Comparative Evaluation of The Effect of Newly Formulated Citrullus Lanatus Dental Varnish with Fluoride and Chlorhexidine Dental Varnish Against Common Oral Microflora

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Abstract
Aim: Caries is a very common disease that affects people all over the world, and although it has become less common, it is still a serious public health issue. Varnishes for the local delivery of antimicrobial drugs have been created and studied in vitro and in vivo over the past ten years. This study aims to compare the antibacterial effect of a newly formulated *Citrullus lanatus* based dental varnish against commercially available fluoride and chlorhexidine dental varnishes.

Materials and Methods: The green synthesis of silver nanoparticles (AgNp) was done using an extract of *Citrullus lanatus.* A new dental varnish was formulated using the synthesized nanoparticles, and the antibacterial activity of the varnish was studied against the available fluoride and chlorhexidine varnishes using the disc diffusion method. The varnish was studied in two different concentrations, and the zone of inhibition was measured after the incubation period using a vernier caliper.

Results: The results of the study showed that the zone of inhibition of the varnish at both concentrations was comparable to that of commercially available fluoride and chlorhexidine varnish, and the activity increased with an increase in concentration.

Conclusions: From this in vitro study, it can be concluded that there was a significant antibacterial activity of the *Citrullus lanatus* based AgNp varnish against common oral microflora such as *S. mutans, C. albicans, E. faecalis, and Lactobacillus sp.*

Clinical Significance:

This newly developed dental varnish will prevent the formation of dental caries, which will be essential in any clinical practice as it is one of the most common oral disorders in the world. The addition of silver nanoparticles increased the antibacterial activity of the varnish against oral microflora.

Keywords: Dental varnish, Oral bacteria, Antibacterial activity, Chlorhexidine, Fluoride, Watermelon, Citrullus lanatus

Introduction

Dental caries, which results from plaque bacteria metabolizing food carbohydrates into acids that release calcium and phosphate ions from hydroxyapatite, is the gradual demineralization of dental enamel (Hap). Caries is a very common disease affecting people worldwide, and although it has become less common, it is still a serious public health issue.[1,2] Dental varnishes are quick and simple to use, and they may safely and effectively administer active ingredients like fluoride or chlorhexidine to the teeth. Fluoride's local activity at the tooth/plaque contact, which encourages remineralization and reduces demineralization, has the most significant anti-caries impact. It also stops S. mutans from producing acid.[3,4] Varnishes have reportedly been utilized for the local transportation of fluor since 1964 and are a reliable and simple-to-operate vehicle. Varnishes for the local delivery of antimicrobial drugs have been created and studied in vitro and in vivo over the past ten years.[5]

The benefit of fluoride varnishes is that they give high fluoride concentrations to tooth surfaces for extended periods of time, increasing the quantity of precipitated CaF2 globules that serve as mineral repositories and release fluoride when cariogenic difficulties arise.[6] The cariogenic bacterium *Mutans Streptococcus* (MS) has a significant impact on how this illness develops. In order to avoid dental caries, it is crucial to suppress and stabilize MS to nonpathogenic levels for prolonged periods of time.[7] However, it appears to be difficult to reduce MS in people who have current caries. For this reason, several antimicrobial medicines have been included in preventative regimens.[8] It has been established that chlorhexidine (CHX) is the most effective chemotherapy treatment for both MS and dental caries. Chlorhexidine has been delivered via a variety of techniques in studies, including mouthwashes, gels, and dentifrices, to prevent dental caries.[9]

Many natural substances have been shown to be useful in treating dental caries in clinical studies; however, catechol, emetine, quinine,

and flavone are the most often mentioned.[10,11] There is a demand for isolated phytochemicals from plants that may be employed as efficient and affordable medicines. But many plant compounds, including spices and herbs, exhibit cell toxicity, necessitating cytotoxicity testing and dosage limits for safe usage.[12,13]

Previous research showed that probiotics, herbs, and spices are natural antibacterial agents that may be useful in preventing dental caries. There have been dental care products on the market for many years that contain plant extracts. Probiotics maintain the integrity of the oral ecology in addition to acting as possible antibacterial agents.[14] Another review claimed that since probiotics and single herbal items each work on a different target, it may be more beneficial to mix probiotics and plants or different plants. Creating functional goods using probiotics and polyphenol extracts might be a promising area for study in the food sector.[15]

This study aims to compare the antibacterial effect of a newly formulated *Citrullus lanatus* based dental varnish against commercially available fluoride and chlorhexidine dental varnishes.

Materials and methodology

Formulation of Dental varnish

Preparation of Citrullus lanatus Extract

Fruits of fresh watermelon Citrullus lanatus were sliced with a sterilized knife. To achieve 100 ml of pure watermelon juice, the seeds from the cut watermelon pieces were removed, and the fruit was then mashed using a mortar and pestle. With the use of sieves and Whatmann grade 1 filter paper, the obtained watermelon juice was further filtered. The amount of material used in the manufacture of the extract can be altered depending on the quantity of the extracted particle required.

Synthesis of Nanoparticles

In order to start the green synthesis of nanoparticles, 0.0169 g of silver nitrate was dissolved in 90 mL of distilled water. To that 10 mL, fresh watermelon extract was added, and the mixture was agitated using a magnetic stirrer for 24 hours. Centrifugation was then carried out at 8000 rpm for 10 minutes. The pellet was kept in an airtight Eppendorf tube at 4 degrees Celsius after the supernatant was discarded. For the antibacterial investigation, the produced nanoparticles were characterized and used. Depending on the quantity of the desired nanoparticle, it is possible to adjust both the amount of material utilized in their synthesis and the time needed to make them.

Dental Varnish Preparation

The varnish was prepared by using nanoparticles synthesized from the components of *Citrullus lanatus*. To prepare the watermelon dental varnish, 4 mL of ethanol was added to 0.9 mL of acetic acid. To the solution obtained, 500 µL of nanoparticles were added. To make up to 10 mL of varnish, 4.6 mL of distilled water was added, which provided us with 10 mL of watermelon dental varnish. The quantity of material used in the preparation of the varnish and the duration taken to prepare it can be altered and will change, respectively, depending on the quantity of varnish required.

In vitro studies have already been conducted using this varnish composition, and its effect against various oral bacteria has been studied in the laboratory, which showed satisfactory

results when compared with the control groups. The effect of the *Citrullus lanatus* dental varnish was compared in the current study to that of the control group containing other commercially available fluoride and chlorhexidine dental varnishes.

Antibacterial Activity

The antibacterial activity of the newly formulated dental varnish was assessed against common oral bacteria such as *S. mutans, C. albicans, E. faecalis, and Lactobacillus sp.* In comparison to commercially available fluoride and chlorhexidine dental varnish, the antibacterial activity of a dental varnish formulation was investigated at two different doses.

For this experiment, MHA agar was used to identify the zone of inhibition. Prepared Muller-Hinton agar was sterilized for 45 minutes at 120 lbs. Sterilized plates were filled with the medium, which was then left to solidify. The test organisms were swabbed after the wells were cut with the well cutter. The plates were incubated for 24 hours at 37 °C. The zone of inhibition was assessed following the incubation period.

Results

The antibacterial activity of the synthesized nanoparticles was studied by assessing the zone of inhibition. The zone of inhibition measured using the vernier caliper is given in the table below (Table 1).

Bacteria sp.	**Dental varnish 50** µL	**Dental varnish 150** µL	**Fluoride dental varnish**	**Chlorhexidine dental varnish**
Lactobacillus sp.	9	11	9	9
S. mutans	10	20	10	11
E. fecalis	9	12	9	10
C. albicans	9	12	10	10

Table 1: Zone of Inhibition assessing the antibacterial property of the dental varnishes.

The antibacterial effect of the two different concentrattions (**50** µL and **150** µL) of newly formulated *Citrullus lanatus* based AgNp Dental varnishes can be assessed using the assessment of zone of inhibition. The zone of inhibition compared with the commercially available fluoride and chlorhexidine dental varnishes against various oral bacteria such as *Lactobacillus sp., S. mutans, E. fecalis, C. albicans* can be seen in the figures (**Figure 1, Figure 2, Figure 3, Figure 4**) respectively.

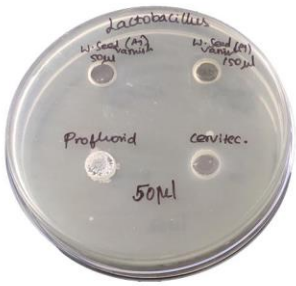

Figure 1: Antimicrobial effect of *Citrullus lanatus*- Dental Varnish on *Lactobacillus sp.*

Figure 2: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *S. mutans.*

Figure 3: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *E. faecalis.*

Figure 4: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *C. albicans.*

Discussions

The results of the current study showed that there was a significant antibacterial activity that was comparable to that of the fluoride and chlorhexidine dental varnishes. The reduction in the bacterial count also signifies an indirect reduction in the formation of dental caries and white spot lesions. According to several studies, people under the age of 18 should apply fluoride varnish every three to six months. Fluoride varnish treatment has been shown to prevent tooth decay by 33% in primary teeth and 46% in permanent teeth.[16,17]

The use of natural and herbal products in oral hygiene maintenance has been researched from earlier times to reduce or overcome the adverse or side effects of conventional oral hygiene reinforcements like chlorhexidine. These adverse effects include staining, taste alterations, numbness, pain in the mouth and tongue, xerostomia, the development of antimicrobial resistance, etc.[18–20] A review of all studies conducted to determine the effectiveness of various herbal products for maintaining oral hygiene revealed that herbal dental products contain ingredients like licorice root, tincture of myrrh, nettle leaves, tea tree oil, chamomile, clove oil, echinacea, eucalyptus, and ginger. Additionally, they demonstrated that Neem was very effective in lowering levels of *Candida albicans* and *Enterococcus faecalis* in the root canals. Halitosis treatment herbal products have also been offered.[21] With proof of its safety and efficacy, bloodroot extract, a *Sanguinaria canadensis* derivative, has previously been used in toothpaste and mouthwash.[22,23]

Various studies have been conducted on the antibacterial properties of *Citrullus lanatus* (watermelon). Even though watermelon is an acidic fruit, it significantly inhibits the growth of the acidogenic bacteria Lactobacillus. They claimed that individuals undergoing orthodontic treatment might be

instructed to drink simple watermelon juice in addition to other oral hygiene products.[24] According to one study, mouthwash made from the *Citrullus lanatus* plant had antibacterial properties that were particularly effective against the oral microflora strains *Lactobacillus* and *Streptococcus mutans*. The antibacterial activity was more pronounced against Lactobacillus than *Streptococcus mutans*.[25] Another investigation on the antibacterial properties of Citrullus lanatus rind ethanolic extract revealed that while Lactobacillus and Streptococcus mutans were not affected by the ethanolic extract of Citrullus lanatus, the juice of the plant had a substantial antibacterial impact. This demonstrates that Citrullus lanatus's antibacterial action was strongly present in the seeds.[26] The conclusion of a study indicated that Citrullus lanatus extract exhibited significant antibacterial activity against Streptococcus mutans and that plant-based alternative medicinal techniques should be preferred over traditional ones because of their accessibility, less side effects, and cheaper cost.[27]

The addition of nanoparticles to dental varnishes was studied earlier by various authors, who showed a significant increase in the antibacterial activity of the varnish and also the remineralizing effect of the varnish on the tooth surface. Further research is required on varnishes with natural product combinations, optimal fluoride concentrations in CS-NPs, ion release, remineralizing capability, as well as clinical efficacy. Although the remineralizing ability of the AuNP dental varnish was significant, it did not outperform the SnF2 dental varnish.[28]

Another study revealed that the CSF-NPs' antibacterial efficacy was not substantially different from that of other natural products, which suggests that these items may be more economical given the high expenses associated with producing the nanoparticles. Additionally, it was said that low molecular weight CS-NPs may provide a stronger antibacterial impact than the medium molecular weight CS-NPs employed in the current work.[29,30] According to research by Harini et al, *Staphylococcus aureus* has the highest zone of inhibition, followed by *Candida albicans*, *Streptococcus* variants, and *Enterococcus faecalis*. Dental varnishes based on nano-zinc oxide and supported by *P. longum* and chitosan will be a superior option for infections brought on by *S. aureus* and *S. mutans*.[31]

In another study, white spot lesions in children were successfully treated with repeated applications of a fluoride varnish containing 0.1% 46 nm AgNPs. The addition of AgNPs to the varnish was a promising treatment for dental remineralization and strengthening enamel to prevent dental caries. They also demonstrated that there was dental remineralization after 3 months of application.[32]

Conclusion

From this in vitro study, it can be concluded that there was a significant antibacterial activity of the *Citrullus lanatus* based AgNp varnish against common oral microflora such as *S. mutans, C. albicans, E. faecalis, and Lactobacillus sp.* and the activity increased with an increase in the concentration of the varnish. The antibacterial activity of the *Citrullus lanatus* varnish was comparable

with that of the antibacterial activity of the fluoride and chlorhexidine dental varnish.

Clinical Significance:

This newly developed dental varnish will prevent the formation of dental caries, which will be essential in any clinical practice as it is one of the most common oral disorders in the world. The addition of silver nanoparticles increased the antibacterial activity of the varnish against oral microflora, which will indirectly reduce the formation of white spot lesions and dental caries.

Limitations

This in-vitro research compares the effects of a newly developed dental varnish with a fluoride and chlorhexidine varnish that is already available. It is crucial to conduct more studies using human clinical trials to verify the study's findings.

Declarations:

Ethical approval: Ethical clearance was obtained from the institutional Human Ethical Committee.

Conflict of interest: The authors have no conflicts of interest to disclose.

Funding: There is no source of funding.

Figure Legends

Figure 1: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *Lactobacillus sp.*

Figure 2: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *S. mutans.*

Figure 3: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *E. faecalis.*

Figure 4: Antimicrobial effect of *Citrullus lanatus* based Dental Varnish on *C. albicans.*

Legends of the Table

Table 1: Zone of Inhibition assessing the antibacterial property of the dental varnishes.

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