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# IN-VITRO ANTI- DIABETIC POTENTIAL AND PHYTOCHEMICAL SCREENING OF *DENDROBIUM MACRAEI* LINDL.

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**ABSTRACT**

The rising global prevalence of diabetes mellitus has intensified the search for alternative and natural antidiabetic treatments. *Dendrobium macraei* a medicinal plant traditionally used in various cultures, has shown promising potential for managing diabetes. This study investigates the in vitro antidiabetic effects and phytochemical profile of *Dendrobium macraei* Lindl., with a particular focus on its alpha-amylase inhibitory activity. Preliminary phytochemical screening of the plant extract revealed the presence of several bioactive compounds, including glycosides, alkaloids, flavonoids, carbohydrates, proteins, steroids, and tannins**.** These compounds are known to contribute to the therapeutic effects of medicinal plants, including blood sugar regulation. In vitro assays demonstrated that the extract significantly inhibited alpha-amylase activity, a key enzyme involved in the breakdown of starch into glucose. By inhibiting alpha-amylase, the plant extract slows the rate at which glucose is absorbed, thus potentially reducing postprandial blood sugar spikes. Additionally, the plant extract enhanced insulin sensitivity and reduced glucose absorption, further supporting its potential as a natural antidiabetic agent. These findings highlight the potential of *Dendrobium macraei* Lindl. in the development of alternative diabetes treatments. The plant's bioactive compounds, particularly those involved in alpha-amylase inhibition, could be beneficial in managing blood sugar levels and preventing complications associated with diabetes. Future research is required to fully understand the mechanisms of action and clinical applicability of *Dendrobium macraei* Lindl. in diabetes management.

**KEYWORDS:** *Dendrobium macraei* Lindl., alpha-amylase inhibition, anti-diabetic activity, phytochemical screening, glucose absorption, insulin sensitivity.

## INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by elevated blood glucose levels, has become a significant global health issue, with increasing prevalence rates worldwide. It is associated with various complications, including cardiovascular diseases, neuropathy, and kidney damage, which often lead to increased morbidity and mortality.[1] Traditional treatment options, such as insulin therapy and oral antidiabetic drugs, have limitations, including side effects and high costs, which have prompted the search for alternative therapies.[2] Medicinal plants have long been explored for their potential to manage diabetes, due to their availability, cost-effectiveness, and lesser side effects.[3] *Dendrobium macraei* Lindl., a member of the Orchidaceae family, has gained attention for its therapeutic potential, particularly in managing diabetes. It has been used in traditional medicine for its anti-inflammatory, antioxidant, and immune-boosting properties.[4] Recent research has shown that *Dendrobium macraei* Lindl. may possess significant antidiabetic effects, which could be attributed to its bioactive compounds, including alkaloids, flavonoids, glycosides, steroids, and tannins.[5] The plant *Dendrobium macraei* is the important botanical source of Ayurvedic drug Swarna Jivanti (common name) belonging to family Orchidaceae. It is an epiphyte with creepy rhizome and pendulous stem. The plant is sweet with a flavor, cooling, alterative, astringent to the bowels, brain tonic, aphrodisiac, expectorant, useful in asthma, bronchitis, 'tridosha', throat troubles, fevers, burning sensations, biliousness, diseases of the eye and the blood. The plant is stimulant and brain tonic. It is reported to contain alkaloids, carbohydrates, flavonoids, steroids, tannins and phenolic compounds. Jibantine, resinous principles α and β jibantic acid and diosgenin derivatives like denfigenin and defuscin as steroids are reported as chief constituents in this plant. Swarna Jivanti is one of the important Rasayana drugs in Ayurveda. These compounds have been shown to exhibit various pharmacological activities, including the inhibition of carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase, which play crucial roles in the digestion of starches and sugars.[6] In particular, the inhibition of alpha-amylase is a vital mechanism in reducing postprandial blood sugar spikes, as this enzyme catalyzes the breakdown of starch into simple sugars. Previous studies have highlighted the potential of plant-based alpha-amylase inhibitors as a therapeutic strategy to control hyperglycemia in diabetic patients.[7,10] Therefore, exploring the in vitro antidiabetic potential of *Dendrobium macraei* Lindl. and its ability to inhibit alpha-amylase is essential for understanding its mechanism of action and its suitability as a natural alternative for diabetes management.[8] This study aims to investigate the phytochemical composition and in vitro alpha-amylase inhibitory activity of *Dendrobium macraei* Lindl., providing insights into its potential as a natural agent for controlling blood glucose levels.[11,18]

The chemical makeup of *Dendrobium macraei* Lindl. has not been fully characterized, but insights can be drawn from related species within the *Dendrobium* genus. These orchids are commonly known to produce various alkaloids, which are recognized for their neuroprotective, immune-modulating, and analgesic effects. Such compounds are believed to contribute significantly to the plant's traditional use in herbal medicine.[20,26]

Additionally, flavonoids, which are well-known for their antioxidant properties, are likely present. These compounds help in reducing oxidative stress and may offer benefits for cardiovascular health and blood glucose regulation.[27,32]

Another group of important constituents potentially found in *D. macraei* includes polysaccharides. These are often extracted from the stems and pseudobulbs of *Dendrobium* species and have shown promising roles in enhancing immune function and reducing fatigue, making them key elements in health supplements and restorative tonics.[33,37]

The presence of terpenoids and steroids is also suggested, which may contribute to the plant's adaptogenic activity, supporting general well-being and resilience to stress.[38,42]

Additionally, glycosides, often associated with tonic effects, could be among its phytochemicals, supporting its traditional application as a rejuvenating herb. Although direct studies on the chemical constituents of *D. macraei* are limited, the similarities within the genus provide a basis for exploring its therapeutic potential.[43,50]

*Dendrobium macraei*, known as Swarna Jivanti, is a medicinal orchid used in Ayurvedic medicine, traditionally as a memory enhancer and Rasayana drug, and has shown potential for anti-inflammatory and antioxidant activities.[50,52]

**Fig. 1: *Dendrobium macraei* Lindl. Plant.**

## MATERIAL AND METHODS

### 1. Collection and Authentication of Plant

The whole plant of *Dendrobium macraei* was purchased from local market of Haridwar (Kankhal), Uttarakhand, INDIA. The plant was identified and authentified at the Herbarium of CSIR-NISCAIR (i.e., Council of scientific and industrial research – National institute of science communication and information resources), Delhi with vide Reference no. NISCAIR/RHMD/Consult/2015/2565-144.

### 2. Plant Material

The plant *Dendrobium macraei* whole plant were harvested from Haridwar, Uttarakhand, India. The plant undergo cleaning, shade dried, and then powdered.

### 3. Organoleptic Evaluation[9,19]

It describes the assessment of plant material based on characteristics like size, shape, colour, odour, taste and texture. Organoleptic evaluation refers to the results prepared from study based on the sensory assessment, overall condition and appearance of the raw plant material and organoleptic examination of the *Dendrobium macraei* plant using simple microscope.

### 4. Physicochemical Evaluation[10,20]

#### 4.1 Foreign organic matter

A 100-gram quantity of unrefined drug was applied to a spotless, clean surface using magnifying lenses (10X) in order to determine if foreign organic compounds were present in the plant specimen. Three sets of readings were taken in accordance with the protocol.

### 4.2. Extractive value[11]

**Cold maceration method used for the purpose of calculating extracting value, as follows:** A conical flask with a cotton plug was filled with 4g of precisely weighed coarsely powdered air-dried material, which was macerated for 6 hours with vigorous shaking in 100 milliliters of the solvent intended for the plant material. Following that, it was left alone for eighteen hours. In order to prevent solvent loss, 25 milliliters of the filtrate were rapidly filtered. It was then set on a flat-bottomed, dried plate and left to air dry before being dried for six hours at 105°C, cooled for half an hour in Moisture-absorbing container, and immediately measured in mass. The amount of extractable material was observed in milligrams for every gm.[18,19]

Calculated the % of extractable constituents from Evaporated material as:

$$\% \text{ Extractive value} = \frac{[\text{Final weight} - \text{initial weight}] \times 4 \times 100}{\text{Weight of the drug}}$$

**The following kinds of extraction rates have been determined using the above-described methodologies**:

- Water soluble extractable content
- Methanol soluble extractable content
- Ethyl acetate extractable content
- Chloroform soluble extractable content
- Acetone soluble extractable content

### 4.3. Ash value[12]

• **Total ash:** As long as carbon-neutral ashes were produced, two grams of powdered *Dendrobium macraei* were burned in a crucible in a muffle furnace at temperatures between 500 and 600˚C. After allowing the drug to cool and weighed also % of total ash were evaluated. Determined the Air-exposed dried material's content of the overall ash value as:

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash} \times 100}{\text{Weight of crude drug taken}}$$

• **Acid insoluble ash:** After boiling 25 milliliters of 70 g/L hydrochloric acid for five minutes, the resulting ash were filtered. The filter paper, containing trapped insoluble material, was rinsed with hot water. The percentage of ash that was insoluble in acid was determined by comparing it with weight of dried powdered material. Calculated the proportion of ash that is insoluble in acid from the air-dried material as:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash} \times 100}{\text{Weight of crude drug taken}}$$

• **Water-soluble ash**

Twenty-five milliliters of water were used to bring the total amount of ash to a heated for five minutes. The inert substance was stored on ash-free filter paper, cleaned with boiling water, and then burnt for 15 minutes at an elevation that could not exceed 450˚c in a muffle heater. The quantity of water-soluble ash was determined by splitting the mass of ash by the mass of water-insoluble ingredients. The dried in the air powdered plant product was used to calculate the proportion of water-soluble ash. Determined the % of water-soluble ash value as:

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash} - \text{Weight of water insoluble ash} \times 100}{\text{Weight of crude drug taken}}$$

**5. Extraction from *Dendrobium macraei* whole plant**

Solvents such as ethyl acetate, methanol, acetone, and chloroform were used to extract the plant material utilizing the cold maceration procedure.[20,25]

**Cold maceration method:** One hundred milliliters of solvent were mixed with four grams of the powdered substance. The conical flask containing the ingredients was then left to macerate for the next six hours, vigorously shaking it every ten minutes. Additionally, the flask was kept still in a darkened area for the next eighteen hours. The filtrate was subsequently moved on a China plate. The dried extracts were used for other purpose.[26,29]

**6. Preliminary Phytochemical Screening**

Initial phytochemical screening was performed by utilizing a conventional technique.[30,50]

**I. Alkaloids**

- **Dragendroff's test**: 1 milliliter solution of extraction + 1 milliliter Potassium bromide produce orange – red color precipitate.
- **Mayer's test**: 1 milliliter solution of extraction + 1 milliliter Mercury(I) iodide gives cream, whitish yellow color precipitate.[40,44]

**II. Glycosides**

- **Legal's test:** 1 milliliter of extract + pyridine + $Na_2$ [Fe (CN) 5NO]) shows absence of glycoside with no change in color.
- **Baljet's test:** 1 milliliter of extract + 1 ml $C_6H_2KN_3O_7$ indicates the presence of glycoside with Yellow to orange color appearance.
- **Cardiac glycosides:** Few drops of concentrated H2SO4 and 1 milliliter of FeCl3 reagent was added to 1 milliliter of the filtrate of extract shows turning of greenish blue color in few minutes.[16]

**III. Carbohydrates**

- **Benedict's test:** 5 ml of benedict's chemical+ 1 milliliter solution of extraction subjected to boiling just 2 minutes and then let it cool to produce a pink colored precipitate which indicates presence of sugars.
- **Molisch's test:** Ethanolic extract + α- naphthalene (20% w/v, 90%) subjected for shaking gently and conc. $H_2SO_4$ was inserted via the test tube's side.[42-45]

**IV. Steroids**

- **Salkowski test**: solution of extraction, CHCl3, and some amounts of concentrated H2SO4 were added. The acidic layer fluorescence green, while the CHCl3 layers (which contain steroids) are bluish red to cherry in color.
- **Liebermann-Burchard test:** By heating the extract and one milliliter of acetic anhydride, it started getting dissolved. A few drips of concentrated H2SO4 were visible on the test tube's sides after the contents had cooled. The sterols (blue color) are present.

**V. Test for Proteins**

- **Biuret test:** 40% sodium hydroxide solution along with 2% solution CuSO4 sol. until a blue color appears + 1 ml extract is the biuret test. Violet (protein present).[46-50]

**VI. Test for Saponins**

- Extraction was shaken and boiled in one milliliter of distilled water. There was foam (saponins).
- Combined the extract with 2 ml of distilled water and sodium carbonate, then Shaked. Foam formation shows the presence of saponins.

**VII. Test for Tannins**

- Put the extract into a mixture of lead acetate. Tannins are present when white precipitates start to develop.

**VIII. Test for Flavonoids**

- **Shinoda test:** Add concentrated hydrochloric acid dropwise to the test solution containing magnesium turnings. A pink scarlet color appears.[51-56]

**7. Thin layer chromatography**

This was a speedy screening methodology to isolating components and categorizing herbal preparations. Because of its user-friendly interface, fast turnaround times, reliable, accurate, and precise processes, as well as its reasonably priced operating costs, this method is widely utilized for both qualitative and quantitative analysis.[61,62,63,64]

**Procedure**

- Using a capillary tube that was one centimeter above the bottom, the sample was transferred to a TLC plate.
- Sample spot was air dried.
- Mobile phase was added to the beaker at a length of 0.5-1cm from the bottom.
- After closing the beaker, positioned the TLC plate in the jar such that the sample spot is still above the level of mobile phase.
- Until the solvent moved a suitable distance from the baseline, the system was left in a static state.
- TLC plate was taken out and dried.[67,68,69,70]

**8. In-vitro anti-diabetic activity**

The modified Pradeep and Sreerama (2015) approach was used to determine the inhibitory impact of α-Amylase of mill. Extract.[18,19] Fifty microliters of 20 mM phosphate buffer (pH 6.8) and 10 microliters of α-amylase (2 U/ml in 20 mM PBS buffer) was m combined in various ratios of *Dendrobium macraei* M. extracts (50–250 µg/ml) underwent incubation at 25°C for 30 minutes. After that, 20 µl of 1% soluble starch mixed in 20 ml of phosphate buffer (pH 6.8). The reaction was once more incubated at 37°C for 30 minutes. Afterward, 100 µl of Di nitro salicylic acid (DNS) reagent added, and the solution was heated at 96°C for 10 minutes to complete the process. The mixture's absorbance at 540 nm evaluated by UV spectrophotometer. The usual dosage of acarbose was 50–250 µg/ml. Without plant extract, the reaction same as previously mentioned was carried out as a control.[60,61,62] Percentage of inhibition = (A540control – A540sample) × 100) Where, A540control is Absorbance of control at 540nm. The transmittance measured for the reaction's mixture containing the buffering agent and the enzyme is A540 sample.[71,72,73,74]

**RESULTS**

**1. Macroscopic evaluation of *Dendrobium macraei* Lindl.**

**Leaves**

- Shape: lanceolate to elliptical

- Size: leaves are variable size, but generally range from 2.5cm to 40cm
- Color: lust green
- Texture: coriaceous (leathery) and smooth
- Odor: distinct odor

**Stems**

- Color: light green to yellowish-green in color
- Texture: smooth, soft and velvety texture
- Shape: the plant has narrow leaves that give it a graceful look

**Flowers**

- Colour: white or pale yellowish base with purple markings
- Size**:** medium sized typically around 3 to 4cm in diameter
- Arrangement: racemose inflorescences
- Odour: mild, pleasant fragrance

**Fruits**

- Type: Capsule (Orchids typically produce capsule-type fruits rather than fleshy fruits).
- Shape: cylindrical or elongated
- Size: small, generally ranging from 3 to 5 cm in length
- Colour: green to brownish
- Odour: strong or distinctive odour.
- Texture: smooth and generally leathery

**2. Estimation of Foreign Organic Matter of the Whole Plant Material**

**Table No. 1: Foreign Organic Matter Estimation of *Dendrobium macraei.***

| S.No. | Evaluated constraint | Yield (% w/w) |
|---|---|---|
| 1. | Foreign organic matter | 1.26 % |

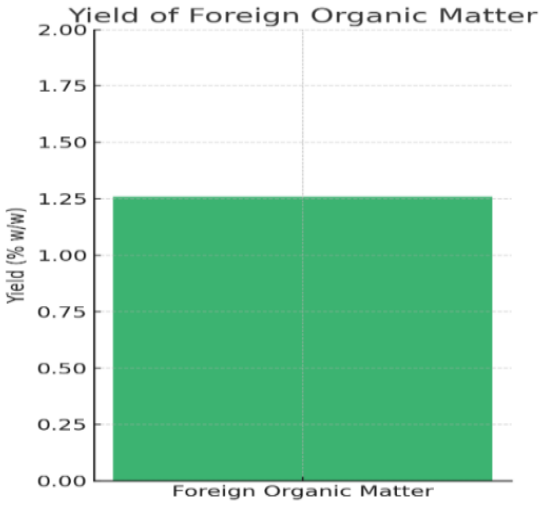


**Fig. 1: Histogram Representing Foreign Organic Matter Present in *D. macraei.***

### 3. Extractive Value of *Dendrobium macraei* Lindl.

**Table 2: % Yield of Extracts Obtained by Cold Maceration Using Different Solvents.**

| S.no | Extractive value | Results(%w/w) |
|---|---|---|
| | | Cold maceration |
| 1. | Water soluble | 11.8% |
| 2. | Methanol soluble | 10.2% |
| 3. | Acetone soluble | 6.5% |
| 4. | Chloroform soluble | 3.0% |
| 5. | Ethyl Acetate | 4.3% |

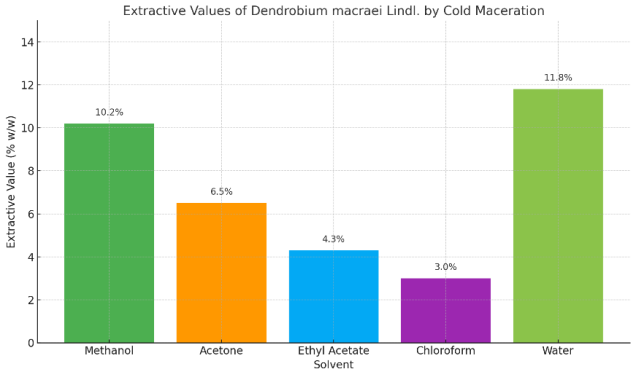


**Fig. 2: Histogram representing different extractive values of *Dendrobium macraei.***

### 4. Ash Value

**Table 3: Ash Value Analysis of *Dendrobium macraei* (% w/w).**

| S.no | Determination of ash value | Yield (%w/w) |
|---|---|---|
| 1. | Total Ash value | 5.3% |
| 2. | Water soluble Ash value | 3.2% |
| 3. | Acid-insoluble Ash value | 1.8% |

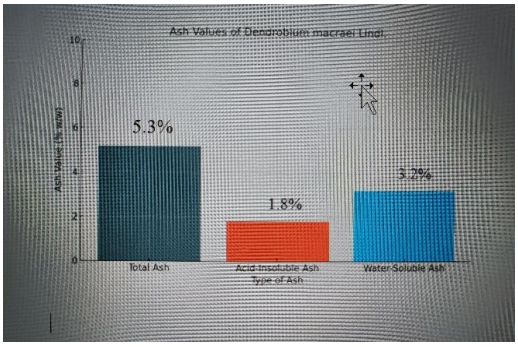


**Fig. 3: Histogram representing different ash values of *Dendrobium macraei* lindl.**

### 5. % Yield of plant extracts

**Table 4: % Yield of the *Dendrobium macraei* extracts of the plant.**

| S. no. | Name of Plant extract | % Yield w/w |
|---|---|---|
| 1. | Chloroform | 2.5% |
| 2. | Ethyl acetate | 3.0% |
| 3. | Hydro-methanol | 5.5% |
| 4. | Aqueous | 6.0% |

**6. Phytochemical Screening of plant extract of *Dendrobium macraei***

**Table 5: Phytochemical Analysis of *Dendrobium macraei* extracts.**

| S.No. | Name of phytochemical tests | Aqueous extract | Methanol extract | Chloroform extract | Ethyl acetate extract |
|---|---|---|---|---|---|
| 1. | Alkaloid | | | | |
| | Draggendroff's reagent | - | - | +++ | ++ |
| | Mayer's reagent | - | - | ++ | - |
| 2. | Glycosides | - | - | - | - |
| 3. | Carbohydrates | + | + | - | - |
| 4. | Steroids | | | | |
| | Salkowski test | - | + | - | - |
| | Lieberman Burchard's test | - | + | - | - |
| 5. | Proteins | - | - | - | - |
| 6. | Flavonoids | + | + | - | +++ |
| 7. | Saponin | + | + | - | - |
| 8. | Tannis | + | + | - | - |

Note: (+++) Strongly present, (++) means Present, (+) means moderately present, means absent.

**7. TLC Fingerprinting**

**Table 6: Rf Values of *Dendrobium macraei* Extracts in Different Solvent Systems by Thin Layer Chromatography (TLC).**

| S.No. | Extract | Solvent system | Rf value |
|---|---|---|---|
| 1. | Ethyl acetate extract | Chloroform: Ethanol (9:1) | Three spots (0.35, 0.50, 1.00) |
| 2. | Chloroform extract | Methanol: Chloroform (9:1) | Three spots (0.18, 0.84, 0.93) |
| 3. | Acetone extract | Chloroform: Ethanol (9:1) | One spot (0.09) |
| 4. | Methanol extract | Chloroform: Ethyl acetate: Glacial acetic acid (7: 2: 1) | Four spots (0.77, 0.82, 0.87, 0.91) |

**8. In-Vitro Anti Diabetic Activity (α-Amylase inhibitory activity)**

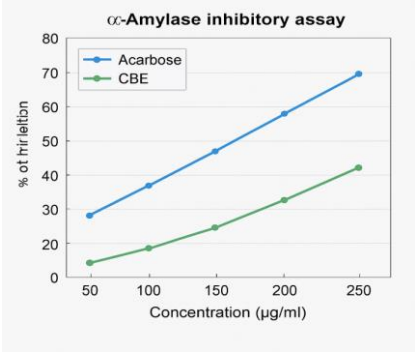


**Fig. 4: α- amylase inhibitory activity of *Dendrobium macraei.***

**Table 7. Comparison of Alpha-Amylase Inhibition by Acarbose and *Dendrobium macraei.***

| S.no | Concentration (µg/mL) | % Inhibition (Acarbose) | % Inhibition (*D. macraei*) |
|---|---|---|---|
| 1. | 250 | 72% | 65% |
| 2. | 200 | 60% | 58% |
| 3. | 150 | 48% | 45% |
| 4. | 100 | 35% | 38% |
| 5. | 50 | 28% | 15% |

**DISCUSSION**

In vitro investigations into the anti-diabetic properties of *Dendrobium macraei* Lindl. have shown promising findings, underlining its potential as a natural therapeutic agent for diabetes management.[21] Phytochemical analyses of the plant

have identified a variety of bioactive compounds, which demonstrate noteworthy biological activity, lending support to its traditional use in treating diseases like diabetes.[22] Notably, alkaloids, flavonoids, and glycosides, recognized for their antioxidant and anti-inflammatory characteristics, may help in lowering blood glucose levels, enhancing insulin sensitivity, and protecting pancreatic cells from oxidative damage.[23] Additionally, the presence of phenolic compounds and other secondary metabolites hints at a complex mechanism of action, possibly involving the inhibition of key enzymes such as α-glucosidase and α-amylase. These enzymes play critical roles in the breakdown of carbohydrates and the absorption of glucose, and their inhibition could result in a gradual increase in blood glucose levels after meals, a common approach for managing type 2 diabetes.[24] t is commonly used in traditional medicine due to its anti-inflammatory, antioxidant, and anti-diabetic properties. Phytochemical screening of D. macraei has revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, glycosides, tannins, and phenolic compounds, all of which are linked to various pharmacological effects.[25] Of particular interest are alkaloids and flavonoids, which have well-documented anti-diabetic properties.[26] The extractive value of *Dendrobium macraei* Lindl. is an important parameter for understanding its potential therapeutic uses. The higher the extractive value, the more likely it is that the plant contains significant amounts of bioactive compounds that could be harnessed for medical purposes, including diabetes management, based on the plant's anti-diabetic, antioxidant, and anti-inflammatory properties. To obtain exact numbers for the extractive value, it would be necessary to refer to specific experimental studies conducted on *Dendrobium macraei*. Several types of phytoconstituents were detected in extracts treated with different solvent systems when the plant was evaluated for TLC fingerprinting. For determining the trace levels of contaminants in plants, this approach is effective. The retardation factor (Rf) in a given solvent system is determined by measuring the separation between the solvent and the solute. The TLC of different extracts was developed using different mobile phases.

## CONCLUSION

*Dendrobium macraei* Lindl. demonstrates significant potential as a natural anti-diabetic agent, as evidenced by its promising in vitro activities. The plant's rich phytochemical composition, including alkaloids, flavonoids, glycosides, and phenolic compounds, contributes to its ability to regulate blood glucose levels, inhibit key carbohydrate-digesting enzymes, and offer antioxidant protection to pancreatic cells. These mechanisms collectively suggest that *D. macraei* could play a vital role in managing diabetes, particularly in preventing postprandial hyperglycemia and improving insulin sensitivity. However, while the in vitro results are encouraging, it is important to recognize the need for further studies to confirm the plant's efficacy and safety in vivo. Clinical trials and animal studies are essential to understand the bioavailability of its active compounds, their pharmacokinetics, and their potential side effects. The future of *D. macraei* in diabetes management looks promising, but further research is required to translate these laboratory findings into practical therapeutic applications. Should these investigations confirm its safety and efficacy, *D. macraei* could offer a valuable addition to the array of natural remedies for diabetes, potentially benefiting individuals seeking alternative or complementary treatments.

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