Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Influence of Extracting Solvent on Pharmacological Activity and Cytotoxicity of *Polygonum minus*, a Commonly Consumed Herb in Southeast Asia

Parayil Varghese Christapher[1,2], Subramani Parasuraman[1], Palanimuthu Vasanth Raj[3], Sultan Ayesh Mohammed Saghir[2], Mohd. Zaini Asmawi[2], Murugaiyah Vikneswaran[2]

[1]Unit of Pharmacology, Faculty of Pharmacy, AIMST University, Semeling, Kedah, [2]Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, [3]Unit of Pharmaceutical Technology, Faculty of Pharmacy, AIMST University, Semeling, Kedah, Malaysia

Submitted: 25-03-2015 Revised: 16-06-2015 Published: 30-09-2016

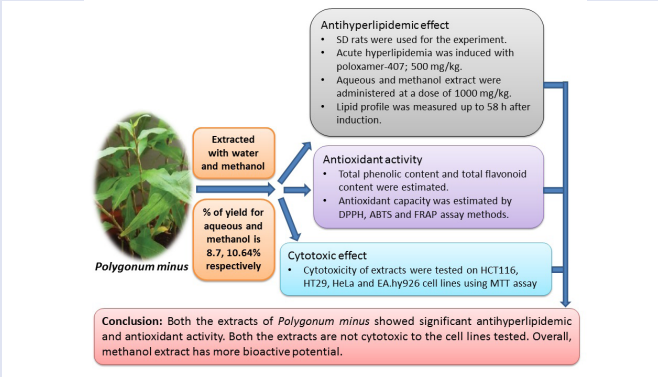
ABSTRACT

Objective: To investigate the antihyperlipidemic, antioxidant, and cytotoxic effect of aqueous and methanol extract of leaves of *Polygonum minus*. **Materials and Methods:** Acute antihyperlipidemic effect was studied on chemically induced hyperlipidemic rat model. Treated groups received aqueous and methanol extract of leaves of *P. minus* respectively (1000 mg/kg; oral) whereas standard treated group received atorvastatin (60 mg/kg; oral) for 3 consecutive days. Blood samples were collected at fixed intervals for lipid profile analysis. Antioxidant effects were studied using 1,1-diphenyl-2-picrylhydrazyl, 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate, and ferric reducing antioxidant power assays. The total flavonoids content and total phenolic contents were also estimated. Cytotoxicity of both extracts was studied on one normal and three cancer cell lines using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay method. **Results:** The methanol extract showed significant reduction in total cholesterol ($P < 0.001$), triglycerides ($P < 0.01$), LDL ($P < 0.05$), VLDL ($P < 0.01$), atherogenic index ($P < 0.001$), and elevation of HDL ($P < 0.05$) levels than the aqueous extract. Similarly, the antioxidant investigations also demonstrated that the methanol extract had higher antioxidant capacity than aqueous extract. Both extracts were not toxic to normal (EA.hy926) as well as to cancer (HCT116, HT29, and HeLa) cells. Significant correlation was demonstrated between total phenolic and total flavonoids contents with the antioxidant activity but not with the antihyperlipidemic effect, suggesting other groups of chemical constituents may be mainly responsible for the antihyperlipidemic effect of this plant. **Conclusion:** The study demonstrated that the presence and extent of bioactivities are influenced by solvents used for extraction. This study confirmed the antihyperlipidemic effect of leaves of *P. minus* in acute hyperlipidemic rat model.

Key words: Antihyperlipidemic, antioxidant, cytotoxicity, poloxamer 407, *Polygonum minus*

SUMMARY

- *Polygonum minus* is an herbaceous flowering plant.
- This plant possess high amount of phenolics and flavonoids
- This study focused on the antioxidant, cytotoxicity and antihyperlipidemic effect of aqueous and methanol extracts of leaves of *P. minus*
- The extracts possess significant antioxidant activity and antihyperlipidemic activity but they are not toxic to normal and cancer cells tested.
- The antioxidant activity is well correlated with phenolic and flavonoids contents but the antihyperlipidemic activity is not correlated with antioxidant effect.



Abbreviations used: CVDs: Cardiovascular diseases, LDL: Low-density lipoprotein, DDPH: 1 ,1-Diphenyl-2-picrylhydrazyl radical, TPTZ: 2,4,6,-tris(1-pyridyl)-5-triazine, ABTS: 2,2′-Azino-di-[3-ethylbenzthiazoline Sulfonate], HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, TC: Total cholesterol, TG: Triglycerides, EC_{50}: Half maximal effective concentration, LD_{50}: Median lethal dose.

Correspondence:

Dr. Murugaiyah Vikneswaran,
Discipline of Pharmacology,
School of Pharmaceutical Sciences,
Universiti Sains Malaysia, Penang, Malaysia.
E-mail: vicky@usm.my
DOI: 10.4103/0973-1296.191451

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Hyperlipidemia, the commonest form of dyslipidemia refers to an increase in the levels of blood lipids, mainly the cholesterol and triglycerides. It is the major risk factor for endothelial damage leading to cardiovascular diseases (CVDs) such as atherosclerosis.[1] CVDs are one of the major causes of health-related death across the globe and their contribution in disease burden has been increasing in the developing and under developed countries.[2] Hyperlipidemia and oxidative stress induced by free radicals contribute to the development of CVDs, in particular atherosclerosis and other chronic diseases.[3] Management of hyperlipidemia can be achieved by both nonpharmacological and pharmacological interventions. Dietary modification is a key factor in the nonpharmacological intervention.[4] Pharmacological way of managing hyperlipidemia relied on two major classes of drugs, such as the statins and nonstatins. Fibrates are the main nonstatin drugs, which helps in

Cite this article as: Christapher PV, Parasuraman S, Raj PV, Mohammed Saghir SA, Asmawi M, Vikneswaran M. Influence of extracting solvent on pharmacological activity and cytotoxicity of *Polygonum minus*, a commonly consumed herb in Southeast Asia. Phcog Mag 2016;12:424-30.

the reduction of blood triglycerides levels but not effective in reducing low-density lipoprotein (LDL). The use of statins are linked with adverse effects, in particular elevated liver enzymes and rhabdomyolysis,[5,6] whereas fibrates are linked to inflammation of liver and muscles and gall stone formation.[7] Thus, the search for effective and well-tolerated lipid-lowering drugs is still on-going.

Natural products play a vital role in the search of new treatment agents for various diseases. About 50% of the drugs introduced in the market during the last 20 years have been derived directly or indirectly from small molecules of natural origin.[8,9] The antihyperlipidemic activities of many natural products have been established using experimental animal models. Commonly available plants such as *Camellia sinensis*, *Tamarindus indica*, and *Garcinia atroviridis* showed potential antihyperlipidemic activity in animal models.[10-12] In addition, medicinal plants and their derived products such as garlic, fenugreek, and curcumin were found to be effective in lowering cholesterol in human trials.[13,14] The excellent antioxidant capacity of many natural products is of advantage, in particular to prevent lipids peroxidation.

In South East Asia, several types of herbs or vegetables are consumed raw as salad for improving general health and as preventive measures against diseases. In Malaysia, these include selom (*Ooenanthe javanic*), pegaga (*Centella asiatica*), and kesum (*Polygonum minus*).[15] Fresh leaves of *P. minus* are usually consumed raw along or it is used in the cooking for flavoring purpose. *P. minus* is traditionally used as for digestive problems and to remove dandruff.[16] The pharmacological activities of *P. minus* were reviewed and reported its anti-LDL oxidation, antimicrobial activity, digestive enhancing activity, antiulcer activity, and immunomodulatory activity. Clinical trial data showed that the combination of *P. minus* and *Eurycoma longifolia* is more effective than placebo in enhancing sexual performance in healthy male volunteers.[17] Despite this, many other potential pharmacological effects of this plant including antihyperlipidemic remain unexplored. This comparative study was carried out to evaluate and compare the influence of extracting solvents (aqueous and organic) on the pharmacological activities of leaves of *P. minus* such as antihyperlipidemic activity and antioxidant. In addition, this study also evaluated the cytotoxicity of those extracts.

MATERIALS AND METHODS

Chemicals, standards, and reagents

1 ,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6,-tris(1-pyridyl)-5-triazine (TPTZ), 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS), poloxamer 407, quercetin, morin, and potassium persulfate were purchased from Sigma (St Louis, MO, USA). Folin–Ciocalteu reagent, methanol, gallic acid, and l-ascorbic acid were obtained from Merck (Darmstadt, Germany). Sodium carbonate, sodium acetate trihydrate, aluminum chloride, ferric chloride hexahydrate, carboxymethyl cellulose (CMC), and ferrous sulfate heptahydrate were purchased from R&M Chemicals (Essex, UK). Atorvastatin was obtained from Ranbaxy Malaysia Sdn. Bhd. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide was purchased from Sigma (St Louis, MO, USA). Normal cell line – human umbilical vein cell line (EA.hy926) and cancer cell lines – human colon cancer cell line (HCT116), human colon cancer cell line (HT29), and human cervical cancer cell line (HeLa) are kind gift from the Oncology and Radiology Science Cluster, Advanced Medical & Dental Institute (AMDI), Universiti Sains Malaysia.

Plant materials

The raw materials of *P. minus* (Polygonaceae) were obtained from the Balik Pulau, Penang Island, Malaysia. A voucher specimen (USM Herbarium. 11542) was deposited at the Herbarium, School of Biological Sciences, Universiti Sains Malaysia.

Preparation of extracts

Fresh leaves of *P. minus* were separated from stems and rinsed thoroughly with water to remove dirt. The leaves were dried in an oven below 50 °C for 3 days. The dried leaves were pulverized using a blender. The powdered leaves were extracted by maceration using distilled water or methanol (powder : solvent ratio of 1:10 [w/v]) for 5 days. The extracts were decanted and replenished with fresh solvent every day. The pooled extracts were filtered through a cotton plug followed by Whatman No. 1 filter paper. The filtrate was then concentrated in a rotary evaporator (Yamato Rotary Evaporator RE300; Yamato Scientific, Tokyo, Japan) and freeze-dried (Thermo Scientific, Massachusetts, USA) to obtain the dry extracts. The extract was kept in refrigerator until further use.

EVALUATION OF ANTIHYPERLIPIDEMIC EFFECT

Animals

Male Sprague-Dawley rats of 14–16 weeks old, weighing initially about 180–220 g were used in this experiment. The animals were kept at room temperature and maintained on a 12 h light/dark cycle. Animals were allowed free access to standard food pellets (Gold Coin, Penang, Malaysia) and drinking water. Animals handling was in accordance with institutional guidelines and approval to use the animals was obtained from the Animal Ethics Committee, Universiti Sains Malaysia (Ref. No. USM/Animal Ethics Approval/2014/(94) (479)) and Animal and Human Ethics Committee, AIMST University (Ref. No. AUHAEC 7/FOP/2013).

Evaluation of antihyperlipidemic in poloxamer 407-induced acute hyperlipidemic rats

The rats were fasted overnight before experimentation with free access to water. Poloxamer 407 solution for administration was prepared by mixing the agent with saline solution and then refrigerated overnight to facilitate its dissolution.[18] Rats were divided into five groups of six animals each; group 1 served as normal control group and given injection of normal saline intraperitoneally (i.p.), whereas animals from groups 2–5 were injected with 500 mg/kg poloxamer 407 i.p. After 6 h administration of poloxamer 407, groups 1 and 2 received 0.5% solution of CMC, group 3 received 60 mg/kg atorvastatin, whereas groups 4 and 5 received 1000 mg/kg aqueous and methanol extracts of leaves of *P. minus*, respectively. The extracts and atorvastatin were prepared in 0.5% CMC and administered by oral route, once daily for 3 days. Blood samples (250 μL) were collected from the retro orbital plexus in eye before and at 10 and 34 h after hyperlipidemia induction, for determination of total cholesterol (TC) and triglycerides, whereas terminal blood samples of 1 mL were collected at 58 h after hyperlipidemia induction for determination of TC, triglycerides, LDL, and high-density lipoprotein (HDL) levels. Blood samples were centrifuged at 5000 RPM for 10 min to obtain the serum, which were stored at −80 °C before analysis. Animals were euthanized and the carcase was disposed through AIMST university bio-waste management system. Serum lipids were determined using commercial kits following the manufacturer's protocol (Thermo Fisher Scientific, Massachusetts, USA). Very low-density lipoproptien (VLDL) concentration was calculated using the equation: (VLDL cholesterol = triglyceride/2.22), whereas the AI was calculated using the formula (TC-HDL)/HDL.[19]

ANTIOXIDANT ASSAY

The *in vitro* antioxidant assays were carried out on aqueous and methanol extracts of *P. minus*. All determinations were done in triplicate. Absorbance was measured using Lab-System Multiscan MS microplate reader (Model No. 354; Helsinki, Finland).

Total phenolic content

Total phenolic content was determined using Folin–Ciocalteu reagent solution following method described by Kumaran and Karunakaran[20] with gallic acid as a standard. In brief, Folin–Ciocalteu reagent, sodium carbonate, standard or test samples (10 mg/mL), and distilled water were mixed in a test tube in a ratio of 5:15:1:79 to the total volume of 200 µL. Absorbance of the reaction mixtures was read at 765 nm after incubation for 2 h at room temperature. The results are expressed as µg gallic acid equivalent/mg dry extract.

Total flavonoid content

Total flavonoid content was determined by the aluminum chloride method, as described by Chang *et al.*[21] using quercetin as reference standard. In brief, 100 µL of standard or extract (10 mg/mL) solutions were mixed with 20 µL of aluminum chloride (10%, w/v), 20 µL of 1 mol/ L sodium acetate, 300 µL methanol, and 560 µL distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The results are expressed as µg quercetin equivalent/mg dry extract.

1 ,1-Diphenyl-2-picrylhydrazyl radical scavenging

DPPH radical scavenging activity was determined by method described by Wang *et al.*[22] Briefly, 100 µL of the test samples (at six concentrations), dissolved in methanol:H_2O (1:1) were added to 100 µL DPPH (200 µmol/L) prepared in methanol and incubated at room temperature for 30 min. Morin was used as reference standard. The amount of remaining DPPH was determined at 517 nm. The results are expressed as EC_{50}, which is the concentration where the scavenging activity is 50%.

2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate] radical scavenging

ABTS radical scavenging activity was measured by the method as described by Re *et al.*[23] Briefly, ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by mixing 14 mmol/L ABTS and 4.9 mmol/L potassium persulfate solutions in equal volumes. The solution was allowed to react in the dark at room temperature for 16–20 h before use. Then, 1 mL of the solution was diluted with 40 mL deionized water to yield working ABTS solution with an absorbance equal to 0.70 ± 0.02 at 734 nm. To 180 µL ABTS working solution, 20 µL test samples (at six concentrations) were added. Absorbance of the samples was read at 734 nm after 6 min. Ascorbic acid was used as reference standard. The results are expressed as EC_{50}, which is the concentration where the scavenging activity is 50%.

Ferric reducing antioxidant power

The FRAP assay was carried out by method of Benzie and Strain.[24] Briefly, 150 µL FRAP working solution (300 mmol/L acetate buffer, pH 3.6, 10 mmol/L TPTZ in 40 mmol/L HCl and 20 mmol/L $FeCl_3$ in a ratio of 10:1:1) was added to 50 µL test samples (10 mg/L). The reaction mixtures were incubated for 8 min before absorbance measurement at 600 nm. Ferrous sulfate ($FeSO_4$ $7H_2O$) was used as reference standard and the results are expressed as nmol Fe^{2+} equivalent/mg dry extract.

CYTOTOXICITY EVALUATION

In vitro cell cytotoxicity of aqueous and methanol extract of *P. minus* was carried out by tetrazolium salt (MTT) assay as described by Denizot and Lang.[25] Four types of cell lines, such as HCT116, HT29, HeLa, and EA.hy926 were used for this study. The cells were cultured in minimum essential medium (MEM). The medium also contains 10% fetal calf serum, penicillin (100 U), and streptomycin (100 µg). Concentrations of extracts used were in the range of 200–12.5 µg/mL, prepared by serial dilution method. Extracts were initially dissolved in 1% dimethyl sulfoxide (DMSO) and further diluted with MEM medium. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using medium. To each well of the 96-well microplate, 0.1 mL of the diluted cell suspension (approximately 10 000 cells) was added. After 24 h, the media was removed, cells were washed once, and 100 µL of plant extracts at different concentrations were added to the cells in microplates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out every 24 h. After 72 h, the drug solutions in the wells were discarded and 50 µL of MTT in MEM was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 50 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the formula

$$\%\,\text{Growth inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}}\right) \times 100.$$

Statistical analysis

The results were presented as the mean ± SEM. The statistical significance of difference was evaluated by analysis of variance, followed by Tukey's *post hoc* test. Pearson correlation analysis was done for total phenolic and total flavonoids contents against antihyperlipidemic and antioxidant activities.[13] A *P*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The leaves of *P. minus* are widely used in cooking as flavoring agent or consumed raw as a salad leaf in South East Asia, other than being used in traditional medicines. The plant is used to relieve stomach ache, body pain, and other aches. Different pharmacological activities of this plant were reported, in particular for aqueous or methanol extracts.[17] Despite the belief among locals that this plant may reduce blood lipids, the antihyperlipidemic potential of this plant was yet to be explored extensively. In this study, we report the influence of aqueous and methanol solvents on potential antihyperlipidemic effect, antioxidant activity, and cytotoxic effect of *P. minus* extracts.

Increased levels of lipids are one of the major risk factors of cardiovascular diseases.[26] In this comparative study, poloxamer 407 induced acute hyperlipidemic rats were used to evaluate the antihyperlipidemic potential of *P. minus*. Hyperlipidemia in experimental animals can be induced chemically using agents such as poloxamer 407 and triton WR1339. Acute hyperlipidemic rat model is preferred due to its convenience, reproducibility, and lack of undesirable underlying pathological conditions.[19,27] However, poloxamer-induced hyperlipidemic rat shows higher total cholesterol and triglyceride levels than that of diet-induced hyperlipidemia. Therefore, this method is a more suitable method for agents that have strong antihyperlipidemic activity.[28] The initial serum TC and triglycerides levels between all groups were not significantly different in this study. Upon induction with poloxamer 407, the hyperlipidemic control animals showed marked increases in TC and triglycerides levels, which were significantly higher than the normal control throughout the study. The antihyperlipidemic effect of aqueous and methanol extracts of *P. minus* leaves in hyperlipidemic rats is summarized in Table 1. The TC of the aqueous and methanol extract treated group was significantly lowered at 34 and 58 h whereas

Table 1: Effect of aqueous and methanol extracts of *Polygonum minus* on lipid parameters of Poloxamer 407-induced hyperlipidemic rats

		Serum TC and TG at various hours after induction of hyperlipidemia								Other serum lipid parameters			
		TC (mmol/L)				TG (mmol/L)				HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	AI
Groups	Dose (mg/kg)	0	10	34	58	0	10	34	58	58 h			
Normal control	-	1.92 ± 0.12	1.99 ± 0.12	1.92 ± 0.07	2.02 ± 0.62	1.37 ± 0.13	1.36 ± 0.11	1.31 ± 0.10	1.21 ± 0.18	1.27 ± 0.08	0.37 ± 0.13	0.55 ± 0.08	1.02 ± 0.07
Hyperlipidemic control	-	1.96 ± 0.17	5.48 ± 0.36**	15.23 ± 0.86***	20.23 ± 0.62***	1.14 ± 0.11	21.28 ± 1.45***	20.46 ± 0.25***	1.52 ± 0.99***	0.44 ± 0.14**	3.48 ± 0.81**	9.78 ± 0.45***	19.23 ± 0.62***
Standard drug (Atorvastatin)	60	2.04 ± 0.13	4.78 ± 0.92	10.10 ± 1.03###	11.24 ± 0.89###	1.57 ± 0.24	14.96 ± 1.96	13.51 ± 1.91##	15.39 ± 0.66##	1.16 ± 0.19##	0.80 ± 0.22##	7 ± 0.30##	10.24 ± 0.89###
Aqueous extract of *P. minus*	1000	1.90 ± 0.13	5.48 ± 0.39	9.73 ± 0.65###	16.01 ± 1.16 #	1.29 ± 0.12	18.82 ± 1.71	16.11 ± 1.33	16.53 ± 0.95#	0.91 ± 0.04	2.21 ± 0.65	7.51 ± 0.43#	15.01 ± 1.16#
Methanol extract of *P. minus*	1000	1.98 ± 0.12	5.61 ± 0.94	8.52 ± 0.56###	13.12 ± 1.02###	1.36 ± 0.25	17.17 ± 1.74	12.6 ± 1.39##	14.59 ± 1.98##	0.98 ± 0.06#	1.36 ± 0.33#	6.63 ± 0.90##	12.12 ± 1.02###

Values are expressed as mean ± SEM ($n = 6$). AI = atherogenic index, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglyceride, VLDL = very low-density lipoprotien. $^{**}P < 0.01$; $^{***}P < 0.001$ compared with normal control. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with hyperlipidemic control.

the triglycerides level showed no significant changes at 34 h but showed significant reduction at 58 h. However, the methanol extract treated group showed significant reduction of both TC and triglycerides levels at 34 and 58 h, and this effect was similar to atorvastatin-treated group.

The effects of aqueous and methanol extracts of *P. minus* leaves on other lipid parameters (LDL, HDL, VLDL, and AI) are shown in Table 1. The LDL, VLDL, and AI levels of hyperlipidemic control animals are significantly higher but the HDL level is significantly lower compared to the normal controls. Hyperlipidemic animals treated with methanol extract has significant reductions in LDL, VLDL, and AI, whereas aqueous extract treated group showed significant reductions of VLDL levels and AI but not LDL. In general, the findings showed that the methanol extract had better antihyperlipidemic activity than the aqueous extract. AI is used as one of investigational tool to evaluate the risk for coronary heart disease. Elevation and oxidation of LDL were related to the formation of plaque in blood vessels, leading eventually to atherosclerosis.[19] In this study, both methanol and aqueous extracts reduced the AI and the results suggest their beneficial role in atherosclerosis and coronary heart disease.

Further study was carried out to evaluate the antioxidant capacity of the aqueous and methanol extracts. The results are summarized in Table 2. A single antioxidant assay is not sufficient to clearly demonstrate the antioxidant capacity of an agent in a biological system.[29] Therefore, in this study, few antioxidant assays were used. The results showed that the antioxidant capacity of methanol extract of *P. minus* leaves were better than aqueous extract, whereby methanol extract had higher phenolic and flavonoids contents, ferric reducing power, and DPPH and ABTS scavenging activities (as indicated by lower EC_{50} values). The antioxidant and free radical scavenging capacity of plant extracts is mainly contributed by the presence of phenolic compounds, in particular the flavonoids and tannins. The redox property of the compounds contributes to the reducing, hydrogen donating and free radical scavenging abilities; other than acting as singlet oxygen quenchers and metal chelators.[30] Previous phytochemical investigations on *P. minus* reported the presence of flavonoids such as myricetin, quercetin, rutin, methyl flavonol (6, 7-4',5'-dimethylenedioxy-3,5,3'-trimethoxyflavone), and flavone (6, 7-methylenedioxy-5, 3',4',5'-tetramethoxyflavone). These compounds possessed antioxidant activity and could contribute at least in part to antioxidant activity of *P. minus* extracts.[31-33] The findings on the antioxidant activity of *P. minus* in this study is in agreement with previous reports, which found that aqueous, ethanol, and methanol extracts of *P. minus* possessed high antioxidant activities. The high polyphenol content, presence of vitamin C, and β carotene was suggested to be responsible for the antioxidant activity of *P. minus*.[34] Similarly, our study also found that the aqueous and methanol extract of *P. minus* had high amount of polyphenolic compounds.

Pearson correlation analysis was used to analyze the correlative relationships between total phenolic and flavonoids contents of the aqueous and methanol extract with their antioxidant and antihyperlipidemic effects. The results are tabulated in Table 3. The analysis showed that total phenolic and flavonoids contents had a significant negative correlation with DPPH and ABTS scavenging activities, and a significant positive correlation with FRAP for both the aqueous and methanol extracts of *P. minus* leaves. In contrast, both total phenolic and flavonoids contents showed no correlation with the antihyperlipidemic effects (any of the serum lipid parameters or AI). Therefore, the findings suggest that the phenolics and/or flavonoids may contribute to the antioxidant activities but not the antihyperlipidemic effect of the *P. minus* aqueous and methanol extracts. The antihyperlipidemic effect of *P. minus* extract

Table 2: Antioxidant activities of aqueous and methanol extracts of *Polygonum minus*

		Assays				
Test compounds	**Yield (%)**	**Total Phenolics (μg gallic acid equivalent/mg extract)**	**Total Flavonoids (μg quercetin equivalent/mg extract)**	**DPPH EC_{50} (μg/L)**	**ABTS EC_{50} (μg/L)**	**FRAP (nmol Fe+2 equivalent/mg extract)**
Aqueous extract of *P. minus*	8.7	74.25 ± 3.32	11.95 ± 0.24	146.41 ± 5.81	249.55 ± 6.65	110.13 ± 3.33
Methanol extract of *P. minus*	10.64	114.66 ± 6.98	53.34 ± 0.33	6.78 ± 0.85	90.21 ± 4.68	253.50 ± 14.90
Ascorbic acid				-	5.22 ± 0.87	
Morin				5.10 ± 1.10	-	

Values are expressed as mean ± SEM ($n = 3$). ABTS = 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate, DPPH = 1,1-Diphenyl-2-picrylhydrazyl, FRAP = ferric reducing antioxidant power, EC_{50}- half maximal effective concentration

Table 3: Correlation between total phenolic content (TPC) and total flavonoids content (TFC) with antioxidant and antihyperlipidemic activity

	Correlation values (*r*)								
	Antioxidant activity			Antihyperlipidemic activity					
Parameters	DPPH	ABTS	FRAP	TC	TG	LDL	HDL	VLDL	AI
TPC	−0.920[a]	−0.933[a]	0.947[a]	−0.179	−0.139	−0.773	−0.005	−0.139	−0.179
TFC	−0.997[a]	−0.993[a]	0.975[a]	−0.312	−0.381	−0.769	0.235	−0.381	−0.312

ABTS = 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate, AI = atherogenic index, DPPH = 1,1-Diphenyl-2-picrylhydrazyl, FRAP = ferric reducing antioxidant power, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglyceride, VLDL = very low-density lipoprotien [a]Correlation is significant at the 0.01 level.

Table 4: *In vitro* cytotoxicity study of aqueous and methanol extracts of *Polygonum minus* against selected cell lines

Extracts	Concentration (µg/mL)	HCT116		HT29		HeLa		EA.hy926	
		% viability of cells	Mean LD_{50}	% viability of cells	Mean LD_{50}	% viability of cells	Mean LD_{50}	% viability of cells	Mean LD_{50}
Aqueous	12.5	98.73	>200 µg/mL	92.79	>200 µg/mL	90.72	>200 µg/mL	70.98	92.2 µg/mL
	25	73.51		88.45		80.64		67.79	
	50	69.99		81.51		74.59		65.69	
	100	64.04		75.78		74.09		46.45	
	200	58.65		70.07		62.12		17.53	
Methanol	12.5	98.24	>200 µg/mL	88.04	>200 µg/mL	92.23	>200 µg/mL	97.52	214 µg/mL
	25	88.54		88.02		87.78		96.46	
	50	86.47		88.04		82.57		93.79	
	100	86.32		81.34		89.15		85.15	
	200	77.44		74.23		82.57		49.57	

Values are expressed as the mean of three consecutive experiments. LD_{50}: Median lethal dose

may be due to the some other chemical constituents or due to the combination of antioxidant principles with other chemical constituents such as myricetin and tetradecanal,[35,36] which are found to be effective in lipid-level modulation.

The cytotoxicity potential of *P. minus* extracts is shown in Table 4. The results showed that both aqueous and methanol extracts are not cytotoxic against all the three cancer cell lines, with LD_{50} values of over 200 µg/mL. At 200 µg/mL, both extracts had maximum cell death of lesser than 42% against HCT116, HT29, and HeLa cells. In the case of normal cell (EA.hy926), the LD_{50} was found to be 92.2 and 214 µg/mL for aqueous and methanol extracts, respectively. Previously, cytotoxicity profile of *P. minus* on normal cell and cancer cell was reported on lung fibroblast cell line (Hs888Lu) and HeLa cells, respectively. The results showed that the extract was not cytotoxic on Hs888Lu cells but cytotoxic on HeLa cells at 0.1 mg/mL.[34] This study demonstrates that both the plant extracts are relatively safe toward living cells even at higher concentrations.

CONCLUSIONS

This study evaluated the influence of different extracting solvent, namely water and methanol on the pharmacological activities and cytotoxicity of *P. minus* leaves extracts. Both aqueous and methanol extracts showed antihyperlipidemic effect but the methanol extract showed better effect and significant reductions in all lipid parameters (except an increase in HDL) and AI. Similarly, the antioxidant investigations also demonstrated that the methanol extract had higher antioxidant capacity than aqueous extract. Both extracts were not cytotoxic on normal as well as cancer cells tested. Significant correlation was demonstrated between total phenolic and total flavonoids contents with the antioxidant activity but not with the antihyperlipidemic effect of *P. minus* leaves extracts, suggesting other groups of chemical constituents may be mainly responsible for the antihyperlipidemic effect of this plant. In conclusion, the study demonstrated that the presence and extent of bioactivities of the extracts are influenced greatly by solvents used for extraction. The antihyperlipidemic effect of *P. minus* in acute model warrants further evaluation of its antihyperlipidemic effect in diet-induced chronic models and elucidation of the possible mechanism of action.

Acknowledgement

The authors also like to thank Dr. Syed Atif Ali, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, Malaysia for his kind help in cytotoxicity studies.

Financial support and sponsorship

This work was supported in part by AIMST University Internal research Grant (AURGC/18/FOP/2013).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mudau M, Genis A,Lochner A, Strijdom H. Endothelial dysfunction the early predictor of atherosclerosis:review article. Cardiovasc J Afr 2012; 23: 222- 31.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3: e442.
3. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr2004; 44: 275-95.
4. Dog TL, Riley D. Management of hyperlipidemia. Altern Ther Health Med 2003; 9: 28- 41.
5. Maji D, Shaikh S, Solanki D, Gaurav K. Safety of statins. Indian J Endocrinol Metab 2013;17: 636- 46.
6. Rader DJ. A new feature on the cholesterol-lowering landscape. Nat Med 2001; 7: 1282- 4.
7. Roberts W. Safety of fenofibrate – US and worldwide experience. Cardiology 1989;76:169- 79.
8. Harvey AL. Natural products in drug discovery. Drug discov today 2008;13:894- 901.
9. Sagar S, Kaur M, Radovanovic A, Bajic VB. Dragon exploration system on marine sponge compounds interactions. J Cheminform 2013;5:1- 7.
10. Yang TT, Koo MW. Chinese green tea lowers cholesterol level through an increase in fecal lipid excretion. Life Sci 1999;66:411- 23.
11. Jindal V, Dhingra D, Sharma S, Parle M, Harna RK. Hypolipidemic and weight reducing activity of the ethanolic extract of *Tamarindus indica* fruit pulp in cafeteria diet-and sulpiride-induced obese rats. J Pharmacol Pharmacother 2011;2:80- 4.

12. Al-Mansoub MA, Asmawi M, Murugaiyah V. Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of *Garcinia atroviridis* a comparative study. J Sci Food Agric 2013;94:1552- 8.
13. Lin TL, Lin HH, Chen CC, Lin MC, Chou MC, Wang CJ. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. Nutr Res 2007;27:140- 5.
14. Tappia PS, Xu YJ, Dhalla NS. Reduction of cholesterol and other cardiovascular disease risk factors by alternative therapies. Clin Lipidol 2013;8:345- 59.
15. Faujan NH, Abdullah N, Abdullah Sani N, Babji AS. Antioxidative activities of water extracts of some Malaysian herbs. ASEAN Food J 2007;14:61- 8.
16. Bunawan H, Talip N, Noor NM. Foliar anatomy and micromorphology of *Polygonum minus* Huds and their taxonomic implications. Aust J Crop Sci 2011;5:123- 7.
17. Christapher PV, Parasuraman S, Christina J, Asmawi M, Murugaiyah V. Review on *Polygonum minus*. Huds,a commonly used food additive in Southeast Asia. Pharmacognosy Res 2015;7:1- 6.
18. Schmolka IR. Poloxamers in the pharmaceutical industry. Florida:. CRC Press 1991
19. Kim HY, Jeong DM, Jung HJ, Jung YJ, Yokozawa T, Choi JS, Hypolipidemic effects of Sophora flavescens and its constituents in poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. Biol Pharm Bull 2008;31:73- 8.
20. Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-Food Sci Technol 2007; 40:344- 52.
21. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods J Food Drug Anal 2002;10:178- 82.
22. Wang KJ, Yang CR, Zhang YJ. Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*). Food Chem 2007; 101: 365- 71.
23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231- 7.
24. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. Anal Biochem 1996; 239: 70- 6.
25. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986; 89: 271- 7.
26. World Health Organization Cardiovascular diseases (CVDs). Fact sheet N?317. Available from http://www.who.int/mediacentre/factsheets/fs317/en/.2015 updated January 2015 and [last accessed on 2015 Aug 20].
27. Johnston TP, Palmer WK. Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. Biochem pharmacol 1993; 46 :1037- 42.
28. Chaudhary HR, Brocks DR. The single dose poloxamer 407 model of hyperlipidemia; systemic effects on lipids assessed using pharmacokinetic methods,and its effects on adipokines. J Pharm Pharm Sci 2013; 16: 65- 73.
29. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005; 53: 4290- 302.
30. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995; 22:375. 83.
31. Imelda F, Faridah D, Kusumaningrum H. Bacterial inhibition and cell leakage by extract of *Polygonum minus* Huds. leaves. Int Food Res J 2014; 21: 553- 60.
32. Hay RK, Waterman PG. Volatile oil crops: their biology, biochemistry and production. New York: Longman Scientific & Technical 1993
33. Urones JG, Marcos IS, Pérez BG, Barcala PB. Flavonoids from *Polygonum minus* Phytochemistry. 1990; 29: 3687- 9.
34. Ali AM, Mackeen MM, El-sharkawy SH, Abdul Hamid J, Ismail NH, Ahmad F. Antiviral and cytotoxic activities of some plants used in Malaysian indigenous medicine. Pertanika J Trop Agric Sci 1996; 19: 129- 36.
35. Liang T, Wang YY, Zhang C, Ling H, Zhao S, Fang X. Hypolipidemic effect of myricetin. Chin J Lab Diagn 2009; 13: 1670- 2.
36. Bushnev AS, Baillie MT, Holt JJ, Menaldino DS, Merrill JR AH, Liotta D C. An efficient asymmetric synthesis of Enigmols (1-deoxy-5-hydroxysphingoid bases), an important class of bioactive lipid modulators Arkivoc. 2010; 8: 263-77.

Vikneswaran Murugaiyah

ABOUT AUTHOR

Dr. Vikneswaran Murugaiyah, is an Associate Professor at Universiti Sains Malaysia, Penang, Malaysia. His research interest is on neurohormetic phytochemicals and cholinesterase inhibitors for neurodegenerative and lipid disorders. He is currently supervising Ph.D and M.Sc students in the field of pharmacology and phytochemistry. He has published a number of research and review articles in reputed impact factor journals.