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Ascertaining the Role of *Piper chaba* Hunter in Functional Dyspepsia by *In Vitro* and *In Silico* Analyses

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Abstract

Background: Functional dyspepsia (FD), a Functional gastrointestinal disorder, involves multiple pathological mechanisms predominated by inflammatory and immune-mediated changes. The existing treatment for FD has certain limitations. *Piper chaba* Hunter. is a commonly used herb in culinary practices and is mentioned in the traditional Indian system of medicine as "*Tuptighna*" (relieves satiety). **Aim:** In the present study, *in vitro* and *in silico* analyses were conducted to understand the possible role of *P. chaba* in FD. **Methods:** *P. chaba* stem ethanolic extract was assessed for total phenols by Folin-Ciocalteu, flavonoids by Aluminium chloride, and antioxidant activity by DPPH method. A network pharmacology and molecular docking approach was applied to elucidate the multitargeted mechanism of action. **Results:** *P. chaba* stem showed phenolic content of 109.8885 mg GAE/gm of extract and flavonoid content of 8.3453 mg QE/gm of extract. The extract showed DPPH free radical inhibition activity with an IC_{50} of 329.94 µg/mL. 23 phytoconstituents were retrieved from IMPPAT 2.0 database, and 92 by literature survey. After ADME screening, the list was narrowed down to 25, which gave 826 unique targets. 1131 targets of FD were obtained by Genecards, intersection with Venny 2.1 revealed 252 common targets. STRING database helped in gene ontology and KEGG pathway enrichment. Cytoscape gave a compound-target-pathway network and hub genes. Chabamide and Piperine showed stable binding energies with hub genes GAPDH, AKT1, ALB1, STAT3, and EGFR. The significantly enriched pathways are the PI3K-Akt signalling pathway and MicroRNAs in cancer. Chabamide and Piperine showed stable docking scores, comparable to that of the standard drug Pantoprazole. **Conclusion:** Modulation involving multiple targets and pathways indicates that *P. chaba* can act by regulation of inflammation and by antioxidant action. The study findings provide a rationale based on an *in vitro* and *in silico* approach, giving a basis for further validation.

Major Findings: *Piper chaba* stem contains phenolic and flavonoid content with antioxidant activity. The phytocompounds Chabamide and Piperine, showed stable binding energies with the core targets of FD addressing inflammatory and immune related molecular targets.

Keywords: Antioxidant Activity, Functional Dyspepsia, *In Vitro*, Molecular Docking, Network Pharmacology, *Piper chaba*

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1. Introduction

Functional gastrointestinal (GI) disorders are common nowadays because of altered food habits and lifestyle, along with stress. These are characterised by chronic GI-related symptoms without any structural abnormality in the gastrointestinal tract. Functional dyspepsia is one such disease which is characterised by early satiety, postprandial fullness in the abdomen, epigastric pain or discomfort and bloating, in the absence of any organic basis. The cause is said to be multifold, to name a few, gastric dysmotility, hypersensitivity in gastrium along with stress and psychological comorbidity[1]. The existing treatment for FD includes the use of pharmacological agents like antispasmodics, proton pump inhibitors (PPIs), Selective serotonin reuptake

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inhibitors (SSRIs), motility agents and antidepressants, along with probiotics[2]. The pathological changes involved in the manifestation of the disease indicate inflammatory and immune-mediated pathological changes. Recently, the alterations in the gut-brain axis have been identified as one of the potential causes for FD[3]. Mitochondrial autophagy has been identified as another cause[4].

The disease FD involves multiple pathological changes, making it a challenging condition to deal with. The current treatment, though, concentrates on the underlying cause; successful treatment of the disease without side effects is still not attained due to some limitations[5]. Functional dyspepsia is a multifactorial disease dominated by inflammatory and immune-mediated pathologies. It is difficult to understand and address multiple pathological mechanisms, and hence, there is a dire need to identify safer drugs that could address the multiple pathological mechanisms involved in the disease manifestation.

Some of the herbal drugs have shown promising action in controlling chronic gastrointestinal symptoms. *P. chaba* Hunter is a commonly used herb in culinary practices throughout India and is mentioned in the traditional Indian system of medicine as "*Tuptighna*" (relieves satiety)[6]. As per the classical texts of *Ayurveda*, *Trupti* results due to aggravation of *Kapha dosha* (one among the *Tridoshas* - three body humours mentioned in *Ayurveda*) and the literal meaning of the term is "satiation". Early satiation or aversion towards food is found as a symptom in many gastrointestinal conditions wherein digestion is hampered due to faulty dietary habits and psychological factors[7]. *Truptighna* means that which destroys the symptom of early satiation. *Truptighna Gana* comprises ten herbal drugs, each of which have their action on the gastrointestinal system. *Ayurveda* highlights the pharmacological actions of these drugs as *Agni Deepana* (enhancer of appetite) and *Pachana* (promoter of digestion)[8,9,10]. In a previous study, *Shunthi* (*Zingiber ossicinale* Roscoe), a constituent drug of *Truptighna Dashemani,* showed positive effects on gastric motility and emptying in FD patients. It stimulated antral contractions and resulted in early gastric emptying when compared to the placebo group[11]. A randomised open clinical study assessing the effect of *Panchakola Siddha Yavagu* (a classical *Ayurvedic* preparation containing *Pippali* - *Piper longum* L. fruit, *Pippali moola* – *P. longum* L. root, *Chavya* – *P. chaba* Hunter, *Chitraka* – *P. zeylanica* L. and *Nagara* – *Z. ossicinale* Roscoe.) in *Agnimandya* (reduced digestive power) showed marked improvement in digestion, reducing the symptoms of dyspepsia[12]. Other drugs like *E. ribes* Burm. f.[13] and *M. tenacissima* (Roxb.) Moon[14] has also shown promising results in maintaining gastrointestinal health.

The phenolic compounds, which are the secondary metabolites present in plants, have a wide range of actions on the human body. They help in removing oxidative products, producing diverse actions like anti-inflammatory, antiproliferative, anti-aging and antioxidant actions. The phenolic fats found in food products reduce the occurrence of chronic illnesses like Diabetes mellitus, Parkinson's disease, Alzheimer's disease, cardiovascular problems and infections by altering the inflammatory cascadeassociate with the development of these diseases. Phenolic compounds are found to act by complementing the actions of NSAIDs. Some phenolic compounds can inhibit the activity or expression of pro-inflammatory markers. They can also upregulate or downregulate the transcriptional elements involved in antioxidant pathways like nuclear factor-κB (NF-κB) or nuclear factor-erythroid factor 2-related factor 2 (Nrf-2)[15]. In FD, anti-inflammatory and immune modulatory actions become relevant, given the pathological changes involved in the manifestation of the disease.

Chavya belongs to the family *Piperaceae*, rich in dimeric alkaloids and alkamides along with phenolic compounds and shows numerous pharmacological activities. Its anti-inflammatory, anti-oxidant and gastroprotective effects are proven[16]. In the present study, *in vitro* analysis was conducted to quantify the total phenols, flavonoids and antioxidant activity of the drug. Further *in silico* study was performed to understand the possible role of *P. chaba* in Functional dyspepsia, particularly on the molecular targets responsible for inflammatory and immune-mediated pathologies. The results of *in vitro* and *in silico* studies were integrated to relate the drug action in FD to see if it can address the molecular targets involved in the manifestation of FD.

2. Materials and Methods

The dried stem of *P. chaba* Hunter was procured from KLE Ayurveda Pharmacy, Khasbag, and was

authenticated in the Central Research Facility, AYUSH-approved drug testing laboratory, KAHER's Shri B. M. Kankanawadi Ayurveda Mahavidyalaya, Shahapur, Belagavi. Preliminary organoleptic and physicochemical analyses were done, followed by quantification studies.

2.1 *In Vitro* Analysis

The stem of *P. chaba* was powdered, and an ethanolic extract was prepared by the cold extraction method (agitation for 6 hours in a flask shaker followed by maceration for 18 hours). The extract was then filtered, and the solvent was evaporated on water bath. This extract was used for further *in vitro* analyses.

2.1.1 Determination of Total Phenols

Total phenol content was determined by the Folin-Ciocalteu colourimetric method[17], Gallic acid was used as a standard. Gallic acid was used to make the calibration curve by dissolving it in methanol and then diluting it to 6.25 µg/ml - 100 µg/ml serial concentrations. A stock solution of extract (1mg/ml) was also prepared with ethanol.

Reaction solutions of 10ml contained a sample extract stock solution/(Gallic acid standard), which was mixed with 10 fold dilute Folin-Ciocalteu reagent and 7.5% sodium carbonate. The tubes were covered with Parafilm and allowed to stand for 30 minutes at room temperature, and the absorbance was read at 760 nm with a Shimadzu UV-1800 spectrophotometer. A blank was prepared similarly by replacing the sample or standard with methanol. A calibration curve using Absorbance *vs*. concentration of gallic acid standard was prepared, and the concentration of total phenols in the sample was determined by using a slope equation that was obtained from the standard graph, and the results for total phenols were expressed as mg of gallic acid equivalent/gm dried extract.

2.1.2 Determination of Total Flavanoids

Total flavonoid content was determined by the aluminium chloride colourimetric method[18], and quercetin was used as a standard. Quercetin was used to make the calibration curve by dissolving in Ethanol and then diluting to 6.25µg/ml - 200 µg/ml serial concentrations. A stock solution of extract(1mg/ml) was prepared with ethanol.

Reaction Solutions of 10 ml contained the sample extract stock solution/(quercetin standard) to which Methanol, 10% Aluminium Chloride, 1 M potassium acetate solution and distilled water were added and mixed well. A sample blank was prepared similarly by replacing Aluminium chloride with distilled water. Sample and standard absorbance were measured at 420 nm with a Shimadzu UV-1800 spectrophotometer. A calibration curve using Absorbance *vs.* concentration of Quercetin standard was prepared, and the concentration of total flavonoid in the sample was determined by using a slope equation that was obtained from the standard graph. Results for total flavonoids were expressed as mg of quercetin equivalent/gm dried extract.

2.1.3 Antioxidant Activity

This was determined by the DPPH method[19]. Chemicals for analysis included Ascorbic acid, plant extract, methanol, and DPPH chemical. Ascorbic acid was used to make the calibration curve by dissolving it in methanol and then diluting it to 6.25 µg/ml - 200 µg/ml of serial concentrations. A stock solution of extract (1 mg/ml) was prepared with methanol and further diluted to serial concentrations (6.25-200 µg/ml).

Reaction Solutions of 5 ml contained sample extract serial concentrations/(Ascorbic acid standard serial concentrations) to which DPPH dissolved in methanol was added and mixed well. Blank contained only DPPH dissolved in methanol. Incubate the tubes for 30 minutes in the darkroom. Absorbance of Blank, sample, and Standard Solutions was measured at 517 nm with a Shimadzu UV-1800 spectrophotometer. Calibration curve using Absorbance *vs* Concentration of sample extract. Serial concentrations and Ascorbic acid standard serial concentrations were prepared, and the percentage (%) of radical scavenging activity in the sample was determined.

2.2 *In Silico* Analysis

2.2.1 Identification of Phytochemicals, Targets, Pathways and Network Construction

Two major plant databases, namely IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics)[20] and Dr. Duke's database[21] were used to compile the bioactive phytoconstituents of *P. chaba* stem. The Canonical SMILES of these bioactives were then

collected from Pub Chem database[22], which were used to screen the Dug likeness, Lipinski violation, absorption in Human intestinal absorption (HIA) and toxicity parameters with the help of ADMET lab 3.0 database[23]. Those bioactive phytochemicals which fulfilled the selection criteria (QED>0.67, Lipinski violation <2, HIA 0-0.3, carcinogenicity 0-0.3 and considering CYP450-mediated interactions, P-glycoprotein interaction analysis, distribution parameters and clearance properties) were selected for the next step.

The SMILES of selected phytochemicals were uploaded into Swiss Target Prediction[24] to obtain the related targets. Genecards database[25] was utilised to procure the protein targets related to the disease Functional dyspepsia, which were later put in Venny 2.1.0[26] to detect the common targets with respect to the plant *P. chaba* and the disease FD.

The common targets retrieved from Venny 2.1.0 were entered into the STRING database[27] to analyse the interactions between the proteins. For this, the species "*Homo sapiens*" was selected. The KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis was performed to obtain the top 10 interacting pathways for these common targets.

Following this step, the networks between Phytochemical-target, target-pathway and combined network were constructed using Cytoscape 3.10.3[28]. For this, the common targets related to plant and disease, the pathways, and bioactive phytoconstituents were uploaded into Cytoscape. This allowed the conduction of topological analysis, followed by the calculation of edge-betweenness and degree layout between the nodes. Using Cytohubba[29], the top 10 targets ranked by degree method were obtained with the calculation of the shortest length between the targets.

2.2.2 Molecular Docking

Molecular docking between the ligands and targets was performed based on the top 10 ranked hub genes and the degree layout. The PDB IDs of respective targets (with minimum x-ray diffraction, minimal chain, with active site and good interactions) were obtained from the RCSB PDB database[30]. The 3D structures of phytochemicals were downloaded from PubChem. Editing of protein and its preparation was done with Discovery Studio 2021[31] and Autodock tool 1.5.7[32]. During this step, water molecules were removed, polar hydrogen bonds and Kollman charges were added. Then, with the help of PyRx[33], molecular docking is performed between the ligand (phytochemical 3D structure) and the target (protein structure) to calculate the binding affinity/energies. The ligands with the best binding energies were visualised via Biovia 2021.

3. Results

The test sample was authenticated with the reference identification number CRF/Auth/88/2025-26. Primary organoleptic and physicochemical analysis results are depicted in Table 1. The results of the estimation of total phenols and flavonoids are depicted in Table 2.

The antioxidant activity analysed by the DPPH method showed an IC_{50} value of 329.94 µg/mL. The results of the inhibition of free radicals are shown in Table 3.

3.1 Results of the *In Silico* Study

When searched in the IMPPAT database using "*Piper retrofractum* (synonym for *P. chaba*)" as the search term and stem as the useful part, 23 phytochemicals were identified. Further, the information on bioactives of *P. chaba* was also obtained from published articles, where 92 phytochemicals were identified[34,35]. After removing the duplicates, the list of phytochemicals was narrowed down to 74. In Dr Duke's database, no phytochemicals related to the drug were identified.

Table 1. Results of organoleptic and physicochemical analyses

Sl. No.	Test	Observation/Result
1	Organoleptic	Stem is greyish brown in colour, possesses an acrid taste and peppery odour.
2	Foreign matter	Nil
3	Total Ash value (TA %)	2.379
4	Acid insoluble ash (AIA %)	0.793
5	Water-soluble extractive value (WSE %)	8.0731
6	Alcohol soluble extractive value (ASE %)	4.7467

Table 2. The concentrations of total phenols and flavonoids

Sl. No.	Test Name	Concentration 1	Concentration 2	Concentration 3	Mean	Standard Deviation
1	Total Phenols	109.8544	110.7743	109.0367	109.8885 (mg GAE/gm of extract)	0.869268
2	Total Flavonoids	8.2890	8.2890	8.4578	8.3453 (mgQE/gm of extract)	0.097413

Table 3. The percentage inhibition of free radicals by the DPPH method

Sl. No.	Concentration of Extract (µg/mL)	Mean Inhibition Percentage of Free Radical
1	15.625	49.42748
2	31.25	50.81107
3	62.5	54.3416
4	125	61.59351
5	250	72.75763
6	500	86.0687

ADME included criteria like QED> 0.67, Lipinski violation <2, HIA 0-0.3, carcinogenicity 0-0.3 and consideration of CYP450-mediated interactions, P-glycoprotein interaction analysis, distribution parameters and clearance properties for selection of compounds for further steps.

CYP450 interaction analysis revealed that several phytoconstituents, particularly piperine, sylvatine, and retrofractamide derivatives, exhibited strong inhibitory activity against multiple isoforms (CYP1A2, CYP2D6, and CYP3A4). This indicated a high potential for drug–drug interactions. In contrast, phenolic acids such as caffeic acid and chlorogenic acid demonstrated negligible CYP inhibition, suggesting a favourable metabolic safety profile. Compounds such as sesamin and isopiperine displayed dual inhibitor–substrate characteristics, indicating complex metabolic behaviour.

P-glycoprotein interaction analysis revealed that the majority of alkaloid constituents exhibited strong inhibitory potential, suggesting their ability to enhance intestinal absorption but also indicating a significant risk of transporter-mediated drug–drug interactions. In contrast, phenolic compounds such as caffeic acid and chlorogenic acid showed negligible interaction with P-gp, indicating a favourable absorption profile. A few compounds, including n-butyl amine and rutin, were predicted to be P-gp substrates, suggesting potential efflux-mediated limitations in bioavailability.

Blood-brain barrier permeability and plasma protein binding indicated that most piperamides have excellent permeability but high plasma protein binding, thereby limiting bioavailability. Phenolics and flavonoids show poor permeability and moderate plasma protein binding.

The plasma clearance (cl-plasma) predictions revealed that most phytochemicals exhibited moderate clearance values (log CL ≈ 5–7), indicating favourable elimination profiles and balanced pharmacokinetics (Piperine, Piplartine, Piperlonguminine, Retrofractamide A, Guineensine, Pellitorine). A subset of compounds showed high to very high clearance (n-butyl amine, n-pentyl amine, Filfiline, Piperchabamide B, Catechin hydrate, caffeic acid, vanillin, versalide), suggesting rapid systemic elimination and limited bioavailability. A few compounds with low clearance values (Sesamin, Syringic acid, Chlorogenic acid, Rutin hydrate, Retrofractamide) may exhibit prolonged retention and possible accumulation risks.

The top five ADMET balanced candidates obtained after screening include Piperine, Piplartine, Piperlonguminine, Retrofractamide A and Pellitorine.

After ADMET screening, the final list of phytochemicals comprised 25 bioactives. Canonical SMILES of each of these 25 phytochemicals were entered in Swiss Target Prediction, which resulted in the identification of 826 unique targets.

The term "Functional dyspepsia" was used in the GeneCards database to get disease-related targets. 1131 targets of the disease were obtained after selecting "protein coding" and with a relevance score cutoff of 2 to include the genes with at least minimal association to the disease. Among these, 17 targets showed GeneCards relevance scores above 20, indicating a very strong association (high priority genes). These are high-priority targets likely playing central roles in the disease pathophysiology and are suitable for focused mechanistic studies or therapeutic targeting. 437 targets showed GeneCards relevance score between 10 and 20,

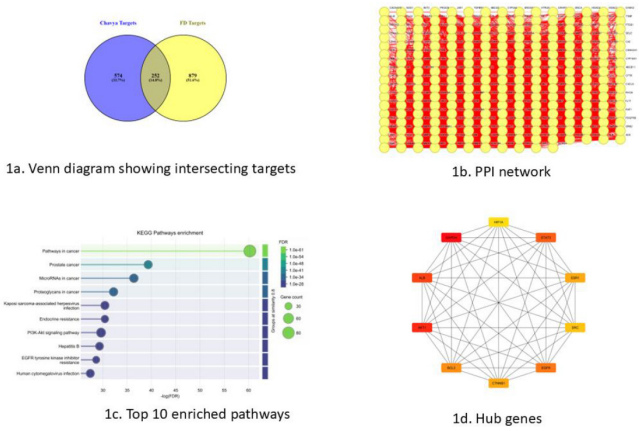


Figure 1. **(a).** Venn diagram with intersecting targets, **(b).** PPI network, **(c).** top 10 enriched pathways and 1d-Hub genes.

indicating a strong association. These are well supported by existing evidence and may contribute significantly to the disease mechanism, but with slightly less specificity. 452 targets showed GeneCards relevance score between 5 and 10, indicating moderate association. These may be involved in secondary pathways, regulatory mechanisms, or indirect biological processes related to the disease. The remaining targets showed weak or indirect association. These include the genes with limited evidence, inferred associations, or involvement in broader physiological processes rather than disease-specific mechanisms. This tiered prioritisation of targets helped in identifying key molecular targets of FD, thereby enhancing the relevance of downstream analyses. This also helped in reducing the noise from weakly associated genes.

The common target count obtained by Venny 2.1.0 was 252 for the said drug and the disease (Figure 1a). Uploading of common targets to the STRING database showed strong interaction between the targets (Figure 1b). The number of nodes was 252. While the expected number of edges was 2618, the number of edges found in the interaction was 5761. The average node degree noted was 45.7, and the PPI enrichment p-value was < 1.0e-16, showing that there are significantly more interactions than expected. The enrichment indicates that the proteins, as a group, are at least partially biologically connected. KEGG analysis identified 202 pathways, among which the top 10 interacting pathways (Figure 1c) were taken into consideration for further network construction.

Table 4. Top 10 targets in the network ranked by the Degree method

Rank	Name	Score
1	GAPDH	181
2	AKT1	172
3	ALB	157
4	STAT3	148
5	EGFR	145
6	BCL2	144
7	ESR1	142
7	CTNNB1	142
9	SRC	140
10	HIF1A	139

Among these, the most interacting pathways were Pathways in cancer, PI3K-Akt signalling pathway, and MicroRNAs in cancer.

The interaction from STRING was exported to Cytoscape, networks were constructed, and the top 10 hub genes were calculated with Cytohubba. The top 10 hub genes (Figure 1d) and their respective ranks are depicted in Table 4.

The 25 phytochemicals, 252 common targets, and the top 10 interacting pathways were considered for compound-target-pathway network construction with the help of Cytoscape. Degree layout was calculated for the said phytochemicals. The highest outdegree value of 100 was seen for two compounds, Chabamide and Piperine. The networks are depicted in Figure 2 (2a: Plant-phytochemical network, 2b: Phytochemical-target network, 2c: pathway-target network, 2d: Phytochemical-target-pathway network).

3.2 Molecular Docking

Docking was conducted with bioactives Chabamide, Piperine, Piplartine, Piperlonguminine, Retrofractamide A, and Pellitorine with targets GAPDH (RCS PDB ID-1u8f), AKT1 (RCS PDB ID-4ekl), ALB1 (RCS PDB ID-7vro), STAT3 (RCS PDB ID-6nuq), and EGFR (RCS PDB ID-3poz). Pantoprazole, which is the standard treatment for FD, was used as a comparator to assess and compare the efficacy of phytoconstituents in *P. chaba* in terms of binding affinity. Chabamide showed the most stable binding energies for all the targets in comparison with the standard drug Pantoprazole. Piperine also showed stable binding energies equal to

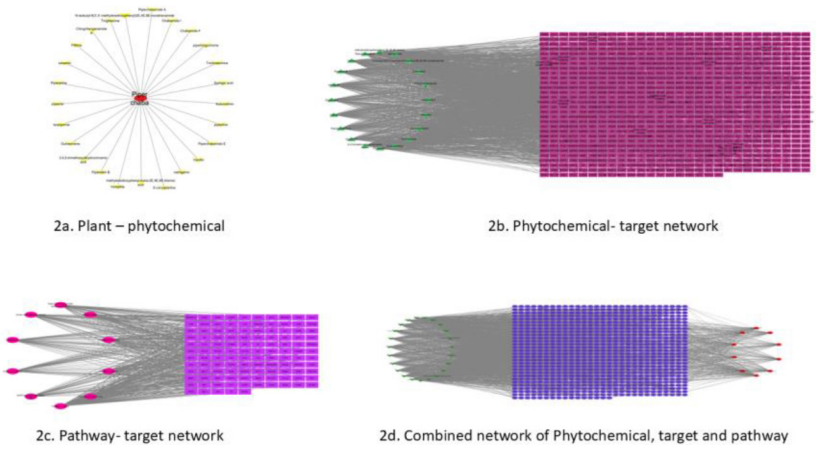


Figure 2. Networks between plant, phytocompounds, targets and pathways. **(a).** Plant-phytocompound network, **(b).** Phytocompound-target network, **(c).** Pathway-target network, **(d).** Combined phytocompound-target-pathway network.

Table 5. The binding energies of *P. chaba* phytoconstituents with different targets

Targets	Chabamide	Piperine	Piplartine	Piperlonguminine	Retrofractamide A	Pellitorine	Pantoprazole
GAPDH (1U8F)	-8.9	-7.2	-7.1	-6.3	-6.2	-5.5	-7.3
AKT1 (4EKL)	-8.9	-7.4	-7	-6.7	-7.6	-5.4	-7.4
ALB1- 7VRO	-10.2	-8	-7.8	-7.6	-7.3	-6.1	-8.2
STAT3- 6NUQ	-8.4	-6.8	-6	-6.1	-6.5	-5.3	-6.8
EGFR- 3POZ	-8.6	-6.9	-6.3	-6.4	-6.1	-5.2	-6.4

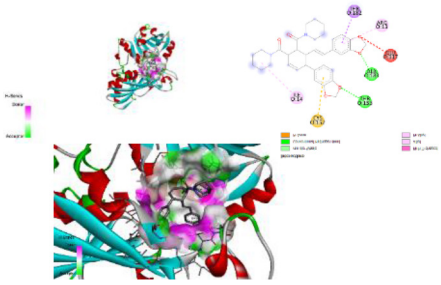


Figure 3. Chabamide with target GAPDH-1u8f.

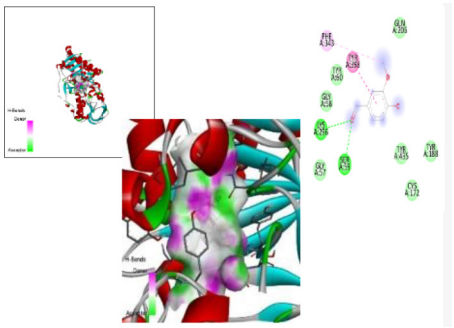


Figure 4. Piperine with target GAPDH-1u8f.

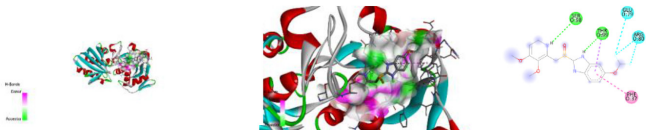


Figure 5. Pantoprazole with target GAPDH-1u8f.

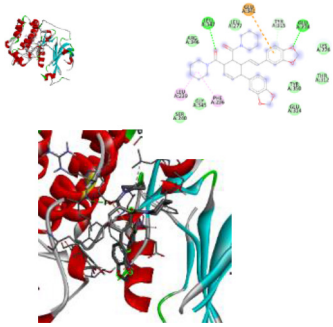


Figure 6. Chabamide with target AKT1-4ekl.

or near those of a standard drug. The binding energies are shown in Table 5.

Visualisation of docking pose of selected targets and phytoconstituents is depicted in Figures 3 to 8.



Figure 7. Piperine with target AKT1-4ekl.

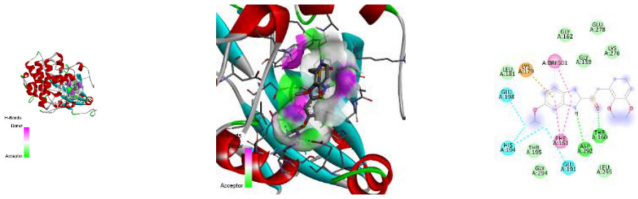


Figure 8. Pantoprazole with target AKT1-4ekl.

4. Discussion

Medicinal plants of *Piperaceae* have carried ethnomedicinal importance and have played a major role as therapeutic agents, especially in treating gastrointestinal conditions. The plants of the *Piper* genus (the largest in the family) contain some significant natural compounds with vast therapeutic action. *Piper* species have hepatoprotective and gastrointestinal effects. These containing natural compounds can be alternatives to synthetically prepared anti-inflammatory agents, which are usually associated with adverse effects[36]. *P. chaba*, known as *Chavya* in *Ayurveda*, is traditionally used to treat abdominal pain, asthma, bronchitis, fever, and piles. The drug has not yet been explored with respect to its mode of action on the GI tract. The present study tried to assess the efficacy of the drug *P. chaba* in Functional dyspepsia, a disease involving multiple pathological mechanisms, by applying *in vitro* and *in silico* analyses.

The stem of *P. chaba* showed physico-chemical parameters within the limits of API (Ayurvedic Pharmacopoeia of India). The ethanolic extract showed total phenolic content of 109.8885 mg Gallic Acid Equivalents/gm of extract and a total flavonoid content of 8.3453 mg Quercetin Equivalents/gm of extract. In support of this observation, a recent study indicated significant levels of phenols and flavonoids in this drug[37].

Polyphenols are a category of secondary metabolites which occur naturally in many fruits, vegetables and cereals. They play a role in the natural defence mechanism of plants by preventing disease attacks. If consumed for a long duration, they can protect our body from diseases like cancer, diabetes, osteoporosis, cardiovascular diseases and neurological disorders. The negative actions of reactive oxygen or nitrogen species formed in our body due to various metabolic activities are prevented by these compounds. Thus, they prevent oxidative stress and act as antioxidants either alone or in combination with vitamins. Further, the phenolic compounds are known to prevent pro-inflammatory mediators from binding and also exert anti-inflammatory action on COX-2 by inhibiting NF-kB[15].

Flavonoids are the secondary metabolites with a benzopyrone ring along with a phenolic or a polyphenolic group at different positions. They are naturally found in vegetables, fruits, cereals, nuts, flowers and seeds. They have found wide applications in medicine due to their pharmacological actions like antitumor, antiviral, antimalarial, antiproliferative and antioxidative agents. Flavonoids are also known to exert anti-inflammatory action by different mechanisms, such as by acting on the inflammatory mediator IL-6, by inhibiting NF-kb, by inhibiting LPS induced TNFα production, by suppressing the activation of nuclear factor jB, inhibiting LPS induced NO production, inhibition of iNOS and COX-2 expression[38].

Antioxidant activity of ethanolic extract of *P. chaba* assessed by DPPH method showed an IC_{50} value of 329.94 µg/mL. That means the concentration of the sample required to scavenge 50% of DPPH radicals is about 330 µg/mL. In a recent study by Md Rubel Al-Mamun *et al.*, the ethyl acetate extract of *P. chaba* stem was tested for its activity using the DPPH method, which showed free radical scavenging activity of 43.85± 1.50 µg/mL[39]. This difference in activity might be due to the solvent used and the method of extraction (cold extraction by agitation and maceration).

In silico analysis of the drug identified 25 bioactive phytoconstituents showing specific targets for the disease Functional dyspepsia. Out of these, Chabamide and Piperine showed the highest outdegree, indicating their downstream influence or regulatory capacity. The highest outdegree tells that these two are multi-target compounds acting on many different proteins.

4.1 Discussion on Core Targets

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multi-dimensional protein which represents one of the most perfect among the class. It exerts a wide variety of functions which are distinct from its classical role of glycolysis/production of energy. Its novel functions are identified as regulating the stability of mRNA, trafficking of intracellular membrane, uptake and transport of iron, metabolism of heme, regulating the gene expression and nuclear tRNA transport, maintaining genomic integrity, DNA repair and apoptosis[40]. It is closely linked to the survival and death of cells and also influences intercellular signalling processes, which impact the progression of cancer and symbiosis of microbes. It ensures metabolic flexibility, which in turn ensures successful interactions between host and microbes. Inhibition of GAPDH can result in a reduction of cancer cell proliferation by disrupting the dependency of malignant cells on glycolysis as a source of energy. Further natural products have shown the capacity to bind to a specific cysteine residue on GAPDH, which results in nuclear translocation. This can enhance cell death in glioblastoma and can be effective in combination with chemotherapeutic agents[41]. In FD, mucosal inflammations lead to oxidative stress in epithelial or immune cells. Since GAPDH is sensitive to oxidative modifications, it can alter its functions under the influence of oxidative stress. Due to an increase in metabolic demands, glycolytic flux might be altered, causing up- or downregulation of GAPDH. As a result, its non-glycolytic roles may become more prominent.

AKT1 regulate a wide range of cellular functions such as cell proliferation, cell survival, cellular metabolism and angiogenesis in both healthy and malignant cells. The PI3K/AKT signalling pathway has a crucial role to play in the survival of tumour cells. The survival factors suppress apoptosis by AKT1 activation, which later inactivates components of apoptotic processes[42]. In FD, there is low-grade mucosal inflammation, and the epithelial barrier is compromised. AKT1 helps in the repair of mucosa by promoting survival signals and reducing apoptosis. Dysfunction of AKT1 signalling might cause impairment of epithelial renewal and increased permeability, further leading to symptoms in FD. While AKT1 interacts with other pathways like PI3K and NF-kB, the changes caused could affect gastric accommodation or immune regulation[43].

4.2 Discussion on Core Phytochemicals

Chabamide is a piperine dimer present in the stem of *P. chaba*. It has shown antimalarial (with IC_{50} values of 2.7 microg/ml) and antitubercular activities (with minimum inhibitory concentration of 12.5 microg/ml) in a previous study[44]. So far, there is a lack of research evidence indicating the role of Chabamide in Functional dyspepsia. In the present study, Chabamide showed the most stable binding energies with the hub genes GAPDH, AKT1, ALB1, STAT3 and EGFR. This means the compound can modify the disease process by acting on these targets. The binding of Chabamide was more stable than the standard drug pantoprazole in all cases. This highlights the possibility that Chabamideis is more potent than standard drugs and can control the pathological mechanisms of FD.

Piperine, an amide alkaloid which is present in all the species of the *Piper* genus, is a well-known bioavailability enhancer[45]. It is also known for its anti-inflammatory, antioxidant, gastroprotective and GI motility modulation effects. It is known to stimulate digestive enzymes in the pancreas and intestine. Further, it helps in reducing FD symptoms by improving digestion and nutrient absorption, thus relieving postprandial discomfort and bloating[46]. Piperine has the ability to stimulate intestinal smooth muscle contractions, and thus it exerts a prokinetic effect by enabling gastric emptying[47]. FD is characterised by low-grade inflammation. Piperine can suppress pro-inflammatory markers and, at the same time, enhance antioxidant enzymes[48]. In the present study, Piperine showed stable binding energies with hub genes. The binding energies of piperine were close to those of Pantoprazole for the said targets. This indicates that piperine is equally potent as the standard drug.

4.3 Discussion on Pathways

The significantly enriched pathways implicated in the study are Pathways in cancer, PI3K-Akt signalling pathway and MicroRNAs in cancer. Cancer is characterised by uncontrolled cell growth and metastasis. The related pathways are PI3K/AKT pathway, MAPK/ERK pathway and NF-kB pathway. On the other hand, FD is a functional disorder without any structural lesions. It involves multifactorial pathology. Since mucosal inflammation is observed in FD, the NF-kB pathway, which mediates inflammation in FD, overlaps with inflammation mechanisms in cancer.

The PI3K-AKT signalling pathway is involved in the regulation of cellular functions (cell growth, metabolism, survival and synthesis). This pathway influences the growth of gastric mucosal and epithelial cells. When abnormal secretion of gastric acid occurs, it disrupts the gastric mucosa. This permits the permeation of hydrogen ions in gastric juice, resulting in FD symptoms. In the PI3K-AKT signalling pathway, PI3K is first activated by growth factors or signal transduction complexes, resulting in the production of PIP3. This inturnactivates AKT, regulates secretion of GI hormones and promotes regeneration of gastric mucosal epithelial cells[49]. GI hormones like gastrin, Cholecystokinin, Ghrelin, GLP (Glucose-dependent insulinotropic peptide) and motilin are responsible for GI motility[50]. In FD, dysmotility is a major symptom which can be overcome through activation of GI enzymes. Thus, activation of this pathway by bioactives in *P. chaba* could help in FD.

MicroRNAs play a crucial role in cancer by regulating gene expression post-transcriptionally. They can either cause oncogenesis or act as tumour suppressors depending on their targets. Dysregulation of MRNAs causes tumour progression by hyperproliferation of cells, avoidance of apoptosis and metastasis. They also modulate immune responses. They are overexpressed in cancer of the lungs, breast and glioblastoma. Recently, the role of MRNA is being explored as a biomarker in FD. Tanaka F *et al*., showed that exosomal MRNAs in gastric juice are downregulated in FD patients as compared to the controls. The expression of MRNA was negatively correlated to the severity of FD symptoms[51]. In another study by Farcas Radu A *et al*., patients with FD (postprandial distress syndrome subtype) showed markedly higher levels of miR-21 and miR-155 and lower miR-203 when compared to FD patients with epigastric pain syndrome subtype. A history of infection with *H. pylori* was associated with miR-203 levels in gastric juice[52]. Thus, there is evidence linking MRNAs to FD, especially in the gastric juice, and they might play a role in deciding the symptom type and severity. But the role of MRNA in the causality and mechanism, or as a therapeutic target in FD, is not yet established.

4.4 Discussion on Docking Visualisation

On visualisation of the docking pose, the ligand Chabamide showed two conventional hydrogen bonds (represented by green dotted lines) with the amino acid residues ALA (O:183) and THR (O:153) for the target GAPDH, with LEU (A:347) and GLU (A:278) for the target AKT1. But the ligand showed one unfavourable bond with the amino acid residue GLU (O:317) for GAPDH. The ligand Piperine showed two hydrogen bonds with amino acid residues LYS (A:296) and SER (A:59) for the target GAPDH, and no hydrogen bonds for the target AKT1. Pantoprazole showed two hydrogen bonds with amino acid residues SER (O:98) and THR (O:99) for the GAPDH target and with ASP (A:292) and THR (A:160) for target AKT1. This confirms the stable binding of ligand Chabamide in comparison to that of pantoprazole for both targets. Whereas Piperine showed stable binding for GAPDH but not for AKT1.

The presence of multiple strong hydrogen bonds increases the binding affinity of the ligand to the receptor, stabilises the complex and suggests favourable and specific interactions. Further Hydrogen bonds help in the proper orientation of the ligand at the active site, determining the best-fit pose. Based on the number of hydrogen bonds, the molecular docking tools assign weightage to the binding interactions. They influence the docking score and help predict affinity. Hydrogen bond visualisation further helps to identify the particular functional group essential for the desired activity. It also helps in the identification of critical amino acids. Thus, it helps in rational drug designing and optimisation studies.

The present study employed an *in vitro* and *in silico* approach to assess the role of phytoconstituents of *P. chaba* in altering the disease process of Functional dyspepsia. The *in vitro* analyses confirmed the antioxidant activity along with the quantitative determination of phenols and flavonoids, which are possibly responsible for the action. FD results due to inflammatory and immune mediated pathological changes, and the pharmacological actions of phenols and flavonoids suffice these effects. Further *in silico* study with molecular docking showed stable binding energies with hub genes. During docking visualisation, the ligands Chabamide and Piperine showed a better docking score, comparable to that of the standard drug Pantoprazole. So the phytoconstituents in *P. chaba* are potent enough to manage the disease. The study has its limitations in not conducting the LC-MS or GC-MS characterisation of the drug and depending on

databases to retrieve the related data. But the present work tried to validate the property of *Truptighna* (alleviating early satiety/dyspepsia) attributed to *P. chaba* (*Chavya*) in the classical literature of *Ayurveda* through a bimodal approach of combined *in vitro* and *in silico* analyses.

5. Conclusions

Based on the observations from this study, we could conclude that the phytoconstituents Chabamide and Piperine can form potential therapeutic agents for Functional dyspepsia, which involves complex pathologies. *P. chaba* might control the inflammatory and immune pathologies involved by acting on the targets GAPDH, AKT1, ALB1 via the pathways PI3K-Akt signalling pathway, Pathways in cancer and MicroRNAs in cancer. Further experimental and clinical validation will help to establish the drug and its specific phytoconstituents as the therapeutic agent for FD.

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7. References

1. Futagami S, Yamawaki H, Agawa S, Higuchi K, Ikeda G, Noda H, Kirita K, Akimoto T, Wakabayashi M, Sakasegawa N, Kodaka Y, Ueki N, Kawagoe T, Iwakiri, K. New classification Rome IV functional dyspepsia and subtypes. Transl Gastroenterol Hepatol. 2018; 3:70. https://doi.org/10.21037/tgh.2018.09.12 PMid: 30363705 PMCid: PMC6182037.
2. Monkemuller K, Malfertheiner P. Drug treatment of functional dyspepsia. World J Gastroenterol. 2006; 12(17):2694-2700. https://doi.org/10.3748/wjg.v12.i17.2694 PMid: 16718755 PMCid: PMC4130977.
3. Fikree A, Byrne P. Management of functional gastrointestinal disorders. Clin Med (Lond). 2021; 21(1):44-52. https://doi.org/10.7861/clinmed.2020-0980 PMid: 33479067 PMCid: PMC7850201.
4. Francis P, Zavala SR. Functional dyspepsia. NCBI Bookshelf. 2024. https://www.ncbi.nlm.nih.gov/books/NBK554563/
5. Gwee KA, *et al*. Herbal medicines in functional dyspepsia—untapped opportunities without risks. Neurogastroenterol Motil. 2021; 33:e14044. https://doi.org/10.1111/nmo.14044 PMid: 33258198 PMCid: PMC7900952.
6. Acharya YT, editor. *Charaka Samhita* of Agnivesha. Chaukhambha Surabharati Prakashan: Varanasi. 2009; pp. 33.
7. Yadavaji Trikamaji, editor. *Charaka Samhita* of Charaka, *Sutrasthana*, chapter 28, verse 9. Reprint edition. Varanasi: Chaukhambha Surbharati Prakashan. 2009; pp. 179.
8. Pandey GS, editor. *Bhavaprakasha Nighantu* of Bhavamishra with commentary by Chunekar KC. Varanasi: Chaukhambha Bharati Academy. 2010; pp. 14, 15, 20, 22, 42, 51, 233, 258, 420, 673.
9. Bapalal G. *Nighantu Adarsha*. Vol 1 and 2. Varanasi: Chaukhambha Bharati Academy. 2007; pp. 35, 36, 345, 364, 707, 796, 802.
10. World Health Organization. WHO international standard terminologies on *Ayurveda*. Geneva: World Health Organization. 2022. https://iris.who.int/server/api/core/bitstreams/bd4c0a7d-f7b2-420e-8c93-0ecb9009f718/content
11. Hu ML, Rayner CK, Wu KL, Chuah SK, Tai WC, Chou YP, Chiu YC, Chiu KW, Hu TH. Effect of ginger on gastric motility and symptoms of functional dyspepsia. World J Gastroenterol. 2011; 17(1):105-110. https://doi.org/10.3748/wjg.v17.i1.105 PMid: 21218090 PMCid: PMC3016669.
12. More SD, Dwivedi RR. A clinical study of *Panchakola Siddha Yavagu* in the management of *Agnimandya*. Ayu. 2011; 32(1):70-75. https://doi.org/10.4103/0974-8520.85733 PMid: 22131761 PMCid: PMC3215422.
13. Jagtap KT, Mulik AR, Pawar SS, Jagtap SD. *In vitro* and *in vivo* comparative evaluation of fruit extracts from *Embelia tsjeriam-cottam* and *Embelia ribes* on ethanol-induced gastric ulcer in rats. Res J Pharm Technol. 2024; 17(9):4543-4548. https://doi.org/10.52711/0974-360X.2024.00702
14. Zhou X, Liu M, Ren Q, Zhu W, Wang Y, Chen H, Chen J. Oral and injectable *Marsdenia tenacissima* extract (MTE) as adjuvant therapy to chemotherapy for gastric cancer: a systematic review. BMC Complement Altern Med. 2019; 19(1):366. https://doi.org/10.1186/s12906-019-2779-y PMid: 31830977 PMCid: PMC6909592.
15. Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, Almikhlafi MA, Alghamdi SQ, Alruwaili AS, Hossain MS, Ahmed M, Das R, Emran TB, Uddin MS. Role of phenolic compounds in human disease: current knowledge and future prospects. Molecules. 2021; 27(1):233. https://doi.org/10.3390/molecules27010233 PMid: 35011465 PMCid: PMC8746501.
16. Islam MT, Hasan J, Snigdha HMSH, Ali ES, Sharifi-Rad J, Martorell M, Mubarak CS. Chemical profile, traditional uses, and biological activities of *Piper chaba* Hunter: a review. J Ethnopharmacol. 2020; 257:112853. https://doi.org/10.1016/j.jep.2020.112853 PMid: 32283191.

17. Pérez M, Dominguez-López I, Lamuela-Raventós RM. The chemistry behind the Folin-Ciocalteu method for the estimation of (poly)phenol content in food: total phenolic intake in a Mediterranean dietary pattern. J Agric Food Chem. 2023; 71(46):17543-17553. https://doi.org/10.1021/acs.jafc.3c04022 PMid: 37948650 PMCid: PMC10682990.
18. Nicolescu A, Bunea CI, Mocan A. Total flavonoid content revised: an overview of past, present, and future determinations in phytochemical analysis. Anal Biochem. 2025; 700:115794. https://doi.org/10.1016/j.ab.2025.115794 PMid: 39894144.
19. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022; 27(4):1326. https://doi.org/10.3390/molecules27041326 PMid: 35209118 PMCid: PMC8878429.
20. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Bharath Chand RP, Aparna SR, Mangalapandi P, Samal A. IMPPAT: a curated database of Indian medicinal plants, phytochemistry and therapeutics. Sci Rep. 2018; 8:4329. https://doi.org/10.1038/s41598-018-22631-z PMid: 29531263 PMCid: PMC5847565.
21. Lans C, van Asseldonk T, Tedje M. Dr. Duke's phytochemical and ethnobotanical databases, a cornerstone in the validation of ethnoveterinary medicinal plants, as demonstrated by data on pets in British Columbia. Medicinal and Aromatic Plants of the World. 2025.
22. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. PubChem 2025 update. Nucleic Acids Res. 2025; 53(D1):D1516–D1525. https://doi.org/10.1093/nar/gkae1059 PMid: 39558165 PMCid: PMC11701573.
23. Fu L, Shi S, Yi J, Wang N, He Y, Wu Z, Peng J, Deng Y, Wang W, Wu C, Lyu A, Zeng X, Zhao W, Hou T, Cao D. ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. Nucleic Acids Res. 2024; 52(W1):W422–W431. https://doi.org/10.1093/nar/gkae236 PMid: 38572755 PMCid: PMC11223840.
24. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. 2014; 42(Web Server issue):W32–W38. https://doi.org/10.1093/nar/gku293 PMid: 24792161 PMCid: PMC4086140.
25. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan-Golan Y, Kohn A, Rappaport N, Safran M, Lancet D. The GeneCards Suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics. 2016; 54:1.30.1-1.30.33. https://doi.org/10.1002/cpbi.5 PMid: 27322403.
26. Oliveros JC. Venny: an interactive tool for comparing lists with Venn's diagrams, Version 2.1.0. 2007-2015. https://bioinfogp.cnb.csic.es/tools/venny/index.html
27. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023; 51(D1):D638–D646. https://doi.org/10.1093/nar/gkac1000 PMid: 36370105 PMCid: PMC9825434.
28. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11):2498-2504. https://doi.org/10.1101/gr.1239303 PMid: 14597658 PMCid: PMC403769.
29. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014; 8(Suppl 4):S11. https://doi.org/10.1186/1752-0509-8-S4-S11 PMid: 25521941 PMCid: PMC4290687.
30. Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, Christie CH, Dalenberg K, Di Costanzo L, Duarte JM, Dutta S, Feng Z, Ganesan S, Goodsell DS, Ghosh S, Hudson BP, Lawson CL, Liang Y, Lowe R, Zardecki C. RCSB Protein Data Bank: immutable global archive for 3D macromolecular structure data. Nucleic Acids Res. 2023; 51(D1):D368–D379. https://doi.org/10.1093/nar/gkac1077 PMid: 36420884 PMCid: PMC9825554.
31. Dassault Systèmes. BIOVIA Software, Version 2021. 2021. https://www.3ds.com/biovia
32. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem. 2009; 30(16):2785-2791. https://doi.org/10.1002/jcc.21256 PMid: 19399780 PMCid: PMC2760638.
33. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Chemical Biology: Methods and Protocols. New York: Springer. 2015; pp. 243-250. https://doi.org/10.1007/978-1-4939-2269-7_19 PMid: 25618350.
34. Sarkar C, Mondal M, Al-Khafaji K, El-Kersh DM, Jamaddar S, Ray P, Roy UK, Afroze M, Moniruzzaman M, Khan M, Asha UH, Khalipha ABR, Mori E, de Lacerda BCGV, Araújo IM, Coutinho HDM, Shill MC, Islam MT. GC-MS analysis and evaluation of protective effect of *Piper chaba* stem bark against paracetamol-induced liver damage in Sprague-Dawley rats: possible defensive mechanism by targeting CYP2E1 enzyme through *in silico* study. Life Sci.

2022; 309:121044. https://doi.org/10.1016/j.lfs.2022.121044 PMid: 36208657.
35. Dey S, Singh AK, Kumar S. Discovery of natural anti-apoptotic protein inhibitor using molecular docking and MM-GBSA approach: an anticancer intervention. Biointerface Res Appl Chem. 2023; 13(5):473. https://doi.org/10.33263/BRIAC135.473
36. Mgbeahuruike EE, Yrjönen T, Vuorela H, Holm Y. Bioactive compounds from medicinal plants: focus on *Piper* species. S Afr J Bot. 2017; 112:54-69. https://doi.org/10.1016/j.sajb.2017.05.007
37. Yesmin S, Lyzu C, Jahan N, Hasan M, Al Mamun MZU, Ahmed KS, Roy DC, Islam D, Akhter H. A comparative study on the nutritional profile, antioxidant potential, bioactive compounds, and therapeutic benefits of *Piper chaba* Hunt and refined *Piper nigrum* L. Pharmacol Res - Nat Prod. 2025; 8:100359. https://doi.org/10.1016/j.prenap.2025.100359
38. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. Important flavonoids and their role as a therapeutic agent. Molecules. 2020; 25(22):5243. https://doi.org/10.3390/molecules25225243 PMid: 33187049 PMCid: PMC7697716.
39. Al-Mamun MR, Maniruzzaman M, Badal MMR, Haque MA. Comparison of piperine content, antimicrobial and antioxidant activity of *Piper chaba* root and stem. Heliyon. 2024;10(19):e38709.https://doi.org/10.1016/j.heliyon.2024.e38709 PMid: 39421366 PMCid: PMC11483291.
40. Sirover MA. Structural analysis of glyceraldehyde-3-phosphate dehydrogenase functional diversity. Int J Biochem Cell Biol. 2014; 57:20-26. https://doi.org/10.1016/j.biocel.2014.09.026 PMid: 25286305 PMCid: PMC4268148.
41. Nature Research Intelligence. Glyceraldehyde-3-phosphate dehydrogenase and its role in cellular mechanisms. 2025. https://www.nature.com/research-intelligence/nri-topic-summaries/glyceraldehyde-3-phosphate-dehydrogenase-and-its-role-in-cellular-mechanisms-micro-131592
42. NCBI Gene. AKT1 AKT serine/threonine kinase 1 [*Homo sapiens*]. 2025. https://www.ncbi.nlm.nih.gov/gene/207
43. Simatupang ST, Dermawan D, Nadia, Tan S, Tjandrawinata RR. Network pharmacology and molecular dynamics studies unveil the therapeutic mechanisms of *Zingiber officinale* against dyspepsia. J Appl Pharm Sci. 2025. https://doi.org/10.7324/JAPS.2025.240961
44. Rukachaisirikul T, Prabpai S, Champung P, Suksamrarn A. Chabamide, a novel piperine dimer from stems of *Piper chaba*. Planta Med. 2002; 68(9):853-855. https://doi.org/10.1055/s-2002-34410 PMid: 12357406.
45. Lambert JD, *et al*. Piperine enhances the bioavailability of the tea polyphenol (-)-epigallocatechin gallate in mice. J Nutr. 2004; 134 (8):1948–1952. https://doi.org/10.1093/jn/134.8.1948 PMid: 15284381.
46. Platel K, Srinivasan K. Influence of dietary spices and their active principles on pancreatic digestive enzymes in Albino rats. Nahrung. 2000; 44(1):42-46. https://doi.org/10.1002/(SICI)1521-3803(20000101)44:1<42::AID-FOOD42>3.0.CO;2-D
47. Capasso R, *et al*. Effect of piperine, the active ingredient of black pepper, on gastrointestinal motility. Pharmacol Res. 2002; 46(3):231-234.
48. Guo G, *et al*. Piperine, a functional food alkaloid, exhibits inhibitory effects on inflammation. Food Sci Nutr. 2020; 8(6):2949-2958.
49. Bai Y, Liu F, Wan Y, Wu X, Luo S, Zhang L, Tang H, Li T, Tang X, Qin W, Gan W, Yang Y, Yin Z, Xie Y, Guo P. Network pharmacology combined with experimental validation reveals the mechanism of action of Erpixing granules on functional dyspepsia. J Ethnopharmacol. 2024; 334:118553. https://doi.org/10.1016/j.jep.2024.118553 PMid: 38992401.
50. Guyton AC, Hall JE. Textbook of Medical Physiology. 15th ed. Elsevier: Philadelphia. 2021.
51. Tanaka F, Takashima S, Nadatani Y, Otani K, Hosomi S, Kamata N, Taira K, Nagami Y, Tanigawa T, Fukumoto S, Watanabe T, Murakami Y, Kawada N, Fujiwara Y. Exosomal hsa-miR-933 in gastric juice as a potential biomarker for functional dyspepsia. Dig Dis Sci. 2020; 65(12):3493-3501. https://doi.org/10.1007/s10620-020-06096-7 PMid: 31974910.
52. Farcas RA, Surdea-Blaga T, Popa Ş, Rusu F, Chira A, Sabo C, Nechita V-I, Budişan L, Zanoaga O, Berindan-Neagoe I, Strilciuc Ş, Dumitrascu DL. Gastric juice microRNAs as biomarkers for functional dyspepsia: an observational case-control study. Front Med (Lausanne). 2025; 12:1636825. https://doi.org/10.3389/fmed.2025.1636825 PMid: 40988736 PMCid: PMC12450937.

Author Contribution

Conceptualisation: D.K. and A.C.; Methodology: D.K.; Software: D.K.; Validation: D.K. and A.C.; Formal Analysis: D.K.; Investigation: D.K.; Resources: D.K. and A.C.; Data Curation: D.K. and A.C; Writing—Original Draft Preparation: D.K.; Writing—Review and Editing: D.K. and A.C.; Visualisation: D.K. and A.C.; Supervision: A.C. All authors have read and agreed to the published version of the manuscript.