


Cite this: *RSC Adv.*, 2025, 15, 33601

# Extraction of secondary metabolites and computational prediction of their anti-inflammatory potential

Sodabeh Gorgi, Roya Kiani-anbouhi,  Rahele Doosti and Melika Asadian

The increasing demand for herbal medicines as an alternative to synthetic drugs has gained significant attention due to their lower side effects and potential therapeutic benefits. This study aims to extract secondary metabolites from *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), and *Valeriana officinalis* (Valerian) using four extraction techniques: Soxhlet, Clevenger, maceration, and hydro distillation. The extracted compounds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), and their interaction with TNF- $\alpha$  (PDB ID: 2AZ5), a key protein involved in rheumatoid arthritis, was evaluated through molecular docking studies using AutoDock software. The results indicated that valerian metabolites exhibited superior anti-inflammatory potential compared to *Curcuma longa* and Ginger, with the lowest binding energy and most stable interactions. These findings highlight the potential use of valerian as a natural inhibitor for autoimmune diseases like rheumatoid arthritis.

Received 20th July 2025  
Accepted 5th September 2025

DOI: 10.1039/d5ra05230k

rsc.li/rsc-advances

## Introduction

For centuries, medicinal plants have formed an essential component of traditional medicine, and their therapeutic potential is being progressively validated within modern pharmacology. Medicinal plants contain bioactive compounds that are important sources of drugs with anti-inflammatory, anti-oxidant, and immunomodulatory activities.<sup>1</sup>

The initial plant chosen for research is *Curcuma longa*. It is a flowering plant, part of the Ginger family (*Zingiberaceae*), with rhizomatous herbaceous perennial native to South Asia.<sup>2</sup> Its anti-inflammatory, antimicrobial, and antioxidant properties have been harnessed, traditionally in Ayurvedic and Chinese medicine.<sup>3</sup> Curcumin is the main bioactive component of turmeric; it is a type of polyphenol responsible for the pigmentation and healing properties of turmeric. Curcumin exhibits strong anti-inflammatory, antioxidative, and immunomodulatory properties, suggesting that it could serve as a promising therapeutic tool for RA.<sup>4</sup> Evidence shows that curcumin functions by blocking NF- $\kappa$ B, a significant transcriptional regulator of the inflammatory cascade, leading to decreased production of TNF- $\alpha$  and other pro-inflammatory cytokines.<sup>5</sup> In clinical trials, curcumin has shown efficacy against symptoms of RA, including joint stiffness and pain.<sup>6</sup> However, the bioavailability of curcumin is still significantly low, and a sophisticated drug delivery method is required.<sup>7</sup>

Species of *Zingiber* are herbs that belong to the *Zingiberaceae* family, various species are widely used in traditional medicine

or as spices to add flavor.<sup>8</sup> It is a perennial herbaceous plant cultivated in warm and humid climates in Southeast Asia. Several biologically active phytochemicals are present in ginger root, the underground rhizome of ginger, which is a chief medicinal source of ginger. It has also been traditionally used for its anti-inflammatory and analgesic properties. The main bioactive compounds of ginger, such as gingerols, shogaols, and paradols, are known for displaying such properties by modulating inflammatory mechanisms through the inhibition of the expression of COX-2 and TNF- $\alpha$ .<sup>9</sup> Molecular Docking studies indicate that 6-gingerol and 6-shogaol have strong binding affinity to TNF- $\alpha$  and can also serve as TNF- $\alpha$  inhibitors.<sup>10</sup> Also, clinical trials indicate that ginger has a significant impact on joint pain and stiffness in RA patients and can thus be used as a supplementary therapeutic agent.<sup>11</sup>

Valerian (*Valeriana officinalis*) is a member of the *Caprifoliaceae* family (previously known as the *Valerianaceae* family), which is a perennial flowering plant that is native to Europe and parts of Asia (Brinker, 2010). It thrives in the temperate climate, typically growing in moist grassy habitats. It is well known for its aromatic rhizome and root, which consist of several bioactive compounds applied in traditional and modern medicine. It is also primarily recognized as having sedative and anxiolytic effects; however, newly published studies also underscore its anti-inflammatory potential.<sup>12</sup> Meanwhile, molecular docking studies have also indicated that valerenic acid and its derivatives significantly interact with TNF- $\alpha$ .<sup>13</sup> These agents have an immunosuppressive influence, decreasing the activation of macrophages and pro-inflammatory cytokines, so they could potentially be beneficial for the RA patient, as inflammation-induced joint damage can be diminished.<sup>14</sup>

Department of Chemistry, Faculty of Science, Imam Khomeini International University, Qazvin, Iran. E-mail: Kiani@sci.ikiu.ac.ir



The increasing worries regarding the side effects of synthetic drugs have triggered laboratory scientists to investigate natural substitutes with effective therapeutic coverage and lower toxicity. One area of study that stands out in particular involves autoimmune diseases, with one such disease being rheumatoid arthritis (RA), which is a chronic inflammatory syndrome that is primarily dependent on the upregulation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).<sup>15</sup> TNF- $\alpha$  inhibition represents an important therapeutic goal in RA, and recent plant-derived secondary metabolites have been proven to play a promising role in modulating this inflammatory mechanism.<sup>16</sup> The bioactive secondary metabolites can be extracted from medicinal plants using several extraction techniques. Standard procedures like Soxhlet extraction, maceration, and hydrodistillation still predominate, however, newer procedures and more expensive ones like supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE) have been employed by researchers to improve extraction efficiency and decrease solvent volume in use.<sup>17</sup> Curcumin is generally extracted using Soxhlet with ethanol as a solvent, whilst hydrodistillation and Clevenger extraction work for the isolation of ginger essential oils and valerian's active compounds.<sup>18</sup> Recent studies have further validated the effectiveness of these methods in extracting secondary metabolites. For example, SFE has been successfully utilized to extract curcuminoids from turmeric with high purity and yield.<sup>19</sup> UAE has shown remarkable efficiency in extracting ginger bioactive, improving antioxidant and anti-inflammatory properties.<sup>20</sup> Similarly, maceration with ethanol has been reported as an optimal method for obtaining valerenic acid from valerian, preserving its pharmacological properties.<sup>21</sup> These findings highlight the growing importance of advanced extraction techniques in optimizing bioactive compound recovery for therapeutic applications.

Molecular docking plays a pivotal role in drug discovery by predicting the binding affinity of bioactive compounds to target proteins.<sup>22</sup> This study aims to explore the potential of secondary metabolites from *Curcuma longa*, *Zingiber officinale*, and *Valeriana officinalis* as natural inhibitors of TNF- $\alpha$ , a key factor in rheumatoid arthritis pathogenesis. By integrating advanced extraction techniques, GC-MS analysis, and molecular docking simulations, this research seeks to identify the most effective plant-derived bioactive compounds for RA treatment. The novelty of this study lies in its comparative approach, evaluating multiple medicinal plants and their respective compounds for their anti-inflammatory potential through computational and analytical methodologies. The findings from this study can contribute to the development of plant-based therapeutic agents with fewer side effects compared to conventional drugs, paving the way for further pharmacological and clinical investigations.

## Materials and methods

### Herbal materials and chemicals

Dried rhizome of *Curcuma longa*, ginger, and dried roots of Valerian were prepared at the University of Tehran. Precise identification of herbs was done at the herbarium lab. Then, the

rhizomes and dry roots of the plants were milled and powdered for essential oil and extract. The chemicals used, including ethanol 96%, chloroform 98%, and normal-hexane 96%, were produced by Dr Mojalali's research team.

### Extraction methods

The plant samples were obtained from verified sources and authenticated. Four extraction methods were used: (1) Clevenger, (2) Soxhlet, (3) maceration, and (4) hydrodistillation.

In the Clevenger method, 60 g of powdered *Zingiber* sample, weighed out with 600 mL of distilled water, was poured into the glass flask of the Clevenger apparatus. After heating for 4 hours, the oily and milky layer of essential oil was collected above the blue layer, the so-called plant sap, and the extraction process was completed. By opening the valve of the apparatus, the essential oil was poured into special containers and kept in the refrigerator at 4 degrees Celsius until the analysis was performed. This process was repeated for the other two plants. The yellow essential oil of *Curcuma longa* was collected after 6 hours, and the greenish-yellow essential oil of *Valeriana officinalis* was collected after 10 hours.

To extract the distillate by the Soxhlet method, ethanol was used. In this method, 60 g of powdered *Zingiber* sample was poured into a thimble and placed in the extraction column. 240 mL of ethanol was poured into a round-bottom flask. The extraction operation was completed after 8 hours, and then the yellow-brown contents of the solvent and the extract in the flask were connected to a rotary device, and after evaporating the solvent, the extract was stored. This process was repeated for two other plants. Red *Curcuma longa* extract was obtained after 17 hours, and brown *Valeriana officinalis* extract after 10 hours.

To extract the distillate, the Maceration method with normal hexane was used. For this purpose, 20 g of each of the ground plant samples was macerated in 100 mL of hexane at room temperature and in the dark for 48 hours. After 48 hours, the organic solvent and the ground plant were separated using filter paper, and the organic solvent was evaporated using a rotary evaporator with a boiling point of 69 °C. The resulting extract was stored in the refrigerator until analysis. This procedure was repeated for the other two plants.

In the hydrodistillation method, 50 g of dry powdered *Zingiber* sample was poured into a 250 mL flask, and 150 mL of water was added to it. By heating the droplets above the flask and the tee, they were collected, and after passing through the condenser, they were collected as a liquid. This juice collected from the plant is in a crystallized form, and to separate these crystallized droplets in the aqueous phase, the liquid-liquid extraction technique must be used. Then, 15 mL of *Zingiber* juice was poured into a separating funnel, and about one-third of the resulting juice was added to it, along with the organic solvent chloroform. The separating funnel was shaken well several times so that the oily and volatile droplets in the aqueous phase entered the organic phase well. Then, by placing the separating funnel, two layers of aqueous and organic phases were observed to be stationary. The organic phase that was on top of the aqueous phase was separated using the screw-in



separatory funnel and stored in a dark container after adding some sodium sulfate to dehydrate it in the refrigerator until analysis. The same process was repeated for the two plants, *Curcuma longa* and *Valeriana officinalis*.

The selection of Clevenger, Soxhlet, maceration, and hydro-distillation methods was based on their wide application in the extraction of essential oils and secondary metabolites from medicinal plants, as documented in several phytochemical studies.<sup>17–21</sup> Clevenger and hydrodistillation are traditional water-based methods suitable for volatile oils, particularly terpenes, whereas Soxhlet is a solvent-based method effective for semi-volatile and non-volatile components such as curcuminoids and phenolic compounds. Maceration, although less efficient in terms of time, is a gentle technique often used to preserve heat-sensitive compounds and is solvent-flexible.

Each method offers specific advantages: Soxhlet ensures exhaustive extraction, Clevenger and hydrodistillation target aromatic fractions, and maceration minimizes thermal degradation. Their inclusion allowed comparative evaluation of method-specific compound profiles. For example, Soxhlet yielded more esterified and phenolic compounds, while hydrodistillation favored sulfur-containing and aromatic components. The diversity and quantity of extracted constituents varied significantly across methods, supporting the importance of a multi-method approach.

Future optimization could include modern techniques such as ultrasound-assisted extraction (UAE) or supercritical fluid extraction (SFE), but the chosen methods offer a balance of accessibility, cost-efficiency, and relevance to traditional practices, making them suitable for this comparative phytochemical analysis. The selection of Clevenger, Soxhlet, maceration, and hydrodistillation methods was based on their widespread application in extracting volatile and semi-volatile compounds from medicinal plants. Each technique targets different classes of phytochemicals. For instance, Clevenger and hydrodistillation are traditionally used to extract essential oils, especially monoterpenes and sesquiterpenes. Soxhlet is effective for extracting less volatile polar compounds like curcuminoids using organic solvents, while maceration is used for temperature-sensitive compounds due to its mild conditions. The choice aimed to encompass a broad spectrum of compound types and to enable a comparative analysis of yield, efficiency, and compound diversity.

### GC-MS and molecular docking analysis

Extracted compounds were analysed using GC-MS to determine their chemical composition. To study the quantitative relationship between the structure and inhibitory activity of a group of secondary metabolites of *Curcuma longa*, *Zingiber*, and *Valeriana officinalis* plants, the molecular docking simulation method was used to analyse the effective interactions between the protein and its inhibitors. The three-dimensional structure of the protein from the Protein Data Bank with the identifier (PDB ID: 2AZ5) and with a resolution of 2.10 Å was selected as the target receptor. So, preparation was performed in the software (Auto Dock 4.2).

## Results and discussion

The GC-MS analysis revealed significant variation in both the quantity and types of secondary metabolites extracted by each method. For example, Soxhlet extraction yielded higher concentrations of esters and phenolic compounds, particularly in *Curcuma longa*, while Clevenger and hydrodistillation methods were more effective in extracting terpenoids and volatile compounds from *Zingiber officinale* and *Valeriana officinalis*. Maceration, although lower in total yield, preserved structurally sensitive compounds and offered a broader range of hydrocarbons and alcohols. The variation is attributed to differences in solvent polarity, temperature, and extraction time, which affect the solubility and stability of target metabolites.<sup>17,18,21</sup> These differences underscore the necessity of method-specific optimization depending on the desired compound class for therapeutic applications.

### Results of GC-MS analysis of the extraction of secondary metabolites from *Curcuma longa*, *Zingiber*, and *Valeriana officinalis* plants using the Clevenger method

Using the Clevenger method, the chromatogram obtained by injecting *Curcuma longa* essential oil into a GC-MS device shows the presence of 27 compounds. Among these identified compounds, 11 unique and specific compounds were obtained using this method compared to other methods, which are alpha. -phellandrene-C10H16, eucalyptol-C10H18O, 2(1H)-naphthalenone,7-ethynyl-4a,5,6,7,8,8a-hexahydro-1,4a-dimethyl-, (1. alpha.,4a.beta.,7.beta.,8a.alpha.)-C14H18, 1,6,10-dodecatriene,7,11-dimethyl-3-methylene-, (E)-C15H24, 3-carene-C10H16, cyclohexene,1-methyl-4-(1-methylethylidene)-C10H16, spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-[1S-(1.alpha.,4.beta.,5.alpha.)]-C15H24, .alpha.-pinene-C10H16, lanceol,cis-C15H24O, 1,6-cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-[s-(E,E)]-C15H24, and bicyclo [3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-C10H16. Fig. 1. Shows a circular chart of the percentage of secondary metabolites essential oil from (a) *Curcuma longa*, (b) *Zingiber*, and (c) *Valeriana officinalis* by the Clevenger method. According to Fig. 1(a), terpenes are the most dominant compound extracted

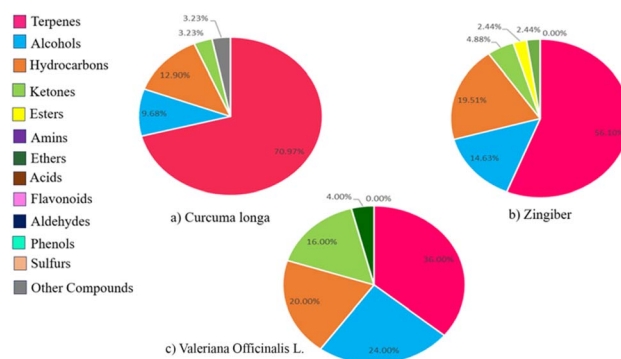


Fig. 1 Circular chart of extraction of secondary metabolites from plants, (a) *Curcuma longa*, (b) *Zingiber*, and (c) *Valeriana officinalis* L. using the Clevenger method.



from *Curcuma longa* secondary metabolites, with a content of 70.97%.

The results obtained from the chromatogram of *Zingiber* essential oil using the Clevenger method show the presence of 40 compounds, of which 25 are unique and specific to this method compared to other methods, including: naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-C15H24, bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)-C10H16, 1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)-C10H16, 1,2,4-metheno-1H-indene,octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.)-C15H24, 3-cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-(S)-C10H18O, naphthalene,decahydro-1,6-bis(methylene)-4-(1-methylethyl)-, (4.alpha.,4a.alpha.,8a.alpha.)-C15H24, beta-myrcene-C10H16, benzene,1-methyl-3-(1-methylethyl)-C10H14, 2-naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-(2R-cis)-C15H26O, azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-,(1S-cis)-C15H24, bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-C15H24, 1H-cycloprop[e] azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b-C15H24, 1,6-octadien-3-ol,3,7-dimethyl-C10H18O, gamma-elemene-C15H24, 2-undecanone-C11H22O, bicyclo[2.2.1]heptan-2-ol,2,3,3-trimethyl-C10H18O, 6-octen-1-ol,3,7-dimethyl-C10H20O, naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-C15H24, caryophyllene-C15H24, cyclohexene,1-methyl-4-(1-methylethylidene)-C10H16, 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-[3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-C15H24, bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-, (1S)-C10H16O, tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-C10H16, bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-, (1S)-C10H16 and 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-C10H18O. According to Fig. 1(b), terpenes are the most dominant compound extracted from Ginger secondary metabolites, with a content of 56.10%. Then, hydrocarbons make up the largest number of compounds with 19.51%.

The results obtained from the chromatogram of *Valeriana officinalis* essential oil using the Clevenger method show the presence of 25 compounds, of which 18 are unique and specific to this method compared to other methods, including 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one-C15H22O, methylhinokiate-C16H24O2, 1,4-methanoazulene-9-methanol,decahydro-4,8,8-trimethyl-, [1S(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9R\*)]-C15H26O, tumerone-C15H22O, 6-(1,3-dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-ene-C15H22O, Ar-tumerone-C15H20O, (+)-epi-bicyclosquiphellandrene-C15H24, .tau.-muurolol-C15H26O, curlone-C15H22O, 2-naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-C15H26O, 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane-C15H22, tricyclo[6.3.0.0(2,4)]undec-8-ene,3,3,7,11-tetramethyl-C15H24, caryophylleneoxide-C15H24O, 1H-cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1 aR(1a.alpha.,4.alpha.,4a.beta.,7-C15H24, azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-

methylethylidene)-,(1S-cis)-C15H24, 1-heptatriacotanol-C37H76O, 4,7-methanoazulene,1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-C15H24, and naphthalene,1,2,3,4-tetrahydro-1,1,6-trimethyl-C13H18. According to Fig. 1(c), terpenes are the most dominant compound extracted from Valerian secondary metabolites, with a content of 36.00%. Then, alcohols make up the largest number of compounds, with 24%, and hydrocarbons are the most common compounds, with a content of 20%.

### Results of GC-MS analysis of extraction of secondary metabolites from *Curcuma longa*, *Zingiber*, and *Valeriana officinalis* plants using the Soxhlet method

The results obtained from the chromatogram of *Curcuma longa* extract by the Soxhlet method show the presence of 23 compounds. Among them, 5 are unique compounds specific to this method compared to other methods, which are 1,2-benzenedicarboxylic acid, diisooctyl ester-C24H38O4, aniline-C6H7N, benzene,2-methyl-1,4-bis(1-methylethyl)-C13H20, bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-C15H24 and benzenemethanol,.alpha.,.alpha.,4-trimethyl-C10H14O. Fig. 2 shows a circular chart of the percentage of secondary metabolites extracted from (a) Turmeric, (b) Ginger, and (c) Valerian by the Soxhlet method. According to Fig. 2(a), Terpenes are the most dominant compound extracted from Curcuma extract using the Soxhlet method, with a content of 69.23%.

The results obtained from the chromatogram of *Zingiber* extract by the Soxhlet method show 32 compounds, of which 19 unique and specific compounds were obtained from this method compared to other methods, including 1,2-benzenedicarboxylic acid, diisooctyl ester-C24H38O4, 6-(3,5-dimethylfuran-2-yl)-6-methyl-hept-3-en-2-one-C14H20O2, 1H-benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-, (R)-C15H24, 9,12-octadecadienoic acid (Z, Z)-C18H32O2, agarospirol-C15H26O, 3-(6-hydroxy-3,7-dimethyl-octa-2,7-dienyl)-4-methoxy-phenol-C17H24O3, bicyclo[4.4.0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-C15H24O2, 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-C15H26O, spiro[4.5]decan-7-

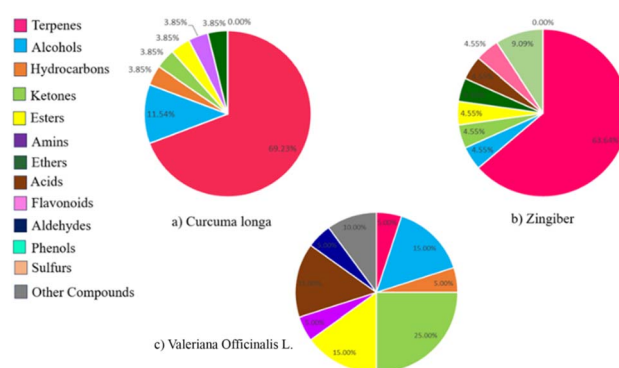


Fig. 2 Circular chart of extraction of secondary metabolites from plants (a) *Curcuma longa*, (b) *Zingiber*, and (c) *Valeriana officinalis* L. using the Soxhlet method.



one,1,8-dimethyl-8,9-epoxy-4-isopropyl-C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>, 2H-pyran-2-one,5,6-dihydro-6-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-4-methoxy-, (S)-C<sub>15</sub>H<sub>18</sub>O<sub>5</sub>, bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-,acetate-C<sub>12</sub>H<sub>18</sub>O<sub>2</sub>, eucalyptol-C<sub>10</sub>H<sub>18</sub>O, beta.-cedren-9- $\alpha$ -ol-C<sub>15</sub>H<sub>24</sub>O, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran-C<sub>10</sub>H<sub>16</sub>O, naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-,(4aR-trans)-C<sub>15</sub>H<sub>24</sub>, bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-C<sub>15</sub>H<sub>24</sub>, 1-naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-,[1R-(1. $\alpha$ .,4. $\beta$ .,4a. $\beta$ .,8a. $\beta$ -C<sub>15</sub>H<sub>26</sub>O, bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-,[1R-(1R\*,4Z,9S\*)]-C<sub>15</sub>H<sub>24</sub> and isocaryophyllene-C<sub>15</sub>H<sub>24</sub>. According to Fig. 2(b), terpenes are the most dominant compound extracted from Ginger extract using the Soxhlet method, with a content of 63.64%. After that, phenol accounted for the highest amount with 9.09%.

The results obtained from the chromatogram of *Valeriana officinalis* extract using the Soxhlet method show the presence of 17 compounds, of which 8 are unique and specific to this method compared to other methods, they are 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one-C<sub>15</sub>H<sub>22</sub>O, 9,12-octadecadienoyl chloride,(Z,Z)-C<sub>18</sub>H<sub>31</sub>ClO, 1,4-hexadien-3-one,5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-C<sub>16</sub>H<sub>22</sub>O, oleic acid-C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>, 2-propanamine, *N*-(phenylmethylene)-C<sub>10</sub>H<sub>13</sub>N, 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde-C<sub>23</sub>H<sub>32</sub>O, propanoic acid, 2-methyl-, (decahydro-6a-hydroxy-9a-methyl-3-methylene-2,9-dioxazulenol[4,5-*b*]furan-6-yl)methyl ester -C<sub>19</sub>H<sub>26</sub>O<sub>6</sub>, and hexadecanoic acid, ethyl ester-C<sub>18</sub>H<sub>36</sub>O<sub>2</sub>. According to Fig. 2(c), about 25% of the compounds are ketones.

### Results of GC-MS analysis of extraction of secondary metabolites from *Curcuma longa*, ginger, and Valerian plants using the maceration method

The results obtained from the chromatogram of *Curcuma longa* extract by the maceration method show the presence of 14 compounds, of which 4 are specific to this method compared to other methods are 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl)ester-C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl)ester-C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>, 7-methoxymethyl-2,7-dimethylcyclohepta-1,3,5-triene-C<sub>11</sub>H<sub>16</sub>O and acetic acid,2-methylene-bicyclo[3.2.1]oct-6-en-8-yl ester-C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>. Fig. 3 shows a circular chart of the percentage of secondary metabolites extracted from (a) *Curcuma longa*, (b) Ginger, and (c) Valerian by the soaking method. According to Fig. 3(a), terpenes are the most dominant extracted compound, with a content of 62.50%.

The results obtained from the chromatogram of *Zingiber* extract by the maceration method show the presence of 26 compounds, of which 12 are unique and specific to this method, including 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester-C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>, butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-C<sub>11</sub>H<sub>14</sub>O<sub>3</sub>, decanal-C<sub>10</sub>H<sub>20</sub>O, hexanedioic acid, bis(2-ethylhexyl)ester-C<sub>22</sub>H<sub>42</sub>O<sub>4</sub>, bicyclo[3.1.0]hexane,4-methyl-1-(1-

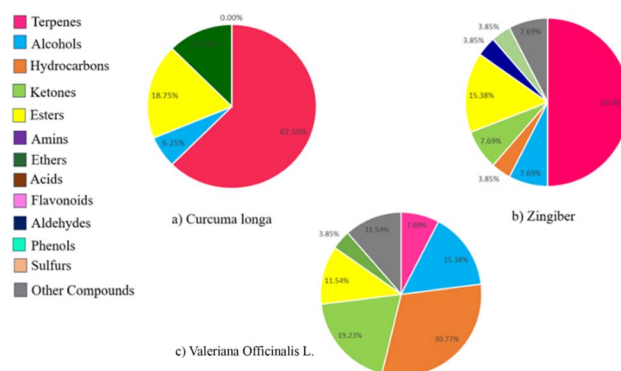


Fig. 3 Circular chart of extraction of secondary metabolites from plants (a) *Curcuma longa*, (b) *Zingiber*, and (c) *Valeriana officinalis* L. using the maceration method.

methylethyl)-,didehydro deriv-C<sub>10</sub>H<sub>16</sub>, tridecane-C<sub>13</sub>H<sub>28</sub>, 3-methylpenta-1,4-diene-3-ol-C<sub>6</sub>H<sub>10</sub>O, di-*n*-octyl phthalate-C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>, 2,6-octadien-1-ol, 3,7-dimethyl-,acetate-C<sub>12</sub>H<sub>20</sub>O<sub>2</sub>, 1,6,10-dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-C<sub>15</sub>H<sub>24</sub>, 6-(*p*-tolyl)-2-methyl-2-heptenol-C<sub>15</sub>H<sub>22</sub>O, and 2-heptanone,3-methyl-C<sub>8</sub>H<sub>16</sub>O. According to Fig. 3(b), terpenes are the most dominant extracted compound, with a content of 50.00%.

The results obtained from the chromatogram of Valerian extract by the maceration method show the presence of 28 compounds, of which 19 are specific to this method, including 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester-C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>, 4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a. $\alpha$ .,4. $\beta$ .,4a. $\beta$ .,7. $\alpha$ .,7a. $\beta$ -C<sub>15</sub>H<sub>26</sub>O, 4,7-octadecadiynoic acid, methyl ester-C<sub>19</sub>H<sub>30</sub>O<sub>2</sub>, germacra-1(10),4,11(13)-trien-12-oic acid,6. $\alpha$ .-hydroxy-, $\gamma$ -lactone, (E,E)- C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>, dodecane-C<sub>12</sub>H<sub>26</sub>, tridecane-C<sub>13</sub>H<sub>28</sub>, naphthalene,decahydro-1,5-dimethyl-C<sub>12</sub>H<sub>22</sub>, undecane-C<sub>11</sub>H<sub>24</sub>, 2(1H) naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-C<sub>15</sub>H<sub>22</sub>O, 3. $\alpha$ .,4. $\beta$ .-dihydroxy-1,5,7. $\alpha$ .(H),6. $\beta$ .(H)-guai-10(15),11(13)-dien-6,12-olide-C<sub>15</sub>H<sub>20</sub>O<sub>4</sub>, cycloundecene,1-methyl-C<sub>12</sub>H<sub>22</sub>, stigmast-4-en-3-one-C<sub>29</sub>H<sub>48</sub>O, patchouli alcohol-C<sub>15</sub>H<sub>26</sub>O, naphthalene, decahydro-1,6-dimethyl-C<sub>12</sub>H<sub>22</sub>, naphthalene,decahydro-2,3-dimethyl-C<sub>12</sub>H<sub>22</sub>, azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1. $\alpha$ .,4. $\alpha$ .,7. $\alpha$ .)]-C<sub>15</sub>H<sub>24</sub>, patchouli alcohol-C<sub>15</sub>H<sub>26</sub>O, acetic acid,3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester-C<sub>17</sub>H<sub>26</sub>O<sub>3</sub>, and 4,7-octadecadiynoic acid, methyl ester-C<sub>19</sub>H<sub>30</sub>O<sub>2</sub>. According to Fig. 3(c), hydrocarbons are the most dominant extracted compound, with a content of 30.77%.

### Results of GC-MS analysis of extraction of secondary metabolites from *Curcuma longa*, ginger, and Valerian plants using the hydro distillation method

The results obtained from the chromatogram of *Curcuma longa* essential oil by the hydro distillation method have reported 3 compounds; the presence of 2 compounds was observed only in



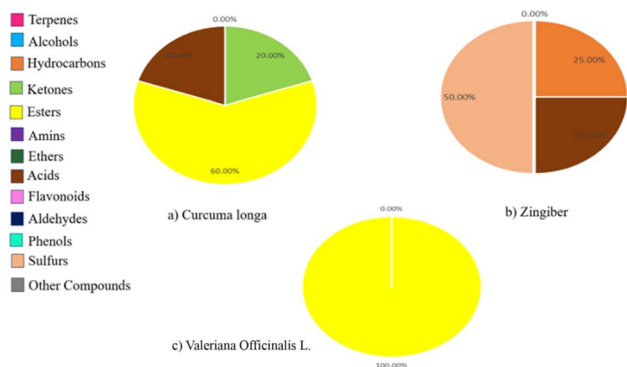


Fig. 4 Circular chart of extraction of secondary metabolites from plants (a) *Curcuma longa*, (b) *Zingiber*, and (c) *Valeriana officinalis L.* using the hydro distillation method.

this method, which are cholest-4-en-3-one-C27H44O and 1,2-benzenedicarboxylic acid, diisooctyl ester-C24H38O4. According to Fig. 3(a), esters are the most dominant extracted compound with a content of 60%, followed by acids and ketones, each with a reported content of 20%. Fig. 4 shows a circular chart of the percentage of secondary metabolites essential oil from (a) *Curcuma longa*, (b) ginger, and (c) Valerian by the hydro distillation method.

The results obtained from the chromatogram of *Zingiber* essential oil by hydro distillation showed that the 3 compounds present in this method are all unique, and these compounds were not observed in other methods, which are nonadecane-C19H40, di-*n*-decylsulfone-C20H42O2S, and sulfurous acid, cyclohexylmethyl octadecyl ester-C25H50O3S. According to Fig. 4(b), sulfur is the most dominant extracted compound with a content of 50.00%; after that, hydrocarbon and acid compounds accounted for the largest amount, with 25% for each compound. The results obtained from the chromatogram of Valerian essential oil by hydro distillation have identified 2 dissimilar compounds, both of which are similar in the hydro distillation and Soxhlet methods, which are 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl)ester-C16H22O4 and 1,2-benzenedicarboxylic acid, diisooctyl ester-C24H38O4. According to Figure 4(c), 100% of the compounds extracted by the hydro distillation method are esters.

### Selection of GC-MS compounds for molecular docking

From the wide range of compounds identified through GC-MS, a subset was selected for molecular docking based on their relative abundance, chemical class (primarily terpenoids, alcohols, and ketones), and known or hypothesized bioactivity in modulating inflammation. This selection was guided by existing literature reporting the anti-inflammatory or immunomodulatory effects of similar compounds. For instance, 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one, tumerone, valerenic acid derivatives, and spiro[4.5]decane-type compounds have previously demonstrated interaction with inflammatory pathways, including NF- $\kappa$ B and cytokine modulation.<sup>12–14,23</sup> Additionally, molecular docking was used as

a predictive screening tool to identify compounds with strong binding affinity to TNF- $\alpha$ . Those showing the most stable interactions (lowest binding energy and hydrogen bonding with active site residues) were considered potential inhibitors and thus highlighted in this study. While *in vitro* or *in vivo* validation is essential for definitive confirmation, this *in silico* approach provides a rational starting point for prioritizing compounds with therapeutic potential. Future work will focus on validating these selected compounds through experimental studies to confirm their anti-inflammatory mechanisms and bioavailability.

### Molecular docking results

**Key binding sites on TNF- $\alpha$ .** Molecular docking analysis revealed that the most active compounds formed stable interactions with key amino acid residues within the binding pocket of TNF- $\alpha$  (PDB ID: 2AZ5). The most frequently involved residues were Ser60, Leu120, Gly121, and Tyr151, which contributed to hydrogen bonding and hydrophobic interactions. For example, 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one interacted with Tyr151, and 1(2H)-naphthalenone bound to Ser60. These residues are located near the receptor's known ligand-binding interface, making them critical for inhibitory potential.

All the results obtained from the molecular docking of Valerian show that it has a higher energy level than the two *Curcuma longa*, and especially *Zingiber*.

Among the docked compounds, the compounds (1(2H)-naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-[4aR-(4a.alpha.,7.beta.,8a.alpha.)]-C15H26O), Fig. 4(a), (2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one-C15H22O) Fig. 4(b) and (1-hexanol, 2-ethyl-C8H18O) Fig. 4(c) with potential energies of  $-5.46 \text{ kcal mol}^{-1}$ ,  $-5.38 \text{ kcal mol}^{-1}$  and  $-5.47 \text{ kcal mol}^{-1}$  had the lowest free energy and were among the best molecules in forming complexes with proteins with the number of cluster ranks of 3, 1 and 2, respectively, as well as the first compound with a hydrogen bond with amino acid Ser60, the second compound with a hydrogen bond with amino acid Tyr151 and the third compound with a hydrogen bond with amino acid Ser60 and they are complexed with the 2AZ5 protein. Fig. 5 shows the molecular docking results of three secondary metabolites from the Valerian plant, along with the target protein.

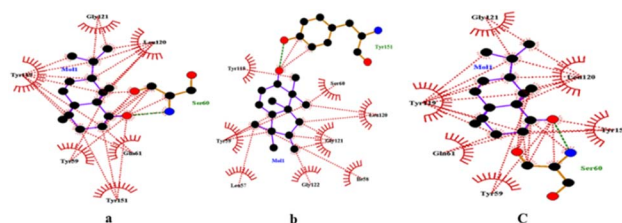


Fig. 5 Molecular docking results of secondary metabolites of Valerian plant (a) 1(2H)-naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-[4aR-(4a.alpha.,7.beta.,8a.alpha.)]-C15H26O, (b) 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one-C15H22O and (c) 1-hexanol, 2-ethyl-C8H18O with the target protein (AZ52).





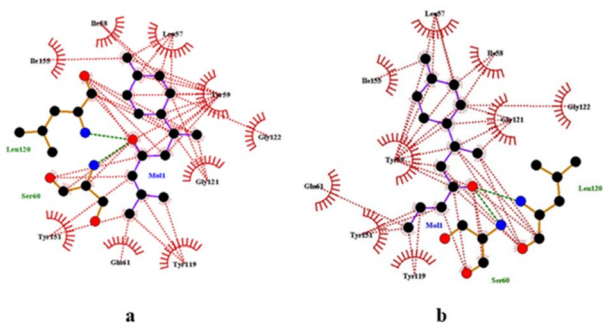


Fig. 6 Molecular docking results of secondary metabolites of the *Curcuma longa* plant, (a) curcuma-C15H22O and (b) curcuma-C15H22O with the target protein (AZ52).

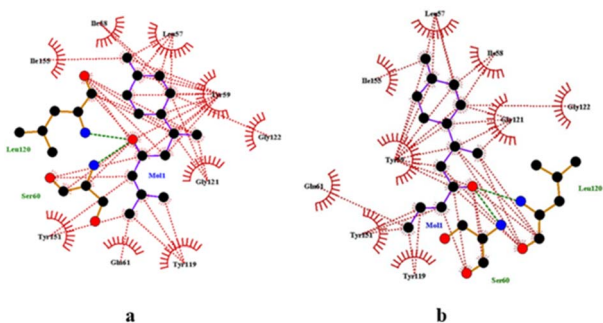


Fig. 7 Molecular docking results of secondary metabolites of *Zingiber* plant (a) 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-(S-(R\*,S\*)))-C15H24 and (b) 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R\*,S\*)]-C15H24 with the target protein (AZ52).

According to the docking results obtained in this project, in general, the secondary metabolites of Valerian were selected as the best inhibitors compared to *Curcuma longa* and Ginger plants due to their stronger interactions with the target protein; in other words, they are better options in inhibiting inflammation and controlling and treating rheumatoid arthritis.

Turmeric (Curlone-C15H22O) Fig. 6(a) and (Curlone-C15H22O) Fig. 6(b), both of which are related to the Curlone compound, had two hydrogen bonds with two amino acids Leu120 and Ser60, with potential energies of  $-5.32 \text{ kcal mol}^{-1}$  and  $-5.17 \text{ kcal mol}^{-1}$ , respectively, and had the lowest Gibbs free energy and was selected as the best inhibitor. Fig. 5 shows the molecular docking results of two secondary metabolites from the *Curcuma longa* plant, along with the target protein.

*Zingiber* plant compounds (1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-(S-(R\*,S\*)))-C15H24 Fig. 7(a) and (1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R\*,S\*)]-C15H24 Fig. 7(b) with potential energy of  $-4.93 \text{ kcal mol}^{-1}$  and  $-4.60 \text{ kcal mol}^{-1}$  had the lowest Gibbs free energy, and (1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R\*,S\*)]-C15H24 with two hydrogen bonds with two amino acids Gly121 and Tyr119 were selected as the best inhibitor among other inhibitors. Fig. 7 shows the molecular docking results of two secondary metabolites from the Ginger plant, along with the target protein.

Table 1 provides an overview of the results obtained from this research, including the best methods used, the plants used, and their best docking results.

**Comparison with previous studies.** The findings align with previous research, which suggests that curcumin and gingerol

Table 1 Overall results of the plants used and the best extraction methods used, along with their molecular docking

| Method                       |                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                        |                                                                                                                                                                                                                                         |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Plant                        | Clevenger                                                                                                                                                                                                                                                                                                                                | Maceration                                                                                                                                                                             | Soxhlet                                                                                                                                                                                                                                 |
| <i>Curcuma longa</i>         | (1) Curlone $\text{C}_{15}\text{H}_{22}\text{O}$ , $-5.17$ and<br>(2) Tumerone $\text{C}_{15}\text{H}_{22}\text{O}$ , $-4.89$                                                                                                                                                                                                            | (1) Ar-tumerone $\text{C}_{15}\text{H}_{20}\text{O}$ , $-5.07$ and<br>(2) 1,3-Benzenedicarboxylic acid,<br>bis(2-ethylhexyl) ester $\text{C}_{24}\text{H}_{38}\text{O}_4$ ,<br>$-4.44$ | (1) Curlone $\text{C}_{15}\text{H}_{22}\text{O}$ , $-5.32$ and<br>(2) Ar-tumerone $\text{C}_{15}\text{H}_{20}\text{O}$ , $-5.09$                                                                                                        |
| <i>Zingiber officinale</i>   | (1) 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-(S-(R*, S*)))- $\text{C}_{15}\text{H}_{24}$ , $-4.93$ and<br>(2) Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- $\text{C}_{15}\text{H}_{22}$ , $-4.67$ and<br>(3) Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, (S-(R*, S*)))- $\text{C}_{15}\text{H}_{24}$ , $-4.17$ | (1) 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*, S*)]- $\text{C}_{15}\text{H}_{24}$ , $-4.60$                                                                     | (1) Gingerol $\text{C}_{17}\text{H}_{26}\text{O}_4$ , $-4.08$                                                                                                                                                                           |
| <i>Valeriana officinalis</i> | (1) 1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a. Alpha.,7. Beta.,8a. alpha.)]- $\text{C}_{15}\text{H}_{26}\text{O}$ , $-5.46$ and<br>(2) 2,2,7,7-Tetramethyltricyclo [6.2.1.0(1,6)] undec-4-en-3-one $\text{C}_{15}\text{H}_{22}\text{O}$ , $-5.38$                                                       | (1) 4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a. Alpha.,4. Beta.,4a. Beta.,7. Alpha.,7a. beta $\text{C}_{15}\text{H}_{26}\text{O}$ , $-5.27$                | (1) 1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a. Alpha.,7. Beta.,8a. alpha.)]- $\text{C}_{15}\text{H}_{26}\text{O}$ , $-5.43$<br><br>(2) 1-Hexanol, 2-ethyl- $\text{C}_8\text{H}_{18}\text{O}$ , $-5.47$ |



exert their anti-inflammatory effects through the inhibition of NF- $\kappa$ B and modulation of cytokines.<sup>23</sup> However, valerianic acid's superior docking score highlights valerian's potential as a less-explored natural source for managing autoimmune diseases. This suggests that further *in vivo* and clinical studies on valerian extracts could lead to new therapeutic formulations. Table 1 generally displays the results of the plant secondary metabolite extraction methods used in this project, along with their best docking results.

## Conclusions

This study investigated the anti-inflammatory potential of secondary metabolites extracted from turmeric, ginger, and valerian, focusing on their interaction with TNF- $\alpha$ , a key inflammatory marker in rheumatoid arthritis. The GC-MS analysis and molecular docking simulations provided strong evidence supporting the use of these plant-derived compounds as natural TNF- $\alpha$  inhibitors. Notably, valerianic acid from Valerian exhibited the highest binding affinity, making it a promising candidate for further pharmacological research. The novelty of this study lies in its comparative evaluation of three medicinal plants, integrating computational and analytical techniques to identify potential anti-inflammatory agents.

### Mechanistic insight into Valerian's inhibition of TNF- $\alpha$

The docking results and literature suggest that certain *Valeriana officinalis* metabolites, such as valerenic acid and its analogues, may inhibit TNF- $\alpha$  activity by stabilizing inactive conformations or interfering with receptor binding. Previous studies have shown that valerenic acid can modulate GABAergic and inflammatory pathways, possibly through NF- $\kappa$ B suppression.<sup>12–14</sup> The interaction of key compounds with Ser60 and Tyr151 within TNF- $\alpha$ 's active site further supports their potential as competitive inhibitors. Further *in vitro* assays are required to validate these pathways.

## Limitations of this study

While molecular docking provides valuable insights into the binding affinity and possible interactions of compounds with a target protein, it is a computational prediction that does not account for the complex dynamics of a biological system. The docking results reported in this study indicate a strong potential for certain compounds—particularly from *Valeriana officinalis*—to interact with TNF- $\alpha$ . However, these interactions should be interpreted as hypotheses requiring confirmation. The actual bioactivity of these metabolites depends on additional factors such as bioavailability, metabolic stability, solubility, and cellular uptake. Therefore, further *in vitro* assays (*e.g.*, TNF- $\alpha$  inhibition ELISA, macrophage cytokine release assays) and *in vivo* models of inflammation are necessary to validate these findings. Moreover, comparison of docking scores with known TNF- $\alpha$  inhibitors (such as etanercept or infliximab peptides) was beyond the scope of this work. Still, it would provide valuable benchmarking in future studies.

## Possibility of mathematical modeling

Although not implemented in this study, future work could employ response surface methodology (RSM) to statistically model the relationship between extraction parameters (*e.g.*, temperature, solvent ratio, time) and extraction yield or bioactive compound concentration. RSM could help predict optimal conditions for maximizing the yield of pharmacologically active compounds and further improve the standardization of herbal extract production.

## Clarification on anti-inflammatory claims

Although the molecular docking results and compound profiles obtained *via* GC-MS indicate potential anti-inflammatory activity, they do not serve as definitive proof of biological efficacy. The current study focuses on the *in silico* exploration of interactions between phytochemicals and TNF- $\alpha$ , a critical inflammatory cytokine in rheumatoid arthritis. As no *in vitro* or *in vivo* bioassays were conducted, we emphasize that the anti-inflammatory potential of these extracts remains hypothetical and requires further experimental confirmation. Therefore, the conclusions drawn in this study are preliminary and should be interpreted with caution.

## Need for experimental validation

While the current study presents GC-MS and molecular docking data suggesting the potential anti-inflammatory activity of selected phytochemicals, no biological assays were conducted to confirm their efficacy. The conclusions regarding anti-inflammatory effects are based on predicted molecular interactions, particularly binding to TNF- $\alpha$ , which is a central cytokine in rheumatoid arthritis pathogenesis. However, these findings must be interpreted as preliminary.

Further experimental validation, such as TNF- $\alpha$  inhibition assays, nitric oxide quantification in activated macrophages, and cytokine profiling *in vitro*, is essential to confirm the activity of these compounds. Moreover, additional mechanistic insights (*e.g.*, NF- $\kappa$ B pathway suppression, COX-2 inhibition) should be explored in future studies to fully characterize their pharmacodynamics. Therefore, while the docking simulations offer promising leads, biological testing is critical to substantiate these predictions and translate them into therapeutic relevance.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The studied protein was downloaded and saved on the Protein Data Bank website with ID AZ52. The Auto Dock 4.2 software from mgltools with the URL (<https://ccsb.scripps.edu/mgltools/>) and the Viewer Lite software with the URL (<https://>





[windowsfileviewer.com/download](https://www.windowsfileviewer.com/download)) were installed and evaluated.

## Acknowledgements

We are grateful to the Imam Khomeini International University for supporting this study.

## References

- 1 T. Efferth, O. Kadioglu, M. Saeed, E. Seoet, A. T. Mbaveng and V. Kuete, *Phytochem. Rev.*, 2021, **20**, 7–53.
- 2 S. Prasad, and B. B. Aggarwal, *Biomolecular and Clinical Aspects*, 2nd edn, 2011.
- 3 J. S. Jurenka, *Alternative Med. Rev.*, 2009, **14**(2), 141–153.
- 4 A. B. Kunnumakkara, H. Choudhary and B. Kishore, *Expert Opin. Drug Metab. Toxicol.*, 2019, **15**(9), 705–733.
- 5 J. H. Kim, C. Gupta, P. Byoungduck, R. Vivek, Y. Bharat and B. Aggarwal, *Mol. Nutr. Food Res.*, 2012, **56**(3), 454–465.
- 6 Y. Panahi, B. Darvishi, N. Jowzi, F. Beiraghdar and A. Sahebkar, *Curr. Pharm. Des.*, 2016, **22**(2), 164–173.
- 7 V. S. Ipar, A. Dsouza and P. V. Devarajan, *Drug Metab. Pharmacokinet.*, 2019, **44**, 459–480.
- 8 *The Genus Zingiber*, ed. P. N. Ravindran and K. Nirmal Babu, 2016, CRC Press, United Kingdom.
- 9 B. Öz, C. Orhan, M. Tuzcu, N. Şahin, İ. H. Özercan, P. D. Öner, S. S. Koca, V. Juturu and K. Şahinet, *Rheumatology*, 2022, **8**(4), 196.
- 10 E. J. Jambi, A. Alamri, M. Afzal, F. Al-Abbasi, S. D. Al-Qahtani, N. A. R. Almalki, A. Bawadood, S. I. Alzarea, N. Sayyed and I. Kazmi, *PLoS One*, 2024, **19**(7), e0305358.
- 11 H. Maleki, H. Azadi, Y. Yousefpoor, M. Doostan, M. Doostan and M. H. Farzaei, *Pharm. Sci.*, 2023, **112**(6), 1687–1697.
- 12 H. Marawne, R. Mohammadhassan, Z. Mohammadalipour and S. Ahmadpour, *Tradit. Med. Res.*, 2022, **7**(5), 47.
- 13 B. Kumari, U. Kumari, D. K. Singh, G. M. Husain, *et al.*, *Endocr. Metab. Immune Disord. - Drug Targets*, 2024, **24**(13), 1506–1517.
- 14 H. Farzaei, M. R. Rahimi and M. Abdollahi, *Curr. Pharm. Biotechnol.*, 2015, **16**(3), 196–210.
- 15 I. B. McInnes and G. Schett, *Lancet*, 2017, **389**(10086), 2328–2337.
- 16 E. A. Ahmed, O. M. Ahmed, H. I. Fahim, T. M. Ali, B. H. Elesawy and M. B. Ashour, *Stem Cells Int.*, 2021, (1), 6665601.
- 17 J. Azmir, I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, *et al.*, *Food Eng.*, 2013, **117**(4), 426–436.
- 18 V. K. Pandey, A. Tripathi, S. Srivastava, A. H. Dar, *et al.*, *Food Sci. Biotechnol.*, 2023, **32**(7), 885–902.
- 19 D. Torres-Ortiz, G. García-Alcocer, L. Berumen-Segura, and M. Estévez, *Sustainable Chemistry for the Environment*, 2024, p. 100157.
- 20 Sh. Ijaz, J. Iqbal, B. A. Abbasi, *et al.*, Bioactive Phytochemicals, in *By-products from Leaf, Stem, Root and Tuber Vegetables*. 2025, p. 281–303.
- 21 E. Stashenko and J. R. Martínez, *Oil Nat.*, 2020, 119.
- 22 M. Asadian and R. Kiani-anbouhi, *Adv. J. Chem., Sect. A*, 2025, **8**(9), 1442–1456.
- 23 N. Liang, Y. Sang, W. Liu, *et al.*, *Inflammation*, 2018, **41**, 835–845.

