IJPSR (2023), Volume 14, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 June 2022; received in revised form, 21 September 2022; accepted 10 November 2022; published 01 March 2023

PRELIMINARY SCREENING OF ANTI-CARIOGENIC PROPERTIES OF SELECTED MEDICINAL PLANTS AGAINST STREPTOCOCCAL DENTAL CARIES

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Keywords:

Antimicrobial agents, Agar well diffusion, Plant extracts, Phytochemical analysis, *Streptococcus mutans*

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ABSTRACT: *Streptococcus mutans* is the primary major causative agent of dental caries, with the ability to produce acid and form dental plaque. *Streptococcus mutans* adheres to the tooth surface *via* extracellular enzymes and produces dental plaque via extracellular polysaccharides synthesized from sucrose *via* glucosyltransferase and fructosyltransferase. This study aimed to look at the alternative antimicrobial agent from plant extracts against *Streptococcus mutans*. *Streptococcus mutans* were used to assess the antimicrobial activities of crude plant extracts. Five different plant extracts were examined using the agar well diffusion method and were found to be antibacterial against *Streptococcus mutans*; Saponin, Proteins, Tannins, Flavonoids, Steroids, and reducing Sugars were found in the phytochemical screening of selected medicinal plants. Among the different extracts, Methanol had the highest antibacterial activity due to its increased solubility of highly active antimicrobial and phytochemical components. The extract has the potential to be a new source of antibacterial agents, and it scientifically justifies the use of plant extracts as an alternative antimicrobial agent against antibiotics, which shows various side effects.

INTRODUCTION: *Streptococcus mutans* is the primary causative agent of dental caries; the process of caries formation involves a sucrose-dependent tight binding of *Streptococcus mutans* to the enamel coating of the tooth surface; this attachment is facilitated by the production of water-insoluble glucans from sucrose, which acts as adhesive compounds to bind bacteria to the teeth; bacteria then digest available carbohydrates into acids, lowering the pH and demineralizing the teeth; bacteria then ferment available carbohydrates to acids [1-3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(3).1202-09

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(3).1202-09

Since, caries is caused by bacteria, simply removing the bacteria or limiting their acid production can minimize the incidence of infection. Dental caries remains the most frequent form of oral infectious illness, despite the adoption of age-old physical and chemotherapeutic treatments. Hence new techniques for dealing with dental cavities are required. Fluoride, iodide, silver nitrate, and xylitol are chemicals used to prevent dental caries. However, increased use of chemicals like fluoride will affect the health of the teeth and bones [4, 5].

Studies find that detergents such as Sodium dodecyl sulfate added from spumes in pastes may cause problems in the mouth. The polishing compounds in pastes may cause teeth to become acidulous. It occurs as a result of enamel damage. Antibiotics like penicillin and vancomycin, and many more, have been used to suppress bacterial

growth. However, the use of these antibiotics for this purpose has been questioned further due to the risk of developing resistant pathogenic bacteria, allergic reactions, and other negative effects. Thus, in light of the evidence of the rapid global dissemination of resistant clinical isolates, new antimicrobial medicines are urgently needed. However, the quick and widespread emergence of resistance to new antimicrobial drugs in the past suggests that even new antibacterial families will face resistance in a short life expectancy [6, 7].

There is a global demand for safe, effective, and cost-effective oral disease prevention and treatment options and products [8, 9]. Verifying the widespread use of such therapeutic herbs is one such technique. Some phytochemicals, including antibacterial compounds, have been isolated from different plants and have shown antibacterial activity against *Streptococcus mutans.* This study aimed to see if the selected medicinal plant had antibacterial action against *Streptococcus mutans* which causes dental caries. In the present study, we isolated the leaves of different plant extracts and studied their antimicrobial activity against the *Streptococcus mutans*. The crude extract of the plant was subjected to phytochemical screening employing qualitative and quantitative assays using various solvents such as Petroleum Ether, Acetone, Methanol, and Aqueous to determine the bioactive components contained in the extract.

MATERIALS AND METHODS:

Sample Collection: Clinical Dental caries samples were collected from the Department of Dentistry, District McGann Hospital, Shivamogga, Karnataka. Dental caries-infected teeth samples were collected and transferred in a 2 ml saline solution (0.4% agar, 0.15% thioglycolate/phosphate buffered saline) to a laboratory where the sample was streaked onto blood agar media. The plates were incubated for 24 to 48 hours at 37°C. Colonies showing α-hemolysis were selected for further biochemical tests. The standard culture of *Streptococcus mutans* (NCIM Accession No: 5660) was procured from NCIM Pune in Lyophilized form for the confirmatory test with clinical isolates.

Characterization of Bacterial Isolates: The isolated cultures were identified using standard culture, morphological characters, and biochemical characteristics based on Bergey's Manual of Systematic Bacteriology [10] and Murray Manual of clinical microbiology 8th edition [11] and *Streptococcus mutans* confirmation was performed by following the methodology Gold *et al*., [12].

Plant Sample Collection: Medicinal plants were collected locally from Thirthalli, Abbe falls, and Kundadri hills of Shivamogga district, Karnataka state, India. The plants collected were identified and authenticated in the Department of Studies and Research in Botany, Sahyadri Science College, Shivamogga district, Karnataka, India and a voucher specimen has been kept in the herbarium of the Department of Studies and Research in Microbiology, Sahyadri Science College, Shivamogga, Karnataka for future reference.

Plant Material: The plant species used for their phytochemical analysis for this study included five different plant species which are *Glochidionellipticum wight*, *Mappiea foetida Lour*, *Homoliareperia*, *Clauesinadenteta (Willd.) Roem*. and *Memecylontaboltianum Brandis* **Table 1.**

TABLE 1: INFORMATION OF SELECTED MEDICINAL PLANT SPECIES USED FOR PHYTOCHEMICAL ANALYSIS

Plant Species	Local Name	Parts Used	Accession Number
Glochidionellipticum Wight	*KaaduKappi*	Leaves	SSCMB-21
Mappiea foetida	*Durvasanemara*	Leaves	SSCMB-22
Homonoia riparia Lour.	*Nirukanigalu*	Leaves	SSCMB-23
Clausena dentata (Willd.) Roem.	*Kadu Karibevu*	Leaves	SSCMB-24
Memecylontalbotianum Brandis	*Chappalu*	Leaves	SSCMB-25

Preparation of Plant Extract: The leaves of the selected plants were plucked and cleaned under running tap water to remove any dust particles. The plant samples were then dried in the shade for a few days, crushed into powder, and stored in an airtight Ziplock container for use. The maceration process was followed for extraction, and 20g of each powdered extract was placed into a flask containing 200ml of various solvents (acetone, petroleum ether, Methanol, distilled water), and

flasks were plugged, well shaken, and left for 48 hours with intermittent stirring. A clean muslin cloth was used to filter the contents of the flasks, followed by Whatman filter paper No.1and the filtrates were then evaporated to dryness at 40^{o}C in the oven. The extracts thus obtained were stored in the refrigerator for further use [13].

Phytochemical Screening: Standard phytochemical analysis was carried out on the freshly prepared extracts to check for the presence of phytoconstituents and these tests were based on the visual observation of color change or the formation of a precipitate after the addition of specific reagents and were carried out using standard procedures [14-18].

Test for Alkaloids: 1 mL of extract was taken in a test tube and 1 mL of potassium mercuric iodide (Mayer's reagent) solution was added and agitated. The presence of alkaloids is indicated by the appearance of a whitish or cream precipitate.

Test for Terpenoids and Steroids: 4 ml of extract were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then a concentrated solution of sulphuric acid was added slowly along the side of the tubes, red-violet color was observed for terpenoids and bluish green color for steroids.

Test for Saponins: 3ml of plant extracts was treated with 5 ml of water and shaken well. Froth formation was observed for the presence of saponins, which was found to be stable for 15 minutes.

Test for Tannins: 1 ml of water and 1 - 2 drops of ferric chloride solution were added to 0.5 ml of extract solution. Gallic tannins were found to be blue in color, while catechol tannins were found to be green and black in color.

Test for Phenols: 4ml of extracts was dissolved in the appropriate solvent, and a few drops of a ferric chloride solution containing 5% ferric chloride were added. The deep blue color was used to indicate the presence of phenols.

Test for Glycosides: For a few drops of extracts in a glacial acetic acid, a few drops of ferric chloride solution and concentrated sulphuric acid were added and observed for a reddish-brown coloration at the junction of two layers, and the bluish-green color in the upper layer.

Test for Flavonoids: 1.5 ml of 50 percent methanol solution was added to four ml of extract solution. The solution was warmed, and metal magnesium was added, 5-6 drops of strong hydrochloric acid were added to this solution, resulting in red color for flavonoids.

Test for Protein: 1ml of plant extract and 4% NaOH solution was added, and 1% of copper sulfite was added appearance of a violet color indicates the presence of Protein.

Test for Carbohydrates: 2 ml of plant extracts and 1 ml of Fehling's solution A, and 1 ml of Fehling's solution B, was added and boiled for a few minutes; the presence of reducing sugars resulted in the development of an orange-red precipitate.

Antimicrobial Activity:

Antimicrobial Screening by Agar well Diffusion Method: By using the standard agar well diffusion method with slight modification, the antibacterial activity of several plant extracts was evaluated [19-24], Mueller Hinton agar (Hi-Media) plates were swabbed with 24 hours old broth culture of a bacterial strain of *Streptococcus mutans* isolates using sterile cotton swabs. With the help of sterile cork borer, wells of 6 mm diameter with 2 cm apart were made aseptically. Different concentrations of 25%, 50% and 75% plant extracts were prepared and about 100 µl of each sample were loaded into respective wells by sterile micropipette syringe. Standard (Streptomycin, 1 mg/ml) and Control (10% Dimethyl sulfoxide) were also loaded into the respective wells. The inoculated plates were incubated at 37 ^{o}C for 24 hrs. in an upright position. The experiment was carried out in triplicate and the zone of inhibition was recorded. and the results were compared with standard strains of *Streptococcus mutans* NCIM No.5660.

Statistical Analysis: Antimicrobial activity was carried out in triplicates for each different solvents of plant extracts and obtained three replicates (n=3) from which the mean and standard deviation (SD) were calculated by using Microsoft Excel 2016.

All data for antimicrobial activity against *Streptococcus mutans* were expressed as [Mean ± Standard Error Mean] and analyzed using Microsoft excel 2016 software, $P<0.05$ was considered significant.

RESULTS AND DISCUSSION:

Isolation of *Streptococcus mutans*from Clinical Dental Caries Positive Sample: The colonies that showed alpha hemolysis were selected and streaked on Brain Heart Infusion (BHI) agar, White, grey, or yellow-colored colonies with a diameter of 0.5-2 mm, as well as rough and irregular colonies were selected and placed on nutrient agar slants for additional testing. Around 25 colonies of *Streptococcus mutans* were isolated from the Dental caries sample. Based on culture, morphological, and biochemical characteristics as described in Bergey's Manual of Determinative bacteriology, the isolates were identified as *S. mutans* **Table 2.**

TABLE 2: CHARACTERIZATION OF *STREPTO-COCCUS MUTANS* ISOLATES

Test	Results
Gramstain	Grampositive
Shape	Cocci in long chains
Motility	Non -Motile
Catalase	Negative
Hemolysis	α and β
Esculin hydrolysis	Positive
Arginine hydrolysis	Negative
Urea hydrolysis	Negative
Voges-Proskauer test	Positive
Optochin sensitivity	Negative
Growth on 6.5%NaCl	Negative
Acid form:	
Sucrose	Positive
Mannitol	Positive
Lactose	Positive
Sorbitol	Positive

Phytochemical Analysis: The crude plant extracts were extracted using different solvents. Table 3 provides some characteristics of the solid extracts obtained after evaporation to dryness as the paste obtained for each extract. As described in the phytochemical analysis of different plant extracts. The crude plant extracts were extracted using different solvents, **Table 3.** Provides some characteristics of the solid extracts obtained after evaporation to dryness as the paste obtained for each different extract. As described in the phytochemical analysis of different plant extracts showed the presence of different phytoconstituents like alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids **Table 4.**

TABLE 3: THE APPEARANCE, CONSISTENCY AND YIELD OF DIFFERENT SOLVENTS OF PLANTS EXTRACTS

Plants Species	Solvents	Appearance, consistency	Yield in Grams
Glochidion ellipticum Wight	Pet Ether	Light yellow; Thick paste	1.27
	Acetone	Dark yellow; jelly paste	1.74
	Methanol	Dark green, Thick paste	2.75
	Aqueous	Dark brown, liquid	10
Mappiea foetida	Pet Ether	Light green, paste	1.29
	Acetone	Dark green, jelly paste	1.5
	Methanol	Dark green, Thick paste	1.25
	Aqueous	Brown, liquid	10
Homonoia riparia Lour.	Pet Ether	Green, paste	1.27
	Acetone	Green, paste	2.75
	Methanol	Dark green, sticky paste	3.69
	Aqueous	Green, liquid	10
Clausena dentata (Willd.) Roem.	Pet Ether	Light green, paste	1.93
	Acetone	Dark green, jelly paste	1.16

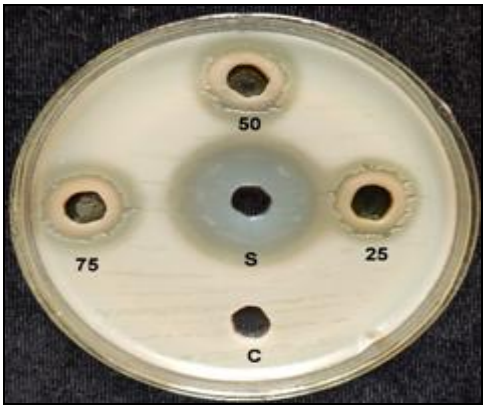
	Methanol	Dark green, thick paste	5.15
	Aqueous	Green, liquid	10
Memecylontalbotianum Brandis	Pet Ether	Green, paste	1.4
	Acetone	Green, paste	1.98
	Methanol	Dark green, sticky paste	1.52
	Aqueous	Green, liquid	10

TABLE 4: PHYTOCHEMICAL ANALYSIS OF DIFFERENT SOLVENTS OF PLANTS EXTRACTS

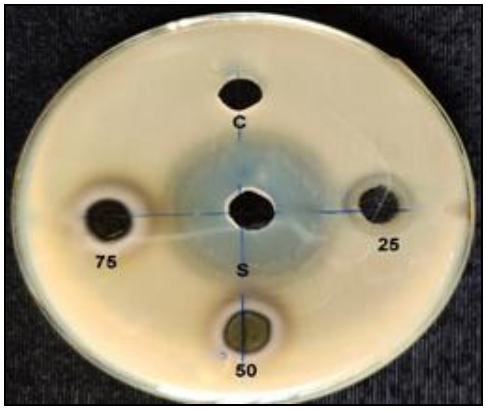
Sl. no.	Plant Name	Solvents Used	A	T	S	Sp	Ta	G	F	P	C
1	*Glochidion ellipticum Wight*	Pet Ether	-	+	-	-	+	+	+	-	+
		Acetone	-	+	-	-	+	+	-	-	+
		Methanol	+	+	-	-	+	+	+	-	+
		Aqueous	-	+	-	-	+	+	+	+	+
2	*Mappiea foetida*	Pet Ether	-		+	-	-	-	-	-	-
		Acetone	-	-	+	-	-	-	-	-	-
		Methanol	-	+	+	-	-	-	-	-	-
		Aqueous	+	-	-	-	-	-	-	-	-
3	*Homonoia riparia Lour.*	Pet Ether	-	-	-	-	-	-	+	-	-
		Acetone	-	+	-	-	-	-	-	-	-
		Methanol	+	+	-	-	-	-	+	-	-
		Aqueous	-	+	-	-	-	-	+	-	-
4	*Clausena dentata (Willd.) Roem.*	Pet Ether	+	+	+	+	+	+	-	+	+
		Acetone	+	+	+	+	+	+	-	+	+
		Methanol	+	+	+	+	+	+	-	+	+
		Aqueous	+	+	+	+	+	+	-	+	+
5	*Memecylon talbotianum Brandis*	Pet Ether	-	-	-	+	-	+	-	-	+
		Acetone	-	-	-	+	-	+	-	-	+
		Methanol	-	+	-	+	+	+	+	-	+
		Aqueous	-	+	+	-	+	-	+	-	+

* A - Alkaloid,T - Tri terpenoids, S - Steroids, Sp - Saponin, Ta - Tannin, G - glycosides, F - Flavonoid, P - Protein, C - Carbohydrate. '+' Present; '-' Absent

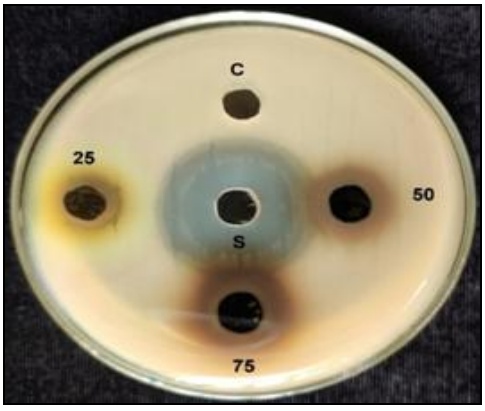
***Glochidion ellipticum* Wight**



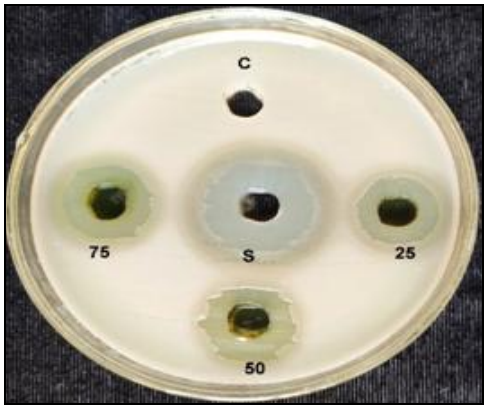
***Homonoia riparia* Lour.**



***Clausena dentata (Willd.)* Roem.**



***Memecylon talbotianum* Brandis**



Mappiea foetida

FIG. 1: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS OF DIFFERENT PLANTS AGAINST *STREPTOCOCCUS MUTANS*. (S - STANDARD ANTIBIOTIC; C - CONTROL)

TABLE 5: ANTIMICROBIAL ACTIVITY OF DIFFERENT PLANT SOLVENT EXTRACTS AGAINST *STREPTOCOCCUS MUTANS* BY AGAR WELL DIFFUSION METHOD

Sl. no.	Plant Name	Plant Extracts	Zone of Inhibition (In mm)				
			Standard	Control (10% DMSO)	25 %	50 %	75 %
1	*Glochidion ellipticum Wight*	Pet Ether	29.83 ±0.08	0.0	11.63±0.31	14.66±0.33	16.93 ± 0.52
		Acetone	29.8 ± 0.08	0.0	12.06 ± 0.12	14.8 ± 0.4	17.03 ± 0.08
		Methanol	29.8 ± 0.08	0.0	13.6 ± 0.3	15.9 ± 0.05	17.9 ± 0.05
		Aqueous	29.8 ± 0.08	0.0	10.8 ± 0.08	15.9 ± 0.05	18.6 ± 0.08
2	*Mappiea foetida*	Pet Ether	29.9 ± 0.06	0.0	12.83 ± 0.4	19.13 ± 0.18	25.06 ± 0.24
		Acetone	29.8 ± 0.05	0.0	15.1 ± 0.2	21.16 ± 0.72	30.43 ± 0.31
		Methanol	29.83 ± 0.08	0.0	17.16 ± 0.2	23.16 ± 0.72	30.43 ± 0.31
		Aqueous	29.8 ± 0.05	0.0	17.1 ± 0.15	24.9 ± 0.26	31.1 ± 0.20
3	*Homonoia riparia Lour.*	Pet Ether	29.9 ± 0.05	0.0	10.2 ± 0.14	14.2 ± 0.14	19.3 ± 0.16
		Acetone	29.8 ± 0.05	0.0	10.1 ± 0.06	14 ± 0.05	18.9 ± 0.21
		Methanol	29.8 ± 0.05	0.0	6.8 ± 0.18	11 ± 0.2	16 ± 0.05
		Aqueous	29.8 ± 0.05	0.0	6.9 ± 0.12	10.7 ± 0.4	15.8 ± 0.18
4	*Clausena dentata (Willd.) Roem.*	Pet Ether	29.9 ± 0.05	0.0	10.3 ± 1.45	14.43 ± 0.29	16.6 ± 0.2
		Acetone	29.63 ± 0.31	0.0	12.13 ± 0.24	17.9 ± 0.20	21 ± 0.5
		Methanol	29.8 ± 0.05	0.0	11.56 ± 0.29	16.16 ± 0.08	22.56 ± 0.29
		Aqueous	19.8 ± 0.05	0.0	10.53 ± 0.24	15.76 ± 0.23	19.13 ± 0.63
5	*Memecylonta lbotianum Brandis*	Pet Ether	29.86 ± 0.03	0.0	10.86 ± 0.08	15.7 ± 0.17	19.86 ± 0.08
		Acetone	29.63 ± 0.31	0.0	7.83 ± 0.12	12.03 ± 0.08	18.43 ± 0.21
		Methanol	29.8 ± 0.05	0.0	5.9 ± 0.20	8.33 ± 0.2	11.33 ± 0.2
		Aqueous	29.8 ± 0.05	0.0	5.8 ± 0.11	7.76 ± 0.14	10.83 ± 0.08

Each value is the mean of three replicate, Mean ± SEM (standard error of the mean). *S mutans: Streptococcus mutans*, DMSO: Dimethyl sulfoxide.

Antimicrobial Screening by Agar well Diffusion Method: Dental caries is one of the major health concerns in the world, especially among lower socioeconomic status groups, and also due to a lack of preventive efforts, and dietary changes on part of the individuals/community concerned. Therefore, we need new and renewed efforts to cut down the drastic increases in dental caries infection. We aimed to find medicinal plants as an anti-caries agent which would effectively replace the commercially available agents. The agar well diffusion method was used to test the antibacterial activity of different plant extracts using different solvents and the results showed variation in the antibacterial studies.

Methanol extracts of *Mappiea foetida* at the concentrations of 75 mg/ml, 50 mg/ml, and 25 mg/ml were reported to have substantial antibacterial activity against *Streptococcus mutans* and the results were tabulated in **Table 5** and **Fig. 1.** *Glochidion ellipticum, Homolia reperia* and *Clauesina denteta* showed moderate effect and *Memecylonta boltianum* showed negligible effect on Streptococcus mutans. The results suggested that only methanol extract possessed the highest

antimicrobial activity on *Streptococcus mutans* and aqueous extracts of plants showed minimum activity on *Streptococcus mutans*. *Mappiea foetida* which showed good inhibitory properties against Streptococcus mutans was also reported as a good anti-cancer agent [25], because of these features which have made it an endangered species [26].

During our preliminary findings, methanol extract showed substantial inhibition at 25% concentration showing 17.16 ± 0.2 mm of inhibition and at 75% concentration showing 30.43 ± 0.31 mm of inhibition compared to the other solvent plant extracts. Wannachot and Rattanakiat [27] in which they investigated the inhibitory effect against *Streptococcus mutans in-vitro*; of the 95% ethanol extracts from five herbs: *M. cochinchinensis* Spreng, *P. guajava* L, *G. glabra* L, *P. retrofractum* vahl and *S. aromaticum* L, the largest inhibition zone 16.7±0.5 mm in diameter was observed in *S. aromaticum* extract. The extract of *P. retrofractum* produced a minimum inhibition zone of 6.7±0.5 mm. In another study conducted by Elgamily *et al.,* [28] methanolic extractions plants Ginger, Clove, Black seed, Cinnamon and Turmeric were investigated against the growth of the *Streptococcus mutans* of which only Clove and cinnamon showed inhibition zones against *Streptococcus mutans* with diameters of 14.00 mm and 12.67 mm respectively.

A similar study by Bharghavi Prabhuswamym, 29 showed that *C. zeylanicum* showed maximum antibacterial activity against *S. mutans* followed by *A. catechu, M. elengi*, and *T. arjuna*. The antimicrobial activity of *C. zeylanicum* is attributed to the presence of cinnamaldehyde. This study reveals that the Methanol extract of *Mappiea foetida* could be used as a source of new antimicrobials as it showed dose-dependent activity.

CONCLUSION: The results of our study indicate that the methanol extracts of *Mappiea foetida* used in this study have an antibacterial effect even at low concentrations, against the *Streptococcus mutans* and improve the effectiveness of oral hygiene practices by combining these extracts of the plants into anti-caries treatments such as Toothpaste and mouthwash. Further studies such as these plants' toxicological and pharmacokinetic properties, need to be conducted to develop these plant products into antibacterial agents for clinical use.

ACKNOWLEDGMENT: The authors would like to thank the Department of Dentistry at District McGann Hospital, Shivamogga, and at Giriraj Dental Clinic Shivamogga, for providing the Dental caries samples and SC/ST Cell, Kuvempu University, Shankaraghatta, Shivamogga for their financial support.

CONFLICTS OF INTEREST: The authors declare no conflict of interest

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How to cite this article:
Vishal M and Mallikarjun N: Preliminary screening of anti-cariogenic properties of selected medicinal plants against streptococcal dental caries. Int J Pharm Sci & Res 2023; 14(3): 1202-09. doi: 10.13040/IJPSR.0975-8232.14(3).1202-09.

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