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Antimicrobial Activity of Medicinally Important Essential Oils against Selected Dental Microorganisms

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ABSTRACT

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Oral diseases are among the major public health problems and the most common of chronic diseases that affect mankind. Essential oils 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 11 essential oils against dental pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus pyogenes*) and fungi (*Candida albicans* and *Candida parapsilosis*) using agar well diffusion method, followed by determination of MIC. Most of the tested essential oils exhibited anticariogenic activity against all tested microbes. 16 formulations were made using them Formulations 10 and 13 showing good activity against *C. albicans* and *C. parapsilosis*. The formulations No. 10 and 13 showed strong antimicrobial activities with MIC ≥ 0.2 mg/ml against *C. albicans*. Active components of oil were separated by TLC. Separation of the compounds of formulation 10 using TLC shows 5 different bands present. Among 5 bands, only 1 band was active against *C. albicans*. These materials can be served as an important natural alternative to prevent microbial growth in dental diseases. The prepared formulation also uses as natural alternative and also less expensive compared to the commercial product.

Introduction

Oral diseases are among the major public health problems and the most common of chronic diseases that affect mankind. Bacteria are the dominant inhabitants of the oral cavity but other microorganisms are also seen which includes species of fungi, viruses and protozoa. The oral cavity is inhabited by more than 700 microbial species and many intrinsic and extrinsic factors affect the composition, metabolic activity and pathogenicity of the highly diversified oral micro flora (Aniebo *et al.*, 2012; Samaranayake *et al.*, 1986; Aas *et al.*, 2005; Nejad *et al.*, 2011). The most

prevalent oral infectious diseases, caries and periodontal disease, are historically the province of dentists for diagnosis and treatment. However, the effect of these oral diseases often extends systemically, particularly in older adults. Hematogenous seeding from an oral source is a dominant cause of bacterial endocarditis and is implicated in late prosthetic joint infection (LPJI). Periodontal disease impairs glycemic control in people with diabetes, and poorly controlled diabetes may exacerbate periodontal disease (Collin *et al.*, 1998;

Taylor *et al.*, 1998). Aspiration of oropharyngeal secretions is the predominant cause of nosocomial pneumonia in elderly persons (Scannapieco *et al.*, 1997). Periodontopathic bacteria in the bloodstream have been linked to atherosclerosis, coronary artery disease, and stroke (Beck *et al.*, 1996).

Dental plaque is formed by the colonization and accumulation of oral microorganisms in the insoluble glucan layer that are synthesized by glucosyltransferase (GTase) from *Streptococcus mutans* (Loesche, 1986). *Actinomyces naeslundii* and *Actinomyces visosus* are usually associated with dental caries particularly human root surface caries. To avoid dental caries due to cariogenic bacteria, inhibition of glucosyltransferase activity by specific enzyme inhibitor (Yanagida *et al.*, 2000), inhibition of initial cell adhesion of *S. mutans* by polyclonal and monoclonal antibodies and inhibition of cell growth of *S. mutans* by antibacterial agents have been investigated (Raamsdonk *et al.*, 1995). Antibiotics such as penicillin and erythromycin have been reported to effectively prevent dental caries in animal and humans but they are never used clinically because of many adverse effects such as hypersensitivity reaction, supra infections and teeth staining (Kubo *et al.*, 1992). Furthermore, *viridians* group *Streptococci* including *S. mitis*, *S. sanguis* and *S. mutans*, the most representative human cariogenic bacteria are moderately resistant to antibiotics (Venditti *et al.*, 1989). These drawbacks justify further research and development of natural antibacterials that are safe for the host or specific for oral pathogens. The natural phytochemicals could offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries and other oral infections. Although, plant products are greatly exploit for therapeutic potential to cure various oral ailments.

Medicinal plants have been recognized as valuable source of therapeutic components for centuries, and about 60% of world's population is known to use traditional medicines derived from medicinal plants. 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 (Pai *et al.*, 2004; Fernandes-Filho *et al.*, 1998). The increasing resistance to available antimicrobials has attracted the attention of the scientific community regarding a search for new cost-effective drugs of natural or synthetic origin (Fine *et al.*, 2000). Essential oils in general demonstrate antimicrobial activity against cariogenic microbes (Takarada *et al.*, 2004) and fungal filaments as well (Prashar *et al.*, 2003). Some studies have pointed out that plant-derived essential oils may be an effective alternative to overcome microbial resistance (Didry *et al.*, 1994). This study was undertaken to determine the *in vitro* antimicrobial activities of 11 essential oils against dental pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus pyogenes*) and fungi (*Candida albicans* and *Candida parapsilosis*) using.

Materials and Methods

Plant materials

The different plant species were selected and collected between May to June (2015), from different areas of Gujarat and surroundings of Ashok & Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), medicinal plant garden of New Vallabh Vidyanagar (Table 1). The plant was identified by Dr. Kalpesh Ishnavia (Plant taxonomist) at Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied

Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India. The leaves and seeds of all the healthy and disease free plants were used for oil extraction for the test of antimicrobial activity.

Extraction of essential oils

Hydro distillation method

Hydro distillation method was used for the extraction of essential oils from the selected plants. Selected plants were collected and washed with tap water. After that leaves were cut into small pieces and weighed 70g. It was placed in a 2-liter round bottomed flask with distilled water (300 ml for 70g fresh material) and the assembly was placed at rotating mantle at 80° C for 3 hours.

The essential oil was extracted and then collected in Eppendorf tubes and stored at room temperature.

The essential oil content was determined on an oil volume to tissue weight. Oil stocks were prepared by using different concentrations 10mg, 30mg, 50mg of oil in 50% DMSO and used for further experiment use (Charles *et al.*, 1990).

Cariogenic microbial strains

A group of microorganisms known to cause tooth decay were selected (*Candida albicans*-MTCC-3017; *Candida parapsilosis*-MTCC-6510; *Lactobacillus casei*- MTCC-1423; *Staphylococcus aureus*-MTCC-96; *Streptococcus mutans*-MTCC-890; *Streptococcus pyogenes*-MTCC-442) and purchased from Microbial Type Culture Collection (MTCC) bank, Chandigarh as a freeze dried pure culture. The microbial cultures were revived by using MTCC specified selective growth medium and preserved as glycerol stocks.

Bioassay for antimicrobial activity

Antibacterial activity

Agar well diffusion method

In the present study, to test antimicrobial activity, eleven different plant essential oils were used. The antimicrobial activity was studied by agar well diffusion method (Perez *et. al.*, 1990). From the stock, 10 mg, 30 mg, 50mg concentrations of essential oils were suspended in one millilitre of Dimethyl sulfoxide (DMSO). In order to make agar plates, the Petri plates were thoroughly washed using detergent, dried and sterilized in autoclave at 15 lbs pressure (121°C) for 15 minutes. Approximately 25ml of sterilized medium was poured into Petri plates and solidified at room temperature. The plates were incubated at 37° C for overnight for sterility testing. A fresh microbial culture of 300 µl was spread on agar plates with glass spreader. A well of 9 mm diameter punched off in Petri plates with sterile cup borer and then 100µl particular plant essential oil was loaded. Plates were placed for 30 minutes in refrigerator for diffusion of oil and then incubated at 37°C for 24 hours or more depending upon the organisms, until appearance of zone of inhibition. The zone of inhibition was measured as a property of antimicrobial activity. In the present study, ampicillin and amoxicillin antibiotics were used as positive control to compare the zone of inhibition with the antibacterial assay.

Minimum Inhibitory Concentration (MIC) determination (for bacteria)

Minimum inhibitory concentration was evaluated by serial broth dilution method (Chattopadhyay *et. al.*, 1998). Essential oils showing more than 08 mm inhibition zone were selected for MIC. Selective broth medium was used for dilutions as well as

preparing inoculums. The bacterial cell density was maintained uniformly throughout the experimentation at 1×10^8 CFU/ml by comparing with 0.5 McFarland turbidity standards. Plants essential oil of 400 μ l from stock solution was taken into first dilution tube containing 1600 μ l of selective medium broth and mixed it well. From these, 1000 μ l were transferred to second tube containing 1000 μ l broth. This step is repeated nine times and from the last tube 1000 μ l was discarded. 100 μ l of test organisms was added in each tube. The final volume of solution in each tube was made up to 1 ml. The MIC was tested in the concentration range between 20mg/ml to 0.2mg/ml. Tubes were incubated at optimal temperature and time in an incubator.

Growth indicator 2, 3, 5-triphenyl tetrazolium chloride solution (100 μ l of 0.1%) was incorporated in each tube to find out the bacterial growth inhibition. Tubes were further incubated for 30 minutes under dark conditions. Bacterial growth was visualized when colourless 2, 3, 5-triphenyl tetrazolium chloride was converted red colour formazone in the presence of bacteria. Each assay was done by using DMSO and selective medium as control.

Antifungal activity

Agar well diffusion method

A drop of fungal spore suspension was placed in the centre of PDA plates and spreader all over with sterile glass spreader. Cups were pored with sterile cup borer and filled with 100 μ l of extract. Plates were place in refrigerator for 10 min and then transferred to incubator held at 28 ° C and incubated for 72 hours then after plates were observed for zone of inhibition. Antifungal activity was measuring by diameter of zone. The

experiment was carried out in duplicate and mean of diameter of inhibition zone was calculated. 100% DMSO used as a control.

Minimum Inhibitory Concentration (MIC) determination (for fungus)

Minimum inhibitory concentration was evaluated by Agar well diffusion method. Essential oils showing more than 08 mm inhibition zone were selected for MIC. From the stock, 10mg, 30mg, 50mg concentrations of essential oils were suspended in one millilitre of Dimethyl sulfoxide (DMSO). In order to make agar plates, the Petri plates were thoroughly washed using detergent, dried and sterilized in autoclave at 15 lbs pressure (121°C) for 15 minutes. Approximately 25ml of sterilized medium was poured into Petri plate and solidified at room temperature. The plates were incubated at 37° C for overnight for sterility testing. A fresh microbial culture of 100 μ l was spread on agar plates with glass spreader. A well of 9 mm diameter punched off in Petri plates with sterile cup borer and then 2 μ l, 4 μ l, 6 μ l, 8 μ l, 10 μ l, 12 μ l, 14 μ l, 16 μ l, 18 μ l, 20 μ l, 22 μ l, 24 μ l, 26 μ l, 28 μ l, 30 μ l and 100 μ l particular plant essential oil formulation was loaded. Plates were placed for 30 minutes in refrigerator for diffusion of oil and then incubated at 37° C for 48 hours or more depending upon the organisms, until appearance of zone of inhibition. The zone was measured and minimum activity zone is considered as the MIC of that essential effect on oral fungal pathogen. Fluconazole was used as a positive control to compare the zone of inhibition with the antifungal assay. DMSO was used as a negative control in both assays respectively.

A preparation of essential oils formulation

Antibacterial and antifungal activity evaluate of the 11 essential oils (Table 2). 11 out of selected essential oils based on the criteria of

minimum inhibitory concentration (MIC) of bacteria and fungus selected. 7 out of 11 essential oils selected for the preparation of the formulation. Essential oils showing more than 08 mm inhibition zone were selected for MIC. Formulations were made by using seven different essential oils for antimicrobial assay.

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 percolated TLC plates (Merck, silica gel 60 F254 plate, 0.25mm). Take the 0.1ml essential oil and 0.9ml formulation is diluted with 0.9 ml toluene prepared sample. This sample further used of the separation of the compound in thin layer chromatography. The 5µl sample is used for TLC for separation of the compound. The Adsorbent - Silica gel 60F₂₅₄- Percolated TLC plates used. The system is Toluene: ethyl acetate: (93:7) used for the separation of compound from the selected formulation. After the run the plate observed under the UV trans-illuminator at 265 nm and 365 nm of TLC plate. Spray reagent Vanillin-Sulphuric acid is used for the detection of the compound present in the formulation. Some other spray reagents apply for the detection of the compound on the TLC plate. After that the plate is evaluated and not down the R_f value. Iodine vapours use for the developed the TLC bands in iodine chamber.

Bio autography

Out of 11 essential oils tested for antimicrobial activity, only one showing maximum growth inhibition against *Candida albicans* was selected and used for bioautography. By using capillaries 5 µL of essential oil of formulation no. 10 (100mg/mL stock solution) was spotted on to 0.25mm thick precoated silica gel 60 F254 plate

(Merck, Germany). The band length was 2mm thick. After air drying the TLC plate was run using pre-standardized solvent system, toluene: ethyl acetate: (93:7). The chromatogram was observed under UV illumination and used for bioautography. Organism specific agar medium, seeded with specific organism *Candida albicans* was overlaid on to the silica gel plate loaded with sample and incubated at 37°C for 24 hrs. On the next day, the plate was flooded with 2, 3, 5-Tri phenyl tetrazolium chloride (0.1%) to visualize growth inhibition. The area of inhibition zone was appeared as transparent against reddish background (lawn of living fungus).

Results and Discussion

Essential oils are rich sources of biologically active compounds which possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties against microorganisms. These essential oils are considered as non-phytotoxic compounds and potentially effective against several microorganisms including many fungal pathogens (Pandey *et al.*, 1982). Conner (1993) found that cinnamon, clove, pimento, thyme, oregano, and rosemary plants had strong inhibitory effect against several bacterial pathogens. It has been also reported that essential oils extracted from some medicinal plants had the antibacterial effects against all the oral pathogens due to presence of phenolic compounds such as carvacrol, eugenol and thymol (Kim *et al.*, 1995). The essential oils and their components have been used broadly against moulds. The essentials oils extracts from many plants such as basil, citrus, fennel, lemon grass, oregano, rosemary and thyme have shown their considerable antifungal activity against the wide range of fungal pathogens (Kivanc, 1991). Therefore, use of essential oils is increased for treatment of oral infection.

In the present study the antimicrobial assay of plant essential oils and different formulation made from the effective oils is carried out for the purpose of checking the sensitivity of oral pathogens. The different concentration of 11 essential oils was screened against selected oral pathogens and formulation was prepared from them.

Antimicrobial activity of essential oils

10 out of 11 essential oils against *C. albicans* give good antifungal activity. The diameters of the inhibition zones are presented in figure 1. The results showed that the isolates sensitivity was increased with the increase of antifungal concentration ($p < 0.05$). The range of the 10 to 31mm zone of inhibition observed. *A. indica* not give any antifungal activity. Maximum activity showed in the *S. aromaticum* against all the selected concentration and also pure sample of the oil. Maximum activity showed in the *S. aromaticum* in pure oil sample.

05 out of 11 essential oils against *C. parapsilosis* give good antifungal activity. The diameters of the inhibition zones are presented in figure 1. The results showed that the isolates sensitivity was increased with the increase of antifungal concentration ($p < 0.05$). The range of the 14 to 32mm zone of inhibition observed. *A. indica*, *E. globuls*, *C. citrates*, *O. sanctum* and *M. elengi* not give any antifungal activity. Maximum activity showed in the *C. martini* against all the selected concentration and also pure sample of the oil. Maximum activity showed in the *S. aromaticum* in pure oil sample.

The activity is compared with negative control DMSO. Which show no zone of inhibition against microorganisms as compared to antifungal and antibacterial positive controls used. Amoxicillin and ampicillin are used as a positive control.

The action of mechanism of phenolic compounds was related to the ability of phenolic compounds to alter microbial cell permeability, thereby permitting the loss of macromolecules from the cell interior, could help explain some of the antimicrobial activity. Another explanation might be that phenolic compounds interfere with membrane function and interact with membrane proteins, causing deformation in structure and functionality (Bajpai *et al.*, 2008).

02 out of 11 essential oils against *S. aureus* give good antibacterial activity. The diameters of the inhibition zones are presented in figure 2. The results showed that the isolates sensitivity was increased with the increase of antibacterial concentration ($p < 0.05$). The range of the 10 to 37mm zone of inhibition observed. *V. negundo* and *S. aromaticum* give antibacterial activity and rest of the oils not give any activity. Maximum activity showed in the *V. negundo* and *S. aromaticum* against all the selected concentration and also pure sample of the oil. Maximum activity showed in the *S. aromaticum* in pure oil sample.

05 out of 11 essential oils against *S. mutans* give good antibacterial activity. The diameters of the inhibition zones are presented in figure 2. The results showed that the isolates sensitivity was increased with the increase of antibacterial concentration ($p < 0.05$).

The range of the 10 to 27mm zone of inhibition observed. *V. negundo*, *S. aromaticum*, *O. sanctum*, *M. elengi* and *P. pinnata* give antibacterial activity and rest of the oils not give any activity. Maximum activity showed in the *V. negundo* and *S. aromaticum* against all the selected concentration and also pure sample of the oil. Maximum activity showed in the *S. aromaticum* in pure oil sample.

06 out of 11 essential oils against *L. casei* give good antibacterial activity. The diameters of the inhibition zones are presented in figure 2. The results showed that the isolates sensitivity was increased with the increase of antibacterial concentration ($p < 0.05$). The range of the 10 to 27mm zone of inhibition observed. *P. granatum*, *V. negundo*, *S. aromaticum*, *O. sanctum*, *M. elengi* and *P. pinnata* give antibacterial activity and rest of the oils not give any activity. Maximum activity showed in the *V. negundo* and *S. aromaticum* against all the selected concentration and also pure sample of the oil. Maximum activity showed in the *S. aromaticum* in pure oil sample.

03 out of 11 essential oils against *S. pyogenes* give good antibacterial activity. The diameters of the inhibition zones are presented in figure 2. The results showed that the isolates sensitivity was increased with the increase of antibacterial concentration ($p < 0.05$). The range of the 14 to 27mm zone of inhibition observed. *V. negundo*, *S. aromaticum* and *C. martini* antibacterial activity and rest of the oils not give any activity. Maximum activity showed in the *S. aromaticum* against all the selected concentration and also pure sample of the oil. Maximum activity showed in the *S. aromaticum* in pure oil sample.

Essential oils have been tested for *in vivo* and *in vitro* antimicrobial activity and some have demonstrated to be possessing potential antimicrobial potential. Their mechanism of action appears to be predominantly on the cell membrane by disrupting its structure thereby causing cell leakage and cell death, secondary actions maybe by blocking the membrane synthesis; and inhibition of cellular respiration (Cristiane *et al.*, 2008). They readily penetrate into the cell membrane and exert their biological effect because of high volatility and

lipophilicity of the essential oils (Inouye, 2003).

The elimination of cariogenic bacteria from the oral cavity using antibacterial agents is one of primary strategies for prevention of dental caries. Herbs are being widely explored to discover alternatives to synthetic antibacterial agents. Essential oils have been shown to possess antibacterial, antiviral, insecticidal and antioxidant properties. Similar to antifungal activity of essential oils oral bacteria are also screened for sensitivity assay. The results obtained from our study shows that the five essential oils have got a very good antibacterial activity against *Streptococcus mutans*. Regardless of which agent is the drug of choice for the treatment of oral diseases, dental scientists are still searching for new therapeutic applications to prevent and treat them. Toxicity, mucosal ulceration, and development of resistant bacterial strains are the adverse effects found with several other antibacterial agents. Collectively, these adverse effects of dental medications motivate dentists to use conventional natural therapeutics for the oral cavity ailments (Takahashi *et al.*, 2003).

In this study, the essential oil of *Syzygium aromaticum* was obtained, eugenol was identified as a compound and its antimicrobial activity was assessed, agreeing with what has been reported in several studies (Chaieb *et al.*, 2007). Its activity against *Streptococcus mutans* was observed, agreeing with several studies which reported its growth inhibitory activity in oral pathogens (Ayoola *et al.*, 2008). Many essential oils have been advocated for use in complementary medicine for bacterial infections. However, few of the many claims of therapeutic efficacy have been validated adequately by either *in vitro* testing or *in vivo* clinical trials. From the above results the most effective seven essential oils are used for preparing different

formulations which are further used to check anticariogenic activity of the formulations.

Antimicrobial activities of formulation of essential oils

C. albicans

15 out of 15 essential oils formulation against *C. albicans* give good antifungal activity. The

diameters of the inhibition zones are presented in figure 3. The range of the 18 to 30mm zone of inhibition observed. Maximum activity showed in the Formulation No. 14 and Formulation no. 15 (30 mm). Maximum activity showed in the selected oral microorganism out of *C. albicans* against the Formulation No. 14 and Formulation no. 15.

Table.1 Plant selected for oils extraction and antimicrobial activity

Sr. No	Botanical Names	Local Names	Part Use
1	<i>Azadirachta indica</i>	Neem	Leaf
2	<i>Pongamia pinnata</i>	Karanj	Seed
3	<i>Eucalyptus globus</i>	Nilgri	Leaf
4	<i>Cymbopogon citratus</i>	Lemon grass	Leaf
5	<i>Punica granatum</i>	Dadam	Seed
6	<i>Vitex negundo</i>	Nagod	Leaf
7	<i>Syzygium aromaticum</i>	Lavige	Fruit
8	<i>Oscimum sanctum</i>	Tulsi	Leaf
9	<i>Cymbopogon martini</i>	Palm roza	Leaf
10	<i>Mimusops elengi</i>	Borsalli	Seed
11	<i>Jatropha curcas</i>	Ratanjot	Seed

Table.2 Different formulation of essential oils

Formulations	Neem (µl)	Eucalyptus (µl)	Tulsi (µl)	Lemon Grass (µl)	Palm Roza(µl)	Clove (µl)	Dadam (µl)
F1(10mg/ml)	200	200	100	100	200	100	100
F2(10mg/ml)	300	100	200	100	100	100	100
F3(10mg/ml)	100	200	300	100	100	100	100
F4(10mg/ml)	100	100	10	200	200	20	100
F5(30mg/ml)	100	200	100	200	200	100	100
F6(30mg/ml)	100	100	100	100	300	100	200
F7(30mg/ml)	100	100	300	100	100	200	100
F8(30mg/ml)	200	200	100	100	100	100	200
F9(50mg/ml)	100	200	100	100	200	200	100
F10(50mg/ml)	100	100	200	200	100	100	200
F11(50mg/ml)	100	200	200	100	100	200	100
F12(50mg/ml)	100	100	300	100	100	100	200
F13(pure)	100	100	100	100	300	100	200
F14(pure)	100	200	200	100	100	200	100
F15(pure)	100	300	100	200	100	100	100
F16(pure)	100	50	300	50	400	50	50

Fig.1 Antifungal activities of essential oils against *C. albicans* and *C. parapsilosis* and their zone of inhibition (in mm)

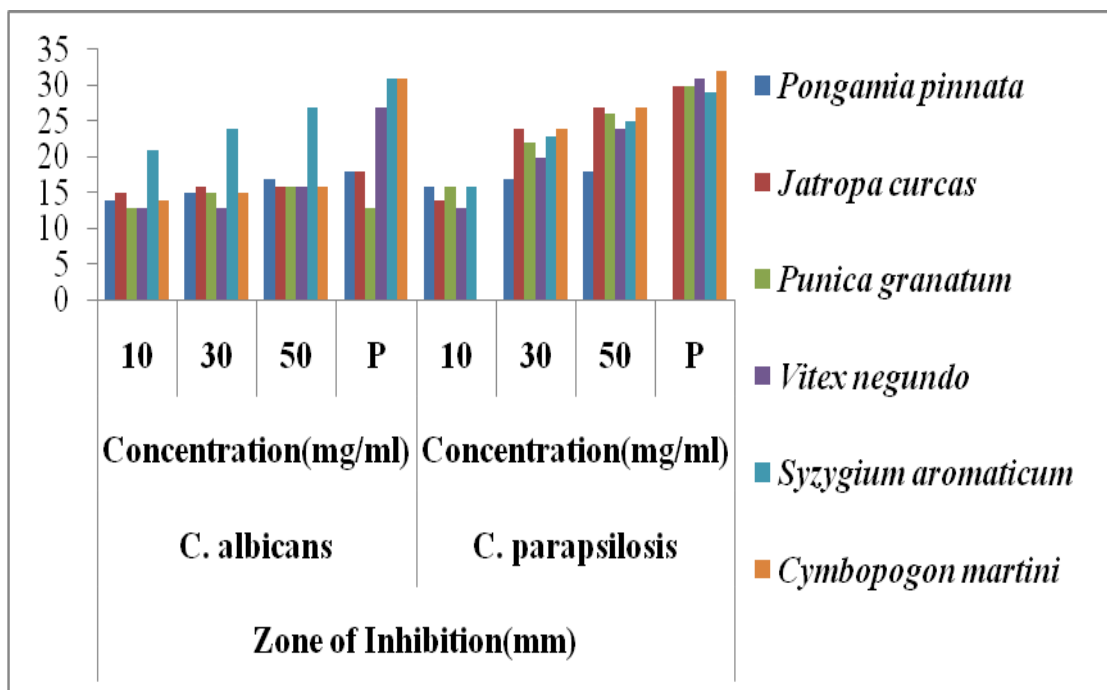


Fig.2 Antibacterial activities of essential oils against *S. aureus*, *S. mutans*, *L. casei* and *S. pyogenes* and their zone of inhibition (in mm)

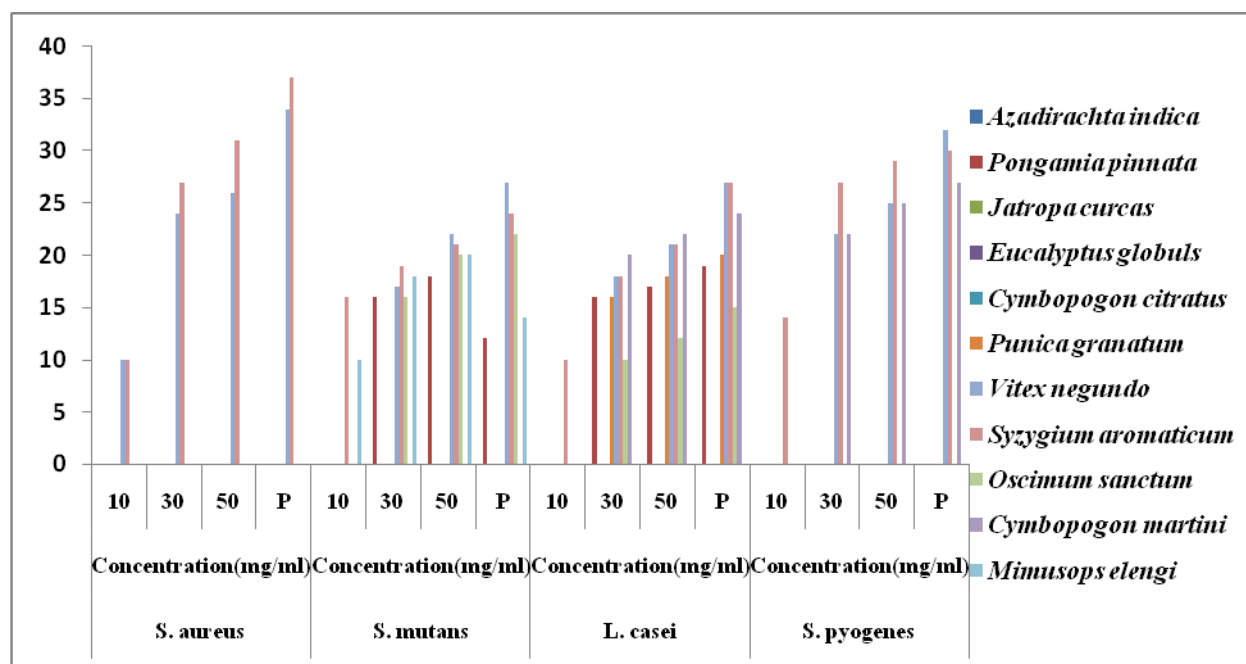
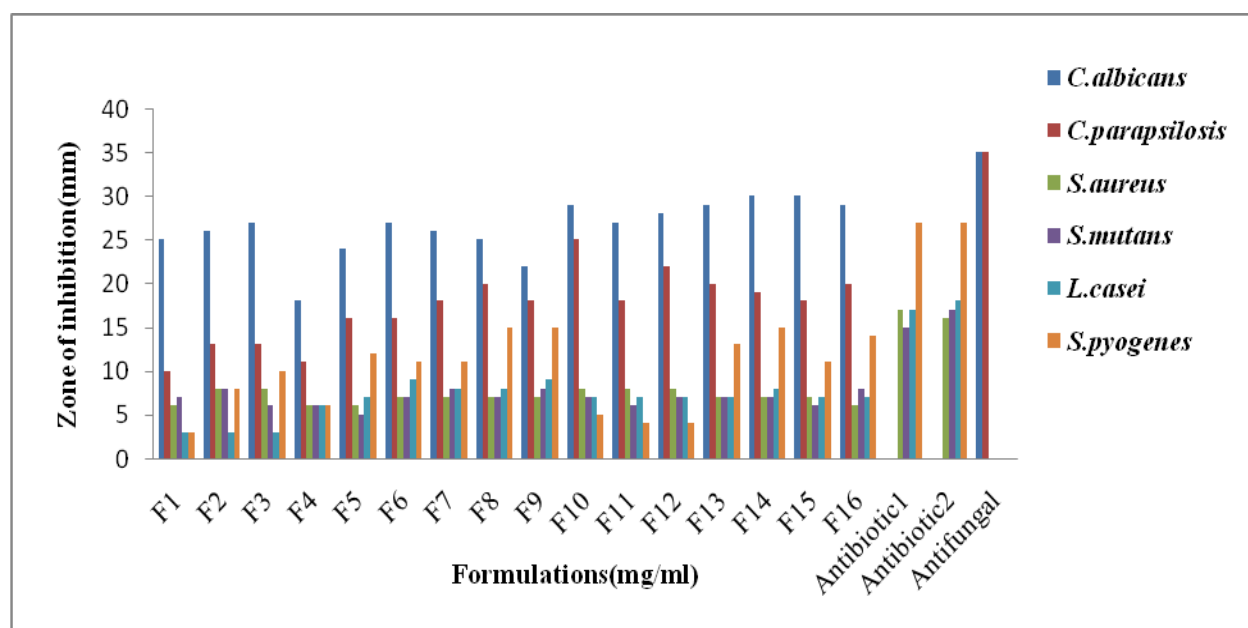


Table.3 The MIC (mg / mL) of selected essential oils formulations against microorganisms

FORMULATIONS	TEST ORGANISMS					
	1	2	3	4	5	6
F6	0	0	0	0	3	0
F8	0	0	0	0	0	3
F9	0	0	0	5	2.5	20
F10	0.2	0.4	2.5	0	0	0
F13	0.2	0	0	0	0	0
F14	0	0	0	0	0	6.5

1- *C. albicans*; 2- *C. parapsilosis*; 3-*S. aureus*;4- *S. aureus*; 5-*L. Casei*; 6- *S.Pyogenes*

Fig.3 Antimicrobial activities of formulation of essential oils against *C. albicans* *C. parapsilosis*, *S. aureus*, *S. mutans*, *L. casei* and *S. pyogenes* and their zone of inhibition (in mm)



C. parapsilosis

15 out of 15 essential oils formulation against *C. parapsilosis* give good antifungal activity. The diameters of the inhibition zones are presented in figure 3. The range of the 10 to 25mm zone of inhibition observed. Maximum activity showed in the Formulation No. 10 (25 mm). Formulation No. 10 compare to *C. albicans* is less active against this organism.

S. aureus

15 out of 15 essential oils formulation against *S. aureus* give moderate antibacterial activity. The

diameters of the inhibition zones are presented in figure 3. The range of the 06 to 08 mm zone of inhibition observed. In this organism showed the moderate activity against all formulation.

S. mutans

15 out of 15 essential oils formulation against *S. mutans* give very poor antibacterial activity among all the selected microorganisms. The diameters of the inhibition zones are presented in figure 3. The range of the 03 to 08 mm zone of inhibition observed. In this organism showed the moderate activity against all formulation.

L. casei

15 out of 15 essential oils formulation against *S. mutans* give moderate antibacterial activity. The diameters of the inhibition zones are presented in figure 3. The range of the 05 to 09 mm zone of inhibition observed. In this organism showed the very less activity against all formulation.

S. pyogenes

15 out of 15 essential oils formulation against *S. mutans* give good antibacterial activity. The diameters of the inhibition zones are presented in figure 3. The range of the 03 to 15 mm zone of inhibition observed. In this organism showed the good activity against all formulation and also among all oral bacteria. Maximum activity showed in the Formulation No. 08, 09 and 14 (15 mm).

Selected microorganisms among antifungal activity give more responded against bacteria. 15 out of the formulation best formulation No. 14 and formulation No.15 among both antifungal and anticariogenic activity. Formulation No. 13, 14 and 15 highly active against *C. albicans* < *C. parapsilosis* < *S. pyogenes* < *S. aureus* < *S. mutans*. Formulation No. 13 include the formulation pure oils more response to other combination of the different concentration of the oils. In this formulation included the Neem (100), Eucalyptus (100), Tulsi (100), Lemon (100), Grass (100), Palm roza (300), Clove, (100) and Punica (200). In this formulation maximum quantity takes for the formulation preparation from the clove oils. Therefore, it is responsible compound available in the clove oils. Components of clove oil are eugenol, eugenol acetate, isoeugenol and caryophyllene. Clove oil is useful for its disinfecting properties, relieving of pain, especially toothache, arthritis and rheumatism. Studies conducted by Dorman *et al.*, (2000) in UK in 2000 and Betoni *et al.*, (2006) in Brazil in 2006 have proved the antimicrobial potential of clove oil. Components of eucalyptus oil that are thought to be responsible for its antibacterial property are pinene, limonene, terpinenol, piperitone and

globulol. Antimicrobial potential of eucalyptus oil has been proved in studies conducted by Sattari *et al.*, (2010) in Iran in 2009 and Filoche *et al.*, (2005) in New Zealand in 2005.

MIC (mg / mL) of selected essential oils formulations against microorganisms

The Minimum Inhibitory Concentration (MIC) values of different formulations of essential oils of all the selected plants showing highest activity against selected organisms was assessed and determined. Examining the MIC values of six formulations of different essential oils generated the data where the maximum MIC value was found to be 20 mg/ml and the minimum value as 0.2 mg/ml.

The Minimum Inhibitory Concentration (MIC) values of essential oil formulation 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 essential oils formulation showed the maximum MIC value was found to be 20 mg/mL and minimum value as 0.2 mg/mL.

The MIC value of essential oils formulation of formulation No.06 against LC is 3 mg/mL.

The MIC value of essential oils formulation of formulation No.08 against SP is 3 mg/mL.

The MIC value of essential oils formulation of formulation No.09 against SM, LC and SP is 5 mg/mL, 2.5 mg/mL and 20 mg/mL respectively.

The MIC value of essential oils formulation of formulation No.10 against CA, CP and SA is 0.2 mg/mL, 0.4 mg/mL and 2.5 mg/mL respectively

The MIC value of essential oils formulation of formulation No.13 against CA was 0.2 mg/mL.

The MIC value of essential oils formulation of formulation No.14 against SP was 6.5 g/mL.

This formulation exhibited moderate MIC values ranging from 3 mg /mL to 20 mg/mL against cariogenic bacteria of SP.

According to these results, Formulation No.13 exhibits good MIC value ranging from 0.2 mg/ml to 20 mg/ml against selected oral pathogenic organisms.

TLC analysis

Based on the MIC value and thin layer chromatography results, formulations are selected for the further study of the characterization of phytochemical constituents using TLC. In this study, formulation No.10 of essential oil used for the characterization. In TLC analysis of formulation no. 10, band no. 4 having active compound against cariogenic fungus *C. albicans*. This band appear as black colored under 254 lower intensity and 365 colored under higher intensity not observed and band. The active compound Rf value is 0.78. In TLC analysis of formulation No. 10 having active compound against cariogenic bacteria *C. albicans*. This band appear as black colored under 254 lower intensity and no color 365 colored under higher intensity. The active compound Rf value is 0.78.

Bio-autographical study

In order to find out active principles present in formulation no.10 of oil, TLC solvent system was standardized (Toluene: chloroform: 0.9: 0.3) and used for subsequent analysis. The bioactive compounds were separated from crude extracts by using TLC technique.

To locate the major active compounds responsible for the anticariogenic activity in formulation no.3, chromatogram was used for TLC – Bioautography against CA. Further chromatographic and spectroscopic analysis of plant formulation extracts is necessary for determination of structures of bioactive compounds.

The present study, the very good inhibitory

potential of essential oils of Neem, Eucalyptus, Tulsi, Lemongrass, Palmrosa, Clove and Punica. The formulations No. 10 and 13 showed strong antimicrobial activities with MIC \geq 0.2mg/ml against *C. albicans*. Active components of oil were separated by TLC. Separation of the compounds of formulation 10 using TLC shows 5 different bands present. Among 4 bands, only 1 band was active against *C. albicans*. The result shows that oils at different concentrations exhibited anti-microbial activity against dental pathogens. These materials could be served as an important natural alternative to prevent bacterial growth in dental diseases. Essential oils have great potential as antimicrobial compound against pathogenic microorganisms, which can be used to treat oral infectious diseases.

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