbs. The total flavonoid content (TFC) was measured as 120.36 ± 0.05 mg quercetin equivalents (QE)/g dry weight in leaves and 87.50 ± 0.04 mg QE/g dry weight in pseudobulbs. The antioxidant activity was further evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant potential (FRAP) assays, demonstrating higher activity for DPPH assay compared to the standard. Gas chromatography-mass spectrometry (GC-MS) analysis identified 88 compounds in leaves and 59 compounds in pseudobulbs. These findings contribute to the establishment of pharmacognostic and phytochemical standards for *D. thyrsiflorum*, aiding in species identification, quality control and standardization of herbal formulations.

Keywords: antioxidant; *Dendrobium thyrsiflorum*; ethnobotany; GC-MS analysis, phytochemical analysis; traditional community healers

Introduction

Plants are one such living organism that has contributed to the human world unaccountably, starting from timber, firewood, fibers, dyes, pesticides, oils, rubber, medicines and many more. In both western and eastern cultures, as well as in highly industrialized and underdeveloped nations, there is evidence of the usage of plants for medical purposes dating back as far as 60000 years (1). Approximately 70000 plant species provide benefits to people today, yet only 7000 of those species are grown in cultivation (2). The age of discovery and conquest brought about a resurgence of interest in the therapeutic potential of plants in Western societies. For instance, the native Indians' use of plants as medicine inspired the Spaniards, who between 1531 and 1536 conquered Mexico and Peru (1).

Orchids are one of the most diverse families of angiosperms, including approximately 25000 to 30000 species classified into 750 to 800 genera (3). Recently, it was stated that there were 28000 currently identified species, 800 subspecies and approximately 763 genera (4). They are mostly grown for their gorgeous blossoms and are well-known for their economic significance rather than for their potential medical benefits (5). Orchid habitat is most abundant in India. According to the first Orchid census of India conducted and released by Botanical Survey of India (BSI) in 2019, India is fortunate to have 1256 orchid species or taxa that belong to 155 genera, 388 of which were reported to be endemic (6–8). The genus *Dendrobium* has 800-1400 species in the world (9). This genus extends from Korea and Japan across Southeast Asia, the Himalayas in the west, the Philippines in the east and Indonesia in the south, Australia, New Zealand and the Pacific Islands (10). The largest genus is *Dendrobium*, which has 58 taxa, 51 of which are monotypic genera that are found in the various regions (11).

In the Northeastern part of India, known for its abundant natural riches, the state of Assam is located at the base of the Himalayas. Due to the high temperatures, high levels of rainfall and humidity in the area, there are huge evergreen and semi-evergreen deciduous forest covers. And as a result, has developed into an ideal

habitat for the growth of many different varieties of orchids. Kokrajhar district in lower Assam has extensive, large-scale forest cover, just like the other districts in Assam. Additionally, this forest area was reported to have several kinds of terrestrial and epiphytic orchids (12, 13).

Ultapani Forest Range has a substantial amount of evergreen and semi-deciduous forest cover. Due to its outstanding biodiversity, this area is progressively growing in popularity amongst tourists. It is one of the 4 territorial ranges under Haltugaon Forest Division under Kokrajhar district. The geographical area of the Ultapani forest range is 224.64 sq. km., located from 26°39′18.1″ N to 26°52′13.5″ N latitude and from 90°14′04.7″ E to 90°21′28.0″ E longitude (14).

Dendrobium thyrsiflorum B. S. Williams is one such exquisite orchid found in this forest region. This species belongs to the family Orchidaceae and it referred to as "Garudi Baha" or "Khejari Baha" among the Santhal folks living in Ultapani Forest Range, BTR, Assam. It is an epiphytic orchid having aching cane stem like pseudobulb of about 5-30 × 1-2.5 cm. The phenology of the vegetative phase of *D. thyrsiflorum* revealed that the new shoots and leaves emerge during May to June, whereas senescence is initiated in the second year of growth, specifically from June and during November to February, no growth is observed. The plant blooms in the month of March to May.

In addition to being an ornamental plant *D. thyrsiflorum* is used by a group of people in Ultapani Forest Range for its medicinal properties. However, no scientific study has been done till date on *D. thyrsiflorum* of this region to specify its medicinal value that is being practiced by the Santhal community traditional healers. The present study was carried out to reveal the ethnobotanical studies, pharmacognostic and phytochemical data (qualitative, quantitative), heavy metal analysis, antioxidant activity and gas chromatography-mass spectrometry (GC-MS) and morphological characterization for the identification of this species of orchid, to ensure quality and purity and standardization of the herbal drug.

Materials and Methods

Plant material collection and identification

A systematic field survey was conducted in the Ultapani Forest Range, Kokrajhar, Assam, India (Global positioning system (GPS) coordinates: N26°46′21.3″, E90°18′29.4″), from 2019 to 2022. Plant specimens were collected directly from their native habitat to ensure authenticity and ecological relevance (Table 1). The voucher specimens were prepared according to standard procedures (15). The preliminary identification was performed by consulting relevant literature and comparing specimens with those deposited at the Department of Botany, Bodoland University. *D. thyrsiflorum* was further verified and authenticated by the Botanical Survey of India (BSI), Howrah (specimen number BUSD-01, dated November 30, 2021). The taxonomic classification of *D. thyrsiflorum* is as follows:

Kingdom: Plantae

Class: Monocotyledons

Order: Asparagales

Family: Orchidaceae

Genus: *Dendrobium*

Species: *D. thyrsiflorum*

Preparation of aqueous extracts

Leaves and pseudobulbs of *D. thyrsiflorum* were washed, air-dried at room temperature and pulverized using an electric grinder. 5 g of each powdered samples were subjected to extraction using 50 mL of double-distilled water (1:10 w/v) for 24 hr at room temperature. The extracts were filtered through Whatman filter paper No. 1 and stored at 4 °C in airtight glass bottles.

Macroscopic examination

Macroscopic features of the plant material were documented through visual and physical examination of whole plants and individual parts against a black background (8).

Ethnobotanical survey

Ethnobotanical information was gathered through semi-structured interviews and questionnaires administered to local residents and traditional healers, focusing on the indigenous knowledge and medicinal uses of *D. thyrsiflorum*.

Pharmacognostic analysis

Organoleptic evaluation

Organoleptic properties (colour, aroma/odour, flavour/taste, texture) of the powdered leaves and pseudobulbs were assessed according to standard procedures (16).

Microscopic examination

Powdered leaf and pseudobulb samples were mounted in water and safranin and examined under a binocular microscope (LaboMed Vision 2000) for fragmented characteristics (17, 18).

Phytochemical screening

Qualitative tests

Aqueous extracts were subjected to qualitative tests for alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, steroids and phlobatannins using established methods (19–23).

Heavy metal analysis

The presence of cadmium, bismuth and lead in the extracts was evaluated using standard protocols (24). Each heavy metal was tested using 2 independent procedures:

- **Bismuth:** Reaction with H_2S (brown precipitate) and NH_4OH (white precipitate).
- **Cadmium:** Reaction with NH_4OH (white precipitate) and potassium ferrocyanide (white precipitate).
- **Lead:** Reaction with dilute HCl (white precipitate) and dilute potassium iodide (yellow precipitate).

Table 1. Details on the parts of the orchid that are used, the vernacular name and the Global positioning system (GPS) coordinates of the collection location

Botanical name	Parts used	Santhal name	Common English name	GPS coordinates
Dendrobium thyrsiflorum B S Williams	Leaves and/or pseudobulbs	Garudi Baha or Khejari Baha	Pinecone-like Raceme Dendrobium	N26^{0}46′21.3″, E90^{0}18′29.4″

Quantitative tests

Total polyphenol content (TPC)

TPC was determined using the Folin-Ciocalteu reagent method, with minor modifications (25). Absorbance was measured at 730 nm and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample in dry weight.

Total flavonoid content (TFC)

TFC was quantified using the aluminum chloride method with quercetin as a standard (26). Absorbance was measured at 510 nm and the results were expressed as milligrams of quercetin equivalents (QE) per gram of sample in dry weight.

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: The extracts and standard were evaluated for their antioxidant activity by measuring their ability to scavenge the stable, DPPH free radical using a standard protocol (23). A 0.006 % w/v solution of DPPH was prepared using a 95 % solvent. The extract and a 95 % freshly prepared DPPH solution were added in test tubes. Subsequently, successive dilutions ranging from 100 to 1000 µg were made in each test tube until the total volume reached 2 mL. After an incubation period of 30 min in the dark, the degree of discoloration was evaluated at a wavelength of 540 nm using a Thermo UV1 spectrophotometer (manufactured by Thermo Electron Corporation in England, UK). Measurements were conducted on a minimum of 3 occasions. Ascorbic acid served as the standard and was diluted in distilled deionized water (DDW) to create the first solution at a concentration of 1 mg/mL. To serve as a control sample, an equivalent volume was prepared without any extract and a blank was created using a solvent consisting of 95 % concentration. The equation provided was utilized to compute the percentage of DPPH-free radical scavenging.

$$S = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample.

In order to determine if the test chemicals reduced absorption, we compared them to the positive controls. Using the dosage inhibition curve, the IC_{50} value was determined.

Ferric ion reducing antioxidant potential (FRAP) assay: The reducing capacity of the extract was assessed following the method described by Oyaizu, with some modifications (27). The extract was mixed with different quantities ranging from 25 to 2500 µg in 1 mL of DDW. This mixture was then blended with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide ($K_3Fe(CN)_6$) (1 %). The solution was subjected to incubation at a temperature of 50 °C for a duration of 20 min. The mixture was thereafter subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 10 min, with the addition of 2.5 mL of trichloroacetic acid (10 %). The top layer solution (2.5 mL) was combined with DDW (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was determined at a wavelength of 700 nm. The increased absorbance of the reaction mixture showed a greater capacity for reducing substances. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as a control. The mean absorbance of the final reaction mixture in 2 parallel experiments was computed, together with its standard deviation.

GC-MS/MS analysis

Phytoconstituent profiling of the aqueous extracts was performed following the standard method using an Agilent 7890B GC and 7000D series Triple Quadrupole mass spectrometer, equipped with an Elite-5MS capillary column (28). The NIST 14 MS Library was used for compound identification.

Statistical analysis

All quantitative data are presented as mean ± SEM (n=3). Statistical comparisons were performed using GraphPad Prism, version 5.0.

Results and Discussion

Macroscopic morphology

It is an epiphytic orchid, was observed to grow to a height of 20–60 cm. It exhibited clustered pseudobulbs arising from a short rhizome. The pseudobulbs were cane-like, pendent, cylindrical, stout, longitudinally ridged, olive-green to yellowish-brown and measured 5-30 × 1-2.5 cm.

The upper portion of the pseudobulbs bore 1-4 distichous, scarcely ovate to elliptic, emerald green leaves (11.5-17 × 3.5-6.5 cm) with obtuse, leathery textures and sheathed bases. Pendulous inflorescences (10–27 cm long) emerged laterally from the apical part of defoliated stems, bearing 4-18 white flowers (approximately 1.5-2 cm across, expanding to 3.5 cm in diameter). The dorsal sepal was white, narrowly ovate and obtuse (2-2.3 × 1.7-1.8 cm). Lateral sepals were white, slightly oblique (2.3-2.8 × 1.7-1.8 cm). Petals were widely elliptic to suborbicular, shortly clawed at the base and as long as the dorsal sepal, with irregularly finely denticulate margins and rounded apices. Sepals and petals displayed a light purple-violet tint dorsally at the base. The lip was bright yellow with white edges, slightly triangular to suborbicular, briefly clawed (2.2-2.7 × 2-2.2 cm). The column and foot were yellow and the anther cap was white (Fig. 1).

Distribution: Native to the Himalayan mountains of India, Northeast, Laos, Myanmar, Burma and Thailand; rare in Vietnam and Assam.

Flowering and fruiting: March–May

Habitat: Epiphytic in semi-deciduous forests.

Status in Assam: Rare

Ecology/occurrence: Epiphytic in semi-deciduous forests. New shoots and leaves emerge during May-June, with senescence occurring in the second year of growth, specifically from June and a period of no growth observed from November–February. GPS coordinates: N26°46′21.3″, E90°18′29.4″.

Specimen examined: Assam: Kokrajhar, Ultrapani forest range, Collection no: 01, 13-03-2020, *Sangita Das*, herbarium information: submitted to CAL, herbarium accession no.: BUSD-01.

Ethnobotanical survey

Ethnobotanical data collected through interviews and questionnaires with Santhal community traditional healers revealed the use of *D. thyrsiflorum* leaves and pseudobulbs for various medicinal purposes (Table 2). Preparations involving *D. thyrsiflorum* with or without *Hibiscus rosa-sinensis* L. and *Shorea robusta* C F Gaertn. were used to treat menstrual problems, abnormal white discharge, otalgia, otorrhea and intestinal worms.

Fig. 1. *Dendrobium thyrsiflorum*. **a)** Habit and whole plant, **b)** Full grown inflorescence **c)** Emerald green leaves, **d)** Pseudobulb **e)** Immature inflorescence **f)** Whole flower **g)** Dissected flower **h. I)** Sepals; **h. II)** Dorsal sepal; **h. III)** Lateral sepal, **i. I)** Petals; **i. II)** Petals as long as dorsal sepal **j)** Lip.

Table 2. Ethnobotanical information collected from Santhal community traditional healers

Scientific name	Parts used	Other ingredient/ supplementary ingredients	Medicinal uses	Processing	Dosage	Herbalist	Preventive measure
Dendrobium thyrsiflorum B S Williams	Leaves and/or Pseudobulbs	*Hibiscus rosa-sinensis* L. flowers (20 %)	Tonic or tablets to treat menstrual problem viz. irregular period; proper blood flow and pre-menopausal issues related to uterine bleeding	Juiced or crushed and made into tablets	2 tablespoon/ 2 tablets per day	TH	Intake of egg, fish, meat or any other allergetic food items is prohibited during medication.
		Bark of *Shorea robusta* C.F.Gaertn. (10 g) and water (10 L)	Treats abnormal white discharge/ leukorrhea	Decoction	1 cup of tonic per day	TH	
		NR	Recovers otalgia	Juiced	2 drops per day	TH, BM	
		NR	Recovers otorrhea	Juiced	2 drops per day	TH	
		NR	Treat worms in intestine	Juiced	2 drops per day	BM	

Herbalists: Mr. Tibru Hembram (TH), Mr. Basu Murmu (BM)

Pharmacognostic analysis

Organoleptic evaluation

Organoleptic analysis of pulverized leaves and pseudobulbs revealed distinct characteristics. Leaves exhibited a granular texture, paddy smell, tastelessness and olive-green colour. Pseudobulbs displayed a spongy granular texture, sweet aroma, tastelessness and wheat-brown colour (Table 3).

Microscopic study

Microscopic analysis of the pulverized plant parts revealed distinct anatomical features in *D. thyrsiflorum*. Fibers were observed in both the leaves and pseudobulbs. Parenchyma cells and stomata were exclusively present in the leaves, while acicular crystals and spiral vessels were identified in the pseudobulbs (Fig. 2).

Parenchyma cells, fundamental to plant metabolism, are involved in various processes, including cellular repair, photosynthesis, gas exchange, energy storage and waste elimination (29, 30). Stomata facilitate gaseous exchange and fibers provide mechanical support within plant tissues (31, 16). Acicular crystals contribute to elasticity and offer support against crushing pressure (32, 33). Spiral vessels, the smallest components facilitating plant movement, play a crucial role in the development of reproductive organs (34, 35).

Table 3. Organoleptic parameters of pulverized plant parts

Parameters	Leaves of *D. thyrsiflorum*	Pseudobulbs of *D. thyrsiflorum*
Texture	Granular	Spongy granular
Aroma	Paddy smell	Sweet
Taste	Tasteless	Tasteless
Colour	Olive green	Wheat brown

Qualitative tests

Qualitative phytochemical screening of aqueous extracts from pulverized *D. thyrsiflorum* plant parts revealed distinct compositional differences between leaves and pseudobulbs (Table 4). Both leaves and pseudobulbs exhibited the presence of alkaloids, carbohydrates, glycosides, terpenoids and steroids. However, flavonoids, phenols, saponins, tannins and phlobatannins were detected exclusively in the leaves.

The observed phytochemical profile suggests potential therapeutic applications, aligning with established literature. Alkaloids, for instance, are known for their diverse pharmacological activities, including anti-allergic, anti-inflammatory, antimicrobial, anticonvulsant and anticancer effects (36–39). Carbohydrates, particularly reducing sugars, contribute to energy provision and may offer cardioprotective benefits (40–42). Flavonoids, found only in the leaves, are recognized for their antioxidant properties, mitigating oxidative damage (43–45). Glycosides exhibit a broad spectrum of therapeutic potential, including analgesic, anti-inflammatory, cardiotonic, antimicrobial, antiviral and anticancer activities (46). Phenols act as antioxidants and possess anti-inflammatory properties, potentially beneficial in conditions like inflammatory bowel disease, rheumatoid arthritis and skin disorders (47). Saponins have been associated with lipid-lowering, cholesterol-reducing and hypoglycemic effects, along with potential applications in hypercalciuria, lead poisoning prevention, dental caries reduction and antiplatelet aggregation (48). Tannins display a range of bioactivities, including anti-inflammatory, antiviral, antioxidant, antimicrobial and anticancer properties and are also implicated in anti-diabetic, anti-wrinkle, anti-cardiac and

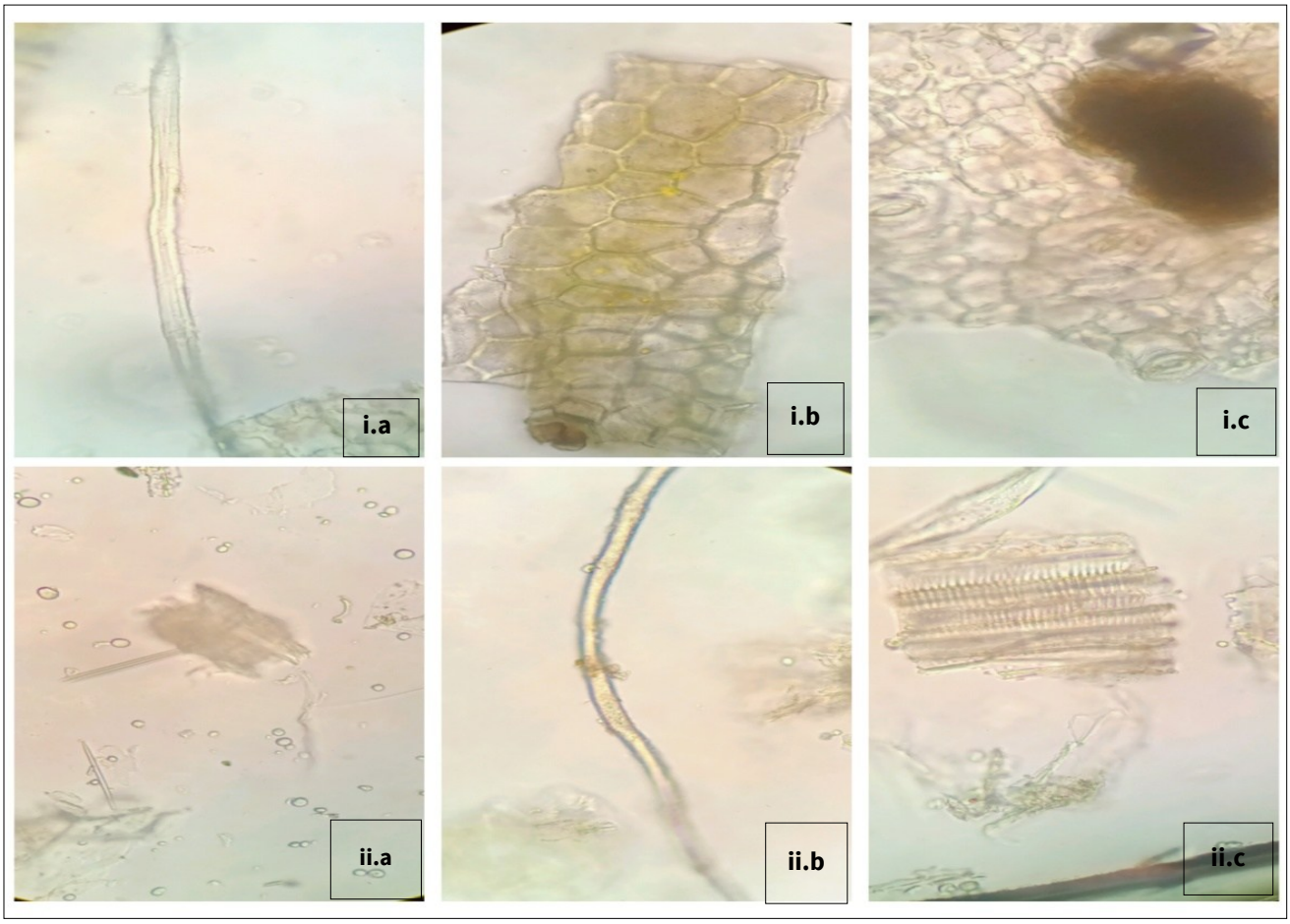

Fig. 2. Microscopy of powdered *D. thyrsiflorum*. (i) Leaves (40X): a) Fibre; b) Parenchyma; c) Stomata; (ii) Pseudobulbs (40X): a) Acicular crystal; b) Fibre; c) Spiral vessel.

Table 4. Phytochemical screening of leaves and pseudobulbs of *D. thyrsiflorum* aqueous extract (Qualitative tests)

Constituent	Chemical test	Leaves of *D. thyrsiflorum*	Pseudobulbs of *D. thyrsiflorum*
Alkaloids	Mayer's reagent test	+	+
	Wagner's reagent test	+	+
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
Flavonoids	Shinoda test	+	-
	Pew's test	+	-
	Alkaline reagent test	+	-
Glycosides	Keller-kiliani test	+	+
Phenols	Ellagic acid test	+	-
	Ferric chloride test	+	-
Saponins	Foam test	+	-
Tannins	Ferric chloride test	+	-
	Bromine water test	+	-
Terpenoids	Salkowski test	+	+
Steroids	Salkowski test	+	+
Phlobatannins	Hydrochloric acid test	+	-

"+" sign and "-" sign indicate the presence and absence of constituent in the sample respectively

anti-diarrheal applications (49). Terpenoids demonstrate anticancer, anti-inflammatory, antimicrobial, antiviral, antimalarial, immunomodulatory, neuroprotective and anti-allergic activities (50). Steroids, essential for various physiological processes, play critical roles in development, growth, energy metabolism, homeostasis and reproduction (51). Finally, phlobatannins, present only in the leaves, have been reported to possess diuretic properties (52).

Heavy metal analysis

Heavy metal analysis indicated the absence of lead, cadmium and bismuth in both leaves and pseudobulbs, suggesting the safety of these plant parts for medicinal use (Table 5).

Table 5. Heavy metal test for *D. thyrsiflorum* leaves and pseudobulbs aqueous extract

Experiment	Observation	Results
Test for Bismuth		
H_2S gas + sample solution	No dark brown precipitate	Bismuth absent
NH_4OH + sample solution	No white precipitate	Bismuth absent
Test for Cadmium		
NH_4OH + sample solution	No white precipitate	Cadmium absent
Potassium ferrocyanide + sample solution	No white precipitate	Cadmium absent
Test for Lead		
Dilute HCl + sample solution	No white precipitate	Lead absent
KI + sample solution.	No yellow precipitate	Lead absent

Quantitative tests

Determination of total polyphenol content (TPC)

Polyphenolic compounds are recognized contributors to antioxidative activity. The TPC of *D. thyrsiflorum* leaves and pseudobulbs was determined to be 27.62 ± 0.03 mg gallic acid equivalents (GAE) per gram dry weight (mg/g) and 17.57 ± 0.02 mg GAE/g dry weight, respectively.

Research on *D. thyrsiflorum* is rare, but several studies have been reported on *Dendrobium* species. For instance, an ethanol extracts of *D. crepidatum* exhibited a TPC of 78.11 ± 0.72 µg GAE/mg extract (53), another study revealed that a TPC of 54.47 ± 0.12 mg GAE/g DW was found in the methanolic stem extract of *in vitro*-raised *D. nobile*, while only 3.25 ± 0.20 mg GAE/g DW was found in the chloroform leaf extract of the same species, highlighting the impact of solvent and plant part on phenolic yield (54).

Determination of total flavonoid content (TFC)

The TFC of aqueous extracts from *D. thyrsiflorum* leaves and pseudobulbs was measured as 120.36 ± 0.05 mg QE per gram dry weight (mg/g) and 87.50 ± 0.04 mg QE/g dry weight, respectively.

Compared to other studies, direct comparative study was rare, however, examining a few *Dendrobium* species reveals that significant amounts of flavonoids were found in *D. crepidatum* extracts, with the hexane fraction having TFC of 82.62 ± 1.13 µg QE/mg (53). Another study found that the TFC of *D. heterocarpum* tissue-culture regenerates was 38.38 mg QE per gram dry weight (mg QE/g DW), whereas young leaves contained 27.27 mg QE/g DW (55).

Determination of antioxidant activity via DPPH scavenging assay

The antioxidant activity of *D. thyrsiflorum* leaves and pseudobulbs aqueous extracts were evaluated using the DPPH radical scavenging assay, providing an assessment of reducing power and total antioxidant capacity. The extracts demonstrated significant antioxidant effectiveness when compared to ascorbic acid, the reference standard. The DPPH assay is based on the principle that antioxidants can reduce the stable free radical DPPH, resulting in a loss of its characteristic deep purple colour and a corresponding decrease in absorbance at 540 nm. This change in absorbance is quantitatively proportional to the antioxidant capacity. The comparative antioxidant activity of the extracts and ascorbic acid across varying concentrations, demonstrating a gradual increase in activity with increasing concentration (Fig. 3). The half-maximal inhibition concentrations (IC50) for *D. thyrsiflorum* leaves and pseudobulbs were determined to be 10.22 and 6.98, respectively.

In other studies, it was found that, at 800 µg/mL, the ethanol stem-extract of *D. crepidatum* scavenged DPPH free radicals with approximately 94.69 % activity, with an IC_{50} value of approximately 73.90 µg/mL (53).

FRAP assay

The reducing capabilities of the *D. thyrsiflorum* leaf and pseudobulb extracts compared to ascorbic acid standard (Fig. 4). The reducing power, which is measured by the conversion of Fe^{3+} to Fe^{2+}, increased with rising extract concentration for both leaf and pseudobulb

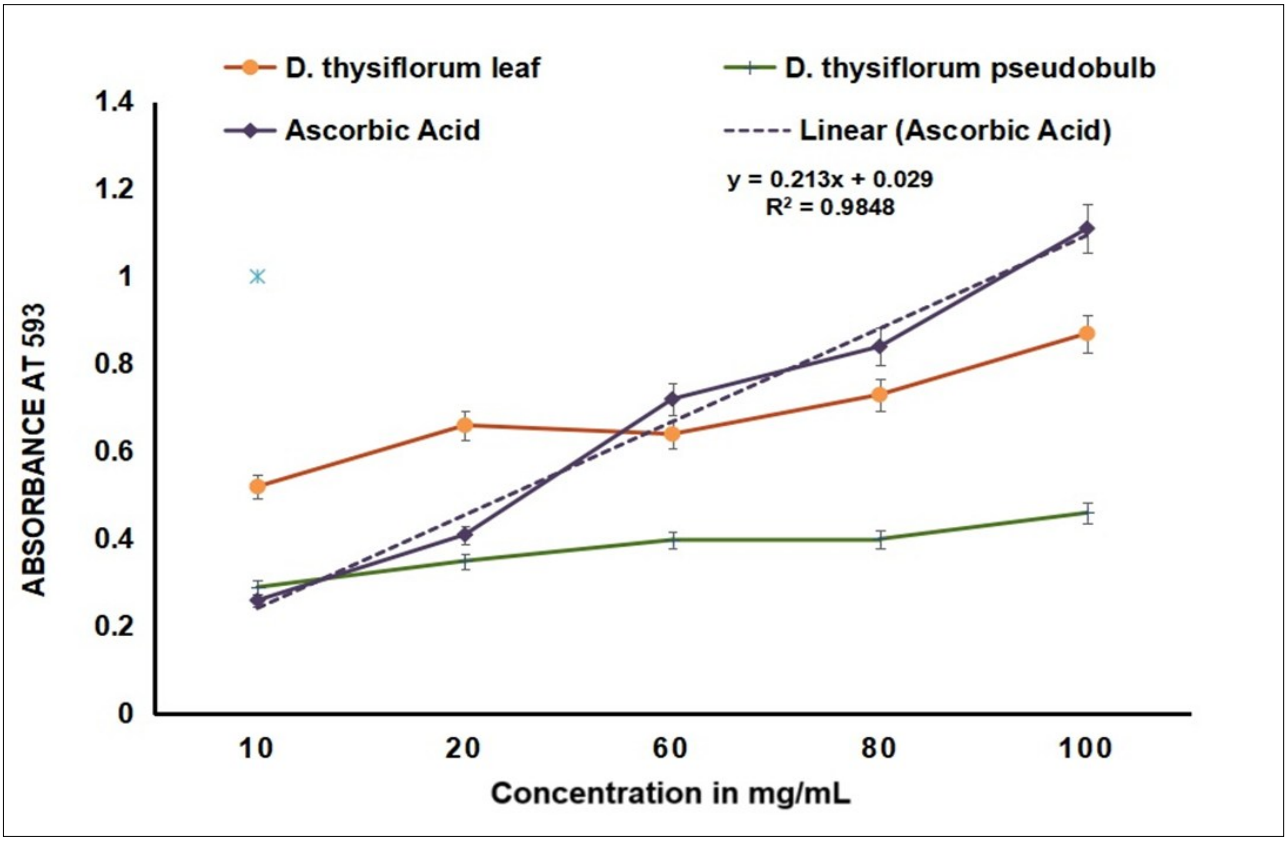

Fig. 3. DPPH Scavenging activity (n=3; mean±SE) of the leaves and pseudobulbs of *D. thyrsiflorum*.

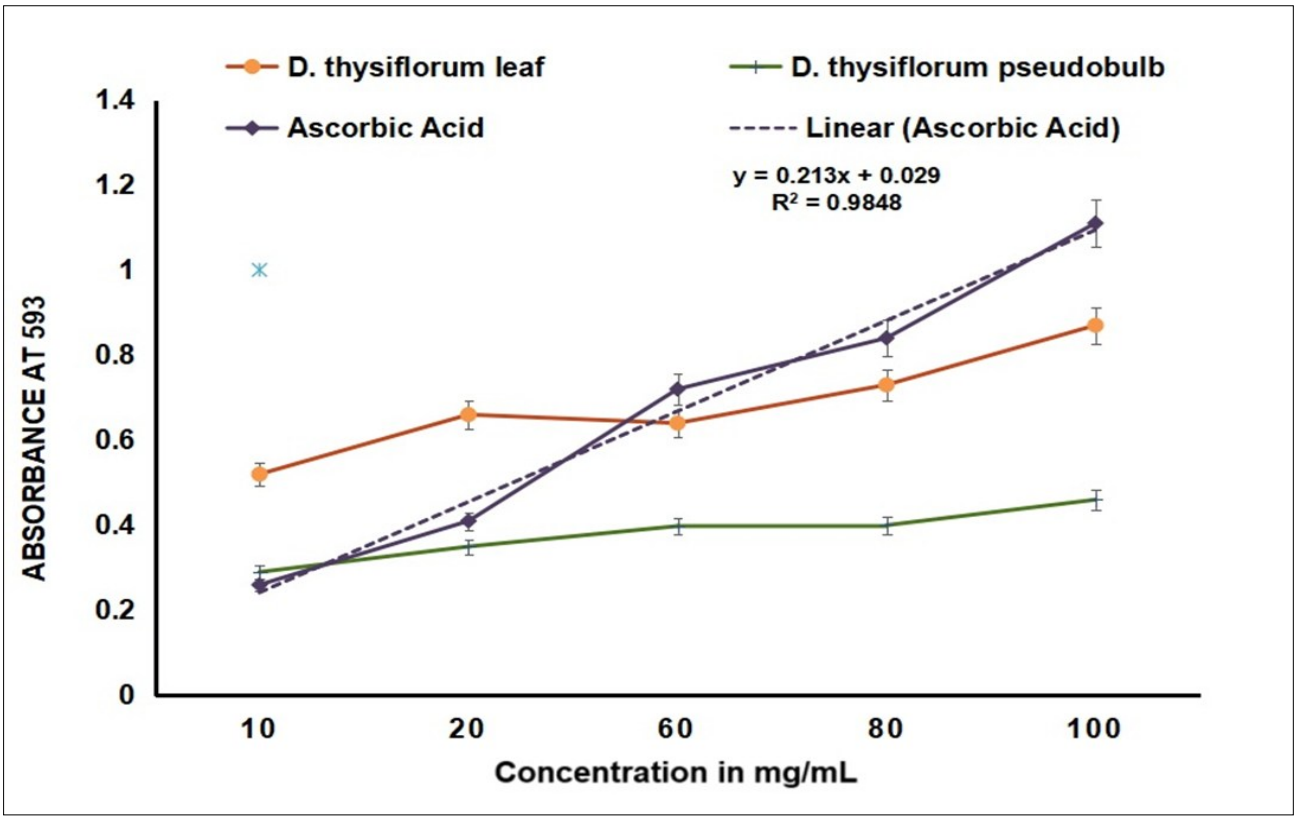

Fig. 4. Ferric reducing-antioxidant power (FRAP) assay (n=3; mean±SE) of the aqueous extract of the leaves and pseudobulbs of *D. thyrsiflorum* in comparison with a standard.

samples.

Specifically, the leaf extract exhibited a higher reducing power than the ascorbic acid standard at lower concentration (10 mg/mL and 20 mg/mL). However, at 60 µg/mL, the reducing power of the leaf extract plateaued or decreased relative to ascorbic acid. Conversely, the pseudobulb extract exhibited consistently lower reducing power than ascorbic acid across all the tested concentrations.

Regarding other species, earlier research on the ethanolic stem, leaf and flower extracts of *D. sulcatum* showed FRAP value of 0.50 ± 0.04 mmol Fe^{2+}/g extract, 0.35 ± 0.05 and 0.12 ± 0.02, respectively (56).

GC-MS/MS profiling

GC-MS analysis was performed on the leaves and pseudobulbs of *D. thyrsiflorum*. The resulting chromatogram is presented in Fig. 5 and Fig. 6 respectively and the identified 88 and 59 phytocompounds of each sample are summarized in Table 6 and Table 7 respectively.

An ethnobotanical survey among the Santhal community revealed the traditional use of *D. thyrsiflorum* leaves and pseudobulbs for treating various ailments. To validate these traditional claims, a scientific investigation was conducted involving qualitative and quantitative phytochemical analyses. Qualitative tests confirmed the presence of diverse compounds in both pulverized plant parts. Subsequently, GC-MS analysis identified 88 compounds in the leaves and 59 in the pseudobulbs. A comparative analysis was performed, correlating the identified compounds with existing literature. Secondary sources reported that several identified compounds, including hexylene glycol, cyclotrisiloxane (hexamethyl-), 1H-indole, 2-methoxy-4-vinyl-phenol, benzaldehyde (3-hydroxy-4-methoxy-), phenol (3,4-dimethoxy-), 2,4-Di-tert-butylphenol, n-hexadecanoic acid, scoparone, hexadecanoic acid (2-hydroxy-1-(hydroxymethyl)ethyl ester), acetic acid, 2(5H)-furanone, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone and phenol (2-methoxy-), possess anti-infectious properties, including antimicrobial, antibacterial, antiviral, anti-inflammatory and antifungal activities (58, 59, 61–63, 65–72, 74–76, 78, 79, 85–88). The presence of these compounds in *D. thyrsiflorum* leaves and pseudobulbs may explain the plant's efficacy in treating ear ailments, such as otalgia and otorrhea, as well as obesity, as reported by the Santhal traditional healers.

Furthermore, cyclotrisiloxane (hexamethyl-), benzaldehyde (3-hydroxy-4-methoxy-), 2,4-Di-tert-butylphenol, 4-ethenyl-2,6-dimethoxy-phenol, n-Hexadecanoic acid, scoparone, hexadecanoic acid (2-hydroxy-1-(hydroxymethyl)ethyl ester), phenol (2-methoxy-), 2,4-Di-tert-butylphenol and hexadecanoic acid (methyl ester) have been documented to possess antioxidant activities (60, 62, 63, 67–69, 71–76, 78, 88, 89). Consequently, the presence of these compounds in the extracts may contribute to the observed DPPH radical scavenging activity.

Conclusion

Our investigation has documented the traditional use of *D. thyrsiflorum* by Santhal healers residing within the Ultapani forest range for the treatment of diverse ailments. To establish a foundation for its medicinal applications, a comprehensive pharmacognostic analysis of the leaves and pseudobulbs of *D. thyrsiflorum* was conducted, encompassing macroscopic, microscopic, organoleptic, phytochemical and heavy metal assessments. The leaves and pseudobulbs exhibited significant antioxidant activity, as evidenced by their ability to scavenge DPPH radicals and reduce ferric ions, comparable to standard antioxidants (ascorbic acid, quercetin and gallic acid). Furthermore, substantial quantities of total polyphenols and flavonoids, known for their antioxidant properties, were detected. While many potential antioxidant and therapeutic compounds were identified, further isolation, purification and targeted bioassays are required to pinpoint the specific molecules responsible for the observed effects. GC-MS profiling revealed the presence of 88 and 59 compounds in the leaves and pseudobulbs respectively, suggesting a rich source of natural antioxidants. Importantly, the absence of detectable heavy metals further supports the potential therapeutic utility of this

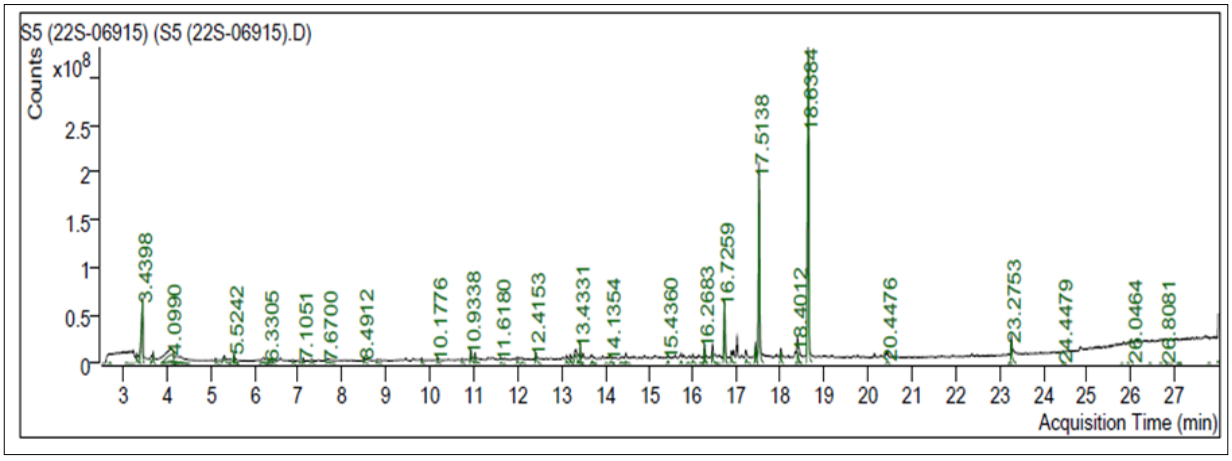

Fig. 5. Gas chromatography-mass spectroscopy (GC-MS) analysis of leaves of *Dendrobium thyrsiflorum* B. S. Williams.

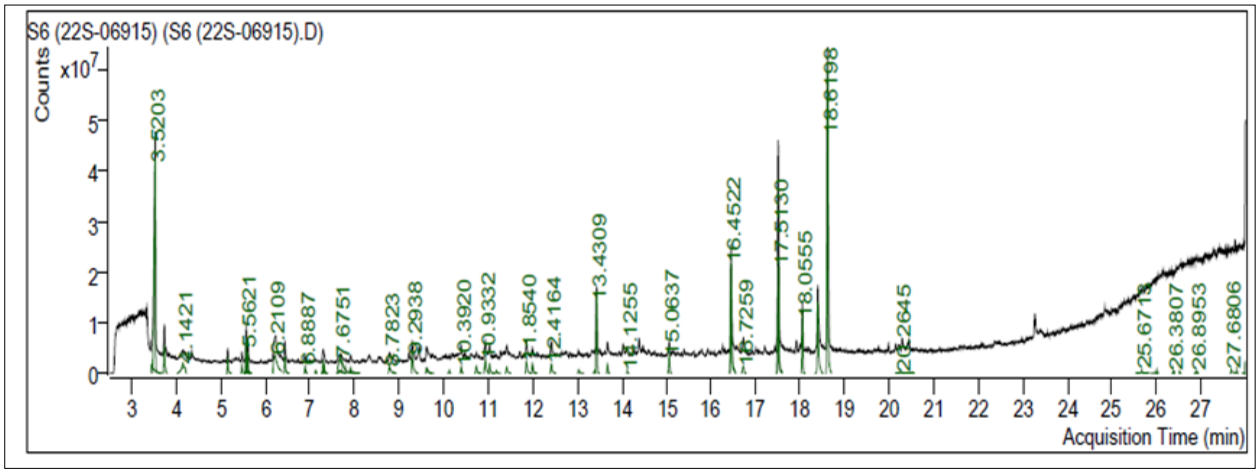

Fig. 6. Gas chromatography-mass spectroscopy (GC-MS) analysis of pseudobulbs of *D. thyrsiflorum* B. S. Williams.

Table 6. Results of GC-MS for Leaves of *Dendrobium thyrsiflorum* B. S. Williams

Component RT	Compound name	Formula	Area	Medicinal value	References
2.6887	2-(ethylthio)-5,6,7,8-tetrahydro- (1) benzothiolo(2,3-d)(1,3) oxazin-4-one	$C_{12}H_{13}NO_2S_2$	685475	NR	
3.0673	trans-(carveylbenzyl) oxide	$C_{17}H_{22}O$	1734248	NR	
3.3060	Methylamine, N, N-dimethyl-	C_3H_9N	11359790	NR	
3.4398	Methyl formate	$C_2H_4O_2$	149700260	NR	
3.6365	methyl-(3R)-(-)-3-ethyl-5-oxopentanoate	$C_8H_{14}O_3$	3152372	NR	
3.6805	Propanoic acid	$C_3H_6O_2$	13476842	1. Lowers fatty acids content in liver and plasma 2. Reduces food intake 3. Exerts immunosuppressive actions 4. Improves tissue insulin sensitivity	(57)
4.0990	6-oxabicyclo (3.1.0) hexane-2,4-diol	$C_5H_8O_3$	102422183	NR	
4.0994	Butanoyl(tert-butyldimethyl) silane	$C_{10}H_{22}OSi$	25082251	NR	
4.2100	1,1-dimethyl-4-tert-butylsemicarbazide	$C_7H_{17}N_3O$	9008720	NR	
4.2201	((1R,4S,5R)-5-((E)-5-(tertButyldimethylsilyloxy)-3- methyl-3-pentenyl)-1- methyl-4-(2- methyl (1,3) dioxolan-2-yl)-2- cyclopentenyl) methanol	$C_{23}H_{42}O_4Si$	6323813	NR	
4.2766	(1R,2R,9R,10R,13S) -(E)-7-Benzenesulfonyl1,5,9- trimethyl-13-(2-methyl (1,3) dioxolan-2-yl)-4- oxatricyclo (10.3.0.0(3,5)) pentadeca-8,14- diene	$C_{27}H_{36}O_5S$	3482635	NR	
4.3010	Propanediamide, 2-amino-	$C_3H_7N_3O2$	8819330	NR	
5.1045	(1R,2R)-1-Methanol-2-acetonitrile-4-cyclohexene	$C_9H_{13}NO$	3552669	NR	
5.3039	2-Pentenoic acid, 4-methyl-	$C_6H_{10}O_2$	19464380	NR	
5.4315	Methyl 3-hydroxy-2-(ptoluenesulfonyloxy) methyl-10-tetrahydropyranyloxydecanoate	$C_{24}H_{38}O_8S$	1354302	NR	
5.5242	Hexylene glycol	$C_6H_{14}O_2$	11737899	1. Used as topical corticosteroids 2. Has antimicrobial properties	(58, 59)
5.5242	2-Methyl-2,4-pentanediol	$C_6H_{14}O_2$	11741559	NR	
5.5656	(Thiomethyl)(4'-nitrophenyl) ((N-methyl) bromopyrrolidinyl)isothioacylamidrazone	$C_{14}H_{19}BrN_4O_2S$	1680393	NR	
6.1692	Propanedioic-1,3-13C2 acid	$C_3H_4O_4$	5690219	NR	
6.2051	(4R,5R)-3-Methylene-4-vinyl-5-n-pentyl-gamma-butyrolactone	$C_{12}H_{18}O_2$	4514457	NR	
6.3305	2-Butenoic acid, 2,3-dimethyl-	$C_6H_{10}O_2$	5284152	NR	
6.3312	Ethane-1,1-d2, 2,2,2-trifluoro-	$C_2HD_2F_3$	880713	NR	
6.4058	Propane, 2-methyl-	C_4H_{10}	3655378	NR	
6.8701	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	$C_6H_8O_2$	1805620	NR	
7.0311	1-Phenylpropane-1,2-diol	$C_9H_{12}O_2$	1574299	NR	
7.1051	3-hydroxy-4,4-dimethyldihydro (2-13C) furan-2-one	$C_6H_{10}O_3$	3732451	NR	
7.3011	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	3683998	1. It has antioxidant, antibacterial, antidiabetic and antimicrobial activities 2. It is essential for scavenging free radicals	(60–64)
7.6700	(+)-3(5)-{(methyl (3'- pinanylmethyl) amino) methyl} pyrazole	$C_{16}H_{27}N_3$	6658720	NR	
8.4912	L-Homoserine lactone, N, N-dimethyl-	$C_6H_{11}NO_2$	8516387	NR	
8.7855	2-Hydroxy-1-phenyl-3-(phenylsulfonyl) propan1-one	$C_{15}H_{14}O_4S$	3134002	NR	
9.8129	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	$C_7H_9NO_2$	3789491	NR	
10.1776	(5R,6R)-6-((3aR,5S,6aS)-2,3,3a,4,5,6ahexahydrofuro(2,3-b)furan-5-yl)-5,6-dimethyl1-cyclohex-2-enone	$C_{14}H_{20}O_3$	4464988	NR	

10.7326	1H-Indole	C_8H_7N	4331171	1. It help to maintain the human intestine's biological barrier 2. Has anti-inflammatory activities 3. Used to treat inflammatory bowel disease, hemorrhagic colitis and colorectal cancer 4. Used to treat diabetes mellitus, inflammation in the central nervous system and vascular regulation	(65)
10.9338	2-methoxy-4-vinyl-phenol	$C_9H_{10}O_2$	20375623	1. Possess anti-inflammatory qualities 2. Has anti-cancer qualities in relation to pancreatic cancer cell lines, Panc-1 as well as SNU-213	(66)
11.0269	1,3-Benzodioxol-5-ol	$C_7H_6O_3$	17865555	NR	
11.6180	Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	$C_{10}H_{12}O_2$	2029140	NR	
11.9943	2H-1-Benzopyran-2-one, 3,4-dihydro-	$C_9H_8O_2$	5864258	NR	
12.1088	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$	2980149	1. They act as plant metabolite 2. Used as an anti-inflammatory, antioxidant and anticonvulsant agent	(67–69)
12.4153	Phenol, 3,4-dimethoxy-	$C_8H_{10}O_3$	17328838	1. It exhibits induction of apoptosis for cancer chemoprevention, cardio protection 2. Photoprotection of the skin 3. It has anti-inflammatory and anti-atherogenic activity	(70)
13.1197	N-(3,4-dimethoxyphenyl) hydroxylamine	$C_8H_{11}NO_3$	5387306	NR	
13.2096	3-Hydroxy-4-methoxyacetophenone	$C_9H_{10}O_3$	7882834	NR	
13.3290	beta-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	38533797	NR	
13.3770	(3R*,5S*,6R*)-3-Isopropyl-5-mesitylamino-6-methyl-6-phenyl-1,2,4-trioxane	$C_{22}H_{29}NO_3$	3348702	NR	
13.4331	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	22999644	1. It has anti-inflammatory, antioxidant and antifungal properties 2. Neurodegenerative ailments are treated using it as a neuroprotective substance	(71, 72)
13.7014	1,3,3a,4,5,6,7,8,9,9a-Decahydro-4,9-epoxybenzo(f)isobenzouran-1,3-dione	$C_{12}H_{12}O_4$	1790109	NR	
13.7066	2-methyl-1-(1,3,5-trimethyl-4-pyrazolyl)-1-propanone	$C_{10}H_{16}N_2O$	4545777	NR	
14.0282	5,6,7,8-Tetrahydro-7,7-dimethyl-6,8-methanoisoquinolin-3-amine	$C_{12}H_{16}N_2$	892878	NR	
14.1354	4-ethenyl-2,6-dimethoxy-phenol	$C_{10}H_{12}O_3$	4874989	1. It has antioxidative and antimutagenic properties	(73)
14.1815	(1E)-2-Methylcyclohexanone oxime	$C_7H_{13}NO$	1237260	NR	
14.3622	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6 atrimethyloxireno (g) benzofuran-5-yl)-	$C_{13}H_{18}O_3$	2284944	NR	
14.4253	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6 atrimethyloxireno (g) benzofuran-5-yl)-	$C_{13}H_{18}O_3$	1420409	NR	
14.4776	Ethyl 2-(1-(N-Ethoxycarbonylamino)-4-tbutylcyclohexyl) prop-2-enoate isomer	$C_{18}H_{31}NO_4$	4756548	NR	
15.4360	2-Cyclohexen-1-one, 3,5,5-trimethyl-4- (3-oxobutyl)-	$C_{13}H_{20}O_2$	4495369	NR	
15.7331	trans-4,5-dihydro-4,4-dimethyl-2- (6-methyl-5-(phenylmethyl)- 1,3-cyclohexadien-1-yl) oxazole	$C_{19}H_{23}NO$	4247809	NR	
15.8967	(E)-3-(7,7-difluoro-5-bicyclo(4.1.0) hepta-1,3,5-trienyl)-2-propenoic acid ethyl ester	$C_{12}H_{10}F_2O_2$	1835194	NR	
16.0034	2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-	$C_{10}H_{12}O_4$	6822476	NR	
16.1778	1H-Cyclopenta(1,3) cyclopropa(1,2) benzen 6 (7H)-one, 3a-(acetyloxy) hexahydro-3b methyl-, (3a. alpha.,3b. alpha.,7aR*)-	$C_{13}H_{18}O_3$	1533535	NR	
16.2683	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7 atetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	30232075	NR	
16.2711	1-Oxaspiro (5.5) undecan-8-one, (.+-.)-	$C_{10}H_{16}O_2$	10094768	NR	
16.4517	8-Methoxytricyclo (6.2.1.0(1,5)) undec-5-en-7-one	$C_{12}H_{16}O_2$	22279273	NR	
16.5366	2-(4-Nitrophenyl)-2-(4-methylphenoxy)-3,3-	$C_{18}H_{19}NO_4$	931654	NR	
16.7259	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl 4 -(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	105272345	NR	
16.8842	(E)-2-acetyl-3-octenoic acid methyl ester	$C_{11}H_{18}O_3$	13132926	NR	
17.2217	3-(3,4-Dimethoxyphenyl) propane-1,2-diol	$C_{11}H_{16}O_4$	9184606	NR	
17.4325	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienyl ester	$C_{17}H_{30}O_4$	25644430	NR	
17.5138	(1,3) dioxolo(4,5-g) chromen-6-one	$C_{10}H_6O_4$	374812281	NR	
18.0131	7-Oxabicyclo (4.1.0) heptan-3-ol, 6-(3-hydroxy1-butenyl)-1,5,5-trimethyl	$C_{13}H_{22}O_3$	20447079	NR	

18.4012	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	27694138	1. It has antioxidant and antibacterial properties	(74)
18.4285	1,1-dipropyl-1-germacyclohexan-4-one	$C_{11}H_{22}GeO$	4110577	NR	
18.6384	Scoparone	$C_{11}H_{10}O_4$	667413188	1. It is a potent and useful ingredient in the herbal treatment Artemisia capillaris Thunb, which has been used to treat cholestasis, jaundice and hepatic dysfunction 2. It has anti-inflammatory, antioxidant, anti-apoptotic, anti-fibrotic activities and hypolipidemic effects 3. Used to treat cholestatic liver disease (CLD), a broad category of hepatobiliary disorders with varied aetiologies that share common characteristics such as periductal fibrosis, ductal reactivity and inflammatory response 4. Used to treat erectile dysfunction (ED)	(75–77)
20.4476	5-Iodo-2-methylbenzofuran	C_9H_7IO	4628379	NR	
23.2251	2-Acetonyl-3-cyano-2,3-dimethylcyclobutane-1- carboxylic acid	$C_{11}H_{15}NO_3$	879412	NR	
23.2753	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	48315450	1. Has antioxidant, anti-inflammatory and anthelmintic properties	(78)
24.4479	cis-6a,7a,8,9,10,11a,11b-Octahydro-4-methoxybenzo(4,5) furo (2,3-c)(1) benzopyran-6- one	$C_{16}H_{16}O_4$	1765024	NR	
24.4481	4-Methyl-9-phenyl-1-nitro-3,8,10-triaza-7-oxatricyclo(4.3.1.0(4,10)dodeca-2,8-diene	$C_{13}H_{12}N_4O_3$	1449610	NR	
25.7993	2H-Pyran-6-carboxylic acid, 3-((4-(4-heptylcyclohexyl) benzoyl) oxy)-3,4-dihydro-2-methyl-4-oxo-, methyl ester, (2S-(2.alpha.,3.beta.(trans)))-	$C_{28}H_{38}O_6$	263173	NR	
25.9210	3-(p-Ethoxyphenyl)-4-(5-(o-chlorophenyl)-.delta.(2)-1,2,4-oxadiazol-3-yl) sydnone	$C_{18}H_{13}ClN_4O_4$	756472	NR	
26.0464	2-(4-Bromophenyl)-5,6-dihydropyrazolo (5,1- a) isoquinoline	$C_{17}H_{13}BrN_2$	465197	NR	
26.4389	tri(propan-2-yl)-((4-(2-tri (propan-2- yl) silylethynyl) phenyl) methoxy) silane	$C_{27}H_{48}OSi_2$	568402	NR	
26.6966	2,8-Bis(dimethylamino)-4,6-bis(propylamino)-5- phenyl-5H-thiopyrano(2,3-d:6,5-d') dipyrimidine	$C_{25}H_{34}N_8S$	727727	NR	
26.8081	3-Methyl-5(Z)-(E)-(3-phenylprop-2-ynylidene)-4-(prop-1-en-2-yl-)-5,6-dihydropyran-2-one	$C_{18}H_{16}O_2$	473464	NR	
26.8489	4-Formyl-3-(p-nitrophenyl) sydnone	$C_9H_5N_3O_5$	843677	NR	
27.0983	2-(2'-Nitro-1-(4"-methoxyphenyl) ethyl)-benzofuran	$C_{17}H_{15}NO_4$	761705	NR	
27.1005	N-Methyl-4'-methoxy benzene sulfena NRide-d5	$C_{14}H_{10}D_5NOS$	892805	NR	
27.1456	5-(di(propan-2-yloxy) phosphoryl-(methylthio)methyl)-2,4-diphenylthiazole	$C_{23}H_{28}NO_3PS_2$	411226	NR	
27.7938	Ethyl 3-methyl-4-triphenylphosphoranylidene4,5-dihydropyrazol-5-one-1-carboxylate	$C_{25}H_{23}N_2O_3P$	742480	NR	
27.9895	3-(N-(2-Hydroxycarbonylmethyl)-carboxamide)- 4-methyl-9H-carbazole	$C_{16}H_{14}N_2O_3$	524240	NR	
27.9895	(E)-beta-(2-Hydroxyphenyl ethylene) benzeneethanol-D2	$C_{15}H_{12}D_2O_2$	666738	NR	

*NR= Not Reported

Table 7. Results of GC-MS for pseudobulbs of *Dendrobium thyrsiflorum* B S Williams

Component RT	Compound name	Formula	Area	Medicinal value	References
3.4407	Formamide, N-(2-methylpropyl)-	$C_5H_{11}NO$	7168491	NR	
3.5203	Acetic acid	$C_2H_4O_2$	84691845	1. Used to treat cancer, diabetes, obesity and cardiovascular diseases 2. Used to treat microbial infections 3. Used to treat hypertension, hyperlipidemia 4. Enhance glycemic control	(79-84)
3.7251	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	8487824	NR	
4.1421	6-oxabicyclo (3.1.0) hexane-2,4-diol	$C_5H_8O_3$	9587146	NR	
5.1476	6-Methylhexahydrocycloprop(a)pentalen-3a,6-diol	$C_{10}H_{16}O_2$	4182521	NR	
5.4677	2(5H)-Furanone	$C_4H_4O_2$	2617535	1. Has the potential to stop different harmful bacteria from forming biofilms	(85, 86)
5.5621	2-Methyl-2,4-pentanediol	$C_6H_{14}O_2$	7146689	NR	
5.5649	Hexane, 2,3-dimethyl-	C_8H_{18}	2678126	NR	
5.5650	Acetic acid, mercapto-, 3-methylbutyl ester	$C_7H_{14}O_2S$	2525333	NR	
5.6013	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	6466340	NR	
6.2109	Methane, nitro-	CH_3NO_2	22037565	NR	
6.4311	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	5638605	NR	
6.8887	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	$C_6H_8O_2$	2169166	NR	
7.1240	4,4-Dideuteriomethoxy cyclohexane	$C_7H_{12}D_2O$	929875	NR	
7.2902	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	4077703	1. Has antimicrobial properties 2. Possess the capacity to act as an anti-infective agent against microbial infections in humans	(87)
7.3106	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	2956558	1. It has antioxidant, antibacterial, antidiabetic and antimicrobial activities 2. It is ssential for scavenging free radicals	(60-64)
7.3123	2-chloranyl-7-methoxy-3-methyl-quinoline	$C_{11}H_{10}ClNO$	2412151	NR	
7.6598	Benzene, ((fluorophenylmethyl)sulfinyl)-	$C_{13}H_{11}FOS$	3444637	NR	
7.6751	Phenol, 2-methoxy-	$C_7H_8O_2$	24525290	1. Possesses the capacity to treat cardiovascular, metabolic and cancerous disorders 2. These compounds assist avoid vascular issues because of their antioxidant and anti-inflammatory properties. 3. Improve general health	(88)

7.7072	Cyclopentadeca-1,2-diene	$C_{15}H_{26}$	2245463	NR	
7.9062	1,3-Propanediamine	$C_3H_{10}N_2$	2118238	NR	
8.7823	O-(1-Carboxyethyl) benzaldoxime	$C_{10}H_{11}NO_3$	4487880	NR	
9.2938	Catechol	C6H6O2	9569857	NR	
9.6144	2-(N-ethyl) imino-3-pentanone	C7H13NO	3211743	NR	
9.6193	4,7-Ethanoisobenzofuran-1,3-dione, 3a,4,7,7atetrahydro-4-methyl-7-(1-methylethyl) -, (3a.alpha.,4.alpha.,7.beta.,7a.alpha.)-	C14H18O3	2788478	NR	
10.1285	1,4-Cyclohexadiene, 1,3,3-trimethyl-6-(2,4,4-trimethyl-2,5-cyclohexadien-1-ylidene)-	C18H24	1052527	NR	
10.3920	2-(Deuteriomethylene)bornane	C11H16D2	2262264	NR	
10.7280	m-Aminophenylacetylene	C8H7N	3200991	NR	
10.9332	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	C9H10O2	6531238	NR	
11.0283	4-Ethyl-5-methoxypyridazine	C7H10N2O	3517340	NR	
11.1806	1-hydroxy-4,4-dimethyl-3-phenyl-2-pentanone	C13H18O2	1071920	NR	
11.4129	2-methoxy-3-methyl-1,4-benzenediol	C8H10O3	2799822	NR	
11.8540	1-(Acetoxymethyl)-3-isopropyl-2,2-	C12H22O2	5554845	NR	
11.9914	3,4-Dihydro-1H-isochromen-1-one	C9H8O2	4065648	NR	
12.4164	1-(5-fluoranyl-2-oxidanyl-phenyl) ethanone	C8H7FO2	4420441	NR	
13.0322	8-fluoranyl-3-methyl-isochromen-1-one	C10H7FO2	1461593	NR	
13.3868	(3R*,5S*,6R*)-3-Isopropyl-5-mesitylamino-6-methyl-6-phenyl-1,2,4-trioxane	C22H29NO3	919705	NR	
13.4309	2,4-Di-tert-butylphenol	C14H22O	21829246	1. It has anti-inflammatory, antioxidant and antifungal properties 2. Neurodegenerative ailments are treated using it as a neuroprotective substance	(71, 72)
13.6787	Benzoic acid, 4-ethoxy-, ethyl ester	C11H14O3	2752578	NR	
14.1255	2-Cyclohexen-1-ol, 2-(phenylethynyl)-, acetate	C16H16O2	700660	NR	
15.0637	1(2H)-Naphthalenone, 3,4-dihydro-6-methoxy-2,2-dimethyl	C13H16O2	5659907	NR	
16.4522	2H-1-benzopyran-2-one, 3,4-dihydro-6- hydroxy -5,7-dimethyl	C11H12O3	42803690	NR	
16.4525	Isopropyl N,P-diisopropylphosphonamidate	C9H22NO2P	28528457	NR	
16.7259	(4E)-6-Methylhepta-4,6-dien-2-one	C8H12O	3473638	NR	
17.5041	1,3-Dimethyl-2-oxo-1,2-dihydro-5. lambda.(5)-pyrido(1,2-a) pyrimidin-5-ylium-4-olate	C10H10N2O2	27022210	NR	
17.5130	(+)-Malbranicin	C11H12O4	38652771	NR	
18.0555	Hexadecanoic acid, methyl ester	C17H34O2	16408615	1. Has antioxidant and anticancer activities 2. Has the potential to treat human colon cancer cells	(89)
18.3984	n-Hexadecanoic acid	C16H32O2	29153153	1. Has antioxidant and antibacterial properties	(74)
18.6198	Scoparone	C11H10O4	104989115	1. It is a potent and useful ingredient in the herbal treatment Artemisia capillaris Thunb, which has been used to treat cholestasis, jaundice and hepatic dysfunction 2. It has anti-inflammatory, antioxidant, anti-apoptotic, anti-fibrotic activities and hypolipidemic effects 3. Used to treat cholestatic liver disease (CLD), a broad category of hepatobiliary disorders with varied aetiologies that share common characteristics such as periductal fibrosis, ductal reactivity and inflammatory response 4. Used to treat erectile dysfunction (ED)	(75-77)
20.2645	syn-(Ra*,1'S*)-N,N-Diethyl-2-(1'-ethoxybut-3'-enyl)-1-naphthamide	C21H27NO2	3643238		
25.6713	i-Propyl-(1,3-diphenylprop-2-ynyl)ether	C18H18O	2232809		
25.9768	6-{((9'- Anthracenyl) methyl) (ethyl) aminomethyl}-2- pyridinamine	C23H23N3	535801		
26.0239	3,4-Dimethyl-11-(3,4-di(carbomethoxy)-5-(cyclohexylimino)dihydrofuran-2- yl) indenoquinoxaline	C30H29N3O5	367496		
26.3807	1-methyl-3,4-bis(trifluoromethylthio)pyrazole	C6H4F6N2S2	624033		
26.5163	(2Z)-2-((6E,7E)-1-keto-6-(2-keto-2-methoxyethylidene)-7-trimethylsilyl-3,4-dihydro-2H-2- benzazecin-5-ylidene) acetic acid methyl ester	C22H27NO5Si	282399		
26.8953	4,12,20-Trideoxyphorbol-13-(2',3'-dimethyl)-butyrate	C26H38O4	865622		
27.6806	5,11,13,14- Tetracarbomethoxytetracyclo (7.3.2.0(2,8).0(10, 11)) tetradeca-2(8),3,6,13-tetraene	C22H22O8	1562368		
27.8083	2-ethyl-3-ferrocenyl-N-phenylpyrrole	C22H21FeN	1029494		
27.9891	(E)-Ethyl 2-benzyl-3-(4'-phenylphenyl) but-2-enoate	C25H24O2	685251		

*NR= Not reported

species. Notably, several compounds identified through GC-MS have been previously reported to possess activities relevant to the treatment of various disorders, including diabetes, cardiovascular diseases, cancer, cholestatic liver disease, obesity and erectile dysfunction. We propose that further investigation into the untapped therapeutic potential of *D. thyrsiflorum* is warranted, aiming to facilitate the development and discovery of valuable herbal products.

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Authors' contributions

AKG conceptualized and designed the study. SD, SB, AKG carried out the research work and acquired the data. SD and AKG analyzed the data and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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