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Exploring the bioactives and the mechanism of Aegle marmelos in the treatment of Inflammatory bowel disease through network pharmacology and molecular docking approach

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Article

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Abstract

Inflammatory bowel diseases (IBD) are recurrent inflammatory conditions that occur in the gastrointestinal tract, and current treatment does not have satisfactory results, we still need newer 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 and belonging 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. 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 the Cyto Hubba plugin of Cytoscape (3.10.0), 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 potent compounds of AM and the molecular docking studies with the hub target depict their higher binding affinity to PI3K, AKT, and EGFR. The Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis suggest that PI3-AKT signaling pathway, EGFR tyrosine kinase inhibitor, and MAP Kinase signaling pathway are the major pathways correlating with AM in combating IBD. The network pharmacological and molecular docking approach 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.

1. 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 incidence of UC, especially in Asia, is increasing in the 21st century[4]. 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[5,6].

IBD is a resilient, relapsing inflammatory condition characterized by immune system dysregulation, an altered cytokine profile, and persistent inflammation of the intestinal mucosa[6]. Since an enormous percentage of UC patients are resistant to or intolerant of typical medication treatments, current options are restricted and the mainstays of UC treatment are medical care and colectomy [7]. There are no satisfactorily approved curative treatments for UC at present times. Treatment methods for UC include using 5-aminosalicylic acid (5-ASA), glucocorticoids, TNF-α blockers, and immunosuppressive medications[8]. 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[9,10].

Several physicians have utilized the Indian traditional medical system of Ayurveda to treat countless gastrointestinal ailments [11]. Alternative therapies from conventional medicine, such as Ayurveda, have been deemed as potential choices in the discovery of new therapeutics. Many bioactives 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 competent to study intricate connections between bioactives, targets, illnesses, and genes [12]. Network pharmacology, also known as computational system biology is an entirely novel approach in computing biology that unifies systems biology, omics, and pharmacological data. It is best to utilize newer drug discovery processes from traditional medicines in a concise duration. By this innovative pharmacological strategy, drugs that target multiple nodes in interconnected systems and are connected to various nodes, generate data for a drug's assessment and may be useful in discovering a multitargeted therapy for chronic disorders[13]. Such computational techniques are helpful in better understanding the disease's pathological states and could accelerate the new drug discovery process. This approach could help mitigate 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[14–20]. AM fruits have many potential health benefits, notably radio-protective properties, peroxidation, antibacterial, lipid-inhibiting, anti-diarrheal, gastroprotective, antiviral, anti-ulcerative colitis, cardioprotective, antioxidant, and hepatoprotective effects due to its biologically active compounds [16,21,22].

Fruit of AM contains fibers, carotenoids, terpenoids, coumarins, flavonoids, and alkaloids, which are responsible for the fruit's health-promoting and protective effects [23,24]. Bael comprises various phytochemicals, and 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 comprises of various biologically active compounds.[14,17,24,25] Numerous additional compounds, including tannin, fagarine, luvangetin, psoralen, cumin aldehyde, citral, lupeol, skimmianine, marmin, marmelide, aurapten, marmelosin, eugenol, citronellal, cineole are biologically effective against both minor and serious gastrointestinal disorders [17,24,26].

Fruit juice of AM and its extract has 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 [27–29]. 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 [30].

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

2. Methods

2.1 Screening of Phytochemical compounds of AM

The list of compounds of AM fruits was 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)[31] 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 [32,33].

2.2 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 bioactives. ADME, which stands for absorption, distribution, metabolism, and excretion, is a crucial step in figuring out the pharmacokinetics of potential medications [34,35]. Oral bioavailability score (OB) and drug-likeliness (DL) as per the violations of Lipinski's rule, Veber rule, and Ghosh rule were chosen among them as pharmacokinetic parameters. Compounds with oral bioavailability score of less than 10% and more than two violations of drug likeliness rules were excluded from the study [36–39]. Rest all the compounds were selected for the screening of their multiple targets.

2.3 Potential target fishing for compounds of AM

Target fishing of the compounds can be done by availing of 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[40]. 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 bioactives [41,42]. Another database used for fetching the targets of the compounds was Super-PRED

(https://prediction.charite.de/subpages/target_prediction.php). Targets were combined and then they were used for further downstream processes [43].

2.4 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 [44–46]. The UniProtKB (https://www.uniprot.org) database validated each target. DisGenNET is a platform for knowledge management that integrates and integrates information about genes and variants linked to disease 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[45]. DrugBank is a web-based database featuring detailed molecular information regarding medications, their mechanisms, their interactions, and their targets[44]. 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 all the targets for IBD.

2.5 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 [47]. Targets with less confidence score 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 screen out the hub targets due to the intricacy of the original network created in the STRING database[48–50]. The common targets of IBD and AM were identified. The Cytoscape network was analyzed using the Network Analyzer tool in Cytoscape and the centrality closeness, betweenness, and degree were explored. The hub targets with more than 11 degree 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 gene 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.

2.6 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 [51]. 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.

2.7 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 to methodically explore the biological processes that AM as a treatment against IBD [52,53]. The putative 80 targets of AM were used for the enrichment analysis. GO terms of top 10 results in bar chart colored by − log10 (P values). Targets involved in biological processes, molecular functions, and cellular functions were extracted from the INPUT database[54–57]. KEGG enrichment analysis was performed and the top 20 pathways were represented in the bubble chart. The 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.

2.8 Molecular docking.

Molecular docking is the most widely used technique in drug design because it can predict the ability of ligands and proteins to bind, as well as the location of binding. The three-dimensional structures of all compounds were downloaded from PubChem [40]. 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/) [58,59]. All these protein targets were chosen for the docking studies. PDB ID and grids are listed in the table. 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(Gu et al. 2020; Xu et al. 2021a; Fan et al. 2023). 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 v21.0.20298 was used to visualize the docked structures[64].

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

3. Results

3.1 Identification of Candidate Compounds in *Aegle marmelos*

Literature surveys and various databases revealed the presence of various compounds in AM fruits. In total of 50 probable compounds of the AM were extracted from databases (Fig. 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 [65]. 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, the rest all the compounds were used for further analysis (Table S1).

3.2 Targets Identification of compounds of *Aegle marmelos.*

The target fishing of all the individual compounds were done using the Swiss Target Prediction 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 (Fig. 2a).

3.3 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. 5140 UC-related genes and 5401 CD-related targets were retrieved from GeneCards, OMIM, and TTD databases (Table S3). A total of 560 targets from various compounds of AM and 6974 targets to UC and CD, they shared 358 targets in common (Fig. 2a, 2b,2c).

3.4 Protein-protein interaction

For the PPI, 358 targets of AM were put into a STRING database. The PPI network comprises 358 nodes and 1183 edges in the interaction network, PPI enrichment p-value was < 1.0e-16 (Fig. 3a). A Confidence interval of 0.9% (highest confidence) was selected for the analysis of PPI. Annotation data of the PPI network of AM targets is shown in detail in Table S4. The PPI network of 358 AM targets was analyzed using Network Analyser, an analytical tool in Cytoscape that was used to analyze the network and to get the targets with the highest degree. The targets with degree more than 11 were selected for further analysis (Fig. 3b). 80 hub targets of AM were opted for the analysis of sub-network construction and were used for the enrichment analysis. Various targets with higher degree score 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). All the 80 hub targets with their degree score are listed in Table 2. Furthermore, the 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 degree (Fig. 4a,b).

Table 2
Putative targets of AM on IBD

Hub targets	Target name	Uniprot id	Degree	Closeness	Betweenness
AKT1	Serine/threonine-protein kinase AKT	P31749	69	0.89	0.05
SRC	Tyrosine-protein kinase SRC	P12931	69	0.89	0.05
MAPK3	MAP kinase ERK1	P27361	67	0.87	0.04
EGFR	Epidermal growth factor receptor erbB1	Q9H2C9	64	0.84	0.02
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase liver	P04406	61	0.81	0.02
HSP90AA1	Heat shock protein HSP 90-alpha	P07900	60	0.81	0.02
MAPK1	MAP kinase ERK2	P28482	60	0.81	0.03
TNF	Tumor Necrosis factor- alpha	P01375	60	0.81	0.02
CASP3	Caspase 3	P42574	57	0.78	0.01
IL6	Interleukin-6	P05231	56	0.77	0.01
VEGFA	Vascular endothelial growth factor A	Q9H1W9	56	0.77	0.01
MTOR	Serine/threonine-protein kinase mTOR	P42345	55	0.77	0.01
ESR1	Estrogen receptor alpha	P03372	54	0.76	0.02
ERBB2	Receptor protein-tyrosine kinase erbB-2	P04626	53	0.75	0.01
MAPK14	MAP kinase p38 alpha	Q16539	51	0.74	0.02
MAPK8	c-Jun N-terminal kinase 1	P45983	50	0.73	0.01
JAK2	Tyrosine-protein kinase JAK2	O60674	48	0.72	0.01
PIK3CA	PI3-kinase p110-alpha subunit	P42336	48	0.72	0.01
S1PR1	Sphingosine 1-phosphate receptor Edg-1	P21453	48	0.71	0.00
PPARG	Peroxisome proliferator-activated receptor gamma	Q15180	46	0.71	0.01
IL2	Interleukin-2	P60568	45	0.70	0.01
MDM2	p53-binding protein Mdm-2	Q53XW0	43	0.69	0.01

Hub targets	Target name	Uniprot id	Degree	Closeness	Betweenness
PTPN11	Protein-tyrosine phosphatase 2C	Q06124	42	0.68	0.01
TLR4	Toll-like receptor 4	Q5VZI9	41	0.68	0.01
CREBBP	CREB-binding protein/p53	Q92793	40	0.67	0.00
MMP9	Matrix metalloproteinase 9	P14780	40	0.67	0.01
FGF2	Basic fibroblast growth factor	Q9UCS5	39	0.66	0.00
HDAC1	Histone deacetylase 1	Q13547	39	0.66	0.01
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1	Q02750	39	0.66	0.00
AR	Androgen Receptor	P10275	38	0.66	0.01
CASP8	Caspase-8	Q9UQ81	38	0.66	0.00
JAK1	Tyrosine-protein kinase JAK1	P23458	38	0.65	0.01
PTK2	Protein tyrosine kinase 2 beta	Q05397	38	0.66	0.00
RPS6KB1	Ribosomal protein S6 kinase 1	P23443	38	0.66	0.00
LYN	Tyrosine-protein kinase Lyn (by homology)	P07948	37	0.65	0.01
IGF1R	Insulin-like growth factor I receptor	P08069	36	0.65	0.00
KDR	Vascular endothelial growth factor receptor 2	P35968	36	0.65	0.00
NFKB1	Nuclear factor NF-kappa-B p105 subunit	P19838	36	0.65	0.00
HSP90AB1	Heat shock protein HSP 90-beta	P08238	35	0.64	0.00
PDGFRB	Platelet-derived growth factor receptor beta	P09619	35	0.64	0.00
ABL1	Tyrosine-protein kinase ABL	P00519	34	0.64	0.00
MET	Hepatocyte growth factor receptor	P08581	34	0.64	0.01
MMP2	Matrix metalloproteinase 2	P08253	31	0.62	0.00
NR3C1	Glucocorticoid receptor	P04150	31	0.62	0.00
PIK3CB	PI3-kinase p110-beta subunit	P42338	31	0.62	0.01
PTPN1	Protein-tyrosine phosphatase 1B	P18031	31	0.62	0.00

Hub targets	Target name	Uniprot id	Degree	Closeness	Betweenness
PRKCA	Protein kinase C alpha	P17252	30	0.62	0.00
TERT	Telomerase reverse transcriptase	O14746	30	0.62	0.00
HSPA5	78 kDa glucose-regulated protein	P11021	29	0.61	0.00
PPARA	Peroxisome proliferator-activated receptor alpha	Q07869	29	0.61	0.01
CDK2	Cyclin-dependent kinase 2/cyclin E1	P24941	28	0.60	0.00
HSPA1A	Heat shock 70 kDa protein 1	P0DMV8	27	0.60	0.00
IKBKB	Inhibitor of nuclear factor kappa B kinase beta subunit	O14920	27	0.60	0.00
JAK3	Tyrosine-protein kinase JAK3	P52333	27	0.60	0.00
PTPN6	Protein-tyrosine phosphatase 1C	P29350	27	0.60	0.00
SYK	Tyrosine-protein kinase SYK	P43405	27	0.59	0.00
PTK2B	Protein tyrosine kinase 2 beta	Q14289	26	0.60	0.00
CDK1	Cyclin-dependent kinase 1/cyclin B1	P06493	25	0.59	0.00
ERBB4	Receptor protein-tyrosine kinase erbB-4	Q15303	25	0.59	0.00
HSPA8	Heat shock cognate 71 kDa protein	P11142	25	0.59	0.00
RXRA	Retinoid X receptor alpha	P19793	23	0.59	0.00
BTK	Tyrosine-protein kinase BTK	Q06187	22	0.57	0.00
NOS2	Nitric oxide synthase, inducible	P35228	22	0.58	0.00
RARA	Retinoic acid receptor alpha	Q96S41	22	0.58	0.00
NR0B2	Nuclear receptor subfamily 0 group B member 2	Q15466	21	0.57	0.00
PLG	Plasminogen	P00747	21	0.57	0.00
PRKCB	Protein kinase C beta	Q9UEH8	21	0.57	0.00
TYK2	Tyrosine-protein kinase TYK2	P29597	21	0.58	0.00
YES1	Tyrosine-protein kinase YES	P07947	21	0.58	0.00

Hub targets	Target name	Uniprot id	Degree	Closeness	Betweenness
PAK1	Serine/threonine-protein kinase PAK 1	Q13153	20	0.57	0.00
MMP1	Matrix metalloproteinase 1	P03956	19	0.57	0.00
HDAC3	Histone deacetylase 3	O15379	16	0.56	0.00
PTPN2	T-cell protein-tyrosine phosphatase	P17706	16	0.56	0.00
BCL2	Apoptosis regulator Bcl-2	P10415	15	0.55	0.00
RXRB	Retinoid X receptor beta	P28702	15	0.55	0.00
HSF1	Heat shock factor protein 1	Q00613	13	0.54	0.00
MAP2K3	Dual specificity mitogen-activated protein kinase kinase 3	P46734	13	0.54	0.00
RARB	Retinoic acid receptor beta	P10826	11	0.53	0.00
RARG	Retinoic acid receptor gamma	Q9H1I3	11	0.53	0.00

3.5 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 (Fig. 4c,d,e,f,g). Details for all the clusters, their targets, and their score are listed in Table S4.

3.6 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 Fig. 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 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).

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 (Fig. 6a) According to the KEGG enrichment most targets were involved in several pathways, and amongst them 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 for using AM in treating IBD. The top 5 KEGG pathways and the gene network was constructed forming the close interaction between genes and pathway (Fig. 6b). Furthermore, the gene distribution feature of the INPUT database provided the Sankey diagram of the top 5 genes and their correlation with the KEGG pathway, biological processes (BP), molecular functions (MF), and cellular components (CC) (Fig. 7). Genes connected to the number of KEGG pathways, BP, MF, and CC are described in detail in Table S5. The network was constructed comprising of the top 6 pathways connected to IBD and their target depicting the interconnection between various pathways (Fig. 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 Fig. 9.

3.7 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 aurapten, 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.3). Figure 10 depicts six compounds with the highest binding energy target. 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.

Table 3
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

4. Discussion

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 [66]. 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 [67]. Even though traditional treatments can be useful in certain cases and have 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 since ancient times. Various fruit extracts of AM are scientifically proven in treating IBD [11,28,68]. 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 [69]. 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 [28]. AM also minimized colonic mucosal damage and inflammation. Several studies are 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 [27,30]. All the studies to date never 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 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 had 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 suppresses the expression of iNOS and COX-2 possesses anti-inflammatory activity through the

JAK/STAT pathway and combats acetic acid-induced IBD [70,71]. Imperatorin is again a putative compound present in AM, a study by Huang MH et al. in 2021 studied 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 [72,73]. It acts on multiple targets including PI3K, AKT-1, NFKB, and several cytokines, and possesses anti-inflammatory activity through PI3K/Akt/ NF-κB pathway[74,75]. Auraptene is a highly pleiotropic molecule that modulates intracellular signaling pathways that control inflammation, cell growth, and apoptosis [76]. 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[77,78]. It also acts on COX-2 ERK1/2 and iNOS [76]. Many such putative compounds of AM act on many such multiple targets and pathways and imbibe 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 various pathways may contribute to treating IBD [79,80]. Various studies stated that the activation of the PI3K/AKT signaling pathways plays a pivotal role in the pathogenesis of IBD. PI3K/AKT pathway is not only crucial for combating IBD, but it is also important for colitis-associated colon cancer (CAC). In this pathway, several 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 the PI3K/AKT pathway in combating IBD.

Studies revealed the interrelationship between HIF, EGFR, and PI3K/AKT pathway. The mammalian target of rapamycin (mTOR) is activated by the PI3K/Akt pathway, which increases the synthesis of HIF-1a [81]. HIF-1a and NF-kB regulate the inflammatory microenvironment, according to studies [82]. One of the hub targets of this study, EGFR, acted as a cell membrane receptor protein that could phosphorylate PI3K and subsequently activate the PI3K/AKT/HIF-1 pathway to transduce inflammatory signals [83]. EGFR suppresses tumorigenesis in chronic colitis by optimizing responses to chronic inflammation and reducing subsequent tumor initiation and progression [84,85]. Thus, EGFR is an important marker of colitis-associated colon cancer (CAC) and EGFR inhibitors could decrease the risk of colitis-associated colon cancer. Researchers utilize the network pharmacology approach to unveil the mechanism of TCM (Traditional Chinese Medicines) used in treating UC[86–89]. Various traditional Chinese medicines used in the treatment of UC have been depicted to act through the EGFR-mediated PIK3/AKT signaling pathway[89–91].

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 a higher binding affinity with PIK3, AKT, and EGFR suggesting interconnection between bioactives and the targets. Taking together, the network pharmacology and molecular docking revealed the targets of AM and its in-depth association in mitigating IBD through the EGFR-mediated PIK3/AKT

signaling pathway. 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.

5. Conclusion

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. The proposed mechanism of the hub targets of AM in treating IBD is displayed in the summary Fig. 11. It demonstrates the way PI3K/AKT, MAPK, and EGFR are cross-linked and how AM interferes with these targets. 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.

Future implications

Computational system biology also known as network pharmacology is a useful tool to accelerate the drug discovery process. The present research uncovered an extensive list of biologically active AM compounds and probable targets. Additional investigation on such bioactives can be further carried out to investigate their scientific role in combating IBD. The association involving AM and multiple KEGG pathways can be utilized for exploring the newer therapeutic role of AM on numerous other medical conditions.

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.

Declarations

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Authors Contributions

Bhagyabhumi Shah: Conceptualization, design of the study, acquisition of data, data analysis, drafting the manuscript. **Nilay Solanki:** supervision, revision of the manuscript, and proofreading of the manuscript.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Ethics declarations

Competing interests

The authors declare no competing interests

Supplementary information

Available in supplementary files.

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Figures

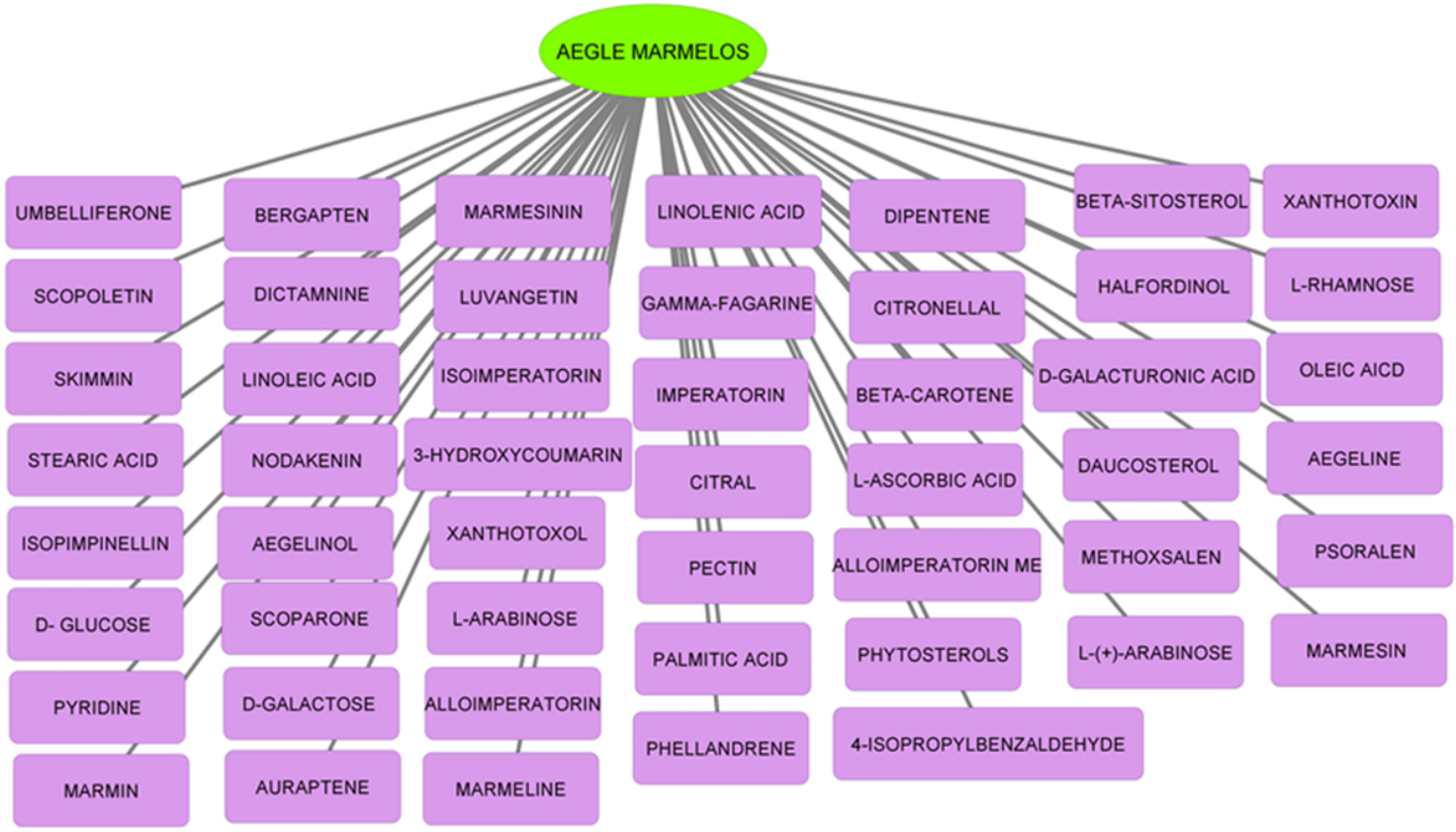


Figure 1

Phytoconstituents present in fruits of Aegle marmelos

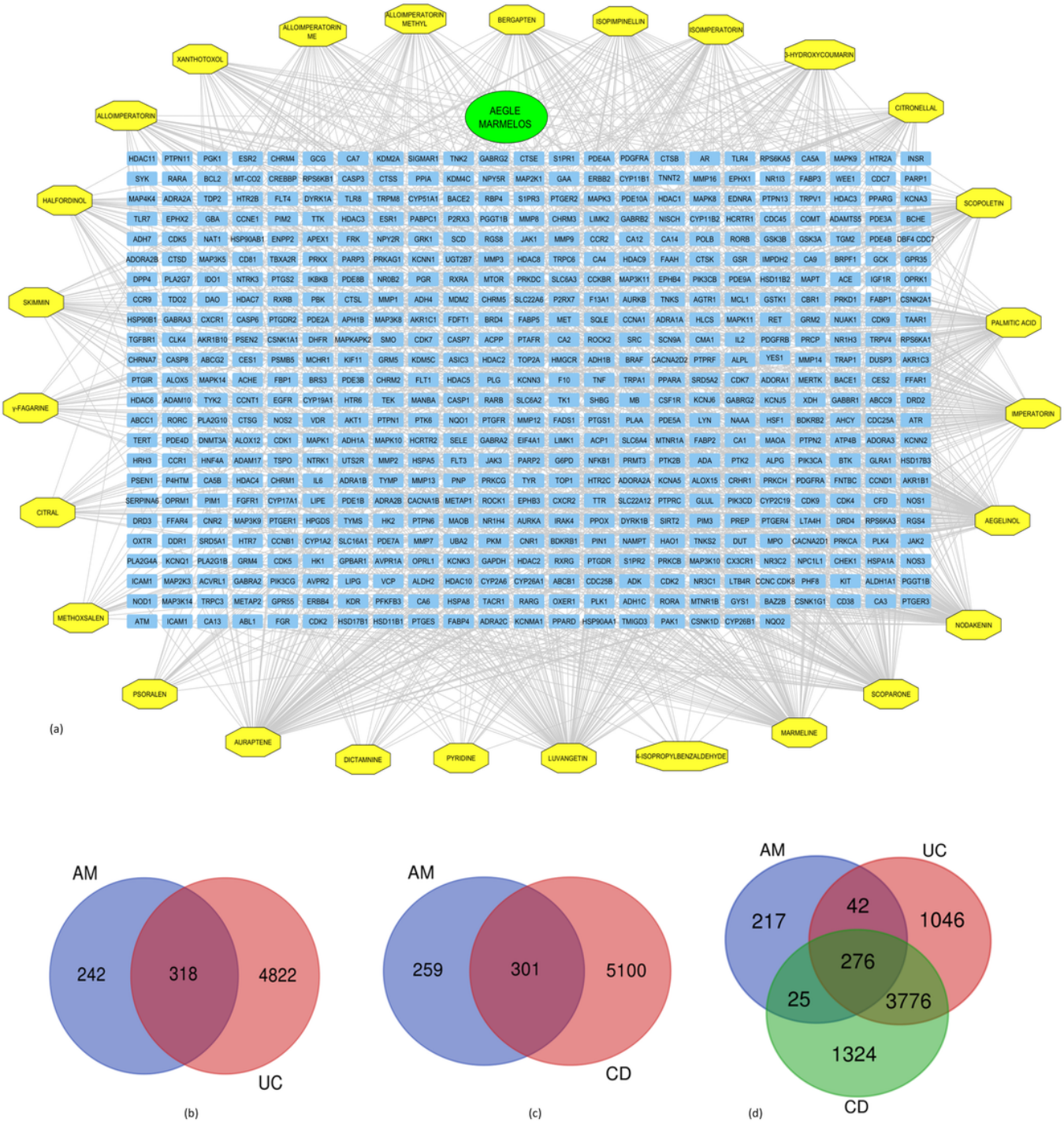


Figure 2

Bioactives of AM and their targets. (a) Interaction network of AM compounds and their putative targets. It comprises of 576 nodes and 1417 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.

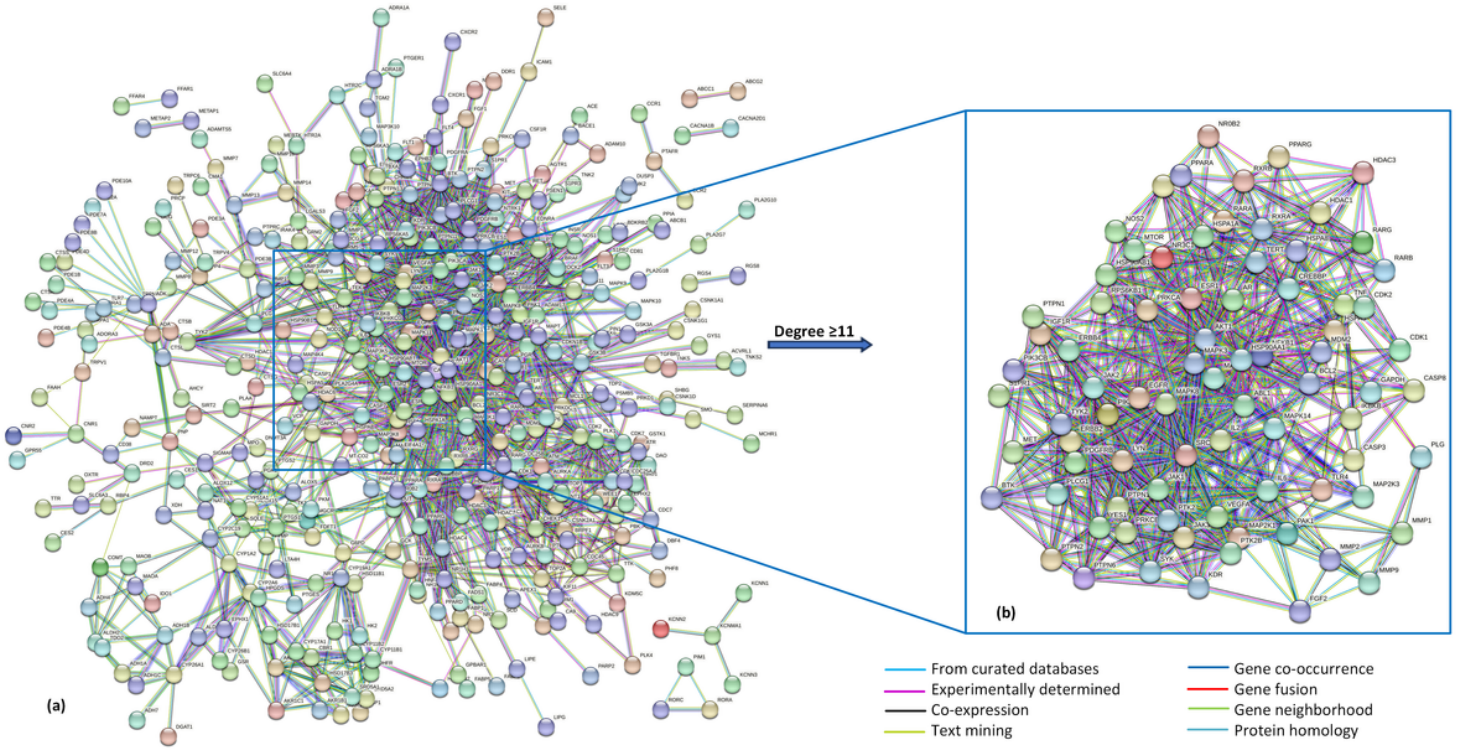


Figure 3

Protein-protein interaction of targets of AM against IBD. (a) The network showcases 358 nodes (all the targets found common with IBD targets) and 1183 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 1391 edges.

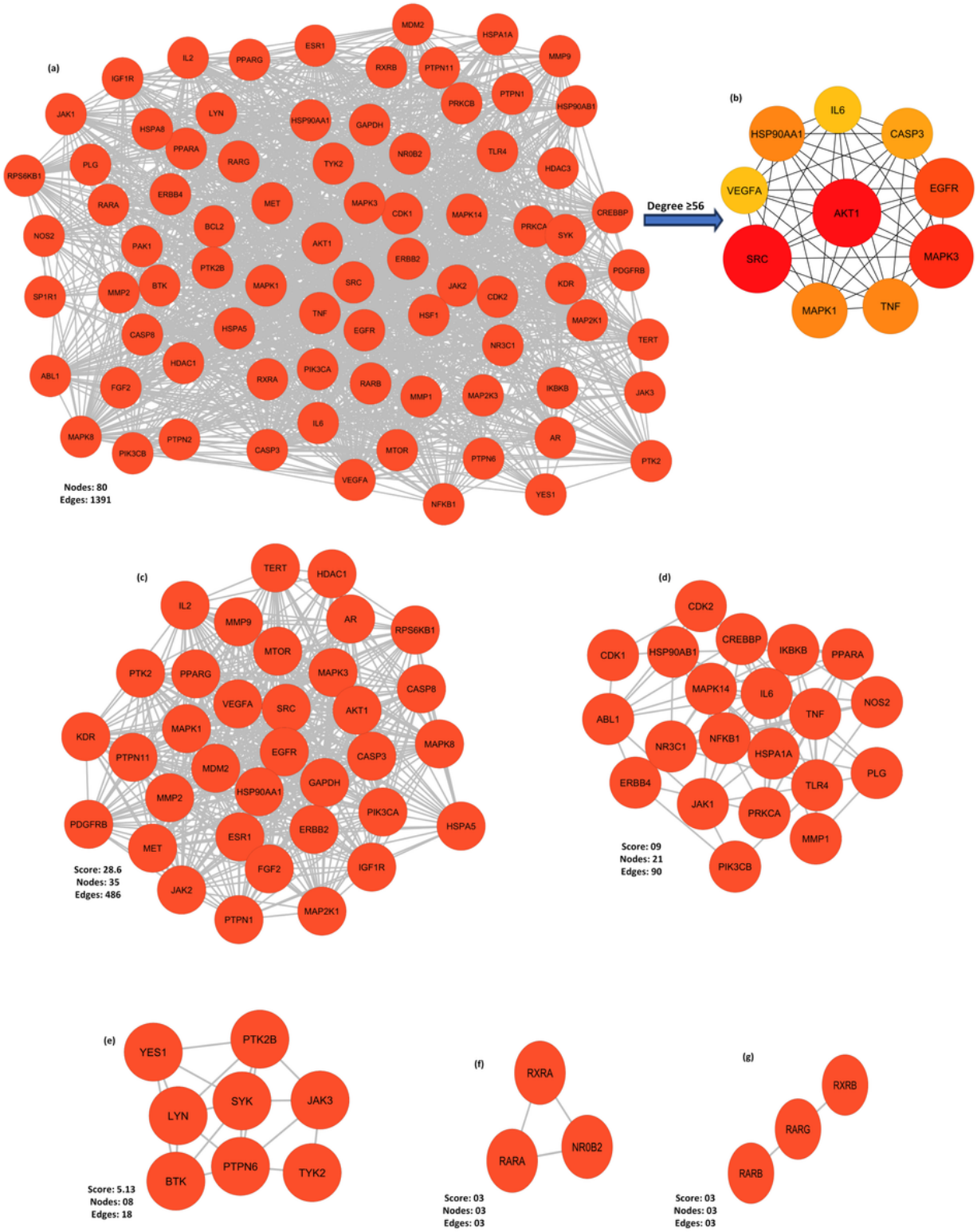


Figure 4

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 the node higher degree amongst all the nodes. MCODE clusters (c,d,e,f,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 3 nodes, 3 edges, and 3 score each.

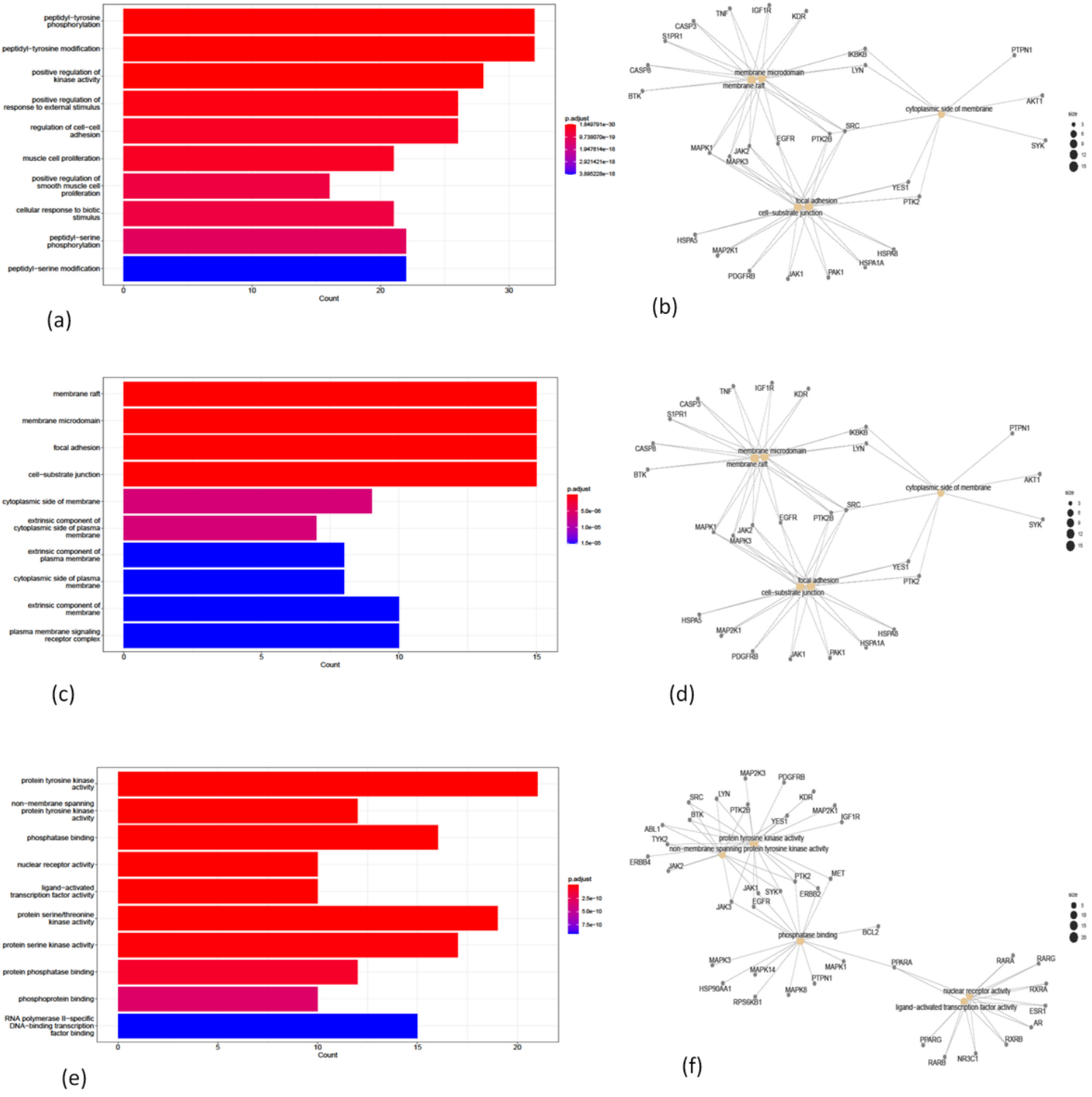


Figure 5

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 rank order according to p-values. The X-axis represents gene

count, while the 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 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 network. (c) and (d) represents the top 10 significantly enriched terms in Molecular Functions (MF) and the top 5 MF and gene networks respectively. (e) and (f) represents the top 10 significantly enriched terms in Cellular Component (CC) and top 5 CC and gene network respectively.

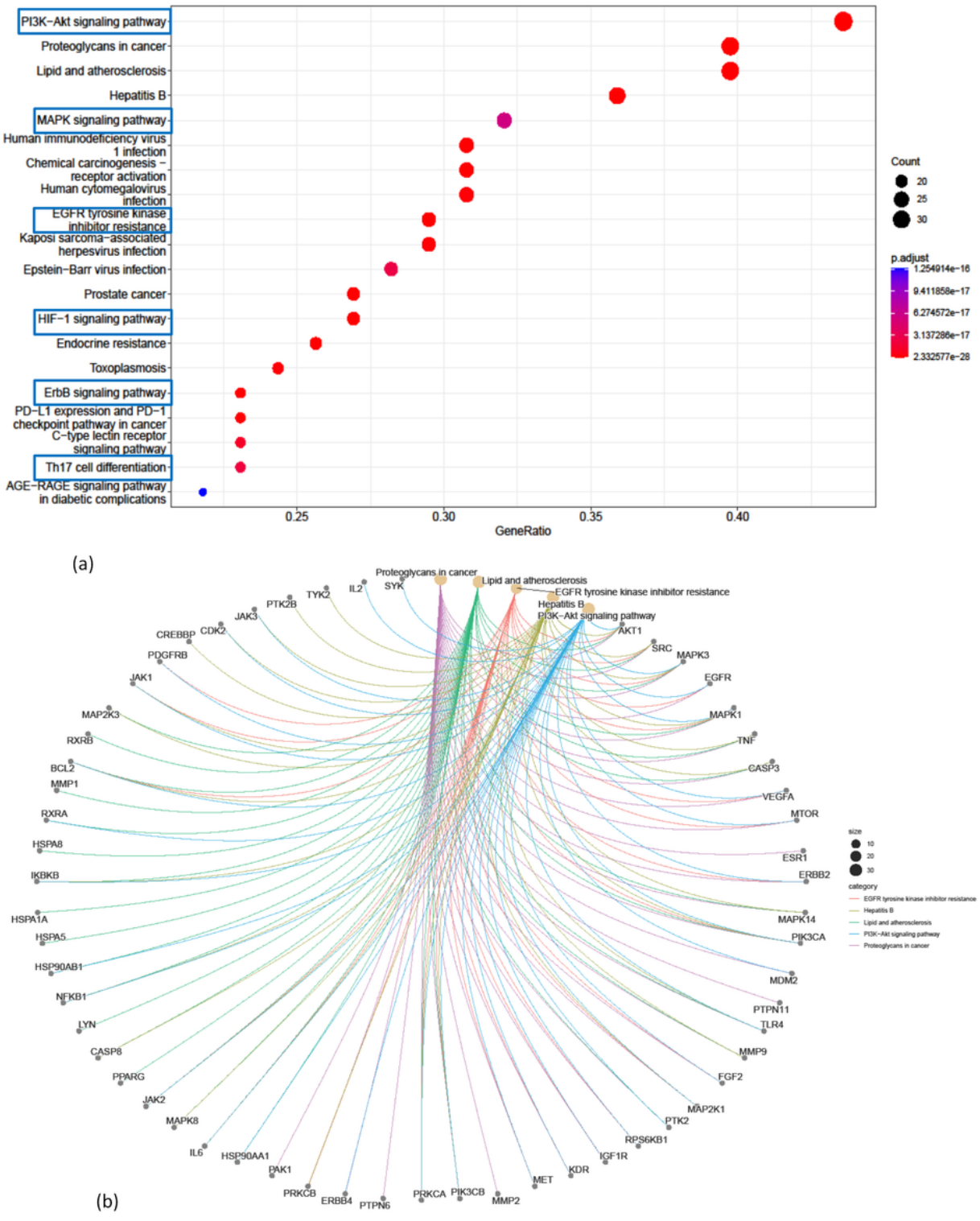


Figure 6

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

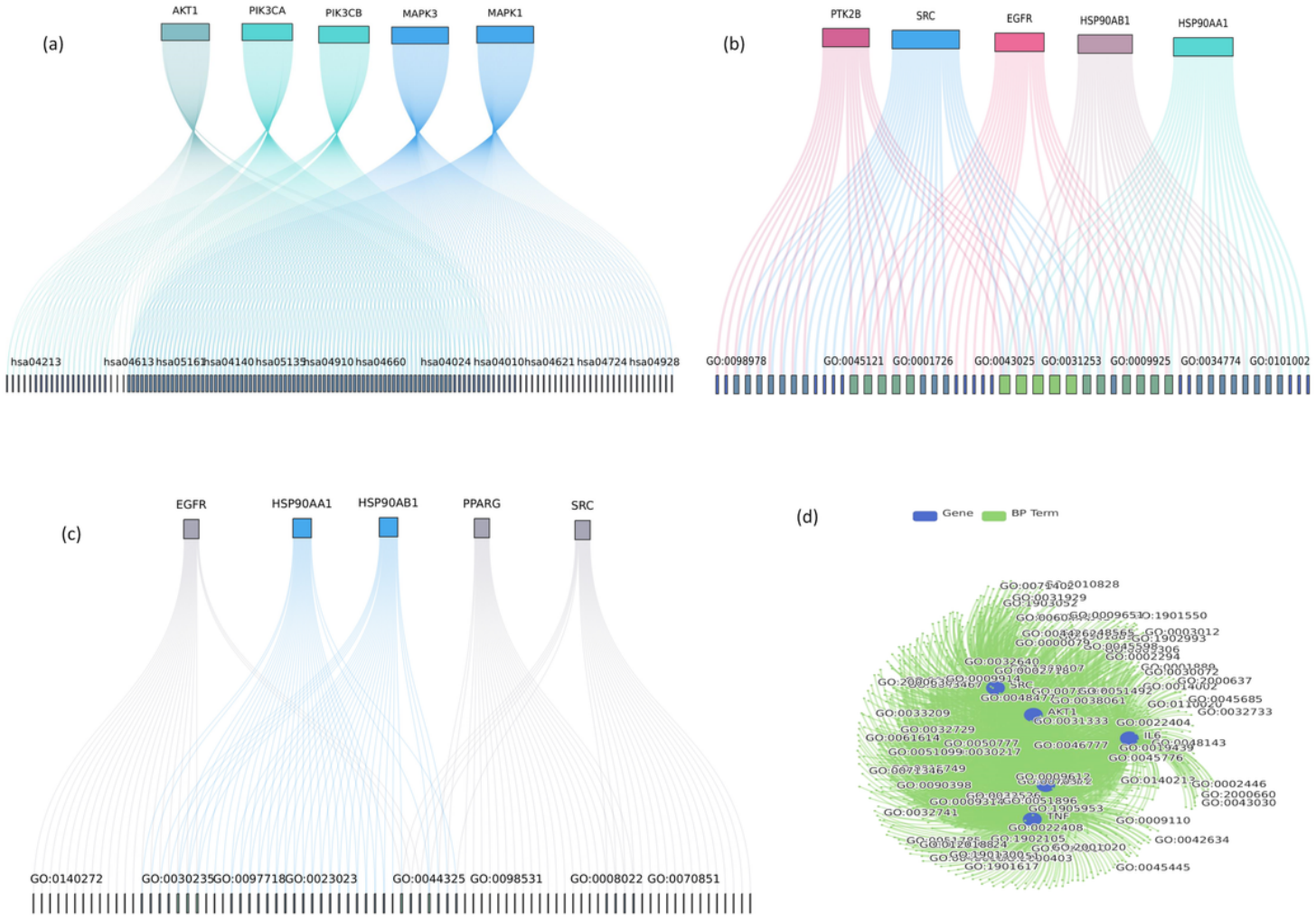


Figure 7

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 more gene involvement in various pathways. Lines with different colors depict different genes and their 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.

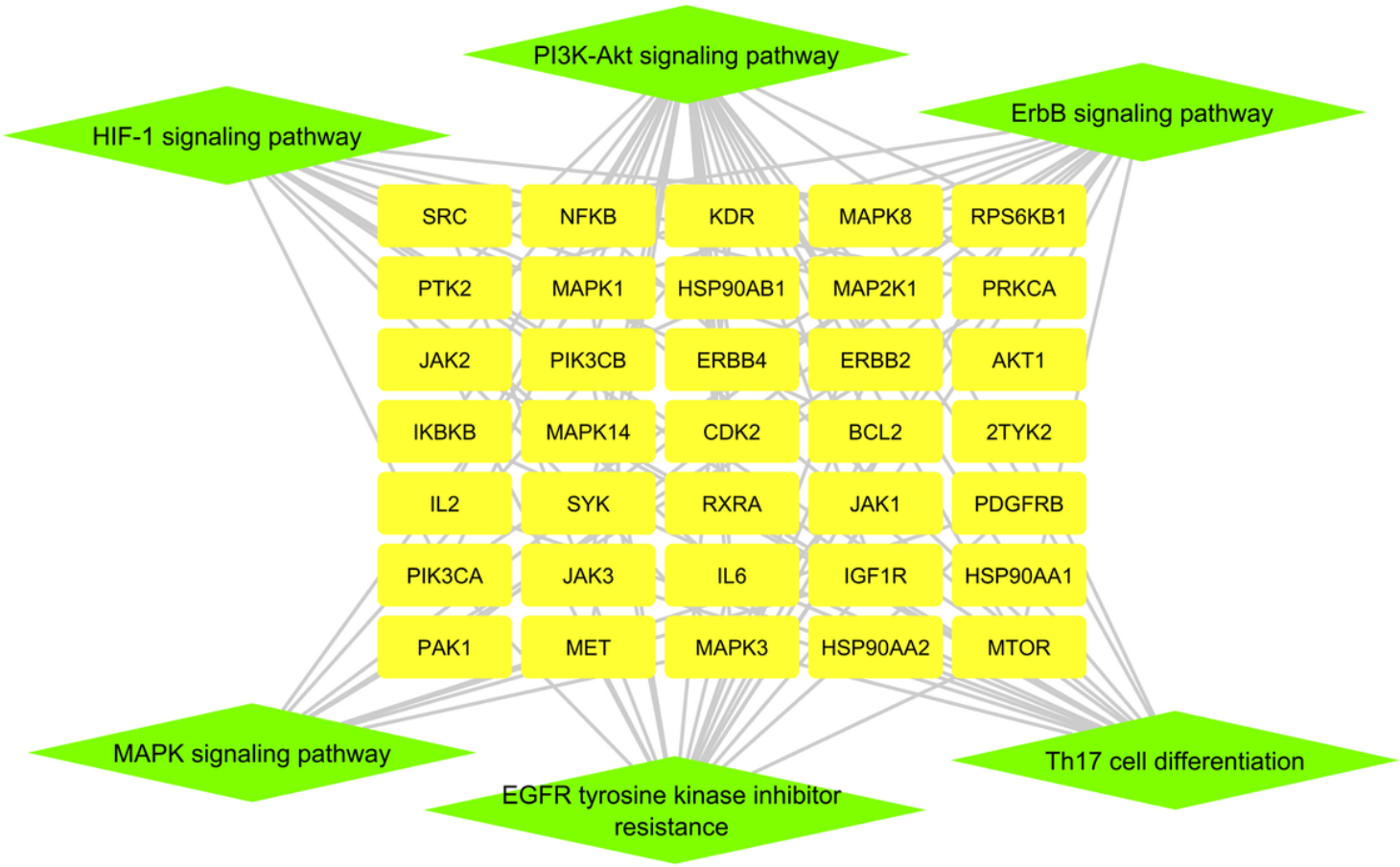


Figure 8

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

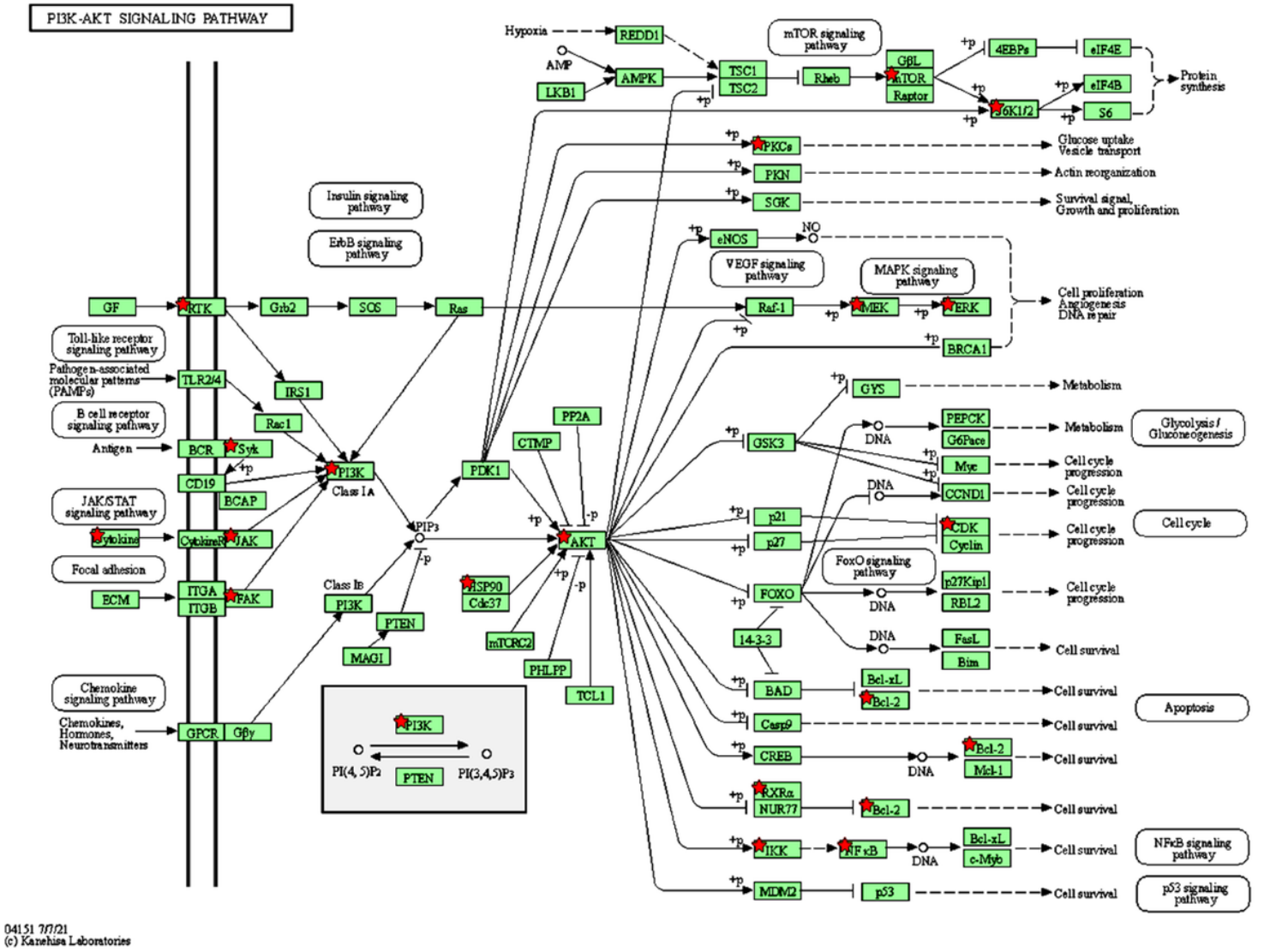


Figure 9

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

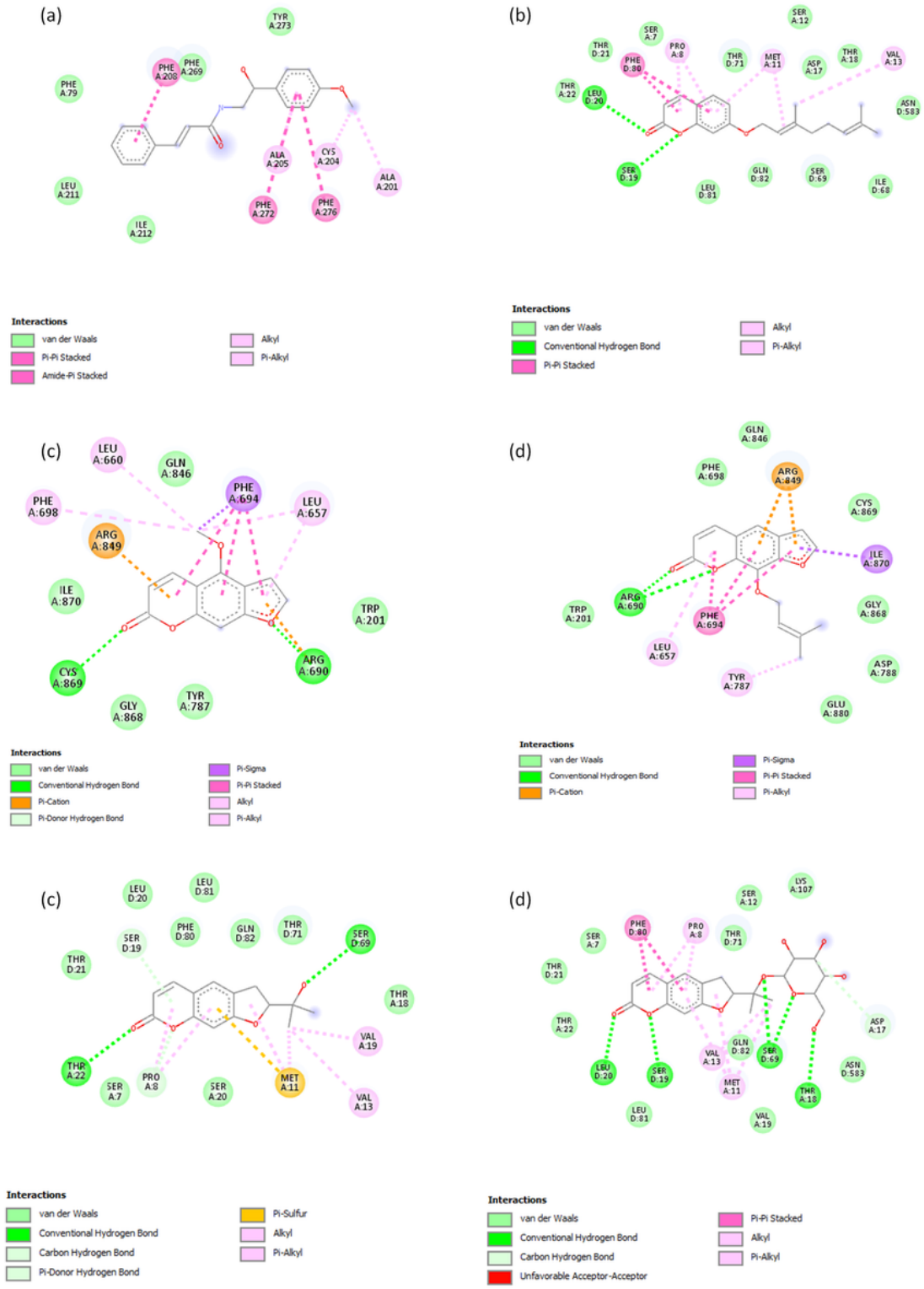


Figure 10

Molecular docking analysis of bioactive compounds of AM towards the putative targets. (a)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. The elaborative information regarding grids and hydrogen bonding interaction and all the other targets and their interaction can be found in Table 3 and Table S6.

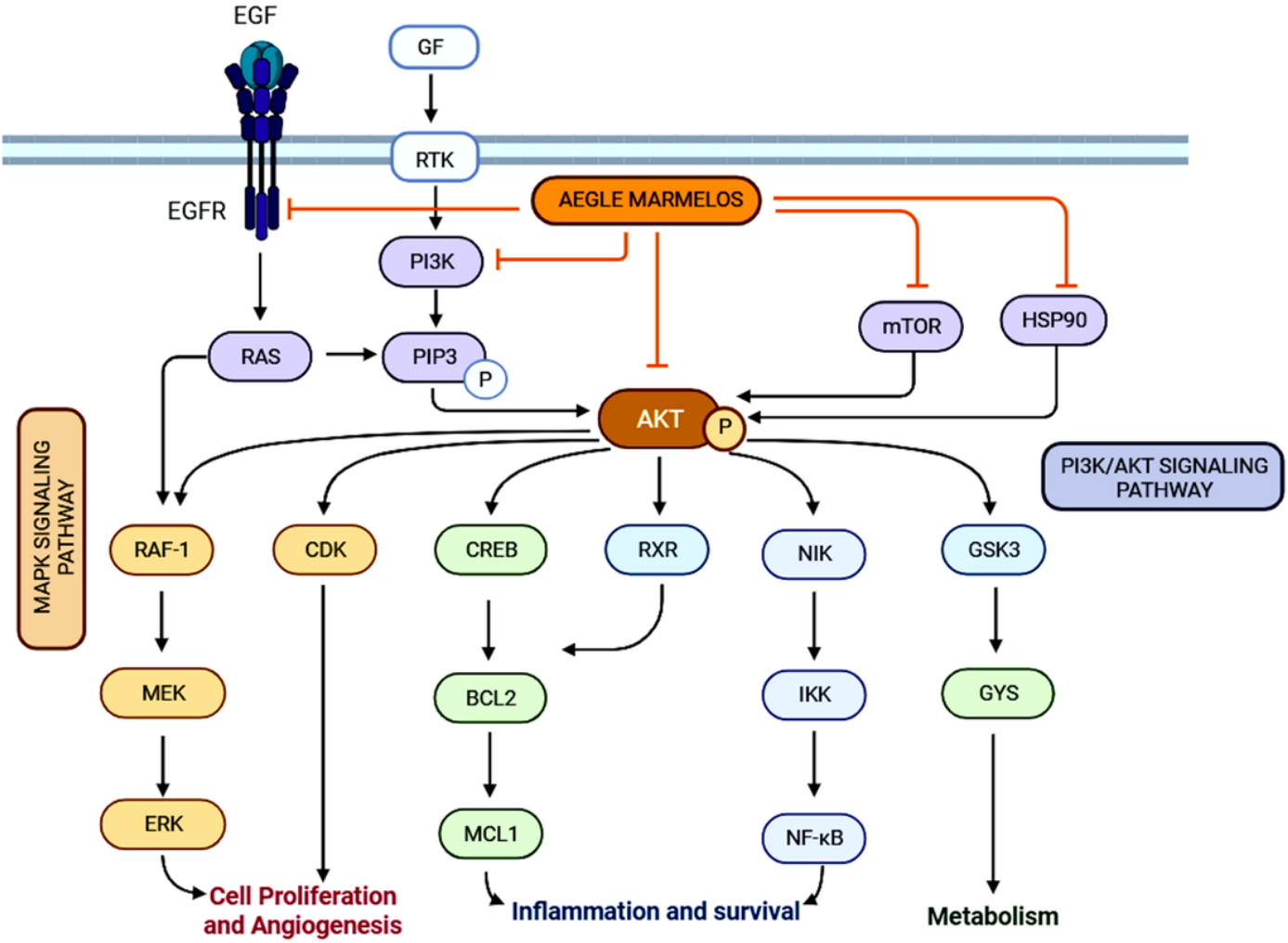


Figure 11

Summary figure. Network pharmacology and molecular docking predicted the pathway of AM in combating IBD. AM targets the EDFR-mediated PI3/AKT pathway along with the 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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.xlsx
- TableS3.xlsx
- TableS4.xlsx
- TableS5.xlsx

- TABLES6.docx
- Graphicalabstract.tif