Anti-cariogenic & Cytotoxic Activity Of Red Sandal (*Pterocarpus santalinus*) Ethanolic Extract

Abstract:

Introduction:
Pterocarpus santalinus, with the common names red sanders, red saunders, red sandalwood, Rakt Chandan, and saunderswood, is a species of Pterocarpus endemic to the southern Eastern Ghats mountain range of South India. This tree is valued for the rich red colour of its wood.The use of ethanolic extract is a cost-effective way to produce a quality extract from a large volume of plants.Pterocarpus santalinus has a characteristic anti-cariogenic and cytotoxic activity.This study aimed to find the Anti cariogenic and cytotoxic activity of *Pterocarpus santalinus* (Red sandal) ethanolic extract preparation.

Materials and Methods:
The anticariogenic activity was done by using agar well diffusion technique and the cytotoxic activity was done by Brine Shrimp Lethality Assay.Pterocarpus santalinus were purchased commercially from an herbal health centre,in Chennai. The obtained powder Pterocarpus santalinus stored in an airtight container. 5 gram of powder is mixed with 50 ml of ethanol and kept in the orbital shaker for 72 hours , after it has boiled in a heating mantle at 62- 70 degree c for 5-10 min. The extract is filtered using whatman filter paper 1.The filter extract again contracted using heating mantle.

Results:
The extract shows very good anticariogenic and cytotoxic activity of *P.santalinus* ethanolic extract by using Brine Shrimp Lethality Assay.

Conclusion:
The Anti-cariogenic and cytotoxic activity of Pterocarpus santalinus ethanolic extract preparation was effective.

Keywords:
Pterocarpus santalinus,ethanolic extract, Anti-cariogenic,Cytotoxic activity

Introduction

Pterocarpus santalinus also known as ‘red sanders’ or ‘red sandalwood’ is a species of Pterocarpus endemic to the southern Eastern Ghats mountain range of South India.It is locally known as ‘Rakta Chandan .Pterocarpus santalinus is a small-to-medium–sized deciduous tree belonging to the Fabaceae family. This tree is valued for the rich red colour of its wood. Red sandalwood is used for treating digestive tract problems, fluid retention, and coughs; and for “blood purification.” In manufacturing, red sandalwood is used as a flavoring in alcoholic beverages.Bioactive compounds present in the plant's heartwood have been shown to have a wide range of biological activities, suggesting the potential of pterocarpus santalinus for the treatment of various diseases.It Contains many other compounds that have medicinal properties.Red sanders has a characteristic anti-cariogenic and cytotoxic activity.Ethanol Extractions is a process used in fine liquor distillation. It is done by soaking raw cannabis in ethanol to pull out a solvent and the cannabis is then removed. The ethanol extraction process is used to filter out alcohol content from extracted material.

Anti cariogenic producing or promoting the development of tooth decay cariogenic foods. Cariogenic bacteria including mutans streptococci and lactobacilli are partly but significantly involved in dental caries development. An effective prevention strategy against dental caries is to decrease the accumulation of this microbiota either in planktonic or in biofilm form. Medicinal plants have been investigated for possible anti-cancer effects to examine the cytotoxic activity of several medicinal plants on different tumor cell lines. Studies on cytotoxicity of Brine shrimp lethality assay utilizing is a more comprehensive and effective test more common to evaluate cytotoxicity of bioactive compounds. Our team has extensive knowledge and research experience that has translate into high quality publications(Veerasamy *et al.*, 2021)(Rajeshkumar *et al.*, 2018)(Vairavel, Devaraj and Shanmugam, 2020)(M. Gomathi *et al.*, 2020)(Rajasekaran *et al.*, 2020)(Santhoshkumar *et al.*, 2019)(R *et al.*, 2020)(Saravanan *et al.*, 2018)(Gheena and Ezhilarasan, 2019)(Ezhilarasan, Sokal and Najimi, 2018)(Ezhilarasan, 2018)(A. C. Gomathi *et al.*, 2020)(Dua *et al.*, 2019)(Ramesh *et al.*, 2018)(Arumugam, George and Jayaseelan, 2021)(Joseph and Prasanth, 2021)(Ezhilarasan, Apoorva and Ashok Vardhan, 2019)(Duraisamy *et al.*, 2019)(Gnanavel, Roopan and Rajeshkumar, 2019)(Markov *et al.*, 2021) The present study aimed to biosynthesize the Anti-cariogenic and cytotoxic activity of red sandal ethanolic extract.

Materials and methods

Collection and preparation of plants

Pterocarpus santalinus were purchased commercially from an herbal health centre,in Chennai. The obtained powder Pterocarpus santalinus stored in an airtight container. 5 gram of powder is mixed with 50 ml of ethanol and kept in the orbital shaker for 72 hours , after it has boiled in a

heating mantle at 62- 70°C for 5-10 min. The extract is filtered using whatman filter paper 1.The filter extract again contracted using heating mantle.

Plant collection and Extraction:

Leaves of Pterocarpus santalinus were collected from Saveetha dental college during July-september 2021. The species was identified and authenticated by a Taxonomist and voucher specimens were deposited. Shade dried and coarsely powdered leaves of Pterocarpus Santalinus (1g) were sequentially extracted with methanol at room temperature for 48 hrs. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator to get completely dried extracts (PSMExt). The yield of the leaf crude extract was about 80 g.

Anticariogenic activity

The anticariogenic activity was done by using agar well diffusion technique. 10µL of fresh microbial cultures such as *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Candida albicans* were inoculated in sterile Hi-Veg broth medium and incubated for 18 hours in an orbital shaker at 120-150rpm. Mueller Hinton agar was prepared (For *Candida albicans* Rose Bengal Agar was used). The antimicrobial activity was done to analyse the efficacy of *Pterocarpus santalinus* Ethanolic extract at different concentrations against oral pathogens. The oral pathogens were swabbed on the surface of each sterile MHA plate (For *Candida albicans* RBA plates) . A gel puncher was used to cut four wells to each plates. The first three wells were loaded with three different concentrations (25µL, 50µL,100 µL) of plant extracts. A standard antibiotic (Amoxyrite) was loaded in the fourth well. The plates were incubated at 37ºC for 24 hours (*Candida albicans*- 48 hours of incubation). After the incubation period, the plates were observed and measured for zones of inhibition around each well.

Cytotoxic activity – BSLA

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). 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.

RESULTS

Figure 1:Preparation of ethanolic extract *Pterocarpus santalinus*.

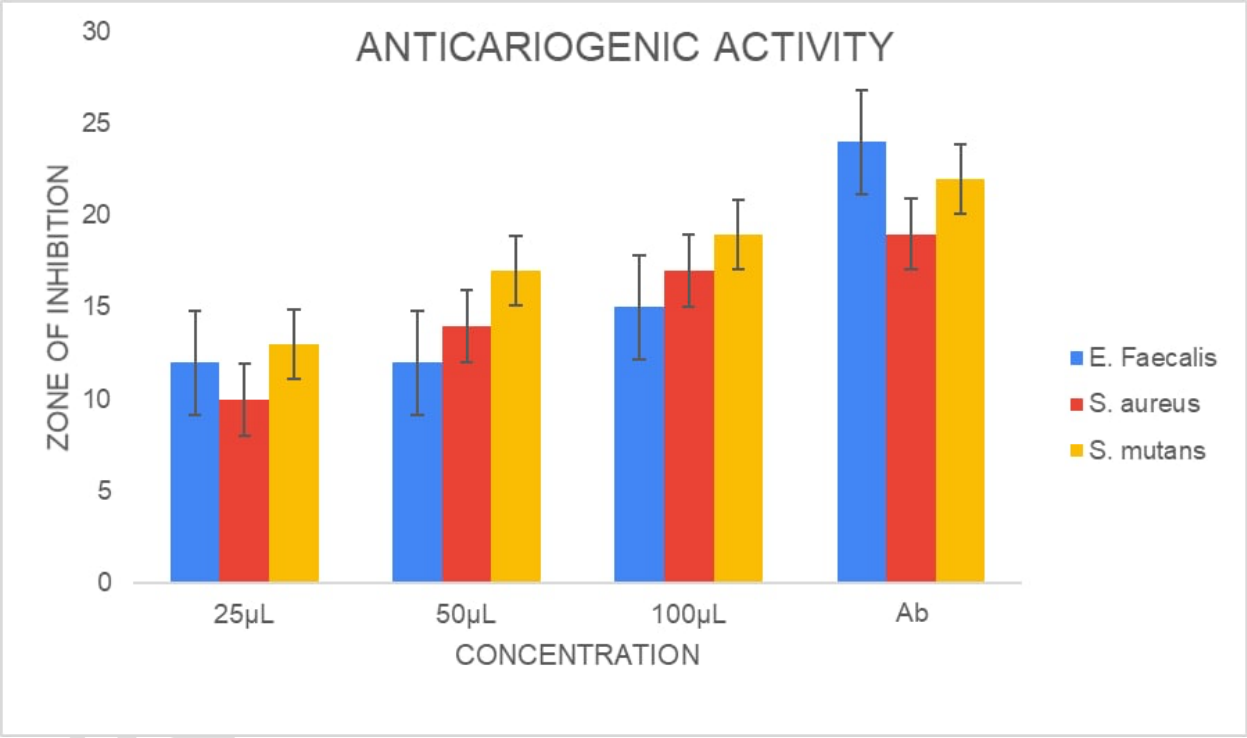

Figure3: The above figure depicts the anticariogenic activity with an increased zone of inhibition with a concentration in microlitres. X axis denotes concentration and the Y axis denotes the zone of inhibition in mm of *pterocarpus santalinus*.

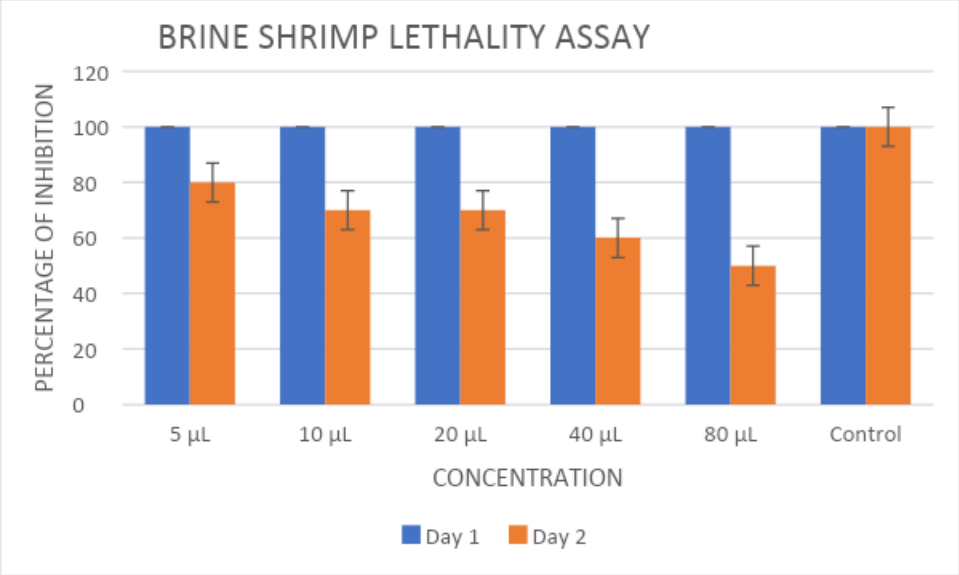

Figure 4 : The above graph depicts the cytotoxic activity of Pterocarpus santalinus ethanolic extract on Brine shrimp Lethality assay increased the percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Pterocarpus santalinus.*

The results of anticariogenic activity and cytotoxic activity were depicted in (Figures 1-2). In the present study, the total anticariogenic activity of Pterocarpus Santalinus ethanolic extract was determined using the Agar well diffusion technique and Brine shrimp lethality of cytotoxic activity in a concentration dependent manner. The result indicated that the PSE Ext significantly (<0.05) inhibited Amoxyrite.Brine shrimp lethality assay is an easy, rapid and sensitive method for the cytotoxic activity of the ethanolic extracts. The present study investigated the anticariogenic and cytotoxic activity of PSE Ext, and expressed the inhibition of Brine shrimp lethality assay using BSLA as standard reference.

Discussion

Pterocarpus santalinus ethanolic extract showed a significant Anti cariogenic and cytotoxic activity by BSLA.The anticariogenic activity of the Pterocarpus santalinus ethanolic extract has been taken. The values were compared to the standard Amoxyrite sodium. It was observed at (25µL,50µL,100µL,Ab) concentration.Anticariogenic activity against caries-causing microbes (S. mutans, E.faecalis, S. aureus, and) at different concentrations and zone of inhibition was measured in millimetre.Cytotoxic activity of Pterocarpus santalinus ethanolic extract on Brine shrimp Lethality assay increased the percentage of inhibition with a concentration in microlitres.It was observed at(5µL,10µL,20µL,40µL,80µL,Control)concentrations.

As compared to this study, it showed effective antimicrobial activity against cariogenic pathogens. The SeNPs synthesized with Brassica oleracea extract can be incorporated in toothpastes, gums, and mouthwashes that are cost-effective and also biocompatible and effective for the prevention of dental caries.(Dhanraj and Rajeshkumar, 2021).As compared to this study,it shows that anticariogenic activity of crude ethanol extracted from *Piper cubeba* seeds, the purified compounds cubebin and its semi-synthetic derivatives were evaluated against oral pathogens.(Ferrazzano *et al.*, 2011).As we compared to this study,it shows that antibacterial activity and cytotoxic activity of medicinal leaf extracts of *Solanum torvum* are a potential source of anti-TB natural products(Nguta *et al.*, 2016).As we compared to previous studies,Cinnamon and sweet basil essential oils with impressive in vitro anti-cariogenic bacteria effects may be proposed as alternative and effective supplements to promote oral health status(Wiwattanarattanabut, Choonharuangdej and Srithavaj, 2017)

CONCLUSION:

Based on our observations, it was confirmed that *Pterocarpus santalinus* (Red sandal) showed strong Anti-cariogenic activity and cytotoxic activity of ethanolic extract.This research is needed to identify biological activity of this medicinal plant. Furthermore, studies are aimed to identify bioactive molecules from the ethanolic extract of *Pterocarpus santalinus.*

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