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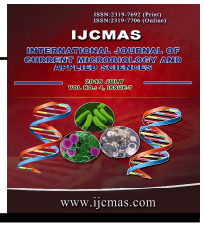

Original Research Article

In vitro Comparison of Antimicrobial Activity of Different Extracts of *Cymbopogon citratus* on Dental Plaque Isolates

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Keywords

Cymbopogon citratus, Medicinal plant, Antimicrobial activity, Mouthwash, Essential oil, Streptococcus mutans

A B S T R A C T

Oral micro-organisms play a significant role in dental caries and control of its activities can promote prevention of dental caries. Use of herbal agents is a notable issue in recent research work in dental caries. The study was designed to evaluate antimicrobial *in vitro* effects of extracts of *Cymbopogon citratus* on bacteria isolated from oral cavity. *Streptococcus mutans* *Streptococcus salivarious* and *Streptococcus mitis* were isolated from dental plaque. Ethanol extract, ethyl acetate extract, hexane extract and essential oil(EO) of *Cymbopogon citratus* were tested against three local dental plaque isolates as well as six standard cultures which included a fungal specie. Results indicated a considerable antibacterial activity of all three extracts. The diameter of zone of inhibition ranged from (15.2 ± 0.6) mm to (7.0 ± 0.4) mm for ethyl acetate extract, (11.6 ± 0.7) mm to (6.3 ± 0.3) mm for ethanol extract, (12.5 ± 0.1) mm to (6.0 ± 0.5) mm for hexane extract in

Introduction

Dental caries and periodontal diseases are among the most important global oral health problems. Over 750 species of bacteria inhabit the oral cavity and a number of these are involved in oral diseases (Jenkinson et al., 2005). The development of dental caries involves acidogenic and aciduric Gram-positive bacteria, primarily the mutans streptococci (*Streptococcus mutans* and *S. sobrinus*), *lactobacilli* and *actinomycetes*, which metabolize sucrose to organic acids (mainly lactic acid) which dissolve the calcium phosphate in teeth, leading to decalcification and eventual decay.

The global need for alternative preventive methods and products for oral diseases that are safe, effective and economical comes from the increase in disease incidence, particularly in developing countries. There is also an increased resistance of pathogenic bacteria to currently used antibiotics and chemotherapeutics, (Trichy et al., 1998)

Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining (Park, 2003; Chung et al.,2006). Other antibacterial agents used in the prevention

and treatment of oral diseases, including cetylpyridinium chloride, chlorhexidine, amine fluorides or products containing such agents, are reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (found in mouthwashes) (Lachenmeier, 2008) have been linked to oral cancer (Rodrigues et al., 2007). Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicines are considered as good alternatives to synthetic chemicals (Prabuseenivasan et al., 2006).

There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. The general antimicrobial activities of medicinal plants and plant products, such as essential oils, have been reviewed previously (Cowan et al.,1999; Kalemba 2003).

Lemongrass (*Cymbopogon citratus***,** *Poaceae*) has plethora of medicinal uses. It is said to have antibacterial, anti-inflammatory, antioxidant, antifungal, antiseptic, astringent, analgesic, antipyretic and carminative properties. Since the herb has not been studied extensively, its effectiveness is based mainly on its centuries-old reputation as a folk remedy.

The need to identify a common and cost effective herbal remedy for the prevention and treatment of sore-throat, mouth sore and dental caries, especially in a developing nation, prompted us to investigate the antimicrobial activity of *Cymbopogon citratus.*

Materials and Methods

Isolations and Identification of isolates

The standard microbial cultures were procured from MTCC as well as were isolated locally from dental plaque samples.

Microbial cultures procured: The microbial cultures in lyophilized form, used for this study were procured from Institute of Microbial Technology (IMTECH), Microbial Type Culture Collection (MTCC) Chandigarh. The cultures included five strains of bacteria *Lactobacillus rhamnosus* (MTCC1408), *Lactobacillus acidophilus* (MTCC10307), *Streptococcus mutans* (MTCC 890), *Streptococcus oralis* (MTCC 2696), *Actinomyces howelii* (MTCC 3048) and one unicellular fungus *Candida albicans* (MTCC4748),

Collection of samples

Subject selection

Subject inclusion criteria:

a. Above 18 years of age
b. Non – smokers
c. Non-pregnant, non-lactating women
d. Good general health
e. All subjects were dentate with at least 24 teeth, excluding third molars or crowned teeth

Subjects exclusion criteria:

a. Subjects with Periodontal therapy in past 6 months.
b. Subjects with Antimicrobial therapy within past 1 month.
c. Subjects using antioxidant soya diet, green tea, supplements containing lemon grass or other polyphenols, were excluded.

d.Subjects with disease that had major inflammation and / or oxidant stress (i.e.diabetes, cancer, arthritis, Crohn's disease, etc).were excluded from the study.

Sample collection

Samples were collected from 50 subjects, 25 males and 25 females between the age group of 18 to 60 years. Samples were collected from different private dental clinics after a written informed consent was taken from the patient. Samples - collected in sterile wide-mouthed screw capped tubes containing 5ml of sterile nutrient medium. Immediately the sampled carried to the laboraory and processed within 2 hrs. of collection.

Samples collected for the isolation of bacteria causing dental diseases

Sample. No.	Source of samples	No. of Samples	No. of Isolates
1.	Supra Gingival	09	09
2.	Dental plaque & caries	41	50

Isolation of organisms

The clinical samples were homogenized by a vortex mixer and 100µl of sample was streaked on sterile Mitis Salivarius (MS) agar plate and sterile Nutrient Agar (NA) plate respectively.

The plates were incubated under aerobic conditions at 37^0C for 24 - 48 hours. All the isolates were purified using sterile MS agar plate and NA agar plates. All the purified isolates were maintained on sterile MS agar and NA agar slants.

Screening of bacterial isolates for further studies

The samples used in study were of dental caries & plaque and supragingival origin, The most common organisms naturally associated with these, belong to genus *Streptococci.*

On the basis of characteristic colony Morphology, Gram Character, distinctive cell shape, selective biochemicals, growth on MS Agar + 1% K-tellurite, out of the 50 isolates obtained, 3 isolates were selected for further identification and studies.

Identification of the isolates

The selected isolates were identified on basis of morphological, cultural & physiological characteristics according to Bergy's Manual of Systematic Bacteriology Vol. I and II.

Plant collection and identification

Leaves of *Cymbopogon citratus* were collected from Pune, India. Authentication and identification was performed at Botanical Survey of India (BSI), Pune. A voucher specimen (BSI-V. No. SOACYC1) has been deposited at BSI. Collected material was shade dried and stored in airtight container.

Shade dried leaves were powdered using a blender and then subjected to successive extraction using solvent of varying polarity such as ethanol, ethyl acetate and hexane. After extraction, the solvent was removed under reduced pressure. Extracted material was stored in air tight container until use. Commercially available pure essential oil of *Cymbopogon citratus* commonly known as Lemon grass oil (LGO) was purchased from a local supplier.

Susceptibility test

Stock solutions were prepared by dissolving known amount of the dried extract in DMSO. From the stock solutions serial dilutions were made to obtain different concentrations.

A standard stock of the bacteria isolates was prepared by suspending a loop full of each microbial growth in about 10mL of nutrient broth. After incubation at 37^{0C} for 24h, the turbidity was adjusted to be comparable with a 0.5 McFarland turbidity standard giving a bacterial load of about 1-2 x 10^8 cfu / ml (Murray et al., 2004).

The agar-well diffusion method prescribed by NCCLS (2000) was employed in the susceptibility testing . Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFarland's standard. Each Mueller Hinton (MH) agar plate was uniformly seeded by means of pour plate technique. Wells of 6mm in diameter, 4mm deep and about 2cm apart were punched in the MH agar with a sterile cork-borer. Approximately 50µl of the extracts were filled into each well which filled them respectively to fullness. The setup was allowed to stabilize before being incubated at 37^0 C for 24h.

The mean zones of inhibition were thereafter measured in mm, for all the individual isolates. A positive control well was filled with chlorehexidine while the DMSO served as negative control. Extracts were diluted to concentrations ranging from 20mg/ml to 2.5mg/ml. Plates were incubated aerobically at 37^0 C for 24h. Zone of inhibition were measured in mm.

Minimum Inhibitory concentration (MIC) was determined using tube dilution test. MIC was determined for all three extracts. MIC of EO also checked.

Results and Discussion

From Local dental plaque sample 3 bacterial species are identified as *Streptococcus mutans* , *Streptococcus salivarious* and *Streptococcus mitis* which are associated with the various degrees of dental caries..Standard cultures obtained include *Lactobacillus acidophilus* and . *Lactobacillus rhamnosus* . Previous authors have described the prevalence of *S. mutans* and *Lactobacillus* in carious teeth (Nishikawara 2006).

The results of mean of zone of inhibition against all three extract is shown in table 1.

The result shows the zone of inhibition ranged from (20.0 ± 0.5) mm to (12.9 ± 0.5) mm for essential oil (LGO) , ethyl acetate extract from (15.2 ± 0.6) mm to (7.0 ± 0.4) mm, ethanol extract from (11.6 ± 0.7) mm to (6.3 ± 0.3) mm, and (12.5 ± 0.1) mm to (6.0 ± 0.5) mm for hexane extract , as compared with (21.4 ± 0.2) mm to (15.0 ± 0.7) mm for chlorhexidine (Table 1), at the various concentrations used. . No inhibition was observed with controls, which proves that solvents could not act as antibacterial agent.

Results indicate a considerable antibacterial activity of all three extracts. The Essential oil was the most effective of all the other extracts. EO was more effective against *Candida albicans* (20.0 ± 0.5) mm but displayed least effective against *L. acidophilus* (12.9 ± 0.5) mm. fig 1

Minimum Inhibitory concentrations of all extracts was in range of 20 mg/ml to 2.5mg/ml and EO was in range from 1:1 to

1:4 (Table 2 and Table 3). MIC is represented graphically (fig 2 and 3)

The present study clearly showed that leaves extracts of *Cymbopogon citratus* has antimicrobial activity against standard cultures as well as local dental plaque isolates which include *Streptococcus mutans*. It is one of the most important oral bacteria and is also known as an initiator of dental biofilm formation and has cariogenic property by demineralizing enamels which plays a major role in dental caries, bacteremia and consequently bacterial endocarditis among predisposed patients. Therefore, to overcome dental carries it is important to control mouth flora especially *Streptococcus mutans* and at the same time control their capabilities to build biofilm. (Loesche, 1986)

Other species present in the mouth include *Lactobacilli*, *Actinomyces* and *Veillonella* species. *Actinomyces* is a Gram-positive, rod-shaped bacterium that occupies the oral cavity. The species has been implicated in periodontal disease, secondary enamel caries and primary root caries. They are some of the first bacteria to occupy the oral cavity and colonize the tooth's surface. This study shows considerable antibacterial activity against this bacteria. The zone of inhibition was observed with LGO (16.0 ± 0.5)mm and ethyl acetate exract is (12.0 ± 0.0)mm.

The Lactobacilli (LB) comprise a diverse group of more than 80 species and for decades, , LB was considered as the major etiological agent of dental caries. LB are prolific lactic acid producers (acidogenic) as well as acid-tolerant (aciduric). They are routinely and consistently isolated from caries-active sites. Lactobacilli can cause dental caries through their highly acidogenic and acid-tolerant characteristics and are frequently detected in deep carious lesions (Vestman N. R., et al, 2013). The two species of *Lactobacillus rhamnosuss* (MTCC1408), *Lactobacillus acidophilus* (MTCC10307),has shown maximum inhibition with LGO and minimum inhibition with hexane extract.

Candida albicans is an opportunistic human pathogen that colonizes the oral cavities of a large proportion of the population without causing disease. Adhesion of *Candida albicans* to saliva-coated surfaces is an important early step in the colonization of the oral cavity. *C. albicans* cells also adhere to several species of oral streptococci including *Streptococcus gordonii*, *C. albicans* can cause a number of mucosal infections, including oral candidiasis.. In this study , LGO shows highest inhibition zone (20.0 ± 0.5) mm against *Candida albicans* (MTCC 4748).

This is one of the first in vitro studies where the antimicrobial effect of EO was tested against the oral microorganisms obtained from local clinical sample as well as MTCC cultures. Many different in vitro studies have been done earlier for testing the antimicrobial effect of EO on many organisms involved in systemic disease.

Prabuseenivasan S et al. studied the *in vitro* antibacterial activity of few plant essential oils by the disk diffusion method. The result obtained for EO for *P. vulgaris* was 14.6 ± 0.7mm 12.1 ± 0.2 mm and 9.5 ± 0.5 mm at 1:1 1:5 and 1:10 dilution and for *P. aeruginosa* was 23.4 ± 1 mm 19.6 ± 0.5 mm and 9.1 ± 0.5 mm at 1:1 1:5 and 1:10 dilution.

K. A Hammer et al. worked on the antimicrobial action of essential oils and plant extracts. They found that Lemon grass, oregano and bay inhibited all organisms at <_ 2.0% (v/v). The oraganisms studied were *S. aureus, E. Coli* and *C. albicans.*

Table showing % present 3 bacterial species in all samples collected

S No	Bacteria identified	%Present in total sample
1	*Streptococcus mutans*	4%
2	*Streptococcus salivarious*	2%
3	*Streptococcus mitis*	2%

Table.1 Rearranged antimicrobial activity of extracts and EO against bacterial cultures

	Organisms (Zone of Inhibition in mm)								
	Standard Cultures						Clinical Isolates		
	Candida albicans (MTCC4748)	*Lactobacillus rhamnosuss* (MTCC1408)	*Lactobacillus acidophilus* (MTCC10307)	*Streptococcus mutans*(MTCC 890),	*Streptococcus oralis*(MTCC 2696)	*Actinomyces howelii* (MTCC 3048)	*Streptococcus mutans*	*Streptococcus mitis*	*Streptococcus salivarius*
EO	20.0 ± 0.5	18.0 ± 0.5	12.0 ± 0.5	14.0 ± 0.5	16.0 ± 0.5	16.0 ± 0.5	14.0 ± 0.5	14.0 ± 0.0	13.0 ± 0.5
Ethyl Acetate Extract	15.0 ± 0.5	12.0 ± 0.5	7.0 ± 0.0	8.0 ± 0.5	12.0 ± 0.5	12.0 ± 0.0	13.0 ± 0.5	12.0 ± 0.5	12.0 ± 0.0
Ethanol Extract	11.0 ± 0.0	8.0 ± 0.5	6.5 ± 0.5	7.0 ± 0.0	10.0 ± 0.0	9.0 ± 0.5	10.0 ± 0.5	10.0 ± 0.5	11.0 ± 0.0
Hexane Extract	6.0 ± 0.5	6.5 ± 0.0	6.0 ± 0.5	7.0 ± 0.0	12.0 ± 00	9.0 ± 0.5	10.0 ± 0.5	9.0 ± 0.	10.0 ± 0.5
Chlorehexidine	21.0 ± 0.5	18.0 ± 0.5	17.0 ± 0.5	18.0 ± 0.0	17.0 ± 0.5	15.0 ± 0.5	15.0 ± 0.0	16.0 ± 0.5	15.0 ± 0.5

Table.2 Minimum Inhibitory Concentration of extracts on cultures / isolates

Cultures	Ethyl Acetate Extract (mg/ml)	Ethanol Extract (mg/ml)	Hexane Extract (mg/ml)
Std cultures			
C. albicans (MTCC4748)	5	5	5
L. rhamnosus (MTCC1408)	10	5	10
L. acidophilus (MTCC10307)	10	10	5
Str. mutans (MTCC 890)	5	5	2.5
Str. oralis(MTCC 2696)	5	10	5
A. howelii (MTCC 3048)	2.5	5	5
Isolates			
Str. mutans	2.5	2.5	5
Str. mitis	5	5	5
Str. salivarious	5	5	2.5

Table.3 Minimum Inhibitory Concentration of Essential Oil on cultures / isolates

Cultures	Essential oil (% , v/v)
Std cultures	
Candida albicans(MTCC4748)	1:1
Lactobacillus rhamnosus(MTCC1408)	1:2
Lactobacillus acidophilus(MTCC10307)	1:2
Streptococcus mutans(MTCC 890)	1:2
Streptococcus oralis(MTCC 2696)	1:2
Actinomyces howelii howelii (MTCC 3048)	1:1
Isolates	
Streptococcus mutans	1:1
Streptococcus mitis	1:2
Streptococcus salivarious	1:2

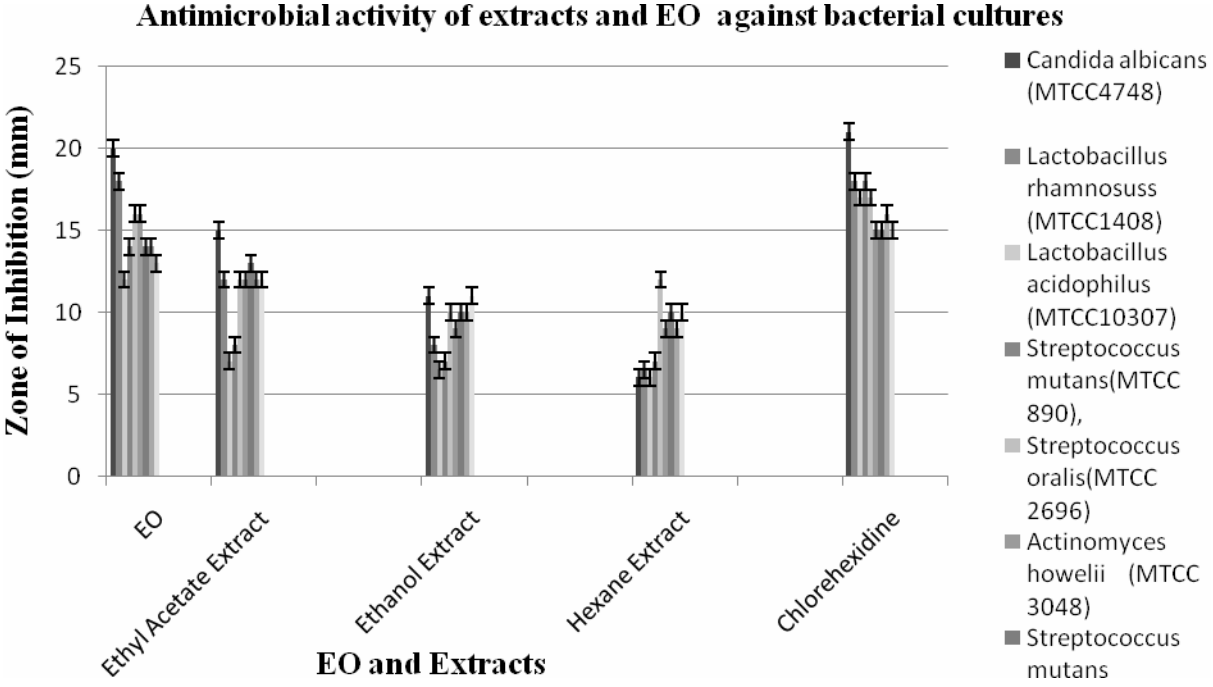


Fig.2 MIC of extracts on Std cultures and isolates

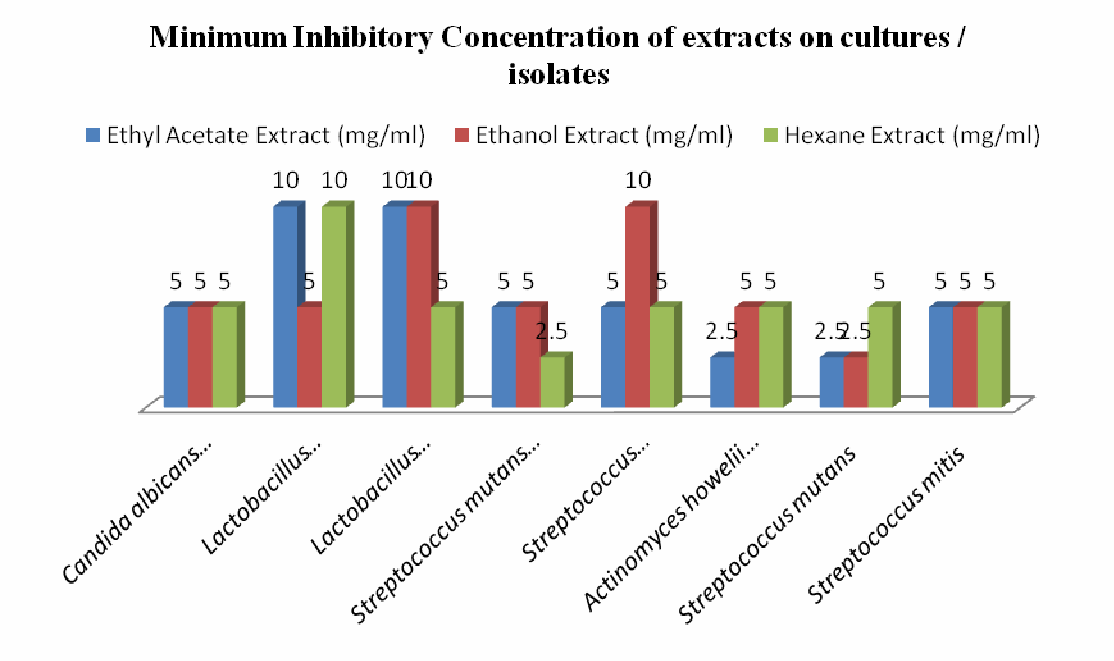
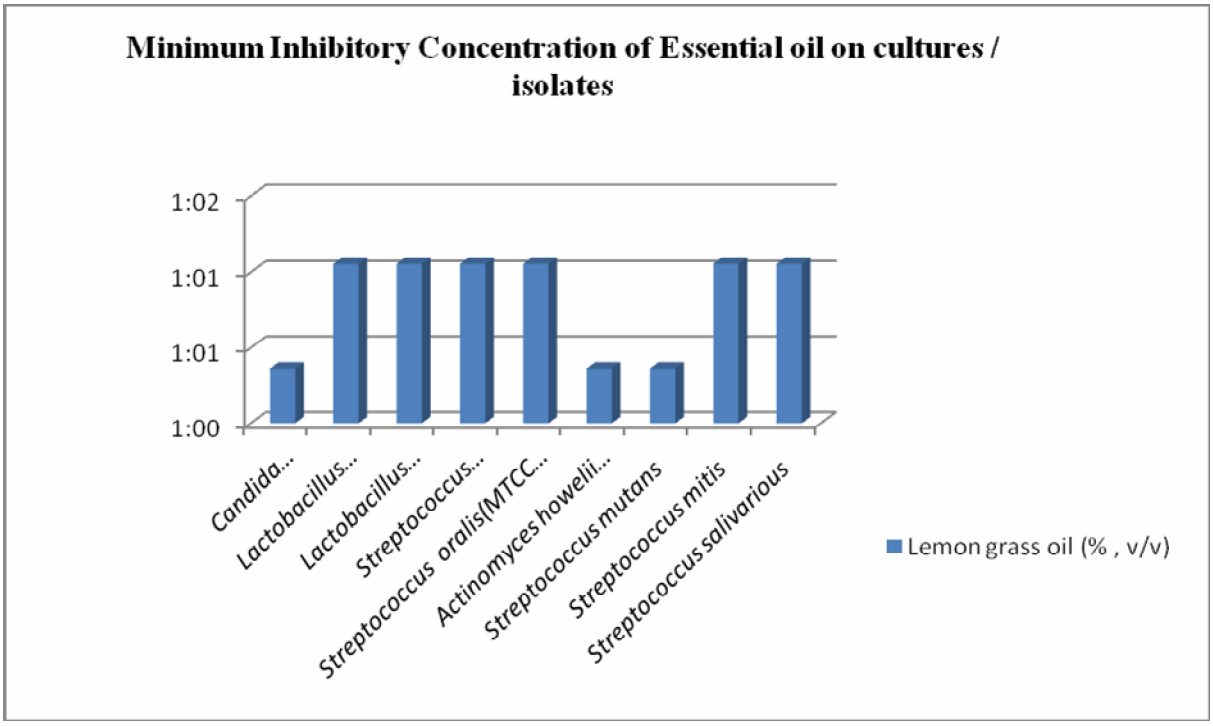


Fig.3 MIC of EO on Std cultures and isolates



Sulaiman Ali Al Yousef studied Lemongrass oil was found to be highly fungicidal, as it showed the lowest MIC and MLC values and the highest growth inhibition; in a range of concentrations (15 to 20 µl/0.4l air space). Lemongrass oil mouthwash can be used as an adjunct along

with the non surgical therapy. (Meena Anand 2011) .

Results indicated a considerable antibacterial activity of all three extracts. The ethyl acetate extract has shown maximum inhibition against the std cultures and local isolates. Ethanol extract was less effective and hexan extact has shown minimum inhibition comparatively. The EO was more effective as compare to the three extracts, inhibition zone ranging between (20.0 ± 0.5) mm to (12.9 ± 0.5) mm. EO of *Cymbopogon citratus* was most effective against *Candida albicans* (20.0 ± 0.5) mm, but least effective against *L. acidophilus* (12.9 ± 0.5) mm as compared to chlorhexidine, Minimum Inhibitory concentrations of all extracts was in range of 20 mg/ml to 2.5mg/ml and EO was in range from 1:1 to 1:4 This study, thus indicates a significant effect of antimicrobial activity by various extracts and EO against dental plaque isolates as well as standard cultures.

The results of this study revealed that, essential oil may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of bacterial infections of oral cavity.

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