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Unveiling Metabolite Compounds and Anti-Inflammatory Properties from *Chromolaena odorata* in Three Geothermal Areas

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ABSTRACT

One of the medicinal plants commonly used as traditional medicine due to its anti-inflammatory properties is *Chromolaena odorata* (L). As invasive plants that have the ability to tolerate extreme heat conditions, the underlying processes that explain their anti-inflammatory actions remain unclear. This study investigates the potential targets of *C. odorata* from three geothermal areas (Ie-Brôuk, Ie-Jue, and Ie-Seu'um) in Aceh Province as anti-inflammatory using network pharmacology and molecular docking. *C. odorata* were extracted using an ethanol solvent and further analyzed using gas chromatography-mass spectroscopy. The antioxidant capacity of each plant was examined using 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH)-scavenging with dose-response models. Next, the structure of metabolite compounds was characterized using cluster analysis with the FragFP descriptor. Targets for the identified compounds were predicted, and protein-protein interaction networks were constructed. To better understand the biological roles of intersecting genes, pathway enrichment analyses were performed using Gene Ontology and the KEGG pathway. The results showed that ethanolic extract of *C. odorata* from Ie-Seu'um, Ie-Jue, and Ie-Brôuk had DPPH-scavenging activities with IC_{50} values of 46.84, 44.79, and 95.78 ppm, respectively. From network pharmacology perspective, this study highlights the intricate molecular interactions involved, focusing on inhibitory protein kinases and their associated genes. The molecular docking analysis underscores the therapeutic potential of ethanolic extracts *C. odorata*, especially those from the Ie-Brôuk area, which contain active compounds such as sakuranin, lupeol, and β-stigmasterol had the highest binding-free energy value. This study offers insightful information about the anti-inflammatory properties of *C. odorata*, paving the way for further research and potential therapeutic applications.

Keywords: *Chromolaena odorata;* Geothermal Seulawah Agam; Network pharmacology; Sakuranin; *Seurapoh*

INTRODUCTION

Aceh Province in Indonesia is known to have significant potential for geothermal energy (Fatony et al, 2021). One of the mountains, Seulawah Agam, exhibits several manifestations, including fumaroles, gases, and hydrothermal activities known as Ie-Brôuk, Ie-Jue, and Ie-Seu'um. Although the three geothermal areas are in the same location in the north zone of Seulawah Agam, they have different manifestations and characteristics (Idroes et al, 2019). The geothermal sites of these locations are surrounded by vegetation that includes a wide variety of plants with potential applications as traditional medicines. However,

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comparative analysis of medicinal plants between three geothermal areas in Aceh Province is still limited (Maulydia et al, 2025).

Acehnese plants are also known to have various therapeutics activities, e.g., *Hippobroma longiflora* as anti-cataract (Imelda et al, 2023), *Calotropis gigantea* as oral antibacterial (Ningsih et al, 2023), and many more. One of the medicinal plants in Aceh is *Chromolaena odorata* (L) (local name: *Seurapoh*). *C. odorata* is a plant that grows abundantly in Aceh Province, Indonesia, and has a long history of usage as an anti-inflammatory in traditional medicine. *C. odorata's* anti-inflammatory properties may be due to the presence of bioactive compounds like chalcones (2′,4-dihydroxy-3′,4′,6′-trimethoxychalcone) and fatty acids (e.g., (S)-coriolic acid methyl ester), which collaborate in preventing chronic inflammation (Dhar et al, 2018; Thi et al, 2011). Many bioactive compounds of *C. odorata* that grow in Aceh, particularly in geothermal areas, have yet to be fully elucidated. An analysis of these metabolites is essential for explaining the process underlying their components. To clarify a more thorough mechanistic perspective of *C. odorata's* anti-inflammatory activity, additional research utilising metabolite profiling and activity-guided compounds is still required (Olawale et al, 2022).

In the current study, ethanolic extracts of *C. odorata* were collected from three geothermal manifestation areas: Ie-Seu'um (locally: hot water), Ie-Jue (locally: boiling water), and Ie-Brôuk (locally: filthy water), and it explores how these compounds interact with molecular targets involved in inflammation. To better understand *C. odorata's* impact on inflammation and its mechanism of action, network pharmacology (NP) was used to screen for associated targets and biological processes. Subsequently, molecular docking analysis was used to validate their activities. This research aims to achieve two primary objectives: (1) to identify the secondary metabolite compounds present in *C. odorata* from three geothermal locations in Aceh Province, Indonesia—specifically Ie-Brôuk, Ie-Jue, and Ie-Seu'um—using gas chromatography-mass spectrometry (GC-MS) and clustering compounds based on their structures (molecular characterization), and (2) to assess their anti-inflammatory capabilities through network pharmacology and molecular docking (biological characterization). This approach underscores the study's significance in the broader context of drug discovery, leveraging the recognised benefits of *C. odorata* plants in Aceh Province, Indonesia.

MATERIALS AND METHODS

Research Design

Three geothermal areas, namely Ie-Brôuk, Ie-Jue, and Ie-Seu'um, were used as sampling locations. *C. odorata's* chemical composition was analysed using gas chromatography-mass spectrometry (GC-MS). Two characteristics, molecular and biological, were characterised using the chemical composition of metabolite compounds from *C. odorata* (Figure 1).

Sampling Point and Preparation Sample

Sampling was conducted at three geothermal manifestation points in Aceh Besar District, Aceh Province, Indonesia, namely Ie-Seu'um (5º32'50''N - 95º32'45''E), Ie-Brôuk (5º31'48''N - 95º37'17''E), and Ie-Jue (5º32'23''N 95º37'45''E). The plant's leaves were properly cleaned before being sliced into small pieces. The leaves were air-dried before being crushed into a ground dried leaf sample. Furthermore, a maceration method was carried out with a 10:1 ethanol-to-plant material ratio, allowing for 5 days of extraction. The extract was filtered and dried with a vacuum evaporator (Büchi Rotavapor, Switzerland) (Maulydia et al, 2023).

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC–MS profiling was performed using a TRACE™ 1310 gas chromatograph coupled to an ISQ™ 7000 single-quadrupole mass spectrometer, fitted with a TraceGOLD™ TG-35MS capillary column (30 m × 0.25 mm × 0.25 µm; Thermo Fisher Scientific Inc., USA). The ethanolic extract of *C. odorata* were injected with the inlet temperature maintained at 250°C, while the ion source was also held at 250°C. The oven program started at 60°C and was ramped to 280°C at 10°C per minute. Helium served as the carrier gas at a constant flow rate of 1 mL/min. Mass spectra were acquired at 75 eV under electron ionization, with a scan range of m/z 40–500.

Antioxidant capacity

The ability of each extract to scavenge free radicals or as antioxidant was assessed using the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) test. The absorbance of the experiment in a 96-well microplate was measured using a BioRad Xmark microplate reader (Hercules, California, USA).

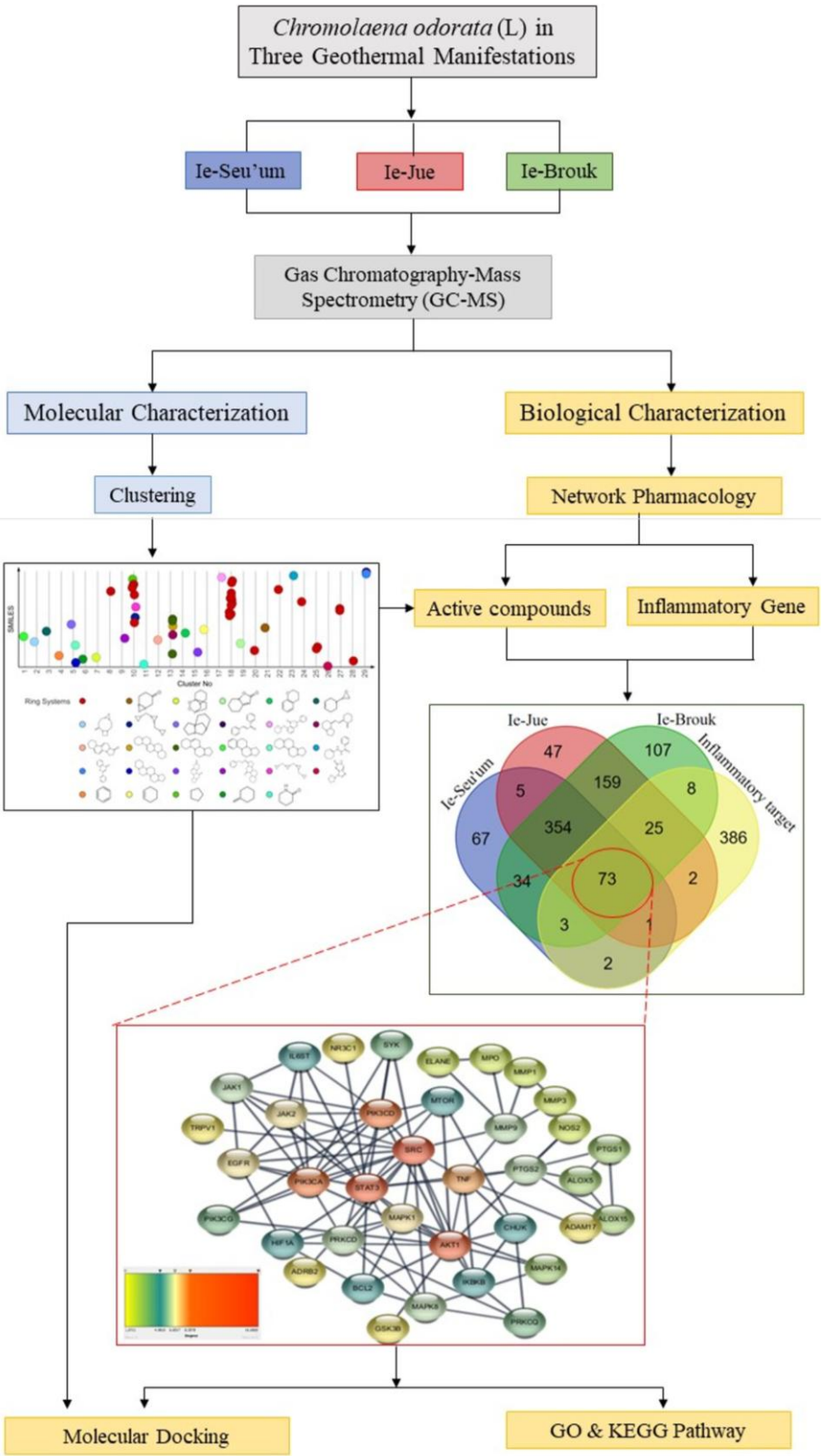

Figure I. The research design in this study.

In each well of the plate, 100 µL of the extract at concentrations of 20; 40; 60; 80; and 100 µg/mL was applied, along with 100 µL of DPPH. Ascorbic acid was used as a standard at concentrations of 2; 4; 6; 8; and 10 µg/mL and treated similarly to samples. The reaction mixture was shaken after 30 min of incubation in the dark at room temperature. DPPH absorbance at 517 nm was measure with a microplate reader (Khairan et al, 2024). In this study, the 50% inhibitory concentration (IC_{50}) value was used to quantify antioxidant activity. The exhibited graphs of scavenging activity vs sample concentration were estimated using a dose-response mode (DRM). In dose–response models, the dependent variable represents the measured response or effect, whereas the independent variable corresponds to the administered dose or concentration. The 4-parameter log-logistic model, also known as the 4PL or LL4 (lower limit equal) model, was used in this study and described by the following equation:

$$f(x) = \mathrm{c} + \frac{d - c}{1 + \exp\left(b(\log x) - (\log(e))\right)}$$

Where f(x) = the inhibition percentage, x = the concentration, b = the slope parameter, c = the lower asymptote (minimum response), d = the upper asymptote (maximum response), and e = the IC_{50} (inhibitory concentration for 50% response).

Molecular Characterization

Clustering of Compounds

The active compounds from GC-MS analysis were characterized using DataWarrior (Sander et al, 2015a). The clustering analysis was done using the simplified molecular input line-entry system (SMILES) and the molecular descriptor namely fragment-based fingerprint (FragFp).

Biological Characterization

Network Pharmacology Analysis

The compounds were evaluated using SwissTargetPredictions (Swiss Institute of Bioinformatics, Switzerland) to determine the macromolecular targets of a small molecule that are most likely to be activated. Additionally, the GeneCards database (Weizmann Institute of Science) was employed with the keyword "inflammation" to compile all targets and eliminate any duplicates, resulting in a total of 500 candidates used as inflammatory targets. The intersection of active substances with the targets from SwissTargetPrediction and GeneCards was depicted using a Venn diagram. The intersecting targets of this study are believed to be metabolite components from the ethanolic extract of *C. odorata* that exhibit anti-inflammatory properties.

Construction Protein-Protein Interactions (PPIs)

The identified overlapping targets were transferred to the STRING protein-protein interaction web database, selecting the human option (*Homo sapiens*). The minimum threshold for the interaction score was set at the highest confidence level (0.900), and non-associated proteins were concealed prior to visualization using open-source software Cytoscape 3.9.1 (United States of America). Network parameters of drug target PPIs, including degree and betweenness, were analyzed to understand the intermediary functions of the drug targets (Raman, 2010). Nodes with high betweenness centrality play a critical role in facilitating interactions between proteins implicated in inflammation (Tallei et al, 2023). The outcome of this process led to the establishment of a protein-protein interaction network for the targets.

Functional Enrichment of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted using the DAVID database (National Institutes of Health, United State of America), which includes the common targets of metabolite compounds from the ethanolic extract *C. odorata* and target inflammations. The primary objective of the GO is to construct an extensive and universal model of biological systems (Thomas, 2017). The composition of GO comprises three ontologies: biological process (BP), molecular function (MF), and cellular component (CC). Next, the KEGG Pathway is a collection of manually drawn pathway maps representing knowledge of the reaction and relation networks for various biological processes (Kanehisa & Goto, 2000). The importance of the enrichment outcome is determined using the -log10 transformation of the *p*-value. As the -log10 (*p*-value) rises, the enrichment outcome is considered more significant, and conversely, it is less significant with a lower -log10 (*p*-value) (Wu et al, 2023).

Afterwards, the collected results were visualized using the SRPlot tool (Chinese Academy of Sciences, China) to aid in their interpretation and analysis.

Molecular Docking Analysis

Molecular docking was performed utilizing the RCSB database (United States of America) to retrieve and download the structure of the key target proteins. The three-dimensional structures of target proteins were sourced from the Protein Data Bank (PDB) and refined by eliminating unnecessary components like water molecules, ions, and co-crystallized ligands. Hydrogen atoms were added, and amino acid protonation states were adjusted to physiological pH. To enhance stability, energy minimization was applied. The three-dimensional structures of the compounds were retrieved from the PubChem database and saved in SDF format. These structures were then processed using Discovery Studio (Dassault Systèmes, French), which involved removing hydrogen atoms and optimizing the molecular structure of the core target protein. Molecular docking was then performed to assess ligand-protein interactions using Autodock Vina (Centre for Computational Structural Biology, United States of America), with the active site identified based on literature or cavity detection methods. The binding site on the target protein was discovered based on predictions made using DoGSite3. DoGSite3 is a grid-based methodology that utilizes a Difference of Gaussian filter to detect potential binding pockets, relying solely on the three-dimensional structure of macromolecules (including protein and nucleic acid chains exceeding 50 atoms) and subsequently partitions them into subpockets. A grid box was established to enclose the active site, guaranteeing adequate coverage for any ligand interactions. Ligands were docked flexibly into the target site, but the receptor remained rigid to replicate actual interactions. Visualization of conformation from the selected compound and receptor was done using PoseView.

RESULTS AND DISCUSSION

GC-MS Analysis

The GC-MS analysis of the ethanolic extracts of *C. odorata* leaves collected from three geothermal manifestation areas in Aceh province of Indonesia showed that there are differences in secondary metabolites produced by *C. odorata* plants from those areas, with plants from Ie-Brôuk producing the most metabolites. The Ie-Seu'um region, which has one of the warmest temperatures, has fewer chemical compounds than the Ie-Jue and Ie-Brôuk areas. This is owing to the plant's survival activities under extreme conditions.

The crude extracts of *C. odorata* leaves collected from Ie-Seu'um, Ie-Jue, and Ie-Brôuk were analyzed by GC-MS to yield chromatogram peaks indicative of the plant metabolites. The number of compounds identified from the geothermal areas were Ie-Seu'um (12), Ie-Jue (29), and Ie-Brôuk (41). This analysis showcased a diverse range of secondary metabolite classes present in the extracts, as outlined in Supplementary Materials I. Geothermal zones exhibit specific metabolite profiles that reliably reflect the different metabolic processes taking place in each environment. These metabolites are produced because of complex biological and chemical interactions that are impacted by the specific geothermal conditions of each place (Noell et al, 2022). The three geothermal areas displayed distinct profiles of secondary metabolites, reflecting the unique biochemical processes that occur in these environments. Ie-Seu'um is characterized by a variety of secondary metabolites including fatty acid esters like methyl 4,7,10,13-hexadecatetraenoate and ethyl 14-methyl-hexadecanoate, and terpenoids such as caryophyllene oxide and phytol. Aromatic compounds (e.g., benzhydrazide derivatives) and alcohols (e.g., 12-methyl-E,E-2,13-octadecadien-1-ol) are also prevalent, alongside cyclic compounds including cyclohexene derivatives and adenosine analogues.

Ie-Jue area displays a rich variety of fatty acid esters, including various forms of hexadecanoic acid and octadecadienoic acid esters. This area also shows a wide range of terpenoids like α-tocopheryl acetate and β-sitosterol, along with steroids and sterols such as androstane derivatives and ergosta-5,22-dien-3-ol acetate. Alcohols identified include various tetramethyl hexadecenols and octadecatetraenols. In Ie-Brôuk, similar fatty acid esters to those in Ie-Seu'um are observed, with compounds like hexadecanoic acid esters and methyl 9-*cis*,11-trans-octadecadienoate. Terpenoids like caryophyllene oxide and *cis*-Z-α-bisabolene epoxide are also present. This area is distinct in its range of steroids and sterols, including estra-1,3,5(10)-trien-17β-ol, and a variety of unique cyclic compounds such as sakuranin and various androstatriene derivatives.

Antioxidant capacity

As one of the heat plants that adapt to geothermal manifestation, information about the antioxidant capacity of ethanolic extract *C. odorata* is still limited. The antioxidant activity of three ethanolic extracts of *C. odorata* from three geothermal locations was determined using DPPH. In this study, Ie-Seu'um, Ie-Jue, and Ie-Brôuk had DPPH-scavenging actities with IC_{50} values of 46.84, 44.79, and 95.78 ppm, respectively (Figure 2). Ascorbic acid was used as a positive control, with DPPH scavenging at 16.63 ppm concentration. The high antioxidant values of each plant suggest that the ethanolic extract of *C. odorata* has antioxidant capabilities and can be employed as an anti-inflammation agent.

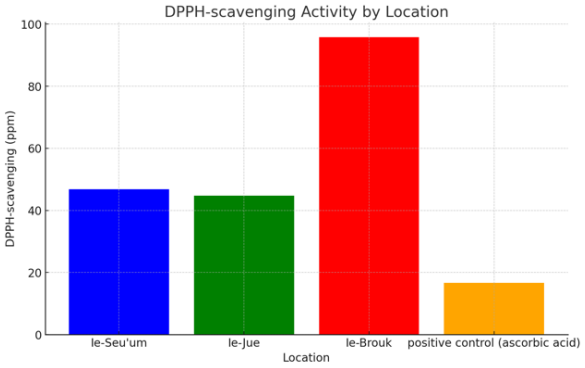

Figure 2. DPPH-scavenging activity of three ethanolic extracts of *C. odorata* and ascorbic acid.

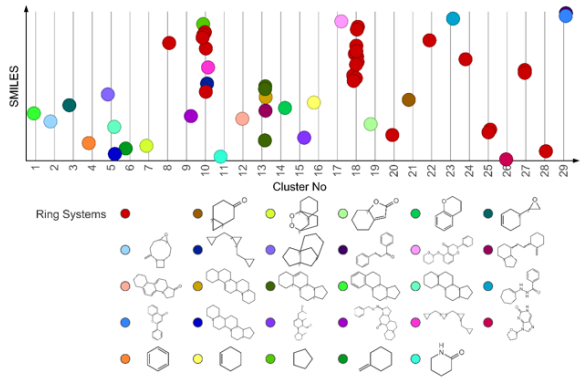

Figure 3. Clustering of active compounds from *C. odorata*

Clustering Analysis

We conducted clustering using DataWarrior based on the structures of the compounds possessed from each geothermal area. The clustering results show that there are twenty-nine groups of bioactive compounds from the ethanolic extract of *C. odorata*. The cyclic ring plays an important role in these clusters by grouping them by main group and side chain. Different dot colors indicate different ring systems (Figure 3). These results show the distribution of chemical structures of compounds into secondary metabolite groups and highlight that geographical conditions also affect the yield of secondary metabolites present in plants. One class of compounds, the fatty acid groups, as mentioned in the red dots, are distributed in clusters 10, 18, 21, 23, 25, 27, and 28, where fatty acid methyl esters are in cluster 18. This group of compounds is known to be distributed in the three regions of Ie-Seu'um, Ie-Jue, and Ie-Brôuk. Details of the clustering are presented in Supplementary Materials II. In general, every geothermal region presents a distinct metabolite profile, highlighting the varied biochemical processes that are taking place in these individual environments. The chemical composition of *C. odorata* extracts may vary due to environmental factors such as soil composition, temperature, and humidity in the three geothermal areas. This variation might influence the reproducibility of results across different regions. The variation in circumstances can be attributed to disparities in soil physicochemical characteristics. Soil temperature has been found to be a significant determinant of microbial communities in geothermal sites (Guo et al, 2020). Microorganisms in geothermal areas have developed distinct metabolic pathways to endure harsh physicochemical circumstances. The intense selection forces present in geothermal areas stimulate the development of distinct metabolic pathways in microbes, which can have an impact on surrounding plant life (Noell et al, 2022).

Network Pharmacology Analysis of Combined Compounds from Three Extracts

The interactions among numerous targets affected by the metabolite from *C. odorata* were scrutinized through a network pharmacology approach. Subsequently, the twelve compounds from the Ie-Seu'um area identified a total of 539 targets, twenty-nine compounds from Ie-Jue exhibited 666 targets, and forty-one compounds from Ie-Brôuk demonstrated 763 targets. The amalgamation of three geothermal areas yielded a cumulative total of 427 targets. Utilizing the Venn diagram for data analysis, it was ascertained that 73 potential targets were identified in the overlapping segment (Figure 4).

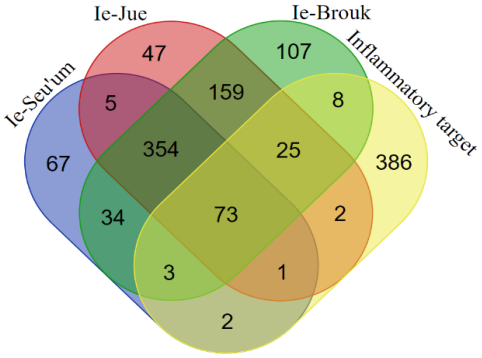

Figure 4. The Venn diagram illustrates the overlap between compounds from Ie-Seu'um, Ie-Jue, and Ie-Brouk and those liked to inflammatory target.

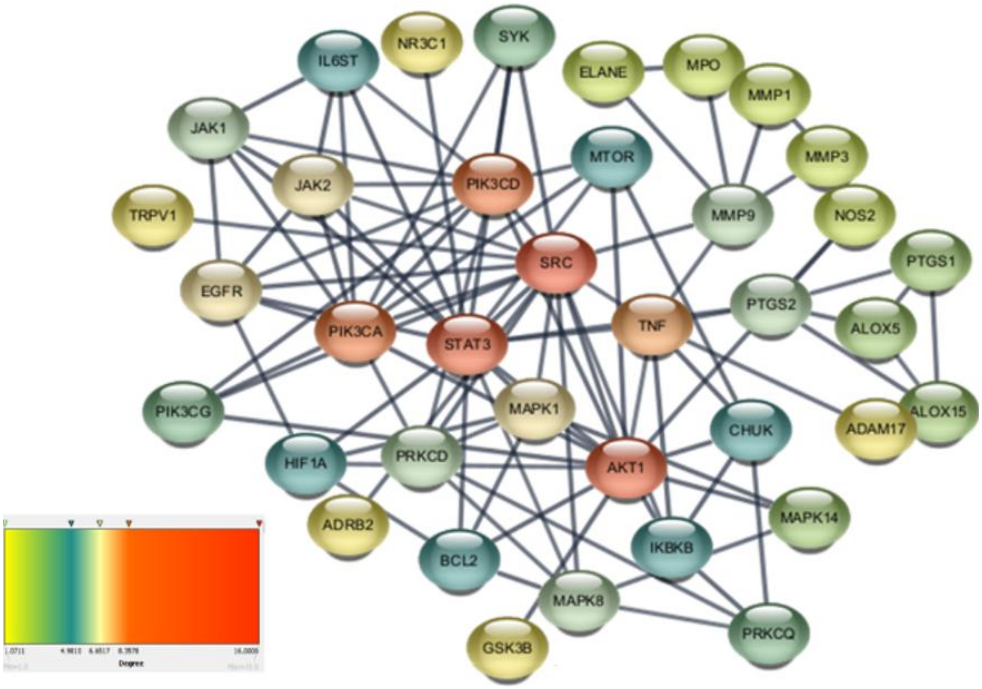

Figure 5. The protein network's construction. The increasing red color indicates higher degrees, while the increasingly yellow color signifies lower degrees.

This suggests the plausible interaction of the active constituents of *C. odorata* with inflammatory mediators, thereby positioning them as potential targets for anti-inflammatory treatment.

Protein-Protein Interaction (PPI) Analysis

The analysis of PPI was conducted on the 73 overlapping targets utilizing the STRING database (Szklarczyk et al, 2023). The PPI analysis results, which have a PPI enrichment *p*-value < $1.0e^{-16}$. This extremely low *p*-value suggests a statistically significant result, indicating that the observed enrichment of protein-protein interactions is not due to random chance but is likely to be biologically meaningful (Figure 5). Based on the results, some of the proteins in the network include: ADAM metallopeptidase domain 17 (ADAM17), adrenoceptor beta 2 (ADRB2), AKT serine/ threonine kinase 1 (AKT1), arachidonate 5-lipoxygenase (ALOX5), arachidonate 15-lipoxygenase (ALOX15), BCL2 apoptosis regulator (BCL2), component of inhibitor of nuclear factor kappa B kinase complex (CHUK), epidermal growth factor receptor (EGFR), elastase neutrophil

expressed (ELANE), hypoxia inducible factor 1 subunit alpha (HIF1A), inhibitor of nuclear factor kappa B kinase subunit beta (IKBKB), interleukin 6 cytokine family signal transducer (IL6ST), janus kinase 1 (JAK1), janus kinase 2 (JAK2), mitogen-activated protein kinase 1 (MAPK1), mitogen-activated protein kinase 8 (MAPK8), mitogen-activated protein kinase 14 (MAPK14), matrix metallopeptidase 9 (MMP9), myeloperoxidase (MPO), nitric oxide synthase 2 (NOS2), nuclear receptor subfamily 3 group C member 1 (NR3C1), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG), protein kinase C delta (PRKCD), protein kinase C theta (PRKCQ), prostaglandin-endoperoxide synthase 1 (PTGS1), Src proto-oncogene non-receptor tyrosine kinase (SRC), signal transducer and activator of transcription 3 (STAT3), spleen tyrosine kinase (SYK), tumor necrosis factor (TNF), and transient receptor potential cation channel subfamily V member 1 (TRPV1). The network was formed using a node's degree as a framework (minimum: 1, maximum: 16), with colors decreasing as the degree decreases. The network's centre serves as an indicator of node centrality and acts as an intersection for correlation among the related genes.

The six genes that have the highest degree (Figure 5). The network's relatively high average node degree indicates significant connectedness, reflecting its complexity. The connections between nodes in protein-protein interactions are very significant, as indicated by the low *p*-value for PPI enrichment. The six genes engaging in betweenness centrality and degree value can be seen in Supplementary Materials III. One type of non-receptor tyrosine kinase protein (NRTK) is proto-oncogene tyrosine-protein kinase (Src), which plays diverse roles in transmitting intracellular signals, contributing to the intricacies of cellular communication, and signalling pathways (Lahiry et al, 2010). The degree of 16 indicates a high level of connectivity, with 16 edges connecting to other nodes. The molecular pathways that are responsible for the activities of Src have been the focus of numerous studies, especially in cancer treatments (Parsons & Parsons, 2004). PIK3CD, the catalytic subunit of phosphoinositide 3-kinase (PI3K), is essential to the PI3K/Akt pathway, a fundamental signalling cascade governing cellular processes. The PIK3CD directly activates AKT1, a downstream effector, which in turn phosphorylates diverse proteins, augmenting cellular growth and survival mechanisms (He et al, 2021). Numerous PI3K inhibitors, such as dual, isoform-specific, and pan inhibitors, are being evaluated at different stages of human clinical studies (Mishra et al, 2021). Beyond the PI3K/Akt pathway, PIK3CD engages with PIK3CA, forming various PI3K complexes with distinct cellular functions. Moreover, its interactions extend to other signalling pathways like Ras/MAPK and mTOR, consequently affecting mechanisms like cell migration and proliferation (Yang et al, 2019). In disease contexts, mutations in PIK3CD are implicated in cancers like breast, colorectal, and lung cancer, where they spur aberrant pathway activation, driving tumor progression (He et al, 2021). PIK3CD emerges as a promising therapeutic target for cancer, autoimmune disorders, and neurological conditions, with ongoing development of drugs aimed at inhibiting its activity and subsequent clinical evaluation (Mishra et al, 2021). This implies that PIK3CD is involved in multiple distinct signalling pathways.

The network also shows that some of the proteins interact with multiple other proteins. It appears that there are several protein-protein interactions that are important for various biological processes. For example, the interaction between NR3C1 and SYK is important for the regulation of inflammation. It is well known that the glucocorticoid receptor gene, NR3C1 gene, inhibits pro-inflammatory transcription factors to produce anti-inflammatory effects, while SYK is involved in immune cell signalling and activation (Escoter-Torres et al, 2019; Wang et al, 2021).

Enrichment GO and KEGG Pathway

The biological processes are represented by a green bar, encompassing five specific processes, namely inflammatory response, peptidyl-serine phosphorylation, protein phosphorylation, cell chemotaxis, and the interleukin-6-mediated signalling pathway (Figure 6a). The highest -log10 (*p*-value) is inflammatory responses, which play a pivotal role in the body's defence against harmful stimuli. The identified gene associated with the inflammatory response exhibits a multifaceted role, serving not only as a regulatory element but also demonstrating intricate interactions within a phosphorylation complex alongside other proteins.

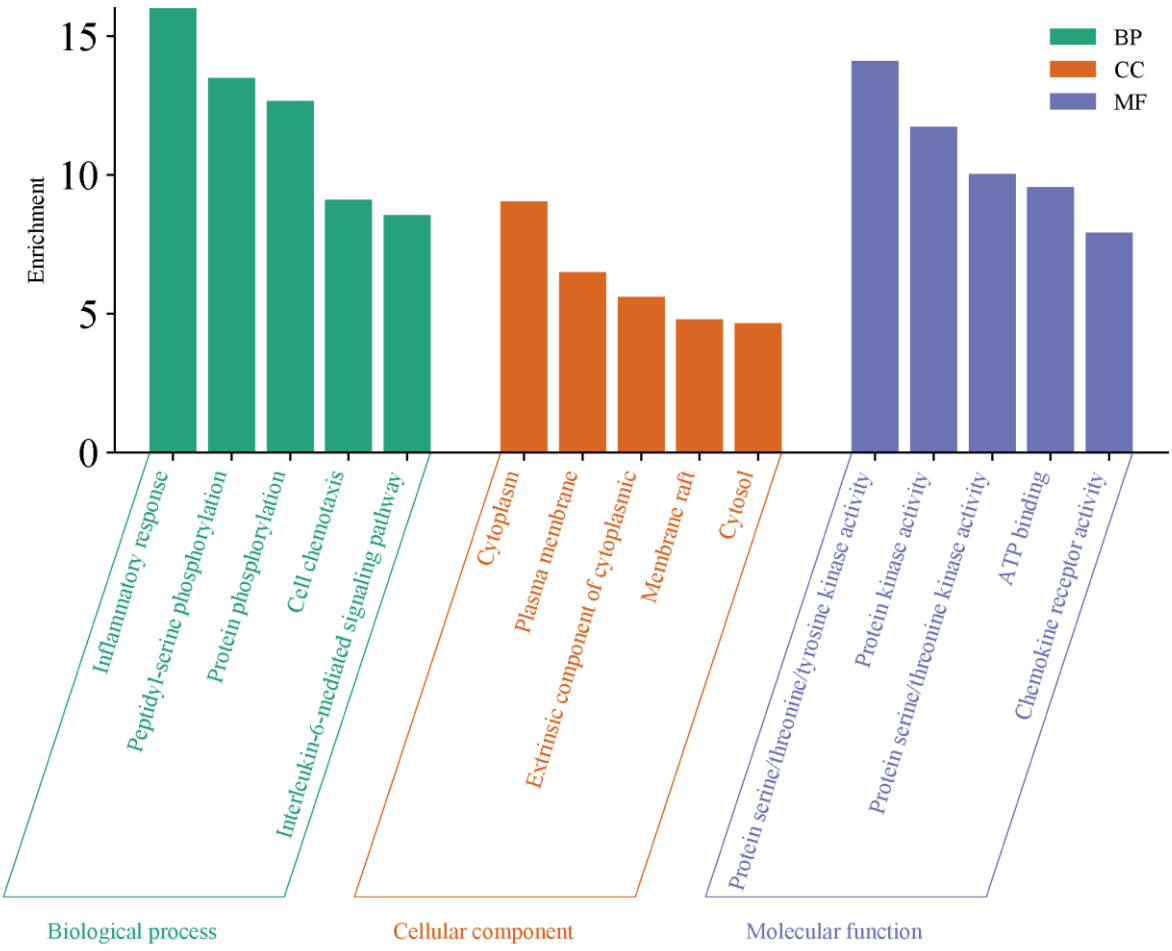

Figure 6. Enrichment of GO: (a) biological process, (b) cellular component, and (c) molecular function. Descriptions: Enrichment is (-log10 (*p-value*))

Based on PPIs network, the six genes with the highest degree are SRC, AKT1, STAT3, PIK3CD, PIK3CA, and TNF. A comprehensive correlation among all six genes is not expressly delineated. Nevertheless, using network pharmacology, it is known that discernible evidence suggests the presence of correlations between specific pairs of these genes. Proteins like tyrosine, serine, and threonine kinases are important in inflammatory activity. The phosphorylation of serine, threonine, and tyrosine residues by these kinases is known to regulate various cellular processes, including the inflammatory response (Yang et al, 2019) and progression from precancerous lesions to cervical cancer (Padilla-Mendoza et al, 2020). The intriguing complexity of the functional linkages of SRC, AKT1, and STAT3 is further enhanced by their intricate interactions with triangle form, which is an intriguing area to investigate. STAT3, a signal transducer and transcription activator, had interactions between the inflammatory response and the interleukin-6-mediated signalling pathway. The production of interleukin 6 (IL-6) is triggered by tissue damage and infections, stimulating acute phase reactions, blood cell production, and immunological responses (Padmi et al., 2021; Tanaka et al., 2014). This nuanced involvement suggests a sophisticated regulatory network of inflammatory response, shedding light on the gene's broader functional significance in the context of inflammatory processes.

A cellular component (CC) is the specific location of a gene product inside cellular structures and compartments. The CC in this network pharmacology system of metabolite compounds in the ethanolic extract of *C. odorata* were cytoplasm,

plasma membrane, extrinsic component of cytoplasm, membrane raft, and cytosol (Figure 6b). Because the cytoplasm is involved in the localization of numerous molecular and biological processes, it is important in the field of network pharmacology. Different cellular processes, such as metabolic pathways, cell signalling, and the flow of materials inside the cell, are all dependent on the cytoplasm, which plays an essential part in all these tasks. These activities have the potential to influence the pharmacological effects of bioactive chemicals. Cytoplasm is the location of unphosphorylated STATs in "off" conditions (Seif et al, 2017). It is necessary to emphasize that cellular components play a vital role in this process. Protein serine, threonine, and tyrosine kinase activity are representations of molecular function (MF) (Figure 6c). Enrichment in genes related to protein kinase activity indicates a focus on cellular signalling pathways, which are often dysregulated in diseases. Targeting kinases is a common strategy in developing therapeutic interventions. One of the gene targets in the inflammatory response is PRKCD. The protein kinases (PKCs) have a broad range of cellular substrates and participate in a wide range of biological processes (Mellor & Parker, 1998). The delta isoform of protein kinase C (PKCδ), a tyrosine kinase, has been identified as a fundamental control mechanism for the inflammatory response in conditions such as sepsis, cancer, diabetes, and neurodegenerative diseases (Yang et al, 2019). Based on the findings of this study, the regulation of protein kinase activity emerges as a promising therapeutic approach for treating inflammation. The study identified significant interactions between *C. odorata* metabolites and inflammatory targets, particularly highlighting the roles of inhibitory protein kinases and their associated genes, such as proto-oncogene tyrosine-protein kinase, protein kinase, and signal transducer and activator of transcription 3.

There are seven different parts that are represented by the KEGG pathway that contain information on molecular interaction and reaction networks (Kanehisa et al, 2012). Twenty pathways with more than ten gene counts are shown in three groups (Supplementary Materials IV). First, environmental information processing with the PI3K-Akt signalling pathway. The KEGG environmental information processing category classifies biological pathways that help organisms interpret environmental information. This category comprises membrane transport, signal transduction, and signalling molecules and interacting routes. The PI3K-Akt signalling pathway is one of the signal transductions that plays a major role as a signalling pathway involved in various types of cancer, controlling hallmarks of cancer such as cell survival, proliferation, and migration (He et al, 2021).

The KEGG organismal systems category encompasses pathway maps for several systems, such as the neurological system, endocrine system, immunological system, digestive system, excretory system, circulatory system, respiratory system, and sensory system. The chemokine signalling pathway, c-type lectin receptor signalling pathway, and T-helper 17 cell differentiation in this KEGG Pathway are components of the immune system, since they are associated with the interactions between cytokine/chemokine receptors and immunological activities. The chemokine signalling route, which has the greatest gene count among organismal systems, plays a critical role in the activation of JAK2/3 and the phosphorylation process of STAT in Jak-STAT signalling pathways. The results showed a significant correlation between the PI3K/AKT pathway and STAT3 phosphorylation, which has implications for the modulation of inflammatory conditions.

The KEGG pathway database's human diseases category comprises approximately sixteen categories, with the largest gene count observed in Kaposi sarcoma-associated herpesvirus infection, which involves twenty genes. Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8) is the pathogen responsible for causing Kaposi's sarcoma (KS), a malignant tumor that affects the connective tissue (Jary et al, 2021). In terms of its lifecycle and pathophysiology, KSHV has multiple associations with JAK, PI3K, STAT3, and SRC genes. The initial step involves the infection of endothelial cells by KSHV, which leads to the persistent activation of STAT3. The STAT3 is a transcription factor that plays a crucial role in the survival of primary effusion lymphoma cells and is consistently active in many tumor cell lines (Punjabi et al, 2007). The continuous stimulation of STAT3 by KSHV may play a crucial role in the viral pathogenesis of Kaposi's sarcoma and primary effusion lymphomas. Subsequently, KSHV initiates the activation of the PI3K/AKT/mTOR signalling pathway as a component of its viral infection mechanism (Bhatt & Damania, 2012). This activation potentially aids the virus in evading the immunological responses of the host and establishing a long-lasting dormant state within the host's body. Furthermore, the K15 protein of KSHV

is produced in a latent state and has been linked to the stimulation of angiogenesis in cultured endothelial cells. It has been suggested the role in specifically targeting Lyn kinase in the membrane raft and stimulating nuclear factor of activated T cells (NFAT) and Activator Protein 1 (AP-1) activities, which are associated with SRC family kinases (Cho et al, 2008). In conclusion, the proteins JAK, PI3K, STAT3, and SRC are significantly correlated with KSHV and have multiple functions in the pathogenesis of the virus, such as immune evasion, angiogenesis, and the formation of a lifelong latent state. In the development of Kaposi's sarcoma and other related illnesses, these associations emphasize the intricate interaction between KSHV and host cell signalling pathways. The relationships between Kaposi's sarcoma and various associated disorders highlight the complex interplay between KSHV and the signalling pathways of host cells. The development of Kaposi's sarcoma and other related disorders highlights the complex interactions between KSHV and host cell signalling pathways, which necessitate a deeper understanding using molecular docking approaches.

Molecular Docking Analysis

Molecular docking analysis were undertaken to explore the interactions between compounds from the ethanolic extracts of *C. odorata* and receptors. This analysis contributes to the understanding of ligand-receptor interactions and informs potential avenues for further investigation in anti-inflammatories. A full understanding of the linked processes of inhibition in protein kinases based on network pharmacology was one of the main goals of this study. The study incorporated four receptors, namely proto-oncogene tyrosine-protein kinase (SRC, PDB ID: 6E6E), protein kinase (AKT1, PDB ID: 4EKL), tumor necrosis factor (TNF, PDB ID: 2AZ5), and signal transducer and activator of transcription 3 (STAT3, PDB ID: 6NJS). These receptors were selected for their relevance to the study's focus on anti-inflammatory agents and the exploration of compound-receptor interactions, each contributing unique insights into the underlying molecular mechanisms. The findings, as depicted in the Supplementary Materials V, showed a gradient arrangement of binding-free energy values (BFE) from the metabolite compounds, with fifteen of them having their highest value, measured in units of Kcal/mol, in the binding-free energy heatmap.

The results shown in Supplementary Materials V revealed clear and specific preferences of the ligands for different receptors. These findings offer useful insights into the potential effectiveness of these ligands as anti-inflammatory drugs. The data above reveals that among the fifteen compounds that have good BFE values, ten compounds were detected in the Ie-Brôuk region, seven compounds in the Ie-Jue region, and three compounds in the Ie-Seu'um region. Notably, chemical compounds originating from Ie-Brôuk were discovered to have higher BFE values compared to the Ie-Jue and Ie-Seu'um locations. This suggests that the activity of *C. odorata*, as assessed by molecular docking, is influenced by the location of its growth zone. Caryophyllene oxide is a compound found in all three geothermal areas; however, its BFE value does not surpass that of the other compounds.

There are four ligands with the highest values in the receptor of SRC (sakuranin (CID: 73607) -10.2 Kcal/mol; stigmasterol (CID: 5280794) -9.4 Kcal/mol; and estra-1,3,5(10)-triene-3,17-diol (CID: 9811784) -9.4 Kcal/mol), AKT1 (stigmasterol (CID: 5280794) -9.4 Kcal/mol; 9,10-secocholesta-5,7,10(19)-triene-3,21,25-triol, (3.beta.,5Z,7E) (CID: 5372266) -9.2 Kcal/mol; and β-sitosterol (CID: 222284) -9.1 Kcal/mol), and STAT3 (α-amyrin (CID: 73170) -8.5 Kcal/mol; lupeol (CID: 259846) -7.9 Kcal/mol; and sakuranin (CID: 73607) -7.4 Kcal/mol). The outcomes of the results offer significant insights into the intricate binding interactions occurring between receptors and their respective ligands. This comprehensive examination contributes to a deeper understanding of the molecular mechanisms governing these interactions, thereby enriching knowledge of the potential pharmacological implications and therapeutic efficacy of the ligands under investigation. Additionally, to augment the findings, a visualization of the molecular docking results is presented in Supplementary Materials VI. These illustrations are used to enhance the understanding and communication of the complex binding interactions between the receptor and ligand. The figure illustrates the hydrogen bonding interactions between the ligand and the receptor, represented by dashed lines, in a comprehensive manner. The significance of hydrogen bonding consists in its pivotal function in maintaining macromolecular connections, hence aiding in the preservation of the protein's three-dimensional structure. The hydrophobic interactions occur to optimize the interaction with hydrophilic areas and

amino acid residues, as shown in the green circle of interactions. This demonstrates that the ligand possesses a favorable capacity to interact with both hydrophobic and hydrophilic substances.

The results showed that sakuranin had the highest BFE value compared to other compounds. Sakuranin is a glycoside form of the flavonoid sakuranetin, which belongs to the group of methoxylated flavanones of the flavonoid class and is found at cluster 17 (Stompor, 2020). The anti-inflammatory study on Sakuranetin showed an inhibitory effect on eosinophilic lung inflammation by suppressing T helper-2 and T helper-17 cytokines and inhibiting MAPK-STAT3 pathways (Santana et al, 2019). In this present study, sakuranin in its molecular interactions establishes a connection with Cys280 by virtue of the methoxy group within its aglycone. Methoxy groups can engage in hydrogen bonding interactions, which can influence the strength of the binding and the positioning of the ligand inside the binding site of the protein. This specific interaction underscores the involvement of sakuranetin as an active compound in the formation of hydrogen bonds, particularly originating from the oxygen atoms within its chemical structure. The other subsequent interactions take place at Ala296, Ile339, Thr341, and Glu313, with the most substantial contribution observed in the hydroxyl group. In this interaction, the hydroxyl group prominently influences the molecular associations at these specific amino acid residues, providing a noteworthy contribution to the overall interaction profile. The hydroxyl group exhibits a capability to engage in three simultaneous interactions: the presence of two lone pairs on oxygen, both capable of acting as hydrogen-bond acceptors (HBA), and the acidic hydrogen atom serving as a hydrogen-bond donor (HBD) (Cramer et al, 2019). This molecular insight provides valuable information regarding the potential contributions of sakuranin to intermolecular forces and bonding patterns, shedding light on its intricate chemical behavior.

CONCLUSION

This study provides a comprehensive analysis of the anti-inflammatory properties of *C. odorata* collected from three distinct geothermal areas in Aceh Province, Indonesia. Using GC-MS, network pharmacology, and molecular docking, the research identified a diverse array of metabolite compounds and elucidated their interactions with key inflammatory targets. The results revealed that *C. odorata* extracts, particularly those from the Ie-Brôuk area, contain valuable compounds such as sakuranin, lupeol, and β-stigmasterol, which exhibit significant binding interactions with protein kinases and other inflammation-related proteins. These findings suggest that the regulation of protein kinase activity by these metabolites could offer innovative therapeutic approaches for treating inflammation. The comprehensive study using network pharmacology and enrichment of GO and KEGG pathways suggests that the ethanolic extracts of *C. odorata* leaves contain protein kinase inhibitors, which are closely associated with its presumed anti-inflammatory effect. The study also showed that four receptors, proto-oncogene tyrosine-protein kinase (SRC), protein kinase (AKT1), tumor necrosis factor (TNF), and signal transducer and activator of transcription 3 (STAT3), have considerable interactions with fifteen compounds from the extracts, eight of which showed strong interactions. While the results are promising, it is imperative to conduct additional studies to gain a more comprehensive understanding of the intricate mechanisms underlying protein kinase inhibition and anti-inflammatory actions of the ethanolic extracts of *C. odorata.*

AUTHOR CONTRIBUTION

Nur Balqis Maulydia: Writing—review & editing, writing—original draft, validation, and data curation. Khairan: Validation, methodology, and data curation. Trina Ekawati Tallei: Software, methodology, and formal analysis. Rinaldi Idroes: Resources, data curation, and conceptualization.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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