HMG-CoA reductase inhibitory activity and phytocomponent investigation of Basella alba leaf extract as a treatment for hypercholesterolemia

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Abstract: The enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase is the key enzyme of the mevalonate pathway that produces cholesterol. Inhibition of HMG-CoA reductase reduces cholesterol biosynthesis in the liver. Synthetic drugs, statins, are commonly used for the treatment of hypercholesterolemia. Due to the side effects of statins, natural HMG-CoA reductase inhibitors of plant origin are needed. In this study, 25 medicinal plant methanol extracts were screened for anti-HMG-CoA reductase activity. Basella alba leaf extract showed the highest inhibitory effect at about 74%. Thus, B. alba was examined in order to investigate its phytochemical components. Gas chromatography with tandem mass spectrometry and reversed phase high-performance liquid chromatography analysis revealed the presence of phenol 2,6-bis(1,1-dimethylmethyl), 1-heptatriacetonol, oleic acid, eicosyl ester, naringin, apigenin, luteolin, ascorbic acid, and α-tocopherol, which have been reported to possess anti-hypercholesterolemic effects. Further investigation of in vivo models should be performed in order to confirm its potential as an alternative treatment for hypercholesterolemia and related cardiovascular diseases.

Keywords: HMG-CoA reductase, Basella alba, phytochemical, GC-MS/MS, RP-HPLC, hypercholesterolemia

Introduction
Atherosclerosis, which is caused by hypercholesterolemia, is a major cause of heart diseases such as myocardial infarction. Elevated levels of plasma cholesterol, particularly low-density lipoprotein (LDL) and triglyceride levels, are mainly responsible for hypercholesterolemia, which can also lead to other diseases such as obesity, diabetes, and cancer.1,2 The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis that catalyzes the conversion of HMG-CoA to mevalonate. The inhibition of HMG-CoA reductase effectively lowers the level of cholesterol in humans and most animals by the activation of sterol regulatory element-binding protein-2, which upregulates the HMG-CoA reductase and LDL receptor that lead to the reduction of cholesterol levels.3 Although statins are well-known HMG-CoA reductase inhibitors, long-term consumption of statins cause severe adverse effects such as muscle and liver damage, rhabdomyolysis, and acute renal failure.4

Due to the side effects of synthetic drugs, attention is now directed to alternative medicines of plant origin.5 Over the decades, the use of medicinal plants represents the interaction between humans and the environment.6 According to the World Health Organization,7 about 80% of the human population depend on alternative medicine for the primary treatment of various diseases. Medicinal plants have been widely reported to have medicinal properties, nutritional value, and pharmacological activities such as antioxidant, anti-thrombotic, anti-inflammatory, anti-atherogenic, and cardioprotective effects.8,9
Phytochemicals in medicinal plants have gained much interest among researchers and the pharmaceutical and food manufacturing industries. Basically, phytochemicals are bioactive compounds that naturally exist in plants and are known as potent effectors of biological processes capable of decreasing disease risk via complementary as well as overlapping mechanisms. Plant flavonoids offer significant protection against the development of chronic illnesses such as diabetes, tumors, cancer, and cardiovascular diseases. Flavonoids have been reported to reduce LDL oxidation, suppress lipid peroxidation, and decrease the progression of atherosclerotic lesions in cardiovascular diseases.

The potential of medicinal plants for the treatment of hypercholesterolemia is still largely unexplored and could be an alternative strategy for the progression of effective and safe anti-hypercholesterolemia drugs. Thus, in this study, the HMG-CoA reductase inhibitory activity of 25 medicinal plant extracts was tested.

Basella alba, locally known as remayung, belongs to the family of Basellaceae and is a wild vegetable that has been employed for the benefit of human health from ancient times. The leaves and stems of B. alba are used for medicinal purposes and it has been proven to have analgesic, antifungal, and antiulcer activities. The hypocholesterolemic effects of B. alba have not been investigated to date. In the present study, phytochemicals present in B. alba leaf extract were determined using gas chromatography with tandem mass spectrometry (GC-MS/MS) and reversed phase high-performance liquid chromatography (RP-HPLC). The phytochemicals of B. alba against hypercholesterolemia and its related cardiovascular diseases have been highlighted based on previous reports.

Materials and methods
Preparation of plant extract
The fresh leaves of the plants were collected from various regions of Selangor, Malaysia. The plants were botanically identified, and the plant voucher specimens were deposited at the Institute of Bioscience, Universiti Putra Malaysia. The leaves were air dried and the sample (500 g) was ground using a blender (Panasonic MX 9967) and subjected to methanol 50% (v/v) distillation for 48 hours. After filtration, the extract was isolated using a separatory funnel. The crude methanol extract of the plants was then concentrated using a rotary evaporator (Heidolph) under reduced pressure at 40°C and freeze dried at −40°C.

Enzyme assay
HMG-CoA reductase inhibitory activity of the plants was determined based on spectrophotometric measurements.

The HMG-CoA reductase assay kit was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The concentration of the HMG-CoA reductase stock solution was 0.5–0.75 mg/mL. Each crude extract (50 μg) was mixed with a reaction mixture containing nicotinamide adenine dinucleotide phosphate (400 μM), HMG-CoA substrate (400 μM), and potassium phosphate buffer (100 mM, pH 7.4) containing potassium chloride (120 mM), ethylenediaminetetra-acetic acid (1 mM), and dithiothreitol (5 mM), followed by the addition of HMG-CoA reductase (2 μL). The reaction was incubated at 37°C, and absorbance was measured at 340 nm after 10 minutes. Simvastatin (Sigma-Aldrich Co.) was used as a positive control, and distilled water as a negative control.

The HMG-CoA reductase inhibition (%) was calculated using the following formula:

\[
\text{Inhibition} \% = \left( \frac{\Delta \text{Absorbance control} - \Delta \text{Absorbance test}}{\Delta \text{Absorbance control}} \right) \times 100
\]

Phytochemical screening
The phytochemical constitutes of B. alba extract were evaluated qualitatively for flavonoids, phenolics, saponins, tannins, alkaloids, triterpenes, and steroids. The phytochemical tests were carried out using freeze-dried B. alba extract.

Test for flavonoids
Ethyl acetate (10 mL) was added to B. alba extract (0.5 mg) and heated for 3 minutes over a steam bath. After filtration, the filtrate (4 mL) was shaken with 10% ammonia solution (1 mL). The formation of yellow color indicates the presence of flavonoids.

Test for phenolic content
B. alba extract (200 μL, 0.5 mg/mL) was mixed with Folin–Ciocalteu reagent (tenfold dilution, 0.75 mL). After incubation for 5 minutes, 6% sodium carbonate solution (0.75 mL) was added, and the mixture was further incubated at room temperature for 90 minutes. A brown coloration indicates the presence of phenolic compounds.

Test for saponins
Distilled water (5 mL) was mixed with B. alba extract (0.5 g) and shaken vigorously. The formation of froth for 15 minutes determines the presence of saponins.

Test for tannins
B. alba extract (0.5 g) was boiled in water (10 mL) and filtered. A few drops of 1% ferric chloride solution were
mixed with the filtrate. Blue–black color formation indicates the presence of hydrolysable tannins, while brownish green precipitate shows the presence of condensed tannins.\textsuperscript{26}

**Test for alkaloids**

*B. alba* extract (0.5 g) was partitioned with chloroform followed by ammoniacal chloroform. The mixture was treated with 10% sulfuric acid and tested with Mayer’s reagent. The formation of white precipitate indicates the presence of alkaloids.\textsuperscript{25}

**Test for steroids and triterpenes**

Chloroform (1 mL) was added to *B. alba* extract (0.5 g) followed by few drops of acetic anhydride and concentrated sulfuric acid. The appearance of green or blue indicates the presence of steroids, while the appearance of brown or red color indicates the presence of triterpenes.\textsuperscript{25}

**Gas chromatography with tandem mass spectrometry (GC-MS/MS) analysis**

*B. alba* leaf extract (1 µL) was analyzed using gas chromatography (TSQ Quantum XLS; Thermo Fisher Scientific, Waltham, MA, USA), which is equipped with a flame ionization detector and a TG-5 MS capillary column (30 m length × 0.25 mm ID × 0.25 μm thickness). Helium was used as the carrier gas at a constant flow rate of 0.8 mL/minute. The oven temperature was held 5 minutes at 40°C and raised 2°C/minute gradually up to 280°C. The injector and flame ionization detector temperature were maintained at 200°C and 250°C, respectively. The mass spectrometer was operated in scan mode from m/z 40–450 Da and the mass spectra were taken at 70 eV with a scan interval of 0.7 seconds. Identification of individual compounds was made by comparing the obtained mass spectra with internal references in the mass spectra library, the National Institute of Standards and Technology (Gaithersburg, MD, USA).\textsuperscript{21,27}

**RP-HPLC analysis of flavonoids and ascorbic acid**

Standard stock solution of eight flavonoid standards (rutin, luteolin, catechin, quercetin, apigenin, naringin, myricetin, and histidine) and ascorbic acid (Sigma-Aldrich Co.) were prepared in methanol at concentrations of 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, and 1.0 mg/mL and filtered through a membrane filter (0.45 mm) (EMD Millipore, Billerica, MA, USA). The standards were subjected to RP-HPLC separately. The linear calibration curve was plotted at the absorbance of 280 nm as the peak area against standard concentration (mg/mL).\textsuperscript{29}

**Gradient RP-HPLC**

The flavonoids and ascorbic acid compounds in the sample were analyzed using an RP-HPLC method, as described by Wang and Helliwell,\textsuperscript{28} with some modifications. The RP-HPLC analyses were performed with a Waters 600 pump controller and 9,486 tunable absorbance ultraviolet detector, and equipped with an Eclipse XDB-C18 reversed phase column (25 cm × 4.6 mm ID × 5 μm) (Supelco; Sigma-Aldrich Co.) at room temperature. The compounds were eluted with a gradient elution of mobile phase solvent A (deionized water, pH adjusted to 2.5 with trifluoroacetic acid) and solvent B (HPLC-grade methanol). The gradient elution program was begun with 100% solvent A at 0 minutes, followed by 70% solvent A and 30% solvent B for the next 10 minutes, 50% solvent A and 50% solvent B for 30 minutes and, finally, with 100% solvent A for 40 minutes. The flow rate was 1.0 mL/minute and the injection volume was 20 µL with a post-time of 15 minutes before the next injection. The detection of flavonoid wavelength was set at 280 nm. Flavonoids in the sample were identified based on comparison with standard retention times of chromatographic peaks.

**RP-HPLC analysis of α-tocopherol**

Standard stock solution of α-tocopherol with the mobile phase composed of methanol:deionized water (92:8) were prepared at concentrations of 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, and 1.0 mg/mL and subjected to HPLC separately. The detection wavelength was set at absorbance 292 nm. The linear calibration curve was obtained by fitting the peak area against the standard concentration (mg/mL).\textsuperscript{29}

**Isocratic RP-HPLC**

An isocratic RP-HPLC method was carried out with some modifications.\textsuperscript{29} The method was performed using an Eclipse XDB-C18 reversed phase column (25 cm × 4.6 mm ID × 5 μm) (Supelco; Sigma-Aldrich Co.). The α-tocopherol compound in the sample was eluted using an elution solvent composed of methanol: deionized water (92:8) with a total run time of 40 min. The sample injection volume was 20 µL with a flow rate of 1.0 mL/minute. The detection wavelength was set at 292 nm. The α-tocopherol compound was identified by matching the retention time against the standard. The laboratory methods performed in this study are summarized in Table 1.

**Results and discussion**

**Inhibitory effect of plants on HMG-CoA reductase**

Among the 25 plant extracts, *B. alba*, *Amaranthus viridis*, and *Piper sarmentosum* showed inhibitory effects of more...
control in this study, simvastatin, showed enzyme inhibition of 85.1%. HMG-CoA reductase catalyzes the rate-limiting step in the synthesis of cholesterol. When human and animal models of hypercholesterolemia are given statins (inhibitors of HMG-CoA reductase), the initial reduction in cholesterol synthesis leads to compensatory responses that start with the activation of sterol regulatory element-binding protein-2. As a result, the expression of HMG-CoA reductase and the LDL receptor is upregulated. This results in normal levels of cholesterol synthesis due to the presence of the inhibitor, which compensates for the high levels of the enzyme. However, the increase in LDL receptor expression causes a reduction in cholesterol levels. Thus, in this study, the inhibition of the enzyme may reflect the potential of B. alba in cholesterol reduction. B. alba is known locally as Indian spinach or remayung and has been used in treating ulcers, hypertension, anemia, digestive disorders, and cancer. Further investigation of the phytochemical constitutes of B. alba were performed in order to determine the possible compounds involved in HMG-CoA reductase inhibition.

### Table 1 Qualitative analysis of phytochemical constitutes

<table>
<thead>
<tr>
<th>Phytochemical constitutes</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolyzed tannins</td>
<td>–</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>–</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>–</td>
</tr>
</tbody>
</table>

Notes: +, detected; –, not detected.

### Table 2 Anti-HMG-CoA reductase activity of plant extracts

<table>
<thead>
<tr>
<th>N</th>
<th>Scientific name</th>
<th>Family name</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carica papaya</td>
<td>Caricaceae</td>
<td>37.3±1.4</td>
</tr>
<tr>
<td>2</td>
<td>Conium tomentosum</td>
<td>Apiaceae</td>
<td>27.1±2.3</td>
</tr>
<tr>
<td>3</td>
<td>Murraya koenigii</td>
<td>Rutaceae</td>
<td>33.7±1.9</td>
</tr>
<tr>
<td>4</td>
<td>Morinda citrifolia</td>
<td>Rubiaceae</td>
<td>29.4±3.2</td>
</tr>
<tr>
<td>5</td>
<td>Piper sarmentosum</td>
<td>Piperaceae</td>
<td>55.1±2.7</td>
</tr>
<tr>
<td>6</td>
<td>Centella asiatica</td>
<td>Apicaeae</td>
<td>8.1±3.8</td>
</tr>
<tr>
<td>7</td>
<td>Orthosiphon grandiflorus</td>
<td>Lamiaceae</td>
<td>3.7±1.1</td>
</tr>
<tr>
<td>8</td>
<td>Cymbopogon citratus</td>
<td>Poaceae</td>
<td>35.2±2.4</td>
</tr>
<tr>
<td>9</td>
<td>Camellia sinensis</td>
<td>Theaceae</td>
<td>4.2±2.4</td>
</tr>
<tr>
<td>10</td>
<td>Spinacia oleracea</td>
<td>Amaranthaceae</td>
<td>29.5±2.5</td>
</tr>
<tr>
<td>11</td>
<td>Dioscorea villosa</td>
<td>Dioscoreaceae</td>
<td>22.2±1.0</td>
</tr>
<tr>
<td>12</td>
<td>Mentha spicata</td>
<td>Lamiaceae</td>
<td>11.3±2.4</td>
</tr>
<tr>
<td>13</td>
<td>Cosmos caudatus</td>
<td>Compositae</td>
<td>13.2±2.0</td>
</tr>
<tr>
<td>14</td>
<td>Aegle marmelos</td>
<td>Rutaceae</td>
<td>9.4±1.4</td>
</tr>
<tr>
<td>15</td>
<td>Ocimum basilicum</td>
<td>Lamiaceae</td>
<td>32.9±3.6</td>
</tr>
<tr>
<td>16</td>
<td>Eclipta prostrata</td>
<td>Asteraceae</td>
<td>24.4±2.4</td>
</tr>
<tr>
<td>17</td>
<td>Acrolpha indica</td>
<td>Euphorbiaceae</td>
<td>19.2±4.1</td>
</tr>
<tr>
<td>18</td>
<td>Azadirachta indica</td>
<td>Meliaceae</td>
<td>5.6±0.8</td>
</tr>
<tr>
<td>19</td>
<td>Cynodon dactylon</td>
<td>Poaceae</td>
<td>2.8±1.7</td>
</tr>
<tr>
<td>20</td>
<td>Hemidesmus indicus</td>
<td>Apocynaceae</td>
<td>26.8±0.5</td>
</tr>
<tr>
<td>21</td>
<td>Alternanthera sessilis</td>
<td>Amaranthaceae</td>
<td>7.5±1.3</td>
</tr>
<tr>
<td>22</td>
<td>Psidium guajava</td>
<td>Myrtaceae</td>
<td>16.9±1.7</td>
</tr>
<tr>
<td>23</td>
<td>Solanum torvum</td>
<td>Solanaceae</td>
<td>6.7±2.8</td>
</tr>
<tr>
<td>24</td>
<td>Amaranthus viridis</td>
<td>Amaranthaceae</td>
<td>69.6±3.4</td>
</tr>
<tr>
<td>25</td>
<td>Basella alba</td>
<td>Basellaceae</td>
<td>74.1±2.3</td>
</tr>
</tbody>
</table>

Notes: Distilled water was used as a negative control (0% inhibition). Simvastatin was used as a positive control (89.2%±3.5% inhibition). All data are presented as the mean ± SD of triplicates. Abbreviation: HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; N, number; SD, standard deviation.

### Methods

#### a) HMG-CoA reductase assay

1. **Flavonoids**: Ethyl acetate test
2. **Phenolic content**: Folin–Ciocalteu test
3. **Saponins**: Frothing test
4. **Tannins**: Ferric chloride test
5. **Alkaloids**: Mayer’s test
6. **Steroids/triterpenes**: Liebermann–Burchard test
7. **Identification of phytocomponents**: GC-MS/MS analysis
8. **Identification of flavonoids**: Gradient RP-HPLC analysis
9. **Identification of α-tocopherol**: Isocratic RP-HPLC analysis

**Abbreviations**: HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; GC-MS/MS, gas chromatography with tandem mass spectrometry; RP-HPLC, reversed phase high-performance liquid chromatography.

#### b) Phytochemical screening

Phenolic compounds, commonly known as polyphenols, have been shown to possess antioxidant properties, raise the antioxidant capacity of human plasma, and inhibit LDL oxidation. Flavonoids and tannins are phenolic compounds; they act as free radical scavengers. Being an antioxidant, flavonoids suppress the oxidation of LDL cholesterol, which is involved in atherosclerotic development. Flavonoids are also reported to exhibit cardioprotective effects such as improvement in endothelial activity and anti-inflammatory action in both in vitro and in vivo.
In addition, flavonoids are also claimed to successfully inhibit platelet aggregation in hypercholesterolemia rabbits. As with flavonoids, tannins have been proven to have strong antiplatelet effects reducing cholesterol absorption in animal studies. Condensed tannins are preferable in therapeutic treatment since they do not interfere with the absorption of iron compared with hydrolyzed tannins, which inhibit iron absorption that may lead to anemia. Several studies on saponins revealed that they inhibit cholesterol absorption in the intestine and decrease the level of plasma cholesterol in various experimental animal models. Saponins isolated from garlic have shown cholesterol-lowering effects by reducing LDL and total cholesterol concentrations without altering high-density lipoprotein cholesterol levels in hypercholesterolemia-induced rats. Furthermore, saponins were also found to reduce the risk of atherosclerosis in rats.

**GC-MS/MS analysis**

GC-MS/MS offers enhanced selectivity and sensitivity compared with gas chromatography–mass spectrometry by the elimination of matrix ion interference through selected reaction monitoring. Selected reaction monitoring is highly specific and can provide identification of low levels of compounds even in the presence of a high matrix background. Compound identification was determined through a comparison of obtained mass spectra with the internal references in the mass spectra library, the National Institute of Standards and Technology. B. alba methanol extract revealed 25 phytocomponents, and their molecular formula, molecular weight, as well as peak area (%) are summarized in Table 4. The major components in the leaves of *B. alba* were vitamin E (peak area 12.337%); l-(+)-ascorbic acid 2,6-dihexadecanoate (peak area 11.611); phenol, 2,6-dimethyl (peak area 9.33); 1-heptatriacotanol (peak area 11.379); (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- (peak area 11.611); phenol, 2,6-bis(1,1-dimethylethyl)- (peak area 11.379); (+)-c-tocopherol, O-methyl- (peak area 8.550). The potential effects of the components involved in the treatment of hypercholesterolemia and its related diseases are presented in Table 5.

**Table 4** GC-MS/MS analysis of phytocomponents identified in the Basella alba leaves

<table>
<thead>
<tr>
<th>N</th>
<th>RT</th>
<th>Compound name</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.23</td>
<td>18,19-Secoyohimb-19-oic acid, 16,17,20,21-tetrahydroxy-16-((hydroxymethyl) , methyl ester, (15α, 16E)-</td>
<td>C_{19}H_{27}O_{12}</td>
<td>352</td>
<td>1.612</td>
</tr>
<tr>
<td>2</td>
<td>9.64</td>
<td>E-8-Methyl-9-tetradecen-1-ol acetate</td>
<td>C_{13}H_{15}O_{3}</td>
<td>268</td>
<td>1.154</td>
</tr>
<tr>
<td>3</td>
<td>9.99</td>
<td>Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-</td>
<td>C_{11}H_{13}F_{3}O</td>
<td>206</td>
<td>2.109</td>
</tr>
<tr>
<td>4</td>
<td>24.61</td>
<td>9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-</td>
<td>C_{17}H_{22}O_{3}</td>
<td>444</td>
<td>1.536</td>
</tr>
<tr>
<td>5</td>
<td>26.65</td>
<td>6,6-Dimethyl-4,5-tetramethylen-2-phenyl-5,6-dihydro-4H-1,3-oxazine</td>
<td>C_{12}H_{15}NO</td>
<td>243</td>
<td>4.679</td>
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<tr>
<td>6</td>
<td>46.17</td>
<td>D-1-Dodecanol</td>
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<td>7</td>
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<td>Phenol, 2,6-bis(1,1-dimethylethyl)-</td>
<td>C_{15}H_{22}O</td>
<td>206</td>
<td>11.379</td>
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<tr>
<td>8</td>
<td>49.08</td>
<td>Bicyclo(3.2.2)nonane-1,5-dicarboxylic acid, 5-ethyl ester</td>
<td>C_{22}H_{22}O_{4}</td>
<td>240</td>
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<tr>
<td>9</td>
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<td>Dicyclopadi-1-methanol, acetate (ester)</td>
<td>C_{17}H_{24}O_{3}</td>
<td>326</td>
<td>1.336</td>
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<tr>
<td>10</td>
<td>58.60</td>
<td>Trans-13-Octadecenoic acid</td>
<td>C_{17}H_{29}O</td>
<td>282</td>
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<td>11</td>
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<td>Estra-1,3,5(10)-tri-en-17α-ol</td>
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<td>256</td>
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<td>12</td>
<td>68.13</td>
<td>Octadecanoic acid, 4-hydroxy-, methyl ester</td>
<td>C_{18}H_{32}O_{3}</td>
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<td>13</td>
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<td>14</td>
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<tr>
<td>15</td>
<td>71.85</td>
<td>Butyl 9-octadecenoate or 9-18:1</td>
<td>C_{22}H_{44}O</td>
<td>338</td>
<td>2.283</td>
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<tr>
<td>16</td>
<td>72.15</td>
<td>l-(+)-Ascorbic acid 2,6-dihexadecanoate</td>
<td>C_{31}H_{44}O_{6}</td>
<td>652</td>
<td>11.611</td>
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<td>17</td>
<td>72.55</td>
<td>Oleic acid, eicosyl ester</td>
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<td>9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-</td>
<td>C_{20}H_{32}O_{3}</td>
<td>884</td>
<td>1.727</td>
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<td>19</td>
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<td>20</td>
<td>74.30</td>
<td>(-)-c-Tocopherol, O-methyl-</td>
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<td>9.330</td>
</tr>
<tr>
<td>21</td>
<td>75.19</td>
<td>Vitamin E</td>
<td>C_{20}H_{32}O</td>
<td>430</td>
<td>12.337</td>
</tr>
<tr>
<td>22</td>
<td>76.34</td>
<td>6-Octadecenoic acid</td>
<td>C_{18}H_{34}O</td>
<td>282</td>
<td>1.162</td>
</tr>
<tr>
<td>23</td>
<td>76.77</td>
<td>Cholestane-3-one, cyclic 1,2-ethanediyl acetol, (5α)-</td>
<td>C_{20}H_{32}O</td>
<td>430</td>
<td>1.189</td>
</tr>
<tr>
<td>24</td>
<td>77.49</td>
<td>9-Octadecenoic acid (Z)-, tetracetyl ester</td>
<td>C_{22}H_{40}O</td>
<td>478</td>
<td>1.156</td>
</tr>
<tr>
<td>25</td>
<td>77.66</td>
<td>9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
<td>C_{22}H_{40}O</td>
<td>356</td>
<td>1.947</td>
</tr>
</tbody>
</table>

Abbreviations: GC-MS/MS, gas chromatography with tandem mass spectrometry; N, number; RT, retention time; MW, molecular weight.

**RP-HPLC analysis**

RP-HPLC is a chromatographic technique widely used in the simultaneous separation and quantification of phenolic compounds. The separation of the compounds was performed with a reversed phase column. The chromatographic separations of...
eight flavonoid standards (rutin, luteolin, catechin, quercetin, apigenin, naringin, myricetin, and histidine) and ascorbic acid at 0.2 mg/mL by gradient elution are shown in Figure 1. The typical HPLC chromatogram of *B. alba* is presented in Figure 2. The compounds detected in *B. alba* leaves were ascorbic acid, luteolin, apigenin, and naringin. In addition, α-tocopherol standard (0.2 mg/mL) was separated by isocratic elution chromatography, as presented in Figure 3A, while the detection of α-tocopherol in the methanol extract of *B. alba* leaves is shown in Figure 3B. The concentration of each flavonoid, ascorbic acid, and α-tocopherol were calculated from the standard calibration curve and presented as the mean of three determinations (Table 6). Ascorbic acid had the highest concentration (0.891 mg/mL), followed by α-tocopherol (0.702 mg/mL), naringin (0.180 mg/mL), apigenin (0.165 mg/mL), and luteolin (0.099 mg/mL).

Based on previous studies, the compounds detected in RP-HPLC can be associated with the prevention and treatment of hypercholesterolemia. Luteolin and apigenin are flavones, a type of flavonoid, and they were demonstrated

### Table 5: Potential effects of the major components in the *Basella alba* leaf extract in the prevention of hypercholesterolemia and cardiovascular diseases

<table>
<thead>
<tr>
<th>N</th>
<th>Retention time</th>
<th>Compound name</th>
<th>Compound nature</th>
<th>Biological activity</th>
</tr>
</thead>
</table>
| 1 | 48.53         | Phenol, 2,6-bis(1,1-dimethylethyl)-         | Aromatic and phenolic compound       | – Anti-inflammatory (animal study)<sup>38</sup>  
|   |               |                                             |                                      | – Antiatherosclerotic, lowers plasma cholesterol level, and inhibits LDL cholesterol peroxidation (animal study)<sup>39</sup> |
| 2 | 71.11         | 1-Heptatriacotanol                          | Alcoholic compound                  | – Suppresses lipid accumulation (animal study)<sup>40</sup>  
|   |               |                                             |                                      | – Antioxidant and lowers triglyceride level (human study)<sup>41</sup> |
| 3 | 72.15         | L-(-)-Ascorbic acid 2,6-dihexadecanoate    | Reductone                           | – Protects LDL against peroxidation and retards the progression of atherosclerosis (animal study)<sup>42</sup>  
|   |               |                                             |                                      | – Inhibits the activity of HMG-CoA reductase (in vitro)<sup>43</sup> |
| 4 | 72.55         | Oleic acid, eicosyl ester                  | Monounsaturated omega-9 fatty acid  | – Lowers total cholesterol and LDL cholesterol (human study)<sup>44</sup>  
|   |               |                                             |                                      | – Inhibits vascular smooth muscle cell proliferation (in vitro)<sup>44</sup> |
| 5 | 73.71         | α-Tocopherol, O-methyl-                      | Alcoholic compound                  | – Antiatherosclerotic effects (animal study)<sup>47</sup>  
|   |               | (+)-c-Tocopherol, O-methyl-                 |                                      | – Most potent antioxidant (human study)<sup>48</sup> |
| 6 | 74.30         | (++)-ε-Tocopherol, O-methyl-                |                                      | – Reduces foam cell formation (animal study)<sup>49</sup>  
|   |               |                                             |                                      | – Suppresses LDL lipid oxidation (animal study)<sup>50</sup> |
| 7 | 75.19         | Vitamin E                                    |                                      | – Possesses protective effects against hypercholesterolemia (animal study)<sup>51</sup>  
|   |               |                                             |                                      | – Most potent antioxidant (human study)<sup>51</sup> |

**Abbreviations:** N, number; LDL, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A.
to inhibit the adhesion of monocytes on oxidized LDL (in human endothelial cells), which indicates their anti-atherogenic properties and effectiveness in treating the initial stage of atherosclerosis. In addition, luteolin and apigenin have vasoprotective effects whereby the compounds protect resistance arteries of rats from injuries by superoxide anions, and they are potentially useful as therapeutic treatments for cardiovascular diseases. Luteolin has been reported to possess antihypercholesterolemic effects since it reduces the concentration of total cholesterol, triglycerides, and free fatty acid, as well as decreases the levels of cardiac marker enzymes, troponin I and troponin T in rats-enzymes that exist during myocardial injury.

Naringin is classified as a flavanone, which is a subgroup of flavonoids. Naringin exhibits important properties that can ameliorate hypercholesterolemia and atherosclerosis. Naringin has been proven to inhibit HMG-CoA reductase and decrease plasma cholesterol, LDL, triglycerides, and hepatic lipid levels without altering high-density lipoprotein cholesterol in rats and rabbits. Naringin also suppresses monocyte adhesion on endothelial cells and smooth cell proliferation, as well as decreasing fatty streak formation. In comparison with lovastatin, a synthetic drug for cholesterol lowering, naringin is nontoxic and possesses hepatoprotective action in mice and rabbits. Ascorbic acid (vitamin C) and α-tocopherol (vitamin E) are known to be effective therapeutics for the treatment of hypercholesterolemia and its related cardiovascular diseases, as mentioned in Table 5.

### Conclusion

The present study provides preliminary data that suggest the B. alba leaf extract is capable of lowering cholesterol levels by inhibiting the HMG-CoA reductase activity. In addition, the compounds of B. alba extract (phenol, 2,6-bis[1,1-dimethylethyl], 1-heptatriacotanol, oleic acid, eicosyl ester, naringin, apigenin, luteolin, ascorbic acid, and α-tocopherol) have been reported to possess beneficial effects in treating hypercholesterolemia and its related diseases. However, the mechanism of B. alba extract in inhibiting the HMG-CoA reductase is unknown. Studies in in vivo models could give further insights into the effects and roles of B. alba as an alternative therapeutic agent in the prevention and management of hypercholesterolemia.

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Disclosure
The authors report no conflicts of interest in this work.

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