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Original Article

Exploring the bioactive properties and mechanism of *Aegle marmelos* in the treatment of inflammatory bowel disease through network pharmacology and a molecular docking approach

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Abstract: Background: Inflammatory bowel diseases (IBD) are recurrent inflammatory conditions that occur in the gastrointestinal tract, for which current treatment does not have satisfactory results, thus we require new therapies to combat the complex pathogenesis of IBD. Herbal medicines have been used for years to cure IBD. One of the plants from Ayurveda, *Aegle marmelos* (AM), commonly known as Bael, which belongs to the family Rutaceae, has ethnomedicinal properties in treating IBD due to its various phytochemicals. However, the mechanisms underlying the effect of AM remain to be elucidated. Methods: In this study, an *in silico* approach, molecular docking, and enrichment analysis were implemented to uncover the potential multicomponent synergistic effect and its molecular mechanism in treating IBD. Putative targets of IBD were obtained through OMIM, GeneCards, and DisGeNET databases. Compounds of AM were screened for their targets using a Swiss target prediction database and SuperPRED database. The common targets amongst AM and IBD were analyzed and the network was constructed using Cytoscape (3.10.0). Protein-protein interactions of target genes of the compounds was carried out through a STRING database. Then, the INPUT database was used to analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Molecular docking of top 6 compounds with hub targets was carried out using Autodock vina. Results: In the study, 46 effective compounds and 358 targets of AM were identified and further analyzed, 80 hub targets depending on the degree were considered effective against IBD. Through CytoHubba we identified AKT1, SRC, MAPK3, MAPK1, EGFR, IL6, TNF, HSP90AA1 and CASP3 as the top 10 hub targets that may contribute to the mechanistic role of AM in treating IBD. Aegeline, auraptene, bergapten, imperatorin, marmesin, and nodakenin were the most potent compounds of AM and those that possess a higher binding affinity to PI3K, AKT, and EGFR. PI3-AKT signaling pathway, EGFR tyrosine kinase inhibitor, and MAP Kinase signaling pathway are the major pathways having a correlation with AM. Conclusion: The study unveils the mechanism of AM in alleviating IBD through the EGFR-mediated PI3K/AKT pathway, stating its multi-component, multi-targeted therapeutic efficacy through multiple pathways.

Keywords: Network pharmacology, *Aegle marmelos*, Bael, inflammatory bowel disease, ulcerative colitis, PI3K/AKT pathway

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), two main types of inflammatory bowel disorders (IBD) are classified as complex, recurrent inflammatory ailments [1]. The common symptoms of IBD include debilitating/severe diarrhea, abdominal pain, weight loss, and chronic fatigue; events that may culminate in life-threatening complications [2]. IBD is associated with an array of factors, including interactions between genetic predisposition, dysregulated immune responses, and environmental factors, although its exact cause is yet unknown [3]. The global burden of IBD is increasing tremendously in more than 195 countries [4]. The global age-standardised incident rate (ASIR) of IBD increased from 4.22 per 100,000 in 1990 to 4.45 per 100,000 in 2021. Global incidence rates of IBD have been increasing from 1990 to

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2021, while the IBD-associated Disability-Adjusted Life Years (DALYs) and mortality have been decreasing [5]. The incidence of UC, especially in Asia, is increasing in the 21st century [6]. The widespread incidence of IBD in India can no longer be regarded as being in its infancy; due to a startling increase in both the disease burden and the population, India is expected to have some of the largest numbers of IBD patients in the world [7, 8].

IBD is a resilient, relapsing inflammatory condition characterized by immune system dysregulation, an altered cytokine profile, and persistent inflammation of the intestinal mucosa [8]. Since an enormous percentage of UC patients are resistant to or intolerant of typical medication treatments, current options are restricted and the mainstays of IBD treatment are medical care and colectomy [9]. Currently, there are no satisfactorily approved curative treatments for UC and CD. Treatment methods for UC and CD include using 5-aminosalicylic acid (5-ASA), glucocorticoids, TNF-α blockers, and immunosuppressive medications [10]. Unfortunately, many patients with IBD do not respond to the available treatments. With the establishment and maintenance of remission, there are significant demands for further therapy. Due to their perceived natural effects, individuals with IBD tend to have greater demands for complementary and alternative medications, particularly for herbal therapies [11, 12].

A number of physicians have utilized the Indian traditional medical system of Ayurveda to treat countless gastrointestinal ailments [13]. Alternative therapies from conventional medicine, such as Ayurveda, have been deemed as potential choices in the discovery of new therapeutics. Many bioactive compounds are present in traditional polyherbal preparations, which can affect numerous disease targets. With the concourse of an innovative approach known as network pharmacology, it is now feasible to study the intricate connections between bioactive molecules, targets, illnesses, and genes [14]. Network pharmacology, also known as computational systems biology, is an entirely novel approach in computing aspects of biology that unifies systems biology, omics, and pharmacological data. It is the best methodology to utilize in newer drug discovery processes from traditional medicines. In accordance with this innovative pharmacological strategy, drugs that target multiple nodes in interconnected systems and are connected to various nodes, generate connectivity data for a drug's assessment and may be useful in discovering a multitargeted therapy for chronic disorders [15]. Such computational techniques are helpful in towards better understanding the disease pathological states and accelerate new drug discovery processes. This approach could be helpful in mitigating chronic inflammatory diseases like IBD.

Aegle marmelos (AM) also known as Bael, is a member of the Rutaceae family of plants. Indian traditional medicine has extensively documented the use of this plant and its parts. There are numerous medical uses for the plant's components, including as an astringent, aphrodisiac, demulcent, hemostatic, antidiarrheal, antidysenteric, antipyretic, antihyperglycemic, anti-cancer, anti-scorbutic activities [16-22]. AM fruits have many potential health benefits, notably radio-protective properties, peroxidation, antibacterial, lipid-inhibiting, antidiarrheal, gastroprotective, antiviral, anti-ulcerative colitis, cardioprotective, antioxidant, and hepatoprotective effects due to its biologically active compounds [18, 23, 24].

Fruit from AM contains fiber, carotenoids, terpenoids, coumarins, flavonoids, and alkaloids, which are responsible for the fruit's health-promoting and protective effects [25, 26]. Bael is comprised of various phytochemicals, coumarins such as marmelosin, marmesin, imperatorin, alloimperatorin, alloimperatorin methyl ether, xanthotoxol, scoparone, scopoletin, umbelliferone, skimming, psoralen, marmelide, several alkaloids such as aegeline, marmeline, dictamine, sitosterol, arabinose, and various biologically active compounds [16, 19, 26, 27]. Numerous additional compounds, including tannin, fagarine, luvangetin, psoralen, cumin aldehyde, citral, lupeol, skimmianine, marmin, marmelide, aurapten, marmelosin, eugenol, citronellal, cineole have been shown to be biologically effective against both minor and serious gastrointestinal disorders [19, 26, 28].

Fruit juice of AM and its extract have been used in curing gastrointestinal disorders since ancient times. It has been scientifically proven that various fruit extracts of AM have mitigated IBD in different animal models [29-31]. One of

our previous studies showed the synergistic activity of AM with *Bombax malabericum* and *Holarrhena antidysentrica* in a polyherbal formulation used for treating dinitrobenzene sulphonic acid-induced IBD in rats [32].

Due to multiple compounds and their multiple targets the mechanism of AM in the treatment of IBD still remains unclear. Various research evidence shows the therapeutic efficacy of AM in combatting IBD, but we are still not aware of the complex relationships between the bioactive properties and targets of AM and its mechanistic role in treating IBD. In line with this objective, the present research work aimed to identify the putative bioactive compounds targeting different pathways with the network pharmacology approach and molecular docking by computational techniques.

Materials and methods

Screening of phytochemical compounds of AM

The list of compounds of AM fruits were collected from Dr. Duke's phytochemical and ethnobotanical Database (DPED) version 1.10.07-Beta_2021-07-14 (https://phytochem.nal.usda.gov/phytochem/search), Universal Natural product database (UNPD) [33] and IMMPAT database (https://cb.imsc.res.in/imppat/). All the databases and the literature data mining provided information for all the compounds found in the fruits of AM [34, 35].

Pharmacokinetics prediction of compounds of AM

The PubChem site (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the canonical SMILES (simplified molecular-input line-entry system) of all the compounds of AM. Then in the next step, Swiss ADME (http://www.swissadme.ch/) was used to archive the ADME data of all the bioactive compounds. ADME, which stands for absorption, distribution, metabolism, and excretion, is a crucial step in figuring out the pharmacokinetics of potential medications [36, 37]. Oral bioavailability score (OB) and drug-likeliness (DL) as per the violations of Lipinski's rule, Veber rule, and Ghosh rule were chosen as pharmacokinetic parameters. Compounds with oral bioavailability scores of less than 10% and more than two violations of drug likeliness rules were excluded from the study [38-41]. The rest of all the compounds were selected for screening of their multiple targets.

Potential target fishing for compounds of AM

Target fishing of the compounds can be done by availing various databases. Query molecules can be imported into the databases by adding their pubchem names, SMILES (The simplified molecular-input line-entry system), structure, or as 2D drawings. PubChem (https://pubchem.ncbi.nlm.nih.gov/), was used to obtain the molecular structure information of screened compounds [42]. Targets for all the compounds present in AM were obtained through two databases. The Swiss Target Prediction database (http://www.swisstargetprediction.ch/) which can be automatically mined to extract information for many compounds, was used to retrieve the protein targets of the bioactive compounds [43, 44]. Another database used for fetching the targets of the compounds was SuperPRED (https://prediction.charite.de/subpages/target_prediction.php). Targets were combined and then they were used for further downstream processes [45].

UC and CD-related target prediction

Different databases, including TTD (https://db.idrblab.net/ttd), DisGeNET (https://www.disgenet.org), GeneCards (https://www.genecards.org), and DrugBank (https://go.drugbank.com), were primarily availed to identify the human genes linked to UC and CD [46-48]. The UniProtKB (https://www.uniprot.org) database validated each target. DisGenNET is a platform for knowledge management that integrates information about genes and variants linked to diseases from many sources. Along with normal and pathological features, it also encompasses the whole range of human disorders. Annotations for all human genes can be found in one place: the GeneCards database [47]. DrugBank is a web-based database featuring detailed molecular information regarding medications, their mechanisms, their interactions, and their targets [46]. The US Food and Drug Administration (FDA) has given the go-ahead for clinical trials for approved UC medicines and drug-like substances. Targets of UC and CD were merged to get the all the targets for IBD.

Protein-protein interaction (PPI)

The STRING database (https://string-db.org/) version 11.5 was used to explore the protein-protein interactions (PPI) of each target, with the species confined to "Homo sapiens" and a confidence score >0.9 [49]. Targets with less confidence scores and non-connected were eliminated. The PPI data was imported into the network visualization program Cytoscape (version 3.10.0) to reconstruct the network for better visualization and to screen out the hub targets due to the intricacy of the original network created in the STRING database [50-52]. The common targets of IBD and AM were identified. The Cytoscape network was analysed using Network Analyzer tool in Cytoscape and the centrality closeness, betweenness, and degree were explored. The hub targets with more than 11 degrees were used for the construction of subnetworks. An interactive network's degree for each node indicates the number of connections to other nodes and represents the significance of each node by counting the edges between each node and other nodes in the network. CytoHubba plugin was used to analyze the top 10 target genes of AM by using MNC (Maximum Neighbourhood Component). The top 10 genes produced by the maximum neighborhood component (MNC) technique were considered the hub genes and they were discovered using the cytohubba plug-in. An interactive network's degree for each node indicates the number of connections to other nodes and represents the significance of each node by counting the edges between each node and other nodes in the network.

Construction of PPI subnetworks

The AM network regions with the highest degree of connectivity, known as MCODE clusters, were identified using the Molecular Complex Detection (MCODE) plugin in Cytoscape [53]. From a large interaction network, the MCODE extracts the densely connected subnetworks. Significant subnetworks were those with an interaction score of at least 2.0 and two or more nodes. Clusters were made by setting parameters such as degree cutoff and K-core = 2 and node score cutoff = 0.2.

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

We used the functional annotation tools DAVID (database for annotation, visualization, and integrated discovery) (https://david.ncifcrf.gov/) and INPUT (http://cbcb.cdutcm.edu.cn/INPUT/) to perform GO and KEGG enrichment analysis in order to methodically explore the biological processes of AM as a treatment against IBD [54, 55]. The putative 80 targets of AM were used for the enrichment analysis. GO terms of top 10 results in the bar chart were colored by -log10 (*P* values). Targets involved in biological processes, molecular functions and cellular functions were extracted from the INPUT database [56-59]. KEGG enrichment analysis was performed, and the top 20 pathways were represented in a bubble chart. KEGG pathway-gene network was constructed to observe the interaction between various pathways and their genes. Networks of genes and the various GO terms were constructed. Further, the gene distribution feature of INPUT was used to analyze the relationship between target genes and their involvement in various other pathways which could be further used for drug repurposing. The network of the top pathways and AM targets was constructed using Cytoscape. It gives the interconnection between pathways and AM.

Molecular docking

Molecular docking is the most widely used technique in drug design because it can predict the ability of ligands and proteins that bind, as well as the location of binding. The three-dimensional structures of all compounds were downloaded from PubChem [42]. The crystal structures of the key targets such as AKT1, PI3K, MAPK1 and EGFR were downloaded from the PDB database (Protein Data Bank) (https://www.rcsb.org/) [60, 61]. All these protein targets were chosen for the docking studies. PDB and grids are listed in the **Table 1**. For the sake of the molecular docking study, the structural files were saved in the PDBQT format. The Scripps Research Institute's AutoDock Vina v1.1.2 was used for the study. Then, using a breakthrough curvature-based cavity detection approach, we determined the centers and sizes of the binding sites, performed ligand docking with Autodock Vina, and acquired the binding activities and Vina score, which represents the binding affinity [62-64]. Vina scores that are more negative imply more stable ligand-receptor binding. The results with the best energy and conformation were chosen for analysis. Discovery Studio visualizer

Table 1. Grid box and sizes used for molecular docking of top proteins

Target Name	Target id	Center			Size		
		X	Y	Z	X	Y	Z
AKT1	7WSW	164.8	189.6	195.2	48	66	67
PI3K	1E7U	23.02	62.41	20.58	42	51	50
MAPK1	4FUX	18.52	6.37	16.73	40	45	50
EGFR	3G5X	23.09	18.13	60.45	40	40	40

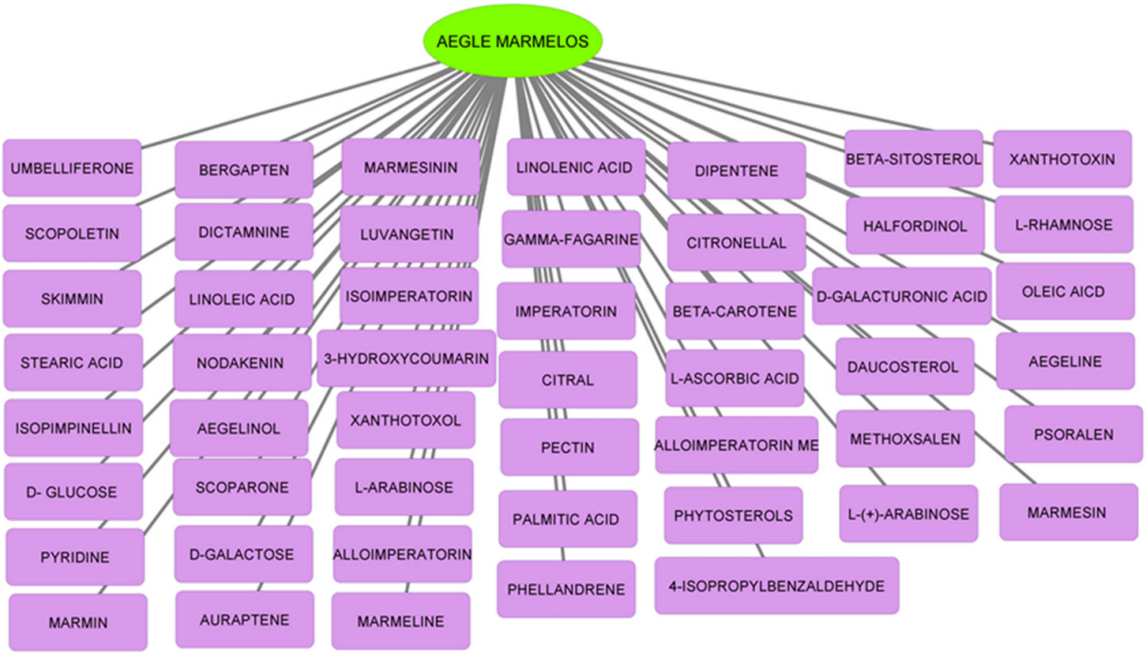


Figure 1. Phytoconstituents present in fruits of *Aegle marmelos*.

v21.0.20298 was used to visualize the docked structures [65].

Results

Identification of candidate compounds in Aegle marmelos

Literature survey and various databases revealed the presence of various compounds in AM fruits. In total, 50 probable compounds of AM were extracted from databases (**Figure 1**). Then they were screened for ADME properties. While comparable, the Abbot Bioavailability Score aims to forecast the likelihood that a chemical will have at least 10% oral bioavailability [66]. After screening the compounds for ADME, all the compounds possessed good oral bioavailability. However, 4 compounds (Linoleic acid, linolenic acid, oleic acid, and stearic acid) do not fit into the criteria of drug likeliness property and those were excluded, and the rest all the compounds were used for further analysis (Table S1).

Target identification of compounds of Aegle marmelos

The target fishing of all the individual compounds was done using Swiss Target Prediction database and Super-PRED database. All the compounds with their specific targets were extracted from the database. After combining all the targets of the compounds 1687 targets were associated with the compounds in AM (Table S2). Interaction between the bioactive compounds of AM and their targets depicted the common targets and the overlapping targets were removed and the network was constructed in Cytoscape. The network showed 560 putative targets of AM forming 576 nodes and 1417 edges (**Figure 2A**).

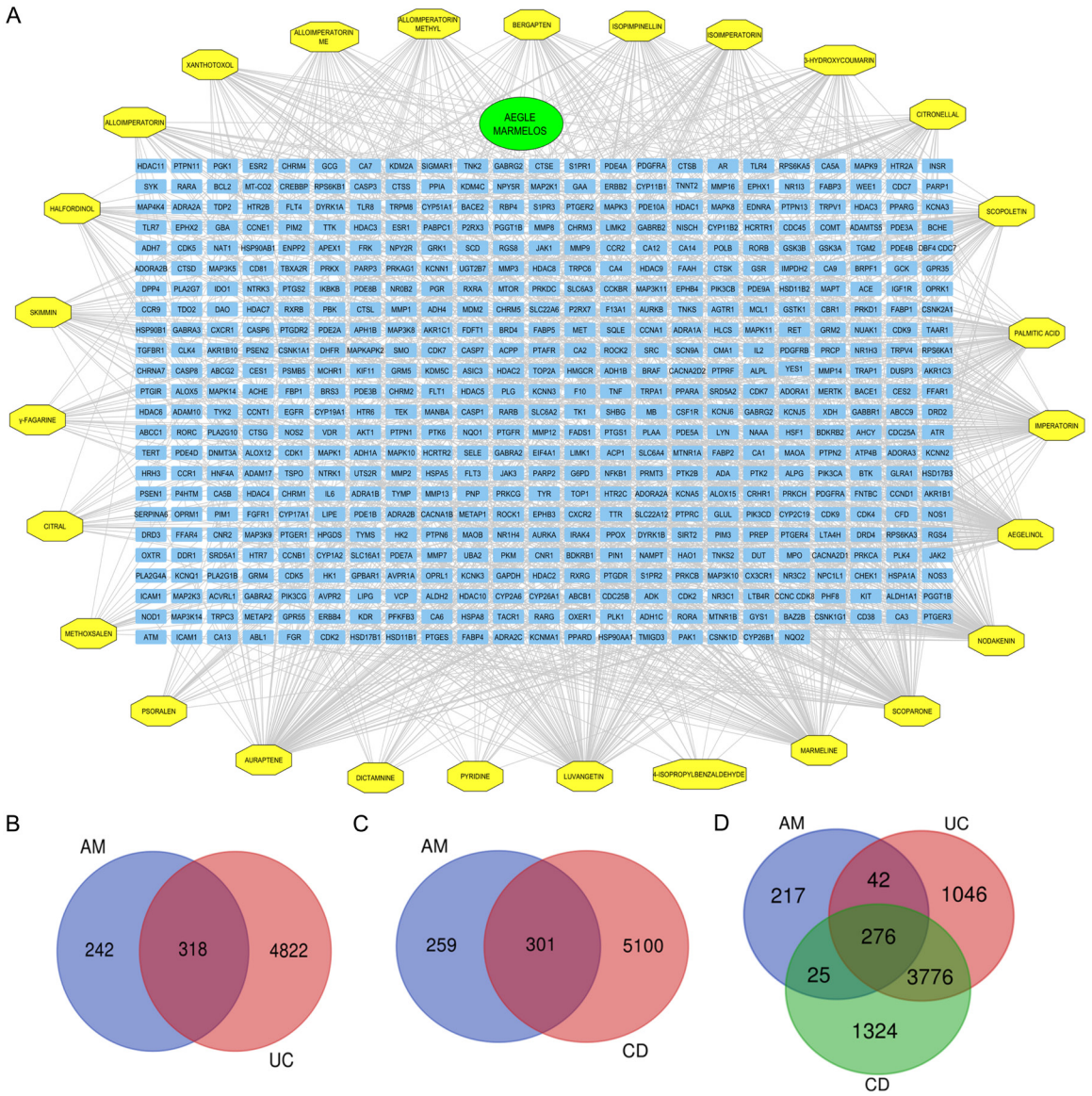


Figure 2. Bioactive components of AM and their targets. A. Interaction network of AM compounds and their putative targets. It comprises of 576 nodes and 1,417 edges. The yellow color nodes are the bioactive compounds of AM and blue color nodes are their targets. B. Venn diagram depicting 318 common targets of AM and UC. C. Venn diagram showing the 301 targets shared with the targets of CD. D. Venn diagram exhibits the coinciding targets of AM,UC, and CD.

Retrieval of potential targets of IBD

A keyword-based search was used to find a total of 1459 UC-related genes and 1383 CD-related genes from the DisGenet database. A total of 5140 UC-related genes and 5401 CD-related targets were retrieved from GeneCards, OMIM, and TTD databases (Table S3). With a total of 560 targets from various compounds of AM and 6974 targets to UC and CD, they shared 358 targets in common (**Figure 2A-D**).

Protein-protein interactions

For the PPI, 358 targets of AM were put into a STRING database. The PPI network comprises of 358 nodes and 1,183 edges in the interaction network, PPI enrichment *p*-value was <1.0e-16 (**Figure 3A**). Confidence interval of 0.9% (highest confidence) was selected for the analysis of PPI. Annotation data of the PPI networks of AM targets is shown in detail in Table S4. The PPI network of 358 AM targets was

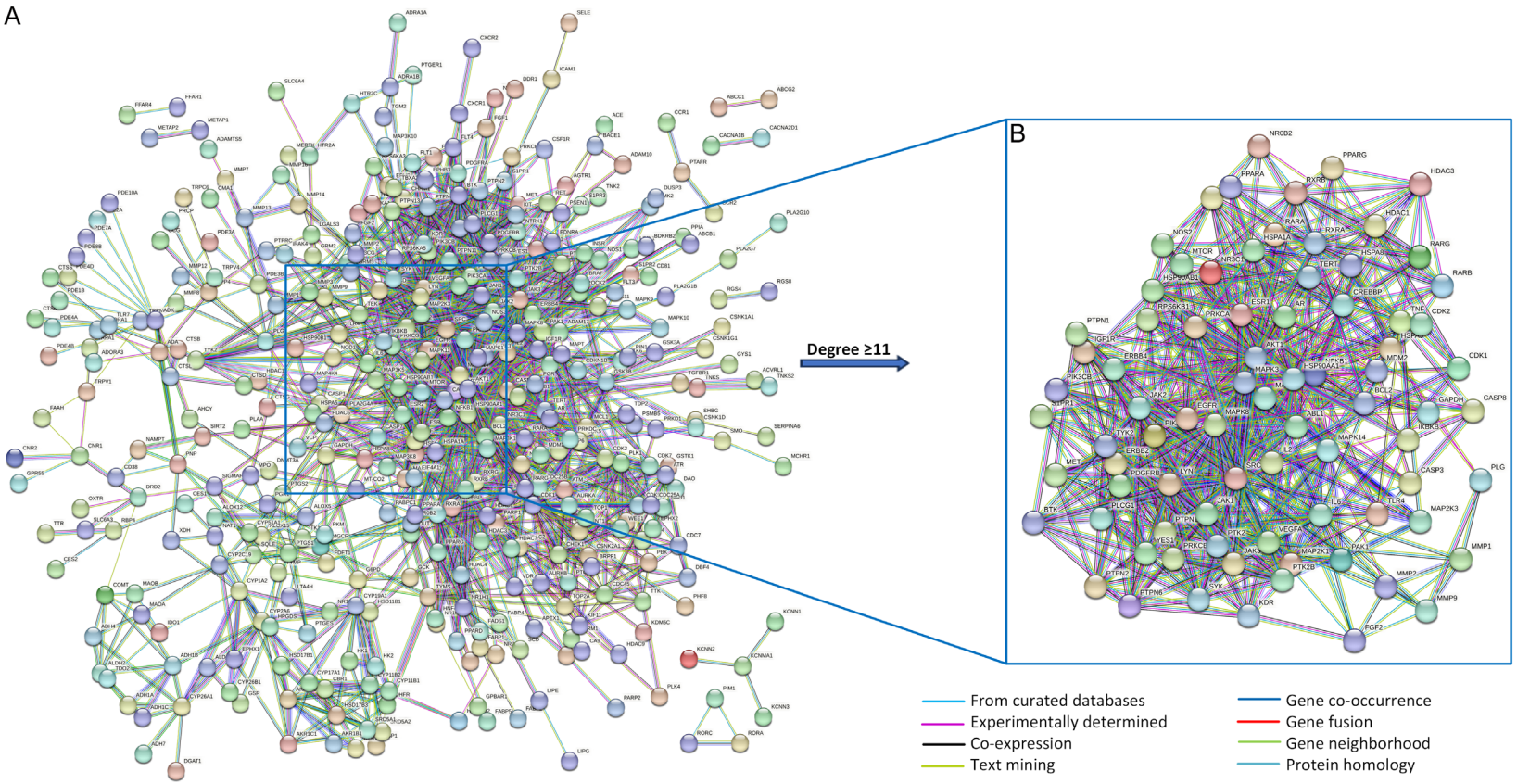


Figure 3. Protein-protein interaction of targets of AM against IBD. A. The network showcases 358 nodes (all the targets found common with IBD targets) and 1,183 edges constructed using STRING with the highest confidence of 0.9 and *p*-value <1.0e-16 (Table S4 shares more details on PPI). The reference for the color of the edges is shown in the left corner of the figure. B. The 80 hub targets of AM with degree more than 11 degrees comprises of 80 nodes and 1,391 edges.

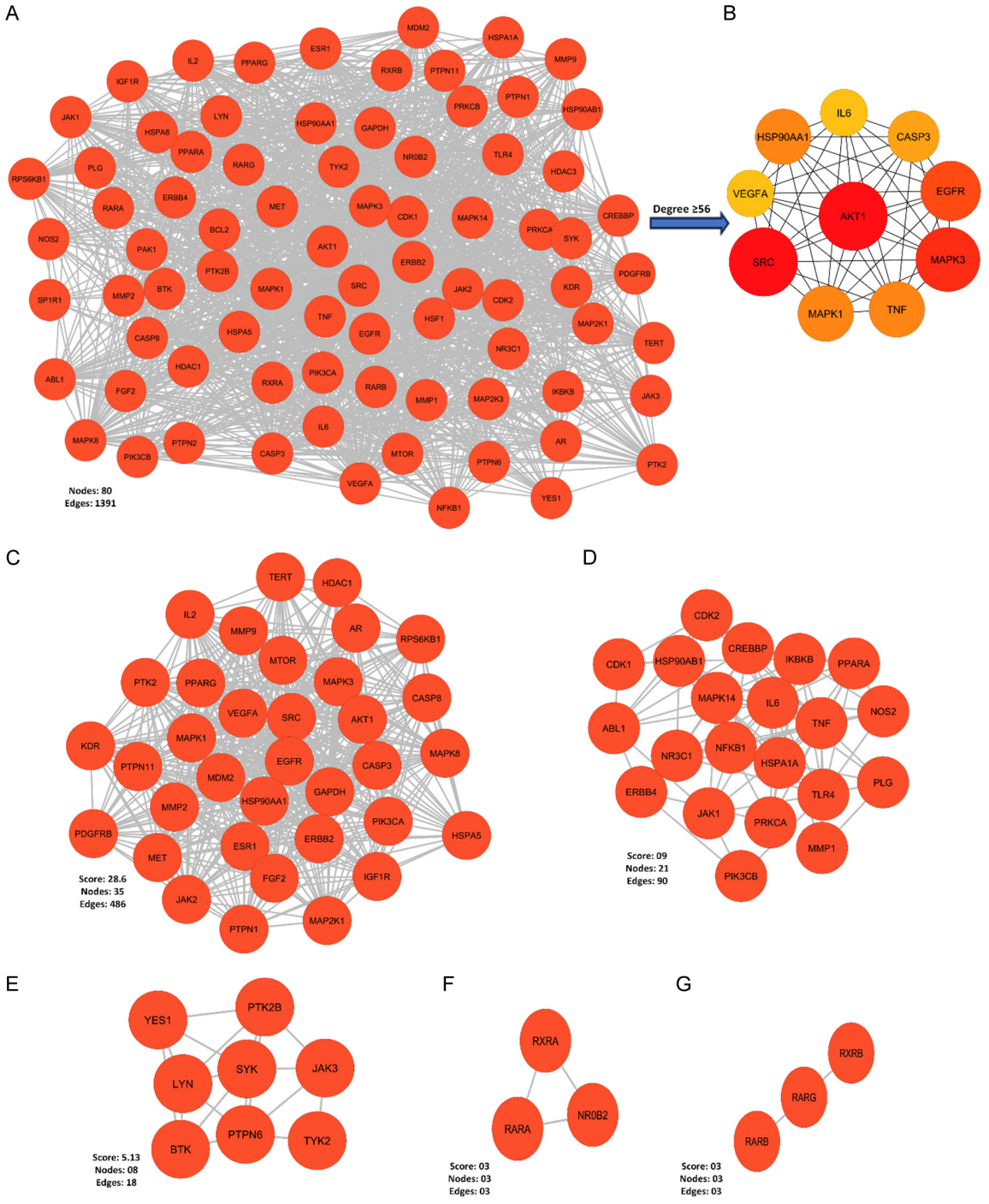
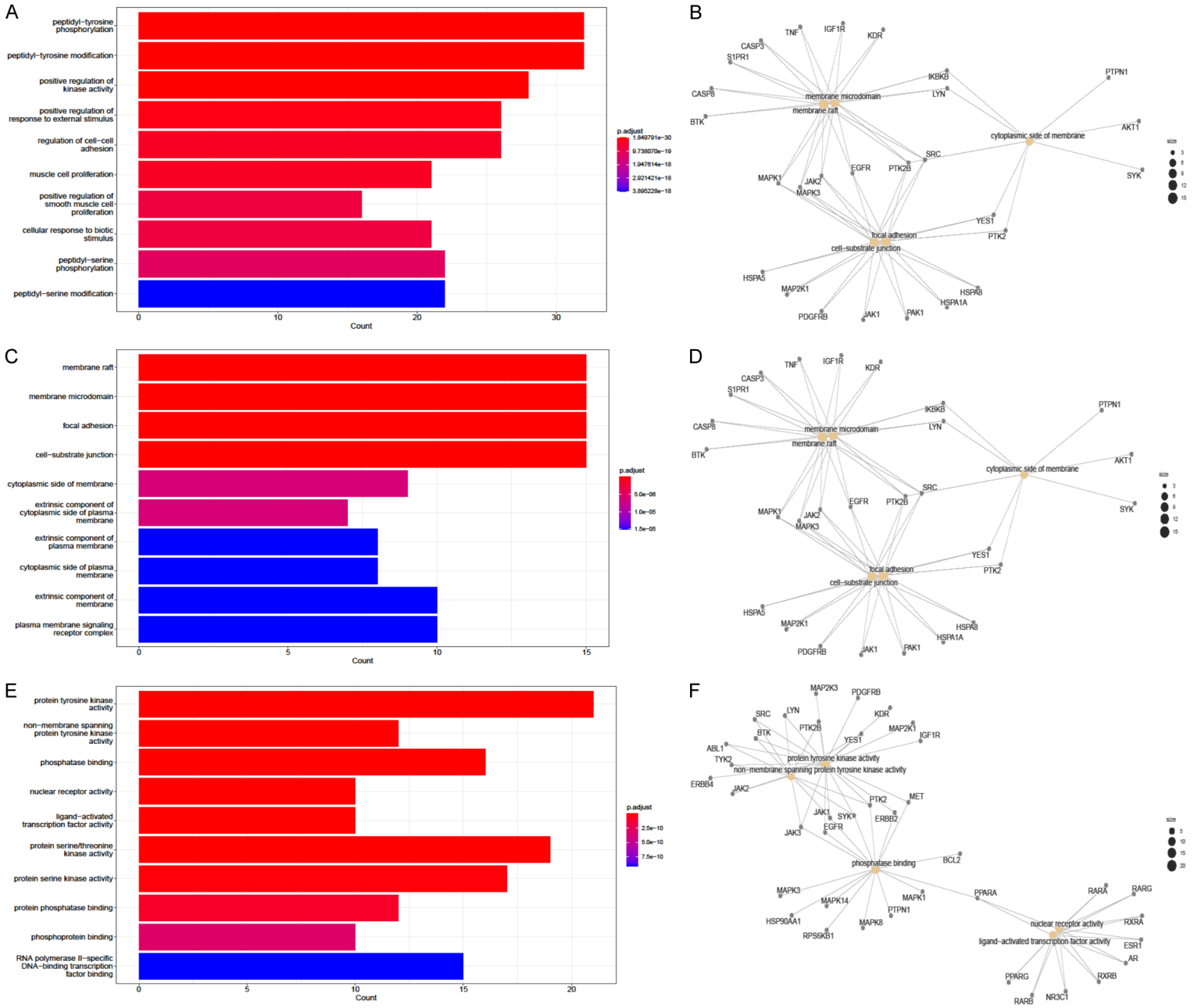


Figure 4. Construction of PPI network and sub-networks of hub targets of AM against IBD. (A) AM PPI network comprises of 80 nodes and 1391 edges connecting to them. (B) Top 10 hub targets of AM analyzed using the CytoHubba plugin and network analysis plugin of Cytoscape 3.10.0. All the targets have more than 56 degree. The degree would depend on the size of the node. Larger nodes have a higher degree amongst all the nodes. MCODE clusters (C-G) were formed as a part of the subnetwork of AM. (C) Cluster 1 comprises of 35 nodes and 486 edges with a score of 28.6. (D) Cluster 2 comprises of 21 nodes with connecting 90 edges and a score of 9. (E) Cluster 3 is constructed with 8 nodes and 18 connecting edges with a score of 5.13. (F) and (G) Cluster 4 and cluster 5 comprises of 3 nodes, 3 edges and 3 score each.

analyzed using Network Analyser, an analytical tool in Cytoscape was used to analyze the network and to find the targets with the highest degree. The targets with degrees greater than


A
peptidyl−tyrosine phosphorylation
peptidyl−tyrosine modification
positive regulation of kinase activity
positive regulation of response to external stimulus
regulation of cell−cell adhesion
muscle cell proliferation
positive regulation of smooth muscle cell proliferation
cellular response to biotic stimulus
peptidyl−serine phosphorylation
peptidyl−serine modification
0
10
20
30
Count
p.adjust
1.849791e−30
9.738070e−19
1.947614e−18
2.921421e−18
3.895228e−18
B
TNF
IGF1R
KDR
CASP3
S1PR1
IKBKB
PTPN1
CASP8
membrane microdomain
LYN
BTK
membrane raft
AKT1
cytoplasmic side of membrane
SRC
SYK
EGFR
PTK2B
MAPK1
JAK2
MAPK3
YES1
PTK2
focal adhesion
cell−substrate junction
HSPA5
MAP2K1
HSPA8
HSPA1A
PDGFRB
JAK1
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membrane microdomain
focal adhesion
cell−substrate junction
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KDR
CASP3
S1PR1
IKBKB
PTPN1
CASP8
membrane microdomain
LYN
BTK
membrane raft
AKT1
cytoplasmic side of membrane
SRC
SYK
EGFR
PTK2B
MAPK1
JAK2
MAPK3
YES1
PTK2
focal adhesion
cell−substrate junction
HSPA5
MAP2K1
HSPA8
HSPA1A
PDGFRB
JAK1
PAK1
size
E
protein tyrosine kinase activity
non−membrane spanning protein tyrosine kinase activity
phosphatase binding
nuclear receptor activity
ligand−activated transcription factor activity
protein serine/threonine kinase activity
protein serine kinase activity
protein phosphatase binding
phosphoprotein binding
RNA polymerase II−specific DNA−binding transcription factor binding
0
5
10
15
20
Count
p.adjust
2.5e−10
5.0e−10
7.5e−10
F
MAP2K3
PDGFRB
SRC
LYN
KDR
BTK
PTK2B
MAP2K1
IGF1R
YES1
ABL1
TYK2
protein tyrosine kinase activity
non−membrane spanning protein tyrosine kinase activity
ERBB4
JAK2
PTK2
MET
JAK1
SYK
ERBB2
EGFR
JAK3
phosphatase binding
BCL2
MAPK3
MAPK14
MAPK1
PPARA
RARA
RARG
HSP90AA1
PTPN1
MAPK8
nuclear receptor activity
RXRA
RPS6KB1
ligand−activated transcription factor activity
ESR1
AR
PPARG
RXRB
RARB
NR3C1
size

Figure 5. Gene Ontology (GO) enrichment analysis of AM targets and the GO terms and gene networks. The top 10 GO terms are represented as bar graphs and ranks order according to *p*-values. The X-axis represents gene count, while Y-axis represents various GO terms. The red color bar shows most genes associated with GO terms followed by magenta and blue color. For the network construction, the bigger the node size the more genes are associated with the GO terms. A. Represents the top 10 significantly enriched terms in Biological Processes (BP). B. Top 5 BP and gene networks. C, D. These images represent the top 10 significantly enriched terms in Molecular Functions (MF) and the top 5 MF and gene networks respectively. E, F. These images represent the top 10 significantly enriched terms in Cellular Component (CC) and top 5 CC and gene network respectively.

11 were selected for further analysis (**Figure 3B**). Eighty hub targets of AM were chosen for the analysis of sub-network construction and were used for the enrichment analysis. Various targets with higher degree scores were alpha serine/threonine-protein kinase (AKT1, degree score 69), tyrosine-protein kinase SRC (SRC, degree 69), Mitogen-activated protein kinase (MAPK3, degree 67), Epidermal growth factor receptor (EGFR, degree 64), Heat shock protein (HSP90AA1, degree 60), Mitogen-activated protein kinase 1 (MAPK1, degree 60), Tumor necrosis factor-alpha (TNF-α, degree 60), Caspase 3 (CASP3, degree 57), Interleukin-6 (IL-6, degree 56). Furthermore, Cytohubba plugin of Cytoscape was used to fetch the top 10 targets of AM by using MNC (Maximum Neighbourhood Component. Those top 10 targets possessed more than 56 degrees (**Figure 4A**, **4B**).

PPI subnetworks analysis

All the 80 hub target network was further subdivided into different sub-networks. The MCODE plugin recognized five different subnetworks as significant in the AM PPI network. For the cluster construction, the degree and K-Core cutoff was 2. MCODE provides the highly connected regions (clusters) of the hub targets (**Figure 4C-G**). Details for all the clusters, their targets, and their scores are listed in Table S4.

Gene ontology and pathway enrichment analysis

Further, GO and route enrichment analysis was carried out to investigate the biological mechanisms of the 80 potential AM targets on IBD. The top 10 most significantly enriched GO terms were exhibited with their *p*-value and gene count. The top 10 highly enriched GO terms were displayed in **Figure 5**. The pathogenesis of UC is highly correlated with the negative regulation of the apoptotic process, protein phosphorylation, cell proliferation, and inflammatory response, which were clearly concentrated in these targets. These findings demonstrated the diverse synergy of AM on cellular mechanisms. The potential targets enriched with GO molecular function mainly include protein tyrosine kinase activity (GO:0004713), non-membrane spanning protein tyrosine kinase activity (GO:0004715), phosphatase binding (GO:0004725), nuclear receptor activity (GO:0004879), ligand-activated transcription factor activity (GO:0098531), protein serine kinase activity (GO:0004712) and protein phosphatase binding (GO:0019903). Cellular components enrichment terms include membrane raft (GO:0045121), membrane microdomain (GO:0098857), focal adhesion (GO:0005925), cell-substrate junction (GO:0030055), and cytoplasmic side of membrane (GO:0098562). The main biological processes include Peptidyl-tyrosine phosphorylation (GO:0018108), peptidyl-tyrosine modification, positive regulation of response to external stimulus (GO:0050731), regulation of cell-cell adhesion (GO:0030155), muscle cell proliferation (GO:0033002).

In order to identify additional potential mechanisms underlying the therapeutic effects of AM, we performed KEGG pathway enrichment analysis on 80 targets and screened 20 pathways using the *p*-value 0.05 cutoff (**Figure 6A**). According to the KEGG enrichment the most targets were involved in several pathways and amongst them the top 20 signaling pathways contains the PI3-AKT signaling pathway (hsa04151), MAP Kinase signaling pathway (hsa04010), EGFR tyrosine kinase inhibitor resistance (hsa01521), HIF-1 signaling pathway (hsa04066), ErbB signaling pathway (hsa04012) and Th17 cell differentiation (hsa04659). All these pathways are connected to IBD and this sets a strong base of using AM in treating IBD. The top 5 KEGG pathways and the gene network was constructed forming the close interaction between genes and pathway (**Figure 6B**). Furthermore, the gene distribution feature of the INPUT database provided the Sankey diagram of the top 5 genes and their correlation

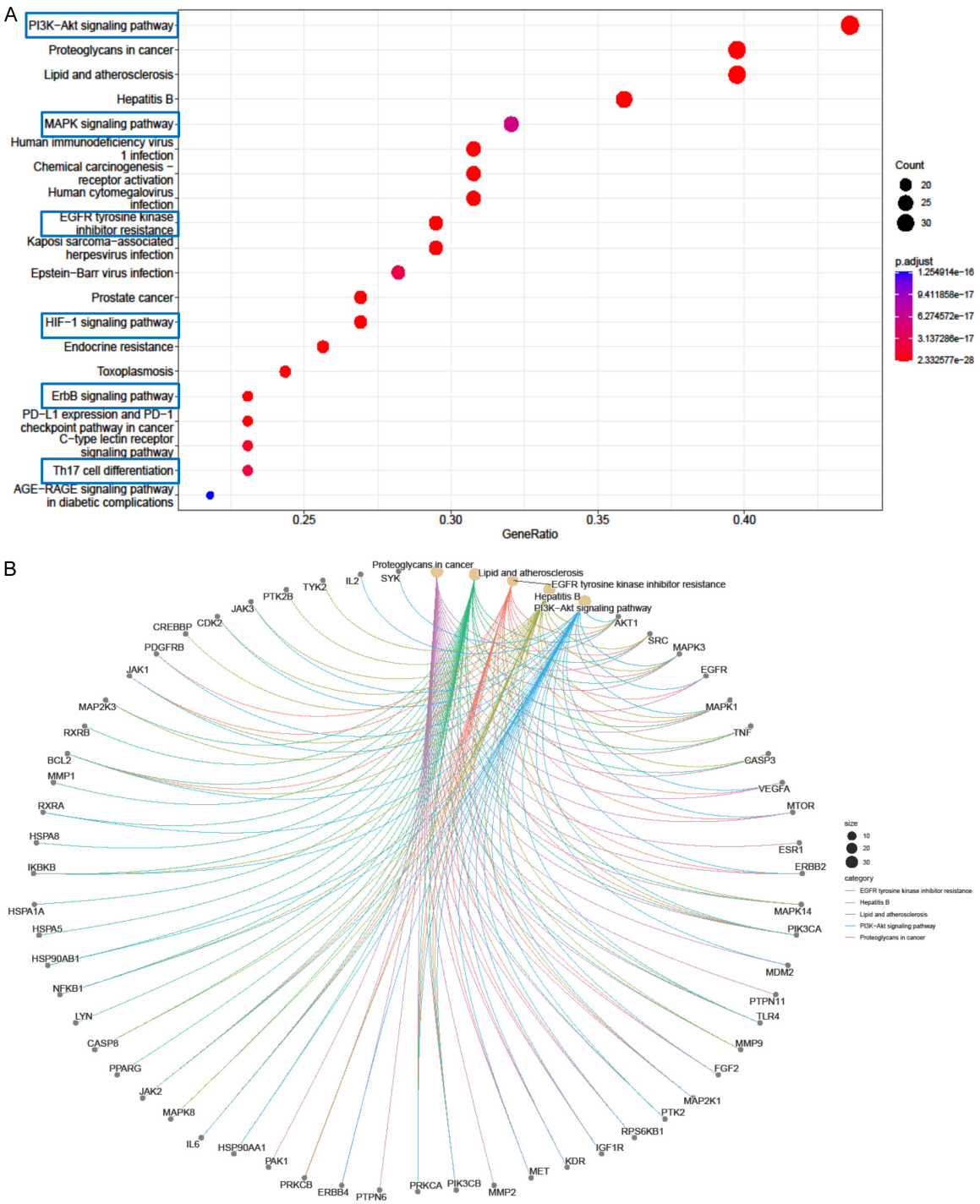


Figure 6. Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis of putative targets of AM. A. Bubble map of top 20 KEGG pathways. X-axis represents the gene enrichment ratio and Y-axis represents the enrichment pathways of the target genes. The depth of the red color and the size of the bubble represents the more targets mapped to the pathway. The blue box highlighted reveals the 6 pathways which may target IBD. B. This image represents the top 5 KEGG pathway and their associated gene network.

with the KEGG pathway, biological processes (BP), molecular functions (MF), and cellular components (CC) (**Figure 7**). Genes connected to the number of KEGG pathways, BP, MF, and CC are described in detail in Table S5. The network was constructed from comprising the top

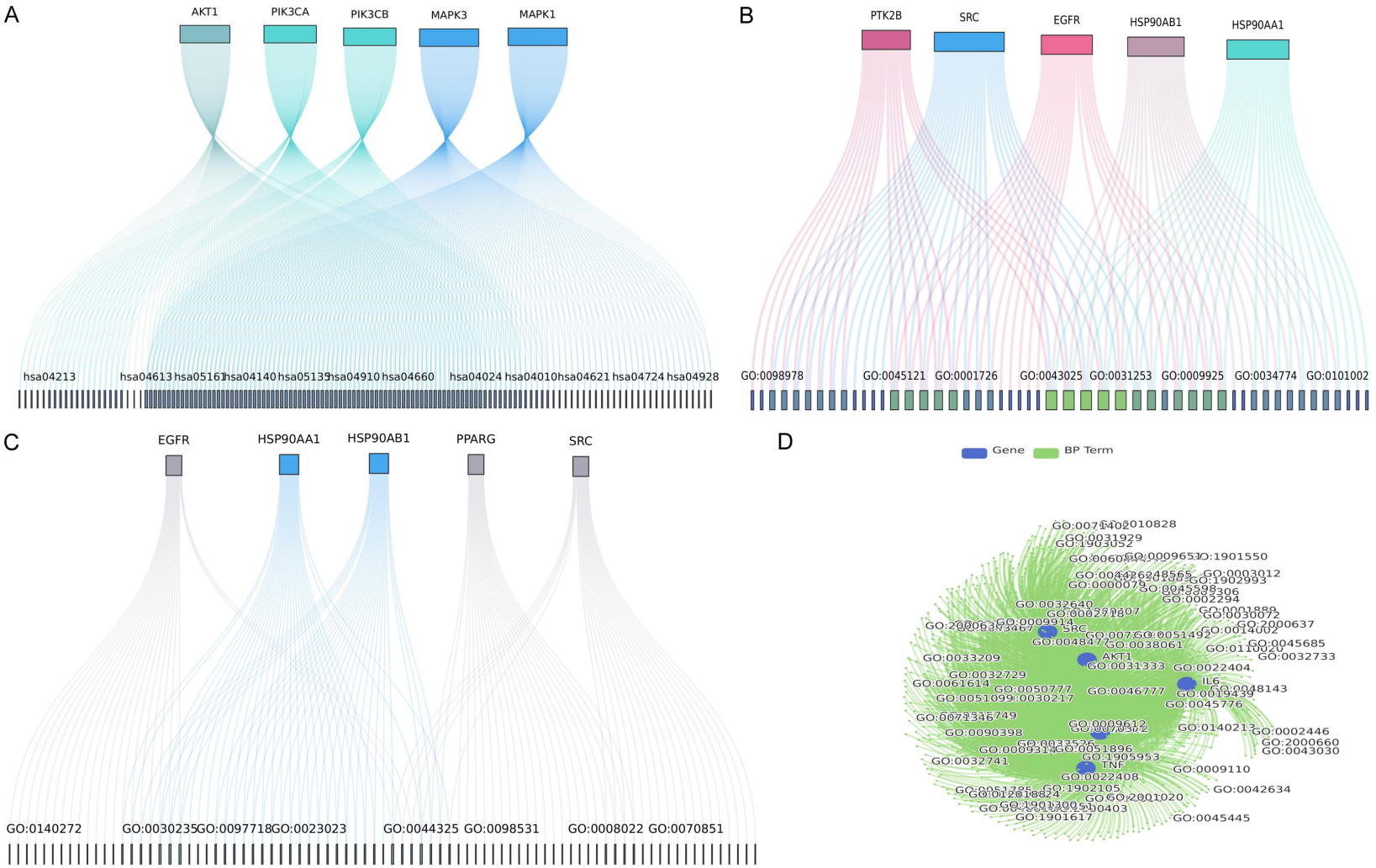


Figure 7. Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment of top 5 putative targets of AM. The greater the degree of overlap, the gene involvement in various pathways has more Lines with different colors depicting different genes and the pathways. A. KEGG pathway enrichment of the top 5 of AM. B. Targets enriched with Cellular Components (CC) of GO terms. C. GO enrichment of targets with Molecular Function (MF). D. Targets of AM and Biological Processes (BP) terms. The details of the GO and KEGG are described in detail in Table S5.

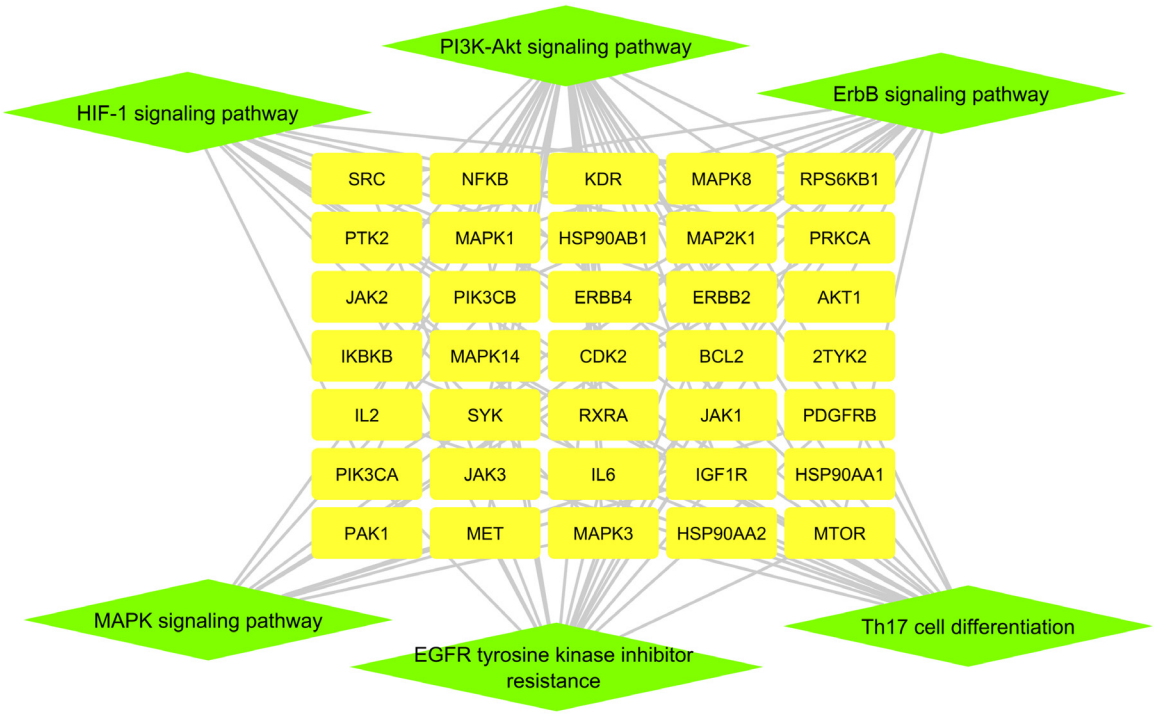


Figure 8. Major pathways targeted by AM and its targets. The network is constructed comprising six major pathways with 35 targets, 41 nodes, and 97 edges.

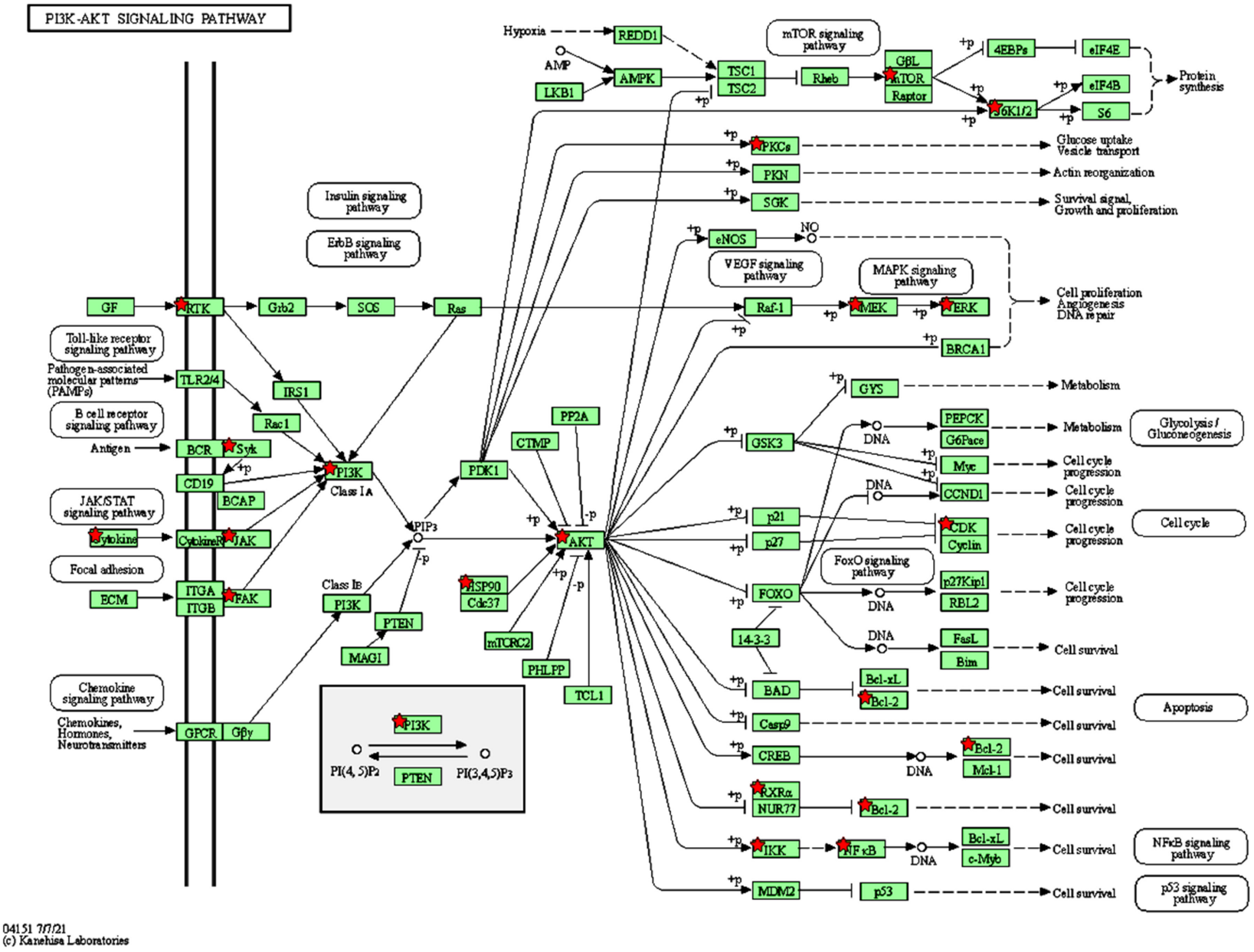
6 pathways connected to IBD and their targets depicting the interconnections between various pathways (**Figure 8**). The targets of AM have deep connections to the PI3K-AKT, EGFR and MAPK pathways. Maximum targets match with the pathway and those are depicted with red stars in **Figure 9**.

Validation by molecular docking

Lastly, the protein-protein interactions, the Cytoscape data, targets, and pathways were verified by using a molecular docking approach. The important active compounds with the highest degree in the network include auraptten, bergapten, imperatorin, nodakenin, marmesin, and aegeline. All those compounds were docked with various hub targets including AKT1, PI3K, MAPK1, and EGFR. According to conventional wisdom, the vina score represents the strength of the binding interaction between the ligand and the receptor, and the lower the vina score, the more stable the chemicals binding to the hub target. All six compounds showed good binding score with an average free energy change of -7.67 kcal/mol (**Table 2**). **Figure 10** depicts six compounds with the highest binding energy targets. All the compounds and their binding energy with all four targets and hydrogen bonding details are described in Table S6. Molecular docking data suggests that the compounds have more affinity towards EGFR and PI3K. We can predict that AM would be acting through the EGFR-mediated PI3K/AKT pathway.

Discussion and conclusion

IBD (inflammatory bowel disease) is a persistent, relapsing condition that affects the gastrointestinal tract. It is a group of diseases involving Crohn's disease and ulcerative colitis, resulting in chronic inflammation of the intestinal tracts [67]. IBD cannot be cured, however, pharmaceuticals can help regulate the condition and enhance the quality of life. The goals of treatment are to lessen symptoms, achieve and sustain remission, and protect against complications. IBD is usually managed through surgery or medication therapy [68]. Despite the fact that traditional treatments can be useful in certain cases and has side effects, they are not always effective. IBD has been reported to respond well to Ayurvedic and herbal remedies. Aegle marmelos (AM) has been traditionally used in curing gastrointestinal problems


PI3K-AKT SIGNALING PATHWAY
Hypoxia
REDD1
AMP
LKB1
AMPK
TSC1
TSC2
mTOR signaling pathway
Rheb
GβL
mTOR
Raptor
4EBPs
eIF4E
eIF4B
S6K1/2
S6
Protein synthesis
PKCs
PKN
SGK
Glucose uptake
Vesicle transport
Actin reorganization
Survival signal,
Growth and proliferation
Insulin signaling pathway
ErbB signaling pathway
eNOS
NO
VEGF signaling pathway
MAPK signaling pathway
GF
RTK
Grb2
SOS
Ras
Raf-1
MEK
ERK
Cell proliferation
Angiogenesis
DNA repair
BRCA1
Toll-like receptor signaling pathway
Pathogen-associated molecular patterns (PAMPs)
TLR2/4
IRS1
Rac1
B cell receptor signaling pathway
Antigen
BCR
Syk
CD19
BCAP
PI3K
Class IA
PIP3
PDK1
PP2A
CTMP
AKT
GYS
Metabolism
GSK3
PEPCK
G6Pace
Metabolism
Glycolysis / Gluconeogenesis
DNA
Myc
CCND1
Cell cycle progression
JAK/STAT signaling pathway
Cytokine
CytokineR
JAK
p21
p27
CDK
Cyclin
Cell cycle
Focal adhesion
ECM
ITGA
ITGB
FAK
Class IB
PI3K
PTEN
MAGI
HSP90
Cdc37
mTORC2
PHLPP
TCL1
FoxO signaling pathway
FOXO
p27Kip1
RBL2
14-3-3
FasL
Bim
Cell survival
BAD
Bcl-xL
Bcl-2
Apoptosis
Casp9
CREB
Mcl-1
RXRα
NUR77
IKK
NFκB
c-Myb
NFκB signaling pathway
MDM2
p53
p53 signaling pathway
Chemokine signaling pathway
Chemokines, Hormones, Neurotransmitters
GPCR
Gβγ
PI(4,5)P2
PI(3,4,5)P3
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(c) Kanehisa Laboratories

Figure 9. PI3K/AKT pathway. The red stars depict 21 targets of AM and its association with the PI3K/AKT, EGFR and MAPK signaling pathway.

Table 2. Molecular docking scores of the top 6 compounds of AM

Target	PDB id	Aegeline	Aurapten	Bergapten	Imperatorin	Marmesin	Nodakenin
AKT1	7WSW	-8.1	-7.7	-6.9	-7.4	-7.7	-8.2
PI3K	1E7U	-7.5	-8.0	-8.2	-8.9	-7.7	-8.8
MAPK3	4FUX	-7.0	-6.1	-6.0	-6.5	-6.7	-8.0
EGFR	3G5X	-7.6	-8.2	-6.8	-7.9	-8.1	-10.1

since ancient times. Various fruit extracts of AM are scientifically proven in treating IBD [13, 30, 69]. An experimental study by Gautam MK et al. in 2013, studied the healing effect of AM and demonstrated the antioxidant effect of ethanolic extract of AM on acetic acid-induced colitis in rats [70]. Furthermore, studies conducted by Ghatule RR et al. in 2014 studied the antibacterial activity of ethanolic extract of AM against intestinal pathogens in TNBS-induced experimental colitis [30]. AM also minimized colonic mucosal damage and inflammation. There are several studies showing the effect of AM in combination with other herbal plants in the polyherbal formulation. Jagtap AG in 2004 and Gandhi T et al. in 2022 studied the combination of herbal plants with AM in various animal models and demonstrated the polyherbal formulation's therapeutic effect in treating IBD [29, 32]. All the studies published up until this date have not demonstrated the mechanistic role of AM in treating IBD. In our study, we systematically demonstrated the molecular mechanism of AM in treating IBD through network pharmacology and a molecular docking approach.

In this study, 46 effective compounds and 358 targets of AM were identified. On further analysis, 80 hub targets depending on the degree were considered effective against IBD. Through CytoHubba we identified AKT1, SRC, MAPK3, MAPK1, EGFR, IL6, TNF, HSP90AA1 and CASP3 as the top 10 hub targets that may contribute to the mechanistic role of AM in treating IBD. Several compounds of AM have been proven to have an impact on the 10 hub targets we identified. Bergapten is a furanocoumarin and it has shown anti-inflammatory activity in in-vitro and invivo animal models. In RAW264.7 cells, Bergapten suppressed the LPS-induced release of TNF-, IL-1, IL-6, PGE2, and NO while upregulating the level of IL-10. It also suppressed the expression of iNOS and COX-2 and possesses anti-inflammatory activity through the JAK/STAT pathway and combats acetic acid induced IBD [71, 72]. Imperatorin is a putative compound present in AM, a study by Huang MH et al. in 2021 revealed the effect of imperatorin on LPS-induced inflammation in RAW 264.7 cells. The study stated that imperatorin hinders the binding of LPS to TLR4 and activates the antioxidative Nrf2 signaling pathway [73, 74]. It acts on multiple targets including PI3K, AKT-1, NFKB, and several cytokines, and possesses anti-inflammatory activity through PI3K/Akt/NF-κB pathway [75, 76]. Auraptene is a highly pleiotropic molecule that modulates intracellular signaling pathways that control inflammation, cell growth, and apoptosis [77]. Kawabata K et al. in 2006 demonstrated the impact of Auraptene on the expression of matrix metalloproteinases such as MMP-2, MMP-7, and MMP-9 in DSS-induced colitis in mice [78, 79]. It also acts on COX-2 ERK1/2 and iNOS [77]. Many such putative compounds of AM act on many such multiple targets and pathways and imbibes the multi-component and multi-targeted mechanism of AM in treating IBD.

We have performed GO functional analysis and KEGG enrichment and the results depicted that the hub targets of AM were primarily related to the PI3K/AKT signaling pathway, MAPK signaling pathway, EGFR tyrosine kinase inhibitor, and HIF-signaling pathway. All these pathways play an important role in targeting IBD. Researchers have proved that there are various pathways that may contribute in treating IBD [80, 81]. Various studies stated that the activation of the PI3K/AKT signaling pathways plays a pivotal role in the pathogenesis of IBD. The PI3K/AKT pathway is not only crucial for combating IBD, it is also important for colitis-associated colon cancer (CAC). In this pathway sev-

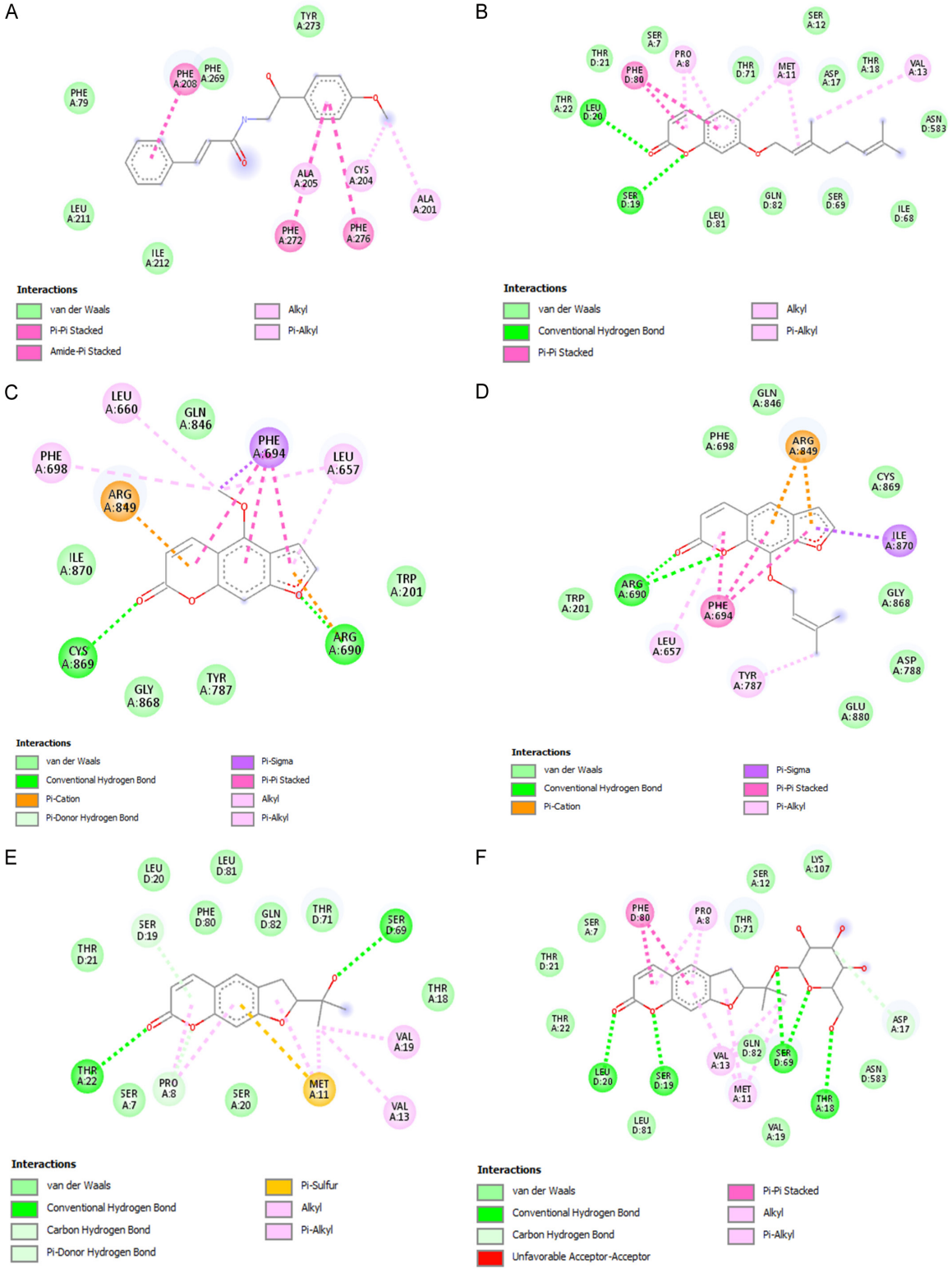

Figure 10. Aegeline-AKT1, vina score = -8.1. B. Auraptene-EGFR, vina score = -8.2. C. Begapten-PI3K, vina score = -8.2. D. Imperatorin-PI3K, vina score = -8.9. E. Marmesin-EGFR, vina score = -8.1. F. Nodakenin-EGFR, vina score = -10.1. Molecular docking analysis of bioactive compounds of AM towards the putative targets. A. The elaborative information regarding grids and hydrogen bonding interaction and all the other targets and their interaction can be found in **Tables 2** and S6.

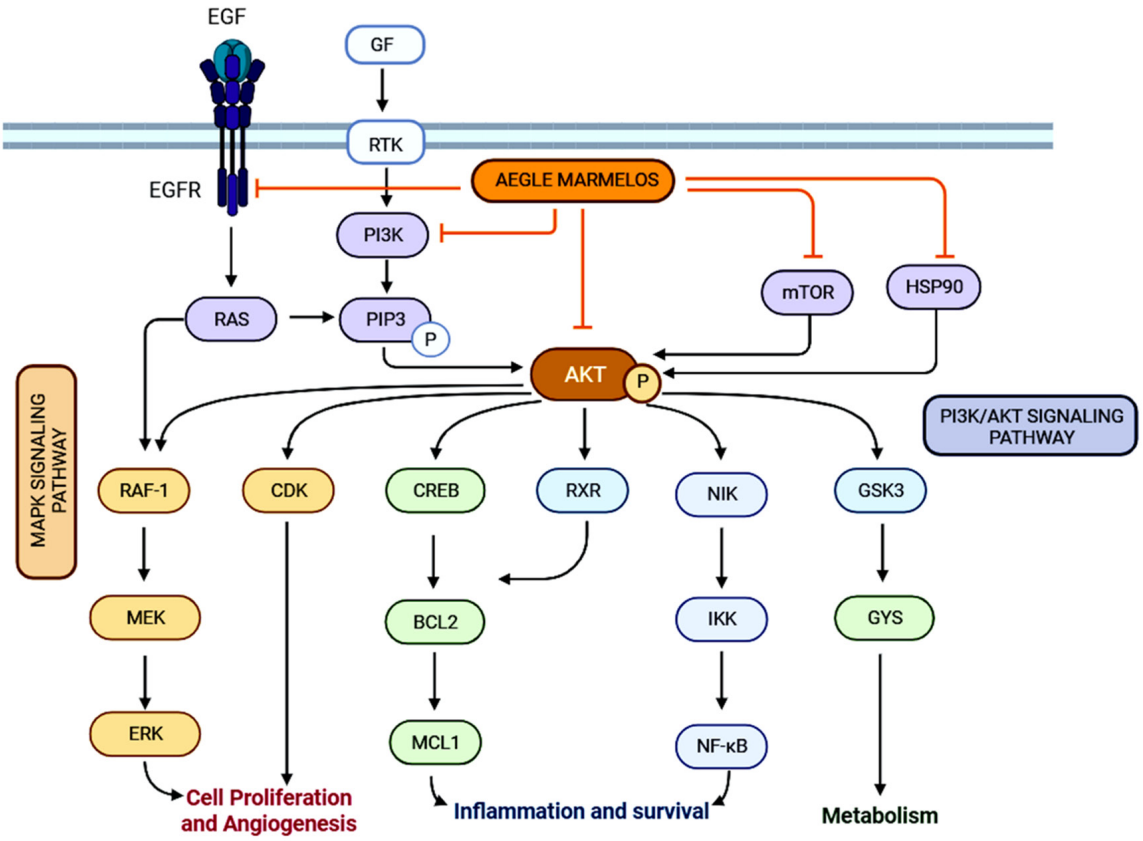

Figure 11. Summary figure. Network pharmacology and molecular docking predicted pathway of AM in combating IBD. AM targets EDFR-mediated PI3/AKT pathway along with MAPK pathway. AM acts on multiple targets and multiple pathways to alleviate IBD. PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; RTK, receptor tyrosine kinase; GF, growth factor; EGF, epidermal growth factor; mTOR, mammalian target of rapamycin. The figure was created with BioRender.com.

eral proinflammatory mediators such as TNF-α, IL-1β, IL-6 and many more are upregulated. Network pharmacological analysis revealed the association of AM with major hub targets from PI3K/AKT pathway in combating IBD.

The present study suggested that AM could treat IBD through various pathways. The results lay the foundation for the extensive role of AM in treating IBD. The molecular docking studies reveal that the compounds have higher binding affinity with PI3K, AKT and EGFR suggesting interconnections between bioactive compounds and the targets. Taken together, the network pharmacology and molecular docking revealed the targets of AM and its in-depth association in mitigating IBD through the EGFR-mediated PI3K/AKT signaling pathway. The proposed mechanism of the hub targets of AM in treating IBD is displayed in the summary **Figure 11**. It demonstrates the way PI3K/AKT, MAPK, and EGFR are cross-linked, as well as how AM interferes with these targets. However, the study needs to be validated in animal models for its various pathways and clinical trials need to be done to assure the impact of AM in treating IBD.

To sum up, we have identified various active compounds from AM in combating IBD. Potential targets of the compounds match an array of IBD pathways, exhibiting a multi-component, multi-targeted therapeutic effect in the treatment of IBD. The present research is the first to predict the mechanism of AM utilizing a computational network pharmacology technique and a molecular docking approach in alleviating IBD. However, as this research is based on predictions made from a variety of databases, further experimental research is still required. Strong preliminary data from the study will assist researchers in focusing on potential targets of AM for treating IBD. The current study strongly implies that AM might have

a focused therapeutic effect in the treatment of IBD.

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Disclosure of conflict of interest

None.

Abbreviations

AM, Aegle marmelos; IBD, Inflammatory bowel disease; CD, Crohn's disease; UC, Ulcerative colitis; OB, oral bioavailability; DL, drug likeliness; PPI, Protein-protein interaction; GO, Gene Ontology; BP, biological processes; MF, molecular function; CC, cellular component; KEGG, Kyoto Encyclopaedia of Genes and Genomes; MNC, maximum neighborhood component; MCODE, Molecular Complex Detection; PDB, protein drug bank; DAVID, database for annotation, visualization, and integrated discovery; CRC, Colitis associated colon cancer.

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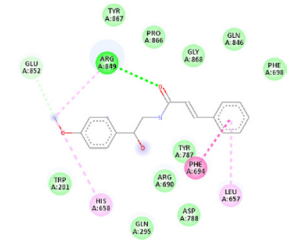
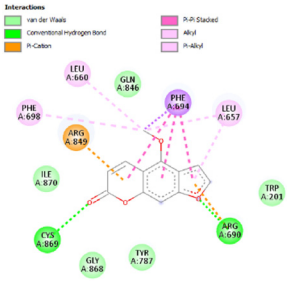
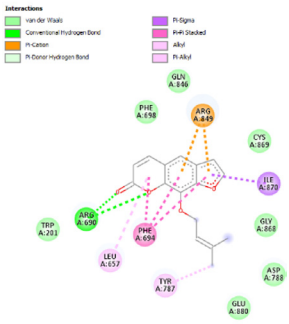
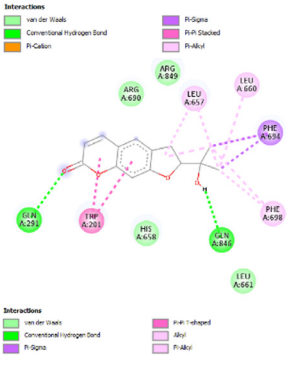
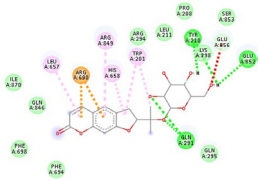
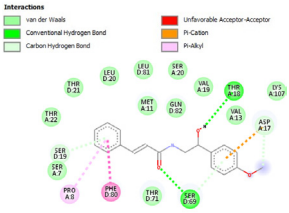
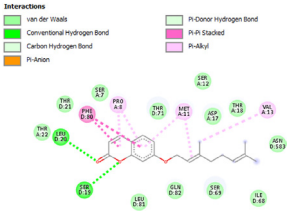
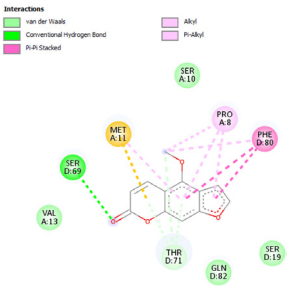
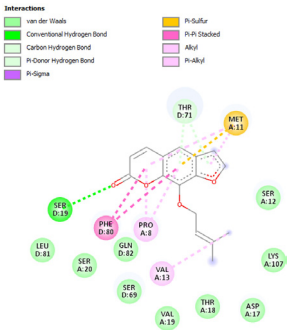
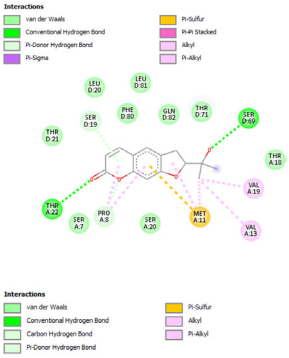
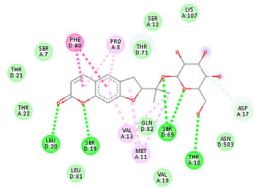
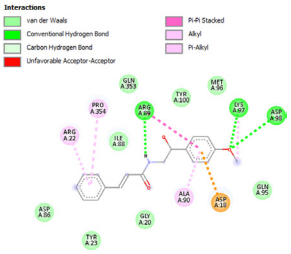
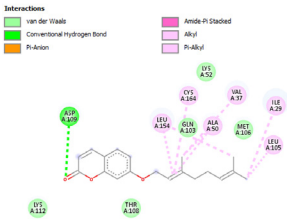
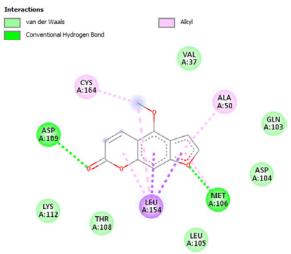
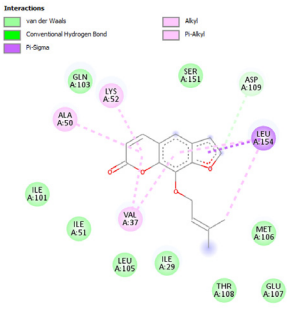
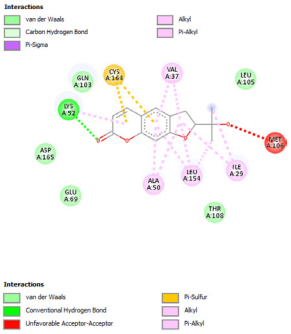
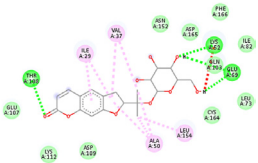
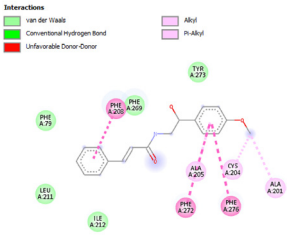
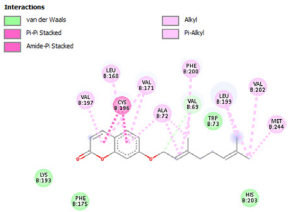
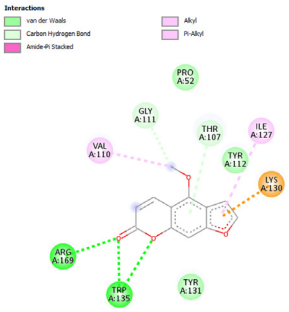
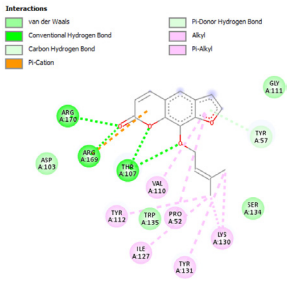
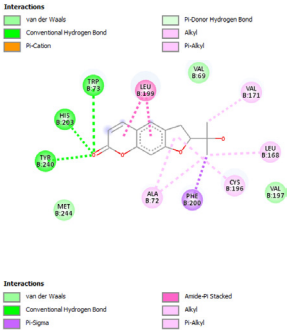
Table S6. Molecular docking of various compounds with hub IBD targets

Protein Name	Protein PDB	Grids	Com-pounds	Docking Score (kcal/mol)	Hydrogen bond interaction	Binding site interactions
PI3K	1E7U	Center_x = 23.028 center_y = 62.413 center_z = 20.582 size_x = 42 size_y = 51 size_z = 50	Aegeline	-7.5	ARG:849	
			Auraptene	-8	CYS: 869 ARG: 690	
			Bergapten	-8.2	CYS: 869 ARG: 690	
			Imperatorin	-8.9	ARG: 690	
			Marmesin	-7.7	GLN: 291 GLN: 846	

			Nodakenin	-8.8	TYR: 210 GLU: 852 GLN: 291	
EGFR (Endothelial Growth Factor Receptor)	3G5X	center_x = 23.094 center_y = 18.130 center_z = 60.451 size_x = 40 size_y = 40 size_z = 40	Aegeline	-7.6	THR: 18 SER: 68	
			Auraptene	-8.2	LEU: 20 SER: 19	
			Bergapten	-6.8	SER: 69	
			Imperatorin	-7.9	SER: 19	
			Marmesin	-8.1	SER: 69 THR: 22	

			Nodakenin	10.1	SER D: 19 SER D: 69 LEU: 20 THR: 18	
MAPK1 (Mitogen Activated Protein Kinase 1)	4FUX	center_x = 18.517 center_y = 6.377 center_z = 16.735 size_x = 40 size_y = 45 size_z = 50	Aegeline	-7.0	ARG: 89 LYS: 97 ASP: 98	
			Auraptene	-6.1	ASP: 109	
			Bergapten	-6.0	ASP: 109 MET: 106	
			Imperatorin	-6.5	--	
			Marmesin	-6.7	LYS: 52	

			Nodakenin	-8.0	THR: 108 LYS: 62 GLU: 69	
AKT1	7WSW	center_x = 164.895 center_y = 189.623 center_z = 195.270 size_x = 48 size_y = 66 size_z = 67	Aegeline	-8.1	--	
			Auraptene	-7.7	--	
			Bergapten	-6.9	ARG: 169 TRP: 135	
			Imperatorin	-7.4	ARG A: 170 ARG A: 169 THR: 107	
			Marmesin	-7.7	TYR: 240 HIS: 203 TRP: 73	

	Nodakenin	-8.2	GLU: 61 ARG: 177 ARG: 58 PRO: 52