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Original Article

Dentistry Section

Effect of Traditionally Used Neem and Babool Chewing Stick (Datun) on *Streptococcus Mutans*: An In–Vitro Study

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ABSTRACT

Purpose: There are various plants, which are used as chewing sticks in different parts of the world. Several studies have been reported on the antimicrobial effects of chewing sticks on oral bacteria. This study was conducted to evaluate the effectiveness of traditionally used neem and babool chewing sticks (datun) extracts on *Streptococcus mutans*.

Materials and Methods: The present invitro study was conducted to assess effectiveness of 5%, 10%, and 50% neem and babool extract on *Streptococcus mutans*. The ditch plate method was used to test the antimicrobial activity. Ditches were prepared on blood agar plates with the help of punch having 6mm diameter. The plates were left for 1h at room temperature and then incubated at 37°C for 48h and examined for zone of inhibition.

Results: There was no zone of inhibition observed with 5% babool and neem aqueous extract. There was significant difference in mean diameter of zone of inhibition of 10% neem and babool extract (p-value 0.001 < 0.05). Similarly the mean difference in 50% neem and babool extract was found to be significant (p-value < 0.001).

Conclusion: Both neem and babool extracts had antimicrobial activity against *Streptococcus mutans*, while antimicrobial activity was significantly higher in neem aqueous extract than babool aqueous extract.

Keywords: Antimicrobial activity, Chewing sticks, Oral bacteria, Zone of inhibition

INTRODUCTION

Oral hygiene measures have been practiced by different populations and cultures around the world since antiquity. The evolution of the modern tooth- brush has its origin in chewing sticks that were used by the Babylonians as early as 3500 BC [1]. The Neem tree originates from northeast India. It is also known as Margosa or the Persian lilac. Neem has been broadly used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine [2]. In India this plant is referred to as the village pharmacy because of its ability to cure many disorders ranging from bad teeth and bed bugs to ulcers and malaria [3]. In rural areas of India, the twigs are used as toothbrush to prevent gingivitis. Neem and Babool (acacia sp) chewing sticks (datun) have been used as oral hygiene measure [4].

Dental caries is one of the most common human diseases that affect the vast majority of individuals. Cross sectional and longitudinal epidemiology surveys have implicated *Streptococcus mutans* in the aetiology of human dental caries [3]. Inhibiting the growth of the *Streptococcus mutans* in the oral cavity would lead to healthier teeth and gums.

Although the anti-bacterial effect of Neem has been proven, there is still a grey area concerning its effective concentration against the *Streptococcus mutans* specifically. After extensive exploration of the literature, we found dearth of data regarding effect of Neem and Babool chewing sticks (datun) extracts on *Streptococcus mutans*. So the present study was undertaken with the aim of evaluate the effectiveness of traditionally used neem and babool chewing sticks (datun) extracts on *Streptococcus mutans* and with objective of comparing the effect of neem and babool chewing sticks aqueous extract on *Streptococcus mutans*.

MATERIALS AND METHODS

The present invitro study was conducted to assess effectiveness of 5%, 10%, and 50% neem and babool extract on *Streptococcus mutans* in the department of microbiology, MS University, Gujarat.

Materials used in the study included Dried chewing sticks of Neem and Babool, Micro-organisms (*Streptococcus mutans*- MTCC – microbial type culture collection), Nutrient Agar plates, Blood agar plates, Petri-dish, Vernier calipers, Punch, Distilled water, Weighing machine, Centrifugal machine, Sterile bottles

Preparation of extracts

Hundred grams each of the chewing sticks were used in the experiment. The chewing sticks were kept sun dried for 2 wks before extract preparation. The sticks were cut into small pieces and ground to powder in a ball mill. The weighted powder i.e. 5 gm, 10 gm and 50 gm was kept separately in sterile, dry screw-capped bottles, which were stored in a dry cool place for one week before aqueous extraction. 10 ml of sterile water was added to each bottle of powder.

The extracts were allowed to soak for 48h before the mixtures were centrifuged at 2,000 rpm for 10 min. The supernatants were passed through a 0.45 mm membrane filter; the extracts were prepared at 5, 10 and 50 % concentrations (v/v) and stored in 5ml portions at 20°C. (Actually, this is a common method for preparing extracts, used by Almas K [4], Prashant GM et al.,[5].

Streptococcus mutans MTCC was procured from Microbial Type Culture Collection (MTCC) Chandigarh,India.

Preparation of Culture Media

Streptococcus mutans MTCC was added to nutrient broth which was incubated at 37^{0}C for 24h. It was then sub-cultured onto nutrient agar plate and incubated at 37^{0}C for 24h. The inoculum for antimicrobial activity was prepared by adjusting the density of organism to approximately 108 colony forming units/ml with the help of 0.5 Mcfarland opacity standards (this is a very standard procedure in microbiology for preparing standard bacterial suspension, it was inoculated on blood agar plate by lawn culture method.

Concentration	Zone of inhibition (mm)				Mean ±SD
	Z_1	Z_2	Z_3	Z_4	
5%	0	0	0	0	0
10%	0.6,	0.7	0.6	0.5	0.6 ± 0.08
50%	1.6,	1.3	1.4	1.4	1.4 ± 0.12

[Table/Fig-1]: Effect of various concentrations of aqueous babool extracts on *Streptococcus mutans*

Concentration	Zone of inhibition (mm)				Mean ±SD
	Z_1	Z_2	Z_3	Z_4	
5%	0	0	0	0	0
10%	1.1	0.9	1.3	1.1	1.1 ± 0.16
50%	3.9	4.1	4.1	4	4.0 ± 0.09

[Table/Fig-2]: Effect of various concentrations of aqueous neem extracts on *Streptococcus mutans*

Concen-trations	Neem Extract Mean ± Sd	Babool extract Mean ± SD	t-value (df)	p-value
5%	0	0	0	
10%	1.1 ± 0.16	0.6 ± 0.08	5.48 (6)	0.001 (S)
50%	4.0 ± 0.09	1.4 ± 0.12	32.89	0.00000005 (S)

[Table/Fig-3]: Comparison of mean zone of inhibitions of various concentrations of neem and babool aqueous extract
S = Significant, df = degree of freedom, level of significane p-value <0.05

Antimicrobial susceptibility testing

The ditch plate method was used to test the antimicrobial activity. Ditches were prepared on blood agar plates with the help of punch having 6 mm diameter. On each petri dish, four ditches were made and labeled for various concentrations of neem and babool extract. 50 µL each of 5%, 10% and 50% babool and neem extracts were introduced into equal sized ditches made on petri dishes. Sterile distilled water was used as control.

The plates were left for 1h at room temperature and then incubated at 37°C for 48h and examined for zone of inhibition. The average of those zones was recorded in millimeters.

STATISTICAL ANALYSIS

SPSS (Statistical Package for Social Sciences) version 16.0 was used. Student t- test was used to compare the mean zone of inhibitions of various concentrations of neem and babool aqueous extract.

RESULTS

Present study was conducted to assess the effectiveness of neem and babool aqueous extracts on *Streptococcus mutans*. Zone of inhibitions were measured from the edge of the punched hole (ditch) to outer border of bacterial inhibition (translucent area) at four different randomly selected perpendicular places. These zones of inhibitions were measured after 24h and 48h but no difference was observed.

[Table/Fig-1] shows effect of various concentrations of aqueous babool extracts on *Streptococcus mutans*. There was no zone of inhibition observed with 5% babool extract. Zone of inhibitions of 0.6, 0.7, 0.6, 0.5 mm were observed with 10% extract with mean zone of inhibition of 0.6mm. Zone of inhibitions 1.6,1.3,1.4,1.4 mm were observed with 50% babool extract with mean zone of inhibition of 1.4 mm.

[Table/Fig-2] shows effect of various concentrations of Aqueous neem extracts on *Streptococcus mutans*. There was no zone of inhibition observed with 5% neem extract. Zone of inhibitions 1.1, 0.9, 1.3, 1.1 mm were observed with 10% neem extract with mean zone of inhibition of 1.1 mm. Zone of inhibitions 3.9, 4.1, 4.1, 4 mm were observed with 50% neem extract with mean zone of inhibition of 4 mm.

[Table/Fig-3] compares the mean zone of inhibitions of various concentration of neem and babool aqueous extract. No zone of inhibition was observed with 5% neem and babool extract. There was significant difference in mean diameter of zone of inhibition of 10% neem and babool extract (p-value 0.001 < 0.05). Similarly the mean difference in 50% neem and babool was found to be significant (p-value < 0.001).

DISCUSSION

An attempt has been made to enrich the knowledge of antibacterial activity of 5%, 10%, and 50% crude extract of Neem and Babool chewing sticks on *Streptococcus mutans*. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents [6,7].

At 5% concentration, Babool extract showed no antimicrobial activity. Some antimicrobial activity was found with 10% babool extract and maximum antimicrobial activity was found with 50% concentration with mean zone of inhibition of 1.4 mm. This is in contrast with the results of study conducted by Almas K [4], in which he found no antimicrobial effect on *Streptococcus mutans*.

The reasons for antimicrobial activity of Babool may include hydrophilic compounds such as polyphenols, gums (poly-saccharides) and tannins. There is increasing evidence to support that the plants of genus Acacia are relatively high in bioactive secondary compound and are thus likely to hold promise for drug discovery. Secondary compounds in Acacia are important for a variety of functions, chief among these are Anti-cancer (triterpenoid and saponins), diuretic (glucosides), natriuretic (glucosides), important nutraceutical (poly-saccaride and gum) anti-digestive disorder (saponins, tannins and flavanoids), anti-oxidant (polyphenols), anti-plasmodial (treptamine, tannins, organic acids and saponins [8].

In our study, no antimicrobial effect was observed with 5% Neem aqueous extract 10% extract showed some antimicrobial effect with a mean zone of inhibition of 1.1mm. The maximum antimicrobial effect was exhibited by 50% extract with a mean zone of inhibition of 4.0 mm. Similar results were found in studies conducted by Chava VR et al., [9], Elangovan A et al., [10], Lekshmi P et al., [11], where antimicrobial activity increased as the concentration of extract increased. Similarly Prashant GM et al., [5] found maximum anitimicrobial activity at 50% concentration, with a zone of inhibition of 3.8 mm.

The reason of this antimicrobial effect include the presence of fluoride, which is known to exert an anticariogenic action, and silica acting as an abrasive and preventing accumulation of plaque; alkaloids, known to exert an analgesic action, also contribute towards dental well-being. The oils have carminative, antiseptic, and analgesic effects.

Bhuyian et al., [12] conducted a study to investigate the effect of crude Neem bark extract on *Streptococcus sobrinus* and found no antimicrobial activity at 5% concentration. Wolinsky et al.,[13] assessed the inhibiting effect of aqueous neem extract upon bacterial properties influencing invitro plaque formation. They reported that water-soluble extracts of the Neem stick affect some bacterial properties which may alter bacterial adhesion and the ability of some *streptococci* to colonise tooth surfaces. Specifically, the Neem stick extracts exhibited broad bacterial aggregating activity among the oral streptococci tested.

Comparison of the degrees of inhibition of the various botanical extracts from different studies cannot be exactly justifiable, since the experiments were performed on different extracts (i.e. aqueous, ethanolic extracts) and not on pure compounds. Further experiments with a narrower range may be proved useful in determining the effective concentrations of the neem and babool chewing stick extracts.

CONCLUSION

Both neem and babool chewing stick extracts demonstrated antimicrobial activity against *Streptococcus mutans*, while antimicrobial activity was significantly higher in neem aqueous extract than babool aqueous extract.

REFERENCES

[1] Wu CD, Darout IA, Skaug N. Chewing sticks: timeless natural toothbrushes for oral cleansing. *J Periodont Res*. 2001; 36: 275-84.

[2] Lekshmi P, Sowmia N, Viveka S, Brindha JR, Jeeva S. The inhibiting effect of Azadirachta indica against dental pathogens. *Asian J Plant Sci Res*. 2012; 2(1):6-10.

[3] Subramaniam SV, Siswomihardjo W, and Sunarintyas S. The effect of different concentrations of Neem (Azadiractha indica) leaves extract on the inhibition of *Streptococcus mutans* (In vitro). *Maj Ked Gigi Dent J*. 2005; 38(4): 176–79.

[4] Almas K. The antimicrobial effects of extracts of Azadirachta indica (Neem) and Salvadora persica (Arak) chewing sticks. *Indian J Dent Res.*1999; 10(1):23-6.

[5] Prashant GM, Chandu GN, Murulikrishna KS, Shafiulla MD. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivavius, Streptococcus mitis*, and *Streptococcus sanguis*: an invitro study. *Indian J Dent Res.* 2007; 18(4):148-51.

[6] Mathur A, et al. Phytochemical investigation and in vitro antimicrobial activity of different parts of Ficus racemosa. *L Der Pharmacia Sinica.* 2011;2 (2): 270-5.

[7] Mathur A, Dua VK, Prasad GBKS. Antimicrobial Activity of Leaf Extracts of Murraya Koenigii against Aerobic Bacteria Associated with Bovine Mastitis. *Int Journal Chem Env Pharm Res.* 2010;1(1): 12-6.

[8] Saini ML, Saini R, Roy S, Kumar A. Comparative pharmacognostical and antimicrobial studies of acacia species (Mimosaceae). *J Med Plant Res.* 2008; 2(12): 378-86.

[9] Chava VR, Manjunath SM, Rajanikanth AV, Sridevi N. The efficacy of neem extract on four microorganisms responsible for causing dental caries viz *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: an invitro study. *J Contemp Dent Pract.* 2012; 3(6):769-72.

[10] Elangovan A, Muranga J, Joseph E. Comparative evaluation of the antimicrobial efficacy of four chewing sticks commonly used in South India: An invitro study. *Indian J Dent Res.* 2012; 23(6):840-45.

[11] Lekshmi P, Sowmia N, Viveka S, Brindha JR, Jeeva S. The inhibiting effect of Azadirachta indica against dental pathogens. *Asian J Plant Sci Res.* 2012, 2 (1):6-10.

[12] Bhuiyan MM, Nishimura M, Matsumura S, Shimono T. Antibacterial effects of the crude Azadiracta Indica Neem bark extract on *Streptococcus sorbinus*. *Paed Dent J.* 1997;7(1):61-4.

[13] Wolinsky LE, Mania S, Nachnani S, Ling S. The Inhibiting Effect of Aqueous Azadirachta indica (Neem) Extract upon Bacterial Properties Influencing invitro Plaque Formation. *J Dent Res.* 1996.;75(2): 816-22.

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