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RESEARCH ARTICLE

Exploring the Phytopharmacognostic Profile of *Tricholepis glaberrima* DC (*Brahmadandi*) Stem

Kalpesh Rajendra Yeole[1], Raju Ratan Wadekar[2*] and Kalpana Sabanna Patil[3]

[1]Shri Vile Parle Kelavani Mandal's Institute of Pharmacy, Dhule - 424001, Maharashtra, India

[2]Department of Pharmacognosy and Phytochemistry, Shri Vile Parle Kelavani Mandal's Institute of Pharmacy, Dhule - 424001, Maharashtra, India; raju.wadekar@svkm.ac.in

[3]Department of Pharmacognosy and Phytochemistry, Tatyasaheb Kore College of Pharmacy, Warananagar - 416113, Maharashtra, India

Abstract

Background: Pharmacognostic evaluations are crucial for the standardization and authentication of crude drugs to prevent adulteration. **Aim:** This study examines the pharmacognostic and phytochemical properties of the stem of *Tricholepis glaberrima* DC, an important medicinal plant in *Ayurvedic* and *Siddha* medicine. **Methods:** Macroscopic and microscopic analyses were performed to document morphological and anatomical features. Physicochemical parameters, including moisture content, ash values, and extractive values, were assessed. Fluorescence analysis under UV light and qualitative phytochemical screening were conducted, alongside quantitative analysis for flavonoid and phenolic content. HPTLC fingerprinting were employed to identify major phytoconstituents. **Results:** The stem's methanolic extract contained flavonoids, tannins, steroids, and glycosides. Total flavonoid and phenolic contents were 38 ±2.0 mg/g and 201 ± 1.0 mg/g of extract, respectively. HPTLC studies revealed quercetin with an Rf value of 0.45. **Conclusion:** The study establishes comprehensive pharmacognostic and phytochemical parameters for *T. glaberrima* DC, supporting its standardization and safe use in traditional and modern medicine.

Major Findings: The stem of *Tricholepis glaberrima* DC demonstrated distinct morpho-anatomical features and was found to contain key phytoconstituents such as flavonoids, tannins, steroids, and glycosides. HPTLC profiling confirmed the presence of quercetin, supporting the plant's standardization and potential medicinal value.

Keywords: HPTLC Fingerprinting, Morpho-Anatomical, Physicochemical, Spectroscopic studies, *T. glaberrima*

Abbreviations: HPTLC: High-Performance Thin Layer Chromatography; METg: Methanolic Extract of *T. glaberrima;* TLC: Thin Layer Chromatography; TG: *Tricholepis glaberrima*

1. Introduction

Pharmacognosy is an applied science concerned with the biological, biochemical, and economic aspects of natural drugs or their constituents[1]. The role of crude drug field knowledge has become an essential field to obtain all information about crude herbs or crude herb extract, pure natural compounds, and foods with health benefits[2]. Pharmacognosy deals with the study of natural origin drug substances from physical, chemical, biochemical as well as biological points of view and trying to obtain novel drugs from natural sources[3]. Interest in phyto therapeutics at the global level has led to a demand for authentic and standardized plant materials[3]. Herbal medicines have been widely used by different cultures all over the world with at least a total 80% of the total population in the developing countries completely relying on these medicines[2]. It is discernible that India being a country with great influence and utilization of Ayush the integration of traditional medicine into modern medicine is increasing[3]. The increasing use and possible exhaustion of potent

*Author for correspondence

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natural drugs call for enhanced plant identification and standardization[1]. Such things as wrong identification, contamination and replacement with substandard materials remain highly vulnerable in therapeutic assembly[1]. Consequently, pharmacognostic studies are indeed essential aid to check plant identity and help in setting standardization parameters to preclude these problems[2]. Plant derived products and secondary metabolites offer a lot of potential in managing diseases inclusive of cancer, cardiovascular diseases, diabetes and infections[4]. Isolation and characterization of nutrient and nonnutrient bioactive compounds is considered one of the key objectives of experimental pharmacology[4,5]. While old pharmacopoeias focus mainly on physicochemical properties of the substance, newer techniques as HPTLC have to be used in order to give proper identification and quantification of the active ingredients[4,6]. HPTLC is widely used for its merits such as simplicity, cost-score, rapidity, higher chemical patterns of resolution that are useful for appraisal and standardization of APA[6]. In its recent publication on the rational use of medicinal plants, the World Health Organization (WHO) called for improved chromatographic and spectral analyses in the standards of these products. hence for the standardization and quality control of the multitude of these formulations, it is imperative that improvements are made on these methods[7,8].

Brahmadandi or *T. glaberrima* DC is an edible wild plant which grows in the tropical and subtropical hilly regions of the world and belongs to the family *Asteraceae* (*Compositae*). The name of the plant was given by joining *Tricholepis*, meaning hair like, and *glaberrima*, meaning having a smooth surfaced. *T. glaberrima* is an annual herb having erect stem and without hair on different parts of the plant, found throughout India and few parts of Afghanistan. Commonly it is found in field bunds, rocky lands, and the grassland regions of Rajasthan, Madhya Pradesh, Maharashtra, Kerala, Karnataka, and some parts of South America[9,10]. The whole plant of *T. glaberrima* has been employed in *Ayurveda* and *Siddha* system of medicine due to its medicational value. It's known to be hot and bitter, thus helps in pacifying the *kapha* and *vata*. This herb is commonly used as a nervine tonic besides being used in leucoderma, inflammatory disease, and fatigue[9,11]. In the same regard, the plant displays great allopathy and has a recognized and experimented potential to cure cancer as well as to treat skin diseases[9,12]. *T. glaberrima* has hepatoprotective, antioxidant, anti-inflammatory, and antidepressant pharmacology attributable to its diverse bioactive compounds[11,12]. Although the plant is reported to possess significant medicare importance, there is a dearth of exhaustive pharmacognostic details for correct identification and authentication of the plant both in the whole and powdered form. Thus, the present work plans to study macromorphology, pharmacognostic analysis, and phytochemical examination of the stem of *T. glaberrima* DC[11,12]. This research aims to add to this scanty literature by developing a reliable monograph on this vital medicinal plant; to create uniformity in the batch.

2. Materials and Methods

The process was carried out in two steps *i.e.* pharmaceutical study and analytical study.

2.1 Pharmacognostical Study

2.1.1 Plant Material Collection and Identification

The plant species, *T. glaberrima*, was identified and authenticated by experts at the Botanical Survey of India, Pune. Samples were collected from the Deopur region in Dhule district, Maharashtra, and a herbarium specimen, labeled SHIGOT3, was archived in the Department of Pharmacognosy at Shri Vile Parle Kelavani Mandal's Institute of Pharmacy. Once authenticated, the stems were carefully dried in open air and processed for subsequent analysis.

2.2 Analytical Study

2.2.1 Chemicals and Instruments

This study utilized high-purity analytical-grade chemicals, including methanol, ethanol, formaldehyde, Folin-ciocalteu reagent, and hydrochloric acid. Standard marker, Quercetin was procured from YUCCA Enterprises, A-246, Antop Hill Warehousing Co., Barkat Ali Naka, Wadala (E), Mumbai. Key equipment included a Zeiss microscope (Germany), a Thermo Electron Microtome (Shandon Finesse model, USA), and a CAMAG HPTLC system (Switzerland), featuring tools such as the Linomat V applicator and TLC Scanner 3, integrated with the WINCATS-4 software.

2.2.2 Macroscopic Analysis

Macroscopic features, including the shape, size, color, texture, taste, and odor of the stems, were documented meticulously. This was done in line with established guidelines outlined by the World Health Organization for quality control of medicinal plant materials[13].

2.2.3 Microscopic Analysis

Fresh stems were rinsed with distilled water and preserved in a solution of 70% ethanol, glacial acetic acid, and formaldehyde for a week. Dehydration was achieved using tertiary butyl alcohol in graded concentrations. Embedded samples were sectioned using a rotary microtome, and histological staining was carried out using phloroglucinol-HCl and iodine. Prepared sections and powdered samples were analyzed under a Zeiss fluorescence microscope with the Ac-Rel-4.5 software, allowing for detailed visualization of plant structures[14].

2.2.4 Physicochemical and Fluorescence Analysis

The physicochemical characteristics of the powdered stem, including moisture content, ash values, and extractive values, were determined using standard protocols. Fluorescence properties were examined under visible and UV light at 254 nm and 365 nm after treating the powder with different reagents, such as sodium hydroxide and sulfuric acid[15].

2.2.5 Preliminary Phytochemical Analysis

The powdered plant material was subjected to continuous Soxhlet extraction with 99% methanol at a controlled temperature of 45-50° C for 7 hours. The solvent was evaporated under reduced pressure using a rotary evaporator, leaving behind a concentrated extract. This residue underwent preliminary phytochemical screening for active compounds, and quantitative tests were conducted to measure total phenolic and flavonoid content[16].

2.2.6 Determination of Phenolic and Flavonoid Content

The total phenolic content was quantified using the Folin-ciocalteu method. A reaction mixture containing the reagent, extract, distilled water, and sodium carbonate was incubated at room temperature, with absorbance measured at 765 nm. Results were expressed in gallic acid equivalents. Flavonoid content was estimated using the aluminum chloride method, with a calibration curve constructed from quercetin standards[16,17].

2.2.7 HPTLC Fingerprinting

The HPTLC analysis was carried out using[18]:

- Solvent System: Toluene, ethyl acetate, and formic acid in a ratio of 50:40:2.
- Stationary Phase: Aluminium-backed plates pre-washed with methanol and heat-activated at 110° C.
- Sample Application: Extract solutions were applied in different volumes using the Linomat V applicator.
- Chromatogram Development and Scanning: Plates were developed in a twin-trough chamber and scanned with a TLC Scanner 3 under UV and visible light.

3. Result

3.1 Morpho-anatomical Features

The woody stems of *T. glaberrima* exhibit a distinct yellowish-brown hue, complemented by an aromatic fragrance and a unique taste as determined through organoleptic evaluation. The stem is erect, slender, and thorny, with a diameter ranging from 6 to 8 mm, as shown in Figure 1.

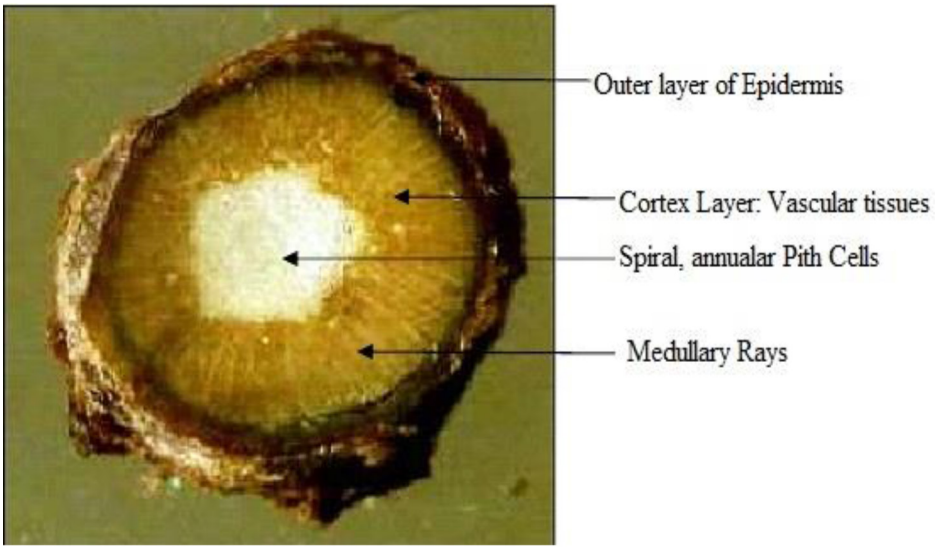
3.2 Histochemical and Microscopic Analysis

Transverse sections of the *T. glaberrima* stem revealed a rounded structure with an undulating surface marked by shallow ridges and furrows. The stem diameter



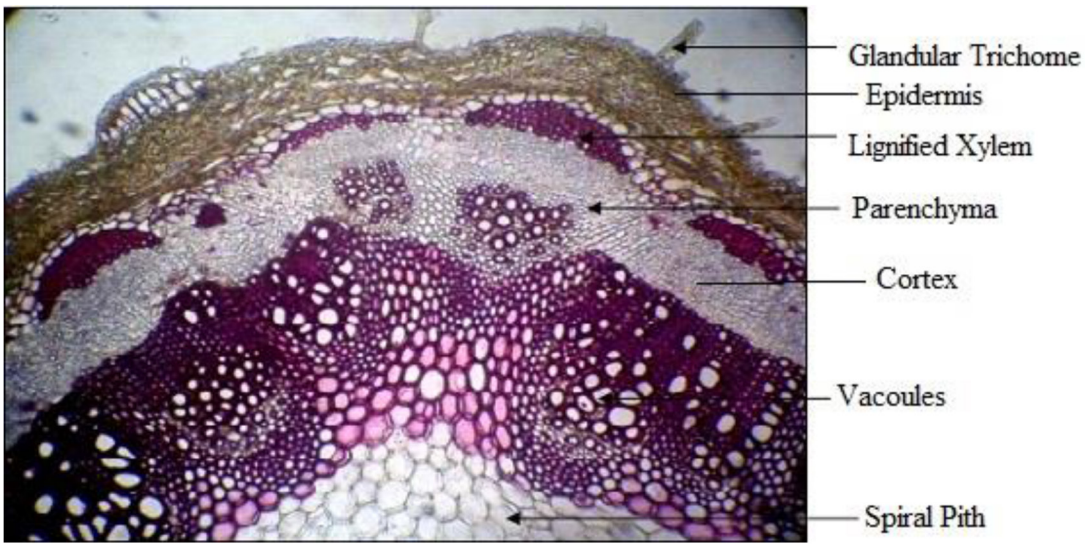
Figure 1. An image of *T. glaberrima* DC (TG) stem and flower.

ranged between 6 and 8 mm. The anatomical structure includes an epidermis with thick rectangular cells, a narrow cortex, a continuous vascular tissue band, and a well-developed central pith. Younger stems exhibit trichomes and stomata. Trichomes, both covering and glandular types, were observed, with covering trichomes being uniseriate and composed of 4-5 thick-walled cells. Glandular trichomes had multicellular stalks and unicellular or bi-cellular glands. The cortical cells showed pigmentation, and the pericycle contained groups of lignified fibers external to the phloem. Vascular bundles were arranged radially, consisting of phloem and xylem tissues, with xylem exhibiting spiral, reticulate, and pitted thickening. The pith comprised large, rounded cells. Figures 2a to 2i illustrate these features



(a)

Figure 2. **(a).** Transverse section (round view) of *T. glaberrima* DC stem.



(b)

Figure 2. **(b).** Transverse section of *T. glaberrima* DC stem.



(c) (d)

Figure 2. **(c).** Covering trichomes of TG stem, **(d).** Glandular trichomes of TG stem.

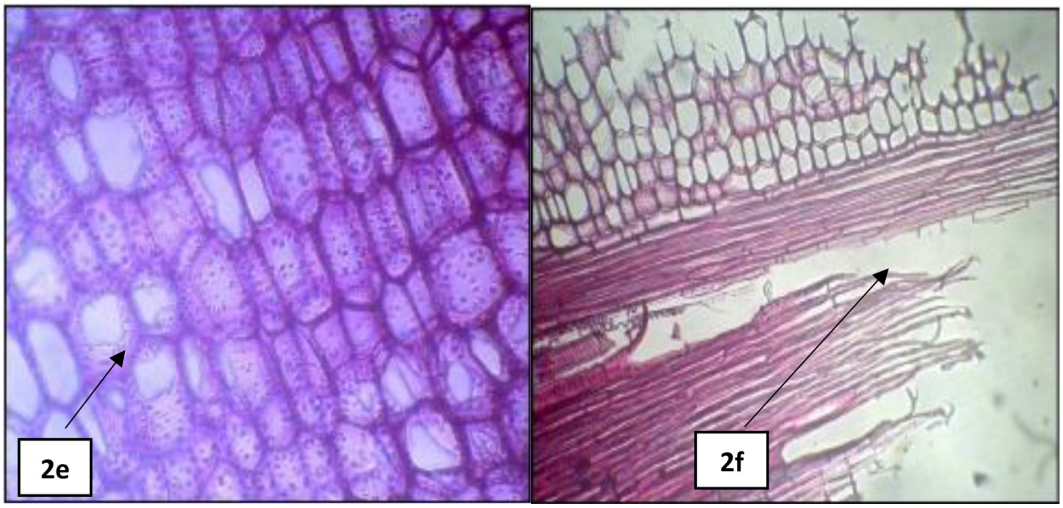


Figure 2. **(e).** Xylem parenchyma of TG stem, **(f).** Fibers of TG stem.

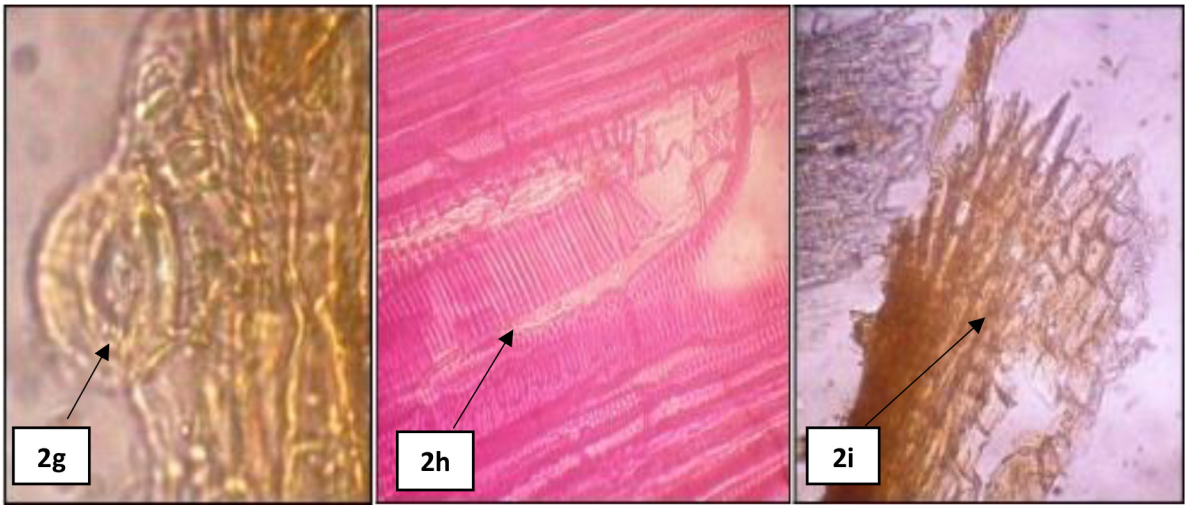


Figure 2. **(g).** Paracytic stomata of TG stem, **(h).** Spiral, annular, and pitted vessels of TG stem, **(i).** Epidermal cells of TG stem.

3.3 Physicochemical Constants and Fluorescence Analysis

The powdered stem of *T. glaberrima* underwent physicochemical evaluation. Results are presented in Table 1. The powder, characterized by a yellowish-brown color and aromatic odor, exhibited specific ash values, moisture content, and extractive values. Elemental analysis indicated the presence of magnesium, calcium, sodium, potassium, phosphorus, and copper. The formation of froth when mixed with water confirmed saponins presence. Fluorescence properties under various reagents are summarized in Table 2.

3.4 Qualitative Preliminary Phytochemical Analysis

Phytochemical screening of the methanolic extract of *T. glaberrima* confirmed the presence of tannins, steroids, polyphenolics, and glycosides. Quantitative analysis revealed flavonoid and phenolic contents of 38 ± 2.0 mg/g and 201 ± 1.0 mg/g of extract, respectively. Extractive yields varied, with petroleum ether (1.07% w/w), chloroform (2.11% w/w), and methanol (5.08% w/w) exhibiting distinct physical properties.

Table 1. Physicochemical evaluation of powder stem of *T. glaberrima* DC

Parameters	% w/w (Mean a ± SEM)
Total ash	2.04 ± 0.12
Acid insoluble ash	1.33 ± 0.03
Water soluble ash	1.04 ± 0.03
Water soluble extractive value	15. 10 ± 0.10
Alcohol soluble extractive value	20. 15 ± 0.18
Moisture content	5.1 ±0.66

3.4 HPTLC Analysis

The methanolic extract (METg) was subjected to HPTLC profiling at 254 nm, identifying 14 components based on their Rf values. The compound with Rf 0.45 was identified as quercetin.

4. Discussion

The present study was conducted to establish detailed guidelines for the morphoanatomical, physicochemical, and phytochemical characterization of the stem of *T. glaberrima* DC using standard analytical techniques. The macroscopic evaluation, a qualitative assessment method, proved effective in differentiating the species and detecting potential adulteration in crude drugs. Microscopic analysis provided a detailed histological profile of the stem, identifying diagnostic characteristics such as its aromatic odor, thorny and slender texture, presence of glandular trichomes, parasitic stomata, and pitted vessels. These features can serve as reliable diagnostic parameters for quality assurance[19].

Physicochemical parameters, including extractive values and ash content, are equally critical in evaluating crude drugs as they indicate the presence of inorganic impurities and quantify phytoconstituents. Variations

Table 2. Fluorescence analysis of powdered stem of *T. glaberrima* DC

Treatment	Observation under Daylight	Observation under UV light (254 nm)	Observation under UV light (366 nm)
Raw powder	Pale yellow	Yellow	Greenish yellow
Raw powder + Nitrocellulose	Light brown	Pale yellow	Bright yellow
Raw powder + 1N NaOH in Methanol	Faint yellow	Yellow	Intense yellow
Raw powder + 1N NaOH in Methanol + Nitrocellulose in Amyl acetate	Fluorescent	Soft fluorescent	Strong fluorescent
Raw powder + 1N HCl	Light pink	Soft brown	Dark brown
Raw powder + 1N HCl + Nitrocellulose in Amyl acetate	Brown	Soft brown	Dark brown
Raw powder + 1N NaOH in water	Pale yellow	Light green	Greenish yellow
Raw powder + 1N NaOH in water, dried, and mounted in Nitrocellulose in Amyl acetate.	Light brown	Faint yellowish brown	Dark brownish yellow
Raw powder + HNO_3 (1:1)	Reddish brown	Reddish brown	Dark red
Raw powder + H_2SO_4 (1:1)	Bright red	Deep red	Reddish brown

in extractive values, which depend on solvent polarity and the composition of phytoconstituents, highlight the need for comprehensive evaluation protocols. Moisture content assessment and fluorescence analysis of stem powder are essential to ensure the stability of the sample and minimize microbial contamination. Fluorescence analysis, under both visible and UV light, provides valuable insights into the chemical nature of the sample[19].

Plants like *T. glaberrima* function as biochemical laboratories, synthesizing secondary metabolites such as alkaloids, glycosides, polyphenols, steroids, and tannins. These metabolites are known to exhibit significant therapeutic activities. The findings of this study confirm the presence of key secondary metabolites in *T. glaberrima*, including flavonoids, tannins, steroids, and glycosides. These bioactive compounds underscore the plant's potential utility in the treatment of various diseases. The therapeutic efficacy of medicinal plants is closely linked to their phytochemical composition, making their identification and quantification critical in standardization efforts[20].

The HPTLC chromatographic analysis demonstrated its reliability as a precise, accurate, and reproducible technique for identifying and standardizing phytochemicals. The results confirmed the presence of quercetin and other polyphenolic compounds in the methanolic extract of *T. glaberrima* DC (METg). The HPTLC profiling provided qualitative and quantitative data confirming the presence of quercetin and other polyphenolic compounds supports the extracts therapeutic relevance[20].

These findings offer robust evidence based on anatomical, physicochemical, and chromatographic data supporting the therapeutic potential of *T. glaberrima* and provide a solid foundation for its future pharmacological investigations[21].

5. Conclusion

Tricholepsis glaberrima DC has been accepted in the traditional medicine practices in treating several invisibility of most work-related ailments in many industries making it compulsory to develop quality control standards. The present investigation proceeded to provide a set of qualitative and quantitative phyto-pharmacognostic parameters in addition to screening markers known as HPTLC finger printing profiles that may be useful for diagnosis to the development of quality including standard, authentication, and identification of the medicinally important plant. Also, it could be helpful for the preparation of an appropriate monograph as well as for the differentiation between the closely related species.

6. Acknowledgements

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7. Authors Contribution

Mr. Kalpesh Yeole: Conducted pharmacognostic studies, physicochemical analysis, and manuscript formatting. Dr. Raju Wadekar: Supervised the study design, phytochemical analysis, HPTLC profiling, and spectroscopic studies. Dr. Kalpana Patil: Contributed to result interpretation and manuscript drafting. All the authors have read and agreed to the published version of the manuscript.

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