Original Article

Synthesis Of A Novel Mouth Rinse Containing Selenium Nanoparticles And Chitosan Colloid And Assessing Its Antimicrobial Property

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Abstract

AIM: The study aimed at synthesising a novel herbal based, alcohol free mouth rinse, containing green synthesised-selenium nanoparticles and chitosan colloid and assessing its antibacterial properties.

MATERIALS AND METHODS: A novel herbal mediated synthesis of selenium nanoparticle synthesis was employed in this study. This study used Stevia leaves for nanoparticle synthesis in combination with Sodium selenite. This mixture was mixed homogeneously using an orbital shaker. Colour change indicated formation of SeNPs. The SeNP's were characterized for shape and size using TEM (transmission electron microscopy) analyses and UV vis spectrophotometry. A novel mouth rinse containing Selenium nanoparticles and Chitosan colloid as made. The activity against microbes of the mouth rinse was evaluated using agar gel diffusion technique. The inhibition zones were measured for: Enterococcus faecalis, Streptococcus mutans, Staphylococcus aureus and Candida Albicans at 25µL, 50µL ,100µL concentration.

RESULTS: Herbal mouth rinse showed excellent antimicrobial activity against S.aureus and C.albicans at all concentrations. Following S. aureus, the mouth rinse was effective against S. mutans at 50 and 100 µL concentration and against Faecalis at 100 µL.

CONCLUSION: The herbal alcohol-free mouth rinse consisting of Selenium Nanoparticles and Chitosan colloid is effecient in inhibiting the activity of oral microorganisms and could be used as an adjunct to mechanical methods of plaque removal in orthodontic patients.

KEYWORDS: herbal mouth rinse, antibacterial activity, Orthodontics, selenium nanoparticle, chitosan,

INTRODUCTION:

The emergence of nanotechnology in recent times has led the way for advancement of treatment options for diseases of varying pathophysiologies(Khurana et al., 2019)(Management Association and Information Resources, 2016; Rajeshkumar and Bharath, 2017)

Nanoparticles possess electrical, optical, magnetic and above all, chemical properties, which cannot be achieved with their bulk counterpart materials. (Gangadoo et al., 2017).Their unique features like reduced size, increased surface area, , high surface chemistry, better solubility and varied functionality make them very strong carriers for the delivery of therapeutic molecules.(Sperling and Parak, 2010).

Since orthodontics involves fixed appliances which is bonded on to the tooth surface, proper cleansing of the oral cavity, removal of remnant food debris from the oral mucosa and tooth surface is a tedious procedure. The

prevalence of calculus, plaque and food debris due to improper oral hygiene is in turn associated with the severity and incidence of white spot lesions.(N et al., 2017). Similarly, the incidence of gingivitis also increases during fixed orthodontic therapy resulting in generalised gingival growth which can propose further oral complications.(Kessler, 1976).Literature reveals that the commercially available mouthwashes which are commonly in use cause less desirable side effects like extrinsic staining , antibacterial resistance to oral microorganisms, and rare but fatal allergic reactions.(Brookes et al., 2020).Thus, the search for an effective and natural mouth rinse overcoming these disadvantages and having effective antimicrobial properties is necessary.

Selenium is extremely essential for humans owing to the fact that it can improve the action of the seleno-enzyme, glutathione peroxidase and inhibit damage to cells and tissues from free radicals.(Zhang et al., 2015)The use of selenium nanoparticle form has attracted attention due to the nano size range property enhancing the photoelectric and biological properties of selenium.(Gangadoo et al., 2017).

Selenium nanoparticles(SeNPs) exhibit less toxicity in comparison to other selenium compounds.SeNPs have been in use to treat cancer, inflammatory disorders, diabetes, liver fibrosis and drug induced toxicities.(Wang et al., 2005; Li et al., 2011; Huang et al., 2013; Kumar et al., 2014).Selenium nanoparticles exhibit increased biological behaviour, bioavailability when compared with organic and inorganic Selenium compounds(Zheng et al., 2012).However, all these properties are rendered when selenium is present in its zero oxidation state-which is highly unstable .(Hosnedlovaet al., 2018).

It is for this purpose, that chitosan colloids can be used along with selenium nanoparticles to stabilize them. Chitosan possesses antibacterial, biodegradable and biocompatible properties which has led to increased application in the fields of drug delivery and biomedicine.(Ahmed, Rahman and Alam, 2018; Rangrazi et al., 2020).

Therefore, this study was aimed at synthesising a novel herbal based, alcohol free mouthwash, containing green synthesised-selenium nanoparticles and chitosan colloid and assessing its antibacterial properties.

MATERIALS AND METHODS:

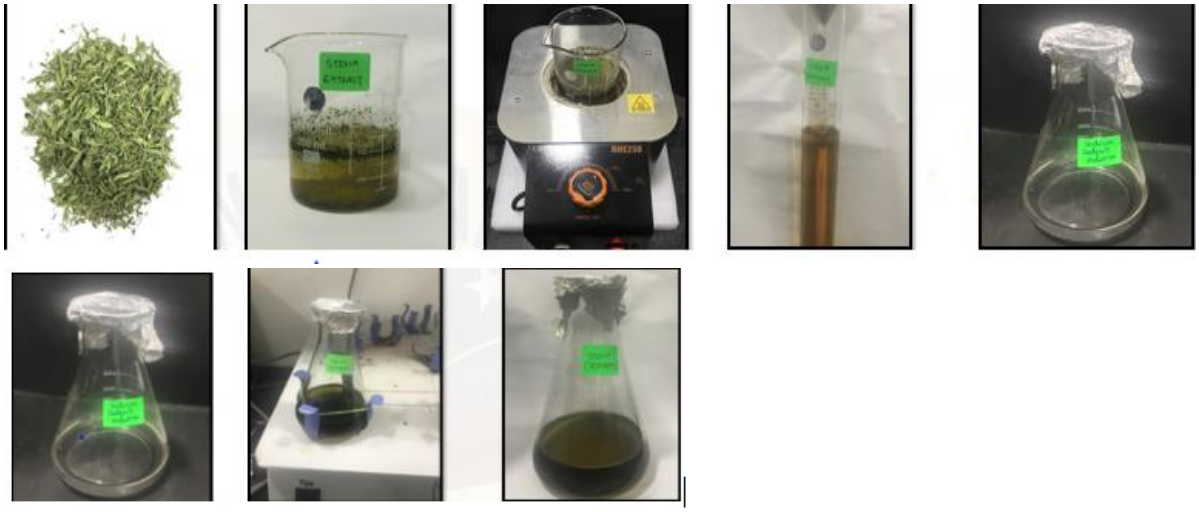
PREPARATION OF STEVIA EXTRACT:

Leaves of Stevia plant were collected and washed with distilled water several times and then dried in an incubator. It was then grounded into coarse particles by means of a mortar. 1 gram of coarse powder was mixed homogeneously with 100 ml distilled water. This solution was heated at 60- 70 ºC on a heating mandrel for 10-15 minutes. Purification of solution was done by filtration using a Whatman filter paper no.1. Residue collected in the filter paper was discarded and the supernatant was collected in a conical flask. (Figure 1)

PREPARATION OF NANO SIZED PARTICLES OF SELENIUM:

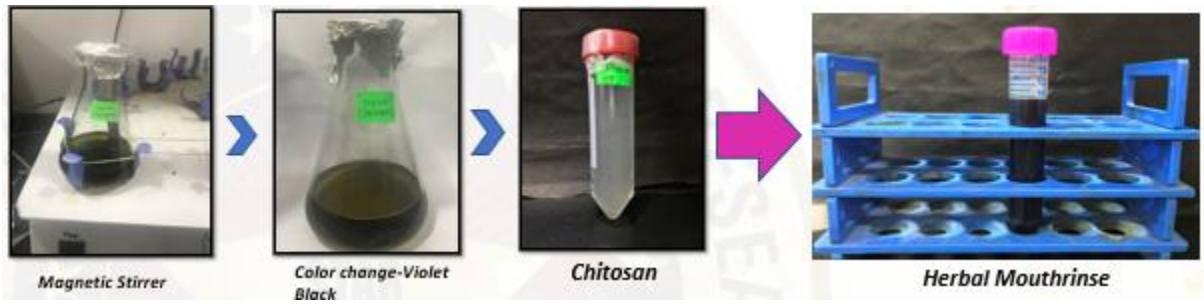
The synthesis of nano sized particles of selenium was obtained by reducing Sodium selenite solution. Stevia plant extract was used as a reducing or capping agent. In the procedure of nanoparticle synthesis,30 milli molar (0.519 grams) of Sodium Selenite was mixed in 50 ml of distilled water. To this solution,50 ml of filtered stevia extract was added and kept overnight on an orbital shaker for homogenous mixing of all particles. The reaction mixture was stirred continuously on a magnetic stirrer till colour change in the mixture was observed. At hourly intervals the synthesis of nanoparticles was monitored by UV – vis spectroscopic analysis. Colour change of selenium nanoparticles indicated its formation at a certain wavelength that was measured on a UV – vis spectrophotometer. Post spectroscopic analysis, the mixture was collected in 5 test tubes and selenium nanoparticles were separated from solution by centrifugation for 20 minutes. (Figure 1)

FIGURE 1:



Herbal aided preparation of Selenium nanoparticles from Stevia leaves.

FIGURE 2:



Preparation of Herbal mouth rinse

PREPARATION OF MOUTH RINSE:

The Selenium nanoparticles prepared using Stevia plant extract were used to the formulation of mouth rinse for its action against plaque producing microorganisms. For preparation of mouthwash, following procedure was employed:

10 mL of distilled water was poured in a test tube. To it 600 µl of nanoparticle solution was added 600µL nanoparticle solution was measured using a micropipette having attached disposable tips which were changed between transfers to prevent contamination and mixing of any solutions at any given point of time. To this solution 0.3 grams of sucrose, 0.005 grams of sodium benzoate were added, 0.01 grams of foaming agent sodium lauryl sulphate was mixed with the above solution and lastly 0.1 ml of peppermint oil was added. (figure 2) .This solution was vigorously mixed until a homogenous solution of the mouth rinse was obtained.(table 1)

TABLE 1:COMPONENTS OF THE MOUTHWASH

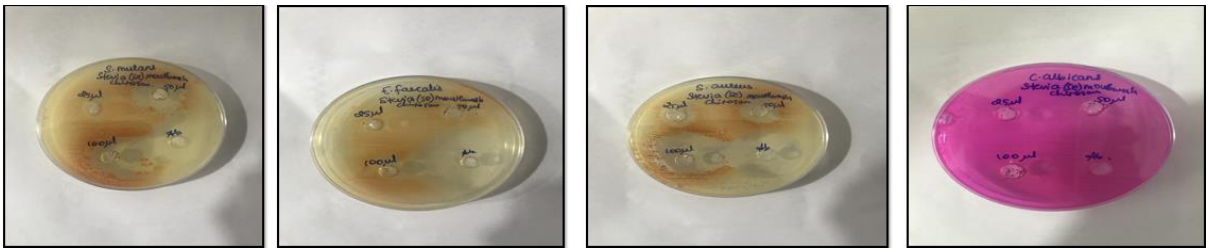
COMPONENT	ACTION
selenium nanoparticles -1ml	antimicrobial activity
chitosan colloid -1ml	antimicrobial activity
sucrose -0.3 mg	sweetening agent

sodium benzoate -0.001 g	preservative
sodium lauryl sulphate -0.01g	foaming agent
peppermint oil-100 mules	flavouring agent.

ANTIMICROBIAL ACTIVITY:

The antimicrobial activities of the synthesized SeNP's and its indigenous mouthwash were tested against S. aureus, S. mutans, E. faecalis and C. Albicans.Agar well diffusion method was used to determine the antimicrobial property. The different concentrations of the AuNP's (25µL, 50µL ,100µL) and its mouthwash (50µL ,100µL, 150 µL) were added to the wells made on nutrient agar plate. Agar plates were incubated at 37 ºC for 24 hours. After 24 hours, the inhibition zone was measured using a Vernier calliper to ascertain the extent of antimicrobial activity. The antimicrobial activity was assessed by taking Amoxicillin as control for bacterial species and Fluconazole as control for fungal species.(figure 3)

FIGURE 3:



Agar well diffusion method for assessment of antimicrobial activity.

RESULTS:

VISUAL OBSERVATION OF NANOPARTICLES AND MOUTHWASH:

Colour change was observed after stirring SeNP's continuously on a magnetic stirrer. The transformation in colour from pale yellow to violet showed the formation SeNP's.

Mouthwash prepared from nanoparticles was violet in colour similar to the colour of SeNP's. (Figure 1 and 2).

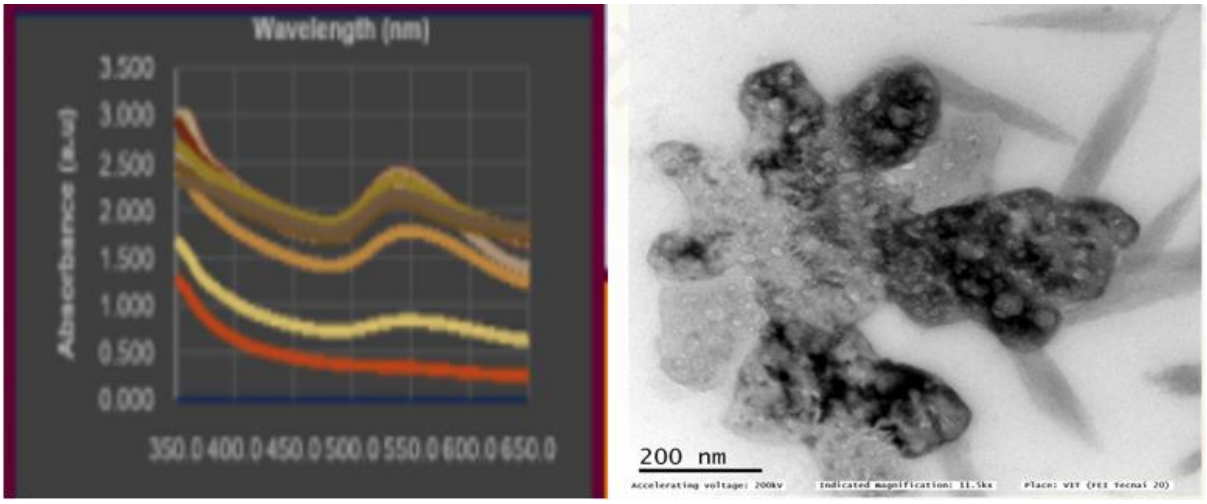
UV-Vis SPECTROSCOPY:

In this study, the colour of gold salt solution was pale yellow and on stirring it using a magnetic stirrer, it turned violet at wavelength 420nm as measured using UV-vis spectroscopy. This was an indication of formation of SeNP's.

TRANSMISSION ELECTRON MICROSCOPE:

TEM analysis was employed for characterization of size and shape of nanoparticles. Ultra-small size (1-100nnm) and large surface area-to-mass-are major advantages of using SeNP's.(Solanke, Ajayi and Arigbede, 2014)Hence, TEM analysis results showed the shape of SeNP's to be spherical and the size observed was in the range of 4-45nm. (Figure 4)

FIGURE 4:



Left : UV-vis spectroscopy :SPR peak at 420nm ; Right:TEM analysis , 4-45nm spherical nanoparticle

ANTIBACTERIAL ACTIVITY OF MOUTHWASH:

The inhibition zones for AuNP's and mouthwash at various concentrations for each of these organisms - S. aureus, S. mutans, E. faecalis and C. Albicans are depicted in the table.SeNP's showed excellent antimicrobial activity against C. Albicans, S. mutans and S. aureus compared to E. faecalis which showed good antimicrobial activity with best activity at 100 µL concentration. Selenium nanoparticle-based mouthwash was efficient against againstS. aureus followed by good activity against, E. faecalis, C. Albicans and S. mutans with the peak activity at 150 µL. At concentrations of 25, 50, 100 µL too, good antimicrobial potential was elicited by SeNP's and Mouthwash. (Table 3)

CONCENTRATION OF MOUTHWASH	25 µL	50µL	100µL	CONTROL
ORGANISMS AND ZONE OF INHIBITION FORMED:				
C.albicans	9	13	15	9
S. aureus	20	20	30	33
S. mutans	12	25	30	20
E. Faecalis	9	11	15	30

Table 3: Antibacterial activity

DISCUSSION:

Selenium is known to have good antibacterial, antioxidant and biodegradable properties. Since selenium nanoparticles are highly unstable at zero oxidation state, Chitosan colloid is used as a stabilizing agent. In this study, a mouth rinse synthesised from selenium nanoparticles and Chitosan colloid was found to have effective antimicrobial properties.

The herbal selenium nanoparticle-based mouth rinse, apart from being found to have effective antimicrobial properties, is also alcohol free. Studies have indicated that alcohol based mouthwashes are contraindicated in patients who are at a risk of ulceration and mucositis.(Sreenivasan et al., 2004).Also (Winn et al., 1991) found a positive correlation between use of alcohol based mouth rinses and development of oral cancer. This further validates the necessity to prevent the use of alcohol based mouth rinses.

The study by (Rangrazi et al., 2020) used ascorbic acid for the production of chitosan-based selenium nanoparticles. They found that the chitosan based SeNPs had effective bactericidal effects against S. aureus and E. faecalis which are in concordance with the results obtained in our study.

According to the results of our study, the herbal selenium nanoparticle-based mouth rinse showed good antimicrobial activity against S. aureus uniformly at different concentrations. S.aureus is a major pathogen of the oral cavity that could results in a wide range of oral and systemic infections.(Tong et al., 2015).Similar results were found in the study by (Tran et al., 2016) wherein they found that selenium nanoparticles had a strong inhibitory effect against S.aureus.(Guisbierset al., 2016) also found a significant inhibitory effect of SeNP's onS.aureus.(Goy, Morais and Assis, 2016) also reported that Chitosan had a significant inhibitory effect against S.aureus which further validate the results of our study.(Tao, Qian and Xie, 2011) studied the effect of chitosan on the cell wall permeability of S.aureus and found that Chitosan had inhibitory effect on S.aureus by increasing the permeability of the cell membrane. All these results indicate that the antimicrobial activity of the herbal mouth rinse is due to the synergistic effect of Selenium nanoparticles and Chitosan colloid.

The herbal mouth rinse also showed significant antimicrobial activity against E.faecalis which is regarded as a major inhabitant of the gastrointestinal tract and is the main causative organism of hospital acquired infections.(Banlaet al., 2018) .Similarly,(Khiralla and El-Deeb, 2015) and (Perelshtein et al., 2013) also found that Selenium nanoparticles and chitosan had effective antimicrobial property against E.faecalis.

C. Albicans is a fungus which has the ability to form biofilms and morphogenetic conversions between yeast and hyphal morphologies contribute to biofilm development and represent an essential virulence factor.(Lara et al., 2015)This is especially of relevance in orthodontic patients undergoing fixed appliance therapy. In our study, the herbal mouth rinse was significantly effective against C. albicans at all concentrations. Similar results were found in a research done by Hwang et al (Hwang et al., 2012) where nano sized particles of silver possessed antifungal properties.

No literature exists regarding the antimicrobial effects of a mouth rinse consisting of green synthesised selenium nanoparticles. However, a study by (Abadi et al., 2013) used silver nanoparticles for formulating an alcohol-free mouth rinse and found that it had effective antimicrobial properties and can be used as an alternative to alcohol-based mouth rinses. Also since routine mouthwashes like chlorhexidine have certain drawbacks like discolouration of enamel, disturbances in taste and irritation of oral mucosa.(Atabeket al., 2012; Newman et al., 2012) ,a study by (Ahrari et al., 2015) assessed the activity of Zinc oxide (ZnO), titanium dioxide (TiO2) and copper oxide (CuO) along with silver nanoparticle containing solutions against Streptococcus mutans and Streptococcus sanguis and compared the results with those of chlorhexidine and sodium fluoride mouthwashes. The study concluded that The mouthwash containing nanoTiO2 is an effecient antimicrobial agent and be used as an alternative to sodium fluoride mouth washes or chlorhexidine . These results further reinforce the objective our study.

Furthermore, a study by (Archana et al., 2013) evaluated the impact of a new mouthwash containing chlorhexidine along with chitosan and found that chlorhexidine-chitosan combination mouth rinse is superior in antimicrobial activity than chlorhexidine or chitosan alone.

Therefore, in summary the herbal alcohol-free mouth rinse consisting of Selenium Nanoparticles and Chitosan colloid is an efficient antimicrobial agent and could be used as a possible adjunct to mechanical methods of plaque removal in orthodontic patients.

LIMITATIONS:

The antimicrobial activity against Lactobacillus, which is a common inhabitant of the oral cavity was not evaluated in this study. Also, Antimicrobial activity of the mouth rinse against various gram-negative microorganisms needs to be evaluated.

SCOPE FOR THE FUTURE:

The mouth rinse could be subjected to further evaluation of antimicrobial activity against gram negative organisms, Antioxidant property evaluation and evaluation of shelf life in future.

List of Abbreviations: NP's: Nanoparticles, SeNP's: Selenium nanoparticles, SPR: Surface Plasmon Resonance, TEM: Transmission Electron Microscope

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Conflict of Interest: None.

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