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Anti-atherosclerosis potency of *Pandanus tectorius* fruit rich by trangeretin and ethyl trans-caffeate, and their cytotoxicity against HepG2 cell line

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Abstract. Hypercholesterolemia can be a major contributing agent to the development of atherosclerosis. Atherosclerosis can provoke to heart attack, is a primary reason of death worldwide and current drug statins is not without adverse side effects. This warrants for alternative remedial candidates from natural resources. Natural products from plant play an important role in production of new potential drugs to the pharmaceutical market. *Pandanus tectorius* (Pandanaceae) is one of the most popular herbs and locally grows in Malaysia and Indonesia is commonly known as 'pandan laut. It has been used traditionally to cure certain diseases. The aimed of study is to find out the potency of fruit rich by tangeretin and ethyl trans-caffeate from *P. tectorius* for atherosclerosis prevention through inhibiting of the HMG-CoA reductase activity. Since the cholesterol mechanism occurs in the liver, the cytotoxicity activity of all extracts and compounds were necessary analysed. The sample was extracted successively using hexane, ethyl acetate and methanol. The compounds profiling was done by thin layer chromatography (TLC). The HMG-CoA reductase kit for anti-atherosclerosis assay (pravastatin as a controlled drug) was purchased from Sigma Aldrich. The cytotoxicity activity was observed by MTT assay. The both data were read by ELISA reader at wavelength of 340 nm and 570 nm, respectively. Result revealed that all extracts have proven to inhibit the activity of HMG-CoA reductase with the highest activity was obtained by PMK ((9 times higher than pravastatin). All samples also showed no cytotoxic activity against HepG2 cell line since they have IC_{50} value more than 30 µg/mL. This Study found that *P. tectorius* fruit rich by tangeretin and ethyl trans-caffeate produces a new candidate for treatment of atherosclerosis.
Keywords: *Pandanus tectorius*, Atherosclerosis, HMG-CoA reductase, cytotoxicity, HepG2

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1. Introduction

Atherosclerosis is one of cardiovascular diseases which have been the leading cause of death in developed countries such as the USA, Europe and Asia [1]. Atherosclerosis is characterized by a gradual thickening and hardening of the arteries that finally cause plaque build-up [2]. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. Among several well-known risk factors, dyslipidaemia is a main contributing factor in the progressing of atherosclerosis. Type of dyslipidaemia which was to be the cause of atherosclerosis is hypercholesterolemia of hyperlipidemia.

Current drugs to prevent atherosclerosis caused by cholesterol abnormality have used 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors popular as statins. Statins are medicines that are commonly used to lower blood cholesterol under permission of a medical doctor. The drugs are able to inhibit the activity of HMG-CoA reductase which catalysing the cholesterol synthesis in the liver. Statin is able to increase high-density lipoprotein (HDL) cholesterol, lower total and low-density lipoprotein (LDL) cholesterol levels, and they have been proven to reduce the risk of coronary heart disease (CHD) such as atherosclerosis [3]. These statin drug groups are obtainable and are effective but they are associated with several adverse side effects such as nausea, diarrhea, myositis and abnormal liver function which severely hindrance their application [4]. Some patients are resistant to or are intolerant of conventional pharmacotherapy. Therefore, alternative approaches with plant-based therapies are eagerly needed, as they are efficacious in reducing lipid levels with negligible or no side effects if they are used in appropriate doses. One of mangrove coastal plants from the Pandanaceae family, *P. tectorius*, consist of nearly 700 species and many of these are important economic crops and are used as folk medicine in curing of numerous diseases. In South China, the native people consume the fruit of *P. tectorius* as a common remedy for the treatment of hyperlipidemia. Some studies reported that the *P. tectorius* fruit extract was rich by caffeoylquinic acids [5, 6] and β-carotene [7-9]. Fruit extract of *P. tectorius* rich by caffeoylquinic acid was reported increases insulin sensitivity and controls hepatic glucose and lipid metabolism in diabetic db/db mice was studied by Wu *et al.* [10]. The extract of roots and stem of *P. tectorius* showed numerous therapeutic effects in curing hepatitis, nephritis, influenza, and urinary tract infection [11]. Isolated compounds of *P. tectorius* with some biological activities were reported as anti-inflammatory [12], antioxidant [13], anti-tumor [14], antiviral [15], cholesterol-lowering activity [16, 17], hypolipidemia [5, 6]. Another phenolics and flavonoids compounds were also already isolated from *P. tectorius* Soland fruit, such vanillin, ethyl-trans caffeate, tangeretin, chrysin, naringenin [18], might contribute to the hypolipidemia effect. The fruits were believed to reduce heart disease, and reduce cholesterol level. Most of the claims are based on traditional practitioner's knowledge and still lack of scientific evidence. There is a need to investigate the potential of the fruits as the source of compounds and new medicinal property i.e. anti- atherosclerosis. Many researchers were concentrate to study on *P. tectorius* leaves, while the researches on its fruit are still limited as well as tangeretin and ethyl trans-caffeate as antiatherosclerosis potential agent via HMG-CoA reductase. Thus, the aims of this research were to investigate the anti-atherosclerosis potency of *P. tectorius* fruit rich by tangeretin and ethyl trans-caffeate, and their cytotoxicity against HepG2 cell line. Cytotoxicity study was needed to be observing since we need to ensure that our samples were not had cytotoxic activity. The activity was also compared to the two commercial compounds, tangeretin and transethyl caffeate which purchased from Sigma Aldrich.

2. Methodology

2.1. Sampling site, Sample preparation and Extraction

Fruit of *P. tectorius* was collected at Teluk Ketapang beach, Kuala Nerus, Terengganu, Malaysia from January to March 2018. The keys of fruits (mesocarb part, excluding seeds) were cut into small pieces and stored for 1-2 days at -80°C. Furthermore, samples were dried using freeze dryer for 2-3 days and, then grinded to be a fine powder. The dry powder of *P. tectorius* fruits was extracted by maceration method using hexane, ethyl acetate and methanol successively. All solvents were of analytical grade and purchased from Sigma Aldrich. These three different solvents were used to extract the chemicals

constituent's content in the sample based on their polarity. Hexane was used to extract non-polar compounds. While, ethyl acetate and methanol were used to extract semi-polar and polar compounds, respectively. Solvents were dried using rotary evaporator to yield the concentrated extracts. All extracts were kept in fridge and labelled as hexane extract (PHK), ethyl acetate extract (PEK), and methanol extract (PMK) to use for further assay. TLC profiling was done on PMK extract and observed under UV_{254} as two bands which have same Rf value with tangeretin and trans ethyl caffeate were only appeared in this extract.

2.2. In Vitro Study: The potency of P. tectorius fruit as an anti-atherosclerosis via HMG-CoA reductase

2.2.1. Citotoxicity Study by MTT Assay. Determination of the cell viability was adopted from Andriani *et al.* [19], by using the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) assay. HepG2 cells at a density of 2.5×10^5 cells/well were seeded on 96-well plates. Samples were prepared in various concentrations by serial dilution in minimum essential medium (MEM) by 60, 30, 15, 7.5, 3.75, 1.875, 0.938 and 0.469 µg/mL on the 96 wells plate. The samples were put into each well and incubate for 72 h at 37°C, 5% CO_2. Then, 20 µL fresh MTT solution was added to each well and incubated for four hours at 37°C, 5% CO_2. After that, medium (170 µL) will be reduced from each well and added DMSO (100 µL) by pipetting 10–20 times. Before reading by enzyme linked immunosorbent assay (ELISA) reader at 570 nm, the plate was left for 30 minutes. Turn on the ELISA reader at least 15-20 minutes before used it. Then, the wavelength was set at 570 nm and samples were put into the ELISA reader to get the absorbance data. Cell viability was calculated as follow:

(Absorbance sample/Absorbance blank) X 100

2.2.2. Inhibitory activity of P. tectorius Fruit on the HMG-CoA reductase enzyme. Determination of the Effect of P. tectorius Fruit on the HMG-CoA reductase activity was analysed by Assay Kit from sigma Aldrich. This kit contains all reagents to detect the HMG-CoA reductase activity. Extracts as well as the compounds were determined their activity in inhibiting of HMG-CoA reductase activity.

3. Results and Discussion

3.1. In Vitro Study: The potency of P. tectorius fruit as an anti-atherosclerosis via HMG-CoA

3.1.1. Cytotoxicity Activity. Fig. 1 shows that all samples, namely PHK, PEK and PMK were showed no cytotoxic activity against HepG2 cell line since the Graph shows they no IC_{50} value at less than 30 µg/mL of concentration. According to Andriani *et al.* [20], samples which have IC_{50} value higher than 30 µg/mL was considered not cytotoxic activity against the tested cells and could proceed to the further bioassay study.

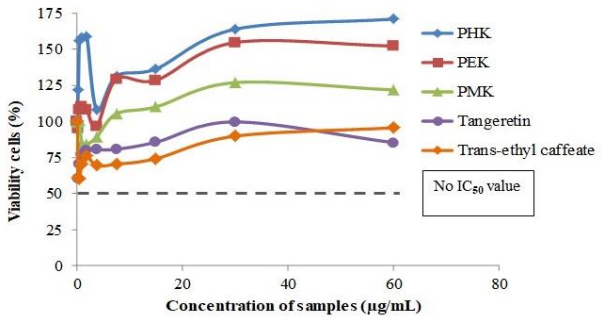

Figure 1. Cytotoxicity property of *P. tectorius* fruits extracts (PHK, PEK, and PMK), Tangeretin and trans-ethyl caffeate against HepG2 cells.

3.1.2. Inhibitory activity of P. tectorius fruit on the HMG-CoA reductase enzyme. Result on Fig. 2 Shows that PHK (a), PEK (b) and PMK (c) of *P. tectorius* fruit inhibited HMG-CoA reductase at all tested concentrations (400, 200, and 100 µg/mL) compare to the Pravastatin as a control drug. From 2nd to 4th minute, it shows the value of HMGR inhibition activity which was conducted by PHK, PEK and PMK at all concentrations. The PEK was revealed good activity at concentration of 400 µg/mL and 3th minute incubation, however its activity is still lower than Pravastatin. For PEK, it is shows the inhibitory activity quite the same with Pravastatin at concentration of 100 µg/mL and incubation time of 3th minute. While, PMK shows very good inhibition activity against HMG-CoA reductase compare to the PHK and PEK start from 2nd to 4th minute of incubation times, The PMK shows highest inhibition activity (9 times higher than Pravastatin as control drug) at concentration of 100 µg/mL and 2nd minute incubation times. High activity at the 100 µg/mL compare to high concentrations could be associated to the antagonistic effect among the compounds content in the samples if they used in the high concentration. However, further study could be needed to confirm this effect on HMG-CoA reductase activity by using of more concentration variations of the sample (less than 100 µg/mL and or more than 400 µg/mL).

We can see on Fig. 2 and table 1 that PMK at 100 µg/mL has highest ability in inhibiting of HMG-CoA reductase via in vitro assay (nine times higher than Pravastatin). This activity could be related to the compounds content in the PMK. Phytochemicals study on the methanol extract of *P. tectorius* fruit was reported by Andriani *et al.* [19]. They have revealed that this extract was rich by phenolics and flavonoids compounds. According to Zhang *et al.* [18], fruits of *P. tectorius* rich by Tangeretin and transethylcafeate and reported that both compound have potency as anti-inflammatory agents. It is known that atherosclerosis is a multifactorial disease triggered by inflammatory [21]. It's mean that compounds which have potency as an anti-inflammatory activity could be have antiatherosclerosis activity, also. Thus, tangeretin and transethylcafeate compounds in our current study could have contribution on regulating of atherosclerosis prevention.

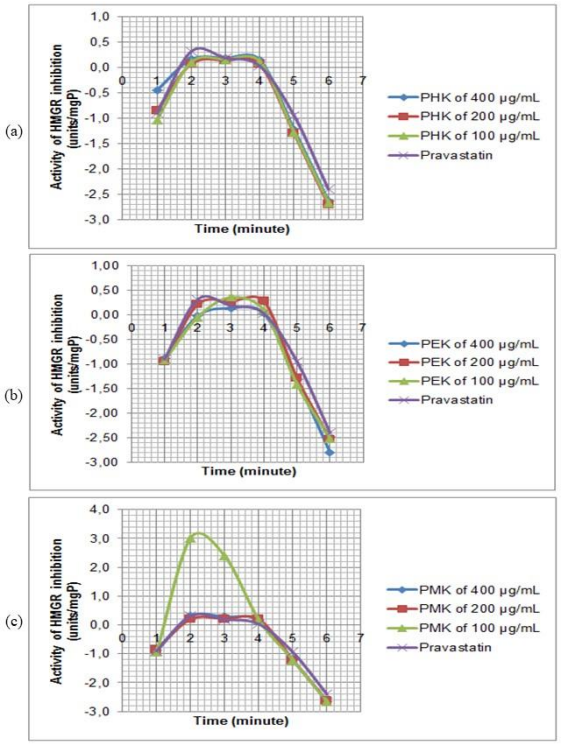

Figure 2. Activity of PHK (a), PEK (b), and PMK extracts of *P. tectorius* fruit in inhibiting of HMG-CoA reductase at 400, 200, 100 µg/mL compare to Pravastatin.

This study was proven that PMK which rich by tangeretin and trans-ethyl caffeate was showed very good activity on inhibiting of the HMGCoA reductase activity at low concentration, 100 µg/mL. The TLC profiling (Fig. 3) was proven that from 3 compounds tested (tangeretin, trans-ethyl caffeate and caffeic guinic acid), only tangeretin and transethyl caffeate were present in PMK. Thus, both compounds were could have responsibility on inhibiting of HMG-CoA reductase activity.

Table 1. The ability of *P. tectorius* Fruit extracts, tangeretin, and trans-ethyl caffeate in inhibiting of HMG-CoA reductase activity compared to Pravastatin.

Incubation Time	Sample	Inhibiting ability
the 2nd minute	Pravastatin	standard drug as a comparison
the 3th minute	Hexane crude extract (PHK) at 400 µg/mL	lower than Pravastatin, but still have activity
the 3th minute	Ethyl Acetate crude extract (PEK) at 100 µg/mL	(same as Pravastatin)
the 2nd minute	Methanol crude extract (PMK) at 100 µg/mL	9 x higher than Pravastatin

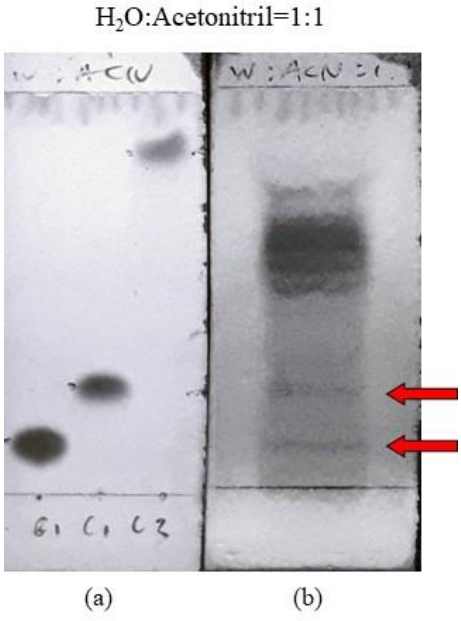

Figure 3. TLC Profile of tangeretin C1, trans-ethyl caffeate C2, and caffeic guinic acid C3 (a) compare to the PMK extract of *P. tectorius* fruits (b).

4. Conclusion

This Study found that *P. tectorius* fruit rich by tangeretin and ethyl trans-caffeate provides a new potential activity for treatment and prevention of atherosclerosis. Further study on molecular level is needed to support the mechanism of action of *P. tectorius* fruit and its compound in preventing of atherosclerosis via inhibiting of HMGCoA reductase enzyme activity.

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References
[1] Fishbein G A and Fishbein M C 2009 Arteriosclerosis: rethinking the current classification *Arch. Pathol. Lab. Med.* **133** 8 1309-16
[2] Xu Y, Xu Y, Bao Y, Hong B and Si S 2011 Identification of dehydroxytrichostatin A as a novel up-regulator of the ATP-binding cassette transporter A1 (ABCA1) *Molecules* **16** 9 7183-98
[3] Rosenson R S 2004 Statins in atherosclerosis: lipid-lowering agents with antioxidant capabilities *Atherosclerosis* **173** 1 1-12
[4] Beltowski J, Wojcicka G and Jamroz-Wisniewska A 2009 Adverse effects of statins-mechanisms and consequences *Curr. Drug Saf.* **4** 3 209-28
[5] Zhang X, Wu C, Wu H, Sheng L, Su Y, Zhang X, Luan H, Sun G, Sun X and Tian Y 2013 Anti-hyperlipidemic effects and potential mechanisms of action of the caffeoylquinic acid-rich Pandanus tectorius fruit extract in hamsters fed a high fat-diet *PLoS One* **8** 4 e61922
[6] Liu H, Zhang X, Wu C, Wu H, Guo P and Xu X 2013 Anti-hyperlipidemic caffeoylquinic acids from the fruits of Pandanus tectorius Soland *J. Appl. Pharm. Sci.* **3** 8 16-9
[7] Englberger L, Aalbersberg W, Fitzgerald M H, Marks G C and Chand K 2003 Provitamin A carotenoid content of different cultivars of edible pandanus fruit *J. Food Compost. Anal.* **16** 2 237-47
[8] Englberger L, Aalbersberg W, Schierle J, Marks G C, Fitzgerald M H, Muller F, Jekkein A, Alfred J and Vander Velde N 2006 Carotenoid content of different edible pandanus fruit cultivars of the Republic of the Marshall Islands *J. Food Compost. Anal.* **19** 6-7 484-94
[9] Englberger L, Schierle J, Hofmann P, Lorens A, Albert K, Levendusky A, Paul Y, Lickaneth E, Elymore A and Maddison M 2009 Carotenoid and vitamin content of Micronesian atoll foods: Pandanus (Pandanus tectorius) and garlic pear (Crataeva speciosa) fruit *J. Food Compost. Anal.* **22** 1 1-8
[10] Wu C, Zhang X, Zhang X, Luan H, Sun G, Sun X, Wang X, Guo P and Xu X 2014 The caffeoylquinic acid-rich Pandanus tectorius fruit extract increases insulin sensitivity and regulates hepatic glucose and lipid metabolism in diabetic db/db mice *J. Nutr. Biochem.* **25** 4 412-9
[11] Peng L, Cheng J, Zhan R and Li J 2010 Research progress on the chemical constituents and biological activities of genus Pandanus *Chin. Med. Mater.* **33** 640-3
[12] Chiang Y M, Lo C P, Chen Y P, Wang S Y, Yang N S, Kuo Y H and Shyur L F 2005 Ethyl caffeate suppresses NF-κB activation and its downstream inflammatory mediators, iNOS, COX-2, and PGE2in vitro or in mouse skin *Br. J. Pharmacol.* **146** 3 352-63
[13] Burri J, Graf M, Lambelet P and Löliger J 1989 Vanillin: more than a flavouring agent—a potent antioxidant *J. Sci. Food Agric.* **48** 1 49-56
[14] Chaumontet C, Droumaguet C, Bex V, Heberden C, Gaillard-Sanchez I and Martel P 1997 Flavonoids (apigenin, tangeretin) counteract tumor promoter-induced inhibition of intercellular communication of rat liver epithelial cells *Cancer Lett.* **114** 1-2 207-10
[15] Nahmias Y, Goldwasser J, Casali M, van Poll D, Wakita T, Chung R T and Yarmush M L 2008 Apolipoprotein B–dependent hepatitis C virus secretion is inhibited by the grapefruit flavonoid naringenin *Hepatology* **47** 5 1437-45
[16] Lee S, Park Y, Bae K, Bok S, Kwon Y, Lee E and Choi M 1999 Cholesterol-lowering activity of naringenin via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyltransferase in rats *Ann. Nutr. Metab.* **43** 3 173-80
[17] Kurowska E M and Manthey J A 2004 Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia *J. Agric. Food Chem.* **52** 10 2879-86

[18] Zhang X, Guo P, Sun G, Chen S, Yang M, Fu N, Wu H and Xu X 2012 Phenolic compounds and flavonoids from the fruits of Pandanus tectorius Soland *J. Med. Plant Res.* **6** 13 2622-6
[19] Andriani Y, Ramli N M, Syamsumir D F, Kassim M N I, Jaafar J, Aziz N A, Marlina L, Musa N S and Mohamad H 2015 Phytochemical analysis, antioxidant, antibacterial and cytotoxicity properties of keys and cores part of Pandanus tectorius fruits *Arab. J. Chem.*
[20] Andriani Y, Effendy M, Sifzizul T and Habsah M 2011 Antibacterial, radical-scavenging activities and cytotoxicity properties of Phaleria macrocarpa (scheff.) Boerl. Leaves in hepg2 cell lines *Int. J. Pharm. Sci. Res.* **2** 7 1693
[21] Fava C and Montagnana M 2018 Atherosclerosis is an inflammatory disease which lacks a common anti-inflammatory therapy: how human genetics can help to this issue. A narrative review *Front. Pharmacol.* **9** 55 1-9