



Research article

Protein-protein interaction networks reveal therapeutic targets in Khlu, Hoary basil and Indian mulberry for inflammation, diabetes and diarrhea

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Abstract

Importance of the work: Khlu, Hoary basil and Indian mulberry leaves have important medicinal properties for treating inflammation, diabetes and diarrhea.

Objectives: To elucidate the roles and molecular mechanisms of Khlu, Hoary basil and Indian mulberry leaf contents based on protein-protein interaction network analysis.

Materials & Methods: Protein-protein interaction networks were constructed for Khlu, Hoary basil and Indian mulberry leaves using databases (ChEMBL, STITCH, STRING and DisGeNET). The developed networks, exhibiting scale-free, small-world and modular characteristics, were analyzed to identify and interpret disease-related modules for inflammation, diabetes and diarrhea.

Results: Leaves from Khlu, Hoary basil and Indian mulberry influenced biological processes linked to inflammatory mediators, insulin resistance and colon fluid secretion, suggesting potential mechanisms to prevent inflammation, diabetes and diarrhea. Active ingredients from these leaves formed interaction networks with characteristics conducive to module analysis, identifying target protein modules for these diseases. Common biological processes —lipid metabolism, metal ion transport and adenosine metabolism —impacted insulin resistance, glucose regulation and inflammatory mediator production.

Main finding: Plausible mechanisms supporting their effectiveness were development for Khlu, Hoary basil and Indian mulberry leaves that are traditionally used by local people for treating inflammation, diabetes and diarrhea. Protein-protein interaction network analysis revealed their influence on inflammatory mediators, insulin resistance and glucose regulation. The identified protein groups could be valuable targets for future research on their medicinal properties.

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Introduction

Khlu (*Pluchea indica* Less), a small shrub in the Asteraceae family, is prevalent in warm-climate locations, such as Malaysia, Indonesia, Australia and Mexico, as well as in coastal areas of Thailand, where its leaves are commonly consumed as a side dish with chili paste and as an ingredient in salads and spicy curries (Suriyaphan, 2014). Beyond its culinary uses, Khlu leaves have medicinal properties, being used for treating inflammation, diabetes, dysentery, arthritis, leucorrhea, mouth and body odor, pus, abscesses and preventing diarrhea (Chan et al., 2022). The roots can treat fever, indigestion, back pain and headaches, while the bark as a decoction can treat hemorrhoids (Roslida et al., 2008; Suriyaphan, 2014). Additionally, Khlu leaves are used to make herbal tea and wet noodles, promoting health due to their rich natural active substances with diverse pharmacological effects (Srisook et al., 2012; Widyawati et al., 2023). Thus, studying the active substances in Khlu leaves is crucial for understanding their roles and molecular mechanisms, potentially leading to the development of effective treatments for various diseases. The important natural active substances in Khlu leaves include phenolic acids (such as chlorogenic acid, caffeic acid and ferulic acid), flavonoids (such as apigenin, luteolin, kaempferol, quercetin and myricetin), as well as anthocyanins and β -carotene (Andarwulan et al., 2012; Chan et al., 2022).

The leaves of Hoary basil (*Ocimum americanum* L.) and Indian mulberry (*Morinda citrifolia*), found in warm climates or specific regions of the world, contain slightly different active substances, suggesting they may have effects similar to those of Khlu leaves (Suriyaphan, 2014). For example, flavonoids, such as apigenin, have anti-inflammatory properties by inhibiting inflammatory mediators, which are major risk factors for chronic diseases (Pan et al., 2010; Ekalu and Habila, 2020). Quercetin impacts diabetes and its complications by reducing blood sugar levels, increasing insulin levels and improving lipid profiles (Yang and Kang, 2018). Despite these useful findings, the molecular mechanisms of these active ingredients in medicinal plants need further elucidation to fully understand their actions. Thus, the current research aimed to bridge that gap by predicting the molecular mechanisms of active ingredients in Khlu, Hoary basil and Indian mulberry leaves.

Molecular mechanisms can be studied by analyzing protein-protein interactions (PPIs), which play a crucial role in signal transduction, metabolic processes and enzyme signaling activities, providing essential information about cellular processes (Braun and Gingras, 2012). This approach is particularly useful for studying the molecular mechanisms of natural active ingredients in medicinal plants (Braun and Gingras, 2012; Xian and Wang, 2024). Bioinformatics data from online public databases can facilitate these studies. For example, research has predicted the molecular mechanisms of substances such as curcumin in turmeric (Gan et al., 2015), fucoxanthin and sargachomenol in brown algae (Sornsiri et al., 2018), active ingredients in the Chinese medicinal plant Qishen Yiqi (Zheng et al., 2015) and Arjunarishta formulation in Indian Ayurvedic medicine (Mahija et al., 2020). Predicting the role of active ingredients in Khlu, Hoary basil and Indian mulberry leaves can be achieved by using target protein information that can then be visualized to analyze the biological network behavior. The analysis and screening of disease-related protein modules enables the identification of relevant modules specific to each disease and plant type, with interpretation of the biological results being used to elucidate the role of these modules at the molecular level.

Materials and Methods

The experimental methodology is summarized in Fig. 1, with detailed steps provided in the following subsections.

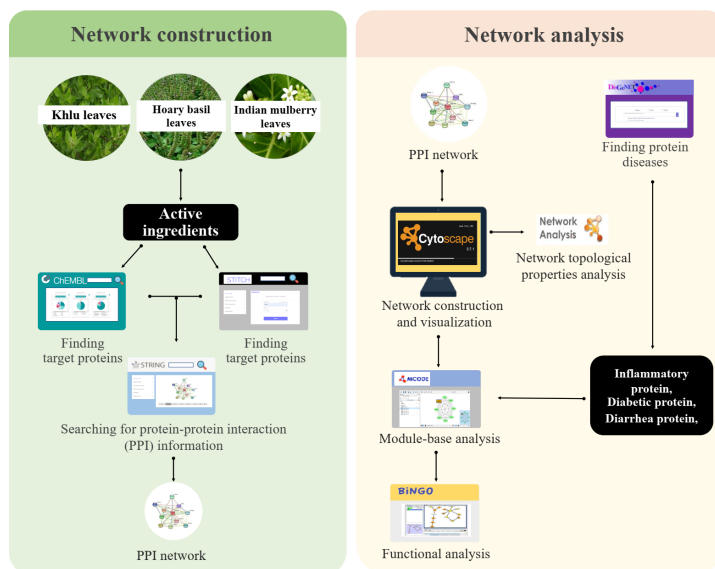


Fig. 1 Experimental methodology

Identifying target proteins of active ingredients

Information on natural active ingredients in the leaves of Khlu (*Pluchea indica* Less), Hoary basil (*Ocimum americanum* L.) and Indian mulberry (*Morinda citrifolia*) was obtained from research articles. This led to identifying active substances such as phenolic acids (chlorogenic acid, caffeic acid, ferulic acid), flavonoids (apigenin, luteolin, kaempferol, quercetin, myricetin), anthocyanins and β -carotene (Suriyaphan, 2014). Then, each active ingredient was matched to target proteins using the ChEMBL database (<https://www.ebi.ac.uk/chembl/>, Davies et al., 2015) and the STITCH 5 database (<http://stitch.embl.de/>, Szklarczyk et al., 2016), selecting only human (*Homo sapiens*) target proteins with a confidence score > 0.7. Protein symbol and amino acid sequence information were stored for subsequent steps, compiling all target proteins found in each type of plant by species.

Identifying protein-protein interactions of target proteins

The identification of PPIs for target proteins was conducted using amino acid sequence information from the STRING 11 database (<http://string-db.org/>, Szklarczyk et al., 2018) database. The organism was set to *Homo sapiens* and a confidence score of 0.7 was required. Interaction data for each target protein were stored and compiled in the Excel software (Microsoft Corp.) recording the interactions for use in subsequent network visualization steps.

Identifying disease-associated proteins

Proteins involved in diseases, such as inflammation, diabetes and diarrhea, were identified using the DisGeNET v.5.0 database (<https://www.disgenet.org/>, Piñero et al., 2017). The search terms “Inflammation”, “Diabetes” and “Diarrhea” were used, specifying *Homo sapiens* as the organism of interest. Then, the collected data were used in the subsequent analysis steps.

Interaction network analysis

Interaction network analysis utilized the PPI data for all target proteins identified in each plant species and visualized using the Cytoscape software environment (Shannon et al., 2003). Target proteins and their interacting partners were identified. Common interactions were selected using the

Advanced Network Merge option and the network behavior was analyzed using the Network Analyzer procedure.

Functional module-based analysis

Module analysis was performed using the MCODE program (Bader and Hogue, 2003) within Cytoscape (Shannon et al., 2003). Disease-related protein data were screened for modules associated with inflammation, diabetes and diarrhea. Target protein modules associated with the diseases of interest were selected, including both common and specific modules for Khlu, Hoary basil and Indian mulberry leaves. Subsequently, these modules were utilized for biological interpretation to identify target proteins related to the diseases. The biological functions of protein targets in these modules were analyzed using the BINGO program (Maere et al., 2005), a plug-in for Cytoscape, where BINGO describes the functions of gene groups using gene ontology (GO), focusing on the Biological Process category and specifying *Homo sapiens* as the organism. This approach facilitated the explanation of the biological functions of the modules of interest.

Results and Discussion

Specifically, 428 inflammation-related proteins, 1,267 diabetes-related proteins and 327 diarrhea-related proteins were discovered. These proteins were used to highlight target proteins and their interacting partners using the Cytoscape program (Shannon et al., 2003). The PPI network results for the active ingredients from the leaves of Khlu, Hoary basil, and Indian mulberry are shown in Fig. 2A, 2B and 2C, respectively. For Khlu leaves, 295 target proteins were identified, with 290 used in STRING, resulting in a network with 1,769 proteins and 8,203 interactions. Hoary basil leaves had 407 target proteins, with 400 used in STRING, leading to a network of 2,163 proteins and 11,520 interactions. Indian mulberry leaves had 317 target proteins, with 312 used in STRING, producing a network of 1,800 proteins and 9,152 interactions (Fig. 2D).

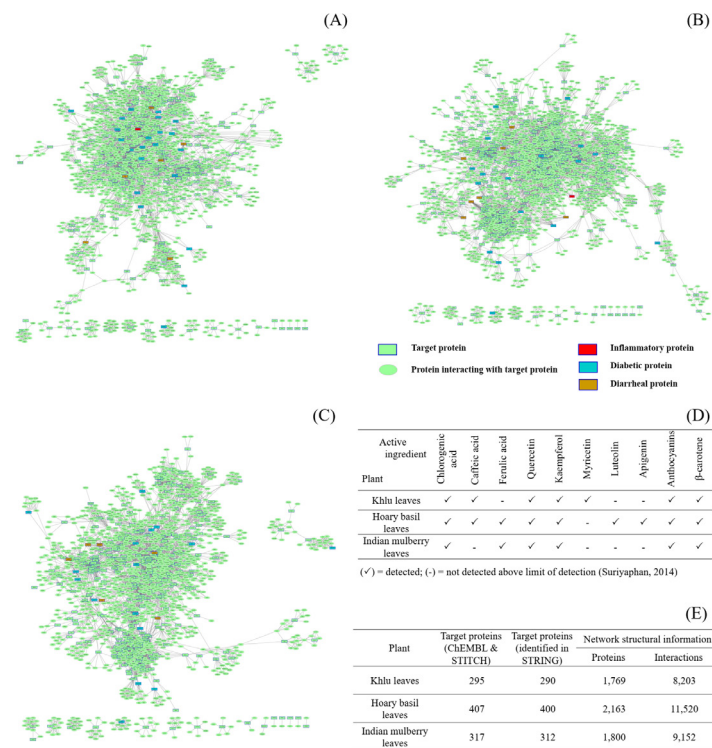


Fig. 2 Protein-protein interaction (PPI) network of active ingredients from leaves of: (A) Khlu; (B) Hoary basil; (C) Indian mulberry, where rectangular nodes represent target proteins of active ingredients, elliptical nodes represent proteins interacting with target proteins, green nodes indicate proteins unrelated to disease of interest, red nodes indicate inflammation-related proteins, blue nodes indicate proteins associated with diabetes and brown nodes indicate proteins associated with diarrhea; (D) table of active ingredients; (E) target proteins and PPI network structures from Khlu, Hoary basil and Indian mulberry leaves

Based on the PPI network analysis of the active ingredients from the leaves of Khlu, Hoary basil, and Indian mulberry, the network behavior was analyzed using Network Analyzer in Cytoscape (Shannon et al., 2003). The topological properties (node degree distribution, shortest path length distribution, average shortest path length and clustering coefficient) were compared to a random network with the same number of proteins and interactions (Fig. 3A–3D). The node degree distribution graph showed the degree distribution for the interaction networks of active ingredients from Khlu, Hoary basil, and Indian mulberry leaves, with equations $y=1640.2X^{-1.716}$, $y=2071.7X^{-1.692}$ and $y=1523.7X^{-1.656}$, respectively, with coefficient of determination values (R^2) of 0.810, 0.837 and 0.830, respectively. These PPI networks had the properties of a scale-free biological network, following the power law distribution rule— $(k) \sim k^{-\gamma}$ ($\gamma < 3$) (Zheng et al., 2015), unlike random networks which have lower R^2 values, where the R^2 values of the PPI network of active ingredients from the Khlu, Hoary basil and Indian mulberry leaves were close to 1, indicating a strong correlation with the power law distribution. This confirmed that nodes with a small number of connections were hubs with many interactions, which is a characteristic of a scale-free network. In contrast,

random networks have R^2 values lower than 0.5, indicating little correlation and few interactions per node, thus not exhibiting scale-free properties, as shown in Fig. 3A–3C (Seo et al., 2013). The shortest path length distribution graph of the PPI networks of active ingredients from Khlu, Hoary basil and Indian mulberry leaves had high frequencies at distances of 4–6, 4–5, and 4–6, respectively, contrasting with random interaction (RI) networks which have more frequent path lengths at distances of 3–4 in all three cases (Fig. 3A–3C). The average shortest path length values for the PPI networks of these leaves were 5.109 for Khlu, 5.078 for Hoary basil and 5.040 for Indian mulberry, which were higher than the corresponding values for RI networks (3.606, 3.515 and 3.496, respectively). In addition, the clustering coefficients of the PPI networks were substantially higher (0.669, 0.658 and 0.677, respectively) than those of the RI networks (0.005 for all three networks), as shown in Fig. 3D (Zheng et al., 2015; Thanasomboon et al., 2017). These findings indicated that the PPI networks of the active ingredients from the Khlu, Hoary basil and Indian mulberry leaves had small-world biological network properties, demonstrating modularity and the potential for functional module-based analysis.

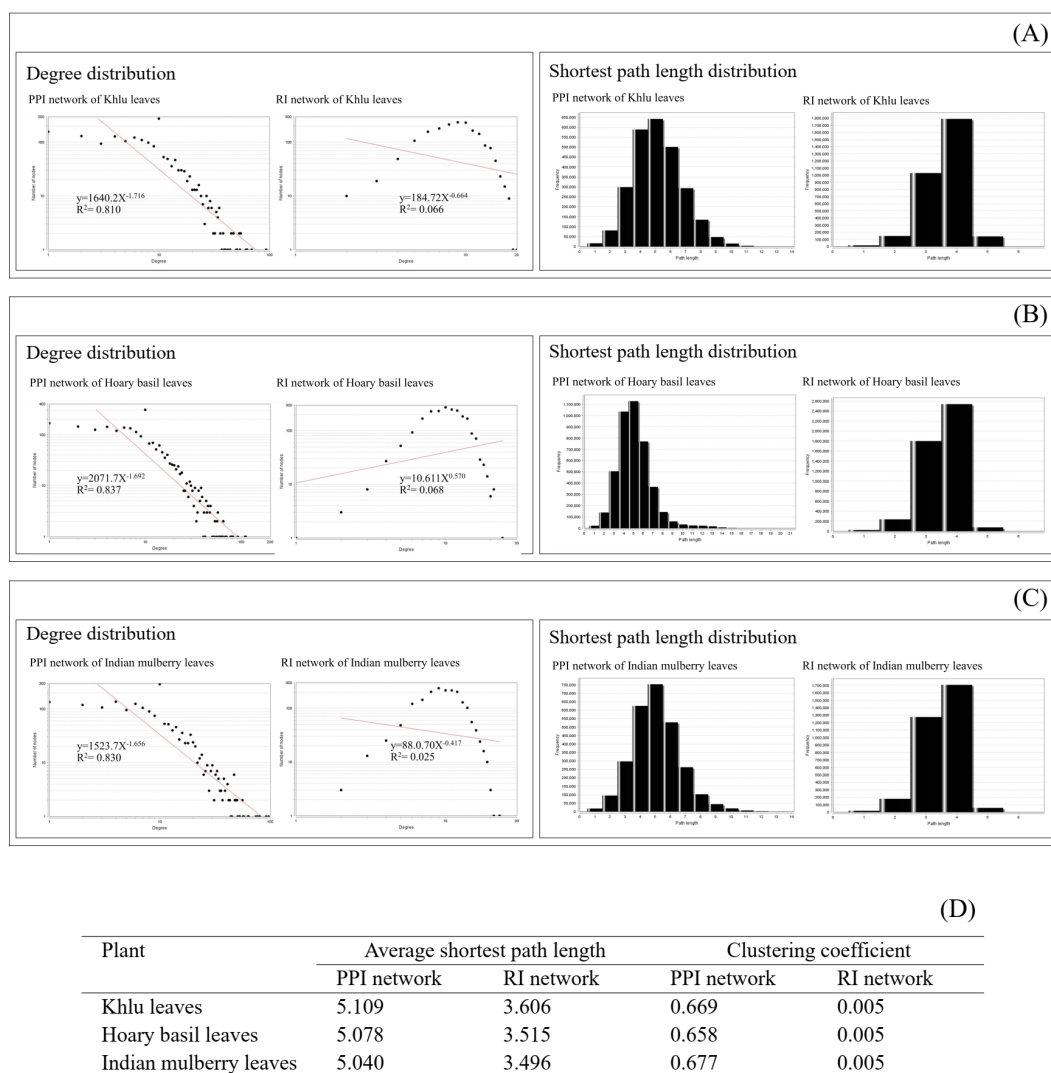


Fig. 3 Degree distribution graph and shortest path length distribution graph of the protein-protein interaction (PPI) networks of active ingredients from leaves of: (A) Khlu; (B) Hoary Basil; (C) Indian Mulberry, compared to their random interaction (RI) networks; (D) table presenting average shortest path length and clustering coefficient values of PPI networks and RI networks of active ingredients from leaves of Khlu, Hoary basil and Indian mulberry

Based on the analysis of modules from the PPI networks of Khlu, Hoary basil and Indian mulberry leaves that were validated as biological networks, there were 106, 109 and 107 modules, respectively. Next, these modules were screened for disease-related protein data associated with inflammation, diabetes and diarrhea, revealing a varying number of modules related to each condition for each plant type. Specifically, Khlu leaf modules associated with these conditions numbered 17, 38 and 12, respectively, while Hoary basil leaf modules numbered 16, 33 and 14, respectively, and Indian mulberry leaf modules numbered 17, 34 and 14, respectively. Among these, modules containing target proteins common to inflammation, diabetes and diarrhea were identified, resulting

in 5, 4 and 7 modules for Khlu, Hoary basil and Indian mulberry, respectively (Figs. 4A, 5A and 6A, respectively). Furthermore, specific modules related to each disease were identified for each plant, with varying counts such as 1, 18 and 5 modules for Khlu leaves in inflammation, diabetes and diarrhea, respectively. However, the biological interpretation was hindered for modules containing the target protein HSPB1, due to potential lack of functional GO information, leaving modules uninterpretable under the BiNGO program (Maere et al., 2005). Detailed GO functions derived from the biological interpretation of Khlu, Hoary basil and Indian mulberry leaves are provided in the tables in Figs. 4C, 5C and 6C, respectively.

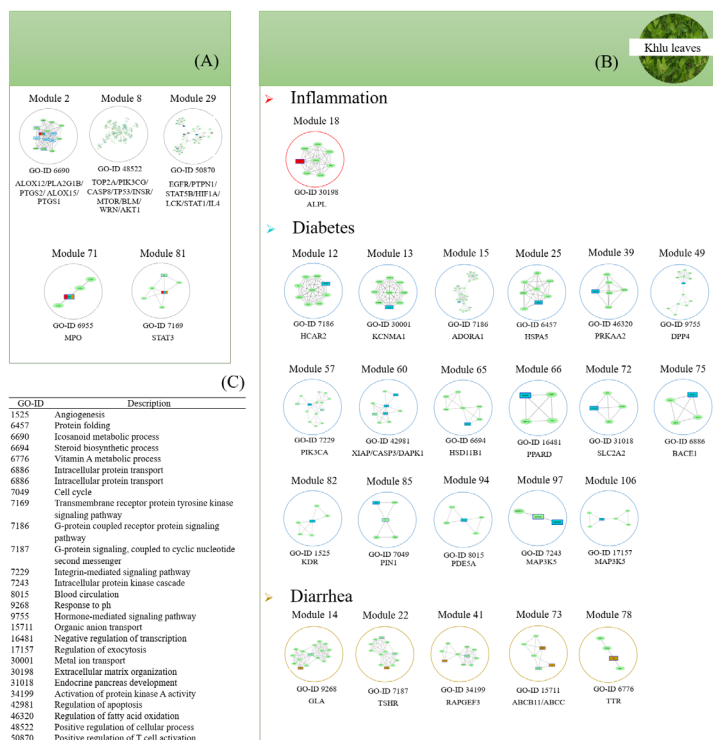


Fig. 4 Functional module-based analysis of protein-protein interaction network of active ingredients from Khlu leaves: (A) common modules identifying target proteins associated with inflammation, diabetes and diarrhea, (B) specific modules identifying target proteins specific to inflammation, diabetes or diarrhea. (C) a table showing details of gene ontology (GO) functions derived from biological interpretation of active ingredients from Khlu leaves.

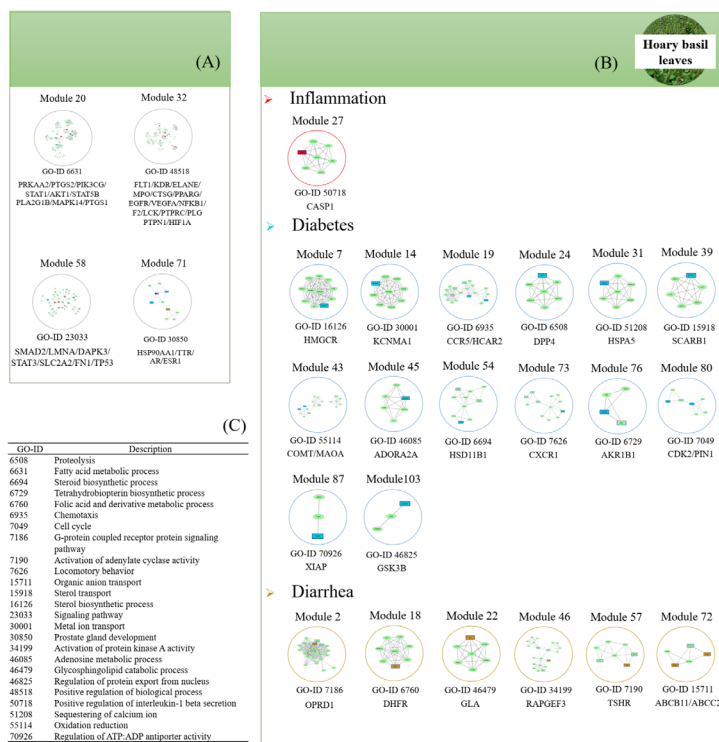


Fig. 5 Functional module-based analysis of protein-protein interaction network of active ingredients from Hoary basil leaves: (A) common modules identifying target proteins associated with inflammation, diabetes and diarrhea; (B) specific modules identifying target proteins specific to inflammation, diabetes or diarrhea; (C) a table showing details of gene ontology (GO) functions derived from biological interpretation of active ingredients from Hoary basil leaves.

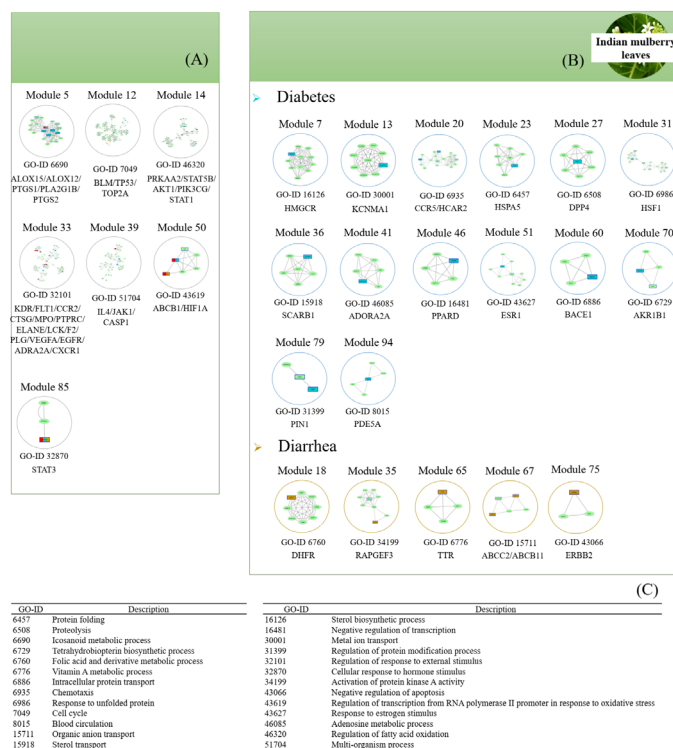


Fig. 6 Functional module-based analysis of protein-protein interaction network of active ingredients from Indian mulberry leaves: (A) common modules identifying target proteins associated with inflammation, diabetes and diarrhea; (B) specific modules identifying target proteins specific to inflammation, diabetes or diarrhea; (C) a table showing details of gene ontology (GO) functions derived from biological interpretation of active ingredients from Indian mulberry leaves.

The biological interpretation of the active ingredients from Khlu, Hoary basil and Indian mulberry leaves revealed diverse GO functions, emphasizing their therapeutic potential across various processes. Khlu leaves have reported involvement in angiogenesis, protein folding, metabolic pathways (such as steroid and vitamin A biosynthesis), intracellular protein transport, cell cycle regulation and signaling pathways such as G-protein coupled receptor and integrin-mediated signaling (Suriyaphan, 2014; Chan et al., 2022), as shown in Fig. 4. Hoary basil leaves have reported functions related to proteolysis, fatty acid metabolism, tetrahydrobiopterin and folic acid biosynthesis, chemotaxis, cell cycle, adenylate cyclase activation, sterol transport and oxidation-reduction processes (Choudhury et al., 2014), as shown in Fig. 5. Indian mulberry leaves have a reported contribution to protein folding, proteolysis, various metabolic processes, chemotaxis, blood circulation, transcription regulation, hormone responses and apoptosis regulation (Shalan et al., 2016; Algenstaedt et al., 2018), as shown in Fig. 6. These findings support the traditional medicinal uses of these plants, highlighting their roles in treating inflammation, diabetes, heart and brain diseases, liver protection and wound healing.

The leaves of Khlu, Hoary basil and Indian mulberry, containing identical active ingredients, shared similar properties and were associated with vital biological processes crucial in inflammatory diseases, diabetes and diarrhea. These processes have been reported to encompass positive regulation of cellular processes, immune response, icosanoid metabolism, fatty acid metabolism and control of fatty acid oxidation, where fatty acid metabolism, particularly arachidonic acid metabolism and the production of inflammatory mediators such as prostaglandins and leukotrienes, is integral to the inflammatory response of the immune system (Namgaladze and Brüne, 2016). Furthermore, dysregulation of fatty acid oxidation can lead to insulin resistance, obesity and type 2 diabetes, emphasizing the importance of these processes in human health (Montague and Rahilly, 2000). Additionally, short-chain fatty acids (SCFAs) derived from colon bacteria play a crucial role in maintaining gut health and preventing diarrhea by regulating fluid production in the colon (Binder, 2010).

In the specific modules related to inflammation found in the Khlu and Hoary basil leaves, Module 18 of Khlu leaves contains the inflammatory protein ALPL, associated with extracellular matrix (ECM) organization that is crucial for

cell function and survival (Millán and Whyte, 2016). Injury-induced ECM degradation and structural changes affect inflammatory cell signaling, including cytokine secretion (Gaudet and Popovich, 2014), as shown in Fig. 4B. Module 27, containing the inflammatory protein CASP1, is related to the positive regulation of interleukin-1 beta (IL1 β) production in Hoary basil leaves, which research has suggested can be inhibited, thereby reducing the production of inflammatory mediators regulated by the NF- κ B signaling pathway (Choudhury et al., 2014), as shown in Fig. 5B.

Specific modules related to diabetes in the leaves of Khlu, Hoary basil and Indian mulberry demonstrated similar biological processes. For example, Module 13 of Khlu leaves (Fig. 4B), Module 14 of Hoary basil leaves (Fig. 5B), and Module 13 of Indian mulberry leaves (Fig. 6B) contained the protein KCNMA1, implicated in metal ion transport, particularly zinc ions, which regulate glycogen synthase kinase-3 β activity, crucial for insulin action and glucose uptake in diabetes treatment (Ilouz et al., 2002). Additionally, processes related to adenosine metabolism were identified in Module 45 of Hoary basil leaves (Fig. 5B) and Module 41 of Indian mulberry leaves (Fig. 6B), involving the protein ADORA2A, which influences the glucose balance by regulating insulin secretion, glycogenolysis and glucose release, thus playing a vital role in diabetes management (Koupenova and Ravid, 2013; Alagbonsi et al., 2016; Nopparat et al., 2019).

Specific modules related to diarrhea in the leaves of Khlu, Hoary basil, and Indian mulberry contained the proteins ABCB11 and ABCC2 that are involved in transporting organic anions, including SCFAs, which in high concentrations can cause diarrhea by affecting salt and water absorption in the colon (Vernia et al., 1988). Additionally, the presence of the RAPGEF3 protein in Modules 41 of Khlu leaves (Fig. 4B), 46 of Hoary basil leaves (Fig. 5B) and 35 of Indian Mulberry leaves (Fig. 6B) suggested involvement in diarrhea through protein kinase A activation, increasing cAMP levels, leading to chloride secretion and fluid loss in the intestines (Hodges and Gill, 2010). These findings underscore the potential of these medicinal plants in preventing or treating inflammation, diabetes and diarrhea, providing valuable insights into their molecular mechanisms for further laboratory studies.

Conclusions

The active ingredients from Khlu, Hoary basil and Indian mulberry leaves form protein-protein interaction networks characterized by scale-free, small-world and modular attributes, enabling detailed module analysis. Disease-related modules were identified as having numerous target protein modules associated with inflammation, diabetes and diarrhea across all three plant types. Common biological processes related to lipid metabolism, metal ion transport and adenosine metabolism influenced insulin resistance, glucose regulation and inflammatory mediator production. These findings highlighted the potential molecular mechanisms of these plant leaves in preventing or mitigating these conditions. However, a limitation of this study was the lack of more extensive information on the active ingredients from additional sources to develop a comprehensive network. Future research should focus on broadening *in vitro* and *in vivo* studies to further validate these results at the laboratory level.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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