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Original Article

IN SILICO SCREENING OF HESPERETIN AND NARINGENIN ESTER DERIVATIVES AS ANTICANCER AGAINST P-GLYCOPROTEIN

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ABSTRACT

Objective: Study the in silico P-Glycoprotein (P-GP) inhibition activity of hesperetin and naringenin ester derivatives. Acyl group substituent was different in the length of the carbon atom chain (acetyl, propionyl, butyryl and valeryl).

Methods: Partition coefficient was predicted by the Chem Draw Ultra program. In silico docking using PLANTS program and visualized by Yasara program. The model of three dimension enzyme structures used in this research was P-Glycoprotein (P-GP) binding pocket with the Protein Data Bank (PDB) code 1MV5. Two dimensions and three dimension conformation models of hesperetin and naringenin ester derivatives and verapamil as the standard P-GP inhibitor generated by using the Marvin Sketch program.

Results: Hesperetin and naringenin have a lower partition coefficient than verapamil. It means that their solubility in the oil phase to cross the cell membrane was lower than verapamil. Trivaleryl hesperetin and trivaleryl naringenin have a higher partition coefficient than verapamil. It means that their solubility in the oil phase to cross the cell membrane was higher than verapamil. Docking score of hesperetin and naringenin as the lead compound was higher than verapamil as the P-GP inhibitor standard compound. It means that hesperetin and naringenin have a weaker interaction to target protein than verapamil. Ester derivatives of hesperetin and naringenin with the increasing the length of the acyl carbon atom chain substituted on hesperetin and naringenin will increase the P-GP inhibition activity. Butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin shows the lower docking score than verapamil as the P-GP inhibitor standard compound. It means that butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin have a stronger interaction to target protein than verapamil.

Conclusion: Increasing of the length of the acyl carbon atom chain substituted on hesperetin and naringenin it will increase the P-Glycoprotein (P-GP) inhibition activity. Trivaleryl hesperetin has the best activity in this study and thus to be a good compound to be synthesized and to be combined with anticancer drug.

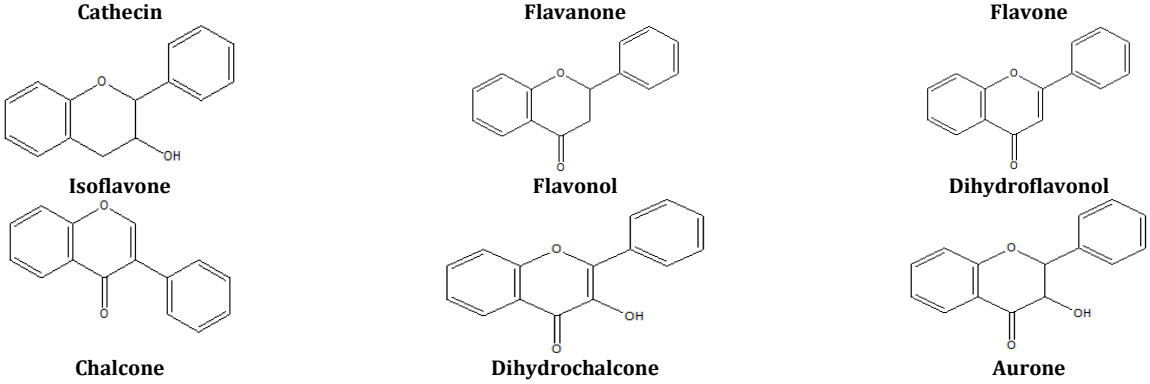
Keywords: In Silico, Hesperetin, Naringenin, Ester Derivatives, Anticancer, P-Glycoprotein (P-GP), 1MV5.

INTRODUCTION

Over expression of P-Glycoprotein (P-GP) efflux pump is a major cause of chemotherapy failure because it will cause resistance of some anticancer drugs against cancer cells. P-GP works to remove anticancer drugs out of the cancer cells. So that needs to be developed P-GP inhibitors are safe and effective to prevent anticancer drug resistance mediated by P-GP [1]. Drugs that can inhibit P-GP can be used as a complementary therapy in conjunction with a cancer chemotherapy drug that is commonly used. Inhibition of P-GP, it will cause the intracellular concentration of anticancer drugs to be high because it was not removed by P-GP [2].

Secondary metabolites which makes it possible to inhibit P-GP efflux pump strongly [3]. Inhibition of P-GP by verapamil can increase drug levels inside of the intracellular cells [4]. Drug compounds that can inhibit P-GP is verapamil, which has been tested preclinical and clinical, but failed to increase the efficacy of the drug therapy. This is due to the relatively low affinity between drug compounds with P-GP [5]. So it is important for researchers to investigate the new compounds of natural ingredients that are safe and can inhibit P-GP to prevent anticancer drug resistance [6].

Flavonoids can inhibit P-GP efflux pump, causing intracellular accumulation of anticancer drugs in cancer cells. Flavonoids are components found in vegetables and also in a variety of medicinal plants that can be combined with anticancer drugs. Flavonoids consist of several subclasses such as: catechin, flavanone, flavone, isoflavone, flavonol, dihydroflavonol, chalcone, dihydrochalcone and aurone [7]. Fig. 1 below shows the basic structure of various flavonoid derivatives.



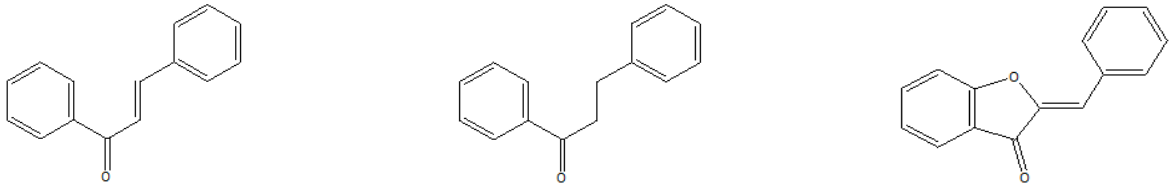
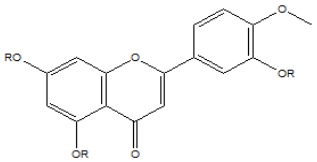


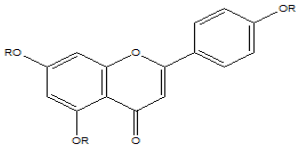
Fig. 1: Basic structure of various flavonoid derivatives

Flavonol widely available in abundance at the various types of fruit, while the flavanone and flavone more limited and often found in groups of citrus. Flavone found in citrus fruit in a relatively low amount compared to flavanone. Hesperidin and naringin are a form of flavanone glycones, while hesperetin and naringenin are a form of flavanone aglycones; generally found in citrus fruits such as oranges, lemons, limes, and are also present in tomatoes. The use of a combination of flavonoids and anticancer drug allows beneficial effects [7].

Flavonoids are widely used in the pharmaceutical field, the use of flavonoids is limited due to the hydrophilic nature of flavonoids, which resulted in flavonoid compounds have low solubility and low stability in lipophilic media. Increased lipophilic properties of flavonoids it will be followed by an increase in biological activity of flavonoids. Because compounds that are lipophilic will more easily penetrate cell membranes and intracellular work on. Compounds derived flavonoids in the form of esters will lead to the enhancement of the lipophilic (hydrophobic) and allow it to provide better penetration into cancer cells. Techniques to improve the lipophilic properties of flavonoid compounds are by esterification (acylation) hydroxyl group of flavonoids [8]. Semisynthesis flavonoid derivatives by esterification (acylation) flavonoids with several kinds of acyl group substituent to the hydroxyl group will increase the overall lipophilicity properties of lead compound flavonoids [9].

Lead discovery was the main components of today's early pharmaceutical research. The aim of target discovery is the identification and validation of suitable drug targets for therapeutic intervention. Computational methods are being developed to predict the drug likeness of compounds. Thus, drug discovery is already on the road towards electronic research & development. In silico approaches contribute significantly to early pharmaceutical research and are especially important in target discovery and lead discovery. The need for timely adaptation and application of in silico approaches in pharmaceutical research has clearly been recognized and is expected to improve further the overall efficiency of drug discovery [10]. The longer the carbon atom chain in the acyl group substituted flavonoids is enhanced lipophilic properties and more easily penetrate across the cell membranes. Lipophilic compounds that enter into the intracellular of cancer cells are expected to inhibit P-GP. Thus, increasing the anticancer drug accumulation in cancer cells. The purpose of this research is to study the in silico P-GP inhibition activity of hesperetin and naringenin derivative lipophilic compounds obtained through esterification (acylation) overall hydroxyl groups. Increasing of the length of the acyl carbon atom chain substituted on hesperetin and naringenin (acetyl, propionyl, butyryl and valeryl) will be observed. Fig. 2 below shows the structure of hesperetin and naringenin derivatives obtained through esterification (acylation) overall hydroxyl groups.





Hesperetin	R = H	Naringenin	R = H
Triacetyl Hesperetin	R = CH_3 – CO –	Triacetyl Naringenin	R = CH_3 – CO –
Tripropionyl Hesperetin	R = C_2H_5 – CO –	Tripropionyl Naringenin	R = C_2H_5 – CO –
Tributyryl Hesperetin	R = C_3H_7 – CO –	Tributyryl Naringenin	R = C_3H_7 – CO –
Trivaleryl Hesperetin	C_4H_9 – CO –	Trivaleryl Naringenin	C_4H_9 – CO –

Fig. 2: Structure of hesperetin and naringenin derivatives obtained through esterification (acylation) overall hydroxyl groups

MATERIALS AND METHODS

Fujitsu T Series (T4310) operated by Windows 7 Home Premium, Intel® Core™ 2 Duo CPU T660 @ 2.20 GHz, 32-bit, hard disk drive 320 GB, and RAM memory 4.00 GB was used to run the molecular docking process. Partition coefficient was predicted by the Chem Draw Ultra program.

In silico docking using PLANTS program and visualized by Yasara program. Connector for Windows Operation System to Linux Operation System was done by Co Pen Drive Linux KDE program. The model of three dimension enzyme structures used in this research was P-Glycoprotein (P-GP) binding pocket with the Protein Data Bank (PDB) code 1MV5 obtained through the website http://www.rcsb.org/pdb. Two dimensions and three dimension conformation models of hesperetin and naringenin ester derivatives and verapamil as the standard P-GP inhibitor generated by using the Marvin Sketch program.

RESULTS AND DISCUSSION

Adenosine Triphosphate (ATP) which was crystallized in the structure of 1MV5 P-Glycoprotein (P-GP) binding pocket was extracted and docked again into its original P-GP binding pocket. The Root Mean Square Deviation (RMSD) values resulted from these ligand docking was 1.2839 Å. The RMSD obtained was less than 2.0000 Å, a value typically used in evaluating the success of docking algorithms, indicating the docking methods were valid [11]. Fig. 3 shows the docking of ATP into the 1MV5 P-GP binding pocket.

In silico docking between hesperetin and naringenin ester derivative compounds into the 1MV5 P-GP binding pocket is resulting the docking score. table 1 show the docking score result of the ligand into the 1MV5 P-GP binding pocket.

Hesperetin and naringenin have a lower partition coefficient than verapamil. It means that their solubility in the oil phase to cross the cell membrane was lower than verapamil. Trivaleryl hesperetin and trivaleryl naringenin have a higher partition coefficient than verapamil. It means that their solubility in the oil phase to cross the cell membrane was higher than verapamil. Partition coefficient only means the ability of the drug to soluble in the oil phase (cell membrane) to reach the inside of the cell, but the activity of the drug to bind with the binding pocket not depend to the partition coefficient but depend on the structure.

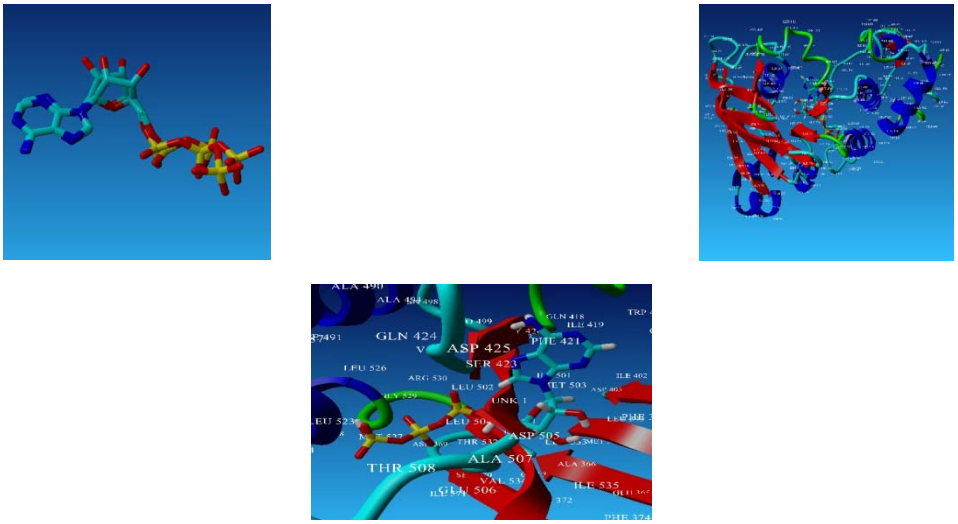


Fig. 3:Docking of ATP into the 1MV5 P-GP binding pocket

Table 1: Docking score of the ligand into the 1MV5 P-GP binding pocket

Number	Ligand name	Partition coefficient	Length of acyl carbon atom chain substituent	Docking score
1	Verapamil	5.69	-	– 85.7743
2	Hesperetin	1.78	0	– 68.5068
3	Triacetyl Hesperetin	1.70	2	– 78.3176
4	Tripropionyl Hesperetin	3.67	3	– 82.3938
5	Tributyryl Hesperetin	4.92	4	– 92.0405
6	Trivaleryl Hesperetin	6.17	5	– 98.6398
7	Naringenin	1.90	0	– 63.8825
8	Triacetyl Naringenin	1.83	2	– 78.9283
9	Tripropionyl Naringenin	3.79	3	– 80.5335
10	Tributyryl Naringenin	5.04	4	– 89.5179
11	Trivaleryl Naringenin	6.30	5	– 95.8587

The docking score represents the binding affinity of the ligand to the target protein, smaller docking score value shows stronger interaction. Docking score of hesperetin and naringenin as the lead compound was higher than verapamil as the P-GP inhibitor standard compound. It means that hesperetin and naringenin have a weaker interaction to target protein than verapamil [12]. Esterification (acylation) of hesperetin and naringenin to overall hydroxyl groups it will increase the lipophilicity property and will easier to penetrate across the cell membrane, but the P-GP inhibition activity was based on the structure. Based on the results it is also known that an increase in the length of acyl carbon atoms chain substituted on hesperetin and naringenin it will be decreasing the docking score, thus increase the P-GP inhibition activity. Butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin shows the lower docking score than verapamil as the P-GP inhibitor standard compound. It means that butyryl and valeryl substituted as the acyl substituent to the hesperetin and naringenin have a stronger interaction to target protein than verapamil.

Fig. 4: Visualization of interaction between interaction between trivaleryl hesperetin and P-GP as the target protein

Increasing costs of drug development and reduced number of new chemical entities have been a growing concern for new drug development in recent years. Therefore, there is a need for the use of alternative tools to get answers on the efficacy and safety faster, with more certainty and at lower cost. One such alternative tool is the in silico drug design or the computer aided drug design (CADD). In silico drug design can play a significant role in all stages of drug development from the preclinical discovery stage to late stage clinical development [13]. The results obtained in silico screening have shown that it represents the best way to get accurate results in a very short time period and saving manner [14].

From the in silico screening results known that trivaleryl hesperetin was the most potential drug to inhibit the P-GP. Trivaleryl hesperetin is expected to be combined with anticancer drugs to increase the effectiveness of therapy, because the inhibition of the P-GP will accumulate the anticancer drug inside of the cancer cell. Fig. 4 below shows the visualization of interaction between interaction

between trivaleryl hesperetin and P-GP as the target protein. Although the application of docking and scoring has led to some remarkable successes, there are still some major challenges ahead [15]

P-GP inhibits the apoptosis of tumor cells in addition to participating in the efflux of intracellular chemotherapy drugs. P-GP which is a serious obstacle in chemotherapy, has also been implicated in causing apoptosis of tumor cells, which were shown to be another important mechanism of anticancer drug resistance recently. P-GP inhibitors also enhanced cell cycle arrest in cancer cell and also inhibit the anticancer drug resistance in cancer cell [16]. Thus very important to find the new P-GP inhibitor; combination of P-GP inhibitor with the anticancer drug can enhance the efficacy of anticancer drug by accumulating the anticancer drug in the intracellular of cancer cell and inhibit the anticancer drug resistance.

CONCLUSION

Hesperetin and naringenin were flavonoid compound with the flavanone subclasses. Hesperetin and naringenin are abundantly contained in citrus fruits. They show the lower P-GP efflux pump inhibition activity than verapamil as the standard P-GP inhibition drug. But the modification of the hydroxyl group with the acyl substituted by esterification will be increasing the P-GP inhibition activity. Increasing of the length of the acyl carbon atom chain substituted on hesperetin and naringenin it will increase the P-GP inhibition activity. Butyryl and valeryl be substituted as the acyl substituent to hesperetin and naringenin shows better activity than verapamil. Trivaleryl hesperetin has the best activity in this study and thus to be a good compound to be synthesized and to be combined with anticancer drug. Stronger P-GP inhibition activity might accumulate the anticancer drug in the intracellular of cancer cell, the treatment also might be more successful.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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