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ORIGINAL RESEARCH

A Preliminary Evaluation of the Antibacterial Activity of Lemon Fruit Juice, *Mondia whitei* Ethanolic Extract, and Their Combination Against *Streptococcus mutans*

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Background: Dental caries has gained momentum as one of the main public healthcare concerns worldwide. Although the occurrence of dental caries in Uganda is on the rise, little attention has been paid to promoting oral healthcare in the country. Thus, this study aimed to evaluate the *citrus lemon* extracts, and *Mondia whitei* root bark ethanolic extract as candidate alternative therapeutic agents for *streptococcus mutans,* the causative agent of dental caries.

Methods: In this study, the citrus lemon juice, pulp citrus lemon juice, and *Mondia whitei* ethanolic extract were screened for phytochemicals. Furthermore, the anti-*Streptococcus mutans* activity of the citrus lemon juice, citrus lemon pulp juice, and *Mondia whitei* ethanolic extract was determined by the agar well diffusion method while the minimum inhibitory concentration and minimum bactericidal concentration were determined by serial broth dilution.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, and tannins in the *Mondia whitei* ethanolic extract and citrus lemon juices, while glycosides were only detected in lemon extracts. The zones of inhibition of *Mondia whitei* ethanolic extract, citrus lemon juice, citrus lemon pulp juice, and the cocktail were 13.67 ± 0.33 mm, 18.67 ± 0.33 mm, 18.33 ± 0.67 mm, and 18.00 ± 0.58 mm, respectively. The citrus lemon juice and citrus lemon pulp juice exhibited significantly lower MIC of 0.195 mg/mL, and 0.391mg/mL, respectively. The efficacy of the extract/juices increased with an increase in the concentration.

Conclusion: The study findings revealed that *Mondia whitei* ethanolic extract and lemon extracts have potent antibacterial activity against *streptococcus mutans,* the main causative agent of dental caries; thus, can be further explored to formulate a herbal concoction for the prevention and treatment of oral cavity infections in resources-limited low-income communities.

Keywords: oral healthcare, dental caries, citrus lemon juice: *Mondia whitei* ethanolic extract

Background

Streptococcus mutans is a Gram-positive bacteria belonging to phylum Firmicutes, Bacilli class, order lactobacillales, and family *streptococcaceae*.[1] The bacterium mainly colonises the mouth and through carbohydrate metabolism, an acidic medium is created that is responsible for causing dental caries.[2] The bacterium synthesizes an extracellular polysaccharide (EPS) that facilitates the establishment of plaque biofilm which is the basis of carcinogenicity. Thus, *Streptococcus mutans* is the leading cause of dental caries worldwide and is considered the most pathogenic of all the eight species of oral *streptococci*.[3]

Despite attempts to improve oral health worldwide, dental caries is still a major public health concern. Dental caries impacts the social life of people reducing the quality of life, and limiting school attendance and performance at work in adults, while in kids it reduces the intake of food which affects growth and development.[2] Indeed, the WHO termed it a silent pandemic especially in school-aged children and most adults with a prevalence of around 60–90%.[4] The survey

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done in 2011 showed that 91% of the American adults between 20 and 64 had suffered from dental caries and it affected their quality of life and social relations.[5] The incidence and severity of dental caries have been low in Uganda until recently when a marked change in the trend mainly attributed to poor oral health coupled with the growing consumption of sugars was reported.[6] Despite the increasing prevalence and impact, dental caries has attracted less attention and investment from the government; for instance, less than 0.1% of the Gross Domestic Product is allocated for direct oral healthcare and the dentist-to-population ratio is 1:158,000 people in Uganda.[7] With this insufficiency, there is a marked need to explore natural remedies for the prevention of dental caries.

Citrus lemon is a flowering plant species that belongs to the family Rutaceae native to Asia, primarily Northeast India, and is commonly grown for commercial purposes across the world.[8,9] Citrus lemon is a rich source of ascorbic acid, flavanones, and flavones.[10] Chemical analysis of citrus lemon juice shows the presence of flavonoids, ascorbic acid, tocopherols, and citric acid, while its microbiological study revealed its antibacterial effect on *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans, Staphylococcus aureus*.[10] Furthermore, a study by[11] showed that fresh citrus lemon juice is most effective against *S. Paratyphi B, and S. sonnei*. Eating fresh lemon fruits in addition to taking juice from the fruits has been associated with overall gum health and protection from oral periodontal diseases.[9]

Mondia whitei (Periplocaceae) is an aromatic softwood climbing plant found in low rain forests with off-white flowers, and its roots and root bark have a vanilla-like scent.[12] The roots are treasured as aphrodisiacs and are used to; (a) treat premature ejaculation, (b) enhance sperm production and (c) manage erectile dysfunction. Furthermore, the root infusion is widely used to treat several ailments such as general pains, gastrointestinal problems, and malaria, and can be used as an appetite stimulant.[13] It is also effective in the treatment of gonorrhea and paediatric asthma.[12] Earlier studies demonstrated that the ethanolic extract of *Mondia whitei* roots is effective against several oral *Streptococci* like *S. pyogenes, S. sobrinus,* and *S. mitis*.[13] *Mondia whitei* and citrus lemon are being used by the local communities of Bushenyi District and many other areas of western Uganda to treat and prevent dental caries, but their efficacy is yet to be documented. Therefore, the purpose of this study was to evaluate the antibacterial activity of the citrus lemon juice, ethanolic extract of *Mondia whitei* root bark, and the cocktail of citrus lemon juice and *Mondia whitei* root bark ethanolic extract against *Streptococcus mutans.*

Materials and Methods

Study Design and Site

The study was an in-vitro quantitative experimental study where the antibacterial activity of citrus lemon juice, citrus lemon pulp juice, *Mondia whitei* ethanolic root extract, and a combination of *Mondia whitei* ethanolic root extract and juice were evaluated. The study was carried out from the Institutional Biomedical Research Laboratory, Kampala International University Western Campus.

Plant Collection and Identification

Fresh fruits of citrus lemon and *Mondia whitei* fresh roots were obtained from Rukararwe Medicinal Gardens, Bushenyi District. Using newly collected branches with leaves and flowers, the plants were identified by Dr. Eunice A. Olet, a botanist at the Department of Biology, Mbarara University of Science and Technology. Voucher specimens were archived at the Mbarara University of Science and Technology Herbarium, Faculty of Science, Department of Biology with accession numbers MK001 for citrus lemon and MK002 for *Mondia whitei*.

Preparation of Citrus Lemon Juice

The citrus lemon juice extraction was carried out following an adjusted protocol previously outlined by.[8] Briefly, the fresh *citrus lemon* fruits were rinsed under clean tap water, and their surfaces were wiped with cotton wool soaked in 70% alcohol. The pericarp was peeled off using a spatula followed by straining off the juice from the fleshy pulp into a sterile 100 mL beaker. Approximately 100 g of the pulp was crushed in distilled water using an electric blender, and the obtained extract was sieved and dispensed into a sterile 100 mL beaker. Both the citrus lemon juice and citrus lemon pulp juice were evaluated for antibacterial activity on the same day of preparation.

Preparation of the Ethanolic Root Extract of Mondia whitei

Mondia whitei ethanolic bark extract was prepared by modifying the methods outlined by.[13] Briefly, the fresh roots of *Mondia whitei* were washed under running tap water to remove entrapped soil on its surface. The roots were then air dried to a constant weight and then later ground into a fine powder using mortar and pestle. The resultant powder was weighed and stored in an air-tight container until use. The total powder yield was 137 gm. The ethanol root extract was obtained using a ratio of 1:10 of the powder material to 80% ethanol in a Soxhlet extractor incubated at 78°C for one hour. The obtained extract was collected in a reagent bottle and later concentrated using a rotary evaporator. The percentage extract yield was estimated using the formula below;

$$Mondia\ whitei\ ethanolic\ extract\ percentage\ yield = \frac{Weight\ of\ Extract\ obtained}{Mondia\ whitei\ powder\ initial\ weight} X100$$

Phytochemical Screening

Phytochemical screening of the ethanolic extract of *Mondia whitei* root barks and citrus lemon juice was carried out to determine the presence of alkaloids, glycosides, terpenoids, flavonoids, and tannins. The analysis was carried out according to the standard method described by[14] outlined below;

Alkaloids

To test for alkaloids, 3 mL of hydrochloric acid was dispensed to 5 mL of the extract and citrus juice while shaking to obtain uniform solutions. This was followed by adding a Meyer's reagent which contains potassium mercuric iodide solution as the main component. A yellowish precipitate was formed indicating that the samples contained alkaloids.

Glycosides

The presence of glycosides was evaluated by adding the *Mondia whitei* ethanolic extract and citrus juice to equal volumes of distilled water (5 mL) followed by 0.5 mls of lead acetate solution. The mixture was thoroughly agitated and sieved. To extract the phytochemicals, the filtrate was then treated with an equal volume of chloroform, and thereafter, the solvents were driven off using a rotary evaporator. The resultant residue was then liquified in glacial acetic acid (3 mL), iron (III) chloride solution (2 mL), and concentrated sulphuric acid (4 mL). A reddish-brown layer that turned to bluish-green indicated the presence of glycosides.

Flavonoids

To detect the presence of flavonoids, five drops of concentrated hydrochloric acid were added to 5mls of ethanolic extract of *Mondia whitei* root bark and citrus lemon juice. The immediate development of the red colour indicated the presence of flavonoids.

Terpenoids

About 2 mL of chloroform was added to 0.5 mL of the ethanolic extract of *Mondia whitei* root bark and citrus lemon juice followed by 3 mL of conc. Sulphuric acid which formed a layer. Reddish-brown discoloration of the boundary revealed the presence of terpenoids.

Tannins

To identify tannins, 2 mL of 5% iron (III) chloride solution was added to 5 mL of the ethanolic extract of *Mondia whitei* root bark and citrus lemon juice. A greenish-black precipitate indicated the presence of tannins.

Saponins

To confirm the presence of saponins, 1 mL of the ethanolic extract of *Mondia whitei* root bark and citrus lemon juice was diluted with 20 mL of distilled water and shaken in a graduated cylinder for 15 minutes. The formation of a one-centimetre foam indicated that saponins were present.

Preparation of Different Concentrations of the Extracts

Preparation of different concentrations of the plant extracts for antimicrobial activity evaluation was performed using methods outlined by.[15] Briefly, 2 g of the ethanolic extract of *Mondia whitei* was mixed with 2 mL of 100% freshly prepared Citrus lemon juice on a clean sterile glass plate, and the mixture was stirred for complete dissolution, then covered with sterilised aluminum foil. This gave a concentration of 1000mg/mL of *Mondia whitei* and 100%v/v of citrus lemon in the extract. Additionally, 1 mL of the combination was added to 1 mL of sterile distilled water, to obtain 500 mg/mL of *Mondia whitei* ethanolic extract and 50% V/V of *citrus lemon juice*. The same procedure was used to prepare 1000 mg/mL and 500 mg/mL of *Mondia whitei* ethanolic extract alone. Furthermore, 50% V/V of fresh citrus lemon juice was prepared by adding 2 mL of fresh citrus lemon juice of 100% v/v to 1 mL of sterile distilled water. The same procedure was applied to obtain 50% V/V pulp juice preparation. Ampiclox 30µg/mL was used as a control since it is the first choice antibiotic used to treat dental caries.[15]

Experimental Microorganisms

Streptococcus mutans isolates were obtained from the Microbiology Laboratory, Kampala International University. The samples were then inoculated on a blood agar base using a sterile swab and incubated overnight for 18 hours at 37.8°C to obtain multiple colonies. On a separate blood agar plate, the bacteria were inoculated again by streak method to obtain a pure culture and then stored at 4°C till when used.[15]

Antimicrobial Screening

Standardized test *Streptococcus mutans* suspension (0.5×10^{8} McFarland standard) was inoculated uniformly on the entire surface of freshly prepared blood agar by streaking, and four wells measuring 6 mm in diameter were made using a sterile cork borer on each plate. The following volumes and concentrations were used; 50µL of concentrations (1000 mg/mL and 500 mg/mL)/100% V/V and 50%) of the combination extract, 50 µL of *citrus lemon juice* extract (100% V/V and 50% V/V), 50µL of *citrus lemon pulp* juice with concentrations of (100% v/v and 50% v/v), 50 µL of *Mondia whitei* ethanolic root extract (1000 mg/mL and 500 mg/mL), and 50µL of 30µg/mL Ampiclox were used as the positive control, while 50µL of sterile distilled water as the negative control. The experiments were performed in triplicates. The streaked plates were left in a laminar flow hood for 30 min to allow the extract to diffuse. Thereafter, the plates were transferred to a bacteriological incubator set at 37.8°C for 22 hours. This was followed by recording the diameter of bacterial growth inhibition zones in millimeters (mm), and the results were construed using the Clinical and Laboratory Standards Institute (CLSI) guidelines.[16]

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the ethanolic *Mondia whitei* roots extract, citrus lemon juice, citrus lemon pulp juice, and the combination extract of citrus lemon juice and *Mondia whitei* ethanolic extract were determined for *Streptococcus mutans* using a two-fold serial micro-broth dilution method on 96 well microtiter plates using the Brain Heart Infusion nutrient broth (BHIB). All tested extracts/juices exhibited potent antimicrobial activity at all concentrations. Therefore, a cocktail of 1000 mg/mL of ethanolic *Mondia whitei* extract and 100% V/V of citrus lemon juice (preparation A), 100% citrus lemon juice (preparation B), 100% citrus lemon pulp juice (preparation C), 1000mg/mL of *Mondia whitei* ethanolic root extract (preparation D), and 30 µg/mL Ampiclox (preparation E) were used for the determination of MIC. Briefly, 100µL of sterile BHIB was pipetted into 14 wells of microtiter plates labeled preparation A, B, C, D, and E. A two-fold serial dilution was done by dispensing 100µL of preparation A into the first well of the plate labeled preparation A. The BHIB and preparation A were thoroughly mixed into a uniform solution. This was

followed by transferring 100µL of the mixture from the first to the second well. This step was repeated until the 14th well. This was followed by dispensing 1.0 µL of fresh bacterial culture of *Streptococcus mutans* at 0.5 McFarland's turbidity standard and 20µL of resazurin into the 14 wells. This procedure was repeated for preparations B, C, D, and E. The 14 wells of plate labeled positive control were filled with 200µL of BHIB. The plates were then incubated at 37.8°C for 24 hours, then plating the samples from each well onto the blood agar. The plates were left to stand all night at 37.8°C under aerobic conditions. The lowest concentrations of each preparation at which there was the least growth were taken as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC of the extract/juice preparations was estimated using the MIC microtiter plates with no visible bacterial growth following 24 hours of incubation. The samples were inoculated on blood agar base plates by the streak method and then incubated at 37.8°C for 24 hours. The plates were later observed for any bacterial growth. MBC was taken as the concentration of the extract/juice preparation in the well before the one with growth.

Statistical Analysis

Data was then analyzed with Graph Pad Prism 6.01 version. Comparison of mean zone bacterial growth inhibition, MIC, MBC, of the different extract/juice preparations was done by the one-way analysis of variance (ANOVA). Statistical significance was considered at $P \leq 0.05$

Results

Mondia whitei Ethanolic Extract Percentage Yield

About 21.72 g of the ethanolic extract was obtained from 130.7 g of *Mondia whitei* powder giving a yield of 15.85%.

Phytochemical Profiling

Phytochemical screening revealed that both the *Mondia whitei* ethanolic extract and citrus lemon juice had alkaloids, flavonoids, terpenoids, and tannins. However, glycosides were detected only in citrus lemon and pulp juice, whereas saponins were not found in any.

Antimicrobial Susceptibility Assay

The antimicrobial activity of the *Mondia whitei* ethanolic extract, citrus lemon juice, citrus lemon pulp juice, and the cocktail of the ethanolic extract and the citrus lemon juice at different concentrations are shown in Figure 1 and Table 1. The findings revealed that all the extracts/juice exhibited potent antimicrobial activity against *Streptococcus mutans*. The susceptibility of *Streptococcus mutans* to extract/juices significantly differed ($P < 0.05$). Inhibition activity of the citrus lemon juice, citrus lemon pulp juice, and a cocktail of citrus lemon juice and *Mondia whitei* ethanolic extract was considerably higher than that of *Mondia whitei* ethanolic extract alone and lower than ampiclox (positive control). The antimicrobial activity of citrus lemon juices, a cocktail of citrus lemon juice, and *Mondia whitei* and *Mondia whitei* ethanolic extract significantly increased with an increase in concentration, Table 1 and Figure 2. Two-way ANOVA using side's multiple comparison tests revealed that the antibacterial activities between the citrus lemon juice, citrus lemon pulp juice, ethanolic extract of *Mondia whitei*, the combination of *Mondia whitei* extract and citrus lemon juice and ampiclox were considerably different ($P \leq 0.05$), Figure 2.

Minimum Inhibitory and Bactericidal Concentrations

The MIC and MBC of *Mondia whitei* ethanolic extract, citrus lemon juice, and the cocktail of the ethanolic extract and citrus lemon juice are summarized in Table 1. The MIC and MBC for the citrus lemon juice and citrus lemon pulp juice were significantly ($P < 0.001$) lower than the MIC and MBC for the *Mondia whitei* ethanolic extract; thus, citrus lemon juices exhibited superior anti-Streptococcus *mutans* activity.

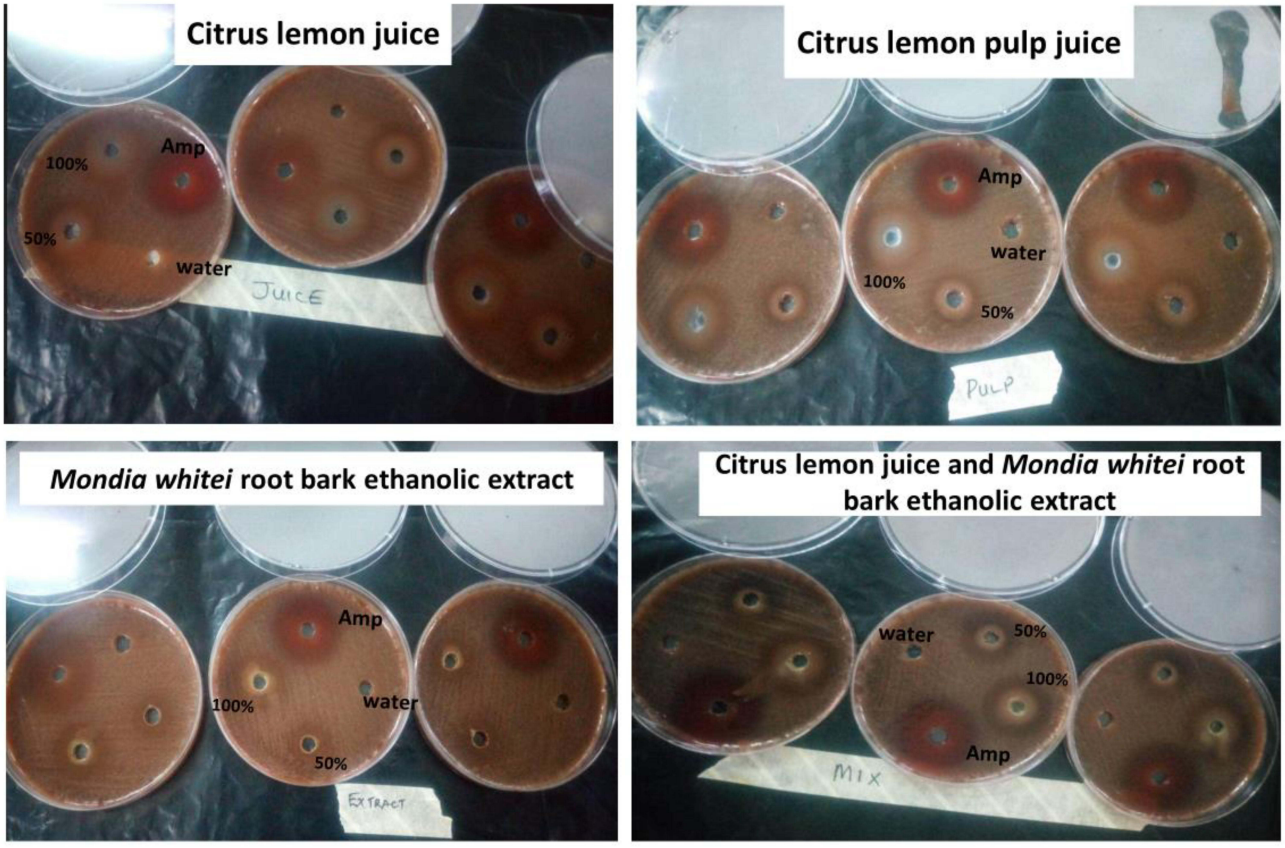

Figure 1 Zones of growth inhibition of the different plant extracts. Amp represents Ampiclox.

Discussion

Approximately 60–90% of the children below the age of 15 and a big percentage of adults are inflicted by dental caries.[17] Less priority is given to dental infections in relation to other communicable diseases in Africa.[4] In Uganda, oral health services are highly distributed in urban centres leaving the rural areas underserved.[18] In most low-income countries, the cost of treating dental decay alone could easily exhaust a country's total health-care budget. Thus, the Ugandan

Table 1 Antimicrobial Activity of Mondia whitei Ethanolic Extract and Citrus Lemon Juice Represented by Mean Zone of Growth Inhibition, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC)

Plant Extract/juice		Mean Zone of Growth Inhibition (mm)	MIC (mg/mL or %v/v)	MBC (mg/mL or %v/v)
Extract/juice type	Concentration			
Citrus lemon juice (Lemon extract)	100% V/V	18.67 ± 0.33^{A}	0.195	3.125
	50% V/V	17.00 ± 0.00^{A}	–	–
Citrus lemon pulp juice (Lemon extract)	100% V/V	18.33 ± 0.67^{A}	0.391	3.125
	50% V/V	14.33 ± 0.67^{B}	–	–
Mondia whitei ethanolic extract	1000 mg/mL	13.67 ± 0.33^{B}	15.63	62.5
	500 mg/mL	11.00 ± 0.00^{B}	–	–
Mixture of *Mondia whitei* ethanolic extract and citrus lemon juice	1000 mg/mL /100% V/V	18.00 ± 0.58^{A}	3.906/0.391	62.5/6.25
	500 mg/mL/ 50% V/V	12 ± 0.00^{B}	–	–
Ampiclox (positive)	30µg/mL	28.67 ± 0.00^{C}	0.015	0.059
Distilled water (negative)	–	0	–	–

Notes: Mean values in each column accompanied by the same letter (such as A) are not significantly different ($p > 0.05$) (Tukey Multiple Comparison), and values accompanied by letter (s) which are not similar (A, B, and C) are significantly different ($p < 0.05$).
Abbreviations: MIC, Minimum inhibitory concentration, MBC, Minimum bactericidal concentration of the different plant extracts and juices.

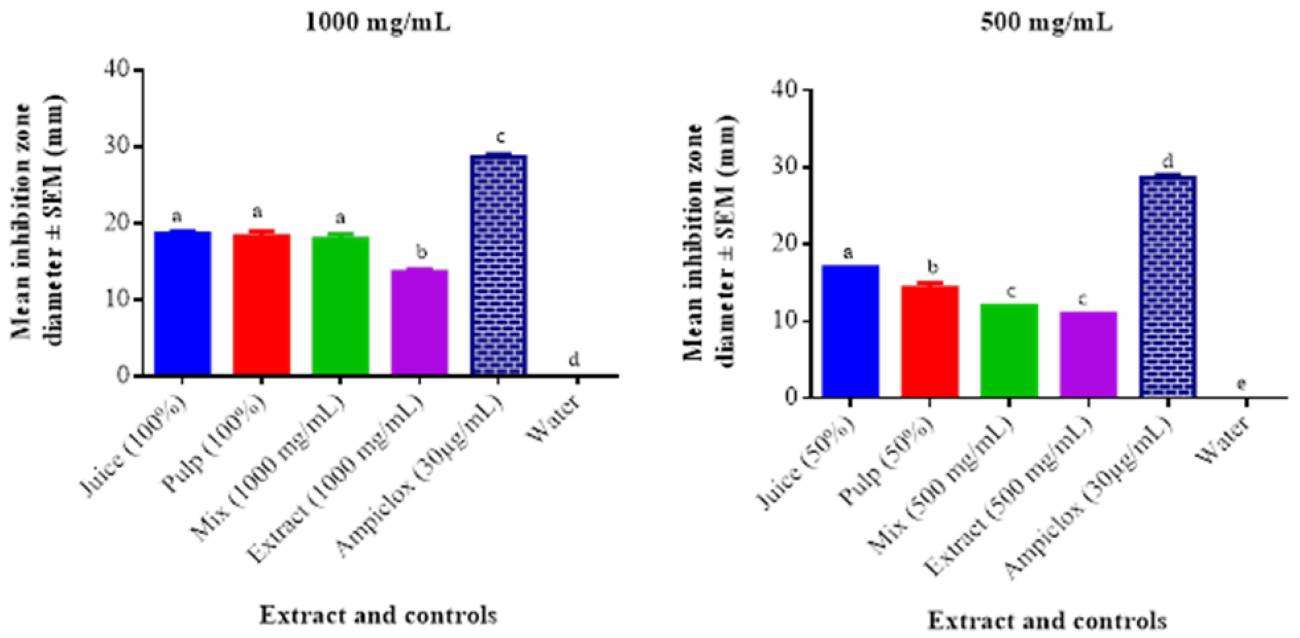

Figure 2 Comparison of the susceptibility of Streptococcus mutans to extract/juices of different concentrations. Mean growth inhibition of each bar accompanied by the same letter (a or b) are not significantly different ($p > 0.05$) (Tukey Multiple Comparison), and values accompanied by letter (s) which are not similar (a and b) are significantly different ($P < 0.05$).

government with limited resources has prioritized funding several pressing health issues giving limited attention to less life-threatening conditions like oral health.[7] Furthermore, the use of artificial toothpaste is associated with exposure to fluoride which may cause side effects like osteoporosis, tooth decay, kidney damage, and fluorosis (change in the appearance of the colour of the enamel), especially in children who swallow the commonly used toothpaste.[19] Furthermore, the removal of one's tooth affects the mastication process and further exposes one to a risk of systemic infection in addition to intraoperative complications that include bleeding, destabilizing surrounding teeth, and injury to tissues.[20] With that background, the search for effective, safer, and affordable alternatives for low-income communities like herbal medicines against *Streptococcus mutans,* the dental caries causative organism is called for.

This study obtained a *Mondia whitei* ethanolic extract yield of 15.85%; however, there is no data from previous studies for comparison purposes. Phytochemical profiling revealed the presence of alkaloids, flavonoids, terpenoids, and tannins in both the *citrus lemon juice* and *Mondia whitei* ethanolic extract but negative for saponins. *Citrus lemon juice* further demonstrated the presence of glycosides which were absent in *Mondia whitei* ethanolic root bark extract. This was in agreement with results from a study conducted by[12] which reported the presence of alkaloids, flavonoids, terpenoids, and cardiac glycosides in *citrus lemon* and *citrus lemon pulp* juice but negative for tannins. Another study conducted by[21] on citrus lemon juice documented the presence of flavonoids in high concentrations that were classified as flavonols-limocitrin and spinacetin; flavones-orientin vitexin and eriocitrin. Additionally, the presence of flavanones such as eriodictyol, hesperidin, hesperetin, and naringin; flavones (apigenin and diosmin), and flavanols-quercetin in citrus lemon fruits was observed by.[10] Furthermore,[22] and[23] identified all the phytochemicals detected in the *Mondia whitei* root ethanolic extract by this study in addition to phenols, saponins, coumarins, iridoids, anthocyanins, tannins, and anthraquinones. The observed differences in the phytochemicals may be attributed to differences in soil content and climatic conditions in the different geographical regions.

The study evaluated the antibacterial activity of citrus lemon juice, pulp citrus lemon juice, *Mondia whitei* root bark ethanolic extract, and a cocktail of *Mondia whitei* ethanolic root bark extract and citrus lemon juice against *Streptococcus mutans*. There is no available *Mondia whitei* ethanolic extract antibacterial data for comparison purposes but for citrus lemon juices, the findings corroborate with the results reported by[10] who found that the juice at concentrations of 10%,

5%, 3.3%, and 2.5% (V/V) had potent inhibitory activity against *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella sonnei*, and *Streptococcus mutans*.

Furthermore, the citrus lemon juice, citrus lemon pulp, and the cocktail of citrus lemon juice and *Mondia whitei* root extract exhibited significantly higher antimicrobial activity against *Streptococcus mutans than Mondia whitei ethanolic* extracts but substantially lower than that of Ampiclox, the preferred antibiotic for the treatment of dental caries as revealed by the ANOVA analyses. The antimicrobial activity in plant extracts is mainly attributed to flavonoids.[21] However, in this study, phytochemical screening exhibited the presence of flavonoids in all plant extracts. Therefore, the significantly superior antibacterial activity in the citrus lemon juice and pulp lemon may be due to the presence of glycosides. Studies by[24] and[25] fractionated individual phytochemicals of *Graptophyllum grandulosum* using Column chromatography and found that flavonoids conjugated to glycosides were responsible for the potent antibacterial activity exhibited by the extract and the mode of action is owed to bacteriolysis with remarkable irreversible leakage of the cytoplasmic content. Furthermore, the antibacterial activity of flavonoid glycosides is more pronounced at high concentrations as there was a remarkable increase in the antibacterial activity with an increase in the concentration of the plant extracts/juices.

Conclusion

The ethanolic extract of *Mondia whitei* root bark extract and citrus lemon juice demonstrated a potent antibacterial activity against *Streptococcus mutans,* the causative agent of dental caries individually and in combination. The combination thus represents a potential remedy for both the treatment and prevention of dental caries and further supports the use of this herbal concoction for oral healthcare in the local communities of Bushenyi District in Uganda. However, there is a need to study the stability and toxicity profile of the two plant extracts before formulating them for general public use.

Data Sharing Statement

Data associated with this study has been incorporated in this manuscript.

Ethical Approval and Consent to Publish

The study was approved by the Scientific Institutional Ethical Review Board of Kampala International University Western Campus, consisting of biomedical scientists who approved the study for implementation. *Streptococcus mutans* were obtained from the Department of Microbiology, Kampala International University archives, hence no need for approval for patients' consent to participate. Furthermore, no additional approvals were required to conduct research using *Mondia whitei* and citrus lemon plant materials.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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