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RESEARCH ARTICLE

Preparation of Mouthwash Using Red Sandal Mediated Silver Nanoparticles and Its Antimicrobial Activity - An In Vitro Study

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ABSTRACT

Introduction: The Indian native herb Pterocarpus santalinus L. is being used to manage a variety of ailments.The potent properties of various phytochemicals found in P. santalinus L. and its antimicrobial activity interested us to analyse their efficiency. The aim of the study is to prepare mouthwash using red sandal mediated silver nanoparticles and evaluate its antimicrobial activity

Materials and Methods: Silver nanoparticle is synthesized by using Pterocarpus santalinus L., followed by characterization of nanoparticles with UV-VIS spectrophotometer, Mouthwash was prepared from its and tested for antimicrobial activity of using agar well diffusion method.

Results: Mouthwash had a better effect on S.Aureus, with a maximum inhibition zone of 23mm at 100µL and on C.Albicans, at 100µL, it had a 27mm zone of inhibition. Thus as the concentration increases the zone of inhibition also increases.

Conclusion: In conclusion, we have demonstrated that 0.1% mouthwash prepared from Pterocarpus santalinus mediated silver nanoparticles exhibit excellent dose dependent inhibition activity against the pathogens Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans. Hence, red sandal mediated silver nanoparticles proves to be a safer alternative for infections caused by oral pathogens Streptococcus mutans, and fungal species like Candida albicans.

KEYWORDS:
Antimicrobial Activity, Agnps Pterocarpus Santalinus, Herbal Mouthwash, Oral Pathogens, Green Synthesis..

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INTRODUCTION

In India, the majority of people suffer from periodontal disease and tooth decay (1). Periodontitis, in particular, is a chronic bacterial infection marked by chronic inflammation, connective tissue loss, and alveolar bone damage, all of which are mediated by pro-inflammatory mediators(2). Plaque can be removed effectively with mechanical methods such as brushing teeth(3). However, it is extremely reliant on the abilities of the user. Furthermore, adequate brushing can be challenging in disabled or traumatised patients(4). Plaque formation has been found to be reduced by using adjunctive strategies such as mouthwashes(5). Regular mouthwashes, such as chlorhexidine, have been associated with enamel discoloration, taste changes, and mucosal irritation(6). As a result, seeking a different antimicrobial agent with less adverse effects seems to be a viable option.

India has a rich ancient medicine system that dates back over three thousand years, and herbal combinations may have been known even before that. However, for many years, people have relied on modern medicines, and the value of traditional medicine has been overlooked(7). Several herbal remedies have been shown to help treat dental plaque buildup when combined with other oral hygiene practises such as brushing and flossing. Natural ingredients are used in the manufacture of over half of modern medicines, so they play an important role in drug development. Natural ingredients have been used in herbal medicine for thousands of years world wide. Many of them have antimicrobial, anti-inflammatory, and cytostatic properties. They have proven to be efficient in human medicine. Neem, tulsi, and propolis are among the herbs used to make mouthwash(8). The Legume family's Pterocarpus santalinus L. (Fabaceae), also known as "red sanders" or "red sandalwood," is a commercial tree. This species is common in well defined forest regions in Andhra Pradesh, one of India's southernmost states. It is highly valued on the international market and is mostly exported from India to Japan and other nations. The aromatic red sandalwood is prized for its use in furniture as well as as a source of colourants and dyes. P. santalinus was already used for inflammation, and to treat fever, headache, ulcers, cancer, skin infections, boils, scorpion stings, and to enhance sight. Ethanol extract of bark was reported to have antihyperglycemic effects(9). According to a critical review of the literature Pterocarpus santalinus L.f. has remained unexplored for several therapeutic properties claimed(10).

Nanotechnology is being used in dental materials in recent years, with nanoparticles being integrated into the structure of dental composites and disinfection solutions due to their bacteriostatic and bactericidal effects(11). Recent developments in nanotechnology and nanoscience have revolutionised the way we detect, cure and prevent diseases across the globe. Silver nanoparticles are useful in nanoscience and nanotechnology, especially in nanomedicine. They have been used for various applications due to unique properties like antibacterial agents in dressings for wounds, including bandages. According to a clinical study, chlorhexidine and herbal mouthwash have similar impacts on anaerobic periodontal pathogen counts and gingival health (12). As per another report by Kariminik, Nanosil mouthwash is more efficient than chlorhexidine at destroying bacteria(13). However, in contradiction to this, in vitro study by Ahrari et al. found that chlorhexidine mouthwash had a statistically significant outcome in antimicrobial activity as compared to nanoparticle mouthwash (14). The antibacterial efficacy of P. santalinus L mediated silver nanoparticles has only been studied in a few studies up until now. Our team has extensive knowledge and research experience that has translated into high quality publications(15–27),(28–32),(33),(34). Therefore it would be of interest to know the antimicrobial activity of red sandal mediated silver nanoparticles. Hence, the aim of this research is to prepare mouthwash with silver nanoparticles mediated by Pterocarpus santalinus and test its antimicrobial properties.

MATERIALS AND METHODS

Preparation Of Plant Extract

Pterocarpus santalinus extract is purchased commercially. 1g of the extract is diluted with 40 mL distilled water in a conical flask and then boiled at 60-70°C for 20 minutes. The extract is filtered using Whitman filtered paper and allowed to remain undisturbed for 20 minutes. 30 ml of filtered extract is obtained (figure 1)

Synthesis Of Silver Nanoparticles

1 m Mol of $AgNO_3$ is weighed and mixed with distilled water of 70ml and mixed with 30ml filtered extract. To obtain synthesis of silver nanoparticles, the extract is allowed to stand in the stirrer for 1 hour, the solution was dark brown in color which was subjected to orbital shaker for uniform dispersion of the reaction mixture and colour changes were visually observed at various time periods. On a routine basis, UV Spectrometers were used to check the reduction of $AgNO_3$ to AgNps.

Preparation Of Mouthwash

The mouthwash was prepared using 60ml of synthesised red sandal mediated silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, Sodium dodecyl Phosphate with 0.1% in concentration. Silver nanoparticles are the main constituent, ethanol acts as a solvent to solubilise the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavouring agent

Fig.1: Image showing the Synthesis of red sandal mediated silver nanoparticles mouthwash

Test Pathogens

Enterococcus faecalis, Candida albicans, Streptococcus mutans, Staphylococcus aureus

Antimicrobial Activity of Red Sandal Mediated Nanoparticles Mouthwash

50µL, 100µL, 150µL of red sandal mediated silver nanoparticles were loaded into the wells in the Mueller-Hinton agar plates and rose bengal agar previously streaked with E.faecalis, Streptococcus mutans, Staphylococcus aureus and Candida albicans and incubated for 24 and 48 hours at 37°C respectively. Amoxicillin and Fluconazole was used as the standard.For each pathogen, the zone of inhibition was measured and mean values were reported.

RESULTS

The mixture of AgNO3 solution and red sandals extract was subjected to UV-visible (UV-vis) spectroscopy analysis in the recorded spectra, which showed a detectable peak at 440 nm (figure 2). The Different Concentration of 0.1% Red Sandal Mediated Silver Nanoparticles mouthwash Showed Dose Dependent Antimicrobial Activity against the tested Streptococcus Mutans, Streptococcus Aureus, E.Faecalis, Candida Albicans (figure 3). Descriptive statistics was used. Mouthwash had a better effect on S.Aureus, with a maximum inhibition zone of 23mm at 100µL and on C.Albicans, at 100µL, it had a 27mm zone of inhibition. Thus as the concentration increases the zone of inhibition also increases. (figure 4)

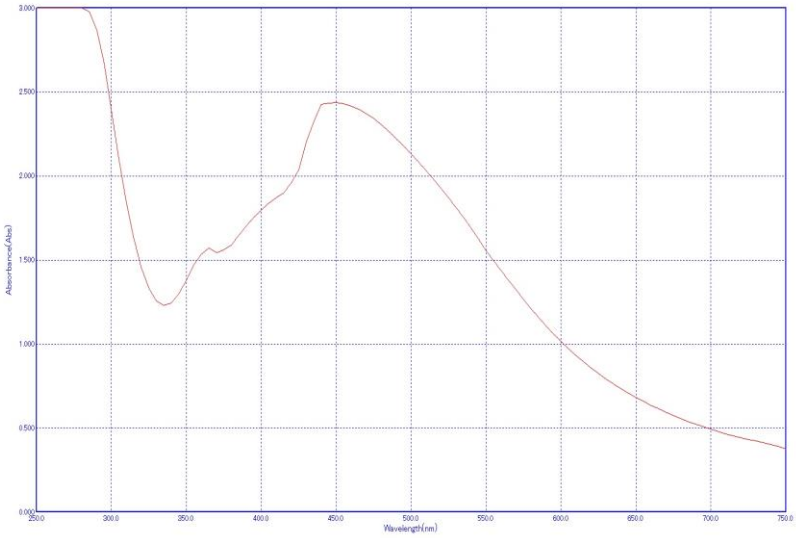

Fig.2: Graph depicting UV-Visual spectroscopy of silver nanoparticles mediated by red sandals. Silver nanoparticles' surface plasmon resonance peak was at 440 nm with a high absorbance, indicating that silver nanoparticles were synthesised.

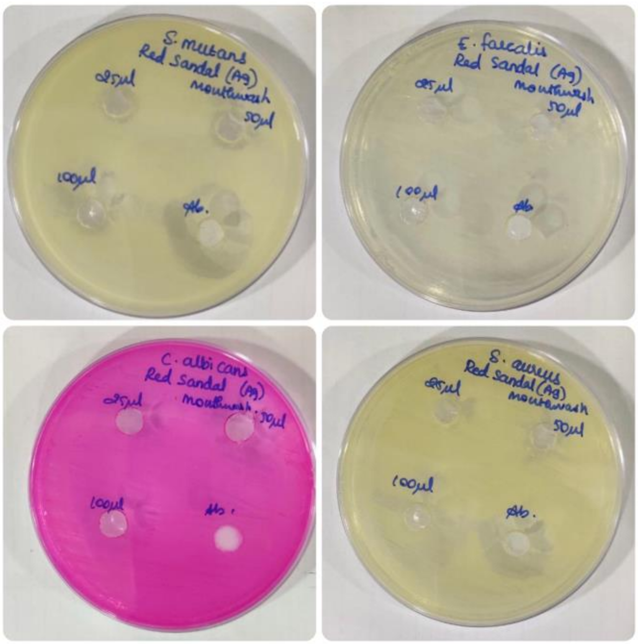

Fig.3: Antimicrobial action of 0.1% red sandal silver nanoparticles in mouthwash against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans at various concentrations

Table 1: showing zone of inhibition of oral pathogens by red sandal silver nanoparticles in 0.1% red sandal nano mouthwash.

Organism	**25 µL**	**50 µL**	**100 µL**	**Ab**
S.aureus	13	16	25	24
S.mutans	14	17	23	30
E.faecalis	11	15	16	28
C.albicans	17	25	27	12

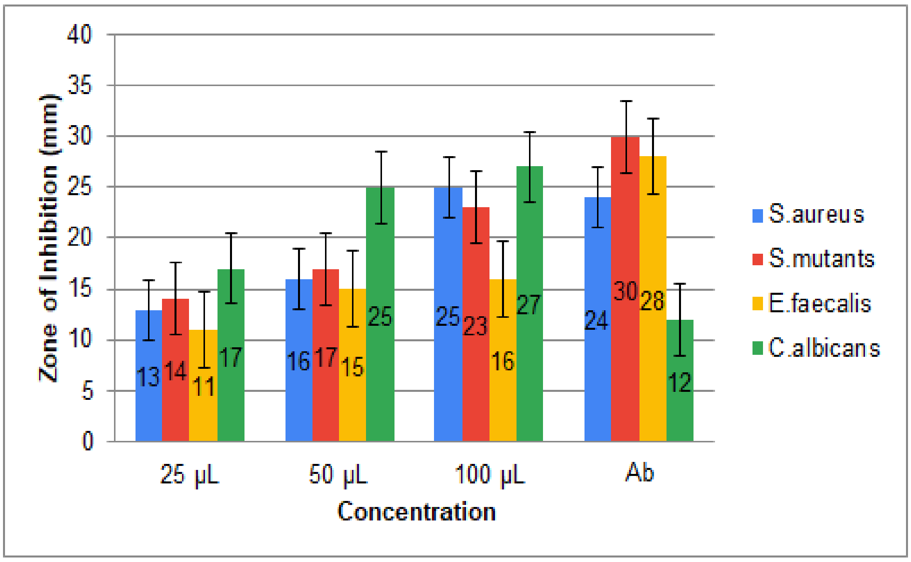

Fig.4: Depicts the zone of inhibition of oral pathogens by red sandal silver nanoparticles in 0.1% mouthwash. The X axis depicts the various concentrations of silver nanoparticle mouthwash, while the Y axis depicts the zone of inhibition. Blue denotes S.aureus, Orange denotes S.mutans, yellow denotes E.faecalis and green denotes C.albicans. Mouthwash had a better effect on S.Aureus, with a maximum inhibition zone of 23mm at 100µL. on C.Albicans. At 100µLL, it had a 27mm zone of inhibition. Thus as the concentration increases the zone of inhibition also increases.

DISCUSSION

Half of Indian adults have periodontal disorder. According to the Oral Health Foundation, the approach to improving oral and dental health is preventative measures and oral hygiene. (35) In the current the study 440 nm wavelength in the UV-Visual spectroscopy correlates to the surface plasmon resonance of silver nanoparticles, and the high absorbance indicates silver nanoparticle synthesis. Several experiments have shown that silver nanoparticles have a resonance peak in this area (36). The formation of a peak about 400–500 nm suggests the reduction of Ag+ to Ag0 (metallic) and is due to formation of conjugation between electron shuttles with the presence of NADH-dependent reductase enzyme in plant extract (37).

Only a few of the 200 to 300 bacterial species that live in the oral cavity cause dental decay (caries) or periodontal disease. The use of nanoparticles to inhibit bacterial growth is becoming more common as a way to prevent and cure infections(38). Previous research has shown that Pterocarpus santalinus stem bark and leaves extracts have broad antibacterial activity against pathogens such as E.coli, S.aureus, Pseudomonas aeruginosa, and others(39). In a similar manner to the current study, Gopinath's research concluded that silver nanoparticles controlled by Pterocarpus santalinus had strong antibacterial ability against both the strains. The antibacterial activity zone of inhibition is determined by the concentration of Silver nanoparticles. As concentration of the silver nanoparticles increases, the zone of inhibition grows as it binds to cytoplasmic membranes and destroys bacteria.

Silver nanoparticles synthesised in this study had an antimicrobial effect on oral pathogens and inhibited the growth of S.mutans and Candida albicans. Silver nanoparticles typically interfere with bacterial membranes and disturb membrane integrity; Silver ions bind to essential biological molecules such as sulphur, oxygen, and nitrogen, thereby inhibiting bacterial growth. It also stops bacteria from making proteins and replicating their DNA(40). The Synthesized AgNps had dose-dependent antimicrobial activity indicating that Pterocarpus santalinus extract can be widely used in the development of effective antibacterial AgNPs for biomedical applications. Furthermore, the red sandals extract can be used to synthesise other metal and metal oxide nanoparticles, and a clinical trial with the mouthwash can be performed to assess its efficiency against other oral pathogens.

CONCLUSION

In conclusion, we have demonstrated that 0.1% mouthwash prepared from Pterocarpus santalinus mediated silver nanoparticles exhibit excellent dose dependent inhibition activity against the pathogens Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans(41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) ((51,52) (53) (54). Hence, red sandal mediated silver nanoparticles proves to be a safer alternative for infections caused by oral pathogens Streptococcus mutans, and fungal species like Candida albicans.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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