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A New Perspective on the Use of *Phaleria macrocarpa* in the Management of Cardiovascular and Metabolic Diseases: A Research Review

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ABSTRACT

Phaleria macrocarpa is one of the plants originating from Indonesia that has been empirically known for its medicinal properties. The medicinal properties have also been scientifically revealed for the management of conditions related to chronic diseases, including hyperglycemia, dyslipidemia and renal disorders. With regards to increased blood glucose, studies have proven that P. macrocarpa helps to lower blood glucose levels. These studies have proven that P. macrocarpa helps to lower blood glucose levels by reducing the activity of α-glucosidase, an enzyme involved in the digestion of carbohydrate, thus further reduced the rate of glucose absorption. P. macrocarpa treatment was also able to significantly improve blood lipid profiles and its related parameters, including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride. It also showed improvements in genes and proteins that are associated to the regulation of cholesterol transport and homeostasis, including LDL-R, PCSK9 and CETP. Nephroprotective effect of P. macrocarpa was revealed by reduction in VEGF and TGF-α that are known to regulate angiogenic and cells proliferation. Effects of P. macrocarpa through various parameters and pathways making it highly favorable to be developed as the treatment of cardiovascular and metabolic diseases risk factor, especially in managing hyperglycemia, dyslipidemia, as well as protecting the kidneys.

Keywords: *Phaleria macrocarpa*, hyperglycemia, dyslipidemia, diabetes, cardiovascular.

INTRODUCTION

Traditional herbal medicine has been in existence for centuries before the development and spread of modern scientific medicine and it has been a part of cultural heritage up until now. During the past few years, there has been a growing interest in the use of natural products, especially herbal medicines which were derived from plants. The most common reason for using herbal medicines is due to the concern of adverse effects that may be occurred during the use of conventional (chemical and synthetic) medicines. The use of herbal medicines is increased when conventional medicines are ineffective in the treatment of chronic diseases [1]. Growing interest in the use of natural products is supported by the fact that Indonesia is one of the centers of biodiversity around the world. Indonesia has around 30,000 species of plants live in the archipelago, out of 40,000 species of plants live in the world. Several studies have been conducted to investigate the potentialities of various medicinal plants for the treatment of chronic diseases. Pharmacological effects of various medicinal plants have been revealed, including DLBS3233, bioactive fraction combination of *Cinnamomum burmannii* and *Lagerstroemia speciosa*, for its effect to lower glucose levels [2-3]. DLBS4847, bioactive fraction of *Curcuma mangga*, has been found to be a potential candidate in the treatment of benign prostate hyperplasia [4]. DLBS1033, bioactive fraction isolated from earthworm *Lumbricus rubellus*, also showed thrombotic and thrombolytic activities which could be used as a promising thrombolytic drug [5]. Anti-inflammatory, anti-angiogenic and pro-apoptotic activities were revealed in DLBS1442 and DLBS1425, bioactive fractions of *Phaleria macrocarpa* fruit, which showed potential treatments of endometriosis and breast cancer, respectively [6-7].

Phaleria macrocarpa, locally known as Mahkota Dewa, is one of the plants originating from Indonesia that has been discovered for its medicinal effects. The complete tree of *P. macrocarpa* consists of fruit, leaves, flower and stem. Its ripe fruit shows a red skin and eclipse-shaped fruit with a diameter of 3 cm. Seeds are exist in the fruit, about 1 to 2 seeds per fruit. The seeds are brown, ovoid and anatropous. The leaves show green color with a length of 7-10 cm and a width of 3-5 cm [8]. The major chemical constituents of *P. macrocarpa* fruit are flavonoids, with the presence of alkaloids, saponins, tannins and terpenoids in much less amounts [9]. From the fruits of *P. macrocarpa*, various components have been isolated, including icariside C3, phalerin and mangiferin. The components also exhibited pharmacological activities [8, 10].

Several parts of *P. macrocarpa* tree have been known empirically for their medicinal properties. Various researchers have also scientifically revealed the medicinal properties of *P. macrocarpa* and its several chemical constituents, including anti-hyperglycemic, anti-hyperlipidemic, atheroprotective, vasorelaxant, anti-cancer, anti-inflammatory and nephroprotective properties [6,8-11]. It also previously reported that *P. macrocarpa* extract has an antioxidant property [8]. Based on the results of previous studies that were focused on the effects of *P. macrocarpa* on managing variety of chronic diseases, this article aims to review the effects of *P. macrocarpa* on lowering blood glucose levels and cholesterol levels, as well as interfering other related supporting parameters.

Effect of *Phaleria macrocarpa* in the management of hyperglycemia

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Several pathogenic processes are ranging from autoimmune destruction of β-cells of pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action [12]. Insulin deficiency and insulin resistance will result in hyperglycemia due to the absence or abnormality of insulin action to uptake excessive glucose in the body [13]. Chronic hyperglycemia in diabetes will further lead to long-term damage, dysfunction and failure of different organs, including eyes, kidneys, nerves, heart and blood vessels [12]. Various interventions have been done to prevent and control hyperglycemia that attempt to decrease the likelihood of tissues harmed by hyperglycemia [14].

P. macrocarpa is one of medicinal plants that have been investigated for its effect to manage hyperglycemia. Various *in vivo* studies have been done by researchers to investigate the hypoglycemic effect of *P. macrocarpa* seeds, leaves, fruits pericarp and whole fruits. In particular, *P. macrocarpa* was able to reduce blood glucose and plasma insulin concentration in rats. It also improved glucose tolerance in intraperitoneal glucose tolerance test. The studies have been done in both normal and diabetic-induced animals [15-16].

Several researchers have performed various studies related to the hypoglycemic effect of *P. macrocarpa*. Study of various extracts of *P. macrocarpa* fruits pericarp was done to evaluate the hypoglycemic and anti-hyperglycemia effect. The results showed that treatment of methanolic extract of *P. macrocarpa* fruits pericarp at a dose of 1 g/kg for 12 days was able to significantly lower blood glucose on streptozotocin-induced diabetic rats by 56.25% and 58.33% compared to diabetic control group and pre-treatment value, respectively ($p<0.05$). It also lowered plasma insulin concentration by 75% and 50% compared to diabetic control group and pre-treatment value. It showed that the lowering effect of plasma insulin concentration of methanolic extract of *P. macrocarpa* fruits pericarp was higher than insulin-treated group [15]. The methanolic extract of *P. macrocarpa* fruits pericarp was further fractionated and investigated for its antidiabetic activity. Results showed that n-butanol fraction of *P. macrocarpa* methanolic extract treatment for 12 days was able to decrease blood glucose levels by 66.67% compared to control group ($p<0.05$) and it was comparable to metformin and glibenclamide treatment group. Study using n-butanol subfraction also revealed a significant reduction of blood glucose level on glucose tolerance test for 2 hours and repeated treatment for 12 days [16].

In vitro studies were done to investigate the proposed mechanism of action of *P. macrocarpa* to reduce blood glucose level. Previous studies have revealed its significant effect to reduce the activity of α-glucosidase, a key intestinal enzyme involved in the digestion of carbohydrate. Inhibition of α-glucosidase activity will result in delayed carbohydrate digestion, prolonged overall carbohydrate digestion time, thus it will reduce the rate of glucose absorption [17-19]. Results of α-glucosidase inhibitory activity evaluation showed that n-butanol extract of *P. macrocarpa* fruits pericarp exhibited highest α-glucosidase inhibition by 71.11%. Blood glucose level of various extracts of *P. macrocarpa* treatment group also showed no significant differences compared to acarbose treatment group on oral glucose tolerance test for 3 hours [20]. Acarbose was used as positive control in the previous studies, since acarbose acted as an α-glucosidase inhibitor that inhibits the digestion and absorption of carbohydrates in small intestine. Through this mechanism of action, acarbose has the ability to inhibit digestion and absorption of carbohydrate in small intestine, thus reducing the increase in blood glucose concentration after carbohydrate load [21].

Other *in vitro* study in BRIN-BD11 cell culture, a glucose-responsive clonal insulin-secreting cell line, showed α-glucosidase inhibition activity of ethanol extract of *P. macrocarpa* fruit at a concentration of 1.5% by 29.22%. The extract in various concentrations also increased insulin secretion *in vitro* by 1.3 to 2 folds compared to control in the presence of high glucose [19]. In addition to *P. macrocarpa* fruits and fruits pericarp, *P. macrocarpa* leaf was also investigated on *in vitro* intestinal glucose absorption activity by everted intestinal sac technique. Subfraction of n-butanol extract of *P. macrocarpa* leaf showed the highest significant α-glucosidase inhibition activity by 86.30% on intestinal glucose transport compared to control ($p<0.05$). Glucose absorption level (mg/g tissue weight) of subfraction of n-butanol extract of *P. macrocarpa* treatment group on everted intestinal sac examination showed a significantly low concentration of glucose absorbed compared to control group ($p<0.01$) and it was lower than acarbose treatment group [16]. Everted intestinal sac technique is an *in vitro* method that is commonly used to evaluate the absorption of a substance by transporter. In the previous study, this method was used in the measurement of fluid and glucose transport [22-23].

In addition to its potency to lower blood glucose and plasma insulin concentration, *P. macrocarpa* extracts also exhibited a protective effect on pancreatic islets. *P. macrocarpa* leaf extract at a dose of 500 mg/kg was given to streptozotocin-induced diabetic rats for 14 days and histology examination was done in the end of the study. On histology examination, it was showed that pancreatic islets of diabetic rats treated with *P. macrocarpa* extract revealed better restoration of the size of Langerhans islets and increased number of islet cells along with β-cells repair, which was statistically significant compared to diabetic control ($p<0.05$). Significant improvement was found in diabetic rats treated with *P. macrocarpa* extract, which recovered the histology and morphology appearances closer to non-diabetic group [24].

P. macrocapa extract was also studied for its effect to insulin sensitivity. Result of hyperinsulinemic-euglycemic clamp technique in rats showed that ethyl acetate fraction of *P. macrocarpa* extract at a dose of 143 mg/kg has the activity to increase insulin sensitivity in hyperglycemic mice. Ethyl acetate fraction of *P. macrocarpa* extract exhibited highest percentage of increased insulin sensitivity by 18.3%, which was not significantly different to metformin group. Increased insulin sensitivity was shown by low insulin necessity to control normoglycemic condition [25]. The hyperinsulinemic-euglycemic clamp technique is commonly used to measure insulin action on glucose utilization [26].

Effect of *Phaleria macrocarpa* in the management of dyslipidemia

Dyslipidemia are disorders of lipoprotein metabolism, including lipoprotein overproduction or deficiency. These disorders may be manifested by elevation of the serum total cholesterol, low-density lipoprotein (LDL) and triglyceride, and a decreased high-density lipoprotein (HDL) [27-28]. Dyslipidemia has become public health concern due to the well-established association between lipid concentrations and cardiovascular risk factors. In addition to lifestyle interventions, medicinal products including pharmaceutical and complementary products are the most common products that have been used to control dyslipidemia [29].

Several researchers have performed *in vivo* studies to evaluate the effect of *P. macrocarpa* in the prevention and management of dyslipidemia. The parts of *P. macrocarpa* tree that have been studied for the effect on dyslipidemia including fruits and leaves. *P. macrocarpa* was able to reduce cholesterol levels and its components, which consist of total cholesterol, LDL cholesterol and triglycerides, and increase HDL cholesterol level. It also found to regulate the levels of various proteins and receptors that are related to cholesterol homeostasis.

Study of hypolipidemic effect of *P. macrocarpa* fruit infusion was done in hypercholesterolemia-induced male rats (*Rattus norvegicus*). *P. macrocarpa* fruit infusion was administered for 56 days together with high-fat diet. The results showed that *P. macrocarpa* fruit infusion treatment at doses equivalent to 194 and 388 mg were able to significantly lower the percentages of cholesterol levels increment compared to control group ($p<0.05$). This study concluded that *P. macrocarpa* might prevent the increase of cholesterol levels which was caused by high-fat diet [30]. Aqueous extract of *P. macrocarpa* fruit was also studied for its effect on various blood lipid parameters. Treatment of *P. macrocarpa* at doses of 20 and 30 mg/kg body weight in hypercholesterolemia-induced Sprague-Dawley rats for 84 days showed significant reductions of total cholesterol, LDL cholesterol and triglyceride levels ($p<0.05$) by 36% and 35%, 38% and 24%, 66% and 71%, respectively [31]. Other study was also found to affect blood lipid parameters in rabbits. LDL cholesterol was decreased by 6.8% and HDL cholesterol was increased by 15.9% compared to baseline level after treatment of *P. macrocarpa* extract for 4 weeks at a dose of 35 mg/1.5 kg body weight. There was an increased level of triglyceride by 3.5% in treatment group. However, increased triglyceride level in treatment group was lower than that in control group [32].

In addition to *P. macrocarpa* fruit extract, extract of *P. macrocarpa* leaves was also investigated for its activity against blood lipid parameters. Treatment of *P. macrocarpa* at a dose of 0.5 mg/kg body weight in

hypercholesterolemia-induced Sprague-Dawley rats for 14 days showed a significant reduction on total cholesterol level by 224.9% compared to cholesterol group ($p<0.05$). In this study, HDL cholesterol level was also increased significantly by 239% compared to cholesterol group ($p<0.05$) [33].

In vivo studies were also done to evaluate the activity of cholesterol-related proteins. Treatment of *P. macrocarpa* fruit extract at a dose of 20 mg/kg showed a significant increase in the levels of hepatic LDL receptor (LDL-R) (160 kDa and 120 kDa) and proprotein convertase subtilisin kexin type 9 (PCSK9) by 115%, 97% and 33%, respectively ($p<0.05$) [31]. LDL-R and PCSK9 are proteins that play important roles in regulating cholesterol homeostasis. LDL-R is a cell membrane protein essential for the uptake of LDL cholesterol and the regulation of plasma lipoprotein levels. Increased LDL-R levels would increase the binding of LDL particles to LDL-R followed by internalization into the peripheral cells via receptor-mediated endocytosis, thus it is closely related to a decreased number of circulating LDL particles [31,34]. PCSK9 is a protease that regulates the degradation of LDL-R. The binding of PCSK9 and LDL-R initiates the receptor-mediated endocytosis pathway, then the complex is routed to lysosomes and degraded, regulating cell surface of LDL-R [31].

Results of previously explained *in vivo* studies were also supported by several *in vitro* studies on cholesterol-related genes. *In vitro* study was done to evaluate LDL-R and PCSK9 protein levels and mRNA expression using HepG2 cells. Western blot analysis showed that LDL-R level in HepG2 cells was significantly increased by the presence of *P. macrocarpa* ($p<0.05$) and maintained LDL-R level similar to control group. PCSK9 levels on HepG2 cells were also increased by the presence of *P. macrocarpa* [31]. The effect of *P. macrocarpa* extract treatment on scavenger receptor class B type I (SR-BI) *in vitro* was evaluated by other researcher. Treatment of *P. macrocarpa* leaves extract on HepG2 cells exhibited higher expression of SR-BI gene and produced higher transcriptional activity of SR-BI promoter compared to untreated samples. *P. macrocarpa* extract at a concentration of 12.5 μg/ml produced the highest increase of SR-BI promoter activity up to 95% of rosiglitazone sample as the positive control at a concentration of 12.5 μg/ml [33]. SR-BI is a protein that plays an important role in mediating the selective uptake of HDL-derived cholesterol and cholesteryl ester (CE) in the liver and steroidogenic tissues. It was well established that HDL plays an important role in reverse cholesterol transport (RCT) by removing plasma CE as well as accumulated CE along the lining of blood vessels to the liver, thus, increased HDL cholesterol levels has beneficial effects to reduce the risk of atherosclerosis [33,35].

Other cholesterol-related genes and proteins that have been investigated by other researchers including cholesteryl ester transfer protein (CETP). *In vitro* CETP gene expression measurement was done in mRNA and protein levels. *P. macrocarpa* treatment was given to HepG2 cells in various concentrations and it was then analyzed using CETP inhibitor drug screening kit. Investigation using PCR showed that *P. macrocarpa* treatment was able to decrease CETP gene expression at mRNA level in a dose-dependent manner. These results were also supported by decreased CETP gene expression at protein level that was analyzed using Western Blot technique. Western Blot analysis results showed that *P. macrocarpa* treatment was able to decrease CETP protein excreted by HepG2 cells to the medium. The results at protein level were in accordance to the analysis results at mRNA level, which showed a reduced CETP expression. It was also in accordance to the result of *in vivo* study, which also exhibited a decreased activity of CETP by 0.3% [32]. CETP is a plasma protein that mediates the exchange of CE in HDL to triglycerides in proatherogenic apolipoprotein B-containing lipoproteins, including VLDL, VLDL remnants, IDL and LDL particles. Decreased CETP activity is closely associated to increased HDL levels and decreased LDL levels, a profile which is known for its antiatherogenic effect [36].

Evaluation was also done on various genes which are involved in cholesterol transport process, including liver X receptor alpha (LXR-α) and sterol regulatory element binding protein-1 (SREBP-1). *P. macrocarpa* treatment on HepG2 cells caused a decreased expression of both genes [36]. LXR-α is a nuclear hormone receptor which induces CETP transcription and maintain the reverse cholesterol transport process. It regulates cholesterol and fatty acid metabolism in liver tissues and macrophages. In the liver, LXR-α mediates cholesterol metabolism by inducing SREBP-1 gene expression. Therefore, decreased LXR-α gene expression will also decrease SREBP-1 gene expression [36-37]. SREBP-1 also plays an important role as lipid synthetic transcription factors especially for cholesterol and fatty acid synthesis. It is a transcription factor which regulates cholesterol synthesis by activating other genes, including CETP. Decreased SREBP-1 gene expression will further decrease CETP gene expression that leads to decreased CETP activity, thus reduced LDL levels and increased HDL levels [36, 38].

In addition to its effect on blood cholesterol levels and its components and cholesterol-related proteins and genes, the constituent of *P. macrocarpa*, icariside C3, was also exhibited vasorelaxant activity on previous study. The study was done *ex vivo* on isolated aorta of male Wistar rat that was given noradrenaline to induce vasoconstriction before treatment. Icariside C3 exhibited a moderate vasorelaxant activity against noradrenaline-induced vasoconstriction on isolated aorta of male Wistar rat [10].

Nephroprotective effect of *Phaleria macrocarpa*

Study has been done to investigate the nephroprotective effect of *P. macrocarpa.* Study was done on alloxan-induced diabetic rats. The diabetic milieu was resulted in the development of severe hyperglycemia and reduced glomerular filtration rate similar to diabetic nephropathy. It further resulted in the increase of vascular endothelial growth factor (VEGF) and transforming growth factor-α (TGF-α), which play important roles in the pathogenesis of diabetic nephropathy. Rats treated with methanolic and aqueous extracts of *P. macrocarpa* fruit showed significant reductions of glomerular VEGF expression compared to metformin-treated group ($p<0.01$), and there were no significant differences between methanolic and aqueous extract of *P. macrocarpa* fruit treatment. VEGF is a signal protein which acts as the main regulator of blood vessel growth and plays an important role in promoting endothelial survival and maintaining the microvasculature. Decreased VEGF will be resulted in decreased proliferation of endothelial cells and also decreased apoptosis, thus it inhibits the loss of renal cells [11,39,40]. The study also showed a significant reduction of glomerular TGF-α expression in rats treated with methanolic extract of *P. macrocarpa* fruit compared to metformin-treated group ($p<0.01$). Lowest level of TGF-α expression is shown by treatment group of methanolic extract of *P. macrocarpa* fruit, as well as normal rats. TGF-α is a gene that encodes a growth factor which is a ligand for the epidermal growth factor receptor, which activates a signaling pathway for cell proliferation, differentiation and development. Decreased TGF-α expression will inhibit cell proliferation and it further inhibits the progression of diabetic nephropathy [11, 41].

CONCLUSION

Phaleria macrocarpa has been proved to be a promising agent to manage chronic diseases, especially those related to hyperglycemia, dyslipidemia and renal disorders. It helps to lower and maintain blood glucose levels through inhibiton of α-glucosidase. *P. macrocarpa* also helps to improve and maintain blood lipid profile through regulation of protein and gene expression related to cholesterol transport and homeostasis. Nephroprotective effect of *P. macrocarpa* was revealed by reduction in gene expressions that regulate angiogenic and cells proliferation. Effects of *P. macrocarpa* through various parameters and pathways make it highly favorable to be developed as the treatment of cardiovascular and metabolic diseases risk factors, especially hyperglycemia, dyslipidemia, as well as protecting the kidneys. However, further study, especially clinical study needs to be done further to ascertain its efficacy and safety in the management of cardiovascular and metabolic diseases.

REFERENCES

[1] IFF Benzie, S Wachtel-Galor. Herbal medicine: Biomolecular and clinical aspects, 2nd Edition, CRC PRess/Taylor & Francis, Boca Raton, **2011**.
[2] OM Tandrasasmita; DD Wulan; F Nailufar; J Sinambela; RR Tjandrawinata. *Int. J. Gen. Med.*, 2011, 4, 345-357.
[3] F Nailufar; OM Tandrasasmita; RR Tjandrawinata. *Biomed. Prev. Nutr.*, **2011**, 1(2), 71-78.
[4] AH Karsono; OM Tandrasasmita, RR Tjandrawinata. *Cancer Manag. Res.*, **2014**, 6, 267-278.
[5] J Trisina; F Sunardi; MT Suhartono; RR Tjandrawinata. *J. Biomed. Biotechnol.*, **2011**, 519652.
[6] OM Tandrasasmita; AM Sutanto; PF Arifin; RR Tjandrawinata. *Int. J. Womens Health*, **2015**, 7, 161-169.
[7] RR Tjandrawinata; D Nofiarny; LW Susanto; P Hendri; A Clarissa. *Int. J. Gen. Med.*, **2011**, 4, 465-476.
[8] MZB Asmawi; MI Umar. *Pharmacogn. Rev.*, 2013, 7(13), 73-80.
[9] MM Lay; SA Karsani; S Mohajerand; SNA Malek. *BMC Complement. Altern. Med.*, **2014**, 14, 152.
[10] S Oshimi; K Zaima; Y Matsuno; Y Hirasawa; T Iizuka; H Studiawan, *et al*. *J. Nat. Med.*, **2008**, 62, 207-210.
[11] E Sulistyoningrum; Setiawati. *Universa Medicina*, **2013**, 32(2), 71-79.
[12] American Diabetes Association. *Diab. Care*, **2011**, 34, S62-S69.
[13] S Riddick-Grisham, LM Deming (ed). Pediatric life care planning and case management, 2nd edition, CRC Press Taylor & Francis Group, Boca Raton, **2011**; 504.
[14] MJ Fowler. *Clin. Diabetes*, **2008**, 26(2), 77-82.
[15] RB Ali; IJ Atangwho; N Kuar; EAH Mohamed; AJ Mohamed; MZ Asmawi; R Mahmud. *J. Med. Plants Res.*, **2012**, 6(10), 1982-1990.
[16] RB Ali; IJ Atangwho; N Kaur; OS Abraika; M Ahmad; R Mahmud; MZ Asmawi. *Molecules*, **2012**, 17(1), 4986-5002.
[17] AJ Scheen. *Drugs*, **2003**, 63(10), 933-951.
[18] H Bischoff. *Eur. J. Clin. Invest.*, **1994**, 24(3), 3-10.
[19] IH Suparto; N Arfianti; T Septiawati; W Triwahyuni; D Iskandriati. *Proceeding of The International Seminar on Chemistry*, **2008**, 285-288.
[20] S Sugiwati; LBS Kardono; M Bintang. *J. Appl. Sci.*, **2006**, 6(10), 2312-2316.
[21] JM Chen; CW Chang; YC Lin; JT Horng; WHH Sheu. *J. Diabetes Res.*, **2014**, 812628.
[22] Z Liu; K Liu. *Asian J. Pharm. Sci.*, **2013**, 8, 151-158.
[23] CHM Versantvoort; CJM Rompelberg, AJAM Sips. *National Institute of Public, Research for Man and*

Environtment, Report 630030001.
[24] ND Salih; N Azmi; HK Gopalan. *Science International*, **2015**, 27(5), 4219-4224.
[25] A Muhtadi; Y Susilawati; AD Zakaria. *Proceeding of The International Seminar on Chemistry*, **2008**, 527-530.
[26] JK Kim. Type 2 Diabetes, Volume 560 of the series Methods of Molecular Biology. Humana Press, New York.
[27] SM Ahmed; ME Clasen; JF Donnelly. *Am. Fam. Phys.*, **1998**, 57(9), 2192-2204.
[28] K Musunuru. *Lipids*, **2010**, 45(10), 907-914.
[29] RH Nelson. *Primary Care*, **2013**, 40(1), 195-211.
[30] Julizar; L Irawati; E Rustam. *Majalah Kedokteran Andalas*, **2012**, 36(1), 51-61.
[31] SC Chong; MA Dollah; PP Chong; A Maha. *J. Ethnopharmacol.*, **2011**, 137, 817-827.
[32] DAS Suciptan; A Aripin; RR Tjandrawinata. US Patent 2014/0099392 A1, April 10, 2014.
[33] Y Andriani; TS Tengku-Muhammad; H Mohamad; J Saidin; DF Syamsumir, *et al*. *Molecules*, **2015**, 20, 4410-4429.
[34] L Zhang; L Fairall; BT Gould; AC Calkin; C Hong; CJ Millard, *et al*. *Genes Dev.*, **2011**, 25, 1262-1274.
[35] G Valacchi; C Sticozzi; Y Lim; A Pecorelli. *Ann. N Y Acad. Sci.*, **2011**, 1229, E1-E7.
[36] PJ Barter; HB Brewer Jr; MJ Chapman; CH Hennekens; DJ Rader; AR Tall. *Arterioscler. Thromb. Vasc. Biol.*, **2003**.
[37] JB Seo; H Moon; WS Kim; YS Lee; HW Jeong; EJ Yoo; *et al*. *Mol. Cell. Biol.*, **2004**, 24(8), 3430-3444.
[38] H Shimano. *Prog. Lipid Res.*, **2001**, 40(6), 439-452.
[39] T Nakagawa; T Kosugi; DA Long. *Diabetes*, **2009**, 58: 1471-1478.
[40] AB Sanz; B Santamaria; M Ruiz-Ortega; J Egido; A Ortiz. *J. Am. Soc. Nephrol.*, **2008**, 19, 1634-1642.
[41] National Center for Biotechnology Information. TGFA: Transforming growth factor alpha [Homo sapiens (human)]. http://www.ncbi.nlm.nih.gov/gene/7039,