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PRELIMINARY SCREENING OF GLUCOSYLTRANSFERASES ENZYME FROM STREPTOCOCCUS MUTANS OF CLINICAL DENTAL CARIES SAMPLE AND INHIBITION BY USING PLANT EXTRACTS

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Keywords:

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ABSTRACT: Dental caries is a persistent disease characterized by acid damage to tooth enamel, which begins with the formation of biofilms on the tooth surface. *Streptococcus mutans* secretes glucosyltransferase, which enables the synthesis of extracellular glucan polymers using ingested starch within the oral cavity, ultimately resulting in acid production, a contributing factor to tooth decay. In this study, we conducted a preliminary screening and partial purification of glucosyltransferase enzymes and evaluated their inhibitory activity against selected plant extracts. We screened 36 isolates of *Streptococcus mutans* for glucosyltransferase enzyme production and identified the SM-18 isolate as having the highest enzyme activity and protein concentration which was selected for further study. Further enzyme optimization studies revealed that the optimal conditions for enzyme activity were pH 6.5, a temperature of 40°C, an incubation time of 18-19 hours, and a substrate concentration of 70 g/L. The extracted enzyme was precipitated at 70% using the ammonium sulphate precipitation method and had a molecular weight of approximately 65 kDa. We also analyzed the inhibitory effects of five different medicinal plant extracts on the activity of the glucosyltransferase enzyme. Our phytochemical screening revealed the presence of various constituents, including alkaloids, saponins, proteins, tannins, flavonoids, and triterpenoids, in the selected medicinal plants. Notably, some of the plant extracts showed a high percentage of inhibition against glucosyltransferase enzyme, suggesting their potential as antibacterial agents against dental caries.

INTRODUCTION: Dental caries, one of the most prevalent diseases occurring on tooth hard tissues, is driven by a disequilibrium in the oral microbial community termed dental biofilm [1]. An important part of the formation of the biofilm is aided by the ability of *S. mutans* to produce glucan polymers from ingested sucrose and starches.

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These extracellular glucan polymers are manufactured by secreted enzymes collectively called glucosyltransferases (Gtfs) as well as glucan sucrases [2]. The sucrose-dependent mechanism mainly relies on glucosyltransferases produced by *Streptococcus* sp, which are responsible for synthesizing glucan from sucrose [3].

One of the prominent pathways is initiated when *S. mutans* produces glycans, high-molecular weight sticky glucosyl polymers *via* glucosyltransferases (Gtfs), which adhere to the tooth surface and then trap other oral bacteria, food debris, and salivary components to create a cariogenic biofilm environment. *S. mutans* possesses three Gtfs, which

are products of GtfB, GtfC, and GtfD, that synthesize mostly insoluble glucans containing more of α-1,3 linked glucans, and GtfC synthesizes a mixture of soluble glucans, whereas GtfD synthesizes predominantly soluble glucans (containing more α-1,6 linked glucans) [4]. Among these Gtfs, GtfD produces only soluble α 1,6-glucan-linked polymers, GtfC produces both insoluble and soluble glucan polymers, whereas GtfB only produces insoluble glucan polymers. Finally, GtfD and Gtfs predominantly forms water-soluble glucan. GtfB and GtfC, in particular, modulate the initial microbial adherence and coherence by binding to the physical integrity and stability of the extracellular matrix by synthesizing water-insoluble glucan [5]. There is a global need for alternative oral disease prevention, and one such alternative would be the use of medicinal plants. The use of medicinal plants or natural products has been one of the most successful strategies for the discovery of new medicines. Some phytochemicals, including antibacterial agents, have been derived from medicinal plants that showed antibacterial inhibition against glucosyltransferase enzyme. In our present study, glucosyltransferase enzyme extracted from *S. mutans* was preliminarily screened and partially purified, and different parameters of enzyme optimization were carried out to inhibit its activity using plant extracts.

MATERIALS AND METHODS:

Bacterial Culture: In our previous study, *Streptococcus mutans* was isolated from clinical dental caries samples. A total of 36 isolates were selected for this enzyme screening study [6].

Screening of Glucosyltransferases Enzyme: Crude glucosyltransferases enzyme was preliminary screened for the quantification of enzyme and protein concentration.

Estimation of Protein: Quantification of protein for the extracted enzyme was carried out by following the methodology of [7], using bovine serum albumin as the standard.

Enzyme Activity: Glucosyltransferase enzyme activity was determined by the Dinitro Salicylate (DNS) method with slight modifications [8].

Dextran Production Test: All clinical isolates were screened for the production of exopolysaccharide dextran by following the methodology of [9].

Enzyme Optimization: The various parameters selected for optimization of glucosyltransferase enzyme include the following:

Effect of pH on Enzyme Activity: To detect the optimum pH for Glucosyltransferase production, the isolate SM- 18 was inoculated into the medium at various pH levels varying from pH 4.5 to pH 8.5 and was incubated at 37^{o}C for 24 hrs. In an orbital shaker incubator at 120rpm. The pH of the media was adjusted by using 0.1N NaOH and 0.1N HCl. The enzyme activity was determined by the DNS method [10].

Effect of Substrate Concentration on Enzyme Activity: The effect of different concentrations of Glucosyltransferase on enzyme activity was determined by inoculating the isolate SM-18 into the media supplemented with different concentrations of glucose (20, 40, 60, 80 up to 200g/L) and incubated at optimized parameters. The enzyme activity was measured, and the concentration of enzyme that showed a high yield was chosen for further studies.

Effect of Incubation Time on Enzyme Activity: The isolate SM-18, was inoculated into Todd Hewitt Broth (Hi media) broth and was incubated at 37^{o}C in an orbital shaker incubator at 120rpm. The enzyme activity was calculated at different intervals of time from 2 hrs to 50 hrs of the incubation period, the broth culture was withdrawn aseptically, and the enzyme activity was determined by the DNS method [10].

Effect of Temperature on Enzyme Activity: To determine the optimum temperature of glucosyltransferase production, the isolate SM-18 was inoculated into the Todd Hewitt Broth (Hi media) broth and incubated at different temperatures ranging from 4°C to 60°C. After the incubation period, the enzyme activity was measured by the DNS method [11].

Ammonium Sulphate Precipitation: Cell lysis was carried out and the lysed content was used for the protein purification, using the ammonium sulphate precipitation technique by following the methodology of [11].

Dialysis: The enzyme precipitate obtained from ammonium sulphate precipitation was dissolved in 0.01M phosphate buffer (pH 6.5) and was dialyzed against the same buffer 0.01M phosphate buffer for 24-48 hours at 4°C with constant stirring and the buffer was changed frequently at every 1-2 hrs, intervals of time. At each step, the enzyme activity and protein concentrations of culture filtrate were estimated.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE): Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was carried out by following the methodology of [12], using 12% cross-linked polyacrylamide gel using a mini slab gel apparatus (Bangalore Genei Pvt. Ltd., India). The crude enzyme preparation was loaded onto the gel, after running the gel they were stained with Coomassie brilliant blue and the molecular weight of the enzyme was determined with reference to the molecular weight marker (Broad Range Marker, Merck Life Sciences).

Extraction of Crude Extracts from Selective Medicinal Plants Collection of Plant Samples: Five different plant samples were collected locally from bhadravathi taluk, Shivamogga district, Karnataka state. The collected plants were identified and authenticated in the Department of Studies and Research in Botany, Sahyadri Science College, Shivamogga and a voucher specimen has been kept in the herbarium of the Department of Studies and Research in Microbiology, Sahyadri Science College, Shivamogga.

Plant Material: The plant leaves samples used in this study include, *Acmella uliginosa, Acaplypha indica* Linn, *Euphorbia maculata, Memecylon edule* Roxb, and *Vitex negundo* Linn **Table 1**.

TABLE 1: INFORMATION OF SELECTED MEDICINAL PLANTS SPECIES USED FOR OUR STUDY

Sl. no.	Plant species	Local name	Parts used	Accession number
1	*Acmella uliginosa*	*Ummalaga*	Leaves	SSCMB- 42
2	*Acaplypha indica* Linn	*Kuppi gida*	Leaves	SSCMB- 43
3	*Euphorbia maculate*	*Dugdhika*	Leaves	SSCMB- 44
4	*Memecylon edule* Roxb	*Ollekudi*	Leaves	SSCMB- 45
5	*Vitex negundo* Linn	*Lakki soppu*	Leaves	SSCMB- 46

Crude Extract Preparations: The fresh plant leaf samples were plucked and cleaned under running tap water to remove any dust particles. The plant samples were dried in the shade for a few days [13], crushed into powder, and stored in an airtight zip-lock bag. The maceration process was used for the extraction. 20g of each powdered extract was placed into a flask containing 200ml of methanol and aqueous solvents. The flask was plugged well and shaken for 48 hours with a rotary shaker. A clean muslin cloth was used to filter the contents of the flasks, followed by Whatman filter paper No. 1. The filtrates were then evaporated to dryness at room temperature, and the obtained extracts were stored in the refrigerator for further use.

Phytochemical Analysis:

Qualitative Phytochemical Analysis: Freshly prepared extracts were subjected to standard phytochemical analysis to determine the presence of phytoconstituents. These tests relied on the visual observation of color change or the formation of a precipitate after the addition of particular reagents and were carried out using standard procedures [14-16].

Test for Alkaloids:

Mayer's Test: A few drops of Mayer's reagent were added to 2mg of extract. The formation of a white or pale-yellow precipitate indicates the presence of alkaloids.

Test for Triterpenoids:

Salkowski's Test: Five ml of extract was mixed in 2 ml of chloroform and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of triterpenoids.

Test for Steroids:

Libermann Burchard's Test: Two ml of acetic anhydride was added to 0.5g extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Saponins:
Froth Test: The extract was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15 minutes. A 2 cm layer of foam indicates the presence of saponins.

Test for Tannins:
Gelatin Test: About 2.5g of the plant extract was dissolved in 5 ml of distilled water filter and ferric chloride reagent was added to the filtrate. A blue-black, green, or Blue-green precipitate was taken as evidence for the presence of tannins.

Test for Phenols:
Ferric Chloride Test: A small amount of the plant extract was taken with 1ml of water in a test tube and 1 to 2 drops of ferric chloride were added blue, green, red, or purple color is a positive test.

Test for Glycosides:
Keller Killoni Test: 5ml of the extract was taken and to this add 2ml glacial acetic acid, a drop of $FeCl_3$ solution and 1ml concentrated H_2SO_4 from a brown ring and often a purple ring appears below.

Test for Carbohydrates:
Benedict's Test: To 0.5ml of the filtrate, 0.5ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 minutes. A characteristic red-colored precipitate indicates the presence of sugar.

Test for Flavonoids:
Alkaline Reagent Test: One to five drops of concentrated hydrochloric acid (HCl) was added to the small amount of extract of the plant material. The immediate development of red color indicates the presence of flavonoids.

Test for Protein:
Biuret Test: 3ml extract, 3ml of 4% sodium hydroxide solution, and a few drops of 1% Copper sulphate is added to form a purple solution.

Quantitative Phytochemical Analysis: There are two types used in the quantitative analysis of plant extracts. They are a determination of total phenolic contents and determination of total flavonoid contents [16].

Determination of Total Phenolic Content: 100mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1ml of this solution was transferred to a test tube, then 0.5ml 2N of the Folin-ciocalteu reagent and 1.5ml 20% of Na_2CO_3 solution was added, and ultimately the volume was made up to 8ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

Determination of Total Flavonoids Content: Briefly, 50µl of crude extract (1mg/ml Methanol) was made up to 1ml with respective solvent and mixed with 4ml of distilled water. Then 0.3ml of $NaNO_2$ (5%) solution was added and after five minutes followed by 0.3ml $AlCl_3$ solution (10%). After the incubation at room temperature for 6min, 2ml of sodium hydroxide was added. The final volume of the reaction mixture was made up to 10ml with distilled water and incubated for 15 min. Then absorbance was read at 510nm against blank.

Screening of Crude Plant Extracts for Glucosyltransferase (GTF) Inhibitory Activity: The effect of plant extracts on GTF activity was indirectly assessed by the inhibition of water insoluble glucose synthesis by following the methodology of [17-19] with modifications.

50µl crude GTF enzyme buffered in sodium phosphate buffer (1mM), pH 6.5, and 50µl of different concentrations in each plant extract. A total 500µl reaction mixture was then incubated at 37°Cfor 18 hrs. The reaction was stopped by placing the tubes in a boiling water bath for 5 min. The water-insoluble glucan produced by the enzyme reaction was suspended by sonication. The turbidity of the suspension was measured at 550nm. The reaction mixture with 50 µl (X %) of ethanol/ sodium phosphate buffer (1mM) instead of plant extracts served as control. A parallel series of mixtures without enzymes were prepared to adjust for quantification errors due to the extracts themselves. All the reactions were carried out in triplicates.

Statistical Analysis: Phytochemical analysis and antimicrobial activity were carried out in triplicates for each different solvent of plant extracts (n=3)

from which the mean and standard deviation (SD) were calculated by using Microsoft Excel 2016 and were expressed as [Mean ± SD].

RESULT AND DISCUSSION:

Screening of Glucosyltransferase Enzyme from *Streptococcus mutans*: A total of thirty-six clinical isolates were screened for the production of glucosyltransferase enzyme (GTF), out of which thirty clinical isolates were found to produce GTF (prevalence rate of 83.33%). Among the isolates, SM-18 was found to be a good GTF producer with an enzyme activity of 0.19 U mol/mL/min and a protein concentration of 0.79 mg/mL **Table 2** and was hence selected for further studies.

TABLE 2: SCREENING FOR THE PRODUCTION OF GLUCOSYLTRANSFERASE ENZYME FROM CLINICAL ISOLATES

Sl. no.	Isolates	Enzyme Activity (U/ml)	Protein Concentration mg/ml	Specific Activity (U/mg)
1.	SM-1	0.19	1.00	0.19
2.	SM-2	0.18	0.97	0.18
3.	SM-3	0.19	0.99	0.19
4.	SM-4	0.19	0.88	0.21
5.	SM-5	0.19	0.98	0.20
6.	SM-6	0.18	0.98	0.18
7.	SM-7	0.20	0.98	0.20
8.	SM-8	0.16	0.77	0.21
9.	SM-9	0.16	0.88	0.19
10.	SM-10	0.18	0.89	0.20
11.	SM-11	0.17	0.88	0.19
12.	SM-12	0.07	0.99	0.07
13.	SM-13	0.18	0.88	0.20
14.	SM-14	0.17	0.99	0.17
15.	SM-15	0.12	0.89	0.14
16.	SM-16	0.19	0.98	0.19
17.	SM-17	0.16	0.90	0.18
18.	SM-18	0.19	0.79	0.24
19.	SM-19	0.14	0.93	0.15
20.	SM-20	0.11	0.89	0.12
21.	SM-21	0.17	0.88	0.19
22.	SM-22	0.18	0.89	0.20
23.	SM-23	0.17	0.87	0.19
24.	SM-24	0.14	0.79	0.17
25.	SM-25	0.14	0.99	0.15
26.	SM-26	0.15	0.79	0.19
27.	SM-27	0.16	0.97	0.17
28.	SM-28	0.14	0.79	0.18
29.	SM-29	0.13	0.78	0.17
30.	SM-30	0.08	0.89	0.09

Dextran Production Test: All clinical isolates produced exopolysaccharide dextran and hence all isolates formed flocculation that indicates the production of dextran **Fig. 1**.

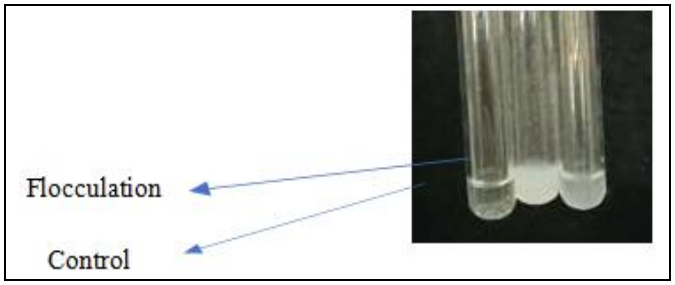

FIG. 1: IN GRAPH Y-AXIS- RELATIVE ENZYME ACTIVITY %

Effect of pH on Activity of the GTF Enzyme: The optimum pH for the GTF enzyme was found to be 6.5 **Fig. 2**, and the highest relative enzyme was found to be 95% using sodium phosphate buffer.

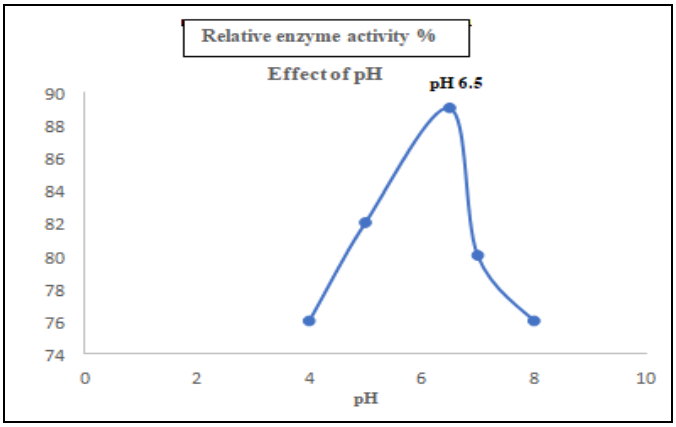

FIG. 2: EFFECT OF PH ON THE ACTIVITY OF THE GTF ENZYME

Effect of Substrate Concentration on Activity of GTF Enzymes: The ideal substrate concentration for enzyme activity was 70 g/L **Fig. 3**.

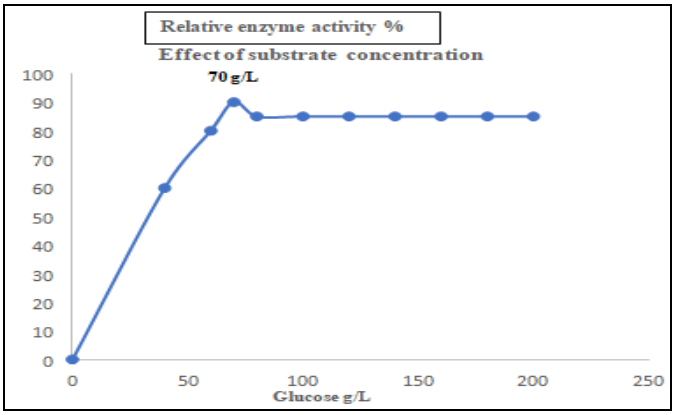

FIG. 3: EFFECT OF SUBSTRATE CONCEN-TRATIONON ACTIVITY OF THE GTF ENZYME

Effect of Incubation Time on the Activity of GTF Enzymes: The optimum incubation time for GTF enzyme was found to be 18-20 hrs, at this time of incubation the enzyme showed good activity further incubation up to 48 hrs, did not affect the enzyme activity considerably **Fig. 4**.

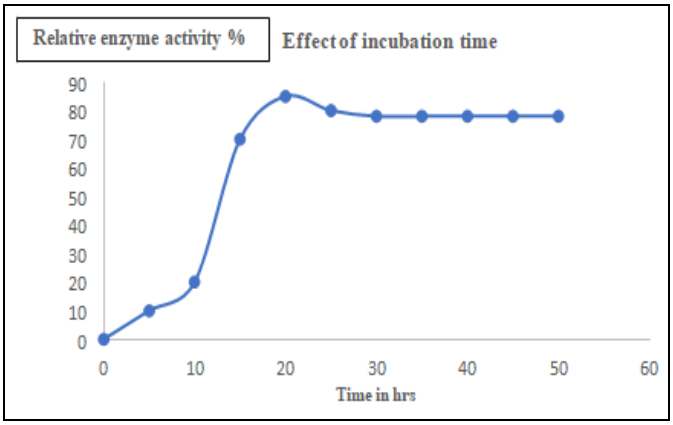

FIG. 4: EFFECT OF INCUBATION TIME ON THE ACTIVITY OF THE GTF ENZYME

Effect of Temperature on Activity of GTF Enzyme: The enzyme activity was determined at various temperatures, the enzyme was observed to be highly active at 40^{o} C at increasing temperatures up to 50^{o}C, the enzyme exhibits residual activity **Fig. 5**.

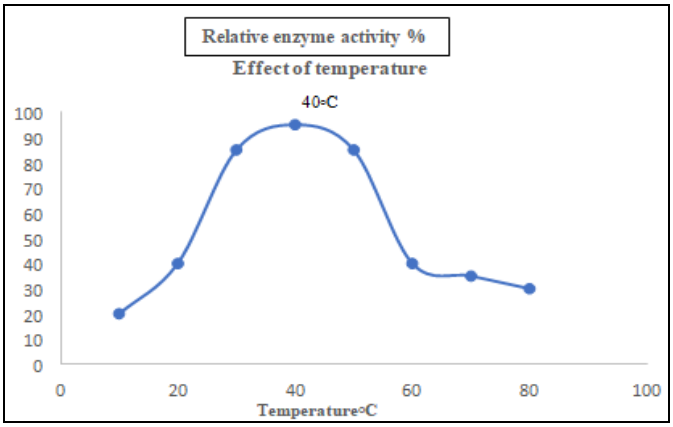

FIG. 5: EFFECT OF TEMPERATURE ON ACTIVITY OF THE GTF ENZYME

Ammonium Sulphate Precipitation: The protein was precipitated using the ammonium sulphate precipitation technique at different concentrations, and maximum precipitation was observed at a concentration of 70%. The precipitated protein was then partially purified using a dialysis membrane.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis SDS PAGE: Different concentration of ammonium sulphate precipitations was analyzed, and partially purified enzymes obtained from the dialysis membrane were also loaded into 12% SDS PAGE and the molecular weight was found to be ~65 KDa **Fig. 6**.

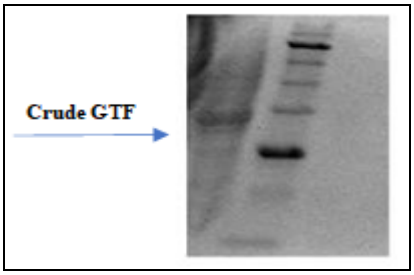

FIG. 6: LANE 1: 48 HR DIALYSIS, LANE 2: LADDER

Phytochemical Analysis: The crude plant extracts were obtained using different solvents and their phytochemical analysis showed the presence of various phytoconstituents like alkaloids, saponins, steroids, glycosides, triterpenoids, and flavonoids. The results of the phytochemical analysis were tabulated **Table 3**.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF METHANOL AND AQUEOUS SOLVENTS OF PLANTS EXTRACTS

Sl. no.	Plant Name	Solvents Used	A	T	S	Sp	Ta	G	F	P	C	Ph
1	*Acmella uliginosa*	Methanol	+	-	+	-	+	+	+	-	-	+
		Aqueous	+	+	+	+	+	-	-	-	-	+
2	*Acaplypha indica* Linn	Methanol	-	+	-	-	-	+	+	-	-	+
		Aqueous	+	-	-	-	-	+	+	-	-	_
3	*Euphorbia maculate*	Methanol	+	-	-	-	-	+	-	-	-	-
		Aqueous	+	+	+	+	+	-	-	+	+	+
4	*Memecylon edule* Roxb	Methanol	+	-	-	+	-	-	+	-	-	-
		Aqueous	+	-	-	+	+	-	+	-	+	-
5	*Vitex negundo* Linn	Methanol	-	+	+	-	-	-	+	-	-	+
		Aqueous	-	+	+	-	-	-	+	-	-	-

* A - Alkaloid, T - Triterpenoids, S - Steroids, Sp - Saponin, Ta - Tannin, G - Glycosidase, F - Flavonoid, P - Protein, C – Carbohydrate, Ph - Phenol '+' Present; '-' Absent.

Quantitative Analysis: The quantitative analysis of total phenolic content and total flavonoid content was carried out for Methanol extracts and results were tabulated **Table 4**.

TABLE 4: TOTAL PHENOLICS CONTENT AND TOTAL FLAVONOID CONTENT OF METHANOL EXTRACT OF DIFFERENT PLANT EXTRACTS

Sl. no.	Plants Name	Total Phenolics Content GAE/g extract	Total Flavonoid Content mg RE/g extract
1	*Acmella uliginosa*	60.4 ± 2.02	13.67 ± 1.87
2	*Acaplypha indica* Linn	10.22±0.22	4.57±0.20
3	*Euphorbia maculate*	4.23±0.25	7.20±0.10
4	*Memecylon edule* Roxb	14.91 ± 1.17	11.65 ± 1.56
5	*Vitex negundo* Linn	8.00±0.01	10.21±0.25

Results are expressed as the mean ± standard deviation (SD) of three determinations.

Determination of Crude Plant Extracts for Glucosyltransferase Activity: In the present study, the methanol extracts of five different medicinal plants were evaluated for their inhibitory activities against GTF. The insoluble glucan synthesized by GTF was measured turbid metrically. In this screening study, all the plants showed inhibitory activity against GTF synthesizing insoluble glucans. Among all the plants tested, *Acmella uliginosa* showed the highest percentage of inhibition, while *Euphorbia maculata* showed the lowest percentage of inhibition. The results are presented in **Fig. 7**.

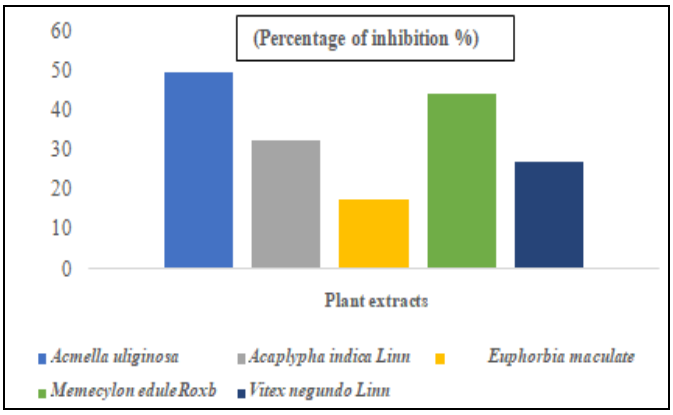

FIG. 7: EFFECT OF CRUDE PLANT EXTRACTS ON INHIBITION OF GTF ACTIVITY

DISCUSSION: In our previous studies, we isolated and identified *Streptococcus mutans* and extracted glucosyltransferase enzyme from it. In this work, we conducted preliminary screening and partial purification of the enzyme and optimized different parameters to inhibit its activity using plant extracts [6].

In our present study glucosyltransferase enzyme has been extracted from *Streptococcus mutans* and was preliminary screened and partially purified in contrast with another study by [20] in which fructosyltransferase enzyme was extracted which is another major enzyme which is responsible for the caries formation.

The production of glucosyltransferase enzyme (GTF) by *Streptococcus mutans* is one of the key virulence factors that contribute to dental caries. In this study, we screened a total of thirty-six clinical isolates for the production of GTF and found that the prevalence rate of GTF production among these isolates was 83.33%. This result is consistent with previous studies that have reported high prevalence rates of GTF production among clinical isolates of *Streptococcus mutans* [21, 22].

Further analysis of the isolates revealed that SM-18 was a good GTF producer, with an enzyme activity of 0.19 U mol/mL/min and a protein concentration of 0.79 mg/ml. These results indicate that SM-18 could be a promising source of GTF for further studies, such as enzyme purification and characterization. Interestingly, the enzyme activity of SM-18 is higher than the values reported in some previous studies [21-22], which could be attributed to differences in the strains of *Streptococcus mutans* used in these studies or differences in the methods used to measure enzyme activity.

Enzyme optimization is a crucial step in improving the efficiency of enzymatic reactions, and the identification of optimal conditions for enzyme activity is an essential aspect of this process. In this study, we screened the optimal conditions for enzyme activity, and the results indicated that the highest enzyme activity was observed at pH 6.5 and temperature 40°C, with a substrate concentration of 70 g/L and an incubation time of 18-19 hours. The findings of this study are consistent with previous research on the optimal conditions for enzyme activity [23] found that the highest activity of a cellulase enzyme was observed at a pH of 6.5 and temperature of 40°C. Similarly, a study by [24] reported that the highest activity of an amylase enzyme was observed at a pH of 6.5 and temperature of 40°C. The optimal substrate concentration reported in this study is also consistent with previous research by [25] found that the optimal substrate concentration for a xylanase enzyme was 70 g/L. The optimal incubation time reported in this study is also in line with a previous study by [26] reported that the optimal incubation time for a cellulase enzyme was 18-19 hours.

Phytochemicals are naturally occurring compounds in plants that have been shown to have potential health benefits. The present study aimed to screen five different plant extracts for the presence of various phytochemicals. The results of the study showed that the methanol extract of *Acmella uliginosa* had the highest content of phytochemicals, followed by *Acaplypha indica* Linn and *Vitex negundo* Linn, while the lowest content was observed in *Memecylon edule* Roxb and *Euphorbia maculate*. The aqueous extracts of various plants also showed the presence of multiple phytochemicals. The findings of this study are consistent with previous studies by [27] reported that the methanol extract of *Acmella oleracea* had a high content of flavonoids, phenols, and tannins. Similarly, a study by [28] found that the methanol extract of *Acaplypha indica* had a high content of flavonoids and phenols.

The phytochemical quantitative analysis study revealed that different plant extracts contain varying amounts of phenolic and flavonoid compounds, which are well-known for their potential health benefits. The total phenolic and flavonoid contents were measured using standard analytical methods. The results indicated that *Acmella uliginosa* had the highest amount of total phenolic and total flavonoid content, followed by *Memecylon edule* Roxb and *Acaplypha indica* Linn, which exhibited moderate levels. The least amount of these compounds was recorded in *Vitex negundo* Linn and *Euphorbia maculate.*

These findings are consistent with previous study by [29] that have shown that phenolic and flavonoid contents vary significantly among different plant species, and even within the same species, depending on factors such as the location, climate, and soil conditions in which they are grown. The high concentration of phenolic and flavonoid compounds in *Acmella uliginosa* may be attributed to its antioxidant properties, which have been associated with a reduced risk of chronic diseases such as cancer, diabetes, and heart disease.

In this study, we also attempted to inhibit the activity of GTF using plant extracts. The results showed that all the plant extracts tested had inhibitory activity against GTF. Among the plants tested, *Acmella uliginosa* showed the highest percentage of inhibition, while *Euphorbia maculata* showed the lowest percentage of inhibition. These findings are consistent with previous studies that have reported the inhibitory effects of plant extracts against GTF activity [30, 22].

CONCLUSION: In this study, we conducted a preliminary screening and partial purification of glucosyltransferase enzymes and evaluated their inhibitory activity against selected plant extracts. In our study, we screened 36 isolates of *Streptococcus mutans* for glucosyltransferase enzyme production

and identified SM 18 to be a good GTF producer with enzyme activity and protein concentration. Inhibition against the Gtfs enzyme by using five different medicinal plant extracts and some plant extracts showed a high percentage of inhibition against the Gtfs enzyme, these plant extracts can be accepted in traditional medicine and natural products as an alternative form of oral health care.

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