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ORIGINAL ARTICLE

Antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* L. methanolic extract from Poly-ethersulphone (PES) membrane separator

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KEYWORDS

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Abstract Antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* L. methanolic extract by using membrane separator were investigated. The extract of *M. citrifolia* L. fruit by methanol was separated into permeate and retentate by Poly-ethersulphone (PES). The effect of temperature in the range of 30–70 °C, and pressure in the range of 0.5–1.5 bar on the antioxidant activity and total phenolic content was studied. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity exhibited a gradual increase in permeates' collection from membrane separation. The total phenolic content was also found to follow the same trend. The optimum magnitudes of DPPH radical scavenging activity and total phenolic content were found to be 55.60% and 43.18 mg GAE/10 gm of sample respectively.

1. Introduction

The global market of trade related to medicinal plants is estimated around US $60 billions per year and is growing at the rate of 7% annually with varying shares of developed and developing countries (Mukherjee, 2006; Quettier-Deleu et al., 2000; Bao-Ning et al., 2004). The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies on traditional medicine for their primary health care needs (Winston, 1999). In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their backbone. Recently, the antioxidant potential of medicinal herbs has been reported (Krishnaiah et al., 2011).

Morinda citrifolia L. has been known for its medicinal value since 2000 years ago. The bioactive components are found to be effective in the prevention of major diseases such as cancer, diabetes, cardiovascular diseases, hypertension etc. This indigenous medicinal plant is of great importance to the health of individual and communities and has a long history of ances-

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tors creating primitive medicines during their struggle against natural calamity and diseases (Ying et al., 2002). Basically, medicinal plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as secondary metabolites of photosynthesis. Oxygen is easily activated by ultra violet (UV) radiation and the heat from sunlight to produce toxic, reactive oxygen species (ROS). Plants produce various anti-oxidative compounds to counteract these ROS in order to survive (Ala et al., 2006). Thus, antioxidants are vital inhibitors of lipid peroxidation, not only for food protection but also as a defense mechanism of living cells against oxidative damage (Zin et al., 2002, 2006).

M. citrifolia L. native to Polynesia is one of the traditional folk medicinal plants that have been used for over 2000 years by polynesians for treating diabetes, high blood pressure, cancer, eye problems and many other illnesses. The bark, stem, root, leaf, and fruit have been used traditionally for many diseases, including diabetes, hypertension, and cancer and are all mentioned as Hawaiian herbal remedies. Many important modern drugs are plant-based or derived directly or indirectly from the plants. But only 6% of all therapeutically important species, which are noted in ancient literature, have been analyzed for their therapeutic potential (Ying et al., 2002; Winston, 1999).

M. citrifolia L. or Mengkudu, which originated in Tropical Asia, seems to be a much valued medicinal plant which has been extensively used in folk medicine and the plant is normally cultivated for its roots, leaves and fruits (Zin et al., 2002). The extract from leaf, fruit and root from Mengkudu exhibited a promising activity in DPPH scavenging and total phenolic content (Dieridane et al., 2005). However, low contents of bioactive components from the extraction and clarification degree of extract may limit the applications of extracts. Membrane processing has replaced conventional filtration methods for clarifying and it has been successfully applied to recovery of phytochemicals in a ceramic membrane system (Krishnaiah et al., 2007; Casaano et al., 2003; Claudio, 2007). The separation of different bioactive components of *M. citrifolia* L. is carried out by clarification and concentrating (Casaano et al., 2003), and membrane separation (Claudio, 2007). The basic properties of membrane operations make them ideal in the recovery of antioxidants with natural quality (Louli et al., 2004). In this study the recovery of bioactive components from a new membrane separator was carried out with the different operating temperatures ranged from 30 °C to 60 °C and pressure varied from 0.5 bar to 1.5 bar.

2. Materials and methods

2.1. Materials

Plant material used in this study is a fresh Mengkudu (*M. citrifolia* L. fruit seedless without core). The samples were washed with tap water and separated before being chopped into pieces. They were oven-dried at 60 °C for 2 days and ground to powder. Analytical grade 2, 2-diphenyl-1-picrylhydrazyl (DPPH), methanol, Gallic acid, Folin–Ciocalteu reagent and sodium carbonate were purchased from Merck, Germany.

2.2. Membrane preparation

Poly-ethersulphone (PES) (Aldrich) was used as the base polymer and *N*-methyl-2- pyrrolidone (NMP) (Acros) was used as the solvent. Distilled water was used as the coagulant. Dope solution was prepared by dissolving 15 wt.% PES in 85 wt.% NMP and stirred at 70 °C in a mechanical glass bottle. After 48 h, the mixture was completely dissolved and homogeneous. The resultant polymer solution in the same glass bottle was placed in a cabinet for 30 h at room temperature of 30 °C for air bubbles removal (Wang et al., 2006). The dope solution thus obtained was poured onto a clean and smooth glass plate at room temperature, and it was cast using a casting knife. The thickness of the membranes was controlled by varying the thickness of adhesive tape at the sides of the glass plate. The glass plate with the casting film was immediately immersed in a 300 mL of distilled water coagulation bath at 28 °C. Phase inversion started after few minutes and the thin polymeric film was separated from the glass plate. The membrane was washed with distilled water and stored in a distilled water bath for two weeks. The defect free membrane thickness was measured by micrometer (Mitutoyo, Japan). In this study, the casting thickness was controlled at 200 μm and the pore size was 7–9 μm measured by scanning electron micrograph as shown in Fig. 1.

2.3. Extraction of antioxidative compounds

Extraction was carried out according to the modified method of Pongnaravane et al. (2006). The ground powder was extracted with methanol using high pressure extractor (HPE) at 30 °C temperature with pressure of 25 bar and extraction time of 6 h. After the hydrostatic pressure extraction, the supernatant was separated from the Morinda powder residue by filtration using Whatman No.4 filter paper and evaporated under reduced pressure to give a dark green viscous mass. The antioxidant activity of this methanol crude extract was measured. The remaining crude methanol extract was further used for membrane separation. Antioxidative activity was then measured after evaporation under reduced pressure (Zin et al., 2002).

2.4. Antioxidant activity

The antioxidant activity was measured in terms of DPPH free radical scavenging activity and is based on the determination of the concentration of 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

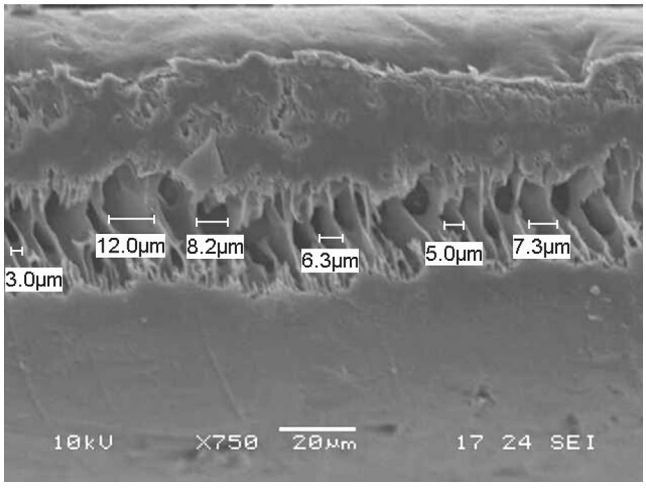

Figure 1 SEM image of PES membrane (average pore size 7–9 μm).

at a steady state in a methanol solution, after adding the mixture of antioxidants. DPPH solution is at maximum at 515 nm, and as its concentration is reduced by the existence of an antioxidant, the absorption gradually decreases with time. Perkin Elmer UV–VIS lambda-25 spectrophotometer was used to estimate the antioxidative activity. The extract was prepared at 100 mg/L concentration. DPPH concentration was 50 mg/L. Methanol was used as black sample and to dissolve DPPH and *M. citrifolia* L. extract (paste form). Then, 3 ml of DPPH solution was mixed with 2 ml of *M. citrifolia* L. extract sample solution and shaken well (quickly). This is because the DPPH and antioxidant reaction begins instantaneously. Then, this solution is quickly moved into cuvette using a pipette. After that, the cuvette was put into the spectrometer and the absorbance was measured at 515 nm. The DPPH radical scavenging in term of percentage is calculated (Krishnaiah et al., 2011):

$$\text{DPPH Scavenging Activity}(\%) = \left(1 - \frac{Abs_{515}\ sample}{Abs_{515}\ \text{DPPH}\ solution}\right) \times 100\%$$

2.5. Total phenolic content analysis

The total phenolic content (TPC) of each sample was estimated using the Folin–Ciocalteu colorimetric method with minor modifications. Appropriately diluted test sample (0.2 mL) was reacted with 0.5 N Folin–Ciocalteu reagents for 4 min at room temperature. The reaction was then neutralized with saturated sodium carbonate (75 g/L) and allowed to stand for 2 h in the dark at room temperature. Later the absorbance of the resulting blue color was measured at 765 nm with a spectrophotometer. Quantification was done on the basis of a standard curve with Gallic acid. The concentration of total phenolic compounds in all plant extracts was expressed as milligrams of Gallic acid equivalents (GAE) per gram dry weight of plant, which was determined from known concentrations of Gallic acid standard prepared similarly (Maisuthisakul et al., 2005).

2.6. Batch mode membrane separator

The experimental setup of the batch mode membrane separator is shown in Fig. 2. The details of setup are shown in Table 1. It consists of l.5 l stainless steel cylindrical vessel, membrane, and a recirculating pump to make the solution homogeneous and to control the temperature. The experimental set up of membrane separator is of batch mode. Separation of bioactive components depends on the membrane characteristics. The yield of each quantity was estimated with respect to different process parameters. The pressure in the separator was maintained by compressed air. The membrane was based on Polyethersulphone (PES). (Rosalam and England, 2004). 1 l of extract of *M. citrifolia* L. is charged into the cylindrical vessel. Then the desired pressure is maintained by the compressed air. The permeate and retentate were collected at different process parameters for analysis of antioxidant activity and total phenolic content. All the experiments were performed in triplicate and the standard deviation was found to be ±0.2%.

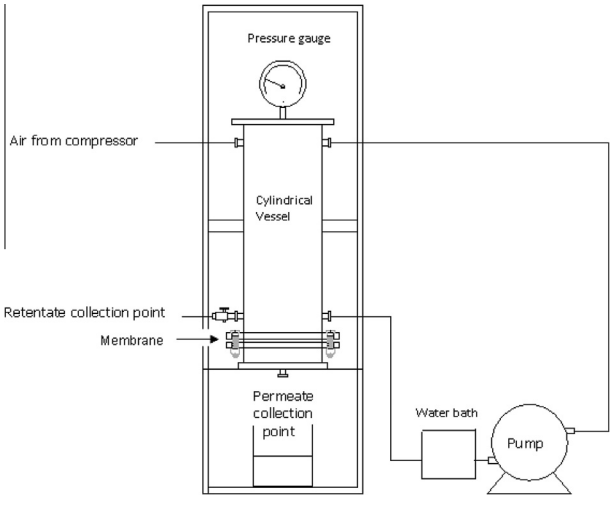

Figure 2 Experimental setup of membrane separator.

3. Result and discussion

3.1. Effect of temperature

The results of various extraction temperatures are shown in Fig. 3. From Fig. 3, it can be observed that DPPH scavenging activity increased from temperature 30 °C to 60 °C and decreased from temperature 60 °C to 70 °C. For an example, the scavenging activity for 6 h extraction period at 30 °C was 20–40% and increased to 55.60% at 60 °C but decreased to 45.30% at 70 °C. This might be due to the denaturation of some heat sensible antioxidants which easily denatured some low molecular weight antioxidants. This is in agreement with Krishnaiah et al. (2012) work in which a loss of 65% radical scavenging activity was found due to heating.

The scavenging activity increased as well as increased in the extraction time from 2 h to 6 h. The overall scavenging activity for extraction period of 2 h was relatively lower than the extraction period for 4 and 6 h. Theoretically, the rate of activity will be doubled as temperature increased by 10 °C. In this case, as the temperature increased with an increase in extraction period, the methanol solvent might diffuse effectively through the Mengkudu fruit and enhance the extraction rate. As a result, more antioxidants are extracted at higher temperatures and longer extraction period. The highest antioxidant activity was obtained at 60 °C for 6 h extraction period with 55.60% radical scavenging activity.

3.2. Antioxidant activity of isolated permeate and retentate fractions

The results of permeate fraction are shown in Fig. 4. From Fig. 4, we found that the permeate side in the separation process exhibited a constant increase in the radical scavenging activity. The radical scavenging activity increased from 40 °C

Table 1 Characteristics of membrane separator.

Parameter	Specification
Vessel material	Stainless steel
Diameter and length	10 cm × 20 cm
Temperature	40–70 °C
Pressure	0.5–1.5 bar
Membrane	Polyethersulphone (PES)
Membrane area	100 cm^2

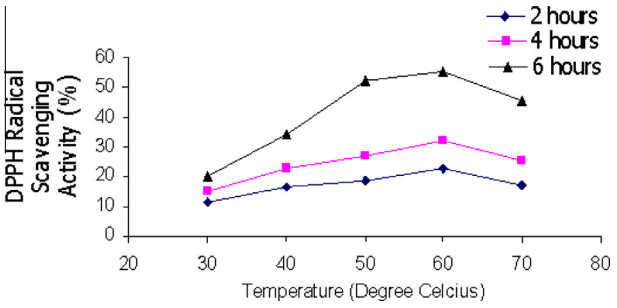

Figure 3 Radical scavenging activity for Mengkudu fruit extract at various extractions.

and reached its peak value at temperature of 55 °C. Somehow it was slightly decreased in its value at 55 °C. This may be due to the denaturation of some low molecular weight antioxidants. The results of retentate fraction are shown in Fig. 5. While the retentate radical scavenging activity decreased constantly as temperature increased from 40 °C to 55 °C. Through the membrane separation, we could observe that the radical scavenging activity in the retentate is an opposite scenario for permeate activity.

3.3. Total phenolic content

Total phenolic content in *M. citrifolia* L. refers to the total amount of phenolic compounds present in a *M. citrifolia L.* sample. For the methanol extract using high pressure reactor, the results are shown in Fig. 7. The highest total phenolic content (TPC) with 43.18 mg GAE/10 gm sample, exhibited at temperature of 60 °C, pressure 1.5 bars with 6 h extraction period. The TPC starts decreasing at temperature 60 °C to 43.09 mg GAE/10 gm sample. It is higher than the value found as 21 mg GAE/10 ml by (Yang et al., 2006). This might be due to the selective separation of antioxidants by the PES membrane.

Figs. 7 and 8 show the TPC of permeate and retentate for *M. citrifolia* L. fruit extract respectively. The pressure effect within the range of study 0.5–1.5 is negligible. The most phenolic compounds belong to the flavonoids. These are considered as a part of the antioxidant members. Thus, phenolic content is interrelated closely to DPPH radical scavenging activity. From the Fig. 6, basically TPC for *M. citrifolia* L. fruit extracts was increased with different increasing operating temperatures and pressures, on the other hand TPC decreased with temperature in retentate. However, TPC decrease is predicted up to 60 °C for all extraction time periods, total phenolic content was also

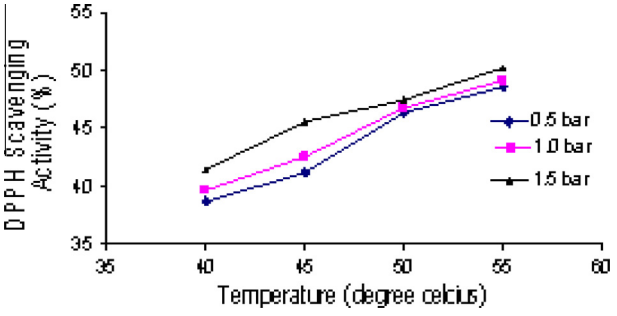

Figure 4 DPPH scavenging activity of permeate of various pressures in Mengkudu fruit extract at different temperatures.

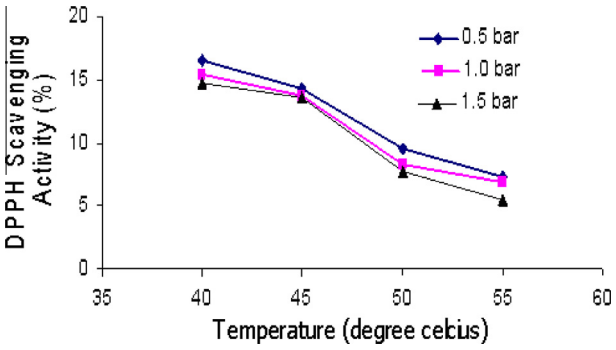

Figure 5 DPPH scavenging activity of retentate of various pressures in Mengkudu fruit extract at different temperatures.

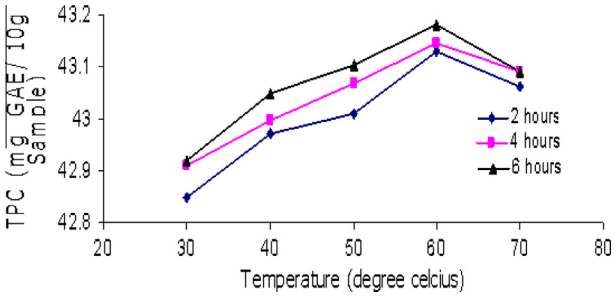

Figure 6 Total phenolic content for Mengkudu fruit extract at various extraction periods.

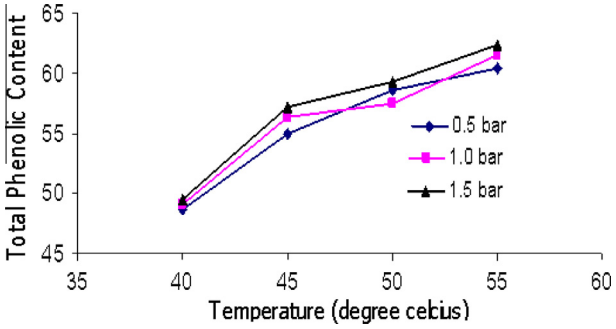

Figure 7 Total phenolic content of permeate in Mengkudu fruit extract at various temperatures and pressures.

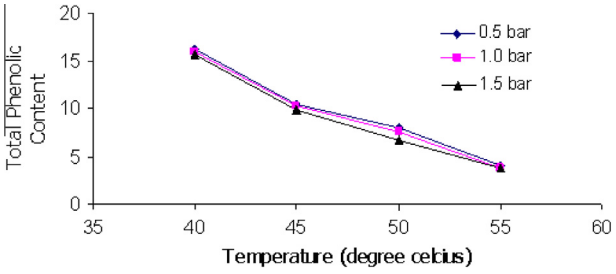

Figure 8 Total phenolic content of retentate Mengkudu fruit extract at various temperatures and pressures.

found to follow the same trend of DPPH radical scavenging activity. However, total flavonoid content was not affected by methanol extraction at temperature of 70 °C and the value increased. Therefore, it can be concluded that some phenolic compounds present in the sample get denatured at 70 °C, there by affecting the DPPH radical scavenging activity.

4. Conclusions

In this study the DPPH scavenging radical activity exhibited a gradual increase in permeates' collection from membrane separation. Total phenolic content was also found to follow the same trend of DPPH radical scavenging activity. Thus, it can be concluded that some phenolic compounds present in the sample influence the DPPH radical scavenging activity. Recovery of permeate instead of retentate is an ideal method in order to get a maximum activity of antioxidants.

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