UNIVERSITI SAINS MALAYSIA

Pejabat Pengurusan Dan Kreativiti Penyelidikan
Research Creativity and Management Office

Universiti Sains Malaysia
Aras 6, Bangunan Canselori
11800, USM Pulau Pinang
T : (604-653 3108/3178/3988/5019
F : (604-656 6466/8470
: (604-653 2350
: www.research.usm.my

No. Fail : F0348
Tarikh : 2 Disember 2011

Prof. Madya Dr. Nornisah Mohamed
Pusat Pengajian Sains Farmasi
Universiti Sains Malaysia

Puan,

LAPORAN AKHIR SKIM GERAN PENYELIDIKAN FUNDAMENTAL (FRGS)
Tajuk Projek : Binding Mechanism of Flavonoids Onto Xanthine Oxidase
No. Akaun : 203/PFARMASI/671158

Dengan hormatnya perkara di atas dirujuk.

2. Terlebih dahulu saya ucapkan ribuan terima kasih di atas satu salinan laporan akhir untuk projek penyelidikan seperti tajuk di atas.

3. Adalah dimaklumkan walaupun projek ini telah selesai, kerjasama Jabatan Bendahari dipohon untuk menguruskan penutupan akaun projek pada selewat-lewatnya **31 Disember 2011**. Tempoh ini bertujuan untuk menyelesaikan semua urusan tuntutan dan bayaran yang telah dibelanjakan di dalam tempoh projek. Walau bagaimanapun, puan dinasihatkan supaya tidak mengeluarkan borang-borang pesanan baru di dalam tempoh ini.

4. Selanjutnya sila ambil perhatian terhadap perkara-perkara berikut sekiranya berkaitan:

(i) Semua penerbitan harus merakamkan penghargaan kepada **Skim Geran Penyelidikan Fundamental (FRGS)** dan puan dipohon mengemukakan satu salinan ke Pejabat ini.

(ii) Bahagian Penyelidikan & Inovasi boleh/akan mengagihkan semula peralatan yang telah dibeli menggunakan peruntukan geran ini seandainya terdapat penyelidik lain yang memerlukan peralatan tersebut.

5. Akhir sekali, tahniah di atas usaha dan kejayaan pihak puan dapat menyelesaikan projek ini dengan jayanya.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"
'Memastikan Kelestarian Hari Esok'

HAH, HAR . SM

Yang menjalankan tugas,

(AMRA OTHMAN)
Penolong Pendaftar
Unit Pengurusan Geran & Kontrak

LAPORAN AKHIR SKIM GERAN PENYELIDIKAN FUNDAMENTAL (FRGS)
Tajuk Projek : Binding Mechanism of Flavonoids Onto Xanthine Oxidase
No. Akaun : 203/PFARMASI/671158

s.k. Dekan Penyelidikan
Pelantar Sains Fundamental
Pejabat Pelantar Penyelidikan
Universiti Sains Malaysia

Dekan
Pusat Pengajian Sains Farmasi
Universiti Sains Malaysia

Timbalan Dekan
(Ijazah Tinggi & Penyelidikan)
Pusat Pengajian Sains Farmasi
Universiti Sains Malaysia

Ketua Pustakawan
Perpustakaan Hamzah Sendut
Universiti Sains Malaysia

} Disampaikan satu salinan laporan akhir projek untuk simpanan Perpustakaan

Penolong Bendahari Kanan
Unit Kumpulan Wang Penyelidikan
Jabatan Bendahari
Universiti Sains Malaysia

Pegawai Sains
Pelantar Sains Fundamental
Pejabat Pelantar Penyelidikan
Universiti Sains Malaysia

} Mohon kerjasama pihak puan untuk menguruskan penutupan akaun projek selewat-lewatnya pada **31 Disember 2011** dan mohon kemukakan satu salinan penyata kewangan terakhir ke Pejabat ini untuk tujuan rekod

F0348

FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)

Laporan Akhir Skim Geran Penyelidikan Asas (FRGS) IPT
Pindaan 1/2010

A

RESEARCH TITLE : **Binding Mechanism of Flavonoids onto Xanthine Oxidase**
Tajuk Penyelidikan

PROJECT LEADER : **Assoc. Prof. Nornisah Mohamed**
Ketua Projek

PROJECT MEMBERS (including GRA) : 1. Prof. Habibah A Wahab
2.
Ahli Projek

PROJECT ACHIEVEMENT *(Prestasi Projek)*

B

ACHIEVEMENT PERCENTAGE			
Project progress according to milestones achieved up to this period	**0 - 50%**	**51 - 75%**	**76 - 100%**
Percentage			✓

RESEARCH OUTPUT

Number of articles/ manuscripts/ books *(Please attach the First Page of Publication)*	**Indexed Journal**	***Non-Indexed Journal***
Conference Proceeding *(Please attach the First Page of Publication)*	**International**	***National***
	Computational Study of Natural Flavonoids fot the Inhibition of Xanthine Oxidase. (2007). Belal Omar, Nornisah Mohamed, Rashidah A Rahim and Habibah A Wahab. Molecular Modelling 2007 (MM2007), Melbourne, Australia. 27-30 November 2007	
Intellectual Property *(Please specify)*		

HUMAN CAPITAL DEVELOPMENT

Human Capital	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	1 Post-Doctoral
PhD Student					
Master Student				1	

Undergraduate Student					
Total				1	

EXPENDITURE *(Perbelanjaan)*

C

Budget Approved *(Peruntukan diluluskan)* : **RM 70,000.00**
Amount Spent *(Jumlah Perbelanjaan)* : **RM 51,623.00**

Balance *(Baki)* : **RM 18,377.00**
Percentage of Amount Spent : 73.75 %
(Peratusan Belanja)

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS
(Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insanian)

D

International		
Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)		
National		
Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)		

PROBLEMS / CONSTRAINTS IF ANY *(Masalah/ Kekangan sekiranya ada)*

E

1. Procurement of standards – time receiving of standards is too long.
2. Degradation of enzyme upon receiving/storing.

RECOMMENDATION *(Cadangan Penambahbaikan)*

F

RESEARCH ABSTRACT – Not More Than 200 Words *(Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan)*

G

In this study, the mechanism of binding of flavonoids onto xanthine oxidase was studied by performing molecular docking for more than 100 natural flavonoids onto xanthine oxidase to screen for the activity of these compounds against XO. After the obtaining the results from molecular docking studies, the enzymatic activity assay will be carried out with some of the top scoring flavonoids which is available in the market to validate the computational technique. The molecular dynamics simulation will be performed with he best flavonoid that binds onto XO to establish the mechanism.

From the docking studies, 125 flavonoids were successfully docked on XO using DOCK 6.0. The top 5 ranked flavonoids are papyriflavonol-A, 3,5,7,2',3'-pentahydroxy-6-methoxy-8-(3-methylbut-2-enyl)flavanone, licoisoflavone-A, sigmoidin-A and quercetagetin. The 35 top ranked compounds were subjected to docking refinement procedure using AutoDock 3.0.5 software. AutoDock 3.0.5 provided more insight in the intra and intermolecular interactions, such as hydrogen bond, hydrophobic, electrostatic and aromatic interactions. The top 5 ranked flavonoids were found to be licoisoflavone-A, erysubin-F, papyriflavonol-A, 5,7-dihydroxy-6-methoxy-8-(3-methylbut-2-enyl)flavanone and sigmoidin-A

Licoisoflavone-A and quercetin were than subjected to the MD silmulation. Quercetin is a well known inhibitor for XO was used as reference. The molecular modelling studies showed that licoisoflavone-A was the most preferable competitive inhibitor among tested flavonoids. The presence of aliphatic chain in the flavonoid structure was responsible in increasing the hydrophobic contribution and thus increasing the binding affinity. The free energy of binding calculated using MM-PBSA method showed that licoisoflavone-A had a lower energy of binding than quercetin, which indicated that licoisoflavone-A was a more potent inhibitor than quercetin.

The enzymatic study was carried out using with a different set of flavonoids as all the top 5 ranked flavonoids in the docking studies are not available in the market. Allopurinol, a potent inhibitor of xanthine oxidase was used as reference. Allopurinol inhibited xanthine oxidase activity with Ki value of 0.5 μM. Apigenin, phloretin and quercetin also exhibited similar inhibition of xanthine oxidase activity as allopurinol with Ki values of 0.56 μM, 0.47 μM and 0.6 μM, respectively. However, the enzymatic studies could not used to be validate the molecular modeling studies as the top 5 ranked flavonoids are not available in the market to be used in the study.

Date : 10 Februari 2011
Tarikh

Project Leader's Signature:
Tandatangan Ketua Projek

COMMENTS, IF ANY, ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)
(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

H .....

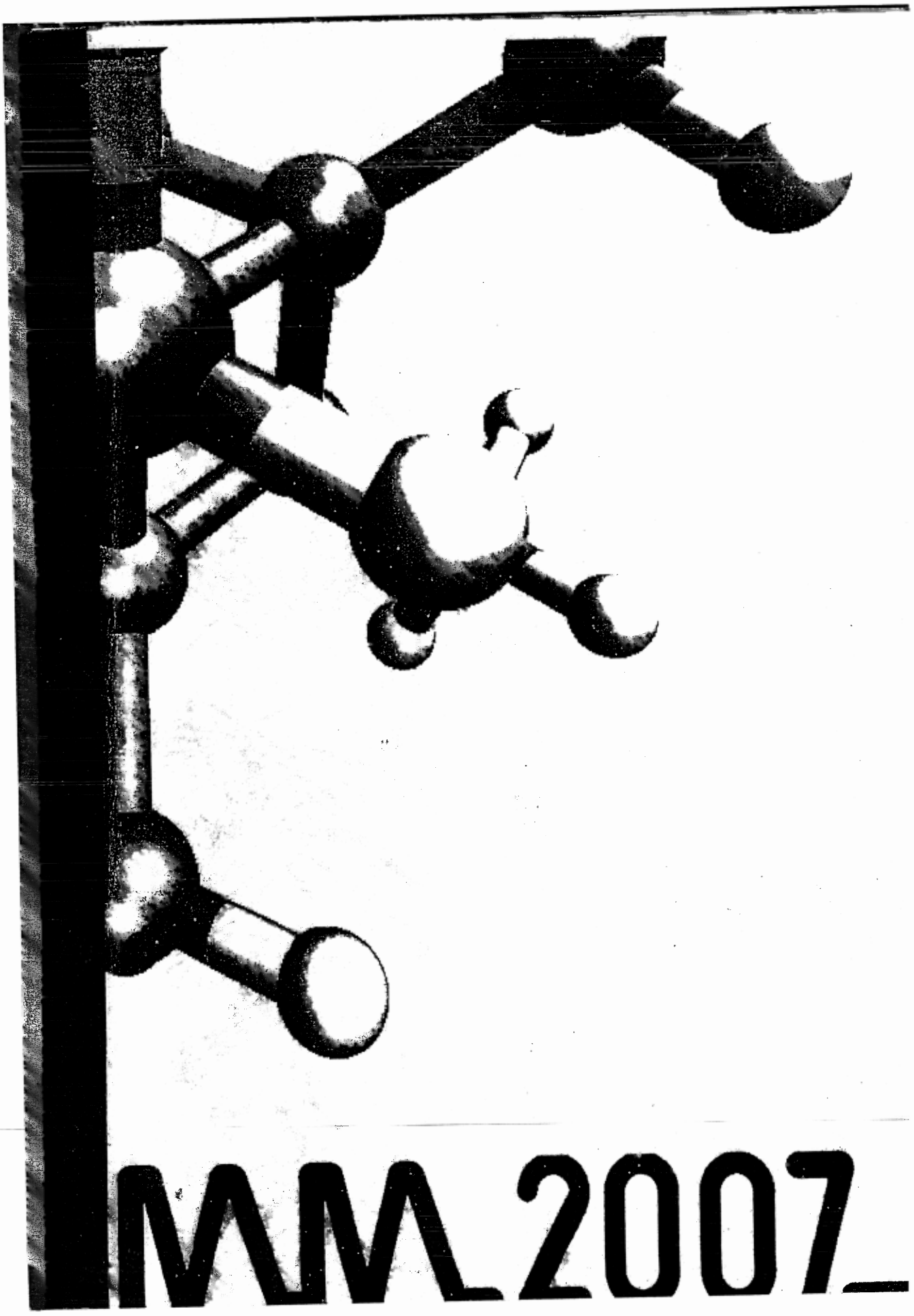
.....

.....

Name:
Nama:

Signature:
Tandatangan:

Date:
Tarikh:



MM_2007_

P33

Computational Study of Natural flavonoids for the inhibition of Xanthine oxidase

Belal Omar[1,2], Nornisah Mohamed[1,2], Rashidah A Rahim[2,3], Habibah A Wahab[1,2]

[1]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang,*
[2]*Makmal Biokristalografi dan Bioinformatik Struktur, Universiti Sains Malaysia, 11800 Minden, Penang,*
[3]*School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang*

Xanthine oxidase (XO) catalyses the conversion of hypoxanthine to xanthine and also xanthine to uric acid. The increase of uric acid level in blood serum, which called hyperuricemia, can lead to major complications such as gout and kidney stones. Flavonoids are known to possess the ability to interact with XO and thus inhibiting the XO activity. Therefore, the current study aims to investigate the potency of flavonoids as XO inhibitors.

The primary stage of the study involved filtering of a library of flavonoids containing 125 compounds using molecular docking software, DOCK 6.0, and AutoDock 3.0.5 was then used for further investigate the 35 top ranked flavonoids obtained from DOCK 6.0. The analysis of the results among all flavonoids in the library (*in silico*) illustrates that licoisoflavone-A showed the most potent activity toward the inhibition of XO. For further understanding of the interaction of the flavonoid toward XO, 4 ns molecular dynamics simulations were performed for licoisoflavone-A. The results indicate that licoisoflavone-A, might have high potential activity to inhibit xanthine oxidase. It has also been observed that the hydrophobic interactions make important contributions to improve binding of flavonoids to XO. Increasing the aliphatic carbons on the flavonoid structure was found to increase the hydrophobicity. Nevertheless, this criteria might not necessarily increase the inhibition activity, as the long chain might prevent the ligand to fit into the active site.

Binding Mechanism of Flavonoids onto Xanthine Oxidase
Final Report

Introduction

Xanthine oxidase (EC1.17.32.2, XO) is a key enzyme that catalyzes the hydroxylation of hypoxanthine to xanthine through the transformation of purine bases which results in the formation of uric acid (Nishino, 1994; Borges *et al.*, 2002). High concentration of uric acid leads to a state of hyperuricemia, a condition which causes serious processes such as gout and kidney stones (Savitz and Leslie, 2003; Nakagawa *et al.*, 2006). Hyperuricemia is usually treated using allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine), an analogue of hypoxanthine which is a specific, readily available potent inhibitor of uric acid synthesis (Fields *et al.*, 1996). Allopurinol is slowly oxidized to oxypurinol, a xanthine analogue which is a more potent inhibitor of xanthine oxidase (Fields *et al.*, 1996).

The most common side effects of allopurinol include rashes, gastrointestinal problems, headache, urticaria and interstitial nephritis. However, the most feared reactions is hypersensitivity syndrome associated with fever, hepatic toxicity, renal failure, a systemic hypersensitivity vasculitis (Arellano *et al.*, 1993; Horiuchi *et al.*, 2000). This reactions most commonly occurs in elderly patients. Although the syndrome is rare, death have also been reported (Horiuchi *et al.*, 2000). Safety in children and during pregnancy has not been established (Borges *et al.*, 2002). Due to these adverse effects, therefore, there is a need for an alternative anti-hyperuricemia agent without the adverse reactions such as those associated with allopurinol.

Flavonoids are an ubiquitous group of polyphenolic substances which are present in most plants, concentrating in flowers, fruit skin or peel, seeds, leaves and bark (Hermann, 1993; White and Xing, 1997). They are characterized by carbon skeleton C_6-C_3-C_6. with a basic structure consists of two phenolic rings linked by a 3-carbon aliphatic chain which normally has been condensed to form a pyran (Cos *et al.*, 1998) (Fig. 1). They frequently attached with sugars moiety, preferred binding site is C-3 and less frequently, in the A-ring at the C-7 position (Herrmann, 1976), to increase their water solubility (Cos *et al.*, 1998; Farkas *et al.*, 2004). Flavonoids are known to possess the ability to interact with XO inhibiting the XO activity (Hayashi *et al.*, 1988; Lin *et al.*, 2002a; 2002b; da Silva *et al.*, 2004).

The crystalline structure of XO determined by Enroth *et al.* (2000) showed that XO forms an asymmetric trimeric complex with a salicylate molecule inserted in the molybdopterin catalytic centre. The oxidation of xanthine takes place at the molybdopterin cofactor and the electrons are distributed to other centres by intramolecular electron transfer (Enroth *et al.*,2000).

To understand the mechanism of binding of flavonoids onto xanthine oxidase, we will perform molecular docking for more than 100 natural flavonoids onto xanthine oxidase to screen for the activity of these compounds against XO. After the obtaining the results from molecular docking studies, the enzymatic activity assay will be carried out with some of the top scoring flavonoids which is available in the market to validate the computational technique. The molecular

dynamics simulation will be performed with the best flavonoid that binds onto XO to establish the mechanism.

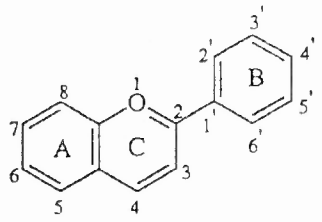


Figure 1 The C_6-C_3-C_6 configuration of flavonoid structure.

Objectives of the research are

1. To perform the binding study of flavonoids against xanthine oxidase using molecular modelling approach.
2. To validate the results by enzymatic studies.
3. To establish the mechanism of enzyme inhibition for the flavonoids.

Molecular Modelling Studies

A group of 125 flavonoids from natural source were selected to investigate their activity toward xanthine oxidase (XO). The molecular modelling studies involve three (3) parts:

(1) filtering of 125 compounds using DOCK 6.0.
(2) docking refinement for 35 top ranked flavonoids obtained from part (1) using AutoDock 3.0.5
(3) molecular dynamic study of the top ranked flavonoid from the molecular docking studies.

Method

(1) Molecular Docking

. The molecular docking studies were performed using two molecular docking softwares; DOCK 6.0 and AutoDock 3.0.5.

(i) DOCK 6.0

The 125 ligand structures were generated and minimised using HyperChem7.5 software (Hypercube, Release 7.5, 2002). The atoms and charges for all the atoms in each ligands were

assigned using InsightII software (Accelrys Inc.). The crystal structure of bovine milk xanthine oxidase was obtained from the Protein Data Bank (PDB, entry ID: 1FIQ). All the heteroatoms and water molecules were removed from the proteins. The study was carried out only on the chain C where the molydopterine is present since this subunit of the protein is responsible for the oxidation reaction. GRID scoring was also performed in order to define the location and the size of the grid to be calculated. The box with 5.0 Å x 5.0 Å x 5.0 Å dimensions was created. The docking simulation was then performed.

(ii) AutoDock 3.0.5

Only 35 top ranked flavonoids were subjected to the Auto Dock 3.0.5 calculations. Autotors program was used to define the torsions of the ligands. The protein was prepared using InsightII software (Accelrys Inc.) and the solvation parameters were added using ADDSOL utility of AutoDock 3.0.5. Autogrid utility program was used to calculate the grid maps of the protein used in the docking experiments. Finally, the docking simulations were performed using AutoDock 3.0.5. The Lamarckian Genetic Algorithm was used to predict and identify the binding conformations of the various flavonoids.

(2) Molecular Dynamic (MD) Simulation

Only two ligands were chosen from AutoDock 3.0.5 calculation were subjected to molecular dynamics simulation.

All the simulations were performed using molecular dynamics program, AMBER 8.0. The starting structure was obtained from crystal structure of bovine milk xanthine oxidase with its competitive inhibitor salicylate (PDB code 1FIQ). The structure was carefully checked and all missing residues, atoms were carefully modelled. The system was hydrated using TIP3P water model whereby the oxygen atom beared a charge of -0.8340 which was balanced by two hydrogen atoms (+0.4170 each atom). The minimisation, equilibration and trajectory collection procedures were carried out at 310 K with the non-bonded cutoff of 12 Å for 4 ns, employing a time-step of 2 fs. The MD trajectories were analysed using MM/PBSA method.

Figure 2 shows the flow of the molecular modelling studies performed.

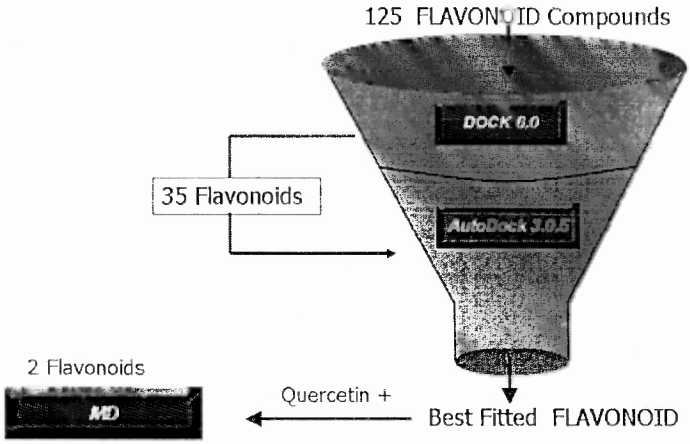


Figure 2

Results and Discussion

The molecular docking program, DOCK 6.0 provided a primary filtering to exclude the ligands that have undesirable physicochemical properties that can not fit in to the active site. The 125 flavonoids were successfully docked on XO using DOCK 6.0. The top 5 ranked flavonoids are papyriflavonol-A, 3,5,7,2',3'-pentahydroxy-6-methoxy-8-(3-methylbut-2-enyl)flavanone, licoisoflavone-A, sigmoidin-A and quercetagetin.

Out of 125 flavonoids, only 35 top ranked compounds were subjected to docking refinement procedure using AutoDock 3.0.5 software. AutoDock 3.0.5 provided more insight in the intra and intermolecular interactions, such as hydrogen bond, hydrophobic, electrostatic and aromatic interactions. The top 5 ranked flavonoids were found to be licoisoflavone-A, erysubin-F, papyriflavonol-A, 5,7-dihydroxy-6-methoxy-8-(3-methylbut-2-enyl)flavanone and sigmoidin-A.

Licoisoflavone-A and quercetin were than subjected to the MD silmulation. Quercetin is a well known inhibitor for XO was used as reference.

Lisoisoflavone-A have been proposed to be the most active flavonoid upon the selected database, due to the following findings

(i) Binding Site (Figure 3):
Results showed that licoisoflavone-A and quercetin bound in the same pocket blocking the active site of the enzyme. The MD simulation showed similar mode of binding, thus increased the conference in the stability of the ligand within the binding pocket.

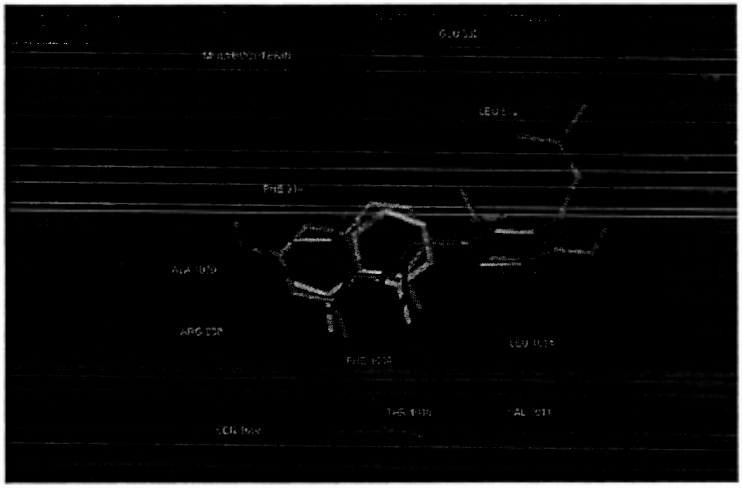


Figure 3: The binding conformation of llcoisoflavone-A (green) and quercetin (red)

(ii) Hydrogen Bonds (Figure 4):

AutoDock 3.0.5 results showed that licoisoflavone-A has the highest number of hydrogen bonds formed among all tested flavonoids, in which four hydrogen bonds have been shown to be formed with the active site amino acids (Glu 802, 1261 and Thr 1010). The same amino acids were also involved in forming hydrogen bonds during MD simulation except for Glu 1261.

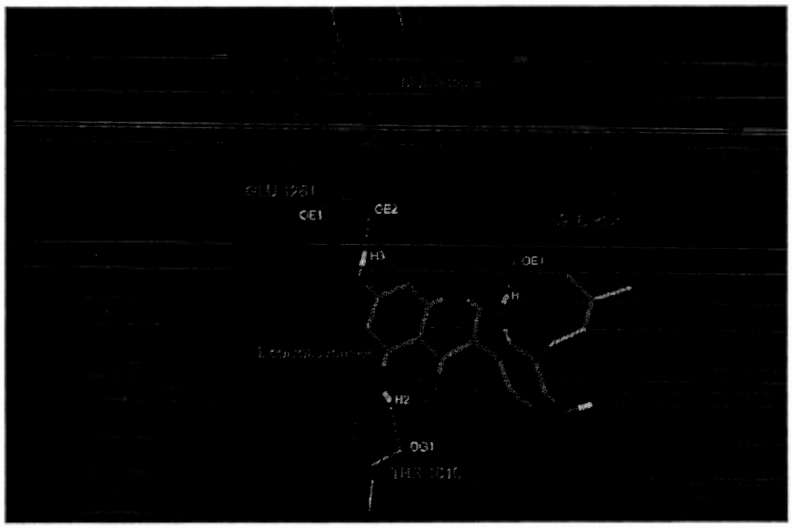


Figure 4: Hydrogen-bonds interaction formed between licoisoflavone-A and Glu 802, Glu 1261 and Thr 1010

(iii) Hydrophobic interaction (Figure 5):

In docking studies, licoisoflavone-A was found to have 20 atoms introduced in the hydrophobic interaction, whereas an 18 atoms was shown to be involved in the representative conformation of licoisoflavone-A during MD simulation

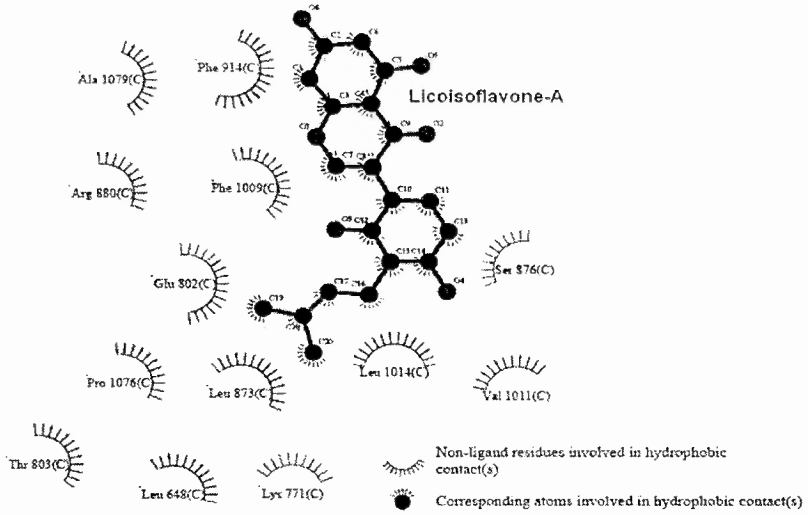


Figure 5: LigPlot representation describing the hydrophobic interaction of licoisoflavone-A in the active site (Molecular docking)

(iv) Aromatic interaction (Figure 6):

This docking result showed the ability of licoisoflavone-A to form strong π-π interaction with Phe 1009 and Phe 914, which also appeared to have the same interaction during the MD simulation.

Figure 6: The π-π stacking of licoisoflavone-A and Phe 1009 and 914

(v) Electrostatic interaction (Figure 7):

The results obtained from AutoDock 3.0.5 showed that licoisoflavone-A hydroxyl groups were aligned beside the guanidinium group of Arg 880, which stabilised the complexes. During MD simulation, licoisoflavone-A hydroxyl groups remained in an orientation which could form electrostatic interaction with guanidinium group of Arg 880 in almost all the simulation period.

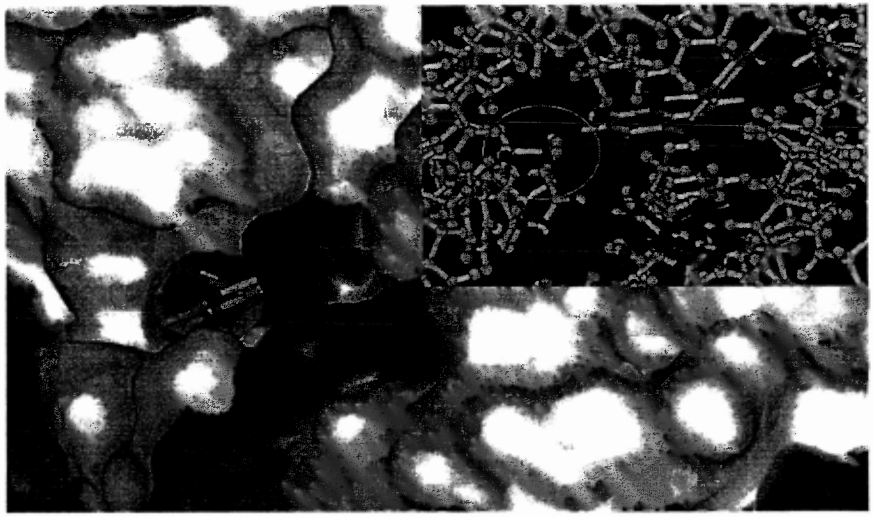


Figure 7: Electrostatic interaction between licoisoflavone-A and guanidinium group of Arg 880, the positively charged residues are colored by blue, red for negatively charge and white for neutral residues.

(vi) Water mediated hydrogen bond (Figure 8):

Water molecules play two important roles in the catalytic reaction. MD simulation showed that WAT 15689 formed two hydrogen bonds; 1) with O2 of licoisoflavone-A and 2) with Arg 880.

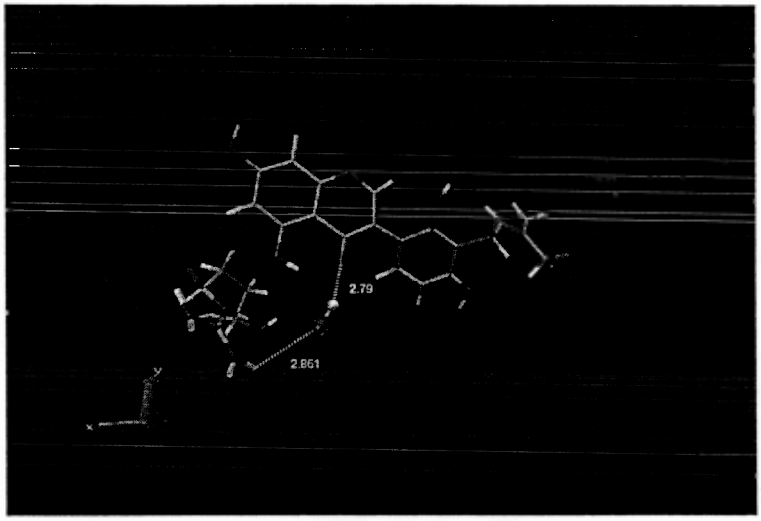


Figure 8: WAT 15689 is responsible ion mediate hydrogen bond between the oxygen number 2 of licoisfoflavone-A and Arg 880

(vii) Free energy of binding :

Table 1 shows the free energy of binding for licoisoflavone-A and quercetin. The calculated binding free energy between licoisoflavone-A and XO was found to be lower than that of quercetin by 1.93 Kcal/mol. These results showed that licoisoflavone-A has higher affinity for binding to XO than quercetin, with a slight domination for hydrophobic contribution for licoisoflavone-A.

Table 1: Results of free energy calculation by MM-PBSA for licosoflavone-A and quercetin, all the energies are in Kcal/mol

	Licoisoflavone-A	Quercetin
ELE	-55.71	-49.73
VDW	-69.90	-61.73
INT	-67.30	-77.50
GAS	-192.91	-189.04
PBSUR	-9.68	-6.10
PBCAL	+195.52	+190.40
PBSOL	+185.84	+183.90
PBELE	+139.81	+140.67
PBTOT	-7.07	-5.14

Enzymatic Study

The enzymatic study was carried out using with a different set of flavonoids as all the top 5 ranked flavonoids in the docking studies are not available in the market. Allopurinol, a potent inhibitor of xanthine oxidase, clinically used for treatment of gout was used as reference compound.

Method

Xanthine oxidase activity will be measured spectrophotometrically by continuosly measuring the formation of uric acid at 295 nm with xanthine as substrate. The assay mixture will contain phosphate buffer, 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), EDTA, xanthine oxidase and xanthine. The assay mixture with or without flavonoids (inhibitors) will be incubated for 3-5 min at 37°C and the absorbance will be recorded at 5 sec intervals. The kinetic assay will be performed and all data obtained will be subjected to the Lineweaver-Burk double reciprocal plots. The K_i values of various flavonoids onto xanthine oxidase will be calculated.

Flavonoids used in the enzymatic studies were as follows:
Allopurinol, apigenin, gavochin, chalcone, morin, obinetin, phloretin and quercetin. Morin, phloretin and quuercetin were compounds ranked top 35 in the docking studies.

Results and Discussion

Table 2 shows the inhibition constant (Ki) values of the flavonoids. From the results, it shows that allopurinol inhibited xanthine oxidase activity with Ki value of 0.5 μM. Apigenin, phloretin and quercetin also exhibited similar inhibition of xanthine oxidase activity as allopurinol with Ki values of 0.56 μM, 0.47 μM and 0.6 μM, respectively. The Ki values obtained for this study is comparable to those carried out by Lin *et al.* (2002b) as shown in Table 2.

Table 2. Ki values for inhibition of xanthine oxidase activity

Flavonoids	Ki values (μM)	Ki values (μM) reported by Lin *et al.* (2002)
Allopurinol	0.5 ± 0.25	0.34 ± 0.22
Apigenin	0.56 ± 0.25	0.61 ± 0.31
Bavochin	0.87 ± 0.4	-
Chalcone	0.68 ± 0.3	-
Morin	0.75 ± 0.3	-
Obinetin	0.72 ± 035	-
Phloretin	0.47 ± 0.2	-
Quercetin	0.6 ± 0.3	1.40 ± 0.78

Lin *et al.* (2002b) showed that in stalibising the flavonoid-XO complex, the hydroxyls linked to the C5 and C7 atoms and to the O9 atom are important. However, substitutions at the C3 atom by hydroxyls or at the C6 atom by the sugar moiety do not favor the formation of the F–XO complex. These has been demonstrated by the Ki values obtained in this study. The Ki values of quercetin and morin are comparable with the docking studies as quercetin as ranked 15 and morin 19.

Conclusion

The molecular modelling studies showed that licoisoflavone-A was the most preferable competitive inhibitor among tested flavonoids. The presence of aliphatic chain in the flavonoid structure was responsible in increasing the hydrophobic contribution and thus increasing the binding affinity. The free energy of binding calculated using MM-PBSA method showed that licoisoflavone-A had a lower energy of binding than quercetin, which indicated that licoisoflavone-A was a more potent inhibitor than quercetin. The enzymatic studies could not used to be validate the molecular modeling studies as the top 5 ranked flavonoids are not available in the market to be used in the study.

References

Arellano, F. and Sacristan, J. A. (1993). Allopurinol hypersensitivity syndrome: a review. *Annals of Pharmacotherapy*, **27**: 337- 343.

Borges, F., Fernande, E. and Roleira, F. (2002). Progress towards the discovery of xanthine oxidase inhibitors. *Current Medicinal Chemistry*, **9**(2): 195.

Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Poel, B. V., Pieters, L., Vlietinck, A. J. and Berghe, D. V. (1998). Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Product*, **61**(1): 71-76.

da Silva, S. L., da Silva, A., Honorio, K. M., Marangoni, S., Toyama, M. H. And da Silva, A. B. F. (2004). The influence of electronic, steric and hydrophobic properties of flavonoid compounds in the inhibition of the xanthine oxidase. *Journal of Molecular Structure (Theochem)*, **684**: 1-7.

Enroth, C., Eger, B. T., Okamoto, K., Nishino, T., Nishino, T. and Pai, E. F. (2000). Crystal structures of bovine milk xanthine dehydrogenase anf xanthine oxidase : Structure based mechanism of conversion. *Proceedings of the National Academy of Sciences of the United States of America*, **97**(20): 10723.

Farkas, O., Jakus, J. and Herbegger, K. (2004). Quantitative structure - Antioxidant activity relationships of flavonoids compounds. *Molecules*, **9**(12): 1079.

Hayashi, T., Sawa, K., Kawasaki, M., Arisawa, M., Shimizu, M. and Morita, N. (1988). Inhibition of cow's milk xanthine oxidase by flavonoids. *Journal of Natural Products*, **51**: 345-348.

Herrmann, K. (1993). Flavonoids in plants foods and their antioxidant activity. *Gordian*, **7**: 108-111.

Herrmann, K. (1996). Flavonols and flavones in food plants: a review. *Journal of Food Technology*, **11**: 433-448.

Horiuchi, H., et al., (2000). Allopurinol induces renal toxicity by impairing pyrimidine metabolism in mice. *Life Sciences*, **66**(21): 2051.

Lin, C. M., Chen, C. S., Chen, C. T., Liang, Y. C. and Lin, J. K. (2002b). Molecular modeling of flavonoids that inhibits xanthine oxidase. *Biochemical and Biophysical Research Communications*, **294**(1): 167-172.

Lin, C. M., Chen, C. T., Wang, L. F., Hui, C. F. and Hwang, J. (2002a). Prevention of cellular ROS damage by isovitexin and related flavonoids. *Planta Medica*, **68**: 365-367.

Nakagawa, T. *et al.*, (2006). Uric acid – A uremic toxin? *Blood Purification*, 24(1): 67.

Nishino, T. (1994). The conversion of xanthine dehydrogenase to xanthine oxidase and the role of the enzyme in reperfusion injury, *Journal of Biochemistry*, 116(1): 1.

Savitz, G. and Leslie, S. W. (2003). The Kidney stone handbook. Four Geez Press, CA, USA.

White, P. J. and Xing, Y. (1997). Antioxidants from cereals and legumes, In: Natural Antioxidants, Chemistry, Health Effects and Applications. Ed. Shahidi, F. AOCS Press, Champaign, Illinois, USA. pp 25-63.