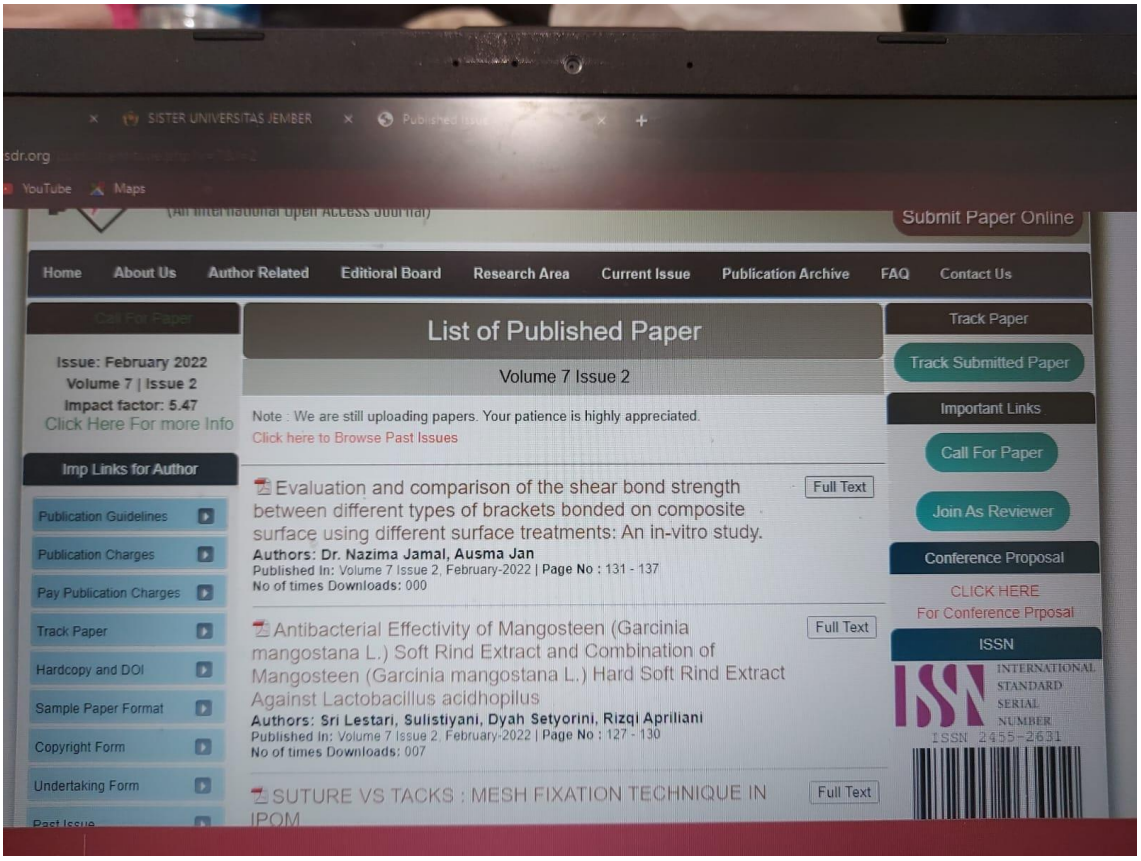
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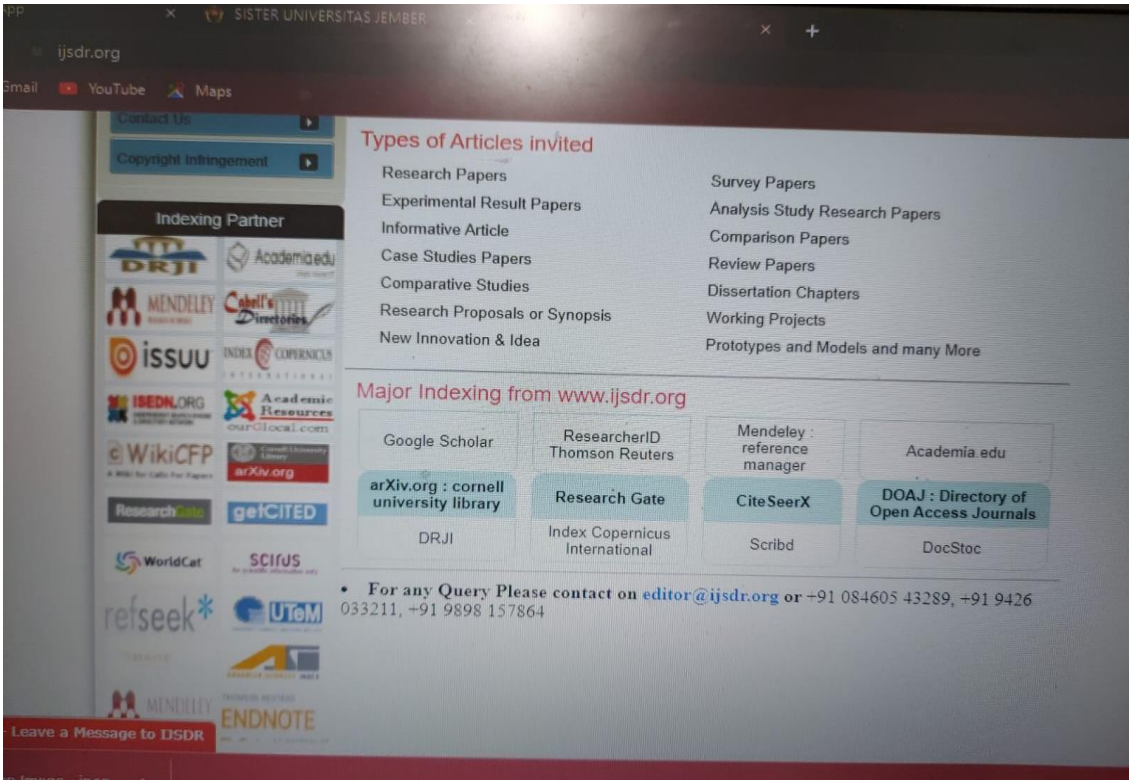
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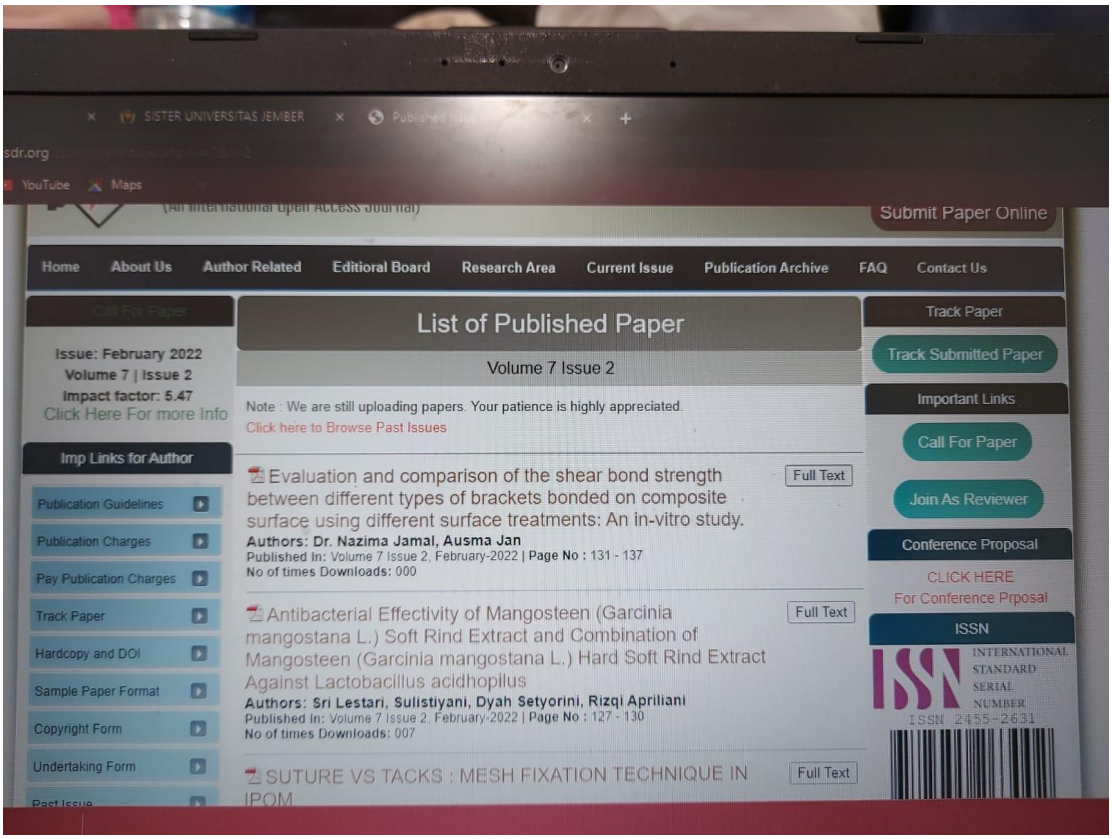
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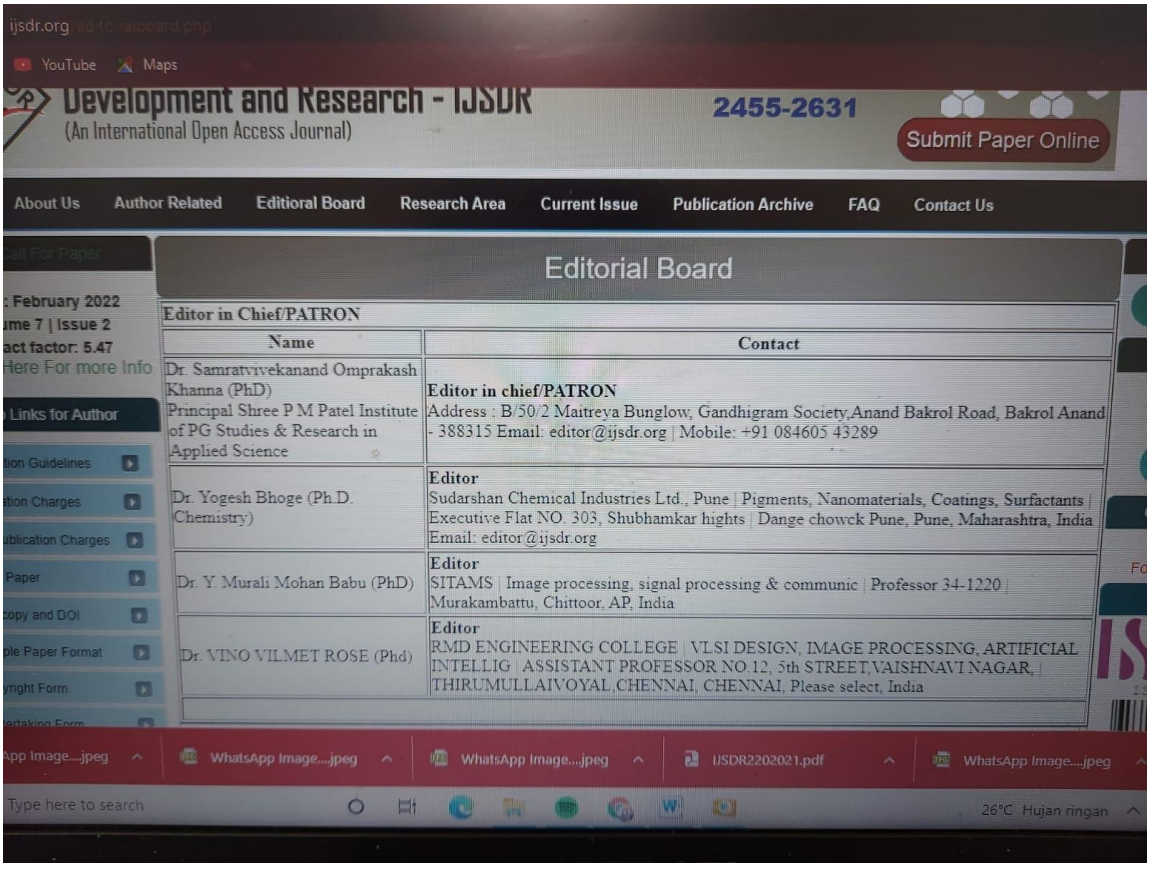
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Antibacterial Effectivity of Mangosteen (*Garcinia mangostana L.*) Soft Rind Extract and Combination of Mangosteen (*Garcinia mangostana L.*) Hard Soft Rind Extract Against *Lactobacillus acidhopilus*

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Abstract

Introduction. Mangosteen rind (Garcinia mangostana L.) is one of the natural ingredients that can be used as an alternative ingredient in the medical field. The mangosteen rind consists of the outer (hard) skin and the inside (soft) which contains antibacterial compounds such as xanton, flavonoids, tannins, and anthocyanins. The aim of the study was to determine the antibacterial efficiency of mangosteen soft rind (Garcinia mangostana L.) and a combination of mangosteen fruit hard-soft skin extract (Garcinia mangostana L.) in inhibiting the growth of Lactobacillus achidopillus bacteria.

Method: The study used the well diffusion method. The number of samples used is 5 petridishes. Each petrid consists of 5 treatment groups, soft rind mangosteen extract concentration 80% and 100%, combination hard-soft rind mangosteen extract extract concentration 80% and 100% and positive control for NaOCl 2, 5%.

Result: The results of the study showed that 100% combination hard-soft rind mangosteen extract (Garcinia mangostana L.) was the most effective treatment group in inhibiting the growth of Lactobacillus achidopillus bacteria for 7 days.

Keywords: antibacterial, irrigation materials, mangosteen rind, Lactobacillus achidopillus

I. INTRODUCTION

The main source of irritation in the root canal is the bacteria found in caries lesions[1]. Gram-negative and anerob species are the most dominant bacteria found in root canal infections, one of the example is Lactobacillus sp[2,3]. Bacteria elimination in the root canal treatment can be done one by root canal irrigation. The most widely used irrigation material today is sodium hypochlorite (NaOCl)[4]. This solution has antibacterial properties, it is inexpensive and capable of dissolving smear layer but its smell is unpleasent and iritative against periapical tissues[1].

One of the natural ingredients that have antibacterial properties is mangosteen rind. The Mangosteen rind consists of hard parts and soft parts. Hard rind is the outer peel that contains the oil and pigment glands. Soft rind is an inner peel that directly contacts the flesh of the fruit[5]. Overall, the skin of mangosteen fruit contains antibacterial compounds such as xanton, antosionine, phenol, tannins, and flavonoids[6]. Palapol et al (2009) reports that the hard mangosteen rind contains anthocyanins compounds more than the soft mangosteen bark[7]. Based on this, the author wants to know the effectiveness of the antibacterial activity of Lactobacillus acidhopilus on the extract of Mangosteen soft rind and a combination of hard-soft rind extract of mangosteen concentrations of 80% and 100%.

II. METHOD

This research is an experimental laboratory research with post test only control group design. The numbers of samples used were 5 for each treatment group. Antibacterial activity test which used in this research is well diffusion method. This research went through several stages, namely the preparation, treatment and measurement stages. Preparation stage consisted of sterilization of tools, preparation of the MRS-B media, preparation of the Lactobacilus acidhopilus culture, Lactobacilus acidhopilus identification test, mangosteen fruit identification test, making the mangosteen rind extract, and preparation of the MRS-A media. The mangosteen rind used for this research is the softer inner part (which has been separated from the harder outer rind) and a combination of hard-soft rind (which the hard and soft parts are not separated) then its dried and mashed to form powder. The powder was macerated with 70% of ethanol for five days then evaporated until the extract became concentrated with semisolid consistency. The extracts in this study were concentrations of 80% and 100%. The next stage is the treatment stage. One cc of bacterial culture was transferred using a syringe into petridish, then the liquid MRS-A media that was allowed to sat until warm (45-50 ° C) was poured into the petridish which already contained bacterial suspension (pour plate method). Petridish is then shaken slowly to mix bacterial culture with the MRS-A media until it is homogeneous.

Lactobacilus acidophilus bacterial suspension was then left for 15 minutes in order to adapt to the media. After the media solidifies, the bottom of the petridish is marked using paper, where the petridish is divided into 5 regions. The label paper reads L1 (for 80% mangosteen soft rind extract), L2 (for 100% mangosteen soft rind extract), K1 (for 80% mangosteen combination rind extract), K2

(for 100% mangosteen combination rind extract) and K(+) for NaOCl 2.5%. A hole was made using a *boorer* to form a 6 mm diameter hole. Each petridish is made into 5 wells. The hole is filled with 15 microliters of each material according to regional division. After all the holes were filled, the petridish was put into the desiccator and then incubated at 37°C for 18-24 hours. After incubated, the diameter of inhibition zone was measured on day- 1st, 3rd, 5th and 7th.

III. RESULT

The results of observations regarding the antibacterial effectiveness of mangosteen (Garcinia mangostana L.) soft rind extract and combination of mangosteen (Garcinia mangostana L.) hard-soft rind extract against Lactobacilus acidhopilus can be seen in the table below.

Table 1. Inhibition zones diameters of Lactobacilus acidophilus in millimeters (mm)

Group	Day-1	Day-3	Day-5	Day-7
L1	19,23	20,56	20,87	20,58
L2	21,87	21,99	23,53	22,48
K1	20,27	22,96	23,41	23,25
K2	20,85	23,71	24,05	24,29
K(+)	14,00	9,41	8,36	7,45

Based on table 1, the average diameter of the largest inhibition zone in the treatment group was obtained, which is the extract of mangosteen hard-soft rind with concentration of 100% (K2) on the 7th day and the smallest inhibitory zone is the mangosteen soft-rind extract with concentration 80% (L1) on the 1st day. The result shows the change of inhibitory zones in each treatment group of the mangosteen rind extract which constantly increased until the 3rd and 5th days then decreases on the 7th day except in the K2 treatment group which increased even until the 7th day. The positive control group (NaOCl 2.5%) experienced a decrease in inhibition zone until the 7th day.

Data which obtained then tested for its normality and homogeneity using the Kolmogorov-smirnov test and the Levene Test. The normality and homogeneity test results obtained was p> 0.05 in all treatment groups, which means that all data are normally distributed and homogeneous. Because the data is normally distributed and homogeneous, the statistical test used is the parametric test (Two Ways Anova) to determine whether there are differences between treatment groups. The anova test result obtained was p<0,05 which indicate that there are differences in all treatment groups, to see the significant differences between the study groups the LSD test (Least Significant Difference) was conducted as a follow up.

IV. DISCUSSION

Mangosteen rind extract is known to have antibacterial properties against various kinds of oral bacteria both gram positive and negative such as *Streptococcus mutans*, *Streptococcus salivarius*, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*[9,10]. In general, the antibacterial properties of the active ingredients contained in natural ingredients is carried out through the inhibitory activity of protein and nucleic acid synthesis, interfere the bacterial cell membranes, and inhibition of enzymes or changes in the protein molecules[6]. The antibacterial propertiesof mangosteen rind extract is caused by ingredients such as xanton, flavonoids, tannins, and anthocyanins. Xanton can bind the amino acids in bacterial cell membranes which trigger an increase in membrane permeability thereby causing the intercellular component to exit the bacterial cell. Flavonoids can prevent the formation of nucleic acids such as DNA and RNA which is an important compound that regulate bacterial cell metabolism, in addition flavonoids also able to trigger potassium loss in bacterial cells that cause direct membrane damage[11,12]. Flavonoids isalso reported to be able to disrupt the exchange of nutrients and metabolites, thus preventing the formation of a bacterial energy supply[13]. Tannins inactivate proteins that used by bacterial cells to stick thmselves to host cells[14]. The anthocyanin antibacterial mechanism is carried out by disturbing the respiration system of the bacterial cells through a decrease in the ATPase enzyme. The ATPase is a compound that convert ATP which stored in the form of ADP in bacterial cell respiration system. This decrease in enzymes can cause the release of ATP from the cytoplasm of thebacterial cells so that it interferes with its metabolic process[15,16].

The formation of the inhibition zones is an indication of material antibacterial propertiesof a activity[17]. Bacterial inhibition zones are formed due to the diffusion process of substances in the agar media. This diffusion process occurs due to the presence of kinetic energy in the form of movement from areas of high concentration to low concentration[18]. According to the Conner and Beuchat classification, the inhibition zone strength standards are categorized into three namely, strong activity> 11 mm, weak activity 6-11 mm, and no activity <6 mm[19]. The results of the average inhibition zone diameter in all treatment groups of the mangosteen rind extract concentration of 80% and 100% in this study showed that the inhibitory activity was relatively strong (> 11mm). The positive control (NaOCl 2.5%) had a relatively strong activity on day 1st, and moderate activity on days 3rd, 5th, and 7th. The results of this study indicate that NaOCl has a smaller inhibitory zone compared to the whole treatment group of mangosteen rind extract and the inhibitory zone decreases after the first day. This can be caused by the nature of NaOCl or sodium hypochlorite which is an unstable compound[20]. One that affects the rate of degradation of this compound is the storage method. Optimal storage for NaOCl is at 4 ° C[21], while in this study the NaOCl used is stored at room temperature so that it affects the release of the active substance. Other factors that may affect the decrease in the NaOCl properties are time and temperature[20]. The increase in temperature affects the pharmacological changes of the active substance contained in NaOCl, therefore in the incubation process at 37 ° C for 7 days in this study is thought to be the cause of the degradation of sodium hypochlorite active compounds which leads to the inhibitory power decreases. The active components of flavonoids and anthocyanins are known to experience no degradation whatsoever despite heating at 40 ° C for 3 days[22]. Flavonoids and anthocyanins are more easily damaged due to the effect of alkaline pH, while the media used in this study are acidic MRS[23], therefore the stability of these compounds can be maintained properly.

The Anova test results showed a value of p <0.05 which means there are differences between all the treatment groups. The difference between the group of soft rind extracts and hard-soft rind extracts is due to differences in the concentration of anthocyanin compounds contained in the rind. Palapol et al (2009) reported that most of the anthocyanin content stored in the hard rind of mangosteen fruit[7]. The total anthocyanin ratio between hard and soft rind in the final stages of development of mangosteen rind color (blackish purple color) reaches 9: 1. The addition of hard rind tothe hard-soft combination extract causes the antibacterial properties of the hard-soft rind extract to be stronger.

The LSD test results showed a significant difference in the treatment group L1 (80% soft rind extract) against K1 (80% hard-rind extract) and K2 (100% hard-rind extract) on days 3, 5, and 7. This can be caused by differences in the anthocyanin content of the rind and also the rate of reaction in the group. There was no significant difference in the change of the inhibition zone diameter of the L1 groupbetween days so it is presumed that the diffusion rate is expected to reach its optimal on the first day, while there is a significant difference in the change of inhibition zone diameter of group K1 and K2 between day1 and 3 which means that with more days the rate of diffusion rate of groups K1 and K2 was still increasing significantly until the 3rd day. Therefore, a significant difference between the groups occurred after K1 and K2 reached the optimal diffusion rate on the 3rd day.

There was no significant difference between the L2 (100% soft rind extract) treatment group against K1 (80% hard-soft rind extract) and K2 (100% hard-soft rind extract) on the same day. This is presumably even though the L2 group contains less total anthocyanins, the total phenol compound content in the soft rind is higher than in hard rind. Research conducted by Chaovanalikit et al (2012) shows that the total phenol compounds (in 100 gr) on soft rindreaches up to 3,404 mg GAE (gallic acid equivalent) whereas on outer rind is 2930 mg GAE[21], so that despite the differences in the total content of antibacterial anthocyanin, the soft rindwith 100% concentration does not have a significant difference with 80% and 100% hard-soft rind concentration.

The results of this study showed an increase in the diameter of the inhibition zones in the mangosteen rind treatment group L1, L2, and K1 until the 5th day, and K2 until the 7th day. This is supported by the statement of Valgas et al (2007) which stated that time is one of the parameters that influence diffusion. The amount of distance that can be achieved by a diffused molecule is roughly proportional to the inverse of the square of time.The decrease in inhibition zone diameter of the treatment group on the 7th day can be caused due to the decrease in diffusion due to the effect of increased interaction of the solutes and solvents (solubility between extracts and agar media)[18]. As for the K2 treatment group, the diffusion process continued to increase until the 7th day (with an insignificant increase in day 5) because it had a lower level of solubility compared to the other treatment groups.

The amount of concentration is the number of particles per unit volume. Dilution with water resulted in the degradation of molecules so that they will be surrounded by solvents. This means that the concentrated solution (high concentration) has a distance between molecules that are closer together than the solution that has been diluted. Increasing the distance between molecules causes the chemical bonds in the substance to weaken[24]. When interacting with other substances, molecules with weak chemical bonds need less energy to split. This causes the dissolved molecules to dissolve more quickly[25], therefore the extract with 80% concentration has decreased on the 5th day because after interacting with the agar media, the molecule decomposes faster than the extract with a concentration of 100%.

V. CONCLUSION

The conclusion of this research is the extract of mangosteen (Garcinia mangostana L.) hard-soft rind with 100% concentration is the most effective treatment group in inhibiting the growth of Lactobacillus achidopillus bacteria in 7 days and mangosteen (Garcinia mangostana L.) soft rind extract with concentration of 80% and 100% as well as hard-soft rind extract of mangosteen (Garcinia mangostana L.) concentrations of 80% and 100% have greater antibacterial power compared to 2.5% conctration of NaOCl.

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