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Research Article

In Vitro Assessment of Inhibition Potential of Ethanomedicinal Plants against Cariogenic Bacteria

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Abstract

Oral diseases major responsible of dental caries that continue to be major health problem through the world. The oral diseases treatment options and products required the global need for alternative prevention method. Medicinal plants extracts could serve as an important natural alternative to prevent microbial growth in oral infection diseases. This study was undertaken to determine the *in vitro* anticariogenic activities of 19 medicinal plants leaves and fruits against dental pathogenic bacteria. *In vitro* antibacterial potential of the ethyl acetate, hexane, methanol and distilled water extracts was evaluated by using four cariogenic bacteria, *Lactobacillus acidophilus* (LA); *Lactobacillus casei* (LC); *Streptococcus mutans* (SM) and *Staphylococcus aureus* (SA). The antibacterial activity study by agar well diffusion method and MIC. The good MIC values showed those all selected plants study for Phytochemical evaluation and TLC – bioautography. The ethyl acetate extract of plant leaves showed the more potent anticariogenic activities against oral pathogen. The fruit extract is more powerful compared to leaves extract as far as growth inhibition in cariogenic bacteria of selected ethanomedicinal plants. High degree of growth inhibition (19 - 25 mm) was found when plant extracts of Coroupita guianensis (Fruit) tested against Lactobacillus casei by various extracts of three plants which is more than that of the standard antibiotic erythromycin (19 mm). The MIC of methanol extract of *Coroupita guianensis* (Fruit) against *Lactobacillus casei* was 0.16 mg/ml. The based on the MIC data selected plants for preliminary phytochemical analysis revealed the presence of alkaloids in the all plants extracts. The methanol extract of *Coroupita guianensis* L. (Fruit) in present the Cardiac glycoside, steroids, Terpenoids and phenolic compound. The resulted chromatogram was used for bioautography against *Lactobacillus casei*. This study revealed that *Coroupita guianensis* is natural alternative to prevent the oral disease.

Keywords: Ethano-Medicinal Plants; Oral Disease; Cariogenic Bacteria; Bioautography

Introduction

Oral diseases continue to be a major health problem worldwide. Oral cavity and teeth diseases are among the most important global level common oral health problems, although conditions such mouth cancers and oral tissue lesions are also major health concerns [1]. The 90% dental caries in school aged children and most adults are affected the people living in industrialized countries, including oral and dental health. The quality of life general well-being and relates to integral of oral health and craniofacial complex. The poor oral health is considerable evidence linking to chronic conditions, for example, periodontal diseases and diabetes there is a strong association [2]. The oral diseases are an important consideration the economic impact with up to 10% of public health expenditure in curative dental care. The expenditure in oral health care is low in developing countries; access to dental healthcare is limited and is usually restricted to emergency dental care. The developed countries worldwide has been marked improvement in oral health, populations of dentally disadvantaged individuals exist in these countries, often those people indigenous and child populations of low socio-economic status, where oral health is weakling [3].

The activities of microbial species and link between oral diseases that oral cavity is well established of part of the microbiota. The inhabit the oral cavity by 750 species of bacterial number of these are implicated in oral diseases [4]. The dental caries development involves aciduric Gram-positive bacteria and acidogenic and, primarily present the *Streptococcus mutans* and *S. sobrinus, Actinomycetes* and *Lactobacilli*, the dissolve the calcium phosphate in teeth because of metabolize sucrose to organic acids, causing decalcification and eventual decay. Dental cavity is thus a supragingival condition [5]. In contrast, anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis, Actinobacillus* sp., *Prevotella* sp. and *Fusobacterium* sp periodontal diseases are subgingival conditions that have been linked. In periodontal diseases, gingival crevice the areas at or below the become infected causing a cellular inflammatory response of the gingiva and surrounding connective tissue. These inflammatory responses can manifest as gingivitis or periodontitis [5].

The oral diseases global need for alternative prevention and treatment options and products that are safe, effective and economical comes from the increasing in disease incidence (par-

ticularly in developing countries), pathogenic bacteria day by day increased resistance to currently used antibiotics and chemotherapeutics, individual's opportunistic infections in immunocompromised is financial matter in developing countries [6]. The commercially several agents available, oral microbiota alter these chemical uses and have unwanted side-effects such as tooth staining, vomiting and diarrhea [7]. Hence, traditional medicine is considered as good alternatives to synthetic chemicals the search for alternative products continues and natural phytochemicals isolated from plants used [8].

The oral diseases use of traditional plants and natural products for the treatment numerous reports. The traditional medicinal systems have many plant-derived medicines used recorded in pharmacopeias as agents used to treatment for disease and a number of these have been recently investigated for their usefulness against oral bacterial pathogens. The medicinal plants and plant products general antimicrobial activities reports, such as leaves extracts, seed extracts, essential oils, have been study previously [9-12]. The inhibit the growth of oral pathogens use the traditional medicinal plant extracts or phytochemicals responsible for reducing the symptoms of oral diseases, influence the adhesion of bacteria to surfaces and development of dental plaque [13,14].

The oral diseases, especially plaque-related diseases such as dental caries recently investigated natural product thoroughly as promising agents for the prevention [15-17]. The antimicrobials have attracted the attention of the scientific community regarding the increasing resistance to a find out the low cost and effective drugs of natural origin [18]. The antimicrobial activity against cariogenic microbes and fungal filaments as well demonstrate the essential oils [19-21]. The plant-derived essential oils study has proven be an effective and alternative to overcome microbial resistance [22]. The traditional medicinal plants against oral pathogens studies the activity have been limited to examination of organic solvent extracts. In most cases, the traditional medicinal use of the plant investigators has simply sought to validate for example, the use of *Drosera peltata* (Droseraceae) leaves which showed that chloroform extracts of the aerial plant parts showed broad spectrum activity against numerous bacteria of the oral cavity as a traditional treatment for dental caries was validated, with high activity against *S. sobrinus* and *S. mutans*. The active component of this extract was identified as Plumbagin. A collection of 27 medicinal and random plants extracts and identified the inhibited the growth of oral streptococci investigated by Tichy and Novak [22].

The present study aimed to screen the 19selected medicinal plant (Leaf materials from 16 plants and fruit materials from 3 plants) extracts for their efficacy against four different tooth decaying bacteria under *in vitro* conditions. The study also extended further to characterize phytochemical constituents at primary level. This information regarding the potential plants will be used for detailed characterization of bioactive compound for their future application.

Materials and Methods

Plant materials

The different plant species were selected and collected from different part of Gujarat and surroundings of Vallabh Vidyanagar between Januarys to February 2010 form (Table 1). The healthy and disease-free leaves of all the plants were used for the anticariogenic activity. The plant material was recognized by plant taxonomist (Dr. Kalpesh Ishnava, at Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India).

No.	Botanical name	Family	Local name	Collection site
1	*Cassia angustifolia* Vahl.	Aval	Caesalpiniaceae	V.V.Nagar
2	*Ficus religoesa* L.	Pipalo	Moraceae	Karamsad
3	*Calotropis procera* (Ait.) R.Br.	Akado	Asclepiadaceae	V.V. Nagar
4	*Coroupita guianensis* L.	Shivingi	Myrtaceae	V.V. Nagar
5	*Annona squamosa* L.	Sitaphal	Annonaceae	Jetpur
6	*Mentha longifolia* L.	Pudina	Labitate	V.V.Nagar
7	*Terminalia arjuna* (Roxb.) W.& A.	Arjun	Combretaceae	Junagadh
8	*Emblica officinalis* Gaertn.	Amala	Euphorbiaceae	Jetpur
9	*Hibiscus rosa-sinensis* L.	Jasud	Malvaceae	Jetpur
10	*Coroupita guianensis* (Fruit)	Shiveligi	Myrtaceae	V.V.Nagar
11	*Moringa oleifera* Lam.	Sargvo	Moringaceae	V.V.Nagar
12	*Vitex negundo* L.	Nagoad	Verbenaceae	Junagadh
13	*Ocimum sanctum* L.	Tulsi	Labiatae	V.V.Nagar
14	*Putranjiva roxburghii* Wall.	Putranjiva	Euphorbiaceae	V.V.Nagar
15	*Streblus asper* Lour.	Hahero	Moraceae	Jetpur
16	*Alstonia scholaris* (L.) R. Br	Saptaparni	Apocynaceae	V.V.Nagar
17	*Salvadora persica* L.	Piludi	Salvadoraceae	V.V.Nagar
18	*Lomonia acidissima* L.(Fruit)	Kothu	Rutaceae	V.V.Nagar
19	*Aegle marmelos* (L.) Corr. (Fruit)	Beeli	Rutaceae	Karamsad

Table 1: Details of selected plants leaves and fruits.

A preparation of plant leaves extracts

First, all plants material was thoroughly washed and blotted and dried under sunlight. For preparation of powder it was grinded in grinder (Maharaja Mixer Ltd). From these, 250 mL of hexane soaked the material of 50 gram of powder for 24 hours at room temperature under shaking 130 - 140 rpm. The help of Whatman filter paper number-1 extract was filtered. The filtrate was collected in petri dish and dried at room temperature. The dried extract from petridish was scraped out and transferred to Eppendorf tube.

After the funnel residual material was dried again and resuspended in 250 mL ethyl acetate for 24 hours at room temperature under shaking condition in130 - 140 rpm. The filtered the extract and collected in petri dish and dried at room temperature. Simi-

larly, re-extracted with same volume (250 mL) of methanol and then distilled water from the residual materials from the funnel are preserved. In both the cases, filtrate was air dried at room temperature of the resultant culture. The dried extract from petri dish was scraped and preserved in eppendorfs tube further study.

Cariogenic bacterial strains

A group of bacteria known to cause oral disease were selected and purchased from MTCC (Microbial Type Culture Collection) bank, Chandigarh. The MTCC specified selective growth medium using bacterial cultures were revived and preserved in glycerol stocks. The details of bacteria responsible for dental caries used for the study of *Lactobacillus acidophilus (*MTCC-447); *Lactobacillus casei* (MTCC-1423); *Streptococcus mutans* (MTCC- 890) and *Staphylococcus aureus* (MTCC- 96).

Preparation of Inoculums

The prepared fresh microbial culture and streaking loopful of bacterial suspension in to organism specific selective media (Himedia) and uniform growth rate of each organism for incubated at optimal temperature to maintain. The 0.5 McFarland turbidity standard compared with bacterial cultures from fresh media, which is throughout the experimentation equivalent to maintained approximately 1 X 10^8 bacterial cell count per mL [23].

Bioassay for Antimicrobial activity

Agar Well Diffusion Method

In the present study, to perform the antibacterial activity of 19 different plant extracts. The antibacterial activity was studied by agar well diffusion method as per reported by Peres., *et al* [24].

Antibiotics Cefadroxil, Erythromycin and Tetracycline were used as standard at a concentration of 100 µg/mL used as positive control and 100% DMSO were used as negative control. Bioassay experiment was performed in duplicate and repeated twice.

Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentration (MIC) was study by the two-fold serial broth dilution method by Chattopadhyay., *et al.* [25]. Plant extracts showing more than 0.9 mm inhibition zone were selected for MIC. Each assay was repeated thrice by using selective medium as control and DMSO.

Phytochemical analysis

Preliminary phytochemical analysis

Qualitative phytochemical analysis (Tannin, Alkaloids, Saponins, Cardiac glycosides, Steroids, Terpenoids, Phenolic compounds) of all the plant leaves extracts selected, based on MIC value was perform as per the methodology of Parekh and Chanda [26].

Analytical thin layer chromatography

To find out suitable solvent system for the development of chromatogram using the analytical TLC. The Chloroform: Methanol: (1:4) solvent mixtures were used for the separation of the compounds on precoated TLC plates (Merck, silica gel 60 F254 plate, 0.25 mm).

TLC- Bioautography

Out of 19 plants leaves extracts study for antibacterial activity, maximum growth inhibition showing only one against *Lactobacillus casei* was selected and used for bioautography. By using capillaries 10 µL of aqueous extract of *Coroupita guianensis* fruit extract (100 mg/mL stock solution) was spotted on to 0.25 mm thick precoated silica gel 60 F254 plate (Merck, Germany) and 2 mm thick band length was prepared. The TLC plate air drying after run using pre-standardized solvent system of Chloroform: Methanol: (1:4). The TLC plate was observed under UV light after used for bioautography. The *Lactobacillus casei* organism seeded specific agar medium was overlaid on to the silica gel plate loaded with sample and incubated at 37°C for 24 hrs. The next day plate visualize growth of inhibition after flooded with 2, 3, 5-Tri phenyl tetrazolium chloride (0.1%). The area of transparent against reddish background (lawn of living bacteria) is inhibition zone was appeared.

Result and Discussion

In the present study, the antibacterial assay of plant leaves extracts against cariogenic bacteria was carried out. The leaves of sixteen plants and three fruits of plant were extracted using hexane, ethyl acetate, methanol and distilled water and used for antibacterial study.

The result of antibacterial sensitivity of oral disease forming bacteria was assessed by visualizing the presence or absence of inhibition zone and measuring the zone diameter. The plant wise results are summarized as under:

Cassia angustifolia Vahl

Hexanolic extract of this plant is slightly active against SMU (4 mm), whereas totally inactive against other three bacteria (LC, LA and SA) (Table 2). Ethyl acetate extract of this plant showed very low activity against SMU (5 mm) and LA (3 mm) while, LC and LA against totally inactive (Table 2). Methanolic extract of this plant was moderately active against SMU (7 mm) and LA (8 mm) and LC and SA against did not show any activity (Table 2). Aqueous extract is totally inactive against all the four bacteria (Table 2). MIC was not determined for this plant.

Ficus religiosa L

Hexane extract of this plant shows low activity against LA (5 mm) and SA (4 mm) and LC and SMU not show activity (Table 2). The LA (8 mm) and SMU (6 mm) against show low activity in ethyl acetate extract and no activity against LC and SA (Table 2). Methanol extract of this plant shows no activity against LC, SMU and SA and very low activity against LA (1 mm) (Table 2). Distilled water extract not shows the activity against SMU, LA, LC and SA (Table 2). Further methanol extract of this plant MIC determination was done, it was 4 mg/ml (Figure 1).

Calotropis procera (Ait.) R.Br

None of the extract was found active against all the selected four bacteria except hexenoic extract, which was moderate inhibition in growth of LA (5 mm) show (Tables 2).

Coroupita guianensis L.

Hexane extract of this plant inactive against SMU, LA and SA and slightly active against LC (5 mm) (Table 2). Ethyl acetate extract of this plant against LC (11 mm) was moderately active, LA (12 mm) whereas slightly active against SMU (6 mm) and inactive against SA (Table 2). Methanol and aqueous extracts of this plant were found inactive against all the four cariogenic bacteria (SMU, LA, LC and SA) (Table 2). Ethyl acetate and distilled water extract of this plant was done MIC determination.

No	Plants Name and Antibiotic	Hexane				Ethyl acetate				Methanol				Distilled water			
		LC	LA	SA	SM	LC	LA	SA	SM	LC	LA	SA	SM	LC	LA	SA	SM
1	*Cassia angustifolia*	-	-	-	4	-	3	-	5	-	8	-	7	-	-	-	-
2	*Ficus religous*us	-	5	4	-	-	8	-	6	-	10	-	-	-	-	-	-
3	*Calotropis procera*	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	*Coroupita guianensis*	5	-	-	-	11	12	-	6	-	-	-	-	-	-	-	-
5	*Annona squamosa*	-	5	-	-	-	9	-	-	-	-	-	-	-	-	-	-
6	*Mentha longifolia*	-	11	-	9	-	12	-	10	-	8		8	-	12	-	**7**
7	*Terminalia arjuna*	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-
8	*Emblica officinalis*	-	-	-	-	4	-	-	-	6	6	6	-	3	-	-	-
9	*Hibiscus rosa-sinensis*	-	-	-	8	-	9	-	8	-	-	-	-	-	-	-	-
10	*Coroupita guianensis*	-	7	-	4	-	-	7	5	25	13	9	8	6	9	-	**8**
11	*Moringa oleifera*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	*Vitex negundo*	12	12	-	10	-	-	-	-	9	9	-	10	-	-	-	-
13	*Ocimum sanctum*	5	5	-	-	7	8	7	-	8	8	-	-	-	-	-	-
14	*Putranjiva roxburghii*	-	-	-	-	6	-	6	-	-	-	-	-	8	8	-	-
15	*Streblus asper*	-	-	-	-	12	12	-	-	-	-	-	-	-	-	-	-
16	*Alstonia scholaris*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	*Salvadora persica*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	*Lomonia acidissima*	-	-	6	-	-	-	6	-	-	-	6	-	20	-	-	-
19	*Aegle marmelos*	-	-	6	-	-	4	-	5	13	-	-	-	-	7	17	**8**
20	Cefadroxil	41	36	31	12	41	36	31	12	41	36	31	12	41	36	31	**12**
21	Tetracycline	41	28	26	28	41	28	26	28	41	28	26	28	41	28	26	**28**
22	Erythromycin	19	23	19	15	19	23	19	15	19	23	19	15	19	23	19	**15**
23	DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Antibacterial activities of crude hexane extracts of plants against cariogenic bacteria (zone in mm). Lactobacillus casei (LC); Lactobacillus acidophilus (LA); Staphylococcus aureus (SA); Streptococcus mutans (SM).

Annona squamosa L.

Both Hexanolic and ethyl acetate extracts showed moderate activity against LA (5 mm and 9 mm respectively) (Table 2). Rest of the extracts are totally inactive against SA, LA, LC and SMU (Table 2).

Mentha longifolia L.

Moderate activity of hexanolic extract of *M .longifolia* was found against SMU (9 mm) and LA (11 mm) (Table 2). Ethyl acetate extract of this plant shows low activity against SMU (10 mm) and LA (12 mm) and no activity against LC and SA (Table 2). Methanol extract of this plant against LA (8 mm) and SMU (8 mm) shows low activity (Table 2). Aqueous extract of this plant against LA (12 mm) shows moderate activity and low activity against SMU (7 mm) and no activity against SA and LC (Table 2). Further hexane extract of this plant MIC determination was done. All the extracts are totally inactive against LC and SA. Further hexanoic extract against SMU (4 mg/mL), ethyl acetate extract against LC and LA (4 mg/ml each) and aqueous extract had 4 mg/ml MIC against LA MIC was determined (Figure 1).

Terminalia arjuna (Roxb.) W. and A

Hexanolic, methanolic and aqueous extract of this plant were totally inactive against all the four bacteria (LA, LC, SA and SMU) (Table 2). Ethyl acetate extract are only active against SMU (7 mm) whereas inactive against LA, LC and SA (Table 2).

Emblica officinalis Gaertn

Hexanolic extract showed no activity against all the four bacteria (Table - 2), while ethyl acetate extract shows activity only against LC (4 mm). Methanolic extract shows moderate and equal activity against LC, LA and SA (6mm in each) (Table 2). SMU against no activity was found. Aqueous extract is slightly active against LC (3 mm) and inactive against SA, LA and SMU (Table 2).

Hibiscus rosa-sinsensis L.

Both Hexanolic and ethyl acetate extract exhibited similar activity against SMU (8 mm) (Tables 2). Ethyl acetate extract also active against LA with zone of inhibition 9 mm, whereas inactive against SA and LC (Table 2). Finally, Methanolic and aqueous extracts of this plant were found totally inactive against all the four bacteria (Table 2).

Coroupita guianensis L. (Fruit)

Hexane extract of this plant showed low activity against LA (7 mm) and SMU (4 mm) and no activity against LC and SA (Table - 2). SA (7 mm) and SMU (5 mm) plant shows low activity against ethyl acetate extract whereas no activity against LA and LC (Table 2). LC (25 mm) highest activity against exhibited methanolic extract and low activity against LA (13 mm), SA (9 mm) and SMU (8 mm) (Table 2). MIC was determined for this extract against LC. LC (6 mm), LA (9 mm) and SMU (8 mm) plant showed low activity against distilled water extract and no activity against SA (Figure 1). Further MIC was determined for methanolic extract [against LA (2 mg/mL), SA (4 mg/mL) and LC (0.16 mg/mL)] and aqueous extract [against SMU (4 mg/mL) and SA (2 mg/mL)] (Figure 1).

Moringa oleifera Lam.

None of the extracts (Hexanolic, Ethyl acetate, Methanolic and Aqueous) from this plant found activity against all the four cariogenic bacteria (LA, LC, SA and SMU) (Table 2).

Vitex negundo L.

Methanolic and hexane extracts of *V. negundo* are inactive only against SA. Hexane extract of this plant shows moderate activity against LC (12 mm), LA (12 mm) and SMU (10 mm) (Table 2). Methanol extract of this plant shows low activity against LC (9 mm), LA (9 mm) and SMU (10 mm). Ethyl acetate and aqueous extracts are inactive against all the four bacteria (SA, SMU, LC and LA) (Table 2). Further MIC was determined for hexane extract of this plant (Figure 1).

Ocimum sanctum L.

Hexanolic extract of this plant showed low activity against LC (5 mm) and LA (5 mm) and did not showed any activity against SMU and SA (Table 2). LC (7 mm) showed moderate activity against Ethyl acetate extract. LA (8 mm) and SA (7 mm) whereas no activity found against SMU (Table 2). LC (8 mm) and LA (8 mm) against Methanolic extract proved its moderate activity and it is totally inactive against SMU (Table 2). SA, LA, LC and SMU bacteria against aqueous extract remains ineffective (Table 2).

Putranjiva roxburghii Wall.

Hexanolic and methanolic extracts of this plant were totally inactive against all the four bacteria (Table 2). Ethyl acetate extract showed similar activity against LC and SA (6 mm each). LA and SMU against not show any activity (Table 2). Finally, LC and LA (8 mm each) against aqueous extract of this plant showed moderately activity whereas SMU and SA against no activity found (Table 2). MIC was not determined for this plant.

Streblus asper Lour.

Both LC and LA (12 mm each) against only ethyl acetate extract showed moderate inhibition in growth, while it was totally inactive against other two (SMU and SA) (Table 2). Rest of the plant extracts (Hexanolic, ethyl acetate, Methanolic and aqueous) not show any activity against selected bacteria (SA, LA, LC and SMU) (Table 2).

Alstonia scholaris (L.) R.Br

All the four extracts are found non-effective against all the four selected cariogenic bacteria (Tables 2).

Salvadora persica L.

Hexanolic, ethyl acetate, Methanolic and aqueous extracts of this plant did not show any inhibition in growth of selected cariogenic bacteria (SA, LA, LC and SMU) (Tables 2).

Lomonia acidissima L. (Fruit)

Hexane and methanolic extracts of this plant are active against SA with equal zone of inhibition (6 mm) in both whereas no activity against SMU, LA and LC (Table 2). SA (6 mm) against ethyl acetate extract of this plant shows low activity and SMU, LA and LC against no activity (Table 2). Aqueous extract of this plant is highly active against LC (20 mm) which was selected for MIC determination. Same extract was totally inactive against LA, SA and SMU (Table 2). Further MIC was determined for aqueous extract against SMU (4 mg/mL), LC (1 mg/mL), SA (2.5 mg/mL) and LA (4 mg/mL) (Figure 1).

Aegle marmelos (L.) Corr. (Fruit)

Variable growth inhibition pattern was found when fruit extract of *A. marmelos* was used. Hexanolic extract very low activity against SA (6 mm) and no activity against SMU, LA and LC (Table 2). Ethyl acetate extract of this plant perform poorly against LA (4 mm) and SMU (5 mm) with SA and LC against no activity (Table 2). LC (19 mm) against methanol extract of this plant shows comparatively high activity and no activity against SMU, LA and SA (Table 2). SA (17 mm) against distilled water extract of this plant showed high activity and low activity against SMU (8 mm) and LA (7 mm) whereas, no activity against LC (Table 2). Methanolic and aqueous extracts of this plant were chosen for MIC determination against SMU (4 mg/mL) (Figure 1).

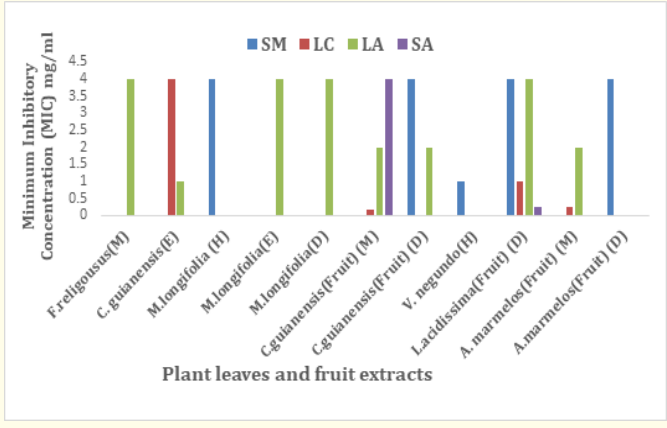

Figure 1: The MIC (mg/ml) of selected plants extract against cariogenic bacteria.

The plants present compounds either kill them or inhibit the growth of pathogen and host cell no toxicity are consider for new antimicrobial drugs development. Toothbrushes and toothpastes of natural tooth cleaning method despite widespread practiced for thousands of years in various countries using plant derived product. The oral diseases prevent natural products have been used, especially dental plaque or caries [27].

Present study on assessment of inhibitory potential of selected ethanomedicinal plants against four cariogenic bacteria revealed that fruit extract is more powerful compared to leaves extract as far as growth inhibition in cariogenic bacteria is consult. In our study, *Lactobacillus casei* by various extracts of three plants which is more than that of the standard antibiotic erythromycin (19 mm) compare to high degree of growth inhibition (19 - 25 mm). Majority of the selected plants are utilized for various human ailments. There are large number of study on the anticariogenic activity of ethanomedicinal plants [28-30]. It is difficult to compare individual plants because of scarcity of literature on selected plants for their anticariogenic activity. But very recently Parimala Devi and Ramasubramaniaraja (2009) reviewed use of medicinal plants in the treatment of dental caries [31].

Preliminary phytochemical analysis shows the presence of alkaloids in the various plants extracts. The tannins, saponins, cardiac glycoside, steroids, terpenoids etc. were present its trace amounts of secondary metabolites in some of the plant extracts (Table 3). Therefore, phytochemical properties and difference among species due to the variable antimicrobial effects of plant species [26]. It is likely that some of the plants found unsuccessfully against cariogenic bacteria. Because they don't have antibiotic properties or insufficient chemical quantity and number of anticariogenic substances. Some of the active chemical constituents are insoluble in water. Change in the during drying time or high light intensity could also be possible and leads to in activity [32].

Plants Name	1	2	3	4	5	6	7
Ficus religousus L. (Meth.)	-	-	-	-	-	-	+
Mentha longifolia L. (Hex)	-	-	-	-	-	-	+
Mentha longifolia L. (EA)	-	-	-	-	-	-	+
Mentha longifolia L. (D/W)	+	+	-	-	-	+	+
Coroupita guianensis (Fruit) Meth.)	-	-	+	+	+	+	-
Coroupita guianensis (Fruit) (D/W)	-	-	-	-	+	-	+
Vitex negundo L. (Hex)	-	-	-	-	-	-	+
Streblus asper Lour. (EA)	-	-	-	-	-	-	+
Lomonia acidissima (Fruit) (D/W)	+	+	-	-	-	-	+
Aegle marmelos (L.) Corr. (Fruit)(D/W)	-	+	-	-	-	-	-

Table 3: Phytochemical analysis of crude extracts of selected plants.

(+) = Present; (-) = Absent;1-Tannins 2-Saponins3-Cardiac Glycosides
4-Steroids 5-Terpenoids 6-Phenolic Compound 7-Alkaloid.

To find out active chemical compound present in methanolic extract of *Coroupita guianensis* fruit extract, first standardized (Chloroform: Methanol (1:4)) TLC solvent system and used for subsequent analysis. The bioactive active compound was separated using TLC technique (Rf value: 0.85). The resulted chromatogram was used for bioautography against *Lactobacillus casei.*

The UV analysis of TLC plate run from crude methanol extract sample of *C. guianensis* (Fruit) showed blue fluorescence at 254 nm and green fluorescence at 365 nm respectively. The presence of band was also confirmed by using iodine vapor. Development of TLC plate using analyte specific reagent may indicate presence of cardiac glycoside, alkaloid, saponin and terpenoids. To the best our knowledge this is a first kind of report on anticariogenic activity *C. guianensis* (Fruit) extract. Further, determination of structure of bioactive compound required for spectroscopic and chromatographic analysis.

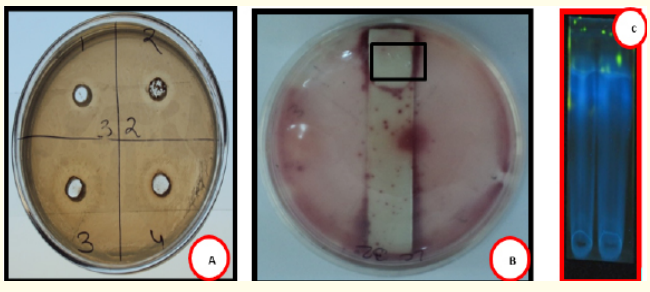

Figure 2: Antibacterial activity of methanolic extracts of Coroupita guianensis (Fruit) against Lactobacillus acidophilus (A-3- Methanol extracts, B- Bioautography, C- TLC -UV-245nm).

Conclusion

The antimicrobial compound against pathogenic microorganisms that can be used to treat infectious diseases for plant extracts have great potential. Some of the ethanomedicinal plants selected in this study are potential source of antibacterial agents. The very good inhibition potential of methanolic extract of *Coroupita guianensis* L. (Fruit) in the present study and its subsequent phytochemical screening indicates presence of multiple anticariogenic substances. This is very interesting, but further chromatographic and spectroscopic characterization of these substances is required for structure elucidation. Moreover, toxicity assay is required to determine the safety level of the plant extract.

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