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Assessment of the antimicrobial effect of a novel Chitosan-Sunflower seed gel – An in vitro study

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ABSTRACT

Background: The development of multidrug resistant microbes has led to increasing interest towards natural products with antibacterial properties. 2 such products are chitosan and sunflower seed oil. Given the importance of discovering novel treatments for patients with wounds, the goal of this study was to assess the antibacterial activity of a novel chitosan-sunflower seed gel in vitro.

Materials and Method: A novel chitosan-sunflower seed gel was prepared. The antimicrobial activity of the prepared gel, in three different concentrations (25, 50 and 100µl), was tested by Kirby-Bauer disc diffusion method against 5 different microbes: Staphylococcus aureus, Candida albicans, Lactobacillus, Enterococcus faecalis and Streptococcus mutans. The inhibition zones were measured using a vernier calliper.

Result: The 100µl dilution of the gel had the best antimicrobial activity against all the tested microorganisms. The antimicrobial activity against Candida albicans was good and was comparable to that of the control antibiotic used (83%), followed by that against Enterococcus faecalis (66.66%), Streptococcus mutans (64%), Staphylococcus aureus (50%) and Lactobacillus (40%) at the 100µl dilution.

Conclusion: The chitosan-sunflower seed gel is a new and propitious alternative with antimicrobial properties against the tested microbial agents. Further studies in a clinical setting are warranted to corroborate the in vitro results of this study so that appropriate treatment protocols can be established.

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Keywords: *Chitosan Gel, Sunflower seed extract, antimicrobial efficacy*

INTRODUCTION

Hemostasis, inflammation, proliferation, and tissue remodelling are the four highly interlinked phases of the wound healing process.(1,2). Non-healing or delayed healing wounds can result when this process fails, resulting in higher healthcare expenses and a higher chance of unfavourable side effects such as secondary infection, excessive use of drugs like NSAIDs or even opioids.(3–5) Drugs that are delivered locally have various advantages over those that are supplied systemically. Since the drug does not have to travel systemically, its formulation can be of a much higher concentration.(6) Locally administered oral antiseptics have been used pre and post surgery to reduce the microbial count and avoid post surgical infections.(7–9) Furthermore, antiseptics in the mouth help to prevent bacteria from migrating via the bloodstream (10,11), lowering the risk of bacterial endocarditis (12,13). For many years, overprescribing antimicrobials has been a big issue. Because of this, multiple drug resistant bacterial strains have emerged.(14) Infections produced by multidrug-resistant microbes are related to increased expenses, longer hospitalization and increased deaths, making antimicrobial resistance a worldwide health issue (15,16). Antibacterial resistance has prompted more investigation into natural compounds that have antimicrobial properties.

Natural products have been employed for various medicinal purposes, including the treatment of inflammations, infections, and skin conditions. Topical application has been documented in human medicine to treat burns (17,18) and pressure sores.(19,20) The antimicrobial activity of vegetable oils has been examined (21–23). Their application in dermatology has been studied (24,25). Sunflower seeds include oleic acid and a variety of unsaturated fatty acids, the most notable one being linoleic acid, a precursor to arachidonic acid (26). The generation of bioactive compounds like prostaglandins, thromboxanes, and leukotrienes is what essentially determines the activity of arachidonic acid, a widely distributed PUFA in the skin. These compounds act as mediators of the inflammatory process (23,24(27)), encouraging regional angiogenesis, migration of cells, proliferation of fibroblasts and production of extracellular matrix.(25,26(28)) Another natural compound of interest is chitosan. Chitosan is renowned for its strong biocompatibility (29–31) and antibacterial (32–34) and antifungal (29–31) characteristics. In addition, It has been described as an agent for wound healing (28,29,33), a drug carrier for the gradual release of several local (34–36) and systemic (37,38) medications, a blood clotting agent (39,40), and a biomaterial for the regeneration of bone (39–41(35)) and cartilage (42,43).

Due to its bioadhesive properties and adhesion to oral mucosa, chitosan is used to delay the delivery of other oral medicinal substances such as chlorhexidine, a property called substantivity. However, the combination of chitosan and sunflower seed oil for wound healing has never been studied. Given the importance of discovering novel treatments for patients with wounds, the goal of this study was to assess the antibacterial activity of a novel chitosan-sunflower seed gel in vitro. Our previous experience has enabled us to perform this research.(36–46).

MATERIALS AND METHODS

Gel Preparation

0.25g chitosan was added to 50ml distilled water to obtain chitosan extract (5% m/v). 50 ml of sunflower seed extract (3% m/v) was prepared by adding 0.15g of sunflower seed powder to 50ml distilled water. The two solutions were mixed to obtain chitosan-sunflower seed extract. To this, 2.5g carbopol was added. The mix was then kept on a stirrer for 48 hours until a homogenized mix of chitosan-sunflower seed gel was obtained.

Antimicrobial Activity Test

The antimicrobial activity of the prepared gel was done by the Kirby-Bauer disc diffusion method.The procedure entails placing paper discs coated with an antibacterial substance on top of agar that has already been sown with the target bacterium. The antimicrobial compound then

permeates the agar, preventing bacterial development in the vicinity of the disc. In this study, agar plates were prepared for five different microbes: Staphylococcus aureus, Candida albicans, Lactobacillus, Enterococcus faecalis and Streptococcus mutans. Different concentrations (25, 50 and 100µl) of chitosan-sunflower seed gel were added to wells in agar plates. Incubation of agar plates was done at 37°C for 24 hours. After 24 hours, measurement of the inhibition zone was done using a vernier calliper.

RESULTS

Results showed that the 100µl dilution of the gel had the best antimicrobial activity against all the tested microorganisms. The antimicrobial activity against Candida albicans was good and was comparable to that of the control antibiotic used (83%), followed by that against Enterococcus faecalis (66.66%), Streptococcus mutans (64%), Staphylococcus aureus (50%) and Lactobacillus (40%) at the 100µl dilution.

FIGURE 1: Chitosan- Sunflower seed gel preparation from Sunflower seed extract and Chitosan extract.

FIGURE 2: Results of the Kirby-Bauer disc diffusion test.

TABLE 1: The inhibition zones (in mm) of different microbes in different dilutions of the Chitosan-Sunflower seed gel.

	25µl	50µl	100µl	Antibody
Candida albicans	9	9	10	12
Streptococcus mutans	10	12	16	25
Enterococcus faecalis	14	16	20	30
Lactobacillus	9	9	10	25
Staphylococcus aureus	9	10	12	24

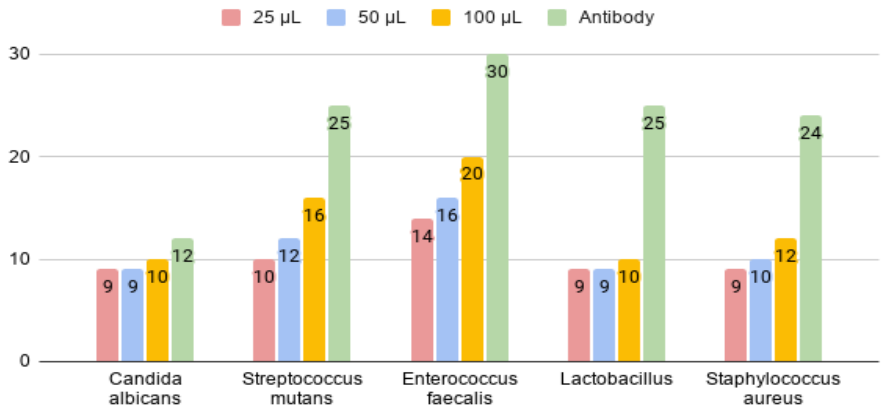

FIGURE 3: Clustered bar graph showing values for zone of inhibition for different microbes in different concentrations of the chitosan-sunflower seed gel.

DISCUSSION

The study was conducted to assess the antimicrobial activity of a novel chitosan-sunflower seed gel against various microorganisms. The results showed that the gel had the best antimicrobial activity against Candida albicans and was also effective against Enterococcus faecalis, Streptococcus mutans and Staphylococcus aureus. However, its activity against Lactobacillus species was only 46% of the control antibiotic used.

The chitosan-sunflower gel studied here showed good antimicrobial activity. This can be substantiated by various studies that have looked into the antibacterial properties of chitosan and sunflower seed oil independently. (44) have examined the ozonated sunflower oil's (Oleozon) antibacterial efficacy against pathogens such S. aureus and Enterococcus faecalis. The results were excellent, with MICs ranging from 1.18 to 9.5 mg/ml. Recently, (9), both in Gram-negative bacterial strains (E. coli and P. aeruginosa) and Gram-positive strains(S. aureus and Micrococcus luteus), this antibacterial property has been demonstrated. Antibacterial and antifungal activity of sunflower seed oil may be attributed to tannins, saponins and extracted flavonoids; which have been shown to inactivate cell envelope transport proteins, enzymes and microbial adhesions.(47) The gel used in the current study showed the best antimicrobial activity against Candida albicans (48). The sunflower seed has a 10 kDa basic polypeptide similar to many plant lipid transfer proteins, called Ha-AP10, which displays high antifungal activity (49). Topical ozonized plant oils have been used as an antifungal agent in treating onychomycosis (50) and vaginal candidiasis (51), and have been shown to be more effective than topical ketoconazole. Unlike oral antifungal medications, it carries no danger of systemic side effects and drug interactions and is a reasonably priced therapy with a mechanism that is probably comparable to that seen in bacterial cells.(52)

Chitosan can have a large number of C2 amino groups with pKa values of B6.5, which can become protonated in mildly acidic environments, depending on the degree of deacetylation. The antibacterial properties are due to its polycationic nature, which allows it to interact with negatively charged microbial cell walls and cytoplasmic membranes. Reduced osmotic stability, membrane rupture, and eventual leaking of intracellular components are all consequences of these interactions.(53–55).Furthermore, chitosan may penetrate the nuclei of bacteria and fungus, where it binds to microbial DNA and inhibits mRNA and protein synthesis (56,57). When seen at a nanoscale, chitosan has a larger surface-to-volume ratio, which means it has a higher surface charge density, a stronger affinity for bacteria and fungi, and more antibacterial activity.(Qi et al., 2005).Chitosan often exhibits stronger antibacterial activity against Gram-positive bacteria and Candida species as compared to

Gram-negative bacteria. In Gram-negative bacteria, divalent cations are displaced by the cationic structure, causing permeabilization of the outer membrane and disruption of lipopolysaccharide- binding (57), while in Gram-positives, there is binding of chitosan to teichoic acids along with a potential extraction of membrane lipids (56). Such events lead to death of the microbial cell.

Limitations and Future Scope

In the current study, only a specific proportion of chitosan and sunflower seed oil was used. It would be interesting to see if different proportions of the gel constituents would yield different results or not. Also, the combination of chitosan with ozonized sunflower seed oil may give rise to superior properties. Further studies should be conducted to prove the antimicrobial action of chitosan- sunflower seed gel against other oral microorganisms. Further studies should also be done evaluating the antimicrobial effect of the chitosan- sunflower seed gel in clinical scenarios after safety assessment.

CONCLUSION

The chitosan-sunflower seed gel is a new and propitious alternative with antimicrobial properties against the tested microbial agents. Further studies in a clinical setting are warranted to corroborate the in vitro results of this study so that appropriate treatment protocols can be established.

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