

Progression of Dental Caries and Evaluation of the Application of Topical Fluoride Combined with Natural Product in School Going Children

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Abstract: Fluoride is a safe and effective agent that can be used to prevent and control dental caries. Fluoride can be delivered topically and systemically. Topical fluorides strengthen teeth already present in the mouth, making them more decay resistant, while systemic fluorides are those that are ingested and become incorporated into forming tooth structures. Systemic fluorides also provide topical protection because fluoride is present in saliva, which continually bathes the teeth. Tea can be used as antioxidant, antimutagenic and anticariogenic. It is used to improve oral health including dental caries, periodontal disease and tooth loss, abolition of halitosis, oral malignancy prevention and regression. Studies on the development of anti-plaque agents in the prevention of dental caries have investigated the effect of some tea preparations and their individual components on the glucan synthesis catalyzed by glucosyl transferase from mutans streptococci. Extracts of tea combined with topical fluoride showed appreciable inhibition of the dental caries prevention. For bacterial screening four Gram-positive and four Gram-negative bacteria was used against crude acetone and chloroforms extracts at a concentration of 200 µg/ml and 400 µg/ml. Upon antibacterial screening, the crude ethyl acetate extract of *C. sinensis L.* extract showed highest activity against *S. mutans* than most of other organisms. A single compound (SR-1) isolated from the crude ethyl acetate extract of *C. sinensis L.* (Black Tea), having RF value 0.73 showed highest antibacterial activity against *S. mutans* among the four Gram positive and four Gram negative bacteria at a concentration of 200 µg/ml and 400 µg/ml. The zone of inhibition are 16 mm and 24 mm when extract used at a concentration 200 µg/ml and 400 µg/ml, respectively. These zone of inhibition are more than that of the standard kanamycin which showed only 13 mm. This is perhaps due to the partial resistance of Kanamycin against *S. mutans*.

I. INTRODUCTION

Dental caries is a progressive irreversible bacterial damage to the tooth tissues. One of the most major causes of all diseases and major causes of tooth loss. It is a biofilm-related oral disease, which continues to afflict the majority of the World's population.

The disease results from the interaction of specific bacteria with constituents of the diet within a biofilm formed on the tooth surface clinically known as dental plaque. Although additional microorganisms may be also involved, *Streptococcus mutans* plays a key role in the pathogenesis of the disease. This bacterium is able to: (i) produce and tolerate acids; (ii) synthesize water-insoluble glucan from sucrose through the activity of glucosyl transferases (GTFs); and (iii) adhere tenaciously to acquired pellicle on tooth surfaces [1]. The combination of these virulence properties allows *S. mutans* to effectively colonize tooth surfaces and modulate the transition of nonpathogenic to highly cariogenic dental biofilms, which leads to caries formation [2,3]. Therefore, approaches aimed at inhibiting the viability and virulence properties of *S. mutans* could be precise and selective for the prevention of dental caries. *Dryopteris crassirhizoma* is a semi-evergreen plant that grows on the deciduous forest floor as a pteridophyte [4]. Considering safe use of herbal medicines, they are gaining importance all over the world and Bangladesh is no exception. Such as Ayurvedic, Unani and other system of medicinal treatment. (Bangladesh has a rich heritage of herbal medicine.

A point that should be mentioned is that the *in vitro* property of green tea has also been much investigated previously, but *in vivo* evidence able to establish its real contributions to caries reduction is not consistent for these reasons a non invasive method of *in vivo* investigation has been developed [5].

The tea plant has shown the antimicrobial efficiency against a variety of pathogenic microorganisms and beneficial effects of tea have been attributed to its strong antioxidant activity which in turn is due to the phenolic compounds [6]. The carotenoids, tocotrienols, flavonoids, cinnamic acid, benzoic acid, ascorbic acid, folic acid, tocopherols are some antioxidants generated by the plants [7]. Different phytochemicals present in green tea are associated with prevention of specific diseases; therefore it is important to consider the preparation method of green tea which generates highest amount of phytochemicals.

II. MATERIALS AND METHODS

This is a retrospective investigation approved by the Institute of Biological Sciences, University of Rajshahi and Ethical Review Board. The study was conducted at Rajshahi

Medical College Dental Unit and Entomology and Insect Biotechnology Laboratory IBSc, RU. This experimental clinical comparative study was conducted by following target groups of patients who had received treatments of dental caries in their primary teeth. Data were collected by reviewing charts, identifying patients who met our criteria, and recalling them for follow-up appointments. After sufficient data were accumulated, survival rates were evaluated, analyzed, and interpreted within the context of a developing treatment protocol.

• **Selection criteria for the examined teeth were:**

- Primary teeth with deep carious lesion: no history of spontaneous or night pain, swelling, presence of fistula or tooth mobility
- Primary teeth with vital pulp exposure as a result of carious process, with hemorrhage at the site of exposure
- Absence of radiographic evidence for internal or external root resorption or radiolucency in furcation area.
- No more than 1/3 physiological root resorption
- Possibility for further tooth restoration

• **Sample**

The subjects selected for this investigation were treated at a pediatric private practice in between January 2017 to December 2018. Informed consent for treatment and academic use was obtained from guardians prior to treatment.

• **Sample-Size Calculation**

The sample size required to achieve 90% power and 0.05 alpha levels using a Fisher Exact test was determined to be 480 patients.

• **Data Collection**

Each patient was designated a numerical code for purposes of maintaining anonymity. Data collected for each numerical entry included date of birth (age), gender, the tooth treated, the treatment date, follow-up time in months, clinical notes regarding the treated tooth at follow-up or recall intervals, and condition of the contralateral tooth. The condition of the contralateral tooth served as a control for the treated tooth, in evaluating exfoliation times. The contralateral tooth was designated as: absent (A), untouched (U), restored with amalgam or stainless steel crown (R), pulpotomy with

stainless steel crown (P), or root canal treatment (E). In the event that the condition of the tooth changed the final condition of the tooth was recorded. The presence and the condition or orientation of the succedaneous teeth on both the treated side and the contra-lateral side were noted. Normal (N) was noted for lack of positional abnormality (P) and uniform or expected radio-density of the succedaneous tooth (I). At recall dates of at least six months, clinical and radiographic observations were noted as per the criteria outlined for success and failure. These observations were noted until the treated and contralateral teeth exfoliated or were extracted. All teeth that demonstrated radiographic failures were followed to exfoliation or extraction. Additional radiographic failures were noted until the teeth were extracted or exfoliated

• **Statistical Methods**

The analysis was carried out using Statistical Product and Service Solution (SPSS) software (20.0 Window, SPSS International, Chicago, Ill).

III. RESULTS

In the target group of 480 participants, were treated with natural compound along with topical fluoride materials between the periods of July, 2018 to December, 2019. A completed consent forms and follow-up appointments were strictly followed for this study. This group of patients was frequently found to be non-compliant and hard to follow-up, changing phone numbers and moving often. Consequently, it was difficult to contact them to return for regular dental appointments.

A. *Characterization of Compound*

Chemical study of the compound showed the negative test for alkaloid and steroid but showed positive test for flavonoid with Salkowski and Liebermann-Burchard reagent. A (12-20), (21-2), (-56) Number test tubes are mixed with each other and dried, a single spot is detected after doing the TLC of 12 to 20 test tubes) B,C (TLC was done of the dried sample of (21-32), (33-56), Number test tube in different solvent ratio and (CHCl₃ :MeOH-9 : 2) Give the best result.

Spectral analysis of the compound SR-2 was presumed to be identical with Phenolic acid and Flavanols type compound isolated previously from the plant *C. sinensis* L.

The structure of the compound is given below-

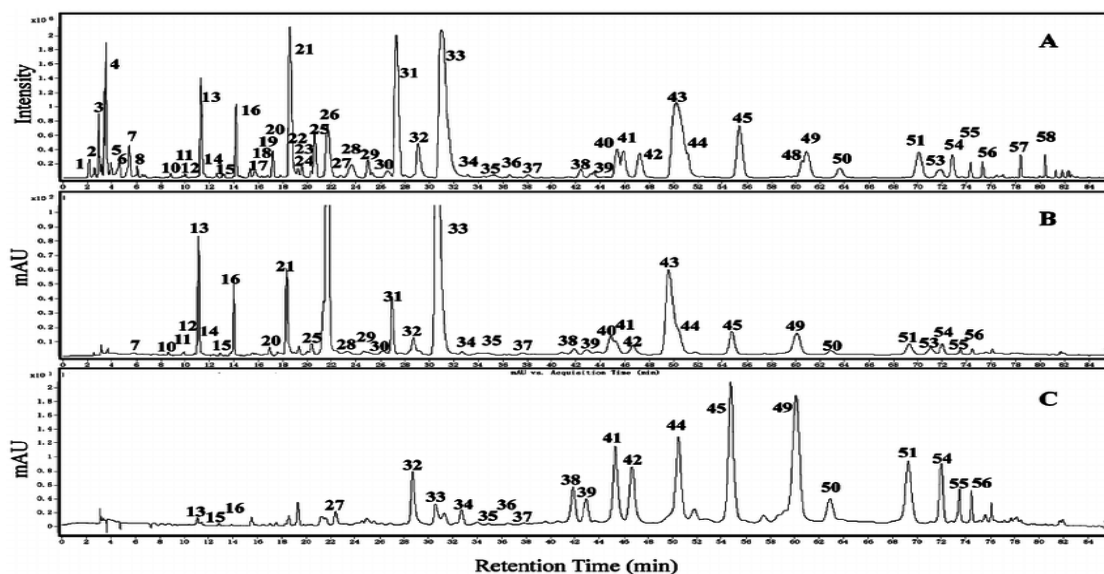


Fig. 1: Phenolic acid and Flavanols.

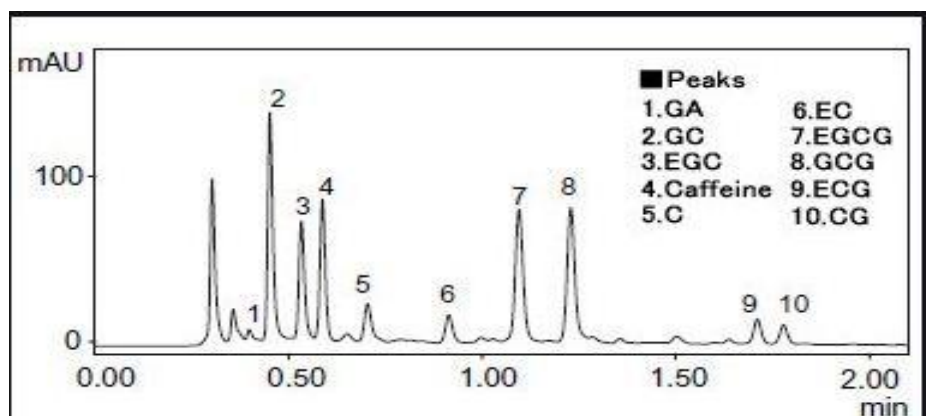


Fig. 2: Phenolic acid and Flavanols from *Camellia sinensis* L

Phenolic acid: Gallic acid Flavanols:

- Catechin (C)
- Gallocatechin (GC)
- Epicatechin (EC)
- Epigallocatechin (EGC)
- Epicatechin gallate (ECG)
- Epigallocatechin gallate (EGCG)
- Gallocatechin gallate (GCG)
- Catechin gallate (CG)
- Caffeine

B. Microbiological Investigation

The most important and effective *in vitro* antibacterial screening of crude extracts or pure compounds is disc diffusion method. This method is simple, using discs of filter paper, put on petridishes containing nutrient broth media where test micro-organisms were cultured. Test

samples were then applied on the filter paper discs in varying concentrations, kept in refrigerator for 12 hours at low temperature (4°C) for maximum diffusion and then incubated at 37°C for bacterial growth. In that case bacterial growth is not observed on the disc. This is known as zone of inhibition. The larger the zone of inhibition the greater the activity of the applied sample and is calculated by measuring the diameter of the zone of inhibition.

The *Streptococcus mutans* bacteria along with others causing dental caries, were isolated from the sample of 480 patients having dental plaque and tested against the chloroform and ethyl acetate extracts of two plants or plant parts for their antibacterial activity (Table-1). Among these two medicinal plants, showed from moderate to highest antimicrobial activity. These two plants were selected for their highest antimicrobial activity against *Streptococcus mutans*.

Specimen (Plant) sample Patient sample	<i>Camelia sinensis</i> L. (Green Tea) Zone of inhibition (mm)	<i>Camelia sinensis</i> L. (Black Tea) Zone of inhibition (mm)
1	13	12
2	12	15
3	14	16
4	19	14
5	22	18

Table 1: Selected plants specimen showing antibacterial sensitivity.

All the two plants showed activity against the dental caries causing bacteria. Here 1 mg/disc were used for the test. Upon antibacterial screening, the extracts showed good activity against the dental plaque causing bacteria. The

processes were continued at a concentration of 2 mg/disc and 3 mg/disc. The results of the antibacterial screening measured in term of zone of inhibition (mm) as shown in table.

Specimen (Plant) sample Patient sample	<i>Camelia sinensis</i> L. (Green Tea) inhibition (mm)	<i>Camelia sinensis</i> L. (Black Tea) Zone of inhibition (mm)
1	15	13
2	16	11
3	13	16
4	14	15
5	17	18

Table 2: Anti bacterial activity test result for selected specimen (2mg/disc). zone of inhibition (mm)

Specimen (Plant) sample Patient sample	<i>Camelia sinensis</i> L. (Green Tea) inhibition (mm)	<i>Camelia sinensis</i> L. (Black Tea) Zone of inhibition (mm)
1	16	15
2	19	17
3	18	20
4	21	23
5	23	19

Table 3: Anti bacterial activity test result for selected specimen (3mg/disc). zone of inhibition (mm)

Plants screened for antibacterial activity against those bacteria causing dental caries, *Streptococcus mutans* bacteria along with others causing dental caries, were isolated and tested with the above plant extracts. Extracts of two plants showed highest antibacterial activity, namely *Camelia sinensis* L. (Green Tea) and *Camelia sinensis* L. (Black Tea.)

To isolate the active compound from the selected plants which show highest activity against the isolated pathogenic bacteria. The structure of the isolated

compounds was determined using spectroscopic methods of analysis.

Upon antibacterial screening, among the crude chloroform and ethyl acetate extracts, the crude acetone extracts of *Camelia sinensis* L. (Green Tea) extract showed highest activity against *Streptococcus mutans* than most of the other Gram-positive and Gram-negative bacteria cited (Table-4). The concentrations was 200 µg/disc and 400 µg/disc. The results of the antibacterial screening measured in term of zone of inhibition as shown (Table-4).

Bacterial strains	Zone of inhibition, diameter in mm				
	Chloroform extract		Ethyl acetate extract		Standard antibiotic kanamycin 30 µg/disc
	200 µg/disc	400 µg/disc	200 µg/disc	400 µg/disc	
Gram positive					
1. <i>Staphylococcus aureus</i>	11	13	12	18	11
2. <i>Streptococcus mutans</i>	13	19	11	16	14
3. <i>Bacillus subtilis</i>	12	15	13	15	10
4. <i>Bacillus cereus</i>	11	16	14	19	12
Gram negative					
1. <i>Escherichia coli</i>	11	14	11	15	13
2. <i>Shigella dysenteriae</i>	12	16	11	14	15
3. <i>Shigella shiga</i>	11	15	12	16	13
4. <i>Salmonella typhi</i>	10	15	13	15	12

Table 4: In vitro antibacterial activity of two fractions of the *Camelia sinensis* L. (Green Tea) extract.

A mixture of two compounds (very close spots on TLC) isolated from chloroform extract of *Camelia sinensis*

L. (Black Tea) was tried to separate using conventional methods, but failed. These mixture (SR-1) of two

compounds showed strong antibacterial activity against *S. mutans*. The result of this test is tabulated (Table-5).

From the table-5 it is seen that the zone of inhibition due to compound (SR-1) is much larger than that of the standard kanamycin. When 200 µg/ml of compound (SR-1) used, the zone of inhibition is 19 mm while in case of 400

µg/ml it is in 24 mm. But in case of Kanamycin (30 µg/ml) the zone of inhibition is only 11 mm. Which indicates, perhaps Kanamycin that was used for the test my partially resistant to *S. mutans*. the mixture of compounds (SR-1) used as for spectral analysis.

Bacterial strains	Zone of inhibition, diameter in mm		
	200 µg/disc	400 µg/disc	kanamycin 30 µg/disc
Gram positive			
1. <i>Staphylococcus aureus</i>	12	16	10
2. <i>Streptococcus mutans</i>	19	24	11
3. <i>Bacillus subtilis</i>	13	15	12
4. <i>Bacillus cereus</i>	11	15	10
Gram negative			
1. <i>Escherichia coli</i>	12	12	10
2. <i>Shigella dysenteriae</i>	12	14	13
3. <i>Shigella shiga</i>	11	15	10
4. <i>Salmonella typhi</i>	13	16	12

Table 5: In vitro antibacterial activity of compound SR-1.

For bacterial screening four Gram-positive and four Gram-negative bacteria was used against crude acetone and chloroforms extracts at a concentration of 200 µg/ml and 400 µg/ml.

Upon antibacterial screening, the crude ethyl acetate extract of *C. sinensis* L. extract showed highest activity against *S. mutans* than most of other organisms. The results of the antibacterial screening measured in term of zone of inhibition are shown in Table-6.

Bacterial strains	Zone of inhibition, diameter in mm				
	Methanol extract		n-Hexane extract		Standard antibiotic kanamycin 30 µg/disc
	200 µg/disc	400 µg/disc	200 µg/disc	400 µg/disc	
Gram positive					
1. <i>Staphylococcus aureus</i>	11	14	10	15	10
2. <i>Streptococcus mutans</i>	12	15	13	17	14
3. <i>Bacillus subtilis</i>	11	12	12	14	12
4. <i>Bacillus cereus</i>	10	11	13	14	14
Gram negative					
1. <i>Escherichia coli</i>	10	13	12	14	10
2. <i>Shigella dysenteriae</i>	11	14	11	13	12
3. <i>Shigella shiga</i>	11	13	11	15	11
4. <i>Salmonella typhi</i>	10	12	12	14	13

Table 6: In vitro antibacterial activity of three fractions of the *Camelia sinensis* L. extract

A single compound (SR-1) isolated from the crude ethyl acetate extract of *C. sinensis* L. (Black Tea), having RF value 0.73 showed highest antibacterial activity against *S. mutans* among the four Gram positive and four Gram negative bacteria at a concentration of 200 µg/ml and 400 µg/ml.

The zone of inhibition are 16 mm and 24 mm when extract used at a concentration 200 µg/ml and 400 µg/ml,

respectively. These zone of inhibition are more than that of the standard kanamycin which showed only 13 mm. This is perhaps due to the partial resistance of Kanamycin against *S. mutans*.

In combined with natural product and topical flouride showed the best performace and used at a concentration of 150 µg/ml disc.

Bacterial strains	Zone of inhibition, diameter in mm			
	200 µg/disc	400 µg/disc	kanamycin 30 µg/disc	Combined with natural product 150 µg/disc
Gram positive				
1. <i>Staphylococcus aureus</i>	13	14	12	16
2. <i>Streptococcus mutans</i>	16	24	13	24
3. <i>Bacillus subtilis</i>	15	19	10	20
4. <i>Bacillus cereus</i>	12	16	12	16
Gram negative				
1. <i>Escherichia coli</i>	10	10	11	12
2. <i>Shigella dysenteriae</i>	12	14	12	15
3. <i>Shigella shiga</i>	10	16	12	17
4. <i>Salmonella typhi</i>	12	16	10	18

Table 7: In vitro antibacterial activity of compound SR-1

IV. DISCUSSION

Dental caries is an important public health predicament. The unique characteristic of dental diseases is that they are universally prevalent and do not undergo diminution or termination if untreated and require technically demanding expertise and time consuming professional treatment. The risk factors should be comprehensively studied so that the occurrence of dental caries can be prevented [8] Many attempts have been made to eliminate *S. mutans* from the oral flora, antibiotics such as penicillin; ampicillin, tetracycline, erythromycin and vancomycin are very effective in preventing dental caries in vivo and in vitro. However, their excessive use can result in alterations of the oral and intestinal flora and cause undesirable side effects. [9]

Tea is an infusion of the leaves of the *Camellia sinensis* plant which grows mainly in South East of Asia. Tea is the most popular beverage in the world after water, Drinking Green Tea, a suggestive of health beverage is common for more than 2000 years. In the last couple of year there is a growing interest in green tea in the western world due to scientific findings that show the health potentials of the beverage. Green tea has a unique composition, which includes proteins such as cellulose, pectin, glucose, fructose and sucrose and lipid components: linoleic and linolenic acids and sterols such as stigmasterol. Besides macronutrients, green tea also includes vitamins, pigments such as chlorophyll and carotenoids. [10] Other important green tea components are the polyphenols which constitute the most interesting group amongst the components of green tea leaves. The main polyphenols in green tea are catechins. The four main catechins are epigallocatechin a 3 gallate (EGCG), epigallocatechin (EGC), epicatechin 3 gallate (ECG), epicatechin (EC). Research suggests that green tea has an antioxidant property. Green tea is a non fermented tea and contains more catechins. Catechins are *in vitro* and *in vivo* strong antioxidants. These catechins possess antimutagenic, antidiabetic, anti inflammatory, antibacterial and anti-viral properties [11].

Green Tea can be used as antioxidant, antimutagenic and anticariogenic. It is used to improve oral health including dental caries, periodontal disease and tooth loss, abolition of halitosis, oral malignancy prevention and regression. It has antihypertensive effect and it reduces cardio vascular disease

risk. It helps in body weight control and also helps in glucose tolerance and insulin sensitivity. Polyphenols constitute one of the most common and widespread groups of substances in plants. The main sources of polyphenols present in the human diet are plants like tea, coffee, cereals and fruits. Subsequent *in vitro* studies on plant extracts suggest an activity against several metabolic activities of *mutans streptococci*, resulting in decrease in growth and virulence. Smullen *et al* have shown that extracts from unfermented green tea have a bacteriostatic effect on *streptococcus mutans* [12]. Studies on the development of anti plaque agents in the prevention of dental caries have investigated the effect of some tea preparations and their individual components on the glucan synthesis catalyzed by glucosyl transferase from *mutans streptococci*. Extracts of tea combined with topical fluoride showed appreciable inhibition of the dental caries prevention.

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