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EXPLORING NEEM, MORINGA, AND THEIR SYNTHESIZED SILVER NANOPARTICLE COATINGS FOR PROLONGED SHELF LIFE AND QUALITY RETENTION OF *Musa sapientum* (BANANA) FRUITS

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Abstract

Musa sapientum (Banana) is a popular fruit worldwide because of its nutritional content. However, post-harvest loss remains a severe challenge in storing this perishable produce. This study aimed to explore the effectiveness of *Moringa oleifera* (Moringa), *Azadirachta indica* (Neem) leaf extracts, and their synthesized silver nanoparticle coatings on the post-harvest shelf life of bananas, and also to isolate and identify the fungi associated with post-harvest spoilage of banana using standard procedures. Thirty-five (35) fully grown green banana fruits were gathered, rinsed, and arranged according to their coatings or treatments. with synthesized silver nanoparticles, moringa, and neem: Control, neem aqueous leaf extract, moringa aqueous leaf extract, neem aqueous leaf extract, and moringa aqueous leaf extract synthesized silver nanoparticles in 1:9 and 6:4 (n=5 each), respectively. Standard procedures were used to assess the bananas' firmness, shelf life, and postharvest degradation percentage. Results showed that the banana fruits coated with *A. indica* were effective in extending the shelf life and maintaining banana physical qualities compared to the coatings with the synthesized silver nanoparticles and moringa aqueous leaf extract. Additionally, three fungi, namely *Aspergillus flavus, Aspergillus niger*, and *Mucor* sp. were identified and isolated as the causal organisms for the post-harvest spoilage of the banana fruit. In conclusion, matured banana fruits' postharvest quality and shelf-life can be retained by coatings with the aqueous neem and moringa extracts and their synthesized silver nanoparticles, although the days of retention differ.

Keywords: *Azadirachta indica*; *Moringa oleifera*; *Musa sapientum*; Post-harvest; Preservatives; Silver nanoparticles; Spoilage.

Introduction

Foods, including crops such as bananas, are essential for maintaining good human health, but extending their shelf life and quality remains a challenge worldwide. Bananas, scientifically known as *Musa sapientum*, are one of the most widely consumed fruits globally. They are native to Southeast Asia but are now cultivated in many tropical regions (Sidhu and Zafar, 2018). Bananas are known for their high nutrient content (Ranjha *et al*., 2022) and their versatility, which makes them popular ingredients in various recipes, from smoothies and desserts to baked goods (Clark, 2019). Banana plays a significant role in agriculture and trade with countries like India, China, and the Philippines being major producers (Evans *et al*., 2020; Gulati *et al*., 2022; Babber, 2023). However, during post-harvest storage, the toxic infection in bananas results in a notable yield loss (Kaushik *et al*., 2021). Thus, it is very important to maintain the quality and shelf life of bananas after transportation and storage. To ensure this, the right temperature and humidity levels are essential (Feyera and Abdo, 2022). Chemical-based methods have been used to retain the postharvest quality of crops for better and extended shelf life, but this method has been associated with serious health concerns (Gulati *et al*., 2022). Therefore, there is an urgent need to use natural products such as plant extracts as an alternative because of their cost- cost-effectiveness, easy accessibility, and non-significant associated health risks.

Medicinal plants have been used since time immemorial as the oldest form of medicine and for other purposes, including the prolongation of the shelf life of harvested foods/fruits. There has been widespread use of plants, including neem, *Aloe vera*, garlic, and moringa (Mahmuda *et al*., 2018). The leaf extracts of neem and moringa are attractive as coating materials because they are rich in antioxidants and exhibit antibacterial properties against a variety of microorganisms (Mitiku and Yilma, 2017; Nwokafor *et al*., 2020; Ahmed *et al*., 2023).

Moringa is a multipurpose plant that has numerous uses in various biological systems (Falowo *et al*., 2018). It is frequently regarded as a rich source of food and food products in many tropical and sub-tropical areas due to its significant nutritional, phytochemical, and antioxidant advantages (Su *et al*., 2020). It contains tannins, terpenoids, alkaloids, flavonoids, etc. (Mulyaningsih *et al*., 2018). Neem, on the other hand, is rich in phenolic compounds, flavonoids, and alkaloids (Othman *et al*., 2019). It has been reported for its hypoglycemic and hypolipidemic properties, and also skin and liver disease treatment. These biological activities have been associated with major active ingredients of neem, azadirachtin (Septiyani *et al*., 2019). The phytochemical components of plant extracts have a significant impact on the stabilization and decrease of metallic ions within a very short time, in contrast to several microorganisms (such as bacteria) that need a longer period of incubation. These plant phytochemicals are crucial components in the synthesis of nanoparticles (Zuhrotun *et al*., 2023). Recently, green synthesis has emerged as a useful platform for the synthesis of nanoparticles. This method has been reported and demonstrated to be cost-effective, easy to use, and non-hazardous (Awote *et al*., 2023a; Ewekeye *et al*., 2024). Coatings with various nanoparticles, especially silver nanoparticles, have been associated with the functional, nutritional, palatability, prolonged shelf life, and retention qualities of products (Odetayo *et al*., 2022). Thus, this study investigated the effectiveness of moringa, neem leaf extracts, and their synthesized silver nanoparticle coatings on the shelf life and quality preservation of bananas.

Materials and Methods

Collection and Extraction of Plant Samples

The leaves of *A. indica* (neem) and *M. oleifera* (moringa)were collected from the vicinity of Lagos State University's sports center and a residential area in Adexson, along Igando, Lagos State, Nigeria, respectively. On the other hand, thirty-five (35) mature and healthy green banana fruits were obtained from a local food and fruits market, Iyana Iba market, Ojo, Lagos State, Nigeria.

The plants were authenticated by a taxonomist, Dr. O. K. Oluwa, at the herbarium of the Department of Botany, Faculty of Science, Lagos State University, Ojo, Lagos State, Nigeria. The dried leaves were blended to a powdery form using a Kenwood electric blender (KC-241B), sieved, and kept in a separate air-tight conical flask. 100 g of each leaf powder was later dissolved in 1000 mL of sterile distilled water, filtered, and the filtrate was stored separately in the refrigerator (Haier Thermocool) until needed (Awote *et al*., 2022a). The resulting solutions were neem aqueous leaf extract (NALE) and moringa aqueous leaf extract (MALE).

Silver Nanoparticles (AgNPs) Synthesis

The synthesis of AgNPs was done based on the modified method of Awote *et al*. (2023b). Briefly, 2 mM silver nitrate solution was freshly prepared and mixed with NALE and MALE, separately in 1:9 (100 mL extract and 900 mL $AgNO_3$) and 6:4 (600 mL extract and 400 mL $AgNO_3$), respectively. The colour change indicated silver nanoparticle synthesis, which was further confirmed by an absorption peak between 400 – 450 nm using a UV-visible spectrophotometer, investigated after 1 hour of reaction mixture. The resulting solutions were synthesized AgNPs using neem (NALE-AgNPs) and synthesized AgNPs using moringa (MALE-AgNPs) at two different concentrations, depending on the mixture ratio.

Treatment of Banana with NALE, MALE, NALE-AgNPs, and MALE-AgNPs

The banana fruits were gently rinsed under running clean water and air-dried at room temperature for a few minutes. Five banana fruits each were coated with either NALE or MALE or their respective synthesized AgNPs by dipping the fruits into the respective prepared solutions. These bananas were left in each solution for 15 minutes before being arranged in a separate container on the Laboratory table at room temperature (27°C). Changes were observed, and data were recorded to ascertain the effects of the extracts and synthesized AgNPs on the bananas. The experiment lasted for 8 days.

Data collection

The collected data were recorded and calculated using post-harvest decay percentage (PDP), marketability, shelf-life, and firmness as follows:

1. Post-harvest decay percentage (PDP) = × 100
2. Marketability= × 100
3. Shelf Life (days)=Total number of days before fruit quality drops below an acceptable threshold
4. Firmness rating scale = 1 - 5

Where 1 = very poor, 2 = poor, 3 = Acceptable, 4 = good, and 5 = excellent.

Isolation and Identification of Spoilage Fungi in Banana Fruits during Storage

The spoilage-causing fungi of banana fruits were isolated using potato dextrose agar (PDA) following the methods of Awote *et al*. (2022b) and Ewekeye *et al*. (2024). Briefly, thirty-nine grams (39 g) of Potato Dextrose Agar (PDA) was dissolved in 1000 mL of distilled water in a sterile glass flask enclosed with cotton wool and aluminum foil. The mixture was well shaken before being autoclaved for 15 minutes at 121 °C and 15 pounds per square inch of pressure. ($15lb/inch^2$). After autoclaving, the medium was allowed to cool to 45 °C before being aseptically dispensed into sterile Petri plates. The medium was supplemented with chloramphenicol to inhibit the growth of bacteria. The workbench was disinfected, and a sterilized cork borer was used to extract pieces from a diseased banana fruit, which were placed into the medium. After 5 days of incubation at 37°C, mixed cultures were re-isolated until a pure culture. Identification was based on morphological features and microscopic examination using Lactophenol cotton blue solution.

Statistical Analysis

The daily weight of the coated banana fruits was recorded in triplicate, and the data were subjected to univariate statistical analysis, mean ± standard deviation (SD) using Statistix 10 software. The means were separated using analysis of variance (ANOVA), and comparisons were made at a significance level of $p<0.05$.

Results

Table 1 shows that the bananas in the control group spoiled and lost their firmness on the 6th day, while those coated with synthesized silver nanoparticles (NALE-AgNPs) solution (6:4) lasted longer until the last day of the experiment. Meanwhile, the other variant of the synthesized AgNPs (1:9) lasted for 7 days before a significant weight loss, leading to the complete deterioration of the banana. Interestingly, the bananas that were coated with NALE were comparable to the bananas coated with AgNPs (6:4) compared to the control. This may imply that the ratio of NALE mixed with the silver nitrate solution to make AgNPs (6:4) extended the banana's shelf life, which can be traced to the increased number of embedded phytochemicals.

Table 1: Effects of NALE and varying NALE-AgNPs concentrations coatings on the weight (g) of *M. sapientum*

Groups/Days	Control	NALE	Nano 1 (6:4)	Nano 2 (1:9)
IWBC	$83.63±3.85^a$	$86.70±2.46^a$	$98.67±5.84^b$	$88.43±11.31^a$
WAC	$83.63±3.85^a$	$86.70±2.46^b$	$98.67±5.84^b$	$88.43±11.31^a$
DAT 1	$83.63±3.85^a$	$86.70±2.46^a$	$98.67±5.84^b$	$88.43±11.31^a$
DAT 2	$81.63±4.19^a$	$103.90±3.31^b$	$95.97±6.63^c$	$84.10±11.51^a$

DAT 3	80.20±2.94^{a}	96.53±3.27^{b}	88.00±9.33^{c}	81.13±11.27^{a}
DAT 4	76.97±2.52^{a}	92.67±2.87^{b}	85.97±7.46^{c}	77.13±9.48^{a}
DAT 5	73.67±2.37^{a}	88.93±2.42^{b}	82.50±7.61^{c}	73.23±7.85^{a}
DAT 6	64.97±2.93^{a}	84.67±2.45^{b}	79.53±7.54^{c}	70.60±7.55^{d}
DAT 7	66.87±13.74^{a}	72.93±0.11^{b}	71.87±4.64^{b}	58.80±5.74^{c}
DAT 8	56.87±2.31^{a}	65.33±0.81^{b}	67.01±3.60^{b}	50.40±9.04^{c}

Mean ± SD values with the same alphabet on the same row are not significantly different at $p<0.05$. Nano 1 = Neem aqueous leaf extract dissolved in $AgNO_3$ solution at 6:4, and Nano 2 = Neem aqueous leaf extract dissolved in $AgNO_3$ solution at 1:9. IWBC = Initial weight before Coating, WAC = Weight after coating, DAT = Day after treatment, Control = not treated.

Table 2 shows that the bananas in the control group spoiled and lost their firmness on the 7th day, while those coated with synthesized silver nanoparticles (MALE-AgNPs) solution at 6:4 and 1:9 lasted longer until the last day of the experiment before deterioration. The MALE-coated bananas lost their firmness and had a significant weight loss on the 8th day of the experiment. This may imply that the coating with AgNPs at different ratios extended the shelf life of the banana, possibly because of the embedded phytochemicals.

Table 2: Effects of MALE and varying MALE-AgNPs concentrations coatings on the weight (g) of *M. sapientum*

Groups/Days	**Control**	**MALE**	**Nano 1 (6:4)**	**Nano 2 (1:9)**
IWBC	84.23 ± 2.81^{a}	78.33 ± 2.45^{b}	79.53 ± 6.74^{b}	96.63 ± 11.88^{c}
WAC	84.23 ± 2.81^{a}	78.33 ± 2.45^{b}	80.50 ± 6.51^{b}	98.17 ± 11.84^{c}
DAT 1	84.23 ± 2.81^{a}	91.03 ± 0.85^{b}	79.00 ± 6.79^{c}	96.23 ± 11.80^{d}
DAT 2	84.23 ± 2.97^{a}	84.50 ± 4.03^{a}	76.93 ± 6.49^{b}	93.90 ± 11.20^{c}
DAT 3	80.20 ± 2.95^{a}	81.73 ± 1.40^{a}	73.33 ± 3.97^{b}	89.43 ± 14.08^{c}
DAT 4	76.97 ± 2.51^{a}	75.83 ± 3.45^{a}	71.57 ± 4.89^{b}	89.07 ± 7.01^{b}
DAT 5	73.67 ± 2.37^{a}	75.70 ± 2.66^{a}	69.83 ± 5.89^{b}	84.03 ± 10.14^{c}
DAT 6	65.13 ± 2.88^{a}	71.33 ± 2.07^{b}	82.33 ± 8.50^{c}	80.00 ± 7.57^{c}
DAT 7	37.47 ± 32.51^{a}	61.87 ± 4.39^{b}	61.53 ± 3.93^{c}	75.07 ± 6.20^{c}
DAT 8	41.93 ± 38.22^{a}	51.80 ± 1.22^{b}	72.53 ± 8.10^{c}	71.13 ± 4.84^{c}

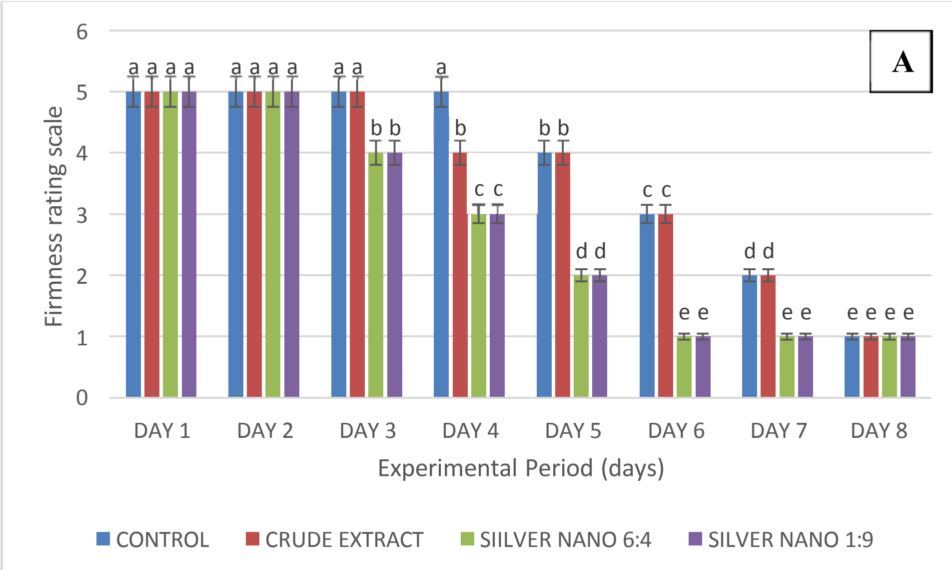
Mean ± SD values with the same alphabet on the same row are not significantly different at $p<0.05$. Nano 1 = Moringa aqueous leaf extract dissolved in $AgNO_3$ solution at 6:4, and Nano 2 = Moringa aqueous leaf extract dissolved in $AgNO_3$ solution at 1:9, IWBC= Initial weight before Coating, WAC= Weight after coating, DAT = Day after treatment, Control= not treated.

Table 3 shows different post-harvest deterioration percentages of bananas, which started on the 5th and 7th day, depending on the extract or varying concentration of synthesized AgNPs compared to the control. Like the control, NALE crude extract showed a lower postharvest deterioration percentage compared with the varying concentrations of synthesized AgNPs using NALE, whose postharvest deterioration percentages are 40 and 60 percent on the 5th and 7th day, respectively. Conversely, both the control and synthesized AgNPs using MALE at different concentrations had a postharvest deterioration percentage of 100 on the 5th and 7th day. However, treatment of the bananas with MALE crude extract lowered the postharvest deterioration to 60% on the 5th day.

Table 3: Post-harvest deterioration (%) of *Musa sapientum* coated with NALE, MALE, and their varying synthesized AgNP concentrations**.**

Extracts	Day 1	Day 3	Day 5	Day 7
MALE crude extract	-	-	60	100
NALE crude extract	-	-	20	40
Moringa oleifera 1:9	-	-	100	100
Azadirachta indica 1:9	-	-	40	60
Moringa oleifera 6:4	-	-	100	100
Azadirachta indica 6:4	-	-	40	60
Control (*Moringa oleifera*)	-	-	100	100
Control (*Azadirachta indica*)	-	-	20	40

Figures 1a and b illustrate the firmness of the banana fruits. It was observed that *Musa sapientum* coated with NALE showed no difference in firmness compared to the control fruits up to day 3. In contrast to the control, the coated fruits showed a significant decrease (p<0.05) in firmness starting from day 4. There was no observed statistically significant (p>0.05) difference in firmness between the control and NALE-coated fruits from days 5 to 8. Additionally, no statistically significant (p>0.05) difference in firmness was observed between the bananas coated with different concentrations of synthesized AgNPs from days 1 to 8 (Figure 1a). Furthermore, the firmness of the four compared groups became the same on the 8th day. Similarly, the trend was observed with coatings with MALE and its synthesized AgNPs. However, the control group lost all its firmness from days 7 to 8 (Figure 1b).



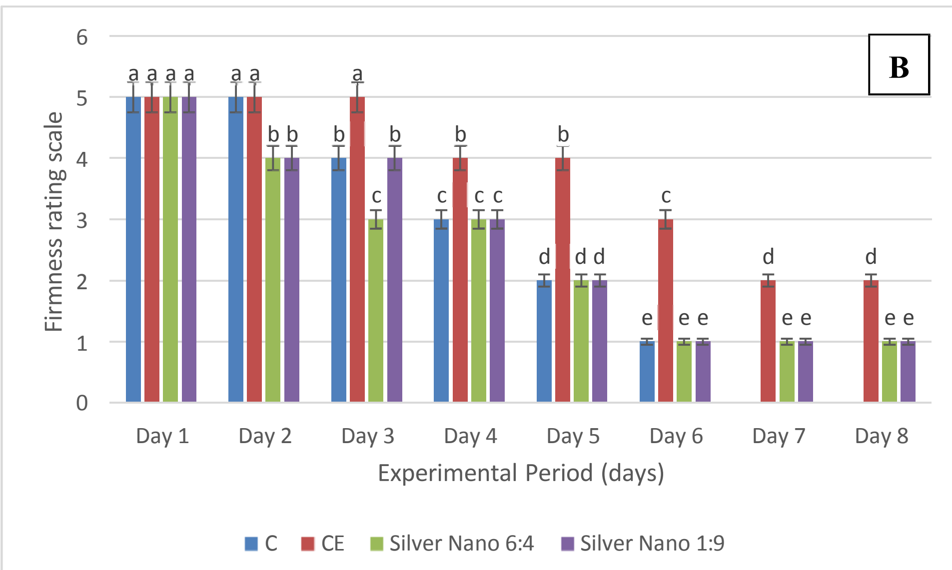


Figure 1: Firmness of *Musa sapientum* with (a) *Azadirachta indica* aqueous leaf extract and its synthesized AgNPs and (b) *Moringa oleifera* aqueous leaf extract and its synthesized AgNPs. 1= very poor, 2= poor, 3= acceptable, 4= good, 5= excellent. CE = crude extract, C = control (not treated), Nano 6:4 = MALE or NALE dissolved in $AgNO_3$ solution at 6:4, and Nano 1:9 = MALE or NALE dissolved in $AgNO_3$ solution at 1:9.

Discussion

Out of the four synthesized treatments incorporated in this study, treatment with *Azadirachta indica*-synthesized silver nanoparticles (NALE-AgNPs) at 6:4 and 1:9 showed a better and higher decay percentage. However, treatment with NALE crude extract showed a decreased decay percentage, which was comparable to the control of *A. indica* leaf extract (NALE). This result agrees with the study of Hossain *et al*. (2023) that the banana treated with *A.*

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indica extract showed a lowered post-harvest rot, thus extending post-harvest shelf life. This observation is also in consonance with the report of El-Khatabi *et al*. (2022), who noted that extracts obtained from medicinal plants like *A. indica* leaf, *M. oleifera, Allium sativum*, and *Mentha arvensis* (Corn mint) leaf were most effective in preserving fruits. Thus, the number of rotten fruits observed in MALE-AgNPs-coated fruits was high, and this is consistent with the findings of Meserat *et al*. (2012) and Hiru *et al*. (2008), who found that fruits lost weight more quickly as storage time increases. The control and treated bananas were fully decayed on the 8th day, which aligns with the findings of Mahmuda *et al*. (2018), who demonstrated that post-harvest treatments with 40% neem leaf extract demonstrated efficacy for prolonging banana shelf life.

A significant increase ($p < 0.05$) observed in the weight and firmness loss in the bananas treated with either NALE crude extract or 1:9 and 6:4 NALE-AgNPs as the postharvest storage days increases, aligns with the findings of Durer-e *et al*. (2023) who found that longer storage periods were linked to higher weight loss percentages. AgNPs coatings serve as semi-permeable barriers to reduce fruit oxidative stress, carbon dioxide release, and water loss percentage while enhancing fruit quality after harvest. During storage, the weight of the banana coated with moringa aqueous leaf extract (MALE) and the control decreased. The moringa leaf was able to form a coating on the banana fruit, thereby lowering the respiration rate since an increase in respiration results in an increase in the metabolic rate and higher weight loss, which is due to the termination of moisture from the fruit (Kator *et al*., 2019). The banana fruits coated with *M. oleifera* crude extract (MALE) had higher marketability than the control and those coated with synthesized nanoparticles. The banana fruits coated with moringa extract were still very marketable on the 6th day of storage. The marketability of the untreated banana fruits and the bananas coated with varying concentrations of silver nanoparticles was reduced significantly ($p<0.05$). In addition, towards the end of the storage duration, not all the banana fruits were marketable. This finding aligns with the reports of Liamngee *et al*. (2019) and Ewekeye *et al*. (2024) on tomato fruits, who reported no significant differences in marketability between the coated tomatoes and the control on days 1, 5, and 9 of the storage period.

The ability of the moringa leaf extract to preserve the marketability of the fruits may be attributed to its capacity to form a protective layer around the fruit, thereby preventing oxygen and moisture loss and inhibiting the activity of microorganisms (Ewekeye *et al*., 2024). It is also not out of place to associate this with the plant's embedded polyphenols. Unlike the post-harvest decay percentage (PDP) and marketability, which have clear mathematical formulas, shelf life does not have a universally standardized formula since it is more of an observational metric rather than a directly calculable one. However, looking to quantify shelf life in a way similar to the other parameters, it could be defined based on thresholds of quality (e.g., firmness or decay levels) as done in this study. *Aspergillus flavus*, *A. niger,* and *Mucor* sp. were identified after isolation. These fungi have also been reportedly found in other fruits, and their association with fruits/foods may suggest their omnipresent, non-host-specific, and non-geographically specific nature (Hosea *et al*., 2017).

Conclusion

This study showed that the aqueous leaf extracts of *Azadirachta indica* (neem) and *Moringa oleifera* (moringa) are effective in extending the shelf life and maintenance of the postharvest quality of banana fruits beyond their usual limits. Furthermore, the synthesized silver nanoparticles using the aqueous extracts of these medicinal plants could also extend the postharvest quality of bananas and other crops, possibly due to the embedded rich phytoconstituents of these plants. Additionally, *Aspergillus flavus*, *A. niger,* and *Mucor* sp. were identified as the spoilage-causing microorganisms of bananas after harvest.

Author Contribution: TSE and OKA designed the study. TSE, OKA, ASA, and OAO wrote the methodology. SRO, ADI, AOS, DOK, and AOA managed the analyses and literature searches. SRO, DOK, and OKA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Not applicable

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