International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 6-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.06.011

In vitro comparative study of ethanol and aqueous extracts of *Garcinia kola* stem bark and seed on *Streptococcus mutans* isolated from oral cavity

Iwueke, Gertrude, C*[1], Obidinma, Sonia[1], Braide, Wesley[2], Kalu, Uchechi Caroline[2], Peter, Chidiebere Okorie[3], Ken Nkemdilim Okeke[4] and Anselem, Dozie Okere[5]

[1]Department of Dental Technology, Federal University of Technology, Owerri, Imo State
[2]Department of Microbiology, Federal University of Technology, Owerri.
[3]Department of Dental Technology, Faculty of Dental Health.
Federal College of Dental Technology and Therapy, Enugu
[4]Department of Dental Technology, Federal University of Technology, Owerri.
[5]Department of Science Laboratory Technology, Federal University of Technology, Owerri.
*Corresponding Author: G.C. Iwueke. Department of Dental Technology,
Federal University of Technology, Owerri.

Abstract

Introduction: The stem bark and seeds of the *Garcinia kola* plant have a history of use in traditional medicine, primarily in the southern regions of Nigeria, particularly for preventing dental cavities.
Aims and objectives: To assess and contrast the antimicrobial effects of ethanol and aqueous extracts from both the seeds and stem bark of the *Garcinia kola* plant against *Streptococcus mutans*.
Materials and methods: Twenty grams of dried, powdered seeds and stem bark of *Garcinia kola* were measured and placed in 250ml flasks. They were then soaked in 100ml of both ethanol and hot water separately. The flasks were sealed with cotton plugs and covered with aluminum foil. The mixtures were left to stand for 24 hours with occasional shaking, after which they were filtered with Whatman 42 filter paper. Ten grams of each plant part dried filtrates were diluted to achieve concentrations of 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml, each in duplicate. Agar well diffusion assay to conduct tests for the zone of inhibition. Minimum inhibition concentration test was done using Mueller Hinton broth suspended in 9 ml of peptone water in which the different concentrations. Minimum bactericidal concentration test against *Streptococcus mutans* was done with Broth from MIC. Gentamycin and mouthwash were used as control.
Results: The ethanol extract from the stem bark exhibited notably stronger inhibitory effects against the test organism compared to both ethanol and aqueous extracts of both the seeds and stem bark.

Conclusion: The aqueous and ethanol extracts from the seeds and stem bark of *Garcinia kola* effectively impede the growth of *Streptococcus mutans* especially at higher concentrations, providing scientific support for their traditional use in preventing dental caries in folk medicine.

Keywords: *Garcinia kola*, *Streptococcus mutans*, dental caries, antimicrobial, zone of inhibition, minimum bactericidal.

Introduction

Research interest has been drawn towards natural substances derived from plants because of their wide-ranging applications in the fields of medicine and nutrition. (Ncube *et al*, 2008). For generations, inhabitants of Africa, particularly those from West and Central regions, have depended on trees and herbal remedies to supply essential sustenance and healthcare solutions for themselves and their animals (Manourova *et al*, 2019). Medicinal plants represent a vast reservoir of resources for traditional remedies, contemporary pharmaceuticals, nutraceuticals, dietary supplements, traditional medicines, pharmaceutical precursors, and chemical compounds used in the synthesis of drugs (Ncube *et al*., 2008). This is because these plants contain abundant secondary metabolites, primarily located in certain plant parts, which give them their pharmacological significance. Some of these edible plants are consumed without awareness of their medicinal properties (Ogunmoyole *et al*, 2012). One example of such a plant is *Garcinia kola*.

Garcinia kola (Heckel) is a perennial plant classified as a dicotyledonous species within the Guttiferae plant family. It thrives in forested regions and is commonly found across West and Central Africa (Iwu *et al*,1990). In Southern Nigeria, *Garcinia kola* is commonly chewed as a masticatory. Among the Igbo tribe in Eastern Nigeria, offering *Garcinia kola* seeds to visitors symbolizes a gesture of peace and hospitality. *Garcinia kola* seeds are called '*aku-ilu'* in Igbo language, '*orogbp*' in Yoruba language, '*namijin goro*' in Hausa language and 'bitter kola' in English language (Ogunmoyole *et al*, 2012). In West Africa, the plant's root serves as bitter chewing-sticks (Otor *et al.*, 2001).

The plant known as *Garcinia kola* or the "wonder plant" has been widely utilized in traditional medicine to address a variety of illnesses. (Taiwo *et al***,** 1999). It has been documented that the seeds are employed for alleviating toothaches and as a preventive measure against dental caries (Ajayi *et al*, 2014). Both the seeds and stem bark of this plant have been found to contain intricate blends of phenolic compounds, including tannins and guttiferins (Etkin, 1981), biflavonoids, xanthenes, benzophenone, kolaflavanone and garcinia flavanone (Iwu *et al*, 1982). All these compounds have antimicrobial activities (Ogunmoloye *et al*., 2012).

Streptococcus mutans is a facultative anaerobic, gram-positive coccus commonly found in human oral cavity (Ryan *et al*, 2004). *Streptococcus mutans* is considered as one of the main etiological factors of dental caries (Madigan *et al*, 2005). *Streptococcus mutans* is a microorganism that resides in the oral cavity as part of the normal microbial community. Its potential to cause harm is influenced by specific environmental factors. This cariogenic bacterium makes up 39% of the *streptococcal* population in the oral cavity and is especially abundant in the crevices and grooves of teeth(Nicolas *et al*, 2011). It is among the rare organisms with specialized receptors that enhance its ability to adhere to tooth surfaces. *Streptococcus mutans* employs an enzyme called glucansucrase to transform sugar into an adhesive extracellular polysaccharide known as dextran, facilitating the formation of plaque. It metabolizes glucose, fructose, and lactose, resulting in lactic acid as the final product. The combination of lactic acid and dental plaque contributes to the development of dental caries (Madigan *et al*., 2005).

Dental caries represents a significant public health issue worldwide and stands as the most prevalent and avoidable ailment (World Health Organization [WHO], 2021). Dental caries is an ailment that spans across all age groups throughout one's life. Approximately 2.3 billion individuals experience dental caries in their permanent teeth, and over 530 million children are affected by dental caries in their primary teeth, according to estimates (WHO, 2021). In low and middle-income nations such as Nigeria, individuals who are economically disadvantaged, vulnerable, and marginalized face limited accessibility to dental caries treatment. This situation contributes to higher rates of occurrence and prevalence of dental caries within these regions. Dental caries can result in tooth loss, infections, and discomfort. *Streptococci* from areas affected by dental caries can enter the bloodstream in case of injury, potentially causing sepsis, the formation of abscesses in the throat, lungs, and liver. Furthermore, it has the potential to lead to cardiovalvulitis and heart-related diseases (Suddik et al., 1990). With this rationale in mind, this research was systematically planned to create various concentrations of ethanolic and aqueous extracts derived from both the stem bark and seeds of *Garcinia kola*. Many studies have been carried out on antimicrobial properties of Garcinia plant parts using various extraction methods. However there haven't been much done on aqueous extract of stem bark on cariogenic organisms such as Streptococcus mutans. The primary objective of this study therefore was to assess and contrast antimicrobial effectiveness of aqueous and ethanol extracts of *Garcinia kola* seed and stem bark against *Streptococcus mutans*, a microorganism associated with dental caries. The ultimate goal is to propose an alternative and cost-effective approach for preventing dental caries, particularly in developing nations with restricted access to dental healthcare services.

Materials and Methods

Collection of plant material

Garcinia kola seeds were purchased from Eke Onuwa market in Douglas, Owerri, Imo State. *Garcinia kola* stem bark was collected from Uzoagba, Ikeduru in Mbaitoli Local Government Area and Egbu in Owerri North Local Government Area, Imo State.

Preparation and extraction of plant materials

Garcinia kola seeds were prepared by removing their hulls, washing, dicing into small pieces, and then sun-dried. Similarly, the stem bark was washed, diced, and sun-dried. To create a fine powder, both the dried seed and stem samples were pulverized using a mechanical grinder and stored in sterile separate containers. For the extraction process, 20 grams of each plant material (seed and stem bark) were accurately weighed and placed in separate 250ml flasks. Subsequently, they were soaked in 100ml of ethanol and hot distilled water, respectively. The flasks were sealed with cotton plugs and further covered with aluminum foil. They were left to stand for 24 hours with periodic shaking. After this period, the flasks were vigorously agitated and then filtered using Whatman's no 42-filter paper. The resulting filtrates were allowed to evaporate until they completely dried in petri dishes which were labeled according to the plant parts.The ethanol and aqueous filtrates of the seeds and stem bark of *Garcinia kola* plant scrapped off from the petri dishes with sterile scalpel into separate sterile containers labeled 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 zone of inhibition. The tests for each plant part and the controls were done in duplicates to obtain the standard deviation. Minimum inhibitory concentration and Minimum bactericidal concentration were also done using different concentrations of plant parts.To obtain different concentrations for testing, a 2-fold dilution method was applied to the ethanol and hot water (aqueous) extracts using 1ml of distilled water. These concentrations included 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml. The process was carried out in duplicate to ensure accuracy, and the standard deviation of the mean values of the tests were calculated.

Source of *Streptococcus mutans* isolate

A sterile swab stick was used to collect a swab sample from both the gum and cavity of a patient at the Dental Clinic. This swab was promptly transported to the laboratory, ensuring its preservation with the use of an ice pack during transit. Upon arrival at the laboratory, the swab stick was introduced into a nutrient broth to revive the microorganisms present within the swab samples.

To prepare for tests, various dilutions of the broth were created by transferring 1 ml of the broth into 9 ml of sterile distilled water. This dilution process continued until the desired concentration of 10^5 was achieved. Subsequently, an aliquot portion measuring 0.1 ml from dilutions 10^1 and 10^2 was aseptically introduced into pre-sterilized Mitis Salivarius agar. The inoculum was evenly spread across the surface of the agar using a sterile glass rod, similar in shape to a hockey stick.

The MS agar plates that were inoculated with the samples were then placed in an anaerobic jar, which was maintained at a temperature of 37°C for a duration of 48 hours. Streptococcus mutans was identified through an assessment of its colonial morphology, gram reaction, and biochemical tests, following the protocol outlined in Cheesbrough (2002).

Minimum Inhibitory Zone Test of *Garcinia kola* seeds and stem bark extracts against *Streptococcus mutans*

The susceptibility of *Streptococcus mutans* to the extracts derived from both the seeds and stem bark of *Garcinia kola* was assessed through the use of an agar well diffusion assay. To initiate this process, four wells, each measuring 6.25 mm in depth, were meticulously created in each agar plates utilizing a sterile cork borer. Each of the four wells were labeled A-D, according to the different concentrations of the ethanol and aqueous extracts of *Garcinia kola* plant parts and their duplicates. These agar plates had previously been inoculated with 24-hour-old standardized cultures of *Streptococcus mutans*.

Subsequently, these wells were filled with varying concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml) of the ethanol and aqueous extracts derived from the seeds and stem bark of *Garcinia kola* according to the labeling on the petri dishes. This entire procedure was performed in duplicates to ensure consistency and accuracy. 80mg/ml of gentamycin and mouthwash (Healthpoint Daily Mouth wash) were employed as controls.

Following the completion of this setup, the agar plates were then placed in an incubator and allowed to incubate for a period of 24 hours at a temperature of 37°C. Upon completion of the incubation period, any clear zones of inhibition that had developed were carefully measured and the measurements were duly recorded in millimeters (mm) with the aid of a transparent ruler.

Minimum Inhibitory Concentration of *Garcinia kola* ethanol and aqueous extracts of the seed, and stem bark against *Streptococcus mutans*

Various dilutions of both the ethanol and aqueous extracts obtained from *Garcinia kola* seeds and stem bark, encompassing distinct concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, were prepared in separate tubes labeled A-D in duplicates.

In parallel, the *Streptococcus mutans* strain was cultivated in Mueller Hinton broth and subsequently suspended in 9 ml of peptone water across different test tubes. To these test tubes, 5 ml of each concentration of the ethanol and aqueous extracts sourced from *Garcinia kola* was introduced. Following this, the test tubes were sealed and subjected to incubation at a temperature of 37°C for a duration of 24 hours.

The assessment of bacterial growth was based on the turbidity observed in the broth tubes. If a tube exhibited cloudiness, it indicated bacterial growth,

whereas if the tube remained clear, it signified the absence of growth. The Minimum Inhibition Concentration (MIC) of the antibiotics or antimicrobial agents refers to the lowest concentration at which growth was not observed.

Furthermore, the bacterial isolates that had grown overnight in the broth underwent additional analysis through spectrophotometric readings to determine the optical density at 340 nm. Each broth tube containing different concentrations of seeds and stembark extracts of *Garcina kola* (labeled 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 *Garcinia kola* ethanol and aqueous extracts of the seed and stem bark

A loopful of wire loop suspensions obtained from the Minimum Inhibition Concentration (MIC) tests were utilized to evenly streak the surface of recently prepared dried and labeled Mueller Hinton agar plates. Subsequently, these plates were subjected to overnight incubation at a temperature of 37°C. This procedure was duplicated for each concentration prepared for the test.

The determination of the Minimum Bactericidal Concentration (MBC) was based on the plate or concentration where the least bacterial growth was observed. This process was carried out in duplicate for each concentration of each plant part.

Results

Table 1 presents the results of the zone of inhibition measurements. For the ethanol extract of *Garcinia kola* seed at a concentration of 500 mg/ml, the mean diameter of the inhibition zone was measured at 15 mm. The largest zone of inhibition was observed at the concentration of 250 mg/ml, with a mean diameter of 17.5 mm. Conversely, the aqueous extract of the seed exhibited the highest mean diameter of 15 mm at a concentration of 125 mg/ml. The smallest zone of inhibition for the seed was recorded at the concentration of 62.5 mg/ml, with a mean diameter of 7.5 mm for the ethanol extract and 5.0 mm for the aqueous extract at 500mg/ml and 62.5mg/ml.

Moving on to the ethanol extract of *Garcinia kola* stem bark, the highest zone of inhibition was observed at the concentration of 250 mg/ml, with a mean diameter of 25 mm. The minimum zone of inhibition was recorded at the concentration of 62.5 mg/ml, with a mean diameter of 15 mm. In the case of the aqueous extract of *Garcinia kola* stem bark, the highest mean zone of inhibition, measuring 22.5 mm, was observed at a concentration of 500 mg/ml, followed by 17.5mm at 250mg/ml while no activity against the test organism was detected at a concentration of 62.5 mg/ml.

Notably, the ethanol extracts of *Garcinia kola* stem bark demonstrated larger zones of inhibition against *Streptococcus mutans* compared to the aqueous extract of the stem bark. Interestingly, both the ethanol and aqueous extracts of seeds and stem bark exhibited their highest inhibitory activities at concentrations of 250 mg/ml and 125 mg/ml, except for the aqueous extract of *Garcinia kola* stem bark with the highest inhibitory zone at 500mg/ml. It's worth mentioning that at a concentration of 80 mg/ml of gentamycin, the zone of inhibition had a diameter of 30 mm, whereas the mean diameter of the zone of inhibition for the mouthwash was 20 mm, as indicated in the data table below. From the table below, ethanol and aqueous extracts of *Garcinia kola* stem bark extracts at 250mg/ml and 500mg/ml respectively have slightly higher zone of inhibition than the mouth wash but lower than gentamycin.

Table 1. Diameter of the Zone of Inhibition of ethanol and aqueous extracts of *Garcinia kola* seed and stem bark, on *Streptococcus mutans*

	Zone of inhibition (mm)					
Concentration (mg)	*Garcinia kola* seed extract		*Garcinia kola* stem bark			
	Ethanol	Aqueous	Ethanol	Aqueous	Gentamycin (80mg)	Mouth wash
500	15±0.0	5.0±5	17.0±7.5	22.5±2.5	30±0.0	20±0.0
250	17.5±7.5	10±0.0	25±0.0	17.5±7.5	30±0.0	20±0.0
125	15.0±5	15.0±0.0	20.0±5	5±5	30±0.0	20±0.0
62.5	7.5±2.5	5.0±5	15.0±5	0±0	30±0.0	20±0.0

Values are the mean of two duplicate± standard deviation.

Table 2 displays the spectrophotometric readings of the minimum inhibitory concentration (MIC) for the ethanol and aqueous extracts of *Garcinia kola* seed and stem bark in their effects against *Streptococcus mutans*. The highest inhibitory concentration was observed at the concentration of 500 mg/ml for the ethanol extract of *Garcinia kola* seed, with a mean value of 2.085 nm. Similarly, the highest inhibitory concentration for the aqueous extract of *Garcinia kola* seed was recorded at 500 mg/ml, with a mean value of 2.107 nm. It's noteworthy that the minimum inhibitory concentration of the aqueous extract of *Garcinia kola* seed was slightly higher than that of the ethanol extract of the seed.

On the other hand, the aqueous extract of *Garcinia kola* stem bark exhibited slightly higher mean MIC values than the ethanol extract across various concentrations, except at the concentration of 125 mg/ml. The data in the table below demonstrates that, in general, the aqueous extracts of both *Garcinia kola* seed and stem bark exhibit greater minimum inhibitory activities against *Streptococcus mutans* compared to the ethanol extracts of the seeds and stem bark.

Table II. Spectrophotometric values (340 nm) of Minimum Inhibitory Concentration of *Garcinia kola* seed Extracts on *Streptococcus mutans*

	Minimal inhibitory concentration			
Concentration (mg)	*Garcina kola* seed extract		*Garcinia kola* stem bark extract	
	Ethanol	Aqueous	Ethanol	Aqueous
500	2.085±0.008	2.107±0.024	2.114±0.075	2.199±0.01
250	1.529±0.048	1.578±0.077	1.183±0.090	1.907±0.028
125	0.948±0.068	1.138±0.132	1.713±0.232	1.386±0.056
62.5	0.565±0.041	0.801±0.090	0.584±0.174	1.135±0.251

Values are the mean of two duplicate± standard deviation.

Table III, presented below, illustrates the bactericidal activities of the ethanol and aqueous extracts of *Garcinia kola* seed and stem bark at different concentrations. At a concentration of 500 mg/ml, the ethanol extract of *Garcinia kola* seed exhibited the highest bactericidal activity, indicated by a minus"-" sign, signifying no bacterial growth. Conversely, the aqueous extract of the seed at 500 mg/ml allowed slight growth of the test organism, denoted by a single +.

Interestingly, the minimum bactericidal concentration for the aqueous seed extract at 62.5 mg/ml demonstrated similar slight growth of the test organism as observed at 500 mg/ml, while the ethanol extract of the seed at 62.5 mg/ml exhibited more substantial growth of the test organism, indicated by a double plus, ++.

However, the ethanol extract of *Garcinia kola* stem bark displayed higher bactericidal activity at various concentrations compared to the aqueous extract of *Garcinia kola* stem bark. It's worth noting that the aqueous extract of *Garcinia kola* stem bark permitted more growth of the test organism than the other extracts. In summary, the ethanol extract of *Garcinia kola stem bark* demonstrated superior bactericidal activities compared to the seed extracts (ethanol and aqueous extracts).

Table III. Minimum Bactericidal Concentration of *Garcinia kola* seed Extracts on *Streptococcus mutans*

	Minimal bactericidal concentration			
Concentration (mg)	*Garcina kola* seed extract		*Garcinia kola* stem bark extract	
	Ethanol	Aqueous	Ethanol	Aqueous
500	-	+	-	++
250	+	+	-	++
125	+	++	+	+++
62.5	++	+	++	+++

- Indicates no growth of *Streptococcus mutans.* + slight growth of *Streptococcus mutans.*
++ noticable growth of *Streptococcus mutans*. +++ overgrowth *Streptococcus mutans*

Discussion

Garcinia kola seed and stem bark have been used in folkloric medicine for the treatment of toothache and prevention of dental caries in Southern Nigeria (Ajayi *et al*, 2014). This is due to the phytochemical constituents such as flavonoids, tannins, saponins, anthraquinone cardiac glycosides, etc (Ezeanya *et al*, 2013). In the current study, a comparative analysis of the antimicrobial effects of ethanol and aqueous extracts obtained from both *Garcinia kola* seeds and stem bark against *Streptococcus mutans* was done. The Zone of inhibition test revealed that the ethanol extract of *Garcinia kola* stem bark exhibited a more substantial inhibitory zone against the test organism, displaying higher activity across various concentrations ranging from 500mg/ml to 62.5mg/ml when compared to the ethanol extract of *Garcinia kola* seeds. Particularly noteworthy was the peak activity at 250mg/ml, where the mean zone diameter reached 25mm. The heightened inhibitory activities observed with the ethanol extract of *Garcinia kola* stem bark align with findings reported by Iyevhobu *et al*. (2022), Nwaokorie *et al*. (2010) and Chukwudi-Emenike *et al.* (20220, both of whom noted that ether and methanol extracts from *Garcinia kola* stem bark and seeds exhibited stronger inhibitory activity than aqueous extracts. This difference may be attributed to the increased solubility of secondary metabolites in organic solvents, which enhances their antimicrobial properties (Ogbulie *et al*., 2007; Mada *et al*., 2013; Ukaoma *et al*., 2013).

Moreover, the aqueous extract of *Garcinia kola* stem bark demonstrated a higher inhibitory activity than the aqueous extract of *Garcinia kola* seeds. However, it did not exhibit any activity against the test organism at a concentration of 62.5mg/ml. This result could be attributed to the presence of phytochemicals such as tannins, resin, saponins, flavonoids, phenols, carbohydrates,

alkaloids, cardiac glycoside, terpenoids and absence of steroids, which conferred antimicrobial property to it (Iyevhobu *et al*., 2022, Amira *et al*., 2020).

It is essential to note that the results contrasted with those reported by Ali *et al*. (2022), who reported that *Garcinia kola* stem bark had lower antibacterial activity against MRSA compared to the seed. This variation could be attributed to differences in the test organism or methodology. Nevertheless, the findings in this study supported the observations of Iyevhobu *et al*. (2022), who reported that *Garcinia kola* stem bark possesses the ability to inhibit bacterial growth.

Results on the minimum inhibitory concentration (MIC), indicated that the aqueous extract of *Garcinia kola* seed demonstrated slightly higher MIC values when compared to the ethanol extract of the seed. Additionally, the aqueous extracts of both *Garcinia kola* stem bark and seed inhibited bacterial growth slightly more at the MIC than the corresponding ethanol extracts. At higher concentrations, it was also noted that the aqueous extracts of *Garcinia kola* stem bark and seed displayed more robust inhibitory effects, with the exception of the concentration of 125mg/ml, where the ethanol extract was slightly higher. The current findings diverged from those of Nwaokorie *et al*. (2010) and Ali *et al*. (2022), who reported that methanol extracts obtained from *Garcinia kola* stem bark and seeds exhibited more robust antimicrobial activity than aqueous extracts when tested against the same concentration of test organisms. However, the present results supported Ali *et al*. (2022) in highlighting the presence of various bioactive compounds such as Alkaloid, Tannin, Saponin, Cardiac glycoside, Flavonoid, Terpenoid, Phenols, Anthraquinone, and Steroid in Garcinia kola plants, including the stem bark. These compounds are known to confer antibacterial properties upon the plant. Moreover, our findings were consistent with the report by Iwu (1985), who suggested that the antibacterial properties of *Garcinia kola* seeds and stem bark could be attributed to kalonones, a secondary metabolite found abundantly in the seeds and stems of the *Garcinia kola* plant.

In the Minimum Bactericidal Concentration assay, ethanol extract of *Garcinia kola* stem bark displayed complete bactericidal activity (no growth of the test organism) at concentrations of 500mg/ml and 250mg/ml. In contrast, the aqueous extract of *Garcinia kola* stem bark showed the opposite outcome, resulting in noticeable growth, and overgrowth at lower concentrations. Meanwhile, the ethanol extract of *Garcinia kola* seeds demonstrated a minimum bactericidal concentration against the test organism at 62.5mg/ml, accompanied by noticeable growth. The aqueous extract of the seed exhibited a minimum bactericidal concentration at 125mg/ml, with slight growth of the test organism, while there was slight growth of the test organism at 62.5mg/ml of the aqueous extract of the seed. These results are in agreement with those of Nwaokorie *et al.* (2010), who reported that the concentrations at which *Garcinia kola* seed extracts inhibited the growth of the test organism were relatively high. Therefore, substantial quantities of the seeds would need to be chewed to effectively inhibit the growth of the test organism. Nevertheless, they suggested that lower concentrations could be applied to dentifrices or used for topical applications at non-lethal doses if the active ingredients were identified.

Conclusion

The conducted studies and their outcomes clearly indicated that both the ethanol and aqueous extracts obtained from the stem and seeds of *Garcinia kola* effectively suppressed the growth of *Streptococcus mutans*, a key contributor to dental caries. Notably, the ethanol extract derived from the stem bark displayed superior antimicrobial properties against the test organism when compared to the other extracts.

These findings suggest that hot water (aqueous) extracts from the seed and stem bark of the plant, particularly the stem bark, can serve as viable alternatives in cases where organic solvents are

either unavailable or contraindicated for the treatment and prevention of dental caries. This in-vitro investigation provides substantial support for the utilization of medicinal plants in traditional medicine practices aimed at addressing dental caries.

Furthermore, the research demonstrates that the aqueous extract of the stem bark contains phytochemical compounds similar to those found in the ethanol extracts, which contribute to its effectiveness against *Streptococcus mutans*. Consequently, it underscores the potential of *Garcinia kola* seed and stem bark extracts as alternatives for promoting oral hygiene and maintaining oral health.

Recommendation

Garcinia kola seed and stem bark extracts have both proven effective in suppressing the proliferation of *Streptococcus mutans*. As a result, the filtrates derived from these extracts hold promise for further purification and potential incorporation into various dental restorative materials. These materials encompass a range of dental products, including dental cement, topical gels, dentifrices, and more.

The integration of *Garcinia kola* extracts into dental materials could offer significant advantages by enhancing the durability and longevity of dental restorations. By impeding the colonization of *Streptococcus mutans* on these restorations, the extracts could contribute to the prevention of bacterial-related issues.

However, considering that the concentrations at which *Garcinia kola* stem bark and seed extracts demonstrated their highest antimicrobial effectiveness were relatively high, it is advisable to conduct further research aimed at refining these extracts. The objective should be to identify doses that can achieve the same level of efficacy without causing adverse effects on oral tissues. Additionally, exploring the potential use of these extracts in the treatment of conventional antibiotic-resistant bacterial strains, such as Methicillin-Resistant *Staphylococcus aureus*, presents an intriguing avenue for future investigations.

Acknowledgments

The authors acknowledge the staff of Anthony Van Leeuwenhoek Research Center**,** Nekede Owerri West and the staff of the department of Dental Technology, Federal University of Technology, Owerri. Imo State of Nigeria.

Conflict of Interest

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

References

Ajayi, TO., Moody, JO., Fukushi, Y., Adeyemi, TA and Fakeye, TO. (2014). Antimicrobial Activity of *Garcina kola* (Heckel) Seed Extracts and Isolated Constituents Against Caries-causing Microorganisms. *Afr. J. Biomed*. **17**:165-171.

Ali, M., Abubakar., UZ, Ahmed., I and lawan, DF. (2018). *In-vitro* Antibacterial Activity and Phytochemical Screening of *Garcinia kola* Extracts against Methicillin Resistant *Staphylococcus aureus* (MRSA). *Journal of Pharmacy and Pharmaceutics*.**5**(1):13-18.

Amira, PO., Daramola, AS., Ayobioloja., AP and Ibukun, SA. (2020). Comparative Studies on the Phytochemical Screening and *In-vitro* Antioxidant Activities of Aqueous Extracts of *Garcinia kola* Stem and Root backs. *European Journal of Biology and Biotechnology*.**1** (3).2020.

Chukkol, IB, Okhale, SE and Muazzam, I. (2016). *Garcinia kola*: The Phytochemistry, Pharmacology and Therapeutic Applications. *International Journal of Pharmacognosy*. **3**(2) 67-81.

Chukwudi-Emenike, NN, Ovie, FO and Onyewuchi, MO. (2022). Evaluation of Aqueous and Ethanolic Extracts of Garcnia kola on Testicular Morphology of Adult male Wistar Rats. International *Journal of Pharmaceutical and Bio-Medical Science*. **1**(6):160-166.

Iwu, MM and Gboko, O. (1982). Flavonoids of *Garcinia kola* seeds. *J. Natural Prod*. **45**:650-651.

Iwu, MM., Gboko, AO and Okunji, CO. (1990). Tempesta MS. Antidiabetic and Aldos Reductase activities of Biflavanones of *Garcinia kola*. *Journal of Pharm*. **42**: 290-292.

Mada, SB., Garba, A., Mohammed, HA and Muhammed, A. (2013). Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of *Momordica charantia* L. leaves. *J. Med. Plants*.**7**:579-586.

Madigan, MM and Martinka, J. (2022). *Brock Biology of Microorganisms*.(11th Ed). Retrieved on June 24, from http://www.wikipedia.com.

Manourova, A., Leuner, O., Tchoundjeou, Z., Van Damme, P., Verner, V., Pribyk, O and Lojka B. (2019). Medicinal Potential, Utilization and Domestication status of Bitter kola (*Garcinia kola* Heckel) in West and Central Africa. *Forest*. **10**(2):124-136.

Ncude, NS., Adolayan, AJ and Okoh AI. (2008). Assessment Techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *Afr. J. Biotechnol*.**7**(12): 1797-1806.

Nicola GG and Lavoic, MC. (2011). *Streptococcus mutans* and oral *Streptococci* in dental plaque. *Canadian Journal of Microbiology*. **57**(1):1-20.

Nwaokorie, F., Coker, A., Ogunsola, F., et al. (2010).Antimicrobial activities of *Garcinia kola* on Oral *Fusibacterium nucleatum* and biofilm. *African Journal of Microbiology Research*. **4**(7):509-514.

Ogbulie, JN., Ogueke, CC and Nwanebu FC. (2007). Antibacterial properties of *Uvaria chamae*, *Congronema latifolium*, *Garcinia kola*, *Vemonia amygdalina* and *Aframomium melegueta*. *Afr. J. Biotech*. **6**: 1549-1553

Ogunmoyole, T., Olalekan, OO., Fatai, O., Makun, JO and Kade, IJ. (2012). Antioxidant and Phytochemical profile of aqueous and ethanolic extract of *Garcinia kola*. *Journal of Pharmacognosy and Physiotherapy*. **4**(5): 66-74.

Ryan, KJ and Ray, CG. (2022).*Multiple Discoveries, a Strategic Research site*. In Merton the Sociology of Science Theoretical and Empirical Investigation. Sherris Medical Microbiology. 4th Edition. Retrieved on July 10, from http://www.researchgate.com.

Suddick, RP and Norman, OH. (1990). Historical perspectives of oral Biology: A series. *Critical Reviews in Oral Biology and Medicine*.**1**(2): 135-151.

Taiwo, O., Xu, HS and Lee, SF. (1999). Antibacterial Activities of Extracts from Nigerian Chewing sticks. *Phytotherapy Res*.**13**(8): 675-679.

World health Organisation. (2021). *Proposed Resolution on Oral Health*. Retrieved on March 14,2022 from www.who.org.

Access this Article in Online

Website: www.ijarbs.com

Subject: Phytotherapy

Quick Response Code

DOI:10.22192/ijarbs.2024.11.06.011

How to cite this article:
Iwueke, Gertrude, C, Obidinma, Sonia, Braide, Wesley, Kalu, Uchechi Caroline, Peter, Chidiebere Okorie, Ken Nkemdilim Okeke and Anselem, Dozie Okere. (2024). *In vitro* comparative study of ethanol and aqueous extracts of *Garcinia kola* stem bark and seed on *Streptococcus mutans* isolated from oral cavity. Int. J. Adv. Res. Biol. Sci. 11(6): 117-126.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.06.011