

# Preparation and Anti-Inflammatory Activity of Mouthwash Prepared Using Red Sandal Mediated Selenium Nanoparticles

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## ABSTRACT

**Introduction:** Application of herbal medicine has been advancing rapidly. Red sandalwood is one such plant with medicinal properties. The wood is used as an astringent and tonic for external application in inflammation. Metal nanoparticles have a special place in the area of nanotechnology as they offer a unique opportunity not only as theranostic agents but possess tremendous potential as carriers for chemotherapeutic agents, proteins etc. One application of nanotechnology in medicine currently being developed involves employing nanoparticles to deliver drugs, heat, light or other substances to specific types of cells.

**Aim:** The objective of this study is to assess the anti-inflammatory activity of mouthwash prepared from red sandalwood mediated selenium nanoparticles.

**Materials and methods:** The plant extract was first prepared and the selenium nanoparticles were added and mixed well. To prepare mouthwash, measured quantities of sucrose, sodium Benzoate, SLS, peppermint oil, distilled water and the prepared extract were added and mixed. To assess the anti-inflammatory activity albumin denaturation assay was performed.

**Results:** From the above study conducted it is evident that keeping diclofenac sodium as the control, and its absorbance value to be 0.172, the absorbance value of the prepared mouthwash from the extract was recorded in different concentrations. After reaching the concentration of 30, 40 and 50 microliters the prepared extract began to show considerably close absorbance values compared to the standard. At 50 microliters concentration the absorbance value of the mouthwash was found to be higher than that of the standard.

**Conclusion:** From the above study we can conclude that the mouthwash prepared from the extract can be used as a good anti-inflammatory agent. The limitations of the study are less sample numbers, comparison with single standards etc. Furthermore developments in this field may lead to production of herbal anti-inflammatory mouthwash.

**Key words:** Anti-inflammatory, *Pterocarpus santalinus*, Albumin denaturation, Percentage of inhibition

**HOW TO CITE THIS ARTICLE:** Obuli Ganesh Kishore S, Lakshminarayanan Arivarasu, Rajesh Kumar S, Preparation and Anti-Inflammatory Activity of Mouthwash Prepared Using Red Sandal Mediated Selenium Nanoparticles, J Res Med Dent Sci, 2022, 10 (11): 186-190.

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**Received:** 07-Sep-2022, Manuscript No. JRMDs-22-44842;

**Editor assigned:** 09-Sep-2022, Pre QC No. JRMDs-22-44842 (PQ);

**Reviewed:** 23-Sep-2022, QC No. JRMDs-22-44842;

**Revised:** 09-Nov-2022, Manuscript No. JRMDs-22-44842 (R);

**Published:** 24-Nov-2022

## INTRODUCTION

*Pterocarpus santalinus*, commonly known as red sanders belongs to the family Fabaceae. It is endangered and endemic to Andhra Pradesh. The wood is used as an astringent and tonic for external application in inflammation; it is also used in treating headache, skin diseases, fever, boils, scorpion sting and to improve sight. The wood and fruit is used in treating diaphoretics, bilious infections and chronic dysentery. The heart wood contains isoflavone glucosides and two anti-tumour lignans, viz. savinin and calocedrin. A triterpene is reported from the

callus of stem cuttings. Ethanol extract of stem bark was reported to possess anti-hyperglycaemic activity [1]. *In vitro* and *in vivo* studies showed that the heartwood and bark have exhibited antidiabetic, antimicrobial, anti-inflammatory, and hepatoprotective activities. In Ayurveda, an Indian system of traditional medicine, it is mentioned that the heartwood of the plant is used as external application for treating inflammation, diabetes, headache, skin diseases, and jaundice, and in wound-healing [2].

Metal nanoparticles of Ag, Au, Ce, Fe, Se, Si, Ti and Zn have a special place in the area of nanotechnology as they offer a unique opportunity not only as theranostic agents but possess tremendous potential as carriers for chemotherapeutic agents, proteins etc. [3]. Nanoparticles possess increased surface areas and therefore have increased interactions with biological targets (such as bacteria) compared with conventional, micron particles. In addition, nanoparticles are much more likely to enter cells

than micron particles. As a result, nano-antibacterial particles will likely exert stronger effects on bacteria than their micro-counterparts [4]. Recently, the biosynthesized SeNPs were used to prevent growth and biofilm formation by six foodborne pathogens including *Bacillus cereus*, *Enterococcus faecalis*, *S. aureus*, *Escherichia coli*, *Salmonella Typhimurium* and *Salmonella Enteritidis* [5]. Herbal extracts are primarily added to cosmetic preparations due to several properties such as antioxidant properties. Apart from that, these herbal extracts have been used as a topical anti-inflammatory agent also, as they block the inflammatory changes [6].

Preparation of mouthwash using *Pterocarpus santalinus* mediated selenium nanoparticles can help in avoiding extrusion of tooth due to periodontitis and gingivitis. Periodontitis and gingivitis are the most common inflammatory conditions of the periodontium, which on progression may lead to reduction in connective tissue support [7]. We know that the NSAIDs are the therapeutic agents in case of inflammation, but there can be side effects. We here biosynthesize metal nanoparticles using plant extracts for various applications as it is eco-friendly, cost effective and efficient [8]. Natural products with anti-inflammatory activity have been used for a long time as a folk remedy for inflammatory conditions such as fevers, pain, migraine, and arthritis. As the inflammatory basis of the disease becomes clearer, anti-inflammatory food and food products become of greater interest [9]. Our team has extensive knowledge and research experience that has translate into high quality publications [10-29]. The objective of this study is to assess the anti-inflammatory activity of the mouthwash prepared from red sandalwood mediated selenium nanoparticles.

## MATERIALS AND METHODS

**Preparation of plant extract:** Dried, crushed and powdered sample of red sandalwood was purchased readymade from the market which was a great advantage. This powdered plant extract was added to distilled water and was mixed well. To the prepared extract the selenium nanoparticles were added and were mixed well. The prepared extract was centrifuged and then it was filtered using a filter paper.

**Preparation of mouthwash:** To 600  $\mu\text{L}$  of the prepared extract, 0.3 g of sucrose was added and mixed well. It was diluted with 10  $\mu\text{L}$  of distilled water and 0.001 sodium benzoate was added and 0.01 g of SLS was added. For the flavour 100  $\mu\text{L}$  of peppermint oil was added. The prepared mouthwash was mixed well and was stored. The major setback was the measurement of minute quantities of liquids. Micro pipetting the solutions showed minor errors.

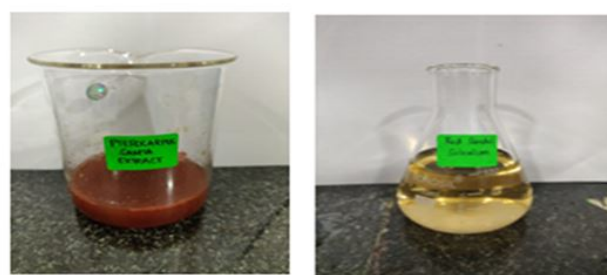
**Anti-inflammatory activity:** The anti-inflammatory activity for selenium nanoparticles was tested by following the albumin denaturation assay. 0.05 mL of Solanum torvum gel of various fixations (10  $\mu\text{L}$ , 20  $\mu\text{L}$ , 30  $\mu\text{L}$ , 40  $\mu\text{L}$ , 50  $\mu\text{L}$ ) was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the

mixture was set to 6.3 utilizing a minimal quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 minutes and then heated at 55°C in a water bath for 30 minutes. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac sodium was used as the standard. DMSO is utilized as a control. Percentage of protein denaturation was determined utilizing the following equation,

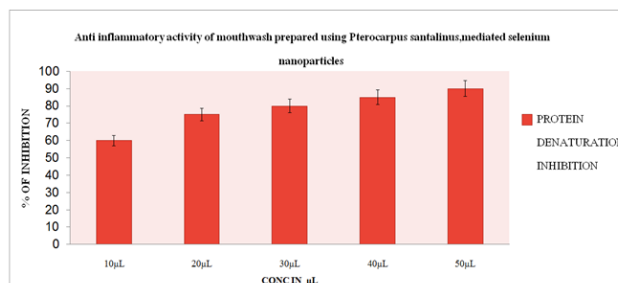
$$\frac{\text{The absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

## RESULTS

The results shows Figures 1 and 2.



**Figure 1:** Extract prepared by adding dried and powdered plant extract to distilled water. This extract was filtered and the selenium nanoparticles were added and filtered.



**Figure 2:** The graph represents the percentage of inhibition of protein denaturation calculated by the formula. The X axis represents various concentrations of the prepared mouthwash. The Y axis represents the percentage of inhibition of protein denaturation, data implies as meanSEM.

## DISCUSSION

From the results obtained, we may discuss that the mouthwash prepared from Red sandalwood mediated selenium nanoparticles is a good anti-inflammatory agent. Absorbance value can be defined as the amount of light absorbed by the particles in a solution. When the concentration of the solution is increased the absorbance value increases as there are more molecules for the light to hit. Here the absorbance value for the prepared mouthwash was measured spectrophotometrically at 660 nm wavelength. At 10  $\mu\text{L}$ , 20  $\mu\text{L}$ , 30, 40 and 50  $\mu\text{L}$

concentration the percentage of inhibition was found to be 60%, 75%, 80%, 85% and 90% respectively. From a similar study conducted, the zinc oxide nanoparticles showed absorbance values of 1.8, 2.2, 2.5, 2.6 and 2.9 at various concentrations in 660 nm wavelength. The formation and the stability of selenium nanoparticles in the prepared solution were assessed by spectrophotometric analysis. UV-V spectroscopy is the most renowned technique to confirm the absorbance of free radicals by the mouthwash subjected to inhibition of protein denaturation [7-9,30,31]. The highest absorbance value was recorded at 50  $\mu$ L concentration at 660 nm wavelength. Even though the value recorded was not significant, it was higher than that of the positive control. From a previous study conducted, the absorption spectrum of the incubated solution showed peaks at 660 nm wavelength which confirmed the formation of selenium nanoparticles. The selenium nanoparticles were spherical in shape and they measured about 5 to 30 nanometres.

The cells subjected to inflammation react by undergoing certain pathological manifestations characterised by heat, redness, pain, and swelling. Denaturation of proteins is a well-known cause of inflammation. Inflammation can be defined as the biological response of the body tissues to harmful stimuli. An anti-inflammatory agent is the substance which has the property to reduce any inflammation or swelling. Therefore we may understand that protein denaturation often leads to inflammation. Hence any anti-inflammatory agent that can inhibit the process of protein denaturation can be considered as a good anti-inflammatory medicine. Here albumin denaturation assay was conducted [32-45].

For the prepared mouthwash, the percentage of inhibition was calculated and is depicted in the form of a graph. The standard diclofenac sodium also was calculated to obtain the percentage of inhibition. At 10, 20, 30, 40 and 50  $\mu$ L concentrations, the percentage of inhibition was found to be 60, 75, 80, 85, and 90% respectively. The highest percentage of inhibition was recorded at 50  $\mu$ L concentration. This means that at 50  $\mu$ L concentration the denaturation of protein was inhibited by 90%, which proved the prepared mouthwash was a potent anti-inflammatory. The highest percentage of inhibition of the extract prepared showed a maximum value of 89% at 50  $\mu$ L concentration [46]. From a study conducted, we may understand that silver when compared with any anti-inflammatory agent can yield potent anti-inflammatory activity [47].

The heartwood of the red sandalwood tree is used as a medicine. It plays an important role in blood purification. India has imposed an export ban on red sandalwood to protect the species in the country. Red sandalwood helps manage edema due to its anti-inflammatory property. A study suggests that by applying a paste of red sandalwood, inflammatory mediators can be arrested. This reduces the build-up of fluids in the tissue. Sandalwood album oil is the most potent anti-inflammatory agent. Selenium acts as a powerful antioxidant. It boosts the immune system and may also

prevent heart diseases. Selenium possesses good anti-inflammatory properties, but the problem is its toxicity. Selenium has been proven to have a protective role in body tissue maintenance, oxidative stress, defence against infections and growth modulation.

The major limitation of this study was that specific concentrations were tested against the standard, and increased concentrations could have been more effective. As mentioned earlier, micro pipetting the solutions leads to minor errors. Various parts of the plants could have been examined for various activities. Further advancements in this study could lead to development of various medicines prepared from red sandalwood. Even if not for anti-inflammatory properties, this mouthwash can be tested for other properties such as antimicrobial, antifungal and so on.

### CONCLUSION

From the results obtained, analysed and discussed, we may conclude by saying that the mouthwash prepared from red sandalwood mediated selenium nanoparticles had good anti-inflammatory properties. Even though it didn't have the absorbance and percentage of inhibition values up to the level of the standard used, some more advancements and rectifications in this study might lead to preparation and marketing of mouthwashes made from natural products.

### ACKNOWLEDGMENT

The authors would like to thank Saveetha Dental College and Saveetha Institute of Medical and Technical Sciences for their kind support to utilize the facilities for the study.

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