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Comparative Study on Phytochemical Screening, Antioxidant, Antimicrobial and Antidiabetic Activity in the Ethanolic Extract of *Azadirachta indica* (Indian Neem) and *Melia dubia* (Malabar Neem) Flowers

Kaviya S.[1]*, Sudha N.[1] and Parimala P.[2]

[1]Department of Biochemistry, Ethiraj College for Women, Chennai 600008, Tamil Nadu, India
[2]Gojan School of Business and Technology, Chennai, Tamil Nadu, India

**Corresponding Author*

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Abstract: India is quite well-known for medicinal plants and its therapeutic benefits since time immemorial. The present study emphases on comparing Ethanolic flower extract (EFC) of *Azadirachta indica* (Indian Neem) and *Melia dubia* (Malabar Neem) for phytochemical screening, quantification, antioxidant, antimicrobial and antidiabetic activity which altogether depicts the antidiabetic potential of both neem flowers. The initial phytochemical screening indicated the presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids, tannins, glycosides in Indian and Malabar neem while quinones was observed in Indian neem only. Next, increased concentration of alkaloids (8.2±0.27 mg of AE/g of extract) in Malabar neem and increased concentration of tannins (2.5±0.01 mg GAE/g of extract), flavonoids (2±0.03 mg QE/g of extract), phenols (3.7±0.03 mg GAE/g of extract) in Indian neem was observed. Next, antioxidant activity was assessed with Nitric oxide radical scavenging assay. The EFC of *Azadirachta indica* and *Melia dubia* exhibited potent antioxidant activity with maximum inhibition of 31% and 35% at 3000 µg/ml, respectively. The antimicrobial activity of EFC of *Azadirachta indica* against two prominent diabetic-dental caries associated organisms, *Candida albicans* and *Enterobacter faecalis* showed zone of inhibition of 12 mm and 13 mm at 1500 µg/ml, respectively. Antimicrobial activity of EFC of *Melia dubia* showed zone of inhibition of 11 mm at 1500 µg/ml against both *Candida albicans* and *Enterobacter faecalis*. The antidiabetic potential was assessed under two parameters, α-amylase and α-glucosidase activity. Under α-amylase activity, the EFC of *Azadirachta indica* showed great antidiabetic potential with maximum inhibition of 119% compared to *Melia dubia* that showed 85% at 3000 g/ml. In α-glucosidase activity, the EFC of *Azadirachta indica* showed great antidiabetic potential with maximum inhibition of 170% compared to *Melia dubia* that showed 145% at 3000g/ml. These findings suggest that ethanolic flower extract of *Melia dubia* showcases potent antioxidant, antimicrobial and antidiabetic activity when compared to that of *Azadirachta indica*.

Keywords: *Azadirachta indica*, Antioxidant, Antimicrobial, Antidiabetic, *Melia dubia*, Ethanolic flower extract

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Introduction

Azadirachta indica, the Neem is popularly referred to as "village pharmacy," nature's drug store in India. It is an evergreen medicinal tree belongs to the family of Mahogony, Meliaceae, commonly known as Indian lilac, nimtree or margosa. Numerous phytochemicals, such as azadirachtin, nimbolide, gedunin, azadirone, and salannin, have been found in the whole neem tree that encompasses many pharmacological activities namely anticancer, antibacterial, antifungal, antiviral, anthelmintic, antimalarial, antipyretic, antidiabetic, analgesic, anti-inflammatory, anti-leishmanial, neuroprotective and cardioprotective properties (Braga *et al*., 2021). Azadirachtin, a primary bioactive component in neem oil, leaves, flowers, and fruits has insecticidal qualities (Chaudary *et al*., 2017). One of the varieties of neem is *Melia dubia*, popularly known as Malabar neem is the largest, deciduous rapidly growing tree which belongs to the family of Meliaceae (Rawat *et al*., 2016). Triterpenoids typically act as antidiabetic agents seem to be found in the leaves, seeds and fruits of this plant (Nazaruk *et al*., 2015). Neem flowers were always not considered for research investigations. Moreover, Indian and Malabar neem were not compared so far and antidiabetic potential of Malabar neem flower is not investigated so far. The present study emphases on comparing basic preliminary phyto-chemical screening, phytochemical quantification, antioxidant activity, antimicrobial and antidiabetic activity ascertained which altogether depicts the antidiabetic potential of both neem flowers.

Materials and Methods

Sample Collection and Processing

Indian and Malabar neem flowers were air-dried, powdered, and dried again. The extracts were prepared with 50% ethanol. After 24 h incubation, the extracts were filtered using Whatman No. 1 paper. 50% ethanol was used to generate the 2% crude extracts, which were then preserved for further examination.

Preliminary Phytochemical Screening

The extract was tested for the presence of bioactive compounds by using the following standard methods given by Varughese and Tripathi (2013).

Phytochemicals Quantification

Quantification of Alkaloids

The extract was mixed with in 2N HCl and filtered. Then, 1 ml of extract was combined with 5 ml of bromocresol green (BCG) solution, 5 ml of phosphate buffer (pH 4.7) and extracted with (5 ml) chloroform. Then, chloroform layer, was diluted up to 10 ml with chloroform, and absorbance at 470 nm was measured. Alkaloid content is expressed as mg AE/g of extract.

Quantification of Tannins

0.1 ml extract was mixed with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution, diluted to 10 ml with distilled water. Absorbance was measured at 720 nm after 30 min incubation with standard GA solutions (20, 40, 60, 80, 100 µg/ ml). The tannin content is expressed as mg GAE/ g of extract.

Quantification of Phenols

Different volumes of aqueous extracts (0.02–0.1 ml) were diluted with distilled water (0.5 ml) and combined with 0.25 ml of Folin-Ciocalteu reagent (1N) and 1.25 ml of 20% sodium carbonate. The absorbance at 550 nm was measured after 40 min incubation. The total phenolic content is expressed as mg GAE/g of extract (Nabavi *et al.*, 2008).

Quantification of Flavonoids

0.5 ml of aqueous extract was combined with 2 ml of distilled water, 0.15 ml of 5% $NaNO_2$, 0.15 ml of 10% $AlCl_3$, 2 ml of 1 mM NaOH and made up to 5 ml with distilled water. After 15 min incubation, the absorbance was measured at 510 nm. The flavonoid content is expressed as mg QE/g (Nabavi *et al.*, 2008).

Assay of Antioxidant Activity

Determination of Nitric Oxide Radical Scavenging Activity (*Griess Ilosvay reaction method*)

2 ml of sodium nitroprusside (10 mM) and 0.5 ml of phosphate buffer (pH-7.4) was mixed with 0.5 ml of samples and vitamin-C (standard); incubated for 150 min at 25 °C. Then, 0.5 ml of nitrite and 1 ml of sulfanilic acid reagent (0.33% of sulfanilic acid in 2% glacial acetic acid) was added and kept for 5 min. Then, 1 ml of 1% naphthyl ethylene diamine dihydrochloride (NEDD) was added and incubated for 30 min at 25 °C. Absorbance of pink colour of the solution was read at 540 nm (Patel *et al.*, 2010).The percentage of nitric oxide inhibition was calculated as follow:

$$(\%) \text{ of nitric oxide radical scavenging assay} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where, A_0 - absorbance of control, and A_1 - absorbance of the treated sample.

Assessment of Antidiabetic Activity

In vitro α -amylase Inhibitory Activity

The substrate solution (0.2 ml) containing starch and pancreatic amylase solution (0.1 ml with a concentration of 2 units/ml) were added to sample solution (0.2 ml) at different concentrations (1000 µg/ml to 3000 µg/ml).The reaction lasted for 10 min at 37 °C before being stopped by adding 0.5 ml of 50% acetic acid to each tube and then centrifuged for 5 min at 4 °C at 3000× rpm. OD of supernatant was measured at 595 nm with acarbose (α-amylase inhibitor) as a positive control (Telagari and Hullatti, 2015). The inhibitory activity of the enzyme was determined using the formula:

$$\text{Activity inhibition} = [(A - B)/A] \times 100$$

Where, A is the control absorbance, and B is the sample absorbance.

In vitro α-glucosidase Inhibitory Activity

A total of 50 µl of samples at different concentrations (1000 to 3000 µg/ml), were prepared and incubated with 10 µl of α-glucosidase (maltase at a concentration of 1 unit/ml) and 125 µl of 0.1 M phosphate buffer for 20 min at 37 °C. 20 µl of substrate p-Nitrophenyl α-D-glucopyranoside (pNPG) at 1 M was prepared and added to preparation and incubated for 30 min. A total of 50 µl of Na_2CO_3 (0.1 N) was added to stop the reaction. OD was measured at 405 nm with acarbose as the positive control (Telagari and Hullatti, 2015). The inhibitory activity of the enzyme was determined using the formula:

$$\text{Activity inhibition} = [(A - B)/A] \times 100$$

Where, A is the absorbance of control, and B is the absorbance of sample.

Evaluation of Antimicrobial Activity

Preparation of Inoculum

The antimicrobial properties of plant extracts were tested against *Candida albicans* and *Enterococcus faecalis.* Bacteria were pre-cultured in Mueller Hinton broth (MHB) overnight in a rotary shaker at 37 °C. Afterward, each strain was adjusted at a concentration of 10^8 cells/ml using 0.5 McFarland standard (Bhalodia and Shukla, 2011).

Antimicrobial Screening

Agar disc diffusion method was used to screen the antibacterial activities of extracts as described by Daoud *et al*. (2015). One ml of fresh bacterial culture was pipetted in the center of sterile Petri dish. Then, 100 µl of each extract (20% w/v) was added to respective discs. The concentration of extracts (20% w/v) has been selected based on previous literature. The plates were placed in the refrigerator for 30 min to let the extracts diffusion well into the agar. Then, the plates were incubated at 37 °C for 18 h. Antimicrobial activity was detected by measuring the zone of inhibition (including the discs diameter) appeared after the incubation period. DMSO at a concentration of 10% was employed as a negative control.

Statistical Analysis

In this study, all the experiments were carried out in three independent biological replicates, and data are expressed as mean ± standard deviation for each set of conditions. Statistical significance of

Table 1: Preliminary phytochemical screening of ethanolic extract of flowers of *Azadirachta indica* and *Melia dubia*

S. No.	Experiments	Inferences	
		Azadirachta indica	***Melia dubia***
1	Test for Alkaloids	+	+
2	Test for Carbohydrates - Molisch's Test	+	+
3	Test for Carbohydrates - Benedict's Test	+	+
4	Test for Carbohydrates - Fehling's Test	+	+
5	Test for Phenols	+	+
6	Test for Flavonoids - alkaline reagent test	+	+
7	Test for Flavonoids - Con. HCl	+	+
8	Test for Sterols	++	++
9	Test for Diterpenoids	+	+
10	Test for Gum and mucilages	_	_
10	Test for Tannins	+	+
11	Test for Quinones	+	_
12	Test for Glycosides	+	+

++ Highly Present; + Present; - Absent (n=2)

the data was tested through one - way analysis of variance (ANOVA) using least significant difference with $p<0.05$, $p<0.01$, $p<0.001$.

Results and Discussion

Phytochemical screening of ethanolic extracts of Azadirachta indica and Melia dubia:

The presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids, tannins, glycosides in *Azadirachta indica* and *Melia dubia* was observed. In addition, quinones was observed in *Azadirachta indica* only (Table 1).

The quantification of phytochemicals in ethanolic extract of flowers of Azadirachta indica and Melia dubia:

The varying concentration of alkaloids, tannins, flavonoids and phenols in ethanolic extract of flowers of *Azadirachta indica* and *Melia dubia* is represented in Figure 1. It showed the increased concentration of alkaloids in *Melia dubia* and increased concentration of tannins, flavonoids, phenols in *Azadirachta indica* (Fig. 1).

Determination of Antioxidant Activity

Nitric Oxide Radical Scavenging Assay:

Antioxidant activity of Indian neem (*Azadirachta indica*) and Malabar neem (*Melia dubia*) flowers revealed the gradual increase in nitric oxide scavenging activity on increasing the concentration (Fig. 2). It also depicted the increased nitric oxide radical inhibition by Malabar neem compared to Indian neem (Fig. 2).

The antimicrobial activity of *Azadirachta indica* and *Melia dubia* flowers against two prominent diabetic-dental caries associated organisms, *Candida albicans* and *Enterobacter faecalis* demonstrating that both imparts effective antimicrobial activity towards tested micro-organisms (Table 2; Fig. 3).

Determination of Antidiabetic Activity

α-Amylase inhibition assay:

The ethanolic flower extract of *Azadirachta indica* showed great antidiabetic activity with maximum inhibition of 119% compared to *Melia dubia* that showed 85% at 3000 g/ml (Fig. 4).

α-Glucosidase inhibition assay:

Ethanolic flower extract of *Azadirachta indica* showed great antidiabetic potential with maximum inhibition of 170% compared to *Melia dubia*, showed 145% at 3000 g/ml (Fig. 5).

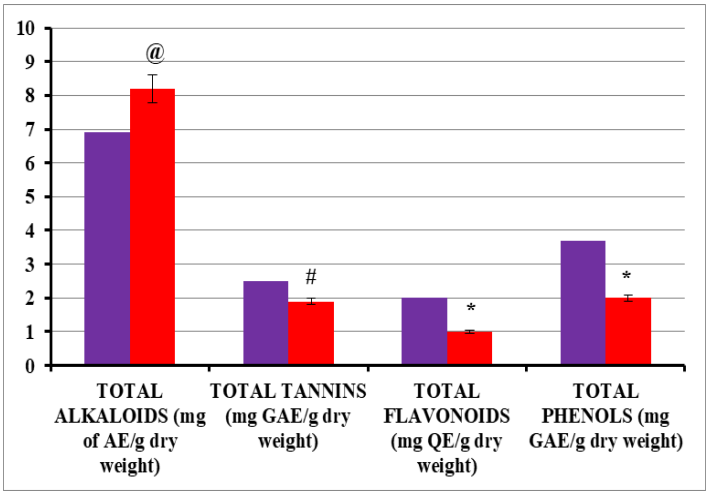


Fig. 1: Quantification of phytochemicals in ethanolic extract of flowers of *Azadirachta indica* (Indian neem) and *Melia dubia* (Malabar neem) (n=4; values are Mean ± SD). Symbols indicate significant differences.

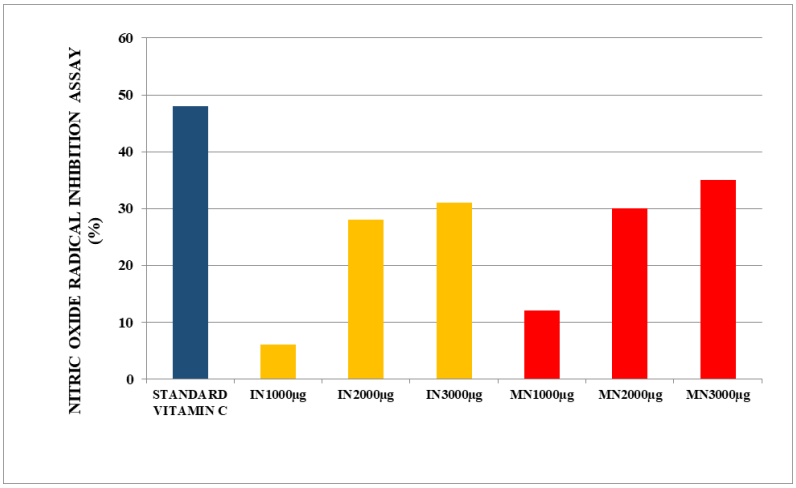
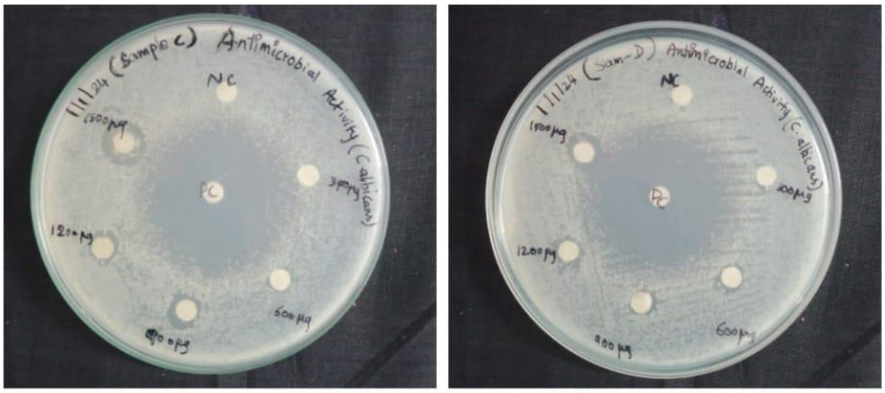
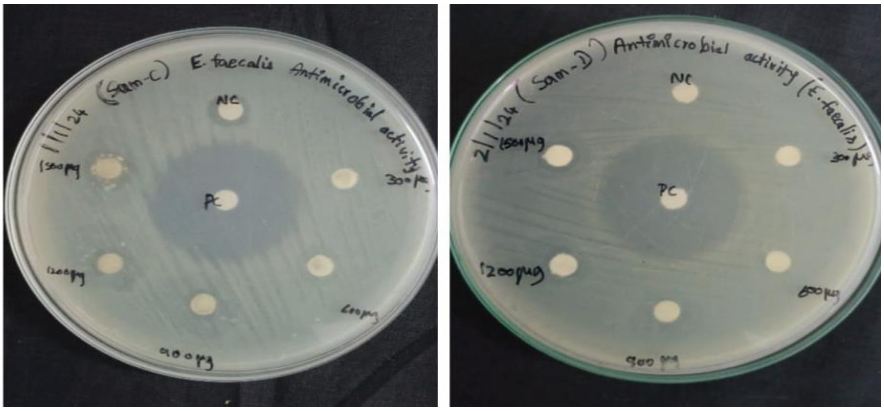


Fig. 2: The antioxidant activity of Indian neem (*Azadirachta indica*) and Malabar neem (*Melia dubia*)flowers showing gradual increase in nitric oxide scavenging activity on increasing the concentration. IN: Indian Neem; MN: Malabar Neem.

Based on qualitative and quantitative research, the current study's findings show that both Malabar and Indian neem are highly phytochemically endowed. Indian and Malabar neem were subjected to phytochemical screening, as shown in Table 1. The study revealed that Indian and Malabar neem include alkaloids, carbohydrates, flavonoids, phenols, sterols, diterpenoids, tannins, and glycosides. Moreover, only Indian neem showed presence of quinones. In the context of phytochemicals quantification. Figure 1 shows the increased concentration of alkaloids in Indian neem and increased concentration of tannins, flavonoids, phenols in Malabar neem when compared to Indian neem. Subsequently, antioxidant activity was exhibited, which indicated a progressive rise in nitric oxide scavenging action as the concentration was increased (Fig. 2). It is also illustrated how Malabar neem inhibited nitric oxide radicals more than Indian neem does as it comprised of phenolic components including flavonoids, phenols, alkaloids, and tannins that typically contributes to anti-cancerous and antioxidant properties.



Candida albicans



Enterobacter faecalis

Fig. 3: The antimicrobial activity of *Azadirachta indica* and *Melia dubia* flowers against *Candida albicans* and *Enterobacter faecalis.* Sample C: Ethanolic extract of flowers of *Azadirachta indica* and Sample D: Ethanolic extract of flowers of *Melia dubia.*

Table 2: Zone of inhibition in mm

Candida albicans						
Sample	300 µg	600 µg	900 µg	1200 µg	1500 µg	PC
Azadirachta indica	NI	8 mm	10 mm	9 mm	12 mm	36 mm
Melia dubia	NI	8 mm	9 mm	10 mm	11 mm	34 mm
Enterococcus faecalis						
Sample	300 µg	600 µg	900 µg	1200 µg	1500 µg	PC
Azadirachta indica	8 mm	10 mm	9 mm	12 mm	13 mm	33 mm
Melia dubia	NI	8 mm	9 mm	11 mm	11 mm	31mm

An adequate oral health and proper glycemic control could help in abolishing the caries risk and its complications. The antimicrobial impact of flowers was tested towards two prominent diabetic-dental caries associated organisms *Candida albicans* and *Enterobacter faecalis* which proved an effective inhibition of microbial growth suggesting their antimicrobial effect and the associated aid in lowering diabetic complications (Fig. 3). Considering this, key dietary carbohydrate

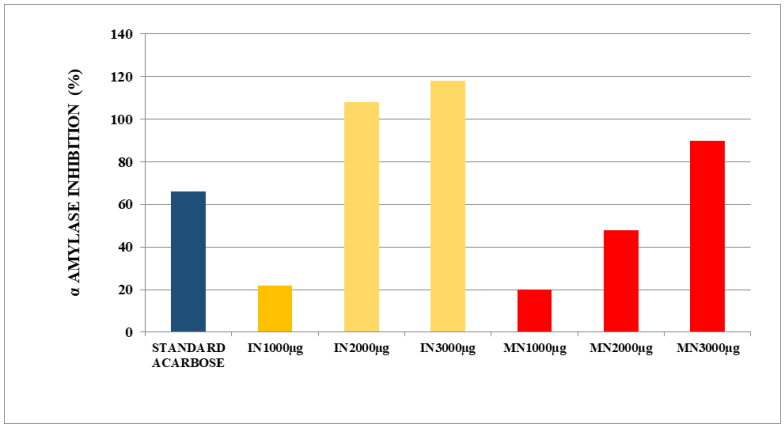


Fig. 4: α-Amylase inhibition assay. IN: Indian Neem MN: Malabar Neem.

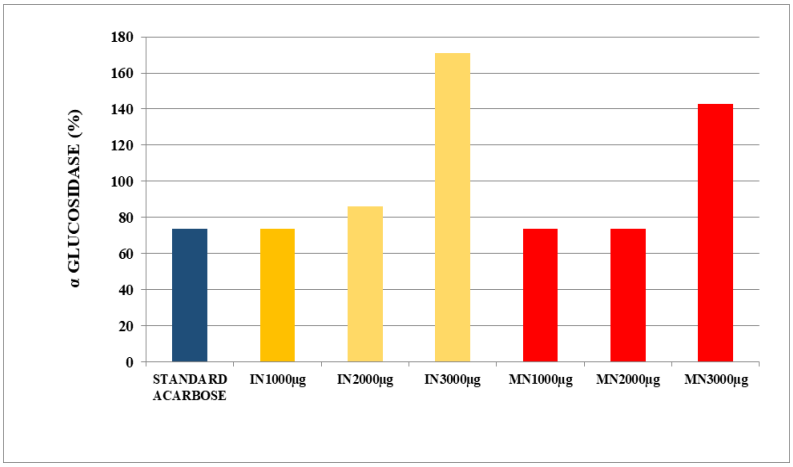


Fig. 5: α-Glucosidase inhibition assay. IN: Indian Neem MN: Malabar Neem.

metabolizing enzymes inhibition (α amylase and α glucosidase) were tested on addition of flowers of Indian and Malabar neem. Both flower extracts were found to inhibit the α amylase and α glucosidase activity highlighting their strong antidiabetic potential while Indian neem flowers had extremely considerable antidiabetic efficacy. But then the difference between Indian and Malabar neem flowers was insignificant (Figs. 4, 5).

The research for alternate remedies (from the plant kingdom) for diabetes mellitus will continue all over the world as the disease poses many challenges not only to the physician but as well as to the researcher. The study anticipates deep insight before endorsing them as a natural treatment or antidiabetic supplement.

Conclusion

Phytochemical analysis of ethanol extracts of flowers of *Azadirachta indica* and *Melia dubia* was carried out. Antioxidant, antidiabetic and antimicrobial activity was also assessed. From the study it was inferred that, Malabar neem showed higher antioxidant activity. Prominent zone of inhibition displayed significant antimicrobial activity in strains of *Candida albicans* and *Enterobacter faecalis*. Substantial antidiabetic potential was detected. Further study was extrapolated to find out the active component present in the flowers of both the plants. Using *in silico* analysis, the active component can be employed in drug designing which can be used for treating various ailments.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Biochemistry, Ethiraj College for Women, Chennai, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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