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Original Article

Lemongrass (*Cymbopogon citratus*) oil nanoparticle synthesis, characteristic, and evaluation of antibacterial and antifungal effects and the influence on hardness of acrylic resin

[Síntesis de nanopartículas de aceite de limoncillo (*Cymbopogon citratus*), características y evaluación de los efectos antibacterianos y antifúngicos y la influencia sobre la dureza de la resina acrílica]

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Abstract

Context: Acrylic resin is used in dentistry as a removable denture base. It can cause various pathologies when not properly cleaned. One of the pathologies is denture stomatitis caused by *Candida albicans* and *Streptococcus mutans* accumulation on the acrylic resin surface. Therefore, microbial agents such as denture cleansers are needed.

Aims: To evaluate the characteristics of lemongrass (*Cymbopogon citratus*) nanoparticles as a better antibacterial and antifungal herbal ingredient and their relationship with acrylic hardness.

Methods: *C. citratus* oil nanoparticles (LON) were synthesized and analyzed by transmission electron microscopy (TEM). Electrospray ionization tandem mass spectrometry (ESI-MS) analysis was used to analyze the characteristics of LON bioactive components. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) against *C. albicans* and *S. mutans* and mechanical hardness test of acrylic were performed.

Results: The LON concentration of MIC and MBC against *C. albicans* and *S. mutans* was 25 and 100%, respectively. One-way ANOVA showed no significant difference between groups of LON with different concentrations (p=0.687). A paired t-test showed significant differences in acrylic resin hardness before and after treatment of LON with 100% (p=0.022) and 50% (p=0.021) concentration. There was no significant difference in hardness before and after treatment of other concentrations of LON and chlorhexidine as positive control.

Conclusions: LON treatment on acrylic resin decreased the growth of *C. albicans* and *S. mutans* without altering the mechanical properties (hardness).

Keywords: acrylic resin; *Candida albicans*; *Cymbopogon citratus*; nanoparticle; *Streptococcus mutans*.

Resumen

Contexto: La resina acrílica se utiliza en odontología como base para prótesis removibles. Puede provocar diversas patologías si no se limpia adecuadamente. Una de las patologías es la estomatitis de la prótesis dental causada por la acumulación de *Candida albicans* y *Streptococcus mutans* en la superficie de la resina acrílica. Por lo tanto, se necesitan agentes microbianos como limpiadores de dentaduras postizas.

Objetivos: Evaluar las características de las nanopartículas de hierba limón (*Cymbopogon citratus*) como mejor ingrediente herbario antibacteriano y antifúngico y su relación con la dureza del acrílico.

Métodos: Se sintetizaron y analizaron nanopartículas de aceite de *C. citratus* (LON) mediante microscopía electrónica de transmisión (TEM). Se utilizó el análisis de espectrometría de masas en tándem de ionización por electropulverización (ESI-MS) para analizar las características de los componentes bioactivos de LON. Se realizaron Concentraciones Mínimas Inhibitorias (CIM) y Concentraciones Mínimas Bacterianas (CBM) contra *C. albicans* y *S. mutans* y pruebas de dureza mecánica del acrílico.

Resultados: La concentración de LON de MIC y MBC contra *C. albicans* y *S. mutans* fue del 25 y 100%, respectivamente. El ANOVA unidireccional no mostró diferencias significativas entre los grupos de LON con diferentes concentraciones (p=0,687). Una prueba t pareada mostró diferencias significativas en la dureza de la resina acrílica antes y después del tratamiento con LON con una concentración del 100% (p=0,022) y del 50% (p=0,021). No hubo diferencias significativas en la dureza antes y después del tratamiento con otras concentraciones de LON y clorhexidina como control positivo.

Conclusiones: El tratamiento con LON sobre resina acrílica disminuyó el crecimiento de *C. albicans* y *S. mutans* sin alterar las propiedades mecánicas (dureza).

Palabras Clave: resina acrílica; *Candida albicans*; *Cymbopogon citratus*; nanopartícula; *Streptococcus mutans*.

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INTRODUCTION

The acrylic resin base is part of the denture, attached to oral soft tissue, and can cause denture stomatitis if oral hygiene is poor (Alqutaibi et al., 2023). Denture stomatitis is when the mucosal tissue covered by the denture becomes inflamed and erythematous. The prevalence of denture stomatitis is approximately 20-67% and is experienced by 2/3 of complete denture wearers (Bukhari et al., 2022; Sartawi et al., 2021). This condition is triggered by decreased salivary flow and oxygen in the tissue underneath the denture, which causes microorganisms to grow. Poor oral hygiene also influences denture stomatitis by facilitating the fungal cells to attach to the denture base and increasing the growth of bacterial colonies in the oral cavity (Inayat et al., 2019; Sartawi et al., 2021). *Candida albicans* and *Streptococcus mutans* are the most common fungi and bacteria that cause denture stomatitis (Archilla and Galan, 2020; Fujiwara et al., 2020; Günther et al., 2020; Volchkova et al., 2020). At the beginning of this disease, the patients will have no symptoms. Nevertheless, as the disease progresses, patients will complain about discomfort, dry mouth with burning sensation, pain, mucosal bleeding, taste disorders, erythematous and mild inflammation (Ayavoo et al., 2021; Bajunaid, 2022; Bansal et al., 2013; Günther et al., 2020; O'Donnell et al., 2015; Sartawi et al., 2021).

Therefore, insufficient cleaning of the denture will retain the fungi and bacteria, which will then cause the formation of plaque and calculus, which will play a role as a reservoir of more complex pathogen microorganisms (Abdurahman et al., 2020; Kaypetch et al., 2023; Sahin et al., 2013). Antimicrobial agents are then used to control the microorganism's growth. However, the efficient dose of this agent usage should be ideally considered. Prolonged denture submersion in the antibacterial agent will influence its mechanical properties. This alteration will cause erosion of the denture surface, a decrease in hardness, and micro-scratches on the surface, increasing microorganisms (An et al., 2018; Barua et al., 2017; Kaypetch et al., 2023; Song et al., 2018; Campos Sugio, 2020).

Using chemical antimicrobial agents has adverse effects on the environment and human health compared to natural agents. Thus, recently, there have been increasing initiatives to utilize natural ingredients in medications (Basera et al., 2019; Jeffrey et al., 2020). These herbal agents can be obtained from various sources, such as animals, sea organisms, and plants, which contain secondary metabolites with antimicrobial potential (Azghar et al., 2023; Jeffrey et al., 2023). These secondary metabolites include tannin, flavonoid, saponin, phenol, steroid, alkaloid, and glycoside, which can provide preventive and therapeutic effects (Barua et al., 2017; Basera et al., 2019; Ribeiro et al., 2019; Campos Sugio, 2020).

Several studies have shown that lemongrass [*Cymbopogon citratus* (DC.) Stapf, family *Poaceae*] has distinct and promising characteristics (Basera et al., 2019; Bossou et al., 2020). *C. citratus* has traditionally been used as a tea, analgesic, anti-inflammatory, antioxidant, insect repellant, and stomachache treatment (Ajayi and Afolayan, 2017; Cherian et al., 2020). Pharmacologically, *C. citratus* has antimicrobial, antifungal, antiprotozoal antioxidant, antimutagenic, and antiinflammation activities (Manvitha and Bidya, 2014). *C. citratus* could also prevent denture biofilm formation by inhibiting bacterial cell membranes, altering ergosterol biosynthesis, and inhibiting fungi proliferation and spore germination (Sahal et al., 2020; Shariati et al., 2022). The bioactive ingredient with an antibacterial function and citral content gives off this smell (Basera et al., 2019; Korenblum et al., 2013). Also, by changing the manufacture of ergosterol, increasing membrane fluidity (which disturbs fungal cells), inhibiting spore germination, fungal proliferation, respiration, and membrane synthesis, it can prevent denture biofilm in dentistry (Monteiro et al., 2021).

The utilization of natural materials' advantages as antimicrobials has the potential to develop into nanoparticles with sizes between 1-100 nm. Nanoparticles not only have superior physical and chemical properties compared to particles with bigger sizes, but the synthesis of nanoparticles is also environment-friendly, cost-effective, non-toxic, and more biocompatible (Ajayi and Afolayan, 2017; Ozdal and Gurkok, 2022; Riyanto et al., 2022). Nanoparticles have unique characteristics based on size, distribution, and particle morphology. For example, smaller particle size will increase particle ability to penetrate bacterial cell membranes, increasing the antimicrobial effect (Rakib-Uz-Zaman et al., 2022; Riyanto et al., 2022). These active biological components, characteristics, and physical properties of nanoparticles will produce the therapeutic results of nanoparticles, which can be applied as broad-spectrum antibacterial and antifungal agents (Li et al., 2014; Sharmin et al., 2021; Wang et al., 2017).

Even though natural components are used, it is necessary to discover ways to make natural ingredients into nanoparticles smaller than 100 nm that can act as antibacterial agents. Its many environmentally beneficial qualities include its many plant sources, low chemical processing, high bioavailability, and active biological components (Ajayi and Afolayan, 2017; Baranwal et al., 2018; Riyanto et al., 2022). Even though natural components are used, it is still necessary to discover ways to make natural ingredients into nanoparticles that can act as antibacterial agents. Its many environmentally beneficial qualities include its many plant sources, low chemical processing, high bioavailability, and active biological components (Ajayi and Afolayan, 2017; Baranwal et al., 2018; Riyanto et al., 2022). A nanoparticle's size, shape, particle dispersion, and phase are the specific characteristics that determine its unique and improved properties. Because smaller particles can more easily enter the intercellular space, their antibacterial effects will be greater. The temperature of the liquid, the concentration of metals, the reducing agent, and the reaction duration throughout the synthesis process can all affect a compound's particle size (Rakib-Uz-Zaman al., 2022; Riyanto et al., 2022).

The physical characteristics of nanoparticles make them suitable for therapeutic applications, as they exhibit broad-spectrum antibacterial action against gram-positive and gram-negative bacteria. In addition, fungal hypha deformation causes the nanoparticle to be able to prevent hypha transition, which results in fungicidal action, so it has antifungal potency (Li et al., 2014; Sharmin et al., 2021; Wang et al., 2017).

Inadequate cleaning might exacerbate oral health issues when wearing dentures made of acrylic resin. *C. albicans* and *S. mutans* surface adhesion is the cause of denture stomatitis, one of the oral health issues. Because *C. citratus* possesses a bioactive antibacterial component, it is necessary to use an antimicrobial treatment when washing dentures. Due to their capacity to enter intercellular spaces, particles of a smaller size will have better antibacterial activity. Therefore, an option for denture cleanser could be nanoparticle antibacterial (Ajayi and Afolayan, 2017; Baranwal et al., 2018; Le Bars et al., 2022; Riyanto et al., 2022; Sartawi et al., 2021).

This research synthesized and analyzed a *C. citratus* oil nanoparticle (LON) with transmission electron microscopy (TEM). The bioactive component was characterized by an electrospray ionization tandem mass spectrometry (ESI-MS) analysis. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed against *S. mutans* and *C. albicans.* Then, after applied to acrylic resin, the hardness was tested to observe the influence of LON on the mechanical properties of acrylic.

MATERIAL AND METHODS

C. citratus extraction

C. citratus extraction was performed in PT Indesso Aroma Pharmaceutical Company, Baturaden, Purwokerto, Central Java, Indonesia, with CoA No Batch. 895850017. The extraction process was part of 'the development of standardized herbal medicine from natural ingredients' project, including *C. citratus* extract as biofilm in acrylic resin. The extraction result was in oil form and then processed for certification of analysis for safety production.

C. citratus oil nanoemulsion (LON) production

Five milliliters of *C. citratus* oil (PT Indesso) was mixed with 5 mL of propylene glycol (Carbowax - X29160), 5 mL of PEG 400 (Carbowax - X29160**),** and 10 mL of glycerin (Merck-104094). All mixtures were stirred with a magnetic stirrer until homogenous at room temperature. After that, 5 mL of chromophore compound was added and mixed with the previous solution.

Transmission electron microscopy (TEM) morphological characteristics testing

Morphology analysis was performed with a TEM analyzer (JEOL JEM-1400) at FMIPA Laboratory, Gajah Mada University, Yogyakarta. Ten µL of the sample were dropped into a grid and left still for 1 minute, and then the remaining liquid was removed with a micropipette. Ten µL of uranyl acetate was dropped into a grid, and the remaining liquid was removed with a micropipette. The grid was dried for 30 minutes and then observed under the TEM. TEM is a preferred nanoparticle size, granule size, size distribution, and morphology measurement method (Smith, 2015).

Electrospray ionization-mass spectra (ESI-MS) measurement

A sample of 0.03 grams was weighed with an analytic balance (Mettler Toledo, Swiss) and dissolved in 90% acetonitrile before being sonicated (DAW, USA) for 30 minutes. After that, quenchers, as substance separators in mass spectrophotometry, were added to separate the sample contaminant and centrifuged for 10 minutes with a velocity of 10000 rpm. Then, the sample was filtered by a 0.2-micron membrane and tested with ESI-MS (TSQ Quantum Access Max) with a velocity of 5 microliters per minute and added eluent methanol with 0.1% formic acid (Akın et al., 2018; De Vijlder et al., 2018).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) analysis of *S. mutans* and *C. albicans*

Culture media preparation

Mueller Hinton Agar (MHA) (Himedia, M096-500G) and Mueller Hinton Broth (MHB) (Himedia, M403-500G) were used as the culture media. Thirty-eight grams of MHA and 21 grams of MHB were dissolved in 1000 mL of ddH_2O with the aid of a microwave (Shivaki, SMW 103). Then, the media were sterilized with an autoclave (HiClave, H-50) with a temperature of 121°C and pressure of 1.5 atm for 25 minutes (Sugiaman et al., 2023).

LON preparation

Thirty mL of 100% LON stock were diluted using the Serial Working Solution method with ddH_2O and 10% DMSO to prepare series concentrations of 3.125, 6.25, 12.5, 25, 50, 100%. After that, the sample concentrations were filtered using a 0.22 µm pore syringe filter (Sartorius, 17845) to obtain sterile samples (Ahmad et al., 2019).

MIC and MBC analysis

S. mutans and *C. albicans* inoculums preparation: Inoculums of *S. mutans* (ATCC-25175) and *C. albicans* (ATCC-10231) were cultured using the direct colony suspension method from the primary culture after 24 hours on MHA and MHB media. The turbidity of the inoculums was adjusted with McFarland 0.5 standard to get bacteria suspensions of 1-5 × 10^8 CFU/mL. Then, inoculum suspensions were dissolved in MHB with a ratio of 1:50 to get 2 × 10^6 – 1 × 10^7 CFU/mL of bacteria suspensions. After that, further dissolving with a ratio of 1:20 was done to get 1-5 × 10^5 CFU/mL of bacteria suspension (Arthington-Skaggs, 2002; Balouiri, 2016; CLSI, 2012).

Broth microdilution method

One hundred µL of inoculum were added to microplate wells and mixed with series concentrations of LON. One hundred µL working solution was added to each concentration until it reached the final concentration. After that, 100 µL of chlorhexidine 0.2% (0011221-C107) was added to wells as the positive control. One hundred µL MHB and 100 µL of inoculum were added to wells as a growth or negative control to antibacterial inhibition. One hundred µL of MHB and 100 µL of working solution of each LON concentration and chlorhexidine 0.2% (0011221-C107) were added to wells as blank groups (CLSI, 2012; Suwonchoochit et al., 2021).

The microplates were incubated for 24 hours at 37°C and then observed with spectrophotometry (Multiskan GO, Thermo Scientific 51119300) with 450 nm wavelength for *S. mutans* and 520 nm wavelength for *C. albicans. S. mutans* and *C. albicans* growth was defined by optical density (OD) comparison between each treatment group and its blank groups. MIC was defined from the lowest LON concentration, which provided inhibition for more than 50% against microorganism growth. MBC was defined from the lowest LON concentration, which provided inhibition against microorganism growth for 99% (CLSI, 2012; Suwonchoochit et al., 2021).

Pour plate method

After the spectrophotometry measurement from each treatment in the microplate, the pour plate method confirmed that the MBC measurement observed could inhibit microorganism growth in agar media (Arthington-Skaggs et al., 2002; Balouiri et al., 2016; CLSI, 2012). Twenty µL from each well were cultured in MHA media with the pour plate method and then incubated (Thermo IH3543) for 24 hours at 37°C. Visual observation was done to count the colony number with the colony counter (Funke Gerber 8500). The MBC was defined from the concentration that showed almost no agar media colony growth. The inhibition percentage was counted with the following formula [1].

$$\text{Inhibition (\%)} = \frac{\text{Negative Control Colony} - \text{Total Treatment Colony}}{\text{Negative Control Colony}} \times 100\% \quad [1]$$

Acrylic specimen fabrication

The wax was sculpted in a flat cylinder with 10 mm diameter and 2 mm thickness with baseplate wax (Cavex) and placed into the metal flask. After boiling out, the heat-cured acrylic resin (ADM, ISO 1567) was manipulated, packed, and pressed into the mould according to the manufacturer's instructions. The metal flasks were placed in a boiler unit for polymerization. After polymerization, the excess resin was trimmed, and one of the surfaces was finished using 180, 220, 360, and 400-grit sandpaper (Hayran et al., 2018). The specimens were randomly assigned to 7 treatment groups (n = 35), which were further divided to six LON treatment (3.125, 6.25, 12.5, 25, 50, 100%) and one group with chlorhexidine 0.2%. The number of specimens in each group (n = 5).

Vickers hardness test

Acrylic resin hardness was measured with a Micro Vickers Hardness Tester (Shimadzu, HMV-G21ST Series) according to ISO 1567, third edition, 1999-02-15. Three random measurements were made on each specimen with a Knoop diamond indenter under a load of 25 g for 5 seconds. The hardness number of each specimen was defined by the mean of the three measurement values obtained (Hayran et al., 2018).

Statistical analysis

Statistical analysis was carried out using Minitab version 20.3 (Minitab LLC). The differences between groups were evaluated using A one-way ANOVA test and a paired t-test to evaluate the hardness before and after LON treatment. Data obtained was expressed as the mean ± standard deviation. The significance level was set up at $p<0.05$.

RESULTS

C. citratus oil nanoemulsion (LON) certificate of analysis (CoA)

CoA was done by observing the fabricated nanoparticle's color and odor. This analysis was one of the important steps in determining product quality and grade. The result obtained from the current study was that LON had a yellow color without a specific odor. LON's physical properties (Table 1) showed that the observation result was within the standard range. Therefore, LON could be used to develop herbal medicine from *C. citratus* as an antimicrobial.

Morphological characteristics analysis with transmission electron microscopy (TEM)

TEM is the gold standard and most efficient method for obtaining the nanoparticle morphological characteristics and size. The method provides accurate characterization, such as shape, structure, and size information. This method will present a two-dimensional nanoparticle illustration, which can be observed for number base shape distribution (Al-Khafaji et al., 2020; Tremi et al., 2021).

Fig. 1 shows that LON sample size measurements were uniformly between 10-100 nm with irregular individual particle shapes. In TEM measurement, the sample observed should be between 60-90 nm thick to penetrate by electron (Tremi et al., 2021). However, TEM is favorable for characterizing nanoparticles because the resolution reaches approximately 0.07 nm (Souza et al., 2016).

Mass spectrophotometry (ESI-MS) result of LON

Determination of the substance's component of LON could be characterized with ESI-MS (TSQ Quantum Access Max). ESI-MS was also used to analyze herbal compounds' nanoparticles, substance composition, and molecule mass (Chen et al., 2019). The chromatogram and substance of LON compounds are presented in Fig. 2 and Table 2.

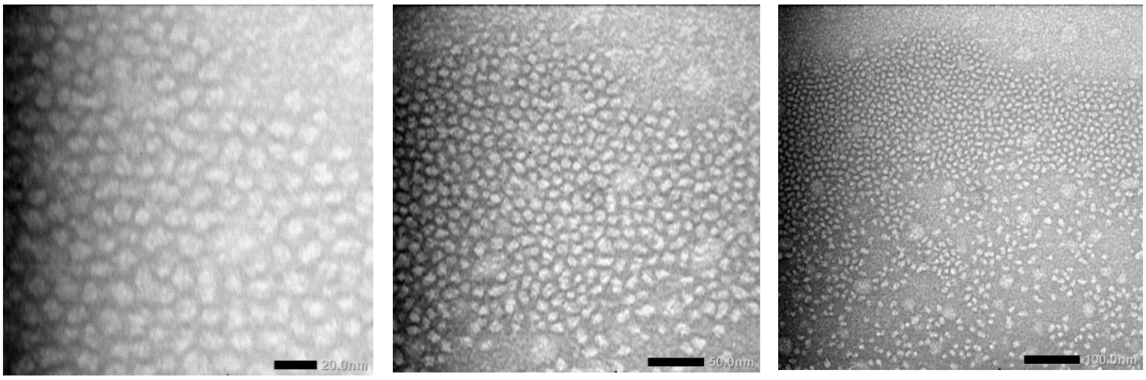
Fig. 2B illustrates that LON contained several compounds, as presented in Table 2.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) results against *S. mutans* and *C. albicans*

The antimicrobial activity of LON against *S. mutans* and *C. albicans* was determined by the MIC and MBC (Tables 3-6). The MIC value of LON against *S. mutans* was at 25% concentration, and the MBC was at 100%. The inhibition level of LON against *S. mutans* growth was considered high because it showed results similar to those of the positive control. The inhibition level was proportional to the concentration; higher concentrations showed more potent *S. mutans* inhibition.

Table 1. *C. citratus* oil nanoemulsion certificate of analysis.

Characteristic	Specification	Result
Appearance	Liquid	Liquid
Color (visual)	Pale yellow-yellow brown	Yellow
Organoleptic (odor)	Conform to standard	Conform to standard
Acid value (titration)	Max 8.0	2.4
Optical rotation (25°C)	(-)6-0	-2
Refractive index (20°C)	1.480-1.493	1.486
Specific gravity (D25/25)	0.872-0.897	0.892



LON 20 nm **LON 50 nm** **LON 100 nm**

Figure 1. Transmission electron microscopy results from *C. citratus* oil nanoemulsion (LON).

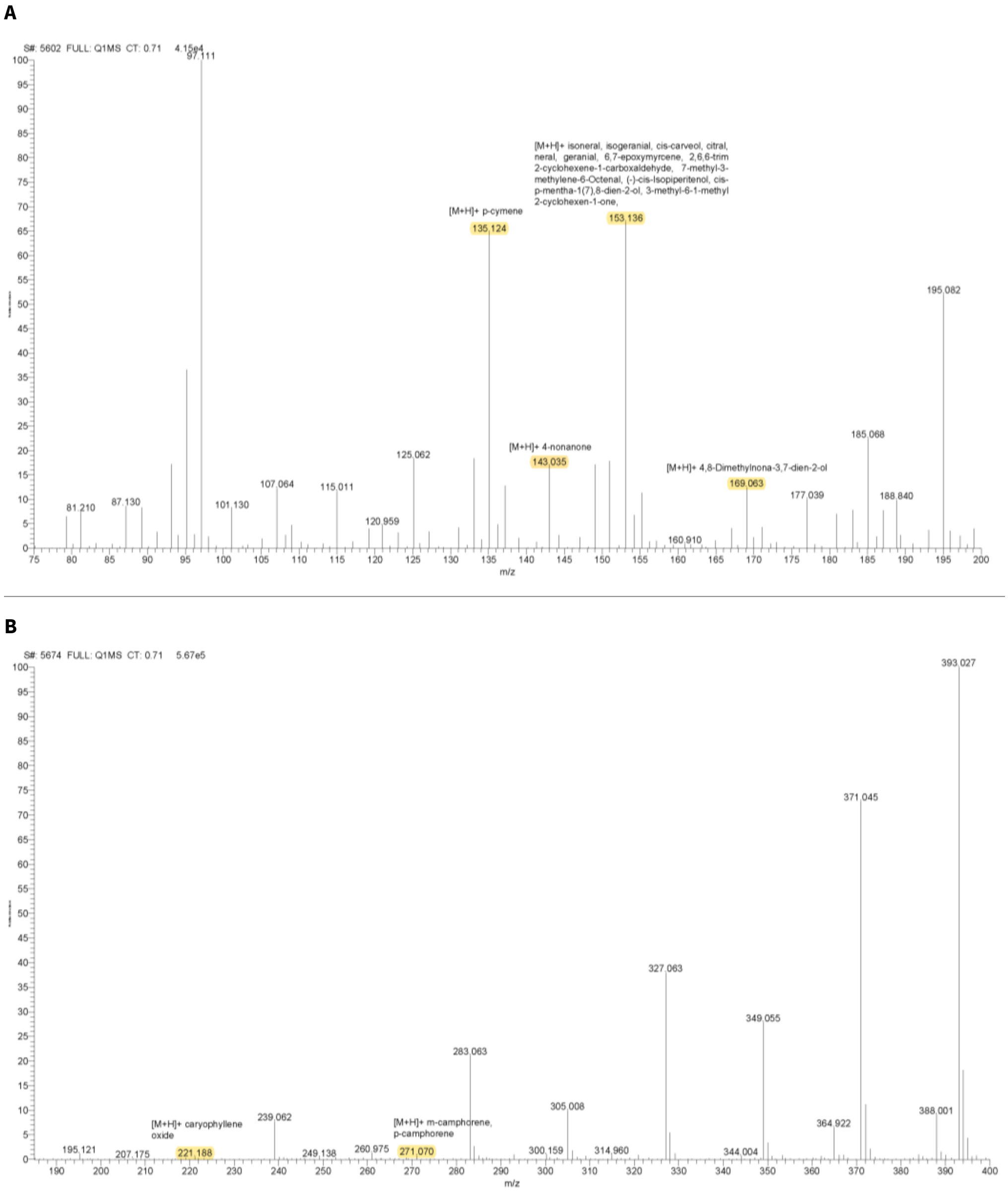


Figure 2. Mass spectra 75-200 m/z **(A)** and 200-400 m/z **(B)** of *C. citratus* oil nanoemulsion (LON) with positive ionization.

Table 2. Target compounds identification of *C. citratus* oil nanoemulsion (LON) with ESI-MS.

Compound	Molecular weight (g/mol)	Mass spectra (MS) $[M\text{-}H]^+$
p-Cymene	134.220	135.124
4-Nonanone	142.240	143.035
Isoneral	152.233	153.136
Isogeranial	152.233	153.136
cis-Carveol	152.233	153.136
Citral	152.233	153.136
Neral	152.233	153.136
Geranial	152.233	153.136
6,7-Epoxy myrcene	152.233	153.136
2,6,6-Trim 2-cyclohexene-1-carboxaldehyde	152.233	153.136
7-Methyl-3-methylene-6-Octenal	152.233	153.136
(-)-cis-Isopiperitenol	152.233	153.136
cis-p-Mentha-1(7),8-dien-2-ol	152.233	153.136
3-Methyl-6-1-methyl 2-cyclohexen-1-one	152.233	153.136
4,8-Dimethylnona-3,7-dien-2-ol	168.280	169.063
Caryophyllene oxide	220.350	221.188
m-Camphorene	272.468	271.070
p-Camphorene	272.468	271.070

Table 3 Viability and inhibition of *C. citratus* oil nanoemulsion (LON) against *S. mutans.*

Treatment	Viability (%)	Inhibition (%)	
LON 3.125%	96.43 ± 0.22^f	3.57 ± 0.22^b	
LON 6.25%	76.35 ± 0.28^e	23.65 ± 0.28^c	
LON 12.5%	70.11 ± 0.19^d	29.89 ± 0.19^d	
LON 25%	$\mathbf{46.80 \pm 0.19^c}$	$\mathbf{53.20 \pm 0.19^e}$	**MIC**
LON 50%	20.93 ± 1.10^b	79.07 ± 1.10^f	
LON 100%	$\mathbf{0.34 \pm 0.19^a}$	$\mathbf{99.66 \pm 0.19^g}$	**MBC**
Negative control	100.00 ± 0.11^g	0.00 ± 0.11^a	
Chlorhexidine 0.2%	1.32 ± 0.45^a	98.68 ± 0.45^g	

Data were presented in average ± standard deviation (n = 3). Different letters show significant differences in the Tukey HSD test (p<0.05).
LON: *Cymbopogon citratus* oil nanoemulsion; MBC: Minimal bactericidal concentration; MIC: Minimal inhibitory concentration.

The MIC value of LON against *C. albicans* was at 25% concentration, while the MBC was at 100% concentration. The inhibition rate of LON against *C. albicans* was proportional to the concentration. Higher concentrations showed a more potent inhibition rate.

Hardness test of acrylic specimen

A hardness test was performed to evaluate acrylic resin hardness differences before and after LON treatment, as shown in Table 7.

A one-way ANOVA test was performed on hardness value, and the p = 0.687, which can be concluded that there were no significant differences between treatment groups. A paired t-test was performed to evaluate the hardness before and after LON treatment. Only the 100% and 50% LON groups showed significant differences between treatments with p-values of 0.022 and 0.021, respectively. However, other groups, including the control group with chlorhexidine 0.2%, showed no significant statistical differences with p>0.05.

Table 4. Colony forming unit (CFU/mL) of *C. citratus* oil nanoemulsion (LON) against *S. mutans*.

Treatment	Dilution factor	Colony count (CC)				CFU/mL				Average (CC)	Average (CFU)
		1	2	3	4	1	2	3	4		
LON 3.125%	10000	210	215	220	218	210×10^3	215×10^3	220×10^3	218×10^3	215.75	215.75×10^3
LON 6.125%	10000	181	185	175	168	181×10^3	185×10^3	175×10^3	168×10^3	177.25	177.25×10^3
LON 12.5%	10000	120	126	123	128	120×10^3	126×10^3	123×10^3	128×10^3	124.25	124.25×10^3
LON 25%	10000	72	71	75	77	72×10^3	71×10^3	75×10^3	77×10^3	73.75	73.75×10^3
LON 50%	10000	31	33	35	33	31×10^3	33×10^3	35×10^3	33×10^3	33.00	33.00×10^3
LON 100%	10000	0	0	0	0	0	0	0	0	0.00	0
Negative control	10000	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Chlorhexidine 0.2%	10000	0	0	0	0	0	0	0	0	0	0

TNTC: Too numerous to count.

Table 5. Viability and inhibition of *C. citratus* oil nanoemulsion (LON) against *C. albicans.*

Treatment	Viability (%)	Inhibition (%)	
LON 3.125%	90.74 ± 0.60^g	9.26 ± 0.60^b	
LON 6.25%	86.92 ± 0.11^f	13.08 ± 0.11^c	
LON 12.5%	68.81 ± 0.12^e	31.19 ± 0.12^d	
LON 25%	$\mathbf{42.96 \pm 0.03^d}$	$\mathbf{57.04 \pm 0.03^e}$	**MIC**
LON 50%	3.55 ± 0.08^c	96.45 ± 0.08^f	
LON 100%	$\mathbf{0.44 \pm 0.04^a}$	$\mathbf{99.56 \pm 0.04^h}$	**MBC**
Negative control	100.00 ± 0.05^h	0.00 ± 0.05^a	
Chlorhexidine 0.2%	2.32 ± 0.04^b	97.68 ± 0.04^g	

Data were presented in average ± standard deviation (n = 3). Different letters show significant differences in the Tukey HSD test ($p<0.05$). LON: *Cymbopogon citratus* oil nanoemulsion; MBC: Minimal bactericidal concentration; MIC: Minimal inhibitory concentration.

Table 6. Colony forming unit of *C. albicans* (CFU/mL).

Sample	Dilution factor	Colony count (CC)				CFU/mL				Average (CC)	Average (CFU)
		1	2	3	4	1	2	3	4		
LON 3.125%	1000	62	61	63	61	62×10^3	61×10^3	63×10^3	61×10^3	61.75	61.75×10^3
LON 6.125%	1000	58	56	51	55	58×10^3	56×10^3	51×10^3	55×10^3	55.00	55.00×10^3
LON 12.5%	1000	43	45	45	48	43×10^3	45×10^3	45×10^3	48×10^3	45.25	45.25×10^3
LON 25%	1000	29	31	33	33	29×10^3	31×10^3	33×10^3	33×10^3	31.50	31.50×10^3
LON 50%	1000	5	2	2	4	5×10^3	2×10^3	2×10^3	4×10^3	3.25	3.25×10^3
LON 100%	1000	0	0	0	0	0	0	0	0	0	0
Negative control	1000	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Chlorhexidine 0.2%	1000	0	0	0	0	0	0	0	0	0	0

TNTC: Too numerous to count.

Table 7. Hardness test of acrylic specimen with *C. citratus* oil nanoemulsion.

Treatment	Before (kg/mm^2)	After (kg/mm^2)
LON 3.125%	21.60 ± 0.38	19.90 ± 1.78
LON 6.25%	21.12 ± 0.73	19.50 ± 2.15
LON 12.5%	21.44 ± 0.75	19.62 ± 1.09
LON 25%	22.38 ± 1.36	21.04 ± 2.01
LON 50%	22.38 ± 0.88	19.24 ± 1.14
LON 100%	22.88 ± 0.56	19.36 ± 2.06
Chlorhexidine 0.2%	21.00 ± 1.69	20.06 ± 1.18

Data are presented as mean hardness value ± standard deviation (n = 3).

DISCUSSION

C. citratus oil was extracted according to the standard and safety production of the certificate of analysis (CoA). The CoA result showed the specification of compounds, such as appearance, color, odor, titration, purity, solubility, and water content (Creff-Froger et al., 2017). The physical properties of LON in current research met the chosen standard. Therefore, *C. citratus* was suitable as an antimicrobial agent from the development of standardized herbal medicine. The *C. citratus* oil was fabricated into nanoparticles with superior characteristics and properties.

The characterization of LON in current research was analyzed using TEM. The results showed that the particles' size and shape characterized the physicochemical properties of LON, which significantly contributed to better particle performance. Other physicochemical characteristics influencing particle performance are texture and surface structure (Rice et al., 2013; Wen et al., 2021). Other than affecting the performance, the characteristics also influence nanomaterials' physical and chemical properties, such as electronic, optic, and catalytic aspects (Lee et al., 2020). By understanding its characteristics, a nanoparticle's stability can be identified by forming particle aggregation because of inter-particle force. This force will induce interaction between particles and create a more extensive cluster (Oktavia and Sutoyo, 2021).

Herbal materials have important antimicrobial roles against pathogens such as bacteria and fungi. The antimicrobial activity depends on the biochemical content and morphology of the materials (Basera et al., 2019; Gunasena et al., 2022; Nitu and Patidar, 2017). The biochemical content of *C. citratus* is usually affected by several factors, such as plants' characteristics, climate, geographical conditions, part of the plant used, ecological conditions, and harvest timing (Basera et al., 2019; Chouhan et al., 2017; Gunasena et al., 2022).

C. citratus essential oil has proved to have the ability to inhibit the growth of bacteria and fungi (Nitu and Patidar, 2017). Essential oil is a mixture of herbal compounds that is easy to evaporate, has low molecular weight, and is hydrophobic. These properties allow the oil to split the lipid component in bacteria cells' membranes and mitochondria, disrupting cell structure and rendering it more permeable. After that, the cell will experience molecular and essential ion leakage and induce cell death (Chouhan et al., 2017; Sharma et al., 2023).

The microbial activities of LON were associated with a high oxygen content of the essential oil, which contains monoterpene and hydrocarbon sesquiterpene, mostly aldehyde and alcohol such as neral/geranial and nerol/geraniol. These contents determined the gram-negative and gram-positive antibacterial properties (Chlif et al., 2021; Islam et al., 2018). Higher oxygen content, such as in geranial, has more effective antimicrobial activity (Hussein and Joo, 2018). The antibacterial activities of essential oil are also affected by a mixture of several complexes, such as monoterpene, sesquiterpene, and the oxygenated derived (Tanhaeian et al., 2020).

According to several studies, there are several components of essential oil which have antimicrobial activities, such as monoterpene ($C_{10}H_{16}$), sesquiterpene ($C_{15}H_{24}$), diterpene ($C_{20}H_{32}$), triterpene ($C_{30}H_{40}$), and other components such as 1,8-cineole, p-cymene, α-terpineol acetate, eugenol, limonene, estragole, menthol, anethole, borneol, thymol, geraniol, cinnamyl alcohol, α-thujone, β-thujone, α-pinene, sabinene, caryophyllene oxide, dan terpinene (Chouhan et al., 2017; Tanhaeian et al., 2020).

LON's ability to inhibit *S. mutans* was similar to chlorhexidine 0.2%, as shown by the number of *S. mutans* formed in LON with 100% concentration, which was 0 CFU/mL. Therefore, current research proved that the essential oil of *C. citratus* has antibacterial effects. Smaller particle sizes also gave more substantial antibacterial effects (Choonharuangdej et al., 2020; Riyanto et al., 2022).

The current study proved that LON had antibacterial and antifungal effects, as shown by the MIC and MBC results, which align with a previous study by Koseki et al. (2018). LON's ability to inhibit the growth of *S. mutans* and *C. albicans* was similar to chlorhexidine by 0.2% (Choonharuangdej et al., 2020; Riyanto et al., 2022). Essential oil in the nanoparticle form of LON has more effective and efficient roles as an antifungal by inhibiting the metabolism process of fungi and then inhibiting its growth (Riyanto et al., 2022).

According to research by Sugiaman et al. (2024), the MBC value of *C. citratus* oil against *S. mutans* and *C. albicans* was determined at 100% concentrations of 99.38% and 99.04%, respectively. This shows that *C. citratus* oil in nanoemulsion form provides better antibacterial and antifungal effects. This is indicated by the MBC value of *C. citratus* oil against *S. mutans* and *C. albicans*, determined at 100% concentrations of 99.66% and 99.56%, respectively.

Older people have a limitation in performing denture mechanical cleaning because of physical deterioration. Therefore, a combination of mechanical and chemical cleaning is suggested. However, this combination sometimes failed to inhibit the growth of *C. albicans* completely (de Lucena-Ferreira et al., 2013).

This failure can be caused by the extracellular matrix polymer material of the denture, which limits cleaning agents' access to microorganisms located far inside the biofilm (Yodmongkol et al., 2014). Therefore, denture biofilm should be adequately cleaned daily because biofilm accumulation can be the source of local and systemic disease (Rocha et al., 2021). Several studies found that there is an accumulation of *Candida* after biofilm formation (Yodmongkol et al., 2014). The porosity of acrylic resin increases the difficulty of mechanical cleaning and infection control in dentures. Thus, denture submersion in disinfectant liquid has become a routine procedure (Pereira et al., 2019). Submersion of dental prosthesis in chemical cleanser aims to deactivate bacteria, viruses, and fungi activity (Kati, 2021). However, the commonly used disinfectant agent has disadvantages such as being toxic (glutaraldehyde), corrosive to metal, and inducing skin irritation and mucosal staining. Therefore, an alternative agent that does not influence the denture's properties is needed (Pereira et al., 2019). Other studies showed that various cleaning agents affect the physical properties of denture bases, such as hardness, transverse strength, roughness, and color (Carvalho et al., 2012). The denture cleaning method should be effective without affecting denture material properties (Porwal et al., 2017).

Hardness is one of the material properties that influences the surface characteristics of acrylic resin. Hardness is also used to evaluate the alteration because of denture cleaning (Lira et al., 2014). Hardness value measurement is an indication of the possibility of polymer matrix degradation. This degradation decreases the hardness value, thereby increasing the possibility of fracture and reducing the length of use of the denture (Ayaz et al., 2014). In the current study, the hardness value of resin acrylic samples after LON treatment declined compared to before treatment. These results were possible because of the water and chemical absorption, which caused decreasing mechanical properties of acrylic resin (Rocha et al., 2021). In contrast, previous studies showed that hardness values increased after applying coated material and before applying a denture cleaner solution (Yodmongkol et al., 2014). Another study showed that the hardness value of conventional acrylic resin increased after submersion in sodium hypochlorite cleaning solution and chlorhexidine, mainly after 120 days, which means the longer the submersion time, the greater the hardness value (Hermana Neppelenbroek et al., 2005). However, another study showed a decreased hardness after one year of submersion in chlorhexidine disinfectant gels (Raszewski et al., 2021). The variety of results can be affected by diverse materials, procedures, and analysis time for each study.

The hardness value of the current research was observed after treatment, which is in line with other studies that observed that chlorhexidine did not significantly decrease the acrylic resin hardness (Kati, 2021). Another study comparing vinegar as a denture cleaning agent and chemical agents showed insignificant differences in hardness values between the two groups (Pereira et al., 2019). This result aligned with current research, which showed insignificant differences in hardness values after the control and LON treatment groups. The negligible differences in hardness values were also observed in another study, which compared four disinfectant agents, including chlorhexidine (Carvalho et al., 2012). Acrylic resin is hydrophilic, easy to absorb water, and acts as a plasticizer; therefore, the decline of hardness value was also possible because of the formation of cracking zones due to the water absorption process and cycle (Mohialdeen et al., 2014).

CONCLUSION

The submersion of acrylic resin in *Cymbopogon citratus* nanoemulsion effectively inhibited the colony growth of *Streptococcus mutans* and *Candida albicans* without affecting the acrylic resin's mechanical properties.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Sugiaman VK	Saputri RI	Naliani S	Amalia J	Jeffrey
Concepts or ideas	x				
Design	x				x
Definition of intellectual content	x	x	x	x	x
Literature search	x	x	x	x	x
Experimental studies	x	x	x	x	x
Data acquisition	x	x	x	x	x
Data analysis	x		x		x
Statistical analysis		x	x		
Manuscript preparation	x			x	x
Manuscript editing	x				x
Manuscript review	x	x	x	x	x

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