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Synergistic therapeutic effect of the volatile oils of Zingiber officinale and Curcuma longa on Selected Advantageous Pathogens

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Abstract

In response to the escalating global drug resistance crisis, this study explores the potential of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) volatile oils as alternative therapeutic agents individually and in combination. These oils were extracted using hydro-distillation and screened for their antibacterial potential against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia* using the Agar well diffusion method. Antioxidant activity of the oils was also explored using DPPH and FRAP. The synergistic antimicrobial effects of the combined volatile oils were evaluated, comparing their ability with conventional antibiotics. The result revealed that the combined oils demonstrated better antimicrobial activity (ZI: 18 mm to 22 mm) than the individual oils (Ginger: ZI: 13 mm to 19 mm, Turmeric: 17 mm to 19 mm) against the bacteria screened. This activity of the combined oils (ZI: 18 mm to 22 mm) was comparable to that of the standard antibiotics: Gentamycin (ZI: 21 mm to 22 mm). DPPH assay revealed a value of 52.22±2.30 µg/mL for the ginger extract and 44.22±2.32 µg/mL for the Turmeric, while FRAP assay revealed a value of 6.22±0.04 µg/g for ginger and 4.10±0.04 µg/g for turmeric. Conclusively, the exploration of volatile oils from ginger and turmeric presents them as viable components in drug formulations for the fight against antimicrobial resistance due to their comparable activity to that of conventional antibiotics, especially in their combined form.

Keywords: Multidrug resistance, Volatile oils, Ginger, Turmeric, Synergy

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1. Introduction

Plants that grow horizontally underground and possess characteristic underground stems unique for vegetative reproduction are rhizomatous plants (1). These plants are valuable in the agricultural system and can be employed for vegetative reproduction. Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) are two rhizomatous plants well-known for their different applications for example, spices, cosmetics, coloring agents, preservatives, pharmacological applications, and in traditional medicine (2). Their rhizome is a storehouse of secondary metabolites, for example, zingiberene, β-bisabolene,

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β-sesquiphellandrene, curcuminoids, gingerols, and terpenoids among others (3, 4, 5). Their therapeutic potential can be attributed to the presence of these compounds which can exert biological activity on different microorganisms via different mechanisms (6, 7, 8). These compounds are either extracted in crude forms or as volatile oils.

Volatile oils or essential oils as they are also called are substances biosynthesized by living organisms. They are widely utilized in the fragrance, food, cosmetic, and pharmaceutical industries. They have been reported to possess numerous health-promoting properties, such as antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. Volatile oils contain a complex mixture of aromatic constituents (9) and are extracted from different plants and plant parts for example fruits, seeds, flowers, leaves, and even in rhizomatous plants (10).

Research suggests that certain bioactive compounds in Rhizomatous plant volatile oils, such as curcuminoids in turmeric and gingerol in ginger, possess potent antioxidant properties by scavenging free radicals and enhancing endogenous antioxidant enzyme activity (11, 12). Volatile oil extracted from ginger contains a complex mixture of bioactive compounds, including gingerol, shogaol, and zingerone, contributing to its distinctive aroma and potential health benefits (13). Curcumin, the primary bioactive compound in turmeric volatile oil, has been shown to modulate inflammatory pathways by inhibiting the activity of pro-inflammatory enzymes and cytokines (8).

The rise of multidrug-resistant bacteria, known as ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), poses a significant threat to modern healthcare due to their ability to evade conventional antibiotics, leading to increased illness, death, and healthcare costs (14). As antibiotic resistance grows, there is increased interest in alternative therapies such as volatile oils, which have shown biological potential.

Volatile oils offer several benefits over traditional antibiotics. They contain multiple bioactive compounds targeting various cellular components, reducing resistance (9, 15, 16), they are generally safe, with fewer side effects, and can synergize with antibiotics to enhance efficacy and reduce resistance (17). A notable factor is their potential to exert a synergistic effect when combined, especially in the treatment of ailments caused by multidrug-resistant bacteria.

Synergy generally involves the interaction of two or more agents to produce a combined effect that is greater than the sum of the effects of individual components (18, 19). The simultaneous administration of different plants and their parts as medicines to patients is commonplace among herbal medicine practitioners. This practice is based on the belief that the plants' therapeutic potentials depend on synergistic interactions of the different components (20, 21) as it is with combination therapy in modern medicine. Despite the inadequacy in the understanding of their mode of action, different instances showcasing better activity of herbal extract than an isolated compound have been reported (18, 20).

Several reports have been made on the synergistic effect of turmeric and ginger plant extracts (21-23), however, reports on the synergistic impact of these plants' oils on multidrug-resistant microorganisms are sparse in the literature. This study therefore aims to extract the volatile oils of ginger and turmeric and evaluate their synergistic biological effect on selected advantageous pathogens.

2. Materials and Methods

2.1. Samples collection

The healthy roots of *Zingiber officinale* (Ginger) and *Curcuma longa* (Turmeric) were collected from Idobi market, Ijebu-ode, Ogun State Nigeria. .

2.2. Extraction of volatile oil

200 g each of chopped dried *Zingiber officinale* and *Curcuma longa* were loaded separately into a Clevenger typed hydro-distillation setup which was previously washed with liquid detergent and distilled water. The hydro-distillation extraction was carried out

continuously for 3 hours. The extract (volatile oil, hexane, and water) was then collected in a glass vial, dried over anhydrous sodium sulphate to remove the water, and evaporated to remove n-hexane. The dry oil was stored in a pre-weighed glass vial and the actual yield of extracted volatile oil was calculated. The oil was stored in an air-tight vial and kept in the refrigerator at 4 °C until further analysis with GC-MS.

2.3. Anti-Microbial Screening of the Extracted Volatile Oils

In-vitro antimicrobial activity of the extracted oils was carried out against three bacterial strains: *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. This was achieved using the agar well diffusion method (24, 25). Gentamycin was the positive control while hexane was the negative control. Nutrient agar plates were seeded with 0.1 mL of an overnight culture of each bacterial strain. The seeded plates were allowed to set and a standard cork borer (7 mm) was used to cut uniform holes on the surface of the agar. A mixture of undiluted ginger and turmeric volatile oil extracts was prepared for the synergistic study following previously reported methods (26) with minor modification. 50 µL each of ginger and turmeric volatile oil extracts were added into the prepared agar plates (in a ratio 1:1). Gentamycin (10 µg/disc) was also added into another agar plates for the positive control studies. In separate agar plates, 50 µL each of the ginger and turmeric volatile oils were added (26). The plates were then incubated for 24 h at 30 ºC. After 24 h, the inhibition zones around the wells (the clear area around the wells) were measured to assess the antimicrobial activity of the volatile oil extracts.

2.4. Antioxidant Screening of the Extracted Volatile Oils

For the antioxidant properties of ginger and turmeric, extracts from the rhizomes were first prepared by extracting 10 grams of each powdered form of the clean rhizomes in 100 mL of methanol. The mixtures of the plant material and methanol are stirred for 24 to 48 hours and then filtered to yield concentrated extracts (27). DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method was employed for the antioxidant assays. This involves mixing diluted extracts with a DPPH solution and measuring the absorbance after incubation to calculate the percentage inhibition, which indicates radical scavenging activity (28). Additionally, the total phenolic content was determined using the Folin-Ciocalteu reagent and a gallic acid standard curve. The absorbance is measured, and the results are expressed in milligrams of gallic acid equivalents (GAE) per gram of extract (29). These methods facilitate the assessment of the antioxidant capacities of ginger and turmeric extracts, which are essential for understanding their potential health benefits in combating oxidative stress (13).

3. Result and Discussion

3.1. Antibacterial activity of the Extracted Volatile oils of Ginger and Turmeric

The result of the antibacterial activity of ginger volatile oils is presented in Table 1. The values of the diameters of zones of inhibition (ZI) against various microorganisms indicate the activity of respective volatile oils as well as that of the positive control (Gentamycin). The microorganisms screened include *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. The ginger volatile oil produced a ZI between 13.00±1.22 mm to 19.00±1.16. In contrast, the turmeric volatile oils had a higher ZI (17.00±1.11 to 19.00±1.26 mm) than ginger across all bacterial strains screened except *E. coli* where the oils exhibited similar ZI values (19.00 mm). The antibiotics, however exhibited a significantly larger ZI (21.00±0.1 to 23.00±0.1 mm) than the two oil extracts.

By comparison, both volatile oils ex-

Table 1. test Antibacterial activity of the individual oils of ginger and turmeric at 50 µL

Microorganisms	Diameters of zone of inhibition(mm)		
	Ginger essential oil	Turmeric volatile oil	Gentamycin
K. pneumonia	14.00±1.22	17.00±1.11	23.00±0.1
E. coli	19.00±1.16	19.00±1.26	24.00±0.4
S. aureus	13.00±1.11	19.00±1.23	21.00±0.1

tracts exhibit significant inhibition against the tested microorganisms, though with some differences. For instance, ginger volatile oil showed inhibition zones of 14.00 mm against *K. pneumoniae*, 19.00 mm against *E. coli*, and 13.00 mm against *S. aureus*. Turmeric oil, on the other hand, demonstrated slightly larger inhibition zones for *K. pneumoniae* (17.00 mm) and *S. aureus* (19.00 mm), indicating a potentially stronger activity against these pathogens (13, 16, 28). Both oils are effective against *E. coli*, with ginger oil showing a comparable inhibition zone (19.00 mm) to turmeric oil. This suggests that both volatile oils have strong antimicrobial properties, particularly against Gram-negative bacteria like *E. coli*. The slightly larger inhibition zones observed for turmeric oil against *K. pneumoniae* and *S. aureus* may indicate a greater overall antimicrobial potency or differences in the specific compounds present in the oils. The observed inhibition zones for turmeric volatile oil are consistent with previous research highlighting its potent antimicrobial properties. The effectiveness of turmeric volatile oil against *E. coli* and *S. aureus* aligns with Singh *et al*. (2011) (27), who reported significant antimicrobial activity of turmeric's oil against eye infection causing pathogens with its major component being Tumerone. The inhibition zones observed in this study confirm the substantial antimicrobial effects that may be attributed to the compounds present in them. This is consistent with findings from earlier studies that documented turmeric oil's effectiveness against similar bacterial strains (16, 27). The results indicate that although antibiotics consistently produce larger zones of inhibition, suggesting stronger antibacterial properties, the extracted volatile oil still shows significant antibacterial activity, particularly against *E. coli*. The broad-spectrum antimicrobial effects of turmeric oil's components reinforce its use in various therapeutic applications (16, 30), complementing the antimicrobial properties observed for ginger oil.

3.2. Synergistic Antibacterial Activity of the Extracted Volatile oils of Ginger and Turmeric

Table 2 represents the observed inhibition zones for the combined volatile oils of ginger and turmeric. The result suggests a significant synergistic effect of the two oils. The inhibition zones were notably larger when the oils were combined (1:1).

On combining the oils, the most pronounced effect was observed on *E. coli*, with a ZI of 22.00 mm, this exceeded the individual inhibition zones for ginger (19.00 mm) and turmeric (19.00 mm) volatile oils suggesting that using the oils in the combined form enhances their antimicrobial potency against this Gram-negative bacterium (29). The observed synergistic effect aligns with existing literature on volatile oil interactions. Research indicates that the combined use of medicinal

Table 2. Synergistic antibacterial activity of the extracted volatile oils of ginger and turmeric (1:1)

Microorganisms	Diameters of zone of inhibition(mm)	
	Combined oil	Gentamycin
K. pneumonia	18.00±1.11	23.00±0.1
E. coli	22.00±1.26	24.00±0.4
S. aureus	20.00±1.23	21.00±0.1

Table 3. DPPH and FRAP antioxidant assay of the extracted volatile oil from Ginger and Turmeric.

Antioxidant Assay	Ginger (volatile oil)	Turmeric (volatile oil)	Ascorbic acid (Positive control)
DPPH Assay ((µg/mL)	52.22±2.30	44.22±2.32	60.24±2.24
FRAP Assay (µg/g)	6.22±0.04	4.10±0.02	4.32±0.02

plants including their oils often results in enhanced antimicrobial activity due to the complementary effects of their constituent compounds (21, 23). For instance, Zingiberene, found in high concentrations in ginger oil (9), and Curcuminoids, prominent in turmeric oil (31) are known for their individual antimicrobial properties and will likely contribute to a broader spectrum of antimicrobial activity. When combined, these compounds may act synergistically to disrupt microbial cell membranes more effectively and inhibit microbial growth (32).

Based on the antibacterial activity of the combined volatile oils of ginger and turmeric, and by comparing their effectiveness against three different microorganisms with that of standard antibiotics, an increase in biological activity was observed, although the standard drug (Gentamycin) still displayed better activity. This observation supports the well-established combination therapy where the activity of a drug component complements that of the other, such as in the case of the antimalarial combination therapy (Artemether-Lumefantrine) hence, resulting in enhanced therapeutic activity against *Plasmodium falciparum*. In the case of *E. coli,* the combined oils demonstrated a ZI of 22.00±1.26 mm, nearly matching the ZI of Gentamycin (24.00±0.4 mm), highlighting a strong antibacterial effect of the combined oils. Whereas, a ZI of 20.00±1.23 mm was obtained for *S. aureus*, which is only marginally different from that of Gentamycin (21.00 ± 0.1 mm), suggesting significant antibacterial efficacy of the combined volatile oils. The results indicate that although standard antibiotics generally produce larger ZI, the combination of ginger and turmeric volatile oils exhibits substantial synergistic antibacterial activity comparable to that of the standard drug, especially against *E. coli* and *S. aureus*. Despite the slightly lesser potency of the volatile oils when used separately, the oils displayed better activity comparable to the activity of the standard antibiotic when combined. This indicated that each oil possesses a contributing effect resulting in an improved effect against the bacterial strains screened.

The synergistic antimicrobial effect observed in this study also supports findings from research on volatile oil combinations, which often demonstrate enhanced efficacy compared to single oils. This enhanced activity is likely due to the combined effects of the diverse bioactive compounds in both volatile oils (18). The increased inhibition zones with the combined volatile oils suggest that their interaction may lead to more effective microbial inhibition than each oil employed individually. The notable antibacterial potential when combined underscores their potential as alternative or complementary antibacterial agents.

3.3. Antioxidant assay

Table 3 shows the antioxidant potential of the volatile oil from ginger, which was assessed using two different assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the FRAP (Ferric Reducing Antioxidant Power) assay. The results were compared with ascorbic acid, which served as the positive control.

In the DPPH assay, the volatile oil from ginger demonstrated a value of 52.22±2.30 µg/mL, while ascorbic acid showed a value of 60.24±2.24 µg/mL. The DPPH assay measures the ability of antioxidants to scavenge free radicals, where lower values indicate higher antioxidant activity. The slightly lower DPPH value for ginger volatile oil suggests that it has a marginally better free radical scavenging capability compared to ascorbic acid. This result

indicates that ginger volatile oil can effectively neutralize free radicals, thereby potentially offering protective effects against oxidative stress. Meanwhile, turmeric volatile oil exhibited a value of 52.22±2.32 µg/mL, this value is even lower than that of ginger volatile oil and ascorbic acid, ultimately indicating that turmeric has a great potential even than ginger to neutralize free radicals, providing a potential protective effect against oxidative stress.

The FRAP assay results further emphasized the strong antioxidant potential of ginger volatile oil. In this assay, ginger volatile oil exhibited a value of 6.22±0.04 µg/g, whereas ascorbic acid value was 4.32±0.02 µg/g. The FRAP value for ginger volatile oil indicates its reducing power, suggesting that it can donate electrons effectively to neutralize free radicals although at a reduced extent compared to the ascorbic acid. The FRAP assay results provide additional insights into the antioxidant properties of turmeric volatile oil. In this assay, while ascorbic acid demonstrated a stronger antioxidant activity (4.32±0.02 µg/g) than turmeric volatile oil, the lather displayed a comparable potential with a value of 4.10±0.02 µg/g. Although the FRAP value for turmeric volatile oil is marginally lower than that of ascorbic acid, it still demonstrates a comparable reducing power. This suggests that turmeric volatile oil has a substantial ability to donate electrons and neutralize free radicals at a comparable threshold to ascorbic acid as supported by previous research finding (16).

In contrast, the FRAP assay revealed a higher antioxidant capacity for Turmeric vola-

Table 4. Chemical Constituents of the volatile oils of Turmeric (*Curcuma longa*).

Compounds	%	RIcal	RIlit
γ-Pyronene	4.59	843	844
Santolina triene	0.24	898	902
(+)-2-Carene	2.03	990	993
Pinane	1.96	1000	1002
sesquiphellandrene	18.77	1025	1021
Terpinolene	13.28	1050	1052
Isopentyl hexanoate	0.83	1220	1218
Ledane	0.25	1377	1380
Aromadendrene	4.80	1390	1386
Aromadendrene	1.34	1435	1439
δ-Cadinene	1.74	1443	1440
Cis-β-farnesene	4.77	1456	1458
α-Zingiberine	7.61	1484	1488
γ-Caryophyllene	3.15	1492	1494
α-Himachalene	1.79	1496	1499
Germacrene	7.43	1511	1515
Epiglobulol	1.19	1536	1530
α –Calacorene	1.78	1540	1537
Nerolidol	0.82	1563	1564
β-Copaene	4.36	1600	1598
Tumerone	18.83	1612	1616
Globulol	1.57	1653	1649
Aromadendrene oxide	5.30	1674	1672
Humulane-1,6-diene-3-ol	0.96	1755	1757

RIcal: Retention index determined relative to n-alkanes (C7-C30) on the HP-5ms column. RIlit: literature retention indices

Table 5. Chemical Constituent of the volatile oils of Ginger (*Zingiber officinale*).

Compounds	%	RIcal	RILit
Camphene	4.76	940	943
Linalool	0.28	1078	1082
α-Terpineol	0.11	1140	1143
3-Thujene-2-one	0.04	1154	1152
γ-Terpinene	0.43	1281	1283
β-Ylangene	1.12	1372	1370
Allo aromadendrene	0.29	1382	1386
Sativene	1.00	1396	1396
Copaene	2.31	1341	1397
Gurjunene	2.01	1403	1406
γ –Muurolene	0.04	1421	1419
Elemene	0.33	1428	1431
δ-Cadinene	4.78	1438	1440
γ-Curcumene	8.32	1484	1480
Zingiberene	13.28	1484	1488
Farnesene	15.51	1496	1496
Cis-β-farnesene	4.36	1496	1499
Elixene	0.33	1510	1514
Cubebene	0.32	1525	1525
Epiglobulol	1.19	1534	1530
β-sesquiphellandrene	3.21	1538	1537
Globulol	1.57	1572	1579
Cubenol	3.42	1584	1580
α-Cadinol	14.36	1625	1627
Humulane-1,6-diene-3-ol	0.96	1753	1757
Spatunelol	2.95	3146	3149

RIcal: Retention index determined relative to n-alkanes (C7-C30) on the HP-5ms column. RIlit: literature retention indices

tile oil (4.10±0.02 µg/g), compared to ascorbic acid (4.32±0.02 µg/g) and ginger volatile oil (6.22±0.04 µg/g) indicating a robust ability to reduce ferric ions to ferrous ions (29). Turmeric's reducing power, as measured by the FRAP assay, remains within a close range with ascorbic acid suggesting that its oil could serve as a potent natural antioxidant with potentials comparable to that of ascorbic acid. The literature supports the findings, noting that ginger and turmeric's volatile oils antioxidant properties are primarily due to their ability to scavenge free radicals (28) and their rich bioactive components. Overall, the potent antioxidant activity of the volatile oils of ginger and turmeric as observed in this study can be attributed to the bioactive compounds that have been previously reported present and which were also observed in the present study, for example, zingiberene, α-curcumene, and sesquiphellandrene, known for their antioxidant properties (28, 32, 33).These findings highlight ginger and turmeric's volatile oils as a valuable natural alternative for food preservation and therapeutic applications.

3.4. Chemical profiles of the extracted volatile oils of ginger and turmeric

The result of the GC-MS analysis of Ginger and Turmeric as reflected in Table 4

and 5 respectively showcased the presence of terpenes and terpenoids with Farnesene being the most abundant (15.51 %) compound in ginger followed by α-Cadinol (14.36 %) and Zingiberene (13.28 %). γ-urcumene was also found in substantial amounts (8.32 %) compared to δ-Cadinene (4.78 %), Camphene (4.76 %) and Cis-β-farnesene (4.36 %). The other compounds were present in small amounts. As for the essential oils of Turmeric, Tumerone was the most abundant (18.83 %) and only marginally greater than sesquiphellandrene by 0.11 % (18.77 %). Terpinolene was also abundant (13.28 %). Other notable compounds are α-Zingiberine (7.61 %), Germacrene (7.43 %), Aromadendrene oxide (5.30 %), Aromadendrene (4.80 %), Cis-β-farnesene (4.77 %), Gamma-Pyronene (4.59 %) and Beta-Copaene (4.36 %).

The observed chemical profiles of ginger and turmeric essential oils suggest that their therapeutic properties may be enhanced through synergistic effects. For instance, in this study, ginger essential oil is primarily composed of Farnesene (15.51 %), α-Cadinol (14.36 %) and Zingiberene (13.28 %). γ-urcumene (8.32 %) and other sesquiterpenes. These compounds collectively contribute to the oil's potent antioxidant and anti-inflammatory properties (28; 31). The presence of zingiberene (13.28%) in ginger essential oil is consistent with previous studies identifying this sesquiterpene as a major component contributing to ginger's therapeutic benefits. Zingiberene plays a significant role in the oil's therapeutic activities, reinforcing the well-documented health benefits of ginger essential oil (28, 29).

Similarly, turmeric essential oil's major constituents, such as sesquiphellandrene (18.77 %) Tumerone (18.83 %), and Terpinolene (13.28 %), work synergistically to exert antimicrobial, anti-inflammatory, and antioxidant effects. The combined effect of Caryophyllene (a dietary cannabinoid with the ability to enhance therapeutic effects (34) with other compounds like α-turmerone and ar-turmerone can amplify turmeric oil's therapeutic benefits, particularly in managing inflammatory conditions and oxidative stress (16, 27). The presence of these key compounds underscores turmeric oil's role in reducing inflammation and oxidative stress, as previously reported. (16).

Additionally, the identification of unique compounds such as spathulenol (2.95%) and humulane-1,6-diene-3-ol (0.96%) in ginger oil, which were not prominently featured in previous studies, could contribute additional or synergistic therapeutic effects that warrant further investigation. Globulol, Epiglobulol, and humulane-1,6-diene-3-ol were also found common to both oils. The presence of all these bioactive compounds suggests that their combined effects may work synergistically to enhance the oil's overall therapeutic efficacy. This synergistic action is supported by the literature, which indicates that the combined effect of multiple compounds often results in greater biological activity than any single component alone (21, 23).

4. Conclusion

The study revealed that while volatile oils from ginger and turmeric have promising antibacterial properties, their potency do not match that of the standard antibiotics. However, when combined, the oils displayed better activity comparable to that of the standard drug pointing to the influence of synergy between the chemical compounds. The significant presence of bioactive compounds like Curcumin in turmeric volatile oil and the added effect from compounds such as gingerol, shogaol, and zingerone in ginger volatile oil suggest their potential to enhance antimicrobial treatments against *S. aureus* and *E. coli*. The results underscore the potential of ginger and turmeric volatile oils as valuable additions in the formulation of antibacterial agents. Their substantial aromatic and therapeutic properties, combined with the presence of diverse bioactive compounds, warrant further research.

Such efforts could enhance the effectiveness of these volatile oils and expand their usefulness in both therapeutic and industrial applications.

Authors' Contribution

Conception and design, by OAD and BOO, the first draft of the manuscript was written by OAD and AOA, Material collection, preparation and extraction were done by AOA, data collection and analysis were performed by NIS, and all authors reviewed the manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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