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Anticariogenic potential of *Potentilla fulgens* extract and its chemical constituents

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Abstract

Streptococci and *Lactobacilli* are the most common bacteria causing dental caries. *Potentilla fuglens*, native of north-east India is used by tribal people to cure teeth and gum problems. Based on its ethomedical use, its anticariogenic potential was evaluated. The crude methanol extract was found to have good anticariogenic potential. Epigallocatechin gallate (4) was found to the most effective inhibitor against the tested bacterial strains. Epiafzelechin (4β 8) epicatechin (8) also demonstrated comparatively better activity than other dimeric compounds (MIC of 1.56, 3.12 and 3.12 µg/mL against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*, respectively). Time-kill studies and biofilm formation inhibition assays showed molecule 8 to be comparable to compound 4 in terms of antibacterial action. The results suggest that these compounds and Potentilla extracts could be employed as natural antibacterial agents in oral health care products.

Keywords: *Potentilla fuglens*, *Streptococcus mutans,* Anticariogenic, Dimeric flavan-3-ols, Time-kill study, Biofilm inhibition

Introduction

Dental caries, a multifactorial complication is an outcome of interaction between dietary factors, oral environment and bacteria. Of these three factors, bacteria, *Streptococci* and *Lactobacilli* are the major players in causing incidence and prevalence of dental caries among the people of different age groups. The bacteria attach to the teeth enamel and form a glycocalyx by synthesizing sticky glucan by action of bacterial enzymes, glucosyl transferases on sucrose resulting in formation of plaque. Bacteria produce acid inside plaque. This low pH demineralises the tooth enamel. These bacteria are very efficient in trapping their substrates, generally carbohydrates from the diet or its left overs in the mouth over a considerable period of time. Several anti-bacterial agents, synthetic as well as natural products, are reported to combat the damage caused by cariogenic bacteria to teeth enamel [1-2]. Chlorhexidine is considered as a gold standard in treatment of dental caries; however its long term use unveils unexpected side-effects, like teeth staining, and taste change, desquamations and soreness in the oral mucosa restricting its routine use [3]. There is a need for continuous research and discovery of newer anticariogenic agents.

Plant polyphenols have attracted substantial attention in past several years as potential anticariogenic agents. Polyphenols are known to prevent oral diseases by inhibiting bacterial replication enzymes, inducing apoptosis in tumour cells, stimulating monocytes/macrophages to produce cytokines, triggering myeloperoxidase-dependent iodination of neutrophils and inactivating bacterial toxins [4-6]. Plants from different families, including Asteraceae, Fabaceae, Rosaceae, Poaceae, Lythraceae, Geraniaceae, Onagraceae, Polygonaceae, Primulaceae, and Verbenaceae are reported to possess bactericidal action. Among these families, members of the Geraniaceae and Rosaceae families are a rich source of polyphenolic compounds with antimicrobial activity [7-9].

Genus *Potentilla,* a member of Rosaceae family, is a rich source of polyphenols, reported to contain a variety of structurally diverse polyphenols including, hydrolysable tannins and flavanoids [10-12]. *Potentilla recta* extracts and its various subfractions obtained by partitioning with solvents of different polarity (aqueous, 50% ethanol, diethyl ether, ethyl acetate and *n*-butanol) have demonstrated *in vitro* inhibitory effects against cariogenic *Streptococcus* spp. strains. These extracts also inhibited the growth of oral *Streptococci* and had inhibitory effect on water-insoluble

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mutan and artificial dental plaque formation. The highest antibiofilm activities especially against *S. sobrinus* GCM 20381, with minimum mutan and biofilm inhibition were observed with ethyl acetate extract at concentrations of 6.25 and 25 µg/mL, respectively. In another study on *Potentilla recta,* a few isolated compounds namely, methyl brevifolincarboxylate, tiliroside (kaempferol 3-O-β-D-(6''-*E*-p-coumaroyl)-glucopyranoside), ellagic acid 3, 3'-di-O-methyl ether 4-*O*-β-D-xylopyranoside, apigenin 7-*O*-β-D-glucopyranoside and luteolin 7-*O*-β-D-glucopyranoside were evaluated against cariogenic *Streptococcus* spp. strains (*Streptococcus mutans* CAPM 6067, *Streptococcus sobrinus* CAPM 6070, *Streptococcus sobrinus* GCM 20381 and *Streptococcus sobrinus*/*downei* CCUG 21020) [13].

Potentilla fulgens, a native of north-east India is a commonly used plant among the traditional folks for treatment of gastrointestinal and respiratory tract ailments [14]. Root powder of this plant is generally consumed to strengthen the teeth and for management of dental complications [15]. Recently, a few reports on its cancer preventing and anti-diabetic properties have been documented [16-17]. We have earlier described isolation and structure elucidation of two new triterpene compounds along with several flavan-3-ol type of compounds from roots of *Potentilla fulgens* [18,19]. In continuation of the phytochemical investigations and recognizing the role of polyphenols in dental caries management, we planned to undertake extensive studies to evaluate anticariogenic effects of the plant and search for anticariogenic phenolic molecules. The present investigation was undertaken to screen different polyphenols from the extracts of *Potentilla fulgens* roots and evaluate its anticariogenic activities with an aim to identify the best bioactive molecule.

Materials and methods

Equipment and apparatus

UV absorbance was recorded on Multiskan (Thermo scientific) 96 well plate reader and UV-3200 double beam spectrophotometer (Labindia). Scanning Electron Microscopy was performed on Hitachi Ion Sputter (JFC-1100, JEOL, Tokyo, Japan). SEM images were analyzed using electron beam of an acceleration potential of 1.2 kV.

Plant Material

Roots of *Potentiala fulgens* were collected from Shillong peak forest area of Meghalaya state in India. A voucher specimen (accession number 11906) has been deposited in herbarium of the Department of Botany, North-Eastern Hill University, Shillong. Roots were crushed to a coarse powder.

Cariogenic bacteria

Cariogenic bacteria were procured from Microbial Type Culture Collection (MTCC), IMTECH Sec-39 C, Chandigarh as a lyophilized powder form. These bacteria were revived by using MTCC specified selective growth medium and preserved as glycerol stocks. *Streptococcus mutans* (MTCC-890), *Lactobacillus acidophilus* (MTCC-10307) and *Lactobacillus rhamnnosus* (MTCC-1423) were used for the study.

Standardization of extract

The extraction of *Potentilla fulgens* roots and isolation of polyphenolic compounds (figure. 1), afzelechin (1), epiafzelechin (2), epigallocatechin (3), epigallocatechin gallate (4), Epicatechin (5), Catechin (6), afzelechin (4β 8) epicatechin (7), epiafzelechin (4β 8) epicatechin (8), catechin (4 8) epicatechin (9), afzelechin (4 8) catechin (10) and afzelechin (4 8) epiafzelechin (11) using semi-preparative HPLC has been reported in our previous communication. The crude methanol extract has been reported to contain monomeric, dimeric, trimeric, pentameric flavan-3-ols and the triterpenes as major constituents. Several flavanols and triterpenes have been estimated in the crude extract by HPLC-UV method. Ursolic acid (0.82 ± 0.05 %) and epicatechin (0.67 ± 0.03 %) were found to be the major compounds in the tested sample of roots [19].

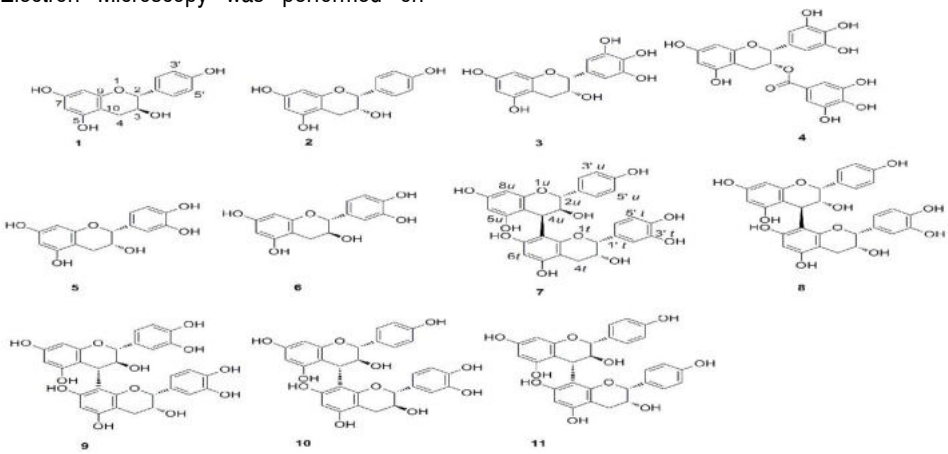

Figure 1: Chemical constituents isolated from ethyl-acetate extract of *Potentilla fulgens*

Inoculum Preparation

Inoculum were prepared freshly by adding bacterial suspension into the bacteria specific selective broth media, enriched brain heart infusion (EBHI) for *Streptococcus mutans* and de Man, Rogosa and Sharpe (MRS) for *Lactobacillus acidophilus* and *Lactobacillus rhamnnosus*. These inoculum were incubated at an optimal temperature (37 C) under shaking condition at 200 RPM in order to maintain approximately uniform growth rate. The bacterial cultures were compared with 0.5 McFarland turbidity standards, which is equivalent to approximately 1 x 10^8 bacterial cell counts per mL.

Antibacterial assay by agar diffusion method

The screening of antibacterial activity of methanol extract and its fractions was conducted using agar well diffusion method [20]. The test samples were dissolved in DMSO to a final concentration of 50 mg/mL. 100µL of prepared culture containing 10^8 CFU/mL of bacteria were spread on EBHI and MRS agar plates using sterile cotton swabs. Wells (6 mm in diameter) were punched off on a spread agar plate using sterile borer. The wells were filled with 50 µL of test sample. DMSO was used as negative control and chloramphenicol (10 µg/mL) was used as a positive control. The resultant plates were incubated for 48 h for bacteria to grow. Antibacterial activity was evaluated by measuring the zone of inhibition against every set of tested bacteria.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Two fold serial broth dilution method [21] was used to determine minimum inhibitory concentration of extracts and pure compounds. Selective broth medium was used for dilutions as well as preparing inoculum. The bacterial cell density was maintained to be 1X10^8CFU/mL by comparing with 0.5 McFarland turbidity standards. Stock solutions of extracts (5 mg/mL) and pure compounds (1 mg/mL) were prepared in DMSO. 100µL from the stock solution was added to first dilution tube containing 900µL of the selective medium broth and mixed well. From this tube, 500 µL was transferred to second tube containing 500 µL broth. This step was repeated nine times and from the last tube 500 µL of the solution was discarded. 100 µL of test organisms was added in each tube. The final volume of solution in each tube was made up to 600 µL. The MIC was tested in the concentration range between 1000-20 µg/mL for extract and 100 - 0.2 µg/mL for pure compounds (1-11). Chloramphenicol was used as a positive control. Tubes were incubated at an optimal temperature and time in an incubator under 200 RPM shaking conditions. MIC was determined spectro-photometrically to check the bacterial growth at 600 nm after 48 h of incubation in each tube. To determine MBC, a culture suspension of 100 µL was taken from each tube showing no visible growth, and subcultured onto Mueller Hinton agar. The plates were incubated aerobically at 37 C for 48 h. The lowest concentration of the extract/pure compound which completely inhibited growth was expressed as the MBC. Each assay was repeated thrice.

Time-Kill Studies

Rate of killing of bacteria causing dental cavities by the crude methanolic extract and isolated pure compounds was studied using a spread plate technique [22]. The extract and test compounds were incorporated into 10 mL Brain Heart Infusion (BHI) broth in 50 mL conical flask at ½ MIC, MIC and 2 MIC. BHI broth without test compound or extract, inoculated with test organism (*Streptococcus mutans* MTCC-890) was used as positive control. The solutions of extract and test compounds were made sterile by filtration with 0.2 µm PVDF filters. The flasks containing 9 mL of BHI broth were inoculated with 1 mL of *Streptococcus mutans* culture (Inoculum density, in range of 10^7CFU/mL). It was verified by total viable count. The flasks were incubated at 37 C on rotary shaker at 200 rpm. A 100 µL aliquot was removed from the culture medium at 0, 4 and 8 h for the determination of CFU/mL by the plate count technique by plating out 20 µL sample. Bacterial colonies were counted, CFU/mL calculated after incubating at 37 C for 48 h. The suspension was then serially diluted and plated out for viable counts. Finally the comparison was made with the total viable count from positive control which was without extract or test compound. All plate counts were performed in duplicate.

Adherence Tests

Adhesion behaviour or inhibition of biofilm formation of *Streptococcus mutans* in the presence of isolated compounds and methanolic extract of *Potentilla fulgens* was examined [23]. Samples (200 µL) of culture of *Streptococcus mutans* containing 10^7 CFU/mL was added aseptically to the well of 96 well plate. The culture medium without isolated compounds was used as the non-treated control. The samples treated with isolated compounds and extract was added in other wells. They were incubated aerobically at 37 C for 36 h. The concentrations of all the compounds utilized for inhibition of biofilm were their respective MIC values. Media from each tube was decanted and planktonic cells were removed by washing with distilled water. Adherent cells were measured by absorbance at 600 nm.

Results and discussion

Various strategies to prevent dental caries include elimination of cariogenic bacteria from the oral cavity, inhibition of their plaque formation and the enhancement of tooth resistance to demineralization. In the first two strategies, plants and their isolated chemical constituents have been widely studied. Several plants are being used traditionally by people to maintain oral

hygiene. Moreover, a number of reports are available on potential inhibition of cariogenic bacteria by plant extracts, which further corroborates importance of phytochemicals in managing dental caries. Sunphenon, a mixture of flavonols isolated from leaves of *Camellia sinensis* comprising (+)-catechin, (+)-gallocatechin, (−)-epicatechin, (−)-epicatechingallate, (−)-epigallocatechin, and (−)-epigallocatechingallate is known to decrease cell viability of *Streptococcus mutans* JC-2 (Ferrazzano *et al.*, 2011). Erycristagallin isolated from *Erythrina variegata* has been reported to possess antibacterial activity against *Streptococci*, *Actinomycetes*, and *Lactobacilli* [7,8].

Preliminary phytochemical screening of *Potentilla fulgens* has shown presence of a variety of polyphenols in the roots of this plant [19]. The roots are traditionally used by people of North-east India to treat dental and gum problems. Hence, based on ethnomedical considerations and the literature review, standardized extract of *Potentilla fulgens* and its chemical constituents were investigated for anticariogenic activity.

Antibacterial assay by agar diffusion method

Effect of methanol extract and its various fractions on the growth of bacterial strains were tested by observing the presence or absence of zone of inhibition. Diameters (in mm) of these zones were measured and are presented in Table 1. Methanol extract displayed 25 ± 0.5, 21 ± 0.6 and 23 ± 0.3 mm zone of inhibition against *Streptococcus mutans, Lactobacillus acidophilus* and *Lactobacillus rhamnnosus* respectively. Further, the four fractions obtained by partitioning of methanol extract when evaluated in same assay also displayed antibacterial activity against cariogenic bacteria. Ethyl acetate extract displayed almost comparable inhibition of bacterial growth as was observed with methanol. Zone of inhibition measuring 23 ± 0.4, 21 ± 0.1 and 20 ± 0.4 mm were observed against *Streptococcus mutans, Lactobacillus acidophilus* and *Lactobacillus rhamnnosus* respectively with this extract.

The observed activity of these extracts can be attributed to the presence of various polyphenolic compounds in the *Potentilla fulgens* extracts.

Table 1. Anticariogenic activity of methanol extract and its fractions

S. No.	Plant extract	Zone of inhibition[a] (mm)		
		Streptococcus mutans	*Lactobacillus acidophilus*	*Lactobacillus rhamnnosus*
1	Methanol	25±0.5	23± 0.6	21±0.3
2	Hexane	7±0.2	9±0.3	7±0.7
3	Ethyl acetate	23±0.4	21±0.1	20±0.4
4	Butanol	19±0.7	16±0.2	18±0.2
5	Aqueous	10±0.5	8±0.7	11±0.5
6	Chloramphenicol	29±0.2	24±0.3	23±0.4

[a]Includes the disc diameter (6 mm), Vehicle control (10μL of DMSO) showed no activity.
Values are expressed as mean± SD (n=3).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Flavanol compounds have been reported to possess activity against Gram-positive bacteria than Gram-negative bacteria. They have potential to influence the process of dental caries formation at several different stages: they may inhibit proliferation of the streptococcal agent, interfere with the process of adhesion to tooth enamel or act as inhibitors of glucosyltransferase and amylase. Flavanols are reported to show inhibition against various bacterial strains (*Escherichia coli*, *Bordetella bronchiseptica*, *Serratia marcescens, Klebsiella pneumonie*, *Salmonella choleraesis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*) by generating hydrogen peroxide and by altering the membrane permeability.

MIC and MBC were determined for all the eleven isolated compounds. The results are summarized in Table 2. Among monomeric flavanols 1–6, compounds 2, 3 and 4 having *cis* stereochemistry at C2-C3 were comparatively more active than compounds 1 and 6 (*trans* stereochemistry at C2-C3). It was found that more the number of free hydroxyl groups in the compound, higher was the activity. Compound 4, epigallocatechin gallate (having eight free hydroxyl groups) containing galloyl units was more active than compound 3 (six free hydroxyl groups) which was in turn more active than compounds 2 and 5, having four and five free hydroxyl groups, respectively. Among dimeric flavanols, compound 8, epiafzelechin (4β 8) epicatechin was more active than isomeric compounds 7 and 10 having same number of hydroxyl groups but *cis* stereochemistry at C2-C3 in both subunits. Compound 8 was more active against *Streptococcus mutans* in comparison to *Lactobacillus acidophilus* and *Lactobacillus rhamnnosus*. All other compounds displayed MIC in the range of 3.12-12.5 μg/mL and MBC of 3.12-25 μg/mL.

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration of tested samples against oral pathogens

Compound	*Streptococcus mutans*		*Lactobacillus acidophilus*		*Lactobacillus rhamnnosus*	
	MIC (µg/mL)[a]	MBC (µg/mL)[a]	MIC (µg/mL)[a]	MBC (µg/mL)[a]	MIC (µg/mL)[a]	MBC (µg/mL)[a]
1	6.25	12.5	6.25	12.5	12.5	25
2	3.12	6.25	3.12	6.25	6.25	12.5
3	1.56	3.12	1.56	3.12	1.56	3.12
4	0.78	1.56	1.56	3.13	3.12	6.25
5	3.12	6.25	12.5	25	12.5	25
6	6.25	12.5	12.5	25	12.5	25
7	12.5	25	12.5	25	12.5	25
8	1.56	3.12	3.12	6.25	3.12	6.25
9	12.5	25	12.5	25	12.5	25
10	12.5	25	12.5	25	12.5	25
11	12.5	25	12.5	25	12.5	25
Extract	125	250	250	500	250	500
Chloramphenicol	2.5	5	2.5	5	2.5	5

[a]Values represent the average obtained from a minimum of three experiments

Time –Kill study

Five isolates and methanol extract from the *Potentilla fulgens* was evaluated for the anticariogenic effects over the *Streptococcus mutans* MTCC 890. Reduction in viability over time period was expressed in the form of Log10 CFU/mL. Traditionally, reduction of 3 Log10 CFU/mL viability termed as good bactericidal activity [24]. Serial dilutions of these compounds restricted the effects on the viability over the BHI agar plates. Three concentrations, ½ MIC, MIC and 2 MIC were used for testing antimicrobial effects. It was observed that after incubation of bacteria with compounds there was reduction in the viable counts of *Streptococcus mutans*. At ½ MIC instead of killing, it favoured the growth which is contrary with results shown at MIC and 2 MIC. Compounds 3, 4 and 8 found to be more efficacious than others, with 4 being most effective. The time-kill curves are presented in figure 2. Since the anticariogenic product or formulation should be effective within few minutes, readings were recorded after 10 min (0.6 h) of incubation. Crude methanol extract showed reduction by 0.32 Log_{10} CFU/mL after 10 min, while the isolated molecules were more effective as reduction by 0.8, 0.6, 1.7, 0.5, and 1.1 Log_{10} CFU/mL was observed with compounds 2, 3, 4, 5 and 8 at their respective MIC values. Compound 4 showed bacteriostatic effects over growth of *Streptococcus mutans* as observed by the reduction of 2.87 and 4.49 Log_{10} CFU/mL at MIC and 2 MIC respectively, after 8h. Compound 8 reduced the viability by 2.60 Log_{10} CFU/mL at MIC and 3.26 Log_{10} CFU/mL at 2 MIC after 8 h, indicating its almost comparable bacteriostatic action to that of compound 4. On further incubation, bacteriostatic effects of compounds started to fade away and growth appeared. Bacteriostatic effects were more prominent and time dependant at 2 MIC values of isolated compounds. This led to prediction that using multiples of MIC may have better activity in minimum exposure of time. The dental anticaries agents or formulation can only be successful if active agents would be effective within few minutes.

Results from the time of kill studies exhibited by the isolates and extract against *Streptococcus mutans* has shown both concentration and time dependent which supports the earlier literature [13][25].

These isolates may prove worthy as some of the antimicrobial agents like chlorhexidine, triclosan, xylitol, cetylpyridinium chloride, which are generally used in oral cavity may cause discoloration of teeth and tongue and drug resistance.

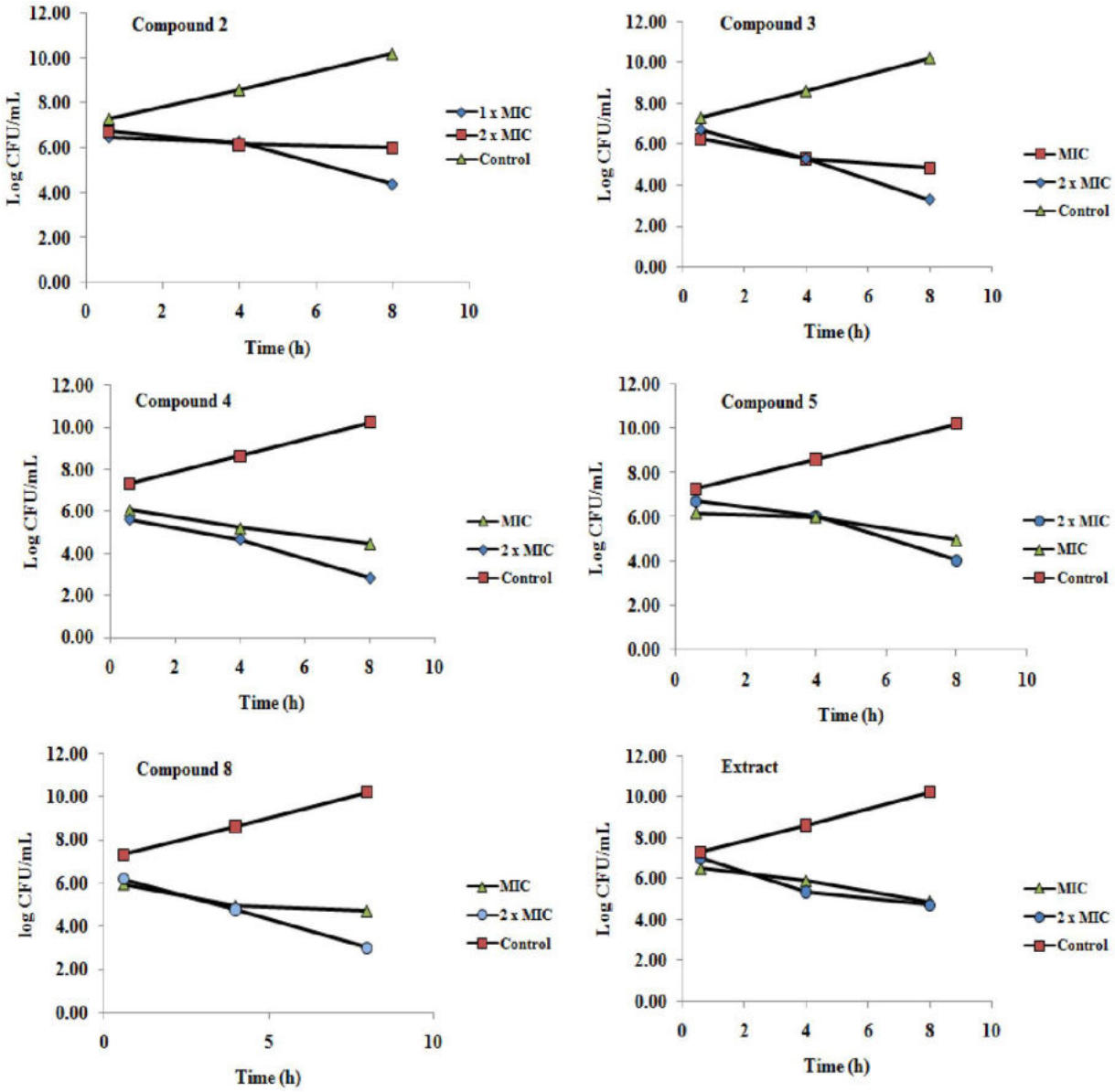

Figure 2: Time-kill curves of methanol extract of *Potentilla fulgens* and isolated molecules

Adherence study

Streptococcus mutans produce glucan called mutan which is found to present in a common extracellular matrix of biofilm of dental plaque. This biofilm enables the bacteria to hide inside and become resistant to anticaries agents [26]. Adhesion of *Streptococcus mutans* was inhibited (figure 3), suggesting the inhibition of glycan synthesis by it. Compound 3, 4 and 8 were effective in inhibition of biofilm formation showing percentage inhibition of 27.9, 38.9, 32.9, respectively at their MIC.

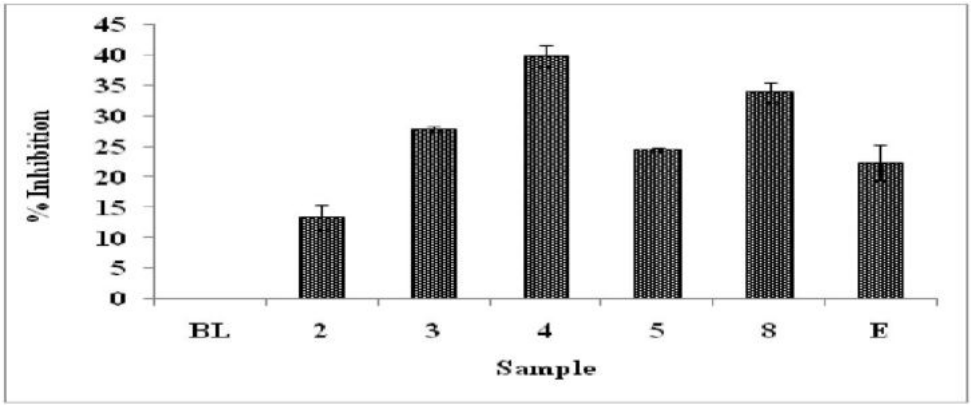

Figure 3: Percentage inhibition of biofilm formation in response to compounds (2, 3, 4, 5 and 8) and extract (E) at MIC

Morphological Studies

Scanning electron microscopy was performed to observe morphological changes effected by the exposure of compound under investigation. The images provided better understanding of complex biofilm formation. It was observed that bacterial cells were embedded inside the matrix of mucoid polysaccharides (figure 4A). Morphological change in shape of the cells and reduction in the cell density was observed on exposure of compound 4 (MIC) to the bacterial cells after 4 h of incubation (figure 4B).

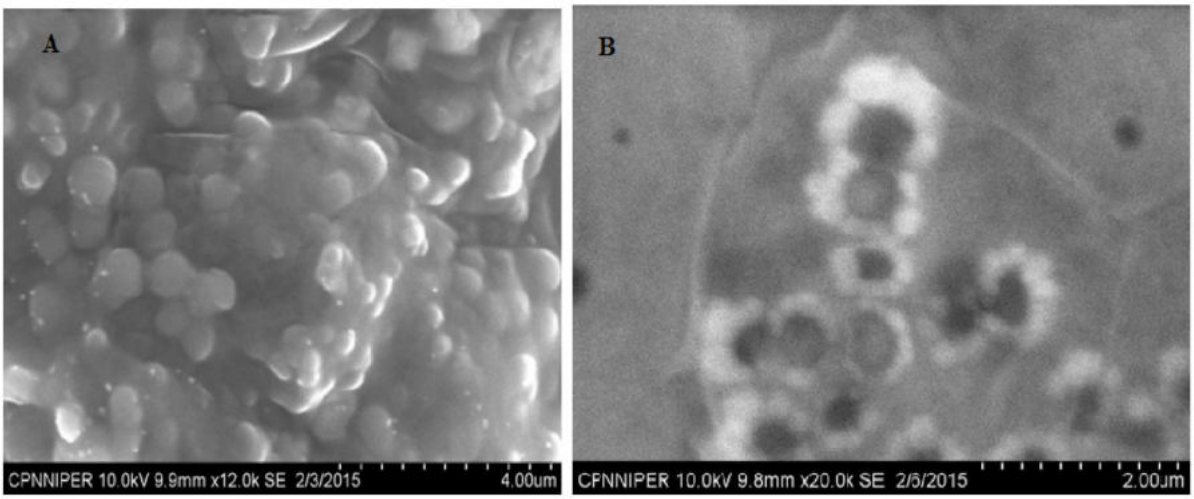

Figure 4: (A) Cocci embedded in the biofilm of *Streptococcus mutans* (B) Effect of compound 4 on morphology of *Streptococcus mutans*

The antibacterial activity exhibited by flavanols can be explained by the presence of a hydrophobic moiety, and a hydrophilic region possessing two hydrogen-bond-donor groups. These structural features may be responsible for an optimal insertion of these compounds into cell membranes through a non-specific interaction with membrane phospholipids, destabilizing the non-covalent interactions between the fatty acids of the lipidic bilayer, and thus interfering on the cellular development. Studies have indicated that flavanols with *cis* stereochemistry more readily partition into phospholipid memberane than their *trans* configured counterparts and hence are more active [27,28]. Thus, the structural features conferring anticariogenic activity to flavanols are hydroxylation of ring B and relative stereochemistry of ring C.

Perusal of studies on flavanols show that these molecules have potential to modulate membrane structure and thereby influence a large number of membrane-dependent cellular processes such as cell signaling and cell cycle, arachidonic acid metabolism and cell proliferation, and apoptosis pathways or mitochondrial functionality. Several studies have emphasized to correlate these activities with the membrane physical properties and with the molecular interactions taking place between membrane phospholipids and flavanol molecules [29].

The biological activities of flavanols have been linked to the occurrence of galloyl and gallic moieties in their structure. The galloylated compounds, epicatechingallate (ECG) and epigallocatechingallate (EGCG) are more effective than their homologous compounds lacking the galloyl group in demonstrating antibacterial, anticarcinogenic, and radical-scavenging activity. These facts have been useful in postulating hypotheses related to structure-activity relationship of flavanols [30].

Conclusions

Various extracts and isolated polyphenolic compounds were screened for anticariogenic activity using agar well diffusion method and MIC of pure compounds was calculated by broth microdilution method. Compounds 3, 4 and 8 were found to exhibit better anticariogenic profile than other compounds as indicated from time-kill studies and inhibition of biofilm formation assays. Compound 8 was found to possess comparable anticariogenic effects as of compound 4. The results suggest that *Potentilla fulgens* can be considered as a source of anticariogenic agents for maintaining oral hygiene.

Acknowledgements

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Conflict of interest

Authors declare no conflict of interest.

Compliance with Ethical Standards

'This article does not contain any studies with human participants or animals performed by any of the authors.'

References

[1]. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369:51–59.

[2]. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. Journal of dental research. 2004;83(suppl 1):C35-8.

[3]. Paula VAC, Modesto A, Santos KRN, Gleiser R. Antimicrobial effects of the combination of chlorhexidine and xylitol. Br Dent J 2010, 209:E19.

[4]. Ferrazzano GF, Amato I, Ingenito A, De Natale A, Pollio A. Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). Fitoterapia 2009:255–262.

[5]. Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A. Plant polyphenols and their anti-cariogenic properties: A review. Molecules 2011:1486–1507.

[6]. Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. Antimicrob Agents Chemother 2011; 55:1229–1236.

[7]. Sato M, Fujiwara S, Tsuchiya H, Fujii T, Iinuma M, Tosa H, Ohkawa Y. Flavones with antibacterial activity against cariogenic bacteria. J Ethnopharmacol 1996; 54:171–176.

[8]. Sato M, Tanaka H, Fujiwara S, Hirata M, Yamaguchi R, Etoh H, Tokuda C. Antibacterial property of isoflavonoids isolated from Erythrina variegata against cariogenic oral bacteria. Phytomedicine 2003; 10:427–433.

[9]. Muroi H, Kubo I. Combination effects of antibacterial compounds in green tea flavor against Streptococcus mutans. J Agric Food Chem 1993;41:1102–1105.

[10]. Tomczyk M, Latté KP. Potentilla-A review of its phytochemical and pharmacological profile. J Ethnopharmacol 2009:184–204.

[11]. Jaitak V, Kaul VK, Himlata, Kumar N, Singh B, Dhar J, Sharma OP. New hopane triterpenes and antioxidant constituents from Potentilla fulgens. Nat Prod Commun 2010; 5:1561–6.

[12]. Jaitak V, Sharma K, Kalia K, Kumar N, Singh HP, Kaul VK, Singh B. Antioxidant activity of *Potentilla fulgens*: An alpine plant of western Himalaya. J Food Compos Anal 2010;23:142–147.

[13]. Tomczyk M, Wiater A, Pleszczy ska M. *In vitro* anticariogenic effects of aerial parts of *Potentilla recta* and its phytochemical profile. Phyther Res 2011; 25:343–350.

[14]. Laloo D, Prasad SK, Krishnamurthy S, Hemalatha S. Gastroprotective activity of ethanolic root extract of *Potentilla fulgens* Wall. ex Hook. J Ethnopharmacol 2013;146:505–514.

[15]. Bhattarai NK. Folk medicinal use of plants for respiratory complaints in central Nepal. Fitoterapia 1993:163–

169.

[16]. Syiem D and Majaw S. Effect of *Potentilla Fulgens* L. Methanolic extract on sorbitol dehydrogenase in normal and alloxan-induced diabetic mice. Pharmacologyonline 2010, 2:671–680.

[17]. Radhika M, Ghoshal N, Chatterjee A. Comparison of effectiveness in antitumor activity between flavonoids and polyphenols of the methanolic extract of roots of *Potentilla fulgens* in breast cancer cells. J Complement Integr Med 2012, 9:Article 24.

[18]. Choudhary A, Mittal AK, Radhika M, Tripathy D, Chatterjee A, Banerjee UC, Singh IP. Two new stereoisomeric antioxidant triterpenes from *Potentilla fulgens*. Fitoterapia 2013, 91:290–297.

[19]. Choudhary A, Radhika M, Chatterjee A, Banerjee UC, Singh IP. Qualitative and quantitative analysis of *Potentilla fulgens* roots by NMR, matrix-assisted laser desorption/ionisation with time-of-flight MS, electrospray ionisation MS/MS and HPLC/UV. Phytochem Anal 2015, 26:161–70.

[20]. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat protoc 2008, 3:163–175.

[21]. Bennett J V, Brodie JL, Benner EJ, Kirby WM. Simplified, accurate method for antibiotic assay of clinical specimens. Appl Microbiol 1966, 14:170–177.

[22]. Olajuyigbe OO, Afolayan AJ. In vitro antibacterial and time-kill assessment of crude methanolic stem bark extract of *Acacia mearnsii* de wild against bacteria in shigellosis. Molecules 2012, 17:2103–2118.

[23]. Smullen J, Koutsou GA, Foster HA, Zumbé A, Storey DM. The antibacterial activity of plant extracts containing polyphenols against *Streptococcus mutans*. Caries Res 2007, 41:342–349.

[24]. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Infect Dis 2004, 38:864–870.

[25]. Yano A, Kikuchi S, Takahashi T, Kohama K, Yoshida Y. Inhibitory effects of the phenolic fraction from the pomace of Vitis coignetiae on biofilm formation by *Streptococcus mutans*. Arch Oral Biol 2012, 57:711–9.

[26]. Ahn SJ, Ahn SJ, Wen ZT, Brady LJ, Burne RA. Characteristics of biofilm formation by Streptococcus mutans in the presence of saliva. Infect Immun 2008;76:4259–68.

[27]. Taylor PW, Hamilton-Miller JMT, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull 2005; 2:71–81.

[28]. Arakawa H, Maeda M, Okubo S, Shimamura T. Role of hydrogen peroxide in bactericidal action of catechin. Biol Pharm Bull 2004; 27:277–281.

[29]. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 2005:343–356.

[30]. Caturla N, Vera-Samper E, Villalaín J, Mateo CR, Micol V. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radic Biol Med 2003; 34:648–662.