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Screening of herbal formulation for anticariogenic activity

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Abstract
Dental caries and periodontal disease are considered to be most important global oral health burdens. The present study is an attempt to prepare an oral tooth powder herbal formulation effective against microbial flora of oral pathogen. 10 different herbal tooth powder formulations and extracted in four (04) solvent (DMSO, Hexane, Ethyl acetate and Methanol) and its efficacy against four (04) oral (*Staphylococcus aureus, Streptococcus pyogenes, Lactobacillus acidophilus* and *Lactobacillus casei*) pathogenic bacteria. Agar well diffusion method and minimum inhibitory concentration were used for this purpose. Among all the solvents, ethyl acetate proved to be the most prominent solvent for extraction of anticariogenic substances from the selected tooth powder herbal formulations. In *Staphylococcus aureus,* 2 solvents out of 4 have good activity and methanolic extract of formulation no.10 showed highest activity (18 mm). *Streptococcus pyogenes,* 3 solvents out of 4 have good activity and ethyl acetate extract of formulation no.6 showed highest activity (15 mm). *Lactobacillus acidophilus,* 2 solvents out of 4 are good activity and methanolic extract of formulation no.5 showed highest activity (22 mm). *Lactobacillus casei,* 2 solvents out of 4 have good activity and ethyl acetate extract of formulation no.5 showed highest activity (18 mm). Ethyl acetate extracts exhibited moderate MIC values ranging from 0.0156 to 0.5 mg/mL against selected cariogenic bacteria in formulation no.10. Therefore, the herbal tooth powder is effective and has cheaper rate so it is affordable to urban and rural community.

Keywords: Ethnomedicinal, herbal tooth powder, oral health, oral pathogen

1. Introduction
Oral health plays an important role in good health. Many dental diseases can cause oral health problems like the toothaches, Throbbing pain, dental caries, teeth borer and tooth sensitivity. They may range in severity from a simple tooth pain to a common tooth cavity, or up to mouth cancer. Oral diseases continue to be a major health problem worldwide (Petersen, 2005) [25]. There is considerable evidence linking poor oral health to chronic conditions, for example, there is a strong association between severe periodontal diseases and diabetes (Petersen, 2003) [26]. The economic impact of oral diseases is an important consideration with up to 10% of public health expenditure in developed countries related to curative dental care. Dental caries and periodontal disease are considered to be most important global oral health burdens. In some countries, oral diseases are the one of the most expensive diseases to treat.

Dental caries is very important dental diseases by infectious bacterial biofilm which is expressed in a produce pathologic oral environment (Liljemark, and Bloomquist., 1996) [19]. As the enamel loses its minerals, it starts to break down resulting in the formation of a cavity. Sticky foods are more harmful than non-sticky foods because they remain on the surface of the teeth. In a healthy mouth the pH is 6.2 to 7.0. A pH of 7 is neutral and most commonly. Thus mouth with problem starts when the pH is less than 5.5 than created the acid environment, and starts demineralization.

The association between oral diseases and the oral bacterial group is well established. It is more than 750 species of bacteria that inhabit the oral cavity, a number are implicated in oral diseases. The development of dental caries involves acidogenic and aciduric Gram-positive bacteria (mutans streptococci, lactobacilli and actinomycetes). Periodontal diseases have been linked to anaerobic Gram-negative bacteria like *Porphyromonas gingivalis, Actinobacillus, Prevotella* and *Fusobacterium* (Zero *et al*., 2009) [37]. *Streptococcus mutans* is a Gram-positive bacteria, facultative anaerobic bacterium mostly found in the human oral biofilm and is a significant contributor to tooth decay. The tooth surface is mainly present the *Neisseria* spp. and streptococci, including *S. mutans*. *S. sobrinus* and *S. mutans* plays a major role in tooth

decay, metabolizing sucrose to lactic acid. Sucrose is utilized by *S. mutans* to produce a gummy material, extracellular, dextran-based polysaccharide that allows them to stick to each other forming plaque. *S. mutans* produces dextran via the enzyme dextransucrase use the sucrose as a substrate in the following reaction:

(Glucose) $_n$ + n fructose ⟶ n Sucrose

The oral gram-positive bacteria *Streptococcus sanguis* and the gram-negative periodontal pathogen *P. gingivalis* have been shown to induce platelet activation and aggregation through the expression of collagen like platelet aggregation associated proteins. All cariogenic bacteria require condition in which sugar is present to express their virulence. *S. mutans* can adhere to salivary agglutinin, other plaque bacteria, extracellular matrix, and epithelial cell-surface receptor (Haynes and Stanford, 2003) [14]. The pathogenesis of periodontal disease is thought to be due to accumulation of dental plaque (bacteria in subgingival biofilms) with consequent mucosal infection and inflammation. Untreated dental caries with associated discomfort or toothache contributes to weight gain, growth and quality of life as well as the cognitive development of young children. Particularly for root caries diseases, the presently closed associated bacteria frequently identified is *Lactobacillus acidophilus, Actinomyces viscosus Nocardia spp*., and *Streptococcus mutans.* These collect around the teeth and gums in a sticky, creamy-coloured mass called plaque, which serves as a dental cavity.

Many chemicals and antibiotics like vancomycin, chlorhexidine, sporamycin, etc. are being used for antibacterial agents against *S. mutans* to reduce dental cavity mediated diseases including dental caries (Gjermo *et al*., 1973) [13]. However, these chemicals possess many side effects such as microorganisms, vomiting, diarrhea, getting tolerance and teeth staining (Chen *et al*., 1989). Also these antibiotic treatments are very costly and big problem for developing countries. Therefore, traditional medicinal plants used to treat dental diseases.

Medicinal plants have been used in traditional folk medicines for thousands of years and have shown promise as a source of components for the development of new antibacterial drugs. Naturally derived compounds have made considerable contributions to human health and have been a source of inspiration for novel drug development (Newman *et al*., 2003; Mahady, 2005) [23, 20]. Numerous traditional medicinal plants have been evaluated for their potential application in the prevention or treatment of oral diseases. A number of studies have investigated the activity of plant extracts and products against specific oral pathogens, which is a primary event in the formation of dental plaque and the progression to tooth decay and periodontal diseases (Steinberg *et al*., 2004) [31]. The different medicinal plant extracts also used traditionally for the control, prevention and treatment of oral problems and their ailments, as well as opportunity for selection of bioactive compound in antimicrobial activity (More *et al.,* 2008) [22].

Natural products have been recently investigated more thoroughly as promising agents for the prevention of oral diseases, especially plaque-related diseases such as dental caries. Many of the plants herbs and spices used by humans to season food yield useful medicinal compounds (Tapsell, 2006) [33]. Many traditional herbal formulations for dental care comprise of various types of medicinal plant extract and chewing sticks made out of young woody stem or root pieces. *Acacia catechu* bark paste is used to treatment of tooth bleeding and hypersensitivity. *A. nilotica* fresh twig is commonly used as tooth brush for keeping the tooth healthy and clean. *Achyrathes aspera* twing is used for brushing teeth and many treatment to the dental problems. *Azadirachta indica* fresh twig is used as tooth brush to prevent gum diseases and pyorrhoea. The increasing resistance to available antimicrobials has attracted the attention of the scientific community regarding a search for new cost-effective drugs of natural origin. The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors (Aliero *et al.,* 2008; Yesmin *et al*., 2008) [2, 36]. Essential oils and leaf extract such as *Eucalyptus globules* and their derivatives are effective against dental caries (Ishnava *et al*., 2013; Bairwa *et al.,* 2012) [16, 4]. Present study is focus on plants or plant products used in folk dental practices or prescribed in Ayurvedic remedies. Despite several dental caries agents (Tooth pest, Tooth Powder, Mouth freshener, Mouth wash etc) being available in the market, the search for an effective agent still continues. Several undesirable side effects associated with these agents stimulated the search for alternate agents. There are also numerous reports on the components of plants, which have revealed antibacterial activities against microorganisms which are widely known as a cause of dental caries (Brandy *et al*., 2003) [6]. The present research problem is an attempt to prepare an oral tooth powder herbal formulation effective against bacterial flora of oral cavity followed by its comparison with other marketed tooth powder brands.

2. Material and method

2.1 Plant materials

Based on the ethnomedicinal data different plant species were selected and collected during 2013-2014 from different parts of Gujarat, India. The different parts of plants like fruits, seeds, leaves, barks used to make plant formulations and to test the antimicrobial activity and compare to market product sample (CMF-1, CMF-2, CMF-3, CMF-4, and CMF-5) (Table1). The plant specimens were identified by Dr. Kalpesh Ishnava (Plant taxonomy) at Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidhyanagar, Gujarat, India.

2.2 A preparation of plant formulation extracts

First of all the required parts of selected plants were thoroughly washed with running tape water, blotted and dried under sunlight. For the purpose of making powders it was grinded in grinder. The extraction procedure was done using the maceration method of Tiwari *et al.,* 2011 [34] (Tiwari *et al.,* 2011) [34]. Different solvents like Hexane, Ethyl acetate, Methanol were selected for extraction. This material used for the anticariogenic activity. Same procedures followed with five selected different market tooth powder product (Table 1).

2.3 Oral Pathogenic Strains

A group of bacteria known to cause tooth decay were selected and purchased from Microbial Type Culture Collection (MTCC) bank, Chandigarh as a freeze dried pure culture. The bacterial cultures were revived by using MTCC specified selective growth medium and preserved as glycerol stocks. The bacteria responsible for dental caries used the *Lactobacillus acidophilus* (LA) (MTCC-*447), *Lactobacillus casei* (LC) (MTCC-1423), *Staphylococcus aureus* (SA) (MTCC-96) and *Streptococcus pyogenes* (MTCC - 442) for the study.

2.4 Preparation of Inoculums
Fresh microbial cultures were prepared by streaking loopful of bacterial suspension in to organism 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.

2.5 Bioassay for Antimicrobial activity
2.5.1 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 by Perez *et al*., 1990 [24] (Perez *et al*., 1990) [24]. From the stock, 100 mg of each plant extract were suspended in one milliliter of Dimethyl sulfoxide (DMSO). Antibiotic, Erythromycin, Amoxicillin and Tetracycline 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.

2.5.2 Minimum Inhibitory concentration
The minimum inhibitory concentration (MIC) was evaluated by the two fold serial broth dilution method by Chattopadhyay *et al*., 2001 [7] (Chattopadhyay *et al*., 2001) [7]. Plant formulation extracts showing maximum zones of each solvent were selected for MIC. The MIC was tested in the concentration range between 2.0 mg/mL to 0.0156mg/mL. Each assay was repeated thrice by using DMSO and selective medium as control.

3. Result and Discussion
Oral infections are one of the common health problems associated with majority of people around the globe. The prevalence of these infections is upto 90% in the school going children and even adults are also affected by them (Peterson *et al*., 2005) [25]. Dental treatment is usually a high expense remedy and is out of reach of common man in developing countries. Various agents used for the treatment are antiseptics, quaternary ammonium-antiseptics, phenolic antiseptics and other remedies such as oxygenating agents, and metal ions Addy, 1986) [1]. Bacterial resistance has been developed to various antibiotics commonly used for the treatment of oral infections such as penicillins, cephalosporins, erythromycin and tetracycline (Bidault *et al*., 2007) [5]. Therefore the search for alternatives products continues to be a major issue and natural phytomolecules isolated from plants used in traditional medicines proved to be a good alternative to chemical remedies (Prabu *et al*., 2006) [27]. There have been numerous citations regarding the use of traditional plants and the products obtained from them in maintenance of oral hygiene. Many plants have been reported in the Indian traditional systems which have efficacy to treat oral bacterial infections (Cowan, 1999; Kalemba *et al*., 2003) [11, 17].

The results of anticariogenic activity of different tooth powder formulation extracts and their potency were quantitatively accessed by the presence and absence of zone of inhibition and diameter respectively (Table 2). The different tooth powder formulation extracts compare with other marketed toothpowder brands (Table 2). Three different organic solvents (Hexane, ethyl acetate and Methanol) were used for extraction of anticariogenic substances. Among all the solvents, ethyl acetate proved to be the most prominent solvent for extraction of anticariogenic substances from the selected plant formulations (Table 2).

3.1 *Staphylococcus aureus*
3 solvents out of 4 have good activity. DMSO showed no activity against this organism. Methanolic extract of formulation and ethyl acetate extract of formulation are showed highest activity. Methanolic and ethyl acetate extract of formulation no.10 showed highest activity 18 mm) and 17mm respectively (Table 2) against this bacterium. The range of the activity of methanolic and ethyl acetate solvent is 8-18 mm zone of inhibition. Methanolic extract of all formulation give good activity. Compare to herbal tooth powder formulations, marketed products of herbal tooth powders are less active. In this formulation *Ocimum* sanctum (10gm) and *Quercus infectoria* (10gm) are used in maximum quantity. It is highly responsible for killing oral bacteria. Herbal medications in the form of essential oils have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to natural preparations as compared to synthetic pharmaceuticals. With the reduced costs of essential oils preparation, makes the search for natural therapeutics an attractive option. *Quercus infectoria* plant fruit traditionally use in the cure of the oral problem. Chursi and Voravuthikunchai (2009) [10] reported the mechanism of *Quercus infectoria* (nutgalls) extract and its components were investigated for antimethicillin-resistant *Staphylococcus aureus* (MRSA).

The formulated toothpowder consisting of specific amount of antimicrobial constituents like glycosides, alkaloids, flavonoids, phenols, tannins and saponins exhibited a synergistic effect against dental pathogens. The toothpowder formulation no. 10 was found more effective than commercial products like commercial marketed formulation (CMF) CFM-1, CFM-2, CFM-3, CFM-4 and CFM-2 (Table 2). The herbal toothpastes showed a reduction in oral bacterial count which may be due to the presence of active ingredients, natural extracts and blends of natural oil ingredients which may have antibacterial effects (Silva *et al*., 1996) [30].

O. sanctum leaves essential oils possess antimicrobial properties and is supposed due to the presence of mono-terpene components mostly phenolic in nature which exert membrane-damaging effects to microbial strains and stimulates leakage of cellular potassium ions which is responsible for a lethal action related to cytoplasmic membrane damage. Herbal medications in the form of essential oils have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to natural preparations as compared to synthetic pharmaceuticals. With the reduced costs of essential oils preparation, makes the search for natural therapeutics an attractive option.

3.2 *Streptococcus pyogenes*
3 solvents out of 4 have good activity. Methanolic extract of formulation and ethyl acetate extract of formulation are showing highest activity. Ethyl acetate extract of formulation no.6 showed highest activity (15 mm) against this bacterium (Table 2). The range of the activity of methanolic and ethyl acetate solvent is 5 – 15 mm zone of inhibition. Ethyl acetate extract of all formulation give good activity. Compare to herbal tooth powder formulations, marketed products of herbal tooth powders are less active (Table 2). Only one brand CMF- 4 tooth powder have good activity but lesser than herbal tooth powder formulation no.6. Ethyl acetate extract of formulation no. 6 include *Foeniculum vulgare* (3gm),

Cinnamomum zeylanicum (0.8 gm), *Punica grantum* (10 gm), *Calotropis* procera (0.5 gm), *Rosa* indica (0.9 gm), *Ocimum* sanctum (6.5 gm), *Syzygium cumini* (04 gm), *Azadirachta* indica (0.5 gm), *Quercus* infectoria (06 gm), *Myristica fragrans* (0.5 gm), *Achyranthes aspera* (0.5 gm), *Phyllanthus emblica* (7.5 gm) *and Mimusops elengi* (9.3 gm) for preparation of the herbal tooth powder. It is highly responsible for killing oral bacteria. There are several studies reporting antibacterial potential of extracts prepared from different parts of *M. elengi*. Two antibacterial compounds *viz*. 2, 3-dihyro-3, 3'4'5, 7-pentahydroxyflavone and 3, 3', 4', 5, 7-pentahydroxyflavone from the seeds of *M. elengi* showed strong inhibitory activity against Gram-positive and Gram-negative bacteria (Hazra *et al*., 2007) [15].

3.3 *Lactobacillus acidophilus*

2 solvents out of 4 have good activity. DMSO and Hexane extract showed no activity against this organism. Methanolic and ethyl acetate extract of formulation no.5 showed highest activity 22 mm respectively against this bacterium. The range of the activity against methanolic and ethyl acetate solvent is 5-22 mm zone of inhibition. Compare to herbal tooth powder formulations, marketed products of herbal tooth powders are less active. Only one brand CFM-1i tooth powder have good activity but lesser than herbal tooth powder formulation no.5.

Ethyl acetate extract of formulation no. 5 include *Foeniculum vulgare* (11.5gm), *Cinnamomum zeylanicum* (0.6 gm), *Punica grantum* (2 gm), *Calotropis* procera (0.4 gm), *Rosa* indica (0.8 gm), *Ocimum* sanctum (7 gm), *Syzygium cumini* (03 gm), *Azadirachta* indica (0.4 gm), *Quercus* infectoria (05 gm), *Myristica fragrans* (0.4 gm), *Achyranthes aspera* (0.4 gm), *Phyllanthus emblica* (10 gm) *and Mimusops elengi* (8.5 gm) for preparation of the herbal tooth powder (Table 1). In this formulation *Foeniculum vulgare* (11.5 gm) and *Phyllanthus emblica* (10 gm) are used in maximum quantity. It is highly responsible for killing oral bacteria.

Foeniculum vulgare effectively work against various pathogenic bacteria (Friedman *et al.*, 2004) [12]. In the present study, *Foeniculum vulgare* seed has shown nearly equal antimicrobial effects on both gram positive and gram-negative bacteria in suspension culture. Might be another possibility that essential oils may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells after massive ion leakage. It may also happen due to hydrophilic nature of bacterial cell wall (Knobloch *et al*., 1986) [18]. *Phyllanthus emblica* fruit is rich source of the vitamin C. It has good antioxidant activity. Its major role is to kill the bacteria because of the fruit contain present phenol in maximum amount.

3.4 *Lactobacillus casei*

2 solvents out of 4 have good activity. Methanolic extract of formulation and ethyl acetate extract of formulation are showing highest activity. Ethyl acetate formulation no.5 showed highest activity (18 mm) against this bacterium (Table 2). The range of the activity against methanolic and ethyl acetate solvent is 5-18 mm zone of inhibition (Table 2). Ethyl acetate extract of all formulation give good activity. Compare to herbal tooth powder formulations, marketed products of herbal tooth powders are less active. Only one brand CDF-4 tooth powder have good activity but lesser than herbal tooth powder formulation no.10. Methanolic extract of formulation no 10.include *Foeniculum vulgare* (5.2gm), *Cinnamomum zeylanicum* (2.1 gm), *Punica grantum* (0.2 gm), *Calotropis* procera (0.9 gm), *Rosa* indica (1.3 gm), *Ocimum* sanctum (10 gm), *Syzygium cumini* (6.5 gm), *Azadirachta* indica (1 gm), *Quercus* infectoria (10 gm), *Myristica fragrans* (0.9 gm), *Achyranthes aspera* (0.9 gm), *Phyllanthus emblica* (8 gm) *and Mimusops elengi* (3 gm) for preparation of the herbal tooth powder (Table 1). All contents are taken in gram. It is highly responsible for killing oral bacteria. *Quercus* infectoria plants contain high amounts of tannins (20%) present. The formulated toothpowder consisting of specific amount of antimicrobial constituents like glycosides, alkaloids, flavonoids, phenols, tannins and saponins exhibited a synergistic effect against dental pathogens. The tooth powder was found as effective as compare to commercial product of the different brands.

The antibacterial activity may be also due to the presence of several metabolic toxins or broad-spectrum antibiotics. Several metabolites from herb species, including alkaloids, tannins and sterols have been associated with anti microbial activity. The sites and number of hydroxyl group on the phenol components may increase in the toxicity against the microorganisms. Yao and Mollering (1995) [35] suggested that the antimicrobial properties of tannins might be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins, their complexity with polysaccharides and their ability to modify the morphology of microorganism. Several reports have been shown that bioactive compounds isolated from plant extracts have inhibitory effect on pathogens strains (Sharma *et al.*, 2011) [28].

3.5 Antibiotic

Antibiotics which are in commercial use have few well defined targets on bacteria (Table 2). 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 well defined, there is a rapid evolution of bacterial drugs. Moreover, there is increasing resistance to available antimicrobials. This mechanism is widely present in the bacterial system and became world health problem. The formulation No. 10 of methanol and ethyl acetate extracts compared with broad spectrum antibiotics against all the selected bacteria (Table 2).

3.6 MIC Values of selected plant formulation extracts

The Minimum Inhibitory Concentration (MIC) values of formulation extracts of all selected formulations showing highest activity against selected organisms was assessed and summarized in Table 3. Examining the MIC values of six samples of various methanol and ethyl acetate extracts showed the maximum MIC value was found to be 0.5 mg/mL and minimum value as 0.0156 mg/mL.

The MIC value of methanolic extract of formulation No.10 against LC and SA was 0.0625 mg/mL and 0.0312 mg/mL respectively. The MIC value of methanolic extract of formulation No.5 against LA was 0.5 mg/mL. The MIC value of methanolic extract of formulation No. 6 against SP was 0.125 mg/mL. The MIC value of ethyl acetate extract of formulation No.10 against LC and SA was 0.0625 mg/mL and 0.0156 mg/mL respectively.

The MIC value of ethyl acetate extract of formulation No.5 against LA was 0.5 mg/mL. The MIC value of ethyl acetate extract of formulation No.6 against SP was 0.125 mg/mL. As compared to above solvents, ethyl acetate extracts exhibited moderate MIC values ranging from 0.0156 to 0.5 mg/mL against selected cariogenic bacteria.

The cost of different market product compare with our formulation. The cost of formulation no.10 raw material is around Rs. 24=00 and total cost of fine product is Rs. 35 = 00. So it is observed that formulation no.10 having low cost than commercial product used and also it possesses great activity against cariogenic bacteria. In allopathy, the treatment of dental problems is expensive and cannot be afforded by poor people. So, these types of herbal formulations are made. Out of 10 formulations, ethyl acetate extract of formulation no. 10 is more effective against selected four oral pathogenic bacteria.

Table 1: Details of various plant powders used in different concentrations for making 10 different herbal tooth powder formulations and 5 different commercial tooth powders.

Sr. No	Botanical Name	Part Used	Formulation (50 gram) (in gram)									
			1	1	2	3	4	5	6	7	7	8	8	9	9	10
1	*Foeniculum vulgare*	Fruit	10		12	11	14	11.5	3	4		5		6		5.2
2	*Cinnamomum zeylanicum*	Bark	2		0.1	0.2	0.4	0.6	0.8	1		1.2		1.4		2.1
3	*Punica grantum*	Fruit	1.5		4	6	5	2	10	0.5		1		0.1		0.2
4	*Calotropis procera*	Leaves	2		0.1	0.2	0.3	0.4	0.5	0.6		0.6		0.8		0.9
5	*Rosa indica*	Leaves	0.4		0.5	0.5	0.4	0.8	0.9	1		1.1		1.2		1.3
6	*Ocimum sanctum*	Leaves	5		8	5	6	7	6.5	11		12		9.1		10
7	*Syzygium cumini*	Seeds	5		1	2	3	3	4	2		3		6		6.5
8	*Azadirachta indica*	Leaves	3		0.1	0.2	0.3	0.4	0.5	0.6		0.7		0.8		1
9	*Quercus infectoria*	Fruit	1		4	3	2	5	6	11		8		9		10
10	*Myristica fragrans*	Fruit	8		9	10	8	9	10	8		10		8		9
11	*Achyranthes aspera*	Seeds	1		0.1	0.2	0.3	0.4	0.5	0.6		0.7		0.8		0.9
12	*Phyllanthus emblica*	Fruit	9		10	11	10	10	7.5	11.1		11		10		8
13	*Mimusops elengi*	Bark	9.1		10	10.5	8	8.5	9.3	6		5		4		3
	TOTAL		50		50	50	50	50	50	50		50		50		50
	Commercial Products															
14	CMF-1		50		-	-	-	-	-	-		-		-		-
15	CMF-2		50		-	-	-	-	-	-		-		-		-
16	CMF-3		50		-	-	-	-	-	-		-		-		-
17	CMF-4		50		-	-	-	-	-	-		-		-		-
18	CMF-5		50		-	-	-	-	-	-		-		-		-

Table 2: Antibacterial activities of solvent extracts of different formulations on oral pathogen and their zone of inhibition (in mm)

No	Formulations, Commercial products & Antibiotics	*Staphylococcus aureus*				*Streptococcus pyogenes*				*Lactobacillus acidophillus*				*Lactobacillus casei*			
		ME	EE	HE	DM	ME	EE	HE	DM	ME	EE	HE	DM	ME	EE	HE	DM
1	Formulation-1	11	8	0	0	8	13	2	0	12	13	0	0	7	7	0	0
2	Formulation-2	11	12	5	0	10	11	2	0	10	15	0	0	12	12	0	0
3	Formulation-3	12	10	0	0	10	10	2	0	7	10	0	0	12	11	0	0
4	Formulation-4	10	8	5	0	8	7	0	0	10	10	0	0	8	5	0	0
5	Formulation-5	10	12	5	0	10	10	2	0	22	22	0	0	12	15	0	0
6	Formulation-6	10	16	6	0	13	15	0	0	12	17	0	0	10	10	0	0
7	Formulation-7	15	15	8	0	10	13	0	0	15	17	0	0	12	13	0	0
8	Formulation-8	12	10	0	0	10	13	2	0	15	15	0	0	12	12	0	0
9	Formulation-9	15	12	10	0	10	12	2	0	10	12	0	0	11	16	0	0
10	Formulation-10	18	17	0	0	9	13	3	0	8	18	0	0	17	18	0	0
11	CMF-1	5	8	8	0	5	6	0	0	15	15	0	0	8	7	0	0
12	CMF-2	10	10	0	0	11	11	0	0	10	13	0	0	12	5	0	0
13	CMF-3	5	8	7	0	5	11	4	0	8	9	0	0	5	15	5	0
14	CMF-4	5	11	7	0	2	5	5	0	8	12	7	0	10	11	0	0
15	CMF-5	8	8	4	0	2	2	2	0	8	5	0	0	5	0	0	0
16	Erythromycin	32				30				22				25			
17	Amoxicillin	13				00				14				20			
18	Tetracycline	30				35				32				35			

Table 3: MIC (mg/ml) of selected plant formulation extract against cariogenic bacteria.

Sr No.	Formulation	Minimum Inhibitory Concentration (MIC) (mg/ml)			
		LA	LC	SA	SP
1	Formulation-10(ME)	-	0.0625	0.0312	-
2	Formulation-10(EE)	-	0.0625	0.0156	-
3	Formulation-5(ME)	0.5	-	-	-
4	Formulation-5(EE)	0.5	-	-	-
5	Formulation-6(ME)	-	-	-	0.125
6	Formulation-6(EE)	-	-	-	0.125

4. Conclusion

The present research is an attempt to prepare an oral tooth powder herbal formulation effective against microbial flora of oral cavity followed by its comparison with other marketed toothpowder brands (CFM-1, CFM-2, CFM-3, CFM-4 and CFM-5). Among all the solvents, ethyl acetate proved to be the most prominent solvent for extraction of anticariogenic substances from the selected tooth powder herbal formulations. In *Staphylococcus aureus*, Methanolic and ethyl acetate extract of formulation no.10 showed highest activity 18 mm and 17 mm respectively against this bacterium. In *Streptococcus pyogenes*, Ethyl acetate extract of formulation no. 6 showed highest activity (15 mm) against this bacterium. In *Lactobacillus acidophilus*, 2 solvents out of 4 are show good activity. Methanolic and ethyl acetate extract of formulation no.5 showed highest activity (22 mm) against this bacterium. In *Lactobacillus casei*, Methanolic and ethyl acetate extract of formulation no.10 and 05 showed highest activity 17 mm and 18 cm respectively.

The methanol and ethyl acetate extracts of formulation no. 10 compare with broad spectrum antibiotics. The comparison showed that the herbal tooth powder formulation has good antibacterial activity against all the selected bacteria. Ethyl acetate extracts exhibited moderate MIC values ranging from 0.0156 to 0.5 mg/mL against selected cariogenic bacteria in formulation no.10. In present study, commercial herbal tooth powder compared with formulation no 10 which has half price than commercial products and also has comparative good activity against all selected pathogenic bacteria. Therefore, the herbal tooth powder is effective and has cheaper rate so it is affordable to urban and rural community.

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