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Uncovering anti-inflammatory potential of *Lantana camara* Linn: Network pharmacology and in vitro studies

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Abstract

Lantana camara Linn contains a diverse array of metabolites that exhibit therapeutic potential. The aim of this study was to evaluate the potential of *L. camara* leaves, which were collected at the Ie-Seu'um geothermal area in Aceh, Indonesia, as an anti-inflammatory through network pharmacology and in vitro analysis. The ethanolic extract derived from *L. camara* underwent identification utilizing gas chromatography-mass spectrometry (GC-MS) to verify chemical constituents for drug-likeness properties. The evaluation of anti-inflammatory activity included network pharmacology and a series of in vitro investigations using two methods: protein inhibition and albumin denaturation assays. The findings revealed that the extract contained a domination of terpenoids and fatty acids class, which met the evaluation criteria of drug-likeness. Network pharmacology analysis identified the top five key proteins (peroxisome proliferator-activated receptor gamma, prostaglandin G/H synthase 2, epidermal growth factor receptor, hypoxia-inducible factor 1-alpha, and tyrosine protein kinase-Janus kinase 2) involved in inflammation-related protein-protein interactions. Gene ontology enrichment highlighted the predominance of inflammatory responses in biological processes (BP), cytoplasm in cellular components (CC), and oxidoreductase activity in molecular functions (MF). In vitro analysis showed that the extract inhibited protein activity and protein denaturation with inhibitory concentration (IC_{50}) values of 202.27 and 223.85 ppm, respectively. Additionally, the extract had antioxidant activity with DPPH- and ABTS-scavenging IC_{50} values of 140 ppm and 163 ppm, respectively. Toxicological assessment by brine shrimp lethality assay (BSLA), yielding a lethal concentration (LC_{50}) value of 574 ppm (essentially non-toxic) and its prediction via ProTox 3.0 that indicated non-active in hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. These results suggested that *L. camara* holds noteworthy effectiveness as a potential candidate for complementary medicine in the realm of inflammatory agents, warranting further investigation in clinical settings.

Keywords: *Lantana camara,* Ie-Seu'um, protein inhibition assay, geothermal area, *tembelekan*

Introduction

*I*nflammatory diseases have become a significant health concern, impacting a large portion of the global population and increasing the risk for various serious conditions [1,2]. Inflammation

is a multifaceted biological process involving the carefully coordinated activation of numerous signaling pathways. These pathways control the expression of genes in local cells and immune cells drawn from the bloodstream that encode pro- and anti-inflammatory mediators [3]. Several chronic diseases, such as metabolic syndromes, neurodegenerative diseases, and cardiovascular diseases, are attributed to important pathways involving inflammation and oxidative stress [4-7]. As a result, the discovery of natural compounds with anti-inflammatory properties holds great promise for the development of novel treatment approaches to address these common health issues.

At present, there is a growing body of research that focuses on the identification and development of therapeutic agents derived from natural sources, with a particular emphasis on plant-based materials due to their ubiquity and untapped potential [8-10]. The use of secondary metabolite compounds as supplemental therapy has gained interest in recent years, especially because of their potential as anti-inflammatory agents [11-13]. Numerous studies have demonstrated that plant-derived metabolite compounds exhibit significant activities against inflammation, especially in *Lantana camara* Linn [14,15]. *L. camara*, a member of the Verbenaceae family, was first mentioned by Linnaeus in 1731 and its flower colors are varied, including violet, white, pink, yellow, and red [16]. This plant has several synonyms, such as *raimuniya* (Hindi) [17], wild sage or red sage (English) [16,18], and *tembelekan* (Indonesian). From an ethnopharmacological perspective, *L. camara* is widely distributed and has a rich history of traditional use in various countries, including Brazil, India, Kenya, Thailand, Mexico, Nigeria, Australia, and throughout Southeast Asia with activities in rheumatism, fever, and inflammation [19,20].

Indonesia's geothermal system, characterized by boiling liquid discharge, is found in quarter vulcanism and active volcanoes, such as Seulawah Agam mountain in Aceh Province, Indonesia [21-24]. Research has revealed the prospect of researching the geothermal manifestation, which includes geochemistry substance, its bioactivity prediction, and heavy metal concentration, especially from Ie-Seu'um geothermal area [25-27]. Many bioactive compounds, particularly the anti-inflammatory agents from *L. camara* that grow in geothermal areas, have yet to be fully evaluated. The screening of these metabolites remains a crucial step in drug development [13]. The significant ability of this plant as an anti-inflammatory is believed to stem from the presence of a diverse array of biologically active substances, such as compounds belonging to the flavonoid, phenolic acid, and triterpenoid families [14,28]. However, there is still room to broaden our understanding of the potential of *L. camara*. The gap in this field leads us to use a combination approach by combining network pharmacology and in vitro studies (protein inhibition and protein denaturation assay). The aim of this study was to identify and characterize the secondary metabolite compounds in *L. camara* extract from Ie-Seu'um geothermal area, Aceh Province, Indonesia, and evaluate the metabolite compounds' activity as anti-inflammatory agents using network pharmacology and in vitro analysis.

Methods

Sample collection and preparation
L. camara leaves collected from the Ie-Seu'um geothermal area (geographical coordinates: 05°32'50''N–95°32'45'E) [21,29]. The leaves were harvested, cleaned, and then cut into small pieces. After being allowed to dry naturally, the leaves were ground into a grounded dried leaf sample. The maceration process involved soaking the leaves in ethanol 1:10 (w:v) for approximately three days. After the maceration process, the extract was carefully filtered to produce the ethanolic extract of *L. camara*, which was then dried using a rotary evaporator (Rotavapor Büchi, Flawil, Switzerland) [26].

Gas chromatography-mass spectrometry (GC-MS) analysis
To examine the chemical compounds from ethanolic extracts of *L. camara*, GC-MS analysis was conducted using a TRACE 1310 GC coupled with an iSQ 7000 single quadrupole MS, (Thermo Fisher Scientific - Waltham, Massachusetts, US). The ion source was kept at 250°C, the same temperature as the injector, with an injection volume of 1 μL. For the column, TraceGOLD TG-

35MS column was used with a temperature gradient program, which increased the temperature by 10°C per minute, starting at 60°C and ending at 280°C. The carrier gas, helium, was used at a flow rate of 1 μL per minute. With an ionization energy of 75 eV, the mass spectrometer was run in electron ionization mode [30].

Drug-likeness properties

To evaluate the drug-likeness properties of each identified metabolite compound of *L. camara* from GC-MS analysis, the existing criteria were employed. Through the SwissADME [31], simplified molecular input line entry system (SMILES) for each compound was evaluated based on criteria of previous studies by Egan [32], Ghose [33], Lipinski [34], Muegge [35] and Veber [36]. Every criterion has distinct physical and chemical parameters as well as a maximum number of violations. Egan's rule is a criterion that evaluates the drug-likeness of compounds based on their molecular properties. It includes parameters such as molecular weight (MW>500 g/mol), lipophilicity (MLog P<4.15), hydrogen bond acceptors (HBA<10), hydrogen bond donors (HBD<5), and the number of rotatable bonds ≤10 [32]. Ghose's criteria focus on the molecular weight (160≤MW≤480), lipophilicity (-0.4≤WLOGP≤5.6), molar refractivity (40≤MR≤130), and the number of atoms in the molecule (20≤number of atoms≤70) [33]. Based on Lipinski's rules, a compound must meet to have optimal drug-like properties as follows: MW>500 g/mol, HBA<10, HBD<5, and MLog P<4.15 [34,37]. Muegge's rule is a more comprehensive criterion than Lipinski's. This rule includes additional parameters such as the number of hydrogen bond acceptors (≤7) and donors (≤2), and the number of rotatable bonds (≤10) [35]. Veber's rule is similar to Lipinski's rule but includes an additional parameter, which assesses the topological polar surface area of the molecule (TPSA≤140) [36]. Compounds satisfy Lipinski's criteria if their deviation is under 2, whereas Ghose, Veber, Egan, and Muegge demand a deviation of less than 1. Lipinski's rule is often used for evaluating drug-likeness because its parameters are more lenient than the others.

Toxicology assessment using brine shrimp lethality assay (BSLA) and ProTox 3.0

Toxicology assessment was used to initially screen the compounds for further more in-depth investigation [38]. In this study, two methods were used to evaluate the toxicity of ethanolic extract *L. camara*: cytotoxicity using brine shrimp lethality assay (BSLA) and toxicity prediction using ProTox 3.0. The cytotoxicity of ethanolic extracts from *L. camara* was tested on brine shrimp nauplii of *Artemia salina* to estimate a lethal concentration of 50% (LC_{50}) of the extract [39]. The LC_{50} value represents the concentration at which 50% of the test organisms, brine shrimp nauplii in this case, are expected to die. Extracts were made with concentrations of 10, 100, and 1,000 mg/mL. Each concentration (2 mL) was placed in a vial, evaporated for 48 hours, and then redissolved in dimethyl sulfoxide before the nauplii were added. Eggs of brine shrimp incubated and hatched in a saline solution inside an aerated divided rectangular container. The eggs were placed in the larger, darker section of the container. After 48 hours, mature nauplii moved to a smaller, brighter section. Ten nauplii were then placed in each vial, and saline solution was added to bring the total volume to 2 mL. The vials were kept at 25°C for 24 hours, and then the surviving nauplii were counted [38,40]. The data was corrected using Abbott's formula, which states that the percentage of death = ((test−control)/control) × 100%.

Following this, the SMILES of compounds from GC-MS analysis were evaluated for toxicity class (I to VI), LD_{50} (mg/kg), and activity (active or inactive) using five criteria, namely hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity using ProTox 3.0 [41]. Toxicity classes are defined according to the Globally Harmonized System of Classification of Labelling of Chemicals (GHS). This method classifies the substances according to their toxicity levels into six classes: (1) class I: fatal if swallowed ($LD_{50}≤5$); (2) class II: fatal if swallowed ($5<LD_{50}≤50$); (3) class III: toxic if swallowed ($50<LD_{50}≤300$); (4) class IV: harmful if swallowed ($300<LD_{50}≤2,000$); (5) class V: may be harmful if swallowed ($2,000<LD_{50}≤5,000$); and (6) class VI: non-toxic ($LD_{50}>5,000$) in mg/kg [42]. In predicting toxicity, ProTox 3.0 combines molecular similarity, fragment propensity, and machine learning to determine whether a compound result is "active" or "inactive" [41].

Network pharmacology

Network pharmacology consists of potential active compounds and targets, protein-protein interactions (PPI), enrichment of Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

Potential active compounds and targets

The metabolite compounds of *L. camara* were evaluated using SwissTargetPrediction (Swiss Institute of Bioinformatics, Lausanne, Switzerland) [43] to identify the target protein/enzyme associated with metabolite compounds of ethanolic extract *L. camara*. Additionally, the GeneCards database (Weizman Institute of Science) was employed with the keyword "inflammation" to compile all targets and eliminate any duplicates of protein or enzyme related to inflammation, resulting in a total of 500 candidates used as inflammatory targets [44]. The intersection of active substances with the targets from SwissTargetPrediction and GeneCards was depicted using a Venn diagram.

Construction protein-protein interactions (PPIs)

The aim of PPIs in network pharmacology is to figure out the complex relationships between proteins and their roles in various biological processes. The intersecting targets of the metabolites from the ethanolic extract of *L. camara* and the protein targets were analyzed using the STRING database (https://string-db.org/) [45]. The threshold for the maximum confidence level for the interaction score was set at 0.900, and non-associated proteins were concealed prior to visualization using open-source software Cytoscape 3.9.1 (Cytoscape Consortium, San Diego, USA) [46]. The PPI network parameters of drug targets, including degree, betweenness centrality, closeness centrality, and clustering coefficient, were analyzed to understand the intermediate functions of compounds from ethanol extract L. *camara* and protein/enzyme targets related to inflammation [11,47].

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

Enrichment of the GO and KEGG pathways aims to identify and analyze functional associations between genes, biological processes, and molecular pathways [48]. The GO enrichment score is calculated using the hypergeometric test *p*-value for a gene set consisting of a gene, its direct STRING neighbors, and a GO term (biological process (BP), molecular function (MF), and cellular component (CC)) [49]. Next, the KEGG enrichment score is also calculated using the hypergeometric test *p*-value for a gene set that includes a gene, its STRING direct neighbors, and a KEGG pathway. Enrichment of GO was conducted using the DAVID database (National Institutes of Health, Bethesda, USA) [50]. The highest *p*-value from the GO terms and KEGG pathway were then determined using the $-\log_{10}$ (*p*-value) [51]. Afterwards, the collected results were visualized using the SRPlot tool (Chinese Academy of Sciences, Beijing, China) to aid in their interpretation and analysis [52].

In vitro analysis

The protein inhibition assay and the protein denaturation assay were employed in vitro to assess the potential anti-inflammatory properties of the ethanolic extract of *L. camara*.

Protein inhibition assay

To evaluate the ability of the ethanolic extract of *L. camara* to inhibit protein activity related to the inflammation process, the protein inhibition assay was conducted. The assay solution was prepared by mixing 1 mL of 20 mM Tris HCl buffer (pH 7.4), 1 mL of extract at various concentrations (100, 200, 300, 400, and 500 μg/mL), and 0.06 mg of trypsin. This mixture was incubated for 5 minutes at 37°C. After the initial incubation, 1 mL of 0.8% (w/v) casein was added to the solution, and it was incubated again for 20 minutes. To stop the reaction, 2 mL of 70% perchloric acid was added. The suspension was then centrifuged, and the absorbance of the supernatant was measured at 210 nm using a blank buffer as a reference [53]. The percentage inhibition of protein inhibition was measured using the formula = ((absorbance control–absorbance sample)/absorbance control) × 100%.

Inhibition of albumin denaturation
To evaluate the ability of the ethanolic extract of *L. camara* to inhibit the denaturation of protein, the inhibition of albumin denaturation assay was conducted. The assay solution, which included extracts at concentrations of 100, 200, 300, 400, and 500 μg/mL and a 1% aqueous solution of bovine albumin, was prepared and its pH was adjusted using a small amount of 1N HCl. This mixture was then incubated at 37°C for 20 minutes. Following this initial incubation, the sample extracts were heated to 51°C for an additional 20 minutes. After allowing the samples to cool, the turbidity was measured at 660 nm [54]. The percentage inhibition of protein denaturation was calculated using the formula = ((absorbance control−absorbance sample)/absorbance control) × 100%.

Antioxidant capacity
The 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were employed to assess each extract's ability to scavenge free radicals. A BioRad Xmark microplate reader (Hercules, California, USA) was used to measure the absorbance of the experiment, which was carried out in a 96-well microplate. In each well of the plate, 100 μL of the extract at concentrations 20, 40, 60, 80, and 100 μg/mL and 100 μL of DPPH solution and ABTS solution were added.

As a standard, ascorbic acid was utilized at 2, 4, 6, 8, and 10 μg/mL and was handled similarly to the samples. After 30 minutes of room temperature incubation in the dark, the reaction mixture was shaken. Using a microplate reader, the absorbance was determined for DPPH at 517 nm and ABTS at 745 nm. The inhibitory concentration 50% (IC_{50}) value served as a representation of the antioxidant activity. The plotted graphs displaying scavenging activity against sample concentration were calculated with percentage antioxidant capacity = ((absorbance control−absorbance sample)/absorbance control) × 100%.

Statistical analysis
The lethal concentration for 50% was calculated by the probit method, for comparison of the toxicity of the ethanolic extract of *L. camara*. The percentages of mortality were transformed into probits, the regression of the logarithm of the dose versus the probits of mortality to determination of the LD_{50}. In in vitro analysis, all measurements were recorded as mean ± standard error of the mean (SEM) and statistical analysis was done using Student's t-test with *p*-values of <0.05. Data from each experiment were statistically analyzed using R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Gas chromatography-mass spectrometry (GC-MS) identification of phytocompounds
The GC-MS analysis of the ethanolic extract of *L. camara* leaves from Ie-Seu'um revealed the presence of 22 distinct peaks (**Figure 1**). The results indicated a diverse range of secondary metabolites, which was primarily consisting of terpenoids and fatty acids. The percentage area of the chromatogram revealed that 10 compounds had an area larger than 1%. These compounds were β-acorenol (1.91%), (1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol (3.41%), germacrene B (3.83%), dodecanoic acid, 3-hydroxy- (3.51%), neophytadiene (4.6%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (7.82%), *n*-hexadecanoic acid (9.02%), β-caryophyllene (10.72%), germacrene D (12.35%), and phytol (15.6%). These compounds were selected to investigate the drug-likeness of metabolite compounds from the ethanolic extract of *L. camara*.

Drug-likeness properties
Ten of the metabolite compounds from the ethanolic extracts of the leaves of *L. camara* meet Lipinski's rules with deviations not exceedingly over two (**Table 1**). The additional parameters showed that two compounds (dodecanoic acid, 3-hydroxy- and 9,12,15-octadecatrienoic acid, (Z,Z,Z)-) fulfilled the requirements without any breaches, suggesting their adherence to drug-like

characteristics. Conversely, Muegge's rules exhibited numerous deviations compared to others. In certain compounds, multiple heteroatoms failed to satisfy these rules. Overall, the results indicated that the whole 10 metabolite compounds from ethanolic extract of *L. camara* might be good candidates for future drug development.
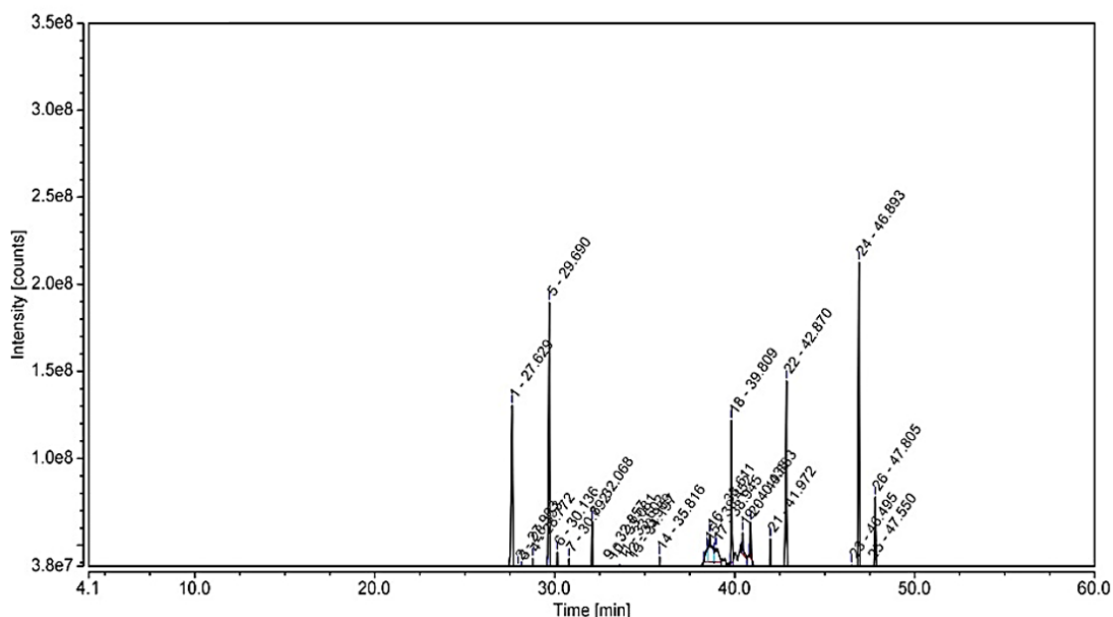

Figure 1. Chromatogram of the metabolite compounds of the ethanolic extract of *Lantana camara* using gas chromatography-mass spectrometry (GC-MS) analysis.

Table 1. Drug-likeness analysis of the bioactive compounds in *L. camara* leaves

Compounds	Lipinski [a]	Ghose [b]	Veber [b]	Egan [b]	Muegge [b]
β-caryophyllene	1	0	0	0	1
Germacrene D	1	0	0	0	1
β-acorenol	0	0	0	0	1
Germacrene B	1	0	0	0	1
(1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol	0	0	0	0	1
Dodecanoic acid, 3-hydroxy-	0	0	0	0	0
Neophytadiene	1	1	1	1	2
n-hexadecanoid acid	1	0	1	0	1
Phytol	1	1	1	1	2
9,12,15-octadecatrienoic acid, (Z,Z,Z)-	0	0	0	0	0

[a] Meets the criteria if the deviation is <2
[b] Meets the criteria if the deviation is <1

Toxicology assessment

The Probit analysis of BSLA results indicated that the extract of *L. camara* had an LC_{50} value of 574 ppm. According to the BSLA toxicity classification, if the LC_{50} value is more than 500, the substance is considered essentially non-toxic [38]. All 10 of the metabolite compounds from ethanolic extract of *L. camara* were inactive in five different parameters, but three compounds were active in immunotoxicity ((1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol, β-caryophyllene, and germacrene D) (**Table 2**). Ethanolic extract of *L. camara* was categorized into toxicity classes IV through VI, with hexadecanoic acid found to have the lowest dose at 900 mg/kg, whereas 9,12,15-octadecatrienoic acid, (Z,Z,Z)- was found to have the highest dose at 10,000 mg/kg. Based on these results, the metabolite compounds obtained from the ethanolic extract of *L. camara* leaves appear to have less potential for toxicity.

Table 2. Toxicity assessment of the bioactive compounds present in *L. camara* leaves

Compounds name	LD$_{50}$ (mg/kg)	Toxicity class	HTx	CGn	ITx	MGn	CTx
Hexadecanoic acid	900	IV	In	In	In	In	In
β-acorenol	2,000	IV	In	In	In	In	In
(1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol	4,300	V	In	In	A	In	In
Germacrene B	4,390	V	In	In	In	In	In
Dodecanoic acid, 3-hydroxy-	4,820	V	In	In	In	In	In
Phytol	5,000	V	In	In	In	In	In
Neophytadiene	5,050	VI	In	In	In	In	In
β-caryophyllene	5,300	VI	In	In	A	In	In
Germacrene D	5,300	VI	In	In	A	In	In
9,12,15-octadecatrienoic acid, (Z,Z,Z)-	10,000	VI	In	In	In	In	In

A: active; CGn: carcinogenicity; CTx: cytotoxicity; HTx: hepatotoxicity; In: inactive; ITx: immunotoxicity; MGn: mutagenicity

Network pharmacology

Potential active compounds and targets

Using the Venn diagram for data processing, 36 potential targets were detected in the overlapping segment between SwissTargetPredictions and GeneCards (**Figure 2**). This suggested that the active chemicals of *L. camara* can interact with inflammatory mediators, making them potential targets for anti-inflammatory treatment.
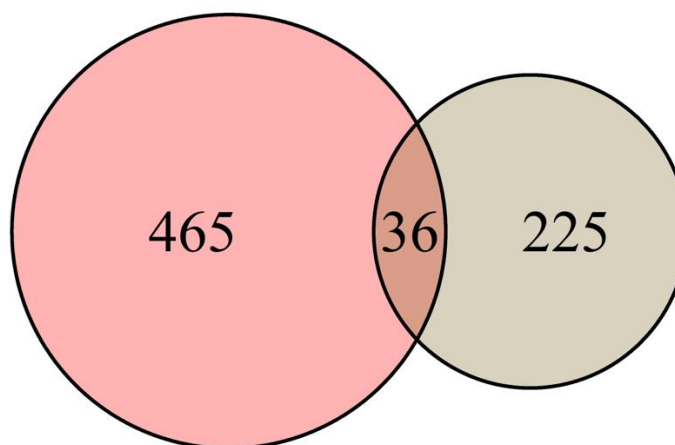

Figure 2. The intersection of data interactions from Genecards (pink punch) and SwissTarget (brown peanut) using a Venn diagram.

Protein-protein interactions (PPIs)

The PPI analysis was performed on 36 overlapping targets using the STRING database [45]. The PPI analysis results are presented in **Figure 3**. The 10 genes' results in degree, betweenness centrality, closeness centrality, and clustering coefficient value can be seen in **Table 3**. The network's relatively high node degree indicated significant connectedness and reflected its complexity in the network. The top five degrees in the networks linked to inflammation are *PPARG* (23), *PTGS2* (22), *EGFR* (18), *HIF1A* (16), and *JAK2* (16). The gene with the highest degree is *PPARG* or peroxisome proliferator-activated receptor gamma with 23 degrees, 0.229 of betweenness centrality, 0.780 closeness centrality, and 0.328 cluster coefficient. Next, an enzyme involved in pain and inflammation is called prostaglandin G/H synthase 2 (*PTGS2*), or cyclooxygenase-2 inhibitors (COX-2). It displayed a high value of 22 degrees, suggesting numerous direct interactions with other genes. The other parameter from *PTGS2*, namely closeness centrality, is high, which was at 0.761, suggesting ease of interaction with other nodes. The clustering coefficient is 0.3766 and was higher than that of *PPARG*, suggesting a higher tendency to form clusters. Furthermore, betweenness centrality was at 0.151, which is moderate but still significant for network connectivity. There is still potential for other genes with less direct interactions in the network, such as epidermal growth factor receptor (*EGFR*), hypoxia-inducible

factor 1-alpha (*HIF1A*), and tyrosine protein kinase-Janus kinase 2 (*JAK2*). However, *PPARG* and *PTGS2* are particularly important in this study because of their high centrality and connectivity.
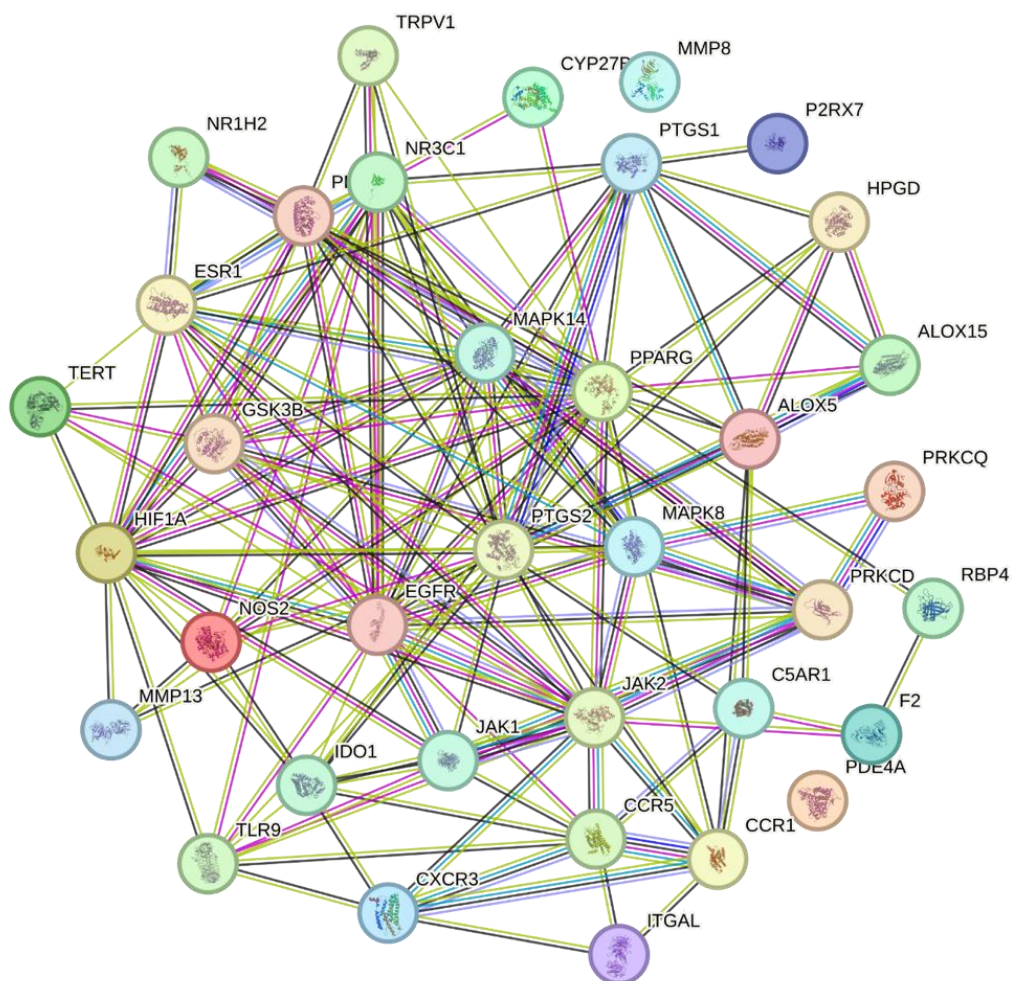

Figure 3. Topological network of anti-inflammation targets with 36 associated proteins from protein-protein interactions (PPI).

Table 3. Genes associated with the highest degree value

Display name	Description	Degree	Betweenness centrality	Closeness centrality	Clustering coefficient
PPARG	Peroxisome proliferator-activated receptor gamma	23	0.229	0.780	0.328
PTGS2	Prostaglandin G/H synthase 2	22	0.151	0.761	0.3766
EGFR	Epidermal growth factor receptor	18	0.064	0.695	0.509
HIF1A	Hypoxia-inducible factor 1-alpha	16	0.032	0.653	0.5667
JAK2	Tyrosine-protein kinase JAK2	16	0.106	0.667	0.4416
NR3C1	Glucocorticoid receptor	14	0.031	0.615	0.571
ESR1	Estrogen receptor	13	0.019	0.615	0.667
PPARA	Peroxisome proliferator-activated receptor alpha	12	0.025	0.581	0.575
MAPK8	Mitogen-activated protein kinase 8	12	0.051	0.603	0.636
GSK3B	Glycogen synthase kinase-3 beta	11	0.007	0.592	0.745

Enrichment of gene ontology and Kyoto encyclopedia of genes and genomes
Enrichment in network pharmacology is divided into biological processes (BP), cellular components (CC) and molecular functions (MF). The significance of the enrichment result was calculated by the $-\log_{10}$ (*p*-value). Based on GO analysis, the value of $-\log_{10}$ (*p*-value) for the top five of BP is inflammatory response (10), positive regulation of pri-miRNA transcription from RNA polymerase II promoter (7), negative regulation of gene expression (7), signal transduction (7), and positive regulation of cytosolic calcium ion concentration (7) (**Figure 4A**).
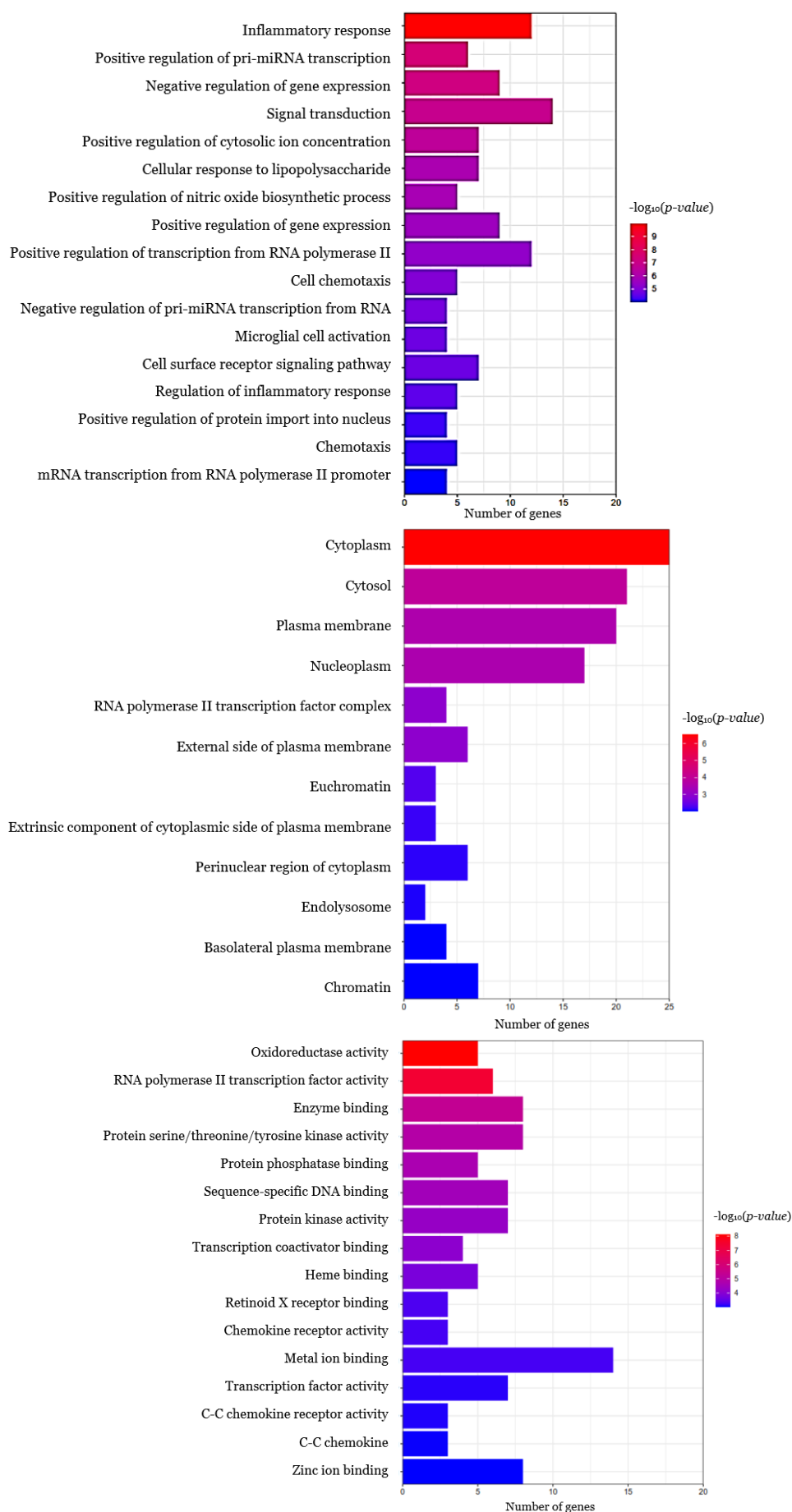
Figure 4. Enrichment of gene ontology: (A) biological processes, (B) cellular components, and (C) molecular functions.

The cellular component refers to the specific location of a gene product within cellular compartments and structures. Based on GO enrichment, cellular components in the system of network pharmacology from ethanolic extract of *L. camara* were cytoplasm (6), cytosol (4), plasma membrane (4), nucleoplasm (4), and external side of the plasma membrane (3) (**Figure 4B**). Molecular function found in oxidoreductase activity (8), RNA polymerase II transcription factor activity (7), enzyme binding (5), protein serine/tyrosine kinase activity (5), and protein phosphatase binding (5) (**Figure 4C**).

Based on the bubble chart (**Figure 5**), the KEGG pathways for the interactions are Kaposi sarcoma-associated herpes virus infection (9), programmed death ligand 1 (PD-L1) expression and programmed death 1 (PD-1) checkpoint pathway in cancer (7), pathway in cancer (12), toxoplasmosis (7), and T helper 17 (Th17) cell differentiation (6). Although the pathway in cancer has more protein counts, based on the $-\log_{10}$ (*p*-value), it is known that the main target in the KEGG pathway is Kaposi sarcoma-associated herpes virus infection.
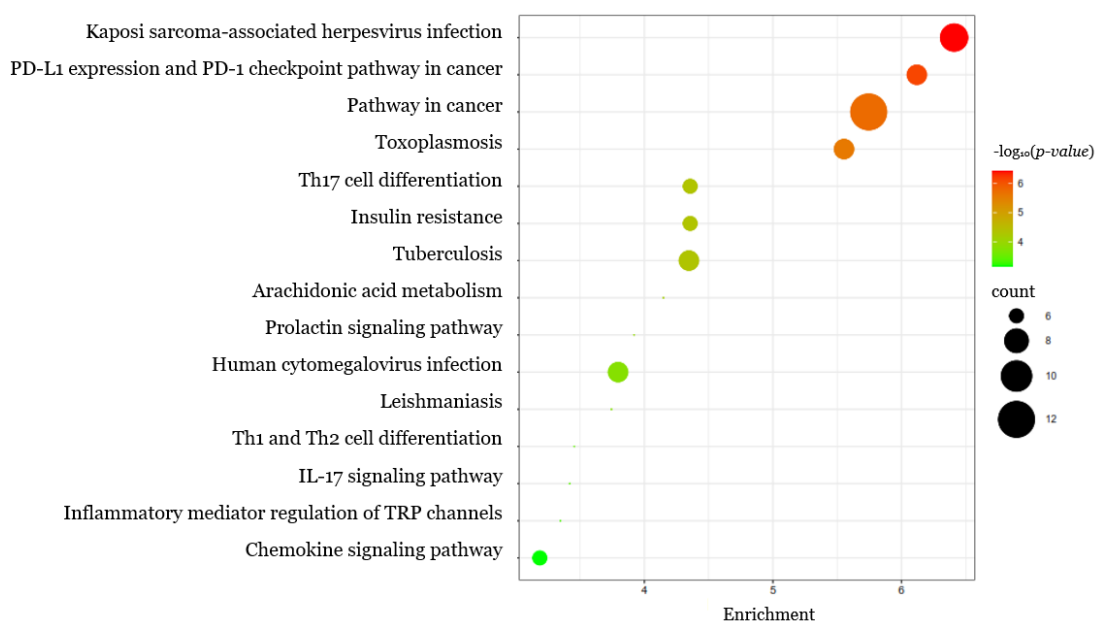

Figure 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

In vitro analysis

The protein inhibition assay and the protein denaturation assay were employed in vitro to assess the potential anti-inflammatory properties of the ethanolic extract of *L. camara*. These assays are supported by the molecular function of network pharmacology, which demonstrated that MF is enriched in protein-associated inflammation activity. Notably, the investigation revealed a clear relationship between the increasing concentration of *L. camara* extract and the corresponding rise in the percentage of inhibition observed (**Figure 6)**. Based on the linearity of percentage inhibition, *L. camara* has IC_{50} values of 202.27 and 223.85 ppm when pretending as an inhibitor of protein and protein denaturation, respectively. These results distinctly highlighted the substantial anti-proteinase activity of *L. camara* extracts across a range of concentrations in the protein inhibition assay. Additional analysis with a Student's t-test suggests that no statistically significant difference was found between the aspirin utilized as a positive control at a significance level of *p*<0.05.

The effectiveness of the total ethanol extracts of *L. camara* in scavenging free radicals was quantitatively assessed through a DPPH and ABTS assay. The IC_{50} value denotes the concentration of the extract necessary to achieve a 50% inhibition of activity in free radical scavenging. The findings demonstrated that the positive control, ascorbic acid, exhibited an IC_{50} value of 16.63 ppm, while the ethanolic extract of *L. camara* showed an IC_{50} value of 140 ppm in DPPH and 163 ppm in ABTS scavenging.
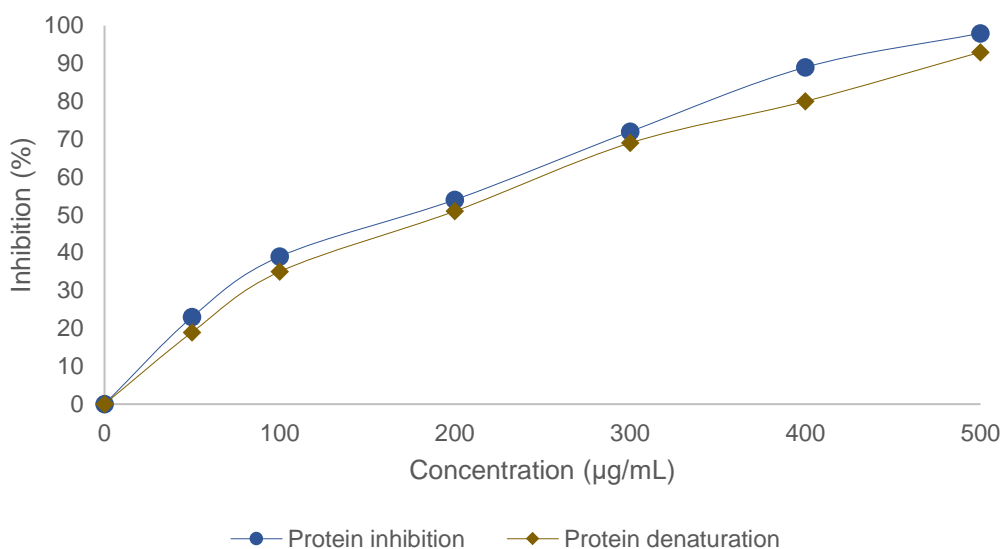
Figure 6. Effect of an ethanolic extract of *L. camara* leaves on in vitro analysis. The blue node is protein inhibition, and the brown node is protein denaturation. Each point represents the mean of inhibition.

Discussion

Inflammation is the body's natural response to injury or infection, characterized as redness, swelling, and pain at the site of injury. In this current study, *L. camara* from the Ie-Seu'um geothermal area was used as a candidate for anti-inflammatory using network pharmacology and in vitro analysis. The various compounds found in *L. camara* range from simple terpenoid to toxic flavonoid glycosides, and its pharmacological activities indicate that it has a lot of potential as a medicinal plant [16]. Our data indicated that the main components present in *L. camara* plants were sesquiterpene compounds from the terpenoid class and some fatty acids.

Protein-protein interactions (PPIs) play an essential role in the creation of macromolecular structures and enzymatic complexes, both of which are fundamental to almost every biological process [55]. These networks facilitate the identification and confirmation of pharmacological targets, which are essential in biomedical research and the advancement of novel pharmaceuticals. In this study, we find that the top five degrees in networks associated with inflammation are *PPARG*, *PTGS2*, *EGFR*, *HIF1A*, and *JAK2*. From the network pharmacology approach, it is known that discernible evidence suggested the presence of correlations between specific pairs of these genes to control the inflammatory response. According to the highest degree of connectivity, *PPARG* is the most connected with 23 degrees. *PPARG* or PPARγ is a member of the steroid receptor superfamily's PPARs, which function as ligand-activated nuclear hormone receptors (NRs) [56]. *PPARG*'s high degree and betweenness centrality suggested that it is an important node in the network. It appeared to act as a central mediator in the network, facilitating interactions between other nodes in addition to interacting directly with numerous other proteins, based on its moderate clustering coefficient and high closeness centrality. This may suggest that *PPARG* is essential to many biological processes and that it should be a major target for therapeutic interventions in conditions where these processes are hampered. In order to control inflammation, PPARγ is essential. Research has demonstrated that PPARγ reduces inflammation by preventing macrophage activation and the release of inflammatory cytokines such as TNF-α, IL-6, and Il-1β [56,57]. Activation of PPARγ has been linked to inflammation, and its ligands have shown promise in treating inflammatory diseases like ulcerative colitis and Crohn's disease [57]. According to this study, protein interactions have multiple faces based on network connectivity, implying that complex mechanisms, rather than a single protein. *PPARG* and *PPARA*, for instance, bind to particular response elements in the promoters of pro-inflammatory genes to inhibit their expression [58]. Next, by activating transcription factors involved in the inflammatory response, *EGFR* and *HIF1A* can similarly induce the expression of genes that promote inflammation. From this research, it is known that pro-inflammatory

mediator suppression is mediated by *PPARG*, prostaglandin synthesis is mediated by *PTGS2*, cell growth, and differentiation is mediated by *EGFR*, the response to hypoxia is mediated by *HIF1A*, and pro-inflammatory cytokines are produced by *JAK2* [58]. A study by El-banna *et al.* [14] showed differences in the main inflammation-related genes of *L. camara*, including protein kinase c alpha (*PRKCA*), transcription factor p65 (*RELA*), interleukin-2 (*IL2*), mitogen-activated protein kinase 14 (*MAPK14*) and proto-oncogene c-Fos (*FOS*).

The GO enrichment showed that the highest enrichment ($-\log_{10}$ of p-value) is inflammatory responses, which play a pivotal role in the body's defense against harmful stimuli. A multitude of signaling pathways that control the expression of pro- and anti-inflammatory mediators in both resident tissue cells and leukocytes drawn from the bloodstream are coordinately activated during an inflammatory response [3,59,60]. The cytoplasm is crucial in network pharmacology, due to its role in the localization of a variety of molecular and biological processes. Different cellular processes, such as metabolic pathways, cell signaling, and the flow of materials inside the cell, are all dependent on the cytoplasm, which plays an essential part in all these tasks. Oxidoreductase activity is a critical component of the inflammatory response, with different oxidoreductases being regulated by distinct Toll-like receptors (TLRs) stimuli and influenced by reactive oxygen species (ROS) and reactive oxygen species (RNS) [61]. Understanding the specific roles of these enzymes can provide valuable insights into the development of therapeutic strategies for inflammatory diseases.

The ability to hinder protein denaturation serves as an indicator of a substance's effectiveness in anti-inflammatory actions. Denatured proteins have the ability to activate cellular inflammatory pathways, which in turn cause the release of mediators that promote inflammation [62]. These mediators attract immune cells to the damaged area, which results in inflammation. According to the in vitro analysis, *L. camara* has IC_{50} values of 202.27 and 223.85 ppm when acting as an inhibitor of protein and protein denaturation. *L. camara* is a strong-smelling shrub with yellow flowers that grows in the Ie-Seu'um geothermal area. In this study, through antioxidant capacity, the extract's antioxidant values of DPPH-scavenging at 140 ppm and ABTS-scavenging at 163 ppm. It was found that the yellow variety of *L. camara's* extract showed enhanced antioxidant qualities and may be used as a natural antioxidant to treat conditions brought on by free radicals [63]. Four distinct varieties of *L. camara*, whose leaves are rich in phenolic compounds, have been extracted with methanol and shown to have strong antioxidant, free radical scavenging, and in vitro lipid peroxidation inhibition properties [59]. This discovery suggests that *L. camara* may have antioxidant qualities and be a viable anti-inflammatory candidate. The identification of specific terpenoids and a detailed safety profile, including the LC_{50} value and absence of significant hepatotoxic, mutagenic, or cytotoxic effects, strengthen the validity of the findings and potential therapeutic relevance. Another study showed that cell cytotoxicity (CC_{50}) of *L. camara* extract is 382.5 µg/mL and indicates the extract is safer than piroxicam as a drug of inflammation [14]. In vivo examination of *L. camara's* five different sections—root, stem, leaf, flower, and fruit—also confirmed this result by showing that the plant's root extract was its most toxic component compared to another [39]. The study's novelty lies in exploring *L. camara* from a unique geothermal region, which could result in distinct chemical properties and potential therapeutic effects [64].

Conclusion

The aim of this study was to explore the anti-inflammatory potential of *Lantana camara* Linn leaves collected from the Ie-Seu'um geothermal area in Aceh, Indonesia, using network pharmacology and in vitro analysis. Through GC-MS analysis, the ethanolic extract of *L. camara* was identified to contain predominantly terpenoids and fatty acids, exhibiting drug-like properties. Network pharmacology identified key proteins involved in inflammation, highlighting the potential therapeutic targets. The interaction of multiple proteins from phytocompounds of ethanolic extract *L. camara* and protein-related inflammation suggests that anti-inflammatory action involves interconnected molecular mechanisms. This action is through multiple molecular targets, including pro-inflammatory cytokines, transcription factors, prostaglandin synthesis, membrane receptors, and other inflammatory mediators. In vitro assays demonstrated that the extract inhibited protein activity and denaturation, with IC_{50} values of 202.27 ppm and 223.85

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ppm, respectively, indicating significant anti-inflammatory effects. Additionally, the extract had antioxidant activity with a DPPH-scavenging IC_{50} value of 140 ppm and ABTS-scavenging with an IC_{50} value of 163 ppm. Toxicological assessments suggested the extract's safety, with a lethal concentration (LC_{50}) value indicating non-toxicity and predictions indicating non-activity in various toxicological parameters. The findings of this study suggest that the regulation of protein activity could potentially offer therapeutic approaches for the treatment of inflammation. This, in turn, facilitates the exploration and utilization of the therapeutic potential of *L. camara* for various health applications.

Ethics approval

Not required.

Acknowledgments

None.

Competing interests

All the authors declare that there are no conflicts of interest.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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References

1. Santana FPR, Da Silva RC, Grecco SDS, *et al.* Inhibition of MAPK and STAT3-SOCS3 by sakuranetin attenuated chronic allergic airway inflammation in mice. Mediators Inflamm 2019;2019:1356356.

2. Scotece M, Conde-Aranda J. Inflammation in health and disease: New insights and therapeutic avenues. Int J Mol Sci 2022;23(15):8392.

3. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol 2009;1(6):a001651.

4. Furman D, Campisi J, Verdin E, *et al.* Chronic inflammation in the etiology of disease across the life span. Nat Med 2019;25(12):1822-1832.

5. Noviandy TR, Maulana A, Idroes GM, *et al.* QSAR-Based stacked ensemble classifier for hepatitis C NS5B inhibitor prediction. 2023 2nd International Conference on Computer System, Information Technology, and Electrical Engineering (COSITE): IEEE; 2023.

6. Suhendra R, Husdayanti N, Suryadi S, *et al.* Cardiovascular disease prediction using gradient boosting classifier. Infolitika J Data Sci 2023;1(2):56-62.

7. Noviandy TR, Maulana A, Idroes GM, *et al.* Integrating genetic algorithm and LightGBM for QSAR modeling of acetylcholinesterase inhibitors in Alzheimer's disease drug discovery. Malacca Pharm 2023;1(2):48-54.

8. Tallei TE, Fatimawali, Yelnetty A, *et al.* An analysis based on molecular docking and molecular dynamics simulation study of bromelain as Anti-SARS-CoV-2 variants. Front Pharmacol 2021;12:717757.

9. Rachmawati R, Idroes R, Suhartono E, *et al.* In silico and in vitro analysis of tacca tubers (Tacca leontopetaloides) from Banyak Island, Aceh Singkil Regency, Indonesia, as antihypercholesterolemia agents. Molecules 2022;27(23):8605.

10. Imelda E, Idroes R, Khairan K, *et al.* Natural antioxidant activities of plants in preventing cataractogenesis. Antioxidants 2022;11(7):1285.

11. Tallei TE, Fatimawali, Adam AA, *et al.* Molecular insights into the anti-inflammatory activity of fermented pineapple juice using multimodal computational studies. Arch Pharm (Weinheim) 2024;357(1):e2300422.

12. Tallei TE, Fatimawali, Yelnetty A, *et al.* Evaluation of the potential for immunomodulatory and anti-inflammatory properties of phytoconstituents derived from pineapple [Ananas comosus (L.) Merr.] peel extract using an in silico approach. Philipp J Sci 2022;151(1):397-410.

13. Maulydia NB, Khairan K, Tallei TE, *et al.* Exploring the medicinal potential of Blumea balsamifera: Insights from molecular docking and molecular dynamics simulations analyses. Malacca Pharm 2024;2(1):33-40.

14. El-Banna AA, Darwish RS, Ghareeb DA, *et al.* Metabolic profiling of Lantana camara L. using UPLC-MS/MS and revealing its inflammation-related targets using network pharmacology-based and molecular docking analyses. Sci Rep 2022;12(1):1-17.

15. Ashal TF, Ifora I, Oktavia S. Potential anti-inflammatory effects of Lantana camara L.: A review. Int Res J Pharm Med Sci 2020;3(6):1-4.

16. Ghisalberti EL. Lantana camara L. (Verbenaceae). Fitoterapia 2000;71(5):467-486.

17. Battase L, Attarde D. Phytochemical and medicinal study of Lantana camara Linn. (Verbenaceae) - a review. Asian J Pharm Clin Res 2021;14(9):20-27.

18. Girish K. Antimicrobial activities of Lantana camara Linn. Asian J Pharm Clin Res 2017;10(3):57-67.

19. Darwish RS, El-Banna AA, Ghareeb DA, *et al.* Chemical profiling and unraveling of anti-COVID-19 biomarkers of red sage (Lantana camara L.) cultivars using UPLC-MS/MS coupled to chemometric analysis, in vitro study and molecular docking. J Ethnopharmacol 2022;291:115038.

20. U.S. Department of Agriculture. Dr. Duke's Phytochemical and Ethnobotanical Databases. 1992. Available from: https://phytochem.nal.usda.gov/. Accessed: 1 May 2024.

21. Idroes R, Yusuf M, Saiful S, *et al.* Geochemistry exploration and geothermometry application in the north zone of Seulawah Agam, Aceh Besar District, Indonesia. Energies 2019;12(23):4442.

22. Idroes GM, Hardi I, Rahman MH, *et al.* The dynamic impact of non-renewable and renewable energy on carbon dioxide emissions and ecological footprint in Indonesia. Carbon Res 2024;3(1):35.

23. Hochstein MP, Sudarman S. History of geothermal exploration in Indonesia from 1970 to 2000. Geothermics 2008;37(3):220-266.

24. Idroes GM, Hardi I, Hilal IS, *et al.* Economic growth and environmental impact: Assessing the role of geothermal energy in developing and developed countries. Innov Green Dev 2024;3(3):100144.

25. Lala A, Yusuf M, Suhendra R, *et al.* Characterization of geochemical and isotopic profiles in the southern zone geothermal systems of Mount Seulawah Agam, Aceh Province, Indonesia. Leuser J Environ Stud 2024;2(1):30-40.

26. Maulydia NB, Khairan K, Tallei TE, *et al.* GC-MS analysis reveals unique chemical composition of Blumea balsamifera (L.) DC in Ie-Jue geothermal area. Grimsa J Sci Eng Technol 2023;1(1):9-16.

27. Abubakar A, Yusuf H, Syukri M, *et al.* Heavy metals contamination in geothermal medicinal plant extract; Chromolaena odorata Linn. Glob J Environ Sci Manag 2023;9(4):995-1004.

28. Wu P, Song Z, Wang X, *et al.* Bioactive triterpenoids from Lantana camara showing anti-inflammatory activities in vitro and in vivo. Bioorganic Chem 2020;101:104004.

29. Maulydia NB, Idroes R, Khairan K, *et al.* Ecotoxicological insight of phytochemicals, toxicological informatics, and heavy metal concentration in Tridax procumbens L. in geothermal areas. Glob J Environ Sci Manag 2024;10(1):369-384.

30. Maulydia NB, Khairan K, Noviandy TR. Prediction of pharmacokinetic parameters from ethanolic extract mane leaves (Vitex pinnata L.) in geothermal manifestation of Seulawah Agam Ie-Seu'um, Aceh. Malacca Pharm 2023;1(1):16-21.

31. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:1-13.

32. Egan WJ, Merz KM, Baldwin JJ. Prediction of drug absorption using multivariate statistics. J Med Chem 2000;43(21):3867-3877.

33. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J Comb Chem 1999;1(1):55-68.

34. Lipinski CA. Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. Adv Drug Deliv Rev 2016;101:34-41.

35. Muegge I, Heald SL, Brittelli D. Simple selection criteria for drug-like chemical matter. J Med Chem 2001;44(12):1841-1846.

36. Veber DF, Johnson SR, Cheng HY, et al. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 2002;45(12):2615-2623.

37. Tumilaar SG, Fatimawali F, Niode NJ, et al. The potential of leaf extract of Pangium edule Reinw as HIV-1 protease inhibitor: A computational biology approach. J Appl Pharm Sci 2021;11(1):101-110.

38. Clemen-Pascual LM, Macahig RAS, Rojas NRL. Comparative toxicity, phytochemistry, and use of 53 Philippine medicinal plants. Toxicol Rep 2022;9:22-35.

39. Pour BM, Sasidharan S. In vivo toxicity study of Lantana camara. Asian Pac J Trop Biomed 2011;1(3):230-232.

40. Meyer BN, Ferrigni NR, Putnam JE, et al. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982;45(1):31-34.

41. Banerjee P, Kemmler E, Dunkel M, et al. ProTox 3.0: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 2024;9999(1):1-8.

42. Banerjee P, Eckert AO, Schrey AK, et al. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 2018;46(W1):W257-W263.

43. Daina A, Michielin O, Zoete V. SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res 2019;47(W1):W357-W3664.

44. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: From gene data mining to disease genome sequence analyses. Curr Protoc Bioinforma 2016;54:1.30.1-1.30.33.

45. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res 2023;51(D1):D638-D646.

46. Shannon P, Markiel A, Ozier O, et al. Cytoscape: A software environment for integrated models. Genome Res 2003;13(22):426.

47. Raman K. Construction and analysis of protein-protein interaction networks. Autom Exp 2010:2(1):2.

48. Thomas PD. The gene ontology and the meaning of biological function. Methods Mol Biol 2017;1446:15-24.

49. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: Tool for the unification of biology. The gene ontology consortium. Nat Genet 2000;25(1):25-29.

50. Sherman BT, Hao M, Qiu J, et al. DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022;50(W1):W216-W221.

51. Wu S, Chen R, Chen J, et al. Study of the anti-inflammatory mechanism of β-carotene based on network pharmacology. Molecules 2023;28(22):7540.

52. Tang D, Chen M, Huang X, et al. SRplot: A free online platform for data visualization and graphing. PLoS One 2023;18(11):e0294236.

53. Oyedapo OO, Famurewa AJ. Antiprotease and membrane stabilizing activities of extracts of Fagara zanthoxyloides, Olax subscorpioides and Tetrapleura tetraptera. Int J Pharmacogn 1995;33(1):65-69.

54. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968;20(3):169-173.

55. Murakami Y, Tripathi LP, Prathipati P, et al. Network analysis and in silico prediction of protein-protein interactions with applications in drug discovery. Curr Opin Struct Biol 2017;44:134-142.

56. Decara J, Rivera P, López-Gambero AJ, et al. Peroxisome proliferator-activated receptors: Experimental targeting for the treatment of inflammatory bowel diseases. Front Pharmacol 2020;11:1-18.

57. Kostadinova R, Wahli W, Michalik L. PPARs in diseases: Control mechanisms of inflammation. Curr Med Chem 2005;12(25):2995-3009.

58. He W, Shi F, Zhou ZW, et al. A bioinformatic and mechanistic study elicits the antifibrotic effect of ursolic acid through the attenuation of oxidative stress with the involvement of ERK, PI3K/Akt, and p38 MAPK signaling pathways in human hepatic stellate cells and rat liver. Drug Des Devel Ther 2015;9:3989-4104.

59. Samudra D, Sumarno, Santoso S, et al. The effect of Escherichia coli on pro-inflammatory mediator level and kidney and liver function of sepsis in Rattus novergicus. Indian J Forensic Med Toxicol 2020;14(4):4302-4305.

60. Putra AK, Sa'Adi A, Widjiati, et al. The downregulation of pro-inflammatory cytokine Interleukin-6 after oral administration of Lactobacillus plantarum IS-10506 in mice model for endometriosis. Indian J Anim Res 2023;57(5):620-625.

61. Li Y, Deng SL, Lian ZX, et al. Roles of toll-like receptors in nitroxidative stress in mammals. Cells 2019;8(6):576.

62. Seidler NW, Yeargans GS. Effects of thermal denaturation on protein glycation. Life Sci 2002;70(15):1789-1799.

63. Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC Res Notes 2014;7(1):1-9.

64. Imelda E, Khairan K, Lubis RR, *et al.* Impact of environmental and geographical position on the chemometric classification of ethanol extracts from Isotoma longiflora leaves. Glob J Environ Sci Manag 2024;10(1):1-14.