The determination of quercetin in *Plectranthus scutellarioides* (L.) R.Br. leaves extract and its *In Silico* Study on Histamine H4 Receptor

Penentuan kuersetin dari ekstrak metanol daun jawer katok dan studi in siliconya pada reseptor histamin H4

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Abstract

Plectranthus scutellarioides (L.) R.Br., or jawer kotok, Family Lamiaceae, grows widely in Indonesia, and has a long history of therapeutic usage in Indonesian traditional *jamu* to cure various diseases. The brownish purple leaves of Plecranthus contain alkaloids, saponin, flavonoids, tannin, volatile oils, and quercetin which has been proven to exert antiinflammatory activity. In this research, a determination of quercetin in Plecranthus leaves extract was performed and followed by a study of its interaction with histamine H4 receptor to understand its anti-inflammatory activity. The dry leaves were macerated by using a mixture of methanol and water (1:1) for 48 hours and the solvent was evaporated at low temperature (40-50^{o}C). Analysis of quercetin in the extract was performed by using reversed-phase HPLC method LC-10AT VP (Shimadzu), Atlantis Hilicsilica C18 (Waters®) 150 mm x 4.6 mm, 5 µm as stationary phase and a mixture of acetonitrile, phosphoric acid, and methanol (40:50:10), flow rate 0.8 mL/minute. *In silico* study of quercetin with histamine H4 receptor was performed by using AutoDock Tools 3.0.5. Histamine H4 receptor (H4R) belongs to G protein-coupled receptors which is involved in arthritis, asthma, and inflammations. The 3D structure model of H4R was built by using MODELLER 9v7. Quercetin contained in Plecranthus leaves extract was 0.05 %. This compound interacted with H4R *via* hydrogen bond formation with Lys158 (2.006 Å) and Glu182 (2.048 Å), and van der Waals interaction with Trp90, Leu91, Asp94, Tyr95, Phe168, Thr178, Ser179, Tyr319, Phe344, and Tyr340, therefore Plecranthus leaves extract might have a chance to be used as histamine H4 receptor inhibitor.

Key words : histamine H4 receptor**,** *in silico study,* Plecranthus leaves, *Plectranthus scutellarioides,* quercetin

Abstrak

Plectranthus scutellarioides (L.) R.Br., atau jawer kotok, Keluarga Lamiaceae, tumbuh di berbagai tempat di Indonesia, dan merupakan tanaman yang secara empirik digunakan sebagai jamu untuk mengobati berbagai penyakit. Daun jawer kotok yang berwarna ungu kecoklatan mengandung alkaloid, saponin, flavonoid, tanin, minyak atsiri, dan kuersetin yang terbukti memiliki aktivitas antiinflamasi. Pada penelitian ini diltentukan kadar kuersetin di dalam ekstrak daun jawer kotok dilanjutkan dengan telaah interaksinya dengan reseptor histamin H4 untuk mengetahui aktivitas antiinflamasinya. Daun kering yang telah dideterminasi dimaserasi dengan campuran metanol dan air (1:1) selama 48 jam, kemudian pelarut diuapkan pada suhu rendah (40-50^{o}C) hingga diperoleh ekstrak kental. Analisis kuersetin di dalam ekstrak dilakukan

menggunakan KCKT fase-terbalik dengan instrumen LC-10AT VP (Shimadzu), fase diam kolom Atlantis Hilicsilica C18 (Waters®) 150 mm x 4,6 mm, 5 µm dan campuran asetonitril, asam fosfat, dan metanol (40:50:10) sebagai fase gerak, dengan laju alir 0,8 mL/menit. Studi *in silico* kuersetin dengan reseptor histamin H4 dikerjakan menggunakan perangkat lunak AutoDock Tools 3.0.5. Reseptor histamin H4 (H4R) termasuk ke dalam kelompok *G protein-coupled receptors* yang berperan dalam penyakit artritis, asma, serta inflamasi. Struktur model 3D H4R telah dibuat menggunakan perangkat lunak MODELLER 9v7 dan disimpan di dalam *data base* untuk digunakan pada penelitian ini. Kuersetin terbukti terkandung di dalam ekstrak daun jawer kotok dengan kadar 0,05 %. Senyawa ini dapat berinteraksi dengan H4R melalui pembentukan ikatan hidrogen dengan Lys158 (2,006 Å) dan Glu182 (2,048 Å), serta interaksi van der Waals dengan Trp90, Leu91, Asp94, Tyr95, Phe168, Thr178, Ser179, Tyr319, Phe344, dan Tyr340, oleh karena itu ekstrak daun jawer kotok dapat dijadikan sebagai kandidat obat penghambat H4R.

Kata kunci: reseptor histamin H4, studi *in silico,* daun jawer kotok, *Plectranthus scutellarioides,* kuersetin

Introduction

Plectranthus scutellarioides (L.) R.Br. (family: Lamiaceae), grows widely in Indonesia, and has a long history of therapeutic usage in Indonesian traditional *jamu* to cure various diseases. The brownish purple leaves of Plecranthus (Figure 1) contain alkaloids, saponin, flavonoids, tannin, and volatile oils.

Quercetin (Figure 2) is a member of the bioflavonoid family, and is widely found throughout the plant kingdom (http://www. immunesupport.com/92fal004.htm). It has been reported contained in the water extract of *Trachelospermum jasminoides*, Apocynaceae (Sheu, *et al,* 2009), in the hydroalcoholic extract of *Nymphaea stellata*, Nymphaceae (Rakesh, *et al*, 2009), in the ethanol extract of *Plectranthus scutellarioides* (L.) R.Br., Lamiaceae (Moektiwardoyo, 2010), and in the methanol extract of *Juglans regia* L., Juglandaceae (Nabavi, *et al*, 2011).

Quercetin has been proven to exert antiinflammatory activity (Morikawa *et al*, 2003). Morikawa and colleagues examined the effect of quercetin on the inflammatory response induced by carrageenan in the rat. The rats were treated with either vehicle or quercetin at a dose of 10 mg/kg one hour before carrageenan challenge. Fourty-eight hours after carrageenan challenge, PGE2, TNF-alpha, RANTES, MIP-2 and the mRNA for cyclooxygenase-2 were proved to be suppressed in the quercetin-treated animals compared to those from vehicle-treated animals. The study indicated that the flavonols modulated the inflammatory response, at least in part, by modulating the prostanoid synthesis as well as cytokine production (Morikawa, *et al*, 2003).

Figure 1. *Plectranthus scutellarioides* (L.) R.Br.

Quercetin also has a strong affinity for mast cells and basophils. It tends to stabilize their cell membranes, preventing them from spilling their pro-inflammatory histamine/ serotonin into the surrounding blood and tissue in response to the IgE antibody (http://www .immunesupport.com/92fal004 .htm). The immune system plays an essential role in the homeostasis. In allergic diseases, however, the immune system recognizes harmless antigens as causative agents. Since the number of people suffering from allergy increases gradually, there is an unmet need for new, more potent antiallergic agents.

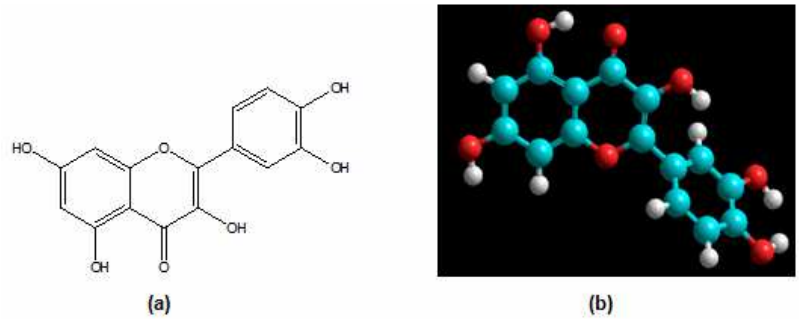

Figure 2. (a) 2D and (b) 3D structures of quercetin (Red balls represent oxygen atom, blue balls represent carbon atom, white balls represent hydrogen atoms, and sticks are the bonds between the atoms)

Literature data suggest that histamine H1 and H4 receptors are potential therapeutic targets against allergy. H1 and H4 antagonists may be used separately or in combination representing an effective therapeutic option for allergy and other immunological diseases (Kiss, 2008).

In this research, a determination of quercetin in Plecranthus leaves extract was performed and followed by a study of its interaction with histamine H4 receptor to understand its antiinflammatory activity.

Methodology

Plant material and extraction method

The fresh leaves of *Plectranthus scutellarioides* were collected from Bandung, West Java, Indonesia in 2010 and identified and authenticated at The Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Bandung, West Java, Indonesia. The leaves were sorted, washed, and dried at room temperature for three days, and were weighed (50 g) and ground before prior extraction. Extraction method was carried out in two steps by using a mixture of methanol and water (1:1) for 2 x 24 hours. The resultant extracts were concentrated in a rotary evaporator at 40-50°C until a viscous extract was obtained (30.8%). Flavonoids were separated by hydrolyzing the extract with a mixture of methanol and HCl 2 N (1:1) at 70°C for 30 minutes.

Determination of quercetin

Quercetin reference standard was purchased from Sigma-Aldrich and was dissolved in methanol for HPLC. The quercetin standard solution was prepared by transferring 1 mg of quercetin, accurately weighed, in a 100 mL volumetric flask and dissolved it in methanol to obtain a 10 µg/mL solution. Acetonitrile, phosphoric acid, and methanol for HPLC and other reagents used in this research were purchased from Merck.

Quercetine in the extract was determined by using reversed-phase HPLC method LC-10AT VP (Shimadzu), Atlantis Hilicsilica C18 (Waters®) 150 mm x 4.6 mm, 5 µm as stationary phase and a mixture of acetonitrile, phosphoric acid, and methanol (40:50:10) as mobile phase, flow rate 0.8 mL/minute. The detection was set at 339 nm. The chromatographic peak was confirmed by comparing the retention time of the Plecranthus leaves extract with that of quercetin standard.

In silico study of quercetine with histamine H4 receptor

The 3D structure model of H4R was built by using MODELLER 9v7. This step was performed by Ardiansyah *et al* and the pdb file of the protein was stored in the data base of Faculty of Pharmacy Universitas Padjadjaran (Ardiansyah, *et. al.,* 2010). *In silico* study of quercetine with histamine H4 receptor was performed by using AutoDock Tools 3.0.5 (http://mgltools.scripps.edu) at the active site of H4R. The receptor's active site was calculated by using Q-SiteFinder (http://www.modelling.leeds.ac.uk/qsitefinder).

Results and Discussion

The result of phytochemistry screening of the extract indicated that flavonoid was present in the extract. This data supported the theory that quercetin is a member of the bioflavonoid family which is widely found throughout the plant kingdom (http: //www. Immune-support.com/92fal004.htm).

The peak purity of quercetin was assessed by comparing the retention time of the

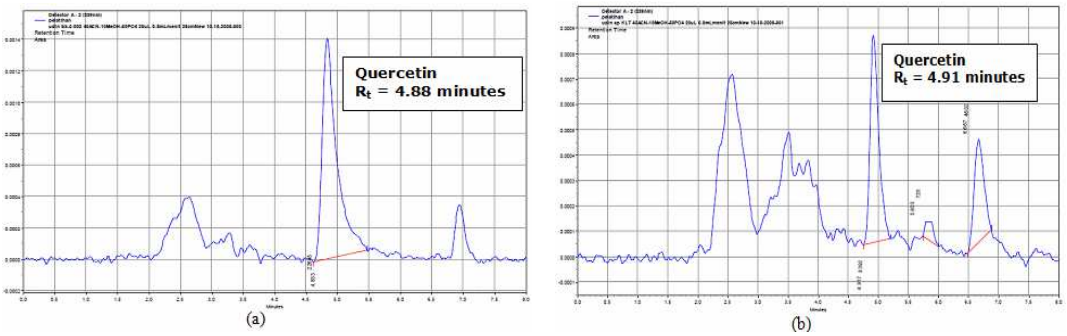

Figure 3. HPLC chromatograms of (a) quercetin standard and (b) Plecranthus leaves extract.

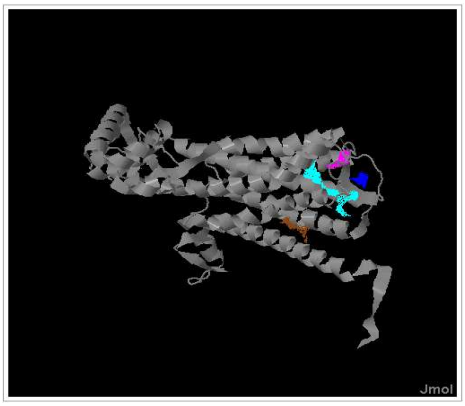

Figure 4. The active site of histamine H4 receptor (green color) as calculated by Q-SiteFinder

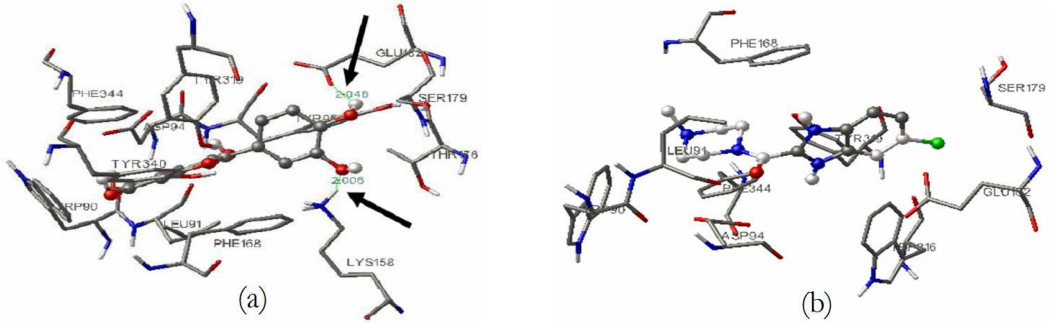

Figure 5. (a) Hydrogen bonds formed between quercetin and H4R (indicated by black arrows)
(b) Interaction of VUF6002 with H4R

chromatograms at peak start, peak apex and peak end positions. The identity of the quercetin band in the Plecranthus leaves extract's chromatogram was confirmed by the chromatogram obtained from the quercetin standard solution (Figure 3a and 3b) and by comparing their retention times.

The peak of quercetin standard was observed at 4.88 minutes, while quercetin in the extract was eluted at 4.91 minutes. The similarity of the retention time positively confirmed that quercetin was contained in Plecranthus leaves extract. Based on the calculation of area under curve, quercetin contained in Plecranthus leaves extract was 0.05%.

The 3D structure model of H4R, which was built by using MODELLER 9v7 (Ardiansyah, 2010), was uploaded to http://

Table I. *In silico* study of quercetin with H4R compared with VUF6002

Compound	Parameters Observed			
	Interaction energy (kcal/mol)	Ki (nM)	Hydrogen Bond	VDW
VUF6002	-10.74	70.7	No hydrogen bond formed	Trp90, Leu91, Asp94, Phe168, Ser179, Glu182, Tyr319, Phe344, Trp316
Quercetin	-10.25	86.1	H : O Glu182 (2.048 Å) O : HN Lys158 (2.005 Å)	Trp90, Leu91, Asp94, Tyr95, Phe168, Thr178, Ser179, Tyr319, Phe344, Tyr340

www .modelling .leeds.ac.uk/qsitefinder to be calculated its active site's position (Figure 4).

In silico study of quercetin with histamine H4 receptor was performed by using AutoDock Tools 3.0.5 (http://mgltools. Scripps.edu) at the active site of H4R and compared with VUF6002, a synthetic inhibitor which has been proved to inhibit H4R (de Esch, 2005).

The study showed that quercetin interacted with H4R *via* hydrogen bond formation with Lys158 (2.005 Å) and Glu182 (2.048 Å), and van der Waals interaction with Trp90, Leu91, Asp94, Tyr95, Phe168, Thr178, Ser179, Tyr319, Phe344, and Tyr340 (Figure 5 and Table I). The energy and inhibitory constant (Ki) of this interaction is comparative to that of VUF6002, means that quercetin might have a chance to inhibit histamine H4 receptor.

Conclusions

Quercetin contained in *Plecranthus leaves* extract was 0.05%. This compound is able to interact with H4R *via* hydrogen bond formation with Lys158 (2.006 Å) and Glu182 (2.048 Å), and van der Waals interaction with Trp90, Leu91, Asp94, Tyr95, Phe168, Thr178, Ser179, Tyr319, Phe344, and Tyr340, therefore Plecranthus leaves extract might have a chance to be used as histamine H4 receptor inhibitor.

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