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Antimicrobial role of Lavandula angustifolia towards Candida albicans, Streptococcus mutans, Staphylococcus aureus and anti-adherence effect on denture base resin

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ABSTRACT

Objectives: The objective of the study was to determine the antimicrobial efficacy of Lavandula angustifolia (True Lavender extract) towards Candida albicans, Streptococcus mutans, Staphylococcus aureus pathogens. Varying proportions of the extract was incorporated into polymethyl methacrylate (PMMA) denture base resin and the anti-adherent effect was investigated.

Methods: An in-vitro study was performed after pure extract was obtained from Lavandula angustifolia (LA) flowers using a solvent based hot extraction process. Chromatographic analysis and computational molecular docking were done to analyze its phytoconstituents with potential target-ligand bond. Tests of antimicrobial susceptibility, minimum inhibitory concentration, minimum bactericidal and fungicidal dosages and in-vitro cytotoxicity were performed. Different proportions of LA extract (0, .5, 1.0, 1.5, and 2.0 %) were added to PMMA resin to assess anti-adherence property. Obtained data were statistically analyzed with One-way ANOVA followed by Tukey post-hoc tests.

Results: The reports revealed significant antimicrobial susceptibility against the test pathogens compared to control drugs ($P > 0.05$). A minimum concentration of .02 mg of lavender extract inhibited microbial growth with low cytotoxicity ($P < 0.05$). The highest anti-adherent activity was observed in the .5 % LA-incorporated PMMA resin group ($P = 0.0001$).

Conclusions: Incorporating lavender extract into denture base resin demonstrated promising antimicrobial properties. This investigation encourages further research to understand its effects on mechanical and physical properties of reinforced dental resins.

1. Introduction

Continuous prevalence of denture stomatitis (DS) influenced researchers to analysis different strategies to fight against the infection. Regular practice of physical brushing,[1] soaking of dentures in disinfectant solutions,[2] use of denture cleansing tablets,[3] microwave sterilization of infected prosthesis,[4] intake of antifungal, antibacterial drugs as tablets,[5] topical powders,[6] lozenges,[7] pastilles,[8] denture creams,[9] antimicrobial oral rinses,[10] lasers[11] and photodynamic therapy[12] were undertaken. Furthermore, control of plaque formation was tried with addition of inorganic chemicals namely oxides of silver, titanium, magnesium and zinc.[13,14] Antifungals like nystatin,[8] amphotericin,[7] and azole group of drugs was added with tissue conditioners to combat the infection.[6] Intake of synthetic medications posed threat of side-effects, altered taste, host-drug reaction, allergy, and ineffective in resistant microbes. This paved way to develop alternative organic antimicrobials.[15]

In recent years, there is an upsurge in use of phytomedicines with dental polymers to fight against the denture infections.[16] Every part of plant from stem, bark, leaves, seeds, fruits, and flowers are shown to have antimicrobial effect. Extracts in the form of powders, pastes, oils, gels, and disinfectant solutions are tried. Benefits from these herbal medicines is attributed to unique phytoconstituents that have shown the ability to inhibit or kill the pathogens.[17] Advantages of native availability, easy preparation and usage, negligible side effects, less cost, and drug interaction has increased its application in present-day research.

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Shui et al., in his meta-analysis, inferred that many studies proved phytomedicines to be better than antifungal drugs with fewer toxicity and more patient comfort.[17–19] Droplet of Zataria multiflora was comparable to effects produced by nystatin in DS management.[20] Chitosan and curcumin were tried as mouthrinse solution by Mustafa et al.[21] Azizi Z et al. evidenced ginger and nystatin mouthwash to show similar effect.[22] Tay et al. demonstrated use of Uncaria tomentosa gel as a supplement to DS treatment.[23] Antifungal effect by Ricinus communis was observed by Pinelli et al.[24] One % hortensis oil as gel formulation, Pelargonium gel, Artemisia mouthwash, topical Punica granatum, powders of henna, curcumin, cinnamon, neem extracts and tea tree oil are few other phytomedicines experimented till date.[25,26]

An increasing growth in the field of medicinal and aromatic plants (MAPs) is recognized owing to the many health benefits observed. Amongst the many aromatic herbs

available, lavender flower, scientifically called as Lavandula angustifolia (LA) is one of the most valuable and used across many countries including India, France and Spain where it grows natively. Its application was popularized due to its pleasant, soothing fragrance, that served as an antianxiety agent.[27] It was used in dentistry as mouth rinse ingredient, agent to reduce inflammation and pain.[28,29] Increased levels of antioxidants and enriched bioactive compounds in LA drew keen interest in its testing. Till date, negligible studies are performed to experiment the efficacy of lavender in the field of prosthetic dentistry to limit denture stomatitis.

This in-vitro study determined the antimicrobial role of Lavandula angustifolia (Lavender) extract towards three common denture stomatitis causative pathogens such as Candida albicans, Streptococcus mutans and Staphylococcus aureus and its anti-adherent effect on dental PMMA. Null hypothesis of the study stated, there would be no antimicrobial effect of Lavender on PMMA resin.

2. Materials and methods

This in-vitro study was commenced after approval from the Institutional Review Board (xxxxx/IRB/2018/PhD/No.101). Different proportions of Lavender extract as 0 % control, .5 %, 1.0 %, 1.5 % and 2.0 % was incorporated into heat-activated PMMA polymer (DPI Heat cure, Dental products of India, Mumbai, India). Totally two hundred and twenty-five specimens were prepared, based on standardized guidelines ($n = 10$). The sample size was calculated with an α error/probability set at $\leq .05$ using G*Power version 3.1.9.3 software (University of Kent). Until the experimentation process, the finished specimen was stored in 37 °C distilled water for 48 h, while the LA extract was preserved in an airtight vacuum desiccator at room temperature.

2.1. Preparation of lavender extract

2.1.1. Plant sample collection & initial processing

One hundred grams of organic freshly picked flowers of Lavandula angustifolia (Lavender or true lavender) that was cultivated natively in Kashmir, India was purchased (Urban Platter dried lavender buds, lot number 701098214705, Mumbai, India). It was dried at a stable temperature of 23 °C in a dark room. It was then made into a fine dry powder using domestic processor (Nutri-Blend Wonderchef mixer, grinder, blender, H.P, India) at 22000 rpm for 3 min giving a break of 2–3 min after every 60 s to avoid heating of motor. Grinding was pulsed in small volumes instead of bulk, to obtain even powder. A fine mesh sieve (.60–0.85 mm) was used to remove large unground particles. To additionally, reduce the powder particle size and to get a homogenized material, it was subjected to planetary ball milling (PM 100 CM, Retsch GmbH, India) at 300 rpm for 30 min to achieve initial 62 gm of raw powder extract. This was reserved in a vacuum desiccator until further use.[30]

2.1.2. Reagents used

Analytical grade reagents and chemicals was used (HiMedia Co.Ltd, India). Solvents such as ethanol (Sigma, #32221) and methanol (Sigma, #34966) were purchased from Sigma-Aldrich for extraction process.[29]

2.1.3. Extraction method

Initial yield of lavender powder was weighed in a cellulose thimble and was subjected to hot extraction process using Soxhlet apparatus (Singhla Scientific Industries, India) at 80 ° C for 48 h with a condenser. Ethanol and methanol extract was condensed in a rotary evaporator and pooled extract was reserved at 4 °C. Obtained extract was strained in a Whatman No.1 filter paper to remove unsolvable particles. Refined filtrate was treated to lyophilization process at −45 °C for 3 days ((TSI, freeze dryers, India) to get final powder. Glass vials dried at 80 ° C for 12 h was used to measure dry residue of extract. Analytical weighing balance (Mettler Toledo, India) was used for estimation of final yield of lavender powder. A final yield of 8.23 gm in powder form was received from 100 gm of raw lavender flower buds. It was stored in vacuum desiccator until further use to avoid ingress of moisture and contamination.[31]

2.1.4. Phytoconstituent analysis

High performance liquid chromatography – mass spectrometry (LC-MS) analytical method was applied in this study to segregate, detect, qualify and quantify the phytochemicals in complex mixture of lavender extract (410 Prostar Binary LC with 500 MS IT PDA detectors, IIT Mumbai, India). From the chromatograph, each compound eluted, to enter the mass spectrometer, that ionized the molecules and was measured as mass-to-charge ratios (m/z). Mass spectrometer generated a spectrum for each compound, showing the m/z ratios, retention times and their respective intensities. The obtained spectra were analyzed with advanced mass spectral database (m/z Cloud™, Slovakia, UK) and chemical structure, molecular formula was curated with databased owned by the Royal Society of Chemistry (ChemSpider, US).[32]

2.1.5. Fabrication of study samples

Lavender extract was incorporated into the heat-cure polymer of PMMA denture base resin at varying proportions (dry wt %/wt %). A digital weighing balance (Mettler Toledo, India) was used for the measurement of material. To affirm uniform homogenization of both the extract and the polymer powder, it was subjected to planetary ball milling for 30 min at 300 rpm (PM 100 CM, Retsch GmbH, India). The reinforced material was then utilized for final processing of the specimen with "lost-wax process." In this study, three dimensionally (3D) printable wax-based resin (Siraya Tech 3D, USA) was used for pattern preparation. Standard tessellation language (STL) format of specimen was created with computer aided software (Autodesk 123D Design, USA). Digital light processing (DLP) 3D printer (Anycubic Photon Ultra DLP, USA) was used for pattern printing at a 0-degree direction, thickness of 30 μm and each layer were cured at 6.4 s. On completion of 3D printing, the patterns were cleaned in an ultrasonic bath containing isopropanol. Following which the patterns were post-polymerized in a UV light curing unit for 30 min (Delta Blue Lux light curing unit, China). Fabricated patterns were then invested, de-waxed, packed and heat cured by long polymerization cycle. Processed samples were finished with acrylic trimmers, sandpapered with different grits of 100, 150, 220, wet grinding on emery sheet of 600,1000, 1500 grit size, 2000 grit and dry polishing with diamond polishing paste (Chennai Metco, 3–4 Mic diamond paste) was done on a twin disc grinder/polisher at 600 rpm for 1 min (Metco Bainpol VTD, India). A digital vernier caliper of 0.01 mm accuracy was used to measure the size of the samples. Final prepared samples were stored in distilled water at 37 °C for 48 ± 2 h prior to testing.[33]

2.2. Antimicrobial tests

2.2.1. Determination of antimicrobial susceptibility

Agar well diffusion method was employed for evaluating the antimicrobial activity of Lavender extract. Three test microorganisms were deployed in this study such as strains of Streptococcus mutans ATCC 25175 (S. mutans), Candida albicans ATCC 14053 (C. albicans) and Staphylococcus aureus ATCC 29213 (S. aureus). Fresh culture of the test organisms was retrieved in sterile Brain Heart Infusion broth for Streptococcus mutans and Staphylococcus aureus. Sabouraud dextrose broth was used for Candida albicans. The turbidity was adjusted to .5 McFarland standards. Lawn cultures of the broth suspensions were made on to the surface of the sterile Muller Hinton agar (MHA) for S. aureus, Mutans sanguis agar (MSA) for S.mutans and Sabouraud Dextrose agar (SDA) for C.albicans. Wells were cut with sterile agar cutter and 50 µL of the Lavender extract was added into the appropriate wells. The plates were incubated at 37 °C for 24 h. After incubation, the zone of clearance was observed, and the zone of diameter was recorded (mm). In this test, drugs ampicillin 10 µg for S.mutans, vancomycin 30 µg for S.aureus and nystatin 100 µg were used a positive control agents.[34]

2.2.2. Determination of minimum inhibitory concentration (MIC) value

Lavender extract was diluted into varying concentrations from 10^{-1} to 10^{-6} 200 mg/mL to .002 mg/mL (w/v) in a microtiter well plate. The 3 test microorganisms were adjusted to .5 McFarland standards and was added to each of the appropriately diluted wells with Brain Heart Infusion broth for S. mutans and S. aureus and Sabourauds dextrose broth for C. albicans. The plates were concealed and incubated at 37 °C for 24 h. The growth in the form of turbidity was measured in ELISA plate reader at 630 nm and the percentage of inhibition of the growth was recorded with standard formula (growth in percentage = sample OD value/Control OD value) x 100. The inhibition percentage more than 50 % was considered as the MIC value of the LA extract.[34]

2.2.3. Determination of minimum bactericidal and fungicidal concentration (MBC & MFC) value

The MBC and MFC value indicate the lowest concentration of LA extract that killed 99.9 % of bacteria and fungi. It was determined by subculturing broth dilutions that inhibited the growth of bacteria and fungus, at a value above the MIC. The broth dilution was streaked onto MHA, MSA and SDA, followed by incubation for 24–48 h.[34]

2.2.4. In-vitro cytotoxic assay

The cytotoxicity of the LA extract was tested using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT assay). The extract material was dissolved in dilute dimethyl sulfoxide (DMSO). It was further diluted in water to prepare 100 µg/mL concentration. Serial dilutions were made using water and used for MTT testing. Vero cells (Vero 76) authenticated cell lines obtained from National Centre for Cell Science (NCCS, Pune, India) Pune were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % foetal bovine serum and 1 % antibiotic antimycotic (Thermofisher scientific). Vero cells were enumerated and seeded into the flask at approximately 1 x 10e4 cells/well (100 µL culture medium) in 96 well plates and incubated for 72 h at 37 °C. After incubation, the old media was aspirated, 100 µl of growth media was added to all wells and incubated for a further 24 h. The MTT reagent was reconstituted with phosphate buffered saline (PBS) at a pH 7.4 and prepared a stock solution of 5 mg/mL. From the stock, 100 µL of MTT solution was added to each well (10 mL working solution per plate) to achieve .5 mg/mL. The plates were further incubated for 4 h at 37 °C and 5 % CO2. Carefully MTT solution was aspirated from each well and formazan precipitate was dissolved by addition of 100 µL solubilization buffer (DMSO). The absorbance was read at 570 nm in microplate ELIZA reader (Multiskan™ FC Microplate Photometer, Thermo Fisher Scientific, USA) which was corrected for background using blank wells.[34,35]

2.2.5. Anti-adherent activity

Fifty discs shaped (15 mm diameter, 3 mm thickness) specimens as per the different LA extract proportions were tested for biofilm formation. Prior to testing, the discs were ultrasonically cleaned (Codyson Ultrasonic cleaner CD 4820, India) for 20 min in distilled water. Discs were then UV sterilized for 20 min in cabinet (Roson UV dental sterilizer cabinet, China). Test discs were immersed in sterile brain heart infusion and Sabouraud dextrose broth with fresh suspension of the test organisms such as S.mutans, S.aureus and C.albicans. The samples were incubated at 37 °C for 72 h for the formation of biofilm. After incubation, the samples were gently agitated to remove the non-adherent bound cells and swabs were collected and the lawn culture was made on the sterile Mutans Sanguis agar, Muller Hinton agar and Sabouraud Dextrose agar. The plates were incubated at 37 °C for 24–48 h. Following the incubation, the viable colonies were counted, and their number was recorded as colony forming units per mL (CFU/mL).[36]

2.2.6. Computational molecular simulation test

Extracted Lavender was subjected to molecular docking test against Glucansucrase (GSase) from S.mutans (PDB ID: 3AIB), Dihydrofolate Reductase (DHFR) from S.aureus (PDB ID: 4LAG) and Cytochrome P450 lanosterol 14α-demethylase from C.albicans (PDB ID: 5V5Z) with the Discovery Studio version 2019. The compound structures were drawn with ChemBioDraw Ultra (Chemical Structure Drawing Standard, USA). Protein data bank was used for downloading the protein-ligand complex structure. Binding energy (kcal/mol) to reflect the strength of interaction between ligand of LA extract and receptor in test pathogen was reported. Predicted inhibitory constant (Ki) value was observed to estimate the concentration of LA extract needed to inhibit the target by 50 %. PubChem compound with compound unique identifier (CID) was reported to use this data to predict the bio-interaction between LA extract and biological targets.[32]

3. Results

Determination of zone of inhibition revealed that the lavender extract had moderate antifungal activity (11 mm) against Candida albicans and was less effective compared to nystatin (16 mm). The lavender extract had antibacterial activity against Streptococcus mutans (14 mm), though it was less potent than ampicillin (23 mm). The lavender extract exhibited strong antibacterial activity (18 mm) against Staphylococcus aureus, even more potent than vancomycin (13 mm) (Fig. 1a and b). Mann-Whitney *U* test revealed insignificant difference

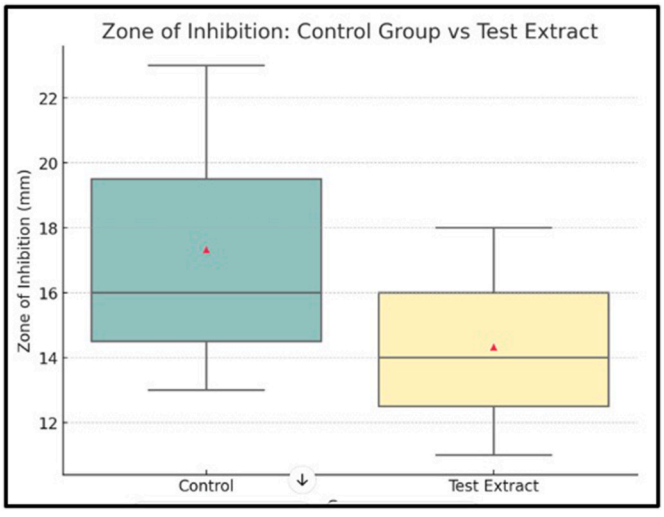

Fig. 1a. Comparison of bacteriostatic potential of LA extract against positive control synthetic drugs.

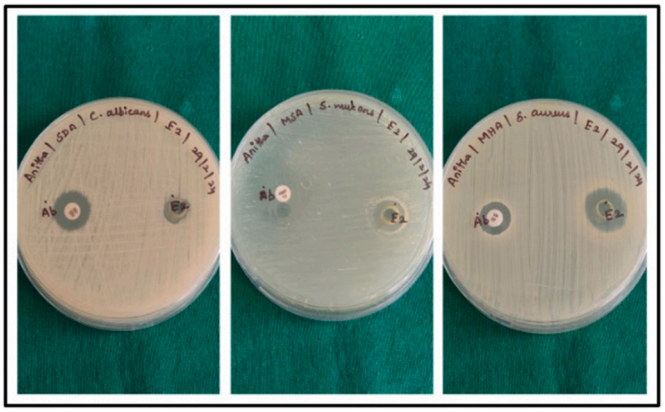

Fig. 1b. Measurement of zone of inhibition to determine antimicrobial susceptibility of Lavandula angustifolia extract.

between control and test extract as the P value was .7.

The efficacy of lavender extract (in percentage) at various dilutions against Streptococcus mutans, Staphylococcus aureus, and Candida albicans to determine MIC showed that the positive control was 100 % effective. Against S.mutans, 92 % efficacy at 200 mg, 89 % at 20 mg and 100 % efficacy at 2 mg was observed. While testing for S.aureus, it was proven that the LA extract was 97 % effective at 200 mg, 95 % at 20 mg, 92 % at 2 mg and 100 % effective at $\leq$ .2 mg. Against C.albicans, LA extract showed 100 % efficacy at all dilutions except, 91 % efficacy at 200 mg (Fig. 2a and b). MIC of LA extract was found to be .2 mg/mL. The minimum bactericidal and fungicidal concentration (MBC/MFC) of LA extract was 2 mg/mL, in which it was highly effective at killing 99.9 % of test pathogens.

The cell viability over 48 h with different concentrations of LA extract was plotted after MTT assay test. The line plot included error bars representing the standard deviation for each concentration. 80–90 % cell viability was observed till 20 mg/mL. Higher drug dosage showed less cell viability indicating cytotoxic effect of mammalian cells. The standard deviations were relatively small, indicating consistent measurements across the three trials at each concentration. This visualization proved increasing concentrations of LA extract impacted cell viability (Fig. 3).

The anti-adherent effect of different LA concentrated PMMA resin discs tested against three pathogens are furnished in Tables 1 and 2. For all the three test pathogens, a significant decline in cell adherence was observed at 2 % concentration compared to control group (P value

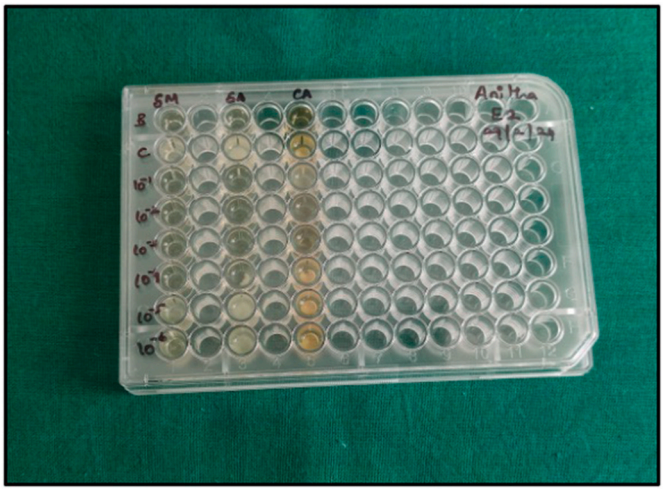

Fig. 2a. Serial dilution test for determination of the Minimum inhibitory concentration assay using 96-well microtiter plate.

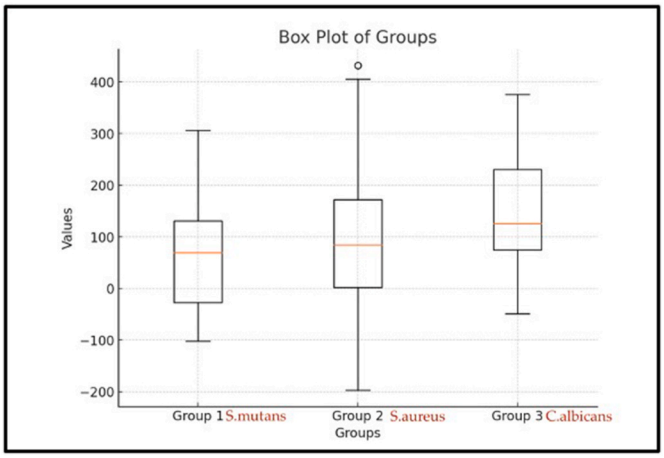

Fig. 2b. Investigation of minimum inhibitory concentration of Lavandula angustifolia extract against S. mutans, S. aureus and C. albicans.

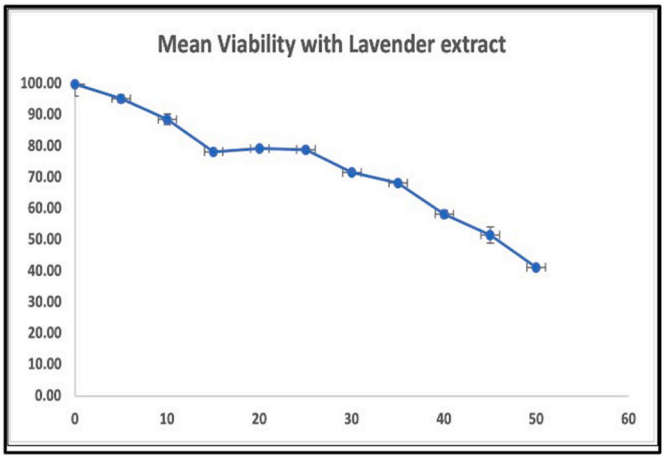

Fig. 3. In-vitro cytotoxicity effect of Lavandula angustifolia extract at various dilutions.

Table 1
Determination of Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC) in mg/mL.

Dilutions	Lavender extract		
	S. mutans	S. aureus	C.albicans
Positive control	NG[a]	NG[a]	NG
10^{-1} (200 mg)	NG[a]	NG[a]	NG
10^{-2} (20 mg)	NG[a]	$.48 \times 10^{2}$	10^{8}
10^{-3} (2 mg)	10^{8}	2.63×10^{2}	10^{8}
10^{-4} (.2 mg)	10^{8}	10^{8}	10^{8}
10^{-5} (.02 mg)	10^{8}	10^{8}	10^{8}
10^{-6} (.002 mg)	10^{8}	10^{8}	10^{8}

[a] NG, No Growth.

.0001). For C.albicans, a statistically significant difference (P = 0.0001) of mean 6.02 ± .04 CFU/mL in control group and in all other concentrations (.5 %, 1 %, 1.5 % & 2 %) there was no growth of fungus observed.

From the chromatographic results, it was observed that a prominent peak appeared at retention time (RT) 24.21 min (Fig. 4). To identify the compounds respective to each peak, mass spectrometry (MS) analysis was performed to obtain the mass-to-charge ratio (m/z) and was compared with known standards. Phytochemical analysis with mass spectrometry revealed its enriched presence of scutellarin, genistin,

Table 2
Intergroup comparison of Biofilm formation.

Test organisms		Mean	SD	95 % Confidence Interval for Mean		F	P value
				Lower Bound	Upper Bound		
S. mutans	Control	9.0970	.15585	8.9855	9.2085	8.95	.0001
	.5 %	6.7820	.03521	6.7568	6.8072		
	1 %	3.7710	.09803	3.7009	3.8411		
	1.5 %	2.2000	.16865	2.0794	2.3206		
	2 %	.0000	.00000	.0000	.0000		
S. aureus	Control	5.0670	.08152	5.0087	5.1253	139.32	.0001
	.5 %	8.1140	.07891	8.0576	8.1704		
	1 %	7.1000	.07288	7.0479	7.1521		
	1.5 %	2.6620	.30911	2.4409	2.8831		
	2 %	1.1100	.10893	1.0321	1.1879		
C. albicans	Control	6.0250	.04767	5.9909	6.0591	159632	.0001
	.5 %	.0000	.00000	.0000	.0000		
	1 %	.0000	.00000	.0000	.0000		
	1.5 %	.0000	.00000	.0000	.0000		
	2 %	.0000	.00000	.0000	.0000		

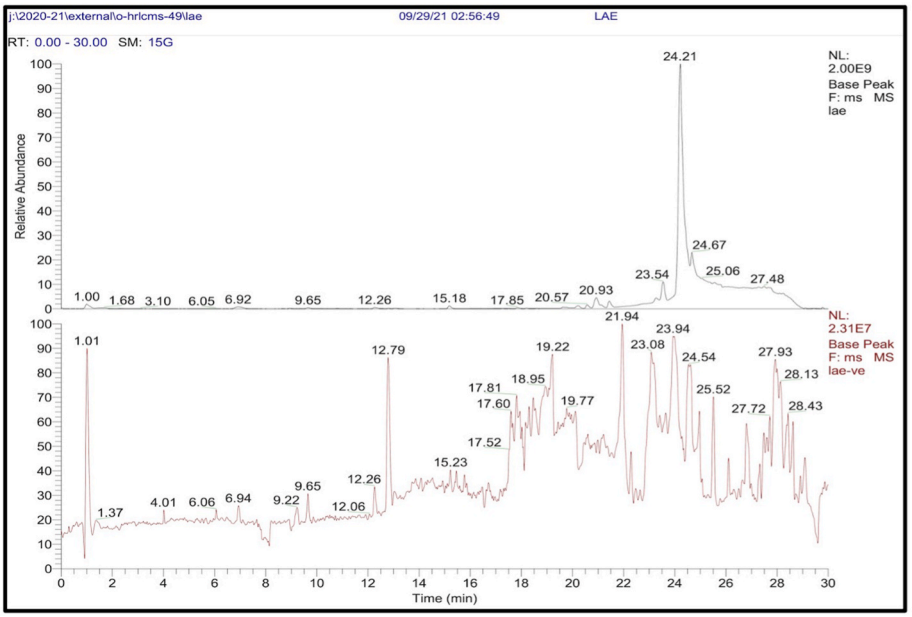

Fig. 4. Phytochemical analysis of Lavandula angustifolia extract with chromatogram.

alpha-cyclodextrin, polyphenols, flavonoids, tannins, terpenoids, linalyl acetate, linalool and terpinenes. Highest abundance of 1-Stearoylglycerol (C21H42O4) with RT of 23.534 min and calculated molecular weight of 358.30827 at a maximum area was observed. It had full match in predicted compositions in mzCloud, and ChemSpider, with delta mass of −.11 ppm (very close to theoretical value) produced best match mzCloud of 94.8 with 84.1 confidence as illustrated in Table 3.

Docking report displayed that, for Glucansucrase (GSase), the compounds such as camphor (CID_2537) showed the lowest predicted inhibitory constant (2.35 μM), indicating strong inhibitory potential, while1,8-cineole (CID_2758) showed weaker inhibition (87.79 μM) (see Table 4). For Cytochrome P450, the 1,8-cineole (CID_2758) had the lowest binding energy (−6.46 kcal/mol) and a relatively low predicted inhibitory constant (18.38 μM), indicating strong inhibition. For DHFR, the terpinenol-4 (CID_11230) had the lowest predicted inhibitory constant (29.85 μM), suggesting it had a strong inhibitory effect on this target (Figs. 5–7).

4. Discussion

The objective of the present study was to investigate the antimicrobial effect of Lavandula angustifolia extract on heat activated polymethyl methacrylate denture base resin. The proposed null hypothesis, that lavender extract would not have any antimicrobial potential on PMMA resin was rejected. Lavender showed antimicrobial activity against all the three tested S.aureus, S.mutans and C.albicans pathogens. There was no significant variation when compared against controls such as vancomycin, ampicillin and nystatin (P = 0.546). Comparable results were evidenced in previous studies done with Lavender essential oil. It was attributed to presence of rich phytochemical compounds such as linalool, linalyl acetate, camphor, and 1,8 – cineole. It possessed phenolic compounds that had the ability to disrupt the microbial cell walls and derange enzyme function. Terpenes in lavender, damaged the cell wall, causing outpouring of contents leading to cell death.[37,38]

A 100 % antimicrobial effectiveness against S.mutans, S.aureus and C.albicans even at lower concentrations of 10^{-6} dilution with MIC value of 0.002 mg/mL was observed in this study. It was found that LA extract was highly effective in killing the three microorganisms at a high concentration of 10^{-1}, 200 mg, with moderate effect at dose of 20 mg and least impact at higher dilutions until 10^{-6}. This unveiled that lavender extract was more bacteriostatic than bactericidal/fungicidal. Previous

Table 3
One-way ANOVA followed by Tukey's post-hoc test for group comparison.

Test organisms	Group	Group compared	Mean Difference	P value
S.mutans	Control	.5 %	2.31500*	.000
		1 %	5.32600*	.000
		1.5 %	6.89700*	.000
		2 %	9.09700*	.000
	.5 %	Control	−2.31500*	.000
		1 %	3.01100*	.000
		1.5 %	4.58200*	.000
		2 %	6.78200*	.000
	1 %	Control	−5.32600*	.000
		.5 %	−3.01100*	.000
		1.5 %	1.57100*	.000
		2 %	3.77100*	.000
	1.5 %	Control	−6.89700*	.000
		.5 %	−4.58200*	.000
		1 %	−1.57100*	.000
		2 %	2.20000*	.000
	2 %	Control	−9.09700*	.000
		.5 %	−6.78200*	.000
		1 %	−3.77100*	.000
		1.5 %	−2.20000*	.000
S.aureus	Control	.5 %	−3.04700*	.000
		1 %	−2.03300*	.000
		1.5 %	2.40500*	.000
		2 %	3.95700*	.000
	.5 %	Control	3.04700*	.000
		1 %	1.01400*	.000
		1.5 %	5.45200*	.000
		2 %	7.00400*	.000
	1 %	Control	2.03300*	.000
		.5 %	−1.01400*	.000
		1.5 %	4.43800*	.000
		2 %	5.99000*	.000
	1.5 %	Control	−2.40500*	.000
		.5 %	−5.45200*	.000
		1 %	−4.43800*	.000
		2 %	1.55200*	.000
	2 %	Control	−3.95700*	.000
		.5 %	−7.00400*	.000
		1 %	−5.99000*	.000
		1.5 %	−1.55200*	.000
C.albicans	Control	.5 %	6.02500*	.000
		1 %	6.02500*	.000
		1.5 %	6.02500*	.000
		2 %	6.02500*	.000
	.5 %	Control	−6.02500*	.000
		1 %	.00000	1.000
		1.5 %	.00000	1.000
		2 %	.00000	1.000
	1 %	Control	−6.02500*	.000
		.5 %	.00000	1.000
		1.5 %	.00000	1.000
		2 %	.00000	1.000
	1.5 %	Control	−6.02500*	.000
		.5 %	.00000	1.000
		1 %	.00000	1.000
		2 %	.00000	1.000
	2 %	Control	−6.02500*	.000
		.5 %	.00000	1.000
		1 %	.00000	1.000
		1.5 %	.00000	1.000

studies have indicated that the minimum inhibitory concentration (MIC) values of lavender essential oils range from .5 to 2.5 μL/mL against various bacteria and fungi. MBC/MFC of LA essential oil was between 2.5 and 7.0 μL/mL. The reasons for this could be because of variation in influencing factors such as choice of Lavandula species, its growth or harvest conditions, extraction method employed, and most importantly the form of Lavender extract studied. In most studies, lavender essential oil was experimented over other forms.[39] In the current analysis, lavender flower extract was chosen over essential oil as flowers contain a wider range of phytochemicals, including flavonoids, phenolic acids, tannins, and other secondary metabolites that are negligible in the

Table 4
Phytoconstituent analysis of Lavandula angustifolia (Lavendar) extract with LC-MS.

Name	Formula	Annot. Delta Mass [ppm]	Calc. MW	RT [min]
1-Stearoylglycerol	C21H42 O4	−.11	358.30827	23.534
Choline	C5 H13 N O	.26	103.09974	.991
Scutellarin	C21H18 O12	1.18	462.08037	12.317
Oleamide	C18H35 N O	.66	281.27205	22.815
Genistin	C21H20 O10	1.46	432.10628	13.026
Phloroglucinol	C6 H6 O3	1.64	126.0319	1.755
NP-021018	C12H18 O4	.84	226.1207	9.648
Dibutyl maleate	C12H20 O4	1.15	228.13642	18.236
α-Cyclodextrin	C36H60 O30	.58	972.3175	1.045
Methyl caffeate	C10H10 O4	1.81	194.05826	12.793
(3S,4R)-7-hydroxy-3-[(3S,4R)-7-hydroxy-4-(4-hydroxyphenyl)-2-oxo-5-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-3,4-dihydro-2H-1-benzopyran-3-yl]-4-(4-hydroxyphenyl)-5-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-3,4-dihydro-2H-1-benzopyran-2-one	C42H42 O20	4.41	866.23076	.989
2-Hydroxycinnamic acid	C9 H8 O3	1.06	164.04752	6.91
4-(3,4-dihydroxyphenyl)-7-hydroxy-5-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-2H-chromen-2-one	C21H20 O11	1.32	448.10115	12.318
NP-021018	C12H18 O4	.84	226.1207	9.937
NP-018730	C21H20 O10	1.46	432.10628	12.15
Phloroglucinol	C6 H6 O3	1.64	126.0319	1.539
1-Stearoylglycerol	C21H42 O4	−.01	358.30831	23.674
Scutellarin	C21H18 O12	1.18	462.08037	13.946
D-(+)-Proline	C5 H9 N O2	.77	115.06342	1.054
NP-019988	C10H10 O4	1.81	194.05826	8.935
2-Amino-1,3,4-octadecanetriol	C18H39 N O3	.55	317.29317	20.145
D-Glucosamine	C6 H13 N O5	.63	179.07948	1.015
2-Hydroxyphenylalanine	C9 H11 N O3	1.47	181.07416	1.172
Phloroglucinol	C6 H6 O3	1.64	126.0319	1.088
NP-015114	C11H19 N O6	.57	261.12139	1.107
13(S)-HOTrE	C18H30 O3	.22	294.21956	20.623
Oleamide	C18H35 N O	.66	281.27205	24.202
Muscone	C16H30 O	1.01	238.22991	22.812
Muramic acid	C9 H17 N O7	.4	251.1006	1.02

(*continued on next page*)

Table 4 (*continued*)

Name	Formula	Annot. Delta Mass [ppm]	Calc. MW	RT [min]
DL-Arginine	C6 H14 N4 O2	.99	174.11185	1.004
13(S)-HOTrE	C18H30 O3	.22	294.21956	19.172
NP-016582	C20H35 N O	.81	305.27211	22.675
15-Deoxy-Δ12,14-prostaglandin D2	C20H30 O4	.62	334.21462	20.077
Cholecalciferol	C27H44 O	.25	384.33931	25.321
NP-018730	C21H20 O10	1.46	432.10628	11.564
3-Methoxybenzaldehyde	C8 H8 O2	1.58	136.05264	10.138
4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-benzodioxole	C11H12 O3	1.52	192.07894	21.714
Genistein	C15H10 O5	1.32	270.05318	15.565
L-Pyroglutamic acid	C5 H7 N O3	1.63	129.0428	1.197
(+)-ar-Turmerone	C15H20 O	1.41	216.15172	14.771
Choline	C5 H13 N O	.26	103.09974	1.215
1,4a-dimethyl-9-oxo-7-(propan-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid	C20H26 O3	−.51	314.18804	19.991
12-oxo Phytodienoic Acid	C18H28 O3	.73	292.20406	16.728
Verrucarol	C15H22 O4	.81	266.15203	10.302
2-Hydroxycinnamic acid	C9 H8 O3	1.06	164.04752	10.183
(2R,3R,4R,5R,6S)-2-{[(2R,3S,4S,5R,6R)-6-(benzyloxy)-3,4,5-trihydroxyoxan-2-yl] methoxy}-6-methyloxane-3,4,5-triol	C19H28 O10	−4.95	416.16619	11.324
NP-019988	C10H10 O4	1.81	194.05826	11.132
6-hydroxy-4a-(hydroxymethyl)-5-methyl-3-(prop-1-en-2-yl)-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-one	C15H22 O3	1.09	250.15717	11.452
Adenine	C5 H5 N5	1.32	135.05467	1.084
Estriol	C18H24 O3	.7	288.17275	17.421
Phloroglucinol	C6 H6 O3	1.64	126.0319	1.341
Oleamide	C18H35 N O	.66	281.27205	24.296
Estriol	C18H24 O3	.7	288.17275	17.294
Luteolin-3′,7-Diglucoside	C27H30 O16	1.07	610.15404	11.078
NP-019811	C6 H7 N O2	1.09	125.04782	1.034

essential oil. This compound enhanced the overall antimicrobial activity and showed synergistic effects against broader spectrum of microbes.[40] Non-volatile compounds that existed in flowers and not in oils possessed anti-microbial property.[41] In this study, the need for large quantities of plant materials for oil extraction, which typically requires substantial resources and time, was eliminated. Additionally, use of minimal extraction process, easy material handling, feasibility to homogenize with PMMA resin polymer, eliminating adverse effects while mixed with monomer liquid were considered as advantages of Lavender flower powder extract over essential oil.[39,40]

Cytotoxicity outcomes showed that there was an evident dose-dependent decrease in cell viability with increasing concentrations of the lavender extract. Higher concentrations of the extract led to lower cell viability. Significant decline in viability began at around 15 μg, with more pronounced effects observed at concentrations of 30 μg and above. Considering this, a concentration of .02 mg of LA extract, which showed 100 % effectiveness in inhibiting the growth of all three pathogens with negligible cytotoxic effect on cells, was incorporated into the study. Past studies affirmed this cytotoxic effect of lavender with higher dosages, and it was associated to the presence of high levels of bioactive compounds that can cause oxidative stress, induce apoptosis, damage cellular membranes, interfere with key enzymes, and produce cytotoxic metabolites.[37,40]

The LA incorporated PMMA resin discs showed significant anti-biofilm activity perceived as reduction in the mean colony forming units of all the 3 pathogens. With higher LA concentrations there was a gradual decline in CFU/mL against S.mutans and S.aureus. For C.albicans, even in the lowest concentration of .5 %, the mean CFU/mL was observed to be zero, indicating strong anti-adherent effect. Supportive studies consistently demonstrated that Lavandula angustifolia essential oil exhibited significant antibiofilm activity against various microorganisms, including Staphylococcus aureus, Pseudomonas aeruginosa, and multi-drug-resistant bacteria.[47–50] It inhibited biofilm formation and disintegrated established biofilms, making it a potential natural agent for combating biofilm-associated infections.

Comprehensive LCMS analysis of Lavandula angustifolia flower extract was performed to better understand the profiles and their biological activities. Observed bioactive compounds were comparable to the previous studies done by Batiha et al. who identified a range of anti-inflammatory elements in it.[27] Antioxidant effects were detected owing to the presence of phenolics and anthocyanins as ingredients in dried lavender flowers.[41] In the present study, abundant quantity of scutellarin, which is a polyphenolic flavone was identified. It is a compound with high therapeutic potential, primarily known for its anti-inflammatory, broad spectrum antimicrobial and antioxidant ability.[42] Furthermore, there was high levels of cyclodextrin in the extract. It is well recognized compound to increase the solubility of poorly water-soluble drugs, enhancing bioavailability and therapeutic efficacy. It has the potential to form inclusion complexes with hydrophobic molecules, encapsulating them in their hydrophobic cavity while remaining soluble in water due to their hydrophilic exterior. It preserves other molecules from degradation due to light, heat, oxidation, and hydrolysis by confining them within their structure.[43] This prospect may be valued towards, sustained retention of the extract material without leaching from the resin matrix, while making the dentures.[44] In the current investigation, highest quantity of stearoyl glycerol was identified in the chromatogram, which is a well-known compound to stabilize the active ingredients. Capacity to facilitate uniform distribution and maintain steady efficacy of the reinforced PMMA resin material could be attributed to this phytochemical.[45]

For further understanding of the drug-target mechanism, molecular docking of the lavender extract was performed. Proteins or enzymes responsible for source of infection caused by S.aureus, S.mutans and C. albicans was studied to correlate with predicted inhibitory constant value (K_i) of the phytochemical in the extract. It is well proven that enzyme glucansucrase produced by S.mutans has the potential to colonize on tooth surface, forming dental plaque and act as a scaffold for biofilm formation.[46] From the study, it was discerned that presence of camphor in the extract showed least K_i value suggestive of strong ligand bond affinity that attacked the targeted enzyme. It also showed more negative binding energy values (−5.04 kcal/mol) that indicated stronger, favorable binding interactions, enabling a stable complex formation with the target.[47] Cytochrome P450 lanosterol 14α-demethylase (CYP51) from C.albicans is a critical enzyme involved in ergosterol biosynthesis needed for formation of cell membrane in fungus.[48] Results of this analysis showed 1,8-cineole in the extract, had lowest K_i with −6.46 kcal/mol binding energy demonstrating the strong binding to disrupt the fungus cell membrane. With regards to S.aureus,

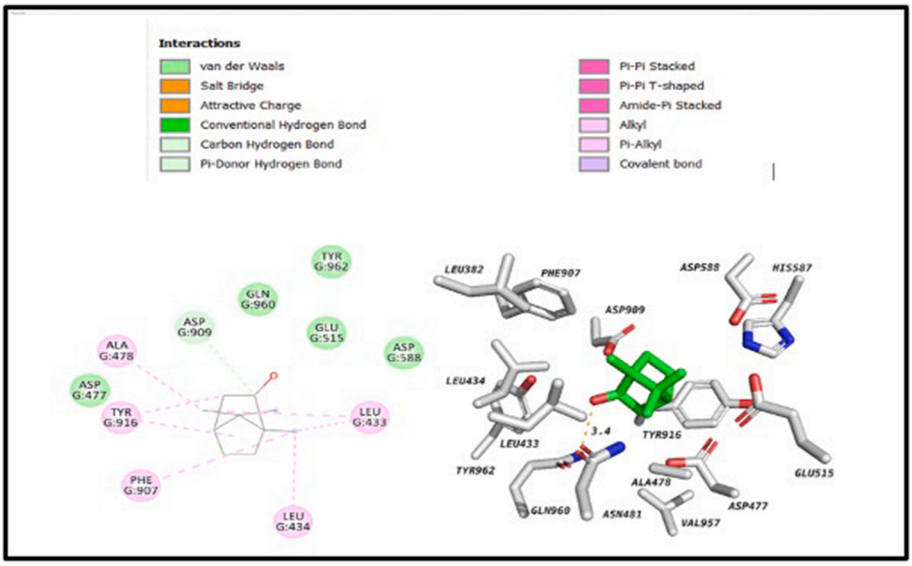

Fig. 5. 2D and 3D interaction between Glucansucrase (GSase) from S. mutans (PDB ID:3 AIB) and camphor from lavender.

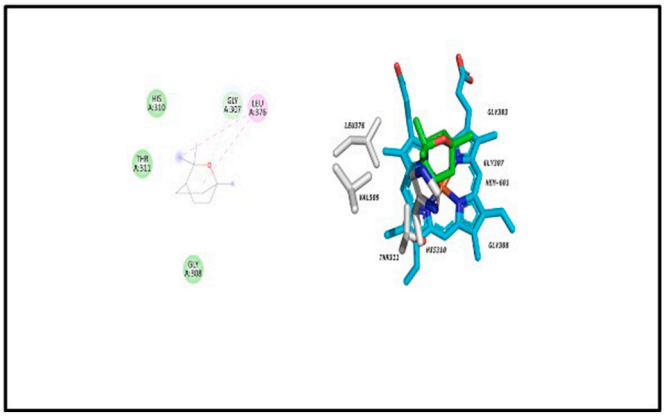

Fig. 6. 2D and 3D interaction between cytochrome P450 lanosterol 14 alpha-demethylase from C. albicans (PDB ID: 5V5Z) and 1,8-cineole from lavender.

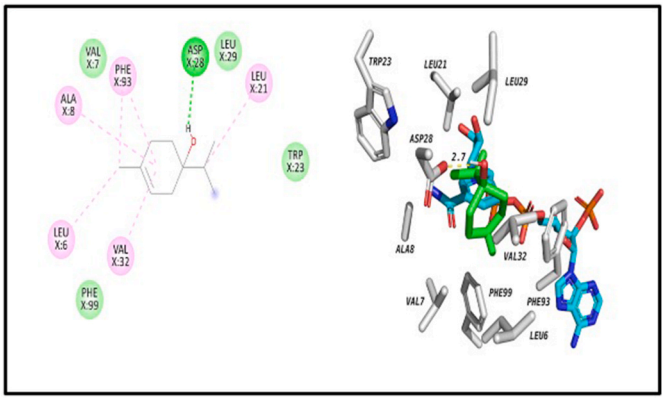

Fig. 7. 2D and 3D interaction between Dihydrofolate Reductase (DHFR) from S. aureus (PDB ID: 4LAG) and Terpinenol-4 from lavender.

Dihydrofolate reductase (DHFR), which is an important enzyme involved in folate metabolism was studied. It plays a major role in the synthesis of nucleotides, DNA replication and cell proliferation.[49] Presence of Terpinenol-4 in the extract furnished relatively less K_i value with strong affinity with target protein as the binding energy was −6.17 kcal/mol. By this testing, clear understanding into the interactions between LA extract ligand and its target protein was possible.[50] The mechanisms of binding were better substantiated, providing way for the development of effective and specific antimicrobial therapeutics with LA extract.

The study detected a natural alternative to synthetic antimicrobials which is timely, relevant to the growing concern over antibiotic resistance and is the major strength of the investigation. It also assessed the safety profile of lavender extract, this would be a notable observation, as it would provide a more comprehensive understanding of its potential as a therapeutic agent. LA antimicrobial potentials are influenced by determinants such as choice of plant species, source, harvesting, storage conditions, extraction method, technique used for phytochemical identification, microbial strain, assay methods used, employment of aseptic microbial testing, understanding of drug absorption, leaching, interaction, bioavailability and drug sustainability are major challenges in the study. This investigation was an in-vitro study, and simple thermal extraction process was employed. Such limitations can be overcome by use of advanced extraction techniques. Anti-adherent property of LA reinforcement PMMA was study up to 2 %. The chance of high LA dose to produce more effectiveness exist. It is important to balance the extract concentration to leverage the antimicrobial benefits while minimizing cytotoxicity to host cells. Impact of lavender reinforcement into dental materials may affect other physical & mechanical properties and provides scope for further research.

5. Conclusions

Within the limitations of this in-vitro study, the following conclusions were drawn.

1. Lavandula angustifolia (Lavender) flower extract showed antimicrobial susceptibility against S.mutans, S.aureus and C.albicans while compared with proven drugs such as ampicillin, vancomycin and nystatin.
2. A concentration of .02 mg of LA extract, inhibited the growth of all three pathogens with minimal cytotoxic effect.
3. LA incorporated heat-cure PMMA denture base material showed significant anti-adherent effect ($P = 0.0001$) with highest impact at 2 %.

4. Enriched phytoconstituent presence of phenolic compound, flavonoids, terpenoids, cyclodextrins and stearoyl glycerol in LA extract was perceived with liquid chromatography and mass spectrometry. Mechanism of action of LA extract's antimicrobial activity was justified with computerized molecular docking study.

Patient consent

The study was an in-vitro analysis and did not involve any patient or guardian in the research.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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