Phytochemical analysis and antioxidant activity of *Mezzetia parviflora* Becc. Woodbark extract

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INTRODUCTION

Free radicals, formed by various environmental chemicals as well as by endogenous metabolism, can cause oxidative damage to DNA, so as to cause mutation and chromosomal damage, oxidize cellular thiols (resulting inhibition of key enzymes), and abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids. This damage was believed to be underlying cause of cancer, cardiovascular problems, and immune system decline.¹²

*Mezzetia* comprises 4 species and indigenous in the Andaman island, Thailand and Malaysia peninsular, Sumatra, Borneo and the Moluccas. All 4 are present within Malaysia¹⁰¹¹. Ongkea, *Mezzetia parviflora* Becc. (*Annonaceae*), have been used empirically in Southeast
Sulawesi, Indonesia, as a traditional medicine for their antidiabetic, antitumor, antihypertension, anticholesterolemic and antiasthma properties. Cui (1998) isolated seven cytotoxic acylated oligorhamnosides from *Mellea leptopoda*,

but literature survey on *M. parviflora* suggest no report about its chemical constituent nor its biological activity. The present study has been carried out to investigate the antioxidant activity of *M. parviflora* extracts. Phytochemical and physico-chemical analysis was conducted to serve as a means for appropriate identification of *M. parviflora* and to ascertain the possible compound responsible for its activity.

### SUBJECTS AND METHODS

#### Plant material

The woodbark of *Mellia parviflora* Becc. were collected from Buton forest, Southeast Sulawesi and identified at the Herbarium Bogoriense, Bogor. Voucher specimens were deposited at the Department of Pharmacognosy and Phytochemistry, Hasanuddin University as reference materials.

#### Phytochemical screening and physico-chemical studies

*M. parviflora* woodbark (100 g) in powdered form were extracted with 70% ethanol using a maceration method, and last traces of the solvent were evaporated in a rotary evaporator and freeze dryer. The dry crude ethanol extract was 24 g that was then subjected to partition using acetone yield acetone extract and insoluble in acetone extract. All of extract were subjected to phytoconstituents identification using thin layer chromatography method by different detecting reagents. Whereas the crude extract was analyzed for physico-chemical properties, such as water and ethanol-soluble extractive matters, water content, loss on drying, total ash content and acid-insoluble ash content. Total condensed tannins were assayed by the butanol-HCl method using purified quebracho tannins as the standard.

#### Antioxidant activity

The free-radical scavenging activity of the extracts was measured by the decrease in absorbance of ethanolic solution of DPPH. An ethanol solution of the DPPH radical (final concentration of DPPH was 0.4 mM) was prepared. An aliquot (1 ml) of solution containing different concentrations of extract was added to 3.8 ml of DPPH solution. The mixture was then shaken and left to stand for 30 minutes. Following the reaction, the absorbance was measured at 517 nm, and compared with the ascorbic acid as a standard. The DPPH solution without sample solution was used as control. 95% ethanol was used as blank. The percentage of DPPH radical scavenging was calculated by using the formula:

\[
% \text{DPPH radical scavenging} = \left(1 - \frac{As}{Ac}\right) \times 100.
\]

Here, Ac = absorbance of control, As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was calculated.

#### Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically. Sodium nitroprusside (5 mmol/L) in phosphate buffered saline pH 7.4, was mixed with different concentrations of the extract (250–2500 mg/mL) prepared in methanol and incubated at 25°C for 30 min. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylene-diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

### RESULTS

#### Physico-chemical analysis of *M. parviflora* woodbark extracts

Physicochemical analysis of ethanol extracts will provide referential physicochemical for correct identification of this plant extract (Table 1).

#### Table 1. Physico-chemical analysis of ethanol crude extract.

<table>
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<tr>
<th>Parameters</th>
<th>Values obtained w/w on dry weight extract</th>
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<tr>
<td>Organoleptic</td>
<td>Reddish brown dry powder, slightly astringent to the taste, and characteristic odor</td>
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<tr>
<td>Water-soluble extractive matters</td>
<td>34.47% ± 4.12%</td>
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<tr>
<td>Ethanol-soluble extractive matters</td>
<td>58.30% ± 7.59%</td>
</tr>
<tr>
<td>Water content (%W)</td>
<td>9.89 ± 0.78%</td>
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<tr>
<td>Loss on drying</td>
<td>12.62% ± 0.27%</td>
</tr>
<tr>
<td>Total ash content</td>
<td>5.59 ± 0.42%</td>
</tr>
<tr>
<td>Acid-insoluble ash content</td>
<td>0.78 ± 0.10%</td>
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The crude ethanol extract was subjected to partition using acetone yield very few components that extracted by acetone (4.91% w/w) and the residue which insoluble in acetone was 91.83% w/w, this suggests that the main components of the extract are polar compounds. This partition step will facilitating the phytochemical analysis.

Examination of the acetone extract by thin layer chromatography (TLC) using stationary phase silica gel GF254 and mobile phase n-hexane-ethyl acetate 8:2 revealed that the extract contain steroid (five purple spots with Rf 0.51, 0.47, 0.41, 0.07 and 0.04 after vanillin-sulfuric acid spraying and chromatogram heating at 120°C) and alkaloid (one spot with Rf 0.14 on Dragenthof spraying). The insoluble in acetone extract TLC profile using stationary phase reversed-phase RP-18 silica gel F254 and mobile phase toluene-acetone-formic acid 3:6:1) under vanillin-HCl spraying showed four red spots of condensed tannin. Quantitatively analysis of condensed tannin with buthanol assay was shown in Table 2.

**Antioxidant activity**

The DPPH and NO radical scavenging activity of the extracts presented in Table 3.

**DISCUSSION**

The physicochemical constants would be serve as a means of assessing the purity and quality of the extract as well as for identification. The ash value of the extract was 5.59 ± 0.42% w/w which is represent the inorganic salts naturally occurring in the extract. The moisture content of the extract was lower than 10%. This lower content of water will inhibit encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. The ethanol-soluble extractive matters were greater than the water-soluble extractive matters. This extractive value was a parameter of the extract purity.[7]

Relatively stable organic radical DPPH has been widely used in the determination of antioxidant activity of single compounds as well as the different plant extracts.[10,11,14,15,16] DPPH produces a violet solution in ethanol and could be reduced in the presence of an antioxidant molecule, and caused the uncolored ethanol solutions. The IC_{50} values were found to be 21.79, 30.22, 60.73, and 262.55 μg/ml for insoluble in acetone extract, ascorbic acid, ethanolic and acetone extracts, respectively. The insoluble in acetone extract displayed higher DPPH scavenging activity as compared to ascorbic acid as a positive control. These results indicated that the polar fraction was potential as an anti-free radical.

But here we found that NO free radicals scavenging activity of the *M. parviflora* extracts was approximately 10 times lower than its DPPH scavenging activity. These results were different to the ascorbic acid activity which achieved the same inhibition potency against DPPH and NO radicals. It can be inferred that the *M. parviflora* extracts antioxidant activity is thought to be due to their hydrogen donating ability.

The high activity of insoluble in acetone extract is related to the high levels of phenolic condensed tannin. This result was justified by higher extractable condensed tannin content of polar extract than non polar extract. The condensed tannin is not detected in acetone extract.

Preliminary study indicated the occurrence of phenolic condensed tannin in the woodbark. Tannins known as the group of phenolic compounds are the significant plant secondary metabolites. Tannins in vascular plants occur as two types, the condensed and the hydrolysable.[11,17] Vanillin-HCl is could be used for determination of condensed tannins. When these are present, pink spots are obtained. But this method is not specific because its method was measures condensed tannins as well as simple flavonoids. The quantitative examination of condensed tannin in this study, therefore, used the butanol-HCl. Hydrolyzable tannins do not react in the assay.[8,9,11]
To minimize the problems from use of inappropriate standards, we use standard from the plant materials i.e purified quebracho tannin.

Consequently, the extracts of *M. parviflora* can be a potential resource of natural condensed tannins with possible application as an antioxidant in prevention of some diseases. Furthermore, comprehensive studies comprising isolation, characterization of phytochemicals, pharmacological and biochemical investigation are essential to characterize the *M. parviflora* extracts as natural antioxidants.

**CONCLUSIONS**

Woodbark extracts of *Mezzettia parviflora* Becc. showed very good DPPH radical scavenging which related to their phenolic, condensed tannin, content. Further the pharmacological activity of the extract is to be carried out to validate the ethnobotanical claims.

Conflicting Interest

All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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**REFERENCES**


