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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY ASSESSMENT OF YELLOW WOOD ROOT (*ARCANGELISIA FLAVA* (L.) MERR.) USING THE FRAP AND ABTS METHOD

Maria Sheryll Oktaverlyn[1], Noer Saelan Tadjudin[2*], Eny Yulianti[3], Frans Ferdinal[4]
[1]Undergraduate Medical Study Program, Faculty of Medicine, Tarumanagara University, Jakarta
[2]Department of Mental Health Sciences, Faculty of Medicine, Tarumanagara University, Jakarta
[3,4]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia

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Corresponding Author:
E-mail: noert@fk.untar.ac.id

ABSTRACT

Background: Yellow wood root/ *Arcangelisia flava*, a traditional medicinal plant commonly used in Kalimantan, Indonesia, has been found to possess strong antioxidant properties. Although previous studies have reported that this plant contains certain phytochemicals and exhibits antioxidant activity, other specific phytochemical composition of the root and its antioxidant capacity using the FRAP and ABTS method remain poorly understood. **Objective:** This study aimed to examine the phytochemical content and antioxidant activity of the methanol extract from *Arcangelisia flava* (yellow wood) root. **Methods:** The research was conducted using an *in vitro* experimental approach to evaluate the presence of secondary metabolites and antioxidant capacity through the *Ferric Reducing Antioxidant Power* (FRAP) and *2,2'-azinobis-(3-ethylbenozothiazoline-6- sulfonate)* (ABTS) assays. Extraction was performed on 75 grams of dried yellow wood root simplicia using methanol as the solvent, yielding 21.57 grams of extract (28.76%). **Results:** Phytochemical analysis detected a range of secondary metabolites including alkaloids, terpenoids, phenolics, quinones, saponins, tannins, coumarins, betacyanins, cardiac glycosides, flavonoids, and glycosides, while steroids and anthocyanins were not detected. The antioxidant activity was measured at extract concentrations of 10-30 μg/mL. In the FRAP assay, the extract reduced Fe^{3+} to Fe^{2+} with an IC_{50} value of 17.48 μg/mL, while in the ABTS assay, it neutralized free radicals with an IC_{50} of 21,99 μg/mL. Both results indicate a strong antioxidant potential and are comparable to the Trolox standards (IC_{50} = 10.54 μg/mL and 13.27 μg/mL, respectively). **Conclusion:** These findings support the traditional use of *Arcangelisia flava* (L.) Merr., suggesting its potential as a source of natural antioxidants. Further research is recommended to explore its mechanisms and potential applications in health and disease prevention.

BACKGROUND

The transformation of healthy organ function and its systems into disease is caused by oxidative stress, which damages cell structures within human organs. During normal cellular activity, free radicals and oxidants are formed internally, though they may also originate from environmental sources such as pollution, radiation, cigarette smoke, and pharmaceutical agents. However, when produced in excess, this condition is referred to as oxidative stress, a condition that can damage cell membranes and other cellular components such as proteins, lipoproteins, lipids and DNA. It arises when there is an imbalance between the production and elimination of reactive oxygen species (ROS) or reactive nitrogen species (RNS). This indicates a pathological condition.[1]

A group of compounds known as antioxidants function to stabilize free radicals and reactive oxygen species (ROS), thereby protecting cells from oxidative damage, which is essential for optimal physiological function. Antioxidants can be found in various plants and traditional medicines. Compounds such as phenolic acids, flavonoids, carotenoids, and

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anthocyanins can provide protective effects against the toxic impact of excessive ROS.[2] Consuming antioxidant-rich foods is vital for maintaining health and preventing illness.

Indonesia, as an archipelagic country, is known for its high biodiversity, encompassing a wide variety of flora and fauna. One well-known plant that is commonly used by the Dayak community in Kalimantan is the yellow wood root, or *Arcangelisia* flava in Latin, which belongs to the *Menispermaceae* family. Traditionally, local people use the yellow wood root to treat fever, hepatitis, malaria, infections, digestive disorders such as dysentery, and cancer wounds. Several studies have shown that preparations made from yellow wood root possess antimalarial, antibacterial, antioxidant, antidiabetic, and anticancer properties.[3] The medicinal potential of yellow wood root may be due to the presence of compounds such as phenolics, alkaloids, flavonoids, saponins, and tannins. Additionally, the roots contain glycosides and alkaloids, especially isoquinoline-type alkaloids including berberine, jatrorrhizine, and palmatine.[4] These compounds act as antioxidants that react with various free radicals, thereby neutralizing cellular imbalances.

Berberine is a golden-yellow alkaloid found in the roots and stems of this plant. Its anti-inflammatory effects are achieved through the suppression of pro-inflammatory cytokines, including IL-1β and TNF-α. In addition, yellow wood root also offers hepatoprotective effects due to the presence of palmatine, a compound recognized for reducing liver damage by suppressing oxidative stress and preventing apoptosis in hepatocytes.[5] Moreover, according to previous studies from the Center for Development of Advanced Sciences and Technology at the University of Jember, yellow wood is a source of flavonoids with therapeutic potential for metabolic disorders. Furthermore, the phenolic extract of yellow wood has demonstrated antioxidant activity comparable to those of standard vitamin C.[6]

The Ferric Reducing Antioxidant Power (FRAP) assay, developed by Benzie and Strain[7], is a widely used colorimetric method to evaluate antioxidant capacity by measuring the reduction of ferric (Fe^{3+}) to ferrous ions (Fe^{2+}). This reduction converts the (Fe(TPTZ)3+) complex into a blue-coloured (Fe(TPTZ)2+) complex, with color intensity measured spectrophotometrically. Performed under acidic conditions, the FRAP assay is simple, fast, and suitable for analyzing plant extracts and biological samples. On the other hand, the ABTS assay, introduced by Miller et al., is known for assessing the total antioxidant capacity in biological fluids. It uses the ABTS radical cation ($ABTS^{\bullet+}$), which is blue-green and becomes decolorized when neutralized by antioxidants. The extent of decolorization is measured by spectrophotometry.[7]

Both FRAP and ABTS are effective, rapid, and complementary methods for measuring antioxidant potential across a wide range of sample types, including natural products and functional foods, as well as medicinal plants.

Nevertheless, research on other phytochemical compounds such as terpenoid, quinones, saponins tannins, coumarins, betacyanins, cardiac glycosides and glycosides, as well as the antioxidant potential of yellow wood root using the FRAP and ABTS method, remains limited. Therefore, this study aims to further investigate the various phytochemical content and antioxidant capacity of *Arcangelisia flava* (L.) Merr. Root extract using both the FRAP and ABTS assays.

METHODS

This study is an experimental research with an *in vitro* and bioassay approach, conducted at the Laboratory of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University, West Jakarta, from December 2024 to May 2025. The sample used was the root of *Arcangelisia flava* (yellow wood), obtained in a dried condition from Pangandaran, West Java. The plant material was taxonomically determined and authenticated at Lansida Herbal Technology, Yogyakarta, Indonesia. The research process began with the preparation of yellow wood root simplicia, followed by extraction using the percolation method with methanol as the solvent. The resulting extract was evaporated to separate the solvent, and part of it was used in phytochemical screening to identify various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tannins, and others using each specific reagents.[8]

The antioxidant capacity assay was performed using the Ferric Reducing Antioxidant Power (FRAP) method. The FRAP solution was prepared by combining 25 mL sodium acetate, 2.5 mL $FeCl_3$, and TPTZ solution (100 mL distilled water and 150 mg

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TPTZ was dissolved in 40 mM HCl), and used in the evaluation of extract's antioxidant activity at various concentrations (10–30 µg/mL). The absorbance was recorded at a wavelength of 594 nm using a spectrophotometer and compared to a standard Trolox solution. Antioxidant activity percentage was determined based on the absorbance readings and the IC_{50} value through linear regression analysis. The Inhibitory Concentration (IC_{50}) value refers to the concentration of antioxidant required to reduce Fe ion activity by 50%.[9] The collected data were statistically analyzed using GraphPad Prism v9.0 and presented in the form of graphs and tables. The formula for calculating percentage inhibition is shown below:

$$(\%) = \frac{Abs.Sample(\lambda) - Abs.Control(\lambda)}{Abs.Sample(\lambda)} \times 100\%$$

On the other hand, antioxidant capacity using the ABTS method was performed by combining potassium persulfate ($K_2S_2O_8$) and ABTS powder, dissolved in distilled water, incubated in the dark for 12 hours, and used to measure the antioxidant activity of the extract at various concentrations (10–30 µg/mL). Using a spectrophotometer, absorbance values were taken at 734 nm and compared to a standard Trolox solution. The percentage of antioxidant activity was calculated using the obtained absorbance results and the IC_{50} value through linear regression analysis. The Inhibitory Concentration (IC_{50}) value refers to the concentration of antioxidant required to inhibit 50% of ABTS radical activity.[10] The collected data were statistically analyzed using GraphPad Prism v9.0 and presented in the form of graphs and tables. The formula for calculating percentage inhibition is shown below:

$$(\%) = \frac{Abs.Control(\lambda) - Abs.Sample(\lambda)}{Abs.Control(\lambda)} \times 100\%$$

RESULTS

Yield

Yield is the ratio between the initial weight of the simplicia and the amount of extract obtained through extraction. The yield percentage is calculated by comparing the weight of the plant extract to the weight of the simplicia, multiplied by 100%. A total of 1000 grams of *Arcangelisia flava* (yellow wood) roots were used in this study. The drying process took approximately 2–3 days, after which the roots were ground using a blender to obtain 75 grams of simplicia. The simplicia was then extracted using the percolation method with methanol as the solvent, resulting in 21.57 grams of extract. The percentage yield obtained was 28.76%, calculated using the following formula:

$$\text{Yield Percentage (\%)} = \frac{\text{Weight of methanol extract}}{\text{Weight of simplicia}} \times 100\%$$

$$\text{Yield Percentage (\%)} = \frac{21{,}57}{75} \times 100\% = 28{,}76\%$$

Phytochemical Screening

Phytochemical screening of the yellow wood root extract showed positive results for several secondary metabolites, including alkaloids, terpenoids, phenolics, quinones, saponins, tannins, coumarins, betacyanins, cardiac glycosides, flavonoids, and glycosides. Negative results were obtained for steroids and anthocyanins (Table 1).

Table 1. Phytochemical Content of Yellow Wood Root Extract

Phytochemical	Method	Result
Alkaloids	Mayer-Dragendorff	+
Steroids	Liebermann-Burchard	−
Terpenoids	Liebermann-Burchard	+
Phenolics	Folin-Ciocalteu	+
Quinones	H_2SO_4	+
Saponins	Foam test	+
Tannins	Ferric Chloride	+
Coumarins	NaOH + Chloroform	+
Anthocyanins & Betacyanins	NaOH	− and +
Cardiac Glycosides	Keller-Kiliani	+
Flavonoids	NaOH	+
Glycosides	Modified Bornträger	+

Total Antioxidant Capacity Test Using the FRAP Method

1. Wavelength and Control Absorbance

In the FRAP test using the Genesys 30-Vis spectrophotometer, the maximum wavelength was found to be 594 nm, with the control absorbance recorded at 0.095.

2. Test Results of Yellow Wood Root Extract

Various concentrations of yellow wood root extract were reacted with FRAP solution, and absorbance values were measured in triplicate using a spectrophotometer. Absorbance data were then utilized to determine the inhibition percentage, and a curve was plotted to obtain the linear equation Y = 1.590X + 22.21 with R^2 = 0.9821, the extract

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concentration is shown on the X-axis, with the inhibition percentage displayed on the Y-axis (Fig. 1). From the linear equation, the IC_{50} value of the extract was calculated to be 17.48 µg/mL (Table 2).

Table 2. FRAP Test Results of Yellow Wood Root Extract

Extract Concentration (µg/mL)	Average Absorbance	Inhibition (%)	IC_{50} (µg/mL)
10	0,109 ± 0,003	37,615 ± 1,719	
15	0,125 ± 0,004	45,600 ± 1,588	
20	0,157 ± 0,005	56,688 ± 1,381	17,48
25	0,170 ± 0,005	60,000 ± 1,178	
30	0,228 ± 0,005	70,175 ± 0,654	

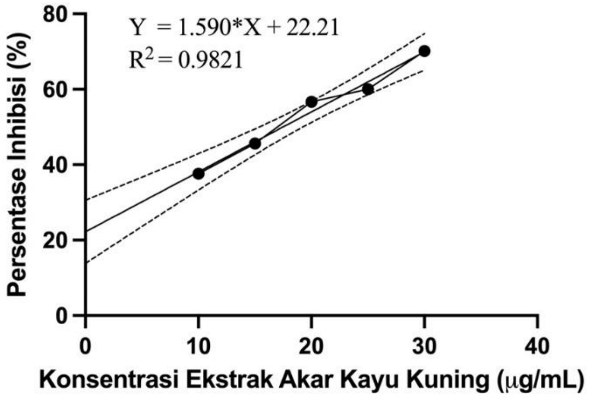

Figure 1. FRAP Assay Curve of Yellow Wood Root Extract

3. Results of Trolox Standard Comparison

Trolox at various concentrations was reacted with the FRAP solution, and absorbance values were measured using a spectrophotometer. These values were used to calculate inhibition percentages, and a curve was plotted with the linear equation Y = 1.813X + 31.12 and R^2 = 0.9949 (Fig. 2). The IC_{50} value of Trolox was determined to be 10.54 µg/mL (Table 3).

Table 3. FRAP Trolox Standard Comparison

Trolox Concentration (µg/mL)	Average Absorbance	Inhibition (%)	IC_{50} (µg/mL)
5	0,156 ± 0,005	39,103 ± 1,807	
10	0,189 ± 0,003	49,735 ± 0,798	
15	0,232 ± 0,004	59,052 ± 0,640	10,54
20	0,283 ± 0,003	66,431 ± 0,356	
25	0,404 ± 0,003	76,485 ± 0,154	

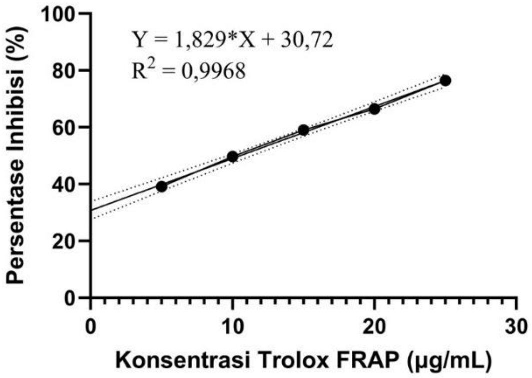

Figure 2. FRAP Assay Curve of Trolox Standard

Total Antioxidant Capacity Test Using the ABTS Method

1. Wavelength and Control Absorbance

In the ABTS test using the Genesys 30-Vis spectrophotometer, the maximum wavelength was found to be 734 nm, with the control absorbance recorded at 0.546.

2. Test Results of Yellow Wood Root Extract

Various concentrations of yellow wood root extract were reacted with ABTS solution, and absorbance values were measured in triplicate using a spectrophotometer. The absorbance values were then used to calculate the percentage of inhibition, and a curve was plotted to obtain the linear equation Y=3.002X – 16.01 and R^2 = 0.9638, the extract concentration is shown on the X-axis, with the inhibition percentage displayed on the Y-axis (Fig. 3). From the linear equation, the IC_{50} value of the extract was calculated to be 21.99 µg/mL (Table 4).

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Table 4. ABTS Test Results of Yellow Wood Root Extract

Extract Concentration (µg/mL)	Average Absorbance	Inhibition (%)	IC_{50} (µg/mL)
10	0,337 ± 0,005	18,005 ± 1,217	
15	0,314 ± 0,003	23,601 ± 0,843	
20	0,220 ± 0,004	46,472 ± 0,973	21,99
25	0,187 ± 0,004	54,501 ± 0,973	
30	0,092 ± 0,004	77,616 ± 0,973	

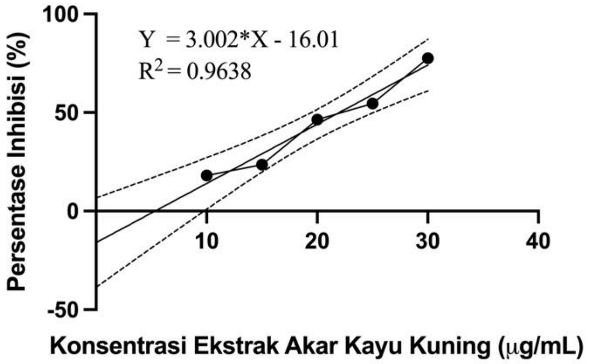

Figure 3. ABTS Assay Curve of Yellow Wood Root Extract

3. Results of Trolox Standard Comparison

Trolox at various concentrations was reacted with the ABTS solution, and absorbance values were measured using a spectrophotometer. These values were used to calculate inhibition percentages, and a curve was plotted with the linear equation linear Y= 1.542X + 29.54 with $R^2 = 0.9987$ (Fig. 4). The IC_{50} value of Trolox was determined to be 13.27 µg/mL (Table 5).

Table 5. ABTS Trolox Standard Comparison

Trolox Concentration (µg/mL)	Average Absorbance	Inhibition (%)	IC_{50} (µg/mL)
5	0,341 ± 0,001	37,546 ± 0,183	
10	0,302 ± 0,003	44,689 ± 0,484	
15	0,261 ± 0,004	52,198 ± 0,660	13,27
20	0,213 ± 0,004	60,989 ± 0,733	
25	0,175 ± 0,003	67,949 ± 0,485	

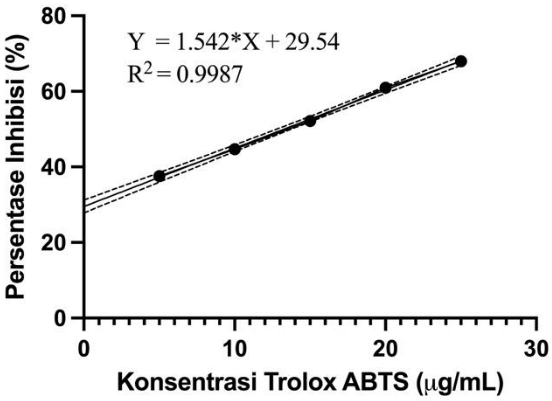

Figure 4. ABTS Assay Curve of Trolox Standard

DISCUSSION

Extraction Results and Phytochemical Screening of Yellow Wood Root Extract

Phytochemical screening was conducted to identify the secondary metabolite compounds present in the natural material, namely yellow wood root. The screening results revealed the presence of phytochemical compounds such as alkaloids, terpenoids, phenolics, quinones, saponins, tannins, coumarins, betacyanins, cardiac glycosides, flavonoids, and glycosides in the extract. These findings are consistent with the study by Kimberly M. Delica et al., which reported the presence of saponins, tannins, flavonoids, and alkaloids in methanol extract, as well as saponins, terpenoids, and cardiac glycosides in ethyl acetate extract. A study by Lilik Duwi Wahyudi et al. found positive results for phenolics and highlighted the role of flavonoids in neutralizing free radicals. Skunda Diliarosta et al. also identified alkaloids, terpenoids, flavonoids, phenolics, saponins, and steroids in yellow wood root. The results of the current study differ slightly, as steroids were not detected, possibly due to factors such as sample collection and quality. Additionally, this study found positive results for quinones, coumarins, betacyanins, and glycosides, although previous studies have not yet explored these specific compounds in yellow wood root.[3,6,11] Flavonoids and phenolics are known to have antibacterial, antiviral, and anticancer properties.[12] The secondary metabolites found in yellow wood root are traditionally used in Kalimantan as herbal medicine with antimicrobial, antifungal, antidiabetic, antihypertensive, analgesic, and anticancer effects.[13]

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Total Antioxidant Capacity Test Results of Yellow Wood Root Extract

In this study, the IC_{50} value indicating the inhibitory concentration was calculated to assess antioxidant capacity. The effectiveness of an antioxidant compound is inversely related to its IC_{50} value: the lower the IC_{50}, the higher its antioxidant activity. A compound is categorized as having very strong antioxidant activity if $IC_{50} < 10$ µg/mL, strong if 10–50 µg/mL, moderate if 50–100 µg/mL, weak if 100–250 µg/mL, and inactive if >250 µg/mL.[14] The FRAP method assesses antioxidant activity based on the extract's ability to reduce Fe^{3+} to Fe^{2+}. Trolox was used as the standard, showing an IC_{50} value of 10.54 µg/mL. The yellow wood root extract showed an IC_{50} of 17.48 µg/mL, which is lower than the values typically obtained using the DPPH method. This difference is attributed to the FRAP method's ability to reduce compounds in an acidic environment, particularly converting Fe^{3+} (green) to Fe^{2+} (dark blue). Compared to Trolox, the extract's IC_{50} is not significantly different, and both fall within the 10–50 µg/mL range, indicating strong antioxidant potential although the extract showed slightly weaker activity. This suggests that yellow wood root extract is capable of neutralizing free radicals, particularly Fe^{3+}. Another study conducted by Kimberly et al. (2024) in the Philippines reported FRAP values of 0.653 ± 0.086 mmol TEAC/g (methanol extract) and 0.107 ± 0.005 mmol TEAC/g (ethyl acetate extract). Although direct comparison is limited due to different units and methods (IC_{50} vs. TEAC), the variation may relate to solvent type, geographic origin, and extraction method. The lack of extract concentration data in the study also prevents accurate conversion to µg/mL.

In the total antioxidant capacity test using the ABTS method, the ABTS radical cation was reacted with antioxidants, and the inhibitory concentration (IC_{50}) was used to evaluate free radical neutralization. Trolox, used as the reference standard, showed an IC_{50} value of 13.27 µg/mL, while the yellow wood root extract had an IC_{50} value of 21.99 µg/mL. To date, no further studies using the ABTS method on yellow wood root have been reported. Both values fall within the 10-50 µg/mL range, indicating strong antioxidant activity. This suggests that the extract effectively neutralizes ABTS radicals, as evidenced by the fading of the blue-green color in the solution. In addition to its antioxidant properties, the extract may inhibit carbohydrate-digesting enzymes, helping to regulate blood glucose levels and offering potential antidiabetic benefits.

Although the ABTS assay covers a broader range of radical scavenging mechanisms, its IC_{50} value was slightly higher than that of FRAP (21.99 µg/mL vs. 17.48 µg/mL). This difference may be attributed to variations in assay sensitivity and reaction conditions. The FRAP method, which relies strongly on electron transfer under acidic conditions, favors phenolic compounds with high reducing power, thereby often yielding lower IC_{50} values. In contrast, while ABTS can capture both electron transfer and hydrogen atom transfer mechanisms and thus detect a broader spectrum of antioxidant compounds, not all constituents in the extract react optimally with the ABTS radical cation ($ABTS^{+\bullet}$), particularly larger or less polar molecules, resulting in slightly higher IC_{50} values. Nevertheless, both methods consistently classify the yellow wood root extract as having strong antioxidant activity, and the small variation observed highlights the complementary nature of these assays in evaluating overall antioxidant potential.

CONCLUSION

Phytochemical analysis of the yellow wood root extract revealed various secondary metabolites, including alkaloids, terpenoids, phenolics, quinones, saponins, tannins, coumarins, betacyanins, cardiac glycosides, flavonoids, and glycosides.

Total antioxidant capacity test of the yellow wood root extract:

a. Using the FRAP method, the IC_{50} value was found to be 17.48 µg/mL, indicating that the yellow wood root extract has strong antioxidant potential in this study.
b. Using the ABTS method, the IC_{50} value was found to be 21.99 µg/mL, indicating that the yellow wood root extract has strong antioxidant potential in this study.

ETHICAL APPROVAL

No ethical approval was required for this experimental study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest in this study.

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AUTHOR CONTRIBUTIONS

Sampling, data collection, data analysis, MSO; corresponding, validation and review of the manuscript, NST; methodology and interpretation of data, MSO, EY; writing- review and editing, MSO, NST; supervision and resources, EY, FF.

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