Preparation and Cytotoxic Effect of *Pterocarpus santalinus* Selenium Based Mouthwash

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Introduction:** A mouthwash or rinse cannot replace the regular oral hygiene routine of twice-daily tooth brushing and daily flossing. A mouthwash or rinse freshen breath, and control the bad breath. A mouthwash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens. The medicinal plants have their application in pharmaceutical, cosmetic, agricultural and food industries. Medicinal plants act as a natural source for bioactive medicinal compounds. Selenium is a vita micronutrient that has excellent antimicrobial, anti cancerous, antidiabetic, and anti-inflammatory properties. The biological method of synthesis of Selenium nanoparticles has extensive application in the field of biomedicine due to low toxicity, targeted delivery of Nano drugs and stability.

**Materials and Methods:** *Pterocarpus santalinus* plant extract and mouthwash was prepared and cytotoxicity activity was performed.

**Results:** On day 1 all the 10 nauplii were alive in all the concentrations. On day 2 when the concentration was 5, 10, 20, 40, 80, control the number of live nauplii was 10, 8, 8, 8, 7, 10

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respectively. Results were tabulated and graphically analyzed in SPSS software. Correlation analysis was done using SPSS software.

**Conclusion:** Increased concentration of selenium nanoparticles was not toxic to living nauplii as it did not cause the death of it. But the *Pterocarpus santalinus* extraction based selenium nanoparticles have less to no cytotoxicity. This study shows that mouth rinses from medicinal plants as a source of an excellent alternative with less common side-effects to chemical-based oral antimicrobial products.

**Keywords:** *Pterocarpus santalinus*; cytotoxicity; mouthwash; selenium.

### 1. INTRODUCTION

A mouthwash or rinse cannot replace the regular oral hygiene routine of twice-daily tooth brushing and daily flossing. The main function of most mouthwashes is to freshen breath, and control the bad breath [1]. A mouthwash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens [2]. In ancient times the mouthwash was formulated with a mixture of decoct extracted from the olive tree leaves, milk, wine and oil, pomegranate peelings, nutgalls and vinegar. This paved way for mouthwash with traditional methods and herbs. Since then a variety of herbal remedies are available triphala, tulsi patra, jyeshtamadh, neem, clove oil, pudina, ajwain, white oak bark, horsetail herb, plantain leaf, alo vera, organic echinacea angustifolia root, myrrh gum, organic lobelia herb and seed, organic peppermint leaf, wildcrafted goldenseal root, clove essential oil, peppermint essential oil, tea tree essential oil [3].

The medicinal plants have their application in pharmaceutical, cosmetic, agricultural and food industries. The use of the medicinal herbs for curing disease has been marked in the past Saga of all civilizations [4]. Humans in the prehistoric era were not aware about the health hazards and their association with irrational therapy. With the commencement of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the disease [5]. Before the synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With the introduction of scientific procedures, medical practitioners were able to understand about toxic principles present in the green flora. Medicinal plants act as a natural source for bioactive medicinal compounds [6]. *Pterocarpus santalinus* (red sandalwood) is one of the medicinal plants used in traditional medicine, and is rich in flavonoids and phenols. Significant antidiabetic activity by reducing the elevated blood glucose levels and glycosylated hemoglobin, improving hyperlipidemia and restoring the insulin levels includes three new sesquiterpenes - namely isopterocarpolone, pterocarpiol and pterocarpdiolone [7].

Selenium is a vita micronutrient that has excellent antimicrobial, anti cancerous, antidiabetic, and anti-inflammatory properties [8,9]. Nevertheless, it is in the traditional form, it has a low degree of absorption and high levels of toxicity [10]. Nano-sized selenium has excellent biocompatibility with enhanced biological effects. The biological method of synthesis of Selenium nanoparticles has extensive application in the field of biomedicine due to low toxicity, targeted delivery of Nano drugs and stability [11]. Seaweeds or marine algae are permanent sources of chemical compounds which consist of a plethora of biologically active secondary metabolites [12]. They are considered as a potential source of antibiotic substances. Ulva lactuca is an edible green marine algae (Chlorophyta), which holds an antibacterial activity against oral pathogens. Ulvan is the sulfated polysaccharide of the algae Ulva Spp is declared to be responsible for its antibacterial activity and has no toxicity [13]. Traditional oral antimicrobial agents in the form of mouth rinses or dental varnish are chemical-based [14] These chemicals based mouthwashes cause many side effects. This emphasized the need for non-toxic natural products based mouth rinses which is also effective in reducing the bacterial load [15]. Our team has extensive knowledge and research experience that has translate into high quality [16-29]. The Present Study is aimed at the preparation and cytotoxic effect of *Pterocarpus santalinus* selenium based mouthwash.

### 2. METHODS AND MATERIALS

#### 2.1 Preparation of Plant Extract

Commercially available dry powder of *Pterocarpus santalinus* was used for this
experiment. This experiment was conducted in Saveetha Dental College, Chennai, Tamilnadu. This experiment was carried out by dissolving 1g of \textit{Pterocarpus santalinus} in 100ml of water. This moisture was then boiled in a heating mantle at 70 degrees celsius for up to 10 minutes. The boiled mixture was then filtered using Whatman number 1 filter paper to obtain the plant extract. Then 40ml of plant extract was measured using a measuring cylinder and the mixture was added to 60ml of 1mM selenium dissolved in 60 ml distilled water.

2.2 Preparation of the Mouthwash

To a eppendorf tube, 10 ml of distilled water was taken then 0.3g of sucrose was measured using an electrical weighing scale and was added to the tube containing the water, the mixture was mixed well and then 0.01g of sodium lauryl sulphate and 0.001 g of sodium benzoate were added and mixed well. Then 12 drops of the plant pellet were added to the above mixture followed by adding 2 drops of peppermint oil and was mixed well.

2.3 Cytotoxicity Activity - Brine Shrimp Lethality Assay

2.3.1 Salt water preparation

Iodine free salt 2g was weighed and dissolved in 200 ml of distilled water. Then 6 wells of the ELISA plates were taken and 10-12 ml of saline water was filled respectively. To that 10 nauplii were slowly added to each well (20, 40, 60, 80, 100).

Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for the number of live nauplii present and calculated.

![Fig. 1. 1g of \textit{Pterocarpus santalinus} powder weighed and taken](image1)

![Fig. 2. Prepared \textit{Pterocarpus santalinus} selenium nanoparticles based mouthwash](image2)
3. RESULTS

![Graph showing cytotoxic activity of Pterocarpus santalinus selenium nanoparticles](image)

Fig. 3. The bar graph represents the cytotoxic activity of the *Pterocarpus santalinus* selenium based mouthwash. X axis represents the concentration and Y axis represents the no. of live nauplii. The blue bar represents day 1 and the orange bar represents day 2.

The present study observed that in figure 3, on day 1 all the 10 nauplii were alive in all the concentrations. On day 2 when the concentration was 5, 10, 20, 40, 80, control the number of live nauplii was 10, 8, 8, 8, 7, 10 respectively.

4. DISCUSSION

It is eminent that the oral ailments are a global health apprehension with a substantial impact on the general public. Additionally, the amplified intake of sugars in the diet, consumption of tobacco, inadequate exposure to fluorides, and lack of approach to dental care, are the expected incidence of dental caries and periodontal disease which will continue to rise [30]. *S. mutans* is the foremost etiological agent of dental caries [31]. *E. faecalis* is an opportunistic pathogen, *S. aureus* and *C. albicans* are secluded from persistent apical periodontal lesions. Oral microflora is considered as a Vital factor in both caries and periodontal disease, and causes the ailment pathogenesis by producing the biofilms [32]. Therefore, the use of various types of mouth rinses are given a proper importance to proceed against the harmful microorganisms associated with oral diseases or infections [33]. Conversely, apprehensions regarding the development of antibiotic-resistant strains and hostile effects of contemporary mouth rinses have directed the attention in the use of non conventional or alternative medicines and plant extracts. Many previous reports have recommended the possible use of plant extracts in oral care for effective and efficient inhibition of microflora by natural antimicrobials (31,34)(10). Nevertheless, the potential benefits of chemotherapeutic formulations in mouth rinses provide thrust for research in verdict effective mouth rinses for oral care [35,36].

The previous study done by Mohanakumari [37], any antimicrobial agents are expected to have minimal cytotoxic effect on host cells. This previous study results demonstrated the cytotoxic effect of both CP and LS on cultured HGF [38]. Nevertheless, no significant differences were observed in the reduction of HGF cells by irradiated and non irradiated mouth rinses. Importantly, an earlier report on the effect of E-beam on sodium dodecyl sulfate demonstrated the reduction in toxicity of the surfactant [39]. The previous study demonstrated the similar result concluded as in the present study.

Microculture tetrazolium assays are sensitive, quantitative, and reliable methods to assess the cellular metabolic activity, where methyl thiazolyl tetrazolium obtains a dark purple colored
formazan through cellular mitochondrial dehydrogenase enzyme. A study done by Geethashri Anand [40], the percentage survival rate of HGF cells and V79 cells dealt with cashew and mango established a significant PI-based mouth rinses than the CHX-based. This indicates less toxicity and long time usage of active components of these plants as an alternative to commercial mouth rinses which is in accordance with the present study.

In a recent study, 1-h exposure to as low as 0.1% Persica solution produced irreversible cytotoxic effects on the cells engaged in the wound healing process [41]. While the presence of FCS (10%) offered a protection from drug toxicity, the diminution in the cytotoxic effect of Persica in the presence of FCS is due to the binding of potent toxic compounds of the mouthwash to serum proteins [42]. Our team has extensive knowledge and research experience that has translate into high quality publications [43–47],[48–53], [45,54,55],[56–60] [61,62] . It is pragmatic that the toxic compounds of Persica solution put forth their effects through irreversible binding to cellular proteins, thereby reassuring their function. The aim of the Present study is set out with the same result [63].

5. CONCLUSION

Increased concentration of selenium nanoparticles was not toxic to living nauplii as it did not cause the death of it.But the Pterocarpus santalinus extraction based selenium nanoparticles have less to no cytotoxicity [64-73].A Previous study done by Ryeeo-Woon Kim [74] discussed that the cytotoxicity assay conducted in this study demonstrated that the Dendropanax morbifera Léveille extracts retained high cell viability (> 60%) at all concentrations. The cytotoxicity test performed using human keratinocyte cells in a previous study revealed that the Dendropanax morbifera Léveille leaf extracts exhibited no cytotoxicity at concentrations lower than 50 µm/mL. This result was in concurrence to the result obtained in the present study. This study shows that mouth rinses from medicinal plants as a source of an excellent alternative with less common side-effects to chemical-based oral antimicrobial products.

NOTE

The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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