Original Article

In-Silico Analysis of Costunolide and Dehydrocostuslactone Interactions with NAFLD-Related Proteins

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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 are compounds found in Saussurea costus, exhibiting antioxidant activities that include anti-hepatotoxic, anti-inflammatory, and immunostimulant properties, which have been proven both in vivo and in vitro. This study aims to identify the bioactive ingredients of S. costus that affect NAFLD and explore its therapeutic targets through pharmacological networking. Various tissue databases were utilised to obtain the bioactive material from S. costus and identify potential therapeutic targets for NAFLD. Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to enrich the functions and molecular pathways of common targets.

Methods: The analysis was conducted using a Structure-Activity Relationship (SAR) search to evaluate the biological potential of the studied compounds. The design of this study involved selecting costunolide and dehydrocostuslactone compounds as the subjects of analysis, with data collection conducted through public databases and relevant literature. SAR assessment was conducted using reporting standards, such as STITCH, to ensure transparency and reproducibility in the analysis. The score range used was 0-1, where the closer to 1, the better the value obtained. This process allows the identification of significant relationships between chemical structure and biological activity, as well as providing deeper insight into the potential of the compounds analysed. Thus, this method not only assesses the effectiveness of the compound but also provides a basis for further research in the development of therapy

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.

Conclusion: Costunolide and dehydrocostuslactone showed significant potential as anti-inflammatory agents and NF-κB transcription inhibitors. These findings indicate that both compounds may be promising candidates for NAFLD therapy, particularly through the mechanism of inhibition of the NF-κB transcription pathway. The implications of these results suggest the need for further studies to explore the efficacy and safety of these compounds in a clinical context, as well as their potential in the development of novel

therapies for non-alcoholic fatty liver disease.

Keywords: Costunolide; Dehydrocostuslactone; Non-alcoholic Fatty Liver Disease; NF-κB; Saussurea costus

Implications for Practice:

- Costunolide and dehydrocostuslactone show potential as natural anti-inflammatory agents for NAFLD by inhibiting the NF-κB transcription pathway.
- Despite limited effects on fat metabolism, their ability to target key inflammatory mediators like TNF-α and IL-6 supports their relevance in treating inflammation-related liver damage.
- Further experimental validation is needed to confirm their efficacy and safety as candidates for NAFLD therapy.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) has been considered as one of the main causes of cryptogenic cirrhosis and chronic liver disease. NAFLD is a common chronic liver disease with high prevalence in developed countries, with a global prevalence diagnosed by imaging of around 25.24%. The highest prevalence of NAFLD was reported from the Middle East (31.79%) and South America (30.45%), while the lowest prevalence was reported from Africa (13.48%). Fat accumulation occurs in 10-15% of normal individuals and 70-80% of people with obesity (Chalasani et al., 2018; Lestari et al., 2021; Setiono et al., 2022).

Fat accumulation in the liver is the initial stage of NAFLD, which occurs due to a large accumulation of triglycerides due to an imbalance between the entry and synthesis of free fatty acids in the liver, the β-oxidation process, and transport out of cells, influenced by various causative factors. The next stage is hepatic steatosis, which can cause inflammation of liver cells and the formation of scar tissue or fibrosis (Berardo et al., 2020; Godoy-Matos et al., 2020; Han et al., 2022).

NAFLD management requires long-term monitoring. Supportive therapy is carried out by reducing weight for obese individuals and changing their lifestyle. Pharmacological treatment is only given to those who do not show improvement with lifestyle changes, and the results are not always satisfactory. Various studies have been conducted to find the most effective therapy in treating NAFLD, including the potential of natural ingredients that play a role in preventing liver disease (Beiriger et al., 2023; Byrne & Targher, 2020; Parlati et al., 2021). Many efforts to prevent liver disease are carried out by utilising various natural ingredients such as ginger, mango peel, red fruit, and pomegranate. One of the natural ingredients that is also considered an alternative medicine is S. costus, which has been proven in vivo and in vitro to have antioxidant, antihepatotoxic, and anti-inflammatory activities (Barghchi et al., 2023; Karamalakova et al., 2019; Tejaswi et al., 2018).

The active substance content in S. costus mainly consists of terpene compounds, anthraquinones, alkaloids, and flavonoids. Important elements in the S. costus plant are sesquiterpene lactones, namely costunolide and dehydrocostus lactone. The dried roots of this plant are used in Unani medicine in powder form to treat various diseases, including asthma, joint pain, dysentery, skin diseases, nervous diseases, liver diseases, and intestinal parasites. S. costus has Anti-cancer, antiviral, antiarthritic, anti-inflammatory, antiulcer, anticonvulsant, and hepatoprotective properties that have been proven in vivo and in vitro. S. costus extracts have shown Anti-cancer potential for

breast, colorectal, and liver cancers (Nadda et al., 2020; Shati et al., 2020). Costunolide and dehydrocostus lactone can inhibit human Hepatoma Hep3B cells, 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., 2023; X. Liu et al., 2021).

Although many studies have been conducted on the therapeutic potential of S. costus, the interaction of active compounds contained in S. costus, especially the main terpenes, namely costunolide and dehydrocostus lactone, with proteins involved in the development of NAFLD disease has not been definitively studied. Previous studies, such as those conducted by AlSaadi et al., (2018) exploring the hepatoprotective effects of S. costus, Belahcene et al., (2023) examining the anti-inflammatory activity of its active compounds, Mishra et al., (2022) analysing the Anti-cancer potential, and Noor et al., (2022) using a pharmacological network approach for natural compounds. Therefore, this study was conducted to identify bioactive compounds from S. costus that have the potential to interact with NAFLD-related proteins, as well as to explore the possibility of S. costus in NAFLD therapy through a more in-depth bioinformatics analysis approach. Thus, this study is expected to provide new contributions in the development of more effective S. costus-based therapies compared to previous pharmacological network studies.

Methods

Study Design

This study employed an in silico experimental design using virtual screening and molecular docking to evaluate the interactions between bioactive compounds and NAFLD-related proteins. The design focused on analysing the structure-activity relationship (SAR) to determine the therapeutic potential of costunolide and dehydrocostuslactone derived from *Saussurea costus*.

Participants

As this is an in silico study, there were no human or animal participants involved. Instead, the "participants" in this context refer to selected target proteins related to NAFLD pathogenesis—namely NF-κB, TNF, and IL-6—obtained from publicly available biological databases such as GeneCards and DisGeNET.

Instruments

The main software tools used in this study included PyRx version 0.8 for molecular docking and Open Babel version 2.4.1 for ligand preparation and structure optimisation. Compound and protein data were sourced from PubChem, GeneCards, and DisGeNET databases. Ligand structures were converted from SMILES to 3D conformation and optimised for docking.

Data Collection

Data were collected from publicly accessible databases. The compounds costunolide and dehydrocostuslactone were retrieved from PubChem (CIDs 6436243 and 73174). Target proteins (NF-κB, TNF, and IL-6) were selected based on relevance to NAFLD pathogenesis from GeneCards and DisGeNET. All relevant molecular structures were downloaded and prepared for docking analysis.

Data Analysis

The docking process was conducted using PyRx to evaluate the binding affinity between the bioactive compounds and target proteins. SAR analysis was performed using a scoring system ranging from 0 to 1, where scores >0.5 indicated strong potential interaction. Scores were interpreted in terms of potential anti-inflammatory, hepatoprotective, and lipid metabolism regulation properties. Data were tabulated and discussed in the context of biological significance.

Ethical Considerations

As an in silico study utilising secondary data from publicly available sources, this research did not involve human or animal subjects and therefore did not require ethical approval. All data sources were properly cited and obtained from verified scientific databases.

Results

The bioactive ingredients *of S. costus* were taken from the PubChem CID 6436243 and CID 73174 databases. The active ingredients to be analysed 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 5 (Toda et al., 2017).

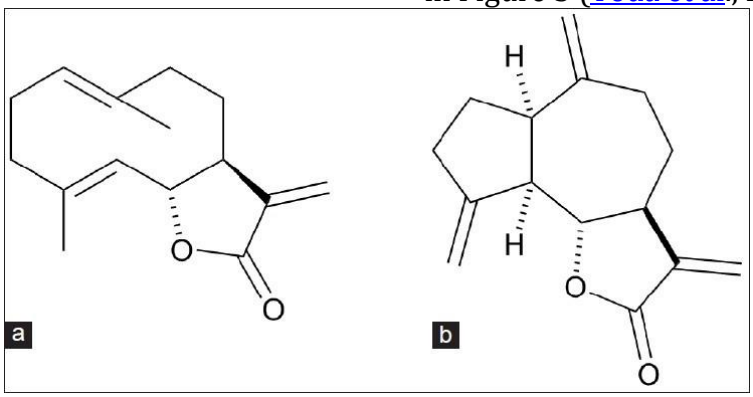

Figure 1. Chemical Structure of Costunolide and Dehydrocostuslactone.

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 analysed 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 5.1, 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 & Devillers, 2024; Toda et al., 2017) **(Figure 1).**

Based on the Structure-Activity Relationship (SAR) analysis, costunolide and dehydrocostuslactone compounds showed potential as hepatoprotectors with scores of 0.426 and 0.372, respectively. Both compounds have low affinity, with scores below 0.5, indicating limited potential. Their potential as regulators of fat metabolism is also restricted, with scores of 0.422 for costunolide and 0.312 for dehydrocostuslactone. In addition, the potential as TNF-alpha inhibitors and Interleukin-6 antagonists also showed less than satisfactory results. Costunolide recorded a score of 0.278 for TNF inhibitor expression and 0.204 as an IL-6 antagonist, while dehydrocostuslactone produced a score of 0 for both aspects **(Figure 2).**

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

Compounds	ID	Canonical SMILE	Isomeric SMILE	Hepato-protectant	Lipid metabolism regulator	Antiinflamatory	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

Both active compounds from S. costus, namely costunolide and dehydrocostuslactone, showed significant potential as anti-inflammatory agents and NF-κB transcription inhibitors, with scores of 0.803 and 0.857, respectively. These findings are in line with their potential in NAFLD therapy through both pathways. In addition, oxidative stress due to cellular redox imbalance can cause various diseases, including diabetes, atherosclerosis, and cardiovascular diseases, including fatty liver. Previous studies have shown that the antioxidant activity of costunolide in a streptozotocin (STZ)-induced diabetic rat model resulted in decreased glutathione (GSH) levels in various tissues, such as the brain, heart, liver, pancreas, and kidney (Flieger et al., 2021). Oral administration of costunolide successfully restored GSH levels in the tissue, which in turn could increase the activities of GSH-dependent enzymes, such as glutathione peroxidase (GPx) and glutathione-S-transferase (GST), thereby reducing tissue damage.

Furthermore, oxidative stress can oxidise and damage membrane phospholipids, producing lipid peroxides such as malondialdehyde (MDA) and hydroxyonenal (HNE), which can cause oxidative tissue damage through the formation of additional DNA products. Costunolide has also been shown to decrease the rate of lipid peroxidation and increase the activities of SOD, catalase, and GPx in MCF-7 and MDA-MB-231 cells. In a rat intestinal mucositis (IM) model, costunolide administration restored plasma superoxide dismutase (SOD) levels that were reduced by 5-fluorouracil (5FU) in the intestinal mucosa. In addition, costunolide inhibits hydrogen peroxide-induced ROS (H2O2) production in rat pheochromocytoma (PC12) cells, indicating the potential of costunolide as an antioxidant agent.

On the other hand, persistent tissue inflammation plays an important role in the pathogenesis of various diseases, including the mechanism of fatty liver. Costunolide has shown anti-inflammatory properties in several preclinical studies. One of the transcriptional regulators of proinflammatory gene expression is the transcription factor Nuclear-Kappa B (NF-κB). Costunolide inhibits NF-κB activation through blocking IκBα phosphorylation in lipopolysaccharide (LPS)-stimulated RAW264.7 cells, thereby reducing the expression of pro-inflammatory markers such as inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production. Chen et al. also showed that costunolide treatment inhibited the expression of iNOS, cyclooxygenase-2 (COX-2), TNF-α, and nitric oxide (NO) production induced by 5-

fluorouracil (5-FU) in a mouse model of intestinal mucositis, by blocking NF-κB activation (Chen et al., 2016). However, a study by B. Liu et al. (2019) reported that costunolide did not show significant effects on NF-κB inhibition in different cell models, indicating that the results may depend on the cellular context and tissue type tested. In addition, costunolide reduced the phosphorylation of STAT1 and STAT3 in human keratinocytes induced by IL-22 or IFN-γ.

Overall, costunolide showed significant anti-inflammatory effects, as indicated by the improvement of ethanol-induced gastric ulcers in mice. The study also reported that this 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 inhibiting the transcriptional activity of activator protein (AP-1) through decreasing the phosphorylation of mitogen-activated protein kinase (MAPK). Thus, this study demonstrates the potential of costunolide and dehydrocostuslactone in the treatment of non-alcoholic fatty liver disease through inhibition of NF-κB transcription.

Discussion

This study explored the bioactive potential of costunolide and dehydrocostuslactone compounds extracted from S. costus, focusing on their interactions with NAFLD-related proteins. The results of the analysis showed that both compounds have potential as hepatoprotectors and anti-inflammatory agents, although with scores indicating low affinity. These findings are in line with previous studies showing that these compounds can contribute to the treatment of non-alcoholic fatty liver disease (NAFLD) through inhibition of the NF-κB transcription pathway.

The results of this study are consistent with previous studies showing that costunolide has significant antioxidant and anti-inflammatory activities. For example, Abdulqahar & Hussein (2023) reported that costunolide can restore glutathione levels in tissues affected by oxidative stress. However, the scores obtained in the SAR analysis indicate that the potential of these two compounds as hepatoprotectors and regulators of lipid metabolism is still limited, which is in contrast to several studies that reported stronger effects. This suggests that despite the potential, the clinical efficacy of these compounds may require further research to identify optimal conditions for their use.

This study provides new insights into the potential of costunolide and dehydrocostuslactone as therapeutic agents for NAFLD. Although the results show limited potential, this study challenges the assumption that these compounds have a potent effect in the treatment of NAFLD. Instead, this study highlights the need for a more in-depth approach to understand the mechanisms of action and interactions of these compounds with target proteins. The value added by this study is a better understanding of the structure-activity relationships of these bioactive compounds in the context of liver disease.

The main limitation of this study is the use of in silico analysis, which cannot completely replace in vivo or in vitro experimental studies. In addition, the limited sample coverage of certain proteins may limit the generalizability of these findings. Further studies are needed to test the efficacy and safety of these compounds in animal and human models, as well as to explore their interactions with other proteins involved in the pathogenesis of NAFLD.

Based on these findings, it is recommended that further studies be conducted to explore the therapeutic potential of costunolide and

dehydrocostuslactone in the clinical context. Experimental trials involving both animal and human models are needed to confirm the efficacy and safety of these compounds. In addition, education and training for healthcare practitioners on the potential use of these compounds in the treatment of NAFLD may help improve the understanding and application of herbal-based therapies in clinical practice. Future research should also consider exploring the combination of these compounds with other therapies to enhance treatment efficacy.

Relevance to Clinical Practice

The findings of this study highlight the potential clinical relevance of costunolide and dehydrocostuslactone as adjunctive therapeutic agents in the management of non-alcoholic fatty liver disease (NAFLD). Their demonstrated anti-inflammatory properties, particularly through inhibition of the NF-κB transcription pathway, suggest they may help mitigate inflammation-associated liver damage. Although their effects on fat metabolism were limited, the ability of these compounds to target pro-inflammatory mediators such as TNF-α and IL-6 reinforces their relevance in treating the inflammatory component of NAFLD. If future in vivo and clinical studies validate their efficacy and safety, these compounds could be integrated into therapeutic strategies, offering a more holistic and potentially safer alternative to conventional treatments. This may also influence clinical guidelines, enhance patient care by reducing side effects, and support the growing interest in plant-based therapies in hepatology.

Conclusion

This study addresses the aims and hypotheses of the study regarding the potential of costunolide and dehydrocostuslactone as therapeutic agents for non-alcoholic fatty liver disease (NAFLD). The results of the Structure-Activity Relationship (SAR) analysis indicate that both compounds have potential as anti-inflammatory agents and are worthy of further exploration, especially due to their ability to inhibit the NF-κB transcription pathway. These findings support the hypothesis that these compounds may contribute to the treatment of NAFLD and provide a better understanding of the mechanisms involved in the pathogenesis of this disease. Further studies are recommended to confirm the efficacy and safety of costunolide and dehydrocostuslactone in a clinical context. These additional studies are important to improve the practice of NAFLD treatment and to explore the potential of these compounds as novel therapies in the management of liver disease.

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CrediT Authorship Contributions Statement

Fransiska A: Conceptualisation, Methodology, Supervision, Writing - Original Draft
Hendri P: Software, Validation, Funding Acquisition, Writing - Review & Editing
Agung H: Investigation, Resources, Data Curation, Project Administration
Tatang B: Review & Editing, Visualisation, Formal Analysis

Conflicts Of Interest

There is no conflict of interest.

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