

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

Gunasekaran Baskaran<sup>1</sup>  
Shamala Salvamani<sup>1</sup>  
Siti Aqlima Ahmad<sup>1</sup>  
Noor Azmi Shaharuddin<sup>1</sup>  
Parveen Devi Pattiram<sup>2</sup>  
Mohd Yunus Shukor<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, <sup>2</sup>Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor, Malaysia

**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-dimethylethyl), 1-heptatriacotanol, oleic acid, eicosyl ester, naringin, apigenin, luteolin, ascorbic acid, and  $\alpha$ -tocopherol, which have been reported to possess antihypercholesterolemic 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.<sup>1,2</sup> 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.<sup>3</sup> 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.<sup>4</sup>

Due to the side effects of synthetic drugs, attention is now directed to alternative medicines of plant origin.<sup>5</sup> Over the decades, the use of medicinal plants represents the interaction between humans and the environment.<sup>6</sup> According to the World Health Organization,<sup>7</sup> 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, antithrombotic, anti-inflammatory, antiarthrogenic, and cardioprotective effects.<sup>8,9</sup>

Correspondence: Mohd Yunus Shukor  
Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia  
Email mohdyunus@upm.edu.my

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,<sup>10</sup> tumors,<sup>11</sup> cancer,<sup>12</sup> and cardiovascular diseases.<sup>13,14</sup> Flavonoids have been reported to reduce LDL oxidation,<sup>15</sup> suppress lipid peroxidation,<sup>16</sup> and decrease the progression of atherosclerotic lesions in cardiovascular diseases.<sup>17,18</sup>

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 antihypercholesterolemia 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.<sup>19</sup> 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 8967) 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.<sup>20,21</sup>

### 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), ethylenediaminetetraacetic 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.<sup>2</sup> The HMG-Co A reductase inhibition (%) was calculated using the following formula:<sup>22</sup>

$$\text{Inhibition \%} = \left( \frac{\Delta \text{ Absorbance control} - \Delta \text{ Absorbance test}}{\Delta \text{ Absorbance control}} \right) \times 100 \quad (1)$$

### Phytochemical screening

The phytochemical constituents 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.<sup>23</sup>

### 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.<sup>24</sup>

### 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.<sup>25</sup>

### 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.<sup>26</sup>

### 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.<sup>25</sup>

### 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.<sup>25</sup>

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

*B. alba* leaf extract (1  $\mu$ L) 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  $\times$  0.25 mm ID  $\times$  0.25  $\mu$ m 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).<sup>21,27</sup>

### 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  $\mu$ m) (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).<sup>29</sup>

### 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,<sup>28</sup> 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  $\times$  4.6 mm ID  $\times$  5  $\mu$ m) (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  $\mu$ L 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 $\alpha$ -tocopherol

Standard stock solution of  $\alpha$ -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).<sup>29</sup>

### Isocratic RP-HPLC

An isocratic RP-HPLC method was carried out with some modifications.<sup>29</sup> The method was performed using an Eclipse XDB-C18 reversed phase column (25 cm  $\times$  4.6 mm ID  $\times$  5  $\mu$ m) (Supelco; Sigma-Aldrich Co.). The  $\alpha$ -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  $\mu$ L with a flow rate of 1.0 mL/minute. The detection of  $\alpha$ -tocopherol wavelength was set at 292 nm. The  $\alpha$ -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

**Table 1** Methods used for the investigation of *Basella alba* extract

Purpose	Methods
a) HMG-CoA reductase assay	HMG-CoA reductase kit
b) Phytochemical screening	
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
c) Identification of phytochemicals	GC-MS/MS analysis
d) Identification of flavonoids (rutin, luteolin, catechin, quercetin, apigenin, naringin, myricetin, and histidine)	Gradient RP-HPLC analysis
e) Identification of ascorbic acid	Gradient RP-HPLC analysis
f) Identification of $\alpha$ -tocopherol	Isocratic RP-HPLC

**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.

than 50% on HMG-CoA reductase activity (Table 2). *B. alba* showed the highest inhibition of 74.1%. The inhibitory effect of *B. alba* was higher than *Rosa damascene* (70%) and *Myrtus communis* (62%), which have been reported to be potent inhibitors of HMG-CoA reductase.<sup>2</sup> The positive

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

N	Scientific name	Family name	Inhibition (%)
1	<i>Carica papaya</i>	Caricaceae	37.3±1.4
2	<i>Coriandrum sativum</i>	Apiaceae	27.1±2.3
3	<i>Murraya koenigii</i>	Rutaceae	33.7±1.9
4	<i>Morinda citrifolia</i>	Rubiaceae	29.4±3.2
5	<i>Piper sarmentosum</i>	Piperaceae	55.1±2.7
6	<i>Centella asiatica</i>	Apiaceae	8.1±3.8
7	<i>Orthosiphon grandiflorus</i>	Lamiaceae	3.7±1.1
8	<i>Cymbopogon citratus</i>	Poaceae	35.3±2.4
9	<i>Camellia sinensis</i>	Theaceae	4.2±2.4
10	<i>Spinacia oleracea</i>	Amaranthaceae	29.5±2.5
11	<i>Dioscorea villosa</i>	Dioscoreaceae	22.2±1.0
12	<i>Mentha spicata</i>	Lamiaceae	11.3±2.4
13	<i>Cosmos caudatus</i>	Compositae	13.2±2.0
14	<i>Aegle marmelos</i>	Rutaceae	9.4±1.4
15	<i>Ocimum basilicum</i>	Lamiaceae	32.9±3.6
16	<i>Eclipta prostrata</i>	Asteraceae	24.4±2.4
17	<i>Acalypha indica</i>	Euphorbiaceae	19.2±4.1
18	<i>Azadirachta indica</i>	Meliaceae	5.6±0.8
19	<i>Cynodon dactylon</i>	Poaceae	2.8±1.7
20	<i>Hemidesmus indicus</i>	Apocynaceae	26.8±0.5
21	<i>Alternanthera sessilis</i>	Amaranthaceae	7.5±1.3
22	<i>Psidium guajava</i>	Myrtaceae	16.9±1.7
23	<i>Solanum torvum</i>	Solanaceae	6.7±0.8
24	<i>Amaranthus viridis</i>	Amaranthaceae	69.6±3.4
25	<i>Basella alba</i>	Basellaceae	74.1±2.3

**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.

**Table 3** Qualitative analysis of phytochemical constituents

Phytochemical constituents	Results
Phenolic	+
Flavonoids	+
Hydrolyzed tannins	–
Condensed tannins	+
Saponins	+
Alkaloids	–
Steroids	–
Triterpenes	–

**Notes:** +, detected; –, not detected.

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.<sup>3,30</sup> 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.<sup>19</sup> Further investigation of the phytochemical constituents of *B. alba* were performed in order to determine the possible compounds involved in HMG-CoA reductase inhibition.

## Phytochemical analysis

The phytochemical screening of *B. alba* using methanol as an extracting solvent revealed the presence of medically active constituents such as phenolic compounds, flavonoids, condensed tannins, and saponins, while other constituents such as hydrolyzed tannins, alkaloids, steroids, and triterpenes were not detected (Table 3). Phenolic compounds, commonly known as polyphenols, have been shown to possess antioxidant properties,<sup>31</sup> raise the antioxidant capacity of human plasma,<sup>32</sup> and inhibit LDL oxidation.<sup>33,34</sup> 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.<sup>35,36</sup> Flavonoids are also reported to exhibit cardioprotective effects such as improvement in endothelial activity and anti-inflammatory action in both in vitro and in



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

N	RT	Compound name	Molecular formula	MW	Peak area (%)
1	7.23	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetrahydro-16-(hydroxymethyl)-, methyl ester, (15á, 16E)-	C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	352	1.612
2	9.64	E-8-Methyl-9-tetradecen-1-ol acetate	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268	1.154
3	9.99	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	C <sub>9</sub> H <sub>9</sub> F <sub>3</sub> O <sub>2</sub>	206	2.109
4	24.61	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	C <sub>28</sub> H <sub>44</sub> O <sub>4</sub>	444	1.536
5	26.65	6,6-Dimethyl-4,5-tetramethylene-2-phenyl-5,6-dihydro-4H-1,3-oxazine	C <sub>16</sub> H <sub>21</sub> NO	243	4.679
6	46.17	1-Dodecanol	C <sub>12</sub> H <sub>26</sub> O	186	4.726
7	48.53	Phenol, 2,6-bis(1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	206	11.379
8	49.08	Bicyclo(3.2.2)nonane-1,5-dicarboxylic acid, 5-ethyl ester	C <sub>13</sub> H <sub>20</sub> O <sub>4</sub>	240	1.768
9	51.47	Dasycarpidan-1-methanol, acetate (ester)	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub>	326	1.336
10	58.60	Trans-13-Octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	2.107
11	62.25	Estra-1,3,5(10)-trien-17á-ol	C <sub>18</sub> H <sub>24</sub> O	256	3.094
12	68.13	Octadecanoic acid, 4-hydroxy-, methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>3</sub>	314	1.421
13	69.89	2,3-Dihydroxypropyl elaidate	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356	1.902
14	71.11	1-Heptatriacotanol	C <sub>37</sub> H <sub>76</sub> O	536	8.615
15	71.85	Butyl 9-octadecenoate or 9-18:1	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338	2.283
16	72.15	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	652	11.611
17	72.55	Oleic acid, eicosyl ester	C <sub>38</sub> H <sub>74</sub> O <sub>2</sub>	562	1.259
18	72.97	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	C <sub>57</sub> H <sub>104</sub> O <sub>6</sub>	884	1.727
19	73.71	α-Tocopherol, O-methyl-	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	8.550
20	74.30	(+)-c-Tocopherol, O-methyl-	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	9.330
21	75.19	Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	12.337
22	76.34	6-Octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	1.162
23	76.77	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)-	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	1.189
24	77.49	9-Octadecenoic acid (Z)-, tetradecyl ester	C <sub>32</sub> H <sub>62</sub> O <sub>2</sub>	478	1.156
25	77.66	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356	1.947

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

vivo studies.<sup>35,37,38</sup> In addition, flavonoids are also claimed to successfully inhibit platelet aggregation in hypercholesterolemic rabbits.<sup>39</sup> As with flavonoids, tannins have been proven to have strong antiplatelet<sup>38</sup> and antihypercholesterolemic effects by reducing cholesterol absorption in animal studies.<sup>41,42</sup> 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.<sup>43</sup> 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.<sup>44-46</sup> 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.<sup>47</sup> Furthermore, saponins were also found to reduce the risk of atherosclerosis in rats.<sup>48</sup>

## 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.<sup>49,50</sup> 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 phytochemicals, 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-bis(1,1-dimethylethyl)- (peak area 11.379); (+)-c-tocopherol, O-methyl- (peak area 9.33); 1-heptatriacotanol (peak area 8.615); and α-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.

## 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

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

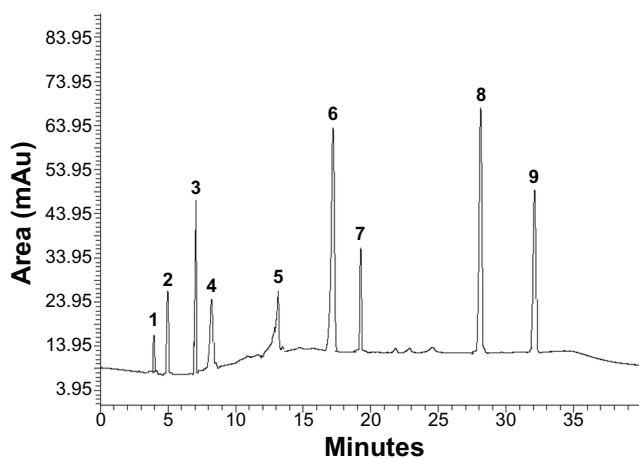
N	Retention time	Compound name	Compound nature	Biological activity
1	48.53	Phenol, 2,6-bis(1,1-dimethylethyl)-	Aromatic and phenolic compound	– Anti-inflammatory (animal study) <sup>58</sup> – Antiatherosclerotic, lowers plasma cholesterol level, and inhibits LDL cholesterol peroxidation (animal study) <sup>59</sup>
2	71.11	1-Heptatriacotanol	Alcoholic compound	– Suppresses lipid accumulation (animal study) <sup>60</sup>
3	72.15	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Reductone	– Antioxidant and lowers triglyceride level (human study) <sup>61</sup> – Protects LDL against peroxidation and retards the progression of atherosclerosis (animal study) <sup>62</sup>
4	72.55	Oleic acid, eicosyl ester	Monounsaturated omega-9 fatty acid	– Inhibits the activity of HMG-CoA reductase (in vitro) <sup>63,64</sup>
5	73.71	$\alpha$ -Tocopherol, O-methyl-	Alcoholic compound	– Lowers total cholesterol and LDL cholesterol (human study) <sup>65</sup>
6	74.30	(+)-c-Tocopherol, O-methyl-		– Inhibits vascular smooth muscle cell proliferation (in vitro) <sup>66</sup> – Antiatherosclerotic effects (animal study) <sup>67</sup>
7	75.19	Vitamin E		– Most potent antioxidant (human study) <sup>68</sup> – Reduces foam cell formation (animal study) <sup>69</sup> – Suppresses LDL lipid oxidation (animal study) <sup>70</sup> – Possesses protective effects against hypercholesterolemia (animal study) <sup>71</sup>

**Abbreviations:** N, number; LDL, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A.

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,  $\alpha$ -tocopherol standard (0.2 mg/mL) was separated by isocratic elution chromatography, as presented in Figure 3A, while the detection of  $\alpha$ -tocopherol in the methanol extract of *B. alba* leaves is shown in Figure 3B. The concentration of each

flavonoid, ascorbic acid, and  $\alpha$ -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  $\alpha$ -tocopherol (0.702 mg/mL), naringin (0.180 mg/mL), apigenin (0.165 mg/mL), and luteolin (0.099 mg/mL).

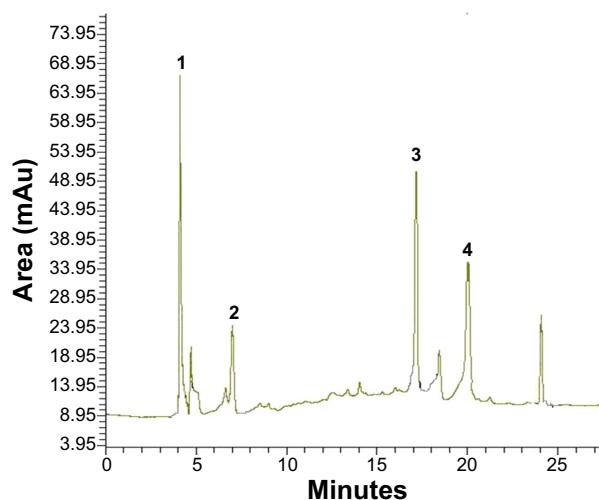
Based on previous studies, the compounds detected in RP-HPLC can be associated with the prevention and treatment of hypercholesterolemia. Luteolin and apigenin are flavones, a type of flavonoid, and they were demonstrated



**Figure 1** HPLC chromatogram for flavonoids and ascorbic acid standards at 280 nm.

**Notes:** 1) Ascorbic acid; 2) rutin; 3) luteolin; 4) catechin; 5) quercetin; 6) apigenin; 7) naringin; 8) myricetin; and 9) hesperidin.

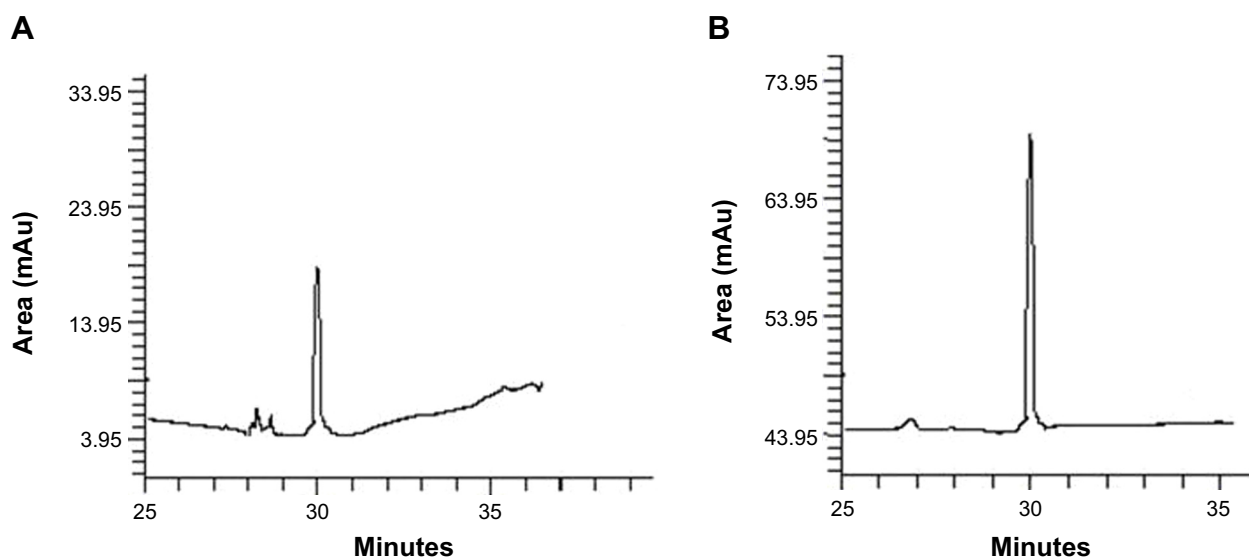
**Abbreviation:** HPLC, high-performance liquid chromatography.



**Figure 2** HPLC chromatogram of *Basella alba* leaves at 280 nm.

**Notes:** 1) Ascorbic acid; 2) luteolin; 3) apigenin; and 4) naringin.

**Abbreviation:** HPLC, high-performance liquid chromatography.



**Figure 3** HPLC chromatogram for  $\alpha$ -tocopherol at 292 nm.  
**Notes:** (A)  $\alpha$ -tocopherol standard; (B)  $\alpha$ -tocopherol compound detected in *Basella alba*.  
**Abbreviation:** HPLC, high-performance liquid chromatography.

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.<sup>51</sup> 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.<sup>52</sup> 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.<sup>53</sup>

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<sup>54</sup> and rabbits.<sup>55</sup> 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<sup>56</sup> and rabbits.<sup>57</sup> Ascorbic acid (vitamin C) and  $\alpha$ -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  $\alpha$ -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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**Table 6** Compounds detected in *Basella alba* leaves

N	Compound	Retention time	Area	Concentration (mg/mL)
1	Ascorbic acid	4.12±0.020	667,500±693.27	0.891±0.0010
2	Luteolin	7.09±0.015	239,510±1,450.46	0.099±0.0015
3	Apigenin	17.20±0.025	511,503±938.27	0.165±0.0012
4	Naringin	19.90±0.014	333,731±1,782.03	0.180±0.0021
5	$\alpha$ -Tocopherol	30.05±0.017	693,125±726.30	0.702±0.0011

**Note:** All data are expressed as the mean  $\pm$  standard error of the mean.

**Abbreviation:** N, number.

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## Disclosure

The authors report no conflicts of interest in this work.

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