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1.	Register pada Journal of International Dental and Medical Research	Melalui email
2.	Bukti konfirmasi submit artikel dan artikel yang disubmit	11 November 2022
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Dear Prof. Dr. Vinna Kurniawati Sugiaman,

Your manuscript entitled " **Antibacterial Differences Effect between the Onion Extract (Allium cepa L.) and Lemon Juice (Citrus limon (L.) Burm.f.) on In Vitro Growth of** *Enterococcus faecalis* " has been successfully submitted to the JIDMR by e-mail and will be considered for publication in **"Journal of International Dental and Medical Research"**.

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Antibacterial Differences Effect between the Onion Extract (Allium cepa L.) and Lemon Juice (Citrus limon (L.) Burm.f.) on In Vitro Growth of *Enterococcus faecalis*

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Abstract

Background *Enterococcus faecalis is a microorganism frequently found after root canal treatment (RCT) and is the common cause of RCT failure. Irrigation is a crucial step in ensuring the success of RCT. Sodium hypochlorite (NaOCl) is a commonly used irrigation agent. However, it can cause unwanted effects such as periapical tissue irritation, cytotoxicity, and tissue destruction. As a result, many companies are currently developing safer alternative irrigation materials. Onion extract and lemon juice contain antibacterial compounds such as flavonoids, phenols, tannins, triterpenoids, alkaloids, and saponins, which make them good candidates for irrigation agents.* ***Objectives*** *This study aims to test the antibacterial activity of onion extract and lemon juice against Enterococcus faecalis bacteria.* ***Methods*** *Antibacterial activity was performed by disc diffusion test for various concentrations of onion extract and lemon juice (100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.125%), positive control (NaOCl 5.25%), and negative control (aquadest).* ***Result*** *Average diameters of the inhibition zones at 100%, 75%, and 50% concentration of onion extract and lemon juice were 7.10mm, 6.58mm, 4.88mm, and*

15.47, 11.93mm, 9.98mm, respectively. ***Conclusions*** *Our result shows that onion extract and lemon juice might inhibit Enterococcus faecalis at minimum 50% concentration as shown by disc diffusion method.*

Keywords: antibacterial, *Enterococcus faecalis*, lemon juice, onion extract
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INTRODUCTION

Trauma, caries, or tooth fracture can result in irreversible or necrotic pulpitis, necessitating root canal treatment (RCT).[1] RCT aims to maintain teeth intact in the oral cavity, eliminate pathogens, and prevent infection in the root canal.[2,3] Cleaning, shaping, and three-dimensional obturation procedures are critical steps in preventing RCT failure.[4]

RCT failure occurs due to various causes, including insufficient root canal cleaning, poor obturation, improper coronal closure, untreated root canal, and persistent bacterial growth. Enterococcus faecalis is one of the bacteria that causes RCT failure.[5] *E.faecalis* is a facultative anaerobe gram-positive cocci bacterium found in tooth root canals as normal flora.[6] This bacteria can cause root canal contamination by colonizing the dentin surface with the help of lipoteichoic acid. Due to *E.faecalis* adhererence to the collagen surface, it is critical to remove all pulp tissue, dentin debris, and microorganisms that live in the root canal during treatment.[5,7]

Enterococcus faecalis was the most common bacteria found in cases of pain and infection after RCT with 90% prevalence.[8] Denny and Mieke (2013) found that 63% of RCT failures were caused by *E.faecalis* reinfection.[6] *E.faecalis* can survive in low-nutrient environments with acidic and alkaline pH (pH 4-11). This bacteria can form biofilm on root canal walls and penetrate as far as 50-300 µm into the dentin, allowing them to avoid instruments and irrigants during chemomechanical preparation.[9,10] This makes them resistant to commonly used antimicrobial agents and difficult to be eliminated from the root canals.[10]

Irrigation solutions are used to lubricate the root canal preparation, remove bacteria and bacterial products, remove the smear layer, and dissolve necrotic pulp tissue during the RCT procedure.[11] A good irrigation material must be able to dissolve organic and inorganic tissues, possess antimicrobial activity, and be low in toxicity. Sodium hypochlorite (NaOCl),

chlorhexidine digluconate, and EDTA have been commonly used as irrigation solutions. However, they may cause a variety of side effects.[12,13]

Sodium hypochlorite (NaOCl) is a commonly used and recommended primary irrigation solution. It possesses broad-spectrum antimicrobial properties and can dissolve vital and necrotic pulp tissue remnants. However, if extrusion occurs, a high concentration of NaOCl can irritate periapical tissue.[14] As a result, it is necessary to develop an alternative, safer root canal irrigation solution with good antimicrobial properties.[15,16]

The use of plants as alternative therapies has grown in popularity among the general public, with one example being the use of traditional medicines such as onions (*Allium cepa* L.) and lemon juice (*Citrus limon* (L.) Burm.f.). Onions have numerous health benefits, including anti-diabetic properties, the prevention of postoperative hypertrophic scars, headache relief, anti-cancer, anti-depressant, anti-allergic, and antibacterial properties.[17] Antibacterial effect of onions is due to several active substances they contain, including flavonoids, saponins, tannins, and steroids.[18,19]

Lemon juice contains many bioactive compounds that have antibacterial properties, including citric acid, saponins, triterpenoids, flavonoids, alkaloids, and tannins. Previous research showed that lemon juice could inhibit Staphylococcus aureus and Porphyromonas gingivalis growth as a representative of positive-gram and negative-gram bacteria, respectively.[20,21]

METHODS

The inhibitory activity of onion extract and lemon juice against *Enterococcus faecalis* was investigated using the Kirby Bauer disc diffusion assay. *Enterococcus faecalis* (ATCC 29212) from the American Type Culture Collection (ATCC) was used in the experiment. Onions (*Allium cepa* L.) were collected from vegetable plantations in Rancabali District, Bandung, Indonesia. Lemons (*Citrus limon* (L.) Burm.f.) were obtained from Padepokan Pandawa Lima plantations in Cibodas, Lembang, West Bandung, Indonesia. Plant determination was carried out at the Biosystematics and Molecular Laboratory, Department of Biology, Padjadjaran University, Indonesia with identification number No:26/LBM/IT/12/2021 and No:24/LBM/IT/12/2021 respectively.

Onion and Lemon Juice Extraction

One kilogram of onion is washed, cut, dried, and ground. Ethanolic extract was prepared by macerating onion powder in 96% ethanol for five days at room temperature. The extract was then concentrated using a rotary evaporator. Lemon juice was made by squeezing the fruit and separating the liquid from the seeds and pulps. For the experiment, onion extract and lemon juice were diluted with distilled water to 75%, 50%, 25%, 12.5%, 6.25%, and 3.125% concentration.

Phytochemical Screening of Onion Extract and Lemon Juice

Visual observation of colour changes or precipitate formation reactions revealed the presence of secondary metabolites. The Lieberman-Burchard reagent (acetic anhydride plus sulfuric acid) is used to characterize steroids (green colour) or triterpenes (red colour). The ferric chloride solution was used to detect phenols and tannins, and the Shinoda reagent (concentrated HCl and granulated magnesium ribbon) was used to detect flavonoids. Saponin is detected if a stable foam forms after the extract was mixed and shaked with concentrated HCl (2N). Alkaloid presence was detected using Bounchardat, Mayer, and Dragendorf reagents.

Disc Diffusion Assay

E.faecalis grown in blood agar overnight was used to make 0.5 McFarland suspension. The suspension was then inoculated onto Mueller Hinton Agar for disk diffusion assay. Paper discs with a diameter of 6 mm were immersed in diluted extracts, 5.25% NaOCl (positive control), and distilled water (negative control). The discs were then placed on the inoculated agar plates and incubated for 24 hours at 37^{o}C. The diameter of the inhibition zone was measured using a calliper. The tests for disc diffusion inhibition zones were performed in triplicates for each extract and the mean of the three values was used as the zone to interpret the result. Inhibitory activity was interpreted based on David and Scout criteria as follow: weak inhibition when the clear zone was less than 5mm, moderate inhibition when the clear zone was 5-10mm, strong inhibition when the clear zone was 10-20mm, and very strong inhibition when the clear zone was > 20mm.[20]

RESULTS

Phytochemical Test of Onion Extract and Lemon Juice

Qualitative phytochemical test result of onion extract and lemon juice can be seen in **Table 1**.

Table 1 Phytochemical Test Results on Onion Extract (*Allium cepa L.*) and Lemon Juice (*Limon citrus (L.) burm.f*)

No	Phytochemical Test	Test Results Onion Extract	Test Result Lemon Juice
1.	Flavonoid	(+++)	(-)
2.	Saponin	(-)	(+)
3.	Phenol	(++++)	(-)
4.	Tanin	(++++)	(-)
5.	Steroid/ Triterpenoid	(-) Steroid (+++) Triterpenoid	(-)
6.	Terpenoid	-	(+)
7.	Alkaloid	(++++)	(-)

Information:

++++ : very high content
+++ : high content
++ : medium content
+ : low content
- : negative content

Zone of Inhibition

Zone of inhibition observed in onion extract treatment can be seen in **Figure 1** and the average size of the diameter per concentration can be seen in **Figure 2**. It can be seen that the inhibition zone was formed at 50%, 75%, and 100% concentration with 4.88 mm, 6.58 mm, and 7.10 mm diameters. Based on the Davis and Scout criteria, the inhibition activity can be classified as weak, moderate, and moderate respectively. Significant difference was observed between groups as tested using Kruskal-Wallis test ($p=0.0002$). The inhibition zone formed around onion extracts were significantly lower compared to the positive control. There was a significant difference of zone of inhibition diameter formed by 50% extract concentration compared to 75% and 100% concentration.

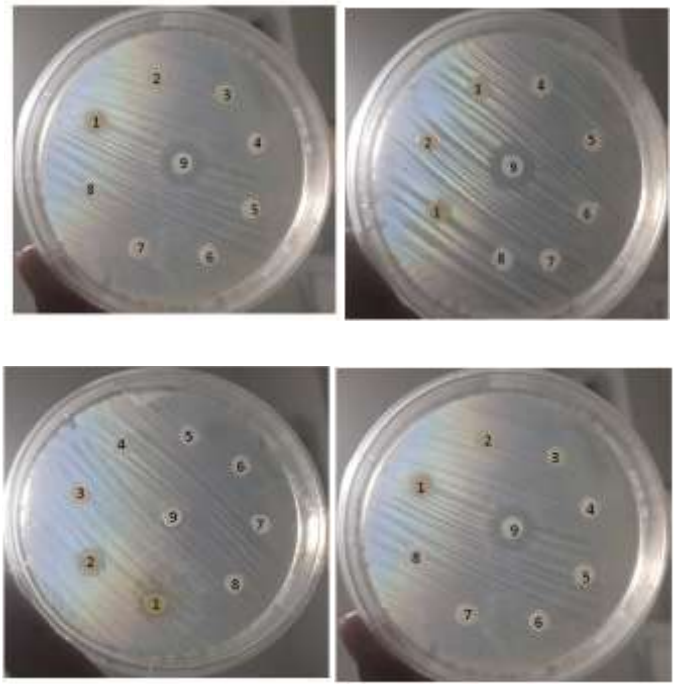

Figure 1. Observation Result of Zone of inhibition Diameter of Onion Extract (Allium cepa L.) against *Enterococcus faecalis*

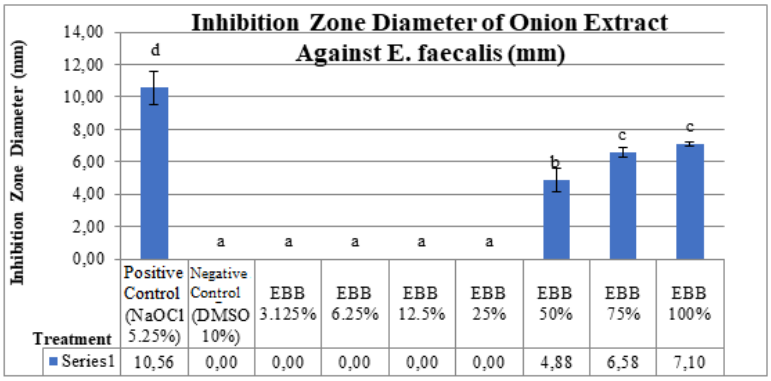

Figure 2. Comparison of Zone of inhibition Diameter of Onion Extract against *Enterococuus faecalis*

Zone of inhibition observed in lemon juice treatment can be seen in **Figure 3** and the average size of the diameter per concentration can be seen in **Figure 4**. It can be seen that the inhibition

zone was formed at 50%, 75%, and 100% concentration with 9.98 mm, 11.93 mm, and 15.47 mm diameters. Based on the Davis and Scout criteria, the inhibition activity can be classified as medium, high, and high respectively. Significant difference was observed between groups as tested using Kruskal-Wallis test (p=0.0000). The inhibition zone formed around 50% lemon juice was statistically equal to the positive control. Significantly higher inhibitory activity was observed for the 75% and 100% concentration.

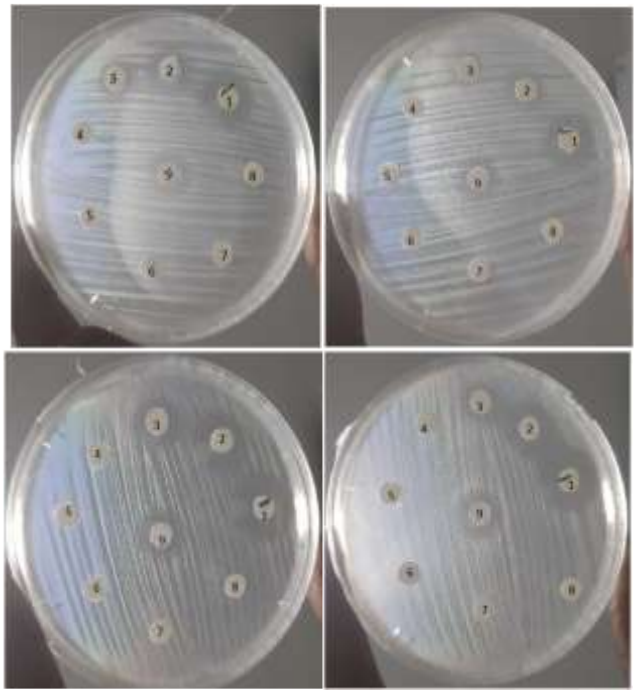

Figure 3. Observation Result of Zone of inhibition Diameter of Lemon Juice (*Citrus limon* (L) Burm. f.) against *Enterococcus faecalis*

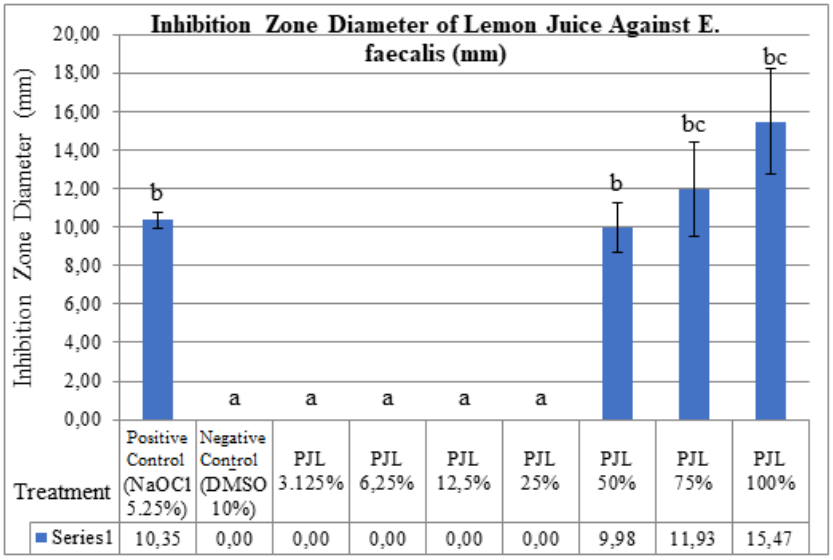

Figure 4. Comparison of the Inhibition Zone Diameter of Lemon Juice against *Enterococcus faecalis*

DISCUSSION

Onion extract and lemon juice formed zone of inhibition around *E.faecalis* inoculum based on disc diffusion assay started at 50% concentration. A dose-dependent response between size of the diameter and extract concentration was observed. This is in linear with higher concentration of active substances present in the extracts that perform the inhibitory activity.[21]

The qualitative phytochemical test showed onion extract contained flavonoids, phenols, tannins, triterpenoids, and alkaloids. Flavonoids have antibacterial, antioxidant, antitumor, anti-inflammatory, antiviral, and cancer-fighting properties.[22,23] Antibacterial activity of flavonoids is due to their energy, cell membrane function, and nucleic acid synthesis inhibitory mechanism. Energy inhibitory mechanism is through the limitation of oxygen use. Meanwhile, membrane function and nucleic acid synthesis disruption are a result of flavonoid complex formation with membrane and dissolved proteins.The hydroxyl group of the flavonoids is the main group reacts with bacterial cellular components which ultimately cause growth inhibition.[26] Phenols possess antioxidant and antibacterial properties. Their structure makes them easy to donate electrons when reacting with radical compounds.[27] Phenols can lyse cells by causing leakage and cytoplasmic coagulation.[28,29] Alkaloids and tannins work against bacteria by interfering with the

peptidoglycan, causing cell wall malformation that leads to cell death.[30,31] Tannins can also cause cell membrane shrinkage[23] and inhibit DNA transcription.[32]

Previous research showed onion had antibacterial activity towards both gram-positive and gram-negative bacteria. Onion extract performed inhibition towards Pseudomonas aeruginosa, a gram-negative bacteria, at 100%, 80%, 60%, and 40% concentrations. Staphylococcus epidermidis, gram-positive bacteria, was inhibited by 40%, 20%, 10%, 5%, 2.5%, 1.25%, 0.625%, and 0.3125% onion extract.[33]

In this study, lemon juice was found to contain saponins and triterpenoids. Saponins are natural surfactants that can reduce bacterial growth by increasing the permeability of the bacterial cell wall. Saponins lyse membranes by dissolving membrane lipids and sterols in water. Membrane disruption affects ion transport, calcium-dependent signalling, and protein and enzyme activities. Ion transport disruption results in cell hyperpolarization leading to cell death. [34]

Triterpenoids are compounds with six isoprene units (2-methyl beta-1,3-diene) carbon skeleton. They are synthesised from acyclic hydrocarbon squalene, a 30-carbon units compound. Triterpenoids have antifungal, insecticide, antibacterial, and antiviral activities.[35] Antibacterial activity of triterpenoids is mainly due to their strong binding to cell membrane porins. This interaction can inhibit bacterial growth as porins are entry and exit points for essential compounds. Porins blockage can result in cell starvation and death.[36]

Conclusion

Based on current findings, we can conclude that onion extract (Allium cepa L.) and lemon juice (Citrus limon (L) Burm. f.) have an inhibitory effect on the growth of Enterococcus faecalis bacteria, as evidenced by the formation of a zone of inhibition at 50% concentration. Compared to onion extract, lemon juice has a stronger antibacterial effect.

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Conflicts of Interest: The authors declare no conflicts of interest.

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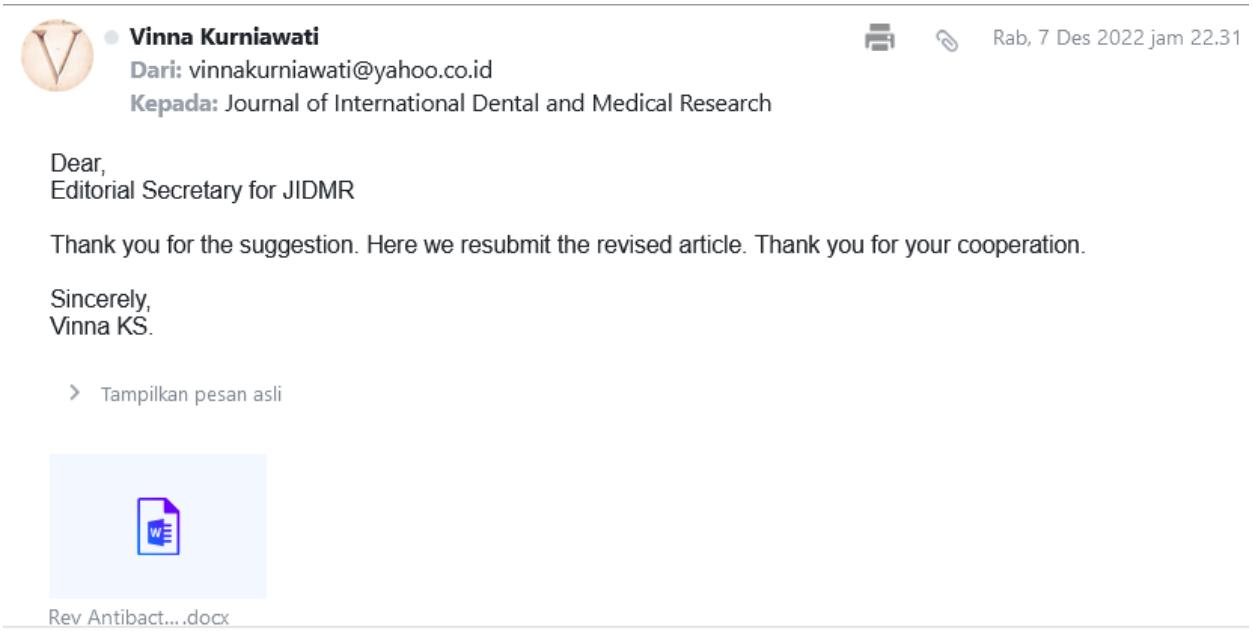

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Dari: jidmreditor@outlook.com
Kepada: Vinna Kurniawati

Subject: Your article has been accepted for Publication. **(Antibacterial Differences Effect between the Onion Extract (Allium cepa L.) and Lemon Juice (Citrus limon (L.) Burm.f.) on In Vitro Growth of *Enterococcus faecalis*. Vinna Kurniawati Sugiaman, Rudy Djuanda, Silvia Naliani, Elsa Alfiyola, Jeanice Winardi, Wayan L Demolsky)**

Dear **Prof. Dr. Vinna Kurniawati Sugiaman,**

It's a great pleasure for me to inform you that your manuscript which titled **" Antibacterial Differences Effect between the Onion Extract (Allium cepa L.) and Lemon Juice (Citrus limon (L.) Burm.f.) on In Vitro Growth of *Enterococcus faecalis* "** has been accepted and will be finalized for **issue 2023; volume 16 number 1** which will be released either late March 2023 or early April 2023.

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