

Evaluation of the antibacterial activity of lemon fruit juice, *mondia whitei* ethanolic extract, and their combination against *Streptococcus mutans*

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Short Report

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Abstract

Objective: Due to the ability to synthesize extracellular polysaccharides that facilitate the formation of plaque biofilm on the teeth enamel which is vital for carcinogenicity, *Streptococcus mutans* has been implicated as the major causative agent of dental caries. Although the occurrence of dental caries in Uganda is on the rise, little or no attention has been paid to promoting oral healthcare in the country. Thus, this study aimed at evaluating the *citrus lemon* fruit extracts and *Mondia whitei* crude ethanolic extract root bark as candidate alternative therapeutic agents for *streptococcus mutans* the causative agent of dental caries.

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

Background

Streptococcus mutans is a Gram-positive bacteria that mainly colonises the mouth. Through carbohydrate metabolism, an acidic medium is created that is responsible for causing dental caries [1, 2]. The bacterium synthesizes an extracellular polysaccharide 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. Indeed, oral periodontal diseases were termed a silent pandemic with a prevalence of around 60–90% worldwide [4]. The incidence and severity of dental caries have been low in Uganda until recently where a marked change in the trend mainly attributed to poor oral health coupled with the growing consumption of sugars was reported [5]. Despite the increasing prevalence and impact, dental caries have attracted less attention from the government [6]. With this insufficiency, there is a marked need to explore natural remedies for the prevention of dental caries.

Citrus lemon is a flowering plant that is commonly grown for commercial purposes across the world [7, 8]. Earlier studies revealed that citrus lemon juice possesses potent antimicrobial activity and eating fresh lemon fruits in addition to taking their juice has been associated with overall gum health and protection from dental caries [9–11]. *Mondia whitei* is an aromatic softwood climber with pale greenish-white or cream flowers and its roots have a vanilla-like scent [12]. Previous studies demonstrated that the *Mondia whitei* crude ethanolic root bark extract (MCERBE) is effective against several oral *Streptococci* like *S. pyogenes*, *S. sobrinus*, and *S. mitis* [13]. *Mondia whitei* and citrus lemon are used by the local communities in 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 citrus lemon juice and MCERBE against *streptococcus mutans*.

Materials and methods

Study design and site

This was an in-vitro experimental study where the antibacterial activity of citrus lemon juice, citrus lemon pulp juice, MCERBE, and a combination of MCERBE and citrus lemon juice was evaluated. The study was carried out from the Institutional Biomedical Research Laboratory, Kampala International University (KIU) Western Campus.

Plant collection and Identification

Fresh fruits of citrus lemon were bought from Ishaka Market while *Mondia whitei* fresh roots were obtained from Rukararwe medicinal Gardens, Bushenyi District. The plants were identified by a botanist at the Department of Botany Makerere University.

Preparation of Citrus Lemon juice

The fresh *citrus lemon* fruits were rinsed under clean tap water and their surfaces wiped with cotton wool soaked in 70% alcohol. The pericarp was peeled off using a spatula and the juice was strained off 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*

The fresh roots of *Mondia whitei* were washed under running tap water to remove entrapped soil. The roots were air dried to a constant weight and then later grounded into a fine powder using an electric blender. The resultant powder was weighed and stored in an air tight container until use [13]. The ethanol root extract was obtained using a ratio of 1:10 of the powder material to absolute ethanol in Soxhlet extractor incubated at 78°C for one hour. The obtained extract was concentrated using a rotary evaporator. The percentage extract yield was estimated using the formula below;

$$\text{Mondia whitei ethanolic extract percentage yield} = \frac{\text{Weight of Extract obtained}}{\text{Mondia whitei powder initial weight}} \times 100$$

Phytochemical screening.

Phytochemical screening of the MCERBE 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 [11].

Preparation of different concentrations of the extracts

Preparation of different concentrations of the plant extracts (PE) for antimicrobial activity evaluation was performed using methods outlined by [14]. Briefly, 2 g of the MCERBE was mixed with 2mls of 100% freshly prepared Citrus lemon juice on a clean sterile glass plate and the mixture was stirred for complete dissolution. This gave a concentration of 1000mg/ml of MCERBE 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 a 500mg/ml of MCERBE and 50%V/V of *citrus lemon* juice. The same procedure was used to prepare 1000 mg/ml and 500 mg/ml MCERBE 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 1ml 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 [14]

Experimental microorganisms.

Streptococcus mutans isolates were obtained from the Microbiology Laboratory, KIU. The samples were then inoculated on blood agar base and incubated overnight for 18 hours at 37.8°C in order to obtain multiple colonies and then stored at 4°C until when used [14]

Antimicrobial Screening

Standardized test *streptococcus mutans* suspension (0.5×10^8 McFarland standard) was inoculated on the 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. This was followed by dispensing 50µl the cocktail (1000 mg/mL and 500 mg/mL) /100% V/V and 50%), *citrus lemon* juice extract (100% V/V and 50% V/V), *citrus lemon* pulp juice (100% V/V and 50% V/V), MCERBE (1000 mg/ml and 500 mg/ml) into their corresponding wells. Ampiclox (30µg/ml) was used as the positive control while sterile distilled water as the negative control. The experiments were performed in triplicates. The inoculated plates were left to stand at room temperature for 30 min for the extract to diffuse into the agar and later, the plates were incubated at 37.8°C for 22 hours. After which, the diameter of zones of inhibition were measured and recorded in millimetres (mm).

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the MCERBE, citrus lemon juice, citrus lemon pulp juice and the combination extract of lemon juice and MCERBE were determined for *streptococcus mutans* using a two-fold serial microbroth dilution method on 96 well microtiter plates using the Brain Heart Infusion nutrient broth (BHIB) as described by [17].

Determination of minimum bactericidal concentration (MBC)

The MBC of the extract/juice preparations was estimated using the MIC microtiter plates with no visible bacteria growth following 24 hour 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 analysed 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 MCERBE and citrus lemon juice had alkaloids, flavonoids, terpenoids and tannins. However, glycosides were detected only in lemon extracts whereas saponins were not found in any.

Antimicrobial activity of the PE

The antimicrobial activity of the MCERBE, citrus lemon juice, citrus lemon pulp juice and the cocktail of the MCERBE and the lemon juice at different concentrations are shown in Table 1, Fig. 1. The findings revealed that all the extracts/juice exhibited potent antimicrobial activity against *Streptococcus mutans*.

Susceptibility of *Streptococcus mutans* to extract/juices significantly differed ($P \leq 0.05$). Inhibition activity of the citrus lemon juice, citrus lemon pulp juice and the cocktail was considerably higher than that of MCERBE alone and lower than Ampiclox. The antimicrobial activity citrus lemon juices, the cocktail and MCERBE significantly increased with increase in concentration, Table 1 and Fig. 2. Two-way ANOVA using side's multiple comparison test revealed that the antibacterial activities between the lemon Juice, lemon pulp juice, MCERBE, the cocktail and Ampiclox were considerably different ($P \leq 0.05$), Fig. 2. The MIC and MBC of MCERBE, citrus lemon juice and the cocktail are summarized in Table 1. The MIC and MBC for the citrus lemon extracts were significantly ($P \leq 0.001$) lower than those of MCERBE; thus, lemon juices exhibited superior anti-*Streptococcus mutans* activity.

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	-	-
<i>Mondia whitei</i> ethanolic extract	1000 mg/ml	13.67 ± 0.33 ^B	15.63	62.5
	500 mg/ml	11.00 ± 0.00 ^B	-	-
Mixture of <i>Mondia whitei</i> 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
Sterile water (negative)	-	0	-	-
Mean values in each column accompanied by the same letter are not significantly different ($p > 0.05$) (Tukey Multiple Comparison), and values accompanied by letter (s) which are not similar are significantly different ($p < 0.05$). MIC, Minimum inhibitory concentration, MBC, Minimum bactericidal concentration of the different plant extracts and juices				

Discussion

In Uganda, the oral healthcare is highly distributed in urban centres leaving the rural areas under served [15]. In most low-income countries, the cost of treating dental decay could easily exhaust a country's total healthcare budget. Thus, the Ugandan government with limited resources has prioritized funding several pressing health issues [6]. Furthermore, the use of artificial tooth pastes is associated with exposure to fluoride which may cause side effects like osteoporosis, tooth decay, kidney damage, and fluorosis [16]. With that background, the search for effective, safer, and affordable alternatives against *Streptococcus mutans* the main dental caries causative organism for low-income communities is called for.

This study obtained MCERBE yield of 15.85%; however, there is not 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 MCERBE but negative for saponins. Also, *Citrus lemon* juice had glycosides which were absent in MCERBE. This was consistent with results from studies conducted by [17–19] which reported the presence of alkaloids, flavonoids, terpenoids, and glycosides in lemon extracts but

negative for tannins. Furthermore, [20–22] identified all the phytochemicals detected in the MCERBE by this study in addition to other phytochemical. The observed differences in the phytochemicals may be attributed to differences in environmental conditions in the different geographical regions.

The study evaluated the antibacterial activity of the PE against *Streptococcus mutans* and result obtained are in agreement with studies conducted by [9, 12, 13]. Lemon extracts and MCERBE exhibited potent inhibitory activity against a broad range of both gram positive and gram negative bacteria [9, 12, 13]. Furthermore, the lemon extracts and the cocktail exhibited significantly higher antimicrobial activity against *streptococcus mutans* than MCERBE but substantially lower than that of Ampiclox the preferred antibiotic for treatment of dental caries The antimicrobial activity of PE is mainly attributed to flavonoids [17]. However, in this study phytochemical screening exhibited presence of flavonoid in all PE. Therefore the significantly superior antibacterial activity of the lemon extracts may be due to the presence glycosides. Studies by [23–24] fractionated individual phytochemicals of PE 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 remarkable increase in the antibacterial activity with increase in the concentration of the PE/juices. Conclusively, the PE demonstrated potent anti-*streptococcus mutans* activity the causative agent of dental caries individually and in combination; thus, represents potential remedies 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 western Uganda.

Limitations

The study never investigated stability of the two PE and the safety profiles of MCERBE yet it is requirement before formulating the concoctions from the extracts for general public use. Furthermore, the study did not conduct quantitative analysis using chromatography to determine the actual active ingredient responsible for the antibacterial activity of the PE.

Abbreviations

MCERBE:	<i>Mondia whitei</i> crude ethanolic root bark extract
KIU:	Kampala International University
PE:	Plant extract
MIC:	Minimum Inhibitory Concentration
MBC:	Minimum Bactericidal concentration
BHIB:	Brain Heart Infusion nutrient broth
V/V:	Volume per Volume

Declarations

Ethical approval and consent to participate

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* isolates were obtained from the department of microbiology, Kampala International University archives hence no need for approval for patients' consent to participate.

Consent for publication

Not applicable

Availability of data and materials

Data associated with this study has been incorporated in this manuscript

Competing interests

The authors declare no competing interests

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Authors' contributions

KS and SN conceptualized the study; MK performed the experiments and wrote the first draft. All the authors analysed and interpreted the data and managed revisions and contributed to the purchase of reagents and other consumables.

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Figures

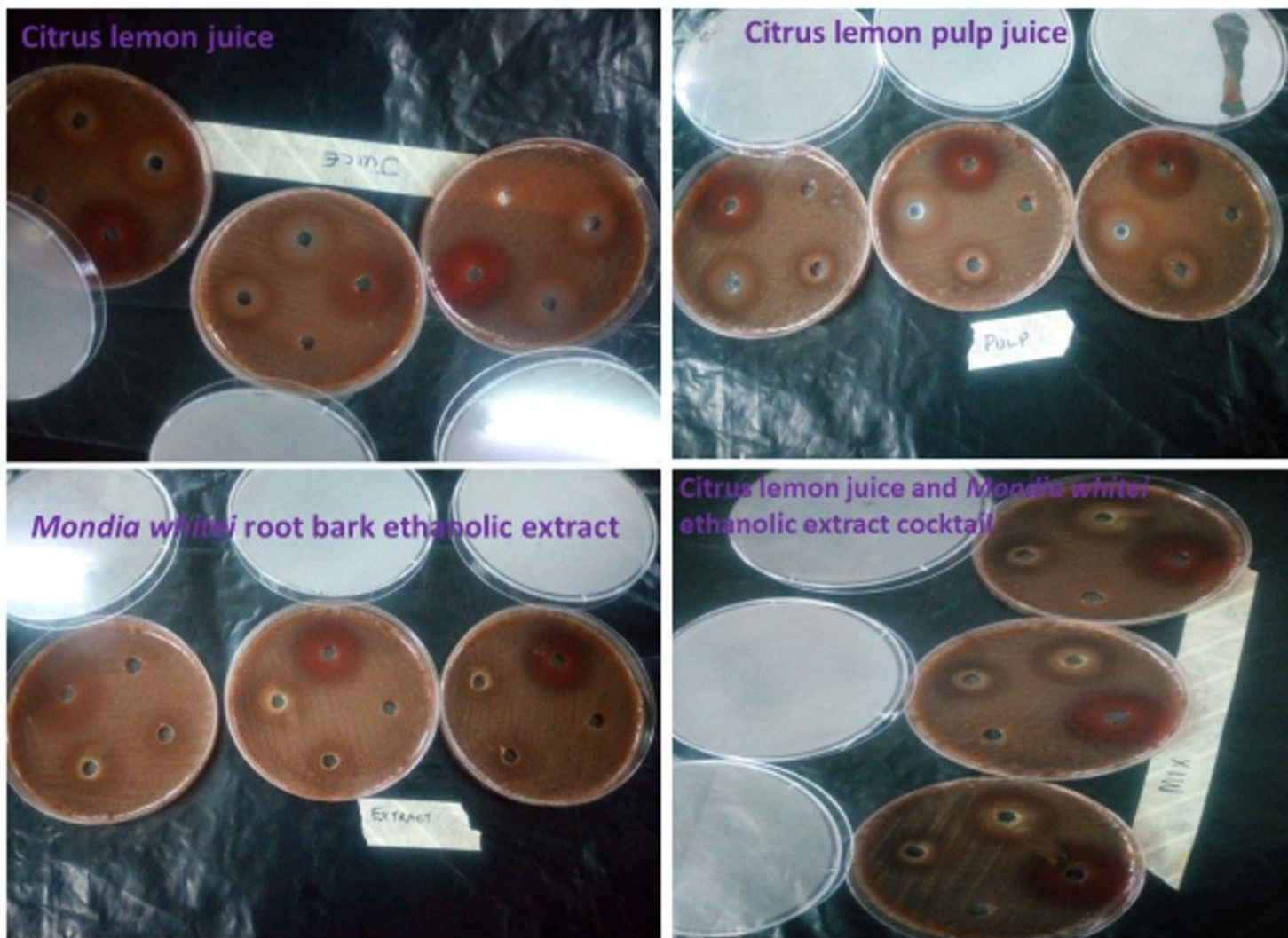


Figure 1

Zones of growth inhibition of the different plant extracts

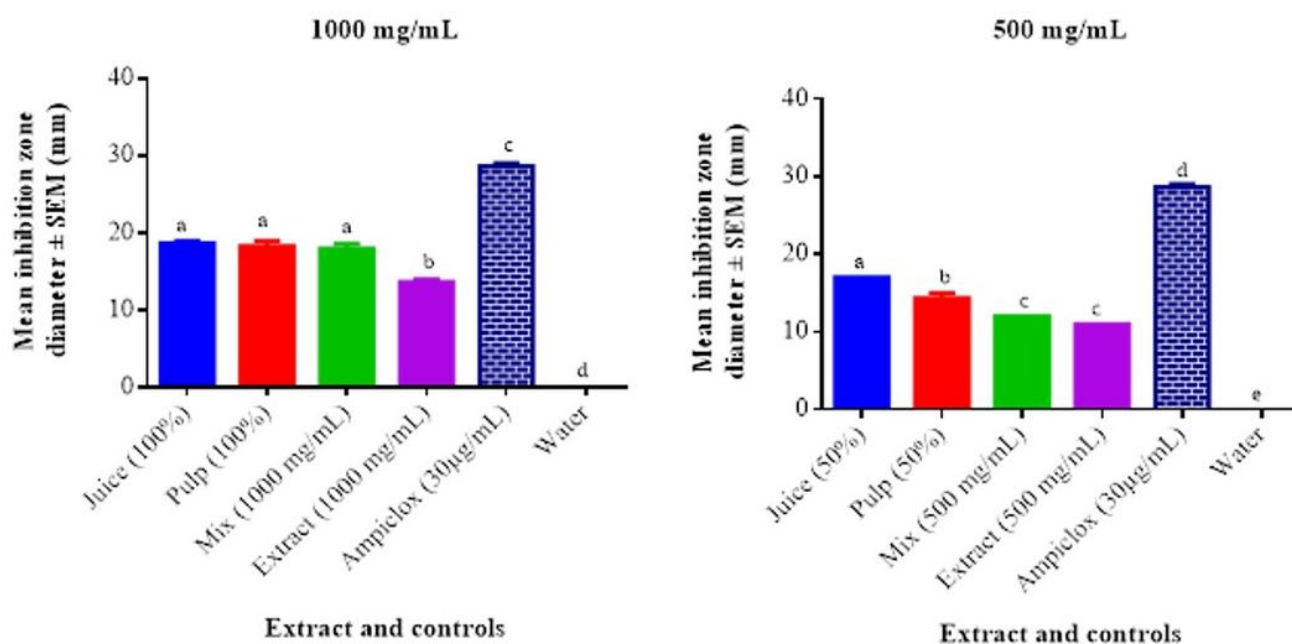


Figure 2

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