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Comparative Analysis of Antimicrobial Efficacy: Harnessing *Cocos Nucifera* Extracts in Novel Toothpaste Formulations

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Abstract

Natural resources, ranging from herbs like neem and clove to minerals like fluoride, are harnessed for their antimicrobial properties in oral care products. These resources provide sustainable alternatives to synthetic chemicals, offering effective yet eco-friendly solutions against oral microorganisms. The present study aims to assess the antimicrobial efficacy of two toothpaste formulations derived from the extracts of Cocos nucifera. Two toothpaste preparations were made from the pulp extracts of Cocos nucifera. Group 1 was prepared from milk extracted from freshly ground coconut pulp. The second extract was obtained by the lyophilization process. Both toothpaste formulations were then analyzed for their antimicrobial efficacy by assessing the zone of inhibition for the following oral pathogens; Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Lactobacillus rhamnosus, Candida albicans by agar well diffusion assay. Statistical analysis was performed using the SPSS software. It was found that the toothpaste derived from the extracts of coconut milk had antimicrobial efficacy against S.mutans, S.aureus and C.albicans in the various dilutions tested and the toothpaste made from the lyophilized extract exhibited antimicrobial efficacy against P.aeruginosa in various dilutions. Toothpaste derived from the coconut milk extract demonstrated significant efficacy against tested oral pathogens, suggesting its suitability for incorporation into oral care products. Its antimicrobial properties highlight its potential for combating oral infections and enhancing oral hygiene routines. Further research and development could optimize its utilization in various dental formulations.

Keywords: *Antimicrobial Efficacy, Green Synthesis, Natural Resources, White Spot Lesions.*

Introduction

Natural resources, ranging from plants and minerals to animal-derived substances, have been harnessed for their therapeutic properties, contributing significantly to pharmaceuticals, medical devices, and treatment modalities [1, 2]. This interdisciplinary approach amalgamates traditional knowledge with contemporary research, offering innovative solutions for various healthcare challenges [3]. The utilization of natural resources in the medical and dental fields has been a longstanding practice, deeply rooted in traditional medicine and evolving alongside advancements in modern science and technology [4].

Moreover, the dental industry benefits from natural resources in various capacities. Traditional oral health practices often involve natural ingredients like neem, clove oil, and myrrh, recognized for their antimicrobial and analgesic properties. These substances are integrated into oral care products, such as toothpaste and mouthwash, to combat plaque,

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gingivitis, and other oral infections effectively [5, 6].

Antimicrobial efficacy in dental oral products is essential to combat oral pathogens, prevent dental caries, gingivitis, and periodontal diseases, and ensure optimal oral health [7, 8]. It helps in reducing plaque formation, controlling bacterial growth, and minimizing the risk of oral infections, ultimately promoting overall well-being and maintaining a healthy oral environment [9]. White spot lesions, common in dentistry and orthodontic patients, signify early enamel demineralization due to plaque accumulation and acid exposure. Preventive measures such as improved oral hygiene and remineralization treatments are crucial to mitigate their progression and preserve dental health [10–14].

Coconut, scientifically known as Cocos nucifera, offers a range of medicinal benefits due to its rich composition of bioactive compounds [15–17]. It possesses antimicrobial properties attributed to lauric acid, aiding in combating various pathogens, including bacteria, viruses, and fungi [18]. Additionally, its antioxidant content contributes to anti-inflammatory effects, supporting skin health and wound healing. Moreover, coconut oil has been associated with promoting heart health by improving cholesterol levels and supporting weight management [19]. These properties together make coconut a versatile natural remedy with potential applications in skin care, wound care, and overall health maintenance. Also, in our previous study, efficacy as a dental enamel remineralizing agent was proven.

By synthesizing traditional wisdom with contemporary scientific advancements, we aim to elucidate the valuable contributions of natural resources to healthcare and inspire further research and innovation in this burgeoning field. The present study aims to assess the antimicrobial efficacy of two toothpaste formulations derived from the extracts of *Cocos nucifera*. By comparing the effectiveness of these formulations against oral microorganisms, the study seeks to provide valuable insights into their potential for promoting oral health and combating dental infections in orthodontic patients.

Materials and Methods

This antimicrobial testing was conducted in a controlled laboratory environment. The research institution served as the study's location. The institutional ethical review board granted ethical permission, and the approval ID is as follows: SRB/SDC/PhD/ORTHO-2007/23/061.

Toothpaste Formulations

Two toothpaste formulations were developed using *Cocos nucifera* as a key ingredient, aiming to leverage its antimicrobial properties for oral health benefits. These formulations represent potential avenues for harnessing the therapeutic potential of *Cocos nucifera* in dental hygiene products. The first toothpaste formulation was directly derived from the pulp extracts of *Cocos nucifera*, while the second utilized lyophilized extracts, indicating diverse approaches to harnessing its beneficial properties for oral care products. *Cocos nucifera* specimens were collected from a local coconut field situated in the Tiruppur district of Tamil Nadu for the study. The pulp was extracted by grating, and the obtained sample was divided into two equal parts. In one group, the extracts were blended without the addition of water, and the resulting coconut milk extract was filtered through a muslin cloth and stored at -4 degrees Celsius. The other half of the sample was subjected to freeze-drying for 24 hours followed by lyophilization for 48 hours, and the resulting extract was preserved in a refrigerator. These variations in formulation may offer insights into optimizing the efficacy and stability of *Cocos nucifera* in dental applications.

Zone of Inhibition

Cultures of the test organisms were freshly inoculated into Brain Heart Infusion (BHI)

broth, de Man, Rogosa, and Sharpe (MRS) broth, and Sabouraud Dextrose broth (SDB). Following inoculation, the turbidity of each culture was adjusted to a standardized level equivalent to 0.5 McFarland standards. This step ensured uniformity in the density of the microbial suspensions across all experimental conditions. Standardizing the turbidity facilitated accurate and reproducible measurements for subsequent analyses. The use of 0.5 McFarland standards allowed for consistent comparisons of microbial growth and susceptibility testing results. Overall, this protocol ensured the reliability and validity of the experimental procedures conducted with the test organisms in various culture media. The broth suspensions were used to create lawn cultures on the sterile Mutans Sanguis Agar (MSA), Muller Hinton Agar (MHA), MRS Agar, and Sabouraud Dextrose Agar (SDA) surfaces.

Both the toothpaste materials were separately diluted in ratios of 1:1, 1:2, 1:4, and 1:8, after which aliquots were dispensed into wells prepared in the agar plate. Subsequently, the plate underwent incubation at 37°C for 24 hours. Following incubation, any zones of clearance were observed, and their respective diameters were recorded if present. The tested pathogens included *S. mutans, S. aureus, C. albicans, L. rhamnosus, P. aeruginosa, and E. coli* (Figure 1 and 2).

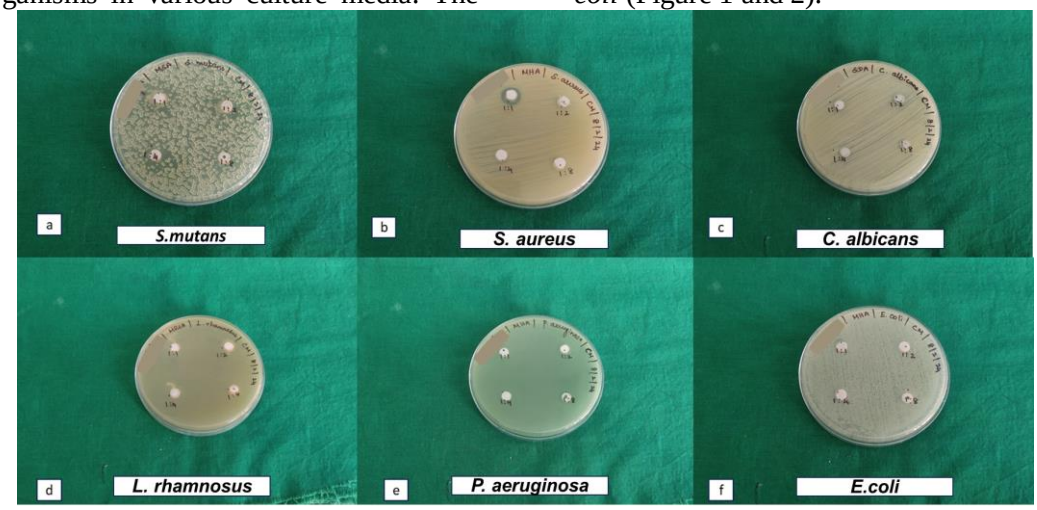

Figure 1. Zone of Inhibition of the CM Toothpaste in a Dilution of 1:1, 1:2, 1:4 & 1:8

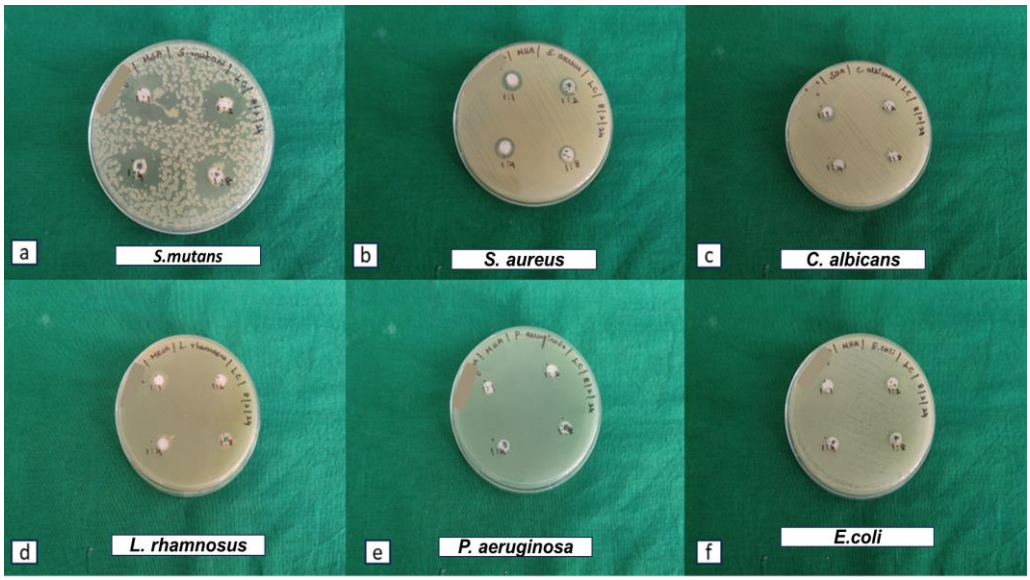

Figure 2. Zone of Inhibition of the LC Toothpaste in a dilution of 1:1, 1:2, 1:4 & 1:8.

Results

The two preparations of toothpaste obtained from the *Cocos nucifera* extracts were separately tested in different cultures for their antimicrobial efficacy against various pathogenic microorganisms, including *S.mutans, S.aureus, C.albicans, L.rhamnosus, P.aeruginosa and E.coli.* Different concentrations of the same toothpaste were tested to identify if there was any dose-dependent variation in antimicrobial efficacy.

From Table 1, it is evident that coconut milk exhibits comparatively more efficacy, which is displayed by the zone of inhibition against *S.mutants, S.aureus, C.albicans and L.rhamnosus* whereas on the other end, the lyophilized coconut-derived toothpaste sample exhibited a zone of inhibition against *P.aeroginosa* only. Intergroup comparisons using the ANOVA revealed that there exists a statistically significant difference between the different concentrations and the two groups of toothpaste tested (Table 2).

Table 1. Zone of Inhibition for the Various Microorganisms in Different Dilutions

Oral pathogen	**Toothpaste sample**	**1:1**	**1:2**	**1:4**	**1:8**	**P value**
Streptococcus mutans	CM	24.33+ 0.57	22.67+ 1.15	20.33+ 0.57	20.67+ 1.52	0.05*
	LC	0.00	0.00	0.00	0.00	-
Staphylococcus aureus	CM	13.66 ± 0.58	11 ± 1	11.33 ± 0.58	10.06± 0.12	0.01*
	LC	12.33± 0.57	0.00	0.00	0.00	-
Candida albicans	CM	18.1 ±0.17	17.33 ±0.57	12.33 ±0.57	10.33 ± 0.57	<0.01*
	LC	0.00	0.00	0.00	0.00	-
Lactobacillus rhamnosus	CM	13.33 ±0.57	0.00	0.00	0.00	<0.01*
	LC	0.00	0.00	0.00	0.00	-
	CM	0.00	0.00	0.00	0.00	-

Pseudomonas aeruginosa	LC	13.33 ±0.57	13.33 ±0.57	11.33 ±0.57	9.33 ±0.57	<0.01*
Escherichia coli	CM	0.00	0.00	0.00	0.00	-
	LC	0.00	0.00	0.00	0.00	-

Table 2. One-Way ANOVA Statistics for Different Concentrations of the CM and LC Toothpastes

ORAL PATHOGENS	**GROUPS- I**		**MEAN DIFFERENCE (I-J)**	**SIG.**
Streptococcus mutans (cm)	1:1	1:2	1.667	.278
		1:4	4.000*	.007*
		1:8	3.667*	.011*
	1:2	1:1	-1.667	.278
		1:4	2.333	.095
		1:8	2.000	.165
	1:4	1:1	-4.000*	.007*
		1:2	-2.333	.095
		1:8	-.333	.978
	1:8	1:1	-3.667*	.011*
		1:2	-2.000	.165
		1:4	.333	.978

Staphylococcus aureus (cm)	1:1	1:2	2.667*	.004*
		1:4	2.333*	.010
		1:8	3.600*	.001*
	1:2	1:1	-2.667*	.004*
		1:4	-.333	.920
		1:8	.933	.355
	1:4	1:1	-2.333*	.010*
		1:2	.333	.920
		1:8	1.267	.156
	1:8	1:1	-3.600*	.001*
		1:2	-.933	.355
		1:4	-1.267	.156
Staphylococcus aureus (lc)	1:1	1:2	12.333*	<0.01*
		1:4	12.333*	<0.01*
		1:8	12.333*	<0.01*
	1:2	1:1	-12.333*	<0.01*
		1:4	.000	1.000
		1:8	.000	1.000
	1:4	1:1	-12.333*	<0.01*

		1:2	.000	1.000
		1:8	.000	1.000
	1:8	1:1	-12.333*	<0.01*
		1:2	.000	1.000
		1:4	.000	1.000
Candida albicans (cm)	1:1	1:2	.767	.319
		1:4	5.767*	<0.01*
		1:8	7.767*	<0.01*
	1:2	1:1	-.767	.319
		1:4	5.000*	.000
		1:8	7.000*	.000
	1:4	1:1	-5.767*	.000
		1:2	-5.000*	.000
		1:8	2.000*	.006
	1:8	1:1	-7.767*	.000
		1:2	-7.000*	.000
		1:4	-2.000*	.006
Lactobacillus rhamnosus (cm)	1:1	1:2	13.333*	.000
		1:4	13.333*	.000

		1:8	13.333*	.000
	1:2	1:1	-13.333*	.000
		1:4	.000	1.000
		1:8	.000	1.000
	1:4	1:1	-13.333*	.000
		1:2	.000	1.000
		1:8	.000	1.000
	1:8	1:1	-13.333*	.000
		1:2	.000	1.000
		1:4	.000	1.000
Pseudomonas aeruginosa (lc)	1:1	1:2	.000	1.000
		1:4	2.000*	.012
		1:8	4.000*	.000
	1:2	1:1	.000	1.000
		1:4	2.000*	.012
		1:8	4.000*	.000
	1:4	1:1	-2.000*	.012
		1:2	-2.000*	.012
		1:8	2.000*	.012

	1:8	1:1	-4.000*	.000
		1:2	-4.000*	.000
		1:4	-2.000*	.012

The mean value is significant at 0.05 level.

Discussion

Toothpaste formulated from the extracts of *Cocos nucifera* were investigated for their antimicrobial activity against common oral pathogens. The study aimed to assess its efficacy in inhibiting bacterial growth and its potential as a natural alternative in oral hygiene maintenance. Results may offer insights into harnessing plant-derived compounds for effective dental care products.

The findings of the conducted study unequivocally demonstrate that the toothpaste derived from *Cocos nucifera* showcases more potent antimicrobial attributes when administered in milk form, effectively combating *S. mutans, S. aureus, C. albicans, and L. rhamnosus*. Additionally, in its lyophilized form, the product exhibits notable efficacy against *P. aeruginosa*, underscoring its potential as a versatile antimicrobial agent across different formulations.

Previous studies have revealed the beneficial effects of various derivatives of *Cocos nucifera*. With its refreshing nature, coconut water offers significant health advantages, with its chemical constituents being vital in the plant industry, biotechnology, and biomedicine. Cytokinins, particularly noteworthy, have garnered attention for their diverse biological functions in plants and humans, with potential implications in anti-cancer therapies [20]. In medicine and dentistry several useful properties of the various extracts of Cocos nucifera had been identified [21]. In a previous study, the researchers compared the antioxidant and nutritional properties of coconut sap with sugar palm and sugarcane juices, finding coconut sap to be rich in antioxidants, vitamins, and minerals, suggesting its potential as a healthier sugar source [22]. Zinc oxide nanoparticles (ZnO NPs) synthesized using *Cocos nucifera* leaf extract displayed antimicrobial, antioxidant, and photocatalytic properties, with potential applications in antibiotics, pharmaceuticals, and photocatalysis. Characterization revealed effective activity against *T. harzianum* and *S. aureus* [23]. Also, in a study, Copper nanoparticles from a herbal preparation obtained from dried ginger, showed an increased zone of inhibition with with increasing concentration [24,25]. Also, As highlighted in a recent study, the green synthesis of silver nanoparticles (AgNPs) using *Cocos nucifera* leaf extract demonstrated substantial antibacterial efficacy against both Gram-positive and Gram-negative bacteria, as noted by the researchers. Characterization methods underscored the formation of AgNPs, indicating their potential as potent antimicrobial agents, and offering promising solutions to combat antibiotic resistance [26]. Several materials have been similarily prepared by green synthesis and mostly exhibited antimicrobial and less cytotoxic effects [27,28]. *Cocos nucifera*, in all its forms, is effective for biomedical applications. This study concentrated on the antibacterial qualities of these extracts in two toothpaste formulations and discovered that coconut milk extract successfully harnesses the antimicrobial capabilities of coconut.

Conclusion

Toothpaste derived from the coconut milk extract demonstrated significant efficacy against tested oral pathogens, suggesting its suitability for incorporation into oral care products. Its antimicrobial properties highlight its potential for combating oral infections and enhancing oral hygiene routines. Further research and development could optimize its utilization in various dental formulations.

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Nil.

Conflict of Interest

Nil.

Author Contributions

NB contributed to research conception, design, data collection, analysis, manuscript drafting, result interpretation, and critical manuscript revision. AS contributed to research conception, design, data interpretation, and critical manuscript revision. Both authors approved the final manuscript and agreed to be accountable for all aspects of the work.

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