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Antimicrobial and Antibiofilm Activity of Citral and its Derivative against Microflora from Dental Plaque

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ABSTRACT

Dental biofilms inhabit the oral cavity in form of dental plaque which then causes dental caries and periodontal diseases worldwide. Lemon grass essential oil (LGEO) has been reported to exhibit antimicrobial and antibiofilm activity against. This study represents the potential of citral and derivatives as antimicrobial and antibiofilm agent against dental microflora. Three bacterial species chiefly responsible for biofilm formation, and five prime colonizer of dental plaque were selected to represent dental microflora. Citral and its derivative *viz*. citral semicarbazone, exhibited antimicrobial and antibiofilm activity against the selected organisms. For the first time, any citral derivative has ever demonstrated to exhibit antimicrobial and antibiofilm activity against the oral microflora. However, study could not established citral or its derivatives as more effective, powerful and better herbal material as compared to LGEO to control the oral microflora associated with dental plaque.

KEYWORDS

Dental biofilm, Citral, Citral semicarbazone, Antimicrobial activity, Antibiofilm activity.

INTRODUCTION

Oral diseases are one of the leading health problems worldwide. Relationship between the oral infection and activities of microbial species that form part of the microbiota of the oral cavity has well been documented [1]. More than 1000 bacterial strains inhabit in the dental plaque (50% are unidentified) and a number of these are associated with oral diseases [2,3].

The current advances in molecular biological approaches have established that dental plaque formation is a complex dynamic process that implicates the early acquisition of an organic film with the subsequent colonization by numerous genetically distinct microbial cells [2]. In humans, more than 65% of hospital acquired infections are originated from biofilm forming bacteria [4-6]. These bacteria generate organic acids as the byproducts, which then causes a carious lesion by dissolution of tooth's crystalline structure [7]. As many plaque infections are not completely prone to synthetic chemicals agents or antibiotics, development of bacterial resistance is very susceptible. Furthermore, synthetic chemicals can alter oral microbiota and have undesirable effects [8]. Thus, the usage of natural

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medicinal plants extracts may be considered as potential alternative for effective suppression of dental plaque formation and biofilm causing oral pathogens [9]. Natural phytochemicals *viz*. tannins flavonoids, alkaloids, and essential oils isolated from medicinal plants used in traditional medicine are good alternatives to synthetic chemicals [10], exhibiting pronounced defensive and remedial activity [11]. It has been documented that that around two million traditional health practitioners have used more than 7500 medicinal plant species [12].

Lemon grass essential oil (LGEO) present in rampantly grown plant, *Cymbopogon citratus*, has been reported to possess remarkable antimicrobial and antibiofilm activity against the dental plaque organism [13]. The major component in the LGEO is citral and it is reported that the effect of citral is greater than that of whole LGEO [20]. The present study aims to explore the antimicrobial and antibiofilm activities of citral and its derivatives against the plaque forming dental flora isolated from healthy individuals. Total eight organisms representing genera *Streptococcus*, *Lactobacillus*, *Staphylococcus* and *Candida* were used as test organisms to study antimicrobial and antibiofilm activity.

EXPERIMENTAL

Citral was procured from Sigma-Aldrich and other chemicals were from SRL Chemicals, Fisher Scientific and Merck, India. Synthesis of citral semicarbazone

Isolation and identification of microflora associated with dental plaque: Dental plaque samples were collected in Pune, India with the help of local dental clinician. The visible plaque present at supragingival and subgingival was collected with the help of sterile probe/explorer in a sterile Eppendorf tubes containing 1 mL of sterile phosphate buffered saline (PBS). These were preserved in 6-10 ºC (ice packs) during transportation and were immediately processed at the laboratory.

Dental plaque samples were homogenized on a vortex mixer. Sample (100 µL) was then inoculated in the liquid enrichment media. The enriched broth/medium was homogenized by vortexing and loopful (10 µL) of sample was streaked on sterile mitis salivarius (MS) agar, sterile de Man, Rogosa and Sharpe agar (MRS), sterile mannitol salt agar, respectively. The plates were incubated under aerobic conditions at 37 ºC for 24-48 h. For MRS medium, plates were incubated under microaerophilic condition at 37 ºC for 24-48 h. After incubation colony characteristics were noted down. Saline suspension of the overnight (24 h) culture was prepared and Gram stained as per the Gram staining procedure. Hanging drop preparation of the above suspensions was observed to check motility of organisms.

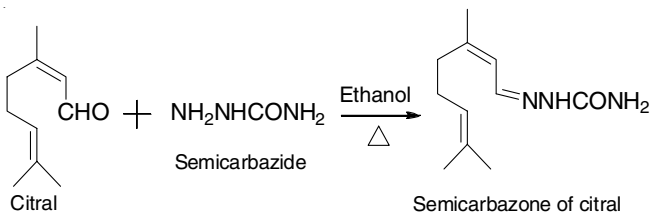
For molecular characterization of selected isolates for identification of organisms, the 16S rRNA gene was amplified from genomic DNA which was purified and sequenced [14]. Bacterial isolates were identified on the basis of 16S rRNA gene sequence homology with the reference sequences available in GenBank. A strain is considered to be a member of species when the observed sequence homology is > 98.2% [15]. From the sequence, three were identified as *Streptococcus agalactiae*, *Staphylococcus epidermidis* and *Lactobacillus fermentum*, which were found to have homology of 99, 99.93 and 99.77%, respectively. The gene sequence data for these three isolates has been deposited to GenBank under the accession number MH793435, MH793436 and MH793437, respectively.

Procurement of microflora associated with dental plaque and finalization of microorganism: In the early stages of biofilm formation various bacterial species take part. In this study, three bacterial species that were identified above, are primarily responsible for the biofilm formation. To explore the complete spectrum of organisms that result in the early biofilm formation, ultimately leading to dental plaque, the remaining most likely organisms were acquired. Accordingly, Microbial Type Culture Collection (MTCC) cultures were included in this study. They were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. The procured microbial cultures received in lyophilized form consisted of 5 microorganisms, of which *Candida albicans* (4748) was fungus while remaining were bacteria. Thus, total 8 organisms referred as test organisms were finalized in this study (Table-1). All cultures were grown and recovered in the various culture media as suggested by IMTECH. *Streptococcus mutans* (890), *Streptococcus oralis* (2696), *Lactobacillus acidophilus* (10307), *Lactobacillus rhamnosus* (1408) and *Candida albicans* (4748) were recovered in brain heart infusion medium, trypticase soy broth, de Man, Rogosa and Sharpe medium and yeast extract peptone dextrose, respectively. All cultures were maintained on their respective solid media.

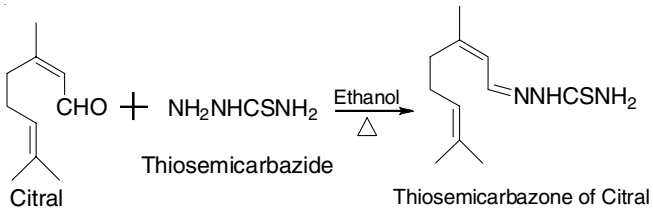
TABLE-1
ORGANISMS FINALIZED FOR THE STUDY

Name of microorganism	Source	GenBank accession number
Streptococcus mutans (890)	MTCC	Not applicable
Streptococcus oralis (2696)	MTCC	Not applicable
Lactobacillus acidophilus (10307)	MTCC	Not applicable
Lactobacillus rhamnosus (1408)	MTCC	Not applicable
Candida albicans (4748)	MTCC	Not applicable
Streptococcus agalactiae	Dental plaque isolate	MH793435
Staphylococcus epidermidis	Dental plaque isolate	MH793436
Lactobacillus fermentum	Dental plaque isolate	MH793437

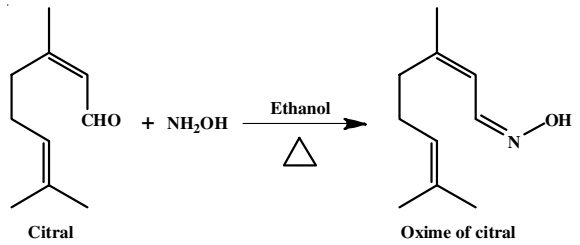
Synthesis of citral derivatives: Synthesis of citral semicarbazone was performed according to the procedure described elsewhere [16]. Citral was reacted with semicarbazide in a proper proportion in presence of ethyl alcohol. In brief, all chemicals were collected together in a round bottom flask fitted with water condenser and refluxed in water bath for 2 h. After every 30 min, TLC was carried out to check the formation of product. When entire citral was converted into derivative the reaction was stopped and product was collected by filtration, washed with ice cold alcohol and dried. Dried product was crystallized from absolute alcohol.



Synthesis of citral thiosemicarbazone: Citral was reacted with thiosemicarbazide in a proper proportion in presence of ethyl alcohol. The process was refluxed using water condenser equipped with guard tube for 2 h. After every 30 min, TLC was carried out to check the formation of the product. When entire citral was converted into thiosemicarbazone derivative, the reaction was stopped and product was collected. Dried product was crystallized from alcohol.



Synthesis of citral oxime: First hydroxyl amine·HCl was allowed to react with NaOH for few minutes. Then hydroxyl amine·HCl and NaOH reacted in presence of ethyl alcohol to neutralize the acid present in Hydroxyl amine·HCl. Then citral was added and reaction for formation of derivative was refluxed using water condenser equipped with guard tube for 2 h. After every 30 min, TLC was carried out to check the formation of product. When entire citral was converted into derivative the reaction was stopped and product was collected. Dried product was crystallized from alcohol.



Determination of antimicrobial activity: Antimicrobial activity of citral and its derivatives against selected isolates was determined by standard disc diffusion assay as per CLSI guidelines [17]. Test organisms were inoculated on respective media and incubated at 37 ºC for 24 h. Saline suspension of 24 h old culture was prepared as per 0.5 McFarland standards. Then 750 µL was mixed with 20 mL of pre-sterilized, cooled Mueller- Hinton agar butt and poured in sterile petri plate. The plates were allowed to solidify at room temperature. Sterile Whatman filter paper discs were soaked (10 µL) in citral or its derivatives and placed on agar surface. All dilutions were carried out using DMSO, which acted as a negative control. Commercially available chlorhexidine gluconate was used as a positive control. Plates were kept at 4 ºC for 30 min for pre-diffusion and later on incubated at 37 ºC for 24 h. All exposures were carried out in triplicates and average value was considered. Diameter of zone of inhibition in mm was measured with the help of HI-MEDIA antibiotic zone measuring scale. Minimum inhibitory concentration (MIC) was also determined.

Determination of growth of biofilm and antibiofilm activity: The quantitative growth of biofilm was determined as per Protocols to study the physiology of oral biofilms described earlier by Lemos *et al*. [18]. The determination is based on the principle that the biofilm which is produced by the organism binds to the crystal violet and the bound crystal violet is later eluted which has the absorbance in proportion to the amount of biofilm.

Test organisms were inoculated on respective media and incubated at 37 ºC for 24 h. Saline suspension of 24 h old culture was prepared as per 0.5 McFarland standards. Biofilm medium containing 1 M glucose (source of carbohydrate) was prepared. Each 0.5 McFarland standards culture (20 µL) was dispensed into each well having 180 µL of biofilm medium. Wells containing 200 µL uninoculated biofilm medium served as negative controls. Wells with 180 µL of medium and 20 µL of chlorhexidine was positive control. Each experiment was conducted in triplicate. Plates were sealed with the help of adhesive microtiter plate sealer and incubated for 24 h at 37ºC without agitation. After the incubation, plates were further processed. The plates were blotted on a paper towel to removed culture media. To remove loosely bound cells, microtiter plates were carefully immersed in a large dish with distilled water. Again plates were blotted on a paper towel. This step was repeated twice. A 0.1% crystal violet (50 µL) was added to the test wells, including the negative control wells. Plates were then incubated at room temperature for 15 min. The washing was repeated. The plates were air dried. Acetic acid solution (33%, 200 µL) was added to the wells to elute the crystal violet, which was bound to the biofilm formed in the wells. Plates were incubated at room temperature for 10 min. Entire content of each well were transferred by multichannel micropipette in the respective wells in a new blank microtiter plate. Measured the absorbance at 570 nm using the Thermo Lab systems ELISA reader Model No. 352. In antibiofilm studies, 160 µL of medium was exposed to 20 µL of culture and 20 µL of inhibitory factor of required strength. All other steps in the protocol remained same as described. The ability of citral and its derivatives to inhibit the formation of biofilm was determined as antibiofilm activity of citral/citral semicarbazone/citral thiosemicarbazone/citral oxime.

RESULTS AND DISCUSSION

In an attempt to search compounds exhibiting higher antimicrobial activity than citral, it was decided to prepare Schiff's bases of citral, as the Schiff's bases usually exhibit remarkable biological activity. Three Schiff bases (citral semicarbazone, citral thiosemicarbazone and citral oxime) were synthesized from citral in laboratory by appropriate chemical reactions under required conditions.

The synthesized citral semicarbazone/thiosemicarbazone/oxime was confirmed by ultraviolet spectrophotometric examination by scanning between the wavelength 200 nm to 800 nm and generating UV spectrum of citral semicarbazone. The UV spectra of citral semicarbazone/thiosemicarbazone/oxime are displayed in Fig. 1, which thereby confirmed the formation of citral derivatives.

Antimicrobial activity of citral: A undiluted citral considered as 100% (v/v) was diluted with DMSO and dilutions up to 0.78% were made by serial dilutions. The antimicrobial activity at each concentration of citral against the 8 test organisms was determined in terms of mean zone of inhibition and is shown in Table-2.

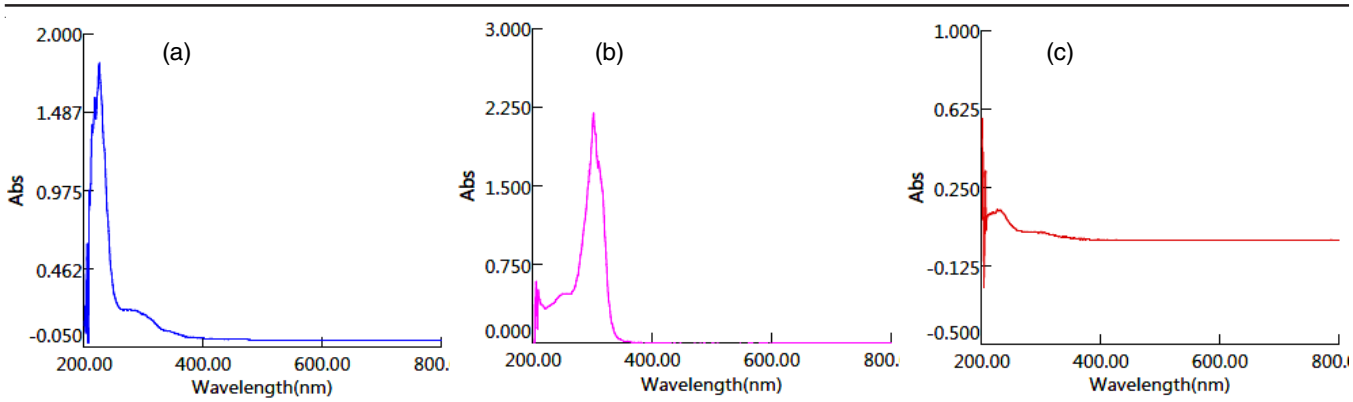


Fig. 1. UV spectrum of citral semicarbazone (a), citral thiosemicarbazone (b), and citral oxime (c)

TABLE-2
ANTIMICROBIAL ACTIVITY OF CITRAL AGAINST TEST ORGANISMS

Test organisms	Zone of inhibition (mm)								
	Chx	Concentration of citral (%)							DMSO
		100	50	25	12.5	6.2	3.1	1.5	0.78	
S. mutans	30	16.33	14.6	13.0	11.0	9.6	0	0	0	0
S. oralis	32	15.33	14.3	12.6	11.0	10.0	0	0	0	0
L. acidophilus	36	16.00	15.3	14.0	12.3	10.3	9	0	0	0
L. rhamnosus	35	20.30	18.3	17.3	14.3	13.0	12.0	0	0	0
C. albicans	34	22.60	19.6	18.0	16.6	14.6	12.3	0	0	0
S. agalactiae	30	18.00	13.6	11.6	11.3	9.6	0	0	0	0
S. epidermidis	29	23.60	21.6	19.3	16.6	13.6	11.6	0	0	0
L. fermentum	30	16.33	14.6	13.0	110	9.66	0	0	0	0

A minimum of 6.2% of citral was found to inhibit all the eight test organisms. However, *S. epidermidis*, *C. albicans*, *L. rhamnosus* and *L. acidophilus* were found to be inhibited by 3.1% of citral. The MIC of citral was found between 3.1% to 6.2% for all the test organisms. The commercially available chlorhexidine was taken as a positive control for comparison and DMSO in which all the dilutions of citral and its derivatives are made was taken as negative control. The inhibitory action by undiluted citral was found to be around 51% of inhibitory action by undiluted chlorhexidine.

Antimicrobial activity of citral derivatives: Out of the three derivatives of citral, only citral semicarbazone demonstrated the antimicrobial activity and therefore the further research was narrowed down to citral semicarbazone. Therefore, various concentrations of citral semicarbazone were subjected to the same set of experiments against the eight test organism to determine its antimicrobial activity, so that it can be compared with that of citral, to know whether citral semicarbazone can be a better antimicrobial agent than citral against the dental oral flora. Mean zone of inhibition at different concentrations of citral semicarbazone are shown in Table-3.

A minimum of 100 mg/mL citral semicarbazone was found to inhibit all the eight test organisms. However, test organisms *C. albicans* and *S. epidermidis* were found to be inhibited at lower concentration of 50 mg/mL (Table-3). The antimicrobial activity of citral and citral derivatives against the test organism, was compared with the antimicrobial activity of LGEO determined against the same eight test organisms in our previous studies [13]. The LGEO antimicrobial activity against dental flora was found better than the citral. This is in total disagreement with the results of Silva *et al*. [19], who reported that the antifungal activity presented by lemongrass oil and citral were similar [19], and also with the studies of Adukwu *et al*. [20] who explained the results on the basis of MIC and MBC that the effect of citral is greater than that of whole LGEO. However, there were recent reports on inhibitory

TABLE-3
ANTIMICROBIAL ACTIVITY OF CITRAL SEMICARBAZONE AGAINST TEST ORGANISMS

Test organisms	Zone of inhibition (mm)							
	Chx	Concentration of citral semicarbazone (% w/v)						DMSO
		40	20	10	5	2.5	1.25	
S. mutans	30	14.66	14.33	12.33	0	0	0	0
S. oralis	32	15.33	13.66	11.66	0	0	0	0
L. acidophilus	36	11.33	10.66	10.33	0	0	0	0
L. rhamnosus	35	12.50	10.33	10.00	0	0	0	0
C. albicans	34	13.33	12.00	10.66	9.66	0	0	0
S. agalactiae	30	15.66	14.66	12.33	0	0	0	0
S. epidermidis	29	17.00	15.33	14.00	10.33	0	0	0
L. fermentum	30	14.66	14.33	12.33	0	0	0	0

and bactericidal concentrations of citral and were reported to be lower than that of LGEO against the isolates of *S. aureus*, [20,21]. The differences in present results of the antimicrobial capacity of LGEO & citral, may be due to the differences in the *Cymbopogon* species and also due to differences in the micro-organisms against, which antimicrobial capacity was tested.

In present study, all the three Schiff's bases of citral did not exhibited the desired antimicrobial activity as reported. Jin *et al*. [22] developed Schiff's base from chitosan and citral reported its antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Aspergillus niger*. Narasimhan *et al*., [16] also reported that the Schiff bases of citral synthesized by reacting citral with amino acid methyl ester hydrochloride possess increased activity than the parent citral molecule and suggested that the Schiff's bases derivatives of citral may come across application in various antimicrobial treatments [16]. The differences in the antimicrobial activity results of citral derivatives in present study may be due to different starting molecules.

The minimum inhibitory concentration (MIC) of citral and citral semicarbazone against eight test organisms was compared with LGEO, which was determined on the same eight test organism (Table-4). LGEO at 1.5% v/v was sufficient to inhibit all the eight test organisms, while a minimum of 3.1% v/v citral for *L. acidophilus*, *L. rhamnosus*, *C. albicans*, *S. epidermidis* and 6.2% v/v citral for *S. mutans*, *S. oralis*, *S. agalactiae* and *L. fermentum* was found to inhibit these organisms (Table-4).

TABLE-4
COMPARISON OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF LGEO, CITRAL AND CITRAL SEMICARBAZONE AGAINST THE 8 TEST ORGANISMS

Test organisms	MIC		
	LGEO (% v/v)	Citral (% v/v)	Citral semi-carbazone (% w/v)
S. mutans	1.5	6.2	10
S. oralis	1.5	6.2	10
L. acidophilus	1.5	3.1	10
L. rhamnosus	1.5	3.1	10
C. albicans	1.5	3.1	5
S. agalactiae	1.5	6.2	10
S. epidermidis	1.5	3.1	5
L. fermentum	1.5	6.2	10

Antibiofilm of citral: The biofilm determination is based on the principle that biofilm produced by the organisms binds to the crystal violet and the bound crystal violet is later eluted which has the absorbance in proportion to the amount of biofilm. Thus higher the absorbance at 570 nm indicates more biofilm. The decrease in absorbance at 570 nm in presence of citral indicated the biofilm inhibitory activity (Table-5), which clearly indicated that higher the concentration of citral, lower is the absorbance indicating higher inhibition of biofilm formation.

In order to explain this fact conveniently, the same results of antibiofilm activity were represented in the form of percentage inhibition as suggested by Jadhav *et al*. [23] using the following eqn.:

$$\text{Biofilm inhibition (\%)} = \frac{\text{Abs without citral} - \text{Abs in presence of citral}}{\text{Abs without citral}} \times 100 \quad (307)$$

The percentage inhibition of biofilm formation by each of the test organism, at the different concentration of citral was calculated (Table-6) from which minimum biofilm inhibition concentration ($MBIC_{50}$) of citral was determined. The $MBIC_{50}$ of citral could be determined only for *L. rhamnosus* and *C. albicans* as 50 and 100%, respectively (Table-6).

All these studies indicated that 50% citral inhibits 50% of the biofilm, which was formed without any antibiofilm agent against *L. rhamnosus*. Further higher concentration was found required for biofilm of *C. albicans*. The other test organisms were inhibited less than 50% and hence could not be quantitated in terms of $MBIC_{50}$.

Antibiofilm of citral semicarbazone: The antibiofilm activity at various concentrations of citral semicarbazone from 0.31 to 20 % w/v was determined (Table-7). These experiments indicated that citral semicarbazone not only has antimicrobial activity but also antibiofilm activity. The percentage inhibition of biofilm formation by each of the test organism, at different concentrations of citral semicarbazone was also calculated (Table-8).

After the comprehensive study of the antibiofilm activity of citral and citral derivatives against the test organism, it was compared with that of LGEO determined in previous study [13]. LGEO was found to exhibit better biofilm inhibitory activity against the eight test organism which represents the dental microflora as compared to the major component citral and its Schiff's derivative citral semicarbazone [13]. This better antimicrobial and antibiofilm activity of LGEO might be due to other components like limonene, citronellal and limonene oxide probably acting synergistically in the overall activity of LGEO.

TABLE-5
ANTIBIOFILM ACTIVITY AT VARIOUS CONCENTRATIONS OF CITRAL AGAINST TEST ORGANISMS

Test organisms	Mean absorbance at 570 nm as index of biofilm formation							
	Concentrations of citral (% v/v)							Chx
	1.50	3.10	6.25	12.50	25	50	100	
S. mutans	0.235	0.231	0.229	0.225	0.217	0.202	0.168	0.1
S. oralis	0.225	0.223	0.219	0.214	0.206	0.195	0.165	0.09
L. acidophilus	0.233	0.23	0.226	0.219	0.215	0.216	0.21	0.1
L. rhamnosus	0.231	0.228	0.221	0.216	0.21	0.201	0.196	0.1
C. albicans	0.222	0.219	0.212	0.206	0.197	0.188	0.153	0.1
S. agalactiae	0.236	0.232	0.228	0.221	0.209	0.193	0.183	0.1
S. epidermidis	0.222	0.219	0.214	0.202	0.189	0.177	0.144	0.09
L. fermentum	0.233	0.229	0.221	0.217	0.214	0.195	0.175	0.1

TABLE-6
PERCENTAGE INHIBITION OF BIOFILM AT VARIOUS CONCENTRATIONS OF CITRAL AGAINST THE TEST ORGANISMS

Test organisms	Percentage inhibition of biofilm						
	Concentrations of citral (% v/v)						
	1.50	3.10	6.25	12.50	25	50	100
S. mutans	22.44	23.76	24.42	25.74	28.38	33.33	44.55
S. oralis	12.10	12.89	14.45	16.40	19.53	23.82	35.54
L. acidophilus	20.20	21.23	22.60	25.00	26.36	28.08	33.21
L. rhamnosus	41.06	43.79	45.48	47.69	49.15	51.33	52.54
C. albicans	35.08	35.96	38.01	39.76	42.39	45.02	55.26
S. agalactiae	8.88	10.42	11.96	14.67	19.30	25.48	29.34
S. epidermidis	4.72	6.00	8.15	13.30	18.80	24.03	38.19
L. fermentum	24.10	25.40	28.01	29.31	30.29	36.48	42.99

TABLE-7
BIOFILM FORMATION INHIBITION ACTIVITY OF CITRAL SEMICARBAZONE AGAINST TEST ORGANISMS

Test organisms	Mean absorbance at 570 nm as index of biofilm formation							
	Concentrations of citral semicarbazone (% w/v)							Chx
	0.31	0.62	1.25	2.5	5.0	10.0	20.0	
S. mutans	0.222	0.214	0.211	0.203	0.197	0.184	0.176	0.10
S. oralis	0.227	0.223	0.222	0.219	0.212	0.191	0.171	0.09
L. acidophilus	0.263	0.243	0.220	0.215	0.212	0.207	0.194	0.10
L. rhamnosus	0.260	0.229	0.218	0.211	0.206	0.193	0.181	0.10
C. albicans	0.218	0.209	0.202	0.196	0.188	0.177	0.162	0.10
S. agalactiae	0.246	0.231	0.224	0.221	0.215	0.196	0.189	0.10
S. epidermidis	0.204	0.194	0.194	0.185	0.176	0.167	0.153	0.09
L. fermentum	0.229	0.222	0.219	0.214	0.202	0.192	0.184	0.10

TABLE-8
PERCENTAGE INHIBITION OF BIOFILM AT VARIOUS CONCENTRATIONS OF CITRAL SEMICARBAZONE AGAINST THE TEST ORGANISMS

Test organisms	Percentage inhibition of biofilm						
	Concentrations of citral semicarbazone (% w/v)						
	0.31	0.62	1.25	2.5	5.0	10.0	20.0
S. mutans	26.73	29.37	30.36	33.00	34.98	39.27	41.91
S. oralis	11.32	12.893	13.28	14.45	17.18	25.39	33.20
L. acidophilus	9.93	16.78	24.65	26.36	27.39	29.10	33.56
L. rhamnosus	37.04	44.55	47.21	48.91	50.12	53.26	56.17
C. albicans	36.25	38.88	40.93	42.69	44.93	48.24	52.63
S. agalactiae	5.01	10.81	13.51	14.67	16.98	24.32	27.02
S. epidermidis	12.18	16.73	16.73	20.60	24.46	28.32	34.33
L. fermentum	25.40	27.68	28.66	30.29	34.20	37.45	40.06

The antimicrobial and antibiofilm activity of undiluted citral was compared with the commercially available undiluted chlorhexidine against the test organisms. Citral was found to have antimicrobial activity lower by mean ± S.D. of 41.62 ± 11.54% as compared to undiluted chlorhexidine. This means that undiluted citral has around 58% capability to exhibit antimicrobial activity as compared to undiluted chlorhexidine against eight test organism. When the antibiofilm activity of both was correlated, then citral was found to have antibiofilm activity lower by mean ± S.D. of 78.54 ± 18.74%.

Citral has been reported to have inhibitory effects on both mycelial and yeast-form growth of *C. albicans* [24]. It is has been established that citral the major components of LGEO, alter cell permeability by penetrating between the fatty acids chains that make the membrane lipid bilayers, disrupting lipid packing and changing membrane fluidity [25,26]. These phenomena [26] might lead to major surface alterations and morphological modifications finally reducing the adherence capacity. The inhibitory effects of citral and its derivative on test organisms and on biofilm formation could be due to the above effects of citral.

In this study, citral showed antimicrobial and biofilm formation inhibition activity at MIC and MBIC50 of 3.1 to 6.2% and 50-100% (v/v), respectively. In previous study [13], we have determined antimicrobial and antibiofilm activity of LGEO which is at much lower concentrations than citral, clearly indicating that LGEO exhibits better inhibitory activity than citral. This suggests that although the major contribution to antimicrobial and biofilm formation inhibition activity in LGEO is by citral, but the other components like limonene, citronellal, and limonene oxide are probably acting synergistically in the overall activity of LGEO [19]. It is well known that herbal extracts show holistic effect and individual components may not show the activity which is exhibited by all its components together [27].

Conclusion

The study demonstrated that the citral, which is the major component of lemon grass essential oil (LGEO) has antimicrobial and antibiofilm activity against the dental plaque organism. Of the three Schiff's bases as derivatives of citral, none of the derivative was found to have better antimicrobial and antibiofilm activity than citral. Only citral semicarbazone demonstrated the antimicrobial and antibiofilm activity, which concluded that no apparent advantage of employing citral or its derivatives as antimicrobial and antibiofilm agent as compared to LGEO. In this study, citral or its derivatives could not be demonstrated to replace LGEO as more effective, powerful and better herbal material to control the oral microflora associated with dental plaque.

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REFERENCES

1. R. Salam, B.K.,Sarker, M.R. Haq and J.U. Khokon, *Int. J. Nat. Social Sci.*, **1**, 1 (2015).
2. R. Saini, S. Saini and S. Sharma, *J. Nat. Sci. Biol. Med.*, **2**, 71 (2011); https://doi.org/10.4103/0976-9668.82317
3. P. Suntharalingam and D.G. Cvitkovitch, *Trends Microbiol.*, **13**, 3 (2005); https://doi.org/10.1016/j.tim.2004.11.009
4. J. Costerton, P.S. Stewart and E. Greenberg, *Science*, **284**, 1318 (1999); https://doi.org/10.1126/science.284.5418.1318
5. R.M. Donlan, *Emerg. Infect. Dis.*, **7**, 277 (2001); https://doi.org/10.3201/eid0702.010226
6. R.M. Donlan and J.W. Costerton, *Clin. Microbiol. Rev.*, **15**, 167 (2002); https://doi.org/10.1128/CMR.15.2.167-193.2002
7. P.D. Marsh, *BMC Oral Health*, **6(Suppl 1)**, S14 (2006); https://doi.org/10.1186/1472-6831-6-S1-S14
8. R. Yadav and D. Yadav, *Asian J. Pharm. Clin. Res.*, **6**, 16 (2013).
9. R. Malhotra, A. Kapoor, V. Grover and D. Saxena, *J. Indian Soc. Periodontol.*, **15**, 349 (2011); https://doi.org/10.4103/0972-124X.92567
10. My M. Ismail, *Res. J. Pharm. Biol. Chem. Sci.*, **1**, 202 (2010).
11. A. Bhardwaj and S.V. Bhardwaj, *J. Intercult. Ethnopharmacol.*, **1**, 62 (2012); https://doi.org/10.5455/jice.20120322035152
12. P. Kanwar, N. Sharma and A. Rekha, *Indian J. Tradit. Knowl.*, **5**, 300 (2006).
13. S.V. Ambade and N.M. Deshpande, *European J. Med. Plants*, **28**, 1 (2019); https://doi.org/10.9734/ejmp/2019/v28i430143
14. V.J. Pidiyar, M.S. Patole, K. Jangid and Y.S. Shouche, *Am. J. Trop. Med. Hyg.*, **70**, 597 (2004); https://doi.org/10.4269/ajtmh.2004.70.597
15. J.M. Janda and S.L. Abbott, *J. Clin. Microbiol.*, **45**, 2761 (2007); https://doi.org/10.1128/JCM.01228-07
16. S. Narasimhan, S. Ravi and B. Vijayakumar, *Int. J. Chem. Appl.*, **3**, 229 (2011).
17. Clinical and Laboratory Standards Institute, *Twenty-Second Informational Supplement.*, **32**, M100 (2012).
18. J.A. Lemos, J. Abranches, H. Koo, R.E. Marquis and R.A. Burne, *Methods Mol. Biol.*, **666**, 87 (2010); https://doi.org/10.1007/978-1-60761-820-1_7
19. C.B. de Silva, S.S. Guterres, V. Weisheimer and E.E. Schapoval, *Braz. J. Infect. Dis.*, **12**, 63 (2008).
20. E.C. Adukwu, M. Bowles, V. Edwards-Jones and H. Bone, *Appl. Microbiol. Biotechnol.*, **100**, 9619 (2016); https://doi.org/10.1007/s00253-016-7807-y
21. E.C. Adukwu, S.C. Allen and C.A. Phillips, *J. Appl. Microbiol.*, **113**, 1217 (2012); https://doi.org/10.1111/j.1365-2672.2012.05418.x
22. J. Xiaoxiao, W. Jiangtao and B. Jie, *Carbohydr. Res.*, **344**, 825 (2008).
23. S. Jadhav, R.M. Shah, M. Bhave and E. Palombo, *Food Control*, **29**, 125 (2013); https://doi.org/10.1016/j.foodcont.2012.05.071
24. S. Abe, Y. Sato, S. Inoue, H. Ishibashi, N. Maruyama, T. Takizawa, H. Oshima and H. Yamaguchi, *Nippon Ishinkin Gakkai Zasshi*, **44**, 285 (2003); https://doi.org/10.3314/jjmm.44.285
25. M. Bard, M.R. Albrecht, N. Gupta, C.J. Guynn and W. Stillwell, *Lipids*, **23**, 534 (1988); https://doi.org/10.1007/BF02535593
26. P.C. Braga and M. Dal Sasso, *J. Chemother.*, **17(supplement 3)**, 109 (2005).
27. P. Rasoanaivo, C.W. Wright, M.L. Willcox and B. Gilbert, *Malar. J.*, **10(S4)**, S4 (2011); https://doi.org/10.1186/1475-2875-10-S1-S4