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FULL PAPER

Unveiling the pharmacological potential of Annona squamosa fruit: A network pharmacology approach

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Annona squamosa, commonly known as sugar apple, custard apple, sharifa, or srikaya is a tropical fruit with significant medicinal value rooted in traditional medicine systems across regions like Indonesia. This study explores the pharmacological potential of Annona squamosa fruit through the lens of network pharmacology. The rich phytochemical composition of A. squamosa, including alkaloids, flavonoids, and tannins, has long been recognized for its diverse therapeutic effects, including antidiabetic, antihypertensive, anticancer, and antimicrobial properties. However, limited research has delved into the molecular mechanisms underlying these benefits. We employed network pharmacology approaches to systematically analyze the interactions between bioactive compounds in A. squamosa and their molecular targets, aiming to elucidate the therapeutic mechanisms and identify potential drug targets. This study identified 12 secondary metabolites in A. squamosa fruit and predicted 122 proteins that interact with these compounds. Network analysis revealed significant enrichment in biological processes associated with central nervous system disorders and diabetes, suggesting potential applications in these areas. Furthermore, gene ontology and KEGG pathway analysis highlighted the involvement of key hub genes, such as GRM5 and GRIA2, in neurological disorders and diabetes. These findings provide valuable insights into the pharmacological properties of A. squamosa and underscore its potential as a natural remedy. Further experimental validation and clinical studies are warranted to harness the therapeutic benefits of Annona squamosa fruit in disease management.

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KEYWORDS

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Introduction

Annona squamosa, commonly known as a sugar apple or custard apple (English), sharifa (Hindi), and srikaya (Indonesia), is a tropical fruit widely cultivated throughout the world, such as in the West Indies, America, Brazil, and Indonesia [1,2]. Beyond its culinary appeal, *A. squamosa* has also been valued in traditional medicine systems for its medicinal properties [3]. The fruit, along with other parts of the *A. squamosa* plant, has been used to treat various ailments due to its rich phytochemical composition, which includes

alkaloids, flavonoids, tannins, and phenolic compounds [4].

In traditional medicine practices, especially in regions like Indonesia, *A. squamosa* has been utilized for its diverse therapeutic effects [1]. The leaf has potentials as antidiabetic [1,5-6], antihypertension [5], anticancer, and antimicrobial [1,3]. The fruit is believed to be used as an antioxidant, antidiabetic, and antiviral [7]. However, more research needs to discuss the benefits of *A. squamosa* fruit.

In recent years, there has been growing interest in exploring the pharmacological potential of herbal plants using network pharmacology approaches [8]. Network pharmacology represents a modern drug discovery and development approach that integrates computational biology, bioinformatics, and pharmacology [9]. It provides a systems-level understanding of how drugs and natural compounds interact with biological networks within the human body [10]. By analyzing the complex interactions between bioactive molecules and their molecular targets, network pharmacology offers insights into the underlying mechanisms of drug action and disease pathogenesis [11]. This holistic approach allows for identifying new drug targets, predicting adverse drug reactions, and exploring synergistic interactions between compounds [12].

By systematically analyzing the bioactive constituents present in *A. squamosa* fruit and their interactions with molecular targets implicated in various diseases, researchers aim to uncover the therapeutic mechanisms underlying its traditional uses. By integrating of network pharmacology with experimental validation and clinical studies, this interdisciplinary approach holds promise for identifying novel drug candidates derived from *A. squamosa* fruit. By elucidating the multifaceted pharmacological effects of *A. squamosa* fruit at a systems level, this study aims to contribute to the development of evidence-based therapeutic interventions rooted in traditional knowledge and modern scientific methods.

Experimental

Tools

The tools used in this research are several online web servers and online tools such as Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), SwissTargetPrediction (http://swisstargetprediction.ch/), and STRING (https://string-db.org/). Apart from that, this research also uses Cytoscape 3.10.1 software with the CytoHubba 0.1 plugin.

Methods

The secondary metabolite compound of *A. squamosa* fruits was searched with Dr. Duke's databases, and then searched for the SMILES code of each secondary metabolite compound in PubChem [13]. Afterwards, the SMILES code was entered into SwissTargetPrediction [14]. The resulting proteins were entered into STRING to determine the pharmacological network between proteins that are predicted to be able to interact with secondary metabolite compounds in *A. squamosal* [15].

Data analysis

The resulting data from STRING was analyzed using Gene Ontology [GO], Kyoto Encyclopedia, and Genes and Gnomes [KEGG], as well as disease-gene associations [DISEASES] enrichment. The data was also imported into Cytoscape [16], and the most important proteins in this network were searched for using the CytoHubba plugin [17].

Results and discussion

From Dr. Duke's databases, *A. squamosa* fruit contains 12 secondary metabolites (Table 1).

Protein that can interact with the secondary metabolite of *A. squamosa* was predicted by SwissTarget Prediction, and proteins with a probability value > 0 will be used for further analysis [8]. It was discovered from the investigation results that 122 proteins were predicted to interact with the secondary metabolite compounds of *A. squamosa*. Network pharmacology analysis was performed using STRING on those proteins. This study aimed to create a network between the chosen target proteins and the biological pathways connected to them [18] (Figure 1). STRING is a database that comprises over nine million proteins that can be utilized for predicting protein-protein interactions (PPI) [19].

TABLE 1 Chemical compound list of secondary metabolite of A. squamosa

No.	Chemical Compound Name
1	Ascorbic-acid
2	Beta-carotene
3	Citrulline
4	Fructose
5	4-aminobutyric acid
6	Niacin
7	Ornithine
8	Pantothenic acid
9	Riboflavin
10	Sucrose
11	Thiamin
12	Pyridoxine

A technique for comprehending biological systems based on a group of genes or proteins obtained from study data is called GO, KEGG, and DISEASES enrichment. Biological process (BP), molecular function (MF), and chemical component (CC) are the three terminologies utilized in GO [20]. A collection of biological signaling pathways manually drawn to represent knowledge about networks of molecular interactions and reactions is known as KEGG enrichment [21]. DISEASES is a systematic analysis tool and a network-based approach successfully applied to identify disease-related genes in various disorders [22]. The False Discovery Rate (FDR) value, or the quantity of potential data that could provide a false positive value that is anticipated to be rejected, serves as the foundation for the analysis's conclusions. Accordingly, the analyst results are more accurate with a lower value [23]. The FDR in this study is expressed as -Log (p-values). The greater the values, the less likely an error possibility (Figure 2). A top 10 BP (Biological Processes), MF (Molecular Function), CC (Cellular Component), KEGG Pathways, and DISEASES were selected in the current study.

The STRING database significantly enhanced the PPI network, which was constructed with all genes in BP. The PPI network has 122 nodes and 616 edges (Figure 1). Cytoscape was used to import PPI data

following the creation of the PPI network. Using several algorithmic topologies, CytoHubba, an application for Cytoscape, is typically used to forecast significant nodes or sub-networks inside a network. The top 10 hub genes in cytoHubba that were selected using the MCC algorithm (Figure 3) [24]. The top 10 genes that contribute to nervous system illnesses, according to the research findings, are GRM5, GRM3, GRIA 2, GRM2, GRIA 1, GRM 1, GRIK5, GRIK2, GRM8, and GRM4.

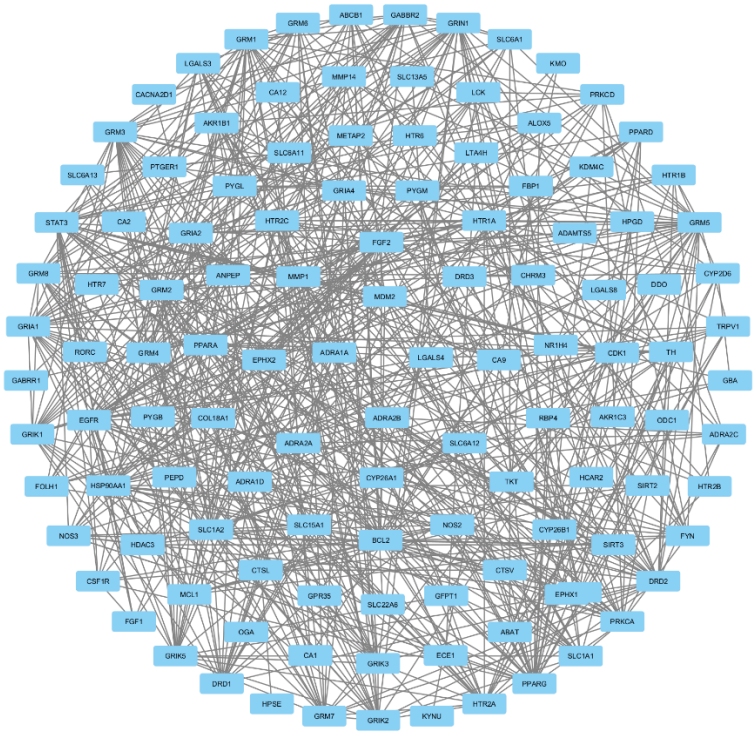

FIGURE 1 Network pharmacology of chemical compound of secondary metabolites of *A. squamosa's* fruit scheme using STRING. It was discovered that 122 proteins from the analysis using SwissTargetPrediction (http://www.swisstargetprediction.ch/) were predicted to interact with the secondary metabolite compounds of A. squamosa. The protein-protein interaction predictions from this picture were carried out using the String-DB platform (https://string-db.org/). The blue box is the protein target, and the lines connecting the boxes indicate connections between proteins with predicted interactions based on gene neighborhood, gene fusion, gene co-occurrence, co-expression, protein homology, or text mining

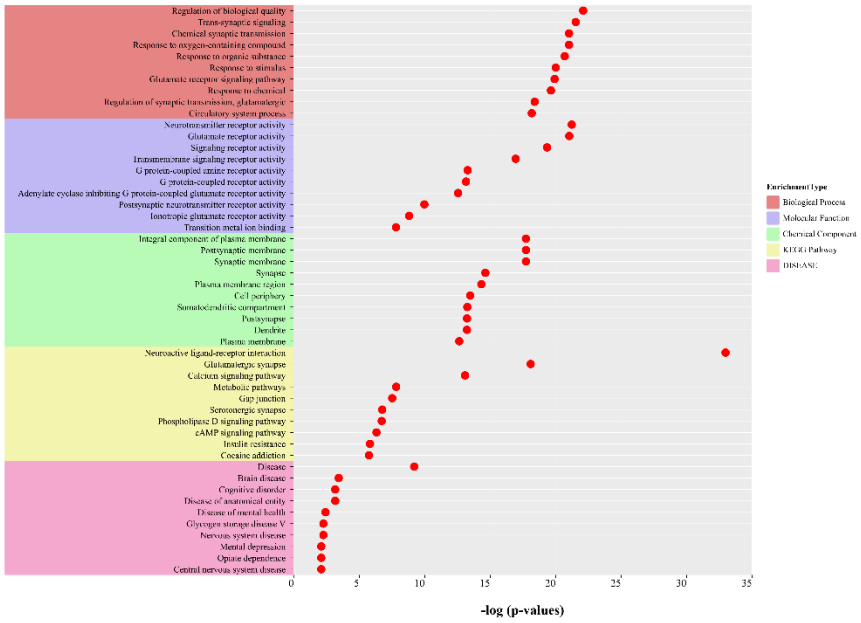

FIGURE 2 GO, KEGG, and DISEASES analysis using the String-DB platform (https://string-db.org/). Analysis of 122 target protein compounds of A. squamosa consisting of five enrichments of candidate targets revealed ten significantly enriched elements in BP (orange), MF (purple), CC (light green), KEGG (light yellow), and DISEASE (pink). The x-axis shows the -log 10 (p-value)

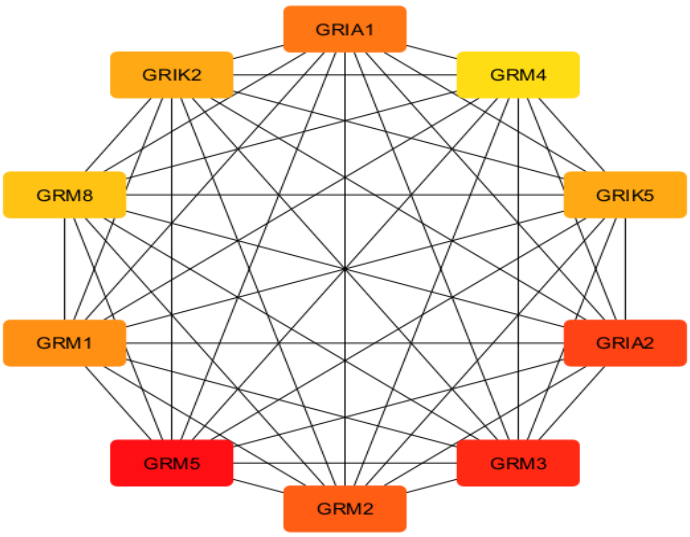

FIGURE 3 The top 10 predicted proteins can interact with the secondary metabolite of A. squamosa's fruit using the MCC algorithm. Boxes indicate predicted protein targets, and lines represent connections between two protein targets. The more lines connecting one target protein to another target protein are indicated by the color gradient of the box from yellow to red

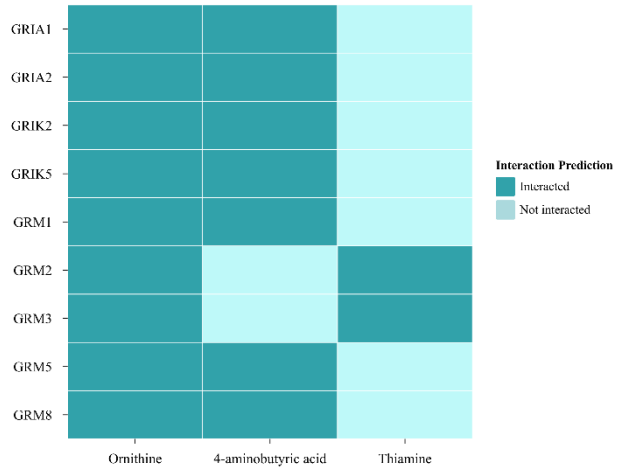

FIGURE 4 The prediction of secondary metabolite compounds of A. squamosa that can interact with the top 10 proteins using the MCC algorithm from CytoHubba shows that these compounds have the potential to bind with specific proteins, which could lead to novel biological activities and therapeutic applications. The dark teal color indicated that the secondary metabolite compound could interact with protein, whereas light teal was otherwise

The results involving many BPs associated with central nervous system disorders. Gene proteins and biological processes can treat various nervous system illnesses. For example, the glutamate receptor signaling pathway contains gene proteins like GRM4 for epilepsy [25] and GRIK3 for schizophrenia [26]. The neuroactive ligand-receptor interaction route, closely associated with GABA (gamma-aminobutyric acid), is the most significantly enriched KEGG pathwamy. In addition to the glutamatergic synapse, the calcium signaling pathway plays a role in network nerves, synaptic plasticity, and regulatory activity.

Glutamate is the primary excitatory neurotransmitter in the nervous system, which is crucial for nociceptive processing and pain regulation [27]. The central and peripheral nervous systems include high expression levels of G-protein-coupled metabotropic glutamate receptors (mGluRs), which are involved in synaptic transmission and neuronal excitability [28,29]. Groups I–II comprise the eight distinct mGluR subtypes discovered thus far. Primarily expressed presynaptically, group Il mGluR2 and mGluR3 negatively couple to adenyl cyclase via Gi/Go proteins and generally block the release of neurotransmitters, including glutamate and GABA [30]. Peripheral, spinal, and supraspinal components of pain-related neuronal processes express group ll MGluRs, which are consistently linked to pain modulation. The nervous system is filled with Group II MGluRs, including areas and circuits crucial for processing emotions, nociceptive signals, and pain regulation [31]. mGluR4, mGluR7, and mGluR8 belong to group III and are identical to mGluRs in group II because they bind negatively to Gi/o-type proteins. Furthermore, mGluR6 couples favorably to the phosphodiesterase cGMP [29,32].

Therapeutic use of compounds acting on group II mGluRs has been suggested for amyotrophic lateral sclerosis, anxiety, depression, and schizophrenia [30], Parkinson's disease [33], drug addiction [34], and pain states [35]. In summary, GRM5, GRM3, GRIA2, GRM2, GRIA1, GRM1, GRIK5,

GRIK2, GRM8, and GRM4 have been identified by the MCC algorithm, indicating their possible importance in the biological network and its consequences for comprehending disease mechanisms and therapeutic targeting.

Upon examination, it was also shown that secondary metabolite of *A. squamosa* can interact with protein associated with diabetes [36]. There is insulin resistance in KEGG pathway result and glycogen storage disesase that association with diabetes. These results follow several preliminary studies involving A. squamosa, which show that this plant can play a role in lowering blood glucose levels [1,5-6]. While there may not be direct evidence linking top 10 mcc genes to diabetes, their involvement in glutamate signaling pathways suggests potential connections to metabolic regulation and insulin sensitivity, which are central to diabetes pathophysiology. Further research would be needed to elucidate the specific roles of these genes in diabetes and related metabolic disorders.

Through an ethnopharmacological approach, several plants from the Annonaceae genus also have antidiabetic and neuriprotectant activity. Leaf extract from *A. reticulata* has been proven to reduce blood sugar levels in induced diabetic rats [37-39], likewise for the seeds [40], and bark [41]. *A. muricata* also has the activity of lowering blood sugar levels [42,43]. Other research also shows that *A. muricata* leaf extract has neuroprotectant activity [44,45].

For further analysis, three secondary metabolite compounds of *A. squamosa* are predicted to interact with the top 10 MCC genes (Figure 4). Ornithine is predicted to interact with all of the top 10 MCC genes, thiamine on GRM2 and GRM3, and 4-aminobutyric acid with the remaining genes.

Ornithine, a non-essential amino acid, has been investigated for its potential therapeutic effects in neurodegenerative disorders, particularly Parkinson's disease. The amino acid is produced by the enzymatic action of arginase on arginine, resulting in the production of urea and the generation of ornithine that has been shown to positively impact mitochondrial function, which is crucial for neuronal metabolism and synaptic activity. This is significant because mitochondrial dysfunction is a crucial feature of Parkinson's disease, contributing to the degeneration of dopaminergic neurons [46].

4-aminobutyric acid, also known as GABA, is the primary inhibitory neurotransmitter in the central nervous system and plays a crucial role in maintaining a balance between excitatory and inhibitory signaling. A disturbance in this balance, known as excitotoxicity, can lead to neuronal damage and cell death [47]. One potential role of 4-aminobutyric acid in neurodegenerative disorders is its involvement in regulating neuronal excitability and inhibiting excessive neurotransmitter activity [48].

Thiamine, also known as Vitamin B1, plays a crucial role in preventing and potentially treating neurodegenerative disorders. These processes are commonly observed in neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease. For instance, plasma thiamine levels are reduced in patients diagnosed with Alzheimer's disease, indicating that some Alzheimer's disease patients may have thiamine deficiency. Thiamine also has a role in preventing neurodegenerative diseases [49]. Supporting vitamin B1 therapy has been found to not only constitute neuroprotection but also have a favorable impact on advanced neurodegenerative diseases such as Parkinson's, Alzheimer's, Wernicke's encephalopathy, and Huntington's disease [50]. In summary, thiamine plays a significant role in preventing and potentially treating neurodegenerative disorders, particularly Alzheimer's disease, by maintaining normal brain function and preventing the development of neurodegenerative processes [49,50].

Utilizing network pharmacology, we have predicted that specific secondary metabolites from this plant can interact with genes involved in neurodegenerative processes, potentially leading to a synergistic effect. This synergy could enhance the therapeutic efficacy of these compounds, making them more effective in againts neurodegenerative disorders [51]. The predicted interactions between the secondary metabolites and genes involved in neurodegenerative processes suggest that these compounds could work together to modulate multiple pathways, leading to a more comprehensive therapeutic approach. This synergistic effect could be precious in the treatment of neurodegenerative disorders, where a multi-faceted approach is often necessary to manage the complex pathophysiological processes effectively involve.

Conclusion

The integration of network pharmacology with traditional knowledge has provided valuable insights into the pharmacological potential of *A. squamosa*'s fruit. Through systematic analysis, this study elucidated the multifaceted therapeutic effects of *A. squamosa*, identifying potential drug targets and pathways associated with central nervous system disorders and diabetes. The top hub genes identified, along with the interaction with proteins involved in diabetes, highlight the promise of *A. squamosa* as a natural remedy. This study underscores the significance of *A. squamosa* in modern pharmacology and suggests avenues for further experimental validation and clinical investigation to harness its therapeutic benefits.

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Authors' Contributions

The authors contributed to this study equally.

Conflict of Interest

The authors declare that there is no conflict of interest in this work.

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