BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

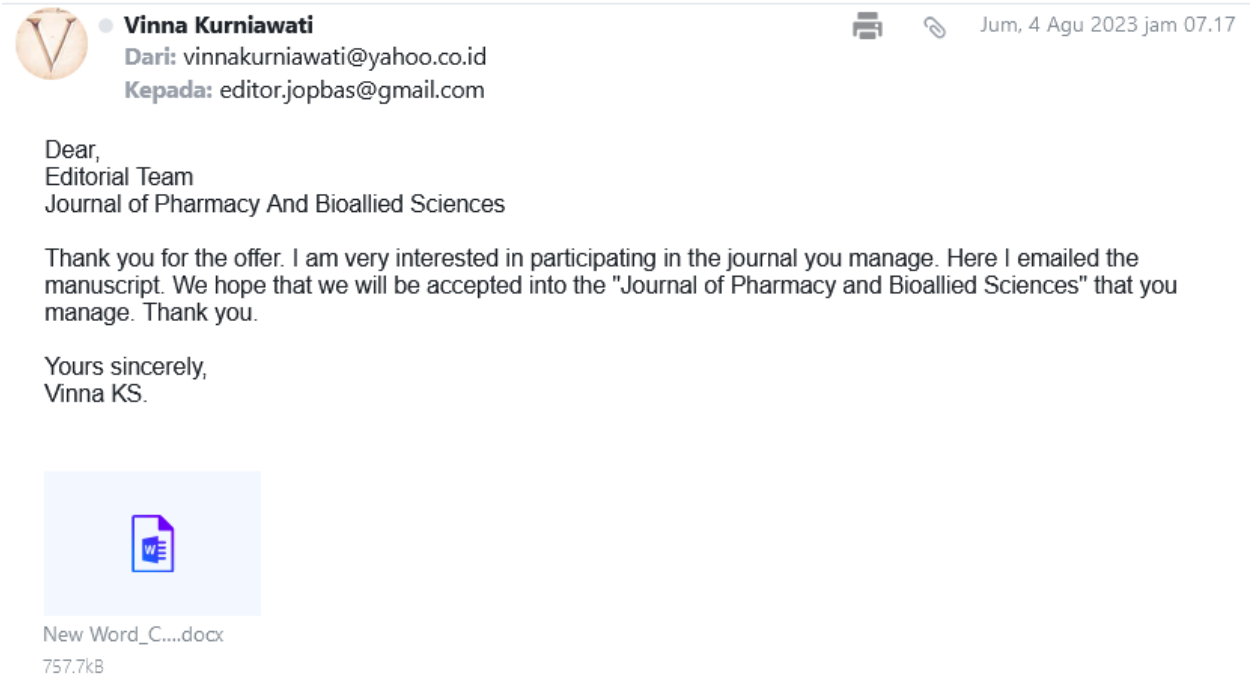
Judul Artikel : Characterization of Lemongrass Extract (Cymbopagon citratus) Nanoemulsion and Its Application as an Antibiofilm Agent in Acrylic Resin

Jurnal : Journal of Pharmacy and Bioallied Sciences

Penulis : Vinna Kurniawati Sugiaman, Rosalina Intan Saputri, Silvia Naliani, Jane Amelia Febriani Wibisono, Jeffrey, Wayan Larissa Demolsky,Wahyu Widowati, Agung Novianto

No	Perihal	Tanggal
1.	Register pada Journal of Pharmacy and Bioallied Sciences	Melalui email
2.	Bukti konfirmasi submit artikel dan artikel yang disubmit	04 Agustus 2023
3.	Bukti melakukan review yang pertama	20 Oktober 2023
4.	Bukti konfirmasi submit revisi pertama yang telah direvisi	24 Oktober 2023
5.	Bukti melakukan review yang kedua	-
6.	Bukti konfirmasi submit artikel yang telah revisi kedua	-
7.	Bukti konfirmasi artikel diterima	17 April 2024
8.	Bukti Galery Proof Manuscript	-
9.	Bukti Publiksi Online Artikel	31 Juli 2024

Bukti konfirmasi submit artikel dan artikel yang disubmit (04 Aguatus 2024)



Vinna Kurniawati
Dari: vinnakurniawati@yahoo.co.id
Kepada: editor.jopbas@gmail.com

Jum, 4 Agu 2023 jam 07.17

Dear,
Editorial Team
Journal of Pharmacy And Bioallied Sciences

Thank you for the offer. I am very interested in participating in the journal you manage. Here I emailed the manuscript. We hope that we will be accepted into the "Journal of Pharmacy and Bioallied Sciences" that you manage. Thank you.

Yours sincerely,
Vinna KS.

New Word_C....docx
757.7kB

Characterization of Lemongrass Extract (Cymbopagon citratus) Nanoemulsion and Its Application as Antibiofilm Agent in Acrylic Resin

Vinna Kurniawati Sugiaman[1], Rosalina Intan Saputri[2], Silvia Naliani[3], Jane Amelia Vebriani Wibisono[3], Jeffrey[4], Wayan L Demolsky[5], Wahyu Widowati[6], Agung Novianto[7]

[1]Department of Oral Biology, Faculty of Dentistry, Maranatha Christian University, Bandung, West Java, Indonesia. Email: vinnakurniawati@yahoo.co.id
[2]Department of Biomedical Science, Faculty of Dentistry, Maranatha Christian University, Bandung, West Java, Indonesia. Email: rosalina.is@dent.maranatha.edu
[3]Department of Prosthodontics, Faculty of Dentistry, Maranatha Christian University, Bandung, West Java, Indonesia. Email: silvianaliani@gmail.com, jane.aw@dent.maranatha.edu
[4]Department of Pediatric Dentistry, Faculty of Dentistry, Jenderal Achmad Yani University, Cimahi, West Java, Indonesia. Email: jeffrey_dent2000@yahoo.com
[5]Faculty of Dentistry, Maranatha Christian University, Bandung, West Java, Indonesia. Email: 2195040@dent.maranatha.edu
[6]Department of Pharmacology, Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia. Email: wahyu.widowati@maranatha.edu
[7]Aretha Medica Utama, Biomedical and Biomolecular Research Centre, Bandung, West Java, Indonesia. Email: agungnovianto@amubbrc.co.id

*** Correspondence:**
Vinna Kurniawati Sugiaman
vinnakurniawati@yahoo.co.id

Keywords: ***Acrylic resin, Antibiofilm, Candida albicans, Cymbopogon citratus, Nanoemulsion, Streptococcus mutans*****.**

Abstract

Background: Using acrylic resin dentures can contribute to oral problems without adequate cleaning. One of the oral problems is denture stomatitis which causes by the surface adhesion of *Streptococcus mutans (S. mutans)* and *Candida albicans (C. albicans).* Therefore, an antimicrobial agent is needed for denture cleaning, such as lemongrass, which has a bioactive antimicrobial component. Smaller particle sizes will provide more potent antimicrobial activity because of the ability to penetrate intercellular space. Thus, nanoparticle antimicrobial is a potential alternative to denture cleaner. **Methods:** This research analyzed lemongrass extract nanoparticles with a Particle Size Analyzer (PSA), Zeta Potential Analysis (ZPA), and biofilm formation inhibition in resin acrylic surface were examined. **Results:** Lemongrass oil nanoemulsion (LON) showed high stability in nanoparticle size. The highest LON inhibition percentage against biofilm was 95.59 ± 0.54% for 100% concentration. A paired t-test on resin acrylic hardness showed LON with concentrations of 100% and 50% had significant differences with a p-value of 0.022 and 0.021. While other concentrations, including chlorhexidine as a positive control, did not show any statistical differences. **Conclusion:** Lemongrass oil nanoemulsion (LON) has proved to be an antibiofilm and effective as a denture cleaning agent because of its ability to inhibit Streptococcus mutans and Candida albicans growth.

1 Introduction

Denture stomatitis affects 20-67% or around two-thirds of removable denture wearers. Common signs of this condition are inflammation and redness on mucosal tissue under the denture, with symptoms like discomfort, burning sensation, pain, and taste disorder. Poor oral hygiene is the most significant risk factor, which promotes bacterial colonization and adhesion in the denture base and oral cavity to form biofilm.[1–4] Thicker and more complex biofilm organization will contribute to more severe condition.[5,6] *Candida albicans* and *Streptococcus mutans* are the commonest fungi and bacteria related to this condition.[3,7–11]

The application of broad-spectrum and long-lasting antimicrobial agents can minimalize or remove biofilm adhesion and prevent denture stomatitis.[9] However, this application has disadvantages, such as high cost, dependence on patient compliance, and antimicrobial resistance potential. Chemical antimicrobial agents also potentially harm the environment and possess toxic effects on human health.[3,14–16]

Recently there has been increasing initiatives to utilize natural ingredient in medication as an alternative to overcome the disadvantages of chemical agents.[17,18] Plants' secondary metabolites or bioactive components, such as flavonoid, tannin, phenol, saponin, alkaloid, steroid, glycoside, dan carbohydrate, showed antimicrobial activities.[3,15,18,19]

Lemongrass is one of the herbal plants which have been widely studied.[18] This herb is a long-living plant with a particular smell that is inexpensive and easily found in the market. Citral is a bioactive component responsible for the smell and has an antimicrobial role.[18,20] Citral can prevent yeast biofilm formation by altering ergosterol biosynthesis, increasing membrane fluidity, and preventing membrane synthesis, spore germination, proliferation and respiration.[18,20–22]

Fabrication of nanoparticles is a solution for low availability of natural products.[23,24] Smaller particle sizes will perform higher antimicrobial effects because of the ability to penetrate the intercellular space.[24,25] Several studies have shown that nanoparticles of natural products provide higher broad-spectrum antibacterial and fungicidal activity through hyphae inhibitory mechanism.[26–28]

Based on these backgrounds, we aim to fabricate lemongrass oil nano emulsion and test its potential against *S.mutans* and *C.albicans* as opportunistic microbes commonly found in denture stomatitis.

2 Materials and Methods

Lemongrass Oil Extraction

Lemongrass oil was produced using standardized herbal medicine extraction protocol in PT Indesso Aroma Pharmaceutical Industry, Baturaden, Purwokerto, Central Java, Indonesia.

Lemongrass Oil Nanoemulsion (LON) Production

Five ml of lemongrass oil (PT Indesso) was mixed with 5 ml propylene glycol (Carbowax-X29160), 5 ml PEG 400 (Carbowax-X29160**),** and 10 ml glycerin (Merck-104094). The mixture was stirred until homogenous. Subsequently, 5 ml of chromophore was added to the mixture.

Measurement of Particle Size Analysis (PSA)

Particle size was measured at HeNe 4 mW and 633nm wavelength laser source in Particle Size Analyzer (Horiba SZ-100). The measurement range (hydrodynamic size) was between 0.6nm– 9000nm, depending on the sample type.

Zeta Potential Analysis (ZPA) Measurement

ZPA was done using Particle Size Analyzer (PSA) (Horiba SZ-100) at 25^{o}C temperature. One milliliters of emulsion was put in a zeta potential cuvette and placed on the PSA holder.

Biofilm Preparation and Inhibition

Lemongrass Oil Nanoemulsion (LON) Preparation

Thirty milliliters of 100% LON stock were serially diluted in 10% DMSO to following concentrations: 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Each solution was then sterilized using a 0.22 µm pore syringe filter (Sartorius, 17845).

S. mutans and *C. albicans* Inoculum Preparation

S. mutans (ATCC-25175) dan *C. albicans* (ATCC-10231) were cultured in Mueller Hinton Broth media for 24 hours at 37^{o}C before being used for broth microdilution assay.

Acrylic Specimen Fabrication

A 5 mm diameter and 2 mm thickness flat cylinder with baseplate wax (Cavex) was placed into a cuvette mould. Next, a cold mould seal (CMS) was smeared into the cuvette, and the wax was boiled out. Heat-cured acrylic (ADM, ISO 1567) was stirred and poured into the cuvette, then cured for 60 minutes at 100°C. The acrylic was finished and polished, yielding a 400 grits acrylic specimen.

S. mutans and *C. albicans* Biofilm Inhibition

S. mutans and *C. albicans* were cultured in Brain Heart Infusion Broth (Himedia-M210) for 24 hours at 37°C. Fifty microliters of *S. mutans* and *C. albicans* (0.5 McFarland) were pipetted to 96 well plates (Costar, 3596) containing heat-cured acrylic. Then, 100uL of LON serial solutions were added. The microplate was then placed in an incubator (Thermo IH3543) for 48 hours at 37°C. After incubation, the remaining liquid was discharged and washed two times with Phosphate Buffer Saline (PBS) (Biowest). The well was then filled with 125 ul Crystal Violet 0.1% (Himedia - TC510), incubated for 10-15 minutes, and rinsed three times with sterile aquadest. Last, 125 uL of 30% acetic acid was added. The absorbance was measured at a wavelength of 625 nm.

Biofilm growth was measured by comparing the Optical Density (OD) of each treatment groups and blanks. OD formula was described as follows:

$$OD = 1 - \frac{(xODs - xODbs)}{(xODp)} \times 100\%$$

Notes:

ODs : OD of the tested sample (405 nm).
ODbs : OD of a blank sample.
ODp : OD of tested solvent - OD of blank solvent.

3 Results

Lemongrass Oil Nanoemulsion Particle Size

Three nano emulsion samples had the following average diameters: 63.5 nm, 62.2 nm, and 61.4 nm. The fabricated LON is categorized as a nanoparticle because the diameter is between 1-100 nm (Haraya, 2022). Particle 3 had the best result of particle distribution index (PDI = 0.555), followed by particle 2 (PDI = 0.578) and 1 (PDI = 0.603). These values showed that our nano-emulsion particles had homogenous particle distribution. PDI > 0.7 indicates an overly broad distribution of particle size and the possibility of sedimentation.

Zeta Potential Analysis (ZPA)

Zeta potential values of 3 LON samples are adequate with the following results: -48.6mV, -49.2mV, dan -47.4mV (Figure 1). It denotes that the fabricated nanoparticles have a high stability because of their charge to prevent particle agglomeration and sedimentation.[29] This also demonstrated how the manufactured nanoparticles would work well in suspension, be more effectively absorbed by the cell membrane, and be less toxic.[30]

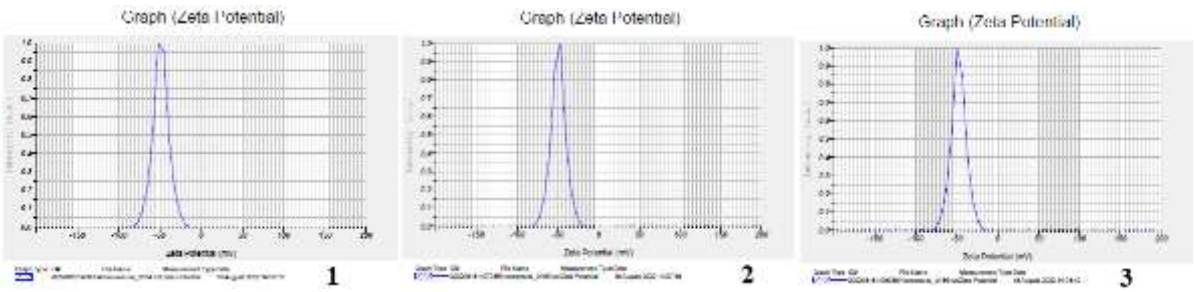


Figure 1 Zeta Potential Measurement of Lemongrass Oil

Biofilm Inhibition of *S.mutans* and *C. albicans*

The highest percentage value of LON inhibition against *S.mutans* and *C. albicans* biofilm was 95.59 ± 0.54% at 100% concentration. This value was nearly at the same rate as the inhibition value of chlorhexidine 0.2%, which was at 99.61 ± 0.01% (Table 1 and Figure 2).

Table 1 Biofilm Inhibition Result of *S. mutans* and *C. albicans*

Sample	Inhibition (%)
LON1	95.59 ± 0.54^{f}
LON2	89.59 ± 1.00^{e}
LON3	68.07 ± 0.08^{d}
LON4	41.19 ± 1.00^{c}
LON5	27.26 ± 0.18^{b}
LON6	8.01 ± 0.74^{a}
Chlorhexidine 0.2%	99.61 ± 0.01^{g}

* Data were presented in average ± deviation standard. a, b, c, d, e, f, g symbols showed significant differences in the Tukey HSD test ($p<0.05$).

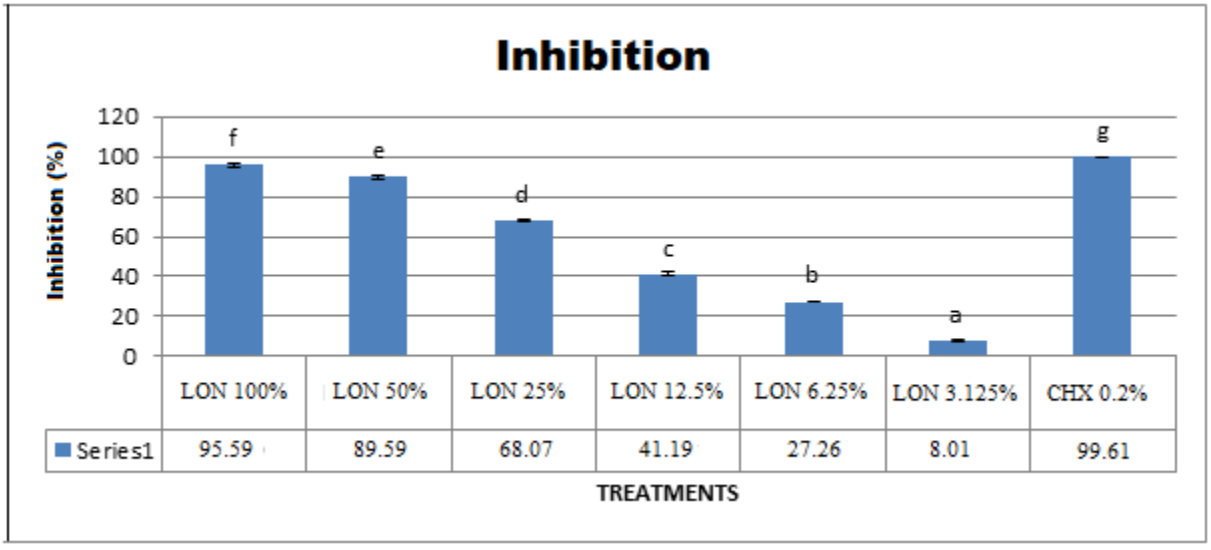


Figure 2 Inhibition percentage of Lemongrass Oil Nanoemulsion (LON) against *S. mutans* and *C. albicans* Biofilm.

4 Discussion

Nanoparticles can keep and protect natural active compounds. It can increase the potential of the extracts as therapeutics by delivering them more effectively and controlling the drug release, thus reducing dose and side effects. Previous studies showed that nanoparticles exhibited excellent stability and promoted the effectivity of antibacterial and antifungal. Smaller particles release drugs faster as they have a wider surface area.[32,33]

Physicochemical properties such as particle size, shape, and surface charge are essential for nanoparticle absorption. Negatively charged particles are absorbed easier by the cell membrane.[34] Particle size and shape from the current study were physicochemical characteristics of LON that significantly influenced particle activity.[35,36]

In addition to activity, these characteristics also influence the physical and chemical properties of the nanomaterial, such as electronic, optic, and catalytic abilities.[37] Surface charge is also related to the ability of nanoparticles to form aggregate. Attractive forces will cause the particles to form a growing cluster.[38] Based on the zeta-potential test, three LON formulas showed high stability because of their negative charge that prevents particle agglomeration and sedimentation.[34,39]

The composition, functional groups, and structure of an active biological component of a plant will have an essential role in determining antimicrobial activity. A previous study from Basera (2019) showed that variation and quantification of the chemical composition of lemongrass were related to the plant characteristics, seasonal and geographical conditions, environmental factors, ecological conditions, type of plant cultivations, harvesting time, part of plant utilized, storage condition, and process of plant extraction.[18,40,41]

A previous study showed that several essential oil constituents from lemongrass have activity as antimicrobials, including monoterpene (C10H16), diterpene (C20H32), sesquiterpene (C15H24), triterpene (C30H40), p-cymene, menthol, limonene, eugenol, anethole, geraniol, estragole, thymol, γ-terpinene, and cinnamyl alcohol.[42,43] Antimicrobial activity is related to the constituent's oxygen contents.[44,45] Higher oxygen compounds, such as in geranial, will provide more effective antimicrobial activity.[46]

The current study showed that LON has antibacterial and antifungal effects based on MIC and MBC tests, which was in line with Koseki *et al*. The ability of LON to inhibit *S. mutans* and *C. albicans* was nearly at the same rate as chlorhexidine, 0.2% against bacteria and fungi.[24,47] LON mechanism as antifungal is through inhibition of fungi metabolism and growth.[24]

Interaction between *S. mutans* and *C. albicans* can induce severe infection, which is challenging to treat. This interaction also increases the adhesion between species in biofilm and can cause microbial resistance.[48–51] Previous studies showed that bacterial biofilm could be inhibited or removed effectively by the active biology component of herbal plants.[52] One of the examples was the essential oil component of lemongrass, which showed various biological activities, including antimicrobial, antioxidant, antifungal, antiinflammation, and antibiofilm.[50,53]

The strategy of natural ingredients development as antibiofilm was to prevent microbial adhesion, eradicate attached microorganisms on the acrylic surface, inhibit quorum sensing, and disturb early biofilm formation.[21,54] Biofilm inhibition can happen in the early phase of attachment by decreasing

planktonic cell adhesion on the acrylic surface. An active biological component of LON, citral, could penetrate and dissolve the exopolysaccharide matrix of mature biofilm.[55,56]

The essential oil component of LON has hydrophobic properties, which can easily penetrate the phospholipid bilayer chain and fatty acid of the bacteria cell membrane. The oil can alter cell membrane permeability and induce ion and intracellular leakage resulting in cell lysis and death. Other known bactericidal mechanism of LON is cell structure disruption, proton pump and ion channel inhibition, membrane potential decrease, cellular component coagulation, and adenosine triphosphate (ATP) formation inhibition.[20,43,57-60] The result is a significant reduction of biofilm formation to approximately 99,79%.[50,53]

p-Cymene [1-methyl-4-(1-methyl ethyl)-benzene], one of the active constituents in LON, is a hydrocarbon monoterpene aromatic with alkyl substitution and benzene ring with methyl and isopropyl groups substitution. This component showed various biological activities, including antibacterial, antifungal, antiviral, antiinflammation, antioxidant, antinociceptive, anticancer, and anxiolytic.[61–63] This component's hydrophobic property causes increase in bacteria cytoplasm membrane and cell wall permeability. Monoterpene can also interact with cell membrane phospholipids which cause cell leakage.[43,61,64,65]

Cis-carveol causes cell membrane function, permeability, and integrity losses, leading to leakage of essential cell components and bacteria cell death.[66,67] Citral, neral (cis-citral, citral B), and geranial (trans-citral, citral A) disrupt the cytoplasm wall, increasing cell permeability that leads to a significant number of proton and ion discharges and cell death.[52,68,69]

Citral and neral were aldehydes formed by geranial monoterpene in cis and trans configurations. These substances damage cell membranes, cytoplasm, and mitochondria and cause DNA leakage.[43,50,53] Citral also cause hyperpolarization of the cell membrane, which influences the pH balance inside and outside the cell, and changes in ATP concentration.[11,70] However, utilization of citral alone or combined, could inhibit planktonic cells and cause bacteriostatic effects on cell biofilm. Citral can disturb bacterial attachment and exopolysaccharide formation by interfering metabolism of carbohydrates, protein, and nucleic acid.[52]

Caryophyllene oxide, a sesquiterpene, has a gram-positive and gram-negative antibacterial effect. This component inhibits microbial growth because of its hydrophobic property, which disrupts membrane permeability. This component also had good antifungal activity.[44,71,72] Therefore, the LON is a favourable antibiofilm because of its ability as an anti-microorganism that inhibits cellular growth.[73]

A combination of mechanical and chemical denture cleaning often failed to remove *C. albicans* from denture biofilm.[74] One of the reasons is extracellular matrix material which prevented penetration of cleaning agent into deep layer organisms.[75] Additionally, acrylic resin porosity makes it difficult to clean mechanically; therefore, denture immersion in a disinfectant agent has been suggested.[76] Regular denture biofilm cleaning can prevent induced local and systemic oral diseases.[77] Dental prosthesis submersion is a chemical cleaning agent intended to inactivate bacteria, viruses, and fungi.[78] These commercial disinfectants contain a toxic component (glutaraldehyde) that is corrosive, irritative, and can stain tissues.[76]

5 Conclusion

Lemongrass oil nanoemulsion (LON) has proved to be an antibiofilm and effective as a denture cleaning agent because of its ability to inhibit Streptococcus mutans and Candida albicans growth.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

VKS: Writing – review & editing, Conceptualization, Project administration; RIS: Writing – review & editing, Conceptualization, Methodology; SN: Writing – original draft, Conceptualization, Methodology; JAVW: Writing – review & editing, Formal Analysis, Methodology; J: Writing – reviewing & editing; WLD: Writing – reviewing & editing; WW: Writing – reviewing & editing; AN: Writing – reviewing & editing.

8 Funding

Funding support from Maranatha Christian University under Grant number 024/SK/ADD/UKM/VII/2022

9 Acknowledgments

The authors would like to express gratitude to Maranatha Christian University for its support throughout the research process.

10 References

1. Le Bars P, Kouadio AA, Bandiaky ON, Le Guéhennec L, de La Cochetière MF. Host's Immunity and Candida Species Associated with Denture Stomatitis: A Narrative Review. *Microorganisms*. 2022;10(7):1-21. doi:10.3390/microorganisms10071437
2. Sartawi SY, Abu-Hammad S, Salim NA, Al-Omoush S. Denture Stomatitis Revisited: A Summary of Systematic Reviews in the Past Decade and Two Case Reports of Papillary Hyperplasia of Unusual Locations. *Int J Dent*. 2021;2021. doi:10.1155/2021/7338143
3. Barua DR, Basavanna JM, Varghese RK. Efficacy of neem extract and three antimicrobial agents incorporated into tissue conditioner in inhibiting the growth of C. Albicans and S. Mutans. *J Clin Diagnostic Res*. 2017;11(5):ZC97-ZC101. doi:10.7860/JCDR/2017/23784.9950
4. Garbacz K, Kwapisz E, Wierzbowska M. Denture stomatitis associated with small-colony variants of Staphylococcus aureus: A case report. *BMC Oral Health*. 2019;19(1):1-4. doi:10.1186/s12903-019-0910-6
5. Sahin C, Ergin A, Ayyildiz S, Cosgun E, Uzun G. Effect of biofilm formation, and biocorrosion on denture base fractures. *J Adv Prosthodont*. 2013;5(2):140-148. doi:10.4047/jap.2013.5.2.140
6. Abdullah SN, Syed Abdurahman SN, Azzimi Nik Zulkifli NMF, Abd Ghafar SA, Abdullah SN. Biofilm Formation between Species Associated with Denture Stomatitis. *Dent Oral Biol Craniofacial Res*. Published online 2020:1-3. doi:10.31487/j.dobcr.2020.06.02

7. Volchkova IR, Yumashev AV, Borisov VV, Doroshina VI, Kristal EA, Repina SI. Influence of Removable Denture Cleaning Agents on Adhesion of Oral Pathogenic Microflora In Vitro: A Randomized Controlled Trial. *Open Dent J*. 2021;14(1):656-664. doi:10.2174/1874210602014010656
8. Fujiwara N, Murakami K, Yoshida K, et al. Suppressive effects of 2-methacryloyloxyethyl phosphorylcholine (MPC)-polymer on the adherence of Candida species and MRSA to acrylic denture resin. *Heliyon*. 2020;6(6):e04211. doi:10.1016/j.heliyon.2020.e04211
9. Günther E, Kommerein N, Hahnel S. Biofilms on polymeric materials for the fabrication of removable dentures. *Dtsch Zahnarztl Z*. 2020;2(4):142-151.
10. Nair VV, Karibasappa GN, Dodamani A, Prashanth VK. Microbial contamination of removable dental prosthesis at different interval of usage: An in vitro study. *J Indian Prosthodont Soc*. 2016;16(4):346-351. doi:10.4103/0972-4052.176536
11. Shi C, Song K, Zhang X, et al. Antimicrobial activity and possible mechanism of action of citral against cronobacter sakazakii. *PLoS One*. 2016;11(7):1-12. doi:10.1371/journal.pone.0159006
12. Bajunaid SO. How Effective Are Antimicrobial Agents on Preventing the Adhesion of Candida albicans to Denture Base Acrylic Resin Materials? A Systematic Review. *Polymers (Basel)*. 2022;14(5). doi:10.3390/polym14050908
13. O'Donnell LE, Robertson D, Nile CJ, et al. The oral microbiome of denture wearers is influenced by levels of natural dentition. *PLoS One*. 2015;10(9):1-22. doi:10.1371/journal.pone.0137717
14. Zhang Y, Chen YY, Huang L, Chai ZG, Shen LJ, Xiao YH. The antifungal effects and mechanical properties of silver bromide/cationic polymer nano-composite-modified Poly-methyl methacrylate-based dental resin. *Sci Rep*. 2017;7(1):1-13. doi:10.1038/s41598-017-01686-4
15. HN K. Use of Natural Products in the Prevention and Treatment of Denture Stomatitis. *Open Access J Biomed Sci*. 2020;1(5):201-206. doi:10.38125/oajbs.000146
16. An S, Judge RB, Wong RH, Arzmi MH, Palamara JE, Dashper SG. Incorporation of the microencapsulated antimicrobial agent phytoncide into denture base resin. *Aust Dent J*. 2018;63(3):302-311. doi:10.1111/adj.12640
17. Okocha RC, Olatoye IO, Adedeji OB. Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Rev*. 2018;39(1):1-22. doi:10.1186/s40985-018-0099-2
18. Basera P, Lavania M, Agnihotri A, Lal B. Analytical investigation of Cymbopogon citratus and exploiting the potential of developed silver nanoparticle against the dominating species of pathogenic bacteria. *Front Microbiol*. 2019;10(FEB):1-13. doi:10.3389/fmicb.2019.00282
19. Ribeiro SM, Fratucelli ÉDO, Bueno PCP, et al. Antimicrobial and antibiofilm activities of Casearia sylvestris extracts from distinct Brazilian biomes against Streptococcus mutans and Candida albicans. *BMC Complement Altern Med*. 2019;19(1):308. doi:10.1186/s12906-019-2717-z
20. E. K, F.R. V, I. A, et al. Antimicrobial action and anti-corrosion effect against sulfate reducing bacteria by lemongrass (Cymbopogon citratus) essential oil and its major component, the citral. *AMB Express*. 2013;3(44):1-8. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L370199622%5Cnhttp://dx.doi.org/10.1186/2191-0855-3-44
21. Sahal G, Woerdenbag HJ, Hinrichs WLJ, et al. Antifungal and biofilm inhibitory effect of Cymbopogon citratus (lemongrass) essential oil on biofilm forming by Candida tropicalis isolates;

an in vitro study. *J Ethnopharmacol*. 2020;246. doi:10.1016/j.jep.2019.112188

22. Shariati A, Didehdar M, Razavi S, Heidary M, Soroush F, Chegini Z. Natural Compounds: A Hopeful Promise as an Antibiofilm Agent Against Candida Species. *Front Pharmacol*. 2022;13(July):1-20. doi:10.3389/fphar.2022.917787
23. Ajayi E, Afolayan A. Green synthesis, characterization and biological activities of silver nanoparticles from alkalinized Cymbopogon citratus Stapf. *Adv Nat Sci Nanosci Nanotechnol*. 2017;8(1). doi:10.1088/2043-6254/aa5cf7
24. Riyanto, Mulwandari M, Asysyafiiyah L, Sirajuddin MI, Cahyandaru N. Direct synthesis of lemongrass (Cymbopogon citratus L.) essential oil-silver nanoparticles (EO-AgNPs) as biopesticides and application for lichen inhibition on stones. *Heliyon*. 2022;8(6). doi:10.1016/j.heliyon.2022.e09701
25. Rakib-Uz-Zaman SM, Hoque Apu E, Muntasir MN, et al. Biosynthesis of Silver Nanoparticles from Cymbopogon citratus Leaf Extract and Evaluation of Their Antimicrobial Properties. *Challenges*. 2022;13(1):18. doi:10.3390/challe13010018
26. Li X, Robinson SM, Gupta A, et al. Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. *ACS Nano*. 2014;8(10):10682-10686. doi:10.1021/nn5042625
27. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int J Nanomedicine*. 2017;12:1227-1249. doi:10.2147/IJN.S121956
28. Sharmin S, Rahaman MM, Sarkar C, Atolani O, Islam MT, Adeyemi OS. Nanoparticles as antimicrobial and antiviral agents: A literature-based perspective study. *Heliyon*. 2021;7(3):e06456. doi:10.1016/j.heliyon.2021.e06456
29. Mohamed RM, Fawzy EM, Shehab RA, Abdel-Salam MO, Salah El Din RA, Abd El Fatah HM. Production, Characterization, and Cytotoxicity Effects of Silver Nanoparticles from Brown Alga (Cystoseira myrica). *J Nanotechnol*. 2022;2022. doi:10.1155/2022/6469090
30. Rasmussen MK, Pedersen JN, Marie R. Size and surface charge characterization of nanoparticles with a salt gradient. *Nat Commun*. 2020;11(1):1-8. doi:10.1038/s41467-020-15889-3
31. Chen T, Yao Q, Nasaruddin RR, Xie J. Electrospray Ionization Mass Spectrometry: A Powerful Platform for Noble-Metal Nanocluster Analysis. *Angew Chemie*. 2019;131(35):12093-12103. doi:10.1002/ange.201901970
32. Bonifácio BV, da Silva PB, Aparecido dos Santos Ramos M, Maria Silveira Negri K, Maria Bauab T, Chorilli M. Nanotechnology-based drug delivery systems and herbal medicines: A review. *Int J Nanomedicine*. 2013;9(1):1-15. doi:10.2147/IJN.S52634
33. Dwi Saryanti, Dian Nugraheni, Nisa Sindi Astuti. Preparation and Characterization of Betel Leaves (Piper betle Linn) Extract Nanoparticle with Ionic Gelation Method. *J Trop Pharm Chem*. 2020;5(1):15-20. doi:10.25026/jtpc.v5i1.224
34. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - A review (Part 1). *Trop J Pharm Res*. 2013;12(2):255-264. doi:10.4314/tjpr.v12i2.19
35. Rice SB, Chan C, Brown SC, et al. Particle size distributions by transmission electron microscopy: An interlaboratory comparison case study. *Metrologia*. 2013;50(6):663-678. doi:10.1088/0026-1394/50/6/663
36. Wen H, Luna-Romera JM, Riquelme JC, Dwyer C, Chang SLY. Statistically representative

metrology of nanoparticles via unsupervised machine learning of tem images. *Nanomaterials*. 2021;11(10):1-13. doi:10.3390/nano11102706

37. Lee B, Yoon S, Lee JW, et al. Statistical Characterization of the Morphologies of Nanoparticles through Machine Learning Based Electron Microscopy Image Analysis. *ACS Nano*. 2020;14(12):17125-17133. doi:10.1021/acsnano.0c06809
38. Oktavia IN, Sutoyo S. Article Review: Synthesis of Silver Nanoparticles Using Bioreductor From Plant Extract As an Antioxidant. *UNESA J Chem*. 2021;10(1).
39. Abdassah M. Nanopartikel dengan gelasi ionik. *J Farmaka*. 2017;15(1):45-52.
40. Voon HC, Bhat R, Rusul G. Flower extracts and their essential oils as potential antimicrobial agents for food uses and pharmaceutical applications. *Compr Rev Food Sci Food Saf*. 2012;11(1):34-55. doi:10.1111/j.1541-4337.2011.00169.x
41. Gunasena MT, Rafi A, Afif S, et al. Mechanism of Action of Essential Oil Extracted from Ginger of Rice. Published online 2022.
42. Tanhaeian A, Sekhavati MH, Moghaddam M. Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens. *Chem Biol Technol Agric*. 2020;7(1):1-11. doi:10.1186/s40538-020-00181-9
43. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines*. 2017;4(3):58. doi:10.3390/medicines4030058
44. Islam M, Amin M, Ahmed M, et al. In-vitro antimicrobial activity of essential oils and different organic extracts of Lippia alba. *J Phytochem Biochem*. 2018;2(1):1-5. https://www.researchgate.net/publication/336133612
45. Chlif N, Ed-Dra A, Diouri M, et al. Chemical composition, antibacterial and antioxidant activities of essential oils extracted from dry and fresh brocchia cinerea. *Biodiversitas*. 2021;22(4):1741-1749. doi:10.13057/biodiv/d220418
46. Hussein KA, Joo JH. Antifungal activity and chemical composition of ginger essential oil against ginseng pathogenic fungi. *Curr Res Environ Appl Mycol*. 2018;8(2):194-203. doi:10.5943/cream/8/2/4
47. Koseki Y, Tanaka R, Murata H. Development of antibacterial denture cleaner for brushing containing tea tree and lemongrass essential oils. *Dent Mater J*. 2018;37(4):659-666. doi:10.4012/dmj.2017-295
48. Jafri H, Banerjee G, Khan MSA, Ahmad I, Abulreesh HH, Althubiani AS. Synergistic interaction of eugenol and antimicrobial drugs in eradication of single and mixed biofilms of Candida albicans and Streptococcus mutans. *AMB Express*. 2020;10(1). doi:10.1186/s13568-020-01123-2
49. Bertolini M de M e., Cavalcanti YW, Bordin D, da Silva WJ, Del Bel Cury AA. Candida albicans biofilms and MMA surface treatment influence the adhesion of soft denture liners to PMMA resin. *Braz Oral Res*. 2014;28(1):61-66. doi:10.1590/S1806-83242013005000025
50. Da Silva NB, Lucena Rangel M De, De Castro RD, et al. Anti-biofilm and hemolytic effects of cymbopogon citratus (dc) stapf essential oil. *Pesqui Bras Odontopediatria Clin Integr*. 2019;19(1):1-10. doi:10.4034/PBOCI.2019.191.103
51. Gabrilska RA, Rumbaugh KP. Biofilm models of polymicrobial infection. *Future Microbiol*. 2015;10(12):1997-2015. doi:10.2217/fmb.15.109
52. Lim AC, Tang SGH, Zin NM, et al. Chemical Composition, Antioxidant, Antibacterial, and

Antibiofilm Activities of Backhousia citriodora Essential Oil. *Molecules*. 2022;27(15):1-20. doi:10.3390/molecules27154895

53. Guimarães R, Milho C, Liberal Â, et al. Antibiofilm potential of medicinal plants against Candida spp. Oral biofilms: A review. *Antibiotics*. 2021;10(9):1-17. doi:10.3390/antibiotics10091142
54. Ariani N, Visser A, Teulings MRIM, et al. Efficacy of cleansing agents in killing microorganisms in mixed species biofilms present on silicone facial prostheses—an in vitro study. *Clin Oral Investig*. 2015;19(9):2285-2293. doi:10.1007/s00784-015-1453-0
55. Mizan MFR, Ashrafudoulla M, Hossain MI, Cho HR, Ha S Do. Effect of essential oils on pathogenic and biofilm-forming Vibrio parahaemolyticus strains. *Biofouling*. 2020;36(4):467-478. doi:10.1080/08927014.2020.1772243
56. Kerekes EB, Vidács A, Takó M, et al. Anti-biofilm effect of selected essential oils and main components on mono- and polymicrobic bacterial cultures. *Microorganisms*. 2019;7(9). doi:10.3390/microorganisms7090345
57. Srivastava A, Kumar V, B VA. *Proceedings of the Conference BioSangam 2022: Emerging Trends in Biotechnology (BIOSANGAM 2022)*. Atlantis Press International BV; 2023. doi:10.2991/978-94-6463-020-6
58. Freires IA, Bueno-Silva B, Galvão LCDC, et al. The effect of essential oils and bioactive fractions on streptococcus mutans and Candida albicans biofilms: A confocal analysis. *Evidence-based Complement Altern Med*. 2015;2015. doi:10.1155/2015/871316
59. Cui H, Zhang X, Zhou H, Zhao C, Lin L. Antimicrobial activity and mechanisms of Salvia sclarea essential oil. *Bot Stud*. 2015;56. doi:10.1186/s40529-015-0096-4
60. Lakehal S, A M. Essential Oil Composition and Antimicrobial Activity of Artemisia herba– alba Asso Grown in Algeria. *Med Chem (Los Angeles)*. 2016;6(6):435-439. doi:10.4172/2161-0444.1000382
61. Marchese A, Arciola CR, Barbieri R, et al. Update on monoterpenes as antimicrobial agents: A particular focus on p-cymene. *Materials (Basel)*. 2017;10(8):1-15. doi:10.3390/ma10080947
62. Balahbib A, El Omari N, Hachlafi N EL, et al. Health beneficial and pharmacological properties of p-cymene. *Food Chem Toxicol*. 2021;153(April):112259. doi:10.1016/j.fct.2021.112259
63. Tian F, Woo SY, Lee SY, Chun HS. p-Cymene and its derivatives exhibit antiaflatoxigenic activities against Aspergillus flavus through multiple modes of action. *Appl Biol Chem*. 2018;61(5):489-497. doi:10.1007/s13765-018-0382-4
64. Li H, Yang T, Li FY, Yao Y, Sun ZM. Antibacterial activity and mechanism of action of Monarda punctata essential oil and its main components against common bacterial pathogens in respiratory tract. *Int J Clin Exp Pathol*. 2014;7(11):7389-7398.
65. Azghar A, Dalli M, Loukili EH, et al. Evaluation of the Antibacterial Activity of Essential Oil of Dysphania ambrosioides (L.) Mosyakin and Clemants Against Clinical Multidrug-Resistant Bacteria. *Asian J Plant Sci*. 2023;22(1):75-81. doi:10.3923/ajps.2023.75.81
66. Guimarães AC, Meireles LM, Lemos MF, et al. Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*. 2019;24(13):1-12. doi:10.3390/molecules24132471
67. Ambrosio CMS, Diaz-Arenas GL, Agudelo LPA, Stashenko E, Contreras-Castillo CJ, da Gloria EM. Chemical composition and antibacterial and antioxidant activity of a citrus essential oil and its fractions. *Molecules*. 2021;26(10):1-16. doi:10.3390/molecules26102888

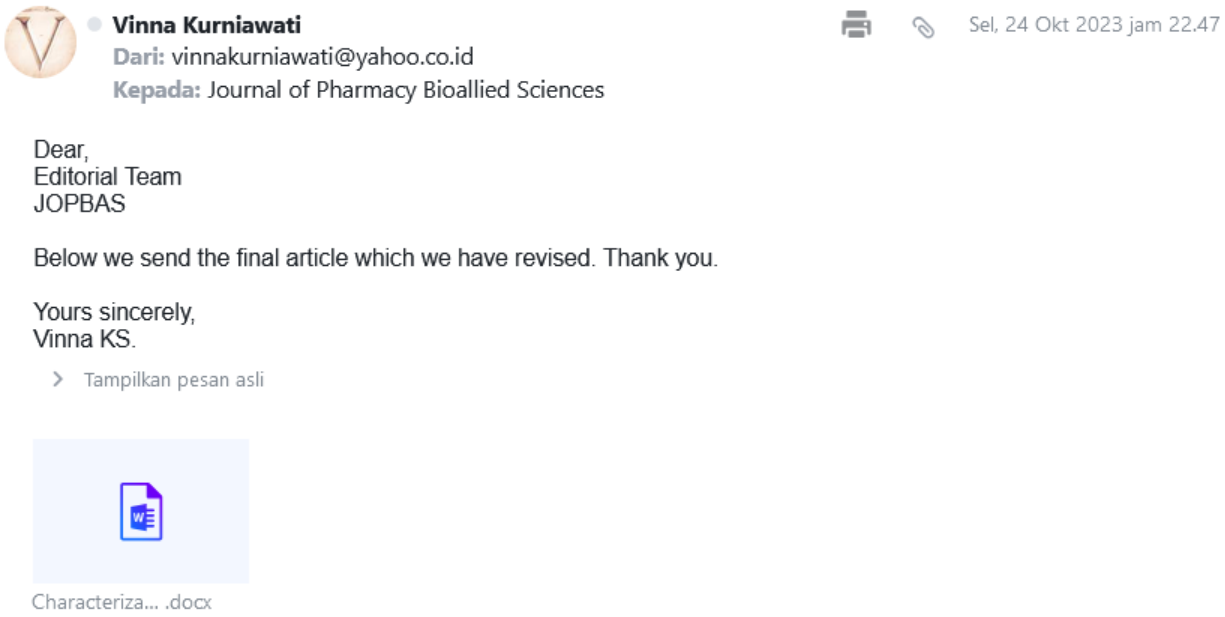
68. Do DN, Nguyen HTT, Huynh TH, Nguyen NP, Luu XC. Chemical composition, antibacterial and antioxidant activities of lemongrass (Cymbopogon citratus) essential oil and its fractions obtained by vacuum distillation. *IOP Conf Ser Mater Sci Eng*. 2021;1166(1):012051. doi:10.1088/1757-899x/1166/1/012051
69. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules*. 2012;17(4):3989-4006. doi:10.3390/molecules17043989
70. Zhang J, Du C, Li Q, et al. Inhibition mechanism and antibacterial activity of natural antibacterial agent citral on bamboo mould and its anti-mildew effect on bamboo. *R Soc Open Sci*. 2021;8(4). doi:10.1098/rsos.202244
71. Sobrinho ACN, de Morais SM, de Souza EB, et al. Antifungal and Antioxidant Activities of Vernonia Chalybaea Mart. ex DC. Essential Oil and their Major Constituent β-caryophyllene. *Brazilian Arch Biol Technol*. 2020;63. doi:10.1590/1678-4324-2020190177
72. Joshri N, Sah GC. GC-MS analysis and antimicrobial activity of essential oil of Nepeta coerulescens. *Int J Res Pharmacol Pharmacother*. 2014;3(1):68-71. http://www.ijrpp.com/view_content.php?quat=3&date=2014&issue=1
73. Cherian T, Ali K, Saquib Q, Faisal M, Wahab R, Musarrat J. Cymbopogon citratus functionalized green synthesis of cuo-nanoparticles: Novel prospects as antibacterial and antibiofilm agents. *Biomolecules*. 2020;10(2). doi:10.3390/biom10020169
74. de Lucena-Ferreira SC, Cavalcanti IMG, Del Bel Cury AA. Efficacy of denture cleansers in reducing microbial counts from removable partial dentures: A short-term clinical evaluation. *Braz Dent J*. 2013;24(4):353-356. doi:10.1590/0103-6440201302183
75. Yodmongkol S, Chantarachindawong R, Thaweboon S, Thaweboon B, Amornsakchai T, Srikhirin T. The effects of silane-SiO2 nanocomposite films on Candida albicans adhesion and the surface and physical properties of acrylic resin denture base material. *J Prosthet Dent*. 2014;112(6):1530-1538. doi:10.1016/j.prosdent.2014.06.019
76. PEREIRA CJ, GENARI B, LEITUNE VCB, COLLARES FM, SAMUEL SMW. Effect of immersion in various disinfectant solutions on the properties of a heat-cured acrylic resin. *RGO - Rev Gaúcha Odontol*. 2019;67:1-5. doi:10.1590/1981-86372019000523090
77. Rocha MM, Carvalho AM, Coimbra FCT, et al. Complete denture hygiene solutions: Antibiofilm activity and effects on physical and mechanical properties of acrylic resin. *J Appl Oral Sci*. 2021;29:1-11. doi:10.1590/1678-7757-2020-0948
78. Kati FA. Effects of chemical disinfectants on surface hardness of heat-cured acrylic resins. In vitro study. *J Oral Res*. 2021;10(6):1-6. doi:10.17126/JORALRES.2021.074

Bukti melakukan review yang pertama (20 Oktober 2023)

AQ1: Kindly provide running head title.
AQ2: Kindly provide revised date
AQ3: Kindly check Ref. Citation 44,45 and 46. References are not available
AQ4: Kindly check caption
AQ5: Kindly provide table in editable format
AQ6: Reference are renumbered in text and list for citation sequence

AQ7: The authors 'Rosalina I. Saputri, Silvia Naliani, Jane A. V. Wibisono, Jeffrey, Wayan L. Demolsky, Wahyu Widowati, Agung Novianto' have not agreed to the copyright terms and conditions which were sent on the author's email address.

Bukti konfirmasi submit revisi pertama yang telah direvisi (24 Oktober 2023)



Vinna Kurniawati
Dari: vinnakurniawati@yahoo.co.id
Kepada: Journal of Pharmacy Bioallied Sciences

Sel, 24 Okt 2023 jam 22.47

Dear,
Editorial Team
JOPBAS

Below we send the final article which we have revised. Thank you.

Yours sincerely,
Vinna KS.

Tampilkan pesan asli

Characteriza... .docx

Bukti konfirmasi artikel diterima (17 April 2024)

Dear Authors,

Vinna Kurniawati Sugiaman
Rosalina Intan Saputri
Silvia Naliani
Jane Amelia Vebriani Wibisono
Jeffrey
Wayan L Demolsky
Wahyu Widowati
Agung Novianto

Faculty of Dentistry, Maranatha Christian University, Bandung, West Java, Indonesia.
Faculty of Dentistry, Jenderal Achmad Yani University, Cimahi, West Java, Indonesia.
Aretha Medica Utama, Biomedical and Biomolecular Research Centre, Bandung, West Java Indonesia.

We are delighted to inform you that your research paper **"Characterization of Lemongrass Extract (Cymbopagon citratus) Nanoemulsion and Its Application as Antibiofilm Agent for Acrylic Resin"** has been accepted for publication in the ***Journal of Pharmacy and Bioallied Sciences***. Your paper has undergone rigorous peer review by experts in the field, and we believe that it meets the high standards of our journal.

Congratulations on your remarkable achievement! Your decision to use our journal to exhibit your work has been greatly appreciated. We are pleased to inform you that your paper has been selected for publication in the upcoming issue of the **JPBAS**.

We would like to express our sincere gratitude for your valuable contribution.

Best regards,

Editor-in-Chief
Journal of Pharmacy and Bioallied Sciences
ISSN:0976-4879; E-ISSN:0975-7406
https://journals.lww.com/JPBS/pages/default.aspx

Bukti Publiksi Online Artikel (31 Juli 2024)