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Original Research Article

In Silico Pharmacological study of the Immunomodulatory and Anti-Inflammatory Mechanism of C*arthamus tinctorius* (Kasumba Turate)

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ABSTRACT

The growing interest in natural products have encouraged the study of traditional medicinal plants as therapeutic candidates. *Carthamus tinctorius*, locally called Kasumba Turate in Makassar, Indonesia, has been used in Bugis–Makassar ethnomedicine. In addition to its traditional use, the plant is known to possess antiviral, antioxidant, anti-inflammatory, anti-allergic, and anticancer activities. Despite these reported benefits, its molecular mechanisms particularly those related to immunomodulation and inflammatory regulation are still not well clarified. This study applied a network pharmacology approach to explore the pharmacological basis of *C. tinctorius*. Bioactive compounds were identified from major phytochemical databases and validated via PubChem. Drug-likeness and pharmacokinetic properties were evaluated using SwissADME and ADMETlab 3.0. Potential targets were predicted through SwissTargetPrediction and SEA. PPI networks were generated using STRING-DB, and hub proteins were determined with CytoHubba in Cytoscape. KEGG pathway enrichment analysis was then conducted, focusing on immunomodulatory and inflammatory pathways. In silico screening yielded 73 drug-like molecules, with 39 showing favorable ADME profiles and potential for good oral bioavailability. Target prediction identified 136 proteins associated with immune regulation and inflammation, with key hubs including EGFR, TNF, NFKB, and AKT1. Enrichment analysis showed major involvement in chemokine signaling, PI3K–Akt, TNF, MAPK, and NK cell-mediated cytotoxicity pathways. These findings support the pharmacological justification for the traditional use of *C. tinctorius* and provide a scientific foundation for its advancement as a multi-target herbal immunomodulator. Further experimental and clinical investigations are needed to confirm the network-predicted interactions and assess its therapeutic potential.
potential.

Keywords: *Carthamus tinctorius, Kasumba turate, network pharmacology, immunomodulator, inflammation.*

Introduction

Public trust in safe therapies using natural ingredients has increased in recent decades. The demand for traditional plant-based medicines is growing due to the perception of low risk of side effects and the sustainability of natural resources. In that context, the exploration of traditional medicinal plants has become very important for the scientific discovery of effective bioactive compounds and their mechanisms of action[1]. *Carthamus tinctorius L*., which is known in the Makassar language as *Kasumba Turate*, is a traditional medicinal plant used by the Bugis-Makassar people to treat chickenpox.

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The plant has also been reported to show antiviral, antioxidant, anti-inflammatory, antiallergic, and anticancer activity[2,3]. Several studies have confirmed the antioxidant activity of *C. tinctorius*. *C. tinctorius* seed extract shows the potential for neutralising free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), as well as Ferric Reducing Antioxidant Power (FRAP). Other studies reported that the phenolic and flavonoid content of . *C. tinctorius* flowers have potent antioxidant activity as well as anti-inflammatory abilities in both in vitro and in vivo models[4,5]. Research on the anti-inflammatory and anti-pain effects of *C. tinctorius* also supports its traditional use. Selected hydroalcoholic extracts and flavonoids (e.g., kaempferol glycosides) showed reduced inflammation in the oedema model and pain test compared to standard controls such as aspirin[6].

The immunomodulatory activity of these plants has also been investigated. The polysaccharide fraction of *C. tinctorius leaves or flowers* increases the proliferation of spleen B cells and activates macrophages in in vitro studies. Antiviral activity of *C. tinctorius* has also been reported. This plant extract decreased the replication of the Kaposi's sarcoma-associated herpesvirus (KSHV) virus in in vitro studies[7]. In addition, the sesquiterpenoid compounds of safflower flowers show the ability to suppress oxidized-low density lipoprotein-induced (ox-LDL-induced) foam cell formation in RAW 264.7 cells and reduce the production of Lipopolysaccharide-induced nitric oxide (LPS-induced NO), which is a sign of anti-inflammatory activity

relevant to cardiovascular disease[8,9].
The chemical content of *C. tinctorius* has also been mapped in detail. The main components of the seed oil are fatty acids such as linoleic (about ~80%), oleic, and palmitic. HSYA (Hydroxysafflor yellow A) compounds, flavonoids, quercetin, rutin, kaempferol, and other phenolic compounds have been identified as active molecules involved in antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects[10,11].
The system biology approach in the study of medicinal plants offers a more holistic way to understand therapeutic effects. This approach combines classical pharmacology (in vitro/in vivo activity tests, doses, and toxicity) with pharmacodynamics (cellular and molecular mechanisms) and bioinformatics/bio-medical systems (regulatory networks, signalling pathways, and gene/protein targets). The combination forms the discipline of "pharmacological systems", which is capable of interpreting the working mechanisms of traditional medicine comprehensively[12].
Based on this scientific description, this study aims to interpret the mechanism of action of compounds sourced from ***C. tinctorius* as an** immunomodulatory **and** anti-inflammatory **therapeutic agent**, as well as its potential role against diseases related to immune dysfunction and chronic inflammation, such as viral infections, autoimmune disorders, or degenerative conditions. This research is vital because scientific evidence on local (traditional) uses, such as chickenpox treatment by the people of Makassar, has not been widely documented molecularly and systematically. Exploration through pharmacological systems can close the gap between traditional uses and modern clinical applications.

Materials and Methods

Compilation of C. tinctorius Bioactive Compounds Database
The bioactive constituents of *C. tinctorius* were compiled from established phytochemical databases, including Knapsack Version 1.200.03, by Nara Institute of Science and Technology, Japan; (https://www.knapsackfamily.com/KNApSAcK_Family/) and Dr. Duke's Phytochemical and Ethnobotanical Databases Last updated June 2025, by US Department of Agriculture (https://phytochem.nal.usda.gov/). Additional compounds were identified through a review of recent scientific literature to ensure a comprehensive and reliable dataset. All collected compounds were then validated and standardized in Simplified Molecular Input Line Entry System (SMILES) format using the PubChem database (National Library of Medicine; 8600 Rockville Pike, Bethesda, MD 20894). This process is carried out to ensure that the digital representation of bioactive compounds can be used in subsequent pharmacoinformatics analysis[13].

Bioactive Compound Screening
Screening was carried out to select bioactive compounds that have the potential to be drug candidates based on drug-likeness. The parameters used include Lipinski's Rule of Five, Veber's rule, and Muegge's criteria. Additional parameters such as molecular weight (MW), Caco-2 permeability, human intestinal absorption, ability to penetrate the blood-brain barrier (BBB), and toxicity (LD50) were also analysed to predict the pharmacokinetic properties and safety of the compound. The analysis was carried out using the SwissADME by the SIB Swiss Institute of Bioinformatics, 2025 version (http://www.swissadme.ch)[14] and ADMETlab 3.0 by Xiangya School of Pharmaceutical Sciences, Central South University, China (2020 version) (https://admetmesh.scbdd.com/) platforms which allows for an integrated assessment of compounds' pharmacokinetics, toxicity, and bioavailability[15].

Target Protein Fishing
The potential target proteins of the active compounds that pass the screening abovewere identified by the in silico approach. Target prediction was carried out using SwissTargetPrediction by the SIB Swiss Institute of Bioinformatics, 2025 version (http://www.swisstargetprediction.ch)[16] and Similarity Ensemble Approach (SEA) by the Shoichet Laboratory, Department of Pharmaceutical Chemistry, University of California, San Francisco (UCSF), 2007[17]. Both platforms allow mapping the relationship between the chemical structure of bioactive compounds and human target proteins through ligand similarity approaches and reported pharmacological interactions. The prediction results were then compiled and analysed to obtain protein candidates relevant to immunomodulatory and anti-inflammatory mechanisms.

Network Pharmacology Construction
The Protein–Protein Interaction (PPI) network was constructed using the STRING database by Academic Consortium (Swiss-Denmark, EURO) Version 12.0 (https://string-db.org)[18]. The resulting networks were then analysed using the Cytoscape version 3.9.1, specifically with the CytoHubba[19] plugin module to identify key proteins (hub proteins) that have a central role in biological regulation. In addition, a network of relationships between bioactive compounds and target proteins were also built to visualise the molecular linkages involved. Biological pathway enrichment analysis was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) by Kanehisa Laboratories, Japan, Version 2025[20]. With a focus on pathways relevant to inflammatory and immunomodulatory mechanisms.

Results and Discussion

Identification of Bioactive Phytochemicals in C. tinctorius
In this study, a total of 151 bioactive chemical constituents of *C. tinctorius* Linn. were identified by retrieving data from the KnapSack and Dr. Duke's Phytochemical Databases, supplemented with information gathered from recent international scientific literature. For clarity and ease of reference, the complete list of compounds obtained from *Carthamus* floral extracts was presented in the supplementary appendix, as the dataset is too extensive to be included in the main manuscript. The pharmacokinetics analysis of Absorption, Distribution, Metabolism and Excretion (ADME) of the compounds were predicted. ADME analysis employed *the SwissADME* (http://www.swissadme.ch/) and *ADMETlab* 3.0 (https://admetlab3.scbdd.com/) web-based webservers, which can be accessed and leveraged for predictive modeling of the physicochemical properties, pharmacokinetics, drug similarities, and medical chemical properties of a compound. The platform has a BOILED-Egg-based analysis approach to estimate absorption and permeability, iLOGP for lipophilicity evaluation, and Bioavailability Radar for an overall picture of molecular bioavailability. Both platforms facilitate the process of selecting potential molecular candidates by predicting critical parameters in drug development. The SwissADME platform http://www.swissadme.ch/ provide an intuitive and accessible web-based interface, facilitating the process of data input and interpretation of predictive results in a simple and efficient way for users from a wide range of skill backgrounds[15,21]. Based on the results of *the* ADME analysis, 73 compounds met the drug-likeness criteria set by Ghose, Veber, Lipinski, Muege, and Egan. The most widely used drug-likeness criteria standard is the Lipinski Rule of Five. The Lipinski Rule of Five provides a simple and efficient guideline for assessing the likelihood of success of a compound as an orally absorbable drug based on physicochemical parameters commonly found in successful oral drugs. Lipinski's Rule of Five includes (i) Molecular Weight <500, (ii) Lipohicity (MLogP) <5, (iii) H-Bond Acceptor <10, (iv) H-Bond Donors <5 and (v) Rotable Bonds <10; and, a maximum Lipinski rule violation of one [22]. Of the 72 compounds that met the drug-likeness criteria, 61 compounds were identified with a maximum violation of 1 of the Lipinski Rule of Five as described by bioavaviability radar.
The next analysis used was the BOILED-Egg analysis, which described the ability of the compounds to penetrate the blood brain barrier (BBB) and absorption in the gastrointestinal tract as a reference to determine whether the compound has the potential to be good as an oral drug[23]. BOILED-Egg Analysis (Figure 1.) shows that there are 39 compounds that have a high gastrointestinal tract absorption, most of which are derived from compounds in the flowers (Quinochalcones**:** Cartorimine; Flavonoids: Acacetin, Apigenin, Kaempferol, Quercetin, Scutellarein, Daphnoretin, Isorhamnetin, and Umbelliferone; Alkaloid: Adenine,

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Thymine, and Uracil; Organic Acid: p-coumaric acid, p-hydroxybenzoic acid, Succinic acid; Other components: Propionate, and Methylsyringin). Then, one compound in the leaf (Flavonoids: Luteolin) and the rest of the compounds in the seed (Alkaloids: N-(p-coumaroyl) serotonin, N-(p-coumaroyl) tryptamine, N-feruloylserotonin, N-feruloyltryptamine, Serotobenin; Organic acid: Caffeic acid, Ferulic acid, Myristic acid, Palmitic acid, Palmitoleic acid, Sinapic acid, Stearic acid, Linoleic acid, Linolenic acid, and Oleic acid; Other component: Arctigenin, Coniferyl alcohol, Matairesinol, Secoisolariciresinol, Sinapyl alcohol, and Trachelogenin) and 12 compounds (Umbelliferone, p-coumaric acid, p-hydroxybenzoic acid, N-(p-coumaroyl) tryptamine, N-feruloyltryptamine, Ferulic acid, Myristic acid, Palmitic acid, Palmitoleic acid, Arctigenin, Coniferyl alcohol, and Sinapyl alcohol) that can be absorbed through the *blood brain barrier*, which means they have a high probability value as drug candidates which means it has superior oral bioavailability. Then, there were 21 compounds (Adenosine, Uridine, Guanosine, N1, N5, N10-(E)-tri-p-coumaroylspermidine, N1, N5, N10-(Z)-tri-p-coumaroylspermidin, Safflospermidine A, Safflospermidine B, Roseoside, Sitosterol, Syringin, 4-[N-(p-coumaroyl)serotonin-4"-yl]-N-feruloylserotonin, 1-Tridecene-3,5,7,9,11-pentayne, 11Z-Trideca-1,11-diene-3,5,7,9-tetrayne, 11E-Trideca-1,11-diene-3,5,7,9-tetrayne, 3E-Trideca-1,3-diene-5,7,9,11-tetray, 3Z,11Z-Trideca-1,3,11-triene-5,7,9-triyne, 3Z,11E-Trideca-1,3,11-triene- 5,7,9-triyne, 3E,11E-Trideca-1,3,11-triene- 5,7,9-triyne, 3E,5Z,11E-Trideca-1,3,5,11-tetraene-7,9-diyne, 3Z,5E,11E-Trideca-1,3,5,11-tetraene-7,9-diyne, and 3E,5E,11E-Trideca-1,3,5,11-tetraene-7,9-diyne) with low gastrointestinal tract absorption indicating low levels of bioviability or poor druglikeness[24].

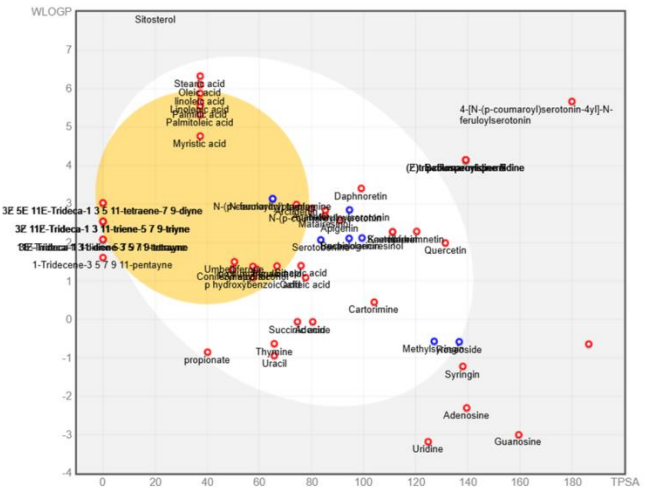

Figure 1: Boiled-Egg diagram showing the distribution of absorption of compounds in the body. The Gray Zone means that the compound is not absorbed by the body. The egg white zone means that the compound can be absorbed by the human intestinal absorption (HIA) and the yolk zone means that the compound can penetrate the Blood Brain Barrier (BBB) zone

Biological activity prediction

The next analysis was biological activity prediction of the 39 compounds contained in *C. tinctorius* flowers. The results of the analysis were carried out using the PASS Online software (Way2Drug; https://www.way2drug.com/passonline/)[25]. Figure 2 shows that the compounds contained in *C. tinctorius* flowers are correlated with a series of immunomudulatory/antiinflamation and antioxidant systems, and have a Pa (the probability that the compound will be active) of more than 0.7, consisting of Cartorimine (Alkenylglycerophosphocholine hydrolase inhibitor, Pa: 0.762; Chlordecone reductase inhibitor, Pa: 0.735), Precarthamin (Monophenol monooxygenase inhibitor, Pa: 0.956, and Anaphylatoxin receptor antagonist, Pa: 0.882). In addition, it relates to the analgesic, inflammatory and anticancer systems i.e. Saffloquinoside B (Vanilloid agonist Pa: 0.933; Antioxidant Pa: 0.825). With regards to drug metabolism and immunomodulation, antioxidants, and inhibition of cell proliferation – Kaempferol (CYP2A4 substrate and CYP2C substrate Pa: 0.714 and 0.716, respectively Anticarcinogenic, Pa: 0.715). Anti-inflammatory and antiproliferative in Quercetin (CYP1A inducer, Pa: 0.951; MAP kinase stimulant and kinase inhibitor, Pa: 0.933), Rutin (Hemostatic, Pa: 0.993**;** Cardioprotective, Pa: 0.988). Immunomudulator and anticancer in Scutellarein (Anaphylatoxin receptor antagonist, Pa: 0.961; HIF1A expression inhibitor, Pa: 0.946), activation of therapeutic, anticancer and antiinflammatory responses – Daphnoretin (CYP2C12 substrate and CYP2 substrates, Pa: 0.941 and 0.903, respectively; HIF1A expression inhibitor, Pa: 0.836), Isorhamnetin (NADPH oxidase inhibitor, Pa: 0.946; Kinase inhibitors and MAP kinase stimulants, Pa: 0.945 and 0.94, respectively). Detoxifying and anti-inflammatory, Umbelliferone (Chlordecone reductase inhibitor, Pa: 0.936; Membrane integrity agonist and Membrane permeability inhibitor, Pa: 0.937 and 0.798, respectively).

Target Fishing Protein, Construction of protein-protein, protein-ligand and disease-related signaling pathways

The 39 compounds from *C. tinctorius* flower that had been obtained, were investigated for their target proteins (CP) through target protein analysis analysis using the Swisstargetprediction (http://www.swisstargetprediction.ch/) platform and 403 target proteins (red circles) were obtained as seen in Figure 3a. Furthermore, the investigation for proteins related to inflammation (IP) was carried out through GeneCard (https://www.genecards.org) with the keyword "inflammation" led to a total of 16,474 proteins (green circle) and proteins related to immunomodulators (MP) with the keyword "immunomodulator" gave 1,682 proteins (blue circle). The proteins identified from the CP, MP, and IP groups were subsequently compared, and their overlapping members were determined through a Venn diagram analysis (https://bioinfogp.cnb.csic.es/alat/venny). The results of the analysis of the venny diagram (Figure 3a.) were 136 "inflammatory, immunomodulatory and target proteins of *C. tinctorius*". Furthermore, the 136 target proteins were constructed in a protein-protein interaction (PPI) network using String-DB (https://string-db.org/), and the visualization of the pharmacological network is presented in Figure 3c.

The subsequent analysis involved assessing protein centrality using the Cytoscape platform, supplemented with the CytoHubba plugin—specifically the Maximal Clique Centrality (MCC) algorithm—to identify the most central target proteins among the 136 previously constructed proteins. The obtained results were 15 proteins based on the MCC Cytohubba algorithm. These fifteen proteins were predicted to be effective inflammatory and immunomodulatory targets of the bioactive compound of *C. tinctorius*. Furthermore, the construction of 15 central proteins and active compounds of *C. tinctorius* flowers were carried out. Target-disease networks on the 15 central target proteins, were employed to construct all the central target proteins and its associated diseases[26]. The results of the analysis of Cytohubba using the MCC method of 15 central target proteins were presented in Figure 3b. The 15 target proteins are, EGFR (Epidermal growth factor receptor) , BCL2 (B-cell leukemia/lymphoma 2) , NFKB1 (nuclear factor-kappa B), HSP90AA1 (Heat shock protein 90kDa alpha (cytosolic), member A1), GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), ESR1 (Estrogen receptor 1), MTOR (mammalian target of rapamycin), TNF (Tumor necrosis factor), MMP9 (Matrix metalloproteinase-9), AKT1 (AKT Serine/Threonine Kinase 1), MMP2 (Matrix metalloproteinase-9), MAPK1 (Mitogen-activated protein kinase 1), PPARG (Peroxisome proliferator-activated receptor gamma), PTGS2 (Prostaglandin-endoperoxide synthase 2) and SRC (Proto-oncogene tyrosine-protein kinase).

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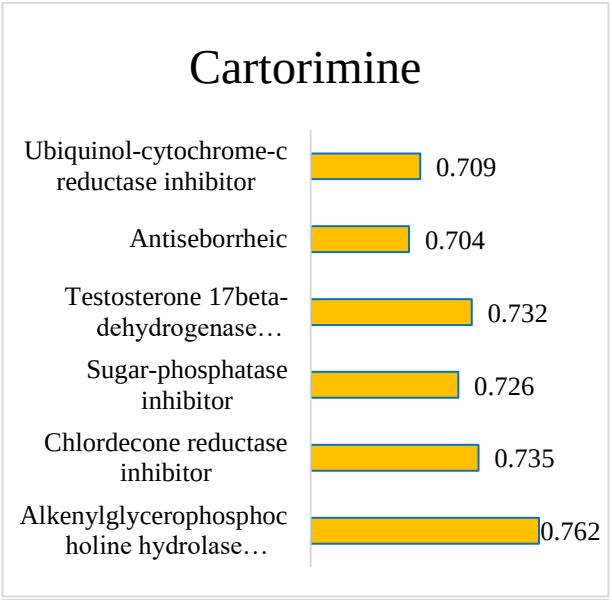

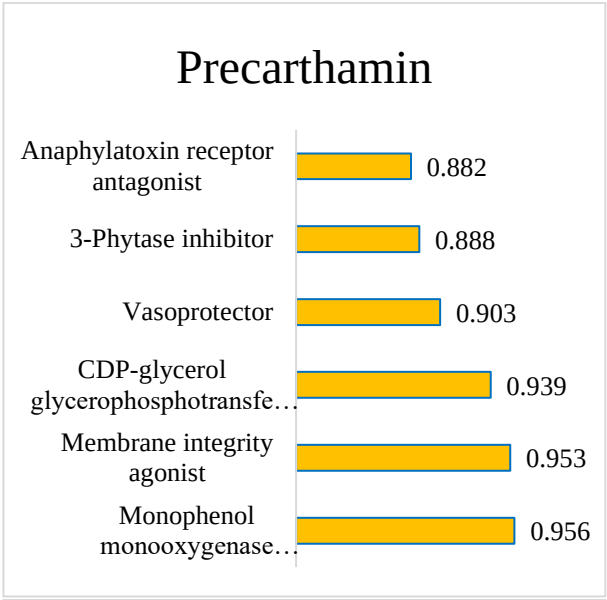

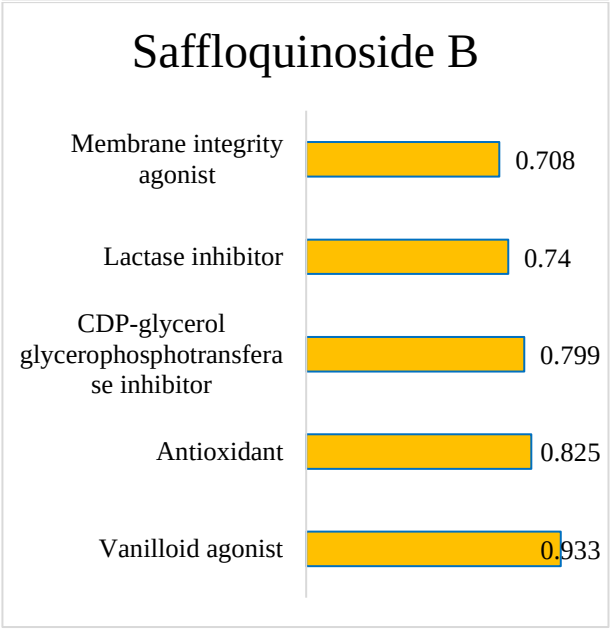

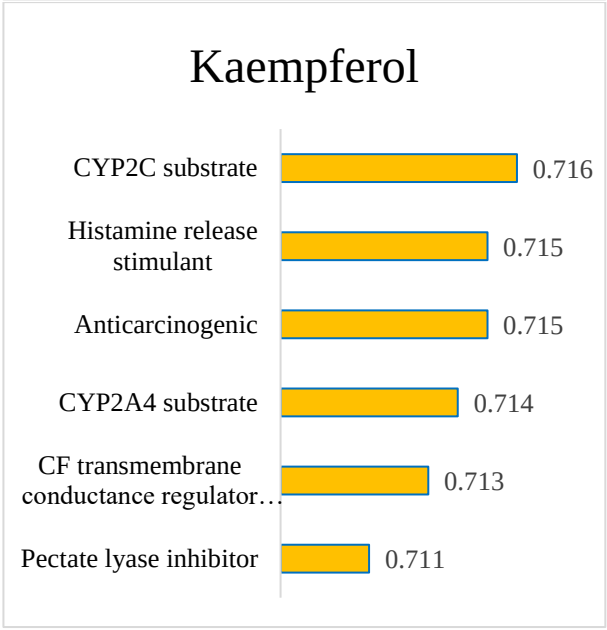

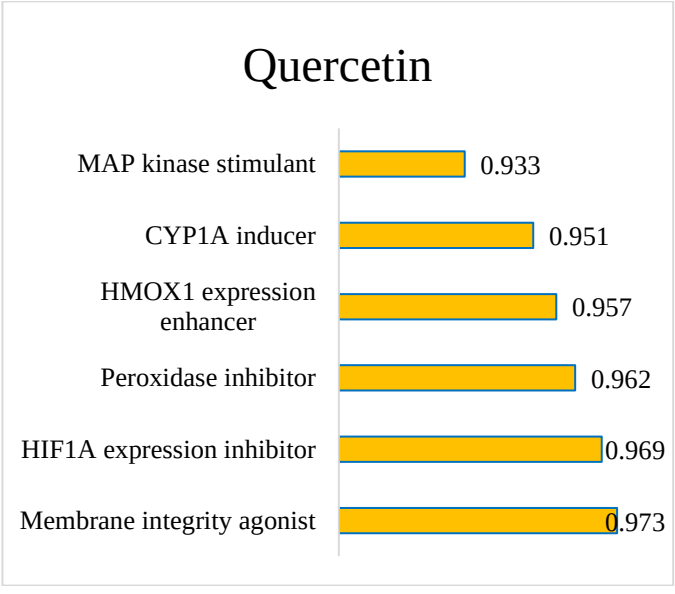

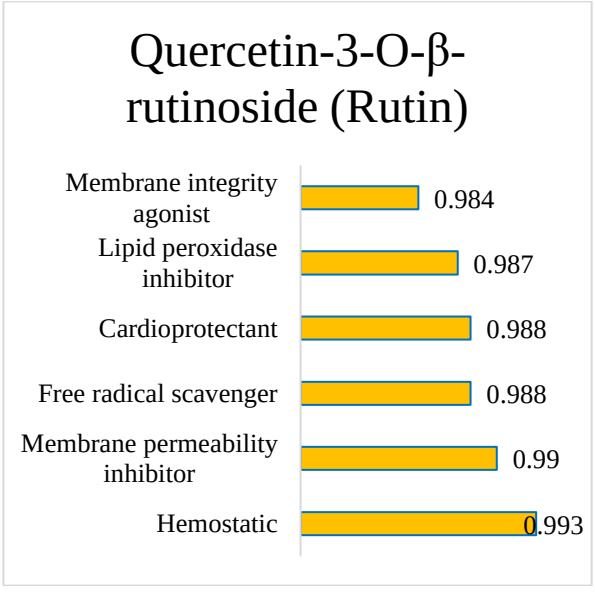

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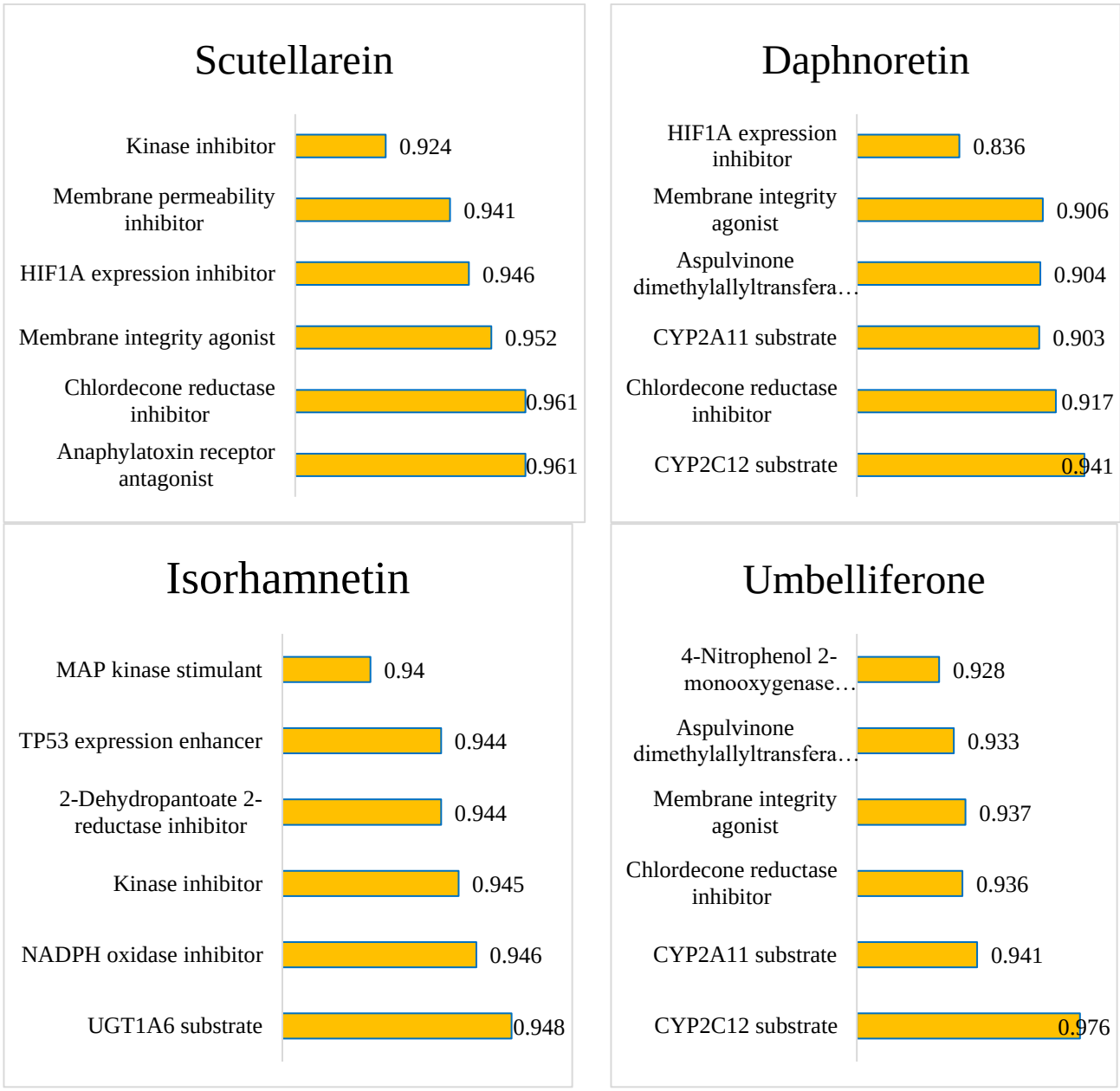

Figure 2: Biological activity prediction percent (Pa), on **39 compounds** contained in *C. tinctorius* flowers. using the analysis of the PASS Software Online (Way2Drug)

The interaction of the active compounds of *C. tinctorius* flower with the 15 target proteins were displayed in the 3d image. The compounds that interacted the most with the 15 target proteins were flavonoid compounds (i.e. Apigenin, Kaempferol and Scutellarrein with 7 interactions). Then, the target protein with the most interactions with the active compounds of *C. tinctorius* flowers is EGFR with 15 interactions.

Based on Figure 4, the results of the analysis of 136 proteins associated with inflammation, immunomodulators and interaction with 39 *C. tinctorius* compounds were found to have 20 pathways. These pathways were obtained from the analysis of interactions between proteins using the string-DB platform and was enriched using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database (https://www.genome.jp/kegg/). Data visualization using the SRPLOT (https://www.bioinformatics.com.cn) platform.

Based on Figure 4, the top five pathways associated with inflammatory activity, immunomodulatory and biological target of compounds of *C. tinctorius* are Chemokin signalling pathway, PI3K-Akt signalling pathway, C-type lectin receptor signalling pathway, TNF signalling pathway, Natural Killer cell mediated cytotoxicity, and MAPK signalling pathway. These pathways were further simplified in Figure 5. The results of the imaging showed several target proteins of *C tinctorius* that are potential targets of its bioactive compounds including JAK2/3 and SRC in the Chemokin signalling pathway, AKT and NFKB in the TNFa signalling pathway, SYK in MAPK signalling pathway, RAF1 C-type lectin receptor signalling pathway, DAP in the Natural Killer cell mediated cytotoxicity pathway.

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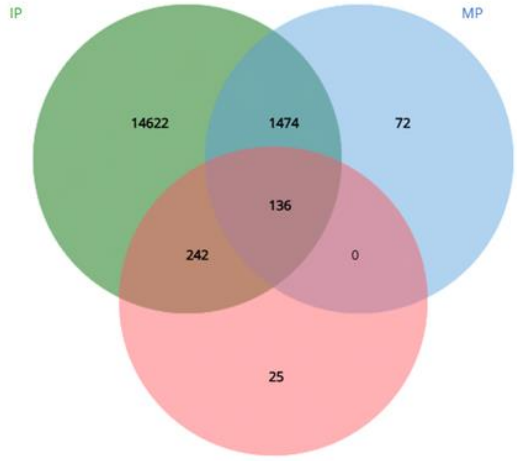

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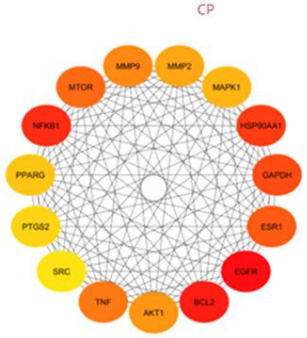
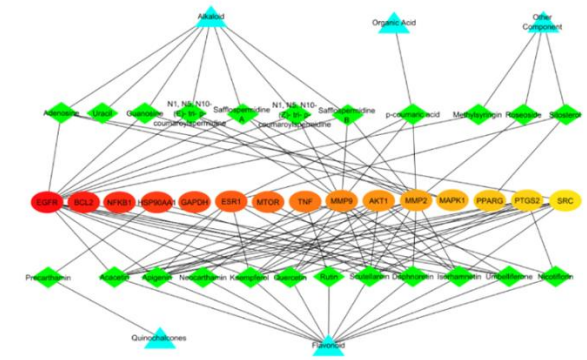


Rank	Protein	Score
1	EGFR	2.93993429429489E+33
2	BCL2	2.93993412297689E+33
3	NFKB1	2.93992941582330E+33
4	HSP90AA1	2.93990929053137E+33
5	GAPDH	2.93988750111091E+32
6	ESR1	2.93941630596875E+33
7	MTOR	2.93894239485149E+33
8	TNF	2.93753213311119E+33
9	MMP9	2.93679252287820E+33
10	AKT1	2.93593774493756E+32
11	MMP2	2.91610803664061E+33
12	MAPK1	2.91388378947384E+33
13	PPARG	2.91308132137134E+33
14	PTGS2	2.90468665543954E+32
15	SRC	2.87162931245076E+34

b



c

Figure 3: Results of tracing proteins related to Inflammation, immunomodulators and target proteins of bioactive compounds of *C. tinctorius.* Figure 3a shows a venny diagram of the inflammation-related proteins (IP), immunomodulatory proteins (MP) and target proteins of the bioactive compounds *C. tinctorius* (CP). 3b shows the 15 most central proteins of the 136 proteins through Cytohubba analysis using the Maximum Clicque Centralities (MCC) approach. 3d. Shows the interaction network of 15 proteins with the bioactive compound *C. tinctorius*

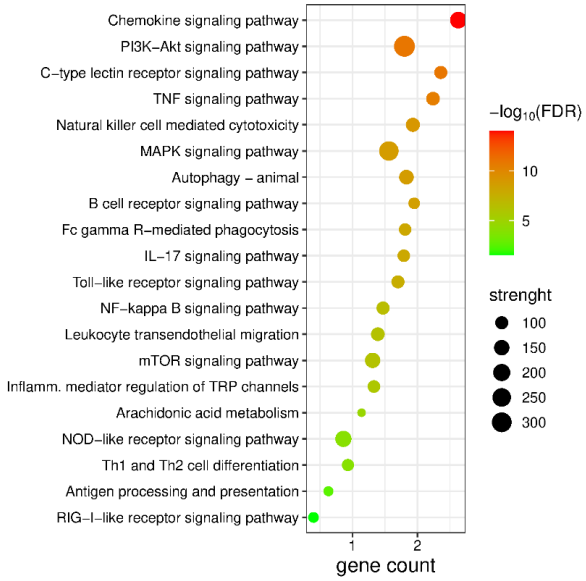


Figure 4: The results of the analysis of 136 proteins involved in the pathway related to inflammation, immunomodulators and included in the target proteins of 39 *C. tinctorius* compounds.

The in silico results showed that of the 151 bioactive compounds of *C. tinctorius* Linn. Identified, 73 compounds met the drug-likeness criteria. Meeting these criteria is important because rule-meeting compounds such as Lipinski, Veber, and Muegge tend to have better pharmacokinetic and pharmacodynamic properties in preclinical trials. The drug-likeness criterion has been proven in many studies to be an effective initial filter for setting aside compounds with low potency in terms of absorption, distribution, metabolism, excretion, and toxicity (ADMET) prior to further laboratory testing.

The BOILED-Egg analysis identified 39 compounds from flowers with high gastrointestinal absorption, one compound from leaves, and several compounds from seeds that showed the capacity to break through the blood-brain barrier (BBB). The research herein found that compounds such as Umbelliferone, p-coumaric acid, Ferulic acid, Myristic acid, Palmitic acid, Palmitoleic acid, Arctigenin, Coniferyl alcohol, and Sinapyl alcohol were able to penetrate BBB suggests which suggest that these compounds have superior oral bioavailability and potential for systemic effects involving central tissues or neuromodulators. The potential for BBB penetration supports the possible use of the compound in neurological diseases or brain inflammation, as reported in a safflower seed-related study that corrected memory deficits in scopolamine models through antioxidant and oxidative stress inhibition mechanisms[27].

Predicting biological activity using PASSOnline for flower compounds with a Pa > of 0.7 indicates a high probability that these compounds will have immunomodulatory, anti-inflammatory, antioxidant, analgesic, and anticancer activities. For example, Cartorimine and Precarthamin exhibit inhibitory activity against enzymes and receptors related to inflammation and allergies. Compounds such as Quercetin, Scutellarein, Isorhamnetin, and Umbelliferone show potential modulation of cellular pathways such as MAP kinase, NADPH oxidase, and cell proliferation regulators. This is consistent with reports that flavonoid compounds from *C. tinctorius* can suppress inflammatory cytokine expression, inhibit iNOS and COX-2 activity [1], as well as activate antioxidant pathways such as Nrf2[28].

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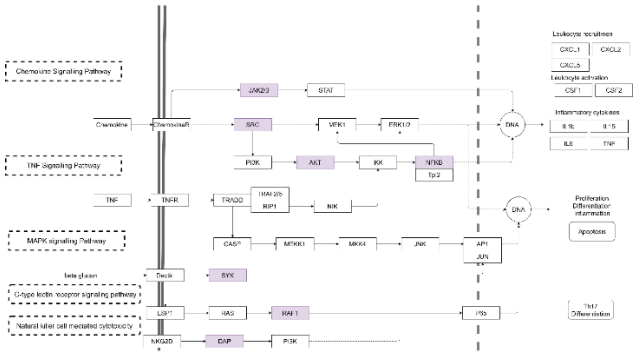

Figure 5: Distribution of *C. tinctorius* protein targets in several immunomodulatory and inflammatory related pathways. Purple nodes indicate potential targets.

Target fishing analysis found 403 potential target proteins for compounds present in the flowers of *C. tinctorius*, and the intersection with the database of inflammatory and immunomodulatory proteins resulted in 136 proteins that were at the intersection of the three domains. The PPI network of 136 nodes and 1746 edges showed that compounds from safflower flower have complex molecular interactions with many proteins that have a high average node degree (~25.7), indicating that the compound's mechanism of action is multifactorial and likely has pleiotropic effects on various biological pathways[29]. Identification of 15 central proteins (hub proteins) specifically EGFR, BCL2, NFKB1, HSP90AA1, GAPDH, ESR1, MTOR, TNF, MMP9, AKT1, MMP2, MAPK1, PPARG, PTGS2, and SRC suggests that the active compounds *of C. tinctorius* flowers are likely to modulate well-known pathways in inflammatory mechanisms, cell proliferation, apoptosis, migration/metastasis, and immune regulation[30].

Pathway analysis based on KEGG showed that the five main pathways associated were Chemokine signalling, PI3K-Akt signalling, C-type Lectin receptor signalling, TNF signalling, Natural Killer cell mediated cytotoxicity, and MAPK signalling pathway. The PI3K-Akt and TNF pathways are specifically often associated with the regulation of proinflammatory cytokine products (such as TNF-α, and IL-6), immune cell overactivation, and the formation of chronic inflammatory responses. Many studies have shown that extracts or compounds from *C. tinctorius* may inhibit the PI3K/Akt/mTOR pathway in the context of liver fibrosis, cancer, or systemic inflammation[31]. For example, *C. tinctorius* was reported to inhibit fibrotic cell activation and PI3K/Akt/mTOR pathways in liver fibrosis studies[32].

Compounds that affect AKT and NFKB as targets (such as Quercetin, Kaempferol, Scutellarein, Daphnoretin, and Isorhamnetin) show that the *in silico* predicted immunomodulation and anti-inflammatory are consistent with preclinical test results that showed decreased proinflammatory cytokine expression, decreased edema, and inhibition of NFKB and iNOS/COX-2. For example, in vivo studies showed that safflower flower extract lowered edema and levels of TNF-α and IL-6 in a mouse model with induction of inflammatory agents Complete Freund's Adjuvant (CFA)[33,34].

The relationship between compounds and the ability to penetrate BBB also offers numerous advantages. Many modern drugs have to cross the BBB for diseases involving the nervous system, while many plant compounds fail at the distribution stage because they are unable to penetrate the BBB. The findings of the research herein suggest that compounds such as Umbelliferone and some phenolic acids and lipid compounds from *C. tinctorius* seeds have a high probability of BBB penetration which indicate therapeutic opportunities for neurological diseases, such as dementia or neuroinflammatory disorders[35].

From the overall data, bioactive compound sourced from *C. tinctorius* showed pharmacokinetic attributes, a biological activity profile, and molecular network interactions that support immunomodulatory and anti-inflammatory activity. These findings confirm its traditional use as a cure for chickenpox or any inflammatory-related disease by the Bugis-Makassar people has a molecular correlation that can be explained scientifically.

Conclusion

This study provides comprehensive network pharmacology evidence that *C. tinctorius* (Kasumba Turate) contains multiple bioactive compounds with strong immunomodulatory and anti-inflammatory potential. In silico screening identified 73 drug-like molecules, of which 39 demonstrated favorable ADME properties and several showed blood–brain barrier permeability, suggesting high oral bioavailability. Target fishing revealed 136 intersecting proteins linking *C. tinctorius* compounds with immune regulation and inflammation, highlighting central nodes such as EGFR, TNF, NFKB, and AKT1. Pathway enrichment further indicated that these targets are primarily involved in chemokine signalling, PI3K–Akt, TNF, MAPK, and NK cell-mediated cytotoxicity pathways—key regulators of inflammation and immune responses. Collectively, these findings reinforce the pharmacological rationale for the traditional use of *C. tinctorius* and establish a scientific basis for its development as a multi-target herbal immunomodulator. Future experimental and clinical studies are warranted to validate these network-predicted interactions and explore therapeutic applications

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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