Antioxidative and cytotoxic effects of prenylated stilbene derivative-rich Melinjo (Gnetum gnemon L.) fruit rind

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Citation: AIP Conference Proceedings 1729, 020057 (2016); doi: 10.1063/1.4946960
View online: http://dx.doi.org/10.1063/1.4946960
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Antioxidative and Cytotoxic Effects of Prenylated Stilbene Derivative-rich Melinjo (Gnetum gnemon L.) Fruit Rind

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Abstract. The identification of fruit-bark of Melinjo was carried out using fractionation column chromatography resulted in 3 isolates A-C with presumption of containing five stilbene derivatives compounds (isorhapontigenin, resveratrol, gnetin D, gnetifolin K, gnetol) and one lignan compound ((+)-lirioresinol B) based on the results of the characterization of UV-Vis, FTIR, and LC-ESI-MS. Isolate C that containing gnetol and (+)-lirioresinol B was reacted through prenylation process with prenyl bromide as a source of prenyl group and K$_2$CO$_3$ as catalyst with reflux system at 60 °C for 24 h. Characterization using LC-ESI-MS showed that gnetol and (+)-lirioresinol B was successfully prenylated with one additional prenyl group. The prenylated stilbene showed comparable antioxidant effect and was slightly lower than the nature stilbene structure, as the impact of the attachment of prenyl moieties at their hydroxyl groups in stilbene, and showed moderate activity against P-388 murine leukemia b cells. The observed inhibition of the bioactivity test provides a reasonable mechanism for the potent cancer chemo preventive activity of prenylated stilbene compounds and may pose this compound as a valuable agent for the treatment of diseases.

INTRODUCTION

The secondary metabolites of diverse living organisms have potent biological activities and produce main compounds in drug discovery. Natural products have been closely linked through the use of traditional medicines [1]. In developing countries such as Indonesia, plants are still the most widely used and accessible source of food and also as primary medicine. Melinjo (Gnetum gnemon L.) is commonly cultivated throughout Indonesia, and the fruits, leaves and flowers are used as an ingredient in many dishes served on important traditional occasions. Melinjo (o gnemon L.) belongs to the family Gnetaceae, native to Indonesia [2]. Natural stilbenoid compounds were found in Melinjo, similar as those found in grapes and red wine, with the chemical constituents of trans-resveratrol, gnetin C, gnetin L, gemonoside A, gemonoside C, and gemonoside D. These derivatives are collectively referred to as “Melinjo resveratrol” [3].

Natural prenylated simple phenolic compounds such as propolis has been reported to have significant bioactivities, such as anticancer, antitumor and antioxidant compared to similar compound in their original phenolic skeleton, cinnamic acid. In case of stilbene skeleton, various stilbenoids have been isolated from natural sources, such as prenylated stilbenes isolated from peanut mucilage [4] and Macaranga alnifolia [5]. Prenylated stilbene has also been reported to be isolated from the root trunk of Indonesian plant, Artocarpus altiss (Moraceae), locally known as Sukun [6]. Since the stilbenoid structure was reported to posses high content of hydroxyl group and no content of prenylated compound, the prenylation reaction is possible for the fruit-rind of Melinjo (Gnetum gnemon L.). Our research is aimed to develop the chemical potency of by-product of Melinjo fruit-rind waste from local food industry, with introducing chemical modification of the “Melinjo resveratrol” content by prenylation reaction to enhance their antioxidant and cytotoxic activity.
MATERIALS AND METHODS

General

All chemicals used were from pro-analytical grade. The fruit bark of Melinjo were collected from local food industry in Banten, Jawa Barat, Indonesia. The UV-Vis measurements were carried out using Varian Cary-50 Bio spectrophotometer. FTIR spectra were recorded on a Bruker ATR ZnSe. Structure confirmation was performed by LC-ESI-MS on a Mariner Biospectrometry-Finnigan instrument.

Isolation, Prenylation and Bioactivity Test

Dried powder of fruit-bark of Melinjo (1.0 kg) was macerated in methanol overnight at room temperature, then it was evaporated under reduce pressure. The crude extract was separated by column chromatography on silica gel and eluted by n-hexane:ethyl acetate in gradient system, and final elution with methanol, resulted in three different fractions, A, B and C. In prenylation reaction, K$_2$CO$_3$ (0.1 g), fraction C and acetone (50 ml) were mixed and stirred at room temperature, then prenyl bromide (0.4 g) was added to the mixture. Finally, the mixture was refluxed for 24h at 60 °C. The product was filtered, and filtrate was concentrated and redissolved in ethyl acetate. The undissolved residue was redissolved in ethanol. The solid product after evaporation was stored at 4 °C. Antioxidant assay procedure was followed the previous method described by Cahyana, et al. [7]. Briefly, 1 ml of prenylated stilbene in different concentrations (1, 2, 4 and 8 mg/ml) was mixed into 1 mL of 0.1 mM DPPH solution, and the mixture was adjusted to 5 ml by adding methanol. Methanol was used as blank solution. The decrease of absorbance at 517 nm was observed by UV-Vis spectrophotometer, and finally, the IC$_{50}$ was evaluated. In cytotoxic assay, murine P-388 leukemia cells was used, and extracted from the previous method [8].

RESULTS AND DISCUSSION

Isolation of Stilbene Derivatives from Melinjo

Three main spots in TLC chromatogram was observed to develop an orange color. Gradient elution resulted in three isolate fractions (A-C). The UV-Vis analysis of isolates were as follows: A (221; 284 nm), B (222; 279 nm) and C (220; 285 nm). Functional group analysis (see Fig. 1) signified the presence of OH group at around 3300 cm$^{-1}$ and C-H olefinic at 3060 cm$^{-1}$. Identification of natural product isolated from Melinjo (Gnetum gnemon L.) was performed and the data is listed in Table 1. Five compounds were identified as stilbene derivatives, including isorhapontigenin, resveratrol, gnetifolin K, gnetin D and gnetol, and another compound has lignin structure, (+)-lirioresinol B.
TABLE 1. LC-ESI-MS analysis of the isolated compounds from Melinjo fruit-bark

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Compound</th>
<th>Spectra data</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Isorhapontigenin</td>
<td>LC (min): 26.77. [M]^+ 258.3 (theoretical 258.0892)</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
<td>LC (min): 27.58. [M]^+ 228.2 (theoretical 228.0786)</td>
</tr>
<tr>
<td>B</td>
<td>Gnetifolin K</td>
<td>LC (min): 33.88. [M]^+ 582.7 (theoretical 582.1949)</td>
</tr>
<tr>
<td></td>
<td>Gnetin D</td>
<td>LC (min): 37.44. [M]^+ 470.4 (theoretical 470.1366)</td>
</tr>
<tr>
<td>C</td>
<td>Gnetol</td>
<td>LC (min): 29.62. [M]^+ 244.4 (theoretical 244.0736)</td>
</tr>
<tr>
<td></td>
<td>(+)-lirioresinol B</td>
<td>LC (min): 31.92. [M]^+ 418.4 (theoretical 418.1628)</td>
</tr>
</tbody>
</table>

![Possible prenylated structure of (a) gnetol and (b) (+)-lirioresinol B](image)

FIGURE 2. Possible prenylated structure of (a) gnetol and (b) (+)-lirioresinol B

TABLE 2. Scavenging activity of isolate C and prenylated isolate C

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration (ppm)</th>
<th>Scavenging activity of isolate C (%)</th>
<th>Scavenging activity of prenylated isolate C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>7.80</td>
<td>16.61</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>12.54</td>
<td>20.00</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>19.66</td>
<td>22.03</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>29.32</td>
<td>40.17</td>
</tr>
</tbody>
</table>

Prenylation Reaction and Biological Test

Isolate C containing gnetol and (+)-lirioresinol B was prenylated using prenyl bromide with potassium carbonate as heterogeneous base catalyst. As a result, gnetol (244.4 g/mol) and (+)-lirioresinol B (418.4 g/mol) were identified to have one additional prenyl group in their structures, based on the analysis using mass spectrometer. At retention time of 30.46 min, the peak was fragmented and m/z of 311.0 was observed, signifying that gnetol molecule have one prenyl group. Another case in (+)-lirioresinol B, m/z of 485.2 indicated the structure have one additional prenyl moiety by comparing with the [M]^+ from natural (+)-lirioresinol B.

In DPPH assay, the antioxidative agents were able to reduce the stable radical form of DPPH to yellow-coloured diphenyl picrylhydrazine. This method is based on the reduction of DPPH in alcoholic solution in the presence of hydrogen donating compound [9]. In biological test, both isolate C and prenylated isolate C have radical scavenging activity (Table 2). Linear plot was obtained between % scavenging and concentration of samples, and finally IC_{50} was found to be 216.14 ppm and 240.13 ppm for isolate and prenylated isolate C, respectively. The increase of IC_{50} in prenylated isolate C can be associated with the decrease in hydrogen phenolic as a result of prenylation reaction. Therefore, the antioxidative capacity of prenylated isolate C is lower than non-prenylated isolate C. The IC50 values for isolate and prenylated isolate C are lower than the previous study performed by Abu-Mellal et al. in conducting the antioxidant evaluation of prenylated cinnamate and stilbenes from Kangaroo island propolis [10].
The cytotoxic properties of prenylated extract were evaluated against murine leukemia P-388 cells and has been conducted with Artonin E as positive control. The prenylated extract compounds was moderately inhibited the cells activity with IC50 values of 25.5 μg/mL. This data suggested that introducing a prenyl group at OH group of resveratrol derivative from Gnetum gnemon increases the cytotoxicity capacity. In case of the prenylated structure of phenolic compound in general, the same tendency was also demonstrated in another research concerning their activities as cytotoxic related with prenyl moeties, such as found in prenylated phenolic from Garcinia xanthochymus [11], prenylated stilbene compounds [12], and in Macaranga mappa plant [13].

CONCLUSIONS

Isolation of the fruit bark of Melinjo (Gnetum gnemon L.) resulted three different fractions (A, B and C) with total six isolated compounds. Five of six compounds were identified as stilbene derivatives prenylation of isolate C of Melinjo under green condition was successfully achieved with K2CO3 as solid catalyst. Moreover, it was shown that the prenylated isolate C (containing gnetol and (+)-lirioresinol B) plays a significant role for scavenging activity of DPPH radical and in cytotoxic properties. This research provides a new route to develop an alternative antioxidative and cytotoxic agent modified from naturally active constituents by prenylation reaction.

ACKNOWLEDGMENTS

Thanks to the Directorate of Research and Community Engagement (DRPM) Universitas Indonesia through research grant “PUPT 2015”.

REFERENCES