Cytotoxicity and phytochemical profiles of *Phyllanthus emblica* stem barks with *in silico* drug-likeliness: Focusing on antidiabetic potentials

**Abstract**

Out of numerous reported medicinal plants, *Phyllanthus emblica* has been reported to possess a strong antidiabetic potential and other pharmacological effects. This research aimed to identify the phytoconstituents in the extracts of *P. emblica* stem barks and hypothesize their antidiabetic potentials based on *in silico* drug-likeliness. Simplicia of *P. emblica* powder was sequentially macerated at room temperature (24 h) using n-hexane, ethyl acetate, and methanol solvents. Phytochemical profiles of the extract were investigated qualitatively using reagents, followed by gas chromatography–mass spectrometry (GC-MS) analysis. All phytocompounds were then analyzed for their pharmacological properties and predicted bioactivities on molinspiration. Cytotoxicity of each extract was evaluated using the brine shrimp lethality test. As many as 18 compounds (from GC-MS), were identified in all extract samples from *P. emblica* stem barks. Based on *in silico* drug-likeliness, methanol extract contained the most potentially bioactive compounds (16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide; 14-β.-H-pregna; and isochiapin B). Isochiapin B was revealed as the only compound that had no violation of the rule of five. All three compounds could hypothetically contribute to the antidiabetic activity of the methanol extract from *P. emblica* stem barks by inhibiting diabetes-related enzymes and interacting with nuclear receptors. Moderate cytotoxicity of ethyl acetate and methanol extract, respectively, further suggests their bioactivities.

**Key words:** Antioxidant, diabetes mellitus, isochiapin B, *Phyllanthus emblica*, phytomedicine, traditional medicine

**INTRODUCTION**

The increasing trend of using and developing plant-based traditional medicines to treat diabetes mellitus is mostly due to the high price, low availability, and inaccessibility of modern drugs.[1] Some people also believe that plant-based therapies have a lower adverse effect, even though this is untrue since current research has proved that plant origins’ compounds could also side effects.[2] With the growing threat of diabetes mellitus burden, especially in developing countries which have problems in fulfilling the availability of modern drugs, there is...
an urgent need to keep investigating and exploring antidiabetic potentials of phytomedicines.\textsuperscript{3,4} Of many plants used as diabetes mellitus therapies, \textit{Phyllanthus emblica} has been reported for its various medicinal benefits including antimicrobial, anti-inflammatory, antioxidant, analgesic, aphrodisiac, and most importantly, antidiabetic activities.\textsuperscript{5} The phytoconstituents profile of a plant extract could provide us a portrayal of its potential bioactivities.\textsuperscript{6,7} We reported the phytocompounds extracted from \textit{P. emblica} stem barks using solvents with various polarities (n-hexane, ethyl acetate, and methanol). The research on the extracts of \textit{P. emblica} stem barks, especially for their antidiabetic activities, is scarcely reported. In an attempt to hypothesize the antidiabetic activities of \textit{P. emblica} stem bark extracts before \textit{in vivo} investigation, we also determined the \textit{in silico} drug-likeness of the extracts. Moreover, we report on the cytotoxicity of each extract from \textit{P. emblica} stem barks to back up their bioactivity’s claims.

**MATERIALS AND METHODS**

**Materials and plant sample**

Solvents used in this study included n-hexane, ethyl acetate, and methanol. Reagents Meyer, Wagner, Dragendorff, and Liebermann–Burchard were used in the phytochemical screening. Other chemicals included HCl, H$_2$SO$_4$, gelatin, and FeCl$_3$. All materials were analytical standard grade and purchased from Merck (Selangor, Malaysia).

\textit{P. emblica} samples were collected in October 2021 from Aceh Besar Regency, Aceh, Indonesia with the following coordinate: 503°1.2’-5045°9.007” N and 95055’43.6”-94059’50.13” E. The plant sample was identified by Dr. Saida Rasnovi in the Laboratory of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala (No. 150/UN11/1.8/TA.00.01/2022).

**Extraction of \textit{Phyllanthus emblica} stem barks**

The stem barks of \textit{P. emblica} were cut into small pieces (3–5 cm) and oven-dried for 24 h at 40\textdegree–50\textdegree C. The simplicia powder was produced from the dried \textit{P. emblica} stem barks using a crushe and sieved (60 mesh) to receive the fine powder. The simplicia powder (3 kg) was then macerated at room temperature in n-hexane for 24 h. The filtrate was separated from the residue, where the residue was re-macerated using ethyl acetate and methanol, sequentially, under the same conditions. Each extracted sample was labeled according to the solvent used; H-PE, EA-PE, and M-PE for samples obtained using n-hexane, ethyl acetate, and methanol solvents, respectively. All filtrates obtained from each solvent were processed separately with rotary evaporation (40\textdegree C) to produce the extract paste. Each obtained extract was qualitatively screened for their major groups of phytocompounds following the previously reported procedures.\textsuperscript{8} Furthermore, more detailed profiles of phytocompounds contained in the extract were obtained from the analysis carried out on the gas chromatography–mass spectroscopy (GC-MS) system (Agilent, Santa Clara, CA, USA).\textsuperscript{9}

**Determination of pharmacological properties and bioactivities \textit{in silico}**

The pharmacological properties and bioactivities of the identified compounds from the extract were analyzed based on the calculation in molinspiration (https://www.molinspiration.com/). The pharmacological properties and bioactivities were obtained by clicking the options on the website interface. LogP was calculated by calculating the total of fragment-based contributions and correction factors using a method developed by molinspiration. Similarly, the molinspiration-developed method was also used to predict the bioactivity of the molecule based on Bayesian statistics. The bioactivities predicted were G protein-coupled receptors (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor.

**Cytotoxicity evaluation**

Cytotoxicity of the extracts from \textit{P. emblica} stem barks was assessed by brine shrimp lethality test assay employing \textit{Artemia salina} larvae. Each extract was diluted into DMSO (dimethyl sulfoxide) with concentrations ranging from 1 to 1000 mg/L. The prehatched \textit{A. salina} larvae were exposed to the prepared extract and left for 24 h under a tubular lamp. The number of dead larvae was used to determine the minimum concentration required to cause 50\% mortality (LC$_{50}$).

**RESULTS AND DISCUSSION**

**Major phytocompounds groups in \textit{Phyllanthus emblica} extracts**

The presence of several groups of phytocompounds in the \textit{P. emblica} extracts was determined qualitatively and the results were presented in Table 1.

\begin{table}[h]
\centering
\caption{Results from the qualitative screening of major phytocompound groups}
\begin{tabular}{|l|c|c|c|}
\hline
Group of compounds & Reagent or testing method & Extract samples & \\
\hline
Alkaloids & Mayer & H-PE & EA-PE & M-PE \\
& Wagner & & & \\
& Dragendorff & & & \\
Steroids & Liebermann–Burchard & + & + & + \\
Terpenoids & Liebermann–Burchard & - & + & \\
Saponins & Shaking & - & + & - \\
Phenolics & HCl and Mg & - & + & + \\
Flavonoids & FeCl$_3$ & - & + & + \\
Tannins & Gelatin + H$_2$SO$_4$ & - & + & + \\
\hline
\end{tabular}
\end{table}

\textsuperscript{(+)} and \textsuperscript{−} symbols represent the presence and nonpresence of the group of compounds in each extract.
Identified phytocompounds in Phyllanthus emblica extracts

Each of the spectral peaks belonged to a compound which was identified in the mass spectrometer and matched with the database [Table 2]. A terpenoid derivative, 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide, appeared with the highest area percentage in EA-PE (75.38%) and M-PE (67.93%). The negative terpenoid content in EA-PE, shown by the qualitative screening, is probably because the compound is a clerodane diterpene which is difficult to be observed qualitatively due to its weak response against Liebermann–Burchard reagent. Another terpenoid compound from M-PE, isochiapin B (a member of sesquiterpene lactones), was also indicated in the GC analysis with a relatively small peak area (4.53%) and weak similarity (77%). M-PE sample was also observed to contain a steroid compound – 14-.beta.-H-pregna with similarity and peak area of 79% and 8.06%, respectively [Table 2]. The structures of 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide; 14-.beta.-H-pregna; and isochiapin B have been presented in Figure 1a-c.

Herein, the presence of phenolics and flavonoids was detected in the qualitative screening. Future studies are warranted to confirm the presence of phenolics and flavonoids because in the screening using GC-MS, they were not identified.

**Table 2: Identified phytocompounds in Phyllanthus emblica extracts based on gas chromatography–mass spectrometry analysis**

| Compounds                                              | Similarity (%) | Retention time (min) | Area (%) |
|--------------------------------------------------------|----------------|----------------------|----------|
| 9-Octadecene                                           | 84             | 13.215               | 14.63    |
| Methyl palmitate                                       | 92             | 14.690               | 17.21    |
| 1-Octadecene                                           | 88             | 15.350               | 14.96    |
| 1-Tetracosanol                                         | 67             | 17.300               | 3.75     |
| Docosanoic acid                                        | 71             | 18.332               | 9.89     |
| Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl | 69             | 19.885               | 39.56    |
| 1-Pentadecene                                          | 94             | 8.311                | 2.01     |
| 1-Hexadecene                                           | 96             | 10.872               | 3.38     |
| 9-Eicosene, (E)-                                       | 96             | 13.220               | 3.99     |
| Neophytadiene                                          | 93             | 13.756               | 4.17     |
| Methyl palmitate                                       | 93             | 14.694               | 2.74     |
| 3-Eicosene, (E)-                                       | 94             | 15.358               | 4.19     |
| Cyclotetracosane                                       | 94             | 17.301               | 2.39     |
| 1-Tricosene                                            | 90             | 19.084               | 1.75     |
| 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide        | 83             | 23.751               | 75.38    |
| 14-.beta.-H-pregna                                     | 79             | 20.647               | 8.06     |
| Glycerine-1-oleate-3-palmitate                         | 76             | 21.040               | 14.77    |
| Isochiapin B                                           | 77             | 21.311               | 4.53     |
| Myristyl oleate                                        | 71             | 22.108               | 4.71     |
| 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide        | 83             | 23.753               | 67.93    |

**Figure 1:** Structures of 16α-hydroxycleroda-3,13 (14)Z-dien-15,16-olide (a); 14-.beta.-H-pregna (b); and isochiapin B (c). Cytotoxic activities (d) of H-PE, EA-PE, and M-PE based on BSLT assay.
not detected. Low quantity of the compound or inaccurate GC-MS analysis (because it relies on similarity) could be the factor as to why phenolics and flavonoids were not observable.

Drug-likeliness of the identified compounds
Using a platform molinspiration, we have obtained molecular properties that could affect the bioavailability and absorbance of the drug candidates, where the results have been presented [Table S1]. A good drug candidate should follow the rule of five,[10] where the molecular weight should be ≤500 g/mol, LogP – ≤5, number of H bond acceptors – ≤10, and number of H bond donors – ≤5. Isochiapin B was revealed as the only phytocompound that did not violate the rule of five. Most of the compounds violate the rule by exceeding the molecular weight of more than 5. However, the other two compounds, 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide and 14-β-H-pregna, had the smallest LogP values (<7).

Platform molinspiration also provided a calculation to predict the bioactivity of the drug candidates. Herein, GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor of the identified phytocompounds from P. emblica were predicted to target nuclear receptors and carbohydrate metabolism-related enzymes as their mechanisms of action. EA-PE and M-PE are potentially bioactive, especially with the evidence from the cytotoxicity screening showing moderate-to-weak cytotoxicity.

CONCLUSIONS
Antidiabetic potentials of the extracts from P. emblica stem barks could be observed through their phytochemical profiles. The methanol extract consisted of most compounds with high bioactivity prediction scores (16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide; 14-β-H-pregna; and isochiapin B). These compounds were predicted to target nuclear receptors and carbohydrate metabolism-related enzymes as their mechanisms of action. EA-PE and M-PE are potentially bioactive, especially with the evidence from the cytotoxicity screening showing moderate-to-weak cytotoxicity.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Kasole R, Martin HD, Kimiywe J. Traditional medicine and its role in the management of diabetes mellitus: “Patients’ and herbalists’ perspectives”. Evid Based Complement Alternat Med 2019;2019:2835691.
2. Chinsembu KC. Diabetes mellitus and nature’s pharmacy of putative antidiabetic plants. J Herb Med 2019;15:100230.
3. Misra A, Gopalan H, Jayawardena R, Hills AP, Soares M, Reza-Albarrán AA, et al. Diabetes in developing countries. J Diabetes 2019;11:522-39.
4. Zahra Z, Ramadhan CT, Mamfaluti T, Pumungkas SR, Firdausa S. Association between depression and HbA1c levels in the elderly population with type 2 diabetes mellitus during COVID-19 pandemic. Narra J 2022;2:e51.
5. Ahmad B, Hafeez N, Rauf A, Bashir S, Linfang H, Rehman M, et al. Phyllanthus emblica: A comprehensive review of its therapeutic benefits. S Afr J Bot 2021;138:278-310.
6. Al-Ishaq RK, Abotalab M, Kubatka P, Kajo K, Busselberg D. Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules 2019;9:430.
7. Unuofin JO, Lebelo SL. Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: An updated review. Oxid Med Cell Longev 2020;2020:1356893.
8. Hasballah K, Sarong M, Rusly R, Fitria H, Maida DR, Iqrahramullah M. Antiproliferative activity of triterpenoid and steroid compounds from ethyl acetate extract of Calotropis gigantea root bark against p388 murine leukemia cell lines. Sci Pharm 2021;69:21.
9. Yahya M, Ginting B, Saidi N. *In vitro* screenings for biological and antioxidant activities of water extract from *Theobroma cacao* L. pod husk: Potential utilization in foods. Molecules 2021;26:6915.

10. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001;46:3-26.

11. Purnama A, Rizki DR, Qanita I, Iqrammullah M, Ahmad K, Mardina V, *et al.* Molecular docking investigation of calotropone as a potential natural therapeutic agent against pancreatic cancer. J Adv Pharm Technol Res 2022;13:44-9.

12. Purnama A, Mardina V, Puspita K, Qanita I, Rizki DR, Hasballah K, *et al.* Molecular docking of two cytotoxic compounds from *Calotropis gigantea* leaves against therapeutic molecular target of pancreatic cancer. Narra J 2021;1:e37.

13. Nazaruddin N, Afifah N, Babi M, Susilawati S, Sani ND, Esmaeili C, *et al.* A simple optical pH sensor based on pectin and *Ruellia tuberosa* L-derived anthocyanin for fish freshness monitoring. F1000Res 2021;10:422.

14. Schulman IG. Nuclear receptors as drug targets for metabolic disease. Adv Drug Deliv Rev 2010;62:1307-15.

15. Poovitha S, Parani M. *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC Complement Altern Med 2016;16 Suppl 1:185.
Table S1: Pharmacology-related molecular properties of the identified compounds from *Phyllanthus emblica* extracts based on molinspiration

| Compounds                                             | Molecular weight (g/mol) | LogP | H bond acceptors (n) | H bond donors (n) |
|-------------------------------------------------------|--------------------------|------|---------------------|------------------|
| 9-Octadecene                                          | 252                      | 8.80 | 0                   | 0                |
| Methyl palmitate                                      | 270                      | 7.37 | 2                   | 0                |
| 1-Octadecene                                          | 252                      | 8.79 | 0                   | 0                |
| 1-Tetracosanol                                        | 354                      | 9.44 | 1                   | 1                |
| Docosanoic acid                                       | 340                      | 9.13 | 2                   | 1                |
| Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl | 238                      | 6.30 | 1                   | 1                |
| 1-Pentadecene                                         | 210                      | 7.68 | 0                   | 0                |
| 1-Hexadecene                                          | 224                      | 8.17 | 0                   | 0                |
| 9-Eicosene, (E)-                                      | 280                      | 9.18 | 0                   | 0                |
| Neophytadiene                                         | 278                      | 7.55 | 0                   | 0                |
| 3-Eicosene, (E)-                                      | 280                      | 9.08 | 0                   | 0                |
| Cyclotetracosane                                      | 336                      | 9.67 | 0                   | 0                |
| 1-Tricosene                                           | 322                      | 9.55 | 0                   | 0                |
| 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide      | 332                      | 5.08 | 3                   | 1                |
| 14β-H-pregna                                          | 288                      | 6.89 | 0                   | 0                |
| Glycerine-1-oleate-3-palmitate                        | 595                      | 10.77| 6                   | 0                |
| Isochiapin B                                          | 336                      | 0.51 | 6                   | 1                |
| Myristyl oleate                                       | 478                      | 10.00| 2                   | 0                |

*The value has exceeded the maximum limits of the rule of five* [10]

Table S2: Predicted bioactivity of the identified compounds from *Phyllanthus emblica* extracts based on molinspiration

| Compounds                             | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---------------------------------------|-------------|-----------------------|-----------------|-------------------------|-------------------|------------------|
| 9-Octadecene                          | −0.08       | 0.02                  | −0.28           | −0.11                   | −0.23             | 0.07             |
| Methyl palmitate                      | −0.11       | −0.05                 | −0.34           | −0.09                   | −0.13             | 0.04             |
| 1-Octadecene                          | −0.14       | 0.01                  | −0.37           | −0.07                   | −0.22             | 0.03             |
| 1-Tetracosanol                        | 0.08        | 0.02                  | 0.01            | 0.12                    | 0.10              | 0.10             |
| Docosanoic acid                       | 0.17        | 0.04                  | −0.10           | 0.23*                   | 0.17              | 0.17             |
| Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylen-3-pentyl | −0.24       | 0.04                  | −0.68           | −0.10                   | −0.35             | −0.00             |
| 1-Pentadecene                         | −0.38       | −0.06                 | −0.66           | −0.32                   | −0.48             | −0.09             |
| 1-Hexadecene                          | −0.29       | −0.03                 | −0.55           | −0.22                   | −0.39             | −0.04             |
| 9-Eicosene, (E)-                      | 0.02        | 0.02                  | −0.16           | 0.01                    | −0.10             | 0.10             |
| Neophytadiene                         | −0.12       | −0.02                 | −0.35           | 0.20                    | −0.11             | 0.14             |
| 3-Eicosene, (E)-                      | 0.08        | 0.0                   | −0.20           | 0.06                    | −0.08             | 0.15             |
| Cyclotetracosane                      | 0.03        | 0.01                  | −0.01           | 0.02                    | 0.01              | 0.02             |
| 1-Tricosene                           | 0.04        | 0.01                  | −0.13           | 0.12                    | 0.02              | 0.06             |
| 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide | 0.31*       | −0.16                 | −0.12           | 0.64**                  | 0.14              | 0.78**            |
| 14β-H-pregna                          | 0.06        | 0.41                  | −0.48           | 0.64**                  | −0.09             | 0.50**            |
| Glycerine-1-oleate-3-palmitate        | −2.56       | −3.46                 | −3.24           | −3.27                   | −1.93             | −2.92             |
| Isochiapin B                          | 0.09        | 0.15                  | −0.34           | 0.77**                  | 0.14              | 0.64**            |
| Myristyl oleate                       | 0.06        | −0.01                 | −0.11           | 0.07                    | 0.06              | 0.10             |

*Moderately active, **Highly active. GPCR: G protein-coupled receptor*