Original Article

COMPARATIVE EVALUATION OF THE EFFECT OF RIND AND PULP EXTRACT OF *CITRULLUS LANATUS* ON *STREPTOCOCCUS MUTANS*

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ABSTRACT

The present study aims to investigate and compare the effects of rind and pulp extracts of *Citrullus lanatus* on *Streptococcus mutans* in vitro. The pulp and rind extracts were prepared from fresh watermelon at four different concentrations of 25µl, 50µl, 100µl, and 150 µl, respectively. Four wells on each of the four MHA plates smeared with Streptococcus mutans were loaded with the prepared extracts such that two plates housed four different concentrations of each extract. They were incubated for 24 hours at 37° C. The zone of inhibition was later evaluated. The mean zone of inhibition observed around the wells loaded with 25µl, 50µl, 100µl, and 150 µl of pulp extract was 27mm, 28mm, 31mm, 36mm, and of rind extract were 26mm, 28mm, 31mm, 35mm respectively. There was a substantial rise in antibacterial activity as the concentration of the extract increased against the *S. mutans* bacteria for both extracts. However, there was no significant difference in the zone of inhibition of *Citrullus lanatus* rind and pulp extracts. It may be inferred from this investigation that the extract of *Citrullus lanatus* had substantial antibacterial activity against *Streptococcus mutans.* Plant alternative therapeutic approaches, instead of conventional methods, are sought after for their accessibility, lower side effects, and affordability. Hence researchers are exploring different herbal and plant-based substrates, such as Citrullus lanatus, as an alternate method for reinforcing oral hygiene with significant antimicrobial activity.

Key words: Antibacterial activity, Streptococcus mutans, Watermelon, White spot lesions.

Introduction

The oral cavity is the source of millions of microorganisms, the most common being the Streptococcus mutans and the Lactobacillus spp [1]. These microorganisms portray the role of either a predisposing or an adjunct factor in the etiopathogenesis of various oral conditions and diseases. Plaque and calculus, whose predominance is undeniable in adults worldwide, are primarily composed of Lactobacillus spp, Streptococcus mutans, and salivary mineral particles [2].

Dental caries, a prevalent infectious disease, affects nearly 60%–90% of children and adults [3]. Its contribution towards the global burden of oral diseases is about 10 times higher than that of periodontal and other common oral conditions. Hence, it is termed a pandemic, characterized by a high percentage of untreated carious cavities causing discomfort, pain, and functional limitations [4]. Governed by multifactorial origin and complex interaction between tooth, bacteria, and time, it dissolves hydroxyapatite crystals and demineralizes the tooth substrate [5, 6].

In recent years, fixed orthodontic therapy has gained popularity owing to the increased number of patients opting for the treatment. Depending on the type of malocclusion, the duration of treatment may vary from one to two years. This long-term treatment further may be associated with other complications. One of the major clinical drawbacks associated is enamel demineralization near orthodontic brackets. Plaque and calculus accumulation around various orthodontic auxiliaries, such as orthodontic brackets, wires, elastomeric modules, and bands, is also a common observation. Further, it is noted that patients undergoing fixed orthodontic treatment experience changes in their bacterial profile within the oral cavity [2].

The role of microorganisms in developing plaque and calculus and in establishing dental caries and white spot lesions in deciduous and permanent dentition has been extensively researched and documented [7]. The literature revealed that in addition to *Streptococcus mutans*, species of *Lactobacillus*, *Veillonella*, *Propionibacterium*, *Bifidobacterium*, low-pH *non-S. mutans streptococci*, *Actinomyces*, and *Atopobium*, also participated in caries development and progression. Further, it was also observed that *non-S. mutans streptococci* and *Actinomyces spp* were involved in initiating dental caries [8]. Karpinski *et al*. stated that *Streptococcus spp* and *Lactobacillus spp* essayed a significant role in caries development [9]. A systematic literature review conducted by Tanzer *et al*. strongly recommended the prominent role of the *Streptococcus*

mutans in caries initiation on smooth surfaces, pits, and fissures and on the root surfaces [10].

Despite Chlorhexidine's powerful antibacterial and antiplaque qualities, its broad and long-term use is limited by its well-documented local adverse effects. Extrinsic discoloration of both natural and artificial teeth is the most common local side effect encountered when used as a mouth rinse, whose severity is directly proportional to its concentration [11, 12].

Traditional medicines are gaining center stage of recognition. Plant products in the therapeutic field are globally relevant due to their decreased side effects, better accessibility, and relative affordability. *Citrullus lanatus, of the family* Cucurbitaceae, commonly known as a watermelon, is a well-known indigenous fruit whose bioactive components, such as cucurbitacin, triterpenes, sterols, and alkaloids, as well as vitamins and minerals, have led to the exploration of its therapeutic potential [13]. Studies have investigated the seeds, rinds, and pulp of *Citrullus lanatus* for its antimicrobial, anti-inflammatory, antioxidant, analgesic, anti-ulcerogenic, and laxative effects with acceptable results.

The peel of a watermelon exhibits better antioxidant and antibacterial activity than the pulp. Apart from being powerful antioxidants and antimicrobials, the rind and the seeds also contain significant amounts of phenolic compounds [14]. However, limited studies are exploring the therapeutic potential of the different parts of *Citrullus lanatus*, especially the anti-bacterial activity. Further, evaluating the effective concentration to perform its function also requires investigation. Therefore, the current study aimed to investigate and compare the antibacterial efficacy of pulp and rind extract of Citrullus lanatus against *Streptococcus mutans* bacteria.

Materials and Methods

This invitro study was conducted in the Department of Pharmacology and Dental Research Cell, Saveetha University, Chennai, India, after receiving the university's scientific research council approval and obtaining clearance from the institutional Human Ethical Committee. The IHEC reference number is IHEC/SDC/ORTHO-1705/21/360. The Kashish variety of *Citrullus lanatus*, the commonest species of watermelon cultivated in Tamil Nadu, was considered for preparing the pulp and rind extract.

Preparation of pulp extract

Freshly obtained pulps of watermelon were cut using a sterile knife, deseeded, and the pulp was crushed using mortar and pestle to obtain 100 ml of pure watermelon juice. To obtain pulp extract, the juice was boiled using Borosil HME 250 heating mantle till a concentrated extract was obtained by reducing100 ml of the juice to obtain 5 ml of pulp extract. Further, four different concentrations of the pulp extract were prepared- 25 microliters, 50 microliters, 100 microliters, and 150 microliters concentrations **(Figure 1)**.

Figure 1. 100 ml of Citrullus lanatus juice obtained from the pulp

Preparation of rind extract

The rind of the freshly obtained watermelon was cleaned, cut into small pieces, crushed into a paste, and combined with 100 milliliters of distilled water. Borosil HME 250 heating mantle was used to boil the preparation, thereby evaporating 100 ml of preparation until it was reduced to 5 ml of rind extract. Further, four different concentrations of the rind extract were prepared- 25 microliters, 50 microliters, 100 microliters, and 150 microliters concentrations **(Figure 2)**.

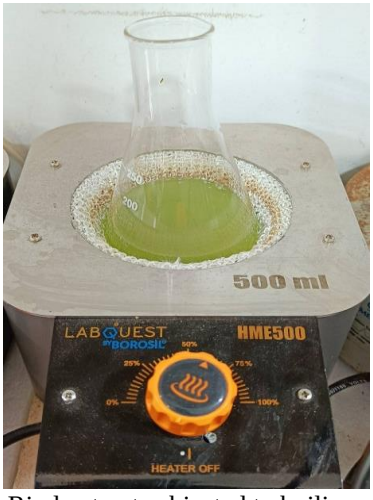

Figure 2. Rind extract subjected to boiling on Borosil HME 500ml heating mantle

Evaluation of antimicrobial activity

Muller Hinton agar (MHA)was prepared and sterilized at 120 lbs for 45 minutes. The media was poured into the four sterilized plates and set aside to solidify. *Streptococcus mutans* procured from cultures that were tested, recognized, and isolated from oral swabs of orthodontic patients for the specific bacteria were considered in the study. The test organisms were then swabbed on the four different MHA

agar plates. This was followed by creating four wells on each plate with a gel puncher. The four different concentrations comprising 25 microliters, 50 microliters, 100 microliters, and 150 microliters of the pulp extract were loaded in each well of two different MHA plates. A similar distribution was followed for rind extract too. Therefore, 2 plates of MHA agar were loaded with four different pulp concentrations and the other 2 plates with rind extract and incubated for 24 hours at 37° C. After the incubation time, the zone of inhibition was measured for both the pulp and rind extracts using a scale. The data was recorded and tabulated, and the means were calculated. Intragroup comparison within the groups and intergroup comparison between the groups was carried out, and the results were analyzed **(Figures 3 and 4)**.

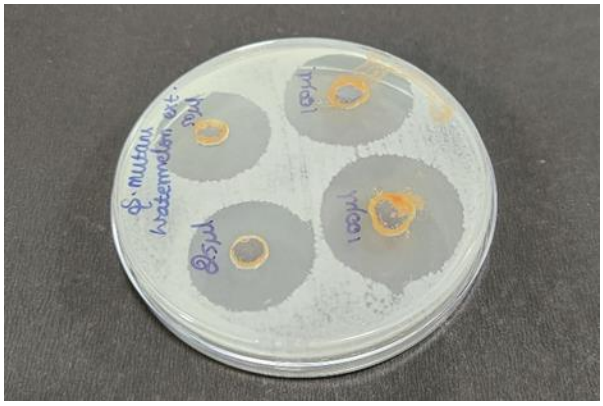

Figure 3. S. mutans swabbed MHA plates with wells loaded with different doses of pulp extract of Citrullus lanatus (25 microliters, 50 microliters, 100 microliters, and 150 microliters)

Figure 4. S. mutans swabbed MHA plates with wells loaded with different doses of pulp extract of Citrullus lanatus (25 microliters, 50 microliters, 100 microliters, and 150 microliters)

Results and Discussion

When examined after 24 hours, the MHA plates presented an evident zone of inhibition in both groups at different concentrations. The mean zone of inhibition observed around the wells loaded with 25µl, 50µl, 100µl, and 150 µl of rind extract was 26mm, 28mm, 31mm, and 35mm, respectively. On intra-group comparison, it is evident that its antimicrobial activity also escalated as the concentration increased. 150µl concentration of rind extract exhibited the maximum inhibitory action in contrast to 25µl, which presented the least action **(Table 1, Figure 5)**.

The mean zone of inhibition observed around the wells loaded with 25µl, 50µl, 100µl, and 150 µl of pulp extract was 27mm, 28mm, 31mm, and 36mm, respectively. On intra-group comparison, it is evident that its antimicrobial activity also surged as the concentration increased. 150µl concentration of rind extract exhibited the maximum inhibitory action in comparison to 25µl, which presented the least action **(Table 1, Figure 5)**.

Table 1. Zone of Inhibition measured in different concentrations of the extract

	25 µL	50 µL	100 µL	150 µL
Rind Extract	26mm	28mm	31mm	35mm
Pulp Extract	27mm	28mm	31mm	36mm

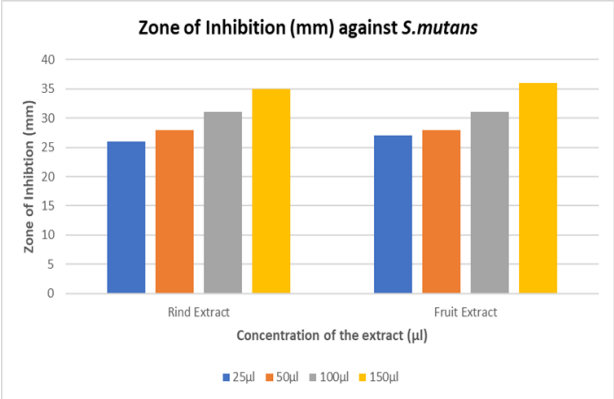

Figure 5. Zone of Inhibition measured in different concentrations of the rind and pulp extracts

On inter-group comparison, it is interesting to note that there was no significant difference between the zone of inhibition of the rind and the pulp extract of *Citrullus lanatus.* Therefore, based on the results of the present study, it can be inferred that both the rind and the pulp extract of *Citrullus lanatus* were effective in exhibiting antibacterial activity at the different concentrations evaluated with a well-defined zone of inhibition on MHA plates.

The results of the present investigation revealed that *Citrullus lanatus* pulp and rind extracts have significant antibacterial activity against *Streptococcus mutans* bacteria for the different concentrations evaluated. The long-term adverse effects, such as staining, and altered taste sensation with the use of antibacterial mouth rinses, manifest the potent need for additional reinforcements in oral hygiene maintenance with minimal or complete absence of adverse effects. Therefore, the present study aimed to explore better

alternatives which were driven by a quest to investigate the efficacy of natural and herbal products in improving oral hygiene.

Researchers worldwide shifted their focus towards natural counterparts whose benefits through well-known but lacked specific scientific documentation of their actions. Different natural products hence were investigated. Cildir *et al*. [15] observed that daily consumption of yogurt containing probiotic bacteria reduced the levels of *S. mutans* bacteria in saliva in patients who were undergoing fixed orthodontic treatment. They further stated that short-term consumption of fruit yogurt daily containing *Bifidobacterium animalis* may reduce the levels of *S.mutans* in the saliva. A study by Khameneh *et al*. [16] stated that a notable source of bioactive substances with strong antibacterial properties is phytochemicals.

While the inhibitory effect of *Andrographis paniculata, Cassia alata*, Chinese black tea, and *Harrisonia perforata* against *S.mutans adherence* was studied by Limsong *et al*. [17], similarly propolis extract also demonstrated a significant effect against the bacteria [18]. According to Philip *et al*., the authors observed and suggested that cranberry extract was not only efficient in inhibiting S.mutans virulence qualities without impacting bacterial viability but also may have an ecological role as a non-bactericidal agent with an ability to modify the pathogenic characteristics of the cariogenic layers on the teeth [19].

Similarly, the different parts of Citrullus lanatus were investigated for antimicrobial activity. Literature reveals the seeds of *Citrullus lanatus* have been utilized for medicinal purposes such as antibacterial, antifungal, antibacterial, anti-inflammatory, and antiulcer values along with the rind and pulp of the fruit, too [20]. Mohammed *et al*. studied the effect of ethanolic extract of *Citrullus lanatus* seed and rind against bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus pneumoniae* and three Gram-negative bacteria *Klebsiella pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa with a* significant zone of inhibition against the bacteria [21]. Hence, the present study evaluated specific streptococcus mutans inhibitory activity of pulp and rind extract of *Citrullus lanatus by agar well diffusion method with favorable results.* Studies have observed that pulp extract obtained by centrifugation of fresh juice of *Citrullus lanatus* showed a significant antibacterial effect against the common oral bacteria, Lactobacillus [1]. The antibacterial activity was in accordance with the present study, however streptococcus mutans was considered instead.

Several researchers also studied the effect of various plant-based green synthesized nanoparticles on different oral and general bacteria with significant inhibitory effects. Data from a study recorded that *Punica granatum* and *Elettaria cardamomum* mediated zinc oxide nanoparticles presented with potent anti-inflammatory and antimicrobial activity [22]. Another study by Kunte *et al*. showed a significant antibacterial effect of pomegranate peel extract against *Streptococcus mutans* bacteria [23]. According to a recent study by Nomura *et al*., extracts obtained from *C. unshiu* fruits have inhibitory effects on S. mutans bacteria, with albedo being one of them suggestive of the former utilization in caries prevention owing to its high polyphenols, neutral pH, and inhibitory properties [24]. Plant-derived exosome-like nanoparticles (PENs) are expected to become effective therapeutic modalities for treating disease or in drug-delivery [25]. All the above studies are following the present study that natural plant-derived substrates do present with antimicrobial activity.

Studies have reported that phytochemical screening of Citrullus lanatus showed the presence of free-reducing sugars and alkaloids in all of its components. Simultaneously, steroids were not identified in any of the extracts. Some alkaloids plant have been demonstrated to have antibacterial activity and hence form a significant medicinal component [26]. Therefore, the presence of alkaloids in the tested extracts of *Citrullus lanatus* could provide a possible explanation for their antibacterial effect displayed. A study by Miranda-Cadena *et al*. stated that Carvacrol, cinnamaldehyde, and thymol are fungicidal against Candida planktonic cells, and phytocompounds are viable alternatives to Candida biofilms [27]. Recent research have revealed that toothpaste components affect oral health-related quality of life and that toothpastes formulated with natural active ingredients have an impact on daily oral cavity cleanliness [28]. The Centre part of the biofilm's bacterial cells are difficult for traditional antibiotic chemotherapy to entirely remove, which exacerbates the condition internationally. Therefore, different approaches like the usage of nanoparticles must be developed and researched in order to combat the drug resistance of bacterial biofilm communities [29].

The major limitation of the present study is its invitro nature. Clinical trials are, therefore, necessary to validate the results of the present study. Further, the antibacterial activity of a standard such as Chlorhexidine was not considered, which could have aided in comparing the efficacy with the extracts. Future studies are required to explore the antimicrobial activity of different parts, their concentration, their action on different microorganisms, and the mode of extraction of Citrullus lanatus. Moreover, the incorporation of nanoparticles in the extracts and the formulation of various dental products such as mouthwashes, powders, pastes, and dental varnishes with it are the areas of interest to look forward to.

Conclusion

Within the limitations of the present in-vitro study, it can be determined that the rind and the pulp extract of Citrullus lanatus have demonstrated a significant antibacterial impact against *Streptococcus mutans* in concentrations of 25

microliters, 50 microliters, 100 microliters, and 150 microliters. Further research on its efficacy on other oral microorganisms, formulation mode, and dispense is required. However, the antibacterial property of this herbal product shows promise to be utilized to reinforce oral hygiene.

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Conflict of interest: None

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Ethics statement: Ethical clearance was obtained from the institutional Human Ethical Committee. The IHEC reference number is IHEC/SDC/ORTHO-1705/21/360.

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