Comparative Pharmacognostical and Phytochemical Evaluation between the Leaves of *Vitex trifolia* Linn. and *Vitex leucoxylon* Linn.

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Research Article

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Abstract

Vitex trifolia Linn (Verbanaceae) is commonly known as chaste tree (English), Nirnochi (Tamil) and jalanirgundi (Sanskrit) and is reported to have good medicinal values in traditional system of medicines. *Vitex Leucoxylon* Linn. (Verbenreae) is found commonly in India. This plant is a large deciduous tree, commonly known as Songarbhi (Marathi). Because of the same species, the plants look alike and it is difficult for the collection and authentication of the plant. This comparative study aims at differentiating the plants on the basis of pharmacognostical and phytochemical evaluations. Morphological studies of leaves showed the presence of various diagnostic characters. In the microscopical studies of both leaves showed the presence of vascular bundle, trichomes, epidermal cells, spongy parenchyma cells etc. Ash value and extractive value was determined for quality standard of drugs. Phytochemical investigation shows the presence of alkaloids, carbohydrate, phenolic compounds, flavonoids, protein and amino acids, tannins, phytosterols, saponins etc.

Keywords: *Vitex trifolia, Vitex leucoxylon, Pharmacognostical evaluation, Physico-chemical parameters, Phytochemical screening.*

Introduction

The World Health Organization (WHO) proves medicinal plants is the best source to obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand the properties, safety and efficacy[1].

Vitex trifolia Linn. belonging to the family of Verbenaceae is commonly known as chaste tree (English), Nirnochi (Tamil) and Jalanirgundi (Sanskrit). It is distributed throughout India in tropical and subtropical regions. Height of the plant is 1-3.5meter. Flowers are appearing in summer or late summer and 6-12 inch long. It is used in the treatment of rheumatic pains, inflammations, sprains, fever and anthelmintic, improves memory, favors the growth of hair, good for the eyes, leucoderma, bad taste in mouth and bronchitis, fever and in amenorrhea[2].

Vitex leucoxylon Linn (Verbenreae) is a large deciduous tree, commonly known as Songarbhi (Marathi), an excellent herbal crude drug found in nature which has the composition of the entire essential constituents required for the normal and good health of human. It is a small to large tree with a thick trunk and spreading crown and is found throughout the Indian Deccan peninsular up to an altitude of 900meters; and extends northwards up to Jhansi and parts of Bihar. The trees are generally found on river banks, streams and ponds. The leaves are smoked for reliving headache and catarrh and are also used for medicinal baths and anaemia[3]. General pharmacological studies revealed anti-psychotic, anti-depressant, analgesic, anti-inflammatory, anti-parkinsonian and anti-microbial activities of aqueous and ethanolic extracts of leaves of *Vitex Leucoxylon*[4,5] have studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves in acute inflammation model[6].

In the present study, an attempt was made to study the comparative pharmacognostical and

phytochemical evaluation of the above 2 species viz., *Vitex trifolia* and *Vitex leucoxylon.*

Material and Method

Collection and authentication

The leaves of *Vitex trifolia* Linn. and *Vitex Leucoxylon* Linn. were collected in the foothills of Yercaud, Salem, Tamil Nadu, in March 2011. The plant materials were taxonomically identified and authenticated by Dr. A. Balasubramanian, Consultant, Central Siddha Research Institute, Salem, Tamilnadu. The plants were deposited in the Herbarium of the Department of Pharmacognosy, Swamy Vivekanandha College of Pharmacy, Tamilnadu, India, for future reference. The leaves were separated and shade dried at room temperature for 10 days and coarsely powdered with a hand-grinding mill and the powder was passed through sieve no. 40 and preserved in the dry air tight container for further studies.

Macroscopic Examination

The leaves of *Vitex trifolia* and *Vitex leucoxylon* were studied individually for its morphological characters such as colour, odour, taste, shape, size, etc.

Preparation of the extract

The powdered material of *Vitex trifolia* and *Vitex leucoxylon* were extracted separately using the Soxhlet apparatus with different solvents[7] and an aqueous solvent for cold maceration. After extraction, the extracts were concentrated under reduced pressure.

Instruments used

Photographs of different magnifications were taken with Nikon Labphot2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background[8].

Microscopic evaluation

i) Preparation of specimen

The healthy leaves were selected carefully for the study of microscopical characters. The specimens were fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of TBA (tertiary- Butyl alcohol)[9]. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

ii) Sectioning of specimen

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12µm. De-waxing of the section was done by customary procedure[10]. The sections were stained with toludine blue (0.25 % having a pH of 4.7) as per the specified method[11]. Since Toludine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also observed. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies. Wherever necessary, sections were also stained with safranin, fast-green and N/10 Iodine to identify the presence of lignified cells and starch grains.

To study the histology of stomata, venation pattern and trichome distribution, paradermal sections (parallel to the surface of leaf) were taken. The clearing of leaf was done with 5% sodium hydroxide solution or epidermal peeling by partial maceration, employing Jeffrey's maceration fluid[9]. The mounting of macerated/cleared materials was performed with glycerin. Powdered materials of different parts were cleared with sodium hydroxide solution and mounted in glycerin medium after staining. Different cell components were studied and measured.

iii) Photomicrographs

Microscopic descriptions of tissues can be described with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto-2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since, these structures have birefringent property under polarized light; they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books[12,13].

Determination of Physicochemical Parameters

The dried powdered leaves were subjected to physicochemical analysis including fluorescence analysis[14,15] moisture content, total ash, water soluble

ash, acid insoluble ash, sulphated ash, alcohol soluble extractive and water soluble extractive[16] to determine the quality and purity of the plant materials.

Preliminary Phytochemical Screening

The dried powdered leaves were extracted with petroleum ether (60-80°C), chloroform and alcohol using soxhlet apparatus and aqueous extraction by cold maceration. The solvents were completely removed and reduced pressure by using vacuum evaporator. All the extracts were screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests[17,18].

Results and Findings

MACROSCOPIC EVALUATION

Vitex trifolia Linn. leaves are variable, some simple and some three foliolate; leaflets elliptic or oblong, obovate, usually obtuse, the terminal leaflet sessile, 5-6.3 by 2.5-3.8cm the lateral smaller, sessile, all glabrous above, very densely white-tomentose beneath, base tapering; common petioles 1.3-1.6cm long. The leaves are dark green in colour and bitter taste. (Fig.1)

Table 1 Determination of physical constants: (Ash values, Extractive values, foreign organic matter and Moisture content)

Evaluation parameters	Yield (% w/w)	
	Vitex trifolia	***Vitex leucoxylon***
Total ash	3.64	11.68
Water soluble ash	1.54	4.58
Acid insoluble ash	2.46	3.64
Sulphated ash	1.89	10.26
Alcohol soluble extractive	8.62	14.97
Water soluble extractive	4.35	23.98
Foreign organic matter	0.1	0.5
Moisture content	4.0	10.34

Vitex leucoxylon Linn. leaves compound, digitate or rarely trifoliate, opposite, decussate; rachis pulvinate, planoconvex in cross-section, minutely pubescent; petiolule 0.5–1.5cm long, canaliculate, glabrous; leaflets 5 (rarely 3), lamina 7–11.5 × 2–3.5cm, elliptic, apex acute to obtuse, base cuneate-attenuate, margin entire, chartaceous or thinly coriaceous, glaucous beneath, glabrous; midrib canaliculate above; secondary nerves 6–14 pairs; tertiary nerves reticulo-percurrent, not prominent. Inflorescence axillary corymbose cymes, minutely pubescent; flowers zygomorphic, sessile; corolla white with purplish pubescent anther lobes purple. (Fig.2)

Table.2 Behavior of the Powder of *Vitex trifolia* and *Vitex leucoxylon* with Different Chemical Reagents

Treatment	Colour	
	Vitex trifolia	***Vitex leucoxylon***
Powder as such	Pale green	Pale green
Powder + Conc.sulphuric acid	Greenish black	Yellowish black
Powder + Conc. nitric acid	Brownish yellow	Yellowish brown
Powder + Conc. Hydrochloric acid	Pale yellow	Brown
Powder + 5% I_2	Brownish yellow	Brownish yellow
Powder + 5M NaOH	Yellowish green	Greenish brown
Powder + glacial Acetic acid	Pale green	Yellowish brown
Powder + 80% H_2SO_4	Black	Yellowish brown

Table 3 Fluorescence Characteristics of Powdered Leaves of *Vitex trifolia*.

Reagents	Day light		UV Light	
	Vitex trifolia	***Vitex leucoxylon***	***Vitex trifolia***	***Vitex leucoxylon***
Drug Powder	Pale green	Pale green	Green	Pale green
Powder +1M NaOH	Greenish yellow	Greenish yellow	Yellowish green	Green
Powder + alcoholic 1M NaOH	Yellowish green	Dark green	Pale green	Pale green
Powder + 1M HCl	Yellowish green	Light brown	Greenish yellow	Faint green
Powder + 50 % HNO_3	Yellowish brown	Light yellow	Pale green	Pale green
Powder + 5% $FeCl_3$	Greenish black	Dark green	Dark green	Greenish yellow
Powder +80% H_2SO_4	Black	Yellowish brown	Greenish brown	Yellowish brown
Powder + Water	Greenish yellow	Greenish yellow	Green	Dark green
Powder+Conc.H_2SO_4	Brownish green	Black	Yellowish brown	Greenish brown

Table 4 Results of Phytochemical screening

Constituents	Pet. Ether		Chloroform		Ethanol		Aqueous	
	VT	VL	VT	VL	VT	VL	VT	VL
Alkaloids	-	-	+	+	-	+	-	+
Carbohydrates	-	-	-	-	+	+	+	+
Glycosides	-	-	-	-	+	-	+	-
Phytosterols	+	+	-	-	+	+	+	+
Phenolic compounds and Tannins	-	-	-	-	+	+	+	+
Flavanoids	-	-	-	-	+	+	+	+
Protein and amino acids	-	-	-	+	-	+	+	+
Gums and mucilages	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	+	-	+	-
Fixed oil and Fats	-	-	-	-	-	-	-	-
Volatile oils	-	-	-	-	-	-	-	-

Fig.1 The Leaves of *Vitex trifolia*

Fig.2 Leaf of *Vitex leucoxylon* Linn

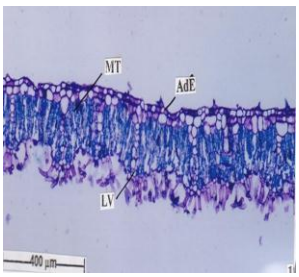
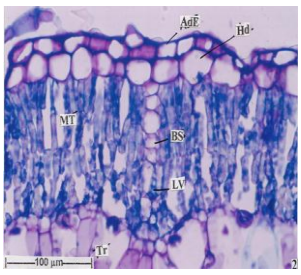


Fig.3.1T.S of Lamina through Lateral Vein Under Low Magnification



3.2T.S of Lamina through Lateral vein under Low magnification Enlarged

MICROSCOPIC EVALUATION

Vitex trifolia Linn. leaflet (Fig.3.1 & 3.2)

The leaflet has smooth and even adaxial surface and densely pubescent uneven abaxial side. It is 140 µm thick along the ridged part of the leaflet and 120 µm thick in between the ridges. The adaxial epidermis is thick and prominent; the epidermal cells are rectangular and tangentially oblong, fairly thick walled and have thin cuticle. These are a subepidermal layer of dilated and hyaline squarish or cylindrical cells. The epidermal layer is 10µm thick; the hypodermal layer is 15µm thick. The abaxial epidermis is narrow with thin walled cylindrical cells. They bear dense non-glandular trichomes. The mesophyll consists of four layers of vertically elongated narrow cylindrical cells. The height of these cells is more along the first row and the height is reduced gradually towards the lower part. All these cells are palisade; and spongy parenchyma cells are not evident.

Vitex leucoxylon Linn. leaflet

The leaflets exhibits dorsiventral symmetry with reference of the structure of the lamina and midrib (Fig.7.1). The midrib is plano – convex; the adaxial side is flat and abaxial side is semicircular. The midrib is 700 µm thick and 700 µm wide. The midrib consists of a thin epidermal layer of small thick walled cells with prominent cuticle. The ground tissue includes 5-9 layers of small, cirular compact parenchyma cells (Fig 7.2).

Vitex trifolia Linn. Midrib (Fig.4.1 & 4.2)

The midrib is thick, projecting prominently on the abaxial side with a short and wide adaxial hump. The epidermal layer is a narrow with small cells. The ground tissue of the midrib consists of circular thin walled compact parenchyma cells. The vascular system of the midrib has a median, wide arc-shaped strand and a group of adaxial accessory strands. The median arc of vascular strand consists of several parallel rows of xylem elements with narrow parenchymatous space in between the xylem rows. In each row of xylem, these are about six cells which are angular and thick walled. The adaxial accessory bundles are circular and collateral. The strand has a small group of xylem elements and wide zone of phloem.

[ADE-Adaxial Epidermis; BS-Bundle Sheath Cells; Hd-Hypodermis; LV-Lateral Vein;
MT-Mesophyll Tissue; Tr-Trichome].

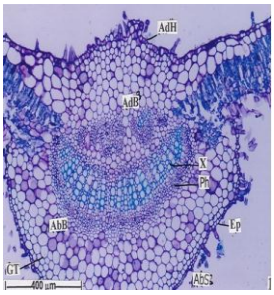


Fig.4.1 T.S of Leaf through Midrib With Lamina

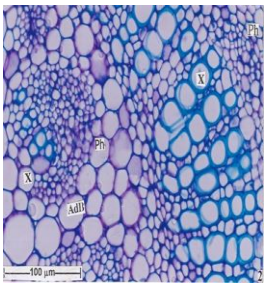


Fig.4.2 T.S of Midrib – Vascular bundle Enlarged

[AbB-Abaxial Bundle; AbS-Abaxial Side; AdB-Adaxial Bundle; AdH- Adaxial Hump; Ep-Epidermis; GT-Ground Tissue; Ph-Phloem; X-Xylem].

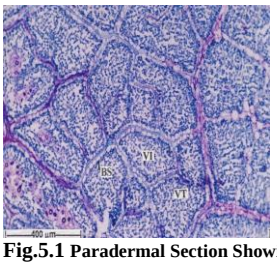


Fig.5.1 Paradermal Section Showing Vein- Islets and Vein-termination

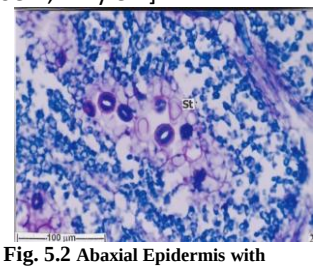


Fig. 5.2 Abaxial Epidermis with Stomata

[Bs-Bundle sheath cells; St-Stoma; VI-Vein-islet; VT-Vein-termination]

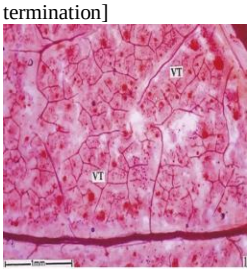


Fig.6.1 Cleared Leaf showing Vein- islets And Vein termination

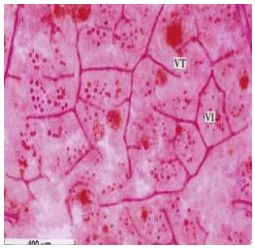
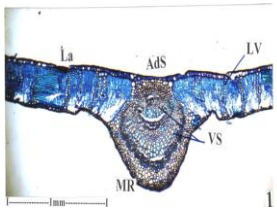


Fig.6.2 Cleared Leaf showing Vein - islets and Vein Termination Enlarged

[VI-Vein-islets; VT-Vein-termination].

Vitex leucoxylon Linn. Midrib

The vascular system of the midrib is somewhat complex. There is a wide, planoconvex sclerenchyma cylinder enclosing a wide prominent arc of abaxial vascular strand and adaxial small groups of irregularly disposed adaxial vascular strands. The abaxial arc consists of several parallel lines of 3–5 xylem elements situated along the abaxial part. Phloem occurs in uniformly thick arc abutting the xylem is on the upper part of the midrib, there is a narrow, thick band of vascular strands with a few xylem elements in short parallel rows and a wide and thick segment of phloem situated on the lower end of the xylem (Fig. 8.1). In addition to this adaxial segment, there are three or four small, less prominent vascular strands with small clusters of xylem and phloem, xylem being placed at the abaxial end (Fig. 8.2). Thus, these are three groups of vascular strands, one abaxial wide, deeply bowl shaped, second adaxial thick flat plate and the third one being small less prominent, three or four nests.



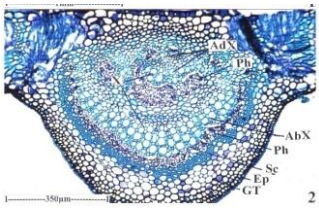


Fig. 7.1 &2 T.S. of *Vitex Leucoxylon* Linn. leaflet through midrib.

(La- Lamina; AdS-Adaxial Side; LV-Lateral Vein; VS-Vascular Strand; MR-Midrib)

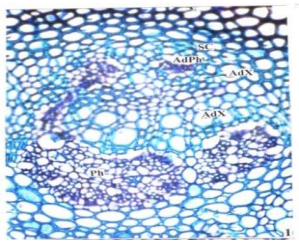


Fig. 8.1 T.S. of midrib- adaxial vascular strands enlarged

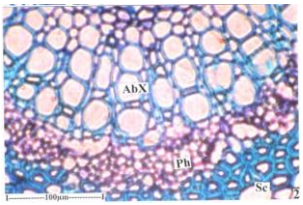


Fig. 8.2 T.S. of midrib – abaxial vascular strand enlarged

(SC-Sclerenchyma; AdPh-Adaxial Phloem; AdX- Adaxial Xylem; Ph-Phloem; AbX-Abaxial Xylem)

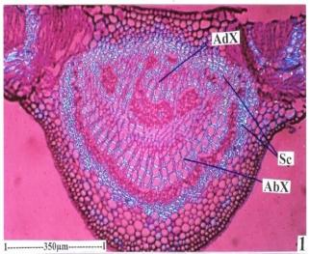


Fig.9.1 T.S. of midrib as seen under polarized light to show the lignified cells

(AdX-Adaxial Xylem; Sc-Sclerenchyma; AbX-Abaxial Xylem)

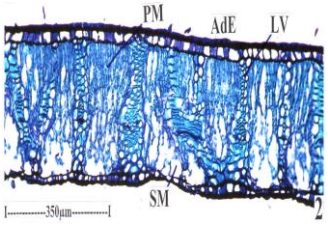


Fig.9.2 T.S. of lamina

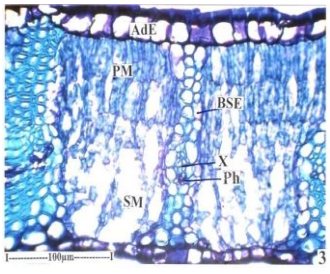


Fig.9.3 Vascular strand of the lamina enlarged

(PM-Palaside Mesophyll; AdE-Adaxial Epidermis; LV-Lateral Vein; SM-Spongy Mesophyll;
BSE-Bundle Sheath Epidermis)

***Vitex leucoxylon* Linn. Lamina**

The lamina has smooth and even surfaces. It is 330 µm thick. It exhibits xeromorphic structure. The adaxial epidermal cells are vertically elongated, thick walled and heavily cuticularized; they are 20 µm thick. The abaxial epidermis is thin; the cells are narrowly rectangular and thick walled, measuring 10 µm thick (Fig 9.1). The palisade mesophyll is 2 layered; the cells are narrow and vertically elongated; the palisade zone is 50 µm in height. The spongy mesophyll consists of 8–10 layers of small, lobed, loosely arranged parenchyma cells with wide intercellular spaces (Fig 9.2). The vascular strands of the lateral veins occur in vertical pillars at regular intervals. They have small collateral vascular bundles of xylem and phloem surrounded a layer of bundle sheath fibres with adaxial and abaxial extensions (Fig 9.3).

***Vitex trifolia* Linn. Venation pattern (Fig.5.1, 5.2, 6.1 & 6.2)**

The venation pattern of the lamina shows uniformly thin and straight lateral veins. The veins are covered by hyaline parenchymatous bundle sheath cells (Fig.5.1). The vein-islets are not well defined; they are open and not distinct. The vein-terminations are distinct; they are long, slender, and straight or slightly bend and unbranched. Stomata are located in the furrows of the abaxial epidermis. They are circular and are anomocytic type.

***Vitex leucoxylon* Linn. Venation pattern**

Venation was studied by paradermal sectioning (Fig. 10.1.). The venation is densely reticulate. Both major and minor veins are thick and straight with prominent bundle sheath cells. The vein islets narrow, polygonal in outline and dense. The vein terminations are absent in most of the islets. When present, the terminations are short, thick and stumpy.

***Vitex leucoxylon* Linn. Stomata**

Stomata are crowded in shallow depression and within the boundary of the vein–islets (areoles) the stomata are mostly cyclocytic type; they are surrounded by a circle of 4–6 subsidiary cells (Fig. 10.2). The guard cells are broadly elliptical, slightly thick walled, with wide stomatal pore. The stoma is 30 µm in length and breadth. The epidermal cells are narrow, thick walled with smooth walls.

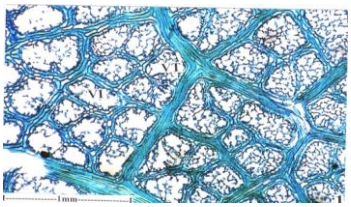


Fig. 10.1 Paradermal sectional view of the venation pattern of the lamina

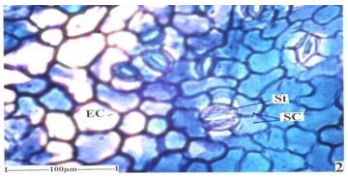


Fig. 10.2 Stomata showing cyclocytic subsidery cells (VI-Vein Islet; VT-Vein Termination; EC-Epidermal Cells; SC-Subsidery Cells)

PHYSICOCHEMICAL PARAMETERS

Fresh leaves of *Vitex trifolia and Vitex Leucoxylon* were collected and subjected to various physicochemical parameters such as moisture content, foreign matters, total ash, Acid insoluble ash, water soluble ash, sulphated ash and various extractive values are shown in Table 1.

Fluorescence Analysis

The fluorescence analysis of powder with various reagents is observed under day light and U.V. light is given in Table 3**.**

Preliminary Phytochemical Screening:

The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of various phytoconstituents like carbohydrate, phenolic compounds, flavonoids, protein and amino acids, tannins, phytosterols and saponins. The results were shown in Table 4.

Conclusion

The present comparative study is related to pharmacognostical, physical constants and preliminary phytochemical screening of *Vitex trifolia* Linn. and Vitex leucoxylon Linn. provided useful information about its correct identity and evaluation. From the above discussion, it was concluded that the results would serve as a useful gauge standardization of the leaf material and ensuring quality formulations. There is an urgent need for the documentation of herbal drugs, systematic phytochemical and pharma-cognostical studies of medicinal plants and their natural products.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.