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Network pharmacology-based findings of the immunomodulatory activity of phytocompounds from *Withania somnifera* and *Aloe barbadensis*

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Abstract

Immunomodulation constitutes a crucial part of individual organisms' defense systems. Moreover, the utilization of plant-based natural products as herbal medicine for immunomodulation has garnered significant interest. Herein, we examined the immunomodulatory potentials of active phytocompounds extracted from *Withania somnifera* and *Aloe barbadensis* by employing ADMET screening, network pharmacology, and molecular docking techniques. This study follows the paradigm in drug discovery, which has shifted from a "one-target, one-drug" mode to a "network-target, multiple-component-therapeutics" mode. Phyto compounds sourced from *W. somnifera* and *A. barbadensis* were mined from online databases, including Dr. Duke's Phytochemical Ethnobotanical Database. After screening these active compounds, their potential targets were predicted through *in silico* ADMET property prediction models. Network pharmacology was utilized to establish a "compound-protein/gene-disease" network and reveal the regulatory mechanism of small molecules in a high-throughput manner through STRING, Cytohubba plugin in Cytoscape, and the g: Profiler software. A molecular docking simulation was performed to examine the binding affinity between the selected hub targets and bioactives. The findings showed that phytocompounds derived from the *W. somnifera* and *A. barbadensis* exhibit immunomodulatory effects by inhibiting specific protein targets, notably AKT1, HCK, JAK2, PDPK1, KIT, and IL2. Molecular docking analysis further revealed the potential of withanolide G, somniferine, and somniferanolide as promising immunomodulatory compounds against HCK, JAK2, and PDPK1 proteins, which are involved in multiple myeloma pathways, encompassing the PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, and Toll-like receptor signaling pathway. In conclusion, these compounds are recommended for further *in vivo* and *in vitro* investigations to ascertain their potential as treatments for multiple myeloma.

Keywords: Immunomodulation; *Withania somnifera*; *Aloe barbadensis*; Phytocompounds; Multiple myeloma; Network pharmacology

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1. Introduction

The human body is protected by various non-specific defense mechanisms against infections and intruders, notably pathogens. On breaching the mucosal surface barrier,

these pathogens encounter two further lines of defense: The innate and acquired immune responses. This robust host defense is adequate to safeguard us against immune-related illnesses and pathogenic invaders, facilitated by the efficient interplay between innate and acquired immunity[1]. In addition, there are alternative avenues for modulating immune responses, including naturally occurring or synthesized compounds capable of modifying these pathways[2].

Natural compounds are a common foundation for the development of therapeutic treatments. In the last four decades, around a quarter of all approved medications (excluding biologicals) were derived from natural sources, and another quarter was found in the environment, often by exploiting the pharmacophore of these molecules[3]. Among these, plant-derived immunomodulators are a fascinating subject of study, offering a multitude of potential avenues[4]. For this study, our focus rests on *Withania somnifera* and *Aloe barbadensis* as the pivotal therapeutic plant candidates for *in silico* analysis.

W. somnifera (also known as Ashwagandha) is a biologically active plant that boasts an extensive spectrum of medicinal compounds, including steroidal lactones (withanolides), sitoindosides, and numerous other beneficial steroidal alkaloids, that have been used for ages to treat a variety of ailments[5]. On the other hand, *A. barbadensis* Miller is a perennial, xerophytic, succulent, and shrubby or arborescent plant in the Asphodelaceae (Liliaceae) family with a pea-green tint. It is primarily found in arid regions across Africa, Asia, Europe, and North America[6]. Both *W. somnifera* and *A. barbadensis* have found use as aphrodisiacs, analgesics, liver tonics, diuretics, hypocholesterolemic agents, anxiolytics, antidepressants, immunomodulatory substances, and anti-inflammatory agents[5,7].

Multiple myeloma, a hematopoietic malignancy, constitutes approximately 10% of all hematologic malignancies. Until the 2000s, limited success and advancements were observed, and achieving protracted survival was unlikely. This malignancy follows a multistep process, originating as a clonal plasma cell cancer[8]. The premalignant stage, termed monoclonal gammopathy of unknown significance, often serves as the initial indicator, heralding the onset of multiple myeloma. This is succeeded by asymptomatic smoldering multiple myeloma and eventually active multiple myeloma[9]. The immune system progressively weakens as the disease evolves. To enhance treatment outcomes, immunomodulatory drugs (IMiDs), proteasome inhibitors (PI), and monoclonal antibodies have been introduced, ushering in an era of improved therapeutic experiences, with immunomodulation exhibiting some level of anti-multiple myeloma efficacy[10].

Computational (*in silico*) approaches are commonly used in drug development. The notable advantage of employing *in silico* approaches lies in their ability to rapidly predict outcomes for a vast array of phytochemicals offering high-throughput predictions. In addition, they are able to predict therapeutically relevant molecules based on chemical structures even before their physical synthesis. As a result, *in silico* approaches find early application in the drug development process, particularly for compounds intended for synthesis. This proves especially important when there lacks an existing drug molecule with curative effects against a human disease. Incorporating computational approaches at the outset also helps reduce the risk of false positives, which could otherwise result in a high attrition rate during the later phases of drug development[11].

One of the key aspects of computational studies is network pharmacology. Through the integration of bioinformatics, cheminformatics, and network biology, network pharmacology has emerged as a dominating approach for elucidating the complicated pharmaceutical mechanism of action associated with the bioactive constituents found within diverse herbs and their pairings[12]. Network pharmacology operates as an efficient tool to construct a "compound-protein/gene-disease" network, shedding light on the regulatory principles orchestrated by small molecules in a high-throughput manner[13]. Moreover, network pharmacology delves into the impact of plant bioactive constituents on both the organism's interactome and the diseasome levels[14].

In this study, a pharmacology network was used to deduce the potential curative benefits and pharmacology activities of the active phytocompounds sourced from two herbal plants, *W. somnifera* and *A. barbadensis*. *In silico* docking was also utilized to analyze their binding affinities at the diseasome level, with a specific focus on multiple myeloma.

2. Materials and methods

The network pharmacology approach was implemented, adhering to a sequence of essential procedural stages. All information relating to bioactives, their targets, immune gene targets, immune system pathways (I-PW), protein-protein interactions (PPi), gene targets relevant to multiple myeloma, the interplay between bioactives and immune targets for multiple myeloma and I-PW-associated interacting proteins (IA-IP) was derived from different knowledge bases (databases). The focal health condition under investigation throughout this study was multiple myeloma.

2.1. Chemical composition of each herbal plant

An exhaustive literature search was conducted for each herbal remedy, aiming to capture all available information regarding their constituents. The compound composition of these plants was further extracted from Dr. Duke's Phytochemical Database (https://phytochem.nal.usda.gov/) and the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) (https://cb.imsc.res.in/imppat/)[15]. Structure files of molecules, provided in mol format, were retrieved from PubChem[16] and ChEMBL[17], including their canonical SMILES.

2.2. Screening of potential active phytocompounds

The attributes of absorption, distribution, metabolism, and excretion (ADME) were recognized as pivotal indicators of herb or potential medication potency. To unveil the possible bioactive components within each of the two herbal plants, three ADME-related models were used, including the evaluation of oral bioavailability (OB) and drug-likeness (DL). The technique also includes the identification and inclusion of the main components present within the plants[17]. OB refers to the percentage of an orally administered dose of a medication that enters the systemic circulation unaltered. High OB is frequently indicative of bioactive compounds possessing drug-like properties suitable for medicinal applications[18]. DL is a qualitative concept employed in drug design to gauge the "drug-likeness" of a prospective product. This assessment aids in the optimization of pharmacokinetic and medicinal characteristics, including solubility and chemical stability. Typically, variables such as OB ≤30 – 33% and/or DL ≤0.1 are widely adopted criteria[19].

The ADMET profiles of all potential substances were estimated using SwissADME and AdmetSar 2.0. In addition, the DL score was estimated based on "Lipinski's Rule of Five" parameters (molecular weight, Log P, hydrogen bond donors, and hydrogen bond acceptors) using the Molinspiration online web server[20,21].

2.3. Targets of the bioactives

Target genes/proteins in humans that interact with active phytochemical substances from two herbal plants were investigated using the Similarity Ensemble Approach (SEA)[22], SwissTargetPrediction[23], and PharmMapper[24].

Data for each protein, including its standard protein name, gene ID and organism (set to *Homo sapiens*), were derived from UniProt[25] ("UniProt: a hub for protein information," 2014) using the UniProt ID provided in BindingDB.

2.4. Related targets in the immune system and the involved pathways

Related targets in the immune system were searched using the innate immune database[25]. Subsequently, the STRING database and the pathway database were utilized to determine the different pathways associated with the immune system. The I-PW were then evaluated and analyzed.

Moreover, the STRING database was used to identify the interacting partners of these bioactive targets searched in the KEGG pathway to find IA-IP.

2.5. Targets of multiple myeloma and control drugs

The multiple myeloma-associated human genes/proteins were obtained from two different databases: GeneCards[26] and DisGeNET[27]. The search query employed the specific term "multiple myeloma," with the search parameters confined to the species "*Homo sapiens*." The targets of control IMiDs (Lenalidomide and Thalidomide) were also retrieved from GeneCards and Disgenet, subsequently overlapping with the targets of the identified bioactives.

2.6. PPi

The overlap of related gene targets of the bioactives and the immune system was regarded as the bioactives' immune targets at the interactome level. Similarly, the overlap of related gene targets of the bioactives' immune targets and multiple myeloma was regarded as bioactive-multiple myeloma's immune gene targets at the diseasome level. These interactions were obtained using Venny 2.0[28]. Subsequently, the STRING database was employed to identify the possible inter-protein interactions[29]. This database compiles both established and predicted PPi. STRING uses five sources to uncover relationships within the database: genomic context predictions, high-throughput lab trials, co-expression, automated text mining, and existing database information. Several variables for STRING-based PPi identification can be used to ensure the reliability of the generated data. These variables include interactions derived exclusively from high-throughput laboratory experiments, a minimum required interaction score of 0.7 (a high confidence score according to STRING), and the highest score of interactors.

2.7. Network construction

A network is a diagrammatic representation that depicts the interactions between numerous components known as nodes. These nodes are interconnected by edges, which are lines that connect them. In this study, the nodes encompass the herbal plants under investigation, the bioactives within these herbal plants, the bioactives' targets, and the

I-PWs associated with these targets. Furthermore, the nodes include the connection between bioactive-multiple myeloma's immune targets.

The bioactive-immune target (compound-target [C-T]) (interactome level) and the bioactive-multiple myeloma's immune gene target (diseasome level) networks were constructed by linking the active compounds and their corresponding targets, and the active compounds, their immune targets and their corresponding multiple myeloma targets, respectively. In addition, the bioactive-multiple myeloma's immune targets-pathway (compound-target-pathway [C-T-P]) network was built by linking the bioactive-multiple myeloma's immune targets with relevant signaling pathways.

These networks were constructed and visualized using Cytoscape 3.2.1, an open-source Java-based software platform designed for visualizing complex networks and integrating them with diverse attribute data[30]. Furthermore, the Cytoscape network analyzer tool facilitated network analysis.

In these networks, nodes represent active phytochemical compounds derived from the herbal plant, targets, or signaling pathways, while edges signify the interactions between the nodes[31]. The degree of a node corresponds to the number of connections it maintains to other nodes within the network[32].

2.8. Functional enrichment analysis (gene ontology [GO]) at the interactome and diseasome levels

The functional enrichment analysis of the bioactives' immune targets (interactome level) and also the analysis of the bioactive-multiple myeloma's immune gene targets (diseasome level) was conducted using two tools: These tools are robust online server-based platforms specialized in functional STRING and g: Profiler profiling of gene or protein sets. To identify the immune pathways and other interacting pathways involving the bioactives, the KEGG database was employed. The GO process involves the identification of biological processes, molecular functions, and cellular compartments associated with these gene sets. It also provided estimations of the degree of enrichment of these gene sets in each of the three different categories[33]. This comprehensive analysis aimed to identify the plants' target-immune pathways, as well as their relevant interacting pathways on the interactome level and also on the diseasome level (which concerns multiple myeloma)[34].

2.9. Molecular docking simulation

The molecular docking simulation of the hub genes (core genes) and their corresponding bioactive components was performed using PyRx software[35], an application that allows for interactively visualizing and analyzing molecular structures and data. To begin, we downloaded the 3D structures of chemicals and proteins from Pubchem and the Protein Data Bank (PDB)[31], respectively. Subsequently, using the Discovery Studio[36] and the Dock Prep plugin (in the Chimera program), the structures of proteins and bioactives were prepared for the docking process. This encompassed replacing missing side chains, as well as adding hydrogens and charges, all integral to the preparation process. Furthermore, the molecular docking was carried out utilizing the PyRx software and the AutoDock Vina plugin[37]. Finally, the Discovery Studio and Ligplot+ were used to visually present 3D and docking models[38].

3. Results

3.1. Chemical composition of each herbal plant

After eliminating duplicates, a total of 67 compounds were identified in *W. somnifera*, and a total of 159 compounds were identified in *A. barbadensis*, resulting in a combined count of 226 compounds.

3.2. Screening of potential active compounds/ingredients

All 226 compounds underwent ADMET and drug-likeness screenings. Among these, 19 bioactives were sourced from *A. barbadensis*, and 18 bioactives were sourced from *W. somnifera*. These bioactives include the major constituents of each plant, even though some did not meet the inclusion criteria. Consequently, 36 compounds were selected as phytocompounds for further analysis (Table 1).

3.3. Targets of the bioactives

Active phytocompounds in *A. barbadensis* bioactives predicted a total of 590 targets, while *W. somnifera* bioactives indicated 684 targets. Using Swiss Target Prediction and Pharmmapper, a total of 820 targets emerged after all duplicates were removed.

3.4. Related targets in the immune system and the involved pathways

A total of 1378 immune genes were identified from the innate immune database. An overlap between these immune gene targets and the bioactives' targets produced a total of 169 bioactive immune targets.

3.5. Targets of multiple myeloma and control drugs

From GeneCards, 3,045 multiple myeloma gene targets were retrieved, and Disgenet contributed a further 1740, culminating in 1088 gene targets associated with multiple myeloma after eliminating duplicates.

Table 1. The values for the chemical descriptors used in the prediction of potential active compound

Compounds	Mol. form.	Mol. weight	nHBA	nHBD	OB	DL	Toxicity
Aloesin*	$C_{19}H_{22}O_9$	394.37	9	5	0.55	0.33	III
Aloesol*	$C_{13}H_{14}O_4$	234.25	4	2	0.55	0.26	III
Aloin*	$C_{21}H_{22}O_9$	418.39	3	9	0.55	0.52	III
Rhein*	$C_{15}H_8O_6$	284.22	6	3	0.55	0.46	II
Steroids*	$C_{22}H_{32}O_3$	344.49	3	0	0.55	1.23	III
Thiamin*	$C_{12}H_{17}N_4OS$	265.35	3	2	0.55	0.87	III
Xylose*	$C_5H_{10}O_5$	150.13	5	4	0.55	0.29	IV
Homonataloin*	$C_{22}H_{24}O_9$	432.42	9	6	0.55	0.35	III
Beta sitosterol*	$C_{29}H_{50}O$	414.71	1	1	0.55	0.78	I
Stigmasterol*	$C_{29}H_{48}O$	412.69	1	1	0.55	0.62	I
10-Hydroxyaloin*	$C_{21}H_{22}O_{10}$	434.39	10	8	0.55	0.86	IV
Casanthranol*	$C_{21}H_{22}O_{10}$	434.39	10	7	0.55	0.61	III
Kampferol*	$C_{15}H_{10}O_6$	286.24	6	4	0.55	0.5	II
7-hydroxyaloin*	$C_{21}H_{22}O_{10}$	434.39	10	8	0.55	0.36	IV
Folic acid*	$C_{19}H_{19}N_7O_6$	441.4	9	6	0.55	1.09	III
Epicatechin*	$C_{15}H_{14}O_6$	290.27	6	5	0.55	0.64	IV
(-)-Desoxyaloin*	$C_{21}H_{22}O_8$	402.39	8	6	0.55	0.47	
L pyroglutamic acid*	$C_5H_7NO_3$	129.11	3	2	0.55	0.3	III
Niacinamide*	$C_6H_6N_2O$	122.12	2	1	0.55	0.37	IV
24-methyldesmosterol**	$C_{28}H_{46}O$	398.66	1	1	0.55	0.76	II
27-deoxywithaferin A**	$C_{28}H_{38}O_5$	454.6	5	1	0.55	0.29	IV
2,3-didehydrosomnifericin**	$C_{28}H_{40}O_7$	488.61	7	4	0.55	0.52	III
Delta-7-alvenasterol**	$C_{29}H_{48}O$	412.69	1	1	0.55	0.25	III
Guggleserone**	$C_{21}H_{28}O_2$	312.45	2	0	0.55	0.72	III
Hydrocortisone**	$C_{21}H_{30}O_5$	362.46	5	3	0.55	1.67	III
Sominone**	$C_{28}H_{42}O_5$	458.63	5	3	0.55	0.39	III
Somniferanolide**	$C_{28}H_{36}O_6$	468.58	6	2	0.55	0.19	I
Somniwithanolide**	$C_{28}H_{38}O_6$	470.6	6	3	0.55	0.5	III
Withaferin A**	$C_{28}H_{38}O_6$	470.6	6	2	0.55	0.37	I
Withanolide G**	$C_{28}H_{38}O_5$	454.6	5	2	0.55	0.5	IV
Withanolide Q**	$C_{28}H_{38}O_6$	470.6	6	3	0.55	0.75	III
Quercetin**	$C_{15}H_{10}O_7$	302.24	7	5	0.55	0.52	II
Somniferine**	$C_{36}H_{36}N_2O_7$	608.68	9	2	0.55	1.09	III
Somnifericin**	$C_{28}H_{42}O_7$	490.63	7	4	0.55	0.54	III
Dihydrowithaferin A**	$C_{28}H_{40}O_6$	472.61	6	2	0.55	0.42	I
Chlorogenic acid**	$C_{16}H_{18}O_9$	354.31	9	6	0.55	0.79	III

Notes: *refers to phytocompounds in *Aloe barbadensis*, while **refers to phytocompounds in *Withania somnifera*. Toxicity class I to VI ranges from fatal if swallowed to non-toxic.

Abbreviations: Mol. Form: Molecular formula; Mol. weight: Molecular weight; nHBA: Number of hydrogen bond acceptors; nHBD: Number of hydrogen bond donors; OB: Oral bioavailability; DL: Drug likeness.

The sum of targets for the control IMiDs, Thalidomide (20 targets retrieved) and Lenalidomide (28 targets retrieved), totaled 48. This set of targets overlapped with the bioactives' immune targets, resulting in a total of 90 bioactive-multiple myeloma immune gene targets.

3.6. Network-based evaluation of the immunomodulatory mechanisms of active phytocompounds

To comprehend the "multi-components, multi-target, and multi-pathway" immunomodulatory mechanisms of *W. somnifera* and *A. barbadensis*, a compound-target network was constructed. This network linked active phytochemical compounds to their corresponding immune targets, which were subsequently analyzed. The compound-target network for these two herbal plants comprised 205 nodes and 1338 edges, encompassing 36 active compounds and 169 targets. Among the active phytochemical compounds, their categorization was based on degrees of connectivity. Notably, withanolide Q (degree = 70), 2,3-didehydrosomnifericin (degree = 69), steroids (degree = 65), hydrocortisone (degree = 63), 27-deoxywithaferin A (degree = 62), withaferin A (degree = 61), somniferine (degree = 59), withanolide G (degree = 58), somniferanolide (degree = 55), Ssominone (degree = 53), chlorogenic acid (degree = 47), somniwithanolide (degree = 47), rhein (degree = 43), gugglesterone (degree = 39), and somnifericin (degree = 39) displayed the highest number of connections with the immune-related targets. This pattern indicated their potential role as primary active compounds responsible for the immunomodulatory activity of *W. somnifera* and *A. barbadensis*. Furthermore, more than 70 targets demonstrated interactions with two or more active phytochemical compounds, demonstrating the multi-compound, multitarget pharmacological properties of these herbal plants.

The STRING database also identified the PPi, forming a network comprising 169 nodes and 1,553 edges, with a PPi enrichment *P*-value of <1.0e-16 (Figure 1). This value indicates that the interactions among these proteins exceed what would be expected in a randomly selected group of proteins with equivalent size and degree distribution from the genome. This enrichment signifies that these proteins are interconnected beyond chance, indicating a cohesive physiological relationship among them. On delving into the disease-gene association, several immune-associated diseases were identified: autoimmune lymphoproliferative syndrome, myeloid neoplasm, combined immunodeficiency, autoimmune disease of the musculoskeletal system, primary immunodeficiency disease, autoimmune disease, immune system disease, and hematopoietic system disease. A subset of these targets also displayed connections to various immune pathways (Table 2), thereby shedding light on the pathways through which the bioactives exert their immunomodulatory effects.

3.7. Network-based analysis of active phytocompounds' immunomodulatory potential in multiple myeloma

To delve into the network-based analysis of the systems-level immunomodulatory properties of the phytocompounds present within the herbal plants, a C-T network was constructed (Figure 2). This network comprised 99 nodes and 447 edges, achieved through associating active compounds with their corresponding multiple myeloma-gene targets. The nodes included 37 active compounds, 2 control drugs, and 60 bioactive-multiple myeloma immune gene targets. Targets not involved in KEGG immune pathways were excluded.

Within this network, 10 active phytochemical compounds, including withanolide Q (degree = 25), hydrocortisone (degree = 24), somniferine (degree = 22), 2,3-didehrdrosomnifericin (degree = 22), 27-deoxywithaferin A (degree = 21), steroids (degree = 20), withaferin A (degree = 19), withanolide G (degree = 17), somniferanolide (degree = 17), and lenalidomide (degree = 16), had the most linkages with multiple myeloma-related targets (Figure 2), thereby implying their pivotal roles as key phytocompounds responsible for the herbal plants' immunomodulatory efficacy against multiple myeloma. Furthermore, 40 targets displayed two or more interactions with the active phytocompounds (Figure 2), demonstrating herbal medicines' multi-compound, multi-target pharmacology.

In the context of the bioactive-multiple myeloma's immune (C-T) network, the hub node target genes were identified. Nodes possessing a degree larger than or equal to twice the network's average node degree were classified as hubs. Core nodes, in particular, have been demonstrated to play an essential role in a wide variety of biological processes[39]. Among the multiple myeloma-associated targets of the bioactives, several stand out as core gene targets with potential roles in the immunomodulatory potential of active phytocompounds in herbal plants (Figure 3). Specifically, these include alpha serine/threonine protein kinase 1 (AKT1) (degree = 29), HCK proto-oncogene (HCK) (degree = 27), 3-phosphoinositide-dependent protein kinase 1 (PDPK1) (degree = 26), Janus kinase 2 (JAK2) (degree = 26), proto-oncogene c-KIT (KIT) (degree = 24), matrix metallopeptidase 9 (MMP9) (degree = 23), growth factor receptor bound protein 2 (GRB2) (degree = 22), interleukin 2 (IL2) (degree = 21), Bruton tyrosine kinase (BTK) (degree = 19), and mouse double minute 2 homolog (MDM2) (degree = 19).

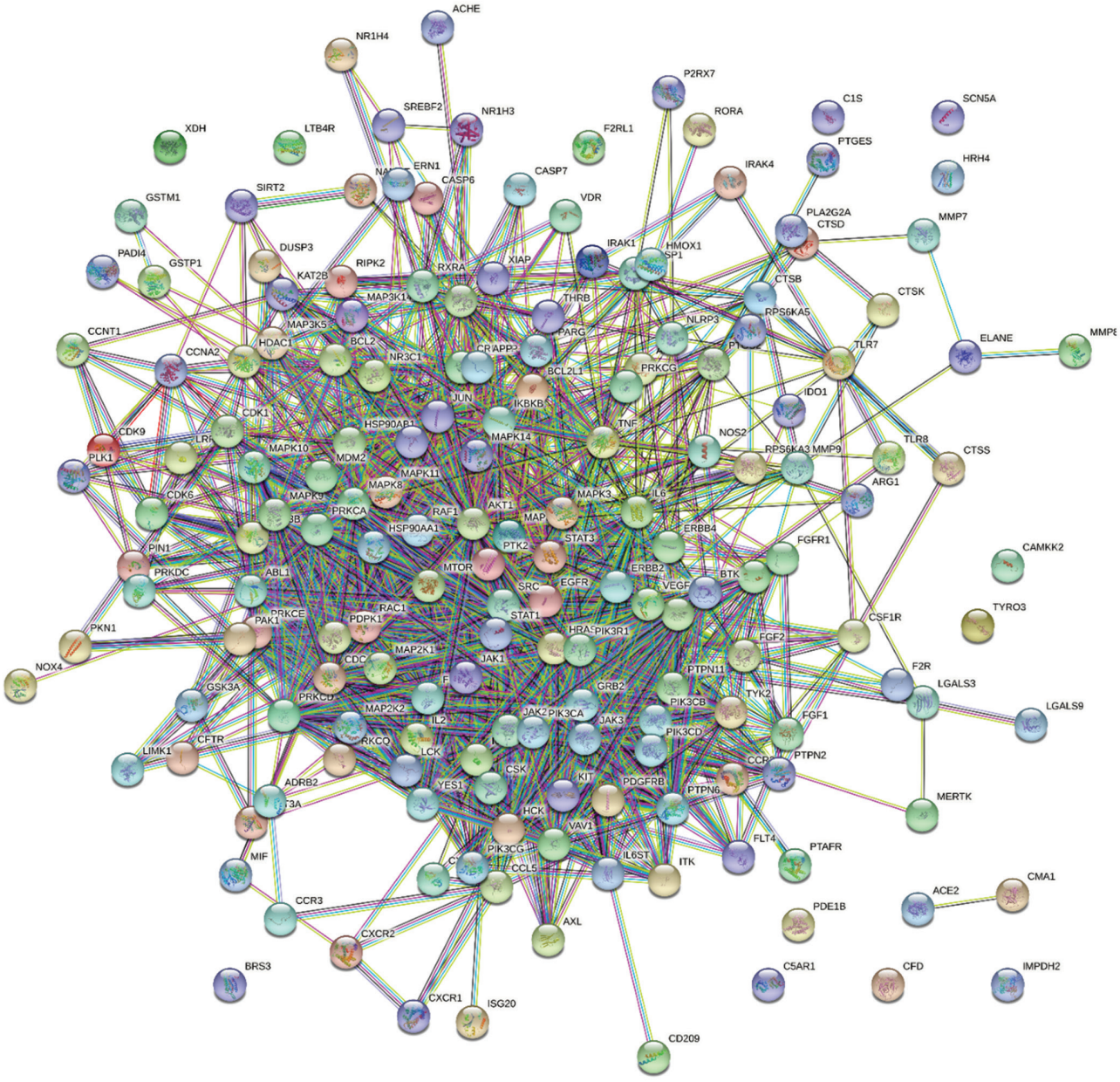


Figure 1. Diagrammatic representation of the protein-protein interaction (with a confidence score of 0.7 and above) between the different bioactives' immune targets. Round globes indicate each bioactive immune target; the lines show their interactions with one another; round globes without lines indicate a confidence interaction score lower than 0.

Furthermore, a PPi interaction was also carried out on the multiple myeloma-related targets of the bioactives, leading to the identification of hub targets. This network comprised 65 nodes and 554 edges, demonstrating a PPi enrichment *P*-value of <1.0e-16. Among these hub targets, key players emerged, including mitogen-activated protein kinase 3 (MAPK3), signal transducer and activator of transcription 3 (STAT3), alpha serine-threonine protein kinase 1 (AKT1), Harvey rat sarcoma virus (HRAS), heat-shock protein 90-alpha (HSP90AA1), growth factor receptor bound Protein 2 (GRB2), phosphatidylinositol 3-kinase regulatory subunit alpha (PIK3R1), interleukin 2 (IL2), and interleukin 6 (IL6).

3.8. Functional enrichment analysis of the active phytocompounds immune targets

GO enrichment analysis was used to investigate the functional roles of the immunomodulatory-associated

Table 2. Depicting all of the bioactives' immune gene targets as well as the immune pathways in which they are involved

KEGG ID	IMMUNE PATHWAY	GENE (NO)	GENE INVOLVED FROM THE LIST
hsa04622	RIG-I-like receptor signaling pathway	23	NFKBIA, NFKB1, MAPK14, TRAF2, RIPK1, IFNA1, CASP10, CXCL10, TRIM25, CASP8, ATG5, CHUK, IFNA2, IFNB1, MAPK8, RELA, CYLD, MAPK9, TNF, IKBKB, TRAF6, TRAF3, IKBKG
hsa04666	Fc gamma R-mediated phagocytosis	20	MAPK1, PIK3R2, RAF1, MAPK3, PIK3CA, FCGR2A, PIK3CB, ARPC5, MAP2K1, SPHK1, GAB2, FCGR3A, SYK, PIK3CD, INPP5D, PRKCA, PTPRC, PIK3R1, HCK, AKT1
hsa04670	Leukocyte transendothelial migration	18	PIK3R2, MAPK14, PIK3CA, ICAM1, PIK3CB, PTPN11, PTK2, CTNNB1, ACTB, MMP9, PIK3CD, CXCL12, ITGB1, PTK2B, ITGB2, CXCR4, PRKCA, PIK3R1
hsa04611	Platelet activation	16	MAPK1, PIK3R2, MAPK14, MAPK3, PIK3CA, FCGR2A, PIK3CB, ACTB, PIK3CG, SYK, PIK3CD, ITGB1, PIK3R1, AKT1, ITGB3, BTK
hsa04640	Hematopoietic cell lineage	14	CD22, EPOR, KITLG, IL4, IL1A, IL1B, CSF1R, KIT, CD14, CD1D, IL6, TNF, CD19, ITGB3
hsa04658	Th1 and Th2 cell differentiation	27	MAPK1, IL2RB, NFKBIA, NFKB1, IL2, IFNG, MAPK14, IL4, MAPK3, NOTCH1, STAT5B, IL13, FOS, STAT5A, JAK1, STAT1, CD247, CHUK, JUN, IL2RG, JAK2, MAPK8, RUNX3, RELA, MAPK9, IKBKB, IKBKG
hsa04664	Fc epsilon RI signaling pathway	27	MAPK1, PIK3R2, MAPK14, IL4, RAF1, KRAS, MAPK3, PIK3CA, PIK3CB, MAP2K1, IL13, PDPK1, GAB2, NRAS, SYK, PIK3CD, GRB2, MAPK8, MAP2K7, MAPK9, TNF, INPP5D, HRAS, PRKCA, PIK3R1, AKT1, BTK
hsa04650	Natural killer cell mediated cytotoxicity	33	MAPK1, PIK3R2, IFNG, KLRK1, TNFSF10, RAF1, KRAS, MAPK3, PIK3CA, ICAM1, IFNAR1, IFNA1, PIK3CB, MAP2K1, PTPN11, CD247, FCGR3A, NRAS, NCR2, SYK, PIK3CD, IFNA2, IFNB1, GRB2, PTK2B, ITGB2, PTPN6, TNF, HLAB, HRAS, PRKCA, MICA, PIK3R1
hsa04062	Chemokine signaling pathway	39	MAPK1, NFKBIA, PIK3R2, CCL2, NFKB1, CCR7, RAF1, KRAS, MAPK3, PIK3CA, STAT3, PIK3CB, CCR2, STAT5B, MAP2K1, CXCL10, CXCL11, CCR4, PTK2, GPR29, PIK3CG, STAT1, NRAS, CHUK, CXCR3, PIK3CD, JAK2, GRB2, CXCL12, PTK2B, RELA, CXCR4, HRAS, PIK3R1, IKBKB, HCK, AKT1, CCL5, IKBKG
hsa04662	B cell receptor signaling pathway	30	CD22, MAPK1, NFKBIA, PIK3R2, NFKB1, RAF1, KRAS, MAPK3, PIK3CA, PIK3CB, MAP2K1, FOS, MALT1, NRAS, CHUK, BCL10, JUN, SYK, PIK3CD, GRB2, RELA, PTPN6, INPP5D, HRAS, PIK3R1, IKBKB, CD19, AKT1, BTK, IKBKG
hsa04657	IL-17 signaling pathway	34	MAPK1, NFKBIA, CCL2, NFKB1, IFNG, MAPK14, IL4, TRAF2, MAPK3, IL1B, HSP90B1, IL13, CXCL10, FOS, MAPK7, HSP90AA1, IL17A, CASP8, PTGS2, S100A9, CHUK, JUN, MMP9, LCN2, MAPK8, RELA, IL6, MAPK9, TNF, IKBKB, TRAF6, TRAF3, TNFAIP3, IKBKG
hsa04659	Th17 cell differentiation	38	MAPK1, IL2RB, NFKBIA, TGFB1, NFKB1, IL2, IFNG, MAPK14, IL4, AHR, MAPK3, IL1B, IL21, STAT3, STAT5B, FOS, SMAD3, HSP90AA1, STAT5A, JAK1, IL17A, STAT1, MTOR, CD247, CHUK, JUN, IL2RG, IRF4, IL6ST, JAK2, MAPK8, RELA, IL6, MAPK9, IKBKB, HIF1A, IL22, IKBKG
hsa04620	Toll-like receptor signaling pathway	47	MAPK1, NFKBIA, PIK3R2, NFKB1, MAPK14, RIPK1, MAPK3, MAP3K8, IL1B, PIK3CA, CD80, IFNAR1, CTSK, IFNA1, PIK3CB, TLR3, MAP2K1, CD14, CXCL10, FOS, CXCL11, CD86, CASP8, STAT1, IRAK1, CHUK, JUN, CD40, TLR4, PIK3CD, IFNA2, IFNB1, SPP1, MAPK8, MAP2K7, RELA, IL6, MAPK9, TNF, MYD88, PIK3R1, IKBKB, TRAF6, AKT1, TRAF3, CCL5, IKBKG
hsa04660	T cell receptor signaling pathway	43	MAPK1, NFKBIA, PIK3R2, NFKB1, IL2, IFNG, MAPK14, IL4, RAF1, KRAS, MAPK3, MAP3K8, PIK3CA, PIK3CB, MAP2K1, CTLA4, FOS, MALT1, CD28, PDCD1, PDPK1, CD247, NRAS, CHUK, BCL10, JUN, PIK3CD, GRB2, MAPK8, MAP2K7, RELA, PTPN6, MAPK9, TNF, HRAS, PTPRC, IL10, NCK1, PIK3R1, IKBKB, AKT1, MAP3K14, IKBKG
hsa04625	C-type lectin receptor signaling pathway	42	BCL3, MAPK1, NFKBIA, PIK3R2, NFKB1, IL2, MAPK14, IRF1, RAF1, KRAS, MDM2, MAPK3, IL1B, PIK3CA, PIK3CB, MALT1, NLRP3, PTPN11, CASP8, STAT1, MAPKAPK2, PTGS2, NRAS, NFKB2, CHUK, BCL10, JUN, SYK, PIK3CD, MAPK8, RELA, IL6, CYLD, MAPK9, TNF, HRAS, IL10, PIK3R1, IKBKB, AKT1, MAP3K14, IKBKG
hsa04621	NOD-like receptor signaling pathway	46	MAPK1, NFKBIA, MEFV, NAMPT, CCL2, NFKB1, MAPK14, TRAF2, RIPK1, MAPK3, IL1B, BIRC3, YWHAE, IFNAR1, IFNA1, IL18, BCL2L1, P2RX7, HSP90AA1, NLRP3, TRPV2, JAK1, CASP8, STAT1, ATG5, CHUK, XIAP, JUN, TLR4, TXN, IFNA2, IFNB1, MAPK8, BCL2, RELA, IL6, MAPK9, TNF, MYD88, IKBKB, TRAF6, TRAF3, CCL5, BIRC2, TNFAIP3, IKBKG
hsa04623	Cytosolic DNA-sensing pathway	16	NFKBIA, NFKB1, RIPK1, IL1B, IFNA1, IL18, CXCL10, ADAR, CHUK, IFNA2, IFNB1, RELA, IL6, IKBKB, CCL5, IKKG
hsa04672	Intestinal immune network for IgA production	14	TGFB1, IL2, AICDA, IL4, CD80, IL15, CD28, CD86, CD40, CXCL12, IL6, CXCR4, IL6, MAP3K14

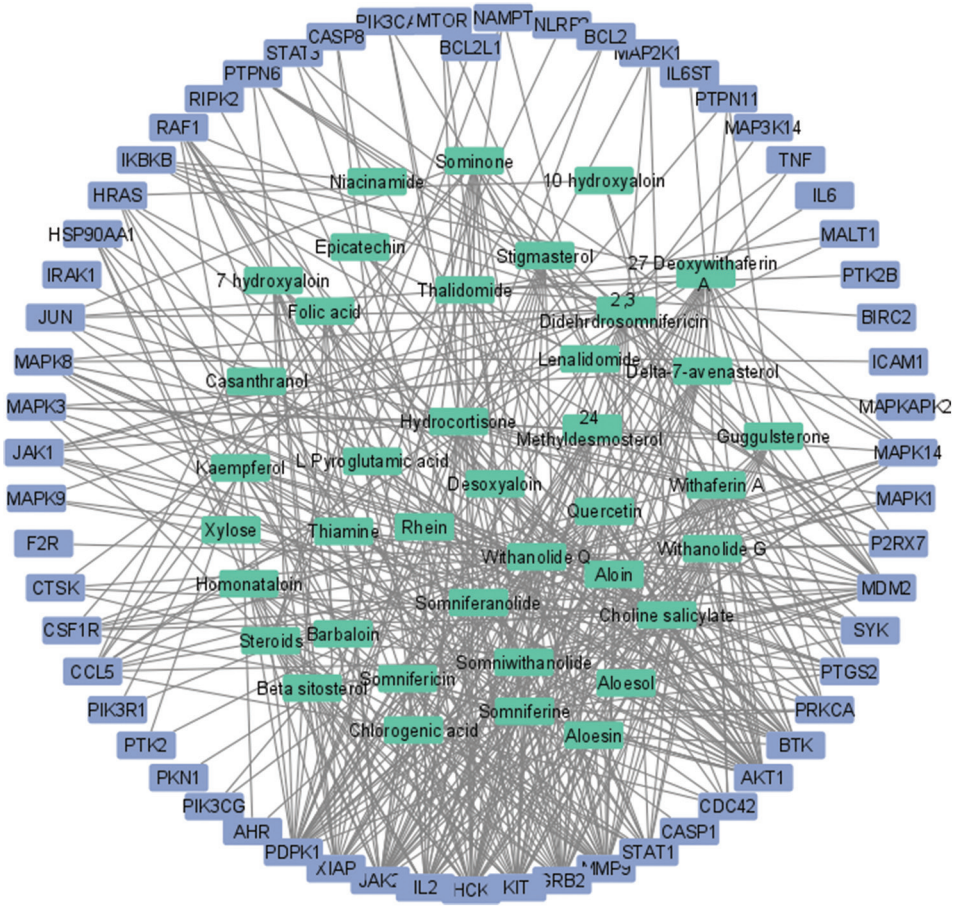


Figure 2. A diagrammatic representation of the Compound-Target Network of the bioactives and the multiple myeloma-associated gene targets. Turquoise boxes indicate the bioactives/compounds; cobalt blue boxes indicate the bioactive-multiple myeloma gene targets; black lines indicate the interactions between the two components.

targets within active phytocompounds in the context of multiple myeloma.

The targets in GO terms associated with the regulation of diverse biological processes (GO: BP) were significantly enriched. These processes include cellular response to chemical stimulus, response to organic substance, cell differentiation, cell proliferation, cell migration, immune response, regulation of programmed cell death, regulation of immune system process, and more.

Similar enrichments were evident with the targets in GO terms associated with molecular functions (GO: MF). These functions include protein kinase activity, phosphotransferase activity, alcohol group as acceptor, catalytic activity acting on a protein, enzyme binding and more.

The significant enrichment of the targets in GO terms associated with their cellular compartments (GO: CC) was also observed. The distribution spanned compartments such as the cytosol, cell periphery, cytoplasm, cytoplasmic vesicle, intracellular vesicle, and beyond.

The immune system is known to be closely controlled by the precise coordination of many important signaling pathways. Deviations in the regulation of immune-related pathways can result in a variety of immunological disorders. To delve further, KEGG pathway enrichment analysis was employed (Figure 4). This pathway-level analysis of the active phytocompounds immune-related targets revealed connections with at least 17 immune pathways and other relevant pathways (Figure 3). These encompass, among others, the PI3-AKT pathway, VEGFA pathway, JAK-STAT signaling pathway, and mTOR signaling pathway.

3.9. Hub gene targets and molecular docking simulation

The top 10 hub genes from the top 40 compound-target networks between the bioactives and the bioactive-multiple myeloma gene targets were identified using

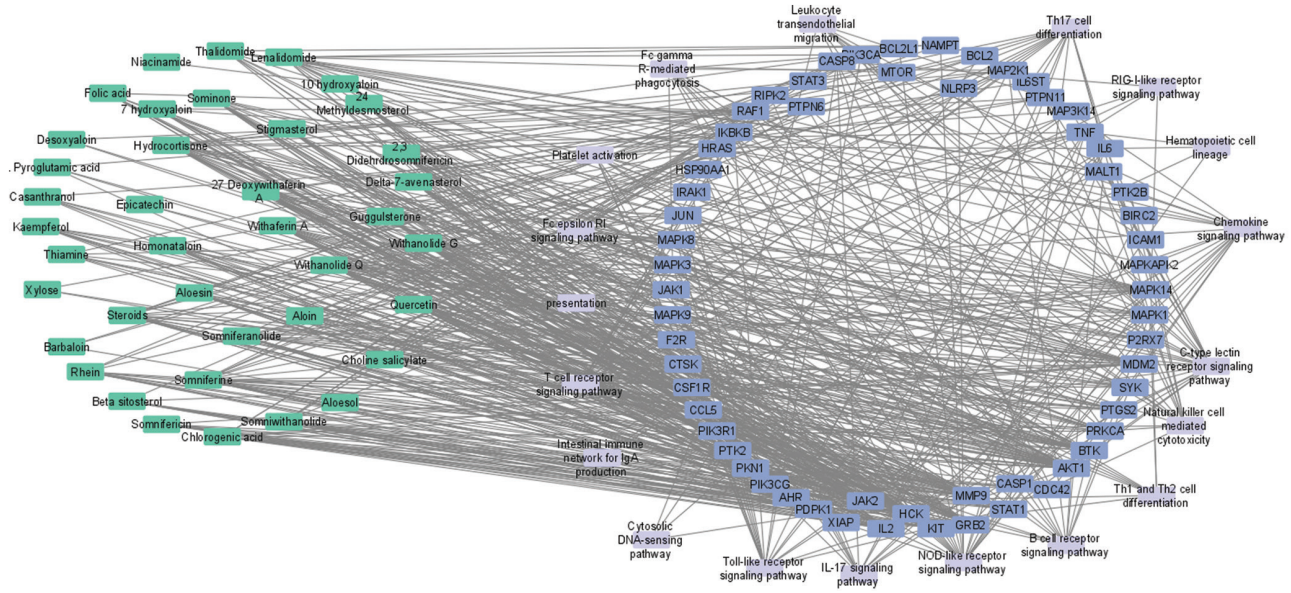


Figure 3. A diagrammatic representation of the Compound-Target-Immune Pathway Network between the bioactives and the bioactive-multiple myeloma immune-associated gene targets. Turquoise boxes indicate the bioactives; cobalt blue boxes indicate the bioactive-multiple myeloma immune-associated gene targets; lilac boxes indicate the immune pathways these gene targets are associated with; the lines represent their interaction.

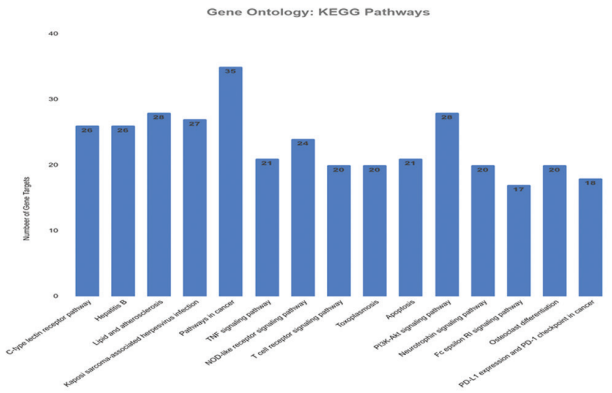


Figure 4. The number of active phytocompounds' gene targets involved in the top 15 signaling pathways, including immune and other important pathways.

Cytohubba. Subsequently, molecular docking simulations were performed using corresponding immunomodulatory bioactives selected from the top 21 bioactives. These bioactives encompassed AKT1, JAK2, HCK, PDPK1, MMP9, KIT, GRB2, IL2, MDM2, BTK, MAPK3, STAT3, HRAS, and HSP90AA1, which were included in the targets designated for docking. The selected bioactives included withanolide Q, hydrocortisone, somniferine, 2,3-didehrdrosomnifericin, 27-deoxywithaferin A, steroids, withaferin A, withanolide G, somniferanolide, somniwithanolide, sominone, quercetin, chlorogenic acid, rhein, homonataloin, 7-hydroxyaloin, delta-7-avenasterol, folic acid, somnifericin, gugglesterone, and stigmasterol. The interaction relationships between the aforementioned hub genes and their corresponding bioactives were analyzed. Simultaneously, lenalidomide and thalidomide were selected as the positive control drugs.

Docking scores were obtained after the binding of phytoconstituents to specific multiple myeloma targets. Lower docking scores indicated higher and stronger binding affinity of components to their respective target. Molecular docking is a technique for predicting energetically advantageous ligand binding conformations within a target protein's active region[40]. This binding was accompanied by the generation of hydrogen bonds, as well as hydrophobic interactions such as Pi-Pi and Pi-cation interactions. These interactions collectively indicated the stability of complexes generated subsequent to ligand binding with the target protein[40]. A total of 21 phytoconstituents of *W. somnifera* and *A. barbadensis* were screened against various targets of multiple myeloma.

The individual docking scores (binding affinity energy) of the selected phytoconstituents are presented in Table 3. Furthermore, detailed insights into the receptor-ligand interactions in both three-dimensional (3D) and two-dimensional (2D) are showcased in Figures 5-7. From these analyses, the top three complexes were selected based on the interactions between the ligand-protein, binding affinity and the ADMET profiles of the compounds. The 2D diagrams present the binding process, highlighting the formation of hydrogen bonds and hydrophobic

Table 3. Illustrating the Target Binding Affinity of the hub targets and some of the highly connected active phytocompounds from the network analysis

Phytochemical	AKT1	HCK	PDPK1	JAK2	KIT	MMP9	GRB2	IL2	BTK	MDM2	MAPK3	STAT3	HRAS
Withanolide Q	−12.4	−9.6	−10.5	−9.8	−8.7	−8.5	−8	−7.6	−9.8**	−8.4	−9.1	−8.3	−9.2
Hydrocortisone	−10	−8.5	−7.8	−8	−8.8	−8	−6.5	−6.3	−8.6	−7.3	−7.8	−7.9	−7.4
2,3−didehrdrosomnifericin	−12.1	−9.4	−9.5	−7.9	−9.2	−8.5	−8	−7	−9.1	−7.6	−9.2	−8.2	−8.8
Somniferine	−8.9	−10.1*	−8.2	−8.4	−9.4	−10*	−8.6*	−7.8	−8	−8.3	−9.3	−9.3	−9.5
Withaferin A	−12.3	−9.3	−9.9	−9.3	−9.5	−9	−8	−7.5	−8.9	−8.2	−8	−8	−9.9*
Withanolide G	12.5*	−9.7	10.7*	10.3	10	−9.1	−8.6*	−7.9*	−9.2	−8.7*	−8.3	−8.3	−8.8
27−deoxywithaferin A	−12.1	−10	−9.9	−10.4	−10.5*	−9.2	−8.4	−7.5	−9.7	−8.1	−9.1	−9.1	−8.9
Somniferanolide	−13.1**	−9.7	−9.6	−10.6*	−10.1		−8.2	−7	−9.2	−8.7	−9.3	−9.3*	−9.9*
Somniwithanolide	−11.3	−8.9	−9.3	−7.1	−8.2	−8.2	−7.2	−6.3	−8.4	−7.1	−8.5	−8.5	−9
Chlorogenic acid	−9.3	−8.3	−8.5	−9.2	−7.9	−7.4	−7	−5.8	−7.6	−7.4	−8.7	−7.8	−8.6
Delta7avenasterol	−11.7	−8.8	−9.6	−9.6	−8.9	−8.5	−7.4	6.5	−8.7	−7.4	−8.9	−7.3	−8.5
Quercetin	−9.8	−9.1	−9.1	−8.8	−9.5	−9.7	−6.7	−6.6	−8.6	−7.1	−8.6	−8.2	−8.3
Rhein	−10.5	9.1	−9.9	−9.3	−9.1	−8.5	−7.1	−6.5	−9.1	−7.4	−9.1	−9.1	−7.7
7−hydroxyaloin	−8.9	−9.3	−8.4	−7.8	−7.8	−8.4	−6.9	−6.2	−8.1	−6.7	−8.1	−8	−7.8
Guggulsterone	−11.3	−8.8	−9.7	−7.3	−9	−8.4	−7.3	−6.8	−9.2	−8.3	−8.2	−8.9	−7.6
Homonataloin	−8.5	−8.4	−7.5	−8.2	−8.6	−8.3	−7	−6.4	−7.7	−7	−7.6	−8.1	−7.2
Somnifericin	−11.2	−9.5	−9.6	−10	−9.1	−8.4	−7.5	−7	−9	−8.4	−8.1	−9	−8.7
Folic acid	−10	−8.9	−9.5	−9.2	8.5	−8.6	−7.3	−6.8	−9.3*	−7.9	−10.1*	−8.7	−9.4
Stigmasterol	−11.4	−9	−9.7	9.3	−9	−8.1	−7.6	−6.5	−8.8	−8	−8	−8	−8.6
Controls													
Lenalidomide	−9.3	−8.9	−7.4	−8.4	−7.7	−7.7	−6.6	−5.7	−7.8	−6.6	−8.1	−7.9	−7.6
Thalidomide	−9.9	−8.8	−7.7	−8.6	−8	−9	−6.9	−6.2	−8.1	−6.9	−8.4	−7.4	−7.8

Notes: *Values with the highest binding affinity with regards to network pharmacology and mostly as a whole except the two instances. **Values with the highest binding affinity regardless of network pharmacology.

interactions between the amino acids of the receptors and the best-docked phytoconstituents.

The binding affinity energy table (Table 3) revealed that withanolide G, somniferine, and somniferanolide consistently emerged as the best-docked phytoconstituents across the majority of the multiple myeloma targets. This is indicative that the phytochemicals have the potential immunomodulatory capabilities in the context of multiple myeloma. With further validation, these compounds could prove to be useful lead compounds.

4. Discussion

This study harnessed the power of network pharmacology to investigate the immunomodulatory potential of *W. somnifera* and *A. barbadensis*. In the trajectory of multiple myeloma, patients experience progressive immunological dysfunction. As a result, several therapy options have emerged, aiming to navigate the immunosuppressive milieu of tumor microenvironments and stimulate the host's immune system, thereby eliciting an anticancer response[41]. While the intricate interplay among varied immunological components is not entirely understood, some advancements have brought us closer to a holistic view of the immune system and its role in host defense[42]. Capitalizing on their potent pharmacological attributes and minimal side effects, herbal medicines have become increasingly popular as therapeutic agents against various diseases[43]. Employing a network pharmacology approach, we investigated the functional roles of the gene targets of the bioactives. This exploration unraveled their immunomodulatory roles and the systems-level pharmacological processes that underpin the immunomodulatory effects of *W. somnifera* and *A. barbadensis* against multiple myeloma. This approach has previously been used in predicting potential lead compounds and understanding the mechanism of actions of different herbal plants in treating human diseases[31].

This study identified a selection of key gene targets, including AKT1, HRAS, HSP90AA1, GRB2, PIK3R1, and IL2. On delving into the interactome relationship

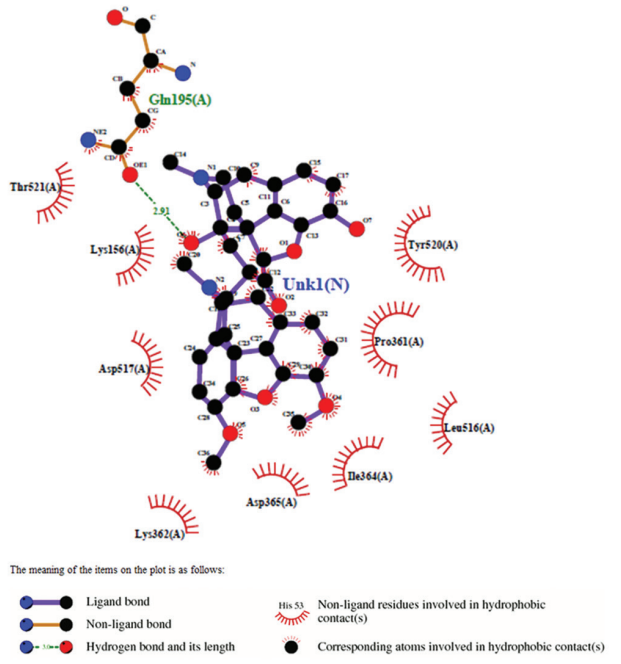


Figure 5. Ligplot+ analysis of the docked complex of the protein receptor HCK and somniferine. Protein residues involved include hydrophobic interactions represented as arcs and hydrogen bonding with dashed lines.

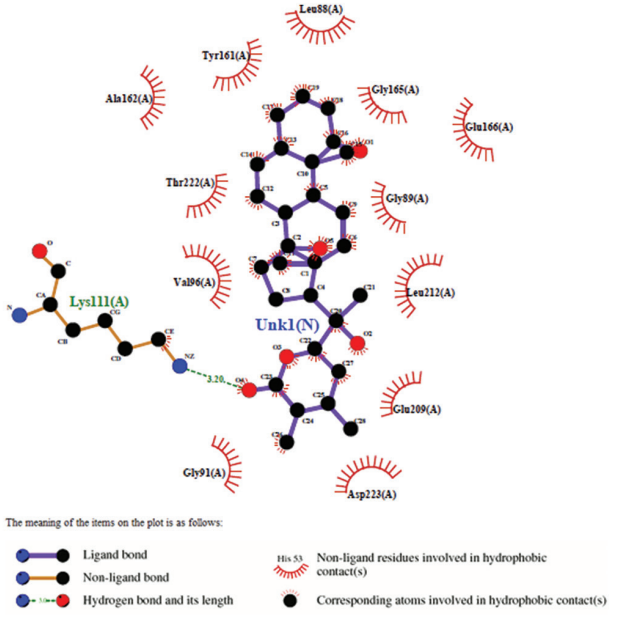


Figure 6. Ligplot+ analysis of the docked complex of the protein receptor PDPK1 and withanolide G. Protein residues involved include hydrophobic interactions represented as arcs and hydrogen bonding with dashed lines.

of the herbs' gene target, the majority of the gene targets exhibited interactions through the Toll-like receptor (TLR) signaling pathway. TLRs activate defense mechanisms that function autonomously from adaptive immunity. They

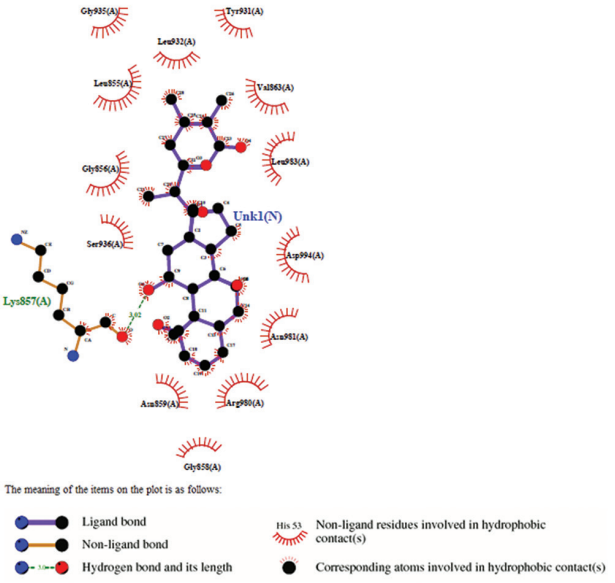


Figure 7. A ligplot+ analysis of the docked complex of the protein receptor JAK2 and somniferanolide. Protein residues involved include hydrophobic interactions represented as arcs and hydrogen bonding with dashed lines.

also play a significant part in the differentiation of naive T cells into effector T cells, a crucial step in adaptive immune responses. Transcription factors within the TLR signaling pathway (such as AP-1) regulate numerous genes, notably those encoding crucial proinflammatory cytokines like tumor necrosis factor-alpha (TNF-alpha), IL-1-beta, IL-6, IL-8, and IL-12. Certain TLRs also trigger interferon regulatory factors (IRFs) such as IRF3, IRF5, and IRF7, which subsequently bolster the production of type 1 (alpha and beta) interferons[44]. Another pathway through which most of the genes interact is the nucleotide-binding oligomerization domain (NOD) signaling pathway. Within this pathway, inflammasome-forming NOD-like receptors (NLRs) contribute to the conversion of procytokines into active IL-1 and IL-18, achieved by triggering caspase-1. TRAF is a group of cytoplasmic signaling adaptor proteins, responsible for packing, regulating and transducing IL1, TLRs, and IL17. These biological processes are vital for regulation of cell survival and immune cell function. These contribute significantly to regulating acute and chronic inflammation (Gissler *et al.*, 2022). Interleukins are involved in cell proliferation, migration, and also immune cell differentiation (Brocker *et al.*, 2010). Studies have shown that NF-κB are important in modulating the adaptive inflammatory response, such as enhanced expression of genes responsible for pro-inflammation. Contrarily, NOD-like receptors focus on regulating the host innate immune response by assembling mitogen-activated protein kinase (MAPK) and NF-κB (Franchi

et al., 2009). The release of inflammatory modulators is regulated by multiple signaling pathways, one of which is mitogen-activated protein kinase (MAPK) pathway. MAPK is responsible for multiple cellular processes, including cell survival, inflammation, stress response, and cell proliferation (Moens, Kostenko and Sveinbjørnsson, 2013).

Myeloid differentiation factor 88 (MyD88), TRIF, and other adaptor proteins bind to TLRs, inciting the activation of NF-κB and the consequent production of inflammatory cytokines[45]. This cascade triggers the expression of proinflammatory cytokines, chemokines, inducible enzymes such as cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), growth factors, immune receptors, and adhesion molecules such as intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) due to NF-κB activation[46]. The STAT protein family constitutes a potent force in immunoregulation. Several proteins, including JAK, GATA3 (a transcription factor that controls Th2 cytokine production), and RAR-related orphan receptor gamma (RORt-Th17 transcription factor), are involved in this process, as elucidated by O'Shea *et al.*[47]. Different STAT proteins also affect the levels of cytokines. Meanwhile, recent studies underscore the pivotal roles of STAT3 in tumor cell growth and proliferation, recommending it as a therapeutic target for inflammation-related diseases and tumor management[48].

The PI3K-Akt/mTOR pathway, which is targeted for anti-multiple myeloma treatment, has been identified as a hub. However, activating mutations in PI3K and AKT have not been detected in multiple myeloma patients[49]. At the same time, a small percentage of multiple myeloma patients experience a deficiency in the phosphatase and tensin homolog (PTEN)[50]. Despite this, the pathway plays a crucial role in the survival and proliferation of multiple myeloma cells. Elevated IL6 secretion by stromal cells, along with the elevated secretion of vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) by multiple myeloma cells due to multiple myeloma interactions, contribute to the pathway's activation. These cytokines activate their corresponding binding sites on multiple myeloma cells, promoting tumorigenesis by upregulating signaling events such as the PI3K/AKT/mTOR, mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways. The complexity of the PI3K/AKT/mTOR pathway is amplified by the presence of multiple feedback loops and interactions with various other pathways. DEP domain-containing mTOR interacting protein (DEPTOR) has been identified as an effective inhibitor of both mTORC1 and mTORC2[51]. Increased expression of DEPTOR inhibited mTORC1, relieving the feedback inhibition of ribosomal protein S6 kinase. Consequently, this results in the activation of AKT and the survival of multiple myeloma cells.

Numerous pathways interact with the PI3K/AKT/mTOR pathway, in addition to the feedback loops within it. The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways exhibit the most intricate and well-documented interaction. Yu *et al.* further discovered that suppressing ERK using the MEK inhibitor U0126 enhances the binding of Grb2-associated binder-1 (Gab1) with PI3K, consequently activating the PI3K/AKT pathway[52]. A more recent study has demonstrated the successful treatment of metastatic and primary colorectal cancers by inhibiting the PI3K/mTOR pathways. This highlights the pathway's potential as a promising route for addressing these lethal cancers[53].

In addition to cell differentiation, proliferation, and survival, the MAPK pathway is an evolutionarily conserved cell regulatory signaling network. Inflammatory cytokines such as TNF-a, IL-6, and IGF1 activate this pathway, triggering subsequent kinase cascades, namely RAS, RAF, MEK, and MAPK, resulting in altered gene expression. The MAPK pathway harbors two main oncogenes, NRAS and KRAS, which are frequently aberrant in many malignancies. Notably, these genes are often affected in multiple myeloma, with a cumulative incidence of 20 – 35%[54]. RAS mutations are thought to be progressive events, as they are uncommon in the initial phases of multiple myeloma but become more common in later disease progression[55].

In terms of compounds, researchers have identified more than ten active phytochemical constituents. Withaferin A, for instance, has been shown to suppress TNF-induced NF-B activation in human myeloid leukemia KBM-5 cells[56]. In both *in vitro* and *in vivo* studies, sitosterol and kaempferol have also demonstrated hematological and immunomodulatory effects[57]. In a kidney cancer cell line, withaferin-A induced dose-dependent apoptotic cell death and PARP cleavage through the downregulation of the STAT-3 pathway[58].

GO enrichment analysis demonstrated that the targets of the active compounds in *W. somnifera* and *A. barbadensis* were involved in diverse immunomodulatory associated-molecular functions. Notably, the protein kinase activity is the most significantly enriched molecular function of the bioactive-multiple myeloma's immune gene target, which holds unique therapeutic potential in the treatment of multiple myeloma[59]. In the realm of multiple myeloma

medication development, the majority of anti-kinase research has centered on protein kinase targets, with a few significant exceptions. Manning *et al*. reported that the human genome encodes 518 distinct protein kinases, each facilitating the transfer of the gamma-phosphate group of ATP to the hydroxyl groups of proteins. These protein kinases are categorized into two types based on the phosphorylated amino acids. They are important components of biological signal transduction pathways that regulate cell proliferation, survival, and migration. While numerous tyrosine kinases (TKs) reside in the cytosolic domains of plasma membrane receptors, multi-capability serine/threonine kinases (S/TKs) can also be located in the plasma membrane; however, the majority is distributed throughout the cytosol and other cellular compartments. This observation aligns with the GO analysis of cellular compartments (CC), which highlights the cytosol as the most significantly enriched cellular compartment among the gene targets[60].

However, this study has a few limitations. First, it relies on the already identified chemical constituents from *W. somnifera* and *A. barbadensis*, potentially overlooking the immunomodulatory effect of unknown chemical constituents. In addition, although favorable binding affinities were observed between the hub targets and corresponding phytoconstituents during the molecular docking simulation, further studies are necessary to authenticate and validate these interactions.

5. Conclusion

The study's approach leverages network pharmacology and molecular docking techniques to uncover active phytocompounds and their potential mechanism of immunomodulation. Network pharmacology analysis revealed that HCK, PDPK1, and JAK2 are crucial immunomodulatory targets for multiple myeloma treatment. These effects are achieved through the inhibition of major signaling pathways that have been constitutively activated, aligning with previous research. Notably, the study highlights significant pathways, including the PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, and TLR signaling pathways. The molecular docking analysis further supports these results, suggesting that with anolide G, Somniferine, and Somniferanolide from *W. somnifera* hold potential as IMiDs leads targeting PDPK1, HCK, and JAK2 hub proteins in multiple myeloma from the network pharmacology construction. These compounds exhibit promise in modulating key pathways in cancer, notably the PI3-AKT pathway, VEGFA pathway, JAK-STAT signaling pathway, and mTOR signaling pathway. Consequently, it is recommended that further *in vivo* and *in vitro* studies be conducted to assess the practical impacts of these lead compounds and their potential impact on immunomodulatory proteins against multiple myeloma.

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None.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Conceptualization: Funmilayo I. D. Afolayan
Investigation: Deborah G. Joseph.
Writing – original draft: Joseph Deborah G.
Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Supporting data can be obtained from the corresponding author following a formal request.

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