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In Silico Studi *Orthosiphon aristatus* as Antioxidant

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ABSTRACT

Orthosiphon aristatus (O. aristatus) is a herbal plant that has antioxidant activity. This study was conducted in a preliminary study of the potential active compound *O. aristatus* with in silico software pyrex autodock vina method, and visualization using biovia discovery studio. Results from the study in silico showed that *O.aristatus* extract has potential as an antioxidant compound. Result value of binding affinity with SOD receptor proteins having more negative values compared to control compounds. The interaction of *O.aristatus* extract is quite potential as an antioxidant, through hydrogen bonding that is equal to control, namely vitamin E.

INTRODUCTION

Free radicals play an important role in aerobic metabolism. Antioxdants Reactive oxygen species (ROS) and other free radicals are responsible for many diseases, such as arteriosclerosis, heart disease, aging, and cancer. Antioxidant agents act as free radicals or reactive oxygen species (ROS) scavengers by stopping radical chain reactions. ROS can trigger lipid peroxidation as a precursor to oxidation process. In the body, there is an intracellular ROS system scavengers to inhibit further oxidation processes; and this includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and others.[1] Free radicals such as ROS, including hydroxyl radicals, superoxide anions, and hydrogen peroxide, play an important role in increasing the destruction of living tissues. The antioxidant activity of phenolic compounds is found mainly because it inhibits its redox through neutralization and free radical cooling.[2] Aerobic organisms normally constantly produce reactive oxygen species (ROS) as a by-product of aerobic metabolism that can play a role in various natural processes in the body. These oxygen derivates under normal circumstances will be neutralized by the enzyme's antioxidant defense system (superoxide dismutase, catalase, glutathione peroxidase) and non-enzyme antioxidants (vitamin C, vitamin E, gluglutation).[3] *O. aristatus* is a herbal plant from the Lamiaceae family that is easy to find in Southeast Asian countries such as the Philippines, Malaysia, Brunei, Thailand and Indonesia. The leaves are extensively processed to treat several dangerous diseases such as gonorrhea, diabetes, or kidney stone. About 116 compounds were isolated from *O. aristatus* and classified as essential oil, favonoid, or phenolic acid.[4] *O. aristatus* main constituents are sinensetin, eupatorine, and rosmarinic

acid which has a lot of pharmacological activity.[5] In addition to *O. aristatus* also contains isosinensetin, orthosiphol A-E, glycosides, kadein, phytosterols, predensin, paclitaxel, tannin, essential oils.[6] *O. aristatus* has a phytochemical composition with various pharmacological activities that can improve the level of human health. *O. aristatus* extract can also be a potential diabetic wound healing phytomedicin for further preclinical and clinical studies [7].

Flavonoid compounds can prevent free radicals and stabilize ROS by bonding with the free electrons of ROS and neutralize species oxygen to a stable form that can delay, prevent, or eliminate oxidative damage to target molecules, for example: proteins, lipids, and DNA due to free radicals.[8] In this preliminary study, researchers wanted to find out how much potential *O. aristatus* extracts have by using the in silico. In silico method began to be ogled because of the advantages of being cheap and the results are faster. In silico is a research method that utilizes computing and database technology to develop further research.[9] The receptor used is superoxide dismutase (SOD). The parameter used is binding affinity to determine the bonding power of exogenous antioxidant compounds with their receptors. Hydrogen bonds are also used in addition to bond strength through interaction with amino acid residues of receptor proteins through hydrogen bonding.[10]

MATERIALS AND METHODS

Research design is descriptive-analytical research. Identification of active compounds from *O. aristatus* extracts was obtained from previous research. The active compounds used are sinensetin, isosinensetin, orthosiphol A, orthosiphol B.[11] The active compounds of *O.aristatus* extract were obtained from previous research. The 3D structure of the active ingredient compound and control is downloaded from the Pub Chem (https://pubchem.ncbi.nlm.nih.gov) server.[12] The 3D structure of each is downloaded in sdf format, SOD receptor protein from human downloaded from (http://www.uniprot.org/).[13] The protein sequence obtained and modeling in https://swissmodel.expasy.org/.[14] The obtained protein is then downloaded and stored in pdb format. The docking process is carried out using a pyrx (autodock vina) program. The docking results are then visualized. To see the interaction between receptors and ligands use the Biovia Discovery Studio program.[15]

Affinity binding results are selected from the most negative values. Negative values indicate ligand conformation with the most stable receptors. The conclusion drawn is seen from the most minus affinity binding values because the smaller the affinity binding value, the binding ability between the ligand used and the stronger the receptor.[16]

RESULTS AND DISCUSSION

Potential Analysis of *O. aristatus* Extract as an antioxidant compound by in silico method.

The study in silico as a preliminary test predicts the potential of active compounds of *O. aristatus* by linking ligands which are the active compounds of *O.aristatus* with its receptor protein SOD. The docking results are shown as in table 1.

Table 1. Analysis of the potential of antioxidant active compounds from *O. aristatus* extracts against its receptor proteins by in silico method

Ligan ID	CID	Receptor	Binding affinity (kcal/mol)
A**scorbic acid***	54670067	SOD	-5,2
Tocopherol*	86472	SOD	-3,6
Sinensetin	145659	SOD	-4,6
Isosinensetin	632135	SOD	-4,7
Orthosiphol A	15385858	SOD	-4.0
Orthosiphol B	15385859	SOD	-5,7

*control

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From table 1 it is seen that the affinity binding values of *O.aristatus* extract have more negative values compared to tocopherol and ascorbic acid. In general, *O.aristatus* extract has a binding affinity value that is more negative and equal to its control. Result *O.aristatus* extract is quite potent as a source of antioxidants.

Analysis of Ligand Interactions of Active Compounds Extracted from *O. aristatus* with Superoxide dismutase as Receptor Proteins

Analysis of the potential of O.aristatus extracts active compounds namely sinensetin, isosinensetin, orthosiphol A, orthosiphol B shows the potential as antioxidant compounds seen from binding affinity values that are more negative and the same compared to the control. In addition to the analysis of its potential judging by the binding affinity value, it also looked at the interaction of ligands with amino acid residues from receptor proteins in silico. Analysis of interactions is done to find out the bond strength between these active compounds. Active compounds that are predicted to have strong bonds with target proteins are active compounds that have hydrogen bonds with amino acid residues of receptor proteins that are the same as the controls. Analysis of ligand interactions with active compounds is shown in table 2.

Table 2. Analysis of ligand interactions of active compounds extracting *O. aristatus* with Superoxide dismutase receptor proteins

Ligand ID	Receptor	Interaction
Ascorbid acid*	SOD	Hydrogen bond : Asp 110, Ser 106, Ser 108, Ile 105
		Van der Waals bond: Ser 103, Val 104, Asn 27, Gln 23, Gly 28, Ser 26, Gly 109, Cys 112
Tocopherol*	SOD	Hydrogen bond : A 3
		Van der Waals bond : Ala 153, Ala 2, Gln 154, Met 1, Phe 51, Glu 50, Lys 4, Glu 22, Lys 24
Sinensetin	SOD	Hydrogen bond : Phe 21, Ala 5
		Van der Waals bond : Val 8, Ala 56, Gly 57, Gly 52, Gly 62, Ile 152
Isosinensetin	SOD	Hydrogen bond : Ser 60
		Van der Waals bond : Lys 4, Gln 154, Asn 54
Orthosiphol A	SOD	Hydrogen bond: Ile 152, **Phe 51, Ala 153, Ala 2, Glu 50, Glu 22, Lys 24**
		Van der Waals bond : **Lys 4**
Orthosiphol B	SOD	Hydrogen bond : Ser 69, His 81, Asn 66
		Van der Waals bond :Pro 63, Arg 70, Lys 71, Thr 136, Glu 133

Bold and underline indicate the existence of a complex (SOD-ligand) has the same interaction with its control
* control

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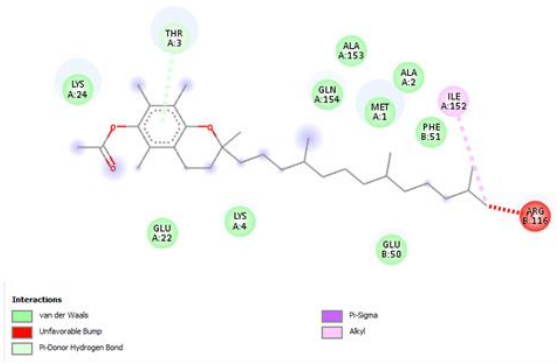

Figure 1. 2D Visualisasion of Sinensetin linking SOD

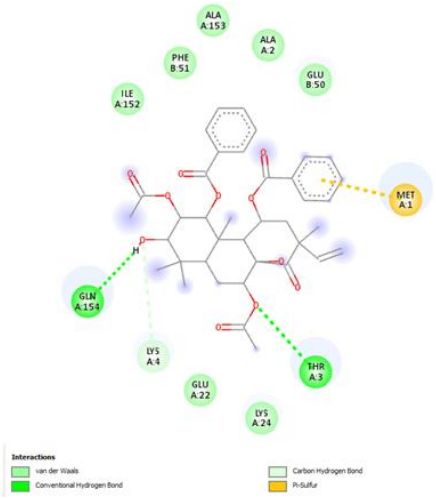

Figure 3. 2D Visualisasion of Orthosiphol A linking SOD

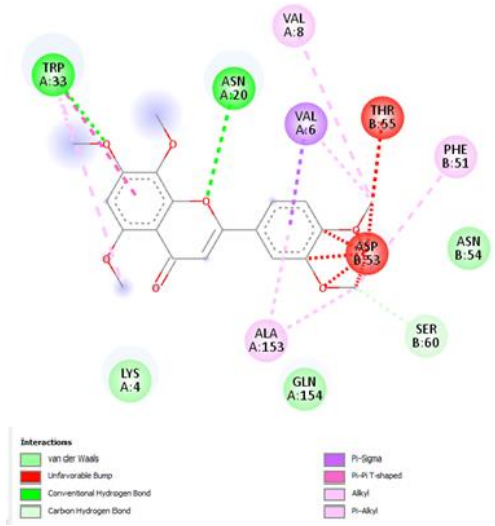

Figure 2. 2D Visualisasion of Isosinensetin linking SOD

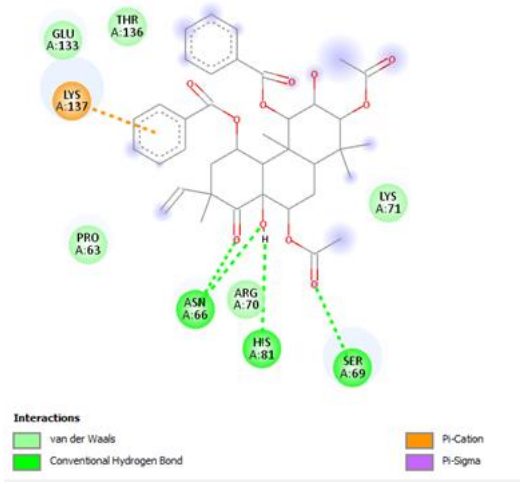

Figure 3. 2D Visualisasion of Orthosiphol B linking SOD

Recently, the molecular basis of the anti-inflammatory action of a flavonoid-rich chloroform extract fraction of O. aristatus leaves and its key bioactive compounds, eupatorin and sinensetin, has been elucidated. Eupatorin and sinensetin alleviated carrageenan-induced in flamation in mice. Furthermore, the two flavonoids attenuated the production of nitric oxide (NO), prostaglandins, and tumour necrosis factor a (TNF-oc) in lipopolysaccharide-activated J774 murine macrophage cells. TNF-oc is a key regulator of inflammatory response and plays a central role in the pathogenesis of inflammatory disorders. The findings thus support the hypothesis that inhibition of the release or production;of inflalftmatory mediators from activated inflammatory cells underlies the anti-inflammatory properties of eupatorin and sinensetin.[17] flavonoids can protect our body of the subsequent reactions of ROS and RNS by capturing ROS, blocking reactions propagation and stimulate the formation of endogenous antioxidants such as GPx, SOD and Catalase and reduce MDA levels due to the absence of lipid peroxidation (PUFA) and reduce 8-OHdG levels because HO* which normally enters the DNA reacts already captured by flavonoids. In addition, flavonoids can function as anti-inflammatory because flavonoids can inhibit formation of pro inflammatory cytokines such as TNF-α, IL-6, IL-1β and interferon-γ.[18]

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Antioxidant and Anti-inflammatory activity, four proteins structure has been selected for target identification and validation. Three targets for evaluation of antioxidant activity was selected based on pathways involving active participation. Research shows that many phytochemicals act as agonists and increase the activity of Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT). SOD, GPx and CAT agonists can increase their activity and thus can overcome stress-related ROS induction. Therefore, these three proteins SOD, GPx and CAT) have been selected as targets of phytochemical antioxidant activity.[1] Sinensetin is a polymethoxylate flavonoid that has strong anticancer activity and various other pharmacological benefits and has promising potential in terms of its intended toxicity activity. In this review, insights into the pharmacological activity of sinensetin and its mechanism of action serve as a useful resource for a more thorough and comprehensive understanding of sinensetin as a potential prime candidate for drug discovery.[16] *O. aristatus* contain a secondary metabolite sinensetin which belongs to the group of flavonoid compounds. Sinensetin has potential as an antiviral agent and immunomodulator.[19] in addition, the content of *O. aristatus* also contains tocopherol molecules that function as antioxidants. Recently, the tocopherol cyclase enzyme has been identified as a key enzyme for tocopherol biosynthesis which plays a key role in antioxidants against free radicals.[20]

CONCLUSIONS

Based on preliminary tests in silico extract *O. aristatus* compound has potential as an antioxidant. It proved by affinity binding values with amino acid residues from SOD have more negative compared to controls. From the interaction data, *O. aristatus* extract is quite potent as an antioxidant, through hydrogen bonding with amino acid residues: Phe 51, Ala 153, Ala 2, Glu 50, Glu 22, Lys 24 with SOD.

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