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The Effectiveness Antimicrobial of Polysaccharide Gel from Durian Peel Ethanol Extract and Chitosan Gel

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Abstract

BACKGROUND: Research on galenic bioactive compounds as antimicrobial agents needs to be developed. Durian peel ethanol extract is a polar polysaccharide consisting of D-galacturonic acid with -1,4 glycosidic bonds and can form a polysaccharide gel. Chitosan can be synthesized through the deacetylation of chitin as a compound (1.4)-2 amino-2deoxy D-glucopyranose and can form a gel in 1.5–2% acetic acid. The properties of chitosan are biodegradable, non-toxic, polycationic antimicrobial, and biocompatible.

AIM: The study aimed to determine the effectiveness of the ethanolic extract of PG durian peel and CG as antimicrobial agents.

METHODS: The research design was an experimental study and conducted at the Microbiology Laboratory, Faculty of Health, Setia Budi University, Surakarta, in August–December 2020. The research stages were the extraction of durian skin polysaccharides, bioformulation of PG durian peel extract and CG, and antimicrobial activity testing using the diffusion method.

RESULTS: The results showed PG durian peel ethanolic extract concentration (100%, 50%, 25%, 12.5%, 1.5%, and CG 1.5% %) or a combination of durian peel extract PG (1.5%) and CG (1.5%) 1:1 ratio; 1:2; 2:1 was antimicrobial against *Staphylococcus aureus* ATCC 2592*3, Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. PG durian peel ethanolic extract concentration of 100%, 50%, 25%, 12.5%, 1.5%, and CG 1.5% or a combination of durian peels ethanolic extract PG (1.5%) and CG (1.5%) ratio 1:1; 1:2; and 2:1 as an antimicrobial *in vitro*.

CONCLUSION: PG durian peels ethanolic extract concentration 100% showed optimum effectiveness as an antimicrobial against *B. subtilis* ATCC 6633. Chitosan 1.5% showed optimal effectiveness as an antimicrobial against *P. aeruginosa* ATCC 27853. The effectiveness of the mixture of PG 1.5% and CG 1.5% ratio 1:2 showed optimum effectiveness against all test cultures.

Introduction

Along with technological advances in the pharmaceutical and health fields, various galenic materials from plants and animals have been developed, including anti-microbial gel preparations as an anti-inflammatory, wound dressings, bio-immunostimulator agents, preservatives, stabilizers, and others. The antimicrobial activity is found in the bioactive compound chitosan and durian peel extract, which were biodegradable, biocompatible, and non-toxic. The potential of these bioactive compounds can be developed in the medical field as a multifunctional and economic galenic preparation. *Durio zibertinus* L. is a tropical fruit originating from Southeast Asia, including Indonesia.

The highest composition of durian fruit is durian skin waste (60–75%). Actually, durian skin contains lignin (15.45%), holocellulose (73.45%), and cellulose (60.45%) which can be used materials for making bio briquettes with a carbon content of 77.87% and second-generation bioethanol, as well as materials for making particleboard partition boards that are economical [1]. Another type of durian peel polysaccharide that can be used is pectin as a polymer of D-galacturonic acid linked by -1,4 glycosidic bonds, which are polar and non-toxic [2], [3]. Durian peel polysaccharides can be extracted as polysaccharide gel (PG), which is multi-functional in the food and pharmaceutical fields as agar and agar preparation, tablets, suspensions, and emulsions [4], [5]. Durian peel PG contains antimicrobial bio-natural compounds that are non-toxic and water-soluble to be developed in the medical and pharmaceutical fields [6]. PG durian peel extract (*Durio zibethinus* L.) can be used as a material for making wound dressing biofilms [7].

Chitosan is a synthesis result of chitin deacetylation, where chitin is widely found in the shells of animals from the Crustaceae family, namely, crabs, shrimp, and clams. Chitosan has been widely used in the biomedical field as a biodegradable pharmaceutical wound dressing, non-toxic, non-immunogenic, and

biocompatible *in vivo* [8]. Chitosan is soluble in acetic acid 1.5–2% as a gel. Chitosan is a polycationic antimicrobial which can suppress the growth rate of diarrhea genic *Escherichia coli in vitro* [9]. The effectiveness of chitosan as an antimicrobial is related to the role of a group of glycan-binding protein complex compounds known as Chitooligosaccharide (COS) derived from chitosan [10]. The results showed that chitosan has multifunctional properties, namely, anti-hypercholesterolemia, anti-hyperlipidemia, anti-inflammatory, and antimicrobial [11], [12]. The combination of 100% snail seromucoid and 1.5% chitosan with a ratio of 1:2 *in vivo* is able to accelerate wound healing and has a synergistic effect *in vitro* as an antimicrobial against *Staphylococcus aureus* [13], [14].

Research on the effectiveness of polysaccharides from durian peel extract and chitosan as antimicrobials has not been carried out. Thus, encouraging authors to carry out scientific developments and novelties, research the combination of galenic plant ingredients, polysaccharide gel, durian peel extract, and or its combination with chitosan gel as an antimicrobial agent need to be done. Based on this, the study aimed to determine the antimicrobial effectiveness of PG and CG durian peel extract on the test culture. The contribution of this research is expected to be developed and applied in anti-inflammatory preparations or preparations for wound dressings.

Method

The research design was an experimental study and conducted at the Microbiology Laboratory, Faculty of Health, Setia Budi University, Surakarta, in August–December 2020. The research stages were the extraction of durian skin polysaccharides, bioformulation of PG durian peel extract and CG, and antimicrobial activity testing using the diffusion method. Medical grade chitosan was obtained from PT Biotech Surindo, Indonesia. Culture media: Vogel Johnson Agar, Muller Hinton Agar, Sabaraud Glucose Agar, Gram stain, physiological NaCl.

The test cultures were obtained from the Microbiology Laboratory, faculty of health, Setia Budi University. The test culture is *S. aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 25922, *Salmonella typhi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 which were obtained from the Microbiology Laboratory, Faculty of Health, Setia Budi University, Surakarta. The raw material for durian skin is obtained from one of the durian fruit sales centers in the Surakarta area. The durian plant specimen used has been determined, verified, and approved by the person in charge of the Plant Determination Laboratory, Plant Systematics Laboratory, Setia Budi University, Surakarta, Indonesia, based on letter number: 114E/DET/UPT-LAB/ 5.10. 2020. The effectiveness of the test preparation was based on the results of the average diameter of the inhibition zone and compared with the positive control.

Extraction of PG durian peel

The white inside of the durian skin is taken and dried, blended, and then dissolved in a 100 mM nitric acid solution with a ratio of 1:40 then heated at 90–100°C for 10 min, then filtered and the filtrate separated. Then, the precipitate was dried at 50°C. The precipitate was redissolved with water, filtered, concentrated, and re-precipitated to obtain PG powder. Furthermore, a polysaccharide gel of durian peel extract was made with various concentrations, as shown in Figure 1 [15], [16].

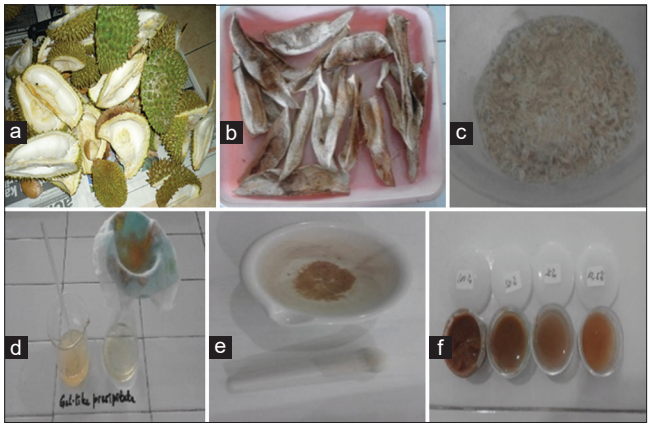

Figure 1: The process of PG synthesis of durian peel extract from (a) raw material (a), (b) dried, (c) blended, (d) precipitated, (e) powdered, and (f) PG durian extract

Preparation of 1.5% chitosan gel (CG)

The chitosan used in this study is the result of the medical-grade chitosan industry, which has standardized physicochemical properties so that no synthesis process is carried out from natural raw materials for Crustaceae skin. Medical-grade chitosan was weighed 1.5 g and dissolved in 2% acetic acid solution to form a chitosan gel. In this study, 1.5% CG was used, referring to the results of the previous research [9].

Formulation of PG and CG

PG durian peel extract made with various concentrations of 100%, 50%; 25%; 12.5%; and 1.5%. The concentration of CG used is 1.5% and a combination of PG (1.5%): CG (1.5%) ratio 1:1; 1:2; and 2:1. The positive control used the pharmaceutical patent product neomycin sulfate as antibacterial and the synthetic imidazole group patent product as an antifungal.

In vitro antimicrobial diffusion method

To determine the effectiveness of the test preparation as antimicrobial, several standardized bacterial and fungal isolates were used from the

Laboratory of Microbiology, Faculty of Health, Setia Budi University, Surakarta. Each test culture was prepared as ac culture suspension with 1 mg/ml concentration of Mc-Farlanc 0.5-1.0 and inoculated aseptically on Muller-Hinton Agar Medium. The media plate, then, was made aseptically wells. Each well was filled with 300 uL of PG, CG, and their mixtures, positive and negative controls. The media was incubated at at 37°C for 48 h. Then, the inhibition zone diameter was measured.

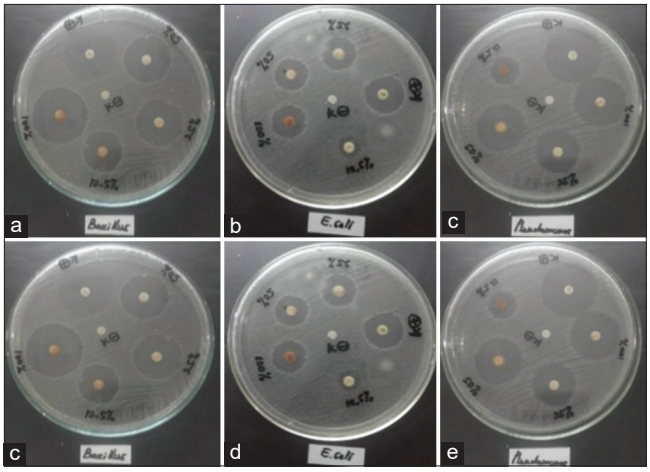

Figure 2: Result sensitivity test to PG durian peel extract (a) Bacillus subtilis ATCC 6633, (b) Escherichia coli ATCC 25922, (c) Pseudomonas aeruginosa ATCC 27853, (d) Staphylococcus aureus ATCC 25923, (e) Salmonella typhi ATCC 13311, and (f) Candida albicans ATCC 10231

Results

The results of the PG effectiveness test of durian peel extract and chitosan gel as antimicrobials *in vitro* are presented in Figure 2. The results of the combination PG durian peel extract and CG effectiveness as antimicrobials *in vitro* are presented in Figure 3.

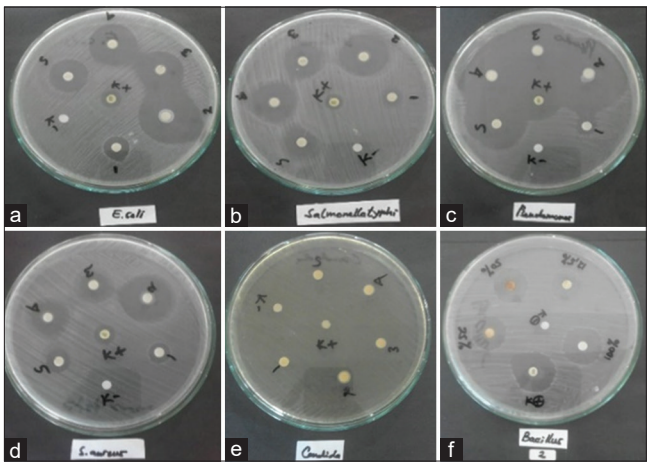

Figure 3: Result sensitivity test combination PG durian peel extract and CG (chitosang gel) ratio 1:1; 1:2; and 2:1 PG durian peel extract (a) Escherichia coli ATCC 25922, (b) Salmonella typhi ATCC 13311, (c) Pseudomonas aeruginosa ATCC 27853, (d) Staphylococcus aureus ATCC 25923, (e) Candida albicans ATCC 10231, and (f) Bacillus subtilis ATCC 6633

The results of the antimicrobial activity of PG durian peel extract (100%, 50%, 25%, 12.5%, and 1.5%); 1.5% CG; a mixture of PG (1.5%): CG (1.5%) with a ratio (1:1; 1:2, 2:1) to the test culture as listed in Table 1 shows that the optimum effectiveness as an antimicrobial of the durian peel extract is PG 100% against B. subtilis ATCC 6633; chitosan 1.5% against P. aeruginosa ATCC 27853; and a mixture of 1.5% PG with 1.5% CG in a 1:2 ratio for all test cultures.

Table 1: The results of the effectiveness of PG and CG as antimicrobial by diffusion method

Material	Mean diameter zone inhibition (cm)					
	SA	BS	EC	ST	PS	CA
PG durian peel extract 100%	2.65	3.95	2.25	2.95	3.82	3.52
PG durian peel extract 50%	2.51	3.45	2.15	2.82	3.25	3.05
PG durian peel extract 25%	1.65	3.15	2.03	2.55	2.85	1.95
PG durian peel extract 12.5%	1.45	2.81	1.65	1.35	1.95	1.05
PG durian peel extract 1.5%	1.40	1.50	1.25	1.05	1.65	0.75
CG (chitosan gel) 1.5%	2.50	4.25	3.75	3.30	5.10	2.45
Ratio (PG 1.5%:CG 1.5%) = 1:1	1.70	3.60	2.60	2.60	4.30	1.75
Ratio (PG 1.5%:CG 1.5%) = 1:2	1.85	3.65	2.95	2.85	4.50	2.15
Ratio (PG 1.5%:CG 1.5%) = 2:1	1.50	3.10	2.35	2.15	3.65	1.85
Positive control	2.45	2.25	2.45	1.55	2.81	2.31

Test culture SA: *S aureus* ATCC 25923, BS: *B. subtilis* ATCC 6633, EC: *E. coli* ATCC 25922, ST: *S. typhi* ATCC 13311, PS: *P.aeruginosa* ATCC 27853, and CA: *C. albicans* ATCC 10231.

Discussion

The results of this study in Table 1 show the potential and effectiveness of PG durian peel extract and CG and the combination of PG and CG ratio 1:2 to microbes due to the presence of bioactive compounds contained in PG durian peel extract and CG. Differences in the effectiveness of PG, CG, or a combination of PG and CG as antimicrobial agents are influenced the structure and physiology of the microbial cell. The diffusion of antimicrobial bioactive compounds to enter microbial cells is highly dependent on the permeability factor of the plasma membrane and cell wall and the physiology of each type of microbe. Differences in cell wall profile and plasma membrane permeability of microbial cells affect the intake or penetration of bioactive compounds to function as antimicrobial agents. In this study, cultures were used for Gram-positive bacteria (*B. subtilis and S. aureus*), Gram-negative bacteria (*E.coli, S. typhi, and P. aeruginosa*), and the fungus *C. albicans*. Gram-positive bacteria cell walls are relatively thick peptidoglycan, and there is no lipopolysaccharide. The yeast cell wall is composed of chitin.

The plasma membrane of each microbe plays an important role in the diffusion of bioactive compounds into the cell so that the mode of action of the bioactive compound is microbiostatic and/or microbicidal. For example, the cell walls of fungi, *C. albicans*, are composed of chitin. On the other hand, Gram-positive bacteria have relatively small peptidoglycan compounds in their cell walls compared to Gram-negative bacteria that lipopolysaccharides in the cell walls of Gram- negative bacteria such as *E. coli*, *S. typhi*, and other pathogenic bacteria are often correlated with the pathogenicity of

microbes that cause infection in humans. Another study showed that chitosan was more effective against Gram-positive bacteria such as *L. monocytogenes, Bacillus megaterium, B. cereus, S.aureus, L. brevis, and L. bulgaris* than Gram-negative bacteria such as *E. coli, Pseudomonas fluorescens, S. typhimurium, and Vibrio parahaemolyticus*. The effectiveness of a galenic material with antimicrobial properties is also influenced by the type and content of bioactive compounds, mode of action of bioactive compounds, and environmental factors. The durian peel polysaccharide extraction process in this study used 66% (v/v) ethanol as solvent. This is done because extracting the active ingredients in galenic materials requires the selection of the type of solvent that can attract the active substance in the extraction process. In general, secondary metabolites are polar, so ethanol as a solvent is generally used to extract active ingredients in a preparation containing these ingredients [17], [18].

The PG of durian peel extract is composed of glucose, rhamnose, fructose and galacturonic acid, and polysaccharides with a molecular weight of 500–1400 kD, which are biodegradable and non-toxic *in vivo* [19]. The results of the extraction of secondary metabolites contained in durian skin (*D. zibethinus* Murr) have the potential to have antibacterial and antioxidant activity, namely flavonoids, alkaloids, saponins, and tannins [20], [21]. As antibacterial, flavonoids work by damaging the permeability of the cytoplasmic membrane so that cells die. The mode of action of saponins is to interfere with the permeability of the microbial cell membrane by changing the structure and function of the plasma membrane, resulting in denaturation of membrane proteins, and resulting in cell damage and lysis. The mode of action of tannins as an antibacterial is astringent, which causes shrinkage of cell walls or cell membranes and induces the formation of enzyme complex compounds of microbial substrates, especially metal ions, which will increase the toxicity of tannins so that cell metabolism is disrupted and cells die. The mode of action of alkaloids as an antibacterial is to damage the peptidoglycan component as a constituent of the bacterial cell wall, causing the cell wall layer to be incomplete, resulting in cell death. Another mechanism of antibacterial alkaloids is the alkaloid component known as DNA interchelator and inhibiting bacterial cell topoisomerase enzymes in the genetic process of microbial cells [22], [23].

The results of another study showed that PG durian peel extract as a film dressing could be used to inhibit the growth of *Staphylococcus epidermidis* and *S. aureus* [24]. Another study showed that 125 g/ml durian peel extract could inhibit the growth of *P. aeruginosa*; durian peel extract with a concentration of 25% can inhibit the growth of *C. albicans* [25], [26]. About 95% ethanol extract of durian peel with 6% concentration was antibacterial against *S. aureus* and *Salmonella enteria serovar typhi* [27]. The 96% ethanol extract of durian fruit (*D. zibethinus* Murr.) is antibacterial against *Salmonella typhi* ATCC 14028 and *Bacillus cereus* ATCC 11778 which cause diarrhea [28]. The research by Pongsamart (2003) [29] showed the antibacterial effect of PG against 11 bacterial strains, seven strains were sensitive to PG inhibition. MIC and MBC PG were 0.64 and 2.56% (w/v) against *S. epidermidis, B. subtilis, M. luteus*, and *E. coli*; PG 1.28% and 2.56% (w/v) against *S. aureus* and 2.56 and 5.12% (w/v) against *L. pentosus*, respectively. The difference in the inhibitory effect of PG durian peel extract on a microbe is also influenced by the type of test media used, because it is related to the growth of the test culture [29].

The effectiveness of chitosan as antimicrobial is influenced by the role of Chitooligosaccharide (COS) compounds which are glyco-binding protein complex compounds that have 1,4-b-glucosamine bonds. COS-derived from chitosan is a potential substance as an “alternative antibiotic” that has a safer value without causing residue [30]. The uniqueness of COS which is polycationic is able to suppress the growth rate of *E. coli* that causes diarrhea *in vitro*. Chitosan in the form of its salt is able to function as a hemostatic agent. Chitosan salts can be synthesized by mixing chitosan with organic acids such as succinic or lactic acid. The way chitosan works as a hemostatic agent is through the interaction between the erythrocyte cell layer has a negative charge and protonated chitosan has a positive charge, thereby encouraging the contribution of platelets to rapid thrombus formation. Chitosan salt can be combined with various ingredients for example by mixing with alginate or other materials that can change the level of solvency and bio-absorbability of chitosan salt. Chitosan salt is biocompatible and biodegradable, so it is valuable as an absorbable hemostat [31]. The mode of action of chitosan bioactive compounds can be influenced by environmental factors such as incubation temperature, pH, humidity, and substrate or growing media. Chitosan is able to interact in the protein flocculation process which is influenced by pH, namely, chitosan is able to absorb metal cations more effectively at high pH conditions [32]. There are three main mechanisms of action of microbicidal chitosan, namely, the first mechanism the interaction between positively charged chitosan molecules and negatively charged microbial cell surfaces. There is a mediated electrostatic force between the protonated NH3+ group and the negative residue, thereby inducing an internal osmotic imbalance resulting in the inhibition of the growth of microorganisms. The second mechanism is through the formation of chitosan bonds with microbial DNA, thereby inhibiting the synthesis of mRNA and protein in the cell nucleus. Chitosan is thought to be able to penetrate the bacterial cell wall which is composed of multilayer murein that is cross-linked and reaches the plasma membrane and inhibits protein synthesis. The third mechanism is as a metal ion chelating agent that completes the basis of microbial cells; as a result, the rate of microbial growth is disrupted [33], [34]. Chitosan 2% was more effective in increasing lymphocyte proliferation *in vitro* than 100% seromucoid and 5% snail mucus cream [35]. The results of this study indicate that durian peel extracts PG and CG as effective antimicrobial agents have the potential

to be developed in further research on bioformulation of pharmaceutical preparations that can be applied in the medical field as safe and effective wound dressing preparations.

Conclusion

PG durian peel ethanolic extract concentration of 100%, 50%, 25%, 12.5%, 1.5%, and CG 1.5% or a combination of durian skin extract PG (1.5%) and CG (1.5%) ratio 1:1; 1:2; and 2:1 as an antimicrobial *in vitro*. PG concentration of 100% durian peel extract showed optimum effectiveness as antimicrobial against *B. subtilis* ATCC 6633. Chitosan 1.5% showed optimum effectiveness as an antimicrobial against *P. aeruginosa* ATCC 27853. The effectiveness of a mixture of PG 1.5% and CG 1.5% ratio 1:2 showed optimum effectiveness for all test cultures.

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Authors' Contributions

Y.S.S was responsible for constructed an idea for research, organized of the study, supervised the project and critical review. M.S. and N.P. were responsible for biological materials, design of methodology, analysis and interpretation of data. A.S.H. and N.P. performed the collection of clinical specimens, isolation of strains, identification and drug susceptibility testing, literature review, and cowriting of the article. Y.S., A.S.H., M.S., and N.P. were responsible for discussing the results and contribution to the final article.

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