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Recovery of Phytochemical Components from Various Parts of *Morinda citrifolia* Extracts by Using Membrane Separator

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Abstract: In this study, extracts from various *Morinda Citrifolia* parts (leaf, fruit and root) by methanol was separated into permeate and retentate fractions using a membrane system equipped with a nanofiltration (NF) membrane. NF was carried on a ceramic membrane with MWCO of 5 kD. Effect of NF transmembrane pressure at 0.1, 0.12 and 0.17 bar was examined at constant temperature 45°C with constant flow rate. The influence of transmembrane pressure on the efficiency of antioxidant activity and total phenolic content of permeate retentate concentration was examined. The antioxidant activities of crude mengkudu extracts, NF permeate and retentate were evaluated by using the DPPH radical scavenging activity and total phenolic content.

Key words: Phytochemical, extraction, membrane separation and nanofiltration

INTRODUCTION

Morinda citrifolia known as Mengkudu or the popular name is Noni was extensively used in folk medicine by the Polynesians for over 2000 years (Samy, 2005; Yanine *et al.*, 2006). Traditionally, the fruit is claimed to prevent and cure several diseases (Bao-Ning *et al.*, 2004; B. Pongnaravane *et al.*, 2006 and Yanine *et al.*, 2006). Therefore it is important to develop the process to recover those phytochemical compounds from various parts of mengkudu plant.

The extraction of antioxidants from *M. Citrifolia* has been accomplished by traditional extraction processes with magnetic stirrer or by using Soxhlet apparatus, such as solid-liquid extraction, using solvents, such as methanol, ethanol and acetone and also through extraction of various plant secondary metabolites using the pressurized hot water (PHW). Pongnaravane *et al.* (2006) reported that the mengkudu root had minimum and maximum antioxidant activity of 79.62 and 81.16% for hexane extraction, 93.42 and 97.94% for Soxhlet extraction and 78.79 to 96.41% for subcritical water extraction.

After the extraction of phytochemical compound, we are interested in its separation. One of the alternative methods is the membrane process which has been successfully utilized in purification, clarification and concentrating of fruit juices (Cassano *et al.*, 2003). The basic properties of membrane operations make them ideal in the recovery of phytochemical compound with high

quality, natural fresh taste and additive-free (Louli, 2004). They are generally, do not involve phase changes or chemical additives; besides, they are simple in concept and operation and characterized by low energy consumption (Mulder, 1996; Rirchard, 2004 and Richard *et al.*, 2002). The membrane separation that will be applied in this study is nanofiltration (NF) which is capable of removing ions that contribute significantly to the osmotic pressure and thus allows operation at pressures that are lower than those needed for reverse osmosis (Braeken *et al.*, 2005; Claudio, 2007). The nanofiltration (NF) is used when membranes remove materials having low molecular weights in the order of 300-1000 Da (Fellows, 2000).

The purpose of this research is to recover the extracts of Mengkudu leaf, fruit and root phytochemical components by using nanofiltration process. In this study, the effect of transmembrane pressure (0.10, 0.12 and 0.17 bar) in the membrane output (permeate and retentate) was investigated.

MATERIALS AND METHODS

Preparation of mengkudu powder: Mengkudu leaf, root and fruit were bought from the Kota Kinabalu Sunday market. The mengkudu fruit, leaf and root were washed with water and dried directly under the sun. After the root was dried, it was cut into smaller chips. The fruit in the other hand was sliced and the fruit seed was removed

before it was sun dried. This process took about 1 to 2 weeks depending on the weather condition. After drying process, the fruit, leaf and root was ground into fine powder. This powder was then kept in close container.

Solvent extraction: The ground powder was extracted with ethylacetate in a water bath at room temperature for 24 h. The solvent was removed by filtration and fresh solvent was then added to the plant material. The extraction was twice repeated. The combined filtrate was evaporated under reduce pressure to give a dark green viscous mass. Antioxidant activity of this methanol crude extract was measured. The remaining crude methanol extract was further extracted and then separated using separating funnels. Antioxidant activity was then measured after evaporation under reduced pressure.

Membrane separation: 0.5 g of mengkudu leaf paste is diluted in 4500 mL methanol. The solution is stirred until the paste is dissolved in the solvent. Then, membrane separation system set up is shown in the Fig. 1 and the details are shown in Table 1. The membrane water bath temperature was set at 45°C. The feed tank was filled with distilled water when the temperature is stable and the

stirrer in the feed tank was started together with pump 1. The distilled water goes through the membrane system and out as permeate (pass through membrane) and retentate (not pass through membrane). Then all the distilled water are withdrawn and dried by opening the tube connection in the membrane outlet (permeate and retentate outlet). The cleaning process was continued for at least two round of fresh distilled water added to the feed tank. For this process, temperature is constant and the pressure is variable.

After that, the mengkudu paste solution was added to the feed tank. The pressure was set at 0.10 bar and the pump was used for circulation. Five hundred milligram of permeate was collected and time was recorded. This process was repeated for pressure at 0.12 and 0.17 bar.

The permeate samples and remaining solutions (retentate) are concentrated using distillation. This process is mainly used to recover back the methanol solvent. Then, permeate and retentate samples are further concentrate by using hot plate. After dried into paste form, the antioxidant and phenolic content are analyzed and compared with the initial extract paste.

Table 1: Ceramic nanofiltration characteristics

Parameter	Data
Type of support	Monolithic Al ₂ O ₃ coated with TiO ₂
Diameter and length	25 and 386 mm
No. of channel	19
Hydraulic diameter of sub-channel	3.5 mm
Operating pressure	up to 10 bar or 160 psi
Service pH	0-14
Operating Temperature	200°C
Solvent	Unaffected
Molecular weight cut	5 kD
Membrane area	816 cm ²

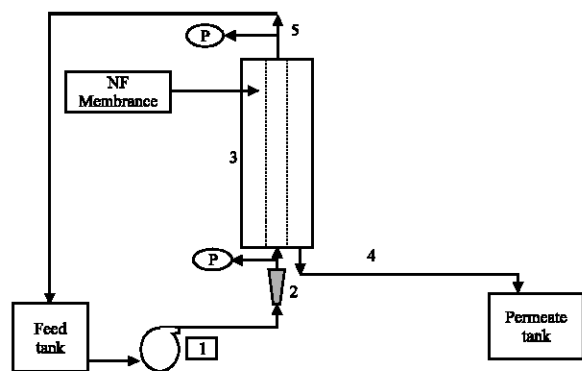


Fig. 1: Experimental set up (1; Pump, 2: Rotameter, 3: Ceramic nanofiltration membrane, 4: Permeate and 5: Retentate)

DPPH Radical scavenging activity: The DPPH free radical method is based on the determination of the concentration of DPPH at steady state in methanol solution, after adding the mixture of antioxidants. DPPH adsorbs at 515 nm and as its concentration is reduced by the existence of the antioxidants, the absorption gradually disappears with time (Louli *et al.*, 2004). One milligram of the mengkudu leaf extract was added to 10 mL of methanol. Then, 2 mL of each diluted extract (100 mg L⁻¹) was added to 3 mL of 50 mg L⁻¹ DPPH (with methanol) solution. The mixture was shaken for 1 min and then the absorbance was recorded in the Cary model UV-Vis spectrophotometer until constant reading of the absorbance was observed. Methanol was used as a reference. The DPPH scavenging activity values were calculated using the following equation.

$$\text{DPPH scavenging activity (\%)} = \left(1 - \frac{\text{Abs}_{515} \text{ sample}}{\text{Abs}_{515} \text{ DPPH solution}} \right) \times 100$$

where Abs₅₁₅ control is the absorbance of control (DPPH solution without sample) and Abs₅₁₅ sample is the absorbance of test sample (DPPH solution plus sample test).

Total phenolic content: TPC was estimated using Folin-Ciocalteu assay and was performed according to (Amin, 2006) procedure with some modification. 1.5 mL of

Folin-Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) was transferred into a test tube and mixed with 2 mL Mengkudu fruit extract sample (5 mg mL⁻¹). The mixture was allowed to stand for 5 min at room temperature. Then, 1.5 mL sodium carbonate (Na₂CO₃) solution was added, followed by 20 mL distilled water. Again the mixture was allowed to stand at room temperature for 2 h. Total phenolic content was determined using a Carry UV/VIS Spectrophotometer at 725 nm. Gallic acid was used as the standard by drawing the standard gallic acid curve. Total phenolic content was expressed as Gallic Acid Equivalent (GAE)/ 10 g sample extract.

RESULT AND DISCUSSION

Extraction of leaf, fruit and root of mengkudu: The results of extraction with different parts of Mengkudu are shown in Table 2. It can be seen that the trends are same where higher antioxidant activity possess higher phenolic content while lower antioxidant activity possess lower phenolic content. The antioxidant activity analysis by using DPPH radical scavenging activity, the mengkudu root exhibited the higher antioxidant activity with 74.9% and total phenolic content is 148.8 mg GAE/10 g of root sample. On the other hand, fruit has the lower antioxidant activity which is 54.2% and total phenolic content is 87.3 mg GAE/ 10 g of fruit sample. Leaf exhibits about 65.8% antioxidant activity. These result indicated that phenolic compound was responsible for the antioxidant activity in the selected mengkudu fraction. Normally, most of the phenolics are classified in two principles groups of phenol carboxylic and flavonoids and the derivatives of flavan (2-phenyl-benzoidihydropyran) (Zin *et al.*, 2006).

Based on results obtained, it is possible that several compounds of different polarity may contribute to the antioxidative activity of each mengkudu part. According to Zin *et al.* (2006) for the ethyl acetate extract, the responsible antioxidative component that may present is alkaloid in nature. In addition, there is more than two components of antioxidant may present in the leaf, fruit and root of antioxidant as reported (Bao-Ning *et al.*, 2004 which are having different polarities.

DPPH scavenging activity analysis: NF was used to study the effect of different transmembrane pressures of 0, 0.12 and 0.17 bar with constant temperature of 45°C to obtain the permeate and retentate efficiency. From Fig. 2, it can be seen that the permeate in the separation process exhibited almost stable radical scavenging activity. The fruit extract shown a slightly decrease at 0.12 bar, this may due to concentration polarization or blockage in the membrane.

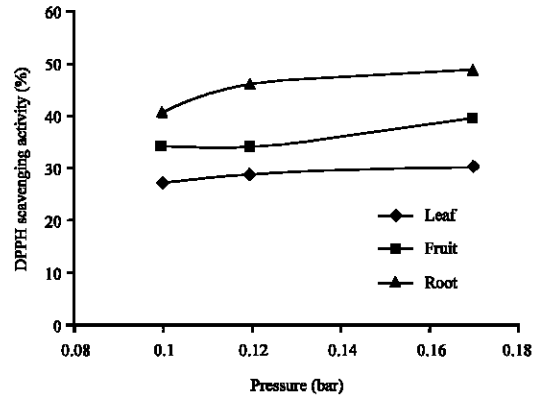


Fig. 2: DPPH Scavenging Activity of permeate of various parts in Mengkudu plant at different pressure

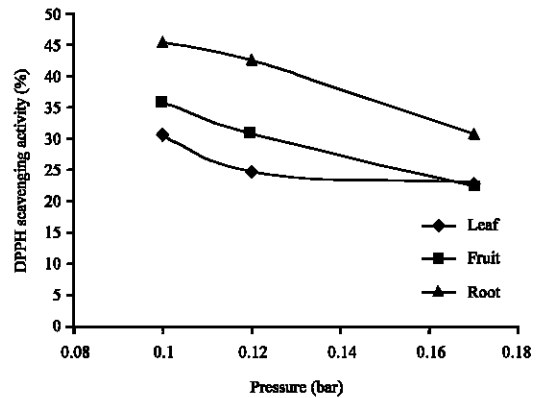


Fig. 3: DPPH Scavenging activity of retentate of various parts in Mengkudu plant at different pressures

Table 2: Contents of mengkudu extract

Property	Leaf	Fruit	Root
DPPH scavenging activity (%)	65.8	54.2	74.9
Phenolic content, mg/GAE / 10 g of sample	90.6	87.3	148.8

The retentate radical scavenging activity decreased as the pressure increased from 0.10 to 0.17 bar as shown in Fig. 3. This trend is similar for all parts of *M. citrifolia* extracts. After membrane separation, we could observe that the radical scavenging activity in the retentate for root and fruit is less than in leaf which is an opposite scenario for permeate activity.

The efficiency of this membrane operation is evaluated based on DPPH scavenging analyses which is shown in the Fig. 4 and 5.

From the figure above, it is observed that for the mengkudu leaf permeate efficiency decreases as the pressure increases and the retentate efficiency increased. For the mengkudu root, the retentate efficiency is decreases as the increase the pressure. Contract to this, the permeate efficiency increases as the pressure is

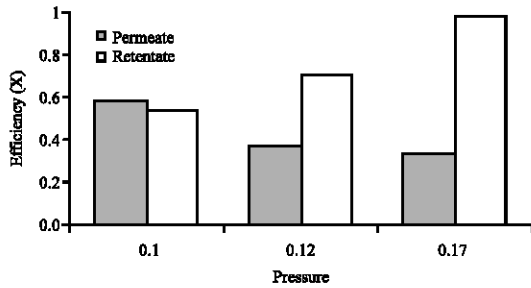


Fig. 4: Comparison of permeate and retentate efficiency for mengkudu leaf extract at constant temperature of 45°C based on DPPH analysis

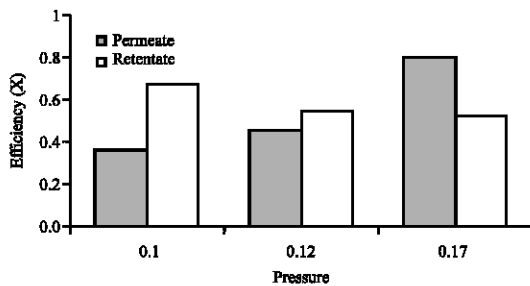


Fig. 5: Comparison of permeate and retentate efficiency for mengkudu root extract at constant temperature of 45°C based on DPPH analysis

increased. This may happen due to the phytochemical components containing different proportions of low and high molecular weight antioxidants which affect the solute rejection. Concentration polarization may also significantly influence the solute rejection and the effective trans-membrane pressure (Gilron *et al.*, 2006).

Total phenolic content analysis: Phenolic compounds in turn are a part of antioxidant families. Therefore, the reason might be due to the existing of both polar and non-polar natural characteristics in root parts compared to non-polar components in fruit and leaf parts of Mengkudu plant. DPPH analysis, the total phenolic content of various mengkudu plant parts were found as similar trend where the TPC content was decreased in permeate for all Mengkudu parts and for the retentate, the de TPC value increased with pressure.

Efficiency of nanofiltration separation in recovering the TPC can be evaluated from the Fig. 8-10. These figures show that the permeate efficiency increased and retentate efficiency decreased for all mengkudu extracts separation. The higher efficiency of TPC was found in permeate. It might due to contribution of molecular size of those phytochemical components (Arsuaga *et al.*, 2006) and suggested that the retention of low molecular weight

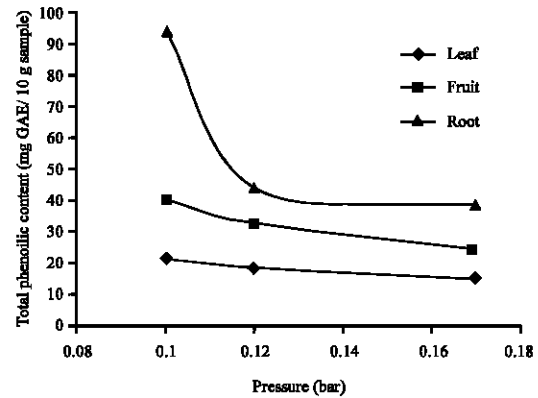


Fig. 6: Total phenolic content of retentate of various parts in Mengkudu plant at different operating pressure

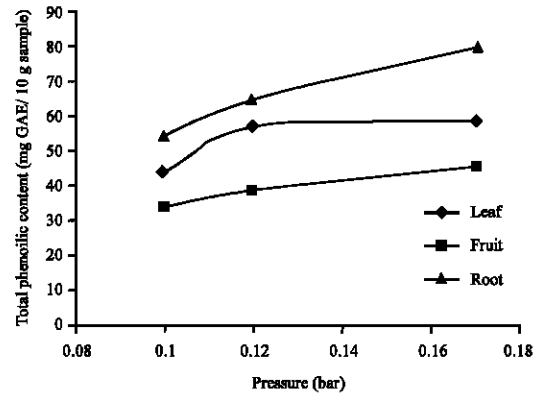


Fig. 7: Total phenolic content of permeate various parts in Mengkudu plant at different operating pressure

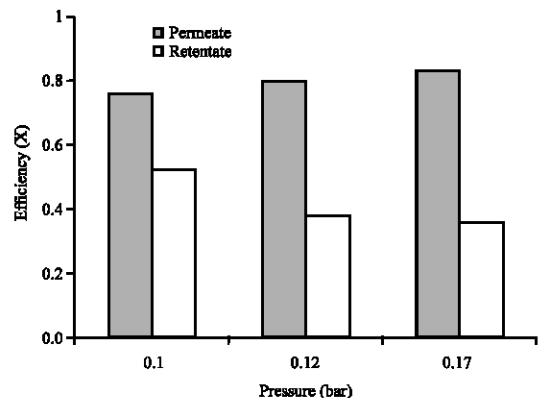


Fig. 8: Efficiency of both permeate and retentate in Mengkudu leaf extract at different operating pressure

organic solutes affected by two other important factors: the ratio of pore diameter/molecule size and the effect

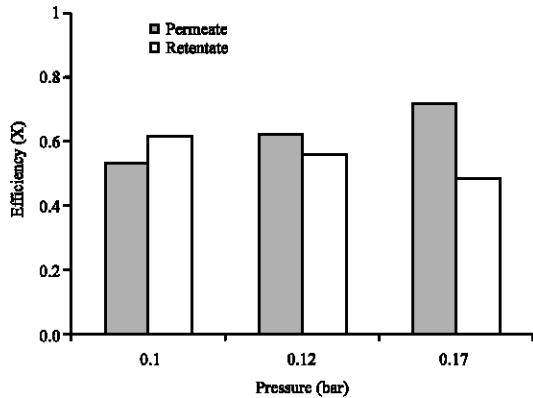


Fig. 9: Efficiency of both permeate and retentate in Mengkudu fruit extract at different operating pressure

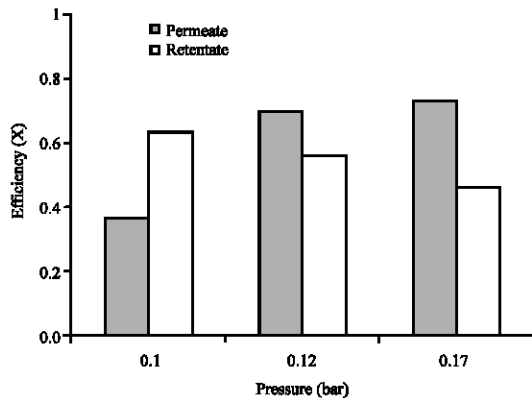


Fig. 10: Efficiency of both permeate and retentate in Mengkudu root extract at different operating pressure

of the interactions in the solvent-membrane-solute interphase. Concentration polarization may also significantly influence solute rejection (Gilron *et al.*, 2006). Higher fraction of polar molecules permeates through the membrane, compared to non-polar molecules with the same size, thus leading to a lower retention of a polar molecule. Furthermore, the retention behavior of an organic molecule is also influenced by charge effects. Depending on the nature of the membrane (charge), a higher or lower retention can be obtained than expected on the basis of molecular weight or any other size parameter (Van der Bruggen *et al.*, 1999).

The antioxidants from the Mengkudu leaf extract can be recovered in both permeate and retentate but the phenolic compound were found more in the permeate product which suggests that the purification of phenolic compounds is possible in the NF separation process. A similar approach was reported (Claudio, 2007).

The Fig. 8-10 indicate that the efficiency for antioxidative components recovery in permeate side is more rather than retentate by increasing the pressure at constant temperature. By comparing the various parts in mengkudu plants, the root exhibited highest TPC. It is followed by leaf and fruit.

CONCLUSIONS

Mengkudu root antioxidant activity and total phenolic content were found to be 74.9% and 148.8 mg GAE/10g, respectively. Whereas fruit antioxidant activity is the lowest one with the value of 54.2% and the total phenolic content of 87.3 mg GAE/10g of samples. For the nanofiltration separation process, the retentate decreased when transmembrane pressure increased which depended on antioxidant components.

REFERENCES

- Amin, I.Y., K.I. Norazaidah and E. Hainida, 2006. Antioxidant activity and phenolic content of raw and blanched Amaranthus species. *Food Chem.*, 94: 47-52.
- Arsuaga, J.M., M.J. López-Munoz, A. Sotto and G. Rosario, 2006. Retention of phenols and carboxylic acids by nanofiltration/reverse osmosis membranes: Sieving and membrane-solute interaction effects. *Desalination*, 200: 731-733.
- Bao-Ning, S., A.D. Pawlus, H. Jung, W.J. Keller, J.L. McLaughlin and A.D. Kinghorn, 2004. Chemical Constituents of the Fruits of *Morinda citrifolia* (Noni) and Their Antioxidant Activity. *J. Nat. Prod.*, 68: 592-595.
- Braeken, L., R. Ramaekers, Y. Zhang, G. Maes, B. Van der Bruggen and C. Vandecasteele, 2005. Influence of hydrophobicity on retention in nanofiltration of aqueous solutions containing organic compounds. *J. Membrane Sci.*, 252: 195-203.
- Cassano, A., E. Drioli, G. Galaverna, R. Marchelli, G. Di Silvestro and P. Cagnasso, 2003. Clarification and concentration of citrus and carrot juices by integrated membrane processes. *J. Food Eng.*, 57: 153-163.
- Claudio, R., 2007. A new membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from Vegetation Waters (VW). *J. Membrane Sci.*, 288: 239-246.
- Fellows, P., 2000. *Food Processing Technology: Principles and Practice*. 2nd Edn., England and USA. Woodhead Publishing Limited and CRC Press LLC.

- Gilron, J.N., Daltrophe and O. Kedem, 2006. Transmembrane pressure in nanofiltration. *J. Membrane Sci.*, 286: 69-76.
- Louli, V., N. Ragoussis and K. Magoulas, 2004. Recovery of phenolic antioxidants from wine industry by-products. *Bioresource Technol.*, 92: 201-208.
- Mulder, M., 1996. *Basic Principles of Membrane Technology*. 2nd Edn., Netherlands. Kluwer Academic Publishers.
- Pongnaravane, B., M. Goto, M. Sasaki, Thitiporn, Prasert. and A. Shotipruk, 2006. Extraction of anthraquinones from roots of *Morinda citrifolia* by pressurized hot water: Antioxidant activity of extracts. *J. Supercritical Fluids*, 37: 390-396.
- Richard, W.B., 2004. *Membrane Technology and Applications*. 2nd Edn., England. John Wiley and Sons Ltd.
- Richard, W. and J.S. Welfoot, 2002. Modelling the performance of membrane nanofiltration-critical assessment and model development. *Chem. Eng. Sci.*, 57: 1121-1137.
- Samy, J., 2005, *Herbs of Malaysia: An Introduction to the Medicinal, Culinary, Aromatic and Cosmetic Use of Herbs*, Shah Alam, Selangor: Times Ed., pp: 154-155.
- Van der, B.B., J. Schaep, D. Wilms and C. Vandecasteele. 1999. Influence of molecular size, polarity and charge on the retention of organic molecules by nanofiltration. *J. Membrane Sci.*, 156: 29-41.
- Yanine, C.B., V.A. Fabrice, M. Perez, M. Reynes, Jean-Marc Brillouet and P. Brat, 2006. Critical Review. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. *J. Food Composit. Analysis*, 19: 645-654.
- Zin, Z.M., A.A. Hamid and A. Osman, 2002. Antioxidative activities of extracts from Mengkudu (*Morinda citrifolia* L.) roots, fruit and leaf. *Food Chem.*, 78: 227-231.
- Zin, Z.M., A.A. Hamid, A. Osman and N. Saari, 2006. Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). *Food Chem.*, 94: 169-178.