**Research Article**

# Exploring the potential therapeutic mechanism of *Bauhinia variegata* and *Commiphora wightii* against colorectal cancer through network pharmacology and docking.

***Jagan Muniyandi [1], Lakshmi Sankaranarayanan[1], Vinoth Vengadesan[1], Sadras Sudha Rani[1]****

[1] Degenerative Disease and Phytotherapy Lab, Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry, India.

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Corresponding Author: ***Sadras Sudha Rani*** Email: ***sadrassudha@pondiuni.ac.in***

## ABSTRACT

Insights on the mechanism of colorectal cancer (CRC) development and progression is critical for accurate diagnosis and better treatment. A comprehensive understanding of traditional medicines may contribute to the development of advanced therapeutic approaches. The kanchanara-guggulu (KG) formulation comprising *Bauhinia variegata* and *Commiphora wightii*, has been extensively used in the Indian Systems of Medicine for treating intestinal disorders. This study presents data on the potential interactions between compounds of KG and CRC-associated proteins using network pharmacology and molecular docking analyses.

Out of 84 compounds of KG that were collected from IMPPAT database, 27 exhibited drug likeness properties. Gene targets for these 27 compounds and CRC were collected respectively from SwissTargetsPrediction and DisGeNET. For the identified 78 mutual targets compound-target network was constructed by Cytoscape 3.9.1 which indicated kaempferol, quercetin, ferulic acid and flavanone as highly interacting compounds. Gene ontology (GO) and KEGG enrichment analyses were performed for the targets which yielded 548 GO terms and 137 pathways. Nine key targets were identified based on protein-protein interaction (PPI) analysis of 78 targets by STRING and topology analysis. Finally, molecular docking analysis demonstrated strong binding interactions for kaempferol, quercetin, ferulic acid and flavanone against five key targets- STAT3, HSP90AA1, CTNNB1, AKT1 and EGFR.

This study provides new insights on the synergistic effect of various bioactive compounds present in KG along with their molecular mode of action against CRC. The data obtained in this study may provide lead to identify potential drug candidate from KG for future therapeutic approaches in CRC.

**Keywords:** Kanchanara, Guggulu, PPI, KEGG

## 1. INTRODUCTION

Colorectal cancer (CRC) is often asymptomatic in its early stages, however in its advanced stages it is characterised by intensive rectal bleeding, anaemia, changes in defecation habits, or abdominal pain. According to global cancer statistics, CRC has been recognized as the third most common malignancy in 2020, and the second leading cause of cancer-related deaths [1]. In the past decade, its global prevalence has been significantly increased in both morbidity and mortality. This rise is primarily attributed to varied factors including genetic predisposition, dietary changes and environmental factors [2].

Although various treatment strategies for CRC such as surgery, radiotherapy, and chemotherapy are currently available, these are often associated with adverse effects, development of drug resistance and also disease recurrence. In the recent years, targeted therapies for cancers that interfere with specific molecular targets associated with tumor growth, progression, and metastasis are on the rise. This strategy has been shown to improve efficacy with minimal damage to normal cells. Advances in genomic

technologies have facilitated targeting of key molecules involved in dysregulated signaling pathways such as PI3K-AKT, TGF-β, RAS/RAF/MEK/ERK, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), MAPK, and Wnt/β-catenin pathways. Some notable inhibitors such as LGK974, ETC-159, vantictumab, ipafricept, PRI-724, cetuximab and panitumumab, that target different signalling pathways have become important therapeutic options for metastatic CRC. The intricate crosstalk among these pathways may influence the immune response, tumor microenvironment along with malignancy and hence may interfere with the therapeutic efficiency [3]. Thus, therapeutic strategies targeting multiple key molecules may have higher efficacy in CRC treatment.

Several medicinal plants such as Acorus calamus (vasambu), Terminalia chebula (kadukkai), Abutilon indicum (thuthi), Glycyrrhiza glabra (adhimadhuram) have been used to treat intestinal disorders in the Indian Systems of Medicines (ISM) like Ayurveda and Siddha. Traditional Ayurvedic formulations are known to possess antioxidant, anti-inflammatory, immunomodulatory, chemoprotective, and radioprotective properties. Several medicinal plants, including *Curcuma longa* (turmeric) and *Withania somnifera* (Ashwagandha) have been demonstrated to exhibit anticancer activities through regulation of apoptosis, oxidative stress, inflammation, angiogenesis, and cell proliferation pathways [4]. Some notable phytocompounds such as curcumin and piperine have been shown to suppress Wnt signalling and thereby inhibiting cell proliferation. The protective effect of silver and gold nanoparticles synthesised from Abutilon indicum leaf extract were demonstrated in CRC-induced Wistar Rats [5]. These findings support the multi-target nature of phytocompounds and provide a scientific basis for their potential application in CRC management. Eventhough Indian traditional medicine does not replace conventional treatments such as surgery, chemotherapy, or radiotherapy, they offer an integrative therapeutic approach for disease prevention, supportive care, restoration of immunity, and improvement of quality of life. Traditional medicinal plants contain diverse bioactive compounds capable of modulating multiple targets and signalling pathways and hence may prove to be advantageous in complex diseases such as CRC.

*Bauhinia variegata* Linn. (*B. variegate*), commonly known as kanchanara in Sanskrit has been used extensively in the ISM like Ayurveda. Particularly, the bark of this plant is known for its astringent effects on the bowels and has been used to treat various conditions including dysentery, diarrhoea, haemorrhoids, piles, oedema, ulcers etc. The resin from *Commiphora wightii* (*C. wightii*), commonly known as guggulu, has been used in Ayurveda to treat ailments such as inflammation, gout, rheumatism and obesity. The tablet formulation of kanchanara and guggulu (KG) has been used in Ayurveda for their gastro-protective properties.

Network pharmacology combines systematic medicine with information science, offering a comprehensive *in silico* approach. Hopkins introduced the concept of network pharmacology in 2007 and further advanced the concept in 2008 and this approach has become increasingly significant in drug discovery. It utilizes a 'protein-compound/disease-gene' network to uncover the mechanisms behind the synergistic therapeutic effects of traditional medicines. [6].

The present study provides a comprehensive data on the interactions between selected active compounds of *B. variegata* bark as well as *C. wightii* resin and CRC-associated target proteins through network pharmacology approach.

## 2. MATERIALS AND METHODS

### 2.1 Identification of bioactive Compounds present in *B. variegata* bark and *C. wightii* resin

The bioactive compounds of *B. variegata* bark and that of *C. wightii* resin were retrieved from Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 (IMPPAT) database (https://cb.imsc.res.in/imppat/) as well as through wide-scale literature mining [7, 8]. The three dimensional (3D) and canonical simplified molecular input line entry system (SMILES) structures of the compounds were downloaded

from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for further analyses.

**2.2 Determination of drug-likeness of bioactive compounds of KG**

The computational examination of drug-likeness (DL) of the compounds was evaluated by uploading the 3D structures of the compounds in the DruLiTo open-source software and checked whether the compounds obey the Lipinski's and Vebers rules [9]. The DL of a compound is usually determined based on the following rules: molecular weight lesser than 500 Da, neutral solubility in both hydrophilic and hydrophobic solvents (log P>5), number of hydrogen bond donors lesser than 5 (HBD>5), number of hydrogen bond acceptors lesser than 10 (HBA>10), number of rotatable bonds more than ten (nRB) and sum of total polar surface atoms or topological polar surface area lesser than 140 (TPSA>140). Those compounds which fulfilled these criteria were selected for further investigation while other compounds were rejected.

**2.3 Determination of ADMET properties of selected compounds**

The pharmacokinetic properties such as adsorption, distribution, metabolism, excretion (ADME) and toxicity (collectively known as ADMET) of the selected compounds were evaluated using the pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/) by uploading the canonical SMILES structures of the compounds [9].

**2.4 Prediction of bioactivity for selected compounds**

The bioactivity of the selected compounds was evaluated by using Molinspiration Cheminformatics online server (https://www.molinspiration.com/). The canonical SMILES structures of the compounds were uploaded in the server and evaluated for various biological activities such as G protein coupled receptors (GPCR) ligands, ion channel modulators, nuclear receptors, enzyme inhibitors, kinase inhibitors and protease inhibitors. Based on these parameters the bioactive scores for all the compounds were obtained [9].

**2.5 Identification of compound targets**

The protein targets of the selected compounds were predicted by uploading their canonical SMILES structures in the SwissTargetPrediction online tool (http://www.swisstargetprediction.ch/).

**2.6 Identification of disease associated targets**

DisGeNET (https://www.disgenet.org/) is a research tool with one of the most extensive publicly accessible collection of genes and variants associated to human diseases. In this study, data on genes associated with CRC was collected from DisGeNET database using the keyword 'colorectal cancer'. Genes with 'score gda' > 0.1 were selected for further analysis. The UniProt database (https://www.uniprot.org/) was used to get standard protein names.

**2.7 Identification of mutual protein targets between selected compounds and CRC**

The mutual protein targets of selected compounds of KG and those associated with CRC were analysed using the online tool Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/).

**2.8 Construction of Compound-Target network**

The interactions between selected compounds and mutual targets of KG and CRC were visualized by importing them into the Cytoscape (3.9.1) software. Each node in the constructed network represent a compound or a target, while edges (connecting lines) represent interactions among them. Topology analysis of the constructed network was carried out to determine degree centrality (DC) and betweenness centrality (BC) for identification of key bioactive compounds of KG.

**2.9 Construction of Protein-Protein Interaction (PPI) network**

The protein-protein interaction (PPI) network for the mutual targets was built using STRING v.11.5 database (https://string-db.org/). For the network construction, species was set as "*Homo sapiens*" and the confidence score was set to be greater than 70. The interactive network information derived from the STRING was exported in TSV format and uploaded to the Cytoscape.

In order to screen the key nodes of the network, topology analysis was carried out in Cytoscape using the plug-in, CytoNCA. The nodes that were having twice the mean of both BC and DC were

considered to be fulfilling the screening criteria and were selected as the key targets of the PPI network [10].

**2.10 GO and KEGG analysis**

The genes encoding the mutual protein targets were subjected to gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses with the help of Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/). GO analysis helps to identify the functional annotations of the genes in three forms: biological process (BP), cellular component (CC) and molecular function (MF) while the KEGG pathway analysis elaborates on the role of target proteins in signal transduction. Data mining was carried out by specifying the p-value to be less than 0.05. Visualisation and interpretation of data was carried out using SRPLOT, an online science and research plotting service (https://www.bioinformatics.com.cn/en).

**2.11 Molecular Docking**

Those compounds which showed highest degree of interaction in the Compound-Target network were chosen for molecular docking analysis. These compounds were checked for binding interaction against those target proteins which showed highest degree of interaction among the key targets in PPI analysis.

The molecular docking experiment involved the following steps:

The 3D structures of receptors were retrieved from Protein Data Bank (PDB) (https://www.rcsb.org/) and adhering to the criteria including organism from "*Homo sapiens*", with better resolution. The structures were then stored in PDB format. The 3D structure of ligands was downloaded as SDF format from the PubChem database and then imported to Open Babel software to obtain PDBQT format. The PDB structure of receptors was imported to AutoDock 4.2.6 software, wherein elimination of water molecules, co-crystallized ligands as well as ions and addition of polar hydrogen and Kollman partial charges were carried out, following which the receptor structure was saved in PDBQT format. Subsequently, the PDBQT structures of ligand were inserted into AutoDock and molecular docking analysis between the ligand and receptor was carried out. The best conformations with the highest binding energies were selected for the visualisation using PyMol software. In molecular docking analysis, binding energy value of less than -5.0 kcal/mol are indicative of excellent binding activity, while those values less than -7.0 kcal/mol represents strong binding activity [11].

## 3. RESULTS

**3.1 Collection of data on active compounds of kanchanara bark and guggulu resin**

Based on data collected from IMPPAT database and literature analysis, kanchanara bark was found to contain 26 compounds and guggulu resin contained 58 compounds (Table S1). A total of 84 compounds were selected for further analyses.

**3.2 Drug-likeness properties of bioactive compounds of KG**

The computational examination for drug-likeness of the collected bioactive compounds of KG was performed as a preliminary step to select the potential compounds. Only 27 compounds obeyed the criteria of Lipinski's and Vebers rule of DL (Table S2). Hence, these 27 compounds were selected as bioactive compounds and their molecular weight was found to be less than 500 Da; especially, 15 compounds had molecular weight less than 200 Da. The log p scores of all 27 compounds were found to be significant.

**3.3 ADMET properties of selected compounds**

In ADMET analysis, absorption factor aids in comprehending the systemic circulation and movement of the medication ingredient. All 27 compounds displayed greater than 90% intestinal absorption value and among them E-guggulsterone, M-guggulsterone, d-Sesamin and flavanone exhibited higher absorption value of 99.65, 98.83, 97.81 and 97.7% respectively.

The steady state volume of distribution (VDss) is the hypothetical volume where the entire drug should be evenly dispersed to achieve the same concentration as in blood plasma. Low and high VDss are 0.71 L/kg and 2.81 L/kg, respectively. Compounds of KG including dihydroquercetin, quercetin, kaempferol showed effective VDss values of 1.638, 1.559 and 1.274 respectively.

A superfamily of haeme-containing enzymes called cytochrome P450 (CYP450) regulates how medicines are metabolised. More than 80% of medicines are detoxified by the CYP450 enzyme during liver first-pass metabolism; therefore, any substance that inhibits P450s may be harmful. In this analysis, 16 out of 27 compounds did not inhibit any isoform of CYP450. Organic cation transporter 2 (OCT2), a renal uptake transporter, is crucial for the renal clearance of drugs and endogenous substances. In this study, none of the 27 compounds were identified as OCT substrates and hence may be considered as safe. Similarly, inhibition of human ether-a-go-go-related gene (hERG) encoded potassium channel causes a fatal ventricular arrhythmia. Out of 27 compounds analysed, all compounds except E-guggulsterone did not exhibit hERG I, hERG II channel inhibition as well as hepatotoxicity (Table S3).

### 3.4 Bioactivity of selected compounds

Bioactivity scores were validated for all the selected 27 compounds using Molinspiration Cheminformatics online tool. Compounds with values more than zero are categorized as active and those with values between -0.5 to 0 are considered moderately active while values below -0.5 are considered as inactive. The data obtained in this analysis indicated that all selected compounds except methylheptanone exhibited appreciable bioactivity scores. Notably, E-guggulsterone, 6-epi-shyobunol, M-guggulsterone, kaempferol, dihydroquercetin and quercetin revealed excellent bioactive scores as nuclear receptor ligand and enzyme inhibitor. Also, kaempferol and quercetin showed good bioactive scores as kinase inhibitors (Table S4).

### 3.5 Selection of Compound Targets and CRC related targets

Data retrieved from SwissTargetPrediction tool indicated a total of 384 targets for the 27 selected compounds, without any duplicates (Table S5). A total of 5474 genes associated with CRC were curated from DisGeNET using the disease ID C0009402 and out of these, only 1110 genes were selected based on their 'score gda' value, without any repeats (Table S6).

A total of 78 proteins were identified as mutual targets for the selected 27 compounds and CRC (Fig. 1 and Table S7). Out of these 27 compounds, 5 compounds namely, myrcene, d-limonene, eucalyptol, d-sesamin, dihydroquercetin did not have mutual genes with CRC. Hence, 22 compounds and 78 targets were used for further analyses.

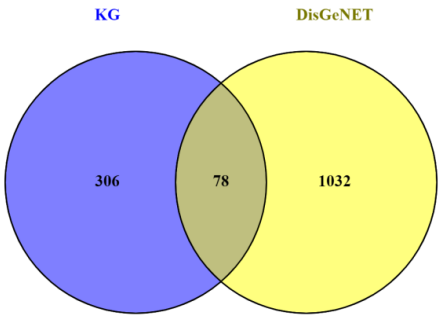



**Fig. 1 Identification of mutual gene targets of CRC and phytocompounds of KG by Venny 2.1.0.**

### 3.6 Construction of Compound-Target network

The Compound-Target network analysis for 22 compounds and 78 mutual targets was constructed using Cytoscape, in which 188 edges (interactions) were observed (Fig. 2). Among the 22 compounds, kaempferol, quercetin, ferulic acid, and flavanone were found to exhibit higher degree of interaction with 29, 28, 25, and 20 targets respectively (Table S8).

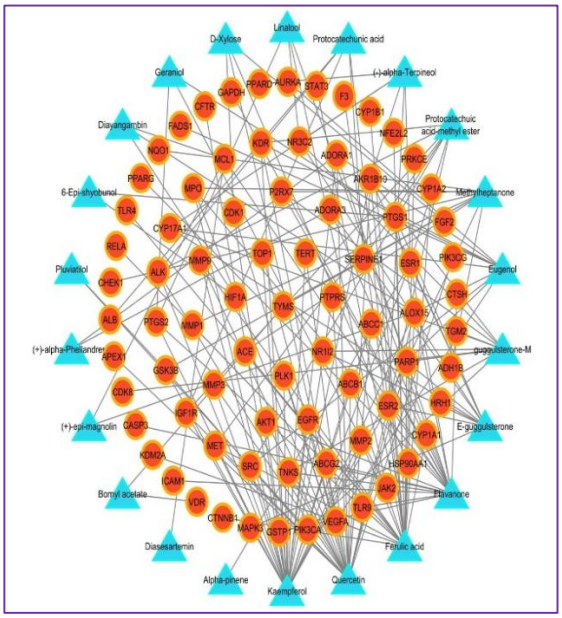



**Fig. 2 Compound-target network analysis by Cytoscape v.3.9.1.**

**(The blue coloured triangular nodes represent the phytocompounds of KG and the red coloured circular nodes represent their corresponding targets).**

### 3.7 Construction of PPI network

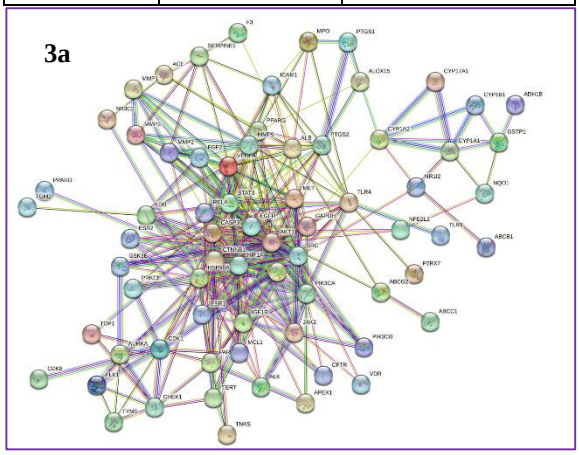
To understand the interaction between the 78 mutual targets, PPI network was built by using the STRING database. The network consisted of 335 edges, with an average node degree of 8.59 with the PPI enrichment P-value <1.0e-16 (Fig. 3a); the independent target protein nodes were hidden. Results obtained in the STRING were imported to the Cytoscape for topology analysis of the network by CytoNCA plug-in which yielded data on DC and BC. In this network interaction, proteins with higher degree may be considered as the important target.

DC is a crucial metric for evaluating the local centrality of a node within a network and a node with high DC score often serves as the central node. The average value of DC observed in this study was 8.5897. BC measures the frequency with which a node serves as the shortest bridge between two other nodes and the average value of BC was 91.5128. Nodes which showed twice the average value of BC and DC were designated as "Key Targets" (Fig.3b). The key targets identified were STAT3 (signal transducer and activator of transcription 3), HSP90AA1 (heat shock protein 90 alpha family class A member 1), CTNNB1 (catenin beta 1), AKT1 (AKT serine/threonine kinase 1), EGFR (epidermal growth factor receptor), SRC (SRC proto-oncogene, non-receptor tyrosine kinase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), CASP3 (caspase 3) and ALB (albumin) (Fig.3c and Table 1).

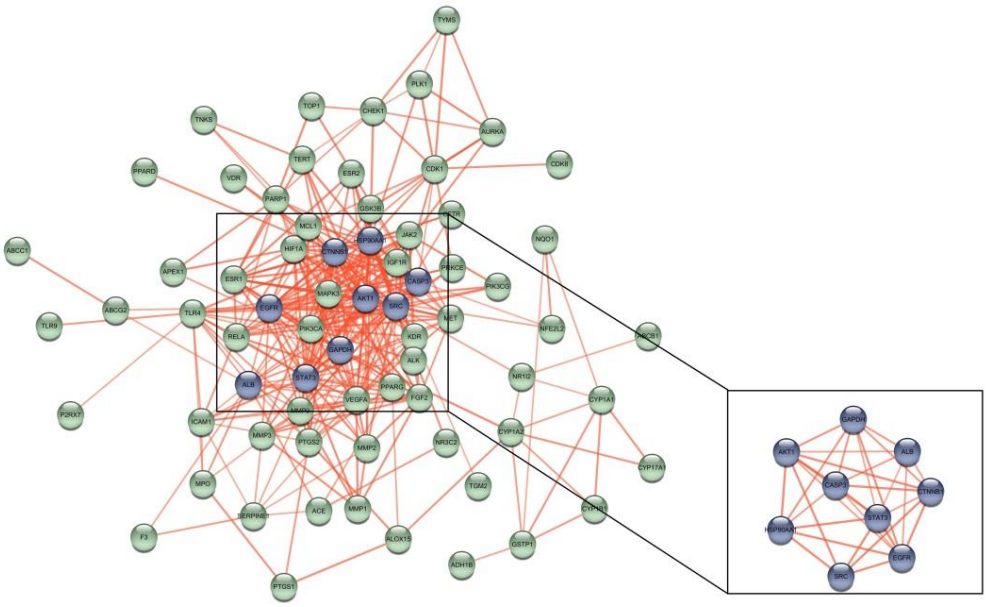
**Table 1: Topological parameters of key targets**

| Key Targets | Degree Centrality | Betweenness Centrality |
|---|---|---|
| STAT 3 | 31 | 267.3314 |
| HSP90AA1 | 29 | 549.76526 |
| CTNNB1 | 28 | 449.45142 |
| AKT1 | 27 | 390.82632 |
| EGFR | 26 | 339.90945 |
| SRC | 26 | 550.91187 |
| GAPDH | 21 | 644.06805 |
| CASP3 | 19 | 251.68976 |
| ALB | 18 | 305.48645 |

**3a**



**3b**



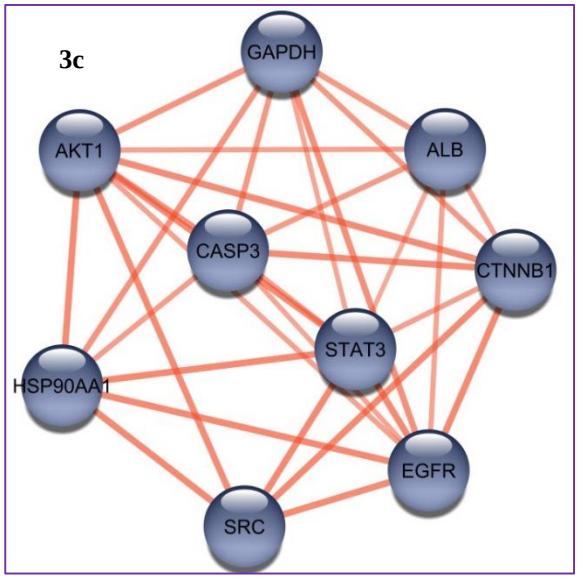



**Fig. 3 Protein-protein interaction network and identification of key targets. 3a. Construction of PPI network by STRING database; 3b. Topology analysis of PPI network by Cytoscape v.3.9.1. (Violet coloured circular nodes represent the key targets); 3c. PPI network among the 9 key targets.**

### 3.8 GO and KEGG analysis

GO and KEGG analyses of 78 mutual targets were carried out by DAVID. GO enrichment analysis yielded 548 GO terms including 402 BPs, 89 MFs and 57 CCs. Subsequently top 10 significantly enriched GO terms with P-value ($p<0.05$) are presented in Fig. 4 and Table S9.

Some of the major BP terms were positive regulation of transcription from RNA polymerase II promoter (GO: 0045944), negative regulation of apoptotic process (GO: 0043066), positive regulation of gene expression (GO: 0010628), and signal transduction (GO: 0007165) (Fig. 4a). CC data indicated that the targets were strongly enriched in nucleus (GO: 0005634), cytoplasm (GO: 0005737), plasma membrane (GO: 0005886), cytosol (GO: 0005829) and nucleoplasm (GO: 0005654) (Fig. 4b). In case of MF, the targets were found to be related to binding with protein (GO: 0005515), ATP (GO: 0005524), zinc ion (GO: 0008270), enzyme (GO: 0019899) and DNA (GO: 0003677) (Fig. 4c).

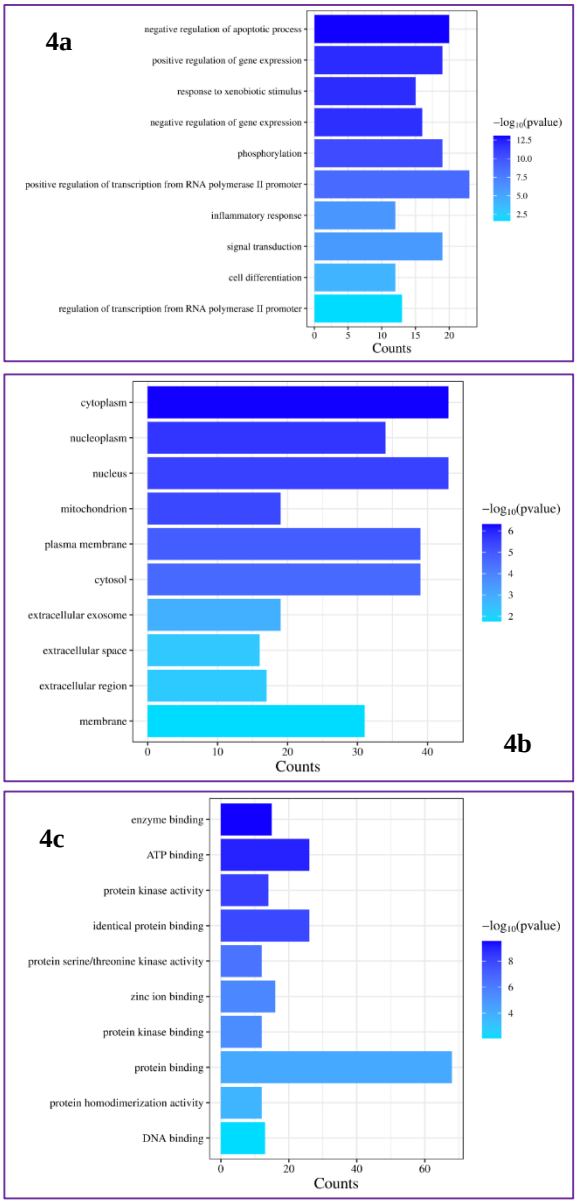



**Fig. 4 Gene ontology analysis of mutual target genes by DAVID (Bubble Plot). 4a. Biological processes; 4b. Cellular component; 4c. Molecular function.**

KEGG enrichment analysis yielded 137 pathways. The top 10 pathways with high gene counts (>10) with p-value <0.05 are presented in Fig. 5., which included Pathways in cancer (hsa05200), Proteoglycans in cancer (hsa05205), Lipid and atherosclerosis (hsa05417), Chemical carcinogenesis- receptor activation (hsa05207), Kaposi sarcoma-associated herpes virus infection (hsa05167) and PI3K-Akt signalling pathway (hsa04151) (Table S8).

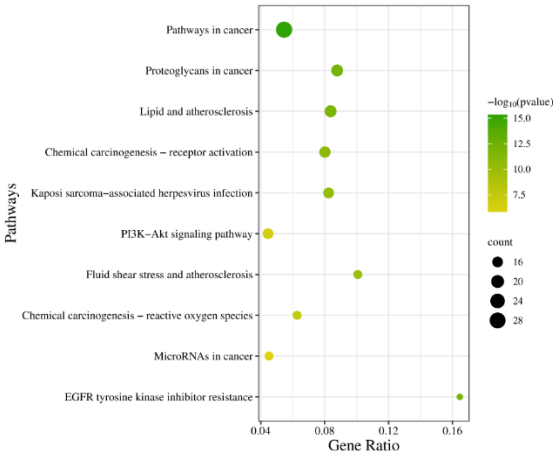



**Fig. 5 KEGG analysis of mutual target genes by DAVID.**

(X-axis represents gene ratio and Y-axis represents pathways; bubble size represents the count of genes enriched in pathways and the intensity of colour represents the p-value).

### 3.9 Molecular Docking

The molecular interaction analysis was carried out for four bioactive compounds that showed highest degree of interaction in the Compound-Target network against top five key targets. They were kaempferol, quercetin, ferulic acid and flavanone and their interaction against STAT3 (PDB ID: 6NJS), HSP900A1 (PDB ID: 4BQG), CTNNB1 (PDB ID: 7AFW), AKT1 (PDB ID: 4BQG) and EGFR (PDB ID: 1M17) was analysed using AutoDock. Most of the 20 conformations were found to display strong binding activity (Fig. 6) and their binding energies are presented in Table 2. Some of the conformations which exhibited efficient binding interactions were HSP900A1-flavanone (-8.62 kcal/mol), AKT1-quercetin (-7.57 kcal/mol), EGFR-kaempferol (-7.45 kcal/mol) and HSP900A1-kaempferol (-7.22 kcal/mol).

**Table 2: Molecular docking binding energy results (kcal/mol).**

| Ligands | Receptors | | | | |
|---|---|---|---|---|---|
| | **STAT 3** | **HSP90AA 1** | **CTNNB 1** | **AKT 1** | **EGF R** |
| **Kaempfero l** | -5.56 | -7.22 | -5.05 | -6.97 | -7.45 |
| **Quercetin** | -5.52 | -7.17 | -5.4 | -7.57 | -7.21 |
| **Ferulic acid** | -5.59 | -5.3 | -4.86 | -5.77 | -5.36 |
| **Flavanone** | -5.55 | -8.62 | -5.5 | -7.09 | -6.57 |

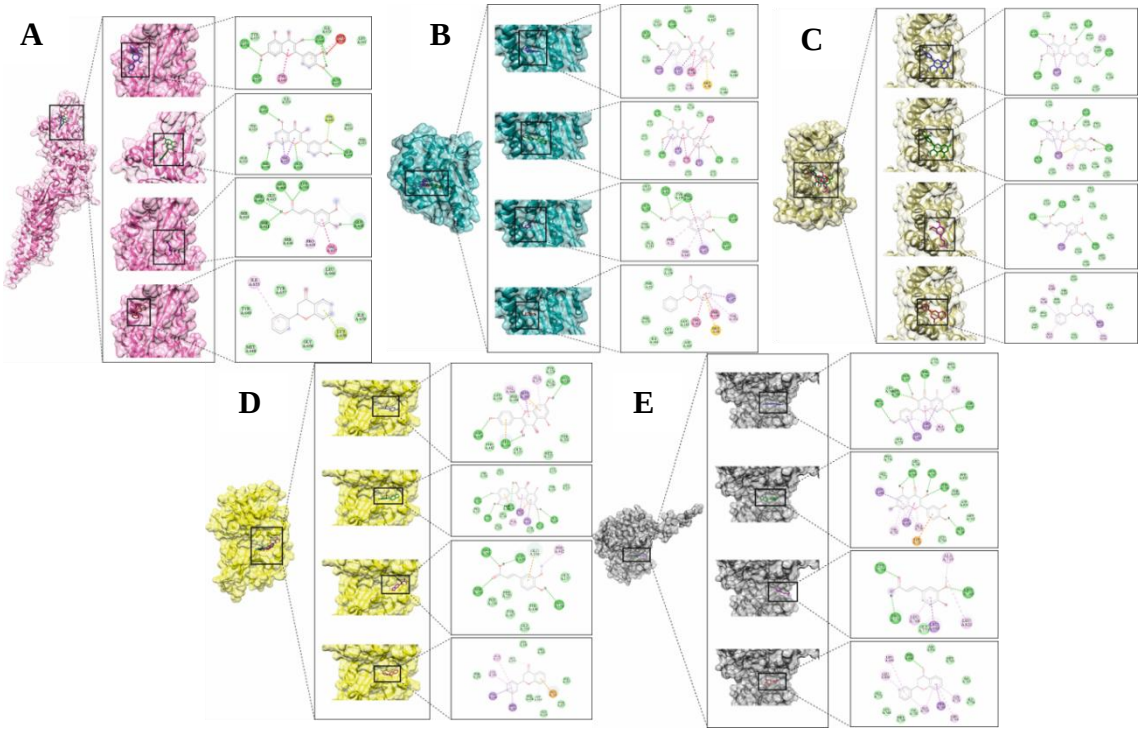





**Fig 6 Docking models (3D and 2D) of kaempferol, quercetin, ferulic acid and flavanone with key targets A. STAT3, B. HSP90AA1, C.CTNNB1, D. AKT1 and E. EGFR**

## 4. DISCUSSION

In recent years, network pharmacology has emerged as an advanced method to analyse the interactions of compounds present in different formulations used in alternative medicines for treating various diseases. Although, kanchanara and guggulu have been used extensively in Ayurveda and Siddha practices for their gastro-protective properties, the molecular basis of their activities needs to be explored. The present study is designed to identify the potential CRC targets of different bioactive compounds present in KG through network pharmacology approach.

In this study, a total of 84 compounds of KG were retrieved out of which 27 were selected based on their DL properties. Further, 78 gene targets were identifying to be mutual for these bioactive compounds and CRC. In order to visually demonstrate the interactions between KG compounds and CRC proteins, a Compound–Target network was constructed for the mutual genes and corresponding compounds through Cytoscape. This analysis revealed that some of the bioactive compounds including kaempferol, quercetin, ferulic acid and flavanone interacted with multiple targets in the network suggesting that these may contribute to a larger extent to the therapeutic potential of KG. Based on the values of DC and BC obtained from topology analysis of PPI, top 5 key targets namely STAT3, HSP90AA1, CTNNB1, AKT1 and EGFR were selected. Finally, molecular docking analysis was performed for these five targets with kaempferol, quercetin, ferulic acid and flavanone.

Among the selected 4 compounds, kaempferol, quercetin, and flavanone belong to the flavonoid group while ferulic acid is a phenolic acid. Flavonoid group encompasses over 4000 different flavonoids including flavanones, flavonoids, isoflavonoids, flavones, and catechins. These biologically active compounds have been demonstrated to exhibit protective effects against diverse types of cancer [12].

Kaempferol, a tetrahydroxyflavone, has been shown to exhibit antioxidant, anti-proliferative, apoptosis-inducing, cell-cycle arresting, as well as anti-metastatic and anti-angiogenic properties [13]. In a recent study, kaempferol treatment caused significant reduction in number of multiple plaque lesions (MPL) and aberrant crypt foci (ACF) along with improved antioxidant, anti-inflammatory and apoptotic effects in 1, 2-dimethylhydrazine (DMH) induced colon cancer in rats [14]. In the present study, EGFR and AKT1 were identified as the protein targets of kaempferol compound-target interaction analysis.

Quercetin, a pentahydroxyflavone, has been demonstrated in a recent study, quercetin was found to cause 50% reduction in tumour numbers and stabilised the antioxidant enzymes in azoxymethane/dextran sodium sulphate (AOM/DSS) induced colon cancer in mice [15]. In the present study, EGFR and AKT1 were observed to be the protein targets of quercetin as observed in the compound-target interaction analysis.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was shown to exhibit anti-proliferative effects in DMH-induced CRC in rats as evidenced by histological and immunohistochemical analyses [16]. In an earlier study, ferulic acid was found to reduce the number of ACF and incidence of colonic carcinoma in AOM-treated rats [17]. Among the five key targets identified in this study, STAT3, EGFR and CTNNB1 were observed to be the

targets of ferulic acid compound-target interaction analysis.

Various flavanone compounds including naringin (trihydroxyflavanone), eriodictyol (tetrahydroxyflavanone) and liquiritigenin (dihydroxyflavanone) have been demonstrated to exhibit protective effects against *in vitro* as well as *in vivo* models of CRC. Naringin caused significant reduction in the number of aberrant crypts and pre-neoplastic lesions in the middle and proximal colon in DMH-treated Wistar rats [18]. Both eriodictyol and liquiritigenin were reported to inhibit cell proliferation, invasion, and epithelial-to-mesenchymal transition (EMT) in HCT 116 cells [19, 20]. In the present study, flavanone was found to target AKT1 through compound-target interaction analysis.

Altogether, all these reports offer convincing evidence for the efficacy of kaempferol, quercetin, ferulic acid and flavanones which are the major components of KG and hence these compounds may contribute to the therapeutic potential of KG against CRC.

Based on the topology analysis of PPI network, STAT3 was observed to be the highly interacting protein followed by HSP90AA1, CTNNB1, AKT1 and EGFR.

STAT3 has long been identified as a therapeutic target and its activated form is known to play a vital role in cancer cell proliferation, invasion and EMT. In a recent study, siRNA mediated selective silencing of STAT3 gene caused significant reduction in the rate of cell invasion and increased apoptotic cell death in colon cancer cell lines LoVo and SW480 [21]. In the present study, in the compound-target network analysis although STAT3 was observed to be targeted only by ferulic acid, docking studies showed significant interaction of STAT3 with all 4 selected compounds.

Increased levels of HSP90AA1 have been demonstrated to be associated with tumorigenesis in *in vitro* and human biopsy samples [22]. In the present study, the compound-target network analysis indicated that none of the selected compounds targeted HSP90AA1. However, data from the molecular docking analysis showed strong binding interaction between HSP90AA1 and flavanone, followed by kaempferol and quercetin.

CTNNB1 is an integral part of the cytoskeleton as well as an important transcription factor. The Wnt/β-catenin pathway has been observed to play a vital role in the progression and metastasis of colorectal cancers. In a recent study, significant increase in β-catenin levels was detected by western blotting analysis in human colon cancer cell lines including HT29, HCT116 and SW620 than in normal colonic epithelial cells (HCoEpiC) [23]. In the present study, data from compound-target network analysis indicated ferulic acid to be the only interacting compound of CTNNB1. However, molecular docking data confirmed significant binding of CTNNB1 with all four selected compounds.

AKT1 has been identified as a target in colorectal cancer, owing to its role in cell proliferation and angiogenesis. In a recent study, shRNA mediated knockdown of AKT1 in different CRC cell lines was shown to decrease cell proliferation, colony formation and cell migration [24]. In the present study, based on the compound-target network analysis, AKT1 was observed as a target for quercetin, flavanone and kaempferol and molecular docking analysis confirmed the same.

The mechanisms by which EGFR promotes tumorigenesis are diverse and involve both cell cycle dysregulation and the promotion of factors that aid in survival of tumours. In a recent study, the inhibitory effect of evodiamine, an indolequinone alkaloid against EGFR protein expression was demonstrated in a human CRC cell line LoVo. Also, the evodiamine nanoparticles targeting EGFR were shown to be more potent in reducing the expression of EGFR, VEGF and MMP2 than evodiamine alone along with suppression of tumour cell invasion and metastasis. Similar findings were observed in BALB/C male athymic nude mice induced with CRC [25]. These findings emphasise that targeting EGFR would prove to be a promising approach for treating CRC. In the present study, the compound target network data indicated the interaction of EGFR with ferulic acid, kaempferol and quercetin. In molecular docking analysis, among these 3 compounds kaempferol and quercetin showed effective binding

interactions with EGFR suggesting their therapeutic effects.

In order to explore the mechanism of KG in the treatment of CRC, GO and KEGG enrichment analysis was performed in this study for the genes encoding the 78 mutual proteins. These data indicated that these genes were involved in multiple signalling pathways including Pathways in cancer, Proteoglycans in cancer, Chemical carcinogenesis- receptor activation, PI3K-Akt signalling pathway, etc. Hence, it may be hypothesised that the compounds present in KG may play a critical role in the treatment of CRC by modulating these pathways. Further experimental investigations are warranted to unravel the efficacy of KG against CRC.

The findings of this study have highlighted the potential of KG in treating CRC. However, the present study has a few limitations. Firstly, the public databases investigated in the study are constantly updated and hence some other bioactive compounds and target genes may not have been included in our analysis. Further studies using *in vitro* and *in vivo* models are warranted to confirm the efficacy of KG against CRC. Despite these limitations, the present study provides comprehensive insights on the therapeutic potential of key bioactive compounds of KG.

## 5. CONCLUSION

This study utilised network pharmacology and molecular docking analyses to investigate the active compounds of kanchanara and guggulu, and their efficacy in treating colorectal cancer. Our findings indicated that quercetin, kaempferol, ferulic acid, and flavanone to be the main active components and play critical role in combating CRC by modulating key proteins such as STAT3, HSP90AA1, CTNNB1, AKT1, EGFR, SRC, GAPDH, CASP3, and ALB. These compounds may exert therapeutic effects by influencing various pathways such as PI3K-Akt signalling pathway. Additionally, molecular docking studies demonstrated that these active compounds exhibited strong binding affinity with STAT3, HSP90AA1, CTNNB1, AKT1, and EGFR. Altogether, this study helps to identify potential compounds of KG for further research in CRC.

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## CREDIT AUTHOR CONTRIBUTIONS

**Jagan Muniyandi** - Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing- original draft, Writing- review & editing; **Lakshmi Sankaranarayanan** - Software, Validation, Visualization, Writing- review & editing; **Vinoth Vengadesan** - Data curation, Software, Visualization; **Sadras Sudha Rani** - Formal analysis, Investigation, Validation, Supervision, Writing- review & editing.

All authors read and approved the final manuscript.

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## DECLARATION OF COMPETING INTEREST

The authors declare that there are no conflicts of interest.

## DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

## GLOSSARY

ACF- Aberrant crypt foci

ADMET- Adsorption, Distribution, Metabolism, Excretion, and toxicity

AMR- Atom molar refractivity

AOM/DSS- Azoxymethane/dextran sodium sulfate

BC- Betweenness Centrality

BP- Biological process

CC- Cellular components

CRC- Colorectal cancer

DAVID- Database for Annotation, Visualization and Integrated Discovery
DC- Degree Centrality
DMH- 1,2 Dimethylhydrazine
EMT- Epithelial-to-mesenchymal transition
Evo- Evodiamine
GO- Gene ontology
HBA- Hydrogen bond acceptor
HBD- Hydrogen bond donor
IMPPAT- Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0
KEGG- Kyoto Encyclopedia of Genes and Genomes
KG- Kanchanara and guggulu
MF- Molecular function
MPL- Multiple plaque lesions
MW- Molecular weight
nRB- Number of rotatable bonds
PDB- Protein Data Bank
PPI- Protein-protein interaction
SMILES- Simplified Molecular Input Line Entry System
TPSA- Topological polar surface area
VDss- The steady state volume of DistributionD

**ABBREVIATIONS**

AKT1- AKT serine/threonine kinase 1
ALB- Albumin
CASP3- Caspase 3
CTNNB1- Catenin beta 1
CYP450- Cytochrome P450
EGFR- Epidermal growth factor receptor
GAPDH- Glyceraldehyde-3-phosphate dehydrogenase
GPCR- G protein coupled receptors
GSK3B - Glycogen synthase kinase 3 beta
hERG- The human ether-a-go-go-related gene
HSP90AA1- Heat shock protein 90 alpha family class A member 1
MMP-2- Matrix metallopeptidase 2
OCT2- Organic Cation Transporter 2
shRNA- Short hairpin RNA
SRC- SRC proto-oncogene, non-receptor tyrosine kinase
STAT3- Signal transducer and activator of transcription 3
VEGF-Vascular endothelial growth factor.

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