

## Original Article

### Effect of an herb root extract,herbal dentifrice and synthetic dentifrice on human salivary amylase

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#### ABSTRACT

**Background:** Salivary amylase is an enzyme, which plays a vital role in formation of dental plaque. It has the ability to bind on the bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids leading to dental caries. Suppression of salivary amylase activity can lead to decrease in risk of dental caries and plaque associated periodontal diseases. The aim of this study was to evaluate the effect of an herb, *Spilanthes calva* (in form of a test dentifrice) on human salivary amylase activity and to compare it with other dentifrices.

**Materials and Methods:** A total of 80 subjects of age 18-35 years were randomly selected and divided equally into 4 groups. Group 1 subjects were assigned to use Test Dentifrice (with *S. calva* root extract), while Group 2, Group 3, and Group 4 subjects were assigned to use Herbal Dentifrice (Arodent™), Synthetic Dentifrice (Colgate®), and Control Dentifrice respectively. Salivary amylase activity was determined by Bernfeld method in each group, before and after using the given dentifrices.

**Results:** Maximum inhibition of salivary amylase activity was found in the group using test dentifrice as compared to others.

**Conclusion:** The present study indicates that, the root extract of *S. calva* possess significant inhibitory activity for salivary amylase. Use of *S. calva* root extract will provide a wider protection against different pathogenic oral microflora. Use of this extract singly or in combination is strongly recommended in the dentifrice formulations.

**Key Words:** Dental caries, dental plaque, dentifrice, *Spilanthes calva*

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## INTRODUCTION

Salivary amylase is a principal digestive enzyme produced by the salivary glands which plays an important role in the colonization and metabolism of Streptococcus, leading to the formation of dental plaque and caries in human beings. It has been identified as a constituent of the acquired pellicle and

also acts as a receptor for microorganism adhesion on tooth surface.<sup>[1-3]</sup> It has the ability to bind on the bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids leading to dental caries.<sup>[4]</sup>

India is rich in natural resources including medicinal plants and herbs. Unfortunately, most of the traditionally used medicinal plants are yet unexplored or have not been fully examined by modern medical science. Therefore, exploring traditionally reported medicinal plants to leverage their potential for betterment of human health is of great importance.<sup>[5]</sup>

One of such medicinal plant is the herb *Spilanthes calva* commonly known as “Akarkarah” or “toothache plant” which belongs to family Asteraceae.<sup>[6]</sup> The

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plants of the genus *Spilanthes* are widely distributed in tropical and subtropical regions of the world. In India, the plants of this genus are reported from some of the regions of south India, Chhattisgarh, Jharkhand and recently reported in Jhalawar district of state Rajasthan.<sup>[5]</sup> *S. calva* is an annual, spreading plant with bicolored, red/gold flower buds. The roots, flower heads and whole aerial part yield a compound known as spilanthol, which are a powerful stimulant, sialogogue and local anesthetic. In Ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, toothache, infections of gums and throat, paralysis of tongue, and remedy for stammering in children.<sup>[6]</sup> Anti-microbial and insecticidal properties of various parts of this plant have also been reported.<sup>[7,8]</sup> Recently, *in vitro* anti-microbial activity of *S. calva* has also been demonstrated.<sup>[9]</sup>

The present study was carried out to evaluate the effect of *S. calva* (in form of a test dentifrice) on human salivary amylase activity and to compare it with herbal dentifrice (Arodent™), synthetic dentifrice (Colgate®) and a control dentifrice without any anti-microbial agent. Suppression of salivary amylase activity can lead to decrease in risk of dental caries and other plaque associated oral diseases.

## MATERIALS AND METHODS

### Plant material

The herbs of *S. calva* were sourced from the local market of Udaipur, Rajasthan (India) in February 2007. The herb along with its roots was identified by the plant taxonomist.

### Preparation of *S. calva* root extracts and test dentifrice

The roots were separated from plant and stored at room temperature. The roots were cut into small pieces and shade dried. The dry material was grounded in an electric grinder and passed through sieve no.240 so as to obtain a powder of 60-mesh size, which was used for extract preparation. 100% methanolic extract was prepared by reflux method in Soxhlet apparatus.<sup>[10,11]</sup> Dried root powder was extracted with 100% methanol in 1:7 ratios. The process was repeated till complete extraction took place. Extracted plant material was vacuum dried and placed in hot air drier. Dried extract was stored in airtight jar and kept in refrigerator.

A dentifrice without any anti-microbial agent was prepared [Table 1] and it was labeled as control

**Table 1: Composition of dentifrices**

Name of dentifrice	Ingredients	Quantity (gm/100 gm.)
Arodent™ Gum and dental paste (IPSA labs Pvt. Ltd., New Delhi, India)	<i>Mentha spicata</i> Linn.	5.0
	Emend. Nethh.	
	<i>Acacia catechu</i> wild	10.0
	<i>Cyperus rotundus</i> Linn	5.0
	<i>Azadirachta indica</i> A. Juss	10.0
	<i>Mimusops elengi</i> Linn	10.0
	<i>Syzygium aromaticum</i> Linn	1.0
	<i>Myristica fragrans</i> Houltt	2.5
	<i>Achyranthes aspera</i> Linn	10.0
	<i>Zingiber officinale</i> Rosc	1.0
	<i>Mesua ferrea</i> Linn	1.0
	<i>Embelia ribes</i> Burm. F	1.0
	<i>Potentilla</i> sp	5.0
	<i>Anacyclus pyrethrum</i> DC	2.0
	<i>Cuminum cyminum</i> Linn	1.0
	<i>Piper nigrum</i> Linn	1.0
	<i>Elettaria cardamomum</i> Maton	1.0
<i>Cinnamomum zeylanicum</i> Breyne	1.0	
Colgate® dental cream (calcium plus minerals) (Colgate-Palmolive India Ltd., Mumbai)	Potash alum	0.5
	Base	q. s
	Calcium carbonate	—
Control dentifrice	Silica	—
	Triclosan	—
Test dentifrice with <i>Spilanthes calva</i> root extract	1,000 ppm fluoride	100 gm
	Calcium carbonate	54.0
	Sodium lauryl sulphate	2.5
	Carboxy methyl cellulose	0.9
	Saccharine sodium	0.1
	Glycerine	0.8 ml
	Liquid paraffin	0.85 ml
	Sterile purified water	44.5 ml
	Methanolic extract	10.00
	Control dentifrice	90.00

SP: Species, DC: De Candolle

dentifrice.<sup>[12]</sup> Test dentifrice containing *S. calva* root extract was prepared by taking dried root extract and mixed in 1:9 ratios (10% concentration) to control dentifrice [Table 1].

The commercial dentifrices; Arodent™ herbal dentifrice (IPSA Labs Pvt. Ltd., New Delhi, India) as well as Colgate® synthetic dentifrice (Colgate-Palmolive India Ltd., Mumbai) were purchased from local market. Dentifrice containing *S. calva* root extract as mentioned above was used as test dentifrice.

### Selection of subjects

The study comprised of a total of 80 healthy male subjects with age range 18-35 years. Subjects with

any obvious oral mucosal lesions, dental caries, and those wearing any prosthetic and orthodontic appliances were not included in the study. All procedures followed were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration. Participants were given verbal and written information on the aim and procedure of the study in their vernacular language, after which written informed consent was obtained. Information regarding socioeconomic status, dietary habits and oral hygiene methods was also acquired. Oral hygiene status was evaluated by a senior periodontist.

### Methodology

Individuals were randomly selected for the analysis and were divided into 4 groups. Group 1 comprised of subjects assigned to use Test Dentifrice (with *S. calva* root extract), while Group 2 comprised of subjects assigned to use Herbal Dentifrice (Arodent™), Group 3 comprised of subjects assigned to use Synthetic Dentifrice (Colgate®) and Group 4 i.e., Control Group comprised of age and sex matched subjects assigned to use Control Dentifrice without any antimicrobial agent.

The subjects were advised not to consume any food for at least 2 h and not to brush in morning (on the same day) before the experiment. Medium sized toothbrushes with nylon bristles (Colgate®) were provided to each subject. They were instructed to use 1 g of dentifrice during the experiment and brush by roll method for 2 min.

### Collection of saliva

Salivary samples were collected between 9.00 am and 12.00 pm. The diurnal variation of saliva is very low during this time. In a review of previous studies, Ferguson *et al.* reported low values of salivary alpha-amylase in early morning and high values in the afternoon.<sup>[13,14]</sup>

The subjects were asked to stimulate chewing action with sterile cotton rolls. The saliva, thus collected over the next 30 s was swallowed by the subjects. This procedure was carried out in order to clear any residual unstimulated saliva. The subjects were then made to chew the sterile cotton rolls again for next 2 min and the saliva thus stimulated was allowed to pool in the mouth. At the end of 2 min, the subjects were made to expectorate into sterile test tubes. Saliva was again collected repeating the same procedure after the use of respective dentifrices.

### Estimation of salivary amylase

Production of maltose from starch by salivary amylase was detected according to Bernfeld method.<sup>[15]</sup> The rate of reaction was measured by the amount of substrate(s) that were utilized and the amount of product that was formed in unit time. Maltose reacts with alkaline dinitrosalicylic acid (DNSA) to give an orange color. Maltose assay is stoichiometric and was used to estimate the amount of maltose formed. Saliva sample was centrifuged at 3000 rpm for 15 min and the supernatant was diluted with distilled water in 1:10 ratios (Saliva 0.025 ± 0.225 ml distilled water) and used as enzyme source. One percent starch solution was used as substrate and it was prepared by adding 1 g of soluble starch powder to 100 ml of phosphate buffer (0.1N, pH 6.7). This solution was mildly heated for dissolving starch and then it was cooled to room temperature and filtered.

Twelve test tubes were taken for each case and those tubes were arranged in “a” and “b” rows of 6 tubes each and labeled accordingly 1a to 6a and 1b to 6b. 1 ml of substrate (10 mg/ml) was poured in each tube and 0.250 ml of supernatant, i.e., enzyme source was added to each tube. Immediately, after addition of enzyme source, 0.5 ml stop reagent was added to 1<sup>st</sup> row in all the tubes and these were labeled as 0 h samples. Tubes in 2<sup>nd</sup> row were incubated for 15 min at 37°C, at the end of which, the reaction was stopped by adding 0.5 ml 2N NaOH. 0.5 ml DNSA reagent was then added to all the tubes and mixed well. All the tubes were kept in boiling water for 5 min. After cooling 20 ml distilled water was added to all tubes. The intensity of the color developed was measured at 520 nm in a Spectrophotometer (Systronics, India). A mixture containing starch solution, distilled water and DNSA reagent was taken as blank and was used to set 100% transmittance. A standard graph of maltose was prepared and used as reference to measure the corresponding amount of maltose formed per ml of saliva (mg/ml).

### Statistical analysis

Statistical analysis was done according to standard methods, and paired *t*-test was applied. Standard deviation was calculated by computerized method using Graph pad prism-4 software. *P* value observed at level of 5% (0.05).

## RESULTS

Salivary amylase activity was assayed before and after

**Table 2: Effect of test dentifrice (containing *Spilanthes calva* root extract), herbal and synthetic dentifrices on human salivary amylase activity (mg maltose/ml)**

Groups	Pre-application	Post-application	Difference	Probability
Group 1 test dentifrice	8.08 ± 0.60	4.11 ± 0.33	3.97 ± 0.46 (maximum)	( <i>P</i> < 0.05) significant
Group 2 Arodent™	8.01 ± 0.66	6.05 ± 0.83	1.96 ± 0.69	( <i>P</i> < 0.05) significant
Group 3 Colgate®	8.22 ± 0.68	6.73 ± 0.95	1.49 ± 0.41	( <i>P</i> < 0.05) significant
Group 4 control dentifrice	7.58 ± 0.69	7.40 ± 0.92	0.18 ± 0.13 (minimum)	( <i>P</i> > 0.05) not significant

treatment with dentifrices. Before using the respective dentifrices, subjects of Group 1 (using test dentifrice), Group 2 (using Arodent™ Herbal dentifrice), Group 3 (using Colgate® dentifrice) and Group 4 (using control dentifrice) showed mean salivary amylase activity of 8.08 ± 0.60, 8.01 ± 0.66, 8.22 ± 0.68 and 7.58 ± 0.69 mg maltose/ml respectively. After treatment with respective dentifrices, there was significant inhibition (*P* < 0.05) of salivary amylase activity in all experimental groups except in Group 4 (using control dentifrice) [Table 2].

Maximum inhibition of salivary amylase activity was found in Group 1 using test dentifrice containing *S. calva* root extract followed by herbal and synthetic dentifrices [Table 2].

## DISCUSSION

Salivary amylase has been considered significant for oral health. It is one of the most important components of human saliva and has distinct biological functions. The enzymatic activity of alpha-amylase undoubtedly plays a role in carbohydrate digestion.<sup>[1,2]</sup>

The fact that, amylase is also found in acquired enamel pellicle suggests a role in the adhesion of alpha-amylase-binding bacteria. All the biological activities seem to depend on an intact enzyme conformation. Binding of alpha-amylase to bacteria and teeth have important implications for dental plaque and caries formation.<sup>[16]</sup> Surface exposed amylase-binding protein A expressed on bacterial surface helps in this binding.<sup>[17]</sup> Amylase bound to bacteria in plaque may facilitate dietary starch hydrolysis to provide additional glucose for metabolism by plaque microorganisms in close proximity to the tooth surface. The resulting lactic acid produced is added to the pool of acid in plaque to contribute to tooth demineralization leading to dental caries.<sup>[16]</sup> Hence, inhibition of salivary amylase is essential for prevention of dental caries and plaque associated oral diseases.

In the present study, exposure to all three experimental dentifrices resulted in reduction of salivary amylase

activity. Maximum inhibition of salivary amylase activity was observed with test dentifrice containing *S. calva* root extract as compared to herbal and synthetic dentifrices [Table 2].

In inhibition of enzyme activity, role of secondary metabolites have been reported. Secondary metabolites like aromatic, polycyclic alkaloids, ellipticine, emetine and phenolics such as ellagic acid, anthraquinones act by binding to DNA and impair various DNA dependent reactions such as those catalyzed by enzymes like RNA polymerase, DNA polymerase, DNA ligase, DNA helicase and topoisomerase I and II.<sup>[18]</sup> Quinones target the microbial cell surface adhesins; cell wall polypeptides and membrane bound enzymes.<sup>[19]</sup> Flavones, flavonoids and flavonols have ability to form complexes with bacterial cell walls and may also disrupt microbial membranes.<sup>[20]</sup>

Role of tannins have also been suggested, Tannins have the ability to inactivate microbial adhesins, enzymes, cell envelope and transport proteins<sup>[21]</sup> whereas lipophilic terpenoids and essential oils are involved in membrane disruption.<sup>[22]</sup> Alkaloids have the ability to intercalate with DNA<sup>[23]</sup> while lectins and polypeptides act by forming ion channels in the microbial membrane.<sup>[24]</sup>

Some antimicrobial agents such as phenolics and polyphenols directly act on cell wall and cell membrane causing leakage and release of protoplasmic contents. Phenolics and polyphenols (e.g., Catechol and Pyrogallol) are toxic to pathogenic microorganisms due to enzyme inhibition or through more nonspecific interactions with the proteins.<sup>[25]</sup>

The inhibitory effect of herbal green tea<sup>[26]</sup> and black tea extract on human salivary amylase has been established by many authors previously.<sup>[27-29]</sup> Green tea is a popular drink throughout the world, and it contains various components, including the green tea polyphenol epigallocatechin gallate (EGCG). Peptide mass fingerprinting indicated that the major proteins precipitated by EGCG were alpha-amylase, S100, and cystatins. In addition, EGCG inhibited the activity

of alpha-amylase by non-competitive inhibition, indicating that EGCG is effective at inhibiting the formation of fermentable carbohydrates involved in caries formation.<sup>[30]</sup>

In present study, the comparatively better inhibition of amylase activity by test dentifrice containing *S. calva* root extract has been demonstrated. It has been reported that, alkamide (a secondary volatile metabolite) is present in genus *Spilanthes* which account for most of its enzyme inhibitory activity. However, most abundant alkamide found is spilanthal. The flower heads and root part of the plant genus have been reported to be rich in this active principal content.<sup>[31]</sup> In *S. calva*, another secondary metabolite, i.e., Flavonoid glucoside has also been reported.<sup>[5]</sup> Further studies are needed to know the exact nature of substance causing enzyme inhibition.

## CONCLUSION

Based on our study, there is reason to believe that the *S. calva* root extract possess significant salivary amylase enzyme inhibitory activity. Suppression of salivary amylase activity can lead to decrease in risk of dental caries and other plaque associated oral diseases. Use of *S. calva* root extract singly or in combination with other herbal/synthetic dentifrice will enhance their activity and will provide a wider protection against different pathogenic oral microflora. So, the use of this extract singly or in combination is strongly recommended in dentifrice preparations.

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