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Exploring of Anticariogenic Activity of Herbal Formulation from Selected Five Medicinal Plants Leaves

Parmar B and Ishnava KB*

Ashok and Rita Patel Institute of Integrated Studies and Research in Biotechnology and Allied Sciences, India

***Corresponding author:** Dr Kalpeh B Ishnava, Assistant Professor, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), Sardar Patel University, New Vallabh Vidyanagar-388121, Gujarat, India, Tel: 919824918606; Email: ishnavakb203@yahoo.com

Review Article

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Abstract

Background: Oral diseases continue to be a major health problem worldwide. Plant leaves extracts of formulation have great source as anticariogenic compound against oral pathogenic microorganisms, which can be used to treat infectious diseases.

Objective: The leaf and their formulation extracts were tested for their effect on anticariogenic organisms under in vitro condition. Attempt was also made to characterize bioactive compounds at primary level.

Material and Methods: The dried plant leaves materials are extracted by cold extraction using hexane, ethyl acetate, methanol, and distilled water. The solvents were evaporated, and the dried masses were suspended in dimethyl sulfoxide and used for anticariogenic activity by agar well diffusion method. 12 different herbal formulations prepared and evaluation their their efficacy against anticariogenic bacteria. Minimum inhibitory concentration (MIC) was evaluated by two-fold serial broth dilution method. Preliminary phytochemical analysis of effective formulation was carried out by thin-layer chromatography (TLC).

Results: Plant leaves (*Lantana camara var.aculcata* (L.) Mold; *Manilkara hexandra* (Roxb.) Dub; *Mangifera indica* L; *Piper betle* L; *Syzygium rubicundum* Wight & Arn extracts and twelve different formulation prepared by leaves extracts was analysis for their anticariogenic activities against oral microorganisms. All the crude plant leaves extracts and different formulation prepared by leaves extracts exhibited varying degree of growth inhibition in all the oral microorganisms. The activity was determined against, *Lactobacillus acidophilus, Lactobacillus casei, Staphylococcus aureus and Streptococcus pyogenes.* Maximum zone of inhibition (22 mm) was found when plant leaves extracts of formualtion tested against *Lactobacillus casei.* Preliminary phytochemical analysis of formulation present the Tannins, Saponins, Steroids and phenolic compound are present.

Conclusion: The very good activity of plant leaves extracts formulation will be useful in the future development of effective for toothpaste or mouth washer against oral microorganisms.

Keywords: Oral diseases; Anticariogenic Activity; Leave Extracts; Herbal Formulations; *Lantana camara var.aculcata; Manilkara hexandra; Mangifera indica; Piper betle; Syzygium rubicundum*

Introduction

Oral health is integral to general well-being and relates to the quality of life that extends beyond the functions of the craniofacial complex. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns. Over 750 species of bacteria inhabit the oral cavity and a number of these are implicated in oral diseases. The mouth contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental caries: *Streptococcus mutans* and *Lactobacilli* among them. *Lactobacillus acidophilus*, *Actinomyces viscosus*, *Nocardia spp.*, and *Streptococcus mutans* are most closely associated with caries, particularly root caries. *Lactobacillus acidophilus* is the most common bacteria present in the oral pathogen [1].

Poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities. Synthetic dentifrices commonly used contain chemical agents, which are known to produce harmful side effects on prolonged use [2]. Bacteria are particularly efficient in enhancing the effect of resistance not only because of their ability to multiply rapidly but also because they can transfer their resistance genes, which can pass on to other related bacteria through replication [3]. As a result some bacterial infections are now essentially untreatable with antibiotics. In a 2003, Institute of medicine report, microbial threat to health, antimicrobial resistance was noted as a paramount microbial threat of the twenty first century, some strain of bacteria are now resistant to essentially available antimicrobial drugs and some remain susceptible to only one. The lack of new drug classes is a consequence of difficulties in discovery of new compounds that has persisted for many years.

Herbal Medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native people. Many drugs commonly used today are of herbal origin for control the oral disease [4-9]. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Chemical compounds such as amino acids, carbohydrates and proteins, are products of primary metabolism and are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids, are products of secondary metabolism and have toxicological, pharmacological and ecological importance [10-12]. However, the main classes of bioactive compounds from plants include flavonoids, terpenes, alkaloids, saponins, and coumarins [13]. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances [4,14-16].

In India particularly Gujarat state is endured with rich source of medicinal plants used by various tribal communities. About 750 species of medicinal plants being used by tribal people residing in the remote area [17]. Therefore, present study aimed for selected medicinally important plants against anticarogenic activity and preparation of the herbal formulation. We selectively 5 different plant leaves extracts for their potential against four different cariogenic organisms. The objective of this study was then to investigate the inhibitory effect of the crude extracts from some traditional medicinal plants on anticariogenic organisms under in vitro condition. The reason for this is to provide a scientific validity for their use for controlling oral organisms. There is a less information regarding bioactivity of leaf extracts and their formulation of medicinal plants against cariogenic microorganisms. The leaf and their formulation extracts were tested for their effect on anticariogenic organisms under in vitro condition. Attempt was also made to characterize bioactive compounds at primary level. These information will may be useful to search for new cost-effective drugs of natural origin in future.

Material and Methods

Plant Materials

The different plant species were selected and collected between Jun to July, 2011 form different part of Gujarat and surroundings of Vallabh Vidyanagar (Table 1). The leaves of all the healthy and disease free plants were used to test the antibacterial activity. The plant specimens were identified by Dr. Kalpesh Ishnava (Plant Taxonomist) at Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India.

Sr. No.	Plant Name	Family	Local Name
1	Lantana camara var.aculcata (L.) Mold	Verbenaceae	Indradhanu
2	Manilkara hexandra (Roxb.) Dub.	Sapotaceae	Rayan
3	Mangifera indica L.	Anacardiaceae	Kari
4	Piper betle L.	Piperaceae	Nagarvel pan
5	Syzygium rubicundum Wight & Arn.	Myrtaceae	Jambu

Table 1: Plants leaves used for formulation.

A Preparation of Plant Leaves Extracts

First of all the leaves of respective plants were thoroughly washed with running tap water, blotted and dried under sunlight. For the purpose of making powder it was grinded in grinder (Maharaja Mixer Ltd). From these, 50 grams of powdered material were soaked in 250 mL of hexane for 24 hours at room temperature under shaking condition (130-140 rpm). The extract was filtered with the help of Whatman filter paper number-1. The filtrate was collected in Petridis and dried at room temperature. The dried extract from Petridis was scraped and transferred to eppendorf tube.

The residual material from the funnel was dried again and suspended in 250 mL ethyl acetate for 24 hours at room temperature under shaking condition (130-140 rpm). The extract was filtered and collected in petridish. It was dried at room temperature. Similarly, the residual materials from the funnel are preserved and re-extracted with same volume (250 mL) of methanol and then distilled water respectively. In both the cases, the resultant culture filtrate was air dried at room temperature. The dried extract from petridish was scraped and transferred to eppendorf tube.

Preparations of Herbal Formulation

The preemption of 12 different formulation of selected four extract of four different plants extract using different organic solvent like methanol, hexane, ethyl acetate and dimetyl sulfonate. In this formulation also use the distilled water extracts. The all extracts are prepared the stock solution of 100 mg/ml. The stock solutions are used for the preparations of the formulation. The 12 different formulations are prepared as per Table 2.

Sr. No	Formulation of extracts
1	10µl(1) + 20µl(2)+ 30µl(3) + 40µl(4) + 900µl(5) =1ml
2	20µl(1) + 40µl(2) + 60µl(3) + 80µl(4) + 800µl(5) =1ml
3	30µl(1) + 80µl(2) + 120µl(3) + 160µl(4) + 610µl(5) =1ml
4	60µl(1) + 160µl(2) + 240µl(3) + 320µl(4) + 220µl(5) =1ml
5	50µl(1) + 100µl(2) + 150µl(3) + 200µl(4) + 500µl(5) =1ml
6	100µl(1) + 150µl(2) +200µl(3) + 250µl(4) + 300 µl(5) =1ml
7	150µl(1) + 200µl(2) + 250µl(3) + 300µl(4) + 100 µl(5) =1ml
8	50µl(1) + 50µl(2) + 50µl(3) + 50µl(4) + 800 µl(5) =1ml
9	100µl (1) + 100µl(2) + 100µl(3) +100µl(4) + 600 µl(5) =1ml
10	150µl(1) + 150µl(2) + 150µl(3) + 150µl(4) + 400µl(5) =1ml
11	200µl(1) + 200µl(2) + 200µl(3) + 200µl(4) + 200µl(5) =1ml
12	25µl(1) + 250µl(2) + 250µl(3) +250µl(4) +00 µl(5) =1ml

Table 2: Different formulation of extracts.
1 – Distilled water extract of *Mangifera indica,* 2- Methanol extract of *Manilkara hexandra,*
3 –Hexane extract of *Piper betle,* 4 – Ethylacetate extract of *Piper betle,* 5- DMSO

Cariogenic Bacterial Strains

Preparation of Inoculums: Fresh microbial cultures were prepared by streaking loopful of bacterial suspension in to organism (*Lactobacillus acidophilus* (LA)- MTCC No.:*447; *Lactobacillus casei* (LC) - MTCC No.:1423; *Staphylococcus aureus* (SA) - MTCC No.: 96; *Streptococcus pyogenes* (SP) - MTCC No.:442) specific selective media (Hi-media) and incubated at optimal temperature in order to maintain approximately uniform growth rate of each organism. The bacterial cultures from fresh media was compared with 0.5 McFarland turbidity standard, which is equivalent to approximately $1X10^8$ bacterial cell count per mL was maintained throughout the experimentation [18].

Bioassay for Antimicrobial Activity

Agar Well Diffusion Method: In the present study, to test antibacterial activity, twenty different plant extracts were used. The antibacterial activity was studied by agar well diffusion method [19]. From the stock, 100 mg of each plant extract were suspended in one milliliter of Dimethyl sulfoxide (DMSO). A well of 10 mm diameter punched off at previously marked petriplates in to agar medium with sterile cup borer and then it was filled with 100 µl of respective plant leaves extract. Plates were placed for 30 minutes in refrigerator for diffusion of extracts and then incubated at 37°C (or specified temperature) for 24 hours or more depending upon the organisms, until appearances of zone of inhibition. The zone of inhibition (excluding well diameter) was measured as a property of antibacterial activity. Antibiotic, Amoxycillin, Deoxycline and Cyclophosphamide was used as standard at a concentration of 100 µg/mL and 100% DMSO were used as positive control and negative control respectively. Bioassay was performed in duplicate and repeated twice.

Minimum Inhibitory Concentration (MIC) Determination: Minimum inhibitory concentration was evaluated by the two fold serial broth dilution method [20]. Plant extract of 40 µl from stock solution (100mg/mL) was taken in to first dilution tube containing 960 µl of selective medium broth and mixed well. From these, 500 µl were transferred to second tubes containing 500 µl broths. This step was repeated nine times and from last tube 500 µl solutions was discarded. The 100 µl of test organisms was added in each tube. The MIC was tested in the concentration range between 4.0mg/mL to 0.0031 mg/mL. Tubes were incubated at optimal temperature and time in an incubator. Bacterial growth was visualized when colorless 2,3,5-triphenyl tetrazolium chloride was converted in to red color formazone in the presence of bacteria. Each assay was repeated thrice by using DMSO and selective medium as control.

Phytochemical Analysis

Preliminary Phytochemical Analysis: Qualitative phytochemical (Tannin, Alkaloids, Saponins, Cardiac glycosides, Steroids, Terpenoides and Phenolic compounds) analysis of all the plant leaves extracts selected, based on MIC value was perform as per the methodology of Parekh and Chanda, 2007 [21].

Analytical Thin Layer Chromatography: Analytical TLC was performed to find out suitable solvent system for the development of chromatogram. The following solvent mixtures were tried on precoated TLC plates (Merck, silica gel 60 F254 plate, 0.25mm) of Toluene: Ethyl acetate (5.0: 5.0).

HPTLC Analysis: For chemical profile analysis, leaves extract formulation of Formulation – 11 was mixed with 1 ml DMSO. The sample used for HPTLC analysis (Camag system equipped with a sample applicator Linomat-5, twin development chamber, TLC scanner-3 and integration software, documentation system Reprostar-3 with G5 digital camera) (Camag, Switzerland). HPTLC aluminum sheet pre-coated with silica gel 60 (1.05547 E Merck) was used as the adsorbent. Toluene: Ethyl acetate (5.0: 5.0) was used as the mobile phase. The chromatographic development chamber was saturated with mobile phase for 10 min prior to placement of the plates. The plates were run up to 8 cm height and derivatized (10% H_2SO_4 in methanol). The derivative plates were heated at 100°C for 4 min, bands were observed and scanned at 366 nm and photographs taken for record.

Result and Discussion

In present study, the anticariogenic assay of plant leaves extract and different plant leaves extract formulation against oral pathogenic organisms was carried out for the purpose of checking sensitivity of cariogenic bacteria. The crude hexane, ethyl acetate, methanol and distilled water extract of 5 plants were screened in present study and based upon this study those extract are selected for preparation of different formulations against cariogenic bacteria. The result of sensitivity of cariogenic organisms (LA, LC, SA, and SP) was assessed by visualizing the presence or absence of inhibition zone and measuring the zone of diameter.

The results are summarized as under

Anti-Cariogenic Activity

The result of anticariogenic assay showed sensitivity of bacteria which can be assessed by presence or absence of inhibition zone and zone diameter. The anticariogenic activities of crude plant leaves extracts against four different selected oral pathogenic organisms are summarized in Table 3.

S. no.	Plant Name & Antibiotics	Hexane				Ethyl acetate			Methanol				Distilled water			
	(100 µg/Ml)	LA	LC	SA	SP	LA	SA	SP	LA	LC	SA	SP	LA	LC	SA	SP
1	Lantana camara	-	7	6	8	-	-	5	-	2	2	5	-	5	-	-
2	Manilkara hexandra	6	-	2	-	5	-	-	15	22	2	2	-	-	-	-
3	Mangifera indica	2	-	-	-	19	5	2	16	6	2	2	-	-	-	20
4	Piper betle	8	10	8	14	20	18	14	-	-	-	-	-	-	-	2
5	Syzygium rubicundum	2	-	-	2	-	-	-	-	-	-	-	-	-	-	2
6	Amoxycillin	20	-	15	-	20	15	-	20	-	15	-	20	-	15	-
7	Deoxycline	20	22	30	25	20	30	25	20	22	30	25	20	22	30	25
8	Cyclophosphamide	20	20	35	21	20	35	21	20	20	35	21	20	20	35	21

Table 3: Antibacterial activity of organic solvent extracts of leaves and antibiotics against cariogenic microorganisms**.**
LA- *Lactobacillus acidophilus*; LC- *Lactobacillus casei*; SA- *Staphylococcus aureus*; SP- *Streptococcus pyogenes*

Hexane Extracts

Only 60 % of plants give rise to anticariogenic substances out of 20 selected plant leaves extracts, as extracted with hexane and tested against four selected cariogenic bacteria LA, LC, SA and SP) show in Table 2. Amongst them, *Piper betle* was found to be active against all the four selected cariogenic bacteria, with maximum (14 mm) growth inhibition in SP, followed by LC (10 mm), SA and LA (8 mm). Hexanolic extract of *Mangifera indica* and *Syzygium rubicundum* were found inactive against both LC and SA. The least active anticariogenic compounds was found when extract from *T. peruviana*, *N. tabacum* and *E. nivulia* (3-4 mm) were used. Slight zone of inhibition was observed in *E. globules* and *E. nivulia* against SA (4–5 mm) [11]. There were 40% of total plant leaves extracts which showed no activity at all. There are many reports on the activity of medicinal plants against various bacteria from India [11,21,22], but there is meager information specifically against cariogenic bacteria.

Ethyl Acetate Extracts

Ten (i.e. 50% plant leaves extracts) of 20 selected plants leaves extracts demonstrated broad spectrum of anti-bacterial activity against 4 selected bacteria, when ethyl acetate was used for extraction of anticariogenic substances. Among these, two plants *Mangifera indica* and *Piper betle* were found to be active against all the four selected cariogenic bacteria, in which *Piper betle* showed maximum zone of inhibition (20 mm) against LA and LC show in the Table 4.

Sr. No.	Chemical constitutions	Formulation No - 11
1	Tannins	+
2	Saponins	+
3	Cardiac Glycosides	-
4	Steroids	+
5	Terpenoids	_
6	Phenolic Compound	+
7	Alkaloid	+

Table 4: Pytochemical analysis of crude seed extracts of selected plants.
"+" = Present, "-" = Absent.

Ethyl acetate extract of *P. grantum, C. papaya, T. patula, N. tabacum* and *E. nivulia* were found moderately active against all the selected bacteria. The least active anticariogenic compounds were found in *T. peruviana, N. tabacum* and *E. nivulia* when tested against SMU and LC with 2 - 4 mm zone of inhibition) [11].

Slight zone of inhibition was observed in *Lantana camara* and *Manilkara hexandra* SP and LA respectively. Ethyl acetate extracts of rest of the plant leaves extract (i.e. 50%) didn't show any anticariogenic activity. Sato, et al. [23] studied antimicrobial activities of flavones with antibacterial activity against cariogenic bacteria. Shyla, et al. [24] reported the antimicrobial activity of few medicinal plants against clinically isolated human cariogenic pathogens.

Methanol Extracts

Eleven (i.e. 55% plant leaves extracts) of 20 selected plants leaves extracts demonstrated broad spectrum of anti-bacterial activity against 4 selected bacteria, when methanol was used for extraction of anticariogenic substances show in the Table 3 .There was an nearby equal ratio of plants showing to those not showing anticariogenic activity against selected cariogenic bacteria, when methanol was used as a solvent for extraction of anticariogenic substances from 5 selected plants. Amongst them, *Lantana camara, Manilkara hexandra* and *Mangifera indica* were found to be active against all the four selected cariogenic bacteria. Here also *Manilkara hexandra* showed maximum zone of inhibition against LA (15) and LC (22 mm). Methanol extract of *Piper betle* and *Syzygium rubicundum* were found inactive against all the selected bacteria with no zone of inhibition observed in the leaves plant extracts. The least active anticariogenic compounds were found in *Manilkara hexandra* against LC (22 mm).

Above result obtained in *Manilkara hexandra* and *Mangifera indica* in methanolic leaf extract is very much effective in comparison to those which were carried out by Jigyna [25] on *M. hexandra* methanolic extract of stem on selected anticariogenic bacteria. Mahesh [11] reported the 20 selected medicinal plant leaves extract showing good activity in methanolic extracts.

The study show the maximum extracts activity plant leaves extracts higher amount of individual extracts requirements in the preparation of formulation. Therefore, formulation are more active against all anticariogenic bacteria again formulation. Antibiotic which are in commercial use have few well defined target on bacteria and fungi. Disruptions of cell wall, inhibition of DNA replication or protein synthesis are some of the common mechanism for antibiotic activity. Since the target of these antibiotics is few well defined, there is a rapid evolution of bacterial drugs. Moreover, there is increasing resistance to available antimicrobials. This bacterial mechanism is widely present in the bacterial system and became world health problem. Antibiotics such as ampicillin, chlorhexidine, erythromycin, penicillin, tetracycline and vancomycin have been very effective in preventing dental caries [26]. However, excessive use of these chemicals can result in dearrangements of the oral and intestinal flora and cause undesirable side effects such as microorganism susceptibility, vomiting, diarrhea and tooth staining. Sanguinarine is an alkaloid isolated from the rhizome of Sanguinaria canadensis, which shows a broad spectrum against various oral bacteria [27]. It has been used as an anticariogenic agent in a wide range of oral care products such as toothpastes and mouthwashes due to its strong antibacterial effectiveness [28]. Its industrial application, however, had been greatly reduced as sanguinarine was reported to be associated with oral leukoplakia [29]. These problems necessitate further search for natural antibacterial agents that are safe for humans and specific for oral pathogens if the any biologically active compound having novel target it will be useful in the long term control of pathogenic bacteria.

There has been revival of great interest in herbal medicine. This is due to increased awareness of the limited ability synthetic pharmaceutical products to control major diseases and need to discover new molecular standard as lead compound from the plant kingdom [30]. The present study therefore is both significant and relevant since plant synthesize array of secondary metabolites they may served as future reservoir of novel drugs and therapeutic agents.

Aqueous Extracts

Finally, when distilled water was used as a solvent for extraction of anticariogenic substances and tested, 20% plant leaves extracts exhibited anticariogenic activity from 20 selected plant leaves extracts against LA, LC, SA and SP show in the Table 3. Nearly, 80% of the plants leave extracts showed no anticariogenic activity. Aqueous extract of *Mangifera indica s*howed the highest activity against SP (20 mm). *Lantana camara*, showed very little activity against the LC selected anticariogenic bacteria. The rest of the cariogenic bacteria are no activity observed. Lowest zone of inhibition was observed in *Piper betle* and *Syzygium rubicundum* (2 mm) against SP. Our results from aqueous extracts are totally contradictory to the findings of Dabur, et al. [31] where aqueous extracts were considered to be more active than hexane, ethyl acetate and methanol.

Recently, antimicrobial properties of plants are being increasingly reported from all parts of the world because of emergence of multiple drug resistance of modern pharmaceuticals to human pathogenic organisms [32]. The compound present in the plants either inhibits the growth of microbial pathogen or kill them and have no toxicity to host cells are considered for developing new antimicrobial drugs. Different parts of plants supplying low cost medicine to population have been used in Indian traditional system for the treatment of various human diseases. Natural products have been used to prevent oral diseases, especially plaque-related diseases, such as dental caries [33]. Our result showed that crude extracts of

hexane, ethyl acetate and methanol of *Manilkara hexandra, Mangifera indica and Piper betle.*

Different Formulation Extracts Anticariogenic Activity

46 plant extract formulations (i.e. 76.66 % plant leaves formulation extracts) of 60 selected plants formulation extracts demonstrated broad spectrum of anti-bacterial activity against 4 selected bacteria, when prepared formulation was used for check the anticariogenic activity (Figure 1). There was an nearby very less ratio of formulation extracts (23.33% plant leaves formulation extracts) for showing to those not showing anticariogenic activity against selected cariogenic bacteria, when different plant leaves formulation was used as a mixing the solvent for anticariogenic substances from 5 selected plants (Table 1). Amongst them, formulation -1 and Formulation-2 are very less activity against all anticariogenic bacteria (Figure 1). Rest of the Formulation – 3 to Formulation- 12 all are the active against all selected anticariogenic bacteria (Figure1). The maximum activity of Formulation-11 and Formulation-10 against LC (22 mm) and LC (20 mm) against cariogenic bacteria respectively zone of inhibition (Figure 2). Maximum activities against all the selected formulation against LC (Figure 2). The range between 10 to 22 mm zones of inhibition. All the selected formulations against minimum activity against SP bacteria. It is only 4 formulations are active and rest of the not active against SP bacteria. The least active anticariogenic compounds were found in Formulation - 11 against LC (22 mm) (Figure 2).

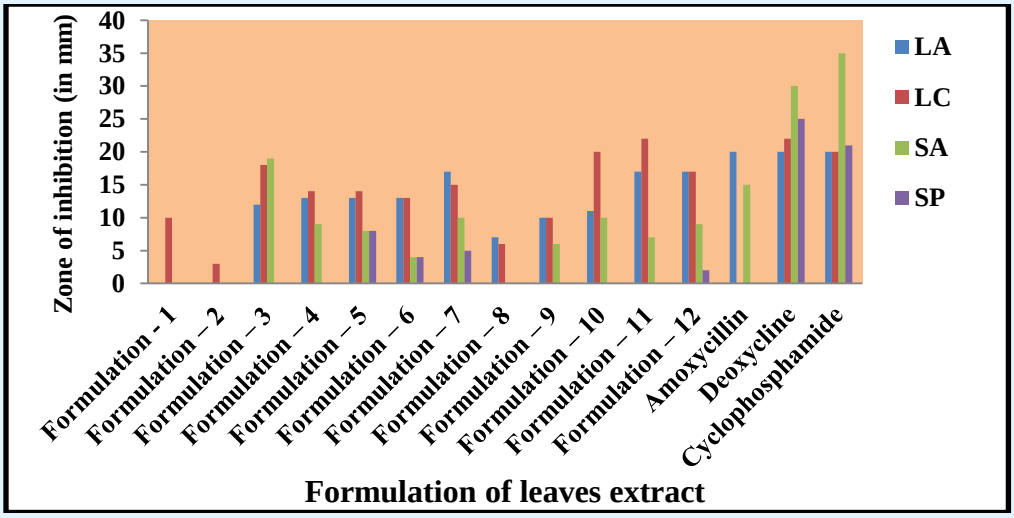

Figure 1: Antibacterial activity of 12 different leaves extract formulation extracts against cariogenic microorganisms.

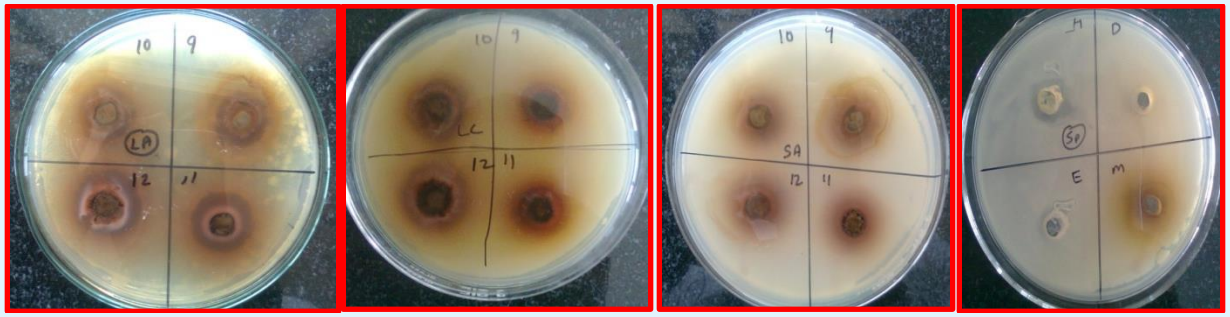

Figure 2: Antibacterial activity of different leaves extract formulation extracts against cariogenic microorganisms.

The formulation no. 3 (LC and LA), 5 (SP), 10 (LC and SA), 11 (LA and LC) and 12 (LA) are more than 15 mm zone of inhibition against anticariogenic bacteria show in the figure1 and figure 2. Therefore, all the selected

formulation for the further study for minimum inhibitory concentration (MIC) and phytochemicals analysis.

The herbs used in the formulation *Azadirachta indica*, was reported to be used widely in oral care formulations. *Terminalia chebula, Terminalia belerica, Emblica officinales* appear to be synergistic to the antimicrobial activity of *Azadirachta indica* in maintenance of oral hygiene. *Terminalia arjuna*, and *Mangifera indica* were selected because of their astringent and antioxidant properties in addition to antimicrobial activity. Extraction was done for individual herbs and was evaluated for antimicrobial activity against dental micro flora. The results showed that all the plants used in the study have antimicrobial activity [34].

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Antibiotic which are in commercial use have few well defined target on bacteria and fungi. Disruptions of cell wall, inhibition of DNA replication or protein synthesis are some of the common mechanism for antibiotic activity. Since the target of these antibiotics is few well defined, there is a rapid evolution of bacterial drugs. Moreover, there is increasing resistance to available antimicrobials. This bacterial mechanism is widely present in the bacterial system and became world health problem. If the any biologically active compound having novel target it will be useful in the long term control of pathogenic bacteria.

MIC Values of Selected Formulation

The Minimum Inhibitory Concentration (MIC) values of different formulation of leaves extracts of all the selected plants showing highest activity against selected organisms was assessed and summarized in Figure 3. Examining the MIC values of nine samples of various extracts generated the data where the maximum MIC value was found to be 3.33 mg/mL and the minimum value as 0.083 mg/mL. The MIC value of leaves extract formulation of Formulation -3 against LC and SA was 1. 66 mg/mL respectively. The MIC value of leaves extract formulation of Formulation -5 against SP was 3.33 mg/mL and Formulation – 12 against LC is 3.33 mg/mL. The MIC value of of leaves extract formulation of Formulation -10 against LC and SA was determined to be 1.66 mg/mL. The MIC value of formulation of leaves plant extract Formulation – 11 against LA and LC is 1.66 and 0.083 mg /L respectively. As compare to above formulation, Formulation – 11 extracts exhibited good MIC values ranging from 0.083 to 3.33 mg/mL against selected cariogenic bacteria (Figure 3). As compared to above solvents, methanolic extracts exhibited moderate MIC values ranging from 0.5 to 2 mg/mL against selected cariogenic bacteria.

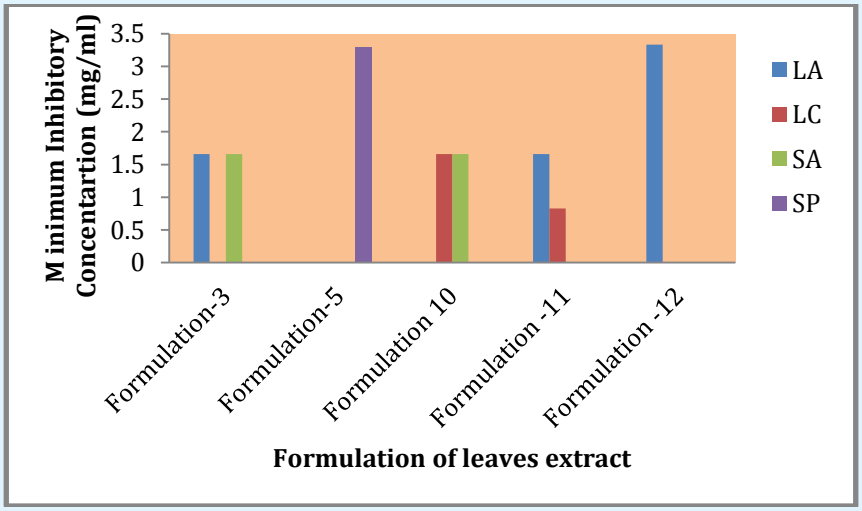

Figure 3: MIC (mg/ml) of selected different leaves extract formulation extracts against cariogenic microorganisms.

Pytochemical Analysis of Formulation - 11

Preliminary phytochemical analysis revealed the presence of alkaloids in the Formulation -11 extracts. The other secondary metabolites like tannins, saponins, steroids, phenol etc, were present its trace amounts in Formulation-11extracts (Table 4). Therefore the variable antimicrobial effects of plant species are due to phytochemical properties and difference among species [21]. It is possible that some of the plants found ineffective against cariogenic bacteria. Because they don't have antibiotic properties or insufficient quantity of anticariogenic substances. Some of the active constituents are insoluble in water. Change in the conformation during drying could also be possible and leads to in activity. Further, spectroscopic and chromatographic analysis is required for determination of structure of bioactive compounds.

HPTLC Analysis of Leaves Plant Formulation of Formulation -11

HPTLC profiles of formulation -11 of plant leaves formulation are showing the figure-4. The HPLC profile are under the fluoresces light 366 nm and UV light showing in the figure 4. The Formulation -11extract total no of peak 5 are present showing in the figure 4.The HPTLC profile maximum % of height presents in the peak no 1 is 17890.1 and end Rf value is 0.55. The leaves extract formulation of spectral composition shown in the figure 5. The chemical profile is further use for characterization and isolation of compound.

Figure 4: HPTLC of leaves extract formulation of chromatogram of Formulation – 11.

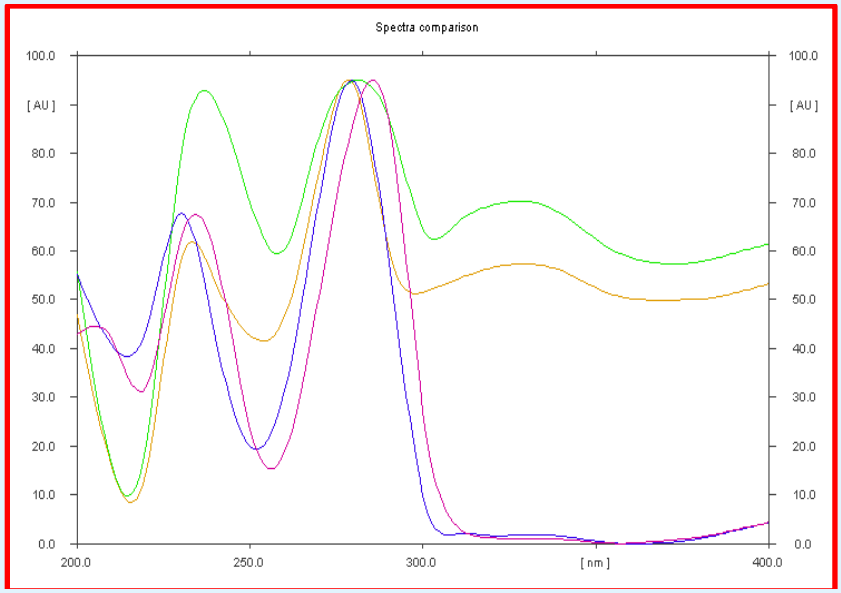

Figure 5: Leaves extract formulation of spectral composition Formulation – 11.

Conclusion

Plant leaves extracts of formulation have great source as anticariogenic compound against oral pathogenic microorganisms, which can be used to treat infectious diseases. The very good activity of plant leaves extracts formulation of Formulation – 11 will be useful in the future development of effective for toothpaste or mouth washer

against oral microorganisms. It indicates that plants have the potential to generate herbal metabolites. The plant leave extracts formulation demonstrating anticariogenic activity could result in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases. Further phytochemical studies are required to establish the types of compounds responsible for the anticariogenic effects of these medicinal plants.

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