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Original Article

Anti-rheumatoid arthritis potential of *Acorus calamus* L. extract by interleukin-17 inhibition: Molecular insights through an *in silico* study

[Potencial anti-artritis reumatoide de extracto de *Acorus calamus* L. por inhibición de interleucina-17. Perspectivas moleculares mediante estudio *in silico*]

Parawansah[1,2], Rizki Rahmadi Pratama[3], Nuralifah[4], Arfan[4], Ruslin[4], Retno Widyowati[5], Sukardiman[5*]

[1]Doctoral Program, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.
[2]Department of Farmacology, Faculty of Medicine, Halu Oleo University, Kendari, Indonesia.
[3]Magister Program, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.
[4]Faculty of Pharmacy, Halu Oleo University, Kendari, Indonesia.
[5]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.
*E-mail: sukardiman@ff.unair.ac.id

Abstract

Context: Interleukin-17 (IL-17) is a pro-inflammatory cytokine that plays a crucial role in immunity and inflammation.

Aims: To evaluate the potential therapeutic effects of two compounds, magnosalicine and neotatarine, derived from the ethanolic extract of *Acorus calamus* L. rhizome, against IL-17.

Methods: Utilizing LC/MS analysis combining molecular docking simulations, drug likeness, ADME and toxicity analysis. This study explored the molecular interactions and binding affinities of identified compounds from *A. calamus* with crucial residues of IL-17, including Tyr62, Pro63, Ile66, Gln94, Ile96, Leu97, and Leu99.

Results: The results revealed that magnosalicine and neotatarine exhibited remarkable binding affinities of -10.16 kcal/mol and -9.53 kcal/mol, respectively, indicating their strong interactions with IL-17. Moreover, both compounds displayed superior binding energies compared to other extract constituents. Interestingly, this study highlighted that all terpenoid compounds from the *A. calamus* rhizome extract were capable of interacting with these key residues of IL-17, resembling the interactions observed with the natural ligand (RMK) and methotrexate. Meanwhile, the analysis results revealed a safer ADME and toxicity profile for neotatarine compared to magnolalicine.

Conclusions: This research unveils the promising potential of neotatarine as candidates for further exploration in therapeutic interventions targeting IL-17-related pathways. These findings shed light on the molecular insights of *Acorus calamus* L. compounds, providing valuable information for developing novel treatments for IL-17-associated disorders.

Keywords: *Acorus calamus*; cytokine; inflammation; molecular docking; rheumatoid arthritis; terpenoid.

Resumen

Contexto: La interleucina-17 (IL-17) es una citocina proinflamatoria que desempeña un papel crucial en la inmunidad y la inflamación.

Objetivos: Evaluar los posibles efectos terapéuticos de dos compuestos, la magnosalicina y la neotatarina, derivados del extracto etanólico del rizoma de *Acorus calamus* L., contra la IL-17.

Métodos: Utilizando análisis LC/MS que combinan simulaciones de acoplamiento molecular, semejanza de fármacos, ADME y análisis de toxicidad. Este estudio exploró las interacciones moleculares y afinidades de unión de los compuestos identificados de *A. calamus* con residuos cruciales de IL-17, incluyendo Tyr62, Pro63, Ile66, Gln94, Ile96, Leu97, y Leu99.

Resultados: Los resultados revelaron que la magnosalicina y la neotatarina mostraron notables afinidades de unión de -10,16 kcal/mol y -9,53 kcal/mol, respectivamente, lo que indica sus fuertes interacciones con la IL-17. Además, ambos compuestos mostraron energías de unión superiores en comparación con otros constituyentes del extracto. Curiosamente, este estudio puso de relieve que todos los compuestos terpenoides del extracto de rizoma de *A. calamus* eran capaces de interactuar con estos residuos clave de la IL-17, asemejándose a las interacciones observadas con el ligando natural (RMK) y el metotrexato. Mientras tanto, los resultados del análisis revelaron un perfil ADME y de toxicidad más seguro para la neotatarina en comparación con la magnolalicina.

Conclusiones: Esta investigación desvela el prometedor potencial de la neotatarina como candidatos para futuras exploraciones en intervenciones terapéuticas dirigidas a las vías relacionadas con la IL-17. Estos hallazgos arrojan luz sobre los conocimientos moleculares de los compuestos de *Acorus calamus* L., proporcionando información valiosa para el desarrollo de nuevos tratamientos para los trastornos asociados a la IL-17.

Palabras Clave: *Acorus calamus*; citocina; inflamación; acoplamiento molecular; artritis reumatoide; terpenoide.

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AUTHOR INFO
ORCID: 0000-0002-5503-342X (P) 0000-0003-1974-5021 (R) 0000-0002-5275-7211 (RRP) 0000-0002-6166-1289 (RW) 0000-0002-4802-9553 (N) 0000-0001-9689-5088 (S) 0000-0003-3004-7101 (A)

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder worldwide, causing progressive joint damage and potential lifelong disability (Smolen et al., 2016; Srirangan and Choy, 2010). RA is characterized by inflammation and synovial hyperplasia, leading to the production of autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA), cartilage and bone destruction, as well as skeletal disorders (McInnes and Schett, 2011). RF and ACPA are important serological markers for diagnosing RA and predicting treatment response and progression of bone damage (Wu et al., 2021). These markers form immune complexes with citrullinated proteins, activating macrophages and triggering the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 (Wu et al., 2020). Various cytokines like IL-1β, IL-6, IL-17, IL-18, IL-33, and TNF-α play a dominant role in the inflammatory and proliferative cascade in apondyloarthropathies (SpA), especially those involving the axial skeleton (Chyuan and Chen, 2018; Kondo et al., 2021).

Interleukin-17 (IL-17) is a pro-inflammatory cytokine that plays a significant role in immunity and inflammation (Mills, 2023). The IL-17 family comprises six isoforms with 20-30 kDa molecular weights, mainly acting on innate immune and stromal cells to promote inflammation (Ge et al., 2020). IL-17 interacts with the type I cell surface receptor IL-17R, activating several signalling cascades that induce chemokines responsible for recruiting immune cells, such as monocytes and neutrophils, to the inflammation site (Meehan and Wang, 2022).

IL-17 is primarily believed to be produced by a unique subset of helper T cells called T helper 17 (Th17) cells, different from Th1 and Th2 cells. Other T cell subsets, like natural killer T (NKT) cells, have also produced IL-17 in response to innate stimuli (Song and Yang, 2017). Growing evidence indicates that IL-17 and IL-17-producing cells are involved in the pathogenesis of various diseases, including allergies, inflammation, autoimmune diseases, allograft transplantation, and even malignancies (Mills, 2023).

Biological drugs such as disease-modifying antirheumatic drugs (DMARDs) have been developed as therapeutic agents, and other immunological conditions are currently under further investigation (McInnes et al., 2016). Methotrexate is currently the first-line DMARD in the treatment of RA, idiopathic arthritis, psoriasis, multiple sclerosis, vasculitis, and systemic lupus erythematosus (Bedoui et al., 2019; Chan and Cronstein, 2010). Common side effects observed with methotrexate use include organ toxicity in the gastrointestinal tract and liver. Additionally, methotrexate can lead to immunological disturbances and lymphoproliferative disorders (Bedoui et al., 2019). Hence, the discovery and development of drugs to address inflammation-mediated RA remain highly needed.

The diversity of compounds found in natural resources provides an excellent opportunity for drug discovery and development, particularly as guidance for modern medications (Mathur and Hoskins, 2017). Various compounds have been successfully isolated from plants to be developed and used as medicines for treating different diseases. Currently, the search for new drug compounds from multiple plants continues, and some of these compounds include calamenone, cis-asarone, and β-asarone, which are abundant in the rhizomes of *Acorus calamus* L., family *Acoraceae* (Rajput et al., 2014). β-asarone has been reported to exhibit anti-adipogenic, antimicrobial, fungicidal, insulin sensitivity/antidiabetic, neuroprotective, immunosuppressive, and anti-inflammatory activities (McGaw et al., 2002; Omer et al., 2022). The essential oil derived from *A. calamus* rhizomes has been reported to possess anti-inflammatory activity by inhibiting albumin protein denaturation with an IC_{50} value of 172.41 µg/mL (Loying et al., 2019). Furthermore, *in vitro* tests on *A. calamus* leaf extracts have demonstrated anti-inflammatory activity against human keratinocyte HaCaT cells, inhibiting the expression of IL-8 and IL-6 and suppressing NF-κB and IRF3 activation (Kim et al., 2009).

Ethnobotanically, the *A. calamus* plant is reported to possess anti-rheumatoid arthritis activity (Alamgeer et al., 2018). In relation to this information, IL-17 is known to mediate cartilage and bone damage occurring in RA, primarily through its actions on synoviocytes and osteoblasts. Therefore, inhibition of IL-17 by *A. calamus* may provide insights into the potential activity of this plant against IL-17-related diseases, including RA (Robert and Miossec, 2019). However, to date, studies on the benefits of *A. calamus* rhizomes in inhibiting IL-17 remain limited. Thus, by identifying potential compounds from *A. calamus* and their molecular interactions against key residues of IL-17, detailed molecular insights are expected to be provided, guiding more effective drug design.

Currently, drug development is not solely based on experimentation but also involves computational chemistry (Garofalo et al., 2020). Computational chemistry has several advantages in drug development, such as reducing costs and saving research time (Lin et al., 2020). Computational chemistry methods, particularly *in silico* techniques, are frequently used in drug discovery and development to predict a mole-

cule's three-dimensional placement of pharmacophores (active functional groups) (Arba et al., 2018; Beroza et al., 2022). One of these *in silico* methods used to analyze the affinity of a drug molecule is molecular docking. Molecular docking is widely used to provide insights into the interactions, bindings, and affinities of a ligand (drug) with its receptor or an enzyme with its substrate or inhibitor (Pinzi and Rastelli, 2019). Based on this description, this study was conducted to investigate and explore the anti-inflammatory activity of compounds derived from *A. calamus* using LC-MS analysis combined with in silico methods targeting IL-17. This research is expected to provide scientific information as an alternative to developing new and effective drugs for RA treatment from natural sources.

MATERIAL AND METHODS

Plant material and extraction process

The rhizomes of *A. calamus* were obtained from Mokoau Village, Kambu Sub-district, Kendari City, Southeast Sulawesi, Indonesia (4°02'31.8" S 122°32'48.8" E). According to the Van Steenis Library, CGGJ. 2008 FLORA, the *A. calamus* plant was identified by the Batu Herbal Materia Medica Laboratory UPT, Malang City, East Java, Indonesia, under registration number 000.9.3/436/102.20/2024. The *A. calamus* rhizomes were wet-sorted to separate impurities, washed, thinly sliced, and air-dried before pulverization. The powder of *A. calamus* rhizomes was weighed and then subjected to maceration with 70% ethanol solvent (3 × 24 hours). The extract was concentrated using a rotary evaporator to obtain a dense extract.

LC-MS/MS QToF analysis

The compound content analysis of the *A. calamus* extract was conducted using LC-MS/MS QToF. The sample was dissolved in methanol, and a 5 µL sample was injected using a syringe into the LC-MS/MS QToF instrument. The obtained measurements would generate mass spectra chromatograms (Pavia et al., 2001).

Ligands and receptors collection

In this study, ten compounds were identified from the ethanolic extract of *A. calamus* rhizomes through LC-MS/MS analysis. The three-dimensional structures of these compounds were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Additionally, methotrexate was utilized as a reference compound, and its structure was obtained from the same database as the test compounds. Furthermore, the three-dimensional structure of the IL-17 receptor (PDB ID 7AMA) (Andrews et al., 2022) was obtained from the RCSB Protein Data Bank website (https://www.rcsb.org/pdb).

Molecular docking study

To assess the binding affinity and interaction of the identified compounds from *A. calamus* with IL-17, the AutoDock 4.2.6 software was used, applying the Lamarckian genetic algorithm with a population size of 100. To validate the docking procedure, RMK (the natural ligand) was redocked to IL-17, ensuring that the root mean square deviation (RMSD) was below 2 Å (Pitaloka et al., 2021). During the docking simulations, water molecules and bound ligands were excluded from the system. Additionally, AutoDock Tools v.1.5.6 was used to protonate IL-17 by adding polar hydrogen atoms and Kollman charges, while the identified compounds (ligands) were hydrogenated and assigned Gasteiger charges (Morris et al., 2009).

To specify the position of the binding site, the RMK coordinates were used as a reference by setting the cubic lattice area to 40 × 40 × 40 Å with a distance of 0.375 Å. Finally, the Discovery Studio Visualizer software was employed to analyze and visualize the molecular interactions between IL-17 ligands.

ADME and toxicity and drug-likeness analysis

A computational approach was employed to predict the pharmacokinetic properties (absorption, distribution, metabolism, and excretion) of the identified compounds in *A. calamus*, utilizing the SwissADME webserver (http://www.swissadme.ch/index.php) (Daina et al., 2017). Additionally, the drug-likeness properties of the compounds were studied by applying Lipinski's rule of five principles from the same website (Benet et al., 2016). Furthermore, the toxicity of the compounds from *A. calamus* extract was predicted using the admetSAR webserver (http://lmmd.ecust.edu.cn/admetsar2/) (Yang et al., 2019). This predicted toxicity profile includes carcinogenicity, mutagenicity, hepatotoxicity, reproductive toxicity, and nephrotoxicity.

In silico data analysis

Data analysis was conducted by screening the chemical constituents of *A. calamus* to identify its bioactive compounds using LC-MS/MS QTOF. Meanwhile, *in silico* data analysis for drug discovery involved several steps using specific computational tools. Molecular docking began with the preparation of ligand and receptor structures and the validation of docking methods by applying RMSD criteria. The binding energy resulting from the docking results

was compared with methotrexate as a reference. Prediction of ADME and drug-likeness of compounds was carried out using SwissADME webserver, and toxicity was assessed using admetSAR webserver. Molecular interactions such as hydrogen bonds and hydrophobic contacts of potential compounds from *A. calamus* against IL-17 were visualized using Discovery Studio Visualizer software.

RESULTS AND DISCUSSION

Extraction process

The wet sample weight of *A. calamus* ethanol extract was 7 kg, resulting in a dry sample of 1,796 kg and obtaining 300 g of extract. Fractionation was carried out using hexane, ethyl acetate, and water as solvents, with yield values of 40.3617, 7.4112, and 184.5581 g, respectively.

Metabolomic profiling of *A. calamus* extract

The LC-MS/MS analysis yielded valuable insights, identifying ten probable compounds within the *A. calamus* extract, including benzoylformic acid, neotatarine, acoramol, asarylaldehyde, berberrubine, tritylamine, acoronene, β-asarone, magnosalicin, and calamusenone (Table 1). The structure of the identified compounds in the *A. calamus* ethanolic extract can be seen in Fig. 1. General and individual chromatograms are shown in supplementary data.

Molecular docking analysis

Molecular docking simulation proves highly valuable in drug discovery and development, as it allows us to observe and study the interactions between a compound and its target receptor responsible for a particular disease. Molecular docking can be utilized to predict the best conformation and binding interactions and estimate a molecule's binding energy with its target receptor (Fitriani and Ansory, 2021). To be effective as a drug candidate, a compound must possess the lowest free binding energy (Qayed et al., 2022).

Before applying the docking method to assess the binding capabilities of test compounds, it is essential to conduct validation to calibrate the docking parameters and replicate the conformation of natural ligands with the target receptor using the RMSD parameter. The docking procedure is valid and can be applied to docking all identified compounds if the RMSD value is below 2 Å. The validation process involves redocking the natural ligand RMK to IL-17, following the ligand's binding site to the target receptor. The results of the docking validation in Fig. 2 reveal that the docking parameters can be applied to all test compounds, as they exhibit an RMSD value of 0.601 Å. A lower RMSD value indicates the docking method's ability to reproduce the x-ray conformation of the natural ligands with the target receptor.

Molecular docking predicts the binding conformation between the receptor and ligand molecules. The outcome of this molecular docking process is the free binding energy, which represents the stability parameter of the interactions between the compounds from the *A. calamus* and IL-17. The free binding energy reflects the capability of a compound or ligand to bind with its target receptor (Arfan et al., 2022). A smaller binding energy indicates a higher affinity between the ligand and receptor (Citra et al., 2023). The primary focus of this study was to assess the ability of the identified compounds to interact with residues in the active site of IL-17, as these specific residues play a critical role in stabilizing the interaction between the compounds and receptors. The analysis demonstrates that the co-crystal ligands (RMK) exhibit significantly stronger affinities towards the receptors than methotrexate and the identified compounds, with energies of -13.28 kcal/mol (Table 2).

Based on the results obtained from the molecular docking simulation, it was observed that a group of ten compounds, identified through LC-MS/MS analysis, exhibited a range of binding affinities towards IL-17, ranging from -4.76 kcal/mol to -10.16 kcal/mol (Table 1). Two compounds sourced from the *A. calamus*, namely magnosalicine, and neotatarine, which displayed significantly enhanced binding affinities to IL-17, with respective binding energies of -10.16 kcal/mol and -9.53 kcal/mol. These results indicate that magnosalicine and neotatarine exhibit superior affinity energies compared to methotrexate, demonstrating a binding energy of -9.17 kcal/mol. The noteworthy implications of these findings underscore the potential therapeutic relevance of these compounds for IL-17-associated conditions, thereby presenting promising avenues for further exploration and pharmaceutical development.

The formation of interactions with specific amino acid residues in a target receptor plays a pivotal role in determining a compound's binding affinity and its potential to inhibit the receptor's activity (Nedyalkova et al., 2021). In molecular terms, a receptor possesses a three-dimensional structure, and within this structure lies the active site, which serves as a docking site for substrates or ligands. The binding of a ligand to the active site involves various types of interactions, including electrostatic interactions, hydrogen bonds, and hydrophobic interactions (Kuhn et al., 2011).

Table 1. Metabolites profile observed in *A. calamus* extract from LC-MS analysis.

No.	Retention time (R.T.)	Mass (m/z)	Calculated (m/z)	Fragmentation ion mayor	Tentative compound	Identified compound	Reference
1.	1.106	183.0652	183.0654	94. 110.	$C_8H_6O_3$	Benzoylformic acid	Zhang et al. (2013) https://pubchem.ncbi.nlm.nih.gov/compound/11915
2	1.717	279.1492	279.1383	97. 113. 152. 216. 229.	$C_{18}H_{15}NO$	Neotatarine	Li et al. (2017)
3	4.797	225.1121	225.1115	105. 125. 168. 193.	$C_{12}H_{16}O_4$	Acoramol	Bai (2022) https://pubchem.ncbi.nlm.nih.gov/compound/70682800
4	6.969	197.0808	197.0812	123. 167. 169.	$C_{10}H_{12}O_4$	Asarylaldehyde	van Alphen (1927) https://pubchem.ncbi.nlm.nih.gov/compound/20525
5	7.208	322.1074	322.1068	115. 191. 221. 280. 294.	$C_{19}H_{15}NO_4$	Berberrubine	Yang et al. (2017) https://pubchem.ncbi.nlm.nih.gov/compound/72704
6	9.718	295.1211	295.2271	115. 167. 169. 233.	$C_{19}H_{17}N$	Tritylamine	https://pubchem.ncbi.nlm.nih.gov/compound/Tritylamine
7	10.020	235.1693	235.1697	135. 161. 181. 217.	$C_{15}H_{22}O_2$	Acoronene	Tribudi et al. (2022) https://pubchem.ncbi.nlm.nih.gov/compound/15558294
8	10.442	209.1172	209.1184	103. 147. 179. 194.	$C_{12}H_{16}O_3$	β-Asarone	Lee et al. (2004); McGaw et al. (2002); Sya'diyah et al. (2014) https://pubchem.ncbi.nlm.nih.gov/substance/482040397
9	11.012	433.2221	433.2234	203. 275. 316. 343. 365. 381.	$C_{24}H_{32}O_7$	Magnosalicin	Tsuruga et al. (1991); Mori et al. (1986); Ware et al. (2023) https://pubchem.ncbi.nlm.nih.gov/compound/442895
10	11.652	219.1743	219.1740	91, 118, 121, 125	$C_{15}H_{22}O$	Calamusenone	Serafini et al. (2018) https://pubchem.ncbi.nlm.nih.gov/compound/91748856

Electrostatic interactions occur between charged amino acid residues and polar groups of the ligand (Klebe, 2013). Hydrogen bonding entails the creation of hydrogen bonds between hydrogen atoms of the ligand and electronegative atoms, commonly nitrogen or oxygen, found in the side chains of amino acids (Klebe, 2013). Hydrophobic interactions arise when nonpolar regions of the ligand associate with nonpolar amino acid residues, which typically cluster together to minimize exposure to the surrounding water molecules (Klebe, 2013).

When a ligand binds to the receptor's active site and interacts with specific amino acid residues, it undergoes conformational changes that can either activate or inhibit the receptor's function (Heldin et al., 2016). In inhibiting inflammation, for example, a ligand that binds to key amino acid residues in the active site of an inflammatory receptor, such as IL-17, may prevent the receptor from binding to its natural substrates, inhibiting downstream inflammatory signaling pathways (Kuwabara et al., 2017).

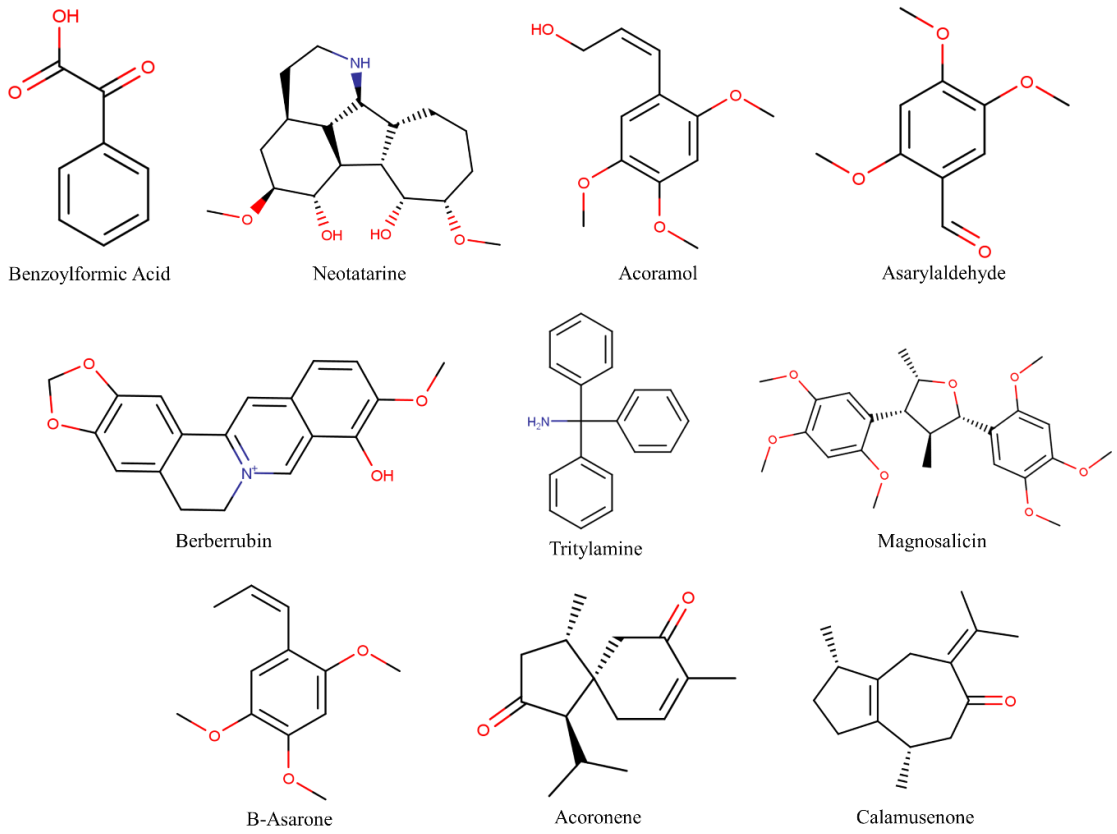


Figure 1. The structure of the identified compounds in the *A. calamus* extract.

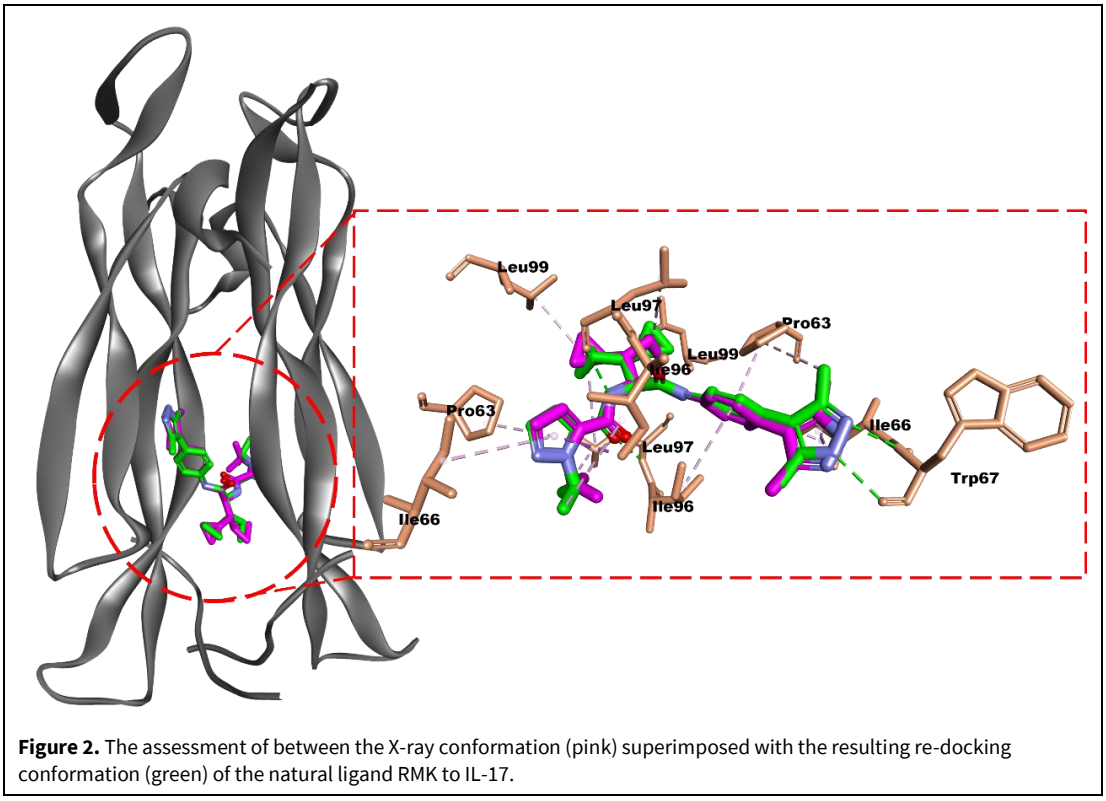


Figure 2. The assessment of between the X-ray conformation (pink) superimposed with the resulting re-docking conformation (green) of the natural ligand RMK to IL-17.

Table 2. Summary of binding affinity energies of the identified compounds in *A. calamus* extract with IL-17.

No.	Identified compound	Binding affinity (kcal/mol)	Inhibition constant
1	Ligand native (RMK)	-13.28	184.45 pM
2	Magnosalicine	-10.16	35.70 nM
3	Neotatarine	-9.53	102.69 nM
4	MTX	-9.17	188.88 nM
5	Berberubine	-9.04	234.64 nM
6	Tritylamine	-8.93	283.54 nM
7	Calamusenone	-8.92	287.03 nM
8	Acoronene	-8.83	339.57 nM
9	Acoramol	-6.17	30.00 µM
10	β-Asarone	-6.08	34.98 µM
11	Asaryldehyde	-5.06	196.82 µM
12	Benzoyl formic acid	-4.76	325.07 µM

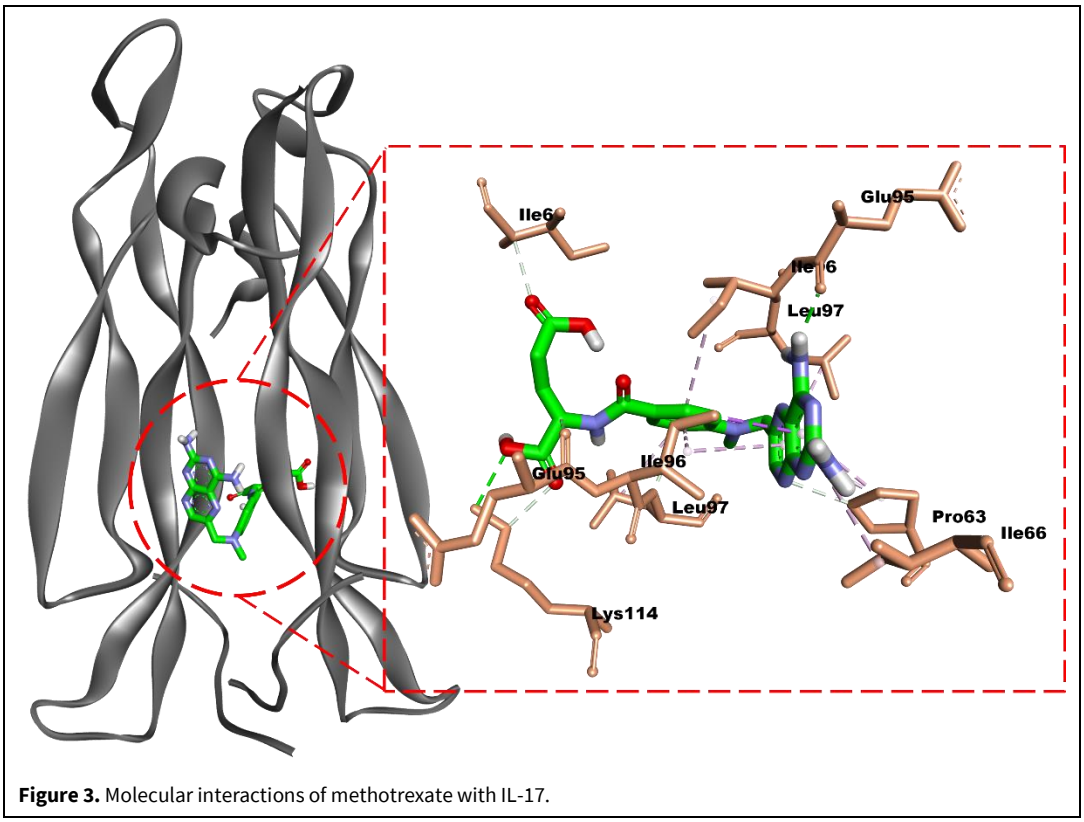


Figure 3. Molecular interactions of methotrexate with IL-17.

The natural ligand RMK forms four hydrogen bonds with amino acid residues Trp67, Gln94, and Leu97 at the IL-17 binding site. Methotrexate, used as a reference compound, interacts with different amino acid residues, forming five hydrogen bonds with Lys114, Glu95, Leu9, Pro63, and Ile66. RMK and methotrexate also establish hydrophobic interactions with specific residues, Pro63, Ile66, Ile96, Leu97, and Leu99 of IL-17 (Fig. 3). These interactions collectively contribute to the stability of the ligand-receptor complex. The observation of similar interactions between RMK and methotrexate with IL-17 suggests that these compounds bind to the same site on the receptor, potentially interfering with its function comparably (Andrews et al., 2022).

Magnosalicin and neotatarine, extracted from *A. calamus*, have emerged as promising candidates with potent inhibitory activity against IL-17, surpassing the

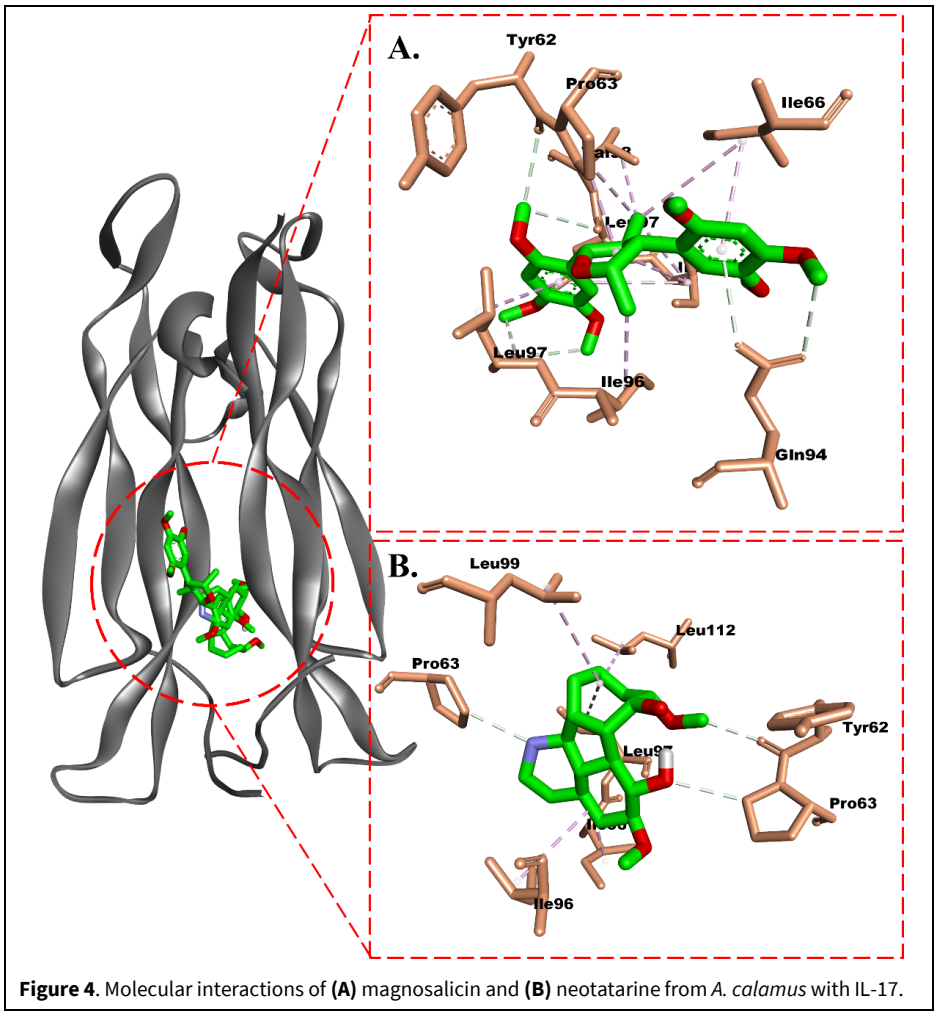


Figure 4. Molecular interactions of **(A)** magnosalicin and **(B)** neotatarine from *A. calamus* with IL-17.

performance of other compounds in the study. Delving into their molecular interactions sheds light on their inhibitory potential. In the case of magnosalicin, the observations reveal the presence of crucial hydrogen bonds with specific amino acid residues of IL-17, including Tyr62, Gln94, and Leu97. Notably, the methoxy group within the magnosalicin molecule takes charge of facilitating the formation of all hydrogen bonds at the active site of IL-17 (Fig. 4A). This methoxy-mediated interaction plays a crucial role in enhancing the binding affinity and stability of the magnosalicin-IL-17 complex (Andrews et al., 2022).

On the other hand, neotatarine demonstrates its inhibition by engaging in unique molecular interactions with IL-17. The pyridine ring and methoxy group in neotatarine contribute to forming specific hydrogen bonds with residues Pro63 and Tyr62 from the IL-17 receptor (as shown in Fig. 4B). These interactions significantly reinforce the binding between Neotatarine and IL-17, bolstering its potential to inhibit IL-17 activity. Magnosalicin and neotatarine exhibit hydrophobic interactions similar to those observed in the reference compounds RMK and methotrexate. These hydrophobic interactions further stabilize the ligand-receptor complexes and fortify their inhibitory effect on IL-17 activity.

Moreover, an intriguing observation is the unique interactions displayed by magnosalicin and neotatarine. Magnosalicin demonstrates an additional hydrophobic interaction with Val98, while neotatarine forms an exclusive bond with residue Ile112, which is not present in the RMK and methotrexate. These specific hydrophobic interactions confer additional stability and contribute to the exceptional inhibitory potential of magnosalicin and neotatarine against IL-17. Overall, the molecular insights obtained from analyzing the interactions of magnosalicin and neotatarine with IL-17 reveal their prowess as potent inhibitors. By targeting crucial amino acid residues at the receptor's active site and forming specific hydrogen bonds, these compounds effectively hinder the inflammatory activity of IL-17, offering promising prospects for their utilization in therapeutic interventions targeting IL-17-related conditions.

In the berberubine, tritylamine, acoramol, β-asarone, and asaryldehyde form a single hydrogen

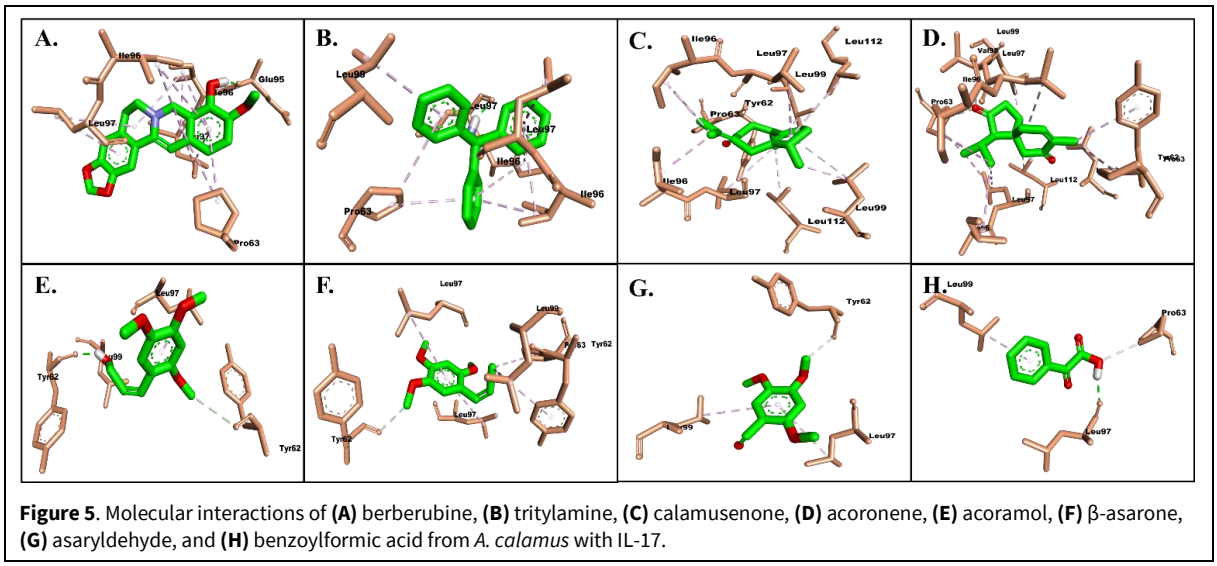


Figure 5. Molecular interactions of **(A)** berberubine, **(B)** tritylamine, **(C)** calamusenone, **(D)** acoronene, **(E)** acoramol, **(F)** β-asarone, **(G)** asaryldehyde, and **(H)** benzoylformic acid from *A. calamus* with IL-17.

Table 3. Summary of molecular interactions of the identified compounds in *A. calamus* extract with IL-17.

No.	Identified compound	Hydrogen bonds (H-bonds)	Hydrophobic interactions
1	Ligand native (RMK)	Trp67, Leu97, Gln94	Ile96, Pro63, Ile66, Leu99
2	Magnosalicine	Gln94, Leu97, Tyr62	Ile96, Pro63, Val98, Ile66
3	Neotatarine	Tyr62, Pro63	Ile96, Leu97, Ile112, Leu99
4	MTX	Lys114, Glu95, Leu97, Pro63, Ile66	Ile96, Ile66, Pro63, Leu97
5	Berberubine	Glu95	Pro63, Ile96, Leu97
6	Tritylamine	Leu97	Ile96, Leu99, Pro63
7	Calamusenone	-	Leu99, Leu112, Leu97 Tyr6B, Pro63, Ile96A, Ile96
8	Acoronene	Val98, Pro63	Leu112, Leu99, Pro63, Tyr62, Leu97, Ile96, Ile96
9	Acoramol	Tyr62	Leu97
10	β-Asarone	Tyr62	Leu99, Pro63, Tyr62, Leu97
11	Asaryldehyde	Tyr62	Leu97, Leu99
12	Benzoyl Formic Acid	Leu97, Pro63	Leu99

bond with Glu95, Leu97, and Tyr62 residues, respectively. On the other hand, acoronene and benzoylformic acid demonstrate a more robust interaction, each establishing two hydrogen bonds: acoronene with residues Pro63 and Val98 and benzoylformic acid with Pro63 and Leu97. However, particularly intriguing is the compound calamusenone, which, despite not forming any hydrogen bonds, shows an impressively strong binding affinity of -8.92 kcal/mol towards IL-17. Diverse and varied hydrophobic interactions with residues Leu99, Leu112, Leu97, Tyr62, Pro63, and Ile96 likely support this intriguing phenomenon (Fig. 5).

All the compounds derived from *A. calamus* generally demonstrate similar patterns of hydrogen bonds and hydrophobic interactions with IL-17. The only exception to this pattern is observed in acoramol and benzoyl formic acid, as they each display only one hydrophobic interaction, specifically with residues Leu97 and Leu99, respectively. A summary of the interactions of all identified compounds in the ethanolic extract of *A. calamus* with IL-17 can be seen in Table 3.

ADME properties

The analysis of ADME properties of compounds from *A. calamus* extract is presented in Table 4. Interestingly, all identified compounds exhibited notable solubility except for magnosalicine and tritylamine, which were categorized as having moderate solubility. Solubility is a critical factor in drug development,

Table 4. The ADME properties of the identified compounds from *A. calamus* extract.

Compound	Log S (ESOL)	GI absorption	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
Magnosalicine	-4.91 (Moderate)	High	No	No	No	Yes	No
Neotatarine	-2.12 (soluble)	High	No	No	No	No	No
Berberubine	-3.57 (soluble)	High	Yes	No	No	Yes	Yes
Tritylamine	-4.26 (Moderate)	High	Yes	Yes	No	Yes	No
Calamusenone	-3.17 (soluble)	High	No	Yes	Yes	No	No
Acoronene	-2.89 (soluble)	High	No	No	Yes	No	No
Acoramol	-2.27 (soluble)	High	Yes	No	No	No	No
β-Asarone	-3.05 (soluble)	High	Yes	Yes	No	No	No
Asaryldehyde	-2.29 (soluble)	High	No	No	No	No	No
Benzoylformic acid	-1.88 (very soluble)	High	No	No	No	No	No

as it determines the ability of a molecule to dissolve in bodily fluids and be absorbed by the body. This property indicates the potential of the compounds in the drug development process, as it enhances their effectiveness and ability to progress through various stages, from formulation to successful absorption into the body (Ottaviani et al., 2010; Ritchie et al., 2013). Additionally, the chemical constituents of this plant extract demonstrated a high gastrointestinal (GI) absorption profile, suggesting that these compounds could be readily absorbed through the gastrointestinal tract. This finding may have influenced their bioavailability and distribution throughout the body (Vinarov et al., 2021).

Overall, the compounds from *A. calamus* extract exhibited interactions with critical enzymes from the cytochrome P450 (CYP) family. Neotatarine, asaryldehyde, and benzoylformic acid compounds are estimated not to inhibit isoforms of body metabolism enzymes. Meanwhile, magnosalicine only inhibits CYP2D6 isoform. These enzymes play a crucial role in drug metabolism and elimination. Inhibiting these enzymes can lead to significant alterations in drug metabolism, potentially resulting in higher drug concentrations throughout the body (Hollenberg, 2002; Kirchmair et al., 2015).

Toxicity prediction

The toxicity profile analysis of compounds derived from *A. calamus* extract, as shown in Table 5, provides substantial information into potential side effects, which is an important factor in drug development efforts. The identified compounds in this plant, magnosalicine and neotatarine, exhibit contrasting toxicity profiles, necessitating thorough evaluation.

Magnosalicine, for instance, is associated with a spectrum of potential risks, including carcinogenicity, mutagenesis, hepatotoxicity, reproductive toxicity, and nephrotoxicity. In contrast, neotatarine exhibits a comparatively safer profile, with toxicity risks primarily associated with reproductive processes. Carcinogenicity refers to the ability of a substance to induce cancerous growth, which is a paramount concern in pharmaceutical research due to its severe implications for human health (Liu et al., 2023). Similarly, mutagenesis denotes the capacity to induce genetic mutations, potentially leading to adverse hereditary effects (Rosendahl Huber et al., 2021). Hepatotoxicity pertains to the liver's susceptibility to damage, posing challenges in drug development due to the organ's vital role in metabolism and detoxification (Rusyn et al., 2021).

Reproductive toxicity is another significant consideration as it pertains to adverse effects on reproductive organs and processes (Dubey et al., 2022). In drug development, compounds with reproductive toxicity risk may pose challenges in regulatory approval, particularly concerning potential impacts on fertility and prenatal development (Parkinson et al., 1997). Nephrotoxicity, involving harm to kidney function, is also a critical concern as it can lead to renal dysfunction and compromised drug elimination, influencing dosage regimens and patient safety (Al-Naimi et al., 2019).

Comprehending these toxicity profiles is crucial in the process of drug development, as it guides choices regarding the selection of compounds, optimization of dosages, and assessments of safety (Hughes et al., 2011). By conducting thorough evaluations and employing strategies to mitigate risks, scientists can effectively manage the intricacies associated with the use of natural compounds, thus optimizing their therapeutic benefits while reducing potential risks.

Table 5. Prediction of the toxicity properties of the identified compounds from *A. calamus* extract.

Compounds	Carcinogenicity (binary) (p-value)*	Carcinogenicity (trinary) (p-value)	Ames mutagenesis (p-value)	Hepatotoxicity (p-value)	Reproductive toxicity (p-value)	Nephrotoxicity (p-value)
Magnosalicine	No (0.8707)	Yes (0.3357)	Yes (0.6363)	Yes (0.5962)	Yes (0.5667)	Yes (0.7082)
Neotatarine	No (0.9800)	No (0.7402)	No (0.7700)	No (0.6250)	Yes (0.9444)	No (0.7182)
Berberubine	No (0.9800)	No (0.4327)	No (0.6100)	No (0.7000)	Yes (0.8111)	No (0.6900)
Tritylamine	Yes (0.5500)	No (0.5520)	No (p=0.8000)	Yes (0.8750)	No (0.8667)	No (0.6194)
Calamusenone	No (0.9400)	No (0.5461)	No (0.8579)	Yes (0.8243)	No (0.5889)	No (0.5935)
Acoronene	No (0.7930)	No (0.4818)	No (0.5800)	Yes (0.7125)	Yes (0.5444)	Yes (0.4668)
Acoramol	No (0.7978)	No (0.7268)	No (0.5500)	No (0.5625)	Yes (0.5444)	No (0.6990)
β-Asarone	No (0.7721)	No (0.4991)	Yes (0.6600)	Yes (0.6625)	Yes (0.6667)	No (0.6055)
Asaryldehyde	No (0.7643)	No (0.6020)	No (0.8600)	Yes (0.6284)	Yes (0.7556)	Yes (0.6057)
Benzoylformic acid	No (0.5668)	No (0.7301)	No (0.9600)	Yes (0.7427)	No (0.7333)	No (0.5743)

*The "p-value or probability value" is the probability that a compound will possess certain characteristics regarding absorption, distribution, metabolism, or excretion. These probability values can provide a clearer understanding of the reliability of the model predictions regarding these properties. The higher the probability value, the more confident the model is in the accuracy of the prediction.

Table 6. Lipinski's rule estimation of identified compounds from *A. calamus* extract.

Compound	Molecular weight	MLOGP	No. H-bond acceptors	No. H-bond donors	Lipinski rules
Magnosalicine	432.51	1.77	7	0	Yes; 0 violation
Neotatarine	325.44	1.05	5	3	Yes; 0 violation
Berberubine	323.34	2.00	4	1	Yes; 0 violation
Tritylamine	259.34	4.33	1	1	Yes; 1 violation: MLOGP>4.15
Calamusenone	218.33	3.46	1	0	Yes; 0 violation
Acoronene	234.33	2.54	2	0	Yes; 0 violation
Acoramol	224.25	1.12	4	1	Yes; 0 violation
β-Asarone	208.25	1.97	3	0	Yes; 0 violation
Asaryldehyde	196.20	0.55	4	0	Yes; 0 violation
Benzoylformic acid	150.13	0.70	3	1	Yes; 0 violation

Molecular weight (criteria ≤500 g/mol), MLOGP (criteria Log $P_{o/w}$ ≤4.15), Number of hydrogen bond (H-bonds) acceptors (criteria ≤10), Number of hydrogen bond (H-bonds) donors (criteria ≤5).

Drug likeness properties

In particular, Lipinski's rules prediction of compound contents from *A. calamus* extract was illustrated in Table 6. Interestingly, all compound contents from this plant exhibited properties that fulfilled Lipinski's criteria. Lipinski's criteria offer a fundamental framework for evaluating the likelihood of

molecules becoming orally active drugs, particularly with regard to solubility and absorption (Mermer and Vakal, 2021). Furthermore, the results of the Lipinski properties identified the most promising compounds as potential candidates for oral drug development, demonstrating enhanced bioavailability and thereby improving their potential efficacy and safety upon oral administration (Doak et al., 2014).

Further exploration of the search results revealed fascinating insights into the multifaceted role of IL-17 as a pro-inflammatory cytokine with profound implications for immunity and inflammation (Li et al., 2019). This multifunctional cytokine is synthesized by both adaptive and "innate" T cells, and its impact extends far beyond merely instigating inflammation. Instead, IL-17 is a pivotal orchestrator of gene expression, intricately linking innate and adaptive immune responses to create a unified defense against invading pathogens (Dong and Ma, 2016).

Central to the biological activity of IL-17 is its interaction with the type I cell surface receptor IL-17R, which triggers intricate signaling cascades. These cascades culminate in the induction of various chemokines, critical for recruiting diverse immune cells, including monocytes and neutrophils, to the site of inflammation. The orchestrated influx of immune cells is vital for timely and targeted responses to infectious agents and inflammatory triggers (Dong and Ma, 2016).

Furthermore, mounting evidence substantiates the substantial involvement of IL-17 and IL-17-producing cells in the pathogenesis of a broad spectrum of diseases (Chyuan and Chen, 2018). Notably, IL-17's influence spans conditions ranging from allergic reactions to autoimmune disorders, allograft transplantation, and malignant transformations (Huang et al., 2023; Wen et al., 2023). This broad impact underscores the significance of comprehending IL-17's intricate roles in health and disease, presenting opportunities for innovative therapeutic interventions targeting IL-17-related pathways (Rafael-Vidal et al., 2020).

Inhibition of IL-17 presents a promising avenue for modulating the NF-κB pathway and attenuating inflammatory responses (Sønder et al., 2011). By interfering with the activation of this crucial signaling cascade, the transcription of pro-inflammatory genes, such as IL-1β, IL-6, and TNF-α, can be effectively reduced, leading to a dampening of the overall inflammatory process (Liu et al., 2017).

Investigating the functions of IL-17 sheds light on the intricate web of immune responses and inflammatory processes. Inhibition of IL-17 and its downstream effects on the NF-κB pathway offer potential avenues for managing diverse inflammatory conditions and related diseases (Liu et al., 2017).

CONCLUSION

Molecular docking simulations of the compounds identified from the LC-MS/MS analysis of the ethanolic extract of *A. calamus* rhizome against IL-17 reveal that magnosalicine and neotatarine are predicted to exhibit the best binding affinities and can interact molecularly with IL-17 with binding energies of -10.16 kcal/mol and -9.53 kcal/mol, respectively. These compounds demonstrate superior binding energies compared to other compound constituents. Meanwhile, all compounds from this plant's rhizome ethanolic extract are capable of interacting with crucial residues of IL-17, similar to the interactions observed with the natural ligand (RMK) and methotrexate, such as Tyr62, Pro63, Ile66, Gln94, Ile96, Leu97, and Leu99. Meanwhile, the analysis findings indicated that neotatarine sourced from *A. calamus* exhibited a more favorable ADME and toxicity profile compared to magnolalicine.

Overall, the molecular docking simulations have provided valuable insights into the favorable binding affinities and molecular interactions of certain compounds, notably magnosalicine and neotatarine, derived from the ethanolic extract of the *A. calamus* rhizome. These compounds have demonstrated interactions with critical residues of IL-17, suggesting their potential as promising candidates for further investigation in therapeutic approaches aimed at targeting IL-17-related pathways.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Parawansah	Rizki RP	Nuralifah	Arfan	Ruslin	Retno W	Sukardiman
Concepts or ideas	x					x	x
Design				x		x	x
Definition of intellectual content	x						x
Literature search	x	x	x				
Experimental studies		x			x		
Data acquisition		x		x	x		
Data analysis	x			x			
Statistical analysis		x					x
Manuscript preparation	x					x	x
Manuscript editing	x					x	x
Manuscript review	x	x	x	x	x	x	x

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Supplementary data

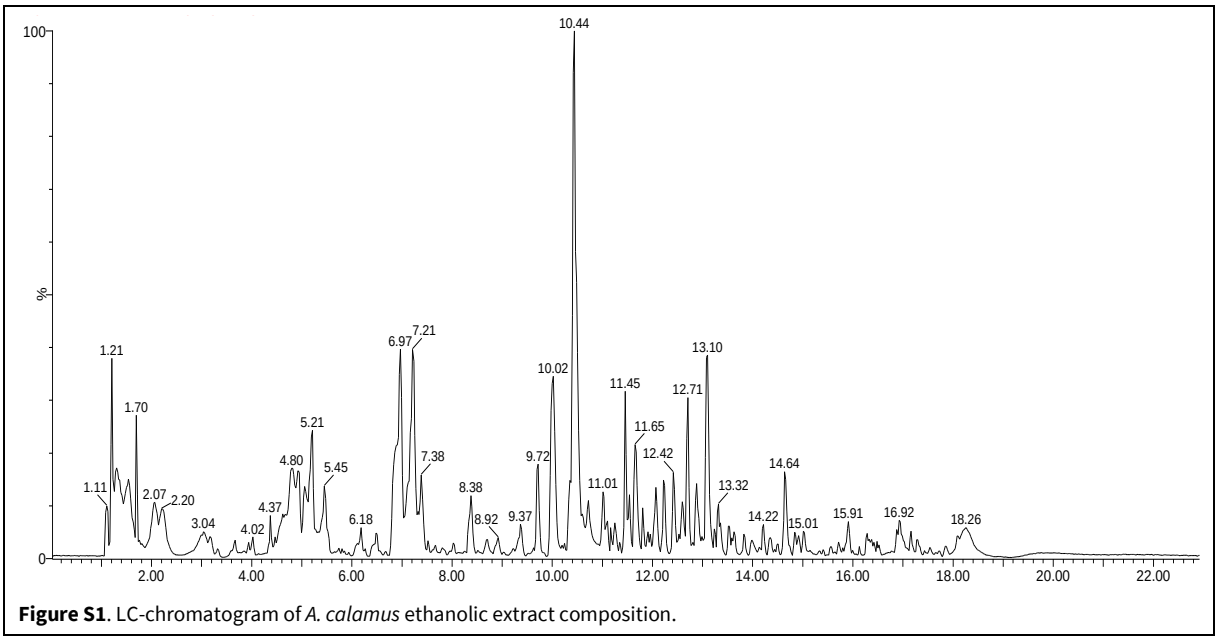


Figure S1. LC-chromatogram of *A. calamus* ethanolic extract composition.

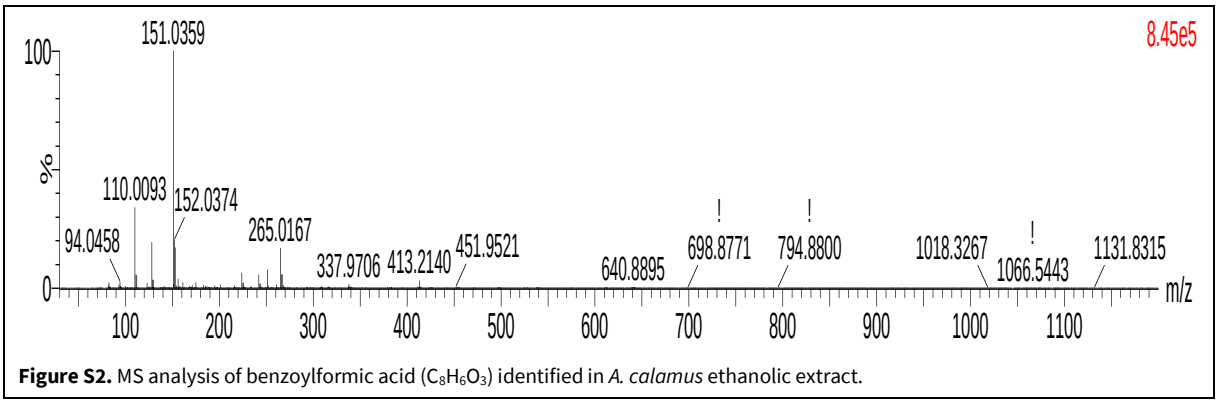


Figure S2. MS analysis of benzoylformic acid ($C_8H_6O_3$) identified in *A. calamus* ethanolic extract.

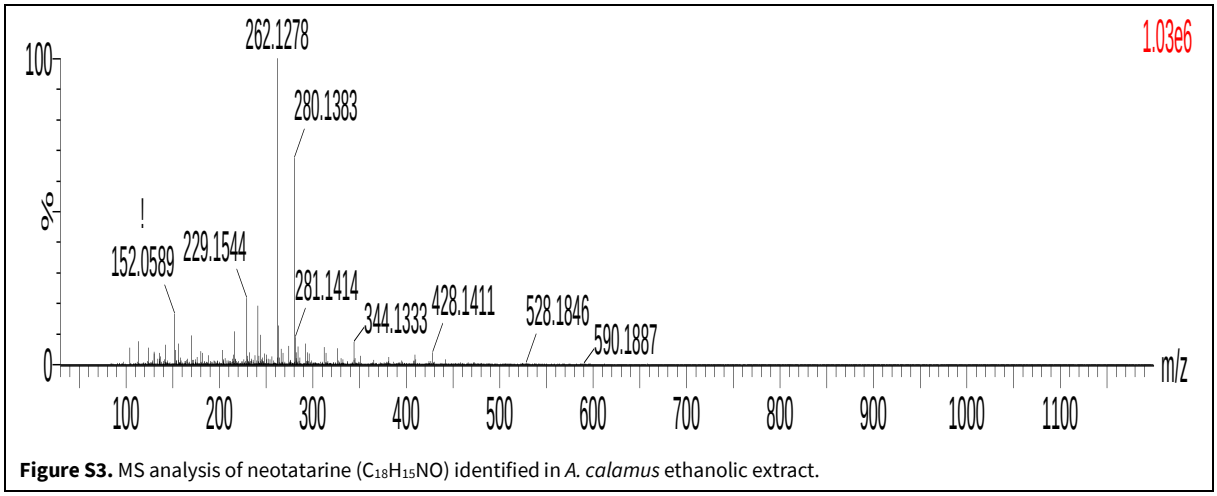


Figure S3. MS analysis of neotatarine ($C_{18}H_{15}NO$) identified in *A. calamus* ethanolic extract.

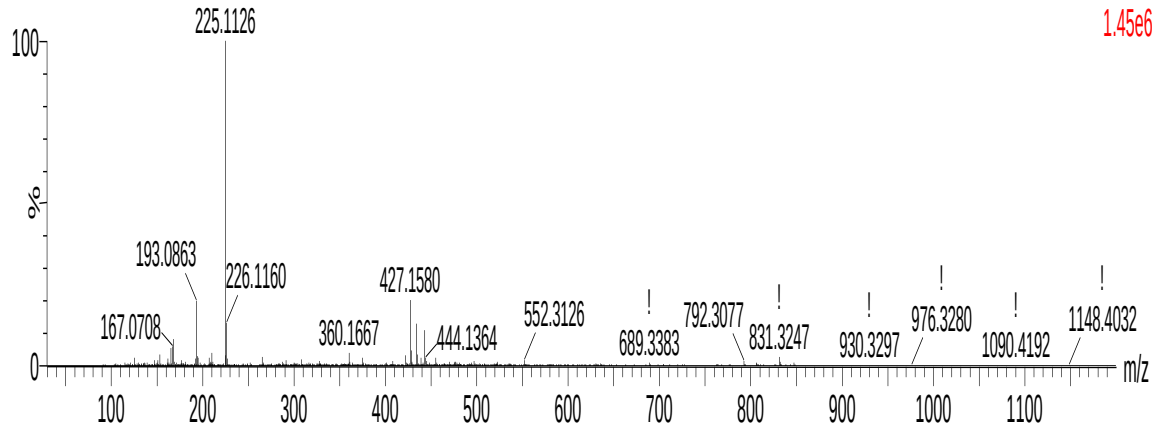


Figure S4. MS analysis of acoramol ($C_{12}H_{16}O_4$) identified in *A. calamus* ethanolic extract.

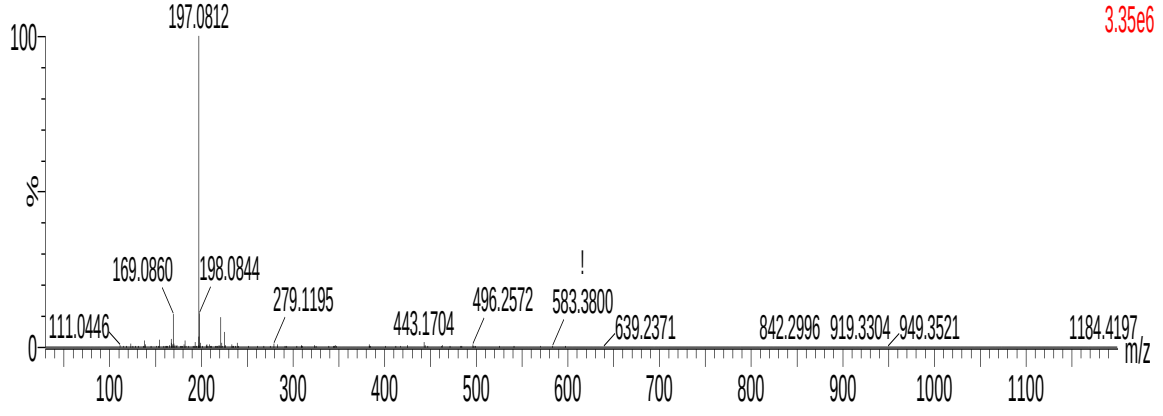


Figure S5. MS analysis of asarylaldehyde ($C_{10}H_{12}O_4$) identified in *A. calamus* ethanolic extract.

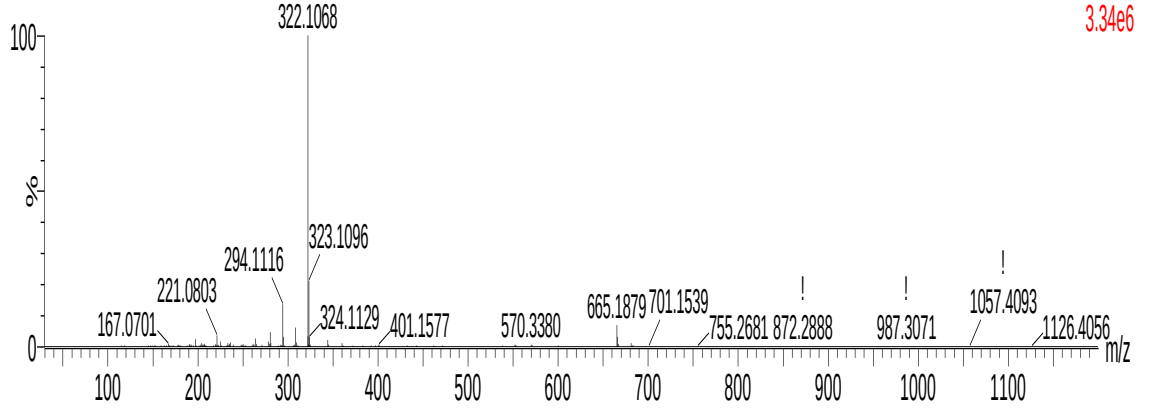


Figure S6. MS analysis of berberrubine ($C_{19}H_{15}NO_4$) identified in *A. calamus* ethanolic extract.

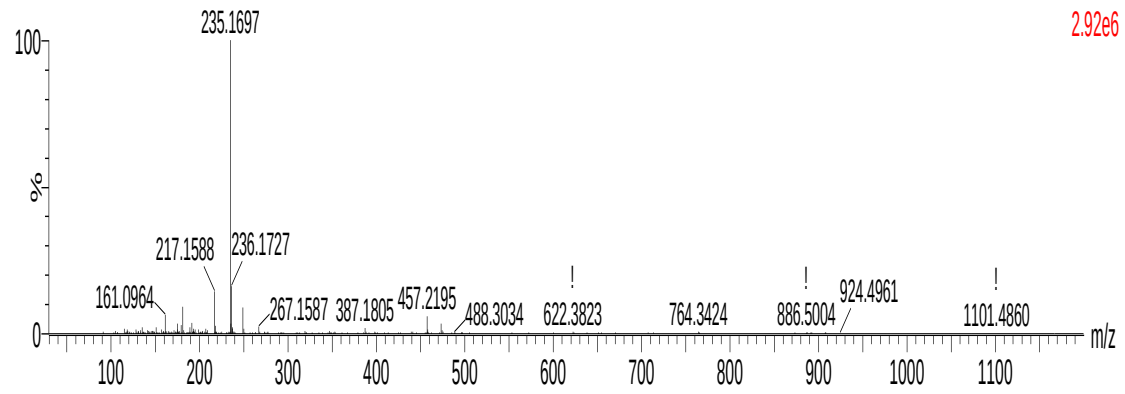


Figure S7. MS analysis of tritylamine ($C_{19}H_{17}N$) identified in *A. calamus* ethanolic extract.

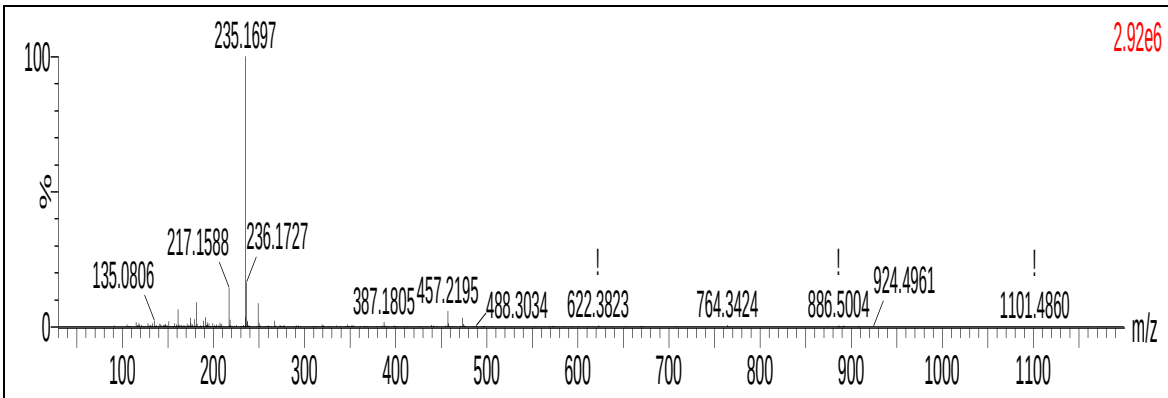


Figure S8. MS analysis of acoronene ($C_{15}H_{22}O_2$) identified in *A. calamus* ethanolic extract.

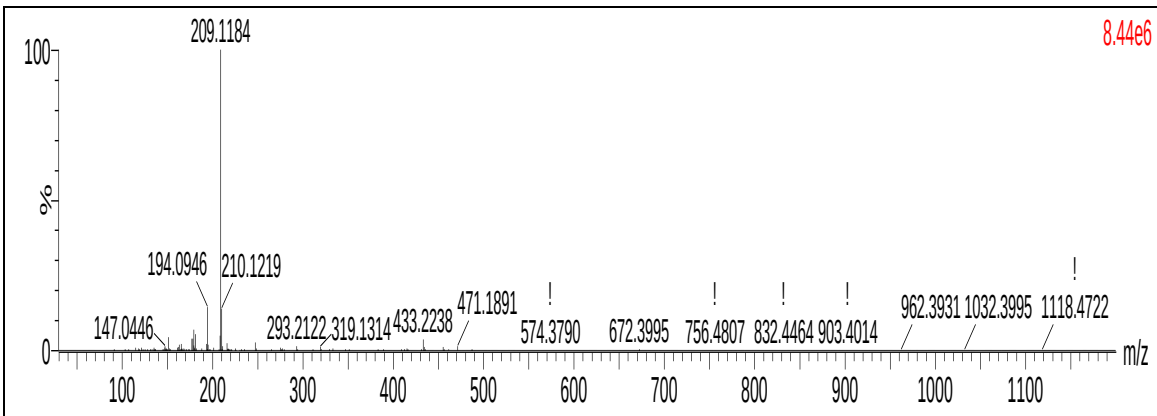


Figure S9. MS analysis of β-asarone ($C_{12}H_{16}O_3$) identified in *A. calamus* ethanolic extract.

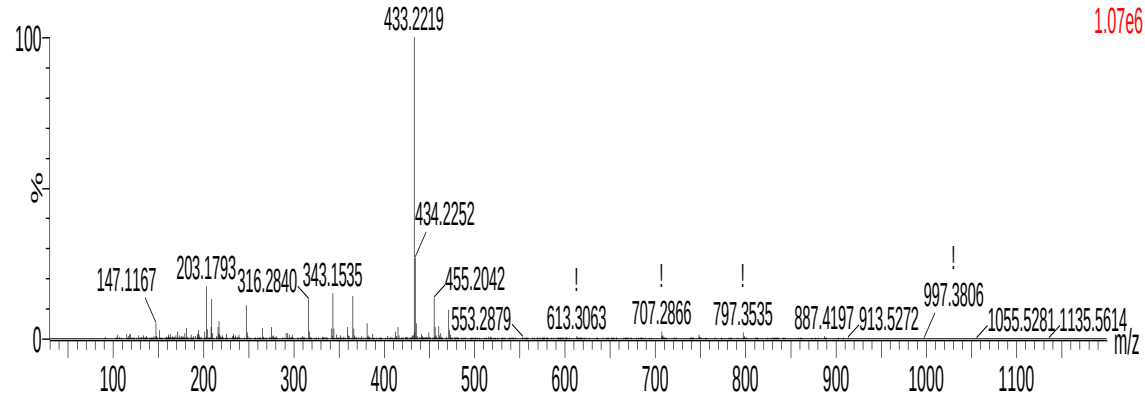


Figure S10. MS analysis of magnosalicin ($C_{24}H_{32}O_7$) identified in *A. calamus* ethanolic extract.

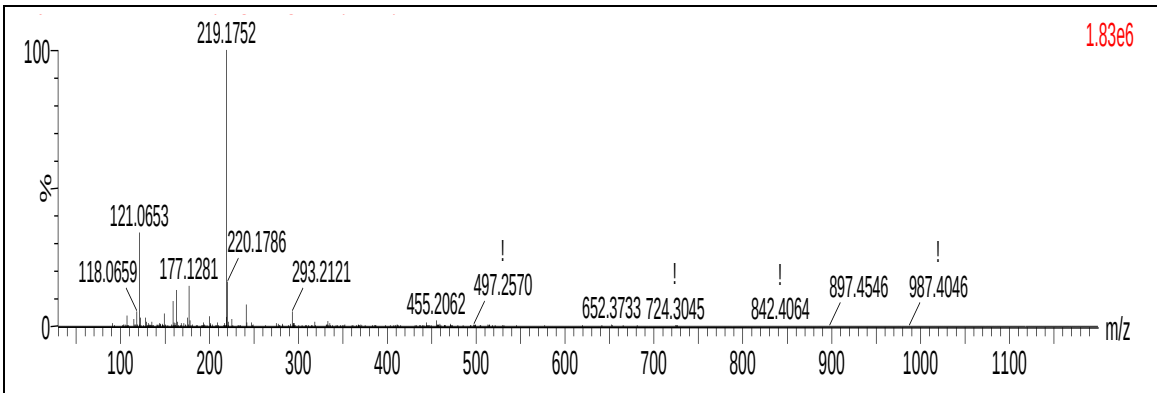


Figure S11. MS analysis of calamusenone ($C_{15}H_{22}O$) identified in *A. calamus* ethanolic extract.