In Silico Prediction of the Bioactive Profile and Metabolites of *Satureja nepeta* in Diseases Related to the Excessive Production of Interleukin-6

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ABSTRACT: Inflammatory bowel diseases are caused by an abnormal reaction of the immune system, which becomes hyperactive because the mechanisms responsible for regulating it get out of control. For an effective immune response, many proinflammatory cytokines are secreted, particularly interleukin-6 (IL-6) keystone cytokine inflammation. Many synthetic and natural compounds targeting IL-6 have been studied. The genus *Satureja* of the Lamiaceae family is generally known for its many virtues, in particular anti-inflammatory properties. However, the mechanism of action is unclear. This study aims to predict the impact of characterized bioactive molecules of Moroccan *Satureja nepeta* in the potential control of inflammatory response mediated by IL-6 cytokine. A list of 9 previously characterized natural compounds of *S. nepeta* was compiled, and a list of 6 potential protein targets involved in intestinal inflammation was made. The 2 lists of natural compound-target proteins were analyzed by the STITCH software (http://stitch.embl.de/) to develop protein-compound and protein-protein interaction networks (PPINs). An ontological enrichment (GO) analysis was performed by the Clue GO plugin to evaluate the PPIN generated by STITCH; finally, the molecular docking to predict the mode underlying the anti-inflammatory effects. STITCH results revealed direct and indirect interactions of *S. nepeta* chemical compounds with a key protein target IL-6. The array results by ClueGO showed that most compounds involved in the regulation of several biological processes related to IL-6 such as inflammation apoptosis, cell differentiation, and metabolic regulation. The targets directly related to IL-6 have been used for molecular docking. Quercetin, catechin, and gallic acid have a strong affinity with the IL-6 receptor (respectively −7.1; −6.1; −5.3). This study strongly suggests that the bioactive compounds of *S. nepeta* could constitute a new therapeutic alternative in the treatment of diseases related to IL-6. However, to validate the results obtained in this work, it is necessary to explore the mechanism of action of potential bioactive molecules by experimentation.

KEYWORDS: *Satureja nepeta*, bioactive compounds, intestinal inflammatory diseases, IL-6, IL-6 receptor antagonist

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Introduction

S. nepeta, an excellent medicinal plant used by the Moroccan population is very rich in phenolic compounds, flavonoids, and tannins;[1] it also has certain biological properties, such as antimicrobial and antioxidant activity. In addition, recent experiments have confirmed their potent antiproliferative effects on human tumor lines as well as anti-inflammatory properties.[1–3] Interleukin-6 (IL-6), also known as interferon β2, is a key cytokine in the regulation of acute and long-term inflammation, and it is produced in response to infections. This pleiotropic cytokine produced by a variety of cell types (fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, T and B lymphocytes) is the messenger between cells involved in the inflammation process.[4] The receptor complex that mediates IL-6's biological processes is made up of 2 membrane-bound glycoproteins: an 80 KDa receptor subunit (IL-6R, CD126) and a 130 KDa signal transducing element (gp130, CD130).[5] A gp130 transmembrane expression can be found in a variety of organs, including the heart, lungs, kidneys, liver, placenta, brain, and intestine.[6] The IL-6R, however, has a limited cellular distribution and is mostly expressed in hepatocytes and leukocyte subpopulations (monocytes, neutrophils, T cells, and B cells). The gp130 was first thought to be the signaling component of IL-6; however, it has been shown to be a signal transduction component of other proteins as well.[7] High levels of IL-6 could disrupt many physiological processes as well as the homeostasis of many cell types causing several diseases such as Crohn's disease, celiac disease, and irritable bowel syndrome.[4,7,8] The objective of this research is to analyze *in silico* the bioactive compounds of *S. nepeta*, which could affect the processes and the signaling pathways involved in IL-6.

Material and Methods

Chemical components of S. nepeta

The chemical compounds of *S. nepeta* are previously characterized by high performance liquid chromatography coupled to ultraviolet (HPLC-UV),[1] then identified in PubChem (https://pubchem.ncbi.nlm.nih.gov) and/or traditional Chinese

Table 1. Chemical composition of *S. nepeta*.

PUBCHEM CID	NAME	COMPOSITION (%)	MOLECULAR WEIGHT (G/MOL)	MOLECULAR FORMULA
370	Gallic acid	18.12	170.12	C7H6O5
8468	Vanillic acid	4.89	168.15	C8H8O4
9064	Catechin	23.25	290.27	C15H14O6
10742	Syringic acid	4.48	198.17	C9H10O5
445858	Ferulic acid	5.36	194	C10H10O4
5280343	Quercetin	21.14	448	C15H10O7
5280805	Rutin	7.23	610.5	C27H30O16
689043	Caffeic acid	6.21	180.16	C9H8O4
102261219	Caffeic	2.33	180	C9H8O4

Table 2. Information on the target of proteins associated with diseases.

DISEASE	PROTEIN NAME	UNIPROT ID
Irritable bowel syndrome	Gamma-aminobutyric acid type B	Q9UBS5
	5-hydroxytryptamine	P28335
	IL-6	P08887
Crohn's disease	Interleukin-6	P08887
	Interleukin-1	P27930
Coeliac disease	Interleukin-6	P08887
	Interleukin-18	O95256
	Interleukin-21	Q9HBE5

medicine systems pharmacology database and analysis platform (TCMSP) (http://lsp.nwu.edu.cn/tcmsp.php) databases (Table 1).

Inflammatory protein targets

Three inflammatory bowel pathologies were targeted and key proteins were determined and verified using the UniProt database (http://www.uniprot.org) forming a list of 6 potential targets (Table 2).

STITCH analysis

The active components of *S. nepeta* and the determined targets have been included in the STITCH 5.0 database (http://stitch.embl.de/) to classify them into direct and indirect interaction networks. The interaction network was used to evaluate the mode of action of *S. nepeta* and its pharmacodynamics components and to obtain information about the interactions between the active compounds and the protein targets.

ClueGO enrichment analysis

Ontological (GO) enrichment analysis was performed to hierarchize and identify the top functions of target proteins. ClueGO plugin of Cytoscape Software 3.8.2 was used to provide biological processes and molecular function as described by Merico et al.[9] The *P* value threshold was set at .05. Also, metabolic pathways involving target proteins and chemical compounds of *S. nepeta* were determined by KEGG pathway enrichment analysis.

For the ClueGO study, the *p* value threshold was set at .05. In addition, a 2-sided hypergeometric test with Bonferroni correction was used in this investigation, as well as an average network type option. The average network, in comparison to the global and comprehensive network types, shows GO words that originate at levels 4 to 8, with an average number of related genes and an average percentage of downloaded genes. To show the network's functionality, an organic layout approach was recently applied.

Molecular docking analysis

A molecular docking analysis with AutoDock Tools (ADT) version 1.5.6 was performed to obtain a closer insight into the interaction of major constituent of *S. nepeta* with IL-6 receptor as a protein target. This approach consists in studying the binding affinity and the type of interactions between 3 major compounds from *S. nepeta* (catechin, gallic acid, and quercetin) and the key protein IL-6R involved in inflammation as described by Nouadi et al.[10] The chemical structures were obtained from the PubChem database in SDF file and then converted to PDBQT format using Open Babel GUI (version 2.4.1). The crystal structure of IL-6 (id: NA2750) was downloaded in PDB format from Protein Data Bank (PDB) (http://www.rcsb.org). The preparation of the target protein was done by AutoDock Tools deleting all heteroatoms and water molecules surrounding the receptor to avoid the hindrance in docking calculations adding polar hydrogen atoms and assigning Kollman charges to compute pdbqt format. The grid size was set to $x = 90$, $y = 90$, $z = 90$, and grid spacing of 0.375 Å well the center of grid was set to $x = 2.668$, $y = -19.933$, and $z = 8.838$. The rest of the

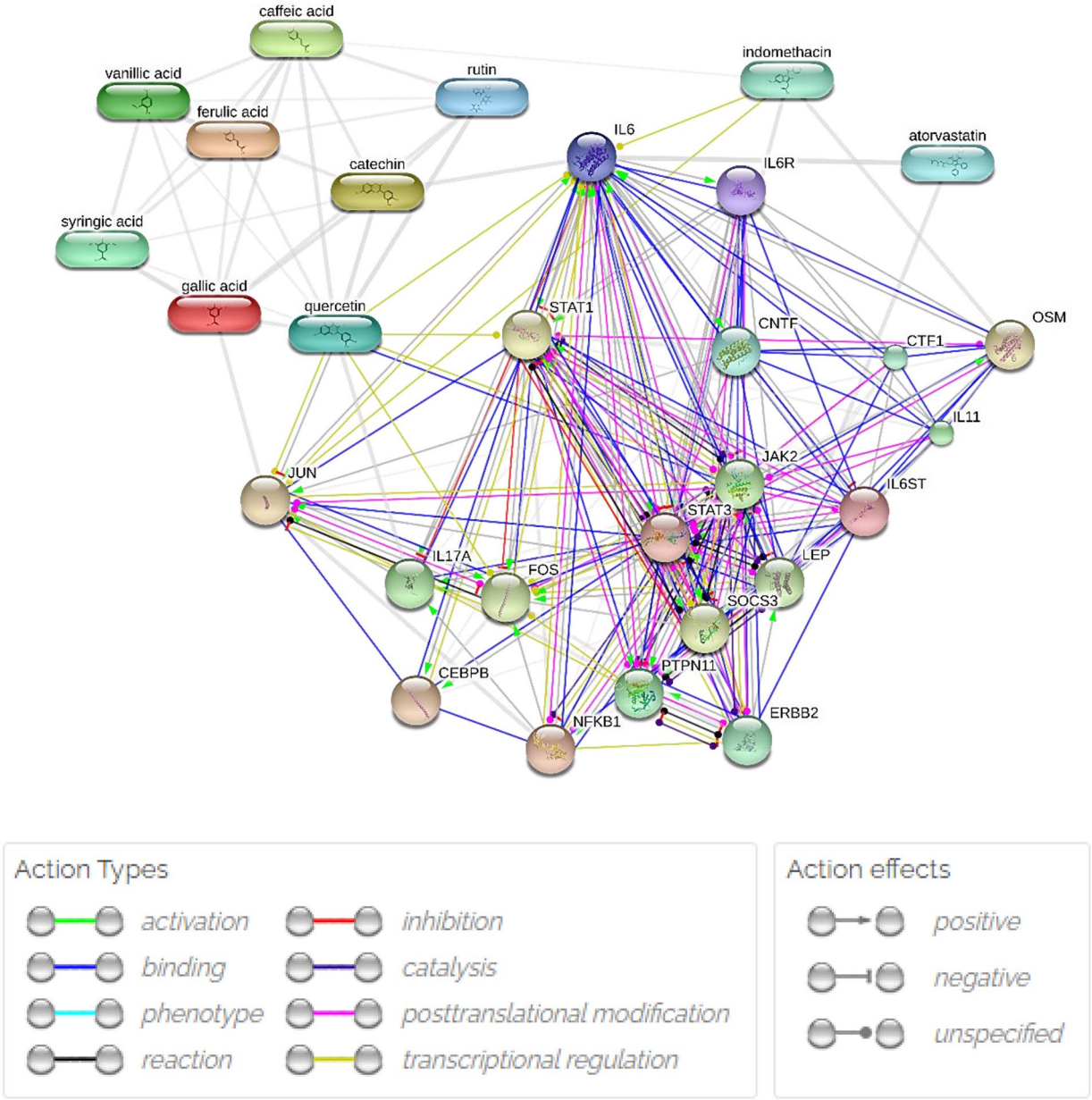


Figure 1. Network of protein-compound interactions predicted by STITCH, the molecular action of molecules-protein or between protein-protein indicates an activation or inhibition interaction.

docking parameters were kept as default, and postmodeling analysis was performed using Discovery Studio 2021 program (Dassault Systèmes BIOVIA Corp, San Diego).[10]

Results and Discussion

Recovery of chemical ingredients and their targets

A previous study characterized the phenolic compounds of *S. nepeta*, for the aqueous extract by HPLC-UV.[1] Among the various compounds characterized by HPLC-UV (rutin, quercetin, catechin, caffeic acid, vanillic acid, ferulic acid, and gallic acid), we found that quercetin, catechin, and gallic acid were the 3 major compounds representing more than 60% of the total composition (Table 1). These 3 compounds are known for their anti-inflammatory effect.[11–13] Moreover, targeted inflammatory bowel diseases in this study share IL-6 as a keystone inflammation protein (Table 2).

STITCH analysis

The protein-protein interaction network (PPIN) and chemical compounds of *S. nepeta*-protein network were constructed through STITCH and the STRING database to predict the interaction and determine the molecular action of these associations (Figure 1) and binding affinity (Figure 2).

The chemicals are represented as pills, the proteins as spheres, and the edges represent the types of interactions, and the number "degree" represents the strength of the activity, the

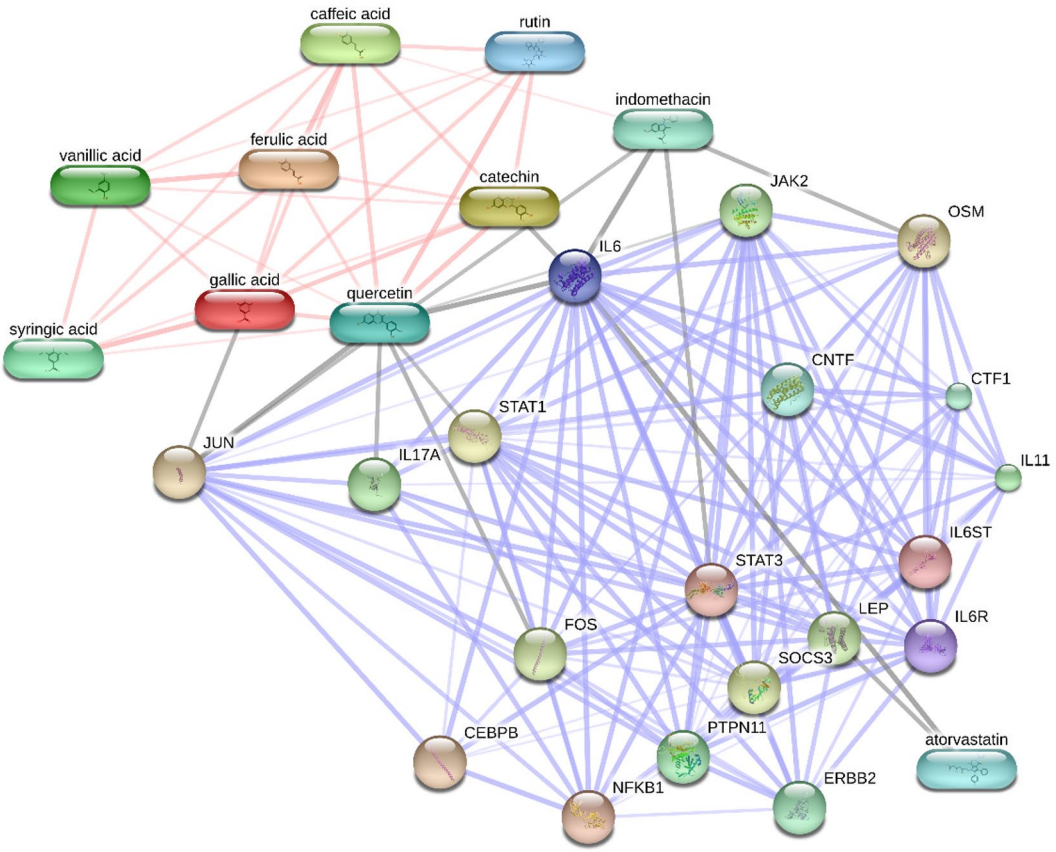


Figure 2. The network of interactions of *S. nepeta* bioactive molecules with protein targets predicted by STITCH 5.0. Molecules are represented by pill-shaped nodes, while proteins are represented by spheres. The nodes that are associated with each other are linked by an edge. The length of the line refers to the bond affinities with each other. Higher affinity means a shorter edge between chemicals and proteins and vice versa.

larger the node the higher the degree value, and the higher the degree value the more the color changes from light green to yellow. A medium probabilistic confidence level of 0.400 was used to build the PPIN (Figure 1).

The network consisted of 41 nodes and 383 edges. The nodes of the network act as representatives of the protein targets or their relevant genes. The lines connecting various nodes indicate the interaction between the corresponding proteins and the bioactive molecules of *S. nepeta*. The *P* value for PPIN enrichment was very significant (.001).

Protein-protein interaction networks revealed the presence of 2 types of interaction with IL-6: direct (catechin and quercetin) and indirect (rutin, ferulic acid and gallic acid).

Direct interactions

The direct interaction between quercetin-IL-6 may explain a possible inhibition of IL-6 functions by decreasing mRNA expression.[14] Quercetin has been shown to inhibit the expression of proinflammatory cytokines in the human mast cell lineage, by blocking the activation of p38, MAPK (mitogen-activated protein kinases), and nuclear transcription factor-kappa B.[14] Similarly, quercetin was shown to induce an inhibition of the immunologic release of inflammatory mediators such as histamine, tryptase as well as the production of cytokines, such as tumor necrosis factor (TNF)-a, IL-6, and IL-8 in cultured human mast cells.[15] Quercetin can also interact with proteins other than IL-6 (Figure 2) such as cytochrome P450 (CYP1A1) and Janus kinase 2 (JAK2). Janus kinase 2 is a nonreceptor tyrosine kinase that regulates cell proliferation, differentiation, and histone modifications. It mediates key signaling mechanisms in both innate and adaptive immunity.[16]

Catechin has an affinity with IL-6, thus regulating the expression of proinflammatory cytokines, such as IL-6 and IL-1β[17] (Figure 2). The PPINs revealed also that catechin could reduce lipopolysaccharide-stimulated inflammation by inhibiting the activation of NF-kB pathway.[18] Catechin shows anti-inflammatory activity in other pathologies by acting on the expression of IL-1 and IL-6 and their signaling pathways.[19]

Quercetin and catechin may reduce intestinal inflammation by regulating immune cell infiltration and proliferation as well as the deterioration of intestinal lesions.[20] In addition, quercetin and catechin have been shown to reduce intestinal inflammation by regulating proinflammatory cytokines (IL-6, TNF-) and inflammation-related signaling pathways, (NF-kB, MAPK, and activator of STAT1/3 transcription pathways).[20,21] Indeed, these 2 bioactive molecules have been shown to reduce inflammation in the gut by increasing the proliferation of intestinal

microbiota including *Bifidobacterium*, and *Lactobacillus* genera and phylum Bacteroidetes.[21,22]

Indirect interactions

The STITCH network shows indirect interaction of *S. nepeta* bioactive molecules with IL-6 (Figure 2). The rutin induces a decrease in IL-6 production through the inhibition of TNF, IL-1b, and iNOS (inducible nitric oxide synthase).[23] In addition to inflammatory bowel diseases, rutin exerts an effect on neurodegenerative diseases by inhibiting glial cell activation and attenuating the production of inflammatory cytokines. Thus, the ability to stimulate microglial proliferation and to induce microglial polarization suggests that it could be used as a potential alternative in the treatment or prevention of diseases.[23,24] Gallic acid interact with IL-6 through JUN which codes for Jun Proto-Oncogene, AP-1Transcription Factor Subunit protein which directly interacts with specific target DNA sequences to regulate gene expression.[25] The network also shows that all the bioactive compounds interact with each other, which strongly suggests a synergistic action of these compounds on the regulation of IL-6 (Figure 2). Polyphenols tend to complex, which increases their molecular weight, thus the interactions intensify. Indeed, this complexation of polyphenols increases the number of hydroxyl groups enhancing the polyphenol-polyphenol interaction and the polyphenol-target protein affinity.[14,26]

GO enrichment analysis

ClueGO database-mediated enrichment connects the GO database to Cytoscape, which is an important plug-in for the Cytoscape visual analysis software. ClueGO apps analysis allows to target product function from different databases. This app classifies and analyzes GO terms for biological processes, molecular functions and cellular components, as well as KEGG pathway enrichment ($P \leqslant .05$). This analysis predicts a potential mechanism of interaction between bioactive compounds and IL-6.

Our results suggest that the major components of the plant have direct (bioactive molecule-protein) and indirect (protein-protein) interactions with IL-6. The GO annotation for the identified proteins demonstrates their association with many biological processes, cellular components, and molecular functions (Figure 3). The target proteins are involved in different important functions, notably regulation of intestinal inflammation, positive regulation of tyrosine and STAT protein phosphorylation (Signal Transducers and Activators of Transcription) and cell and regulation of metabolism. The regulation of intestinal inflammation is mediated by signaling pathways such as the regulation of interferon-gamma response and the IL-6-mediated signaling pathway (Figure 3). These pathways are involved in the regulation of gastrointestinal inflammation by enhancing both innate and adaptive immune responses to maintain intestinal immunological homeostasis.[27]

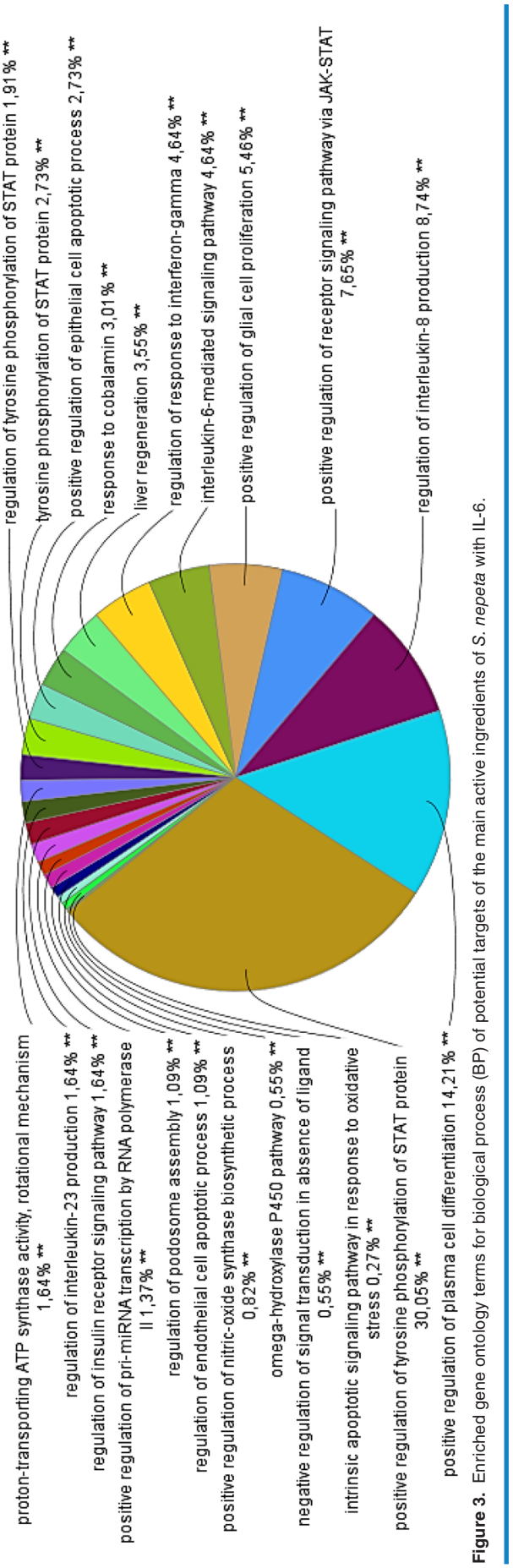
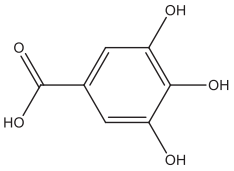
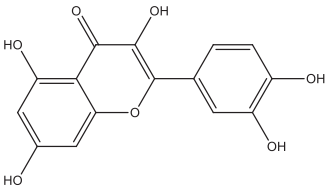
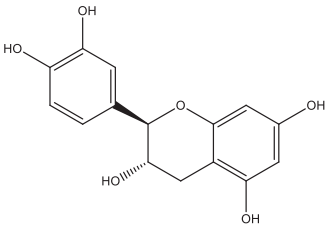


Figure 3. Enriched gene ontology terms for biological process (BP) of potential targets of the main active ingredients of *S. nepeta* with IL-6.

Table 3. Structure of predominant bioactive molecules of *S. nepeta* and docking score values of IL-6R ligand.

DOCKING SCORE (KCAL/MOL)			
COMPOUNDS	INFORMATION	CHEMICAL STRUCTURE	DOCKING SCORE (KCAL/MOL)
Gallic acid	MW: 170.12 g/mol MF: C7H6O5 H-bond donor: 4 H-bond acceptor: 5 PubChem ID: CID 811292		−5.3
Quercetin	MW: 302.23 g/mol MF: C15H10O7 H-bond donor: 5 H-bond acceptor: 7 PubChem ID: CID 5280343		−7.1
Catechin	MW: 290.27 g/mol MF: C15H14O6 H-bond donor: 5 H-bond acceptor: 6 PubChem ID: CID 9064		−6.1

H-bond, hydrogen bond; MF, molecular formula; MW, molecular weight; chemical structures were retrieved from PubChem database.

In fact, studies have demonstrated that the major active compounds of *S. nepeta* have significant therapeutic potential for inflammatory bowel diseases such as Crohn's disease and irritable bowel syndrome.[1,28]

Rutin and quercetin may be useful in the treatment of such diseases by regulating the expression of tight cell junction genes. These bioactive molecules improve the intestinal barrier function of the tight junction.[29]

Gallic acid has also shown anti-inflammatory effects at the gut level by directly inhibiting NF-kB expression via suppression of proinflammatory activators, such as iNOS and COX-2 (cyclooxygenase 2).[30] Overexpression of iNOS contributes to the appearance of early intestinal inflammation, which could lead to carcinogenesis of the colon. Similarly, overexpression of COX-2 induces ulcerative colitis.[30]

Quercetin has been shown to have anti-inflammatory effects, by inhibiting the JAK/STAT cascade.[31,32] It can decrease the expression of ICAM-1, IL-6, IL-8, and MCP-1 mRNAs. In addition, it could reduce the production of key proinflammatory mediators (prostaglandin E2) and of IL-1, IL-6, and TNF. At the same time, it could stimulate the secretion of IL-10 and tumor growth factor (TGF)-β.[33,34]

Catechin may have anticancer therapeutic potential due to its biological activities, including antioxidant, anti-inflammatory, and antitumor properties.[35]

The bioactive compounds, quercetin, gallic acid, and catechin have different biological activities modulating the expression of IL-6, and therefore impacting several metabolic pathways in different types of pathologies of inflammatory origin.[36–38]

Molecular docking study of identified compounds

To see the details proper mechanism by which the identified compounds of *S. nepeta* bind to IL-6R, we performed a molecular docking study. The docking results are shown in Table 3, while the interactions between the most active compounds and the targets are shown in Figure 4. Catechin, gallic acid, and quercetin representing the predominant compounds of *S. nepeta* are the most important active compounds which directly interact with IL-6R.

Docking results showed that quercetin fit perfectly into the IL-6R binding site with the lowest binding energy of −7.1 kcal/mol (Figure 4). In contrast, lower binding energy was obtained with catechin and gallic acid (−6.1 and −5.3 kcal/mol, respectively). The extracellular portion of the gp130 signal transducer consists of 6 domains. It has recently been shown that the 3 distal membrane domains bind to the IL-6/IL-6R complex.[39] A structural model of the IL-6/gp130 and IL-6R complex allowed to propose amino acid residues in these domains of gp130 interacting with IL-6 bound to its receptor.40 The amino acid residues of the IL-6 receptor involved in the interaction with IL-6 are located in the B′C′ loop (Val252) and in the F′G′ loop (Glu106, Lys150) with the score between -7.84 and -10.81 kcal/mol.[40]

According to Figure 4, quercetin forms 3 hydrogen bonds with ASP34 and ARG30. On the other hand, catechin interacts

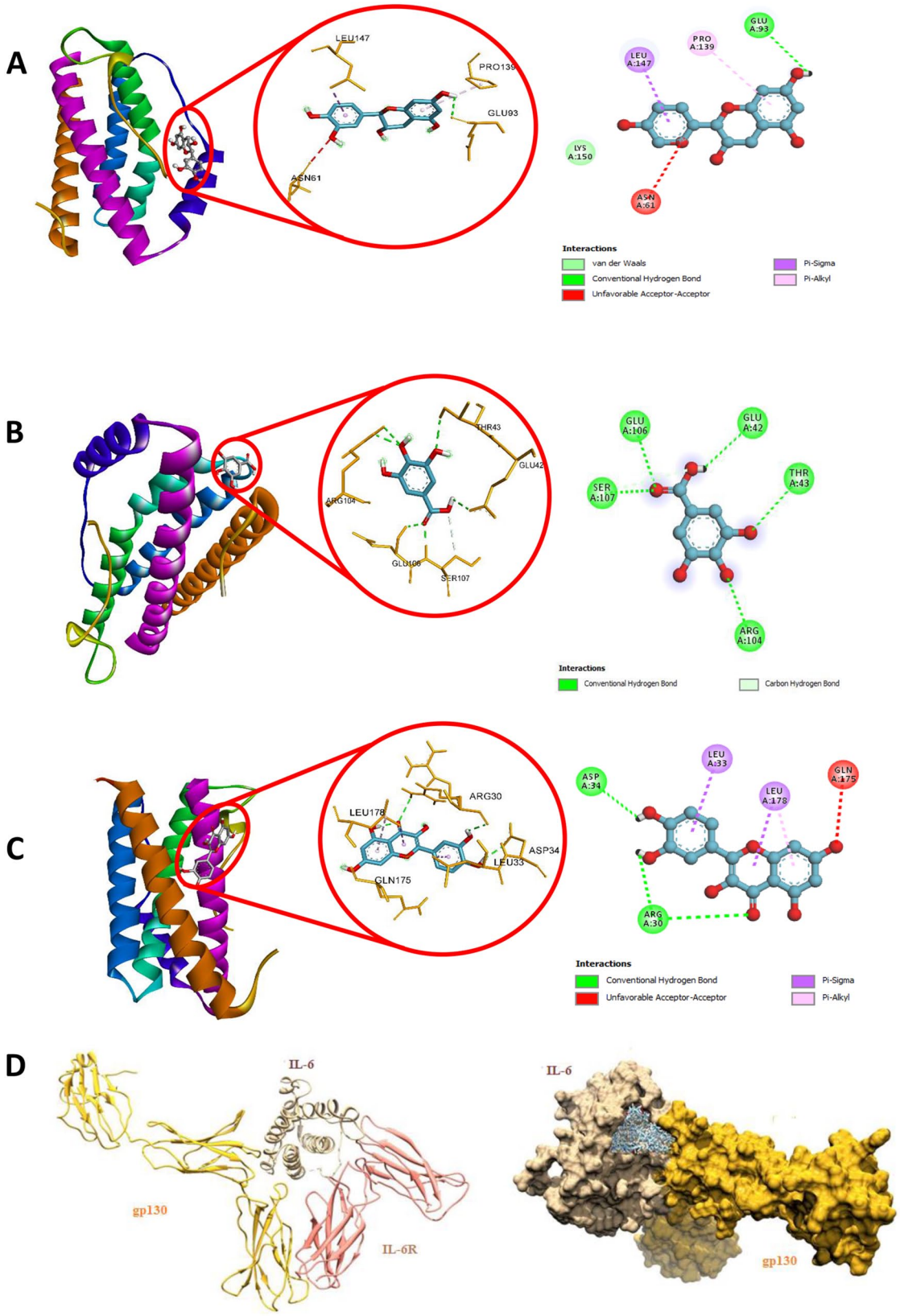


Figure 4. The 3D and 2D detailed view of the binding mode between catechin (A), gallic acid (B), quercetin (C), and IL6/gp130 with IL-6R (D).

with amino acid LYS150 and a hydrogen bond with GLU93 via Van der Waal forces. While gallic acid forms 5 hydrogen bonds (SER107, GLU106, GLU42, THR43 and ARG104) with IL-6. However, antagonistic action on the active site of IL-6R or masking of the IL-6 binding site by gallic acid, quercetin, and catechin could be possible mechanisms for the inhibition of

IL-6 action by *S. nepeta*.[41] A study has shown that quercetin could demonstrably bind to the IL-6R by partially (30%-35%) blocking IL-6 binding. It has been reported that the lys and glu residues provoke a complete loss of ligand binding to the IL-6R.[42] Therefore, the interaction between quercetin, catechin, gallic acid, and the IL-6R is likely to inhibit IL-6 ligand binding to the receptor by altering an affinity or protein conformation of the receptor.[42] The engagement of polyphenols with IL-6R is shown to control many biological processes including the activation of several intracellular signaling pathways JAK/STAT, regulation of insulin receptor signaling, regulation of epithelial cell apoptotic process, and the activation or inhibition of transcription factors.[43–45] Thus, blocking connections between IL-6 signaling pathways or the IL-6R could stop signaling that causes inflammatory diseases due to elevated IL-6 expression. This mechanism could constitute a therapeutic alternative for inflammatory bowel diseases.

Conclusion

The results have revealed that identified bioactive components of *S. nepeta* interact directly and indirectly with IL-6, respectively (quercetin, catechin, rutin, and gallic acid). These high-affinity interactions could confer on these bioactive molecules potent IL-6 inhibitory functions. Docking analysis showed that ligands (quercetin, catechin, and gallic acid) capable of establishing various bonds with IL-6R with high docking scores. Therefore, GO enrichment pathway analysis showed that the interactions of these compounds with IL-6 have a major role in several biological processes, suggesting that *S. nepeta* has the potential to manage intestinal diseases related to excessive IL-6 production. This semipredictive analysis, suggests a possible mechanism underlying the inhibition of intense IL-6 production by identified bioactive components of *S. nepeta*. Thus, an alternative treatment would be possible in pathologies linked to the excessive production of IL-6. The results of this study constitute a very interesting preliminary step in the process of being validated.

Author Contributions

All authors contributed conception and design of the study. Also, all authors contributed to the drafting of the manuscript.

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