In vitro comparative study of the ethanol and aqueous extracts of Spondias mombim leaves, stem bark and charcoal on Streptococcus mutans

ABSTRACT
Background
Plants are increasingly explored for their potential as antimicrobial agents. *Spondias mombin* plants have been used in folkloric medicine in the treatment of dental health and general health issues.

Aims and objectives
To compare zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration of *S. mombin* aqueous and ethanol stem bark, leaves and charcoal extracts on *Streptococcus mutans*.

Material and methods
Twenty grams of each plant part was soaked in 100ml of ethanol and boiled in distilled water and left to stand for 24h with intermittent shaking until evaporated. The dried filtrates were diluted to obtain concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml in duplicates. The zone of inhibition, minimum inhibition concentration and minimum bactericidal concentration tests on *S. mutans* were carried out using a standard procedure.

Results
Ethanol and aqueous extracts of *S. mombin* displayed antimicrobial activity against *Streptococcus mutans*. The leaves and stem bark extracts demonstrated superior potency compared to charcoal extracts. Ethanol extracts (32.4mm) consistently exhibited stronger inhibitory effects than aqueous extracts (25mm).

Conclusion
The salient feature of this study justifies the folkloric medicinal use of *Spondias mombin* plants in the treatment of toothaches and prevention of dental caries.

Keywords
*Streptococcus mutans*, *Spondias mombin*, dental caries, dextran, zone of inhibition, minimum bactericidal

INTRODUCTION
*Streptococcus mutans* is classified as a gram-positive facultative anaerobe. It is an important pathogen in dental caries formation. *Streptococcus mutans* can be isolated from active and non-active sites in the oral cavity. The pathogenicity of *Streptococcus mutans* is more associated with the presence of dietary sucrose. *Streptococcus mutans* produces complex extracellular polysaccharide (dextran) and glycoproteins from sucrose which helps *Streptococcus mutans* and other associated microbes bind tightly to the teeth and the surrounding structures leading to a large colony of *Streptococcus mutans* producing acids from sucrose. The acidic environment resulting from acid production from the metabolism of carbohydrate substrates on the teeth surface leads to the formation of lesions on the teeth enamel. Untreated dental caries can cause difficulties in chewing, sleeping, pain, systemic infections, stunted growth in children and absence from work and school, thus impairing quality of life. The breakdown of pulpal tissues can lead to intense pain. It can also cause halitosis, foul taste, sensitivity, discomfort when chewing, difficulty in facial movement, jaw pain etc. 

Several studies have revealed that dental caries can be prevented by eliminating and reducing the growth of *Streptococcus mutans* in the oral cavity by preventing formation of dental plaque inhabited by dextran-mediated bacterial aggregation. This can be achieved by mechanical means such as brushing, which may not be sufficient. Chemical agents such as xylitol and chlorhexidine have proven to be effective in reducing the growth of *Streptococcus mutans*. However, prolonged use of chlorhexidine is associated with altered microbiota, taste alteration, staining of teeth and restorations and mucosal desquamation.

The folkloric medicinal plants have been suggested as an alternative to synthetic chemical agents since...
they have little or no side effects on the oral tissues.


All parts of the *Spondias mombin* tree are medically important in folkloric medicine. The fruit decoction is used as a diuretic and febrifuge. Tea made of *Spondias mombin* leaves and flowers relieves stomachache, biliousness and inflammation. Decoction of young leaves is used to treat diarrhoea and dysentery. Crushed leaves and the powder of dry leaves are applied to wounds. They were also used for their antimicrobial, antiviral, abortifacient anti-inflammatory properties.

The Ivorians use the roots as febrifuge. The stem is used as a purgative and local treatment for leprosy. The decoction of stem bark is used for the treatment of severe cough, serves as emetic, remedy for diarrhoea, dysentery, hemorrhoids, gonorrhoea and leucorrhoea.

The decoction of astringent stem bark is used to expel calculi from kidneys. The gum in the trees is used as expectorant and to expel tapeworms. The Igbos used the decoction of the leaves to wash swollen faces. The antimicrobial properties of parts of the *Spondias mombin* tree – leaves, stem bark and fruits – have been confirmed by numerous phytochemical studies. Plants and plant-derived products have been used for centuries to improve human health, even before contemporary medicine.

The medical use of *Spondias mombin* has been mostly through oral communication but there has not been any report of adverse effects through the use of the leaves. The purpose of this study is to determine the part of *Spondias mombin* plant that has greater antimicrobial activities against *Streptococcus mutans* in order to encourage developing countries to incorporate *Spondias mombin* in plant-based therapies in their healthcare systems for the prevention and treatment of dental caries.

**MATERIALS AND METHODS**

*Collection of plant materials*

Fresh leaves, stem bark and wood of *Spondias mombin* for the charcoal were obtained from Ezioobodo in Owerri, Imo State, Nigeria.

*Preparation and extraction of Spondias mombin plant*

The leaves, stem bark and wood were sundried. The leaves and stem barks were pulverised using a mechanical grinder. The dried wood was burnt to obtain charcoal which was pulverised using a mechanical grinder. Twenty grams of each plant material was weighed with a digital weighing balance and soaked in 100ml of ethanol and hot water in 250ml Uniscope flasks. The flasks were covered with a cotton plug and then wrapped with aluminium foil and allowed to stand for 24h in an electronic shaker. Filtration was done using Whatman 42 filter paper. The filtrates were allowed to evaporate to dryness in as many petri dishes that could contain the leaves, stem bark and charcoal solution and labelled accordingly on the base of the petri dishes. The ethanol and aqueous filtrates of the leaves, stem bark and charcoal extracts of *S. mombin* were scraped off the dish with a sterile scalpel into separate sterile containers labelled appropriately and covered, ready to be used. 80ml of Gentamycin infusion and mouthwash were used as the control to compare the effectiveness of the plant against *Streptococcus mutans* in zones of inhibition. The tests for each plant part and the controls were done in duplicate to obtain the standard deviation. Minimum inhibitory concentration and minimum bactericidal concentration were also done using the concentrations of plant parts.

**Source of Streptococcus mutans isolate**

Tooth swabs were collected from the gum and cavity of a patient in a dental clinic using a sterile swab stick. The swab stick was immediately transported to the laboratory in an ice pack. The swab stick was transferred into a nutrient broth to resuscitate the organisms in the swab stick. One-tenth millilitre (0.1ml) from dilution 10⁴ was inoculated into pre-sterilised MS agar, and inocula spread evenly and incubated anaerobically in an anaerobic jar maintained at 37°C for 48h. *Streptococcus mutans* was identified according to the method of Cheesbrough (2002).

**Antimicrobial susceptibility tests of Spondias mombin ethanol and aqueous extracts of the leaves, stem bark and charcoal**

Susceptibility of *Streptococcus mutans* to the extracts of *Spondias mombin* leaves, stem bark and charcoal was done by means of agar well diffusion assay. Four wells 6.25mm deep labelled A-D were made with a sterile cork borer on each four (4) Mueller Hinton Agar petri dishes previously seeded with the 24h old, standardised cultures (*Streptococcus mutans*). For this test, dried ethanol and aqueous filtrates of *Spondias mombin* leaves, stem bark and charcoal were each introduced into a test tube (labelled appropriately) with 1ml of distilled water using a pipette and then shaken. These were then used to obtain different concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml using 2-by-2 dilution method in duplicate for each plant part (labelled A-500mg/ml, B- 250mg/ml, C-125mg/ml, D-62.5mg/ml). The four wells on each petri dish (A-D) were filled with different concentrations (500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml) of ethanol and aqueous extracts of *Spondias mombin* leaves, stem bark and charcoal drawn with a pipette according to the strength of 6.25mm deep labelled A-D were made with a sterile cork borer on each four (4) Mueller Hinton Agar petri dishes previously seeded with the 24h old, standardised cultures (*Streptococcus mutans*). For this test, dried ethanol and aqueous filtrates of *Spondias mombin* leaves, stem bark and charcoal were each introduced into a test tube (labelled appropriately) with 1ml of distilled water using a pipette and then shaken. These were then used to obtain different concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml using 2-by-2 dilution method in duplicate for each plant part (labelled A-500mg/ml, B- 250mg/ml, C-125mg/ml, D-62.5mg/ml). The four wells on each petri dish (A-D) were filled with different concentrations (500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml) of ethanol and aqueous extracts of *Spondias mombin* leaves, stem bark and charcoal drawn with a pipette according to the alphabetic order. 80mg/ml of Gentamycin and mouthwash were introduced to two wells on the fourth petri dish as controls. This was done in duplicate. The plates were incubated for 24h at 37°C. Clear zones of inhibition were measured after incubation and recorded in millimeters (mm) using a transparent ruler.

**Minimum inhibitory concentration (MIC) of Spondias mombin ethanol and aqueous extracts of the leaves, stem bark and charcoal**

Serial dilutions of the ethanol and aqueous extracts of *Spondias mombin* leaves, stem bark and charcoal (representing different concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml) were made in two-fold dilution in nutrient broth. 0.5ml of standardised *Streptococcus mutans* cultures were introduced into the broth containing the different concentrations of the extract and incubated at 37°C for 24h. The procedure was repeated for the extracts. Broth tubes that appear turbid are indicative of bacterial growth while tubes that remain clear indicate no growth. The MIC of the antibiotics/antimicrobial agents is the lowest concentration that does not show growth. The overnight grown isolates in
the broth were further subjected to spectrophotometric reading to determine the optical density at 340nm. Each broth tube containing different concentrations of leaves, stem bark and charcoal extracts of *Spondias mombin* (labelled according to the plant parts and concentrations) was placed into a spectrophotometer (machine), which had its readings reset to zero, using distilled water. The spectrophotometer, when switched on, allowed the passage of light rays through the broth tubes. Readings of last figures on display were recorded. Broth tubes with clearer solution recorded higher figures on the machine. This also indicated no growth of test organism. Turbid broth tubes recorded lower figures on the machine, indicating growth of test organism.

**Minimum bactericidal concentration of *Spondias mombin* ethanol and aqueous extracts of the leaves, stem bark and charcoal**

A loopful of wire loop suspensions from MIC (with different concentrations of leaves, stem bark and charcoal extracts of *Spondias mombin*) were streaked equally on a freshly prepared surface of dried Mueller Hinton agar plates labelled according to the plant parts and concentrations (30 plates in all), and incubated overnight at 37°C. Each concentration of leaves, stem bark and charcoal extracts of *Spondias mombin* were in duplicate which resulted in 24 plates for ethanol extracts and 24 plates for aqueous extracts. The streaking with loopful of wire loop suspensions from MIC was carried out on all the plates. MBC was indicated as the plate/concentration with the least bacteria growth. The different concentrations were duplicated.

**Data analysis**

Using Statistical Package for Social Science (SPSS version 29, IBM), paired sample t-test was applied to test the mean zone of inhibition (mm) effects between aqueous extracts and ethanol extracts for each of the concentrations. One-way analysis of variance was applied to compare the mean zone of inhibition (mm) for 500mg leaf extracts, Gentamycin (80mg) along with the standard deviation to show the variability within those duplicates. The results in Table I revealed interesting patterns. At a concentration of 500mg, both *Spondias mombin* bark and leaves ethanol extracts exhibited substantial antimicrobial activity, with zone of inhibition values ranging from 22.5±3.5mm to 32.5±3.5mm, respectively. The ethanol extract of *Spondias mombin* leaves showed the highest zone of inhibition (32.5±3.5), suggesting it had a stronger inhibitory effect on *Streptococcus mutans* compared to aqueous extracts (25±0.0). This difference is not statistically significant – ie t-test p-value=.102.

As the concentration of the extracts decreased to 250mg and 125mg, the zone of inhibition also reduced. However, the extracts still demonstrated noticeable antimicrobial activity, especially the ethanol extract of *Spondias mombin* leaves at 125mg concentration, which showed a zone of inhibition of 17.5±3.5mm against the aqueous extract of *Spondias mombin* leaves.

The antimicrobial activities of the stem bark, and ethanol extracts of the leaves and charcoal, are nonexistent at the concentration of 65.2mg. The aqueous extracts of the leaves and charcoal show some antimicrobial activity, 7.5 μg/mL ± 3.5 and 7.5 μg/mL ± 0.0 respectively.

**RESULTS**

**Antimicrobial activities of *Spondias mombin* against *Streptococcus mutans***

For each Spondias mombin ethanol and aqueous extracts of stem bark, leaves and charcoal, multiple concentrations were tested against *Streptococcus mutans*. The zone of inhibition values were recorded as the mean of duplicates, along with the standard deviation to show the variability within those duplicates.

The Spondias mombin leaves ethanol extract exhibited a zone of inhibition of 32.5mm at 500mg/ml against the test microorganism. This suggested that the extract at this concentration had a significant inhibitory effect on the growth of the test organism.

The Spondias mombin leaves aqueous extract showed a slightly reduced zone of inhibition of 25mm at 500mg/ml. While the inhibitory effect was slightly lower compared to the ethanol extract, it still demonstrated substantial antimicrobial activity against the microorganisms.

**Table I. Diameter of the Zone of Inhibition of ethanol and aqueous extracts of *Spondias mombin* stem bark, leaves and charcoal against *Streptococcus mutans***

<table>
<thead>
<tr>
<th>Concentration (mg)</th>
<th>Spondias mombin stem bark ethanol</th>
<th>Spondias mombin leaves ethanol</th>
<th>Spondias mombin leaves aqueous</th>
<th>Charcoal ethanol</th>
<th>Charcoal aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>22.5±3.5</td>
<td>32.5±3.5</td>
<td>25±0.0</td>
<td>10±0.0</td>
<td>0±0.0</td>
</tr>
<tr>
<td>250</td>
<td>15±0.0</td>
<td>22.5±3.5</td>
<td>16.5±2.1</td>
<td>6.5±0.0</td>
<td>0±0.0</td>
</tr>
<tr>
<td>125</td>
<td>6.5±2.1</td>
<td>17.5±3.5</td>
<td>12.5±3.5</td>
<td>2.5±3.5</td>
<td>5±7.1</td>
</tr>
<tr>
<td>62.5</td>
<td>0±0.0</td>
<td>7.5±3.5</td>
<td>0±0.0</td>
<td>7.5±0.0</td>
<td>0±0.0</td>
</tr>
</tbody>
</table>

Values are the mean of two duplicates ± standard deviation. P>0.05 (t-test), 0a= t cannot be computed because the standard deviation of the difference is equal to 0.
Figure 1 shows that the ethanol extract of *Spondias mombin* leaves has a larger zone of inhibition compared to Gentamycin (32.5mm vs 30mm). Nevertheless, Gentamycin exhibited substantial zones of inhibition of 30mm at 80mg/ml while the leaves extracts performed stronger at higher concentration. Mouthwash also showed zones of inhibition of 25mm at 80mg/ml than both ethanol and aqueous extracts of the plant parts at lower concentrations (250mg/ml, 125mg/ml and 62.5mg/ml).

In summary, the results demonstrated that *Spondias mombin* leaves ethanol and aqueous extracts, at a concentration of 500mg, exhibited notable antimicrobial activity against the test microorganism. However, the antimicrobial activity was stronger in the ethanol leaves extract.

The data in Table II shows the Minimal Inhibitory Concentration (MIC) of different extracts from *Spondias mombin* bark, *Spondias mombin* leaves and *Spondias mombin* charcoal against *Streptococcus mutans*. The MIC is the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism.

At the highest concentration of 500mg, the *Spondias mombin* bark and leaves extracts exhibited MIC values ranging from approximately 1.53 μg/mL to 1.98 μg/mL against *Streptococcus mutans*. Among these extracts, the ethanol extract of *Spondias mombin* leaves showed the most potent inhibitory effect, having an MIC of 1.46 μg/mL against *Streptococcus mutans*.

As the concentration of the extracts decreased to 250mg and 125mg, the MIC values generally increased, indicating that higher concentrations were required to achieve the same level of inhibition. However, the extracts still demonstrated considerable antimicrobial activity, with the ethanol extract

### Table II. Minimum Inhibitory Concentration of ethanol and aqueous extracts of *Spondias mombin* stem bark, leaves and charcoal against *Streptococcus mutans*

<table>
<thead>
<tr>
<th>Concentration (mg)</th>
<th><em>Spondias mombin</em> bark</th>
<th><em>Spondias mombin</em> leaf</th>
<th>Charcoal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>P value</td>
</tr>
<tr>
<td>500</td>
<td>1.5310±0.01</td>
<td>1.1145±0.05</td>
<td>0.065</td>
</tr>
<tr>
<td>250</td>
<td>1.4420±0.01</td>
<td>1.3050±0.07</td>
<td>0.181</td>
</tr>
<tr>
<td>125</td>
<td>1.4970±0.56</td>
<td>0.9825±0.19</td>
<td>0.409</td>
</tr>
<tr>
<td>65.2</td>
<td>1.0775±0.56</td>
<td>0.9180±0.24</td>
<td>0.603</td>
</tr>
</tbody>
</table>

Values are the mean of two duplicates ± standard deviation. P>0.05
Table III. Minimum bactericidal concentration of ethanol and aqueous extracts of Spondias mombin stem bark, leaves and charcoal on Streptococcus mutans

<table>
<thead>
<tr>
<th>Minimal bactericidal concentration</th>
<th>Spondias mombin stem bark</th>
<th>Spondias mombin leaves</th>
<th>Charcoal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
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<td>-</td>
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<td>++</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
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</tbody>
</table>

of Spondias mombin leaves showing the highest MIC of approximately 2.04 μg/mL against Streptococcus mutans.

At the lowest concentration of 65.2mg, the MIC values decreased further, showing that the antimicrobial activity decreased at this concentration. However, the extracts remained effective against the microorganism, with the ethanol extract of Spondias mombin leaves having an MIC of approximately 1.46 μg/mL against Streptococcus mutans.

The charcoal extract showed varying MIC values across different concentrations and extraction medium but generally exhibited weaker inhibitory effects compared to the Spondias mombin bark and leaves extracts.

Overall, the data in Table III indicates that both Spondias mombin bark and leaves extracts possess notable bactericidal properties, with the ethanol extracts demonstrating stronger bactericidal effects compared to the aqueous extracts. The MBC values provide valuable information about the effectiveness of each extract and concentration against the tested microorganism.

Indicates no growth of Streptococcus mutans. + slight growth of Streptococcus mutans. ++ noticeable growth of Streptococcus mutans. +++ overgrowth of Streptococcus mutans.

DISCUSSION

This study compared the zone of inhibition (ZOI), minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol and aqueous extracts of Spondias mombin stem bark, leaves and charcoal against Streptococcus mutans, which is a cariogenic organism. Many studies have shown that plants contain antibacterial and even antifungal substances. Spondias mombin is among the numerous plants of medicinal interest due to its use in folkloric medicine in the treatment of various ailments including dental health issues.17

In this study, Spondias mombin stem bark, leaves and charcoal ethanol and aqueous extracts all exhibited antimicrobial activities at varying concentrations (with the exception of 62.5mg/ml of ethanol and aqueous extracts of the stem bark and leaves extracts) in zone of inhibition assay. This result from zone of inhibition was similar to the research carried out by Garga, Garasin18 and Mada, Garba19 who reported that the higher the concentrations of both extracts, the higher the antibacterial activities against test organism.

The stem bark extracts showed concentration dependent activity. The result contrasted the findings of Osuntokun11 who reported that aqueous extracts of stem bark of Spondias mombin were less active against the test bacteria than ethanol extract. He attributed his findings to the partial dissolution of some of the active components by water. However, the result of these findings could be attributed to the use of hot distilled water instead of cold distilled water which could mean that hot water is a better solvent than cold water, and so justifies the use of hot water as extractant by traditional healers.20

The result from this study revealed that ethanol leaves extract of Spondias mombin had higher antibacterial activities in zone of inhibition with mean value of 32.5mm at 500mg/ml (higher than Gentamycin (30mm) and mouthwash (25mm). The present results confirm the finding from a study by Cordeiro, de Mendonç21 where they discovered that ethanol extracts of Spondias mombin leaves had more inhibitory activities against Streptococcus mutans while using chlorhexidine digluconate as gold standard. Further, this study supports the finding by Maduka, Okpogba22 who reported that ethanol extract of Spondias mombin leaves inhibited growth of Staphylococcus aureus more than hot and cold aqueous leaves extract of Spondias mombin. A study by Amadi, Oyeka23 did not notice any noticeable antimicrobial activities of Spondias mombin ethanol and hot water extracts against Streptococcus mutans in zone of inhibition diameter. However, they reported higher zone of inhibition diameter of the cold-water extracts of Spondias mombin leaves and Baphia nitida leaves combined than Baphia nitida alone against Streptococcus mutans. Kudi, Umoh24 suggested that plants extract with zone of inhibition of 6mm and above against a selected pathogen is considered to have antimicrobial activities while Adeleye, Omadime25 suggested 10mm and above. In this study, the stem bark, the leaves and charcoal ethanol extracts all demonstrated zone of inhibition diameter greater than 6mm and above, at varying concentrations. The aqueous extract of charcoal had a zone of inhibition diameter of 7.5mm at 62.5mg/ml. The ethanol and hot water extracts of Spondias mombin charcoal had the least zone of inhibition diameter among the parts of Spondias mombin tree. The hot water extracts of charcoal were noticed to have some inhibitory diameter at lower concentration than ethanol extracts.

The spectrophotometric readings of minimum inhibitory concentrations of ethanol extract of leaves of Spondias mombin showed more inhibitory activity against test organism at higher concentrations (500mg/ml to 250mg/ml) but, at 125mg/ml, ethanol stem bark extract demonstrated slightly higher inhibitory activity. This result corroborates the
finding by Maduka, Okpogba who reported that stem bark and leaves ethanol extracts of Spondias mombin inhibited E. coli, Pseudomonas, Klebsiella and Staphylococcus. The ethanol and aqueous extracts of the leaves showed highest inhibitory activities at 500mg/ml, while aqueous extracts of the charcoal exhibited more inhibitory action against the test organism than ethanol extracts of charcoal. This finding confirmed the result by Cordeiro, de Mendonca and de Lima, Alves who reported that the aqueous and ethanol extracts of Spondias mombin leaves exhibited bactericidal actions in the first two hours after initial contact with test organisms. It was suggested that hydroethanolic extracts of Spondias mombin leaves in clinical test as mouthwash. Chlorhexidine was found to exhibit bactericidal effect for up to 12h. Bactericidal actions decreased with a decrease in concentration. However, aqueous extracts of stem bark exhibited bactericidal action at 250mg/ml, barest growth at 500mg/ml and noticeable increase at decreased concentrations. The bactericidal activities of aqueous extract of the leaves reduced with decreased concentration. The aqueous extracts of the charcoal had the least bactericidal activities against test organisms. All the extracts exhibited concentration dependent bactericidal activities. These findings provide valuable insights into the antimicrobial properties of Spondias mombin leaves extract and the effectiveness of Gentamycin and mouthwash as antimicrobial agents. The study highlights the potential applications of Spondias mombin leaves extract in various contexts where inhibiting the growth of the Streptococcus mutans is desired, such as in the development of pharmaceuticals, oral care products and antimicrobial agents. Further research and analysis may be required to explore the mechanisms underlying the observed antimicrobial activities and to optimise the concentrations of the treatments for specific applications. Moreso, since dental treatment is expensive in most underdeveloped countries, Spondias mombin could be refined and employed in the primary health centres for preventive treatment of dental caries. This would greatly support the current trend of preventive dentistry.

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Conflict of interest

The authors declare no conflict of interest. The authors alone were responsible for the content and the writing of the paper.

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