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PRELIMINARY SCREENING AND EVALUATION OF *COMMIPHORA CAUDATA* LEAF EXTRACT ON CARIOGENIC PATHOGENS

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ABSTRACT: Cariogenic organisms are responsible for producing and developing tooth decay, which ends up in dental caries. It is commonly seen in both children and adults. The aim was to evaluate anticariogenic activity of *Commiphora caudata* leaf extract. Two different solvents such as acetone and 70% ethanol used against *Lactobacillus casei* and *Streptococcus mutans*. Agar well diffusion method and minimum inhibitory concentration (MIC) was used for this purpose. Ethanolic extract showed significant antibacterial activity against the tested organisms. Preliminary phytochemical screening of the extract revealed the presence of steroids, phenols, flavonoids and coumarins in both acetone and ethanol extract. The above observations remarkable cariogenic inhibition, which may be due to these active secondary metabolites. This may potentially treat dental caries and be used as lead(s) molecules in the development of new antimicrobial drugs against cariogenic pathogens.

INTRODUCTION: Plants are an important source of medicine. Herbal medicine occupied a vital position in Indian culture and folklore therapy [1]. World Health Organization (WHO) reports that 80% of the world population depends on medicinal plants for the prevention and treatment of diseases. Even today, people depend on traditional hand medicine for their preliminary health care and treatment [2].

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The knowledge received from generations is widely used to manage and treat various diseases. Bioactive components from different parts of the plants such as leaves, stem, latex, bark, root, flower and seeds are known as secondary metabolites involved in plants' defense mechanisms against microorganisms, insects and herbivores.

Plants possess various bioactive molecules such as tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, phenolic compounds and many more [3]. It is a known fact and a belief that phytoconstituents obtained from the medicinal plants serve as probe molecules in the recent medications. Currently, 25% of herbal drugs in the modern pharmacopoeia are plant-based and several synthetic drugs are manufactured by

using chemical substances isolated from plants. It is also believed that folklore medicine may have good efficacy and cultural acceptability, be cost-effective, affordable and have less undesirable effects than modern medicines [2, 4]. One such traditional herb is "*Commiphora caudata*" or commonly called as "Hill Mango" which is belongs to the family of Burseraceae [5]. In Tamil, it is known as "Pachai Kiluvai" [6]. Chewing the *Commiphora caudata* fresh leaves as a remedy for oral health issues is followed by the rural folks of Erode district. This indigenous routine created an interest for us to analyze the anti-cariogenic properties of this plant extract. Analgesic and anti-inflammatory properties of the *Commiphora caudata* leaf extract had been evaluated already. This plant is found to be non-toxic, known for its antispasmodic activity, cytotoxic activity, hypothermic activity, analgesic, anti-inflammatory, antibacterial, antiulcer, antiviral, anticancer, antidiabetic activity, antiarthritic, anti-hyperlipidemic, antioxidant, anti-lipid and peroxidation. Also, it is well documented that leaves have been used in folk medicine and possess astringent, sweet, cooling, aphrodisiac properties [7, 8-9].

Dental caries is one of the most important preventable global infections of the mouth caused by oral microbiota [10]. *Streptococcus mutans* is known as the primary pathogen of dental caries [11]. It is a multifactorial disease in which diet, nutrition, microbial infection and host response all play important roles. Their key role in the etiology of periodontitis and dental caries, the most prevalent diseases in the world, is well established [12]. The formation of dental caries is caused by the colonization and accumulation of oral microorganisms, and adherence is the first step in the colonization process. Poor oral hygiene and nutritional deficiencies increase the cariogenic activity of *Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus mitis, Streptococcus oralis, Lactobacillus, actinomycetes* and various other organisms in viable count [10]. Along with dental caries, various other oral diseases such as periodontal disease, gingival inflammation and tooth loss may significantly affect overall health [13].

Antibacterial and antiseptic agents such as cetylpyridinium chloride, chlorhexidine, fluorides, and phenol derivatives are widely used in dentistry to combat bacterial growth. However, increased incidence of dental caries due to high sugar consumption, antibiotic resistance, supra infections, allergic reactions and various adverse effects are seen. Mouthwashes mainly help remove plaque, gingivitis, and the removal of infections during extraction of a tooth, intraoral surgical procedures, or immune suppression during cancer therapy. Chlorhexidine is an anticaries agent used for the prevention of dental caries by decreasing the number of microorganisms or inhibiting the formation of dental plaque. However, its regular use, which results in several side effects such as unpleasant taste and staining, invigorates the search for alternate agents [14]. Using natural alternatives to replace inorganics has been trending in the contemporary biological field of study [15].

AIM: To do the preliminary phytochemical screening and evaluation of *Commiphora caudata* leaf extract on cariogenic pathogens.

MATERIALS & METHOD:

A. Plant Material Collection: Fresh leaves of *Commiphora caudata* were collected from the surroundings of Erode district and kept in zip lock bags with appropriate labeling and stored in an ice cooler until being transported to the laboratory for extraction.

FIG. 1: DRIED KILUVAI LEAVES

FIG. 2: POWDERED KILUVAI LEAVES

B. Authentication: The collected leaves were identified and authenticated by the Plant taxonomist Prof. Dr. P. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu (PARC/2019/4119). For future reference, the voucher specimen was deposited in the Central Research Lab, Meenakshi Ammal Dental College and Hospital, Chennai.

C. Cariogenic Organism: In our study, human dental pathogens such as *Lactobacillus casei(NCIM 5304)* procured from the National Collection of Industrial Microorganisms, Pune, Maharashtra and *Streptococcus mutans MTCC 497, (*Microbial Type Culture Collection, Gene Bank, Chandigarh, India*)* by Central Research Laboratory(CRL), Meenakshi Ammal Dental college and Hospital (MADCH), Chennai, Tamil Nadu. The isolates were Sub-cultured on nutrient agar plates, bring to pure culture and maintained at 5°C [16].

D. Standard Solution: The chlorhexidine (0.12%) mouthwash was used as a standard to compare with the leaf extract was purchased from the local market in Chennai, Tamil Nadu.

E. Preparation of Plant Extract: The collected samples were first washed under running tap water, distilled water and air-dried in the shade at room temperature. Using a home mixer, the dried leaves were ground to a fine powder. The fine dividend Powder was sieved and used for the further extraction procedure. 25 grams of *Commiphora caudata* leaf powder dissolved in 75 ml of acetone and 70% ethanol, respectively, at room temperature for three successive days. The mixture was shaken in an orbital shaker at 50rpm. The supernatant was filtered through Whatman filter paper (No 2) on a Buchner funnel, while the residues were used for a second (50 ml) and third (30 ml) extraction. The dissolved parts were filtered and stored in a preweighed flask each day before drying. After the third extraction, the filtrates were decanted and combined. The procedure was repeated for 70% Ethanol sequentially [11]. The crude extract was collected in a vial, used for the antimicrobial assay.

TABLE 1: EXTRACTION YIELDS OF THE PLANT EXTRACT

S. no	Solvent Used	Extract Yield (gms)
1	Acetone	1.058
2	70%Ethanol	2.967

F. Crude Extract: Crude extract prepared by dissolving 10mg (Acetone filtrate) and 10mg (70% Ethanol filtrate) plant yield in 50µl DMSO, respectively, along with standard chlorhexidine mouthwash (positive control) and DMSO (Negative control).

F. Growth Media used: Nutrient agar (NA), Mutans-Sanguis (MS) agar, and Mueller-Hinton agar (MHA) were used as growth media to examine the microbial growth. Nutrient agar (NA) contains per liter of deionized water: 5g Hi veg peptone, 1.5g Hi veg extract, 1.5 g yeast extract, 5 g sodium chloride, agar 15 g with pH of 7.4 at 25°C. Mutants-Sanguis (MS) agar contained per liter of deionised water: v Tryptone 15 g, Yeast extract 5 g, L-Cysteine 0.2 g,Sodium sulphite 0.10 g, Sodium chloride 1 g, Disodium hydrogen phosphate 0.8 g, Sodium bicarbonate 2 g, Sodium acetate 12 g, Sucrose 50 5, Agar 12 g with pH of 7.3 at 25°C. Mueller-Hinton agar (MHA) was used to determine the susceptibility of microorganisms against antimicrobial agents, which contained per liter of deionized water: meat infusion 300 g, casein hydrolysate 17.5, starch 1.5, agar-agar 17 g with pH of 7.3 at 25°C [11].

G. Antimicrobial Assay of *Commiphora caudata* Leaf Extract: Antimicrobial assay was performed by agar well diffusion method. 20 ml of Mueller Hinton Agar (MHA) was poured into sterile petriplates and allowed to solidify. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving the final inoculums of 1.5×10^8 CFU/ml [17].

MHA plate was lawn cultured with standardized microbial culture broth. Six agar wells of 6 mm were bored in the cultured media with the help of a sterile cork borer (0.65 cm). Wells were filled with different concentrations of the extract ranging from 20µl, 30 µl, 40 µl, 50 µl, respectively, and allowed to diffuse into the medium for about 45 minutes. Negative control was maintained with DMSO and the positive control with standard chlorhexidine mouthwash at a concentration of 30 µl. same procedure was repeated for 70% Ethanol. Treated and control plates were incubated at 37°C for 24 h. The experiment was performed in strict aseptic conditions.

Microbial growth was determined by measuring the zone of inhibition (mm) using a metric scale. Each extract was analyzed in triplicate to obtain the mean values.

H. Phytochemical Screening: Acetone & 70% alcohol extract of the leaves of *Commiphora caudata*, subjected to qualitative phytochemical analysis for detecting the chemical compounds in it. The leaf extract's phytochemical components were screened using standard procedures [18, 19, 20, 21, 22, 23].

Test for Alkaloids: About 2ml of the filtrate was taken in three different test tubes and few drops of dil. HCl were added in each of the tube, followed by Mayer's, Hager's and Wagner's reagent. Presence of alkaloids is indicated by the appearance of cream precipitation, yellow precipitation, and reddish brown colored precipitation after adding Mayer's, Hager's, and Wagner's reagent, respectively.

Test for Steroids: (Salkowsky Test): About 0.5gm of the filtrate dissolved in 2ml of chloroform. 2ml of conc. Sulphuric acid is added to form a lower layer (chloroform layer). A reddish-brown colour at the interface indicates the presence of steroids.

Test for Triterpenes: About 0.5gm of the filtrate dissolved in 2ml of chloroform (chloroform solution).

The chloroform solution is shaken with sulphuric acid on standing yields yellow color indicating the presence of triterpenes.

Test for Terpenoids: (Lieberman Burckhardt Test): Chloroform solution of the extract added with few drops of acetic acid and one ml concentrated sulphuric acid along the sides of the test tube. The appearance of red-brown color indicates the presence of triterpenoids.

Test for Triterpenoids (Lieberman Burchardt Test): Chloroform solution of the extract added with a few drops of acetic acid and one ml concentrated sulphuric acid along the sides of the test tube. The appearance of blue-green color indicates the presence of triterpenoids.

Test for Phenols: Filtrate treated with a few drops of 15% acetic acid and a few drops of sodium nitrate solution. Muddy or Niger brown color indicates the presence of phenols.

Test for Quinones: About 1ml of the filtrate treated with 1ml of sulphuric acid. The appearance of red color indicates the presence of quinones.

Test for Anthraquinones: About 5ml of filtrate mixed with 10ml benzene and 5ml of 10%NH3 solution. The appearance of pink, red or violet color in the lower phase indicates the presence of anthraquinones.

Test for Flavonoids: About 1 ml of the filtrate was treated with a few 10% lead acetate drops. The appearance of yellow-orange colour indicates the presence of flavonoids.

Test for Tannins: About 1 ml of the filtrate treated with 0.5ml of 10% lead acetate. The appearance of white precipitate indicates the presence of tannins.

Test for Phlobatannins: About 2ml of filtrate boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate indicates the presence of phlorotannins.

Test for Saponins: About 0.5gm of the filtrate was shaken with water in a test tube. Frothing, which persists on warming, was taken as preliminary evidence for the presence of saponins.

Test for Carbohydrates: About 2ml of filtrate added with 2 drops of molish reagent and a few drops of conc sulphuric acid. The appearance of violet or reddish color indicates the presence of carbohydrates.

Test for Glycosides: Dissolve a small amount of filtrate in 1ml water and add sodium hydroxide solution. The appearance of yellow color indicates the presence of glycosides.

Test for Cardiacglycosides: About 0.5gms filtrate added with 2ml of glacial acetic acid, ferric chloride and conc sulphuric acid.

Test for Coumarins: about 1ml of extract, add 1ml of 10% sodium hydroxide. The appearance of yellow color indicates the presence of coumarins.

Test for Proteins (Millon' s test): To 2ml of plant extract, added 2ml of Millon s reagent and observed for two minutes for the formation of white precipitate. On gentle heating, which may be turned to red, indicates the presence of proteins in it.

Test for Amino Acids (Ninhydrin Test): To 2ml of plant extract, added 2ml of Ninhydrin reagent. Violet color indicates the presence of amino acids.

RESULTS & DISCUSSION: Numerous studies were demonstrated to identify the valuable drug components with high efficacy. The quantitative determination of the *Commiphora caudata* extracts' anticarcinogenic activity was assessed using two solvents exhibiting high antibacterial activity against *Streptococcus mutans* and *Lactobacillus casei.* Both the solvent extracts showed a good zone of inhibition **Table 2** and **Fig. 3** equal to the comparator chlorhexidine mouthwash (12mm). Comparatively, more potency can be seen in 70% ethanol than acetone. Examining the MIC values of samples of extracts, *Streptococcus mutans* showed MIC at 1.53 µl and 25 µl for acetone and ethanolic extract, and *Lactobacillus casei expressed the* MIC at 25 µl and 50ul for acetone and ethanol extract, respectively. When the extracts' concentration was decreased, it showed a slight decrease in inhibition zones. The results are tabulated in **Table 3**. This study is a preliminary assessment to identify the naturally available potential therapeutics that are effective in treating dental caries. It may act as an alternative to the regular mouth rinse in restricting the progression of the disease process as plant medicine has proved its effectiveness in various microbial disease processes [24]. This study revealed the presence of common phytochemical constituents such sterols, phenols, and coumarins in both extracts and triterpenoids, tannins specific in ethanolic extract **Table 4**. These secondary metabolites are reported to have many biological and therapeutic properties [25]. The *Commiphora caudata* leaf extract possesses these secondary metabolites in high concentration, which may also account for the various significant medicinal values. Since this screening study is very basic, it may still require more to explore the many pharmacological properties.

TABLE 2: ZONE OF INHIBITION (MM) OF *COMMIPHORA CAUDATA* LEAF EXTRACT AGAINST SELECTED CARCINOGENS-AGAR DIFFUSION METHOD

Organism	Zone of Inhibition(mm)		
Sreptococcus mutans	**Concentration (µl)**	**Acetone**	**70% Ethanol**
	20	6	7
	30	7	8
	40	10	10
	50	12	11
	Postitive Control (Chlorhexidine)	12	22
Lactobacillus casei	20	8	8
	30	9	10
	40	10	12
	50	6	14
	Postitive Control (Chlorhexidine)	10	10

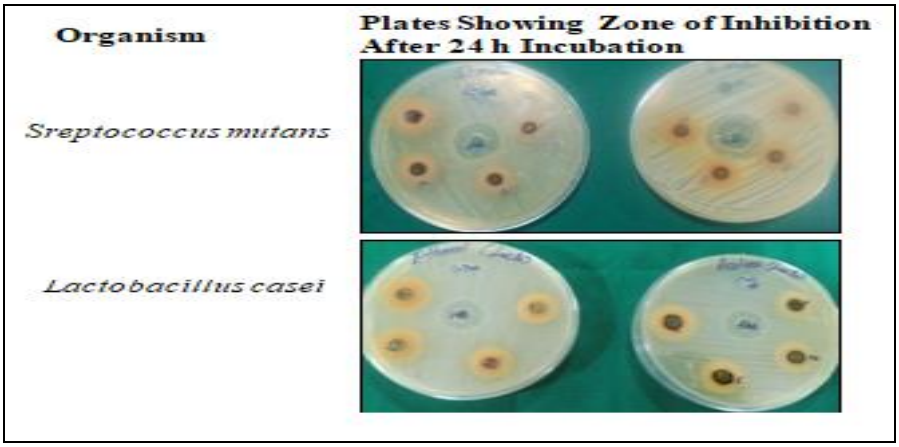

FIG. 3: ZONE OF INHIBITION

TABLE 3: MINIMUM INHIBITORY CONCENTRATIONS

Acetone		100µl	50 µl	25 µl	12.5 µl	6.25 µl	3.0625 µl	1.53 µl
	Sreptococcus mutans	×	×	×	×	×	×	×
	Lactobacillus casei	×	×	✓	✓	✓	✓	✓
70%Ethanol	*Sreptococcus mutans*	×	×	×	✓	✓	✓	✓
	Lactobacillus casei	×	×	✓	✓	✓	✓	✓

✓- Growth Seen × – No Growth.

TABLE 4: RESULT OF PHYTOCHEMICAL SCREENING OF *COMMIPHORA CAUDATA* LEAF EXTRACT

S. no	Phytochemical Tests	Acetone extract	70% Ethanol extract
1	Alkaloids	-	-
2	Steroids	+	+
3	Triterpenes	-	+
4	Terpenoids	-	+
5	Triterpinoids.	-	+
6	Phenols	+	+
7	Quinones	-	-
8	Anthraquinones	-	+
9	Flavanoids	+	+
10	Tannins	-	+
11	Phlobatannins	-	-
12	Saponins	-	-
13	Carbohydrates	-	-
14	Glycosides	-	-
15	Cardiacglycosides	-	-
16	Coumarins	+	+
17	Proteins	-	-
18	Amino Acids	-	-

- + (Positive) – (Negative)

CONCLUSION: The study was a preliminary assessment of the early available plant components that could be effective in treating dental caries. The leaf extract of *Commiphora caudata* showed more potency in 70% ethanol than acetone.

The study Might add up to the trend of proper use of medicinal plants and identified the new indigenous sources of drugs that could be utilized in the pharmaceutical conclusion, plant extracts were of great value as natural antimicrobials that could be used in the preparations used against dental caries and could used safely as a remedy. Thus naturally available compound might face challenges in dental caries management in the future health care system.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest**.**

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