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Short Communication

Potential Cholesterol-lowering Activity of Selected Plant Extracts

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Cholesterol is an essential component of cell membranes and a precursor of steroid hormones and bile acids (Nelson and Cox 2008). Its homeostasis is maintained by a complex regulatory network that balances its biosynthesis, consumption, and transport, preventing its accumulation (Voet et al. 2013). Increased levels of circulating cholesterol, known as hypercholesterolemia, is a strong risk factor in the development of cardiovascular diseases, such as atherosclerosis (Voet et al. 2013).

Cholesterol biosynthesis involves several enzymatic reactions (Bloch 1965). One enzymatic reaction is the NADPH-dependent reduction of β-hydroxy-β-methylglutaryl-Coenzyme A (HMG-CoA) to mevalonate by the enzyme HMG-CoA reductase (EC 1.1.1.34). This reaction is considered as the rate-limiting step and the point of regulation in the pathway. The rate of cholesterol synthesis is regulated by subjecting HMG-CoA reductase to competitive inhibition, allosteric effects, and hormonal control (Voet et al. 2013). One way to lower cholesterol levels is to inhibit the activity of HMG-CoA reductase with statins (Endo and Hasumi 1993). However, adverse effects from statin usage, such as liver damage, have been reported (Golomb and Evans 2008), underscoring the need for new cholesterol-lowering drugs that are more potent but with minimal adverse effects.

Plants are excellent sources of bioactive compounds due to their natural abundance and availability. The determination of plant-derived compounds and their pharmacological screening provide a basis for drug discovery (Palvai and Urooj 2014). Previous studies have shown that plant extracts are potential sources of HMG-CoA reductase inhibitors (Gholamhoseinian et al. 2010; Reddy et al. 2012; Iqbal et al. 2014).

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Pouteria campechiana from the family Sapotaceae is commonly known as canistel and locally referred to as *tiesa*. Ethyl acetate extracts of *P. campechiana* contain six stilbenes and six flavonoid gylcosides (Chichioco-Hernandez et al. 2008). The fruit extracts exhibited antioxidant and hepatoprotective properties (Aseervatham et al. 2014). Antioxidant properties were also reported for the pulp and peel extracts of *P. campechiana* leaves (Kong et al. 2013).

Barringtonia asiatica of family Lethycidaceae or fish poison tree is locally known as *botong*. The uses of *B. asiatica* in traditional medicine include the treatment of stomachache and rheumatism with its heated leaves, and the removal of intestinal worms with its seeds (Wild Fact Sheets 2013). The whole tree is known to contain poisonous saponin. Two major saponins and a triterpene ester saponin were isolated from the seeds, while a new triterpene was isolated from a freeze-dried bark (Herlt et al. 2002; Rumampuk et al. 2003; Ragasa et al. 2012). Using brine shrimp hatchability and lethality assay, which may correlate with cytotoxic activity, the fruit extracts exhibited higher activity than the leaf extracts (Mojica and Micor 2007). The saponins showed high antifeedant activity toward *Epilachna* sp. (Herlt et al. 2002). The freeze-dried bark extract also exhibited slight antimicrobial activities against *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Ragasa et al. 2012).

Vitex parviflora of family Verbenaceae is commonly known as *molave*. The distribution of *V. parviflora* is documented in the Philippines and Southeast Asia. Traditional uses of *V. parviflora* include the use of its bark extract in wounds, poison, diarrhea, jaundice, and dropsy (Orwa et al. 2009). Phytochemical screening of its methanol extract yielded positive results for alkaloids, flavonoids, terpenoids, phenolic compounds, and saponins. Moreover, the extract also exhibited gastroprotective properties in ethanol and aspirin-induced models in mice (Tarin and Chichioco-Hernandez 2012). Antimutagenic properties were also reported for phytol, lupeol, β-amyrin, sitosterol, and stigmasterol isolated from its ethyl acetate extract (Ragasa et al. 2003).

Antidesma bunius from the family Euphorbiaceae or currant tree is locally known as *bignay*. The *A. bunius* tree grows in China, India, Southeast Asia, and Australia. The leaves of *A. bunius* are sudorific and used to remedy snakebites (Morton 1987). The fruits of *A. bunius* were reported to contain three flavonoids, namely catechin, procyanidin B1, and procyanidin B2 (Butkhup and Samapito 2008). High phenolic content and antioxidant and antimicrobial activities were observed in its mature fruits (Lizardo et al. 2015). Methanol extracts of its fruits and leaves were active in

brine shrimp and hatchability assay (Micor et al. 2005). The leaves were found to contain the polyphenols gallic acid, ferrulic acid, ellagic acid, corilagin, vicinin II, and amentoflavone, and its extracts were shown to possess antioxidant and hepatoprotective properties (Kassem et al. 2013). The aqueous ethanol (80%) extracts of the leaves also inhibited α-glucosidase activity (Lawag et al. 2013). The leaves and bark were also reported to contain dammara-20,24-dien-3β-ol, friedelan-3β-ol,friedelin, and β-sitosterol (Hui and Sung 1968).

Diospyros blancoi of family Ebenaceae or velvet apple is locally known as *mabolo* (fruit) and *kamagong* (tree). The tree is native to the Philippines, and was introduced in Southeast Asian islands, as well as in the United States. In folklore, the mabolo fruit has been used for the treatment of diarrhea and wounds, while the other parts have been utilized for the treatment of respiratory diseases and skin ailments (Morton 1987). The volatile components of the mabolo fruit were studied via GC and GC/MS and 96 compounds were identified, including benzyl butyrate, butyl butyrate, and cinnamyl butyrate (Pino et al. 2008). The ethyl acetate acetate extract of the leaves yielded bioactive terpenes—isoarborinol methyl ether, α-amyrin palmitate, α-amyrin palmitoleate, β-amyrin palmitate, β-amyrin palmitoleate, and squalene. Isoarborinol methyl ether displayed significant antimicrobial activity, whereas β-amyrin palmitoleate showed significant antimicrobial, analgesic, and anti-inflammatory activities (Ragasa et al. 2009). Phytochemical screening of the ethanol extract of the leaves revealed the presence of tannins and alkaloids. The study also reported significant antioxidant, antidiarrheal, antimicrobial, and cytotoxic activities for the ethanol extract of the leaves (Howlader et al., 2012b). A similar study also reported the presence of tannins, alkaloids, reducing sugars, and gums in the ethanol extract, which also possessed antidiarrheal activity (Hossain et al. 2012). In another study, the methanol extract of the leaves revealed positive results for the presence of alkaloids, flavonoids, tannins, sugars, and gums, and possessed significant free radical scavenging activity and dose-dependent antidiarrheal activity (Howlader et al. 2012a). Another report also suggested significant DPPH and hydroxyl-scavenging activities of the ethanol extract of *mabolo* leaves (Lee et al. 2006). Methanol extract of the leaves exhibited significant anti-asthma effects in murine model of allergic airway inflammation (Lee et al. 2012).

In this study, the HMGCR inhibitory actions of *P. campechiana*, *B. asiatica*, *V. parviflora*, *A. bunius*, and *D. blancoi* extracts were evaluated using an established assay protocol.

Collection of Samples

The leaves of *P. campechiana*, *B. asiatica*, *V. parviflora*, *A. bunius*, and *D. blancoi* were collected within the University of the Philippines Diliman campus and submitted to the Dr. Jose Vera Santos Herbarium, Institute of Biology, UP Diliman for verification.

Preparation of Plant Extracts

Leaves of good condition were washed with water and air-dried. The leaves were homogenized using a blender, weighed, and soaked in distilled methanol. The methanol extracts were filtered and concentrated *in vacuo* at 40°C using a rotary evaporator (IKA® RV 10). The resulting methanol extracts were dissolved in 200 mL or 250 mL distilled water (depending on the weight of extract), and exhaustively partitioned between distilled water and hexane at a volume ratio of 1:2. The layers were allowed to settle and the hexane extract was collected. The aqueous layer was partitioned with ethyl acetate (1:2) and the procedure was repeated. The hexane and ethyl acetate extracts were concentrated *in vacuo* at 40°C. The aqueous layer was lyophilized to concentrate the extract.

HMG-CoA Reductase Inhibitory Assay

The HMG-CoA reductase assay kit was purchased from Sigma-Aldrich®. The kit includes the enzyme HMG-CoA reductase, the substrate HMG-CoA, NADPH, and the inhibitor solution (Pravastatin). The enzyme, substrate, and NADPH were prepared with the buffer to make stock solutions of 0.12µM, 150 µM, and 405 µM, respectively.

In 1.5 mL Eppendorf tubes, 1.5 mg each of the methanol, hexane, ethyl acetate, and aqueous extracts were dissolved in 10% DMSO and 90% buffer (pH 7.4 0.1 M phosphate buffer with 0.12 M KCl, 0.001 M EDTA, and 0.05 M dithiothreitol) to make 1 mg/mL samples. Two trials of four replicates for each extract, including the blank control, solvent control, and positive control, were prepared in a 96-well plate.

Each reaction well contains the following: 185 ml buffer, 50 ml NADPH, 50 ml HMG-CoA, 10 ml of enzymes, and 5 ml of either the plant sample, solvent control, or positive control. The final concentrations of the sample/pravastatin, enzyme, substrate, and NADPH were 0.0167 mg/mL, 0.004 µM, 25 µM, and 67.5 µM, respectively.

In each designated well, either the plant sample, pravastatin, or solvent was first added, followed by the buffer, NADPH, and HMG-CoA. The microplate was incubated at 37°C for 5 minutes, after which 10 µL of HMG-CoA reductase was added. The absorbance was read at 340 nm for every 15 seconds (up to 10 minutes) using the Thermo Scientific Multiskan™ GO Microplate Spectrophotometer.

Percent inhibition was calculated using the equation below, where is the rate of change in absorbance. Average values and standard deviations were reported.

$$\%\ Inhibition = \left(\frac{\frac{\Delta abs_{blank}}{\Delta t} - \frac{\Delta abs_{sample}}{\Delta t}}{\frac{\Delta abs_{blank}}{\Delta t}} \right) \times t0 \quad (1)$$

RESULTS AND DISCUSSION

The yields of the methanol extracts of the five plants are reported in Table 1. It should be noted, however, that yields are inconclusive since the plants were not exhaustively rewashed with methanol.

Table 1. Percent yield of methanol extracts and their respective percent inhibition

Plant/Control	Sample (g)	Methanol extract (g)	Yield (%)	% Inhibition
P. campechiana	302.54	36.67	12.12	71.22±0.30
B. asiatica	265.56	26.88	10.12	32.61±9.21
V. parviflora	253.96	23.45	9.23	41.40±4.73
A. bunius	191.29	9.50	4.96	93.21±2.13
D. blancoi	92.98	22.77	24.49	96.17±4.69
Pravastatin (positive control)				90.94±0.37

Results of the HMG-CoA reductase inhibitory assay using the methanol extracts are shown in Table 1. *P. campechiana*, *A. bunius*, and *D. blancoi* exhibited significant inhibition of HMG-CoA reductase activity since their percent inhibition values are greater than 50%. *A. bunius*, and *D. blancoi* were selected for further studies due to the greater degree of inhibition they displayed. Table 2 shows the percent inhibition of hexane, ethyl acetate, and aqueous extracts of *A. bunius* and *D. blancoi*.

The partitioned extracts exhibited significant inhibition of HMGCR, with values ranging from 89.19% to 97.74%. The extracts of *A. bunius* and *D. blancoi* had

inhibition values greater than the positive control (Pravastatin). The inhibitory activity of the two plants may be attributed to the secondary metabolites found in the extracts. The *A. bunius* extracts have been reported to contain flavonoids and phenolic compounds (Butkhup and Samappito 2008; Lizardo et al. 2015), while *D. blancoi* extracts were shown to contain alkaloids and flavonoids (Howlader et al. 2012). Phenolic compounds identified from *Citrus maxima* peels have been shown to inhibit HMG-CoA reductase activity (Ademosun et al. 2016). Similarly, phenolic compounds from grapefruit peels inhibited HMG-CoA reductase activity (Ademosun et al. 2015). *Citrus bergamia* juice contains a high percentage of flavonoids and the rare flavonoids brutieridin and melitidin (Janda et al. 2016). Brutieridin and melitidin were subjected to computational studies, which revealed that the two molecules bind efficiently at the catalytic site of HMG-CoA reductase (Leopoldini et al. 2010).

Plant extracts are great sources of bioactive compounds. It is recommended that the phytochemicals from of *A. bunius* and *D. blancoi* responsible for the HMG-CoA reductase inhibitory activity be isolated and identified for further studies. Additionally, cell lines, such as Caco-2 cell lines, and *in vivo* animal models should be used to predict absorption.

Table 2. Average percent inhibition ± SD of hexane, ethyl acetate, and aqueous extracts of *A. bunius*, and *D. blancoi*

Sample	Hexane extract	Ethyl Acetate extract	Aqueous extract
A. bunius	89.19±0.55	95.54±0.35	96.53±0.55
D. blancoi	91.10±0.17	97.74±0.78	95.15±0.15
Pravastatin	84.38±0.28		

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