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Antibacterial Efficacy of Nanoparticles of Rambutan Peel Extracts (*Nephelium lappaceum L.*) compared to Microparticles against Oral Bacteria

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Abstract: Oral biofilm containing microorganisms is responsible for various oral infections and inflammatory diseases. Bioactive compounds found in rambutan peel extracts (*Nephelium lappaceum L*) has antibacterial properties. Nanoparticle sizes were known to have the greater capability as an a antimicrobial. This study aimed to compare the efficacy of rambutan peel extracts (RPEs) in microparticles with nanoparticles against oral bacteria, i.e. *Streptococcus mutans* and *Staphylococcus aureus*. This was a laboratory experimental study with a post-test-only design conducted by using RPEs in microparticles (62.5 mg/mL and 250 mg/mL) and nanoparticles (26.5 mg/mL). The particle sizes were measured; the active compound screening was carried out with gas chromatography-mass spectrometry (GC–MS); and the antibacterial activities were tested with the disc diffusion method. The particle size distribution for RPEs with microparticles was measured at 2489 nm, whereas the nanoparticle at 7.491 nm. The GC-MS results demonstrated that both microparticles and nanoparticle RPEs contained oleic acid, hexadecanoic acid, and decanoic acid. A higher percentage of oleic acid was found in nanoparticles of RPEs. There was a significant difference between microparticles of RPEs of 62.5 mg/mL (12.83±1.532) and nanoparticles (16.25±1.529). This study demonstrated that inhibitory power increased along with the elevation of RPEs' concentration as the number of chemical components intensified. The ANOVA and post-hoc Tukey HSD test showed significant differences in the effectiveness of RPEs in nanoparticles groups compared to microparticles 62.5 mg/ml against *S. mutans* ($p<0.05$). and *S. aureus* ($p<0.05$). In conclusion, RPEs were more effective in inhibiting *Staphylococcus aureus* than *Streptococcus mutans*.
Keywords: nanoparticles; rambutan peels; *Nephelium lappaceum* L; antibacterial activity; *Streptococcus mutans*; *Staphylococcus aureus*

INTRODUCTION

Poor oral health is a predictive sign of various systemic conditions,[1] including aggravation of neurovascular[2] and cardiovascular disorders.[3] The primary etiology of poor oral health is a biofilm containing microorganisms responsible for various oral infections and inflammatory diseases.[4] The most prevalent pathological conditions in the mouth are dental caries, oral mucosa infections, and periodontal disease.[5] Microorganisms are the agent responsible for oral diseases. There are many species of bacteria in the oral cavity, some of which are the leading causes of diseases in the oral cavity, such as *Streptococcus mutans* and *Staphylococcus aureus*.[6] *Streptococcus mutans* is most commonly found in cavities specific for dental caries and dentinal cavity,[7] producing GTF, Gtf B, -C, and -D, which use glucose and sucrose to synthesize glucose polymers.[8] Meanwhile, *S. mutans* metabolizes carbohydrates and creates an environment for pathogenic bacteria to

thrive.[9] *Staphylococcus aureus* is one of the microorganisms that cause oral infections, such as peri-implantitis, cheilitis, parotitis,[10] oral mucositis, and other oral infections.[11] *Staphylococcus aureus*, a Gram-positive anaerobic bacterium with a low guanine and cytosine nucleotide (GC) content,[12] has a significant role in colonization and biofilm-associated infections.[13]

One way to reduce the number of pathogenic bacteria is using antimicrobial agents, namely: chlorhexidine, clindamycin, fluoride, quaternary ammonium salts, and antimicrobial peptides (AMPs).[14] Herbal ingredients as an alternative to antimicrobial agents have proliferated in recent decades. However, their side effects are challenging its current role as first-line therapy. Thus, antibacterial substances found in plants have been subject to extensive investigation in recent years for their biological activities in countering diseases.[15]

In Indonesia, the environment is tropical and humid, making it the ideal place for traditional medicine. Rambutan fruit (*Nephelium lappaceum L.)* is one of the exotic fruits that received much attention due to its bioactive compounds.[16] Based on Hernández-Hernández et al's study,[17] the substances found in the peel, flesh, and seeds of rambutan fruit have the potential as antibacterial, anticancer, antivirus, antidiabetes, antioxidants, and anti-inflammatory. The dried rambutan flesh (*Nephelium lappaceum L.*) has been commonly applied in traditional medicine, while fruit peel has always been considered waste.[18] Rambutan peel extracts (RPEs) have better antimicrobial potential than other parts,[19] consists of phenolics, flavonoids, tannins, steroids, and other components.[16] Interestingly, phenolic compounds, one of the essential phytochemical constituents, were found abundance in rambutan fruit peels.[20] Its phenolic profile comprises corilagin, ellagic acid, geraniin, and quercetin.[19,21,22] These phytochemical compounds of RPEs showed high biological activities as antimicrobial, antioxidant, antiviral, anti-inflammatory, and cytotoxic, which may be advantageous for further applications.[23] From the research of Phuong et al,[24] it is known that rambutan peel can be an antibacterial against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Listeria monocytogenes*. The study is supported by Tina et al's findings[25] on rambutan Binjai peel extract which acts as an antibacterial against methicillin-resistant Staphylococcus aureus (MRSA).

Particle scale and combination of several biomaterials were being investigated to enhance the antimicrobial effectiveness of the plant extracts. The nanoscale biomaterial, size range from 1–100 nm, increases the surface area's ratio per volume, which is of interest for researchers while exploring the alternatives for antimicrobial agents.[26] The nanoparticle technology is used as an alternative in developing antimicrobial drugs that can increase the therapeutic efficacy of drugs and prevent unwanted side effects[27] by increasing the surface compounds and intensifying the ability to fight bacteria.[28] Nanoparticles substantially affect living cells, which affect absorption efficiency, selection of internalization pathways, and cytotoxicity.[29] Therefore, this study aimed to compare the antibacterial efficacy of rambutan peel extracts (RPEs) in microparticle and nanoparticle sizes against oral bacteria (*Streptococcus mutans* and *Staphylococcus aureus*.

METHODS

This laboratory experiment with a post-test-only research design was conducted using rambutan peel extracts in various sizes and concentrations, i.e., microparticles 62.5 mg/mL; microparticles 250 mg/mL; and nanoparticles. Three groups of each bacteria and six replications were observed. Firstly, the rambutan fruit used as the sample was determined and confirmed as *Nephelium lappaceum L*. Next, the extraction process was carried out using the maceration method. Fresh rambutan peels (14.32 kg) were thoroughly washed, then oven-dried and smashed into powder using a blender. The dried rambutan peel extract powder (5.3 kg) was mixed and soaked with 70% ethanol with a 1:1 ratio, covered with aluminum foil, and shaken for 24 hours using a shaker.

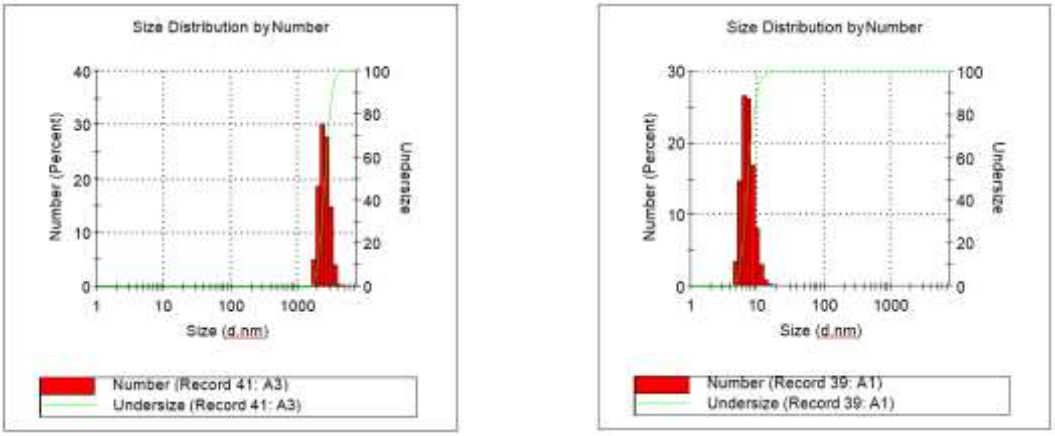
Afterward, the soaking extract was filtered using a vacuum pump to separate the filtrate and residue. The filtrate obtained was thickened using a rotary evaporator for 4-5 hours at 40°C.[30] Half of the rambutan peel extract was diluted in two concentrations, 62.5 mg/mL and 250 mg/ml, and stored in a dark place at room temperature. Afterwards, the nanoparticles were prepared using 1.5 g of rambutan peel extract and mixed with 10 mL propylene glycol and 5 mL isopropyl methyl. The solution was stirred for 15 minutes to form the oil phase. Separately, a 35 mL PEG-400 and 5 mL tween-80 were poured into beaker glass and stirred for 10 minutes. Then the two solutions were mixed and ultrasonicated for 15 minutes.[31,32] The particle size was analyzed after obtaining the microparticles and nanoparticles sizes of rambutan peel extracts. Subsequently, the active compound screening was carried out to examine the phytochemical properties of rambutan peel extracts with gas chromatography-mass spectrometry (GC–MS).[30]

Lastly, antibacterial activit tests were performed with the disc diffusion method. The Mueller Hinton Agar (MHA) medium was poured into sterile Petri dish and allowed to solidify. Bacterial suspension of *Staphylo-coccus aureus* and *Streptococcus mutans* were inoculated separately by streaking a sterile ose on the medium surface. Paper discs were prepared and dripped with 20 µL of microparticles RPEs (*Nephelium lappaceum L.*) in two concentrations (62.5 mg/mL and 250 mg/mL) and nanoparticles (26.5 mg/mL). The Petri dishes were incubated at 37°C for 24 hours, observed, and measured the clear zone formed using caliper expressed in millimeters. Measurements were made in the vertical and horizontal directions.[33]

RESULTS

The particle size distribution for RPEs with microparticles was 2489 nm, and with nanoparticles was 7.491 nm, as shown in Figure 1. Based on the obtained results, the average microparticle size of RPEs analyzed in this study extended from 1718 to 4145 nm (Fig.1A). The nanoparticle sizes ranged from 4.849 to 13.54 nm (Fig.1B). The phytochemical compounds in RPEs were identified by GC-MS (Fig.2), in micro sizes (Fig.2A) and nano sizes (Fig.2B). The compounds' detail was characterized in macroparticles (Table 1) and nanoparticles (Table 2).

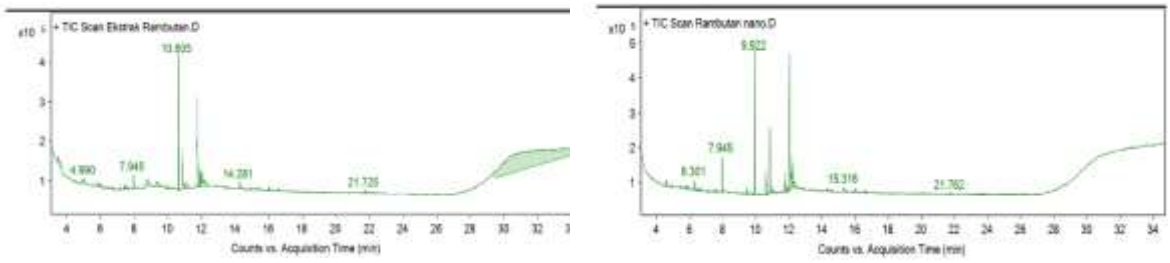
The normality test showed that the data were normally distributed ($p>0.05$), and the variance of data was homogenous ($p>0.05$). The ANOVA was run followed by the Tukey HSD post-hoc test, which showed differences in the effectiveness of RPEs in the three particle size groups (microparticles 62.5 mg/mL, microparticles 250 mg/mL, and nanoparticles) against *S. mutans* ($p<0.05$).



1A **1B**

Figure 1. Particle size distribution of rambutan peel extracts (RPEs) in (1A) microparticles (2489 nm) and (1B) nanoparticles (7.491 nm).

There was no significant difference noticed between microparticle size RPEs 250 g/mL with either microparticles size 62.5 mg/mL or nanoparticles in the *S. aureus* group ($p>0.05$). Thus, it can be concluded that Ho was rejected, and it was proven that there was a difference in effectiveness between the micro and nano-sized of RPEs, in which nanoparticles had tremendous antibacterial potential against oral bacteria.



2A **2B**

Figure 2. The GCMS scan of rambutan peel extracts, in micro sizes (2A) and in nano sizes (2B)

Table 1. GC-MS Identification of rambutan peel extracts in micro sizes

RT	Compound Name	Formula	Area sum (%)
11.75	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid)	$C_{19}H_{36}O_2$	5.29
10.605	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	3.83
11.99	(E)-1-(Methoxymethoxy)-1-tetradecen-3-ol (Juniperic acid)	$C16H32O_3$	2.38
10.845	Hexadecanoic acid	$C_{16}H_{32}O_2$	1.69
3.493	Cyclohexanone, 2-methyl-	$C_7H_{12}O$	1.38
11.898	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	0.6
11.048	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	0.46
7.945	Dodecanoic acid (Lauric acid)	$C_{12}H_{24}O_2$	0.43

Table 2. GC-MS identification of rambutan peel extracts in nano sizes

RT	Compound Name	Formula	Area sum (%)
12.009	9-Octadecenoic acid, (E)- (Oleic acid)	$C_{18}H_{34}O_2$	26.61
9.922	Isopropyl myristate	$C_{17}H_{34}O_2$	13.21
10.845	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	9.66
12.194	Ethyl (9E)-9-octadecenoate (Oleic acid)	$C_{20}H_{38}O_2$	8.05
11.75	6-Octadecenoic acid, methyl ester (Petroselinic acid)	$C_{19}H_{36}O_2$	5.41
7.945	Dodecanamide, N, N-bis (2-hydroxyethyl)- (Lauramide diethanolamine)	$C_{16}H_{33}NO_3$	5.39
12.286	Methyl octadeca-13,14-dienoate (Methyl linoleate)	$C_{19}H_{34}O_2$	3.74
10.605	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	2.39
6.301	Decanoic acid	$C_{10}H_{20}O_2$	1.79
9.46	Myristic acid	$C_{14}H_{28}O_2$	1.46

Table 3. Antimicrobial inhibition power of rambutan peel extracts (RPEs) against *Streptococcus mutans* and *Staphylococcus aureus*

Bacteria	n	Mean ± SD	Rambutan Peel Extracts		p value	
					Within groups	Between groups
Streptococcus mutans	6	12.47±1.587	Microparticle 62.5 mg/mL	Microparticle 250 mg/mL	0.998	0.004
				Nanoparticle	0.000	
	6	12.55± 0.909	Microparticle 250 mg/mL	Microparticle 62.5 mg/mL	0.998	
				Nanoparticle	0.000	
	6	16.10± 0.787	Nanoparticle	Microparticle 62.5 mg/mL	0.000	
				Microparticle 250 mg/mL	0.000	
Staphylococcus aureus	6	12.83±1.532	Microparticle 62.5 mg/mL	Microparticle 250 mg/mL	0.298	0.000
				Nanoparticle	0.003	
	6	14.13±1.294	Microparticle 250 mg/mL	Microparticle 62.5 mg/mL	0.298	
				Nanoparticle	0.058	
	6	16.25±1.529	Nanoparticle	Microparticle 62.5 mg/mL	0.003	
				Microparticle 250 mg/mL	0.058	

DISCUSSION

The particle sizes were analyzed to determine the exact size of each sample and its conformity with predetermined standards. The sizes of microparticle ranged from 0.1-100μm.[34] The microparticle sizes stretched from 1718 nm to 3580 nm (99.8%). The sizes of nanoparticles of the sample analyzed stretched from 4.849 to 15.690 nm, with 87.7% sized below 10 nm. In particular, approximately 52% of the particle size was distributed within 6-7 nm and roughly 17% around 8 nm. Apart from particle size, in line with the study conducted by Rostinawati et al.[35] which demonstrated that inhibitory power increased along with elevation of extract's concentration as the number of chemical components intensified.

The phytochemical analysis using GC-MS on RPEs detected eight secondary metabolites in microparticles, while the nanoparticles group consisted of 10 chemical compounds in large quantities. Several components of nanoparticles were similar to microparticles, i.e., octadecenoic acid and hexadecanoic acid. Nevertheless, the primary chemical compounds in nanoparticle size of RPEs were oleic acid, isopropyl myristate, lauramide diethanolamine, and myristic acid. Several studies have proven that RPEs has antimicrobial and antioxidant capacities due to its secondary metabolites, namely oleic acid, hexadecanoic acid, juniperic acid, lauric acid, decanoic acid, and myristic acid.[36-40] The oleic acid, an omega-9 fatty acid that belongs to a hydrophobic group,[41] consists of more than ten carbon atoms and shows an excellent antibacterial activity by causing lysis to bacteria's protoplasm.[42] Oleic acid functions as a pathogen controller for bacteria and fungi.[43] Lauramide diethanolamine performs as an antiviral and antimicrobial compound.[44,45] Myristic acid has antifungal properties against *C. albicans* and also has potential as an antimicrobial against oral pathogens.[46]

Apart from that, RPEs were known to have bacteriostatic properties from their

flavonoids and tannins contents. Flavonoids are valuable as antimicrobial, antioxidant, antitumor, and anti-inflammation.[47-49] Tannins are polyphenolic compounds widely distributed in plants that are more sensitive for gram-positive bacteria.[50] Phenolic hydroxyl is the content of tannin, which produces antibacterial properties. Tannins have several ways of inhibiting bacterial growth, namely iron chelation, cell wall synthesis inhibition, and cell membrane disruption.[51]

Nanoparticles can cross bacterial membranes and affect cell membranes' shape and function and interact with essential components of bacterial cells such as DNA, lysosomes, ribosomes, and enzymes.[52] Antibacterial investigations of the two oral bacterias, *S. mutans* and *S. aureus*, confirmed that the inhibition zone of the nanoparticles was more extensive than that of the microparticles. Ultimately, smaller particle sizes are better in impeding bacteria.

CONCLUSION

This study demonstrated the capability of rambutan peel extract (RPEs) to impede the most common oral bacteria, *Streptococcus mutans,* and *Staphylococcus aureus*. The statistical analysis showed that RPEs was more effective as an antibacterial against *S. aureus* than *S. mutans* at various concentrations and particle sizes. Rambutan peel extracts have better antimicrobial potential in nanoparticles (26.5 mg/mL) than microparticles (62.5 mg/mL and 250 mg/mL) in inhibiting the growth of both bacteria.

Conflict of Interest

The authors declare no conflicts of interest.

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