Original Research Article

Cytotoxic Effect of *Pterocarpus santalinus* and *stevia*-based mouthwash - A Lab-based analysis.

Running title: The lab based analysis of cytotoxic property of *Pterocarpus santalinus* and *stevia* based mouthwash.

ABSTRACT-

INTRODUCTION:
Red sandalwood called as *Pterocarpus santalinus* is esteemed for the rich red colour of its wood. *Pterocarpus santalinus* used as astringent, analgesic and anti-inflammatory agent. Also helpful in treating skin illness, fistula, haemorrhage and ulcers. *Stevia rebaudina* is a food additive with lingering flavor. The wood isn't aromatic. The tree isn't to be mistaken for the fragrant Santalum sandalwood trees that fill locally in South India. In the present study we have prepared *Pterocarpus santalinus* and *stevia*-based mouthwash and evaluated its cytotoxic activity.

MATERIALS AND METHODS:
1g of *Pterocarpus santalinus and stevia* were measured and 50 ml of distilled water were measured. Both were mixed together to make the aqueous extract. To that 10 nauplii were slowly added and the cytotoxic activity is analysed by the number of live nauplii counts.

RESULTS:
First day, Nauplii were grown in the medium and it hatches out after 24 hours. Second day, Mouthwash was added according to the concentration. Nauplii were collected and for each concentration 10 nauplii were added. After adding the nauplii, cytotoxicity well as undisturbed for one full day to analyze the inhibition of growth. Third day, nauplii were counted and cytotoxicity of mouthwash was evaluated. Statistical analysis showed significant reduction in the nauplii count($P<0.05$).

CONCLUSION:
Medicinal plants cure many severe diseases. Application of medicinal plants in the field of medicine should be improved. Based on the results recorded in the present study, it is concluded that *Pterocarpus santalinus* has a potential cytotoxicity activity. Hence the present study findings provide a beautiful base for some of the medicinal uses of *Pterocarpus santalinus.*

Keywords: *Pterocarpus santalinus*; *stevia*; Brine shrimp; Cytotoxicity; Innovative technique, Greensynthesis, Mouthwash.

INTRODUCTION-

Red sandalwood is derived from the leaves of *Pterocarpus santalinus*. *Pterocarpus santalinus* is a blackish-brown bark which resembles crocodile skin. This tree is esteemed for the rich red colour of its wood. The wood isn't aromatic [1]. The tree isn't to be mistaken for the fragrant Santalum sandalwood trees that fill locally in South India. Red sandalwood is used as an astringent and tonic, and is sweet, cooling, analgesic, anti-inflammatory, and febrifuge [2]. Its decoction is given in persistent dysentery. It is likewise helpful in vitiated states of pitta, consuming sensation, itching, skin illnesses, sickness, ulcers, fistula, and hemorrhages. *Stevia* is derived from the plant species *Stevia rebaudiana.* The status of *stevia* as a food additive or dietary supplement. The body doesn't utilize the glycosides in *stevia*, so it contains zero calories, similar to some counterfeit sugars. *Stevia's* taste has a slower beginning and longer span than that of sugar, and a portion of its concentrates may have a harsh or licorice-like lingering flavor at high fixations[3]. Nanoparticles and green synthetic powder are chemically and physically altered with a higher potency of reaction in the body [4].

Mouthwash is a fluid so it offers the advantage of arriving at regions that a toothbrush can't get to. Adding mouthwash to your brushing routine can clear debris and extricated plaque on your teeth that have been given up[5]. Washing with water would have similar advantages yet one of the advantages of mouthwash is that it refreshes your breath too and that is engaging a few people[6]. A restorative mouthwash can help severe gum diseases, for example, gum disease by decreasing the measure of plaque and microbes present in your mouth. The microscopic organisms from gum diseases can cause certain pregnancy complexities when it enters a women's circulation system and you can diminish this danger by washing with a mouthwash consistently[7-9]. A restorative mouthwash with fluoride can help diminish depressions and demineralization of your teeth when utilized consistently. There are contemplates that have shown that ordinary utilization of mouthwash could build circulatory strain since it takes out a portion of the helpful microscopic organisms found in the mouth [10]. Not all microorganisms are terrible microscopic organisms and mouthwash can wipe out the microbes answerable for delivering nitric oxide that helps in ensuring your cardiovascular system[11-13].

Preparation of *Pterocarpus santalinus* and *stevia*-based mouthwash have many benefits as they are used for analgesic, anti-inflammatory, and febrifuge. The leaf extract of S. rebaudiana is utilized to improve nourishments and is likewise utilized as a dietary enhancement [14]. The significant parts are glycosides, to be specific, stevioside and rebaudioside-A. These mixes show trademark organoleptic appropriate ties and have pleasant forces in excess of multiple times that of sucrose. Leaf extract of this plant has been utilized generally for the treatment of diabetes [15]. A characteristic, noncaloric sugar *stevia* has a lot of interest for use in oral hygiene items as it ends up being a strong antimicrobial without other results. However, there is less knowledge regarding the usage of *Pterocarpus santalinus* and *Stevia*. The probable reason may be lack of economic interest in finding a healthy substitute for sugar [16].

In this present investigation we have prepared the plant extract *Pterocarpus santalinus* and *stevia*. *Pterocarpus santalinus* and *stevia* mouthwash were prepared to evaluate the cytotoxicity. Our team has extensive knowledge and research experience that has translate into high quality publications [17,18-31, 32-36].

MATERIALS AND METHOD-

Preparation of plant extract-

Pterocarpus santalinus and stevia powder were commercially available which has been utilised in this procedure. 1g of *Pterocarpus santalinus and stevia* were measured and 50 ml of distilled water were measured (Figure:1). Both were mixed together to make the aqueous extract. The aqueous extract was kept in the shaker overnight. The aqueous extract was then boiled at 50°C (Figure:2). And then filtered using whattman filter paper. The filtered extract was then again kept in the shaker overnight. This procedure was done under the guidance of technicians. Sampling bias is avoided by Random sampling method were the samples include randomly distributed nanoparticles.

Preparation of mouthwash-

0.3G of sucrose, 0.001g of sodium benzoate, 0.01g of sodium lauryl sulphate, 100 µL peppermint oil, 10µL H2O, 600µL of nanoparticles were measured and mixed together to make the mouthwash. Micropipette cannot be added without the help of the practitioners (Figure:3). The validation of the procedure was done by principal investigators and experts in nanotechnology.

BRINE SHRIMP LETHALITY ASSAY:

Salt water preparation:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10µL,20µL,40µL,80µL and control). 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 number of live nauplii present and calculated by using following formula,

Number of dead nauplii/Number of dead nauplii + Number of live nauplii × 100

Figure:1 The figure represents the measurement of1g of Pterocarpus *santalinus* and 1g of *stevia.*

Figure:2 The figure represents the Preparation of aqueous solution. The aqueous solution was boiled at 50°C.

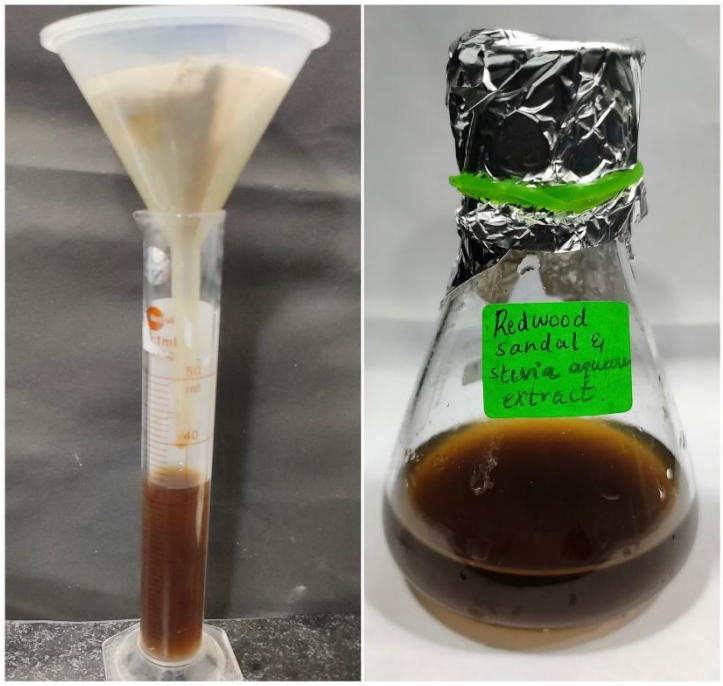

Figure:3 The figure represents the filtered aqueous solution.

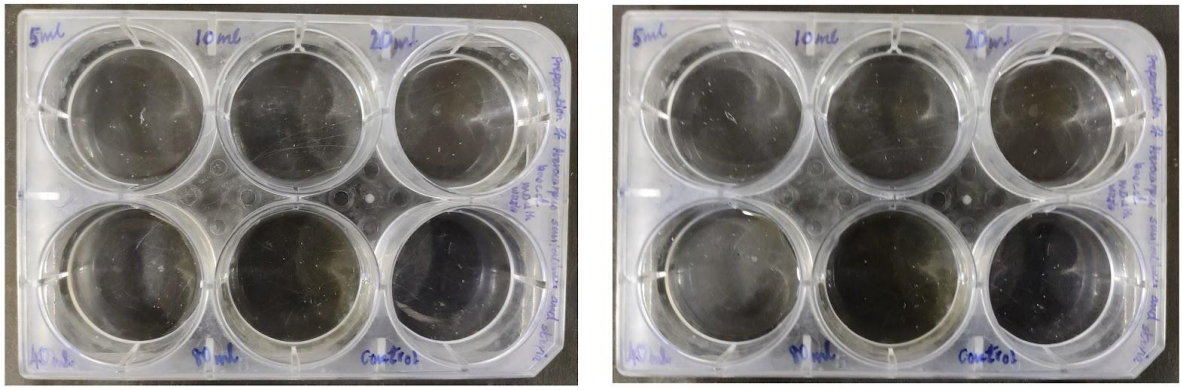

Figure: 4 Thefigure represents thenumber of nauplii present in cytotoxic wells.

This procedure was done for three consecutive days. First day, Nauplii were grown in the medium. Nauplii hatch out after 24 hours. Second day, Mouthwash was added according to the concentration. Nauplii were collected and for each concentration 10 nauplii were added. After adding the nauplii, keep the cytotoxicity well aside undisturbed for one full day to analyze the inhibition of growth. Third day, nauplii were counted and cytotoxicity of mouthwash was evaluated (Figure:4). The results were tabulated and analysed statistically by using IBM SPSS version 23. The Pearson correlation analysis is used as statistical analysis in this study. The spearman correlation analysis was performed to analyse *Pterocarpus santalinus's* cytotoxic activity using SPSS software version 23. and Non-parametric correlation was significant at p value less than 0.05.

RESULTS AND DISCUSSION:

At 5µL, there is no inhibition of growth of the nauplii. So, with little concentration of mouthwash, there is no effect on inhibition of growth. At 10µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 9 at the end of 24 hours. At 20µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 8 at the end of 24 hours. At 40µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 8 at the end of 24 hours. At 80µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 7 at the end of 24 hours. Thus, the growth of inhibition was high at 80µL concentration. Control was kept to avoid the confusion in the count of nauplii (Table:1).

CONCENTRATION	5µL	10µL	20µL	40µL	80µL	Control
DAY 1 LIVE NAUPLII	10	10	10	10	10	10
DAY 2 LIVE NAUPLII	10	9	8	8	7	10

Table:1 The above table shows the growth of inhibition of nauplii at different concentrations (µl) of Pterocarpus *santalinus* and *stevia*-based mouthwash.

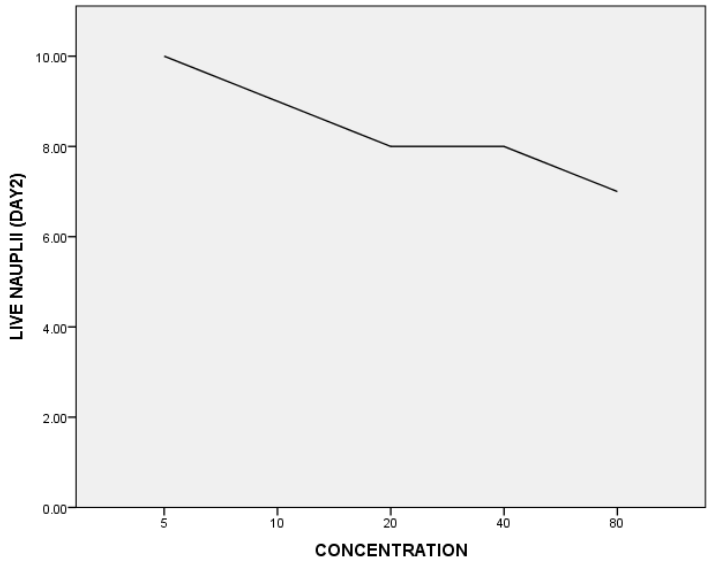

Figure: 5 The figure represents the decrease in number of live nauplii count with increase in concentration

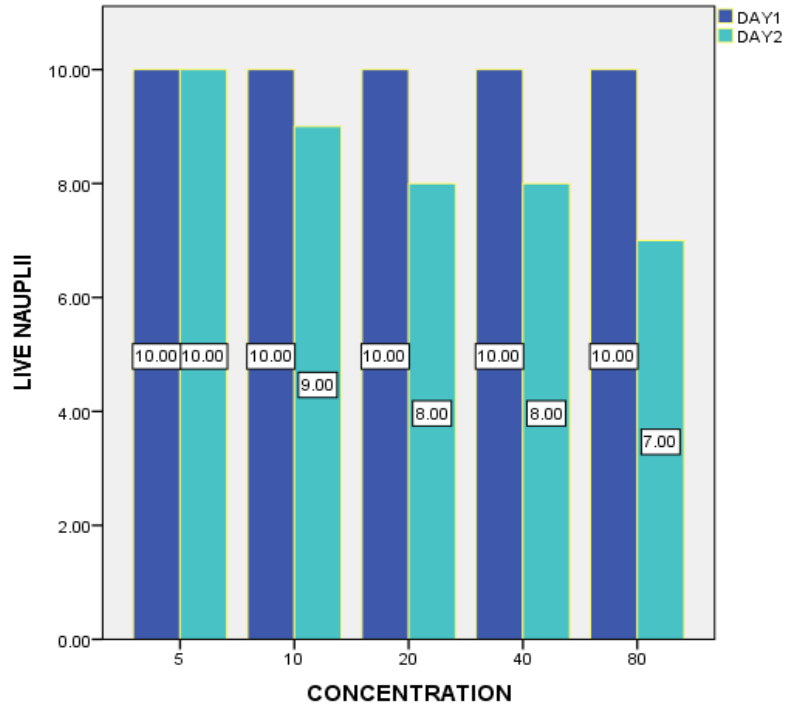

Figure: 6 The bar graph represents the negative spearman correlation (r=-1) of concentration and live nauplii count. Non parametric correlation was significant at p value less than 0.05.

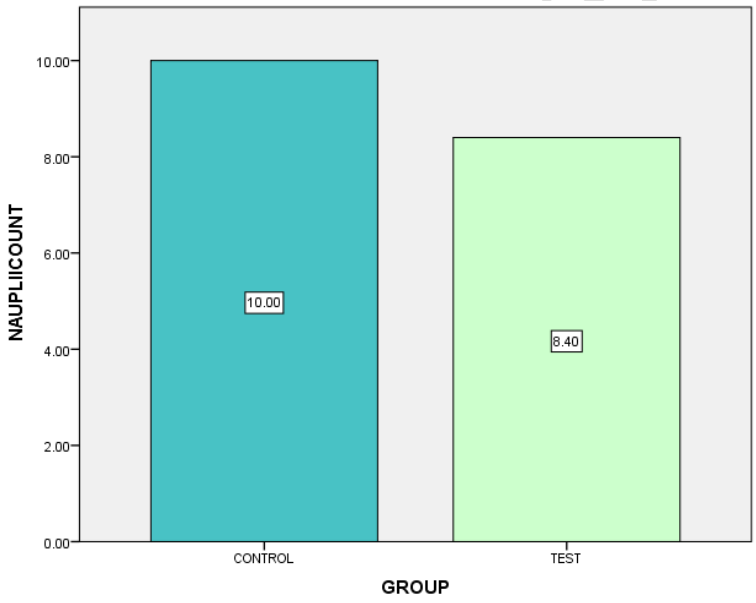

Figure: 7 The bar graph represents the correlation between the control and average of day 2 nauplii count.

A previous study showed that the cytotoxicity of the enzymatic mouthwash was discovered to be lower than that of the chlorhexidine mouthwash. Expanded oxidative pressure was noticed for the mouthwash. Subsequent to presenting the fibroblasts to the mouthwashes, a G2/M stage block was noticed and cell death occurred transcendently by necrosis [37]. The present study showed the same that *Pterocarpus santalinus* and *stevia*-based mouthwash has an

cytotoxic effect against infective organisms which was checked with a larvae species, Nauplii.

A similar study where the author concluded that the mouthwash from juca extract did not promote cytotoxic impact in human fibroblasts. This mouth rinsing plan introduced antimicrobial movement against microorganisms and fungi from the oral cavity[38]. In contrast, the study done by Cunha et al, concluded that the cytotoxicity test was performed on HaCat epithelial cells and evaluated by the 2,5-diphenyl-2H-tetrazolium bromide (MTT) technique. Tested solutions totally hindered the development of the both microorganisms in the adhesion stage. All solutions indicated inhibitory movement against 24 h-biofilm development. However, citronella oil led to more prominent microbial reduction[39]. Green synthetic powder from traditional medicinal plants are investigated against pathogens[40]. Similarly, the present study showed that *Pterocarpus santalinus* and *stevia*-based mouthwash has more cytotoxic effect in higher concentrations.

Cytotoxicity was determined by mitochondrial reductase action with essential gingival fibroblasts, L929 cells, and HSC-2 epithelial cells. Phase contrast microscopy and trypan blue staining were then performed to uncover cell morphology. Cells stayed fundamental after exposure to mouthwashes that were just utilized for cosmetic purposes. Moderate cytotoxic impacts were noticed for mouthwashes containing 0.05% chlorhexidine, ethanol, or pegylated hydrogenated castor oil and sodium dodecyl sulfate[41]. The aqueous seeds extract of Avocado can be utilized effectively for the synthesis of copper nanoparticles at room temperature. The incorporated copper nanoparticles were discovered to be stable at room temperature. The green synthesised technique is convenient, eco-friendly and can be applied in different applications and the utilization of Avocado has an added advantage of interest that the plant has numerous medicinal properties[42]. Ag-NPs blended utilizing herbal formulation of A. vera and A. indica have cytotoxic impacts[43]. The bio synthesization of AgNPs from AgNPs shows promising outcomes for biomedical applications. An absorption peak at 460 nm in UV-vis range demonstrated the development of AgNPs from amla organic product seed extract[44].

Zinc oxide nanoparticles reinforced with clove and cinnamon extract have a potential as a cytotoxicity, anti-inflammatory and anticancer agent and can be used as an alternative to commercially available products[45]. Garlic oil-mediated SeNP shows critical antimicrobial and cytotoxicity. From the previous study, it was concluded that garlic oil-intervened SeNP have a decent antimicrobial and cytotoxicity activity at high concentration[46]. Cytotoxicity was evaluated by testing on shrimp culture. Titanium mini implants when coated with silver nanoparticles have good antimicrobial properties and, thus can be utilized as a biomaterial in orthodontics but further tests are expected to assess the covering during and after arrangement[47]. There was a lower risk pace of SeNPs and significant anti-inflammatory activity which concluded that these nanoparticles can be utilized in different medication planning aspects in future[48]. Enterococcus-inferred AuNPs actuated apoptotic cell death in HT-29 cells and recommended that AuNPs could be utilized as a pro apoptotic agent for colon disease treatment[49]. Solanum trilobatum mediated by selenium nanoparticles indicated an expanded LD 50 in higher fixation on account of cytotoxicity activity. There was a critical impact of the plant extract mediated selenium nanoparticle when contrasted and the standard ascorbic acid in the antioxidant activity. Subsequently, Solanum trilobatum mediated selenium nanoparticles has good cytotoxicity activity[50]. The selenium nanoparticles extracted from Capparis decidua don't have any cytotoxic effect on shrimps. The SeNPs possessed significant antioxidant activity with more concentration. These SeNPs are naturally useful and can be utilized as eco-friendly, cost effective and productive biomedical agents and therapeutics[51].

The corrosive assists the body with engrossing chromium. Randomized controlled preliminaries have neglected to exhibit a connection between chromium supplementation and the counteraction or treatment of type 2 diabetes or debilitated glucose resilience. Chromium supplementation of young men and women doesn't advance muscle gradual addition, fat misfortune, or gains in strength. Physically active people with concern about gathering rules for supplement admission ought to be directed to choose and burn-through food sources with high supplement densities instead of to depend on wholesome enhancements. Chromium picolinate interceded zinc oxide nanoparticles show great outcomes in antimicrobial action just as in cytotoxicity. Chromium picolinate intervened Zn nanoparticles is an effective antibacterial and a potential cytotoxicity agent[52].

Different concentrations of Hyaluronic corrosive intervened zinc nanoparticles are consolidated to the wells. After 24 hrs the outcomes were analysed. Hyaluronic mediated zinc nanoparticles is end up being powerful against a wide scope of foodborne and clinically applicable Gram-positive and Gram-negative bacteria utilizing a few tests, for example, circle dispersion, agar or stock dilution. Hyaluronic corrosive intervened Zinc nanoparticles has high strong cytotoxic potential it had been demonstrated with the assistance of brackish water shrimps. From the noticed outcomes, it has been inferred that Hyaluronic corrosive has a ton of restorative qualities and it has antimicrobial movement and it has great cytotoxic potential[53]. In a previous study, the cytotoxic potential of microbial mediated silver nanoparticles in a cancer cell line was analysed. It was found that Inhibition of PCNA protein expression in AuNPs induces antiproliferative effects[54].

This study was done as an in vitro representation of cytotoxic effects of *Pterocarpus santalinus* and *stevia*-based mouthwash against a cellular organism. This study did not define the cytotoxic effect against the host cells or pathogenic cells properly. This may be considered as the limitations of this study and in future the cell line oriented in vitro study can be conducted to know the cytotoxicity of *Pterocarpus santalinus* and *stevia* against a particular cell which can be cancerous or infective pathogens.

CONCLUSION-

Nowadays, using medicinal plants cure many severe diseases. Application of medicinal plants in the field of medicine should be improved. Based on the results recorded in the present study, it is concluded that *Pterocarpus santalinus* has a potential cytotoxicity activity. Hence the present study findings provide a beautiful base for some of the medicinal uses of *Pterocarpus santalinus*.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

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COMPETING INTERESTS

Authors have declared that no competing interest exist

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