Vol 7 No 3 October 2022

E-ISSN: 2528-410X

RESEARCH ARTICLE

Anti-Cervical Cancer Study of a Labdane-type Diterpene Obtained from Legundi Fruit (Vitex triolia L.) Targeting the Bcl-2 Gene

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Abstract: The development and induction of cervical cancer carcinogenesis are linked to sexually transmitted Human Papillomavirus (HPV) infection, and can affect human genes, particularly those involved in the apoptotic process, one of which is the Bcl-2 gene. Several synthetic compounds, including pyrimidineylpiperazine, phenylpyrazole, kendomycin, and navitoclax, have been shown to be effective in inhibiting the antiapoptotic activity of Bcl-2, but it is the labdane-type diterpene content of the natural legundi fruit plant (Vitex trifolia L.) that has anticancer bioactivity, particularly in the process of genetic mutations, Apoptotic inhibition and therapeutic failure have yet to be discovered, particularly in cervical cancer. The study's goal was to investigate the mechanism of interaction (molecular docking) of labdane type-diterpene compounds with the antiapoptotic protein Bcl-2. In silico (docking molecular) research was used to determine the interaction between legundi fruit ligands and the Bcl-2 target protein in cervical cancer. The docking analysis results will then be visualized with the Discovery Studio 4.1, LigPlot+, and Ligand Scout 3.1 software. The interaction of proteins and ligands was studied to determine the number and type of bonds formed, such as hydrogen and hydrophobic bonds. The results were as follows: Gibbs energy -7.8, pKi 1.91 m, 10 hydrophobic bonds, and hydrophobic residues Leu80, Leu82, Thr83, Thr86, Ala87, Cys90, Met118, Glu121, Ser122, and Leu130. The findings presented here suggest that labdane-type diterpenes are powerful anticancer agents capable of inducing apoptosis and inhibiting the antiapoptotic action of the Bcl-2 gene, which should be investigated further in (pre)clinical studies.

Keywords: Bcl-2, legundi fruit, cervical cancer, Labdane-type diterpene, Vitex trifolia L.

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E-ISSN: 2528-410X

INTRODUCTION

Cervical cancer is the fourth most common malignancy in women, with 604,100 new cases and 341,800 deaths each year.[1,2] The use of adequate therapy despite the fact that therapy failure has been discovered. Local recurrence occurs at a rate of 10-20% in the early stages and 40-60% in the advanced stages.[3] Cancer cells can develop resistance to radiation and chemotherapy. Apoptosis inhibition is one of several mechanisms that can lead to resistance.[4]

Human Papillomavirus (HPV) infection can affect human genes, particularly those involved in apoptosis (Bcl-2, p53, pRb, Bcl-XL, and BAX), the cell cycle, and proliferation (Ki-67, OVCA-1, cyclin D-1, p53, and p63). [5–7] The persistence of the viral oncogenes E6 and E7 as a result of HPV Deoxyribonucleic Acid (DNA) integration into the cervical epithelial genome leads to uncontrolled proliferation and carcinogenesis.[6,7] The antiapoptotic protein Bcl-2 is expressed more frequently in cancer cells when genetic instability and mutations in apoptotic genes, including Bcl-2, are present, indicating the emergence of neoplastic processes.[6,8,9] Positive antiapoptotic Bcl-2 staining is associated with a lower 5-year survival rate in patients with initial and recurring cancer.[10,11]

The use of natural phytochemicals can help to reduce therapeutic problems such as the emergence of resistance, but information is still limited. Legundi is one of the natural agents that has been studied for its anticancer properties (Vitex trifolia L.). Legundi fruit ethanol extract has been shown in skin cancer to inhibit proliferative activity, tumor growth, and p53 expression.[12–14] The flavonoids in legundi leaf extract can inhibit cancer cell proliferation in mice, but it can also activate apoptosis.[15] Another study found that the active ingredient in legundi, rotundifuran (labdane-type diterpene), can inhibit cervical cancer proliferation in HeLa and Siha cells.[16]

The study of labdane-type diterpene of legundi in cervical cancer is still in its early stages, so more research is needed before it can be considered as an adjunct therapy to improve survival rates. In silico interactions (molecule docking studies) on apoptotic induction activity will be reported to demonstrate whether labdane-type diterpene compounds with potential as cervical anticancer agents observed in silico can be followed up with in vivo studies.

METHODS

The legundi fruit (Vitex trifolia L.) used in the study was identified at Universitas Sumatera Utara's Herbarium Medanese (MEDA) Laboratory. The maceration process was then performed by soaking two thousand five hundred grams of legundi in 96% ethanol (1: 10) for three days. After that, it was vacuum filtered with Whatman filter paper no.40 and evaporated with a rotary evaporator T=550C, P=80 mBar.

The type of functional group in an isolated plant tissue was determined using

E-ISSN: 2528-410X

Fourier Transform Infrared (FTIR) Spectroscopy. The spectrum display shows the peaks that correspond to specific groups along with a graph of the absorption of wavenumber to transmittance (%T).

The sample is injected into the injector as a liquid and then evaporated. The carrier gas transports the sample in the form of vapor to the column for separation. After separation, each component will pass through the ionizing chamber and be bombarded with electrons, causing ionization. This procedure is carried out for testing, analysis, gas chromatography, and mass spectroscopy (GC-MS). The final step is to investigate molecular docking with protein ligands using silica analysis.

Looking for amino acids that are part of the target protein

Protein ligand amino acid sequences were obtained from the National Center for Biotechnology Information (NCBI) database, National Library of Medicine (NLM), National Institute of Health (NIH) (http://www.ncbi.nlm.nih.gov). Using OpenBabel software, the 3D structure of the protein ligand in *.sdf file format will be converted into a *.pdb file.[17]

The structure of the labdane-type diterpene active compound from Vitex trifolia L. is being sought.

The PubChem Open Chemistry Database was used to obtain the 3D structure of the active compound component of legundi fruit (Vitex trifolia L.). Using OpenBabel software, the 3D structure of the various compounds in *.sdf file format will be converted into *.pdb files.[17]

3D protein structure modeling

The target proteins' 3D structures were predicted using the SWISS-MODEL webserver and the homology modeling method. The Ramachandran plot was then used to validate the protein's 3D structure.[18]

The docking and visualization between protein-ligand

The docking simulation between Nicotiana tabacum L and the target protein was performed using the HEX 8.0 software. The docking protocol consists of three visualization stages: rigid-body energy minimization, semi-flexible repair, and finishing refinement in an explicit solvent. Chimera 1.6.2 and Discovery Studio 4.1 software are used to visualize the docking results.

Analysis of the binding interaction between protein and ligand

The docking analysis results will then be visualized using Discovery Studio 4.1, LigPlot+, and LigandScout 3.1 software. The interaction of proteins and ligands was studied to determine the number and type of bonds formed, such as hydrogen and hydrophobic bonds.

RESULTS

Legundi fruit extract has undergone maceration, and the GC-MS test has bioactive substances that contribute to inducing apoptosis in the mutated Bcl-2 gene. The outcomes are detailed below.

Legundi Fruit Extract (Vitex trifolia L.)

E-ISSN: 2528-410X

After preparing a 2.5 kg legundi fruit sample, simplicia and 4.8 L of clear brown liquid extract were obtained. Organoleptic testing of Legundi fruit extract yielded a thick extract with a distinct odor, a blackish brown color, and a bitter taste. The extract yielded 38.72 grams and a 13.1% extract yield using the evaporation method.

Test Fourier Transform InfraRed (FTIR) Spectroscopy

The FTIR test produces IR spectra as shown in Figure 1. The results of the identification of legundi fruit extract using an IR spectrophotometer showed a typical absorption in the wave number region of 2964.75 cm-1, 2923.72 cm-1 and 2854.48 cm-1 indicating the presence of C-H bonds, at wave number 1709, 34 cm-1 indicates the presence of a C=O carbonyl group, at wave numbers 1674.31 cm-1 and 1622.01 cm-1 indicates the presence of a C=C group, at wave numbers 1515.67 cm-1 indicates the presence of a C=C group. aromatic, at wave numbers 1444.46 cm-1 and 1375.54 cm-1 indicates the presence of C–H groups, at wave numbers 1220.18 cm-1 indicates groups -N=C=O, at wave numbers 1160.41 cm -1, 1104.29 cm-1 and 1054.72 cm-1 indicated the presence of CO and the absorption at the wave number 3363.99 indicated the presence of an OH group. Legundi was found to contain labdane-type diterpene compounds.

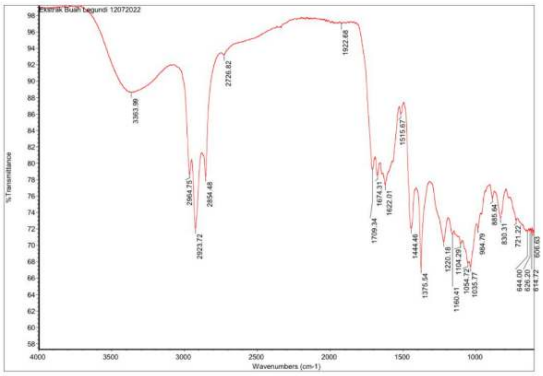

Figure 1. Spectrum of Functional Groups of Legundi Fruit Extract

Gas chromatography and mass spectroscopy tests (GC-MS)

Gas Chromatography Mass Spectrophotometry (GC-MS) was used to identify the compounds contained in Legundi fruit extract (Vitex trifolia L.) qualitatively and quantitatively. The chromatogram's peak spectrum can be seen at number ten. The results are shown in Figures 2 and 3.

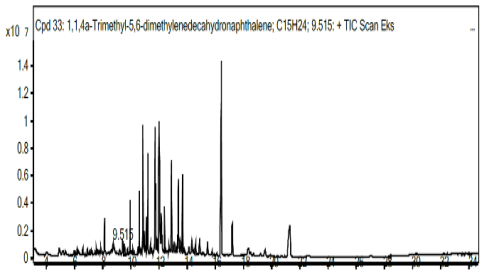

Figure 2. GC-MS Results of Legundi Fruit Extract (Vitex trifolia L.)

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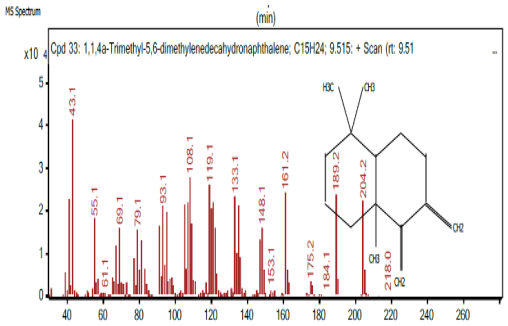

Figure 3. Peak MS Spectrum of Legundi Fruit Extract (Vitex trifolia L.)

Figures 2 and 3 show that the compound 1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene with the molecular formula C15H24 was isolated from legundi fruit (Vitex trifolia L.). At 41152.13, the highest level is seen at 43.1. Table 1 shows the total level of the entire system.

Table 1. The concentration of the compound 1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene identified in legundi fruit extract based on GC-MS data

MS	z	Kadar
41.1		22184.13
43.1	1	41152.13
107.1		21480.77
108.1		27450.42
119.1		25517.6
121.1		21675.61
133.1		22935.91
161.2		23795.27
189.2	1	23278.71
204.2	1	21806.41

Test In Silico

After docking, there was an interaction between the labdane-type Bcl-2 protein receptor and diterpene ligand. The interactions and hydrophobic bonds between the two compounds are depicted in Figures 4 and 5.

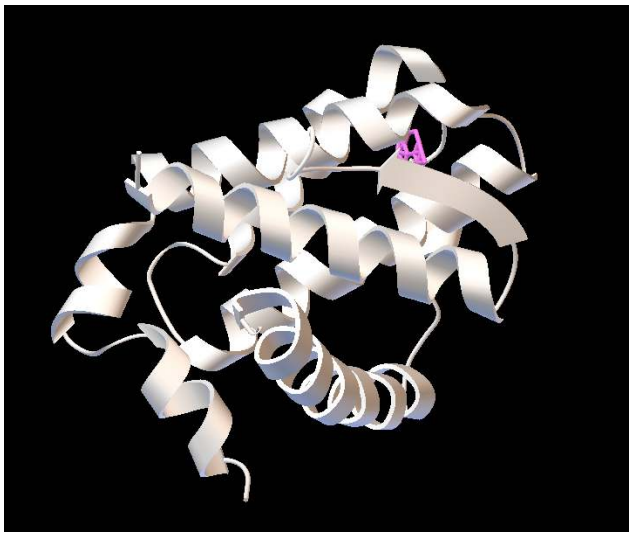

Figure 4. Interaction of labdane-type diterpene ligands and Bcl-2 receptor

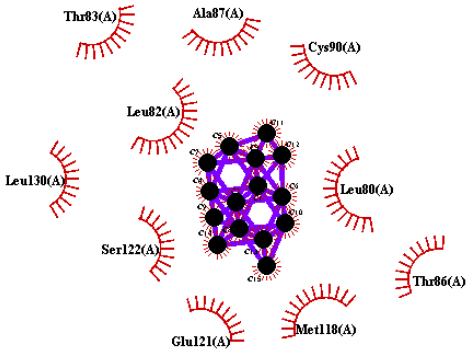

Figure 5. Hydrophobic bond between labdane-type diterpene ligand and Bcl-2 receptor

Table 2 displays the results of docking between receptors and ligands. Based on the findings, it can be concluded that labdane-type diterpenes can interact with Bcl-2. The stronger the binding interaction between the ligand and the receptor, the lower the Gibbs energy. The lower the resistance value, the stronger the ligand-protein bond, and the greater

E-ISSN: 2528-410X

the number of hydrophilic bonds, the stronger the ligand-protein bond.

Table 2. Docking between labdane-type diterpene ligands and Bcl-2 receptors

Labdane-type diterpene	Bcl-2
Gibbs energy	-7.8
pKi	1,91 μM
Hydrogen bond	None (-)
Hydrophobic bond	10
Hydrophobic bond residue	Leu80, Leu82, Thr83, Thr86, Ala87, Cys90, Met118, Glu121, Ser122, Leu130

According to the 2018 Global Cancer Observatory, 36,633 women were diagnosed with cervical cancer, making it the fourth most common cause of death in women worldwide. Given the effect of labdane-type diterpene as an inducer of apoptosis against Bcl-2 gene mutation in cervical cancer, this ligand was chosen for further investigation into the mechanism of inhibiting apoptosis.

Based on previous research, the Bcl-2 family protein is a proto-oncogene that can induce apoptosis through the synthesis of pro-apoptotic genes while inhibiting apoptosis through the synthesis of anti-apoptotic genes. Bcl-2 expression dysregulation can prolong cell survival by preventing apoptosis and participating in malignant transformation.[19]

Reduced pro-apoptotic protein Bcl-2 expression in cancer serves the same functional purpose as increased pro-survival expression. Apoptosis avoidance may promote tumor development, metastatic survival, and therapeutic resistance, all of which may contribute to oncogenic transformation. Gene amplification, chromosomal translocation, increased gene expression/translation, and protein stability via multiple mechanisms are just a few examples of how this can occur.

The in silico test yielded good results with a low Gibbs energy value and a large number of hydrophobic bonds. This suggests that a labdane-type diterpene derived from legundi fruit (Vitex trifolia L.) has the potential to be developed as a new cancer therapy. It is also inextricably linked to assessing individual genetic mutations that influence therapy success. It is critical to have a thorough understanding of in silico studies in order to determine potential target therapies in patients who respond or do not respond to cancer treatment.[20]

Cervical cancer is known to have tumor suppressor gene mutations, overexpression of anti-apoptotic proteins such as Bcl-2, and/or decreased pro-apoptotic proteins. According to the above description, a compound can function as an anti-cancer with a protein target of the Bcl-2 group if it can affect the expression of both proapoptotic and anti-apoptotic proteins, in this study devoted to the Bcl-2 protein. Compounds effects on the expression of pro-apoptotic proteins (Bax, Noxa, PUMA) can be either p53-dependent or p53-independent.[21] This study has limitations because it only looked at Bcl-2's apoptotic inhibition without looking at the proapoptotic protein. More research will be conducted in order to make a more comprehensive assessment of the mechanism of apoptosis in broad terms,

E-ISSN: 2528-410X

including the role of proteins involved in apoptosis, such as p53.

Rotundifuram, a labdane-type diterpene, was found to have a significant antitumor effect on cervical cancer cell lines via the intrinsic induction of mitochondrial-mediated apoptosis in the form of Cyr61, which is a potential target to be triggered by RTF apoptosis by biophysical proteomic method by Gong et al. (2021).[16]

In addition to studying the genes and proteins that cause cancer, it is becoming clear that the genes' regulators, which include not only previously identified tumor suppressor genes and proto-oncogenes, but also non-coding elements and epigenetic factors in general, can play a significant role in the disease.[22,23] One approach to studying cancer and its treatment is to look at complex pathways rather than a single genetic mutation. Some patients' profiles are so diverse that pathway similarity analysis is required to identify phenotypic subclasses associated with cancer-causing genotypes. As a result, additional studies will be developed from various cancer pathogenesis pathways, with the use of herbal plant compounds, of course.

CONCLUSION

Finally, it demonstrates that a labdane-type diterpene induces apoptosis and inhibits the antiapoptotic action of the Bcl-2 gene. This study will help in the future development of labdane-type diterpenes as drug candidates for the treatment of cervical cancer.

ACKNOWLEDGMENT

The authors would like to thank the Directorate General of Higher Education, Research, and Technology of the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, as well as Universitas Muhammadiyah Sumatera Utara, for funding.

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