Available online at https://www.mmpubl.com/

International Journal of Community Dentistry

Original Article

Comparative evaluation of anti-bacterial effect of *Caesalpinia sappan* aqueous extract and 0.12% Chlorhexidine against *Streptococcus mutans* – An in vitro study

Madhivathani S M [1] Umesh K [2] Sangeeta Chavan [3] Palanivel Pandian R [4] Prem Kumar P [5]

1 Post Graduate student, Department of Public Health Dentistry, Best Dental Science College and Hospital, Madurai, Tamil Nadu*

2 Professor and Head, Department of Public Health Dentistry, RVS Dental College and Hospital, Coimbatore, Tamil Nadu

3 Professor and Head, Department of Public Health Dentistry, Best Dental Science College and Hospital, Madurai, Tamil Nadu

4 Reader, Department of Public Health Dentistry, Best Dental Science College and Hospital, Madurai, Tamil Nadu

5 Senior Lecturer, Department of Public Health Dentistry, Best Dental Science College and Hospital, Madurai, Tamil Nadu

How to cite: Madhivathani S M et al. Comparative evaluation of anti-bacterial effect of Caesalpinia sappan aqueous extract and 0.12% Chlorhexidine against Streptococcus mutans– An in vitro study. Int J Comm Dent 2023; 11(2): 61-68. DOI: https://doi.org/10.56501/intjcommunitydent.v11i2.867

Received: 20/06/2023 *Accepted: 26/07/2023* *Web Published: 29/07/2023*

Abstract

Introduction : To evaluate and compare the antimicrobial activity of various concentration of *C. sappan* wood aqueous extract (100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, 500mg/ml and 600mg/ml) and 0.12% chlorhexidine mouthwash using agar well diffusion method against *St. mutans*.

Materials and Methods : A comparative experimental invitro study was conducted to evaluate and compare antimicrobial activity of various concentration of *C. sappan* aqueous extract and 0.12% Chlorhexidine. The study enclosed extract of 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, 500mg/ml, 600mg/ml and 0.12% Chlorhexidine as control. The antimicrobial activity was assessed by agar well diffusion method, were 50µl of each concentration was poured in the prepared agar well and assessed for zone of inhibition.

Results : The antimicrobial potential of test compounds was estimated by measuring the zone of inhibition and all the procedure are done in triplicates and values are assessed using One way ANOVA and Post Hoc analysis. All the concentration used showed zone of inhibition against *St. mutans*. At the concentration, 600mg/ml of aqueous *C. sappan* extract have a larger zone of inhibition (22.41±.32). In agar well diffusion method, at the minimal concentration of 600mg/ml and *C. sappan* aqueous extract was comparatively better than positive control group (0.12%cholorhexidine).

Conclusion : *C. sappan* wood aqueous extract could be preferred for routine oral hygiene practice where it can provide maximal antimicrobial effect without side effects of routine aid.

Key words : Pathimugam, Antibacterial activity, C. sappan wood, Caries prevention

Address for Correspondence:
Madhivathani S M,
Post Graduate student, Department of Public Health Dentistry, Best Dental Science College and Hospital, Madurai, Tamil Nadu.
Email-Id: **madhikuzhali01@gmail.com**

INTRODUCTION

A person's overall health and wellbeing commence with their oral health. The promotion of oral health and the prevention of oral diseases, which have a significant impact on general health, are essential components of any health promotion programme (1). Despite a noticeable increase in oral health status in prosperous nations, the prevalence of oral diseases continues to rise in developing countries. In many underdeveloped nations, dental caries and periodontal disorders are serious public health issues (2). Dental caries and periodontal disease treatment is not highly expensive as negligence. However, the cost of neglect is extremely severe given their well-established link to systemic health (3). Promoting locally developed preventive strategies that are convenient, affordable and effective is precisely needed.

One of the most significant causes of dental caries and gingivitis is oral bacteria. Oral pathogenic strains, notably Streptococcus spp., are the most devious bacteria. Although this genus contains bacteria that are part of the natural flora in the oral cavity, some species, including *Streptococcus mutans* and *Streptococcus intermedius*, are harmful. The most common cariogenic bacteria that cause dental caries are *S. mutans*. They could additionally release acid that dissolves the enamel on teeth (4,5). Studies have shown that removing these harmful microorganisms and the microbial dental plaque biofilm can prevent tooth cavities and gingivitis (6). However, even with routine daily cleanliness, it is difficult to entirely eradicate these oral bacteria from the oral cavity.

Antibacterial agents tend to be the most effective instruments for this prevention, but continued use of these substances has caused the rise of organisms that are multidrug resistant. Natural products with positive effects on health have received a lot of attention nowadays. Numerous reports on novel plant-based medicinal products exist. Different active chemicals that are produced by plants can effectively combat bacterial diseases. There have been numerous reports on the antibacterial activity of putative plant extracts against oral infections. Additionally, reports of the beneficial interactions of secondary plant metabolites with antibiotics or specific bioactive substances exist (7).

The use of antimicrobial mouthwashes in conjunction with mechanical plaque management techniques has been advised. The antiplaque agent that is most frequently utilized is chlorhexidine gluconate. However, prolonged use has been linked to altered taste perception, tooth discoloration, and the emergence of microbial strains that are resistant to it (8). This necessitates the development of some novel tactics to combat the bacteria responsible for dental caries and periodontal disorders. Exploring the enormous variety of therapeutic plants that are abundantly available in the natural world could serve as one such technique.

Caesalpinia sappan is a member of the Leguminosae family. It is known as Pathimugam in Tamil, Sappan wood in English, Sappanga in Kannada, Patunga in Hindi, Chappangam in Malayalam, and Pathang in Marathi. The natural red pigment, Brazilin is the chief active compound present in pathimugam heartwood. Numerous foods, beverage, and cosmetic items have exploited the heartwood of C. sappan as a colouring ingredient. It is significant to note that *C. sappan* has been used traditionally for long periods to treat a variety of infectious disorders. This plant contains a few phenolic compounds, and researchers have discovered some of its pharmacological properties, including antioxidant (9) and anti-inflammatory (10) effects. Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Salmonella faecalis, Staphylococcus aureus, Salmonella ebony, Enterobacter aerogenes, Bacillus subtilis, and Pseudomonas aerogenosa were all reported to be susceptible to the antimicrobial activity of *C. sappan* extracts (11). Additionally, the extract exhibited antifungal efficacy against Candida albicans and Aspergillus niger.

However, up to our best knowledge, there is no scientific evaluation of *C. sappan* aqueous extracts on inhibition of oral pathogenic bacteria *S. mutans*. This study was designed to investigate the antimicrobial activity of *C. sappan* aqueous extract on *Streptococcus mutans*.

AIM

The aim of in vitro study is to compare and evaluate the antimicrobial activity of *C. sappan* wood aqueous extract in various concentration with Chlorhexidine mouthwash (0.12%).

OBJECTIVES

-To assess the antimicrobial activity of 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, 500mg/ml and 600mg/ml of *C. sappan* wood aqueous extract and chlorhexidine mouthwash (0.12%) using agar well diffusion method

-To compare the antimicrobial activity of *C. sappan* wood aqueous extract various concentration with Chlorhexidine mouthwash (0.12%) by measuring the zone of inhibition.

MATERIALS AND METHODS

An invitro experimental study was conducted to compare and evaluate the antimicrobial activity of *C. sappan* wood aqueous extract in varying concentration and 0.12% chlorhexidine using agar well diffusion method against St. mutans. The strains were obtained from American Type Culture Collection (ATCC), Chandigarh, India. The study specimen (C. sappan wood) was prepared and tested against organism in Department of Biotechnology, Madurai Kamarajar University, Madurai, Tamilnadu, India.

Aqueous extract preparation from *C. sappan* wood:

The C. sappan wood approximately 200 gms was sourced from the local market in Madurai. *C.sappan* wood was made to fine chops. The *C. sappan* wood samples were authenticated by an expert botanist and specimens were preserved at room temperature. The *C. sappan* wood chops weigh 10gms was washed and extracted four times with 50ml of water (50x 4=200ml). The plant samples are soaked were soaked in 50ml of water and subjected to shaking (160 RPM, 37ºC, 6hr) and the supernatant solution was separated, and the residue is re-extracted in same way three more times. The aqueous extract was lyophilized to dry powder. The dried extract was weighed and a varied concentration of (100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, 500mg/ml,600mg/ml) solution was prepared by adding distilled water. Then, the extract was filtered using Whatman paper (12).

Microorganism and Growth condition:

Lyophilized Streptococcus mutans (ATCC 25175-0266P) were purchased, rehydrated in brain heart infusion (BHI) broth, and incubated at 37ºC in an aerobic environment for 48 hours. The turbidity standard was used to compare the inoculation broth, and the density of the test suspension was adjusted to be equivalent to 0.5 tube McFarland.

Preparation of medium:

The antibacterial activity was assessed using the Agar well diffusion method. 20ml of MH agar medium for bacteria was poured into petri dishes. Bacteria from broth culture were injected on the surface of the media. A sterile cotton swab was used to streak the inoculums on the plate surface after the inoculated broth was transferred to Mueller-Hinton agar plates. The inoculation plates were allowed to dry at room temperature for a few minutes (13).

Anti – microbial susceptability testing of *C. sappan* aqueous extract against *S. mutans*:

The inoculated plates wells were cut off and *C. sappan* aqueous extract, 50µl of varied concentration (100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, 500mg/ml and 600mg/ml) were added to agar wells using Micro pipettes. Chlorhexidine 0.12% was used as positive control. The plates were incubated at 37ºC for 24 hrs and observed for zones for inhibition, diameter of inhibition zone around each well was measured in millimeter. Each assay was repeated in triplicate, and then the mean inhibition zone was calculated for each set.

Measurement of zone of inhibition:

Zone of inhibition is an area of media where bacteria are unable to grow, due to the presence of a drug that obstructs their growth. Fuzzy zones are avoided, and it is rounded off to nearest mm. It was recorded by measuring the distance between the center of well disc to periphery of the inhibitory zone. The zone of inhibition of tested microorganism by samples were measured using a millimeter scale.

Statistical analysis

The data were calculated using graph pad prism 6.0 software (USA). For statistical analysis data were performed using IBM Corporation, SPSS version 26.0 (Statistical package for social sciences). One way ANOVA and Post Hoc (Tukey) analysis was performed.

RESULTS

The present study was conducted with an attempt to evaluate and compare the antimicrobial effect of *C.sappan* wood aqueous extract at varying concentrations and Chlorhexidine(0.12%) against *St.mutans*. The *C.sappan* wood bark of the plant was consumed and aqueous extract was prepared in varying concentration as 600,500, 400,300,200,and 100mg/ml and 0.12% of Chlorhexidine was used as positive control and all the procedure are done in triplicates and values are assessed.

The antimicrobial potential of test compounds was estimated by measuring the zone of inhibition illustrated in (Table 1). All the concentration used showed zone of inhibition against *St.mutans*. At the concentration, 600mg/ml of aqueous *C. sappan* extract have a larger zone of inhibition (22.41±.32). In agar well diffusion method, at the concentration of 600mg/ml and *C. Sappan* aqueous extract was comparatively better than positive control group(0.12% cholorhexidine).

Table 1 –Intergroup comparisons on zone of inhibition obtained by *C. sappan* aqueous extract at varying concentration against *St. mutans*

Test solution	Maximum Zone of inhibition (mm)						
	N	Mean	Std.Deviation	Std.error	95% C. I		P-value
					Lower bound	Upper bound	
100mg/ml	3	11.10	1.22	.70	8.04	14.15	.000
200mg/ml	3	13.43	.208	.12	12.91	13.95	
300mg/ml	3	14.23	.251	.14	13.60	14.85	
400mg/ml	3	15.10	.173	.10	14.66	15.53	
500mg/ml	3	15.43	.152	.08	15.05	15.81	
600mg/ml	3	22.41	.329	.19	21.59	23.22	
0.12% chlorhexidine	3	22.10	.529	.30	20.78	23.41	

*p value is significant at 0.05.

Table 2- Pairwise comparison on zone of inhibition obtained by *C. sappan* aqueous extract at varying concentration against *St. mutans*

Groups		Mean Difference	Std.Error	p-value	95% Confidence Level	
					Lower bound	Upper bound
100mg/ml	200mg/ml	-2.33*	.442	.002	-3.84	-.821
	300mg/ml	-3.13*	.442	.000	-4.64	-1.62
	400mg/ml	-4.00*	.442	.000	-5.51	-2.48
	500mg/ml	-4.33*	.442	.000	-5.84	-2.82
	600mg/ml	-11.31*	.442	.000	-12.82	-9.79
	Control	-11.00*	.442	.000	-12.51	-9.48
200mg/ml	300mg/ml	-.80	.442	.564	-2.31	.71
	400mg/ml	-1.66*	.442	.027	-3.17	-.15
	500mg/ml	-2.00*	.442	.007	-3.51	-.48
	600mg/ml	-8.97*	.442	.000	-10.48	-7.46
	Control	-8.66*	.442	.000	-10.17	-7.15
300mg/ml	400mg/ml	-.86	.442	.479	-2.37	.64
	500mg/ml	-1.20	.442	.166	-2.71	.31
	600mg/ml	-8.17*	.442	.000	-9.68	-6.66
	Control	-7.86*	.442	.000	-9.37	-6.35
400mg/ml	500mg/ml	-.33	.442	.986	-1.84	1.17
	600mg/ml	-7.31*	.442	.000	-8.82	-5.79
	Control	-7.00*	.442	.000	-8.51	-5.48
500mg/ml	600mg/ml	-6.97*	.442	.000	-8.48	-5.45
	Control	-6.66*	.442	.000	-8.17	-5.15
600mg/ml	Control	.31	.442	.990	-1.20	1.82

*The mean difference is significant at 0.05 level.

DISCUSSION

All around the world, herbs are experiencing a "revitalization" and making a comeback. The substances made from herbs today support for protection. In contrast to natural products, synthetics are thought to be harmful to both people and the environment. Herbal extracts have been utilized in dentistry as anti-oxidant substances, antimicrobial agents, antifungals, antivirals, analgesics, and antiplaque therapeutics to reduce inflammation (7).

The present study was an innovative attempt assessing the antimicrobial activity of *C.Sappan* wood aqueous extract in varying concentration against *St.mutans* in vitro. In Southeast Asia, the medicinal plant *Caesalpinia sappan*, often called Brazil or sappan wood, is commonly known as pathimugam(Tamil) has several benefits. Thai traditional medicine uses red dye, which is frequently derived from plant heartwood, to cure conditions like anaemia, diarrhoea, dysentery, skin infections, and tuberculosis. The main constituents of sappan wood were examined, and several phenolic components, including xanthone, brazilin, brazilein, chalcones, coumarin, flavones, and homoisoflavonoids were identified. Brazilin and brazilein are bioactive substances that are employed as dyes and have been linked to a number of beneficial effects, including anti-inflammatory, antioxidant, anti-photoaging, anti-allergic, anti-inflammatory, antibacterial, and vasorelaxant qualities (14). In India, especially in Kerala, it is common preparation to drink water in which these herbs are prepared, rather than plain water. Very often the two are not considered different and are both referred to as “water” colloquially (15).

One of the most pervasive public health issues is dental disease. Dental caries issues are costly and labor-intensive to address, and they affect people of all ages. (WHO Global Oral Health Status Report) (16). Dental

caries, a common oral disease, is caused by many cariogenic bacteria, including Lactobacillus spp., Streptococcus spp., and Actinomyces spp., which frequently form plaque biofilms on tooth surfaces. The S. mutans GTase first creates dental plaque, and subsequently oral bacteria establish colonies and aggregate in this water-insoluble glucan layer. The synthesis of water-insoluble glucans by S. mutans is an important factor in the development of dental plaque. Controlling or even lowering the levels of the key oral pathogens responsible for dental caries and periodontal disease, such as S. mutans, is an important step in the prevention and treatment of these infectious diseases (17). Mueller M and collaborators (2016) evaluated the anti-inflammatory properties of sappanol, brazilin, (iso-)protosappanin B, protosappanin C, and episappanol isolated from C.Sappan Extract and Brazil had the best anti-inflammatory effectiveness (10).

In the present study the *C. sappan* wood aqueous extract in varying concentration are tested against St.mutans by Agar well diffusion method. The research material was infused into the medium and interacted with test organisms in a plate that had just been seeded with it to determine the antibacterial effect.

In the present study 600mg/ml concentration had comparable effect to the positive control (chlorhexidine 0.12% mouthwash) with a mean value (22.41±.32) of zone of inhibition. It was similar to the study done by N.H.Yim et al (2013) (18) were sappan lingum (same genus) exhibited similar MIC values at 150mg/ml.

In the present study the most effective concentration was 600mg/ml when comparing with the positive control. The study result was in ordinace to the study by Puttipan R et al 2017 (19) were methonolic extract of C.sappan wood showed a MIC of 18.5±0.6 against oral pathogen *St.mutans*.

Yodsaoue O et al did an in vitro investigation and discovered that *C.sappan* wood is antiallergic (20). Saenjum C et al 2010 studied the protective effects of *C. sappan* extract on DNA damage caused by hydroxyl radicals in order to assess the chemopreventive potential of C. sappan extract (9). Budi HS et al 2020 conducted an in vitro study and concluded that sappan wood ethanol extract (*Caesalpinia sappan*) can suppress the development of A. actinomycetemcomitans and P. gingivalis at MIC 1.56% and MBC 3.125% concentrations (21). The plant extracts were discovered to be a good source of metals, vitamins, and secondary metabolites. The extracts were also examined for their ability to inhibit various human pathogenic bacteria. It was discovered that the heartwood's methanol and ethyl acetate extracts were efficient against specific pathogenic bacteria (22).

The result of the present study suggest *C.sappan* aqueos extract has an antimicrobial effect against oral bacteria *St.mutans*. Of the tested concentration 600mg/ml effect of *C.sappan* wood aqueous extract was comparable to control and suggest for its application in treatment of dental diseases.

The strength of the present study all the procedure was conducted in controlled environment and the limitation of the present study was other biological factors impacting microbial count could not be taken into account in the current study, which limits its ability to focus on clinical reproduction. An in vivo investigation would focus more on clinical reproduction.

CONCLUSION

The current study's findings show that a 600mg/ml concentration of *C.Sappan* aqueous extract has a comparable impact to chlorhexidine. As a result, *C.Sappan* wood aqueous extract may be preferable for routine oral hygiene practise, where it can deliver maximum antibacterial action while minimising the negative effects of the routine aid.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest

REFERENCES

1. Department of Human Services. Promoting oral health 2000–2004: strategic directions and framework for action. Melbourne: Department of Human Services; 1999.

2. Listl S, Galloway J, Mossey PA, Marcenes W. Global economic impact of dental diseases. J Dent Res. 2015; 94:1355-1361.

3. Mealey BL, Oates TW. American Academy of Periodontology. Diabetes mellitus and periodontal diseases. J Periodontol 2006; 77:1289-303.

4. Krzyściak, W., Jurczak, A., Kościelniak, D. et al. The virulence of Streptococcus mutans and the ability to form biofilms. Eur J Clin Microbiol Infect Dis 33, 499–515 (2014).

5. Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet. 2007 Jan 6;369(9555):51-9.

6. Addy M. Plaque control as a scientific basis for the prevention of dental caries. Journal of the Royal Society of Medicine. 1986;79(Suppl 14):6.

7. Rao NJ, Subash KR, Kumar KS. Role of phytotherapy in gingivitis: A review. Int J Pharmacol. 2012; 1:1-5

8. Zanatta FB, Antoniazzi RP, Rösing CK. Staining and calculus formation after 0.12% chlorhexidine rinses in plaque-free and plaque covered surfaces: a randomized trial. J Appl Oral Sci. 2010 Sep-Oct;18(5):515-21

9. Saenjum C, Chaiyavat C, Kadchumsang S, Chansakaow S, Suttajit M. Antioxidant activity and protective effects on DNA damage of Caesalpinia sappan L. extract. J Med Plant Res 2010; 4: 1594-608.

10. Mueller M, Weinmann D, Toegel S, Holzer W, Unger FM, Viernstein H. Compounds from Caesalpinia sappan with anti-inflammatory properties in macrophages and chondrocytes. Food Funct. 2016 Mar;7(3):1671-9.

11. Arunkumar Naik Bukke, Fathima Nazneen Hadi, Chandramati Shankar Produtur, Comparative study of in vitro antibacterial activity of leaves, bark, heart wood and seed extracts of Caesalpinia sappan L., Asian Pacific Journal of Tropical Disease, Volume 5, Issue 11, 2015, Pages 903-907.

12. Sahal D. Evaluation of Antimalarial Potential of Kerala Ayurvedic Water “Pathimugam. Available at SSRN 4124886.

13. CLSI, Methods for Antimicrobial Dilution and Disk Susceptibility of Infrequently Isolated or Fastidious Bacteria, Approved Guideline, 2nd. ed., CLSI document M45-A2. Clinical and Laboratory Standards Institute, 950 West Valley Roadn Suite 2500, Wayne, Pennsylvania 19087, USA, 2010.

14. Nirmal NP, Rajput MS, Prasad RG, Ahmad M. Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. Asian Pac J Trop Med. 2015 Jun;8(6):421-30.

15. Thomas G, John AM, Joy J, Mathew SS, David A.Impact of taking thyroxine with herbal brews. J Curr Res Sci Med 2018;4:94-7.

16. https://www.who.int/news-room/fact-sheets/detail/oral-health.cited on 11.06.2023.

17. Venditti M, Baiocchi P, Santini C, Brandimarte C, Serra P, Gentile G, Girmenia C, Martino P. Antimicrobial susceptibilities of Streptococcus species that cause septicemia in neutropenic patients. Antimicrob Agents Chemother. 1989 Apr;33(4):580-2. doi: 10.1128/AAC.33.4.580.

18. Yim NH, Jung YP, Cho WK, Kim T, Kim A, Im M, Ma JY. Screening of aqueous extracts of medicinal herbs for antimicrobial activity against oral bacteria. Integr Med Res. 2013 Mar;2(1):18-24.

19. Puttipan R, Wanachantararak P, Khongkhunthian S, Okonogi S. Effects of Caesalpinia sappan on pathogenic bacteria causing dental caries and gingivitis. Drug Discov Ther. 2017;11(6):316-322.

20. Yodsaoue O, Cheenpracha S, Karalai C, Ponglimanont C, Tewtrakul S. Anti-allergic activity of principles from the roots and heartwood of *Caesalpinia sappan* on antigen-induced beta-hexosaminidase release. Phytother Res. 2009 Jul;23(7):1028-31.

21. Budi HS, Soesilowati P, Wirasti MJ. Antibacterial activity of sappan wood (Caesalpinia sappan L.) against aggregatibacter actinomycetemcomitans and porphyromonas gingivalis. Systematic Reviews in Pharmacy. 2020;11(3):349-353.

22. Senthilkumar, Natesan, Subban Murugesan, N. R. L. Banu, Sultan Supriya and C. Rajeshkannan. “Biochemical Estimation and Antimicrobial Activities of the Extracts of *Caesalpinia Sappan* Linn.” Bangladesh Journal of Scientific and Industrial Research 46 (2012): 429-436.

Published by MM Publishers
https://www.mmpubl.com/ijcd