Potential Interaction of Costunolide and Dehydrocostuslactone Compounds on Proteins Associated With Non-Alcoholic Fatty Liver Disease in An In-Silico Study

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ABSTRACT

Background: *Non-alcoholic Fatty Liver Disease (NAFLD) is a growing health problem but there is no standard drug for its treatment. Costunolide and dehydrocostuslactone is the compound of Saussurea costus, and an antioxidant that has activities as anti-hepatotoxic, anti-inflammatory, and immunostimulants, which have been proven in-vivo and in-vitro.*
Purpose: *This study aims to identify the bioactive ingredients of S. costus that affect NAFLD and explore its therapeutic targets through pharmacological networking.*
Methods: *Various tissue databases were identified to get the bioactive material S. costus and identify NAFLD therapeutic targets. Gene ontology enrichment (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed to enrich the functions and molecular pathways of common targets.* The analysis was carried out through the *Structure-Activity-Relationship (SAR) search*, with the determination of a range score of 0-1, where the closer to 1 the better the value.
Results: *The results of the Structure-Activity-Relationship (SAR) analysis were that the costunolide and dehydrocostuslactone compounds had scored <0.5 as a hepatoprotector and as a regulator of fat metabolism. The potential of these two compounds as TNF-alpha inhibitors and Interleukin-6 antagonists also shows a score <0.5. Costunolide and Dehydrocostuslactone showed a score >0.5 for their activity as anti-inflammatory and NF-κB transcription inhibitors, indicating the existence of anti-inflammatory potential, and the potential as a candidate for NAFLD therapy indicated by the NF-κB transcription inhibitory pathway.*
Conclusion: *Costunolide,* and *Dehydrocostuslactone showed anti-inflammatory potential, and the presumed potential as a candidate for NAFLD therapy was shown to inhibit the transcription pathway of NF-κB*

Keywords: *Costunolide, Dehydrocostuslactone,* NAFLD, NF-κB, S. Costus

INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) has been considered one of the leading causes of cryptogenic cirrhosis and chronic liver disease. NAFLD is a common chronic liver disease with a high prevalence in developed countries. The global prevalence of NAFLD diagnosed by imaging is about 25.24%. The highest prevalence of NAFLD was reported from the Middle East (31.79%) and South America (30.45%), while the lowest prevalence rate was

reported from Africa (13.48%). Fatty liver occurs in 10-15% of normal individuals, and 70-80% of people with obesity (Chalasani, et al., 2017; Lestari, et al., 2021; Setiono, Wantania, dan Polii, 2022).

The accumulation of fat in the liver is the initial stage of NAFLD. This occurs due to the large accumulation of triglycerides caused by an imbalance between the influx and synthesis of free fatty acids in the liver, the process of **β**-oxidation, and transport outside the cell arising from various causative factors. The next stage occurs liver steatosis which leads to the process of affecting and inflamed liver cells and ends with the formation of scars or fibrosis (Berardo, et al., 2020; Godoy-Matos., et al., 2020; Han, et al., 2023).

Management of NAFLD requires long-term monitoring. Supportive therapy is carried out by losing weight for obese people and modifying lifestyles. Pharmacological therapy is only given to those who have not experienced improvement by making lifestyle changes, and the results are not necessarily satisfactory, Various studies have been conducted to be able to find the most effective therapy in the treatment of NAFLD, including the potential of natural ingredients that play a role in the prevention of liver disease (Beiriger, et al., 2023; Byrne, et al., 2020; Parlati, et al., 2021). Many efforts to prevent liver disease are carried out by utilizing various natural ingredients such as Curcuma, manga bark, red fruit, and pomegranate. One of the natural ingredients that is also suspected to be able to use alternative medicine is S. sostus which has been proven in vivo and in vitro to have activities as antioxidants, antihepatotoxics, anti-inflammatory, and immunostimulants (Barghchi, et al., 2023; Karamalakova, et al., 2019; Tejaswi, et al., 2018).

The active content of S. costus is mainly terpene compounds, anthraquinones, alkaloids, and flavonoids. A significant element in *S. costus plants* is sesquiterpene lactones, namely costunolide, dehydrocostus lactone. The dried roots of this plant are used in the treatment of Unani as a powder form and for the treatment of diseases, such as asthma, joint pain, dysentery, skin diseases, neurological diseases, liver diseases, intestinal parasites, and others. *S. costus* has anti-cancer, antiviral, anti-arthritic, anti-inflammatory, anti-ulcer, anti-convulsant, and hepatoprotective properties that have been proven in vivo and in vitro. S. *costus extract* has anti-cancerous potential for the breast, colon, and liver (Nadda, et al., 2020; Shatti, et al., 2020). Costunolide and dehydrocostuslactone can inhibit Hepatoma Hep3Bcells in humans, thereby inhibiting the production of hepatitis B antigen (HBsAg) and inhibiting breast cancer through the c-Myc/p53 and AKT/14-3-3 pathways (Jubayer, et al., 2020; Liu, et al., 2021; Semwal, et al., 2020). The interaction of the active compounds contained in S. costus, especially the main terpenes, namely costunolide and dehydrocostuslactone, against proteins involved in the course of NAFLD disease has not been studied definitively. Therefore, this research was carried out to identify S. costus bioactive materials that have the potential to interact with NAFLD-related proteins, as a step to explore the potential of S. costus for NAFLD therapy through pharmacological networking.

MATERIALS AND METHODS

The study is experimental with a descriptive method. Data were obtained from the results of docking ligands and proteins whose interactions were analyzed. The research object uses ligands and model proteins obtained from the GDP database. The proteins associated with NAFLD were obtained from the GDP database at http://www.rcsb.org/pdb/home/home.do address. Then the costunolide and dehydrocostuslactone were obtained from the PubChem CID 689043 database. Costunolide and dehydrocostuslactone were obtained by reconstructing the three-dimensional shape

using Canonical SMILES available in the PubChem database. Minimize energy and eliminate water molecules in costunolide and dehydrocostuslactone using the *PyRx virtual screening* software *program Open Babel tool*, with PyRx software used to convert SDF protein costunolide and dehydrocostuslactone files into PDB format (Abdulqahar, et al., 2023; Xu, et al., 2021)

Appropriate targets of S. costus bioactive materials were collected from the TCMSP database and the Swiss Target Prediction database (//www.swisstargetprediction.ch/). The GeneCards database (http://www.genecards.org/) and the DisGeNET database (https:// www.disgenet.org/) were searched using the term "NAFLD" or non-alcoholic fatty liver disease (UMLS CUI: C0400966) to identify potential therapeutic targets for NAFLD. The UniProt (https://www.uniprot.org/) database is used to standardize gene information and subsequently to remove duplicate and pseudogene genes. The general target of the bioactive material was manually screened and the interaction tissue was visualized using Cytoscape 3.7.2. software (Abdulqahar, et al., 2023; Xu, et al., 2021). The analysis was carried out through the *Structure-Activity-Relationship (SAR) search*, with the determination of a range score of 0-1, where the closer to 1, the better the value (Devillers, et al., 2024). The first research was carried out at the Laboratory of Bioinformatics, Faculty of Medicine, Jenderal Achmad Yani University.

RESULTS

The bioactive ingredients *of S. costus* were taken from the PubChem CID 6436243 and CID 73174 database (National Center Information, 2024a; National Center Information, 2024a). The active ingredients to be analyzed in this study are costunolide and dehydrocostuslactone which are sequesterpen lactones as the main components of *S. costus* with an overview of the chemical structure shown in Figure 1 (Toda, et al., 2017).

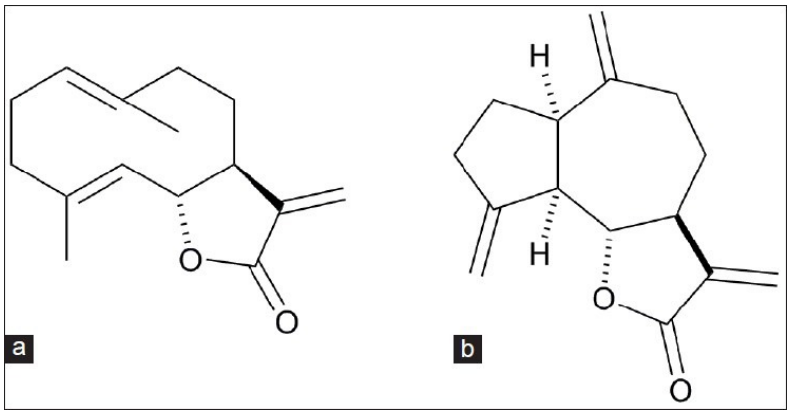

Figure 1. Chemical Structure of Costunolide and Dehydrocostuslactone

Structurally, costunolide is a monocarboxylic acid that has three double bonds that through catalytic hydrogenation produce hexahydro-costunolide. Partial hydrogenation of costunolide produces dihydrocostunolide. Two bioactive ingredients of S. costus were analyzed to find compatibility with NAFLD-related proteins taken from the DisGeNet and Genecards databases. The results of the *Structure-Activity-Relationship (**SAR**) analysis* show the same picture as in Table 1, where the score range is 0 to 1, with the interpretation getting closer to the value of 1, the better the interaction between proteins (Devillers, et al., 2024; National Center Information, 2024a; National Center Information, 2024a; Toda, et al., 2017).

Table 1. SAR Analysis of Costunolide and Dehydrocostuslactone Compounds on NAFLD

Senyawa	ID	Canonical SMILE	Isomeric SMILE	Hepato-protectant	Lipid metabolism regulator	Antiinflammatory	TNF expression inhibitor	Transcription factor NF kappa B inhibitor	Interleukin 6 antagonist
Costulonide	5281437	CC1=CCCC(=CC2C(CC1)C(=C)C(=O)O2)C	C/C/1=C\CC/C(=C/[C@@H]2[C@@H](CC1)C(=C)C(=O)O2)/C	0.426	0.422	0.803	0.278	0.756	0.204
Dehydrocostuslactone	73174	C=C1CCC2C(C3C1CCC3=C)OC(=O)C2=C	C=C1CC[C@@H]2[C@@H]([C@@H]3[C@H]1CCC3=C)OC(=O)C2=C	0.372	0.312	0.857	0	0.715	0

DISCUSSION

Based on the *Structure-Activity-Relationship (**SAR**) analysis,* it can be seen that the compounds *costunolide* and *dehydrocostuslactone* have the potential as hepatoprotectors with a score of 0.426 for costunolide and 0.372 for the dehydrocostuslactone compound, both of which have a score of less than 0.5, which can be concluded that the potential of the two compounds is not good. The potency as a regulator of fat metabolism also showed poor values, where there was a score of 0.422 for costunolide compounds and 0.312 for dehydrocostuslactone. The potential as a TNF-alpha inhibitor *and* Interleukin-6 *antagonist* is also not good. The costunolide compound showed a score of 0.278 for its potency on TNF inhibitor expression and 0.204 as an IL-6 antagonist, while the dehydrocostuslactone compound produced a score of 0 for both aspects.

The two active compounds of S. costus show good value as anti-inflammatory and NF-κB transcription inhibitors. Costunolide showed a score of 0.803 and dehydrocostuslactone showed a score of 0.857 as an anti-inflammatory. Its potency as an NF-κB transcription inhibitor shows a score of 0.756 for costunolide compounds and 0.715 for dehydrocostuslactone. This suggests the potential of costunolide and dehydrocostuslactone compounds in the therapy of NAFLD through both pathways. Oxidative stress due to cellular redox imbalance causes many diseases, such as diabetes, atherosclerosis, and cardiovascular diseases including fatty liver. Previous research studying the antioxidant activity of costunolide in a mouse model of streptozotocin-induced diabetes (STZ), showed a decrease in glutathione (GSH) levels in the brain, heart, liver, pancreas, and kidneys (Flieger, et al., 2021; Santos-Sanchez, et al., n.d; Rahman, et al., 2023). Oral administration of costunolide restores GSH levels in these tissues. Increased GSH levels can increase levels of GSH-dependent enzymes, such as glutathione peroxidase (GPx) and glutathione-S-transferase (GST), thereby reducing tissue damage. Oxidative stress oxidizes and damages membrane phospholipids to produce lipid peroxides, such as malondialdehyde (MDA) and hydroxyonenal (HNE), which by forming additional DNA products can cause oxidative tissue damage (Kim, et al., 2019). Costunolide also decreased lipid peroxidation rates and increased the activity of SOD, catalase, and GPx in MCF-7 & MDA-MB-231 cells. In a mouse intestinal mucositis (IM) model, administration of costunolide restored the levels of 5-fluorouracil (5FU)-depleted plasma superoxide dismutase (SOD) in the mucosa of the rat intestine. Costunolide also inhibits the production of hydrogen peroxide-induced ROS ($H2O2$) in mouse pheochromocytoma cells (PC12) (Kim, et al., 2019; Rao, et al., 2015; Yan, et al., 2019). This proves the potential of costunolide as an antioxidant.

Persistent tissue inflammation plays an important role in the pathogenesis of various diseases including the mechanism of occurrence of fatty liver. Costunolide has shown anti-inflammatory properties in some preclinical studies. One of the transcription regulators of proinflammatory gene expression is the transcription Factor Nuclear-Kappa B factor (NF-κB). Costunolide negates NF-κB activation through blockade of IκBα phosphorylation in lipopolysaccharide (LPS)-stimulated RAW264.7 cells, thereby reducing the expression of proinflammatory markers, such as inducible nitric oxide synthase (iNOS), and nitric oxide (NO) production (Jubayer, et al., 2020; Kim, et al., 2019; Yan, et al., 2019). Chen et al. also showed that treatment with costunolide inhibited the expression of iNOS, cyclooxygenase-2 (COX-2), TNF-α, and 5-fluorouracil (5-FU)-induced nitric oxide (NO) production in a mouse model with intestinal mucositis. by blocking the activation of NF-κB (Chen, 2016). Costunolide reduces the phosphorylation of STAT1 and STAT3 in IL-22 or IFN-γ-induced human keratinocytes. Costunolide showed anti-inflammatory effects as evidenced by the improvement of ethanol-induced peptic ulcers in rats. The study also reported that the compound suppressed the activation and/or induction of NF-κB, TNF-α, NO, iNOS, and COX-2. Costunolide inhibits interleukin (IL)-1β protein and mRNA expression in LPS-stimulated RAW264.7 cells by blocking the transcriptional activity of activator protein (AP-1) through mitogen-activated protein kinase (MAPK) phosphorylation downregulation (Kim, et al., 2019; Rao, et al., 2015; Yan, et al., 2019). This study shows the potential of costunolide and dehydrocostuslactone in non-alcoholic fatty liver disease through the inhibition of NF-κB transcription.

CONCLUSION

Based on the results of the Structure-Activity-Relationship (*SAR) analysis, Costunolide,* and *Dehydrocostuslactone showed anti-inflammatory potential, and the presumed potential as a candidate for NAFLD therapy was shown to inhibit the transcription pathway of NF-κB.*

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