PHENOL EXTRACTION FROM MORINGA OLEIFERA AS BIOFOULING REDUCER AGENT FOR MIXED MATRIX ULTRAFILTRATION MEMBRANES

[1]SHINTA ROSALIA DEWI, [2]LAILATUL MASYRIFA, [3]NI'MATUL IZZA, [4]DIMAS FIRMANDA AL RIZA, [5]YUSUF HENDRAWAN, [6]BAMBANG DWI ARGO, [7]YUSUF WIBISONO

[1,2,3,4,6,7]Program of Bioprocess Technology, [5]Program of Agricultural Engineering,
Faculty of Agricultural Technology, University of Brawijaya, Jl. Veteran No.1 Malang 65145, Indonesia
Email: [1]shinta.rd17@yahoo.com

Abstract - Membrane technology is robust technology to clarify tropical fruit juices. Yet, during clarification process, ultrafiltration membrane suffered from biofouling developed on the membrane surface. The use of antimicrobial agents provides alteration of unwanted microorganism grew on membrane surface and maintain the membrane process. In this study, antimicrobial compounds from Moringa oleifera was extracted using ethanol by maceration. Moringa oleifera (Mo) dried by using oven, milled and meshed. The liquid extracts were then processed by using a rotary evaporator to increase concentration. The concentrated extract then dried by using freeze dryer to obtain powdered Mo. The powdered was then used as filler in the mixed matrix membrane for ultrafiltration of fruit juices. The powdered Mo is acted as biofouling reducer agent in this membrane clarification process. The results showed that Mo extract had total phenolic content of 7.01% and was able to against Escherichia coli in its application on membrane.

Keywords - Moringa oleifera, extraction, maceration, phenolic content, antibacterial

I. INTRODUCTION

Membrane technology is one of technologies that is efficient, low energy and environmentally friendly to process of agricultural commodities into beverage products. Membrane technology has been widely applied in filtration of food productions such as ultrafiltration of pineapple juice (De Barros et al, 2003), purification of kiwi juice (Mondal et al, 2011), ultrafiltration of potato fruit juice (Schmidt, et al, 2016) and sugarcane juice (Li et al, 2017). However, the most problem in membrane technology is the decrease of membrane performance due to biofouling or blockage of pore or membrane surface by biological material (Wibisono, 2014).

Membrane biofouling in either food processing or waste treatment cannot be avoided as the processed materials are biological matter. Thus, some research is needed to reduce biofouling to prolong membrane life and maintain membrane performance. The membrane biofouling can be prevented by coating the membrane with a material that prevents adhesion or using antimicrobial material (Wibisono, 2015). Damayanti et al (2011) reported that activated carbon powder, zeolite and Moringa oleifera as biofouling reducer agents eliminated microbial contaminants on membrane bioreactor of palm oil.

Moringa oleifera (Mo) is a small-medium tree, contains plenty of phenolic compounds, which has nutritional and therapeutic properties (Moyo et al, 2012). Almost all the parts of Mo plant have been used for medicine. Marcus and Nwineewii (2015) reported that Mo leaf extract contained saponins, terpenoids, phenols alkaloids, glycosides, while flavanoids and tannins were absent. The content of alkaloid and phenolic in Mo inhibited bacterial activity (Pandey et al, 2012). The antimicrobial activity of Mo leaf extract against Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumonia (Kalpana, et al, 2013). The presence of antibacterial compounds in Mo extract can be used as biofouling reducer agent to prevent decreasing of membrane performance during ultrafiltration process.

Many studies of phenolic extraction were reported using maceration (Sulaiman et al, 2011) and soxhlet extraction (Chan et al 2011). Maceration is widely used for extraction of phenolic compounds because it is simple methods. It also requires low energy and prevents the damage of extract obtained compared to those uses heating such as soxhlet extraction. In this research, the phenolic compound was extracted from Mo leaf using maceration to prevent the extract damage.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Moringa oleifera leaf were collected from Gresik, East Java, Indonesia. All chemicals and reagents used in this study are analytical grades (pure analysis). Ethanol and sodium carbonate were purchased from Merck. Folin-Ciocalteu phenol reagent was purchased from Sigma-Aldrich Chemicals. Gallic acid was obtained from J.T. Baker. NA, nutrient broth and Escherichia coli used for antibacterial tests.

2.2. Extraction of Moringa oleifera (Mo)

The Mo leaf (1000 g) were washed with distilled water and dried in oven at 60^{o}C for 20 hours. They were later ground using a grinder to yield powder. Powder, then, sieved using molecular sieved of 60

mesh. Mo powder was extracted using ethanol (1:5 %b/v) by maceration method for 48 hours. The extracts were then filtered using vacuum filter and evaporated using rotary vacuum evaporator (Heidolph, 220 V) at 50°C, 65 rpm for an hour. The fresh extract was dried in oven for 48 hours and was characterized by FTIR.

2.3. Determination of total phenolic content (TPC)

Total phenolic content (TPC) of extracts were quantified using Folin-Ciocalteu method with gallic acid as standard. Gallic acid solutions with concentration of 0, 10, 20, 30, 40, 50, 60 and 70 mg/L were prepared as standard solutions. Gallic acid standard solutions were prepared by adding 2.5 ml of Folin-Ciocalteau 10% into 0.5 ml of gallic acid solution and incubated for 5 min. The 2 ml of sodium carbonate 7.5% then was added into these mixtures and incubated for 15 min. For each sample, the TPC of extracts were analyzed by adding 2.5 ml of Folin-Ciocalteau 10% into 0.5 ml of the sample then 2 ml of sodium carbonate 7.5% was added. After 15 min of incubation at room temperature, the absorbance of each sample was read by Spectrophotometer UV-Visible (Genesys 10) at 765 nm.

2.4. Antibacterial tests

Antibacterial tests were done by agar diffusion method (wells). In the media for microbes that have been planted, filled with antibacterial substances. After incubated at 37°C and 24 h, observations were done by looking at the presence or absence of microbial activity.

III. RESULTS AND DISCUSSION

3.1. Characterization of Moringa oleifera (Mo) extract

Mo extract was characterized using FTIR spectroscopy and the spectra shown in Fig 1. Fig 1 proved that Mo extract contains phenolic compounds. Phenolic content was evidenced by the specific peak at 3333.53 cm^{-1} (–OH groups of phenol); 2926.57 cm^{-1} (C-H stretching); 1237.05 cm^{-1} (C-O groups of phenol), and the peak of C=C groups at 1611.21 cm^{-1} and 926.53 cm^{-1}. Furthermore, the peak at 1611.21 cm-1 proved two groups of C=C stretching of alkenes and C=O of carboxylic acid; While the peak at 926.53 cm^{-1} (C-C and C=C of aromatic compound) indicated the phenolic compounds.

The peak of spectra in Fig 1 is accordance with Adebisi et al (2014) studied, reported that at peak of 3116 cm-1 indicated OH groups of phenol compounds; the peak of C=C of alkenes at 1647 cm-1, whereas at 1106.21 cm-1 was the vibration of C-O-H bending and C-O stretching. In another study mentioned that at a range of wavelength of 3500 to 3200 cm-1 was OH groups of phenol, the peak of 1244.40 cm-1 was C-O stretching of phenol, at a range of 900 to 675 cm-1 was C-C and C=C groups of aromatic compounds (Marcus and Nwineewii, 2015).

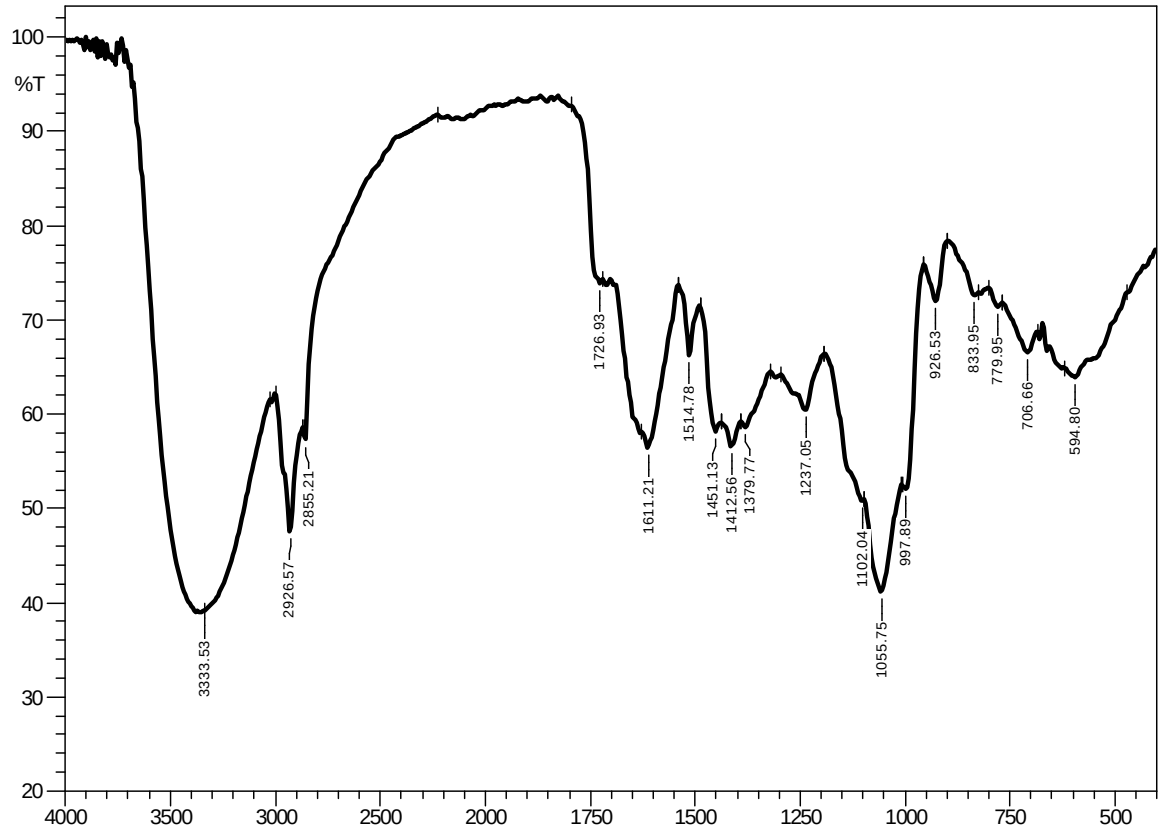


Fig 1. FTIR Spectra of Moringa oleifera extract

3.2. Total phenolic content (TPC)

In this study, Moringa oleifera (Mo) leaf was extracted using ethanol by maceration. The solvent used for phenolic extraction was ethanol because it is polar thus can dissolve phenolic compound which is polar. The total phenolic content (TPC) in Mo extract was determined using Spectrophotometer. The result showed that the TPC values of Mo leaf extract was 7.01%. According to Foild (2007), the content of phenolic in fresh Mo leaf was 3.4% while the phenolic in Mo leaf extract was 1.6%. Moreover, Ojiako (2014) reported that total phenolic content in Mo leaf extract was only 0.19%. It can be concluded that the Mo extract contained higher TPC than in the previous study. It is evident that Mo extract contains high phenolic compound which has potential as antibiofouling in membrane.

3.3. Antibacterial activity

Mo extract contains phenolic compound where phenolic compound has potential as antibacterial. Kalpana et al (2013) reported that Mo leaf extract has ability to inhibit bacteria activity of Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumonia. In this study, the antibacterial activity of Mo extract was tested using Escherichia coli (E. coli). The result of antibacterial tests of Mo extract as antibiofouling agent shown in Fig 2. After incubated for 24 hours, Mo extract showed the inhibition of E. coli. It indicated that Mo extract inhibited E.coli activity.

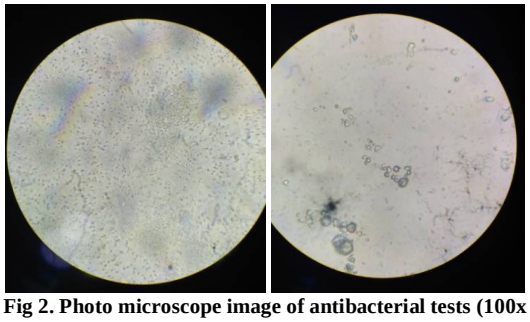


Fig 2. Photo microscope image of antibacterial tests (100x magnification) : membrane without Mo extract (left); with Mo extract (right)

Antibacterial testing method was conducted by dipping the membrane in bacteria culture. From Fig 2, it is found that the area of attachment of bacteria is getting smaller with the addition of Mo extract in membrane. In membrane without the addition of Mo extract, the area of bacterial attachment of 7.18%, then decrease to 1.52% in addition of Mo extract. It is evident that phenolic compounds in Mo extract inhibit E.coli.

Phenolic compounds have an antibacterial activity that works by interacting with bacterial cells through absorption processes involving hydrogen bonds. It interferes cytoplasmic membrane, inhibits the active transport and proton strength, which eventually killed the bacteria. The phenolic compounds will be absorbed into the cell's cytoplasmic membrane (1) and bind to the cell membrane components such as proteins, nucleic acids, and phospholipids thereby causing an increase in membrane permeability and inhibiting the DNA (2, 3). As a result, the cell components will release from cell resulting cell death (Fig. 3).

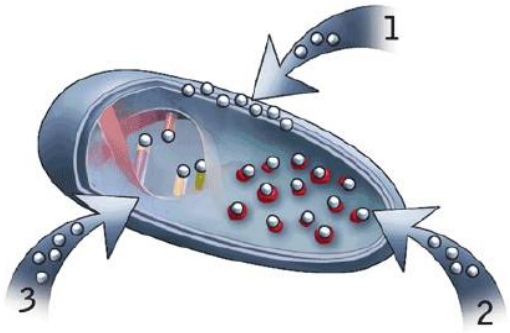


Fig. 3. Mechanism of Mo inhibit bacteria

CONCLUSIONS

The phenolic compound of Moringa oleifera (Mo) has been extracted using ethanol by maceration. The FTIR spectra confirmed that Mo extract contained phenolic indicated by the specific peak of phenolic at 3333.53 cm^{-1} (–OH groups of phenol); 2926.57 cm^{-1} (C-H stretching); 1237.05 cm^{-1} (C-O groups of phenol), 1611.21 cm^{-1} and 926.53 cm^{-1} (aromatic groups). The result is in accordance with the high content of total phenolic content (TPC) of Mo extract was 7.01%. The result of antibacterial tests showed that Mo extract against E. coli which is confirmed by decreasing area of bacteria attachment from 7.18% to 1.52%.

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