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Investigation of catechin's anti-inflammatory activity: A bioinformatics and molecular docking study

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ABSTRACT

Background: Inflammation plays a key role in the progression of many chronic diseases. As a country with rich biodiversity, Indonesia offers numerous phytochemicals with potential for drug development, including catechin, a natural compound with anti-inflammatory properties.

Objective: This study aimed to identify potential anti-inflammatory targets of catechin and evaluate its inhibitory potency through molecular docking simulations.

Methods: Data acquisition and refinement were conducted using the NCBI, STRING, and STITCH databases, with intersections identified through Venn diagrams. Molecular docking was performed using AutoDockTools 1.5.6, and interactions were visualized with BIOVIA Discovery Studio.

Results: Bioinformatics analysis predicted that catechin inhibits three pro-inflammatory proteins: COX-2, HSP90, and IL-2. Catechin's inhibitory potential was indicated by negative binding energies and interactions with amino acid residues critical for target protein activity. Among the targets, IL-2 exhibited the lowest binding energy with catechin (-5.12 kcal/mol), suggesting it as the primary anti-inflammatory target. However, catechin's binding affinity was lower than that of the native ligand (-11.78 kcal/mol).

Conclusion: IL-2 is predicted to be the primary target for catechin's anti-inflammatory activity. Structural modifications of catechin are recommended to enhance its binding affinity and therapeutic potential.

Keywords: anti-inflammation, bioinformatic, catechin, molecular docking

Introduction

Inflammation is a protective response to invading pathogens or tissue damage, characterized by redness, swelling, pain, heat, and loss of tissue function. It involves three primary signaling pathways—NF-κB, MAPK, and JAK-STAT—which trigger the release of inflammatory markers and initiate cellular responses. While inflammation is essential for immune defense, excessive or prolonged inflammation can contribute to the pathogenesis of chronic diseases such as cancer, arthritis, diabetes, inflammatory bowel disease, and cardiovascular disease [1].

The treatment of inflammation typically involves steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). These medications act through various mechanisms, including the inhibition of NF-κB signaling [2] and the suppression of inflammatory markers such as prostaglandins [3,4]. However, concerns about side effects and drug resistance have spurred interest in alternative treatments, particularly natural products.

Natural compounds and their structural analogs have been pivotal in drug discovery. Indonesia, with its rich biodiversity encompassing approximately 30,000 plant species [5], offers an abundance of plant-derived materials that have been used empirically in pharmacotherapy. Among these, catechin—a polyphenolic compound—stands out

for its anti-inflammatory potential. Catechins are secondary metabolites characterized by two aromatic rings and multiple hydroxyl groups (Figure 1). Subtypes include catechin, epicatechin (EC), epigallocatechin (EGC), and epigallocatechin-3-O-gallate (EGCG) [6]. Catechins are found in high concentrations in gambir (*Uncaria gambir*) (73.3%) compared to tea leaves (30–40%) [7].

While most research has focused on the pharmacological actions of EGCG, fewer studies have specifically investigated catechin. Existing evidence suggests that catechin exhibits notable anti-inflammatory effects. For instance, an *in vivo* study reported that catechin hydrate attenuates inflammation in benzo(a)pyrene-induced lung damage in mice [11]. Similarly, catechin demonstrated therapeutic effects on streptozotocin-induced diabetic nephropathy in mice by downregulating inflammatory markers such as NLRP3, ASC, AIM2, caspase-1, IL-1β, and IL-18 [12]. An *in vitro* study revealed that catechin modulates pro-inflammatory and anti-inflammatory cytokines through the NF-κB, AMPK, FOXO3a, and SIRT1 pathways in 3T3-L1 adipocytes [13]. Additionally, network pharmacology and metabolomic studies suggest that catechin mediates ferroptosis in macrophages to exert anti-inflammatory effects [14].

Molecular docking studies have predicted several direct anti-inflammatory targets of catechin, including TNF-α, IL-1β, IL-6, iNOS, COX-2, MPO, and PPAR-γ receptors [15–17]. Despite these findings, no comprehensive study has systematically examined the potential anti-inflammatory targets of catechin. Given the complexity of inflammatory pathways and mediators involved in inflammation-related diseases [1,16,17], further exploration of catechin's anti-inflammatory targets is warranted.

This study aims to identify and refine potential anti-inflammatory targets of catechin using bioinformatics approaches. Databases such as NCBI, STITCH, and STRING were utilized alongside molecular docking and amino acid interaction visualization to predict catechin's mechanism of action. The findings from this study provide valuable preliminary data to guide future *in vitro* and *in vivo* experiments, facilitating the development of catechin as a potential anti-inflammatory therapeutic agent.

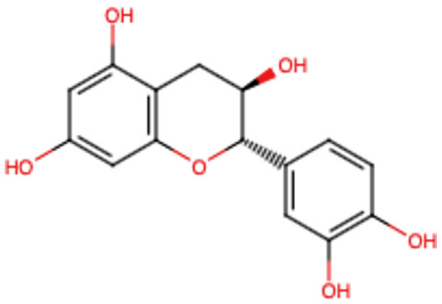


Figure 1. Chemical structure of catechin

Methods

Data acquisition and refinement

Bioinformatics analyses were conducted to identify inflammatory regulatory proteins and catechin target proteins. Inflammatory regulatory proteins were retrieved from the NCBI gene database (https://www.ncbi.nlm.nih.gov/) [18] using the keyword "inflammation" and filtered for *Homo sapiens*. Catechin's direct target proteins were identified using the STITCH database (http://stitch.embl.de/), and indirect target proteins of the top 10 direct targets were identified using the STRING database (https://string-db.org/) with a minimum interaction score of 0.4 and no more than 10 interactions in the first shell.

The intersections between inflammatory regulatory proteins and catechin's direct and indirect target proteins were determined using a Venn diagram generated by Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). The resulting protein-protein interaction network (PPIN) was constructed using the STRING database and analyzed for the contextual roles of the proteins, particularly their involvement in anti-inflammatory processes. The PPIN was exported in TSV format and imported into Cytoscape software for further analysis. The CytoHubba plugin in Cytoscape ranked the proteins based on the "Degree" topological analysis method to identify key catechin anti-inflammatory targets [19–22].

Preparation of ligand

The three-dimensional structure of catechin (ChemSpider ID 8711) was downloaded from the ChemSpider database (http://www.chemspider.com/) in MOL format. The structure was optimized using GaussView 6.0 software [23] with Density Functional Theory (DFT) and a basis set of B3LYP/6-31G [24].

Preparation of target proteins

Crystal structures of target proteins identified through bioinformatics analysis were obtained from the PDB database (http://www.rscb.org/pdb/). Inclusion criteria for protein selection included structures with no mutations, resolution < 3 Å, derived from *Homo sapiens*, and bound to a small molecule inhibitor. Protein structures were prepared in AutoDockTools by removing water molecules, adding hydrogen atoms, and applying Kollman and Gasteiger charges.

Molecular docking of cathecin to target proteins

Molecular docking was performed using AutoDockTools 1.5.6 software. The docking method and grid box size were validated by re-docking the native ligand to the target protein to confirm alignment with the binding site of the native ligand. Validation was considered successful if the Root Mean Square Deviation (RMSD) was ≤ 2 Å [25].

Following validation, catechin was docked to each target protein, with the grid box size adjusted to match the validated parameters. Binding energy between catechin and target proteins was calculated. The interactions between catechin and potential target proteins were visualized using BIOVIA Discovery Studio 2021 [26] by selecting the "Ligand Interaction" menu.

Results

Bioinformatics studies

Bioinformatics analysis was conducted to identify the molecular targets of catechins in inhibiting inflammation. A search of inflammatory regulatory genes in the NCBI database identified

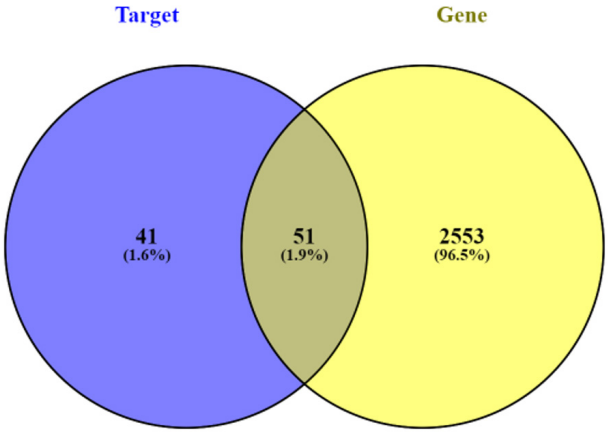


Figure 2. Venn diagram of catechin inflammatory regulatory genes

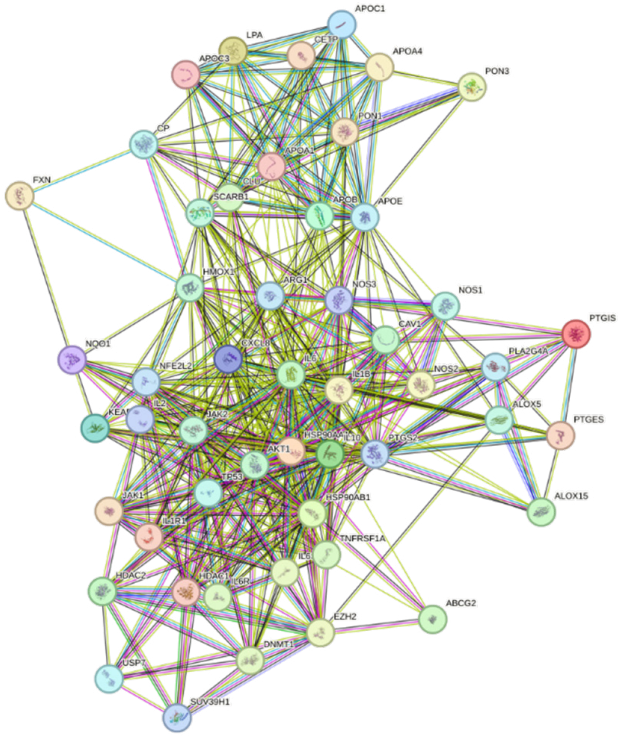


Figure 3. Protein-protein interaction network (PPIN) of catechin potential anti-inflammatory targets

2,604 human (*Homo sapiens*) genes associated with inflammatory regulation, as supported by research articles archived in the database. The STITCH database query for direct catechin target proteins yielded 10 proteins: NOS3, NOS1, IL-6, COX-2, NOS2, DNMT1, HMOX1, SLC47A1, APOB, and PON1. These 10 proteins were subsequently used to retrieve indirect target proteins from the STRING database, with each direct target generating 10 indirect target proteins.

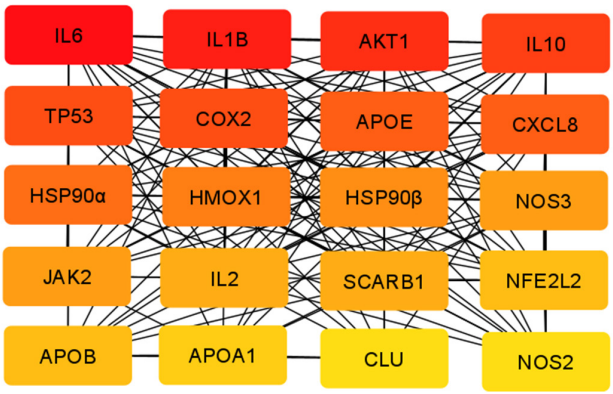


Figure 4. Top 20 catechin target proteins ranked by degree Score using Cytoscape and Cytohubba plugin

Using the Venn Diagram tool (Venny 2.1), 10 direct target proteins, 100 indirect target proteins, and 2,604 inflammatory regulatory genes were analyzed to determine intersecting proteins. Figure 2 shows that 51 proteins are predicted as the target of catechin in inhibiting inflammation, comprising 9 direct target proteins (APOB, DNMT1, HMOX1, IL-6, NOS1, NOS2, NOS3, PON1, and COX-2) and 42 indirect target proteins. A protein-protein interaction network (PPIN) of these 51 proteins was generated using the STRING website (Figure 3).

The top 20 proteins were selected based on their number of interactions (degree scores) in the PPIN, including 6 direct target proteins (APOB, HMOX1, IL-6, NOS2, NOS3, and COX-2) and 14 indirect target proteins. The PPIN for these top 20 proteins is presented in Figure 4, and their degree scores are listed in Table 1.

Among the top 20 proteins, 9 were associated with anti-inflammatory functions (IL10, TP53, CXCL8, NOS3, HMOX1, SCARB1, CLU, APOB, and NFE2L2), while 11 exhibited pro-inflammatory roles (IL1β, AKT1, COX-2, APOE, HSP90α, HSP90β, JAK2, IL-2, APOA1, and NOS2). Crystallographic structures meeting the criteria of no mutations, resolution < 3 Å, and binding to inhibitory molecules were available for 7 pro-inflammatory proteins (AKT1, COX-2, HSP90α, HSP90β, JAK2, IL-2, and NOS2).

Molecular docking

The docking procedure was validated for all selected proteins by re-docking their native ligands.

Table 1. Ranking of target proteins based on degree score

Rank	Protein name	Degree score
1	IL6	43
2	IL1B	39
3	AKT1	35
4	IL10	32
5	TP53	31
6	COX-2	31
7	APOE	28
8	CXCL8	28
9	HSP90α	27
10	HMOX1	25
11	HSP90β	24
12	NOS3	23
13	JAK2	23
14	IL-2	21
15	SCARB1	21
16	NFE2L2	20
17	APOB	20
18	APOA1	18
19	CLU	17
20	NOS2	17

Table 2. Validation results of molecular docking for native ligands with target proteins

Protein (PDB ID)	Grid box			RMSD
	x	y	z	
AKT1 (3O96)	23	44	34	1.03
COX-2 (5IKR)	24	16	22	0.6
HSP90α (2XJX)	20	22	36	1.4
HSP90β (3NMQ)	26	30	23	1.45
JAK2 (6VGL)	20	22	14	0.94
IL-2 (1PW6)	30	23	44	1.59
iNOS2 (4NOS)	12	13	10	0.68

The Root Mean Square Deviation (RMSD) values were ≤ 2 Å, confirming the accuracy of the docking protocol. The grid box size and RMSD values are detailed in Table 2.

Catechin exhibited negative binding energies for all tested proteins except NOS2, indicating strong binding affinities. The binding energies are summarized in Table 3. Visualizations of catechin or native ligand interactions with amino acid residues in pro-inflammatory proteins are presented in Figure 5.

Table 3. Molecular docking results of catechin with target proteins

Protein (PDB ID)	Binding energy (Kcal/mol)		
	Native ligand		Catechin
AKT1 (3O96)	Inhibitor VIII	-12.81	-6.65
COX-2 (5IKR)	Mefenamic acid	-7.6	-1.7
HSP90α (2XJX)	Onalespib	-9.13	-5.49
HSP90β (3NMQ)	EC144	-9.45	-4.82
JAK2 (6VGL)	Ruxolitinib	-8.11	-4.92
IL-2 (1PW6)	FRB	-11.78	-5.12
iNOS2 (4NOS)	S-ethylisothiourea	-2.99	221006.35

Discussion

Our study provides additional insights on the molecular targets of catechin related to its anti-inflammatory effects. Previous research has identified potential direct anti-inflammatory targets of catechin, such as TNF-α, IL-1β, IL-6, iNOS, COX-2, MPO, and the PPAR-γ receptor [15,16]. In contrast, our study employs a different approach by identifying potential targets through the NCBI, STITCH, and STRING databases, with prioritization based on the degree score within a protein-protein interaction network (PPIN). Previous studies have demonstrated that the degree-based ranking is associated with gene essentiality. In other words, proteins with higher degrees are more likely to be essential [21,22].

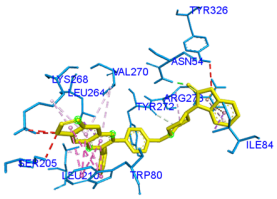
Our study predicts that catechin did not bind to iNOS, as evidenced by the absence of negative binding energy (Table 3). This contradicts prior findings [15], which used the PDB ID 1NSI (an iNOS structure bound only to cofactors) [27]. In contrast, our study employed the PDB ID 4NOS, which includes a small molecule inhibitor, simulating catechin binding to the inhibitor's active site alongside cofactors (heme and tetrahydrobiopterin) [28]. This methodological difference may explain the discrepancy.

Catechin's predicted binding to other targets demonstrates significant potential for anti-inflammatory activity. Catechin interacts with AKT1 at Trp80, a critical residue for the activity of allosteric inhibitor VIII, suggesting catechin could inhibit AKT-1 activation. AKT-1 is central to the PI3K/AKT pathway, which promotes pro-inflammatory cytokines and activates the NLRP3 inflammasome [29,30]. The crystal structure of AKT1 used in the molecular docking study is bound to an allosteric inhibitor, Inhibitor VIII. This inhibitor interacts with AKT1 at the junction of the PH domain and the N- and C-lobes of the kinase domain. A key ring-stacking interaction between Inhibitor VIII and the residue Trp80 has been observed. Notably, alanine substitution at Trp80 in AKT1 abolishes the activity of Inhibitor VIII, highlighting the residue's critical role in binding [30]. Our study revealed that catechin also interacts with Trp80, suggesting that it may similarly inhibit AKT1 activation.

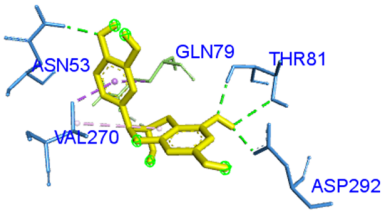
COX-2 plays a significant role in inflammation by producing prostaglandins [31]. In this study, the binding site of catechin to COX-2 was modeled based on the binding site of mefenamic acid to the enzyme. Both catechin and mefenamic acid form hydrogen bonds with Tyr385, a key catalytic residue essential for the cyclooxygenase reaction [32]. Additionally, catechin uniquely forms a bond with Arg120, a residue crucial for arachidonic acid binding, which is not observed with mefenamic acid [33]. These findings suggest that catechin may be more effective than mefenamic acid in inhibiting COX-2 activity, potentially enhancing its anti-inflammatory effects.

HSP90α and HSP90β are members of the HSP90 chaperone protein family, which play critical roles in folding and activating proteins involved in inflammatory processes. Thus, targeting HSP90 for inhibition offers a potential therapeutic approach for inflammatory diseases [34,35]. The binding

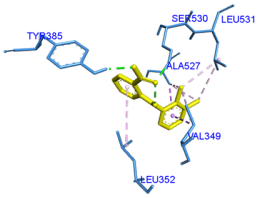
AKT1 and native ligand



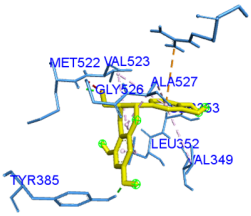
AKT1 and catechin



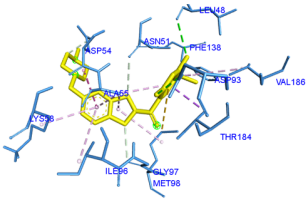
COX-2 and native ligand



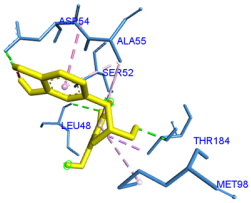
COX-2 and catechin



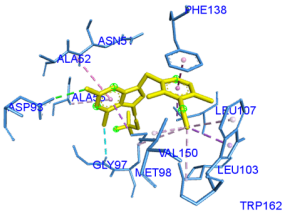
HSP90α and native ligand



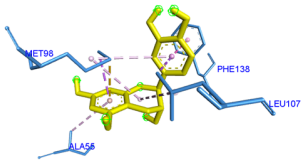
HSP90α and catechin



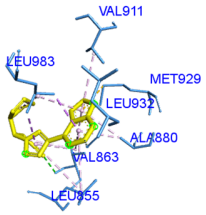
HSP90β and native ligand



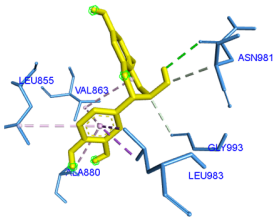
HSP90β and catechin



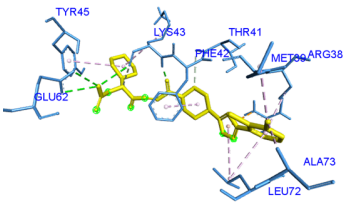
JAK2 and native ligand



JAK2 and catechin



IL-2 and native ligand



IL-2 and catechin

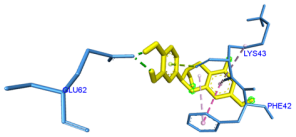


Figure 5. Visualization of interactions between catechin or native ligands and amino acid residues of pro-inflammatory proteins

simulation of catechin to HSP90α was compared to the binding of onalespib, an HSP90α inhibitor. Onalespib interacts with Asp93, a residue critical for ADP binding and ATP hydrolysis, which is essential for HSP90 activity [35]. However, catechin showed no interaction with Asp93, suggesting that its binding to HSP90α may not inhibit its activation.

Both HSP90α and HSP90β are implicated in inflammation but have distinct roles. HSP90α is involved in vascular inflammation [36], while HSP90β regulates NLRP3 inflammasome activity [37]. Interaction studies revealed that ATP binding to HSP90β involves residues such as Ile110, Leu107, Phe138, and Asp93 [38]. Catechin demonstrated interactions with key residues, including Leu107, Phe138, and Ala55, suggesting that catechin binding may inhibit HSP90β activity.

JAK2 activation promotes inflammation by upregulating pro-inflammatory pathways [39]. Catechin's binding to JAK2 was modeled based on the binding site of ruxolitinib, a JAK2 inhibitor. Ruxolitinib forms hydrogen bonds with Glu930 and Leu932 and engages in van der Waals (VDW) interactions with Leu855 and residues in the P-loop [40]. Catechin similarly interacts with Leu855, but no interaction was predicted with Glu930 or Leu932, suggesting that catechin may not effectively inhibit JAK2 ATP binding.

IL-2 is a cytokine that drives inflammation by stimulating T, B, and NK cell proliferation and activation [41]. Phe42 and Glu62 are critical residues for IL-2's interaction with its receptor IL-2Rα, while Leu72 acts as a gatekeeper residue requiring conformational changes for binding [42]. Catechin shares interactions with Phe42 and Glu62, similar to the native ligand FRB, indicating that catechin may inhibit IL-2 activity by preventing receptor interaction.

Molecular docking predicts that catechin has binding potential with several proteins, including AKT-1, COX-2, HSP90α, HSP90β, JAK2, and IL-2. However, amino acid interaction analysis using Biovia Discovery suggests that catechin binding may result in inhibition only for COX-2, HSP90β, and IL-2. While catechin's interaction with COX-2 supports findings from previous studies [15], this study reveals HSP90β and IL-2 as novel anti-inflammatory targets for catechin.

Catechin demonstrated negative binding energy and specific interactions with amino acid residues essential for the activity of COX-2, HSP90β, and IL-2, suggesting inhibitory potential. Notably, no HSP90β inhibitors are currently FDA-approved, though several are in clinical trials [43]. In contrast, COX-2 and IL-2 inhibitors, such as mefenamic acid and aldesleukin, are already FDA-approved for treating inflammatory conditions. Among these targets, catechin exhibited the lowest binding energy with IL-2, indicating it may be the most prominent anti-inflammatory target. However, catechin's binding affinity to these proteins was lower than that of native ligands, emphasizing the need for structural modification of catechin to enhance its efficacy.

Conclusion

This study reveals that catechin's anti-inflammatory activity may be mediated through interactions with AKT-1, COX-2, IL-2, HSP90β, and JAK2, with potential inhibitory effects on COX-2, IL-2, and HSP90β. Despite promising findings, catechin's binding affinity to these targets is lower than that of native ligands. Structural modification of catechin is recommended to optimize its anti-inflammatory efficacy, paving the way for future preclinical and clinical investigations.

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Author contributions

NYS: conceptualization, supervision, funding acquisition, writing – original draft, RF: supervision,

writing – original draft; BRS: investigation, writing – review & editing; F: investigation, writing – review & editing.

Declaration of interest

None.

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