

Leucas aspera: A computational approach to manage co-occurring Autoimmune diseases

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Abstract: Autoimmune diseases such as Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Psoriasis (PS), Multiple Sclerosis (MS), and Sjogren's Syndrome (SS) occur when the immune system inflicts damage to the body in response to a trigger. Although each disease affects different areas of the human anatomy, they share common communities in their inflammatory and immunological pathways, with the involvement of proteins such as mitogen-activated protein kinase 1 (MAPK1), RAC-alpha serine/threonine-protein kinase (AKT1), and epidermal growth factor receptor (EGFR). The current use of anti-inflammatory and immunomodulatory treatments is associated with significant side effects. Thus, the growing interest in traditional medicine and ethnobiology has directed our attention to *Leucas aspera*, which is utilized in Ayurvedic and Chinese medicinal formulations for treating arthritis and psoriasis, suggesting the presence of bioactive phytochemicals capable of targeting critical pathways to mitigate their effects. This study conducted an *in-depth* analysis of bioactive phytochemicals from *Leucas aspera* and performed molecular docking, testing 71 plant-derived compounds against over 350 autoimmune-related gene targets found in selective autoimmune diseases. The most promising compounds were filtered and further examined for their interactions with major hub targets from network analysis by performing molecular dynamics simulations, underscoring their potential as attractive candidates. This study's results provide a novel perspective on *Leucas aspera* in improving treatment protocols for various autoimmune diseases, minimizing adverse effects while increasing efficacy.

INTRODUCTION

Several natural product sources from plants that have been used for centuries in medical practices such as Ayurveda and Traditional Chinese Medicine contain potential therapeutic properties that form the foundation of current research into their medical uses (Moudgil & Venkatesha, 2022). Various studies have demonstrated the effectiveness and efficacy of plant-based remedies in managing autoimmune conditions, highlighting their immunosuppressive and anti-inflammatory properties. Herbal medicines have been shown to exert beneficial effects in autoimmune disorders by modulating immune cell activity and reducing the levels of

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inflammatory markers (Di Sotto *et al.*, 2020). Ethnopharmacology has been crucial for identifying natural compounds to treat chronic inflammatory diseases like rheumatoid arthritis and lupus (Moudgil & Venkatesha, 2023). One such plant is *Leucas aspera* that stands out for its diverse medicinal properties and potential role in managing autoimmune inflammation (Enjamoori *et al.*, 2019). Belonging to the Lamiaceae family, which grows abundantly in areas of tropical and subtropical climate conditions across Asia and Africa, this plant has been used by the tribals from India and Bangladesh for treating diseases due to its efficiency in anti-inflammatory, analgesic, and anti-microbial properties (Nirmala & Kanchana, 2018). This plant is famous for its lanceolate leaves and small white flowers in dense spikes and has a vast ethnobotanical background (Enjamoori *et al.*, 2019). Conventionally, this plant has been used as an antipyretic and insecticide, with the entire plant also being used for treatments of bites, psoriasis, rheumatism, skin eruptions, and snake bites (Enjamoori *et al.*, 2019). Extracts from *Leucas aspera* are immunostimulatory, suggesting its promise for further exploration in treating immunosuppressive conditions (Nirmala & Kanchana, 2018). *Leucas aspera* contains flavonoids and polyphenols, which are phytochemicals that have been shown to possess antioxidant and anti-inflammatory properties. These compounds are involved in countering oxidative stress and inflammation, which are two key aspects of autoimmune diseases (Nirmala & Kanchana, 2018). *Leucas aspera*'s demonstrated efficacy regarding its immunological effects and its capacity to lessen inflammation make it a potential candidate for the development of new therapies for autoimmune diseases.

Autoimmune diseases are chronic inflammatory diseases whereby the body's own tissues are targeted by the immune system, leading to their dysfunction (Song *et al.*, 2025). Autoimmune disorders are becoming more common worldwide, with recent research showing that around 7.6% to 9.4% of people may be affected. These numbers underscore a notable increase, with annual rates estimated to rise by 19.1% in new cases and 12.5% in overall prevalence across various regions and populations (Miller, 2023). These conditions can be managed with the use of anti-inflammatory and immunomodulatory medications (Song *et al.*, 2025). Systemic autoimmune diseases (SADs) are a subset of autoimmune diseases that are complex, chronic, and associated with inflammation affecting the whole body. This type of autoimmune disease includes SLE and systemic sclerosis, where immune dysfunction produces autoantibodies, which infiltrate several autoantigens in multiple organs. These diseases often present with fluctuating symptoms that can complicate diagnosis and management, as well as result in fatal complications, including kidney and lung disease, and therefore remain one of the most significant causes of morbidity and mortality in the general population (Shi *et al.*, 2013). The idea that autoimmune diseases have shared (common) characteristics is aptly illustrated by their tendency to co-occur in a patient or in close family members, either as coexistences or overlaps. Epidemiological research on the co-occurrence of autoimmune diseases has contributed to our understanding of autoimmunity. A predisposition to develop additional autoimmune diseases is observed in about 25 percent of patients with an initial diagnosis of an autoimmune disease (Masood *et al.*, 2014). The phenomenon of the co-occurrence of three or more autoimmune diseases is termed as Multiple Autoimmune Syndrome (MAS) (Masood *et al.*, 2014). In this study, the diseases selected are Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), Psoriasis, and Sjogren's Syndrome (SS) due to the findings stating the co-occurrence of these autoimmune diseases. Type 3 MAS groups together Sjögren's syndrome, systemic lupus erythematosus (SLE), and occasionally Multiple Sclerosis (MS) (Masood *et al.*, 2014). Furthermore, psoriasis and SLE may develop or be exacerbated as a result of therapies for RA (Masood *et al.*, 2014).

Autoimmune diseases can be described as multifactorial processes, which are mediated through genetic, environmental, and immunologic factors that result in the attack of the immune system on the self-antigens (Shi *et al.*, 2013). This breakdown may occur at the central level; for example, immature lymphocytes that recognize self-antigen tissue are eliminated within the thymus and bone marrow, whereas the peripheral level also involves mechanisms that control

mature self-reactive lymphocytes through anergy and suppression by induced Treg cells (Ahsan, 2017). Strong positive associations of particular alleles of the Major Histocompatibility Complex (MHC) to specific autoimmune diseases have been firmly established. An autoimmune response can also be caused, triggered, or worsened by environmental factors, for instance, infection (Ahsan, 2017). Such signals may cause molecular mimicry, or an attack on self-antigens due to cross-reactivity with a pathogen or through abnormal presentation of self-antigens, activation of self-reacting T and B cells (Ahsan, 2017). Despite variations in clinical manifestation, dysregulated signaling often converges on major molecular hubs like mitogen-activated protein kinase 1 (MAPK1), RAC-alpha serine/threonine-protein kinase (AKT1), and epidermal growth factor receptor (EGFR) across autoimmune diseases, revealing similar immune-inflammatory circuitry. These proteins participate in common pathways governing cytokine production, lymphocyte activation, and tissue-damage responses, which explains the overlapping of immunopathological features. This repeated implication emphasizes their role as central community nodes within larger networks and makes them appealing targets for multi-disease therapeutics.

Advancements in computational approaches, such as molecular docking, enable the exploration of interactions between the bioactive constituents of *Leucas aspera* and key molecular targets implicated in autoimmune inflammation (Khan *et al.*, 2022), while molecular dynamics simulation sheds light on the stability and dynamic behavior of the ligand-target complexes, thus providing deeper insight into therapeutic potential (Khan *et al.*, 2022). This integrative approach not only improves our understanding of the therapeutic benefits of traditional medicine but also helps to discover new leads that might be developed into useful drugs. The combination of indigenous knowledge and contemporary scientific approaches could give rise to novel ways of tackling autoimmune diseases and enhancing the results of the therapy (Moudgil & Venkatesha, 2023). This study seeks to identify promising bioactive phytochemicals that can accomplish this by potentially targeting key targets of autoimmune diseases, providing the framework for new drugs to be developed aimed at managing the condition.

METHOD

Ligand Database Preparation

The compounds for the study were identified from literature mining and from the Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT) database (<https://cb.imsc.res.in/imppat/>) (Mohanraj *et al.*, 2018; Vivek-Ananth *et al.*, 2023). These compounds were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) (Kim *et al.*, 2023) and downloaded as a three-dimensional (3D) representation in the SDF structural format (SDF). For the ligands without a 3D structure in the PubChem database, the two-dimensional (2D) representation in the SDF structural format (SDF) was downloaded. The structures of the top compounds were further drawn using the ChemDraw tool by Revvity Signals Software (<https://revvitysignals.com/products/research/chemdraw>).

Pharmacological analysis

The pharmacokinetic behaviors and toxicokinetic parameters of the selected compounds were assessed using Swiss ADME (<http://www.swissadme.ch/>) (Daina *et al.*, 2017) and pkCSM, a pharmacokinetics software (<https://biosig.lab.uq.edu.au/pkcsml/>) (Pires *et al.*, 2015). The various parameters considered included Gastrointestinal (GI) Absorption, Blood-Brain Barrier (BBB) Permeation, Caco-2 Permeability, P-Glycoprotein (P-gp) Substrates, P-Glycoprotein (P-gp) Inhibitors, Cytochrome P450 (CYP) inhibitors, including CYP1A2 Inhibitor, CYP2C19 Inhibitor, CYP2C9 Inhibitor, and CYP3A4 Inhibitor. The results for all ligands were compiled in an Excel file. The Brain or Intestinal estimated permeation (BOILED-Egg) plot between WLOGP and TPSA was generated to predict gastrointestinal absorption and brain penetration of the selected compounds (Daina *et al.*, 2017). A bioavailability radar was generated to

anticipate the oral bioavailability and drug-likeness properties of the selected compounds. The preferred compounds were assessed for AMES toxicity, max. tolerated dose (human), hERG and II inhibition, oral rat acute toxicity (LD50), oral rat chronic toxicity (LOAEL), hepatotoxicity, skin sensitization, T. Pyriformis toxicity, and minnow toxicity (Pires *et al.*, 2015). The drug-likeness score was obtained using the drug-likeness and molecular property prediction tool by Molsoft LLC (MolSoft LLC, n.d.).

Disease targets and intersection targets collection

Databases, OMIM (McKusick, 2007) (<https://www.omim.org/>) And GeneCards (<https://www.genecards.org/>) (Stelzer *et al.*, 2016) were utilized with the search terms “Rheumatoid Arthritis”, “Systemic Lupus Erythematosus”, “Multiple Sclerosis”, “Psoriasis”, “Sjogren's Syndrome”, and “Humans” to obtain a list of potential biological targets for the selected autoimmune diseases. SwissTargetPrediction (<http://www.swisstargetprediction.ch/>) (Daina *et al.*, 2019) was used to predict each compound's potential target using the compound's Simplified Molecular Input Line Entry System (SMILES) from the PubChem database. A Venn diagram was generated using the Bioinformatics & Evolutionary Genomics Web tool to determine the common targets for each of the selected diseases (<https://bioinformatics.psb.ugent.be/webtools/Venn/>). An Excel file was generated for all biologically active targets for each disease, respectively.

Network construction

The intersection targets were uploaded to construct protein-protein interaction (PPI) networks on STRING software (<https://string-db.org/>) (Szklarczyk *et al.*, 2023) for every selected disease, with the species limited to “Homo sapiens” and a confidence score ≤ 0.9 . The STRING networks of genes were exported to Cytoscape 3.10.1 software (<https://cytoscape.org/>) (Shannon *et al.*, 2003). CytoHubba, an extension in Cytoscape, was launched using the MCC method to calculate the node score and obtain the top fifteen genes for each disease.

Prediction of activity spectra for substances (PASS)

PASS (Prediction of Activity Spectra for Substances) was made for the ligands using canonical SMILES from PubChem, which is based on the PASS WAY 2 drug server(<http://www.pharm.aexpert.ru/passonline/>) (Filimonov *et al.*, 2014). The results for all ligands were compiled in an Excel file, and the predictions with probability "to be active" (Pa) higher than 70% were considered (Filimonov *et al.*, 2014).

Molecular Docking

Structures of the common targets were retrieved from the Protein Data Bank (PDB) (<https://www.rcsb.org/>) (Berman, 2000) and AlphaFold Protein Structure Database (<https://alphafold.ebi.ac.uk/>) (Jumper *et al.*, 2021). The protein structures were prepared for molecular docking by removing water molecules, ions, ligands, and additional structures as indicated in PDB using the BIOVIA Discovery Studio by Dassault Systèmes (<https://www.3ds.com/products/biovia/discovery-studio>). The targets were converted to macromolecules using PyRx – Python Prescription Virtual Screening Tool (<https://pyrx.sourceforge.io/>) (Dallakyan & Olson, 2015). Selected ligands, after absorption, distribution, metabolism, and excretion (ADME) analysis, were converted from SDF format to PDB files using Open Babel software included in PyRx, and the energies were minimized.

The PyRx tool was used to perform molecular docking. The docking results displayed nine conformations with their binding energy scores and root mean square deviation (RMSD) values. Three trials of docking were performed for every target and every ligand, and the results were saved as comma-separated value (CSV) documents. The average of values was calculated from the three trials and documented in Excel files and used for further analysis. A heatmap was generated by plotting the average binding affinity scores of the hub targets from each disease against the key compounds. The scores were depicted in a three-color gradient of red, yellow,

and green, with red representing the lower binding energy scores and green representing the higher binding energy scores. Visualization of the protein-ligand interaction in three-dimensional space was performed using PyMOL by Schrödinger (<https://www.pymol.org/>) and in two-dimensional space using LigPlot+ v.2.3 - ligand-protein interaction diagrams (<https://www.ebi.ac.uk/thornton-srv/software/LigPlus/>) (Laskowski & Swindells, 2011).

Molecular Dynamics Simulation

To understand the identified protein-ligand interactions, molecular dynamics (MD) simulations were conducted. Using GROMACS software version 2023.4, MD analyses were carried out. With the aid of UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) and SwissParam (<https://old.swissparam.ch/>), all required input files and ligand topology files were produced (Pettersen *et al.*, 2004; Zoete *et al.*, 2011). MD simulation was performed in GROMACS using the CHARMM 27 force field. The simulation results were subjected to Root Mean Square Fluctuation (RMSF), Root Mean Square Deviation (RMSD), Radius of Gyration (Rg), and hydrogen bond studies. All the trajectory files were extracted, and the diagrams were plotted using a free software, QtGrace version 0.26 (<https://qtgrace.soft112.com/>).

Functional enrichment analysis

The hub genes from every disease were uploaded, respectively, to the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://davidbioinformatics.nih.gov/summary.jsp>) (Sherman *et al.*, 2022), and the gene-set enrichment tool for Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) (Kanehisa, 2019; Kanehisa *et al.*, 2023) enrichment analysis to clarify their regulatory pathways and biological functions, with "Homo sapiens" as the species. Terms with a *p*-value < 0.01, a minimum count of three, and a false discovery rate (fdr) value < 0.05 were considered significant enrichment and were tabulated in an Excel file.

RESULTS

Computational Analysis

Ligands and Predicted Targets of Leucas aspera

A total of 154 phytochemical compounds were obtained from the literature, which were filtered using Swiss ADME to obtain 71 compounds for further study. A total of 765 targets were retained from SwissTarget prediction after processing using a probability score ≥ 0.1 and eliminating the duplicates. The list of the 71 compounds is given in Appendix 1.

Prediction of activity spectra for substances (PASS) analysis

The PASS analysis was conducted for 71 ligands to check the antioxidant, anti-inflammatory, immunomodulant, and immunosuppressant activities. A large number of compounds showed these properties, though the Pa and Pi values, reflecting their effectiveness, were found to be significant in a few compounds only. Baicalein was predicted to have good antioxidant activity; diosmetin, chrysoeriol, and 7-methoxyflavonol were predicted as strong free radical scavengers; and allo-cassaic acid methyl ester was predicted to have immunosuppressive properties, as shown in Table 1. Additional data is given in Additional File 1.

Table 1. Prediction of activity spectra for substances (PASS) of selected phytoconstituents for anti-inflammatory, antioxidant, and immunosuppressant activity.

Phytoconstituent	Pa	Pi	Activity
baicalein	0.801	0.003	Antioxidant
diosmetin	0.808	0.003	Free radical scavenger
chrysoeriol	0.808	0.003	Free radical scavenger
7-methoxyflavonol	0.726	0.004	Free radical scavenger
allo-cassaic acid methyl ester	0.764	0.009	Immunosuppressant

Disease and Intersection targets

A total of 26,116 targets were collected after removing duplicates from GeneCards and OMIM databases, of which 5,828 targets were for Rheumatoid Arthritis, 6,282 for Systemic Lupus Erythematosus, 8,817 for Multiple Sclerosis, 4,695 for Psoriasis, and 1,040 for Sjögren's syndrome. A total of 365 intersection targets were identified by Venn diagrams combining the targets obtained for every disease and eliminating the duplicates. The list of the 365 targets, along with the identifier used to obtain the structure, is given in Additional File 2.

Protein-Protein Interaction Networks and Hub Targets

PPI networks were constructed in STRING for each disease with the highest confidence level (0.9) for the minimum needed interaction score, and dissociative targets were removed to give a total of 365 targets for the study. CytoHubba was used to categorize and rank the top 15 targets as the hub targets for each disease, utilizing the MCC method, where the target nodes, depicted in red, are ranked the highest, and the target nodes colored in yellow are ranked the lowest. A total of 33 targets were finally obtained after eliminating duplicates as the hub targets across the five selected diseases. A list of the top 15 targets for each selected disease with an identifier is given in Appendix 2.

Molecular Docking

Molecular docking was performed for 365 targets obtained with 71 compounds to analyze the broad effects of many phytochemical compounds against the selected diseases. The scores are colored in a three-color gradient, red, yellow, and green, with red representing lower binding energy and more stable binding and green representing higher binding energy and less stable binding. Results suggested that nine compounds showed the lowest binding energies across all targets and hence were selected as key compounds. The compounds 3-hydroxyspirost-8-en-11-one, baicalein, and diosmetin demonstrated high binding affinity with targets like AKT1 and MAPK1, while chrysoeriol and 6-methoxyflavone also showed notable binding. The compounds 3-carboethoxy-4-hydroxybenzo[g]quinoline 5,10-dione, 3-hydroxyflavone, 7-methoxyflavonol, and allo-cassaic acid methyl ester showed relatively lesser binding affinity among the selected compounds, as seen in the heatmap generated by plotting the binding energies of the hub targets against the key compounds in [Figure 1](#). Additional data on the average docking score of the plant compounds and standards is given in Additional Files 3 and 4, respectively.

Molecular Dynamics Simulation

The MD simulation studies were performed to assess the binding stability of the targets MAPK1 and AKT1 and the plant compounds baicalein, diosmetin, and chrysoeriol along with the standard drug Methotrexate using GROMACS software. The RMSF and RMSD were measured to assess the stability of the target and ligand complex. The results of the MD simulation were obtained from the target protein backbone flexibility by plotting the RMSD, RMSF, number of hydrogen bonds formed, and Rg of all of the amino acid residues of the two targets.

MD simulation studies of MAPK1 revealed that while baicalein, diosmetin, and chrysoeriol have Rg values of 2.05–2.125 nm, 2.1–2.2 nm, and 2.1–2.125 nm, respectively, with chrysoeriol being the most stable, methotrexate has an Rg that ranges from 2.105 to 2.18 nm, showing moderate compactness with flexibility. Methotrexate forms 1–6 bonds, which indicates the strongest binding, according to a hydrogen bonding study, while baicalein, diosmetin, and chrysoeriol form 1–2, 1–3, and 1–3 bonds, respectively, suggesting lesser to moderate binding. Methotrexate's strong flexibility (0.5–1.8 nm) is highlighted by RMSD, whereas better structural stability is displayed by baicalein (0.25–0.5 nm), diosmetin (0.3–0.6 nm), and chrysoeriol (0.2–0.4 nm), as seen in [Figure 2](#). Similarly, RMSF shows that methotrexate has a high degree of residue flexibility, whereas chrysoeriol exhibits the lowest and most stable interaction overall, while baicalein and diosmetin show moderate changes, as seen in [Figure 3](#).

MD simulation studies for AKT1 revealed that methotrexate forms 1–3 hydrogen bonds with peaks of 4 and exhibits strong binding. Its Rg value is between 2.1 and 2.2 nm, and it is moderately stable and flexible. Baicalein is stable due to its moderate hydrogen bonding (1–2 bonds), high compactness, and Rg values of 2.05–2.1 nm. Chrysoeriol exhibits less hydrogen bonding (1–2 bonds) but the most stable compactness (Rg 2.1–2.15 nm). Strong hydrogen bonds (2–3 bonds, peaking at 5) and compactness and flexibility (Rg 2.08–2.12 nm) are balanced in diosmetin. The most stable contact (~0.35 nm) is achieved by chrysoeriol; diosmetin retains dynamic stability (0.4–0.5 nm); baicalein demonstrates stable binding (~0.4 nm); and methotrexate exhibits moderate flexibility (0.5–0.6 nm) in terms of RMSD, as seen in Figure 4. Methotrexate's high residue-level flexibility (0.3–0.4 nm); baicalein's enhanced stability (0.25–0.3 nm), chrysoeriol's least fluctuations (<0.25 nm), and diosmetin's balance between flexibility and stability (0.25–0.3 nm) are all highlighted by RMSF, as seen in Figure 5. Reference to Supplementary Material for Rg and H bonds.

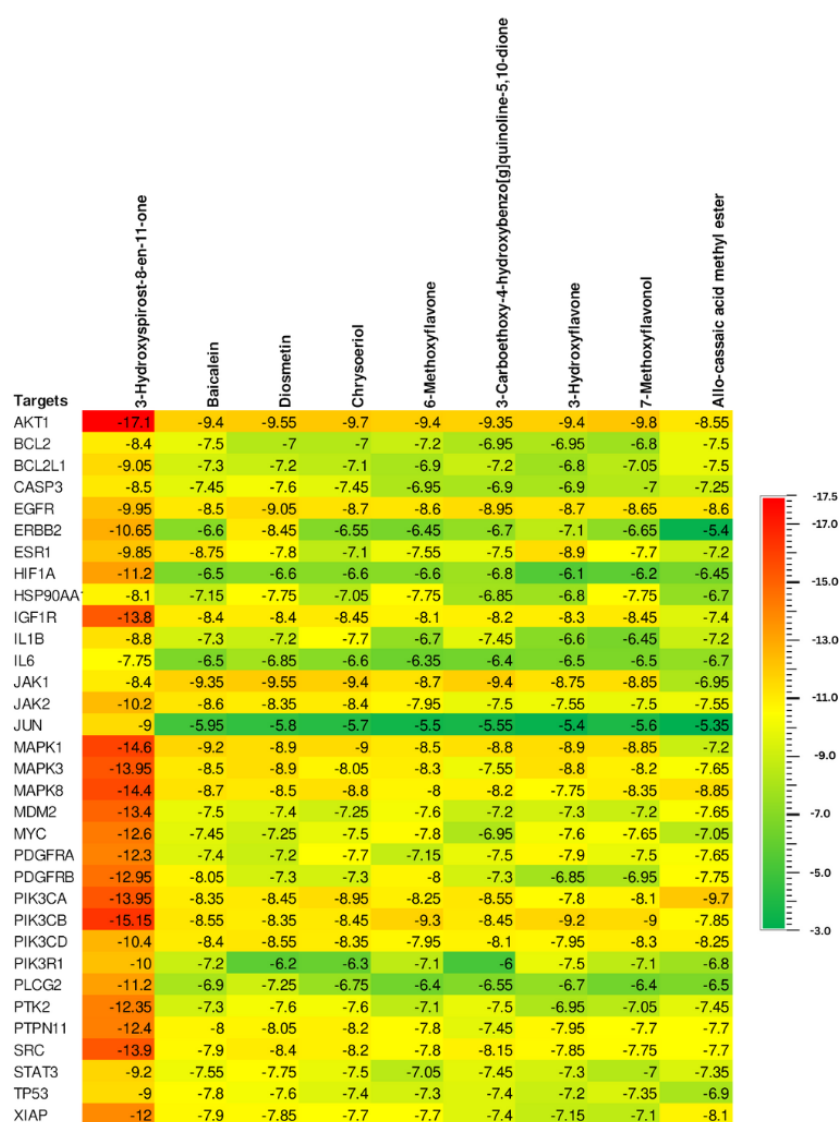


Figure 1. The binding free energy results of molecular docking performed using PyRx are presented as a heat map.

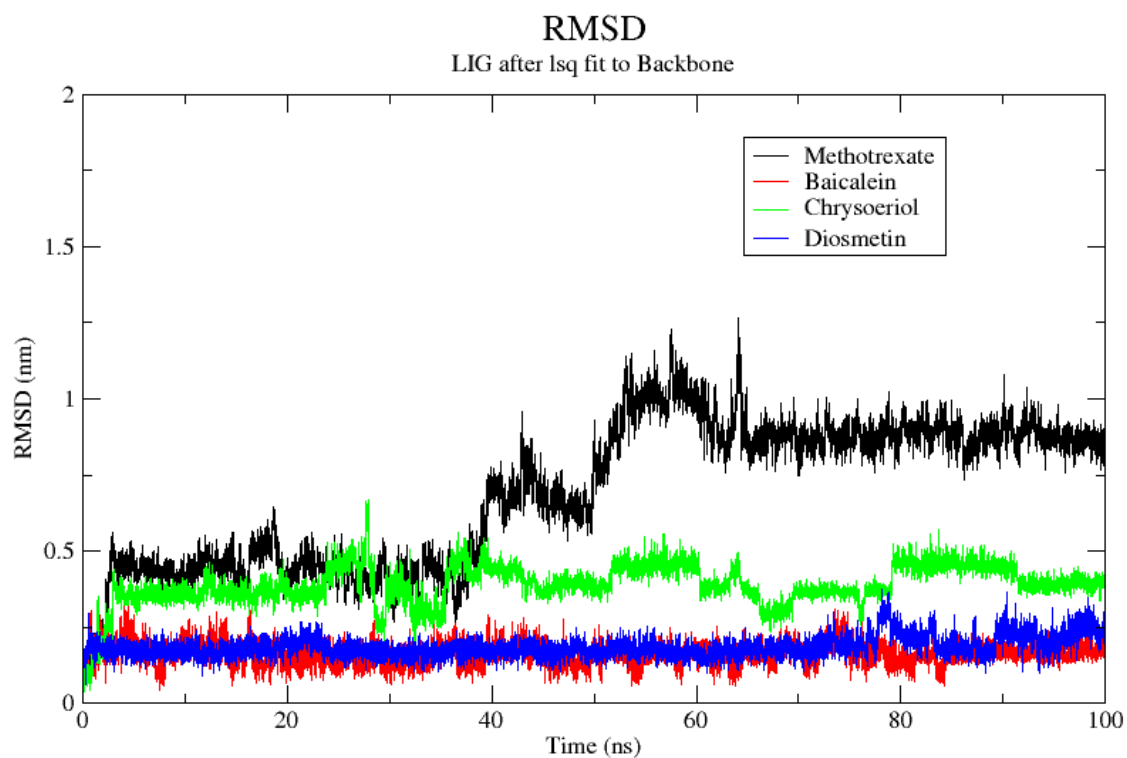


Figure 2. The MD simulation analysis using the RMSD plot for MAPK1

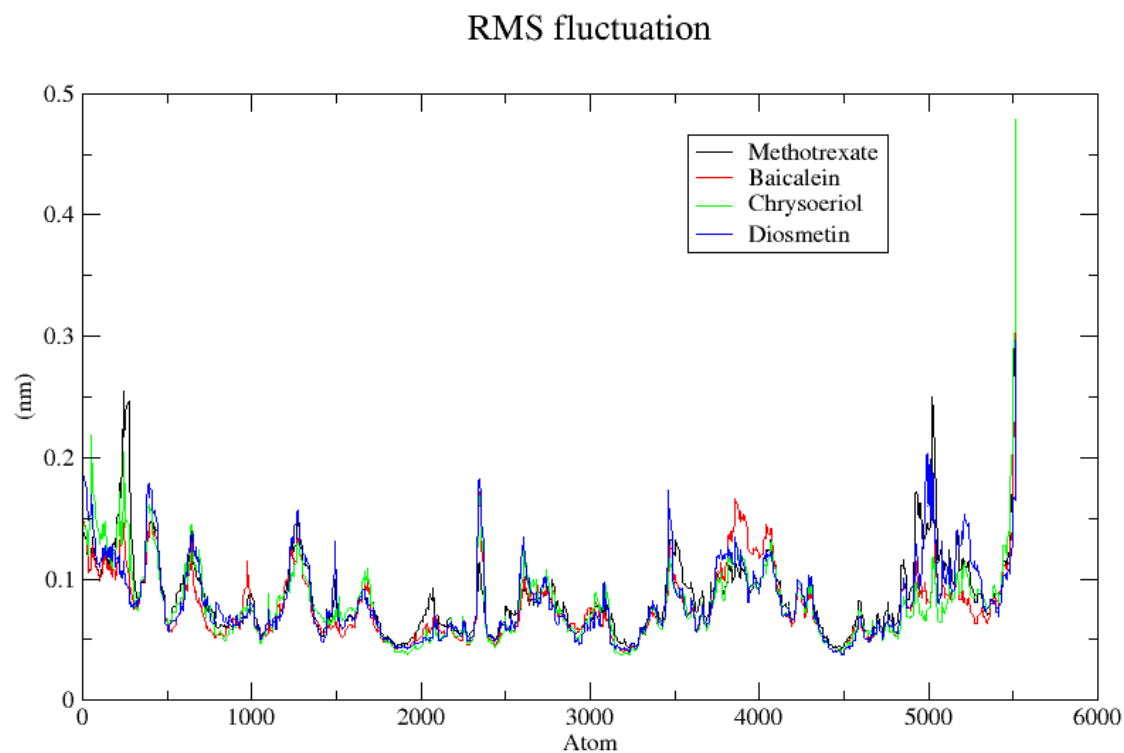


Figure 3. The MD simulation analysis using the RMSF plot for MAPK1

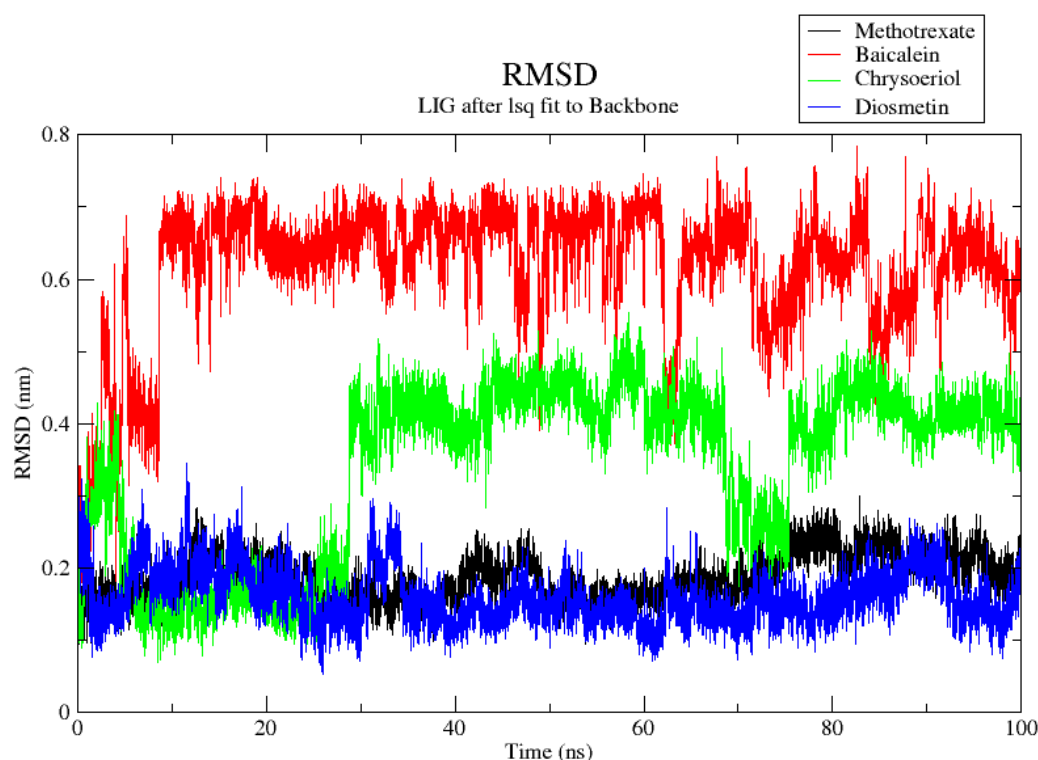


Figure 4. The MD simulation analysis using the RMSD plot for the target AKT1.

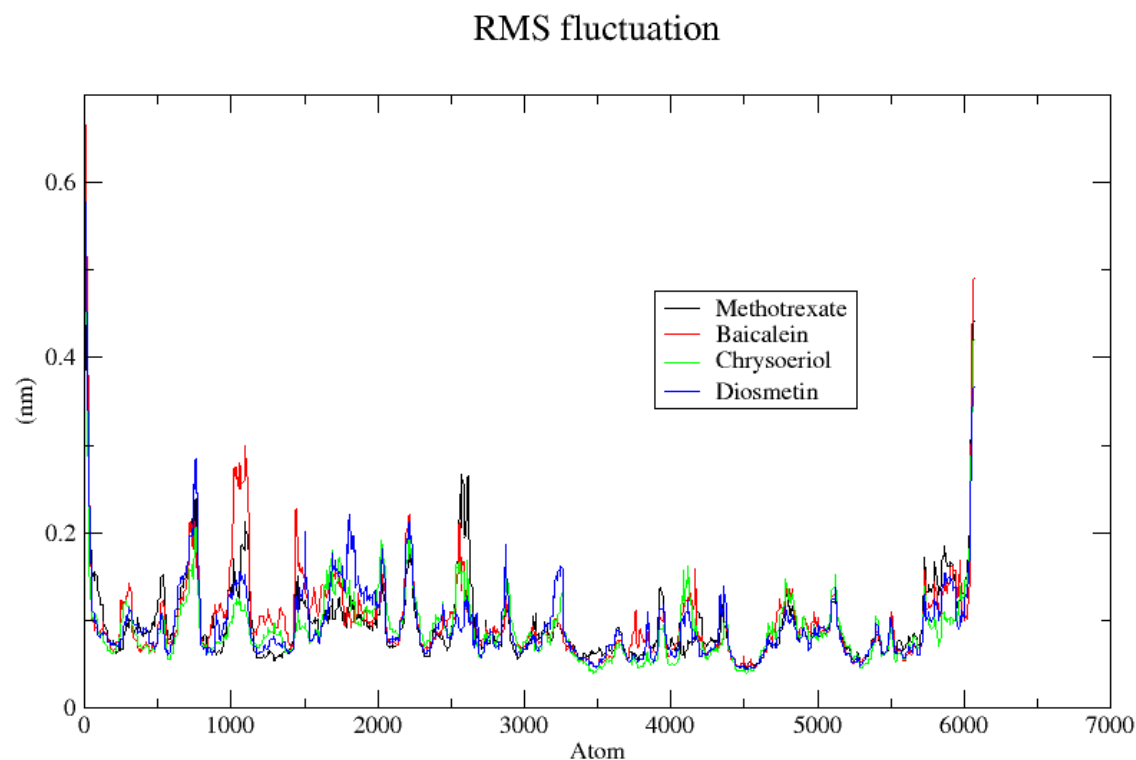


Figure 5. The MD simulation analysis using the RMSF plot for AKT1.

Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis

The biological process (BP), cell components (CC), and molecular function (MF) annotations of 15 hub targets from every disease were identified using the DAVID functional Annotation Tool and Shiny GO. There was a total of 249 GO terms obtained, including 131 from BP, 49 from CC, and 69 from MF. The important biological processes involving the hub targets are signal transduction and phosphorylation, while the hub targets are widely found in the cytoplasm, nucleus, cytosol, and plasma membrane. Due to the largest number of hub targets

being involved in these functions, protein binding, ATP binding, and enzyme binding were found to be significant molecular functions. KEGG pathway analysis showed that the hub targets were involved in a total of 499 pathways, with varied significant pathways in each disease. Additional data is given in Appendix 3.

Physico-Chemical Analysis

The physicochemical characteristics of the selected top bioactive compounds were assessed, and structures were drawn using ChemDraw. Molecular weights ranged from 238.242 (3-hydroxyflavone) to 428.613 (3-hydroxyspirost-8-en-11-one), and Log P values ranged from 1.327 to 5.037, indicating diverse lipophilicity. All compounds except 3-hydroxyspirost-8-en-11-one had 1-5 rotatable bonds. The hydrogen bond acceptors varied between 3 and 6, while the donors were between 0 and 3. Surface areas varied between 102.931 Å² to 186.686 Å². Additional data is given in Additional File 5 and Additional File 6.

Pharmacological Analysis

Toxicokinetic Analysis

Out of the selected compounds, five showed low levels of AMES toxicity, no compound showed hERG I toxicity, and only 3-hydroxyspirost-8-en-11-one is a weak hERG II inhibitor. Similarly, 3-carboethoxy-4-hydroxybenzo[g]quinoline showed low hepatotoxicity, and no compound showed skin sensitization. In addition, the drugs' characteristics varied concerning oral toxicity, minnow toxicity, and T. pyriformis toxicity. Refer to Additional File 5 for the complete values.

Pharmacokinetic Behavior

The evaluated compounds showed diverse pharmacokinetic properties regarding GI absorption, BBB permeation, Caco-2 permeability, P-gp interactions, and cytochrome P450 enzyme inhibition. All compounds show high GI absorption, and BBB permeation is generally low, with 3-hydroxyspirost-8-en-11-one and 6-methoxyflavone being notable exceptions, but none of the compounds are poorly distributed in the brain. Caco-2 permeability is positive across the board except for 3-carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione. Several compounds serve as P-gp substrates, while 3-hydroxyspirost-8-en-11-one is a weak inhibitor of P-gp and allo-cassaic acid methyl ester is a relatively stronger inhibitor of P-gp, suggesting possible drug-drug interactions. CYP inhibition differs among the compounds, with baicalein, diosmetin, and chrysoeriol weakly inhibiting CYP1A2, 3-hydroxyspirost-8-en-11-one specifically weakly inhibits CYP3A4, and 3-hydroxyflavone and 7-methoxyflavonol are weak CYP2C19 inhibitors, all crucial enzymes involved in drug metabolism. Refer to Additional File 5 for the complete values and details.

Drug-Likeness Behavior of the Selected Compounds

The drug-likeness scores of the compounds ranged from -0.66 to 0.31. 3-hydroxyspirost-8-en-11-one had the highest score, indicating the best drug-like properties, while baicalein and diosmetin had negative scores, suggesting less favorable characteristics. Refer to Appendix 4 for the values.

Bioavailability Radar Analysis of the Drugs

Swiss ADME software was used to analyze the bioavailability radar of the compounds, where the pink area represents the optimal range of a particular property. The top compounds were considered for Radar Analysis, a computational tool that provides insights into the rules followed by compounds such as FLEX, LIPO, SIZE, POLAR, INSATU, and INSOLU. These parameters shed light on the drug-likeness behavior of the selected compounds. The LIPO represents lipophilicity as the XLOGP3 between -0.7 and +5.0; SIZE represents the molecular weight of the compound between 150 and 500 g/mol; POLAR (topological polar surface area) represents the polarity between 20 and 130 Å²; INSOLU represents the insolubility in water by log S scale not higher than 6; INSATU represents the instauration as per fraction in the carbon

in the SP3 hybridization not less than 0.25; and FLEX represents the flexibility as per rotatable bonds with no more than 9 rotatable bonds (Daina *et al.*, 2017). The compounds 3-hydroxyspirost-8-en-11-one and allo-cassaic acid methyl ester showed promise on the bioavailability radar.

Boiled Egg Representation

BOILED egg analysis was performed using the Swiss ADME software through which useful information about properties like BBB, HIA, PG+, and PG- can be obtained. The selected top compounds are represented as molecules 1-9, which are 1 3-hydroxyspirost-8-en-11-one, 2 baicalein, 3 diosmetin, 4 chrysoeriol, 5 6-methoxyflavone, 6 3-carboethoxy-4 hydroxybenzo [g]quinoline-5,10-dione, 7 3-hydroxyflavone, 8 7-methoxyflavonol, and 9 allo-cassaic acid methyl ester, respectively. The physicochemical space for highly probable BBB permeability is depicted by the yolk, and the physicochemical space for highly probable HIA absorption is depicted by the white. Active efflux from the central nervous system or to the gastrointestinal lumen is represented by color-coding: blue dots for P-gp substrates (PGP+) and red dots for P-gp non-substrates (PGP-), which together make up the egg-shaped classification plot shown in Figure 6 (Daina *et al.*, 2017). The two compartments are not exclusive of one another, and the molecules represented by the grey area outside the compartment have characteristics that point to low absorption and restricted brain penetration (Daina *et al.*, 2017). The compounds 3-hydroxyspirost-8-en-11-one, 6-methoxyflavone, 3-hydroxyflavone, 7-methoxyflavonol, and allo-cassaic acid methyl ester showed BBB permeability, while the rest of the compounds showed intestinal absorption but not BBB permeability.

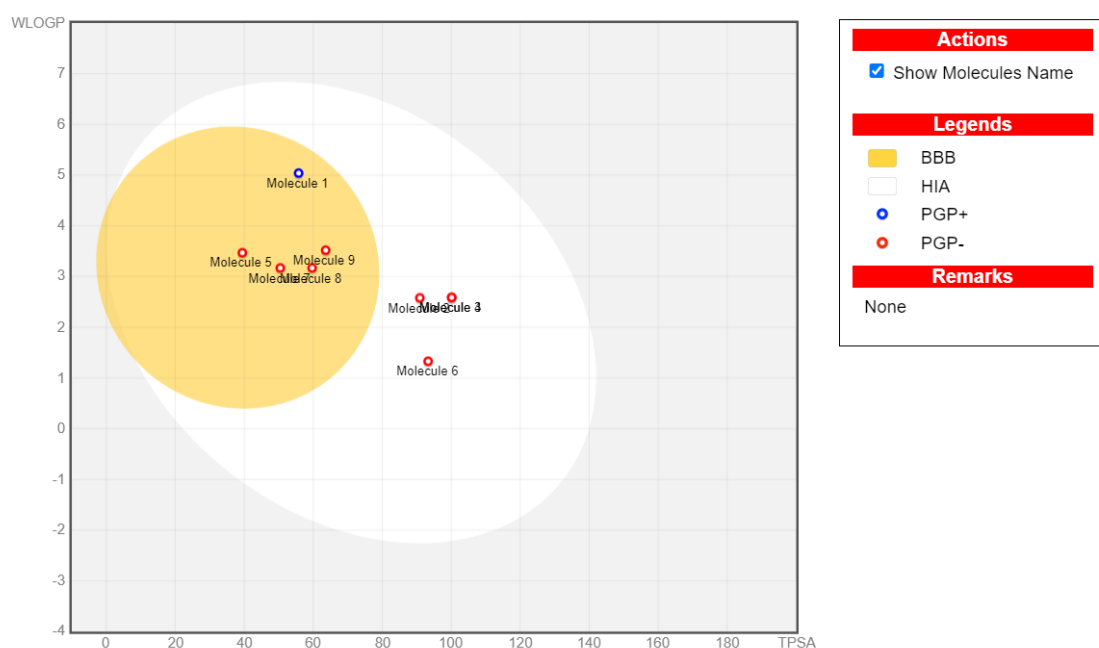
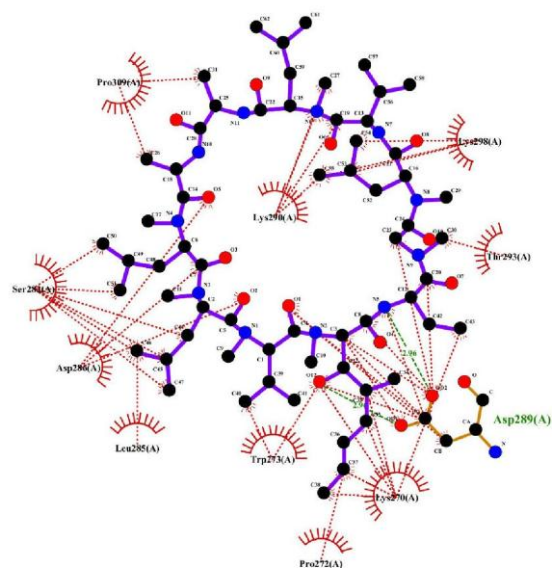


Figure 6. Boiled Egg Analysis of the selected nine compounds performed using the Swiss ADME software.

DISCUSSION and CONCLUSION

The genes related to the aforementioned autoimmune diseases were obtained from GeneCards and OMIM databases using the keywords "Rheumatoid Arthritis", "Systemic Lupus Erythematosus", "Multiple Sclerosis", "Psoriasis", and "Sjogren's Syndrome", and compared with the predicted gene targets of the compounds of *Leucas aspera*. Using a Venn diagram, the common gene targets for every disease were obtained. Among these gene targets, it was observed that some genes, namely AKT1, EGFR, MAPK1, STAT3, were present in most of the selected diseases.

7a)



7b)

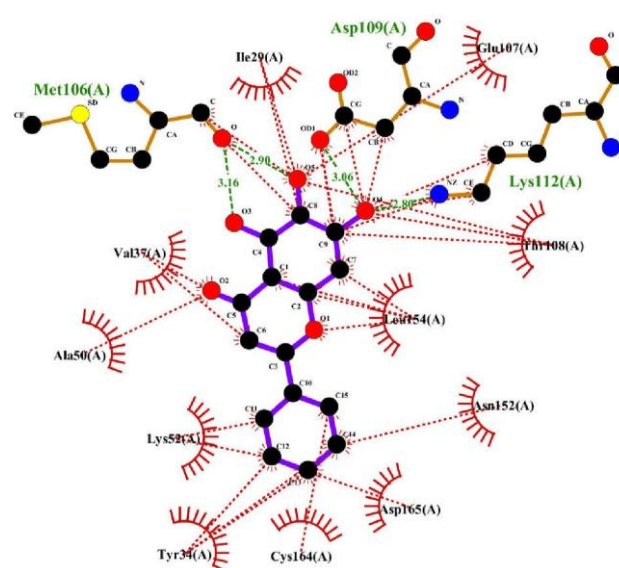


Figure 7a and 7b. The LigPlot visualization of the docked structures of a) MAPK1 with Cyclosporine and b) MAPK1 with baicalein

The current study discovered a large number of active compounds through a review of the literature of GC-MS analyses of the extract of *Leucas aspera*. Of relevance to this study is the fact that some of these compounds exhibit anti-inflammatory, anti-oxidant, and immunosuppressive properties. A condition of temporary or permanent immune system deficit caused by shocks to the immune response that results in an increased immune system's resistance to disease is known as immunosuppression, and the class of medications known as immunosuppressants inhibits or lessens the body's immunological response (Hussain & Khan, 2022). The immune system targets the body's own tissue when an autoimmune disease occurs; thus, immunosuppressive medications suppress this response because they impair immunity (Hussain & Khan, 2022). The most widely prescribed immunosuppressive medications for the selected diseases have been listed. Methotrexate (MTX), a folate antagonist, has been successfully used to treat a number of autoimmune diseases, mainly Rheumatoid arthritis (Tiong *et al.*, 2020). Azathioprine is a prodrug that is broken down into the purine antagonist 6-mercaptopurine (6-MP), which is the drug's active component. The United States Food and Drug Administration (FDA) has licensed it for the treatment of RA and the prevention of rejection from kidney transplants; nevertheless, off-label usage for rheumatic disease remission maintenance, including SLE, polymyositis/dermatomyositis, and a steroid-sparing drug in many other rheumatic diseases, is common. Cyclophosphamide is used off-label as an alkylating drug in CNS vasculitides, neurosarcoidosis, and refractory multiple sclerosis (Tiong *et al.*, 2020). Now recognized as a common immunomodulatory medication, hydroxychloroquine (HCQ), an approved anti-malarial, significantly improved oral symptoms, inflammatory indices (ESR, CRP), and immunoglobulins (IgM) in patients with Sjogren's syndrome (Tiong *et al.*, 2020). Cyclosporine A (CsA) is one of the most effective systemic drugs available for the treatment of psoriasis, as evidenced by the results of several randomized studies and by a prolonged experience in dermatological settings (Tiong *et al.*, 2020). Extracts from *Leucas aspera* are known to have anti-inflammatory and antioxidant qualities, and they have been used to treat skin conditions and chronic rheumatism. A thorough computational analysis was conducted, which is the new focus in drug development, to investigate these features. Finding compounds from *Leucas aspera* that showed promise and may be utilized to treat autoimmune diseases with fewer adverse effects was the major objective of our research. The active compounds' anti-oxidant, anti-inflammatory, and immunosuppressive properties were first verified by PASS analysis. Fifteen hub genes were identified through network

analysis using the MCC method. Enrichment analysis was also performed, but showed varied results across diseases. Hence, only docking results were taken into account to arrive at a conclusion. All gene targets from each disease were subjected to molecular docking studies, and the average docking score ranged from -2.15 kcal/mol with 1-propene, 1,2,3-trichloro-, (Z)- to -19.25 kcal/mol with 3-hydroxyspirost-8-en-11-one. Furthermore, the molecular docking analysis of the top active compounds obtained from the above-mentioned docking with the 15 hub genes from each selected disease showed average docking scores ranging from -2.9 kcal/mol with 3-carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione to -9.3 kcal/mol with allo-cassaic acid methyl ester. The two-dimensional visualization showed multiple interactions via hydrophilic, hydrophobic, and H-bonds as seen in [Figure 7](#). The top active molecules have the potential to function as immunosuppressants that are noticeably more effective when compared to scores obtained by docking the immunosuppressive drugs mentioned. This study indicates that *Leucas aspera*'s active compounds have a great deal of potential for novel drugs in the ADME assessment. However, their effectiveness should be investigated further.

The physico-chemical analysis, toxicokinetic analysis, pharmacokinetic behavior, drug-likeness behavior, bioavailability radar analysis, and boiled egg analysis were conducted using Swiss ADME software and pkCSM software. These methods give an important understanding about the surface area, number of donors and acceptors, rotational bond count, molecular weight, and LogP value of the active compounds. The various physico-chemical behaviors of the selected compounds were shown by computational analysis. The carcinogenicity, hepatotoxicity, skin sensitivity, minnow toxicity, cardiotoxicity using the herG enzyme, and maximum tolerated dose were the toxicity tests that were assessed. Through the AMES toxicity test it was observed that most compounds showed carcinogenic toxicity but at lower levels. Most of the compounds exhibited no toxicity against the liver, and no minnow toxicity was observed. It is crucial to look into a substance's toxicity on vital organs, including the liver, kidneys, and heart, before using it in clinical practice (Khan *et al.*, 2022). Using computational methods, the pharmacokinetic characteristics of the chosen drugs were evaluated (Khan *et al.*, 2022). The pharmacodynamic action of any drug is significantly influenced by its pharmacokinetic properties (Khan *et al.*, 2022). *In Silico* pharmacokinetic studies offer a very reliable, cost-effective method of quickly screening a large variety of drugs. Intestinal absorption, the blood–brain barrier, Caco-2 permeability, cytochrome p450, and P-glycoprotein activity were the different parameters that were evaluated. The selected compounds had variations in their cytochrome p450 and glycoprotein activities, whereas the remaining 10 compounds exhibited primarily low BBB penetration and strong GIT absorption. Similarly, Molsoft software was used to determine the drug-likeness scores for each of the top compounds, which were rather favorable, suggesting some potential as drug candidates. The boiled egg analysis, performed using the Swiss ADME software, evaluated the P-glycoprotein substrate, human intestinal absorption, and BBB. Only a small number of compounds exhibited P-glycoprotein substrate activity and BBB permeability, while most of them showed intestinal absorption.

The potential immunosuppressive activity of the selected phytochemical compounds to treat autoimmune diseases is better understood by this study. Upon further analysis, the compounds with the highest binding energy can be used to treat the chosen diseases. Not all of the symptoms related to the various interactable side effects may be effectively resolved by the several treatments that are available at present. Alternative methods that are safe and effective are therefore required. This study offers valuable insights into the treatment of autoimmune diseases since a few compounds exhibited promising activity against the hub genes, no major toxicity against essential organs, and acceptable pharmacokinetic properties. The current work provided a comprehensive computational assessment of the chosen compounds using molecular docking and ADMET analysis. The hub target's highest affinities, as well as possible binding energies for different molecules, were discovered through computational analysis. For the top

compounds, the ADMET analysis revealed a variety of pharmacokinetic and toxicokinetic characteristics.

The selection of the nine compounds highlighted from the initial 71 analyzed compounds in *Leucas aspera* is strongly justified by their demonstrated anti-inflammatory and immunomodulatory properties. These compounds - 3-hydroxyspirost-8-en-11-one, baicalin, diosmetin, chrysoeriol, 6-methoxyflavone, 3-carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione, 3-hydroxyflavone, 7-methoxyflavonol, and allo-cassaic acid methyl ester - were selected based on their potent anti-inflammatory activities and ability to modulate immune responses. Baicalin has shown remarkable anti-inflammatory and immunomodulatory effects in various studies. It significantly reduced the expression of pro-inflammatory cytokines like TNF- α , IL-1 β , and IL-6, while also inhibiting the NF- κ B signaling pathway (Wen *et al.*, 2023). In experimental autoimmune encephalomyelitis mice, baicalin ameliorated disease severity by suppressing Th1 and Th17 cell differentiation (Wen *et al.*, 2023). Diosmetin exhibited strong anti-inflammatory properties by inhibiting the production of inflammatory mediators such as NO, PGE2, and pro-inflammatory cytokines. It also showed protective effects against acute kidney injury by suppressing inflammatory responses (Yang *et al.*, 2017). Chrysoeriol demonstrated potent anti-inflammatory activity by inhibiting the JAK2/STAT3 and NF- κ B pathways (Yoon & Park, 2021). While limited information was available for some compounds, their selection was based on their unique structural features and potential for novel therapeutic applications in inflammatory and immune-related conditions.

Network enrichment analysis performed in this study revealed multiple pathways involving the hub genes for each disease. It was observed that the pathways varied for each disease, with a few common ones such as the PI3K/AKT signaling pathway. The phosphoinositide 3-kinase (PI3K)/Akt signaling axis plays an important role in cellular growth signaling and proliferation. An increasing amount of data is helping to describe the role of this pathway in lymphocyte development with regard to the immune system, as well as to show how changes that lead to non-regulated activation in this pathway may give rise to systemic autoimmunity or malignancy (Cushing *et al.*, 2012; Roy *et al.*, 2023). Compounds that block the PI3K or Akt isoforms are promising therapeutic targets for the modulation of inflammatory and autoimmune disorders, as well as cancer and cardiovascular diseases. There is increasing evidence that PI3K/Akt signaling plays a role in the regulation of both acute and chronic inflammatory processes (Cushing *et al.*, 2012; Roy *et al.*, 2023). The two targets, AKT1 and MAPK1, involved in the pathway were chosen to perform MD simulation due to their high binding score with the top ligands. It was also observed that both targets were present in four of the five diseases selected for this study. Methotrexate was taken as the standard drug due to its universal use in treating autoimmune diseases. The phytochemicals baicalein, diosmetin, along with methotrexate as the control, were the selected as ligands owing to their high binding scores. For MAPK1, analysis of RMSD, RMSF, Radius of gyration, and number of hydrogen bonds revealed that methotrexate and diosmetin excel in binding strength, while Chrysoeriol provides the highest structural stability, and baicalein maintains a balance between dynamics and binding strength. Whereas for AKT1, the analysis showed that baicalein provides balanced dynamics and binding, chrysoeriol provides the most effective structural stability, while methotrexate and diosmetin excel in binding strength. Through both the results, we can conclude that diosmetin, a phytochemical found in *Leucas aspera*, is the closest to the control, methotrexate, with respect to binding properties and stability.

The molecular docking studies and network enrichment assessments provided strong evidence for these compounds' binding affinities to biological targets associated with inflammatory and immune responses. As revealed by ADMET analysis, the compounds demonstrated favorable pharmacokinetic properties and minimal toxicity risks. Molecular dynamics simulations further validated the stability of these compound-target interactions, reinforcing their potential as anti-inflammatory and immunomodulatory agents.

In Silico research is highly advantageous to drug development for several reasons. By facilitating the screening, design, and therapeutic potential prediction of new drugs, they aid in the process of finding possible drug candidates (Nimgampalle *et al.*, 2021). Additionally, by assisting with toxicity prediction, *In Silico* techniques help researchers save time and money by identifying potentially adverse effects early in the development process. Computational methods such as virtual ligand screening, molecular modeling, and docking-based virtual screening aid in the discovery of new and promising compounds by modelling the binding modes of potential drug candidates and analyzing their interaction patterns (Nimgampalle *et al.*, 2021). Furthermore, early drug development has become more flexible and ethically sound by *In Silico* research, which uses existing knowledge to direct future processes. Owing to *In Silico* drug discovery, numerous therapeutic candidates with great potential have been identified. Common challenges and important factors in the docking process include, but are not limited to, ligand and receptor conformation, flexibility, and cavity detection (Khan *et al.*, 2022). These draw attention to the challenges and restrictions posed by the underlying theories. Another major study drawback is that the compounds were not investigated following computational analysis through *In Vitro* and *In Vivo* analysis.

Scientific studies focusing on *Leucas aspera* have used different computational methods to analyze the plant for phytochemicals and their qualities. For example, one study on *Leucas aspera* bioactive compounds looked at its inhibitory effect against alpha-synuclein, which plays a role in Parkinson's disease, where the study showed positive neuro-protective properties in the consumption of the plant (Menezes, 2022). In addition, conventional drug discovery from *Leucas aspera* was replaced by omics approaches to identify the bioactive compounds from this plant that activate immunity relevant for traditional medicinal remedies (Baruah *et al.*, 2022).

The identified bioactive compounds from *Leucas aspera* show promising potential for therapeutic applications in autoimmune diseases, with the present findings indicating significant translational potential for baicalein, diosmetin, and chrysoeriol as candidate modulators of inflammation-associated pathways in autoimmune diseases. Their high-affinity interactions with central hub proteins such as AKT1 and MAPK1, combined with favorable ADMET characteristics and stable molecular dynamics profiles, suggest that these phytochemicals may act as promising scaffolds for future pharmacological development. Prior studies support this direction: baicalein and its glycoside baicalin exhibit notable anti-inflammatory and immunomodulatory effects across cellular and animal autoimmune/inflammatory models (Wen *et al.*, 2023; Yang *et al.*, 2024); diosmetin regulates NF- κ B and JAK/STAT signaling in both cellular and *In Vivo* models (Fang *et al.*, 2024; Lee *et al.*, 2020); and chrysoeriol directly suppresses JAK2/STAT3 and fibroblast-like synoviocyte hyperproliferation in rheumatoid models (Wu *et al.*, 2022; Yoon & Park, 2021). Given that the PI3K/AKT and MAPK axes are clinically validated therapeutic pathways, the computational interactions identified here offer a route for lead-optimization and target engagement studies (Glaviano *et al.*, 2023; Bobkova *et al.*, 2025). Finally, the successful examples of converting flavonoid scaffolds into improved drug-like derivatives and the growing toolkit for standardizing plant extracts and improving pharmacokinetics demonstrate that the path from computational hits to *In Vitro* validation, *In Vivo* efficacy models, medicinal chemistry optimization, and IND-enabling studies is well-trodden and feasible (Putri *et al.*, 2023; Chaachouay & Zidane, 2024).

The present study both expands and aligns the computational evidence base with prior *In Silico* studies on *Leucas aspera*. Previous studies have used virtual screening of *L. aspera* phytochemicals against diverse targets. Sharavanan *et al.* (2019) demonstrated favorable “lead-likeness” for ligands such as Leucasperone B by performing docking and ADME/toxicity filtering of multiple compounds against bacterial membrane proteins. According to a more recent study, baicalein and Leucasperone A derived from *L. aspera* yielded the best antagonistic docking poses as reported by a computational analysis targeting the neurodegenerative protein

α -synuclein (Menezes, 2022). Similarly, a network pharmacology and docking-based study revealed that *L. aspera* metabolites may target natural killer T-cell function in transplantation-related pathogenesis, autoimmune, and other immunological disorders (Baruah *et al.*, 2022). Compounds extracted from *L. aspera*'s methanolic leaf extract were docked and pharmacokinetically evaluated against the capripox viral RNA polymerase complex in another GC-MS-based study, emphasizing favorable drug-like characteristics and binding scores (Burranboina *et al.*, 2022). Further still, Giri & Parida (2024), confirmed the efficacy of the plant's compound-library for diverse pathogen-directed interventions by implementing *In Silico* antifungal screens of *L. aspera* phytochemicals against target proteins of *Trichophyton mentagrophytes*. These prior studies provide a consistent translational narrative when combined with our docking, ADMET, and MD simulations: *L. aspera* phytochemicals repeatedly demonstrate favorable docking, drug-likeness, and target-engagement potential across multiple disease systems, thereby supporting our current proposition of baicalein, diosmetin, and chrysoeriol as reliable lead entities for further pharmacological investigation.

In Vitro studies should be conducted to evaluate the compounds' effects on specific cellular targets and pathways implicated in autoimmune disorders. These studies can help elucidate the mechanisms of action and validate the computational predictions. Subsequently, *In Vivo* animal models of autoimmune diseases should be utilized to assess the efficacy, safety, and pharmacokinetics of the most promising compounds. Before initiating downstream biological studies, obtaining analytically pure (>98%) baicalein, diosmetin, and chrysoeriol, confirming identity and purity through LC-MS and NMR, and preparing standardized stock solutions to ensure experimental reproducibility can be done. Initial biochemical assays using recombinant AKT1 and MAPK1 would confirm direct inhibition and determine IC₅₀ values (Haubrich & Swinney, 2016). These can be followed by cell-based studies assessing reductions in phosphorylated AKT and ERK in immune or synoviocyte models, applying phospho-protein detection approaches (Molgaard *et al.*, 2016). Functional readouts such as cytokine modulation or suppression of synoviocyte proliferation would further validate biological relevance (Wen *et al.*, 2023; Lee *et al.*, 2020; Wu *et al.*, 2022). Parallel *In Vitro* ADME profiling can be conducted (Qu *et al.*, 2019) and guided by *In Silico* tools like pkCSM. Compounds showing favorable profiles can then be evaluated *In Vivo* using the carrageenan-induced paw-edema model for acute inflammation, collagen-induced arthritis (CIA) model and collagen antibody-induced arthritis (CAIA) models for chronic autoimmune pathology (Rosloniec *et al.*, 2021; Luan *et al.*, 2021; Balkrishna *et al.*, 2019). These studies will allow assessment of clinical, biochemical, and histopathological endpoints, including *In Vivo* suppression of AKT and ERK phosphorylation. Lead molecules demonstrating robust efficacy may then proceed into medicinal chemistry optimization (Putri *et al.*, 2023) aimed at improving stability, bioavailability, and potency, ultimately supporting their progression into early translational research.

The traditional Ayurvedic use of *Leucas aspera* in decoctions and other formulations provides valuable insights for potential drug delivery methods. These traditional preparations could be studied further to develop standardized extracts or isolate specific compounds for pharmaceutical formulations (Savrikar & Ravishankar, 2010). Additionally, synergistic effects of compound combinations, as often found in traditional medicine, should be explored (Savrikar & Ravishankar, 2010). The integration of modern drug discovery techniques with traditional knowledge can accelerate the development of novel therapies.

This study integrates additional computational methods to not only discover potential phytochemicals, but also determine the specific biological activities. This dual strategy helps us to get a balanced view as to how this plant can help to treat autoimmune diseases. By utilizing network pharmacology, we investigate how the bioactive compounds interact with common immune pathways implicated in MAS, revealing similarities that could inform targeted therapeutic strategies. This strategy also entails extensive data extraction and literature

integration, which helps in defining the plant as a multi-faceted modulator of immune responses with shared molecular targets and signaling pathways.

In conclusion, this study highlights the potential of *Leucas aspera* as a source of bioactive compounds for treating autoimmune diseases. Based on the GC-MS analysis and literature review, the study considered 71 compounds but focused on 9 of them that have pharmacological importance, which include 3-hydroxyspirost-8-en-11-one, baicalein, diosmetin, chrysoeriol, 6-methoxyflavone, 3-carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione, 3-hydroxyflavone, 7-methoxyflavonol, and allo-cassaic acid methyl ester.

Molecular docking, ADMET studies, and network enrichment analysis revealed that these compounds exhibited significant binding profiles and favorable effects on the molecular targets associated with autoimmune diseases. These compounds exhibited favorable pharmacokinetic activity and low levels of toxicological effects, suggesting their therapeutic potential. By performing Molecular Dynamics Simulation, we strengthened our computational research and looked more closely at compound-target interactions. This improved our understanding of the different complexes' stability and significant interactions, as well as how it behaves over time.

By performing a more detailed analysis of the results and thorough *In Vitro* and *In Vivo* tests against the targets under study, especially for the compounds with the highest binding energies, the computational analysis can be confirmed. The combination of traditional knowledge and other computer-based approaches creates a viable approach for the development of new plant-derived drugs for autoimmune diseases. This study not only lays a strong groundwork for future drug discoveries but also reveals better opportunities for *Leucas aspera* in the modern world to develop more effective and safer therapeutic strategies for the management of the adverse effects of autoimmune diseases.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJATE belongs to the author(s).

Data Availability Statement

No datasets were generated or analysed during the current study.

Authorship Contribution Statement

A.S.C.: Material preparation, data collection, analysis, and Writing-original draft. **A.G.B.:** Material preparation, data collection, analysis, and Writing-original draft. **S.R.:** Material preparation, data collection, analysis, and Writing-original draft. **R.S.V.:** Conceptualization of the study and Supervision.

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APPENDIX

Appendix 1. PubChem IDs of phytoconstituents.

Ligands	PubChem ID
Olivetol	10377
Spiro (4,5) decan-7-one	10888101
4-Ethoxy-3-methoxybenzyl alcohol	112741
3-Hydroxyflavone	11349
leucasperones A	11843975
Pentyl propionate	12217
Menthol	1254
6-Methoxyflavone	147157
beta-Pinene	14896
Coniferyl alcohol	1549095
Clovanediol	15599878
alpha-Terpineol	17100
Ethyl 2-hydroxy-4-methoxy-6-propylbenzoate	179627
Camphorsulfonic acid	18462
Cyclohexanecarboxylic acid, 2-[[bis(2-methylpropyl)amino]carbonyl]-,4-nitrophenyl ester	22295452
Limonene	22311
Neocurdione	24836956
Thujone	261491
3-Carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione	276891
Decanoic acid	2969
Eugenol	3314
4-vinylguaiaicol	332
7-Methoxyflavonol	344546
2-Cyclohexen-3-ol-1-one,2-[11-phenylundecanoyl]-	365014
Octanoic acid	379
dodecanoic acid	3893
guaiaicol	460
Chrysoeriol	5280666
(E)-beta-ocimene	5281553
Baicalein	5281605
Diosmetin	5281612
2-Nonenal	5283335
2-Decenal	5283345
Neoisolongifolane, hydroxy-	534931
Allo-cassaic acid methyl ester	5379314
Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl-	538938
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	545303
3-Methyl-2-(2-oxopropyl) furan	545772
1,4-Methanoazulen-7[1H]-one-octahydro-4,8,8,9-tetramethyl	572868
5-Bromo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	572870
2,4,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydro-2H-chromene	573147
Cyclopropaneoctanoic acid	57346156
1H-Inden-1-one, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-	585274
4-Aminobenzoylglutamic acid penta-methyl derivative	590578
Bicyclo[4.1.0]heptane,1-(3-oxo-4-phenylthiobutyl)-2,2,6-trimethyl-	591694
chavibetol	596375
3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	610072
3-Hydroxyspirost-8-en-11-one	628694

alpha-THUJENE	637518
Citral	638011
Methyl-alpha-D-glucopyranoside	64947
Linalool	6549
d-Borneol	6552009
Diisobutyl phthalate	6782
Thymol	6989
Syringol	7041
Methyleugenol	7127
2,4-Di-tert-butylphenol	7311
Isoamyl propionate	7772
Citronellal	7794
Hydroquinone	785
Nonanoic acid	8158
2-(3-Pentyl)pyridine	81887
Dodecanal	8194
Linalyl acetate	8294
Nicotine	89594
Trisporic acid B	90470632
beta-Thujone	91456
2-Methyl ester-(4-ethyl-2-acetoxy-5-methoxyphenyl)acetic acid	91724133
4-Fluoronaniline	9731

Appendix 2. Top 15 targets for each of the five selected diseases.

Rheumatoid Arthritis	Systemic Lupus Erythematosus	Multiple Sclerosis	Sjögren's syndrome	Psoriasis
JAK2 (alpha fold)	PTPN11 (alpha fold)	TP53 (alpha fold)	BCL2 (8HOG)	TP53 (alpha fold)
PTPN11(alpha fold)	PIK3R1 (5M6U)	AKT1 (alpha fold)	TP53 (alpha fold)	AKT1 (alpha fold)
EGFR (1XKK)	PIK3CA (6BTY)	MAPK1 (2OJG)	AKT1 (alpha fold)	MAPK1 (2OJG)
STAT3 (alpha fold)	PIK3CB (2ZKM)	STAT3 (alpha fold)	STAT3 (alpha fold)	HIF1A (4H6J)
TP53 (alpha fold)	PIK3CD (alpha fold)	HIF1A (4H6J)	JUN (alpha fold)	MYC (1NKP)
SRC (1YOJ)	JAK2 (alpha fold)	MYC (1NKP)	HIF1A (4H6J)	STAT3 (alpha fold)
AKT1 (alpha fold)	EGFR (1XKK)	JAK2 (alpha fold)	MAPK1 (2OJG)	JUN (alpha fold)
HIF1A (4H6J)	PDGFRB (5K5X)	EGFR (1XKK)	BCL2L1 (1R2D)	BCL2 (8HOG)
MYC (1NKP)	ERBB2 (2A91)	JUN (alpha fold)	MAPK8 (alpha fold)	MAPK3 (2ZOQ)
PIK3CD (alpha fold)	PTK2 (1MP8)	SRC (1YOJ)	MDM2 (alpha fold)	JAK2 (alpha fold)
JUN (alpha fold)	PLCG2 (4FBN)	ESR1 (2POG)	IL6 (4NI7)	HSP90AA1 (alpha fold)
PTK2 (1MP8)	IGF1R (5HZN)	ERBB2 (2A91)	XIAP (alpha fold)	PIK3CA (6BTY)
ESR1 (2POG)	PDGFRA (5K5X)	IGF1R (5HZN)	IL1B (alpha fold)	EGFR (1XKK)
ERBB2 (2A91)	JAK1 (4IVC)	PIK3CD (alpha fold)	EGFR (1XKK)	PIK3CD (alpha fold)
MAPK1 (2OJG)	SRC (1YOJ)	PTPN11 (alpha fold)	CASP3 (2CJY)	ESR1 (2POG)

Appendix 3. Number of Gene Ontology terms and pathways involved in each of the five selected diseases.

Disease	Biological Process	Molecular Function	Cellular Component	Pathways
Multiple Sclerosis	26 terms	17 terms	12 terms	96 terms
Psoriasis	27 terms	16 terms	11 terms	109 terms
Rheumatoid Arthritis	28 terms	16 terms	10 terms	93 terms
Systemic Lupus Erythematosus	24 terms	11 terms	10 terms	109 terms
Sjögren's Syndrome	26 terms	9 terms	6 terms	92 terms

Appendix 4. Drug Likeness score of selected phytoconstituents.

Compound	Drug-Likeness Score
3-Hydroxyspirost-8-en-11-one	0.31
Baicalein	-0.1
Diosmetin	-0.66
Chrysoeriol	0.3
6-Methoxyflavone	0.17
3-Carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione	0.29
3-Hydroxyflavone	0.12
7-Methoxyflavonol	0.17
Allo-cassaic acid methyl ester	0.11