ORIGINAL RESEARCH

Use of an aqueous extract of *Terminalia chebula* as an anticaries agent: A clinical study

Carounanidy Usha, Satyanarayanan R, Velmurugan A

Division of Conservative Dentistry and Endodontics, Rajah Muthiah Dental College and Hospital, Annamalai University, Chidambaram, Tamilnadu - 608 002, India

Received : 31-01-07
Review completed : 13-04-07
Accepted : 20-04-07
PubMed ID : 17938489

ABSTRACT

Plant-derived medicines have been a part of our traditional health care system, and the antimicrobial properties of plant-derived compounds are well documented. The purpose of this study is to evaluate the effect of an aqueous extract of *Terminalia chebula* (a medicinal plant) on salivary samples and its potential for use as an anticaries agent in the form of mouthwash. A concentrated aqueous extract was prepared from the fruit of *T. chebula*. A mouth rinse of 10% concentration was prepared by diluting the extract in sterile distilled water. The efficacy of the mouth rinse was assessed by testing on 50 salivary samples. Salivary samples were collected from subjects assessed to be at high risk for caries. Salivary pH, buffering capacity, and microbial activity were assessed before rinsing, immediately after, and 10 min, 30 min, and 1 h after rinsing. There was an increase in the pH and buffering capacity and decrease in microbial count. An aqueous extract of *T. chebula* used as a mouth rinse seems to be an effective anticaries agent.

Key words: Dental caries, plants, saliva, *Streptococcus mutans*

Management of dental caries has evolved from a centuries-old surgical model to the present medical model. This shift is because of the change in the outlook of the dental profession towards dental caries. Dental caries is now being viewed in a dual perspective—'caries as a disease' and 'caries as a lesion.' The surgical model of management of 'dental caries as a lesion,' namely the triad of removal of the carious lesion, tooth preparation, and restoration, is now being reserved for the lesion that has cavitated and penetrated the dentinal aspect of the tooth structure. The medical model of management of 'dental caries as a disease' includes (a) identifying the risk group for the disease; (b) remineralizing the noncavitated carious tooth, without surgical intervention; and (c) preventing the occurrence / recurrence of the disease in the individual.

The exponential advancements in the field of cariology have reemphasized the importance of prevention. Fluoride has been the most popular preventive material because of its ability to remineralize the tooth and make it acid resistant. Causation of dental caries is multifactorial, involving dietary factors, salivary factors, and microbial factors; therefore, other preventive materials have emerged, targeting these causative factors. For example, the use of antimicrobial agents, like chlorhexidine, is now recognized as yet another useful preventive measure.

There has been a change in thinking globally, with a growing tendency to 'go natural.' Western civilization is now looking to ancient Eastern culture for ways to improve and invigorate their entire lifestyle; this is evident in the widespread interest in spirituality, yoga, healthy organic diets, and the use of medicinal plants.

Indian civilization, as everyone is aware, is very ancient and rich in these resources. The use of herbs and plants for treating diseases has been common practice here since ages. But with the influence of the West and the strong scientific evidence in favor of Allopathy, alternative systems of Indian medicine have been in eclipse. However, phoenix-like, it is now reemerging, strong and sound, and with numerous scientific researches producing evidence in its favor.

Review of literature reveals abundant evidence for the use of plants and plant products in preventing caries.[1,2] In our study we have selected one medicinal plant—*Terminalia chebula*—to study the efficacy of its extract as an anticariogenic mouth rinse.

Correspondence:
Dr. A. Velmurugan,
E-mail: muthu_bds @yahoo.com

AIMS AND OBJECTIVES

The aim of the study was to analyze the efficacy of the extract of *T. chebula* when used as an anticariogenic mouth rinse.

The objective of the study was to observe, in high caries risk individuals, the effect of gargling with the extract of *T. chebula*; the following were measured:

1. Change in the pH of saliva
2. Change in the buffering capacity of saliva
3. Change in the microbial count in saliva

MATERIALS AND METHODS

Preparation of the extract

The dried ripe fruit of *T. chebula* was obtained and ground into a fine powder. It was mixed with 10 times its quantity of sterile distilled water in a round-bottomed flask and the suspension was kept at 4°C for 72 h. The aqueous extract was decanted, clarified by filtration through a muslin cloth, and evaporated in a flat-bottomed porcelain dish at 40°C. The dried extract was again suspended in polyethylene glycol (20% v/v) and distilled water evaporated to get the final concentrate. This concentrate was then diluted with sterile distilled water to get a mouth rinse of 10% (w/v) concentration.[3]

Sample population and size

The sample was selected from among the patients attending the outpatient Department of Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram. Male and female patients in the age group of 18-25 years were assessed for caries risk using the criteria [Table 1] followed at the Division of Conservative Dentistry, Annamalai University.

The risk categories were as follows:

High risk: More than 70% of score in a minimum of two categories, of which one of the categories has to be salivary analysis or clinical examination.

Moderate risk: 50-70% of score in a minimum of two categories, of which one of the categories has to be salivary analysis or clinical examination.

Low risk: Less than 50% of score in all categories.

Fifty patients with high caries risk were chosen. Consent was obtained after the protocol had been clearly explained to them.

Salivary pH analysis

Saliva samples from the 50 patients were taken and the pH analyzed using a chair-side kit (GC saliva check).

Unstimulated saliva was allowed to collect in the floor of the mouth and then transferred to the collecting jar. The pH test paper was dipped in the sample for at least 10 s and the color changes were compared with the chart provided by the manufacturer. The values were recorded.

Table 1: Caries risk assessment criteria

General history	
Susceptible age factor	Yes / No
Past history of extractions of carious teeth	Yes / No
Past history of restoration for carious teeth	Yes / No
Medical history predisposing to caries	Yes / No
Family tendency	Yes / No
Socio economic status and dental awareness	Good / Bad / Moderate
Clinical examination	
Presence of active carious lesions	Yes / No
Presence of more than two proximal caries /anterior caries	Yes / No
Presence of root stumps in need of extraction	Yes / No
Presence of restorations done for carious reason	Yes/No
History of oral habits	
Oral habit status	Good / Bad / Moderate
Oral hygiene status	Good / Bad / Moderate
Oral hygiene habits status	Good / Bad / Moderate
Dietary habits	Good / Bad / Moderate
Salivary analysis	
Salivary flow	High / low / moderate
Salivary pH	High / low / moderate
Buffering capacity of saliva	High / low / moderate
Streptococcus mutans count	High / low / moderate
Lactobacilli count	High / low / moderate

Salivary buffering capacity analysis

The salivary buffer was assessed using a chair-side kit (GC saliva check).

The buffer test was done on stimulated saliva. The patient was instructed to chew on a piece of paraffin wax for 30s. The first saliva that was secreted was discarded and the subsequently secreted saliva was collected for testing. Using the pipette provided, one drop of saliva was placed on each test pad. After 2 min, the color of the strip was compared with the chart provided by the manufacturer, and the values were recorded.

Salivary microbial analysis

Saliva for microbial analysis was collected from 32 patients in the same manner as was done for the buffer test.

The technique used for assessing the microbial content was the dilution and spread plate technique.[4] Salivary samples were taken twice from each of these 32 patients: before rinsing with *T. chebula* extract and 90 min after the rinse. The samples were diluted with saline and then streaked on to petri plates containing the appropriate medium: MSB agar for *Streptococcus mutans* and LB agar for lactobacilli. The plates were incubated for 72 h at 35°C. After incubation the colonies were counted.

The patients were asked to use the 10% concentrated extract as mouthwash for rinsing and to retain it in the mouth for 40 s before expectorating it. Patients were not allowed to rinse with water or to consume anything orally for 90 min following this. The pH and buffer test were repeated at 10, 30, 60 and 90 min intervals. The microbial analysis was repeated at 90 min. The results were recorded.

The values obtained from the pH analysis, buffering analysis, and microbial analyses were tabulated and the means and standard deviations were calculated. Statistical analysis was done using the Student's t test to compare the following:
Group I: The prerinse sample with the 10 min postrinse sample
Group II: The prerinse sample with the 30 min postrinse sample
Group III: The prerinse sample with the 60 min postrinse sample
Group IV: The prerinse sample with the 90 min postrinse sample

RESULTS

Results of pH analysis

From Table 2 it is seen that the pH increases to a peak value of 7.428 at 30 min and then decreases gradually until, at 90 min, it reaches its lowest value of 6.84.

The Student's t test revealed that the results were highly significant for groups 1 and 2 at the probability level of.001, with t values of 17.758 and 17.756. The differences in groups 3 and 4 were less significant at the probability level of.001; the t values were 10.728 and 6.872.

Results of buffering capacity analysis

Table 3 reveals that the buffering capacity increased to a peak value of 10 at 30 min and then decreased gradually until at 90 min, it reached a low of 7.42.

The Student's t test revealed that the results were highly significant for groups 1 and 2 at the probability level of 0.001; the t values were 6.971 and 3.834. The differences in groups 3 and 4 were not significant at the probability level of 0.001; the t values were 0.522 and 0.522.

Results of microbial analysis

Count

The results of microbial analysis revealed that there was a 65% decrease in the microbial count for *Streptococcus mutans* and a 71% decrease for lactobacilli [Table 4].

The Student's t test indicated no significance in the reduction of *Streptococcus mutans* at the probability level of .001; the t value was 2.06. The reduction in lactobacilli was even less significant at the probability level of .001; the t value was 3.549.

DISCUSSION

There is an increasing tendency in the medical field to opt for therapeutic agents from natural sources and this is also reflected in the management of dental caries,[2] which is one of the oldest diseases of mankind. Our review of literature also showed that many studies are being conducted for identifying therapeutic agents from natural sources for the management of dental disease. Most of the agents being evaluated are plant extracts and are aimed at the management of periodontal disease and dental caries. *T. Chebula* is one of the exceptions, in that its extract was being used for the prevention of dental caries. This study was designed to find out the efficacy of *T. chebula* in preventing dental caries.

Tannins are a group of polymeric phenolic substances and the plant extract of *T. chebula*, with tannin as its active compound, is well recognized for its microbial activity and its astringent property.[5,6] In this study we used an aqueous

Table 2: Salivary pH values

No. of samples		Prerinse	Post rinse			
			10 min	30 min	60 min	90 min
50	Mean	6.4200	7.4240	7.4280	7.1040	6.8480
50	Std. Deviation	0.2807	0.2847	0.2857	0.3528	0.3394

Table 3: Salivary buffering capacity values

No. of samples		Postrinse	Prerinse			
			10 min	30 min	60 min	90 min
50	Mean	6.4200	7.4240	7.4280	7.1040	6.8480
50	Std. Deviation	0.2807	2847	0.2857	0.3528	0.3394

Table 4: Reduction in percentage of *Streptococcus mutans* and Lactobacilli

No. of samples	Percentage reduction in *Streptococcus mutans* count	Std deviation in %	Percentage reduction in Lactobacilli count	Std deviation in %
32	65%	50	71%	40

extract, since water is a high polarity solvent and almost all compounds in the plant would be soluble in it. Although it is extracted in water, the presence of tannin or other phenols would create a problem for nucleic acid extraction and, therefore, polyethylene glycol was used. Polyethylene glycol was added to reduce the high affinity of polyphenols and reduce nucleic acid polyphenol complexes. A 10% concentrate was used in this study since it has already been proved to be effective.[7]

The use of saliva as a diagnostic aid is being increasingly recognized in various branches of medicine, including dentistry. This study was done on the saliva samples of patients belonging to a high risk group for caries as assessed by the protocol followed at Annamalai University. This proven protocol has been in use for the past four years in the curriculum as one of the simple means to identify dental caries.

The pH, buffering capacity, and the microbial activity of *Streptococcus mutans* and lactobacilli in the saliva were assessed and the influence of *T. chebula* on these factors were recorded.

T. chebula mouthwash of 10% concentration was utilized following the guidelines and results of the promising works by Jagtap,[3] in which he proved that a 10% concentrate of the extract in the form of a mouthwash was an effective anticaries agent.

The pH and buffering capacity were assessed by the chair-side saliva check kit (GC India), which is a proven and simple protocol. For pH analysis, unstimulated saliva was taken as per the protocol given in the kit; it is known that the resting salivary pH plays a major role in caries initiation. Stimulated saliva was taken for analysis of buffering capacity and microbial analysis since the stimulated saliva is known to play a major role in counteracting the changes due to any stimulant.[8]

The pH and buffering capacity were recorded 10, 30, 60, and 90 min after rinsing with the mouthwash both for reasons of convenience and also to find out how the effect of the mouthwash varied over a period of time. On the other hand, testing for microbial activity was performed at a postrinse interval of 90 min in accordance with the previous study of Jagtap.[3]

The results of the study clearly indicate that the *T. chebula* mouthwash is very effective in increasing the pH of saliva until the end of the test period of 90 min. However, the peak effect (pH of 7.42) was seen at 30 min and was followed by a gradual decrease in pH till it reached a value of 6.8 at 90 min. It is to be noted that at the end of 90 min the pH was higher than in the prerinse sample. Hence, it can be safely assumed that in order to increase the efficiency of the agent over a longer period, either the concentration has to be increased or it must be made available in other forms, such as a gel or varnish. Further research in this direction should be helpful.

The buffering capacity of the 50 samples of saliva from high caries risk subjects that were tested in this study clearly indicate that only seven samples suffered from a low buffering capacity. The effect of *T. chebula* on the buffering capacity was similar to the effect on pH, with increase in buffering capacity at 30 min. Similar to the pH, the buffering capacity then decreased gradually over a period of 90 min. This indicates that the inferences regarding the beneficial effects of *T. chebula* mouthwash on pH hold good for buffering capacity also.

The effects of *T. chebula* on microbial activity is in accordance with the previous study by Jagtap. In our study there was a 65% reduction in *Streptococcus mutans* and 71% fall in lactobacilli count after 90 min. However, the reduction was not statistically significant. Only 32 samples from the total of 50 samples were subjected to microbial analysis. This was because of the lack cooperation from some patients, along with the lack of time and finances. Table 4 shows that the standard deviations for the microbial analysis are very high. Hence, the results are not statistically significant. To increase the sensitivity of this study and to decrease the standard deviation, the sample size would have had to be considerably more. Even though the effect of *T. chebula* on microbial analysis was not statistically significant, both species of bacteria were reduced by 60% or more, and this can be considered as being clinically significant. However, more research is necessary before definite conclusions can be made.

The pilot study conducted to assess this particular medicinal value of *T. chebula* seems to be encouraging. The extract of the fruit definitely has an anticaries effect. Some fine-tuning is necessary with regard to dosage and the form and frequency of rinsing. Further experimentation with other Indian medicinal plant extracts may show the way to the development of a synergetic compound with a long-lasting anticaries action on the tooth surface.

CONCLUSION

T. chebula extract is a potential anticariogenic mouthwash. For better efficiency, a proper vehicle such as gel or varnish form has to be chosen.

REFERENCES

1. Lee SB, Zhang W, Li F. The Antimicrobial potential of 14 herbal dentifrices. JADA 2004;135:1133-41.
2. Badrina FA, Zidan OA. Natural products for dental caries prevention. J Med Food 2004;7:381-4.
3. Jagtap AG, Karkera SG. Potential aqueous extract of *Terminalia Chebula*

as an anticaries agent. J Ethnopharmocol 1999;68:299-306.
4. Brooks GF. Medical microbiology, 19th ed. Apleton and Lange: East Norwak; 1991.
5. Akiyama H, Fujii K, Yamasaki O. Antibacterial effect of several tannins against *Staphylococcus aureus*. J Antibacterial Chemother 2001;48:487-91.
6. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
7. Arcuri PB. Polyethylene glycol and polyvinylpirrolidone effect on bacterial rRNA extraction and hybridization from cells exposed to tannins. Pesq Agropec 2003;38:1073-81.
8. Mount GJ. Preservation and restoration of tooth structure, 2nd ed. Mosby: London; 1999.

How to cite this article: Carounanidy U, Satyanarayanan R, Velmurugan A. Use of an aqueous extract of *Terminalia chebula* as an anticaries agent: A clinical study. Indian J Dent Res 2007;18:152-6.

Source of Support: Nil, **Conflict of Interest:** None declared.