**In silico** molecular docking and **in vitro** analysis of ethanolic extract
*Ocimum sanctum* Linn.: Inhibitory and apoptotic effects against non-small cell lung cancer

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**Abstract**

**Background and Aim:** Lung cancer, especially non-small cell lung cancer (NSCLC), has been identified as the leading cause of cancer deaths worldwide. The mortality rate from lung cancer has been estimated to be 18.4%. Until now, conventional treatments have not yielded optimal results, thus necessitating an investigation into the use of traditional herbal plants as potential candidates for its treatment. This study aimed to determine the inhibitory and apoptotic activity of the ethanolic extract from *Ocimum sanctum* Linn. (EEOS) by *in silico* molecular docking and through *in vitro* studies using NSCLC cells (A549 cell line).

**Materials and Methods:** Dried simplicia of *Ocimum sanctum* was converted into EEOS using the maceration method. Spectrophotometry was then employed to analyze the EEOS compound. The known main active compounds were further analyzed for inhibitory and apoptotic effects on gene signaling using *in silico* molecular docking involving the downloading of active compounds from PubChem and target proteins from the Protein Data Bank; the active compounds and proteins were then prepared using the Discovery Studio software v. 19.0.0 and the PyRX 0.8 program, interacted with the HEX 8.0.0 program, and visualized with the Discovery Studio Visualizer v. 19.0. Finally, an *in vitro* analysis was performed using an antiproliferative-cytotoxic test (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay in the NSCLC A549 cell line).

**Results:** The analysis revealed that the active compounds in the ethanolic extract were dominated by quercetin (flavonoids) (47.23% b/b) and eugenol (phenolic) (12.14% b/b). These active compounds interacted with the active sites (residual amino acids of the αβ3 integrin, α5β1 integrin, caspase-3, caspase-9, and vascular endothelial growth factor). Hydrogen bonds and Pi-cation and Pi-alkyl interactions were involved in the relationships between the active compounds and the active sites and thus may reveal an antioxidant property of the extract. Furthermore, *in vitro* analysis showed the inhibitory and antiproliferative effects of the EEOS against non-small cell cancer (A549).

**Conclusion:** Taken together, our data showed the ability of EEOS as an inhibitor and apoptotic agent for lung cancer; however, further research is needed to determine the exact mechanism of EEOS as an herbal medication.

**Keywords:** *in vitro*, lung cancer, molecular docking, *Ocimum sanctum*.

**Introduction**

Non-small cell lung cancer (NSCLC), a type of lung cancer, has been determined to be a significant cause of cancer death worldwide. Data from the Global Cancer Statistics show that the mortality rate due to lung cancer was 18.4% in 2018, with 1.8 million deaths and 2.1 million new lung cancer cases recorded [1]. Lung cancer is deemed the most fatal compared with other types of cancer. Although lung cancer is strongly correlated with smoking, adenocarcinoma of NSCLC can also occur in non-smokers, thus increasing the prevalence of lung cancer in all populations.

At present, most lung cancer treatments involve conventional therapy approaches (chemotherapy/medication and surgery). Standard medication therapy in the treatment of NSCLC cases is the use of cisplatin in combination with pemetrexed [2]. In addition, treatment with antibody engineering systems such as pembrolizumab, nivolumab, and atezolizumab is now actively used [3,4]; however, the administration of these drugs has been noted to have drawbacks.
Aside from being expensive, these drugs induce a chemoresistant effect that appears in the treatment of lung and breast cancer. In addition, pembrolizumab and nivolumab are known to cause oral mucositis, rash, and pruritus caused by immune-related adverse effects [3,5]. Combination therapy such as radiotherapy with pembrolizumab is known to have side effects that increase the drug’s toxic effect [5].

Because of the disadvantages of these medications, there is a need to explore natural herbal traditional remedies. The system of traditional medicine has been used for thousands of years to prevent, diagnose, and treat several acute and chronic diseases. *Ocimum sanctum* Linn. is a traditional medicine commonly found in Indonesia and Asian countries. Various species of *Ocimum* are known to provide many health benefits, including anti-inflammatory, anti-fatigue, antitussive, antiseptic, antispasmodic, neuroprotective, and neuroproliferative activities [6-11], but until recently, their active mechanisms, safety, and dosage have not been determined.

Thus, in this study, we aimed to determine the dynamic majority content of the ethanolic extract from *O. sanctum* Linn. (EEOS) and analyze the extract’s effect against NSCLC by in silico molecular docking and in vitro studies.

**Materials and Methods**

**Ethical approval**

The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (00053/EC/FKH/Int./2021).

**Study period and location**

The study was conducted from January to June 2021 at the Department of Pharmacology Faculty of Medicine, Public Health, and Nursing and Integrated Laboratory for Research and Testing Laboratory, Universitas Gadjah Mada.

**Preparation of ethanolic extract**

*O. sanctum* Linn. leaves and dried simplicia were derived from the herbal company CV Merapi Farma Herbal, Yogyakarta, Indonesia. The leaves were identified at the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. The dried simplicia was then made into an ethanolic extract at the Integrated Laboratory for Research and Testing Laboratory, Universitas Gadjah Mada.

The ethanolic extracts were made using the maceration technique. A volume of 4000 mL of ethanol 96% (Merck) was added to the simplicia of *O. sanctum*. The mixture was stirred for 30 min, allowed to stand 48 h, and then filtered twice. The filtrate was evaporated using a vacuum rotary evaporator (Buchi, Flawil, Switzerland) at 70°C with occasional stirring. A final extract weight of 26.48 g of *O. sanctum* leaf extract was obtained.

**Spectrophotometry analysis**

**Flavonoid**

EEOS, weighing 50 mg, was placed into a 10 mL test tube, in which 0.3 mL of 5% sodium nitrite (Merck) was subsequently added. After 5 min, 0.6 mL of 10% aluminum chloride (Merck) and 2 mL of 1 M sodium hydroxide (Merck) were added to the solution along with the addition of up to 10 mL of distilled water. The mixture was then transferred into a cuvette and measured through spectrophotometry (Shimadzu, Kyoto, Japan) at a wavelength of 510 nm. Quantification of the total compound was calculated using the following formula:

\[
\text{value (ppm)} = \frac{\text{total compound}}{\text{end volume (mL)}} \times \frac{\text{weight of sample (g)}}{\text{dilution factor}} \times 100\%
\]

**Phenol**

The EEOS weighed 50 mg. To the extract, 0.5 mL of Folin–Ciocalteu (Merck) reagent and 7.5 mL of aquabides were added. The mixture was allowed to stand for 10 min at 24°C followed by the addition of 1.5 mL of 20% sodium carbonate (Merck). Sterile water was then added to achieve a final volume of 10 mL. The solution mixture was transferred into a cuvette and measured on a spectrophotometer (Shimadzu) at a wavelength of 760 nm. Quantification of the total compound was calculated using the following formula:

\[
\text{value (ppm)} = \frac{\text{total compound}}{\text{end volume (mL)}} \times \frac{\text{weight of sample (g)}}{\text{dilution factor}} \times 100\%
\]

**In silico molecular docking preparation**

The two bioactive compounds with the largest amounts in the EEOS were identified as eugenol (CID_3314) and quercetin (CID_5280343), downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

**Protein target preparation**

The target proteins used in this in silico study were integrin \(\alpha v \beta 3\) (Protein Data Bank [PDB] ID: 4g1e), integrin \(\alpha 5 \beta 1\) (PDB ID: 3v13), caspase-9 (PDB ID: 1jxq), caspase-3 (PDB ID: 1nms), and vascular endothelial growth factor (VEGF) (PDB ID: 1eab).
Eugenol and quercetin are the dominant components in the EEOS

The experimental analysis using an ultraviolet-visible spectrophotometer showed that EEOS consisted mostly of the active compound of flavonoid derivatives, that is, quercetin (47.23% b/b), and phenol derivatives, that is, eugenol (12.14% b/b) (Table-1).

Quercetin binds to the active sites of integrins αβ3 and αβ1

Four amino acid residues depict the binding of quercetin and integrin αβ3 at Lys409, Arg261, Tyr224, Arg99, and Ser160, yielded bond energy of 257.3 kJ/mol (Figure-1 and Table-2). Meanwhile, the interaction between quercetin and integrin αβ1 showed five interacting amino acid residues (Thr258, Gly255, Asn256, Leu257, and Ser277) and yielded bond energy of −226.1 kJ/mol (Figure-2 and Table-2). The bonding between quercetin and αβ3 and αβ1 occurred through hydrogen and carbon-hydrogen bonds (Table-2).

Chemical interactions occurred between quercetin and caspase-3/caspase-9

The interaction of caspase-3 with quercetin showed six amino acid residues bind to the amino acid residues of quercetin, namely, Thr270, Arg241, Thr152, Cys183, and Thr182.

Table-1: Bioactive compounds with the largest composition contained in the ethanolic extract of Ocimum sanctum Linn.

| No. | Name of compound | Concentration | Unit | Method |
|-----|------------------|---------------|------|--------|
| 1.  | Quercetin (Flavonoid) | 47.23 | %b/b | Spectrophotometry UV-Vis |
| 2.  | Eugenol (Phenol) | 12.14 | %b/b | Spectrophotometry UV-Vis |
Gly153, Lys271, and Ile187, with total energy formed of −262.9 kJ/mol (Figure-3 and Table-2). In addition, quer cetin bound to caspase-9 at the amino acid residue Glu187 and yielded energy of −115.8 kJ/mol (Figure-4 and Table-2). The interaction between quercetin and caspase-3 and caspase-9 was formed by hydrogen bonds and Pi-cation, P-sigma, and Pi-alkyl interactions (Table-2).

Quercetin binds to the active site of VEGF
Quercetin interacted with the amino acid residues in VEGF, namely, Gln79, Pro49, and Lys48, Gly153, Lys271, and Ile187, with total energy formed of −262.9 kJ/mol (Figure-3 and Table-2). In addition, quercetin bound to caspase-9 at the amino acid residue Glu187 and yielded energy of −115.8 kJ/mol (Figure-4 and Table-2). The interaction between quercetin and caspase-3 and caspase-9 was formed by hydrogen bonds and Pi-cation, P-sigma, and Pi-alkyl interactions (Table-2).

Quercetin binds to the active site of VEGF
Quercetin interacted with the amino acid residues in VEGF, namely, Gln79, Pro49, and Lys48,
### Table 2: Interaction, chemical bond, and binding energy between quercetin and integrins αβ3, integrins αβ1, Caspase-3, Caspase-9, and VEGF.

| Ligands | Protein | Binding energy (kJ/mol) | Point Interactions | Category | Type | Donor | Acceptor |
|---------|---------|------------------------|--------------------|----------|------|-------|----------|
| Quercetin αβ3 | −257.3 | Hydrogen Bond | Conventional Hydrogen Bond | A: LYS409:H22 | :UNK0:O3 | B: ARG261:HH11 | :UNK0:07 |
| Quercetin αβ3 | −257.3 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:O7 | :UNK0:H29 | :UNK0:H29 | :UNK0:O4 |
| Quercetin αβ3 | −257.3 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:O4 | :UNK0:H31 – A: TYR224:0 | :UNK0:H31 | A: TYR224:0 |
| Quercetin αβ3 | −257.3 | Hydrogen Bond | Carbon Hydrogen Bond | :UNK0:CD | :UNK0:04 | A: SER160:CB | :UNK0:06 |
| Quercetin αβ3 | −226.1 | Hydrogen Bond | Conventional Hydrogen Bond | A: THR258:HN | :UNK0:O2 | B: ARG261:HH11 | :UNK0:07 |
| Quercetin αβ3 | −226.1 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:O2 | :UNK0:H28 – A: GLY255:O | :UNK0:H28 | A: GLY255:O |
| Quercetin αβ3 | −226.1 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:O4 | :UNK0:H29 | :UNK0:H29 | :UNK0:O4 |
| Quercetin αβ3 | −226.1 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:H31 – A: ASN256:OD1 | :UNK0:H31 | A: ASN256:OD1 |
| Quercetin αβ3 | −226.1 | Hydrogen Bond | Pi-Donor Hydrogen Bond | A: LEU257:HN | :UNK0 | A: LEU257:HN | :UNK0 |
| Quercetin αβ3 | −226.1 | Hydrophobic Bond | Pi-Alkyl | :UNK0 – A: LEU257 | Biochemistry | B: SER227:HG | :UNK0:O3 |
| Quercetin Caspase 3 | −262.9 | Hydrogen Bond | Conventional Hydrogen Bond | A: THR270:HG1 | :UNK0:05 | B: ARG241:HE | :UNK0:03 |
| Quercetin Caspase 3 | −262.9 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:03 | :UNK0:H29 | :UNK0:H29 | :UNK0:04 |
| Quercetin Caspase 3 | −262.9 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:04 | :UNK0:H32 – A: THR152:O | :UNK0:H32 | A: THR152:O |
| Quercetin Caspase 3 | −262.9 | Hydrogen Bond | Carbon Hydrogen Bond | A: GLY153:CA | :UNK0:O1 | B: ARG241:HE | :UNK0:03 |
| Quercetin Caspase 3 | −115.8 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:H29 | :UNK0:04 | B: GLU187:CG | :UNK0:05 |
| Quercetin Caspase 3 | −115.8 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:O5 | :UNK0:H28 – A: GLN79:O | :UNK0:H28 | A: GLN79:O |
| Quercetin VEGF | −213.4 | Hydrogen Bond | Conventional Hydrogen Bond | :UNK0:H29 | :UNK0:04 | A: PRO49:CD | :UNK0:01 |
| Quercetin VEGF | −213.4 | Hydrogen Bond | Carbon Hydrogen Bond | :UNK0:04 | :UNK0:01 | A: PRO49:CD | :UNK0:01 |
| Quercetin VEGF | −213.4 | Hydrophobic Bond | Pi-Sigma | A: GLN79:O | :UNK0 | A: GLN79:O | :UNK0:01 |
| Quercetin VEGF | −213.4 | Hydrophobic Bond | Pi-Alkyl | A: PRO49:CD | :UNK0 | A: PRO49:CD | :UNK0:01 |
| Quercetin VEGF | −213.4 | Hydrophobic Bond | Pi-Alkyl | :UNK0 – A: PRO49 | Biochemistry | B: GLU187:CG | :UNK0:05 |
| Quercetin VEGF | −213.4 | Hydrophobic Bond | Pi-Alkyl | A: GLN79:O | :UNK0 | A: PRO49:CD | :UNK0:01 |
| Quercetin VEGF | −213.4 | Hydrophobic Bond | Pi-Alkyl | :UNK0 – A: PRO49 | Biochemistry | B: GLU187:CG | :UNK0:05 |
and produced energy of −213.4 kJ/mol (Figure-5 and Table-2). Hydrogen bonds and Pi-cation, P-sigma, and Pi-alkyl interactions were the foundation of the chemical interactions between quercetin and VEGF (Table-2).

**Eugenol binds to the active sites of integrins αvβ3 and α5β1**

Three amino acid residues were visualized from the interaction between eugenol and the αvβ3 integrin, namely, Ser342, Tyr406, and Arg261; these bonds produced affinity energy of 181.6 kJ/mol (Figure-6 and Table-3). Meanwhile, the active compound in eugenol only bound to α5β1’s amino acid residue, Leu257, yielded energy of −169.2 kJ/mol (Figure-7 and Table-3). Hydrogen bonds and Pi-cation interactions established the chemical interactions between eugenol and integrins αvβ3 and α5β1 (Table-3).

**Chemical interactions occurred between eugenol and caspase-3/caspase-9**

The interaction of the eugenol ligand and protein caspase-3 produced one amino acid residue (Arg241) that bound the eugenol ligand (Figure-8), yielded energy of...
Furthermore, the binding of eugenol and caspase-9 involved two amino acid residues that bound to eugenol on the active site of caspase-9, namely, Glu259 and Leu240 (Figure-9). The interaction of eugenol and caspase-9 resulted in binding energy of approximately −90.9 kJ/mol (Table-3). The interaction between eugenol and caspase-3/caspase-9 was established through hydrogen bonds and Pi-alkyl interactions (Table-3).

Eugenol bound to the active site of VEGF

The interaction of the eugenol compounds and the VEGF protein indicated the involvement of three amino acid residues (Leu97, Glu38, and Asn75) that interacted with the eugenol compounds through Pi-alkyl and hydrophobic interactions (Figure-10 and Table-3). This interaction yielded a bond energy of −162 kJ/mol (Table-3).

Decreasing the viability of A549 cells in the presence of EEOS

NSCLC cells were cultured to evaluate the ability of EEOS to inhibit the cells’ proliferation and adhesion to the extracellular matrix. Our results showed that EEOS significantly exhibited a cytotoxic
effect in human A549 cells, demonstrated by the percent-mean viability decrement in a concentration-dependent manner similar to cisplatin, and compared with the untreated control. The optimal concentration of EEOS was 200 µg/mL; at this EEOS concentration, there was a smaller number of viable A549 cells than the number seen with other concentrations of EEOS (50, 70, and 100 µg/mL) (Figures-11 and 12).

**Discussion**

Lung cancer remains the leading cause of cancer death in men and women worldwide [12]. In the past few years, the use of herbal medicine has continued to increase; currently, it is a trend to use herbal medicines concurrently with traditionally established treatments for cancer. One of the most
popular herbs for medicinal uses is *O. sanctum* Linn.; however, not much has been known about its use in the treatment of lung cancer. In this current study, we used *in silico* molecular docking and *in vitro* approaches to determine the mechanism of action of the EEOS.

Spectrophotometry analysis showed that EEOS contained two primary compounds, that is, quercetin and eugenol, belonging to the flavonoid group and the phenol group, respectively (Table-1). Some research has shown that natural sources (eugenol, caffeic acid, gallic acid, apigenin, quercetin, and rosmarinic acid) have therapeutic benefits in treating various diseases, including cancer [13-17]. *In silico* molecular docking analysis showed that quercetin and eugenol were able to bind to the active site of the αvβ3 integrin (Arg99, Arg261, Tyr224, Lys409, Ser342, Tyr406, and Arg261) and α5β1 integrin (Thr258, Gly255, Asn256, Leu257, Ser277, and Leu257). Integrins are known to play a crucial role in mediating the adhesion of epithelial

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**Figure-8:** The visualization of the three-dimensional and two-dimensional interaction between phenolic compounds (eugenol) and caspase-3. (a) Three-dimensional structural interaction between the ligand eugenol (blue) and caspase-3 (yellow). (b) This interaction is established by hydrophobic hydrogen bonds on the active site of caspase-3, namely, Arg241, which is seen in the two-dimensional (b) and three-dimensional (c) structures.

**Figure-9:** The visualization of the three-dimensional and two-dimensional interaction between phenolic compounds (eugenol) and caspase-9. (a) Three-dimensional structural interaction between the ligand eugenol (blue) and caspase-9 (green). (b) This interaction is established by hydrophobic hydrogen bonds on the active site of caspase-9, namely, Glu259 and Leu240, which can be seen in the two-dimensional (b) and three-dimensional (c) structures.
cells to basement membranes. They also contribute to the migration, proliferation, and survival of tumor cells [18]. Furthermore, tumor cell expression of the integrins avβ3, a5β1, a6β4, a4β1, and vβ6 correlates with disease progression in various tumor types and is associated with cancer [18-20]. The adhesion of integrins to the extracellular matrix (ECM) provides the necessary traction for tumor cells’ invasion. Integrins contribute to tumor cells’ invasion by regulating the localization and activity of matrix-degrading proteases, such as matrix metalloprotease 2 (MMP2) and urokinase-type plasminogen activator (uPA). Integrin-mediated migration generally requires focal adhesion kinase and Src family kinase signaling [18,21,22]. Cancer treatment could be promoted by inhibiting the activity of the integrin complex [22,23]. In addition, integrins physically associate laterally with cell membrane proteins (e.g., CD151 or CD47) to elicit or modulate signaling [24,25]. Data from this study indicated the potential for inhibition by the ligand on the integrin avβ3 and integrin a5β1 proteins. Inhibition of the integrin complex would impact the inhibition of the extracellular matrix adhesion (ECM) process and limit tumor cells’ invasion. In addition, inhibition of the process and activity of proteases and activators may occur. As a result, endothelial and epithelial cells rapidly undergo anoikis followed by inflammation and apoptosis when adhesion is disturbed [26,27]. The excessive growth of cancer cells is thus kept in check.

In addition, in silico molecular docking revealed the interaction between quercetin and eugenol with the active sites of VEGF, at positions Gln79, Pro49,
proteins, Thr270, Arg241, Thr152, Gly153, Lys271, interact with the active sites of caspase-3 and caspase-9. It was found that quercetin and eugenol were able to inhibit apoptosis. Based on our molecular docking, α-antioxidant activity of quercetin and eugenol [16,35]. Binding interaction also supported the hydrogen's stability of the bond structure [31-34]. Moreover, the interactions, which significantly contributed to the hydrogen bonds and Pi-cation, Pi-sigma, and Pi-alkyl interactions to VEGF resulted from the interaction of conventional drugs [12]. The binding between the active compounds in EEOS and the VEGF site through conventional tors [12]. The binding between the active compounds in EEOS and the VEGF site through conventional hydrogen bonds is expected to inhibit the activity and stimulation. Our data in line with the previous studies showing that the main contents of EEOS, namely, flavonoids and eugenol, were able to inhibit cells’ attachment to the extracellular matrix. EEOS may thus inhibit the adhesion of the NSCLC (A549) cells in the same way the conventional commercial drug cisplatin (used in this experiment) does. EEOS’s mechanism of action may inhibit adhesion, invasion, and cell migration, thus triggering anoikis and apoptosis in the A549 cell line.

Conclusion

Our data revealed that EEOS could act as both an antiproliferative and apoptotic agent on NSCLC cells in in silico molecular docking and in vitro experiments. Nevertheless, further investigation of the mechanism, dosage, and other potential benefits of EEOS as a possible herbal medication to prevent NSCLC is needed.

Authors’ Contributions

UK and TSDR: Experimental work and data analysis. NDAA: Literature study and wrote the manuscript. HW: Design of the experiment, literature study, wrote the concept of the original manuscript, and reviewing and editing of the final manuscript. DLK: Writing, reviewing, and editing of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.
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