International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Special Issue 3, 2025

Original Article

GC-MS METABOLITE PROFILING, TOTAL PHENOLIC, ANTIOXIDANT ACTIVITY, AND IN SILICO APPROACH IN CHRONIC ANTI-INFLAMMATORY ETHANOL EXTRACTS OF *POLYSCIAS SCUTELLARIA* (BURM. F.) FOSBERG LEAVES

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Received: 15 Apr 2025, Revised and Accepted: 05 Jul 2025

ABSTRACT

Objective: *Polyscias scutellaria* has been recognised as a medicinal herb with therapeutic potential. Various studies have identified bioactive compounds in this plant that exhibit a range of biological activities, including anti-proliferative, anti-inflammatory, anti-parasitic, and anti-diabetic properties.

Methods: This study aimed to profile the Extract Ethanol *Polyscias scutellaria* (EEPS) metabolites using gass chromatography mass spectrometry (GC-MS) and predict their activities against four anti-inflammatory receptors obtained from the Protein Data Bank (RCSB PDB), cycloxygenase-2 (COX-2) (5IKT), interleukin-6 (IL-6) (1ALU), Interleukin-1 beta (IL-1β) IL-1 β (8C3U), and tumor necrosis factor alpha (TNF-α), TNF-α (7JRA) using AutoDock 1.5.6 software. Validation of the native ligands were carried out using the root mean square deviation (RMSD) value, and the total phenolic compounds were also examined using Spectrophotometry Ultra Violet-Visible (UV-Vis). Furthermore, the UV-Vis was also used to test the 2,2-azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) and Ferric Reducing Antioxidant Power (FRAP).

Results: The Total Phenol Content (TPC) is 131.458±8.818 ppm, and during the FRAP assay, EEPS showed the highest IC_{50} value, measured at 54.66±2.35 µg/ml, which was significantly higher than that of ascorbic acid (10.10±0.14 µg/ml). Similarly, in the ABTS assay, EEPS exhibited an IC_{50} of 55.13±1.19 µg/ml, exceeding the IC_{50} of ascorbic acid (10.47±0.29 µg/ml). Although GC-MS analysis identified eight compounds, molecular docking was performed only on the two most abundant and structurally similar compounds: (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and phytol. Native ligands were validated with RMSD values of less than 2 Å.

Conclusion: Molecular docking showed that (S-Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol binds better to the COX-2 and IL-6 receptors than Phytol. However, phytol binds better to the TNF-α and IL-1β receptors. These findings suggest potential anti-inflammatory activity, but further *in vitro* and *in vivo* studies are necessary to confirm their biological effects.

Keywords: Anti-inflammatory, Cytokine, In silico, Molecular docking, *Polyscias scutellaria*

DOI: https://dx.doi.org/10.22159/ijap.2025.v17s3.03 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The *Araliaceae* family is a substantial plant group containing 43 genera and approximately 1,400 species, widely used in both traditional and modern phytotherapy. Plants in this family are known for their diverse secondary metabolites, including triterpenes, triterpenoidal saponins, sterols, diterpenes, cerebrosides, and acetylenic lipids. This family exhibits a broad spectrum of biological activities such as anti-proliferative, anti-inflammatory, anti-parasitic, hypoglycemic, neuroprotective, and cardioprotective effects [1]. *P. scutellaria* leaves contain a variety of active compounds, such as calcium, oxalate, peroxidase, amygdalin, phosphorus, iron, lipids, proteins, vitamins A, B1, and C, tannins, saponins, alkaloids, flavonoids, and terpenoids [2–5].

Traditionally, the leaves of *P. scutellaria* have been used for the treatment of breast inflammation, wound recovery, and urinary system ailments. Scientifically, it has been shown that the leaves of *P. scutellaria* possess *in vivo* anti-inflammatory effects [6, 7], antifungal, antioxidant, antibacterial, and wound healing properties [8–10]. This is because mangkokan leaves contain phenolic compounds and other compounds that exhibit pharmacological activity. Previous study on the determination of total phenols, using 96% ethanol extract, resulted in an average Total Phenolic Content (TPC) of 14.67 mgEAG/g [4]. Phenolic compounds are known antioxidants that can neutralize reactive oxygen species (ROS), thereby preventing oxidative stress-induced cellular damage. More importantly, ROS can activate inflammatory signaling pathways, particularly through nuclear factor kappa B (NF-κB), leading to the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [11–13]. Cytokines are key modulators of inflammation that play a role in both acute and chronic inflammation. Key pro-inflammatory cytokines involved in the inflammatory process include COX-2, TNF-α, IL-1β, and IL-6 [14–16]. Previous studies have reported that *P. scutellaria* (50 µg/ml) can inhibit NO production and suppress the secretion of critical pro-inflammatory mediators (TNF-α, IL-6, IL-1β, and IL-12) in RAW 264.7 cells [17]. Several other plants that are used empirically have the potential to be further developed as anti-inflammatories [18–23]. Apart from phenolic compounds, anti-inflammatory potential has also been shown in terpenoid compounds [24].

Despite the promising pharmacological potential of *P. scutellaria*, previous studies have not comprehensively explored the relationship between the metabolite profile and anti-inflammatory mechanisms at the molecular level. In particular, there is a lack of investigations on the combination of volatile metabolite identification using GC-MS with molecular docking against multiple cytokine targets involved in inflammation [25]. This study addresses that gap by integrating GC-MS profiling of the 90% ethanol extract, TPC measurement, antioxidant assays, and molecular docking of major identified compounds against COX-2, TNF-α, IL-1β, and IL-6. According to findings, this is the first study to systematically investigate the anti-inflammatory potential of *P. scutellaria* through a multi-target molecular approach, aiming to clarify the mechanistic basis of the traditional use and resolve previous inconsistencies in the literature.

MATERIALS AND METHODS

Chemical

Gallic acid (Sigma-Aldrich, USA). Chemicals used were Folin-Ciocalteu reagent (Merck, Germany), sodium carbonate (Merck,

Germany), FRAP (Sigma Aldrich, St. Louis, Mo., 101 USA), and ABTS (Sigma Aldrich, St. Louis, Mo., 101 USA). All can be found at Sigma Aldrich. All other chemicals and solvents used in the study were of analytical grade (Smartlab).

Instrument

Gass Chromatography Mass Spectrum (Shimadzu), Spectrophotometry UV-Vis (Shimadzu), AutoDock 1.5.6 software.

Collection of plant material

The *P. scutellaria* plant was collected from Candimulyo, Magelang Regency, Central Java, Indonesia (7°30'59.6"S 110°16'22.5" E). The identification of the plants was carried out in the Pharmacognosy and Phytochemistry Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, with reference number: 25.20.7/UN1/FFA.2/BF/PT/2023.

Preparation of extracts

During the preparation, 1,000 g of powdered *P. scutellaria* leaves (simplisia) were soaked in 10 L of 90% ethanol solvent 1:10(w/v) and remaceration in a 1:5(w/v) ratio. The maceration was carried out for 72 h at room temperature, and the macerate obtained was separated from the solvent using a rotary evaporator at 50 °C, followed by a water bath (60 °C), and then stored in a refrigerator at 4 °C.

GC-MS analysis

GC-MS analysis was performed using a Thermo Scientific Trace 1310 GC system equipped with an HP-5MS UI capillary column (30m × 0.25 mm in inner diameter and 0.25 µm in film thickness). The maximum temperature applied was 325/350 °C. The GC system was connected to a Perkin Elmer Clarus 600C MS, and Ultra-high purity (UHP) helium, with a purity of 99.99%, was used as the carrier gas and maintained at a constant flow rate of 1.0 ml/min. The temperatures of the injection port, transfer line, and ion source were maintained at 230 °C, while an ionizing energy of 70 electron volts (eV) was used. The electron multiplier voltage was determined through autotuning. The oven temperature was programmed to increase from 60 °C (held for two minutes) to 280 °C (held for eight minutes) at a rate of 3 °C per min. The sample was diluted 1:100 (v/v) using dichloromethane before injection, mixed evenly, and, if needed, centrifuged at 9,500 revolutions per minute for three minutes. Particle-free diluted crude extracts (1 µl) were loaded into a syringe and injected into the injector with a split ratio of 30:1. All the mass spectra data were gathered between 40 and 550 amu and used the peak area to determine what percentage of the crude extract components were made up of each type.

Total phenolic content (TPC)

After carefully 1 ml of sample was added. 0.5 ml of Folin-Ciocalteu reagent (7.5%) were added. After the mixture was allowed to sit at room temperature for 8 min, add 4.0 ml of NaOH 1% and incubation for 1 h. Aquabidest was added to reach a total volume of 10 ml. Dilution was performed as required, and the solution was transferred to a cuvette and analyzed at maximum wavelenght.

FRAP and ABTS assays

The antioxidant activity was determined by adding 0.2 ml of FRAP solution to a test tube, followed by 0.4 ml of sample solution for each concentration. For the ABTS assay, 4.0 ml of ABTS solution was added to a test tube, followed by the addition of 0.2 ml of sample solution for each concentration. The mixture was homogenized using a vortex mixer for one minute and subsequently allowed to stand in the dark according to the operating time of each test solution. The absorbance of the solution was measured at the maximum wavelength.

Molecular docking

In silico molecular docking studies

The protein targets used are COX-2 with code 5IKT, IL-6 (1ALU), IL-1β (8C3U), and TNF-α (7JRA), obtained from the Protein Data Bank (RCSB PDB) (https://www.rcsb.org/). These proteins play important roles in inflammatory processes, making them significant for therapeutic targeting in various diseases. By understanding their structures and interactions, more effective drugs aimed at modulating these inflammatory pathways can be developed.

Preparation of ligands

The two-dimensional structure of the compound was generated using SMILES or the IUPAC name, obtained by searching for the compound name on PubChem (https://pubchem.ncbi.nlm.nih.gov/). This allowed a clear visualization of the molecular geometry and facilitated further analysis of the chemical properties.

Preparation of target macromolecules

The protein target was prepared using AutoDock 1.5.6 software. Water molecules were first removed, and the analysis was conducted to differentiate the protein target from its ligands. The selected ligand was saved separately as a PDB file. The last target macromolecule was changed by adding hydrogen atoms and charges, and it was then saved as the protein target in pdbqt format.

Docking validation

Validation of the molecular docking protocol was conducted by redocking the native ligand into the active site of its corresponding receptor. The accuracy of the docking was evaluated by comparing the binding poses of the redocked and crystallographic ligands, with the root mean square deviation (RMSD) calculated over three replicates. The docking protocol was considered valid when the RMSD value was ≤ 2 Å

Statistical analysis

The data were analyzed using GraphPad Prism version 10.2.3. Comparisons of the outcomes of the solvent group and treatment groups were made using one-way analyses of variance (ANOVA) followed by Tukey's multiple-comparison post-hoc test. *P*-values below 0.05 (*p<0.05) were deemed statistically significant.

RESULT AND DISCUSSION

GC-MS analysis

1 and

Table **1** show the metabolite profile made from the leaves extract of *P. scutellaria* mixed with 90% ethanol. It was found that eight compounds had more similarity index of 90%.

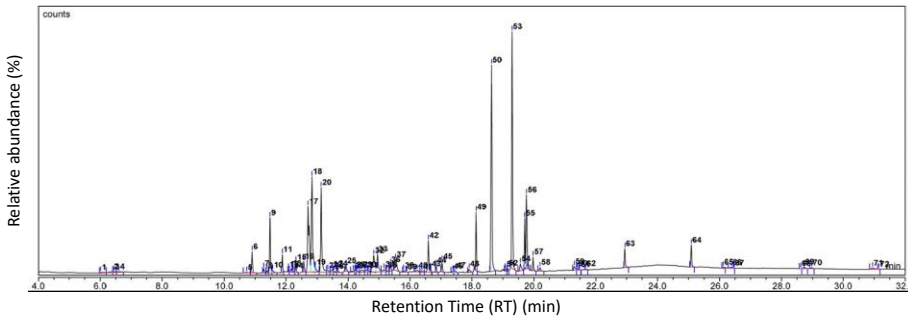

Fig. 1: GC-MS chromatogram of ethanol extract of *P. scutellaria* leaves

Among these eight compounds, (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol (15.74%) and Phytol (16.73%) exhibit relatively high relative abundances

Table **1**. The compounds exhibiting high relative abundance and known anti-inflammatory pharmacological activities were selected for computational analysis against various target proteins or receptors involved in the inflammatory process [26, 27]. 2. (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol or falcarinol. Falcarinol was first isolated from *Panax ginseng* roots by Takahashi [28] and was named panaxynol [29]. Furthermore, Phytol (PYT; 3,7,11,15-tetramethylhexadec-2-en-1-ol) is a chemical that occurs abundantly in nature. As a component of the chlorophyll molecule, it is synthesized by nearly all photosynthetic organisms, including algae [30], plants [31], and bacteria (cyanobacteria) [32].

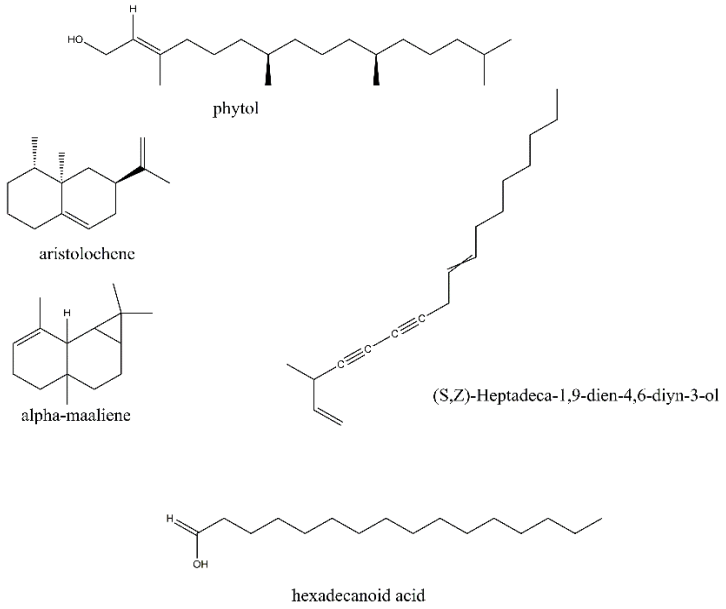

Fig. 2: Top five compounds with high relative abundance and similarity index

Table 1: Chemical composition of ethanol extracts from GC-MS analysis

Compounds	Class/group	Chemical	Retention time (min)	Relative abundance (%)	Similarity index (%)	Biology activity	Reference
Phytol (1)	Diterpene	$C_{20}H_{40}O$	19.30	16.73	91.2*	Antimicrobial Antinociceptive (mice) and antioxidant Anticancer Anti-inflammatory	[33] [26] [27] [34, 35] [29]
(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol (2)	Polyacetylenes	$C_{17}H_{24}O$	18.64	15.74	97.1*	Anticancer and anti-inflammatory	[37]
Aristolochene	Sesquiterpene	$C_{15}H_{24}$	12.71	8.31	94.9	Antimicrobial	[38]
a-Maaliene	Terpenoide	$C_{15}H_{24}$	13.13	6.90	92.7	Anticancer	[39]
Hexadecanoic acid	Palmitic acid ester	$C_{18}H_{36}O_2$	18.14	3.02	91.8	Anti-inflammatory Antibacterial	[40] [41, 42]10/8/2025 1:53:00 PM
9,12-Octadecadienoic acid, ethyl ester	Polyenoic fatty acid	$C_{20}H_{36}O_2$	19.71	2.51	91.9	Antibacterial, Antioxidant	[43, 44]
alfa.-Copaene	Tricyclic sesquiterpene	$C_{15}H_{24}$	10.90	1.45	91.9	Antioxidant, Anticancer	[45]
Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	11.88	1.00	91.7	Neuroprotective Anti-inflammatory Antioxidant Antimicrobial Anticonvulsant	[46, 47] [27–29] [51, 52] [53] [54, 55]10/8/2025 1:53:00 PM

Note: *) Compounds with high relative abundance selected for docking against several target proteins.

Total phenolic (TPC) and antioxidant activity

The TPCs of the different leaf extracts were quantified in terms of gallic acid equivalents (GAE). The TPCs were determined using the linear regression equation derived from the standard plot of gallic acid.

$$Y = 0.005395x+0.1321, r^2 = 0.9663$$

Y represents absorbance, while x denotes the quantity of gallic acid in micrograms (µg).The TPCs were determined using a linear regression equation based on a standard gallic acid solution, yielding a concentration of 132.7382± 3.261 mg GAE/g.

The antioxidant activity is assessed by using two different methods to determine the true antioxidant activity of a sample. A sample is considered to possess antioxidant activity if it has been evaluated using at least two different antioxidant mechanisms [56]. The ABTS and FRAP were selected to conduct the antioxidant assays with three replicates (n=3). In the FRAP assay, EEPS showed an IC_{50} of 54.66±2.35 µg/ml, while ascorbic acid exhibited a lower IC_{50} of

10.10±0.14 µg/ml (table 2). In the ABTS test, EEPS exhibited an IC50 of 55.13±1.19 µg/ml, above the ascorbic acid IC50 of 10.47±0.29 µg/ml (fig. 3). From the test results, ascorbic acid has higher activity as an antioxidant than EEPS. However, the IC_{50} values derived from each assay (EEPS) indicated strong antioxidant activity, with values ranging from 40 to 100 ppm [57]. There was a significant difference between the two after statistical analysis was carried out (p=0.00001, p<0.05).

A few plants have been identified for their abundance of flavonoids and phenolic chemicals, exhibiting various biological actions, including antioxidant and anti-inflammatory effects [58–60]. Phenolic compounds, including flavonoids and terpenoids, showed the ability to reduce ROS produced by carrageenan-induced inflammation [61, 62]. In addition, there is a correlation among total flavonoids, total phenols, and antioxidants [63]. Phenolic compounds exert antioxidant effects by scavenging ROS, thereby reducing oxidative stress. This reduction in oxidative stress can inhibit the activation of the NF-κB pathway, a key regulator of inflammatory responses. Inhibition of NF-κB subsequently leads to decreased transcription and production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β [64, 65]. Therefore, preliminary studies included a molecular docking analysis of chemicals identified using GC-MS (

Table 1) with respect to inflammatory proteins/receptors, specifically TNF-α, IL-1β, IL-6, and COX-2.

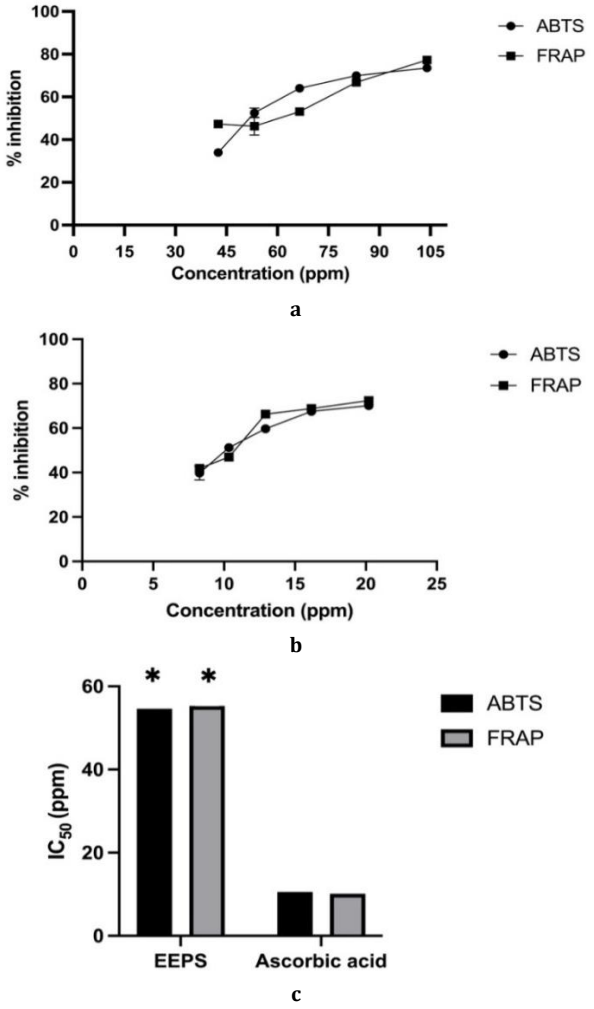

Fig. 3: Antioxidant activity (% inhibition) of EEPS (A) and Ascorbic acid (B) and IC_{50} value (C) with ABTS and FRAP methods. Data are presented as mean ±SD. *#statistically significant (p=0.00001), (p<0.05) difference when compared with the ascorbic acid group

Molecular docking

Molecular docking is an important stage in drug discovery, and the goal is to predict the structure of ligand-receptor complexes using computational methods [66, 67]. Docking studies were conducted on mediators/receptors involved in inflammation, such as cycloxygenase-2 (COX-2), tumor necrosis factor alpha (TNF-α), Interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) [15, 16, 68]. The four receptors were downloaded from the Protein Data Bank with the following IDs: COX-2 (5IKT), TNF-a (7JRA), IL-1b (8C34), and IL-6 (1ALU). All ligand structures were energy-minimized using VegaZZ prior to docking to ensure optimal geometry.

The similarity index and relative abundance of (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and phytol were used to study how they bonded with different receptors, including COX-2 (5IKT), TNF-α (7JRA), IL-1β (8C34), and IL-6 (1ALU). Two ligands found by GC-MS were successfully docked onto four possible target macromolecules to examine how the macromolecules and ligands interact with each other. Molecular docking analysis was used to assess the spontaneity of reactions and the stability of ligands bound to target macromolecules, represented by ΔGbinding values, as well as interactions between ligands and amino acid residues [69]. A more negative or lower ΔGbinding value indicates that the formed ligand conformation requires less energy, resulting in stronger binding. Interactions between the test ligand and target macromolecule can be inferred from the similarity of amino acid residues between the native ligand and the test ligand. The presence of similar amino acid residues suggests that the test ligand, a compound found in plants, has the potential for therapeutic use [70].

Protein targets were validated (redocked) before the docking investigation was finished, and the RMSD values were used as a parameter (Three replicates). RMSD is a characteristic that

demonstrates the ability to replicate the protein and native ligand complex in a suitable configuration during the development process. An optimal RMSD value is less than 1Å; however, a value less than 2Å is also considered acceptable [71]. The validation results for the protein targets are presented in (4-7). RMSD values of COX-2 (5IKT), TNF-a crystal (7JRA), IL-1b crystal (8C34), and IL-6 crystal (1ALU) were obtained as follows: all<1 Å, except for IL-6, which was<2 Å (1.177±0.04). Therefore, the protein and native ligands can proceed to docking.

The native ligand for each target protein was identified from https://www.rcsb.org/, namely COX-2 (5IKT): 2-[(3-chloro-2-methylphenyl)amino]benzoic acid; TNF-α crystal (7JRA): 2-[5-(3-chloro-4-{[(1R)-1-(2-fluorophenyl)ethyl]amino}quinoline-6-yl) pyrimidin-2-yl]propan-2-ol; IL-1β crystal (8C34): cobalamin (B12), dan IL-6 crystal (1ALU): L(+)-tartaric acid.

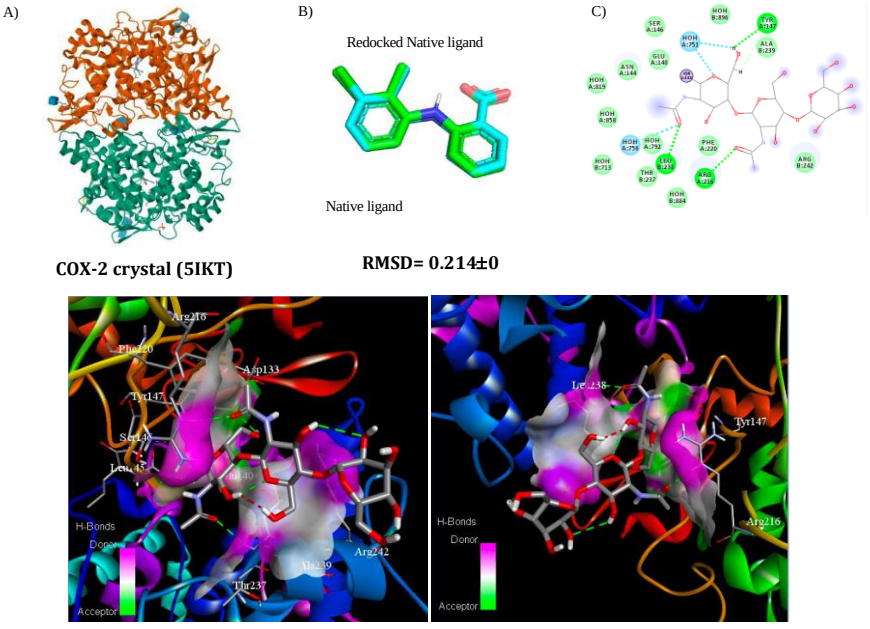

Fig. 4: 3D configuration of (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol with native ligand (COX-2 crystal=5IKT), and 3D interaction of both test ligands with amino acids. (A) 3D structure of COX-2 (5IKT); (B) superposed binding orientation of the native ligand with native ligand; (C) 2D interaction of native ligand with amino acids (D) Docked (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol 3D interaction with the receptor based on surface area hydrogen bonding; (E) Docked Phytol 3D interaction with the receptor based on surface area hydrogen bonding. Native ligand: 2-[(3-chloro-2-methylphenyl)amino]benzoic acid

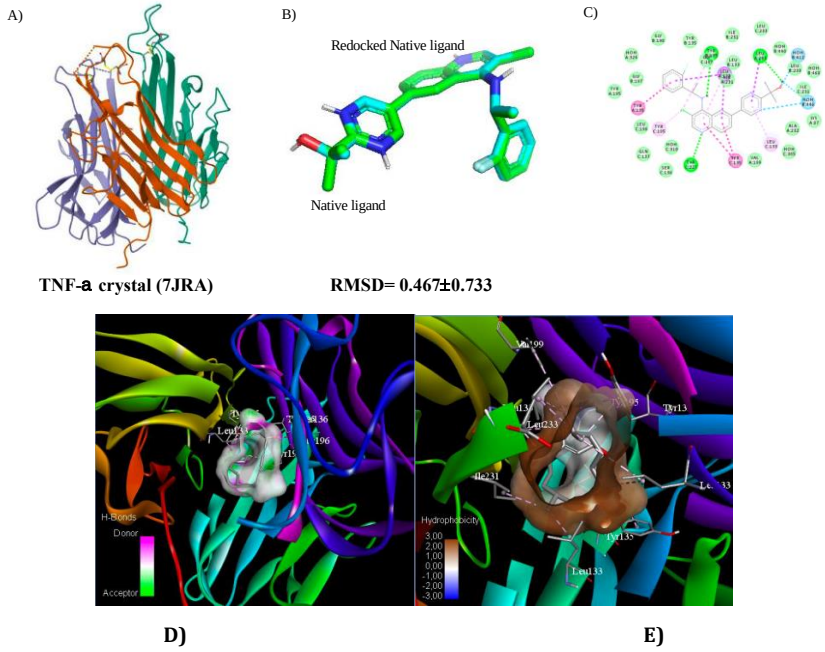

Fig. 5: 3D configuration of (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol with native ligand (TNF-α crystal (7JRA), and 3D interaction of both test ligands with amino acids. (A) 3D structure of TNF-α (7JRA); (B) superposed binding orientation of native ligand with native ligand; (C) 2D interaction of native ligand with amino acids (D) Docked (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol 3D interaction with the

receptor based on surface area hydrogen bonding; (E) Docked Phytol 3D interaction with the receptor based on surface area hydrogen bonding. Native ligand: 2-[5-(3-chloro-4-{[(1R)-1-(2-fluorophenyl)ethyl]amino}quinoline-6-yl)pyrimidin-2-yl]propan-2-ol

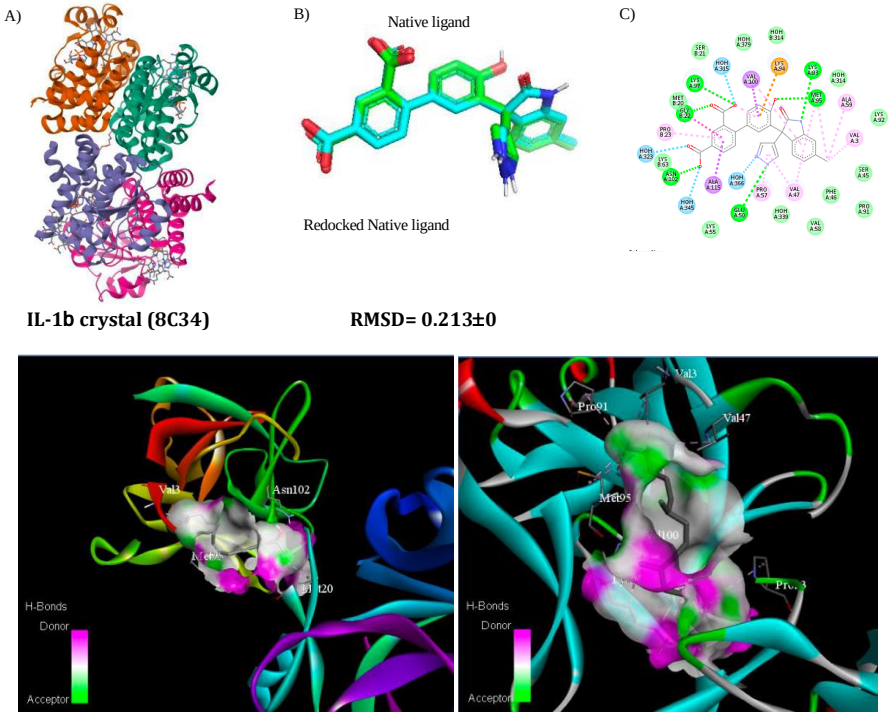

Fig. 6: 3D configuration of (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol with native IL-1β crystal (8C34), and 3D interaction of both test ligands with amino acids. (A) 3D structure of IL-1β (8C34); (B) superposed binding orientation of native ligand with native ligand; (C) 2D interaction of native ligand with amino acids (D) Docked (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol 3D interaction with the receptor based on surface area hydrogen bonding; (E) Docked Phytol 3D interaction with the receptor based on surface area hydrogen bonding. Native ligand: cobalamin (B12)

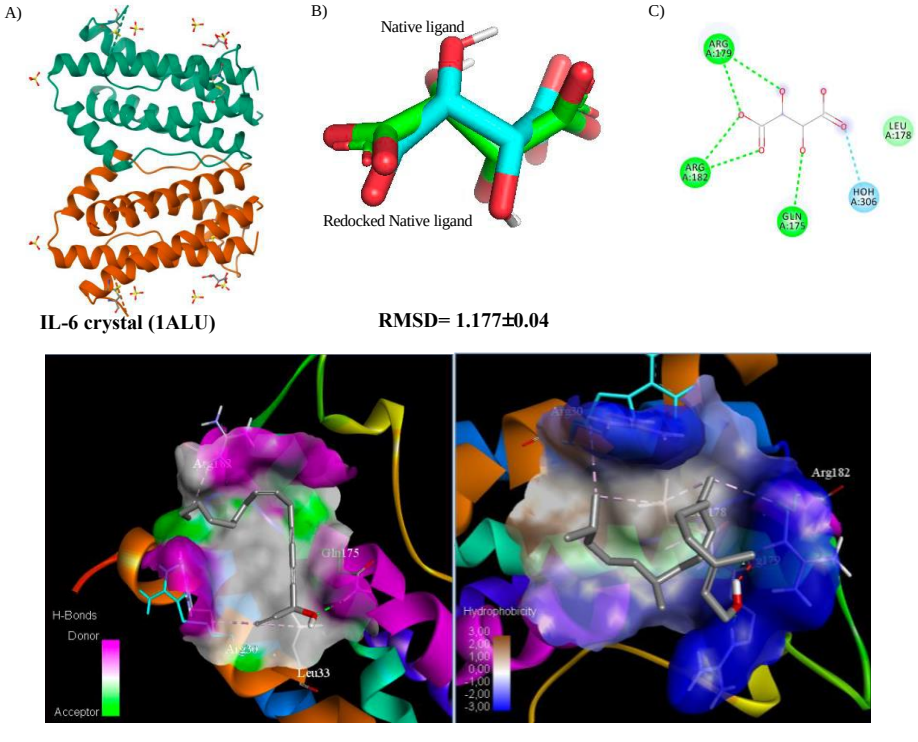

Fig. 7: 3D configuration of (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol with native IL-6 (1ALU), and 2D interaction of both test ligands with amino acids. (A) 3D structure of IL-6 (1ALU); (B) superposed binding orientation of native ligand with native ligand; (C) 2D

interaction of native ligand with amino acids (D) Docked (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol 3D interaction with the receptor based on surface area hydrogen bonding; (E) Docked Phytol 3D interaction with the receptor based on surface area hydrogen bonding. Native ligand: L(+)-tartaric acid

Hydrogen bonds represent the most prevalent structural motifs found within biological systems. Their impact is crucial in establishing the affinity and selectivity of protein-ligand interactions [72]. According to the results of the docking simulation (table 2), the binding affinities of (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol towards the receptors COX-2 (5IKT), TNF-α (7JRA), IL-1β (8C34), and IL-6 (1ALU) were determined. The binding affinity of (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol towards COX-2 (5IKT) was calculated to be-7.37 kcal/mol, which is slightly less negative than of phytol (-6.77 kcal/mol), indicating that (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol has a stronger predicted binding affinity due to its more negative ΔG value. Although the difference in ΔG (0.6 kcal/mol) may seem modest, it could still reflect a meaningful difference in biological activity, which is supported by lower Ki values. Additionally, the presence of stabilizing hydrogen bonds, particularly the hydrogen bond formed between phytol and Tyr195 in the COX-2 binding pocket, may contribute to the observed differences in binding affinity and should be considered in evaluating ligand efficacy. The native ligand, (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol, and phytol all have the same binding structure. This is shown by the fact that they have the same hydrophilic interactions with active site amino acids such as Leu238, Arg216, and Tyr147 (fig. 4). Another docking simulation was performed against the TNF-α receptor (7JRA). The binding affinities of Phytol more negative than (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol), indicating that Phytol has a stronger predicted binding affinity due to its more negative ΔG value. Between the two ligands, Phytol exhibited a lower binding affinity compared to (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol (table 2), indicating that Phytol is more effective/stable than (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol in inhibiting production of TNF-α. Among Phytol and the native ligand, they share the same binding structure, indicated by one hydrophilic interaction with the active site amino acid Tyr^{195} (fig. 5), In contrast, (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol does not exhibit the same hydrophilic interaction with the native ligand, which affects its binding affinity being higher than Phytol. TNF-α is a potent pro-inflammatory cytokine that affects immunity, inflammation, differentiation, and apoptosis [73, 74]. This is in line with previous studies shown in table 1, demonstrating that Phytol has antioxidant, anticancer, anti-inflammatory, and antinociceptive activities [26, 27, 34–36].

Phytol on the IL-1β receptor (8C34) has a stronger binding affinity compared to (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol, with values of-6.88 kcal/mol and-6.29 kcal/mol, respectively (table 2), indicating that Phytol has better/stable activity in inhibiting IL-1β release. However, both Phytol and (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol have higher affinity values compared to their native ligands. Phytol shows the same hydrophilic amino acid interaction similarity with the native ligand, namely Lys^{97}, while there is one amino acid binding structure similarity between the native ligand and (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol, namely Asn^{102} (fig. 6). During the intermediate and advanced stages of inflammation, IL-1β may promote the development of systemic inflammation and organ dysfunction [75]. In this context, Phytol is better at managing sub-chronic and chronic inflammation.

These results are in contrast with the docking results between (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol on the IL-6 receptor (1ALU). (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol exhibits better activity stability than Phytol in inhibiting the release of IL-6 release with binding affinity values of-2.83 kcal/mol and-2.79 kcal/mol (table 2) (fig. 7). Phytol's potential to reduce the production of pro-inflammatory cytokines (TNF-α and IL-1β) indicates the medicinal potential in treatment acute and chronic inflammatory diseases [76–79]. The observed inhibitory effects suggest a synergistic interaction among bioactive compounds targeting various inflammatory pathways. However, it needs to be confirmed again with *in vitro* and *in vivo* anti-inflammatory tests [23].

Table 2: Molecular docking of P. scutellaria metabolites with the 5IKT, 7JRA, 8C34, and 1ALU receptors

COX-2 (5IKT)				
Ligand	**Binding affinity (*ΔGbinding*) (kcal/mol)±SD**	**Inhibition constant (ki)(μM)**	**Amino acid residue interactions**	
			Hydrogen bond	**Non hydrogen bond**
Native ligand	-8.05±0.00	1.25	Leu^{238}, Arg^{216}, Tyr^{147}	-
(S-Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	-7.37±0.00	3.96	Leu^{238}, Arg^{216}, Tyr^{147}	-
Phytol	-6.77±0.00	10.94	Leu^{238}, Arg^{216}, Tyr^{147}	-
TNF-α (7JRA)				
Ligand	Binding affinity (*ΔGbinding*) (kcal/mol)±SD	Inhibition constant (ki)(μM)	Amino acid residue interactions	
			Hydrogen bond	Non hydrogen bond
Native ligand	-10.83±0.06	0.01053	Tyr^{195}, Leu^{233}, Tyr^{227}	Tyr^{135}, Leu^{133}, Tyr^{195}
(S-Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	-7.00±0.00	7.44	Leu^{196}	Tyr^{195}, Leu^{133}
Phytol	-7.27±0.00	4.74	Tyr^{195}	Tyr^{195}, Leu^{133}, Ile^{231}, Val^{199}, Leu^{233}, Tyr^{135}
IL-1β (8C34)				
Ligand	Binding affinity (*ΔGbinding*) (kcal/mol)±SD	Inhibition constant (ki)(μM)	Amino acid residue interactions	
			Hydrogen bond	Non hydrogen bond
Native ligand	-17.14±0.00	2.723	Asn^{102}, Glu^{50}, Lys^{97}, Gly^{22}, Met^{95}, Lys^{93}	Val^{47}, Pro^{57}, Val^{3}, Ala^{59}, Pro^{23}, Val^{10}, Lys^{94}
(S-Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	-6.29±0.00	24.58	Asn^{102}	Val^{3}, Met^{95}, Met^{20}
Phytol	-6.88±0.00	9.01	Lys^{97}, Ala^{115}	Val^{3}, Ala^{59}, Met^{95}, Pro^{91}, Pro^{23}, Val^{47}, Val^{10}
IL-6 (1ALU)				
Ligand	Binding affinity (*ΔGbinding*) (kcal/mol)±SD	Inhibition constant (ki)(μM)	Amino acid residue interactions	
			Hydrogen bond	Non hydrogen bond
Native ligand L(+)-tartaric acid	-5.83±0.01	53.27	Gln^{175}, Arg^{182}, Arg^{179}	-
(S-Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	-2.83±0.09	84.50	Gln^{175}	Arg^{30}, Leu^{33}, Arg^{182}
Phytol	-2.79±0.09	90.20	Arg^{179}	Arg^{30}, Leu^{178}

CONCLUSION

In conclusion, the 90% ethanol extract has a high relative TPC. Metabolite analysis using GC-MS identified eight compounds with similarity values above 90%, including two compounds with high relative abundance, namely (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol and Phytol. Molecular docking based on binding affinity values indicated that (S, Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol exhibits better activity than Phytol on the COX-2 (5IKT) and IL-6 (1ALU) receptors, while Phytol is more effective on the TNF-α (7JRA) and IL-1β (8C34) receptors. The anti-inflammatory potential predicted through in silico analysis requires further validation using *in vitro* and *in vivo* experimental studies to confirm the biological relevance and therapeutic efficacy of these compounds.

ACKNOWLEDGEMENT

The authors would like to thank the Indonesian Education Scholarship (BPI), Center for Higher Education Funding and Assessment Ministry of Higher Education, Science, and Technology of Republic Indonesia, and Indonesia Endowment Fund for Education (LPDP) for their financial support to the first author through a Doctoral Scholarship Scheme (00751/J5.2.3/BPI.06/9/2022).

AUTHORS CONTRIBUTIONS

AS contributed in the writing the initial manuscript, generating the experiment, data analysis, and interpretation; NF contributed in the writing the manuscript, supervising, editing, reviewing; AN contributed in the reviewing and editing the manuscript. All authors have agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

CONFLICT OF INTERESTS

The authors report that they have no financial or other conflicts of interest in this work.

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