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Exploring therapeutic potential of polyherbal churna *via* network pharmacology and *in vitro* digestive assay

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Worldwide, digestive diseases are now a prevalent issue that cause a considerable amount of illness. The available treatments for digestive issues frequently have drawbacks and adverse consequences. The major limitations are rebound effect, addressing the root cause, dependence, addiction, etc. These issues motivate research into traditional herbal treatments as a possible medicinal solution is gaining momentum. Herbs such as betel leaf, liquorice, saunth, cinnamon, fennel, cardamom, marica, pipali, and caraway, have long been used to treat digestive problems. Their combined effects on the modulation of digestive enzymes, however, have not been well investigated. Considering fewer Ayurvedic digestive formulations and formulation available in market a new poly herbal churna formulation was prepared for this investigation. Using lipase, amylase, and protease assays, the formulation was evaluated *in vitro*. The formulation's impact on digestive enzyme activity was compared to standard. The prepared formulation exhibited significant amylase and lipase inhibitory activities, comparable to standard inhibitors. The formulation's digestive enzyme modulation properties were attributed to the synergistic effects of its constituent herbs. A network pharmacology study of the churna's phytoconstituents targeting digestive function and related diseases identified top 10 genes (STAT3, HIF1A, IL6, JUN, TNF, MTOR, SRC, MMP9, ESR1, and NFKB1). KEGG pathway analysis revealed involvement of these genes in pathways related to insulin resistance, inflammatory bowel disease, cancer, pancreatic cancer, and hepatitis. The formulation may be used therapeutically to treat digestive problems because of its capacity to alter the activity of digestive enzymes. To better understand the formulation's mechanisms of action and investigate its potential therapeutic uses, more *in vivo* studies are necessary.

Keywords: Betel leaf, Digestive churna, Gene ontology, *In vitro* digestive model, KEGG pathway, Network pharmacology

Millions of individuals worldwide suffer from digestive diseases, which have a major negative influence on their quality of life. The available treatments for digestive issues, including proton pump inhibitors (PPIs), histamine-2 (H2) blockers, and antacids, frequently offer short-term respite but may have drawbacks and adverse consequences. Since ancient times, traditional herbal treatments have been utilized to treat digestive problems, providing a natural and comprehensive approach to health. The ability of herbal formulations to address the underlying cause of digestive issues has made them popular, especially those based on Ayurvedic principles.

According to ancient classical Ayurvedic documents such as Bhojankutuhalam, betel leaf is a healthy way to manage weight, promote digestion, lessen excessive coughing that accumulates in the mouth, and cleanse the throat after a meal[1,2]. A betel leaf infused with therapeutic herbs is referred to as "paan" in Ayurveda. According to Ayurveda, betel leaf has a strong, sweet, bitter, alkaline, astringent, and hot flavor. It is an aphrodisiac, and it kills worms and additionally boosts the fire in the digestive system[2]. Digestive enzymes can be stimulated by betel leaf obtained from *Piper betle,* which belongs to the family Piperaceae. Effective anti-inflammatory and antibacterial properties have been noted. Vitamins and minerals are present in betel leaves, along with several other essential amino acids, such as arginine, lysine, and histidine; the leaves also contain enzymes like catalase and diastase[3,4].

Fennel (*Foeniculum vulgare*) and cardamom (*Elettaria cardamomum*) are carminative; cinnamon (*Cinnamomum verum*) has digestive and carminative

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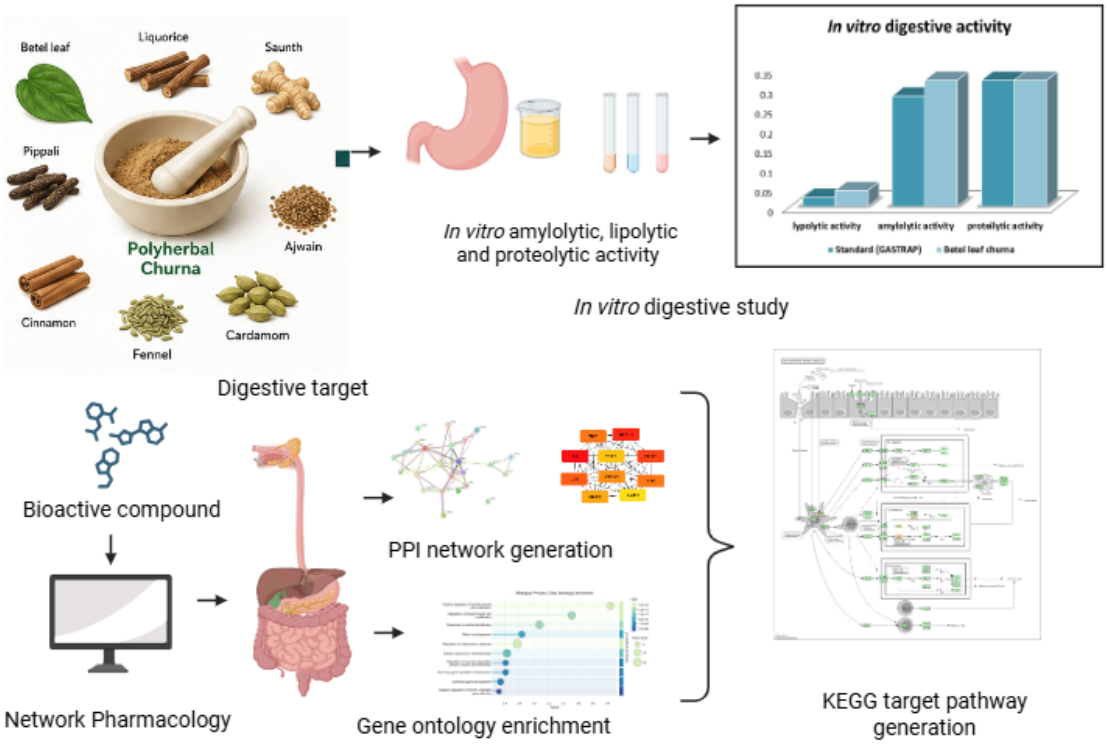


Graphical abstract

effects; and betel leaf is combined with sunthi (*Zingiber officinale*), which is known for its anti-inflammatory qualities and capacity to calm the digestive tract[5]. Maricha (*Piper nigrum*) also increases the carminative effect and bioavailability. While pippali (*Piper longum*) is digestive, antioxidant, and carminative, caraway (*Carum carvi*) is antispasmodic and carminative; liquorice (*Glycyrrhiza glabra*) has antiulcer properties and soothes digestive issues[5-7]. Ayurveda is a well-known ancient system of medicine that incorporates different dosage forms, including churna[8]. Churna is defined as a fine powder of a drug or drugs in the Ayurvedic system of medicine. These medicaments are prescribed generally because of their particle size.

According to literature, some Ayurvedic churnas that use saunth, cinnamon, fennel, cardamom, maricha, pippali, and caraway and have digestive properties include hingvastaka churna, trikatu churna, ajmodadi churna, hingupanchak churna, and panchakola churna[9,10]. However, a significant gap was found: not a single churna gathers betel leaves. However, gandharvaharitaki arishta, tamalpatra asava, and tamalpatra arishta are betel leaf-based formulations used as digestive aids[10]. This study aims to develop and evaluate a novel polyherbal churna formulation comprising these herbs, assessing its digestive enzyme modulation properties and potential as a natural remedy for digestive disorders.

Material and Methods

Physical drug evaluation of crude drugs

The various physical evaluations of crude drugs such as ash value, extractive value, loss on drying, foreign organic matter was performed as per prescribed in The Ayurvedic Pharmacopoeia of India[11,12].

Chemical evaluation of crude drugs

Various chemical tests for primary and secondary metabolites were carried out as per method specified in official books[12-14].

Method of preparation

The row material such as leaves of *Piper betel,* rhizomes of *Zingiber officinale,* bark of *Cinnamomum verum,* seeds of *Elettaria cardamomum, roots of Glycyrrhiza glabra, flower petels of rose,* fruits of *Foeniculum vulgare, Piper nigrum, Piper longum* and *Carum carvi* were used for preparation. All the required raw ingredients were collected from local market of Akluj Tal. Malshiras, Dist. Solapur and it was authenticated by department of Pharmacognosy College of pharmacy, Akluj. The authentication was carried out based upon microscopic and morphological

Table 1 — Formulation and optimization of churna

Ingredients	Biological source	Quantity
Betel leaf	*Piper betel*	3 g
Liquorice	*Glycyrrhizaglabra*	1 g
Fennel	*Foeniculumvulgare*	2.5 g
Elachi	*Elettariacardamomum*	0.6 g
Sunthi	*Zingiberofficinale*	3 g
Marica	*Piper nigrum*	2.4 g
Pippali	*Piper longum*	3.8 g
Caraway	*Carumcarvi*	2.5 g
Rose petals	*Rosa chinensis*	0.6 g
Cinnamon	*Cinnamomumverum*	0.6 g
Rock sugar	-	0.48 g

drug evaluation. The ingredients were dried, grinded and passed through sieve number 44 mixed all ingredients as per ratio specified in formulation (Table 1). Add rock sugar powder and Pack it in tightly closed containers to protect from light and moisture.

Evaluation of churna

a. Physical evaluation of churna.

The bulk density, tapped density, angle of repose and hausner's ratio was estimated by procedure mentioned in official book[15-18].

b. Determination of pH (churna).

The pH of formulated churna was determined using pH meter using 1% solution[19].

c. Determination of % moisture content.

The moisture content in churna was measured using IR moisture balance (wensar)[19].

d. Determination of Ash value (churna).

The total ash value, acid insoluble, water soluble ash value, and sulphated ash value of churna was determined using muffle furnace. The standard procedure mentioned in official books was employed[19,20].

e. Determination of extractive value (churna).

Water soluble and alcohol soluble extractive values were estimated as per procedure mentioned in official books[19,20].

f. Determination of crude fibre content in churna.

g. Determination of heavy metals in churna.

The presence of arsenic, iron, lead, mercury, and sulphate were determined by means of limit test. For this detection the standard procedure mentioned in official books was employed[21].

h. Determination of microbial contamination.

The microbial load of churna was determined as per the standard procedures mentioned in Indian pharmacopoeia 2010 and WHO guidelines. The 1% churna solution was tested for Microbial analysis and total bacterial count, yeast count and mould count were determined. Highlighting the species *E. coli and S. aureus, P. aeruginosa* was selected for screening[22-24].

i. Determination digestive properties.

***In vitro* amylolytic activity**

Test sample preparation – Appropriately 0.1 gm of precisely weighed churna was extracted using a 1:4 ratio of 20% aqueous glycerol and phosphate buffer (pH7.8), filtered, and utilized as a source of enzymes. The GASTRAP (marketed formulation) was considered as standard and procedure for standard preparation was same as that of sample.

1 mL of the substrate (soluble starch 1% in phosphate buffer) was mixed with 1 mL of digestive churna extract and standard which had been incubated separately for 15 min at 27°C. 2 mL of DNS reagent were added, and the enzyme reaction was stopped after five minutes of heating. At 520 nm, the absorbance was measured[23,24].

***In vitro* lipolytic activity**

In the presence of 100 mg of bile salts, 2 mL of castor oil was neutralized to pH 7 and thoroughly mixed with 25 mL of water until an emulsion formed this solution is used as substrate. 5ml phosphate buffer was added in 20 mL substrate and stirred upon magnetic stirrer at 35°C. The pH was adjusted at 7. After the pH reached its starting point, it was maintained for 30 to 60 minutes. Each time, the amount of alkali consumed was recorded[23,24].

$$lipolytic\ activity\ of\ sample = \frac{volume\ of\ alkali \times strength\ of\ alkali}{weight\ of\ sample(mg/ml) \times time\ in\ min}$$

***In vitro* proteolytic activity**

The casein (milk protein) was precipitated out from 500 mL of milk using acetic acid. 1gm of casein was dissolved 100 mL distilled water and used as substrate.

1 mL of calcium chloride and 1 mL of 0.1 M phosphate buffer (pH 7.6) were added to 1 mL of substrate solution. After incubating for 1 hour with 3 mL of a 5% trichloro-acetic acid solution, digestion ceased when 1 mL of sample (churna extract) was added. Centrifugated for ten minutes, and 5 mL of Lowry's reagent were combined with supernatant further staining with diluted Folin-Ciocalteau reagent (1:2). The optical density was measured at 650 nm[24].

The proteolytic activity was then calculated from standard curve in milligrams of tyrosine.

Statistical analysis

The data obtained by evaluation were calculated as mean ± Standard Error of the Mean (SEM). One-way Analysis of Variance (ANOVA) was used to compare the means of multiple groups, followed by p-value was calculated for each analysis. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 8.0.

Network pharmacology

Prediction of target

Based upon a literature search from PubChem, Google Scholar, SpringerLink, ScienceDirect, and Web of Science data, fifteen potential constituents from natural sources were selected such as chavibetol, and chavicol, from *Piper betel*. Gingerol and shogaol from *Zingiber officinale,* cinnamaldehyde, and cinnamic acid are found in *Cinnamomum verum;*1,8-cineole, and α-terpinyl acetate are found in *Elettaria cardamomum,* glycyrrhetinic acid is a major constituent found in *Glycyrrhiza glabra;* fenchone and estragole were observed in *Foeniculum vulgare ;*carvone is the active principle found in *Carum carvi*. Moreover,eugenol is common in *Piper betel* and *Cinnamomum verum,* limonene is commonly found in *Carum carvi* and *Elettaria cardamomum, additionally,* piperine is commonly observed in *Piper nigrum* and *Piper longum.*

Disease gene prediction

For screening of digestive properties of churna, an *in silico* approach was utilized. 15 major constituents from churna preparation were employed for network pharmacology, and five diseases, such as indigestion, carminatives, *diabetes mellitus*, stomach cancer, and colon cancer, were screened. The disease genes were retrieved from the human disease database, such as GeneCard (https://www.genecards.org) and MalaCard (https://www.malacards.org)[25-27].

Target gene prediction of ligand

Further, the target genes of selected phytoconstituents were screened using several molecular recognition databases, such as BindingDB (https://www.bindingdb.org)[28], SEA (https://sea.bkslab.org)[29], PubChem, and SwissTargetPrediction[30]. For all databases, canonical SMILES were required, which were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov). Here the probability score was kept ≥ 0.3, and the gene ID was listed. Further, the identification of common genes between disease genes and targeted compounds is necessary. For this Venny 2.1.0 software was utilized (https://bioinfogp. cnb.csic.es /tools/venny), the listed genes were pasted, and common genes were recognized[31].

Network analysis

The common gene collected from Venny 2.1.0 software was further processed in STRING version 12.0 (https://string-db.org) software using the multiple protein tab[32]. The gene ID was introduced and screened against Homo sapiens. This will help for analysis of protein–protein interaction, enrichment analysis, and gene ontology enrichment analysis. The generated string network was further sent to Cytoscape from the export tab, and the network was observed in Cytoscape[33].

Functional gene network

The functional gene network is built using the Cytoscape interface (https://cytoscape.org). The top gene in the network is ranked using the Maximal Clique Centrality topology analysis approach. Closeness, degree, and betweenness were investigated using CytoNCA software. Several disease-associated genes with ligands were calculated considering the p-value significance. The network of churna formulation and gene ontology enrichment chart was generated using STRING.

KEGG pathway analysis

The top 10 genes from churna were determined, among which the topmost gene was utilized for pathway generation using KEGG[34-36].

Results and Discussion

Physical evaluation of churna was carried out and the findings were observed in (Table 2).

The ash value extractive value, moisture content, and pH were determined and the finding was observed in (Table 3).

The presence of heavy metals in churna were determined using limit tests the finding suggested that churna passes the test for arsenic, iron, lead, mercury, and sulphate.

Determination of microbial content in churna was estimated and finding suggested that there is no growth of microbes after incubation.

***In vitro* digestive activity**

***In vitro* amylolytic activity**

The amylolytic activity of the churna formulation was evaluated and compared to that of a standard commercial digestive enzyme supplement, GASTRAP. The results showed that the churna formulation exhibited a higher amylolytic activity

Table 2 — Characterization of formulated digestive churna

Parameters	Mean ± SEM
Tapped density (gm/ml)	0.694 ±031
Bulk density (gm/ml)	0.520 ±005
Angle of repose (°)	37° ±0.26
Hausner's ratio	1.33 ±0.09
Carr's index	25.07 ±0.28

Table 3 — Physical evaluation of formulated digestive churna

Parameters	Mean ± SEM
Total ash value	10 ± 048
Acid insoluble ash value	3 ±012
Water soluble ash value	0.17 ±037
Sulphated ash value	0.02 ±001
Crude fibre content	9.08 ±0.84
Water soluble extractive value	12 ±0.33
Alcohol soluble extractive value	6.8 ±0.16
Moisture content	4.8 ±0.04
pH of churna	6.3 ±0.02

(0.32±0.015) compared to GASTRAP (0.28±0.017). The comparison between standard and manufactured formulation showed in (Fig. 1).

***In vitro* lipolytic activity**

The lipolytic activity of the churna formulation was evaluated and compared to that of a standard commercial digestive enzyme supplement, GASTRAP. The results showed that the churna formulation exhibited a significantly higher lipolytic activity (0.0397±0.002) compared to GASTRAP (0.0229±0.003). The comparison between the standard (GASTRAP) and manufactured formulation (betel leaf churna) is depicted in (Fig. 1). The results clearly indicate that the churna formulation exhibits a higher lipolytic activity than GASTRAP.

***In vitro* proteolytic activity**

The proteolytic activity of the churna formulation was evaluated and compared to that of a standard commercial digestive enzyme supplement, GASTRAP. The results showed that the churna formulation exhibited a comparable proteolytic activity (0.032±0.004) to GASTRAP (0.032±0.007). The comparison between the standard (GASTRAP) and manufactured formulation (churna) is depicted in (Fig. 1). The results indicate that the churna formulation exhibits a comparable proteolytic activity to GASTRAP, suggesting that the formulation is equally effective in breaking down proteins.

Network Pharmacology

Target and disease gene prediction

Total 15 phytoconstituents such as chavibetol, chavicol, gingerol, shogaol, cinnamaldehyde, cinnamic acid, 1,8-cineole, α-terpinyl acetate, glycyrrhetinic acid, fenchone, estragole, carvone eugenol, limonene, and piperine were retrieved from PubChem and the common gene were identified using SwissTargetPrediction, sea database, binding DB and PubChem. While the disese gene retrieved from genecard and malacard. Further using venny 2.1.0 common disease genes were identified. And finding suggested that amongst all shogaol shows highest common gene in targeted diseases and chavicol shows the least common gene with targeted diseases. Additionally piperine followed by glycyrrhetinic acid, shogaol and cinnamic acid shows more common gene with digestive disease.

Table 4 — Limit test of formulated digestive churna

Parameters	Findings
Limit test for arsenic	Absence of arsenic
Limit test for mercury	Absence of mercury
Limit test for lead	Absence of lead
Limit test for iron	Absence of iron
Limit test for sulphate	Absence of sulphate

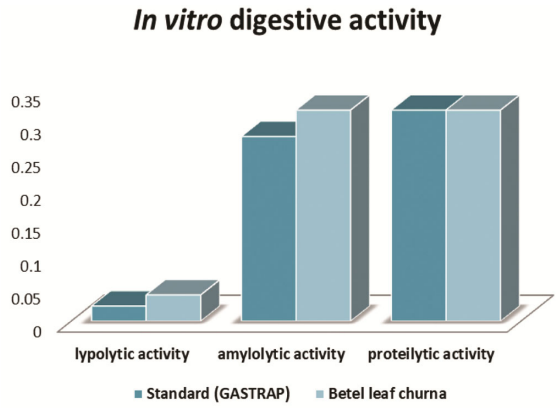


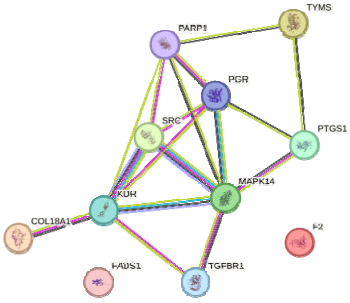
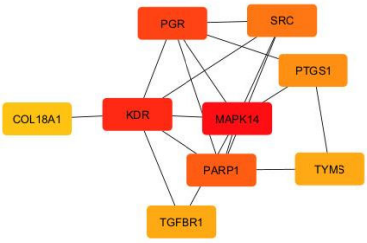
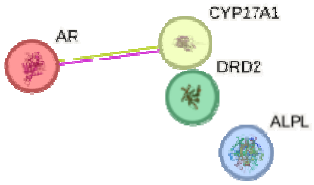
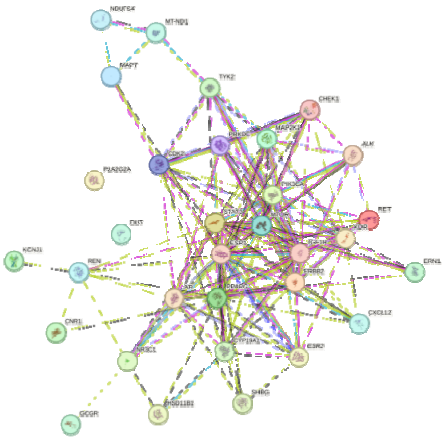
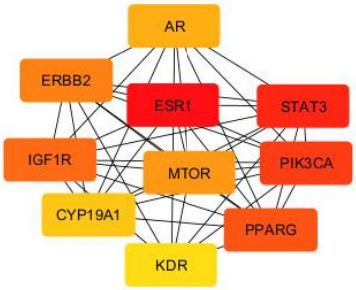
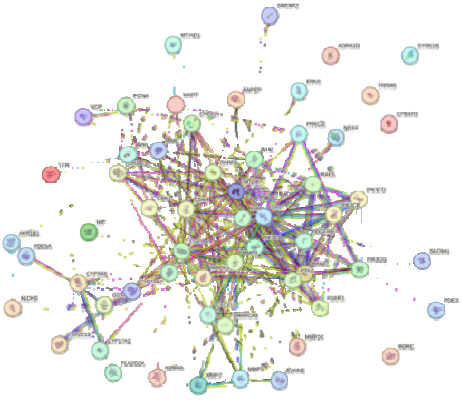
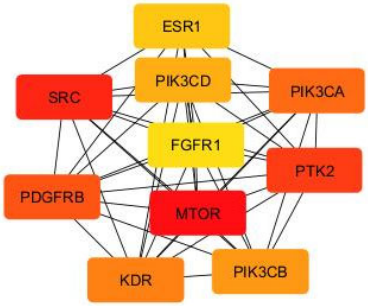
Fig. 1 — Comparison of *in vitro* digestive activity of betel leaf churna and standard. *In vitro* digestive activity comparison between betel leaf and standard. Betel leaf churna exhibited significant amylolytic (P= 0.031) and lipolytic (P= 0.002) activity, but not proteolytic activity (P= 0.95), compared to the standard. as determined by One-way ANOVA

Network analysis

The network between the genes was generated using STRING, and the centralities (closeness, betweenness, and degree) were measured using Cytoscape and CytoNCA; the figures are observed below (Table 4). The top 10 targets were detected *via* CytoHubba, and the images are observed below (Table 5), where the darker red shades characterizes a higher score among the genes.

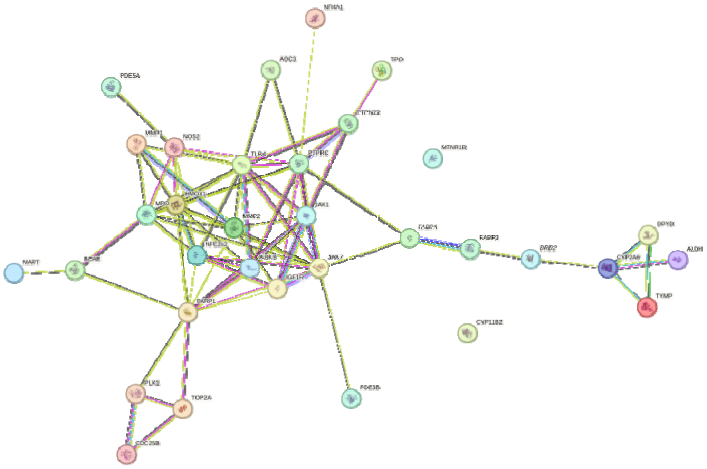
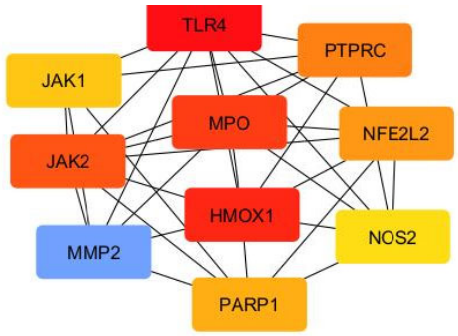
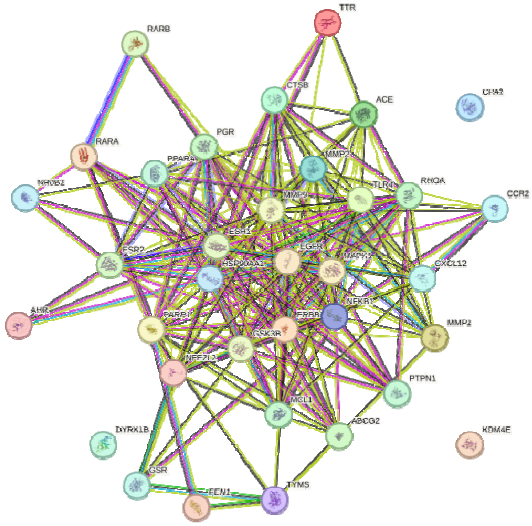
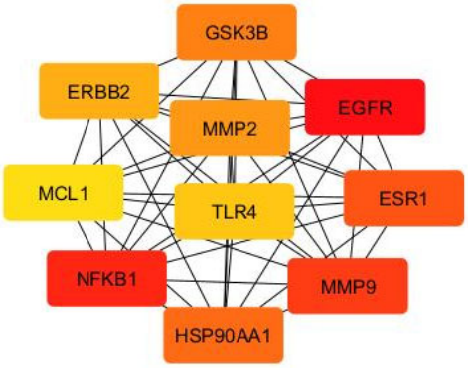
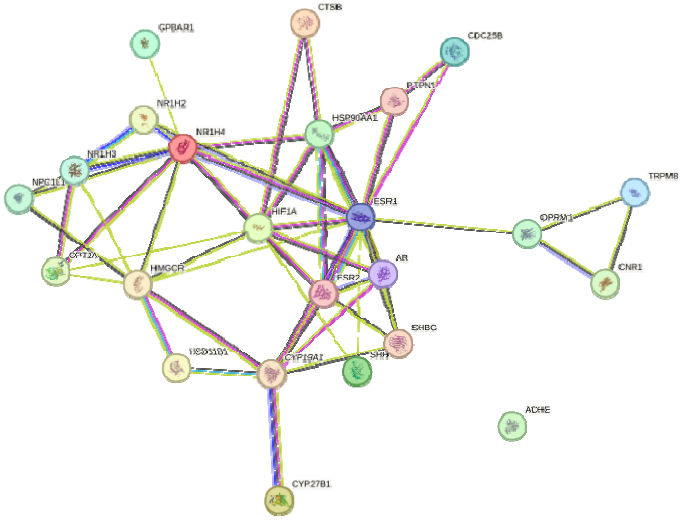
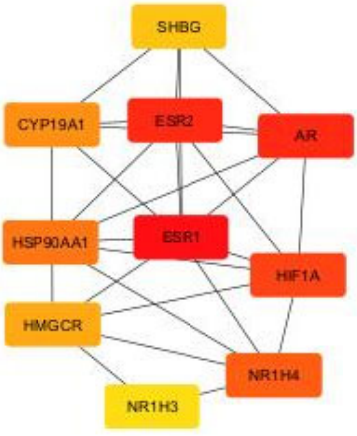
The top genes for chavibetol are KDR, MAPK14, and PGR. For gingerol, ESR1, STAT3, PIK3CA, and PPARG are prominent genes. In the case of shogaol,

Table 5 — Gene network generated by string and top 10 genes observed in Cytohubba

	Gene network	Top 10 genes observed
(a) Chavibetol		
(b) Chavicol		Nil
(c) Gingerol		
(d) Shogaol		

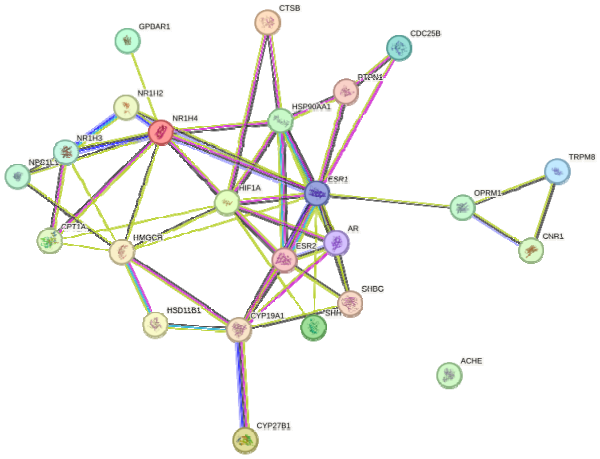
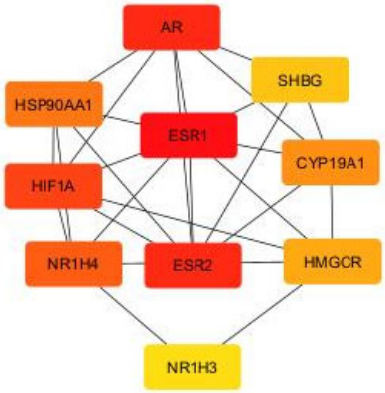
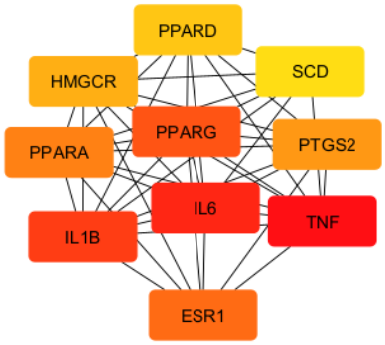
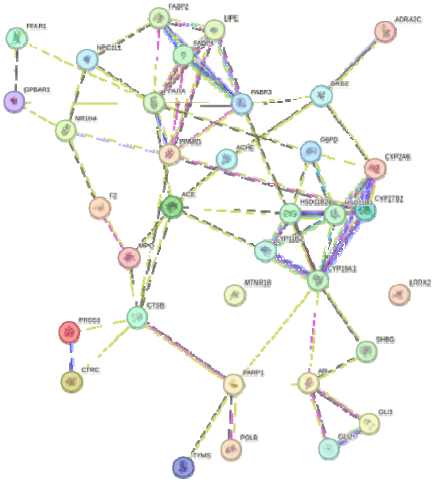
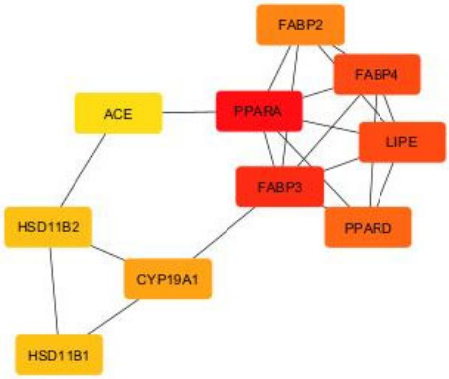
(*Contd.*)

Table 5 — Gene network generated by string and top 10 genes observed in Cytohubba (*Contd.*)

Gene network	Top 10 genes observed
(e) Cinnamaldehyde	
	
(f) Cinnamic acid	
	
(g) 1,8-cineole	
	

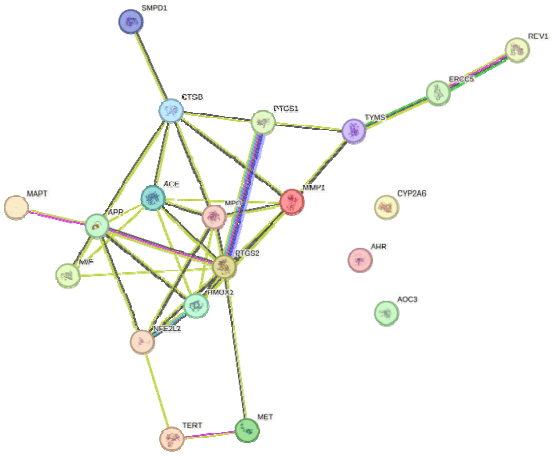
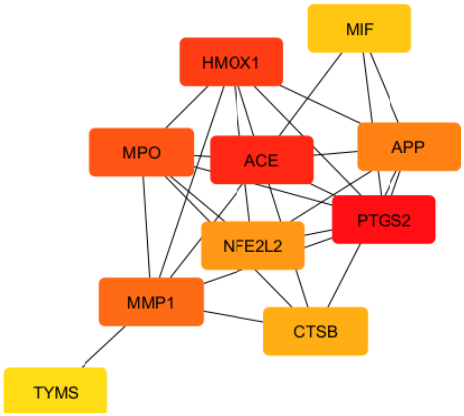
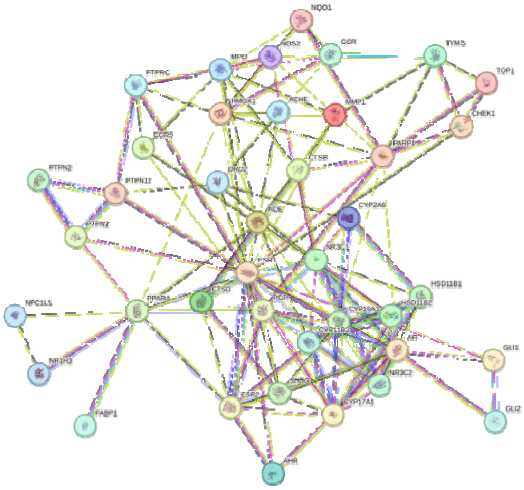
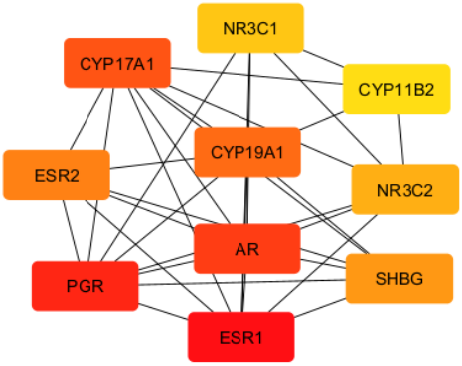
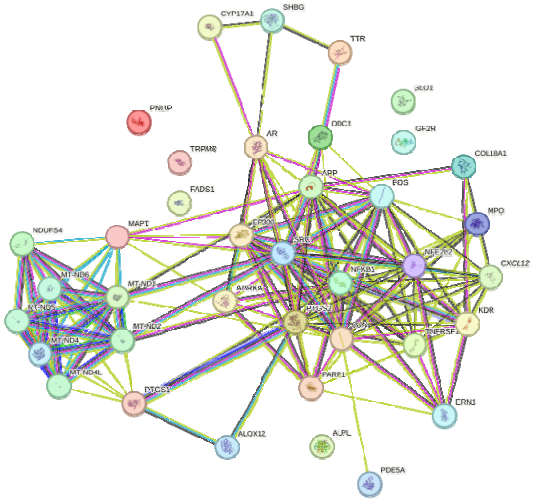
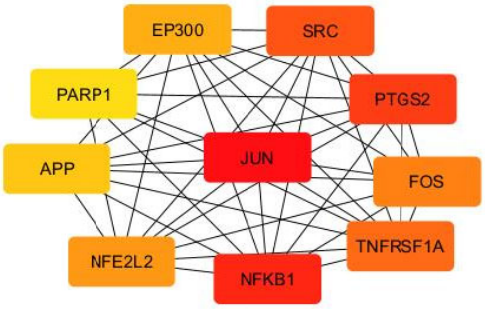
(*Contd.*)

Table 5 — Gene network generated by string and top 10 genes observed in Cytohubba (*Contd.*)

Gene network	Top 10 genes observed
(h) α-terpinyl acetate	
	
(i) Glycyrrhetinic acid	
	
(j) Fenchone	
	

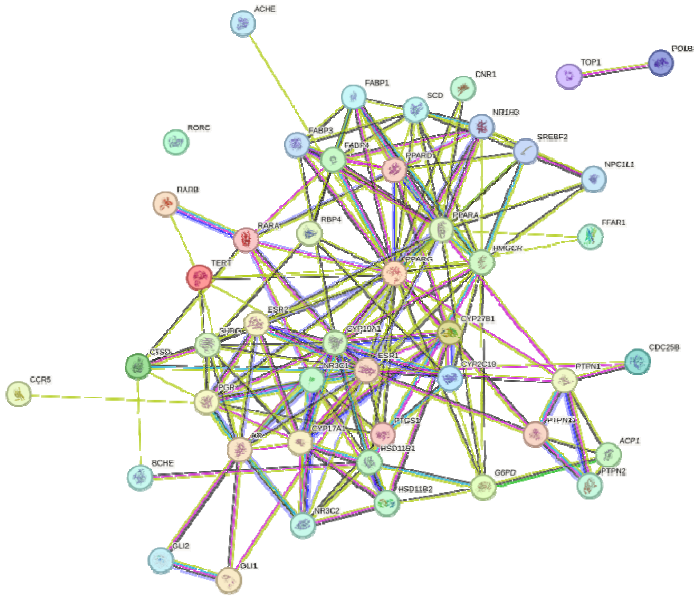
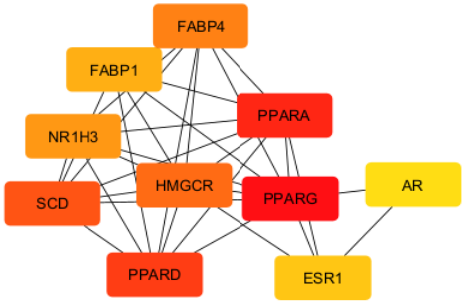
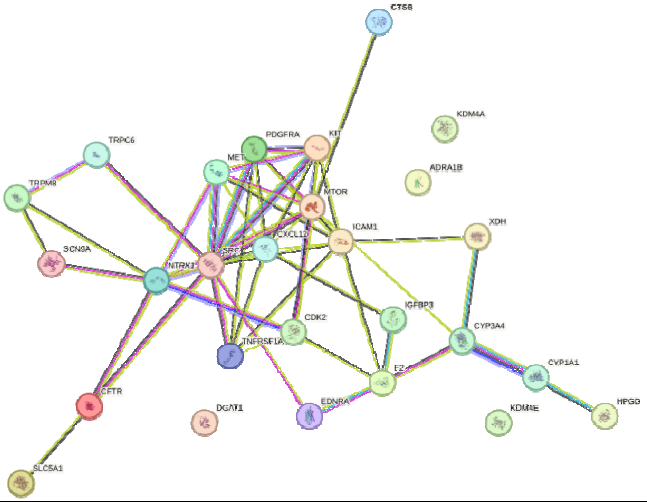
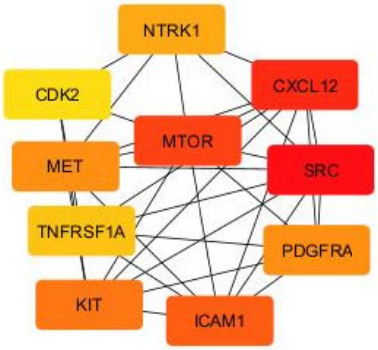
(*Contd.*)

Table 5 — Gene network generated by string and top 10 genes observed in Cytohubba (*Contd.*)

Gene network	Top 10 genes observed
(k) Estragole	
	
(l) Carvone	
	
(m) Eugenol	
	

(*Contd.*)

Table 5 — Gene network generated by string and top 10 genes observed in Cytohubba (*Contd.*)

	Gene network	Top 10 genes observed
(n) Limonene		
(o) Piperine		

SRC, MTOR, PTK3, and PDGFRB genes have higher score centralities. For cinnamaldehyde, TLR4, HMOX1, MPO, and JAK2 are prominent genes. Cinnamic acid has EGFR, NFKB1, MMP9, ESR1, and HSP90AA1 genes holding high score centralities. 1,8-cineole, α-terpinyl acetate, and limonene are commonly observed in *Elettaria cardamomum* amongst that 1,8-cineole and α-terpinyl acetate shows common genes such as ESR1, ESR2, AR, HIF1A and NR1HA prominent genes, while limonene shows PPARA, PPARG, PPARD, SCD and HMGCR are prominent genes. Glycyrrhetinic acid having TNF, IL6, IL1B, PPARG and ESR1 are genes with prominent centralities. In case of Fenchone PPARA, FABP3, FABP4, LIPE, and PPARD genes were found, whereas for estragolePTGS2, ACE, HMOX1, MPO and MMP1 are prominent genes. Carvone having prominent genes ESR1, PGR, AR, CYP17A1, CYP19A1, eugenol shows JUN, NFKB1, PTGS2, SRC, and TNFRSF1A as prominent genes while piperine having SRC, CXCL12, MTOR and ICAM1 are prominent target genes.

A network of churna formulation was constructed using all genes associated with its phytoconstituents, revealing a complex network with 213 nodes. The gene enrichment chart is depicted in (Fig. 2). To

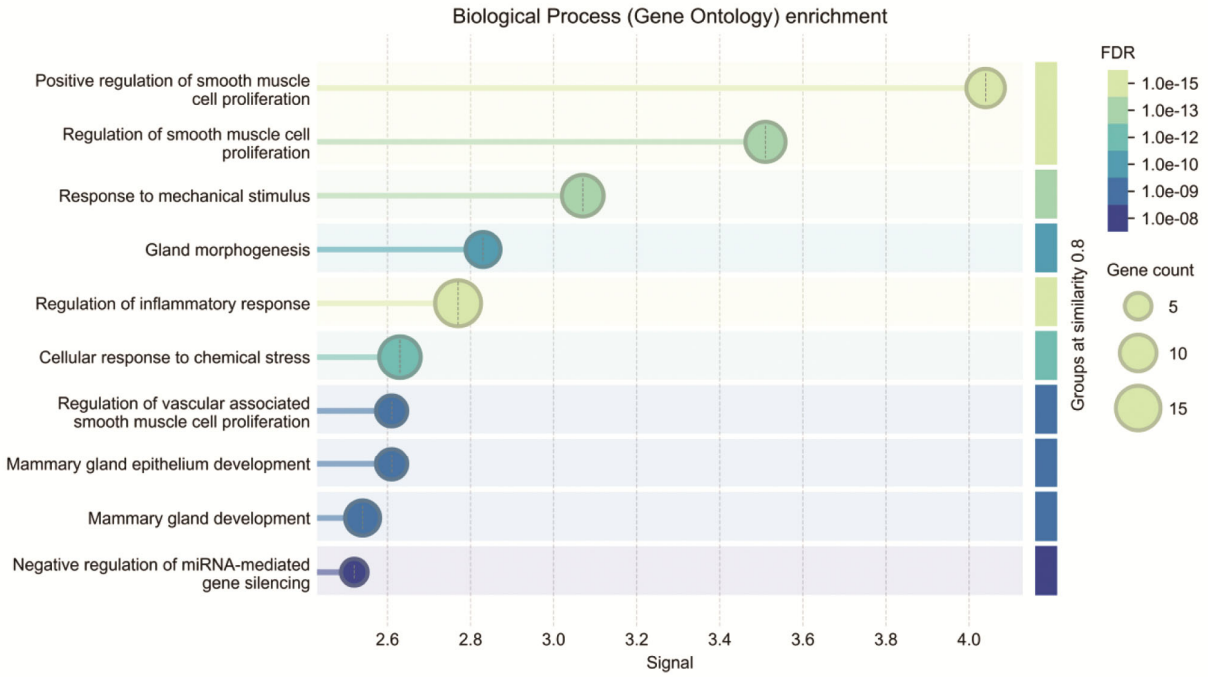


Fig. 2 — Gene Ontology (GO) enrichment analysis of targets associated with churna formulation. The analysis highlights significant Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC) enriched within the identified gene set. The length of the line and position of the node represent the Fold Enrichment. Symbols are color-coded by their adjusted p-values (FDR), where a threshold of $P< 0.05$ was considered significant. Terms were grouped based on a semantic similarity of [0.7] to reduce redundancy

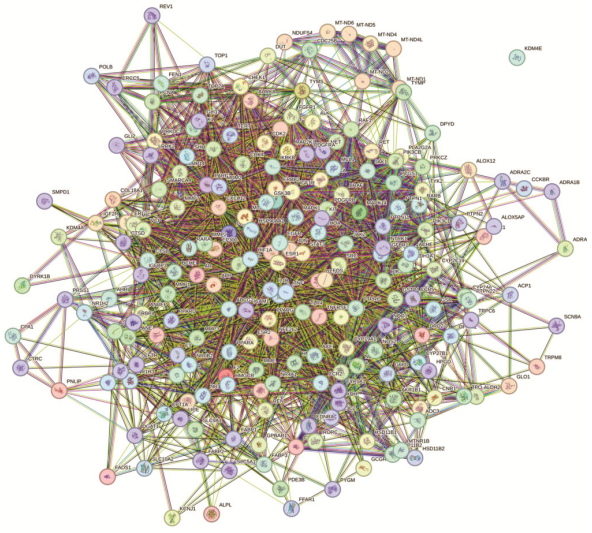


Fig. 3 — Analysis of Gene network of churna formulation using string. Major enriched processes include the positive regulation of smooth muscle cell proliferation, regulation of inflammatory response, and cellular response to chemical stress

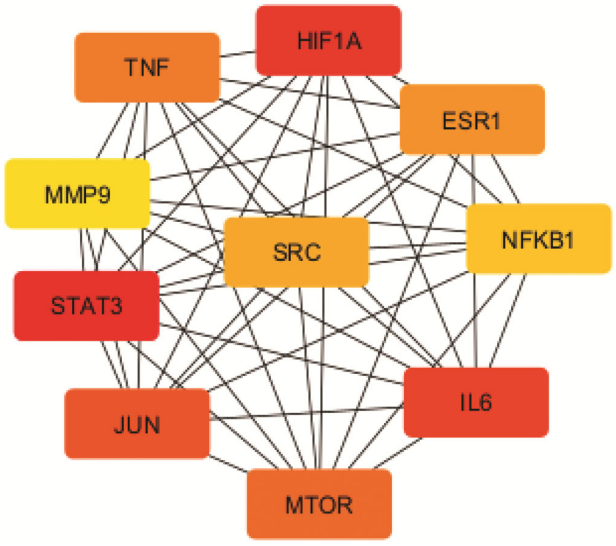


Fig. 4 — Analysis of top 10 Gene network of churna formulation using Cytohubba CytoHubba analysis of Churna formulation identifies top 10 gene networks. The network illustrates the complex interactions between genes and highlights potential targets for further investigation

identify key regulatory genes, the top 10 genes with highest centralities were identified using CytoHubba, which included STAT3, HIF1A, IL6, JUN, TNF, MTOR, SRC, MMP9, ESR1, and NFKB1. And the network was observed in (Fig. 3) while top 10 genes were displayed in image given below (Fig. 4). Notably, STAT3 exhibited the highest MCC centrality, making it a prime candidate for further pathway analysis.

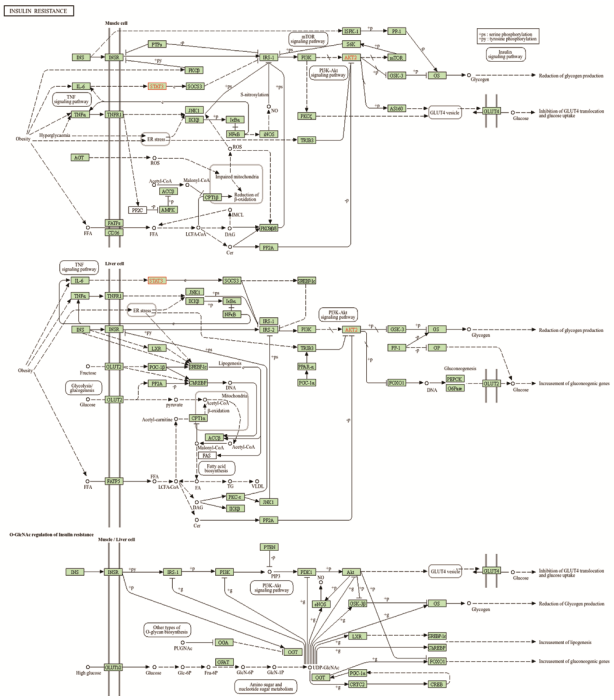


Fig. 5 — KEGG pathway analysis of insulin resistance-related genes. The pathway map highlights key genes and signaling molecules involved in insulin resistance, providing insights into the underlying molecular mechanisms

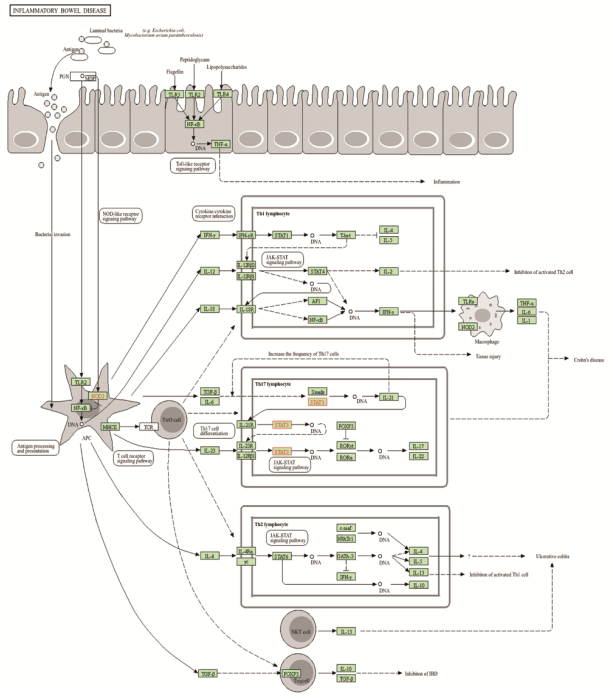


Fig. 6 — Inflammatory bowel disease pathway analysis using KEGG. The figure illustrates the complex interactions between genes and signaling pathways involved in IBD, including key nodes and potential therapeutic targets in the TNF, IL-17, and NF-κB signaling pathways

KEGG pathway analysis

KEGG pathway database employed the genes were observed in Insulin resistance, inflammatory bowel disease, cancer, pancreatic cancer and hepatitis B. All the pathways were displayed in (Figs. 5-9), respectively. The genes STAT3, HIF1A, IL6, TNF, JUN, MTOR, SRC, MMP9, ESR1, and NFKB1 were found to be implicated in several key KEGG pathways, underscoring their potential significance in various disease processes. Notably, these genes are associated with pathways related to cancer, where they may contribute to disease progression and pathogenesis. Additionally, involvement of these genes in pathways such as insulin resistance, pancreatic cancer, inflammatory bowel disease (IBD), Hepatitis B, and Hepatitis C was observed (Table 6).

The involvement of key genes in various pathways was observed as follows. In cancer, the genes STAT3, HIF1A, IL6, TNF, JUN, MTOR, SRC, MMP9, ESR1, and NFKB1 play a role. In the context of insulin resistance, the genes STAT3, IL6, JUN, MTOR, and NFKB1 are involved. STAT3, JUN, MTOR, and NFKB1 are implicated in pancreatic cancer. In inflammatory bowel disease (IBD), STAT3 and IL6 are involved. For Hepatitis B, the genes STAT3, IL6, TNF, JUN, MMP9, and NFKB1 play a part, whereas in Hepatitis C, STAT3, TNF, and NFKB1 are involved.

Discussion

Churna, a traditional Ayurvedic herbal powder, offers numerous benefits for digestion, including stimulating digestive enzymes, relieving gas, bloating, enhancing nutrient absorption, supporting gut health, reducing inflammation, and immune function[37]. Churna is a natural, safe, and cost-effective solution for digestive concerns, offering a holistic approach to digestive health. The present investigation involved the development and evaluation of Betel leaf churna using a multidisciplinary approach, encompassing physical, chemical, pharmacological, and in silico assessments. Initially, the authenticity of the raw material was verified through chemical, physical, and microscopical examinations. Subsequently, the Betel leaf Churna was formulated and subjected to powder characterization studies. The physicochemical parameters, including tapped density, bulk density, angle of repose, Hausner's ratio, and Carr's index, were evaluated and found to be within the stipulated limits[19]. The powder characterization data underscore the critical role of these parameters in

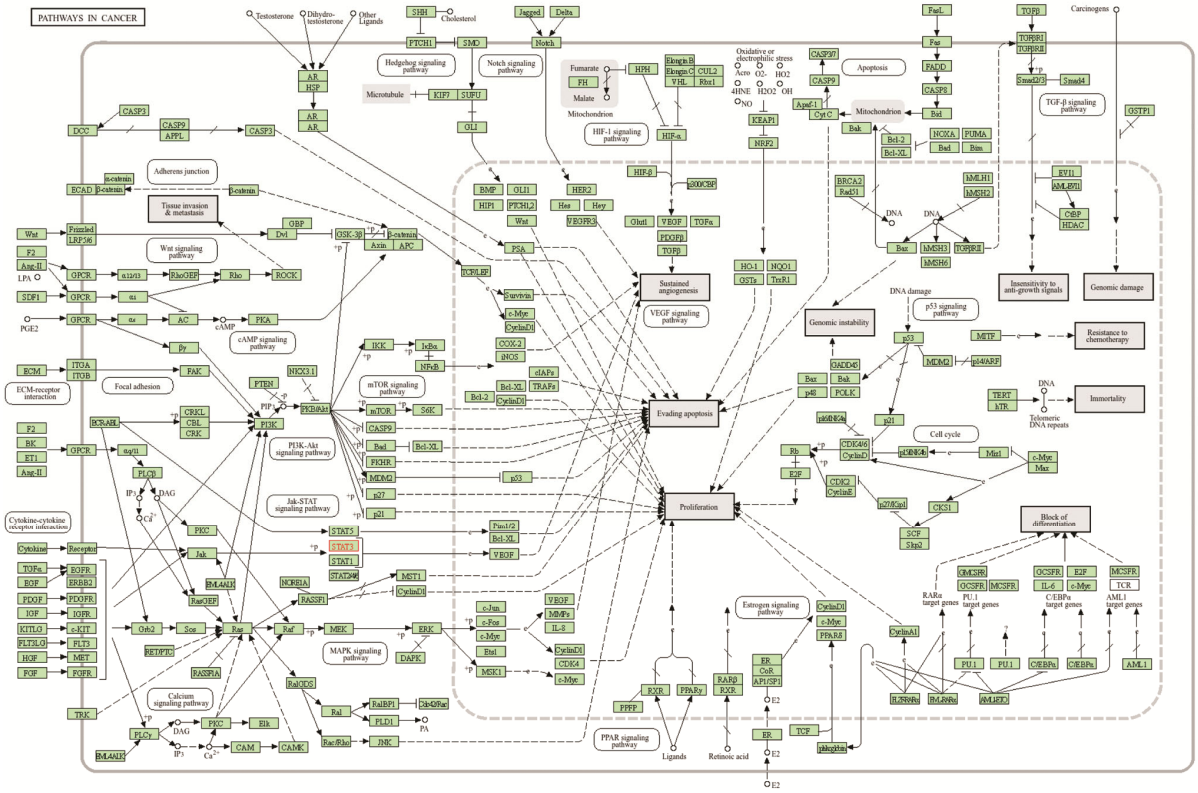


Fig. 7 — KEGG pathway analysis of cancer-related genes. The pathway map highlights key genes and signaling molecules involved in cancer development and progression, including PI3K-Akt signaling, MAPK signaling, and cell cycle regulation

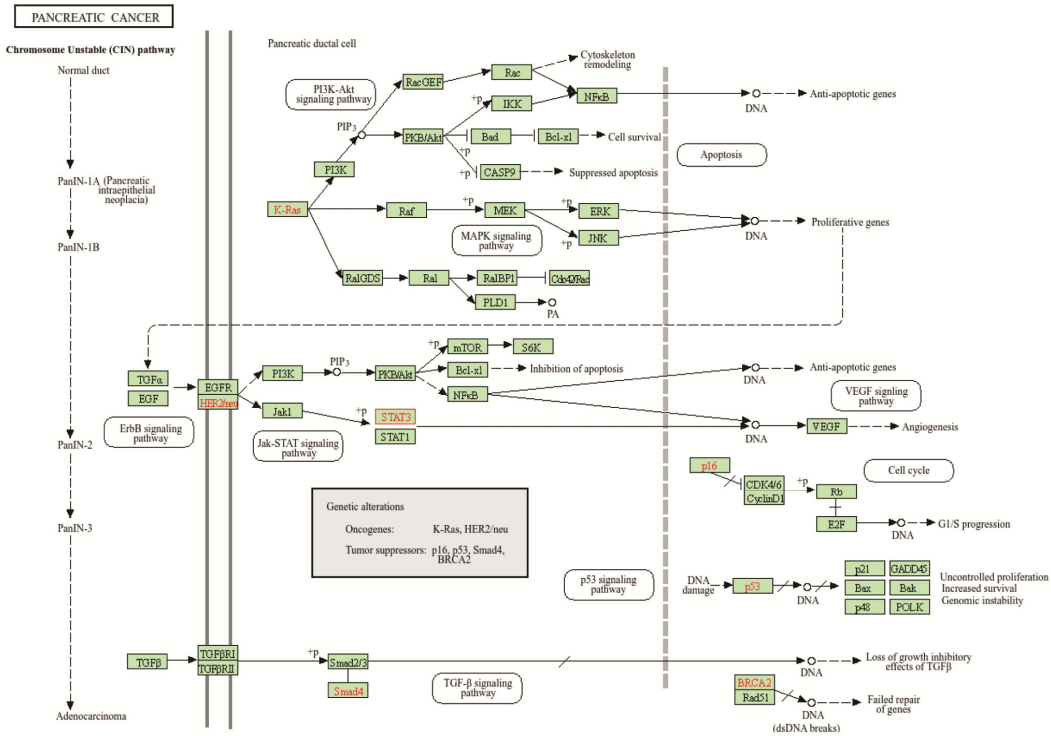


Fig. 8 — KEGG pathway analysis of pancreatic cancer-related genes. The pathway map highlights key genes and signaling molecules involved in pancreatic cancer development and progression, including the PI3K-Akt, MAPK, and p53 signaling pathways

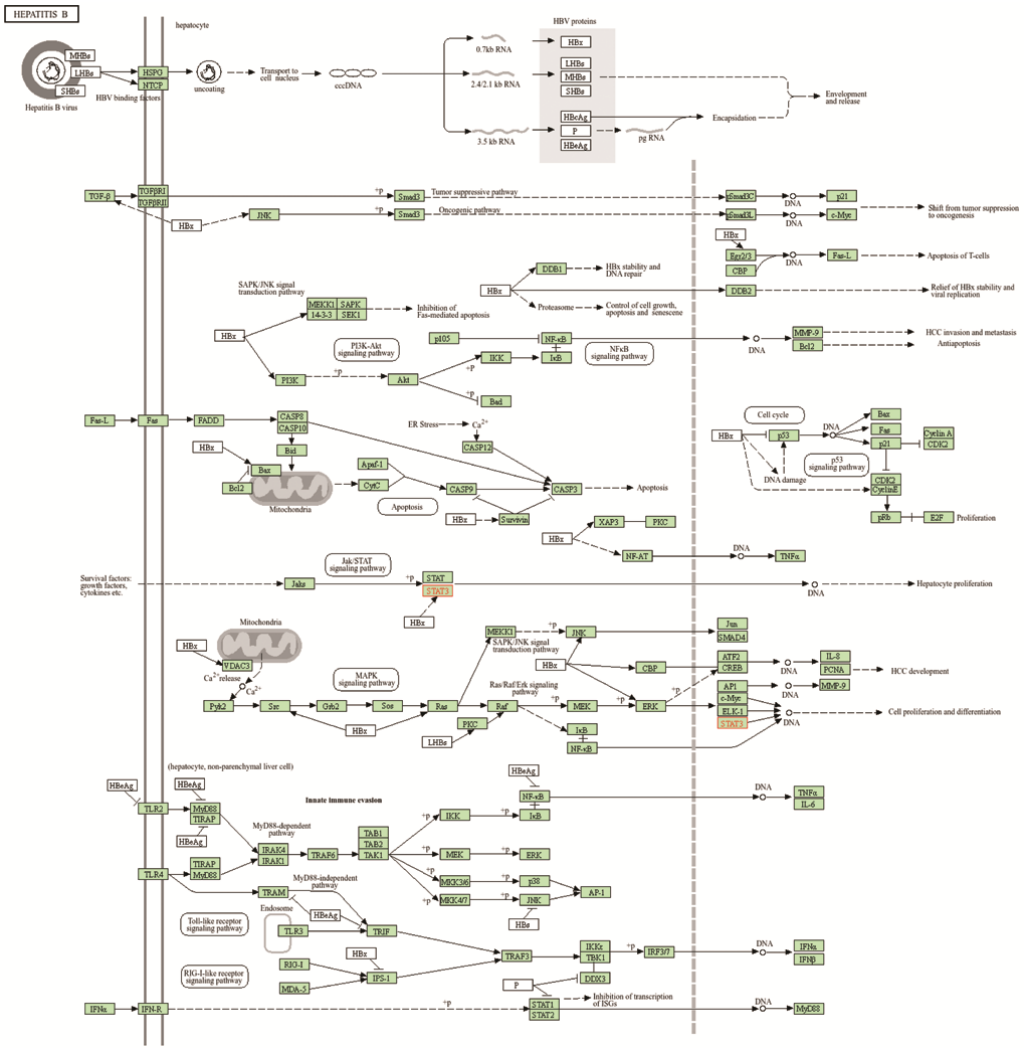


Fig. 9 — KEGG pathway analysis of Hepatitis B-related genes. The pathway map highlights key genes and signaling molecules involved in Hepatitis B infection and pathogenesis, including the TLR, NF-κB, and PI3K-Akt signaling pathways

Table 6 — Top 10 genes in churna and their presence in KEGG pathway

Disease pathway	STAT3	HIF1A	IL6	TNF	JUN	MTOR	SRC	MMP9	ESR1	NFKB1
cancer	STAT3	HIF1A	IL6	TNF	JUN	MTOR	SRC	MMP9	ESR1	NFKB1
Insulin resistance	STAT3	nil	IL6	nil	JUN	MTOR	nil	nil	nil	NFKB1
Pancreatic cancer	STAT3	nil	nil	nil	JUN	MTOR	nil	nil	nil	NFKB1
IBD	STAT3	nil	IL6	nil	nil	nil	nil	nil	nil	nil
Hepatitis B	STAT3	nil	IL6	TNF	JUN	nil	nil	MMP9	nil	NFKB1
Hepatitis C	STAT3	nil	nil	TNF	nil	nil	nil	nil	nil	NFKB1

ensuring optimal mixing, dissolution, and bioavailability of the Churna, thereby influencing its overall efficacy and therapeutic potential. The ash value parameter served as an indicator of the formulation's purity and authenticity, providing insight into the extent of inorganic contamination in betel leaf churna. The extractive values, comprising water-soluble and alcohol-soluble fractions, were assessed to quantify the bioactive constituents extracted from the plant material using respective solvents[10,11]. Notably, both extractive values exceeded the standard reference values, indicating optimal extraction efficiency and absence of exhausted material. Furthermore, the crude fibre content, a proxy for dietary fibre, was evaluated and found to be within the stipulated standard limits,

thereby ensuring compliance with quality control benchmarks[19].

Accurate pH of Churna is crucial for a number of reasons, such as enhancing efficacy, stability of formulation, and digestive comfort. Typically, the ideal pH range for Churna is between 5.5 and 7.5, depending on the specific herbal formulation. Excessive moisture can lead to spoilage, mold growth, and reduced shelf life of churna; hence, the moisture content of betel leaf churna was evaluated and found within the acceptable range[24]. Heavy metals such as lead, mercury, and arsenic are known to induce a range of adverse health effects, including gastrointestinal disturbances, nephrotoxicity, and dermatological disorders. Furthermore, chronic exposure to these metals has been linked to severe health outcomes, including carcinogenesis and neurodegenerative damage. In order to assess the presence of heavy metals, a limit test was conducted. The results of this analysis revealed that the betel leaf churna was devoid of heavy metals, indicating that the material meets the requisite safety standards[21].

Considering the *in vitro* digestive study of churna, the comparative study with GASTRAP was carried out. GASTRAP is a commercially available digestive enzyme supplement that contains a blend of enzymes, including amylase, lipase, and protease. Amylolytic activity is a measure of the ability of an enzyme or formulation to break down starches into simpler sugars. In the context of digestive health, amylolytic activity is crucial for the proper digestion and absorption of carbohydrates. The high amylolytic activity of the churna formulation has significant implications for digestive health. The formulation may be useful in supporting the digestion and absorption of carbohydrates, particularly for individuals with impaired digestive enzyme function or those experiencing digestive discomfort after consuming carbohydrate-rich foods. Lipolytic activity is a measure of the ability of an enzyme or formulation to break down lipids (fats) into fatty acids and glycerol. In the context of digestive health, lipolytic activity is crucial for the proper digestion and absorption of fats. The high lipolytic activity of the churna formulation has been significant implications for digestive health. The formulation may be useful in supporting the digestion and absorption of fats, particularly for individuals with impaired digestive enzyme function or those experiencing digestive discomfort after consuming fatty foods. The proteolytic activity is a measure of the ability of an enzyme or formulation to break down proteins into smaller peptides and amino acids. In the context of digestive health, proteolytic activity is crucial for the proper digestion and absorption of proteins. The comparable proteolytic activity of the churna formulation to GASTRAP has significant implications for digestive health. The formulation may be useful in supporting the digestion and absorption of proteins, particularly for individuals with impaired digestive enzyme function or those experiencing digestive discomfort after consuming protein-rich foods.

Hence, we prepared a churna with natural ingredients commonly used by mankind for culinary purposes. These ingredients can work together to create a comprehensive digestive churna formulation that supports overall gut health and well-being. From network pharmacology, the findings from network pharmacology revealed that ESR1, PPARG, HMOX1, SRC, MTOR, PGR, NFKB1, PTGS2, MPO, and AR are the common genes observed in most of the active constituents from churna. These genes are associated with various diseases, highlighting the potential therapeutic applications of these bioactive compounds. Notably, ESR1, common to gingerol, cinnamic acid, 1,8-cineole, α-terpinyl acetate, glycyrrhetinic acid, and carvone, is linked to breast cancer, myocardial infarction, and estrogen resistance syndrome. PPARG, common to gingerol, limonene, and glycyrrhetinic acid, is associated with thyroid cancer, type 2 diabetes, and genetic obesity. Other common genes include HMOX1 (COPD and heme oxygenase-1 deficiency), SRC (colorectal cancer and various other cancers), MTOR (various cancers and neurological disorders), PGR (breast cancer and other diseases), NFKB1 (common variable immunodeficiency), PTGS2 (esophageal cancer), MPO (various cancers and diseases), and AR (various cancers). These findings suggest potential therapeutic applications of these bioactive compounds and highlight the importance of further research into their molecular mechanisms. A network of churna formulation was constructed using genes associated with its phytoconstituents, revealing a complex network with 213 nodes (Fig. 2). Using CytoHubba, the top 10 genes with the highest centralities were identified as STAT3, HIF1A, IL6, JUN, TNF, MTOR, SRC, MMP9, ESR1, and NFKB1 (Figs. 3 & 4). Notably, STAT3 exhibited the highest MCC

centrality, making it a prime candidate for further pathway analysis. The KEGG pathway database analysis revealed the involvement of genes STAT3, HIF1A, IL6, TNF, JUN, MTOR, SRC, MMP9, ESR1, and NFKB1 in key pathways related to insulin resistance, IBD, cancer, pancreatic cancer, and hepatitis (Figs. 5-9). These genes may contribute to disease progression in these pathways. Further screening can determine their molecular interactions with specific proteins, aiding in understanding mechanisms and potential therapeutic targets.

Conclusion

The present study effectively developed and evaluated Betel leaf Churna, a traditional Ayurvedic herbal powder, using a multidisciplinary approach. The results demonstrated that the Churna possessed optimal physicochemical properties, purity, and authenticity. The formulation exhibited high amylolytic, lipolytic, and proteolytic activities, comparable to a commercially available digestive enzyme supplement. These findings suggest that Betel leaf Churna can be a natural, safe, and effective solution for digestive concerns, supporting overall gut health and well-being. The study validates the traditional use of Betel leaf Churna and provides a scientific basis for its *in silico* and *in vitro* activity.

A network pharmacology study for specific phytoconstituents of the churna was performed targeting digestive function and related diseases and the top 10 genes identified. Further screening for pathway generation revealed that the churna may be suitable for conditions like insulin resistance, inflammatory bowel disease (IBD), and liver disease. These findings suggest potential therapeutic applications of the churna in managing or modulating these conditions through its phytoconstituents' interactions with key genes and pathways. Considering the future scope these genes can be screened further to determine their molecular interactions with specific proteins. This can help in understanding the mechanisms of action and potential therapeutic targets. Additionally, detailed study, including *in vivo* screening, can be possible; formation of vati or avahela using the same ingredient is recommended for improving patient compliance.

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Conflict of interest

All authors declare no conflict of interest.

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