Original Article

Evaluation of antimicrobial properties of *Solanum xanthocarpum* and *Pistacia lentiscus* extracts on *Streptococcus mutans*, *Lactobacillus* species and *Actinomyces viscosus*: An *in vitro* study

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Abstract

Introduction: The Ayurvedic system of medicine is popular in India and has gone through various change and remains as the mainstay of medical relief to a large population. Dental caries is the one of the major issue in the recent time, and there is no definite caries prevention method so far apart from Topical fluoride application, regular oral hygiene measures, pit and fissure sealants and diet measure. In spite of the above mentioned preventive measures the people are still prone to dental caries. In order to find a better solution the present study evaluates the antimicrobial properties widely available and cost effective herbal extracts of Solanum xanthocarpum and Pistacia lentiscus extract on cariogenic oral microbial flora.

Aim: To Evaluate the Antimicrobial properties of Solanum xanthocarpum and Pistacia lentiscus extracts on cariogenic oral microbial flora.

Methodology: The antimicrobial properties of Solanum xanthocarpum and Pistacia lentiscus extracts on cariogenic oral microbial flora (streptococcus mutans, lactobacillus, actinomyces viscosus) by different culture techniques to estimate the zone of inhibition (well diffusion method) and minimum inhibitory concentration (tube dilution method).

Results: The antimicrobial efficacy of Solanum xanthocarpum and Pistacia lentiscus on test bacteria were analysed using kurskal wallis test and values of 0.003 and 0.002 were obtained respectively, since the p values were less than 0.005, indicating that both herbal products possess statistically significant antimicrobial properties.

Conclusion: The antimicrobial effects of the herbal extracts were almost on par with commercially available allopathic agents like chlorhexidine on oral cariogenic microbes. The efficacy should be further validated in a large scale studies and can be utilized for caries prevention in the form of mouth rinses, dentifrices and topical application in the future.

Keywords: Antimicrobial, evaluation, *Lactobacillus* species and *Actinomyces viscosus*, *Pistacia lentiscus*, *Solanum xanthocarpum*, *Streptococcus mutans*

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INTRODUCTION

The Ayurvedic system of medicine predominantly the natural extracts is popular in India since the historic period of human civilization. Although Ayurveda has gone through many changes in the course of its lengthy history, it still remains the mainstay of medical relief to a large section of population of the nation. Due to industrialization and deforestation, Ayurveda is no longer self-contained unit gathering and preparing his own medicines as before.[1]

"Dental caries is an irreversible microbial disease of the calcified tissue of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitations." The word tooth decay was originated from the Latin word denotes "rot" or "decay." Tooth decay is a multifactorial dynamic and complex process, wherein many factors such as acid producing bacteria, fermentable carbohydrate and host factor like saliva plays a role. Even though various microorganisms thought to play an major role in the initiation along with progression of tooth decay among which *Streptococcus mutans*, *Lactobacillus* species and *Actinomyces viscosus* play a vital role. Diagnosing dental caries is the key in treating the condition. Various diagnostic procedures have been developed for precise diagnosis of the condition. Among all the methods, radiographic method seems to easy and cost-effective in diagnosing dental caries, and based on the level of dental caries, treatment planning can be done.[2]

There is no definite caries prevention method so far apart from topical fluoride application, regular oral hygiene measures, pit and fissure sealants and diet measure. In spite of the above mentioned preventive measures the people are still prone to dental caries. Dental caries vaccination is still under trail. Most of the modern preventive measures are based on allopathic medicine which includes fluoride based mouth washes, fluoride tablets and water fluoridation. Few formulations are also available based on chlorhexidine and other mouthrinses which are also based on allopathic medicine. Prolonged usage of the above mentioned formulations may cause side effects such as staining of teeth, oral allergic reaction, suppression of normal oral microbial flora and overgrowth of harmful bacteria leading to few oral infections.

To find out better solution for prevention of the dental caries, various researches are going on utilizing naturally available products like herbal extract. Most of the studies related to the antimicrobial (anticariogenic) properties of the herbal extracts are going in the alternative medicine side when compared with the allopathic medicinal research. Earlier studies in alternate and allopathic medicine proved that many of the herbal extract have antioxidant/anticancer properties and are being utilized in the cancer treatment. Many researchers in the past utilized saliva to test the antimicrobial efficacy of the herbal extracts.

Solanum xanthocarpum (SX) is a perennial low diffused sharply spine scent or under shrub found everywhere and has various medicinal properties including that of antimicrobial property, antifilarial, anti-inflammatory activity, hepatoprotective activity, hypoglycemic activity, apoptosis-inducing activity and hypolipidemia activity. This substance has numerous curative property also but not much of studies have been done, similarly *Pistacia lentiscus (PL)* commonly called as mastic tree is a shrub or small tree of pistacia genus mainly cultivated for its aromatic resin. It is one of the sources for naturally occurring antimicrobial agent that is being extensively used in medicine in recent years in the form of mastic oil and mastic gum, both has antimicrobial properties.

The present study evaluates the antimicrobial properties of *S. xanthocarpum* and *P. lentiscus* extracts on cariogenic oral microbial flora by different culture techniques and also estimates the minimum inhibitory concentration for the same. Moreover, it also emphasizes on the efficacy of antimicrobial activity utilizing widely available and cost-effective herbal extracts so that they may be utilized for the prevention of dental caries in the future.

METHODOLOGY

Study material

Powdered form of the Herbal products *S. xanthocarpum* (fruit) and *P. lentiscus* (mastic gum) were procured from the herbal supplier and was stored in −20°C temperature. Bacterial strains of *S. mutans*, *Lactobacillus* species and *A. viscosus* and material required for the culture media were procured from HiMedia Laboratory (Mumbai). Selective medium for individual strains were prepared as per instruction given in manual.

Study was done during June 2016 and June 2017 in the SRM Dental College Chennai after getting approval from our university institutional review board.

Selective culturing of microbes

Nutritive medium (Enrichment media) used for the selective isolation of *S. mutans* and *A. viscosus*, and for isolation of *Lactobacillus*, the "De Man, Rogosa and Sharpe Agar" were utilized. All the above mentioned culture media were prepared by Suspending 90.07 g of appropriate reagents as mentioned in the manual "in 1000 ml distilled water" and Boiling to dissolve the reagent, Sterilized in autoclave at 15 lbs pressure

for 15 min, 1 ml sterile 1% "Potassium Tellurite" was added to the cooled reagent. This mixture is poured into petri dishes.

The strains of *S. mutans*, *Lactobacillus* species and *A. viscosus* were inoculated separately into the corresponding culture medium at a temperature of 37°C along with suitable environmental conditions required for each bacteria. The cultural characteristics of the growth were observed and recorded between 18 and 48 h [Figures 1-3].

Preparation of herbal extracts

Neat (standard concentration preparation)

Five grams each of the herbal extracts (*S. xanthocarpum* and *P. Lentiscus*) were taken in two separate beaker containing 50 ml of the distilled water and were heated till boiling cooled and filtered to obtain standard concentration (Neat). 0.1 ml each of standard concentration were taken separately and inoculated into corresponding spread plates meant (*S. mutans*, *Lactobacillus* species, *A. viscosus*) to find the zone of inhibition (2 herbal extracts × 3 bacteria).

Determine minimum inhibitory concentration

Well diffusion method

0.9 ml of sterile distilled water was taken in four test tubes, 0.1 ml of the test sample (herbal extract-standard concentration) is added to get 10^{-2} and this is serially diluted to get the 0.9 ml of 10^{-3}, 10^{-4} and 10^{-5} of concentrations of herbal extracts. Thus, four diluted (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) concentration was prepared.

Specific media plates were prepared, and standard two McFarland of inoculums of each test organisms was spread onto the corresponding media plates to achieve the growth of the organism. The agar plates were dried and wells were cut (8 mm in diameter). One hundred microliters herbal extracts were added into the wells of the specific media plates for each of the test organisms and allowed for diffusion and incubated at 37°C for 24 h (overnight incubation) in the respective conditions and the antimicrobial efficacy indicated by the zone of inhibition around the well containing the extracts were measured [Figures 4a-c and 5a-c].

The experiments were done separately for each of the herbal extracts and all the experiments were repeated five times (pentatuple).

Statistical analysis

The following Statistical analysis were done using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA): Since the data did not follow the normal distribution, the median values of each test were utilized for statistical analysis. And, Chi-square test, Mann–Whitney test and Kruskal–Wallis test were done.

RESULTS

Since the $P < 0.005$, indicating that there is a significant difference in antimicrobial efficacy between *S. xanthocarpum* and positive control on test group of bacteria [Table 1].

Since the $P < 0.005$, indicating that there is a significant difference in antimicrobial efficacy between *P. lentiscus* and positive control on test group of bacteria [Table 2].

Since the data did not follow the normal distribution, nonparametric Mann–Whitney test was done to test whether there are any significant differences between the antimicrobial efficacies of the two herbal extracts (*S. xanthocarpum* and *P. lentiscus*) on test bacteria.

Since the $P > 0.005$, indicating that there is a no significant difference in antimicrobial efficacy between *S. xanthocarpum* and *P. lentiscus* on test group of bacteria [Table 3].

Overall antimicrobial efficacy of *S. xanthocarpum* and *P. lentiscus* on test bacteria was analyzed using Kruskal–Wallis test, and values of 0.003 and 0.002 were obtained, respectively, since $P < 0.005$, indicating that both herbal products possess statistically significant antimicrobial properties [Table 4].

Table 1: Comparison of efficacy of solanum xanthocarpum with positive control on test bacteria

Bacterial Strains	Solanum xanthocarpum	Positive control	*P*	Significance
Streptococcus mutans	30	32	0.003	Significant
Lactobacillus	26	28	0.003	Significant
Actinomycosis viscosus	24	26	0.003	Significant

Table 2: Comparison of the efficacy of pistacia lentiscus with positive control on test bacteria

Bacterial Strains	Pistacia lentiscus	Positive control	*P*	Significance
Streptococcus mutans	31	32	0.002	Significant
Lactobacillus	26	28	0.002	Significant
Actinomycosis viscosus	22	26	0.002	Significant

Table 3: Comparison of the efficacy of solanum xanthocarpum and pistacia lentiscus on test bacteria

Bacterial strains	Solanum xanthocarpum	Pistacia lentiscus	*P*	Significance
Streptococcus mutans	30	31	0.89	Not significant
Lactobacillus	26	26	0.27	Not significant
Actinomycosis viscosus	24	22	0.28	Not significant

Table 4: Overall antimicrobial efficacy of solanum xanthocarpum and pistacia lentiscus on test bacteria

Herbal extracts	*P*	Significance
Solanum xanthocarpum	0.003	Significant
Pistacia lentiscus	0.002	Significant

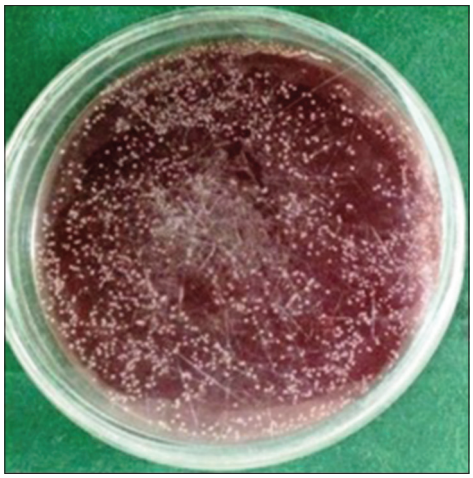

Figure 1: *Actinomyces viscosus* colonies

Figure 2: *Streptococcus mutans* colonies

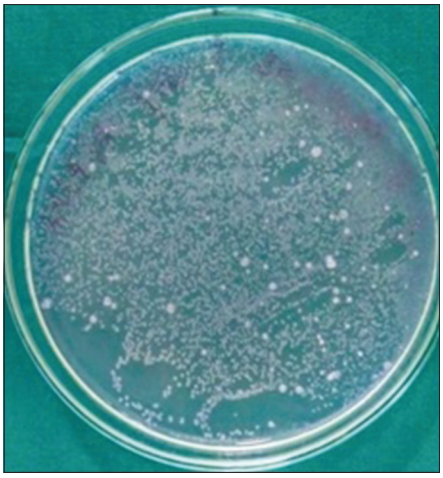

Figure 3: *Lactobacillus* colonies

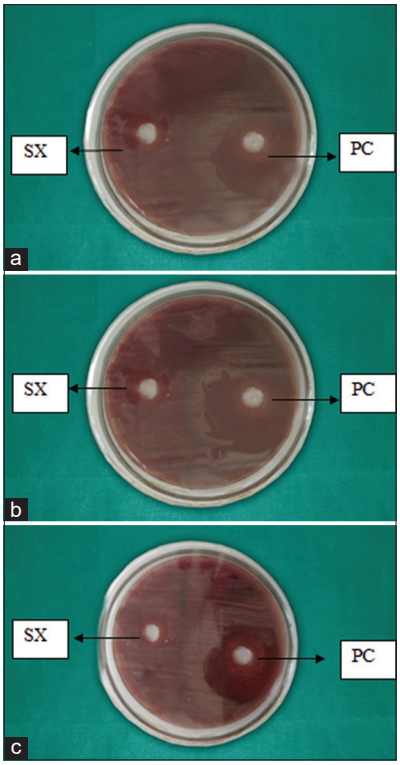

Figure 4: Zone of inhibition produced by solanum xanthocarpum (SX) and positive control (PC) on different bacteria. (a) *Streptococcus mutans,* (b) *Lactobacillus* species, (c) *Actinomyces viscosus*

DISCUSSION

"Natural scaffolds contain the key to bacterial vulnerability." This statement shows the important role played by natural products (plant sources) in the inhibiting the occurrence of microbial disease.

The present study aimed to find the antimicrobial properties of *S. xanthocarpum* and *P. lentiscus* extracts on cariogenic oral microbial flora. The herbal extracts (*S. xanthocarpum* and *P. lentiscus*) in varying dilutions and positive control (chlorhexidine) were incorporated separately in the culture medias each containing *S. mutans*, *Lactobacillus* species and *A. viscosus* separately. The efficacy of herbal extract was evaluated by measuring zone of inhibition and minimum inhibitory concentration.

The two test herbal extracts (*S. xanthocarpum* and *P. lentiscus*) and positive control (chlorhexidine) exhibited antimicrobial activity against dental caries causing bacterial strains (*S. mutans*, *Lactobacillus* species and *A. viscosus*).

All the tests in our study with the standard extract (neat) alone produced a zone of inhibition whereas further dilution of the herbal extract did not produce any zone of inhibition, except in one test where *S. xanthocarpum* produced zone of inhibition on *Lactobacillus* colony in the next corresponding dilution (10^{-2}) in addition to neat concentration. Our study is in accordance with Fahime

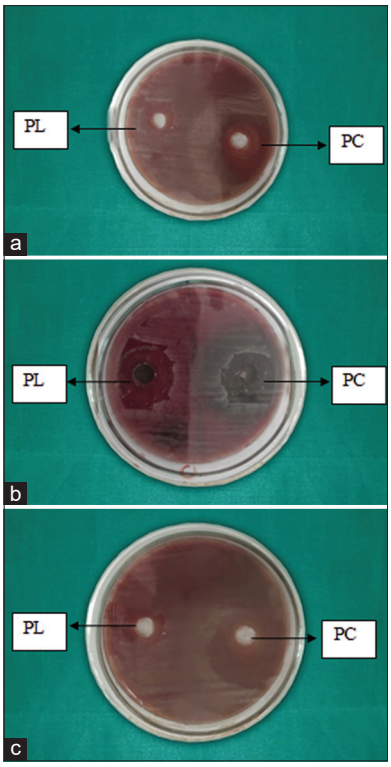

Figure 5: Zone of inhibition produced by Pistacia Lentiscus (PL) and Positive Control (PC) on different bacteria. (a) *Streptococcus mutans*, (b) *Lactobacillus* species, (c) *Actinomyces viscosus*

Kooshki *et al.* (2018) they showed an antimicrobial efficacy of the herbal products on *S. mutans* and *Lactobacillus* species decreases when compared with the chemical substance (control) when the herbal extracts were further diluted.[3]

S. xanthocarpum exhibited antimicrobial activity against *S. mutans* with a zone of inhibition of 30 mm, whereas the positive control exhibited a value of 32 mm. Although the antimicrobial activity of positive control was marginally higher than the *S. xanthocarpum*, the difference was statistically significant. Our study is also in accordance with the study conducted by Saxena *et al.* (2015) the test herbal extract showed lesser zone of inhibition than the positive control against the test bacterial strains. The difference was found to be statistically significant.[4] The reason said was that the larger the zone of inhibition produced by the positive control may be attributed to its high diffusibility and volatile nature in the agar media. Zone of inhibition also depends on various factors like solubility and diffusibility in the agar media, loading dose, method of herbal extraction and volatile nature of the samples. Our study was also accordance with the study conducted by Abbas *et al.* (2014) obtain a zone of inhibition of 12.7 mm on Gram-positive bacteria by *S. xanthocarpum* and compared with the standard drugs (ampicillin) as control and found that even though the zone of inhibition of *S. xanthocarpum* was lesser than the control still the extract possess substantial antimicrobial efficacy.[5]

P. lentiscus exhibited antimicrobial activity against *S. mutans* with a zone of inhibition of 31 mm, whereas the positive control exhibits a value of 32 mm. Although the antimicrobial activity of positive control was marginally higher than the *P. lentiscus*, the difference was statistically significant.

Our study was nearly in accordance with the research conducted by Ali Roozegar *et al.* where they showed a zone of inhibition of 25 mm at the highest concentration of (*P. lentiscus*) which was higher than the zone of inhibition produced by the positive control (amoxicillin) but the difference was not statistically significant. Though in the above mentioned study *P. lentiscus* showed a higher zone of inhibition (25 mm) than the positive control, the zone of inhibition was much lesser than the values obtained in our study (31 mm).[6]

P. lentiscus exhibited antimicrobial activity against *Lactobacillus* species with a zone of inhibition of 26 mm whereas the positive control exhibits a value of 28 mm. Although the antimicrobial activity of positive control was marginally higher than the *P. lentiscus*, the difference was statistically significant.

P. lentiscus exhibited antimicrobial activity against *A. viscosus* with a zone of inhibition of 22 mm, whereas the positive control exhibits a value of 26 mm. Although the antimicrobial activity of positive control was marginally higher than the *P. lentiscus*, the difference was statistically significant. Our study showed that *P. lentiscus* was effective in inhibiting the *A. viscosus* evidenced by a zone of inhibition of 22 mm. Extensive search of literature showed very minimal study done utilizing *P. lentiscus* extract on oral microbial flora and most of them are against *S. mutans* species.

According to studies conducted by Paraschos *et al.* (2007), Aksoy *et al.* (2007), Kajaria *et al.* (2012), Abbas *et al.* (2013), Pol *et al.* (2016), Ali Roozegar *et al.* (2016), and Koychev *et al.* (2017), *S. xanthocarpum* and *P. lentiscus* showed effective antimicrobial properties against oral microbial flora including cariogenic and periodontal pathogens, which was reflected in our study that both the herbal extracts effectively inhibited the *A. viscosus*.[5-11]

As said by the above authors both *S. xanthocarpum* and *P. lentiscus* has got an effective antimicrobial property.

So far, to the best of our knowledge no other studies were done by utilizing the combination similar to our studies (antimicrobial efficacy of *S. xanthocarpum* and *P. lentiscus* on cariogenic microbial flora-*S. mutans*, *Lactobacillus* species and *A. viscosus*).

So many studies were done to prove the antimicrobial efficacy of various herbal combinations on different groups of microbes. Among this a study done Pol *et al.* utilized *S. xanthocarpum* to find its antimicrobial efficacy on *S. mutans* and *Lactobacillus* species and found that the *S. xanthocarpum* is equally effective against both the bacteria.[10]

Overall the herbal extracts (*S. xanthocarpum* and *P. lentiscus*) showed a statistically significant antimicrobial efficacy against all the three bacterial strains (*S. mutans*, *Lactobacillus* species and *A. viscosus*).

Since the outcome of the present study proved that the test herbal extracts (*S. xanthocarpum* and *P lentiscus*) were found to be very effective in inhibiting the cariogenic microbial flora (*S. mutans*, *Lactobacillus* species and *A. viscosus*), the above herbal extracts will be helpful in prevention of dental caries in the future when utilized appropriately.

CONCLUSION

Nowadays herbal medicines are gaining popularity due to their cost effective, ease of availability and effectiveness towards wide variety of microbial infections and eco-friendly nature. In the present study the two herbal extracts (*S. xanthocarpum* and *P. lentiscus*) was proved to be effective in inhibiting the cariogenic microbial flora and the efficacy was found to be statistically significant. The effects of the herbal extracts were almost on par with commercially available allopathic agents like chlorhexidine on oral cariogenic microbes. Though the results were promising, still further research are needed by subjecting these herbal products to further elaborate *in vitro* studies with different strains of cariogenic flora, *in vivo* studies and clinical trials by this their efficacy can be further validated and can be utilized for caries prevention in the form of mouth rinses, dentifrices and topical application in the future.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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