ORIGINAL ARTICLE

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

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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-. beta.-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

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an urgent need to keep investigating and exploring antidiabetic potentials of phytomedicines.[3,4] Of many plants used as diabetes mellitus therapies, *Phyllanthus emblica* has been reported for its various medicinal benefits including antimicrobial, anti-inflammatory, antioxidant, analgesic, aphrodisiac, and most importantly, antidiabetic activities.[5] The phytoconstituents profile of a plant extract could provide us a portrayal of its potential bioactivities.[6,7] We reported the phytocompounds extracted from *P. emblica* stem barks using solvents with various polarities (n-hexane, ethyl acetate, and methanol). The research on the extracts of *P. emblica* stem barks, especially for their antidiabetic activities, is scarcely reported. In an attempt to hypothesize the antidiabetic activities of *P. emblica* stem bark extracts before *in vivo* investigation, we also determined the *in silico* drug-likeliness of the extracts. Moreover, we report on the cytotoxicity of each extract from *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_2SO_4, gelatin, and $FeCl_3$. All materials were analytical standard grade and purchased from Merck (Selangor, Malaysia).

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/UN11/1/8.4/TA.00.01/2022).

Extraction of *Phyllanthus emblica* stem barks

The stem barks of *P. emblica* were cut into small pieces (3–5 cm) and oven-dried for 24 h at 40°–50°C. The simplicia powder was produced from the dried *P. emblica* stem barks using a crusher 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°C) to produce the extract paste. Each obtained extract was qualitatively screened for their major groups of phytocompounds following the previously reported procedures.[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).[9]

Determination of pharmacological properties and bioactivities *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 *P. emblica* stem barks was assessed by brine shrimp lethality test assay employing *Artemia salina* larvae. Each extract was diluted into DMSO (dimethyl sulfoxide) with concentrations ranging from 1 to 1000 mg/L. The prehatched *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 *Phyllanthus emblica* extracts

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

Table 1: Results from the qualitative screening of major phytocompound groups

Group of compounds	Reagent or testing method	Extract samples		
		H-PE	EA-PE	M-PE
Alkaloids	Mayer	–	–	–
	Wagner	–	–	–
	Dragendroff	–	–	–
Steroids	Liebermann–Burchard	+	+	+
Terpenoids	Liebermann–Burchard	–	–	+
Saponins	Shaking	–	–	–
Phenolics	HCl and Mg	–	+	+
Flavonoids	$FeCl_3$	–	+	+
Tannins	Gelatin + H_2SO_4	–	+	+

(+) and (–) 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

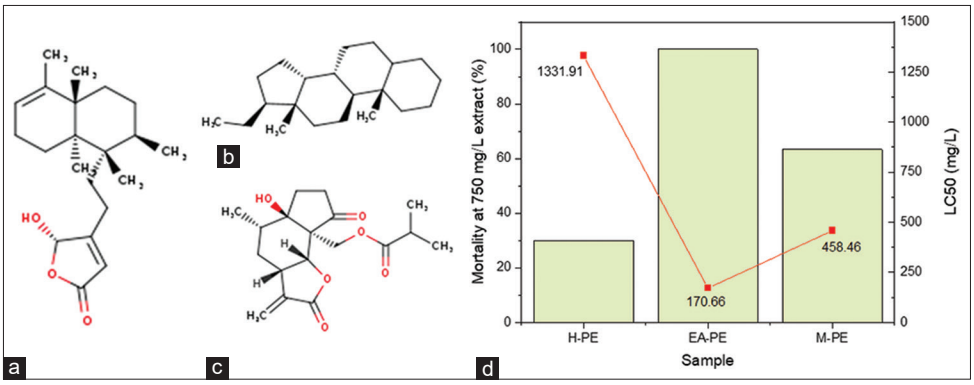

Figure 1: Structures of 16α-hydroxycleroda-3,13 (14)Z-dien-15,16-olide (a); 14-.beta.-H-pregna (b); and isochiapin B (c). Cytotoxic activities (d) of H-PE, EA-PE, and M-PE based on BSLT assay

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

Compounds	Similarity (%)	Retention time (min)	Area (%)
H-PE			
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
EA-PE			
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
M-PE			
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

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-. beta.-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 extract were presented in Table S2. 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide was the only compound having three potential bioactivities (GPCR ligand, nuclear receptor ligand, and enzyme inhibitor).14-.beta.-H-pregna was predicted to significantly capable of interacting with nuclear receptors and enzymes. Similarly, isochiapin B was predicted to act as a nuclear receptor ligand and enzyme inhibitor with scores of 0.77 and 0.64, respectively.

According to the *in silico* analysis of drug-likeliness, three phytocompounds emerged as the potential drug candidates, they are: 16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide; 14-.beta.-H-pregna; and isochiapin B. Efficacy of secondary metabolites could be derived from its molecular targets,[11,12] where each phytocompound could complement one another.[8,13] Based on the bioactivity prediction, the three compounds could act as nuclear receptor ligand and enzyme inhibitors. Treatments targeting nuclear receptors such as proliferator activated receptors and the liver X receptors were found promising for diabetes mellitus.[14] Moreover, as stated earlier, inhibitions of α-amylase and α-glucosidase are useful to control blood glucose in diabetic individuals.[15]

Cytotoxicity of *Phyllanthus emblica* stem bark extracts

Cytotoxic activities of H-PE, EA-PE, and M-PE against *A. salina* larvae have been presented in Figure 1d. When the *A. salina* larvae were exposed to each extract with a concentration of 750 mg/L, the mortality percentages of H-PE, EA-PE, and M-PE were 30, 100, and 63.3%, respectively. From the lowest to the highest, the LC_{50}s of 170.66, 458.46, and 1331.91 mg/L were obtained from EA-PE, M-PE, and H-PE, respectively. These data suggest that EA-PE was moderately active, M-PE – weakly active, and H-PE – nonactive as cytotoxic agents.[7] This is in agreement with our previous hypothesis that EA-PE and M-PE possess bioactivities because both extracts contain *in silico* – predicted bioactive compounds (16α-hydroxycleroda-3,13 (14) Z-dien-15,16-olide; 14-.beta.-H-pregna; or isochiapin B).

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-.beta.-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.

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Conflicts of interest

There are no conflicts of interest.

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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
14-.beta.-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-methylene-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**
14-.beta.-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