

INHIBITORY EFFECTS OF *Pandanus tectorius* FRUIT
EXTRACTS, TANGERETIN, AND TRANS-ETHYL
CAFFEATE ON HMG-CoA REDUCTASE

INTEN PANGESTIKA

MASTER OF SCIENCE
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**Thesis Submitted in Fulfilment of the Requirements for the Degree of
Master of Science in the Institute of Marine Biotechnology
Universiti Malaysia Terengganu**

Dedicated this thesis to:

My supervisor, Yosie Andriani HS, Ph.D.

My co-supervisor, Professor Tengku Sifzizul Bin Tengku Muhammad, Ph.D.

My co-supervisor, Jasnizat Saidin, Ph.D.

My beloved parents and siblings and lastly, R.A.O.

For all their dedication, sacrifice, and endless love.

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Master of Science

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AP 603, #92
Hydroxy methyl glutaryl

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Field of Study : Master of Science in Biochemistry

One of the pathways to reduce cholesterol production in the liver is through the inhibition of HMG-Coa reductase (HMGCR) enzyme generally using statins, but it has side effects when consumed for a prolonged period. Therefore, the current perspective study is to look for a natural agent of HMGCR inhibitor to lower cholesterol levels through the inhibitory mechanism of HMGCR involved in synthesizing cholesterol. Thus, the natural agent can be as an alternative drug in treating hypercholesterolemia and preventing atherosclerosis. The objectives of this study were to investigate the cytotoxicity effects on HepG2 cells and to determine inhibitory effects of *Pandanus tectorius* fruit extracts, tangeretin, and trans-ethyl caffeate on HMGCR. To evaluate the potential of tangeretin as an anti-hypercholesterolemic agent on hypercholesterolemia-induced rats was also the objective of this study. The cytotoxicity evaluation was conducted by MTT assay on HepG2 cells. Inhibitory effects were done using HMGCR *in vitro* kit (Sigma Aldrich) with pravastatin and simvastatin as controls. The hypercholesterolemic effects of tangeretin were investigated in an intervention study with rats. Four experimental groups (n = 8 *Sprague dawley* rats) were fed with a standard or a high cholesterol diet

provided to the normal and intervention groups, respectively. Tangeretin and simvastatin were used to treat the hypercholesterolemic rats for 14 days. The levels of total cholesterol, LDL, HDL and SGOT/SGPT activity of blood plasma were analyzed in this study. The decrease in HMGCR activity was estimated based on a HMGCR *in vivo* assay using the colourimetric method at 540 nm. Besides the SGOT/SGPT analysis, the toxicity study of tangeretin was also carried out by histological observation on the rat liver. The results showed that *P. tectorius* fruit extracts, tangeretin, and trans-ethyl caffeate had no cytotoxic effects against HepG2 cells. All tested concentrations of samples could lower the HMGCR activity compared to the control. The highest inhibition for tangeretin was obtained at a concentration of 400 µg/mL (83.8% inhibition), and its efficacy was almost as good as simvastatin and pravastatin. The levels of total cholesterol and LDL significantly lowered in the intervention group after treating with tangeretin while HDL significantly increased. Moreover, the HMG-CoA/mevalonate ratio on the rat liver showed that the enzyme activity by tangeretin is the lowest of all rat groups. The toxicity study on the SGOT and SGPT levels revealed that there were no significant side effects after treatment with tangeretin compared to the normal group. The histological observations displayed that the treatment of tangeretin reduced fatty degenerations in the liver cells. The results found that *P. tectorius* fruit extracts, tangeretin, and trans-ethyl caffeate are safe and suitable as an alternative control for reducing cholesterol levels via the inhibition of HMGCR activity.

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sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**KESAN RENCATAN EKSTRAK BUAH *Pandanus tectorius*,
TANGERETIN, DAN TRANS-ETIL KAFEAT KE ATAS ENZIM
HMG-CoA REDUKTASE**

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Salah satu cara untuk mengurangkan pengeluaran kolesterol dalam hati adalah melalui perencatan enzim 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGCR) secara amnya menggunakan statin tetapi ia mempunyai kesan sampingan apabila digunakan dalam tempoh yang panjang. Oleh itu kajian perspektif semasa ialah bagi mencari perencat HMGCR agen semulajadi untuk mengurangkan paras kolesterol melalui mekanisme enzim perencatan yang terlibat dalam mensintesis kolesterol. Dengan itu, agen semulajadi ini boleh menjadi ubat alternatif dalam merawat hiperkolesterolemia dan mencegah aterosklerosis. Objektif kajian ini adalah untuk mengkaji sifat sitotoksik pada sel HepG2 dan untuk menentukan kesan perencatan ekstrak sebatian buah *Pandanus tectorius*, tangeretin, dan trans-etil kafeat ke atas HMGCR. Untuk menilai potensi tangeretin sebagai agen anti-hiperkolesterolemia ke atas tikus yang dirangsang berkolesterol tinggi juga menjadi objektif kajian ini. Penilaian sifat ketoksikan dilakukan melalui ujian MTT pada sel HepG2. Kesan perencatan dilakukan menggunakan kit *in vitro* HMGCR (Sigma Aldrich) dengan pravastatin and simvastatin sebagai kawalan. Kesan hiperkolesterolemik tangeretin

disiasat dalam kajian intervensi dengan tikus. Empat kumpulan eksperimen ($n = 8$ tikus *Sprague dawley*) diberi makan dengan diet normal atau diet kolesterol tinggi kepada masing-masing kumpulan normal dan kumpulan intervensi. Tangeretin dan simvastatin digunakan untuk merawat tikus hiperkolesterolemia selama 14 hari. Paras kolesterol total, LDL, HDL, dan paras SGOT dan SGPT plasma darah dianalisis dalam kajian ini. Pengurangan aktiviti HMGR dianggarkan melalui ujian *in vivo* HMGR menggunakan kaedah kolorimetri pada 540 nm. Selain analisis SGOT and SGPT, kajian ketoksikan tangeretin dinilai dengan pemerhatian histologi pada hati tikus. Hasilnya menunjukkan bahawa ekstrak sebatian buah *P. tectorius*, tangeretin, dan trans-etil kafeat tidak ada kesan ketoksikan terhadap sel HepG2. Semua kepekatan sampel yang diuji dapat menurunkan aktiviti HMGR berbanding kawalan. Perencatan tertinggi untuk tangeretin diperoleh pada kepekatan 400 $\mu\text{g/mL}$ (83.8% perencatan), dan keberkesanannya hampir sama dengan simvastatin dan pravastatin. Paras kolesterol total dan LDL jauh lebih rendah dalam kumpulan intervensi selepas dirawat dengan tangeretin, sementara HDL meningkat dengan signifikan. Selain itu, nisbah HMG-CoA/mevalonate pada hati tikus menunjukkan bahawa aktiviti enzim paling rendah dari semua kumpulan tikus setelah rawatan dengan tangeretin. Kajian ketoksikan ke atas paras SGOT dan SGPT menunjukkan bahawa tiada kesan sampingan yang signifikan selepas rawatan tangeretin berbanding dengan kumpulan normal. Pemerhatian histologi menunjukkan rawatan tangeretin menurunkan degenerasi lemak dalam sel-sel hati. Keputusan mendapati bahawa ekstrak sebatian buah *P. tectorius*, tangeretin, dan trans-etil kafeat adalah selamat dan sesuai sebagai kawalan alternatif untuk mengurangkan paras kolesterol melalui perencatan aktiviti HMGR.