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IN SILICO STUDY OF ESTRAGOLE, APIOLE AND MYRISTICIN FROM *Nigella sativa* L. AS ANTIFUNGAL

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Abstract

Black cumin (*Nigella sativa* L.) is a plant that is widely used in world medicine. This is due to the large amount of chemical compounds that can have a positive effect on the body. Therefore, this study wants to see the potential use of chemical compounds from black cumin from the phenyl propanoid compounds, namely estragole, apiole and myristicin. Reverse docking study was perfomed using PyMOL Software v1.7.4.5 (Schrodinger), PyRx 0,8 software, SwissAdme Prediction and Discovery study 2019 client. The results of this study indicate that Myristicin can be a new drug candidate for anti-fungal based on the binding affinity value, namely -8.7 and close to the binding affinity value of e (a compound that is widely used as an anti-fungal).

Keywords : *Nigella Sativa, In Silico, Antifungal.*

Intoduction

Black cumin (*Nigella sativa* L.) is one of the most popular aromatic plants and is widely used as a nutraceutical and also used as medical food. This plant can grow in cold air conditions and can be consumed in the form of black cumin oil (Kiralan et al., 2014). Black cumin has positive effects for health (Sultan et al., 2009). Black cumin is most commonly used for nutraceutical purposes in phytotherapy (Karaman, 2020). This plant can be extracted into oil which has various bioactive compounds and has many health benefits.

Black cumin oil and also black cumin seeds are very influential on the oxidative reactions that occur in the fatty acid components (Solmaz Mohammed et al., 2017) and are very easily damaged during the storage process caused by air, sunlight and heat (Edris, 2011).

Black cumin has been a major subject in pharmaceutical and medicinal research in recent years. Several studies have shown that black cumin compounds can be used in various fields including antibacterial (Haiyan et al., 2016), anti-inflammatory (Al-Ghamdi, 2001), anti-tumor (Wei et al., 2012), analgestic and anti-depressant (Khanna et al., 1993), hypoglycemic (Al-Hader et al., 1993), muscle relaxant (Aqel & Shaheen, 1996), cytotoxic and immuno stimulants (Swamy & Tan, 2000).

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The results of these studies indicate that the compounds from black cumin have great potential in the search for new drugs. Anti-fungal compounds are an object of study that can be developed from the potential of this black cumin plant. Anti-fungal is a compound that functions to inhibit the growth of fungi. Anti-fungal is usually extracted or obtained from secondary metabolites of several plants and one that has the potential is the black cumin plant.

Based on research from (Perrins et al., 2005) on the chemical composition of black cumin, a total of 8 fatty acids and 32 bioactive compounds have been obtained which have great potential for use in pharmacology.

Myconazole is an anti-fungal drug that is often used in medicine to inhibit fungal growth. Its use is based on a certain dose. The antifungal therapy using Myconazole has shown a more efficient result when combined with the chlorhexidine digluconate compound (Andrade et al., 2015).

Estragole, apiole and myristicin are compounds contained in black cumin which are included in the Phenyl propanoid compounds (Perrins et al., 2005). Based on the data on the composition of the compounds contained in black cumin compounds, it is necessary to do an in silico anti-fungal test against estragole, apiole and myristicin compounds in black cumin plants.

Materials and Methods

1. Ligan Preparation

The compound used as an anti-fungal candidate is based on previous research from the GC-MS results of previous studies with details of 3 compound components (estragole, apiole and myristicin). The preparation process is carried out by downloading the test ligand file on the Pubchem website. Then the Pyrx application with the *Open Babel* program was prepared to minimize energy prior to docking.

2. *Target Selection*

To find the target protein candidates, a database search was performed:: Swiss Target Prediction (www.swisstargetprediction.ch). The validation was done using Uniport (https://www.uniprot.org). The protein preparation process was carried out by downloading the protein at RSCB PDB, in this case the protein used was Lanosterol 14 alpha demitylase with the protein code 4LXJ. Then the protein was prepared in the DS Visualizer application by removing the water component, ligand group, heatm to maintain stability during the locking process.

3. *Molecular Docking*

PyRx 0.8 was used for molecular docking. The reverse docking process ws performed using the Vina Wizard function integrated into PyRx 0.8 software, which reacts with protein target proteins Lanosterol 14 alfa demitilase and estragol, apiole dan myristicin as ligands.

4. Molecular Visualisation and small molecule interaction

PyMol v1.7.4.5 software was used to visualize and analyze the interaction between the ligand (estragol, apiol dan myristicin) and protein target (lanosterol 14 alfa demitilase).

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Results and Discussion

Nigella sativa L. produces several bioactive compounds which are very useful in medicine. Some of the compound components contained in black cumin are Nonterpenoid hydrocarbons, Monoterpenoid hydrocarbons, Monoterpenoid alcohols, Sesquiterpenoid hydrocarbons and Phenyl propanoid compounds (Perrins et al., 2005). The contents of these compounds have been tested before and have potential as antibacterial, anti-inflammatory, anti-tumor, analgestic, anti-depressant, hypoglycemic, muscle relaxant, cytotoxic and immuno stimulant. One of the components that need to be developed is Phenyl propanoid compounds. Estragole, apiole and myristicin are compounds from the Phenyl propanoid compounds that have the potential to be tested as anti-fungi.

Estragole (1-methoxy-4-prop-2-enylbenzene) is an organic compound that is widely found in plants. One of them has been extracted from Tarragon (*Artemisia dracunculus*), in addition to other plants such as *Croton zehntneri* and *Pimpinella anisum* (Andrade et al., 2015) (Hu et al., 2019). Several studies have shown that estragole can be used as anti-microbial and anti-bacterial or their antibiotic activity modification (Haiyan et al., 2016), antifungal (Li et al., 2020), antioxidant (Ivanov et al., 2019), as well as insecticide activity (Rosa et al., 2020).

The compound 4,7-dimethoxy-5- (2-propen-1-yl) -1,3-benzodioxole or apiole has been isolated from various types of plants including from Petroselinum sativum which has been studied as potential antitumor (Wei et al., 2012). This apiole compound is also found in black cumin which can be studied as an antifungal candidate.

The latter is a myrsticin compound which is an aromatic compound with possible targets for this component, such as organic acids, esters, and aromatic compounds in bacterial cell walls. Myristicin and elemicin compounds are efficacious compounds in nutmeg seeds. Myristica fragrans seeds have an antibacterial effect (Ibrahim et al., 2013).

Lanosterol-14α-demethylase is a target protein that can be found in fungi, plants, animals, humans, and mycobacteria. This enzyme is required for biosynthesis of sterol in eukaryotes and is the major target for azole antifungal agents (Friggeri et al., 2019). In mammals, lanosterol-14α-demethylase is the enzyme that catalyzes lanosterol to cholesterol conversion, which is necessary to maintain a variety of metabolic functions (Chang et al., 2017). An ideal antifungal agent should have minimal effect on human CYP51 enzymes while keeping potent inhibition of fungal enzyme to reduce the side effects. Lanosterol14α-demethylase consists of an iron protoporphyrin unit in its active site (Monk et al., 2020).

From the results of molecular docking between the test ligands (Estragole, Apiole and Myristisin) against the target protein Lanosterol-14α-demethylase, the binding affinity and RMSD values indicated that myrticin compounds could be candidates for anti-fungal drugs compared to the other two test ligands.

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Table 1. Binding affinity of fruit compounds

Ligan with Receptor	The Binding Affinity
Myconazole and Lanosterol-14α-demethylase	-9,8
Myrtisin and Lanosterol-14α-demethylase	-8,7
Estragole and Lanosterol-14α-demethylase	-6,6
Apiole and Lanosterol-14α-demethylase	-6,4

Table 1. shows the binding affinity value from the docking results of 4 compounds from plants and the syinthetic compound of Jintan Hitam (*Nigella sativa* L.). The binding affinity value shows the stability between the compound and the receptor. A low affinity value indicates a high bond potential or has much better potential as an antifungal.

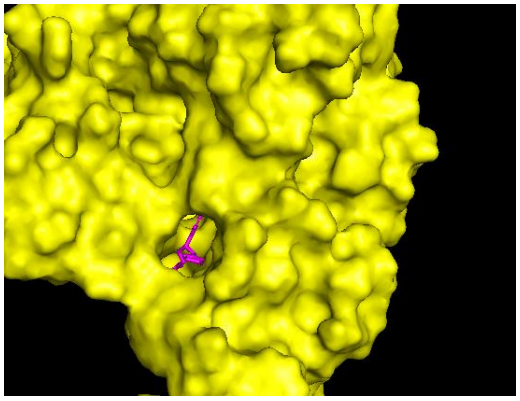

Figure 1. The binding site of Myconazole (Pink) and Lanosterol-14α-demethylase (Yellow)

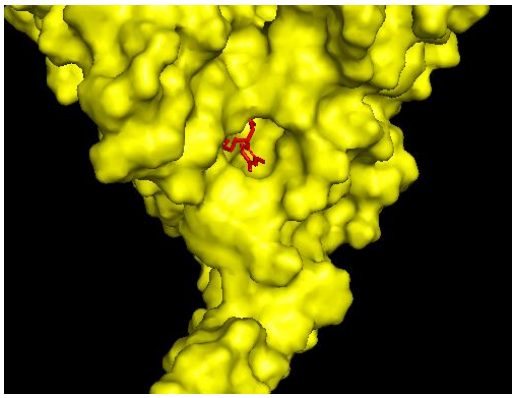

Figure 2. The binding site of Myristicin (Red) and Lanosterol-14α-demethylase (Yellow)

The visualization process of the black cumin compound which has the best binding affinity is done using the Pymol application (Figures 1 and 2). The visualization results show the 3D form of the Myrsticin and Miconazole compounds, where these compounds are very stable bind to the active site of the target protein Lanosterol-14α-demethylase. This shows great potential for Myristicin to be used as an anti-fungal candidate in further treatment.

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Conclusion

This study has proven that Myristicin from black cumin docking on the protein Lanosterol-14α-demethylase has great potential as an anti-fungal drug candidate as indicated by the binding affinity value of -8.7 and this value is close to the miconazole value of -9.7 as a drug. which is often used for anti-fungal. Based on the value of biding affinity and visualization of the bond Myristicin has the potential to be an excellent anti-fungal.

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