Hydrogel synthesis of PVA/O. sativa for antimicrobial activity using freeze-thaw method

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Abstract. This research project was aimed to develop antibacterial hydrogel from blends of PVA and Oryza sativa L by applying freeze-thaw method (later on will refer as PVA/OS hydrogel). Agar well diffusion method was used to test the antimicrobial activity of the hydrogel against Gram-negative (E. coli) and Gram-positive bacteria (Staphy. aureus). The results indicate that PVA20/OS at pH 7 was effective as antimicrobial against both tested organisms while OS/PVA20 at pH 8 was effective for E. coli only. Based on the phytochemical test, the antimicrobial activity probably causes by the present of flavonoids content in the hydrogel. The higher PVA used in the blend’s hydrogel will increase the thickness of hydrogel. PVA40 at pH 7 and pH 8 shows a good result in mechanical properties where the value of thickness is 0.750 and 0.770 mm respectively. PVA/OS blend hydrogel had good antimicrobial activity and good compatibility to form an antimicrobial hydrogel for medical purpose with the used of 20%.

1. Introduction
Nowadays, multifunction hydrogel with a different combination of polymer became a good biocompatibility and high mechanical toughness is extensively investigate for great potential in medical sector especially for artificial skin, regeneration of skin, drug delivery and so on [1,2].

Today, most recent studies on hydrogel are about fabrication [3], degradation process, in vitro and in vivo cell interaction [4] but lacked of report about the antimicrobial activity of hydrogel. Natural antimicrobial has become very important in global health because of their potential as antibiotics for many diseases [5]. Many types of antimicrobials have been created in order to destroy and inhibit the growth of bacteria since last half of the previous century. But now it is become more complicated because of resistance of bacteria for certain vaccination or antibiotic are developing increasingly. A study shown the biofilms represent a target of new compositions for inhibiting the growth of bacteria in medical field [6].

By adding a natural active agent, the quality of a films can be improving. Some studies shown that plants rich in phenolic compounds can be used as active agent because high of antioxidant properties
[7]. *Oryza sativa* (*O. sativa*) can be used as active agents because it contains phytochemical like phenol that can acts as antioxidant besides it has good film forming properties. Other than that, phytochemical like phenol or flavonoid also has an excellent activity against bacteria such as *E. coli* and *Staph. aureus*.

In this study, we reported a simple fabrication of PVA/OS that possessed an antimicrobial activity and nontoxic hydrogel. Started from blended of PVA with *O. sativa* to reinforced networking within the hydrogel, until PVA/OS hydrogel could be obtained. This study provides a new inside of antimicrobial properties of hydrogel, which will improve the biocompatibility of hydrogel.

2. **Methodology**

In this research, two factors are antimicrobial activity and mechanical characteristic have been chosen to evaluate a PVA/OS hydrogel.

2.1 **Materials**

*O. sativa* is collected from Arau, Perlis and grain was dried at temperature 40ºC for 24 hours. After dried, the grain was grinded into powder using mechanical blender.

2.2 **O. sativa solution**

2g of *O. sativa* powder is mixed with 50 ml distilled water and heated at least half an hour with magnetic stirrer at temperature 75ºC by use double boiling method. Then, 7.5 ml of glycerol is added with constant stirring with magnetic stirring for half an hour until get homogenized mixture with an increasing a temperature until 85ºC.

2.3 **PVA solution**

10 g of PVA is mixed with 50 ml distilled. Then, heated and stirred using magnetic stirring at 90ºC [8] until all the PVA is completely dissolve to produce a good film. Then the films are produced by different concentration of PVA from 10% to 40% (w/v).

2.4 **O. sativa / PVA solution**

The *O. sativa* and PVA solution is mixed together. The mixture is then heated by use double boiling technique for at least half an hour with constant magnetic stirring at temperature 85ºC.

2.5 **Casting**

Pipetted the mixture into the plastic petri dish and used flaming to remove air bubbles. The solution then will be dried at 30ºC at least 24 hours for both pH7 and pH8, then follow with freezing process at -20ºC for 6 hours (1 cycle of freeze-dried process) and repeated until 3 cycle of process. The films are then will be removed from the plastic petri dish and will be placed in the desiccator.

2.6 **Agar well diffusion methods**

Nutrient Agar (NA) is used as a medium in agar well diffusion methods. Four grams of NA in 100 ml distilled water until completely dissolve and continue with autoclaving the NA for 15 min, 1 atm at 121ºC. Before the test for agar well diffusion is conducted, 0.5 McFarland bacterial inoculum should be prepared first. Single colony of bacteria from overnight culture are transfer into distilled water and vortex to dissolve the bacteria. The turbidity of bacterial suspension adjusted to 0.5 McFarland or (0.5-2.5) x 10^8 CFU/ml. The bacterial suspension then is diluted in sterile dH₂O to make the concentration become 5x10^5 CFU/ml. Then, 100 ml of NA was cooled at room temperature and inoculated with 1-2 ml of 5x10^5 CFU/ml before pour into petri disc. After 30-45 minutes, four well, 6mm in diameter well was punched aseptically with a sterile tube or tip [9] to develop a well. 100 µl of each sample/sterile dH₂O, 100% DMSO/ 50mg/ml of commercial antibiotic is introduced into respective well. Generally, antimicrobial agent diffuses into the agar and inhibit growth of the target bacteria and the diameter of lysis zones are measured by Vernier calliper.
2.7 Thickness test
The vernier caliper were used to measure the thickness of 5cm x 1cm of PVA/OS hydrogel. The thickness is measured in triplicate for each blended (10%, 20%, 30% and 40%), pH7 and PH8 respectively.

2.8 Morphological studies
The PVA/OS hydrogel are cut into square shape (10 mm x 10 mm) and placed on the Scanning Electron Microscope (SEM) sample holder but must coated with a thin layer of platinum to avoid electrostatic charging and poor resolution during examination.

2.9 Chemical bonding
The spectrum in range from 400 to 4000 cm\(^{-1}\) [8] are recorded using Fourier Transform Infrared Spectroscopy (FTIR). For the films, a square shape (10 mm x 10 mm) of sample was directly used for the FTIR test.

3. Results and Discussions
The results tabulate the PVA/OS with the specified variable parameters condition which obtained from the antimicrobial and phytochemical study of hydrogel and simulated in the Excel software.

3.1 Antimicrobial Activity Test
Antimicrobial value from E. coli and Staphy. aureus agar diffusion was shown in Figure 1 and Figure 2 respectively. The results tabulate the average value for antibacterial activity of each percentage PVA/OS hydrogel.

The results tabulate the average value for antibacterial activity and effect of different pH of each percentage PVA/OS hydrogel against E. coli. Two different pH which is pH7 and pH8 were shown a slightly decrease of antibacterial property from PVA\(_{20}\)/OS > PVA\(_{30}\)/OS > PVA\(_{40}\)/OS. However, PVA\(_{10}\)/OS with a 30% differentiation between pH7 with pH8 and PVA\(_{20}\)/OS was able to inhibit the highest growth of bacteria. O sativa has low hydrophobic binding that can enhance the antimicrobial activity. But pattern was shown with the increment of PVA concentration, cause a slightly decrease of antimicrobial property of hydrogel [9].

**Figure 1.** Anti – E. coli against PVA/OS hydrogel
Figure 2 show the result obtain from Agar Well Diffusion Methods from *Staphy. aureus*. The result obtained for *Staphy. aureus* was a bit differed to *E. coli*. Staphy. aureus is a Gram-positive bacterium which is lacked of protection shield compared to Gram-negative bacteria, *E. coli*. [10][11]. Previous author was reported a difference between antibacterial activity of Gram-positive (*Staphy. aureus*) compared with Gram-negative (*E. coli*) [8].

![Anti- Staphy. aureus against PVA/OS hydrogel](image)

**Figure 2.** Anti – *Staphy. aureus* against PVA/OS hydrogel

The antimicrobial activity for *E. coli* and *Staphy. aureus* agar diffusion was shown a pH8 for PVA/OS hydrogel has the high activities against a target bacterium. Meanwhile, a PVA20/OS of pH7 was shown a slightly highest activity against these two different types of bacteria, which is 0.89mm and 2.243 mm respectively. Thus, this antibacterial activity result was shown PVA/OS hydrogel easier to inhibit growth of *Staphy. aureus* compared to *E. coli*. Besides that, a different pH also shown a different ability to lysis a bacteria cell; decreasing for *E. coli* and slightly increase for *Staphy. aureus*.

**3.2 Characterization test**

SEM, thickness and FTIR test were used to determine a chemical bonding and morphology of PVA/OS hydrogel. A SEM image for the cross section of PVA/OS was shown in Table 1.
Table 1 was showing the SEM image for cross-section in 1000x magnification for PVA₁₀/OS, PVA₂₀/OS, PVA₃₀/OS and PVA₄₀/OS with the increasing the amount of PVA from 10% until 40%, a cross section PVA/OS become roughest due to its brittle structure.

The cross-section for PVA₈₀ show the smooth image because the film contains high amount of PVA. The binding between *O. sativa* and PVA in this blend film was the strongest and it give compact structure for the film. It can be seen clearly that the cross-section for PVA₂₀ was brittle and pores visible in the film. It is because PVA₂₀ has low concentration of PVA.

| PVA/OS   | pH7     | pH8     |
|----------|---------|---------|
| PVA₁₀/OS | ![SEM image](image1) | ![SEM image](image2) |
| PVA₂₀/OS | ![SEM image](image3) | ![SEM image](image4) |
| PVA₃₀/OS | ![SEM image](image5) | ![SEM image](image6) |
| PVA₄₀/OS | ![SEM image](image7) | ![SEM image](image8) |
Table 2. Thickness result of PVA/OS hydrogel

| pH Value | pH7 Thickness (mm) | pH8 Thickness (mm) |
|----------|-------------------|-------------------|
| PVA_{10}/OS | 0.400             | 0.390             |
| PVA_{20}/OS | 0.510             | 0.520             |
| PVA_{30}/OS | 0.625             | 0.655             |
| PVA_{40}/OS | 0.750             | 0.770             |

Thickness of PVA_{40}/OS was the thicker compared to another blend hydrogel because it contains high amount of PVA. This thickness study also shows by the increase the concentration of PVA, cause a thickness of hydrogel structure become thicker.

In this study, the presence of two different peaks of hydroxyl was shown at 3300-3500cm$^{-1}$ and 993.19-996.33cm$^{-1}$ respectively was show in Fig. 3. This result was support with a previous study by Huang Feng et. al [12], which is all the hydrogel produced the appearance of -OH groups within the wavelength 3360.9-3367.2cm$^{-1}$. This PVA/OS hydrogel also show another absorption band at range 2948.4-2952.6cm$^{-1}$ due to anti-symmetric stretching C-H from alkyl groups. Abdullah et. al [13]. Moreover, the peak between 1461.8-1645cm$^{-1}$ was indicate as a stretching C=O and C-O from acetate group in PVA.

Figure 3 was shown a FTIR result for both PH7 and PH8 with a different concentration of PVA/OS hydrogel.
Figure 3 show the presence of C-O vibration of C-O-C groups at peaks 1049.9-1119.1 cm\(^{-1}\). With the concentration of PVA increased from 10% until 40%, the peak intensity of C-O in C-O-C groups will be decreased or almost have a same pattern. This is because of the characteristic of absorption band and asymmetric stretching for pure PVA hydrogel at 1088 cm\(^{-1}\) [10]. In addition, peak in the 100-1200 cm\(^{-1}\) region were assigned to the C-O vibration of C-OH groups indicating the strong hydrogen bonding interaction between PVA with \textit{O. sativa} in hydrogel. The presence a few -OH, C-O and hydrogen bond in this PVA/OS was show the compatibility of this two-material become a good and sustainable hydrogel for medical purpose.

4. Conclusions
This study is helpful in enhancing the quality of hydrogel enforce with \textit{O. sativa} where the objective of fabrication new antimicrobial hydrogel of PVA/OS have been achieved. The result also shows that:
- By using natural source of material such as \textit{O. sativa} will enhance the structure and morphology of hydrogel.
- A new fabrication PVA\textsubscript{20}/OS with pH8 was show the best antimicrobial ability for both Gram-positive and Gram-negative bacteria.

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