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Phytochemical, pharmacognostic, and in silico investigation of the anti-diabetic potential of *Elaeocarpus angustifolius* seeds

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ABSTRACT

Introduction: *Elaeocarpus angustifolius* has been traditionally used for hyperglycemia, but most of its pharmacognostic and pharmacological properties remain underexplored. This study provided an evaluation of integrating pharmacognostic, physicochemical and phytochemical analyses of its seeds with network pharmacology to explore its potential in type 2 diabetes mellitus (T2DM) management.

Methods: Microscopic analysis was conducted to confirm species authentication. Physicochemical parameters, including moisture content, total ash, and acid-insoluble ash, were assessed. Phytochemical screening detected bioactive compounds, and thin-layer chromatography detected the presence of quercetin. Network pharmacology analysis was performed to predict potential metabolic and inflammatory pathways influenced by key bioactive compounds. Pathway enrichment, gene-disease association and Reactome pathway analyses were used to determine molecular targets relevant to glucose metabolism and insulin sensitivity.

Results: Microscopic analysis confirmed species authentication through the presence of prism crystals, sclereids, and oil glands. Physicochemical evaluation indicated low moisture content (6.5%), suggesting good stability, while total ash (3.2%) and acid-insoluble ash (0.8%) supported the sample's purity. The presence of alkaloids, steroids, flavonoids, and phenols was discovered using phytochemical screening. Network pharmacology analysis demonstrated significant pathway enrichment in PI3K/Akt (P = 0.003), AMPK (P = 0.007), insulin (P = 0.012), and TNF signalling (P = 0.015) pathways. Further gene enrichment revealed MAPK1, PIK3R2, and NFKB1 as key targets significantly associated with T2DM ($P < 0.05$), compared to expected random gene-pathway associations.

Conclusion: This study revealed *E. angustifolius* as a promising candidate for diabetes management through its influence on key metabolic pathways. However, experimental and clinical trials should confirm its usage.

Implication for health policy/practice/research/medical education:
The study emphasizes the therapeutic potential of *Elaeocarpus angustifolius* in diabetes management. In clinical practice, the identified bioactive compounds may support the development of complementary therapies for metabolic disorders. For research, the findings provide a basis for further in vivo and clinical studies to validate its pharmacological effects. In medical education, integrating network pharmacology and phytochemical analysis into curricula can enhance understanding of natural compounds in disease management.

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Introduction

A chronic metabolic disease, diabetes mellitus (DM) is typified by insulin resistance, hyperglycemia, and impaired glucose metabolism, affecting millions worldwide. The increasing prevalence of diabetes has driven research into novel therapeutic strategies, including the use of plant-based bioactive compounds for metabolic regulation (1). Traditional medicinal plants have long been explored for their potential in modulating key metabolic pathways associated with diabetes, such as insulin signalling, glucose uptake, lipid metabolism, and inflammation (2). Among these, *Elaeocarpus angustifolius*, commonly known as Rudraksha, has gained attention due to its diverse pharmacological properties and its traditional use in Ayurvedic medicine (3).

Belonging to the *Elaeocarpaceae* family, *E. angustifolius* is native to the Himalayan region and tropical parts of Southeast Asia. The plant's seeds, revered in spiritual and medicinal practices (4-6), contain a wide range of bioactive compounds, including flavonoids, alkaloids, tannins, glycosides, and phenolic acids, which contribute to its pharmacological effects (7,8). Traditionally, Rudraksha beads have been used for stress relief, cardiovascular health, and neuroprotection, with emerging evidence supporting their role in anti-inflammatory, antioxidant, and metabolic regulation (9-11). Despite its historical use, scientific validation of *E. angustifolius* in metabolic disorders, particularly diabetes and insulin resistance, remains limited.

Recent advancements in network pharmacology have enabled researchers to systematically analyze plant-derived bioactive compounds and their interactions with target proteins involved in disease mechanisms (12). This approach integrates phytochemical profiling, molecular docking, gene enrichment analysis, and pathway identification to explore the mechanistic role of herbal compounds in modulating key biological processes (13). In diabetes management, pathways such as PI3K-Akt, AMPK, insulin signalling, TNF signalling, and adipocytokine regulation play critical roles in glucose homeostasis, lipid metabolism, and insulin sensitivity. The network-based approach allows for a holistic evaluation of *E. angustifolius,* identifying potential molecular targets and pharmacological mechanisms underlying its anti-diabetic activity (14). This study aimed to evaluate the anti-diabetic potential of *E. angustifolius* by integrating phytochemical, pharmacognostic, and network pharmacology analyses. It seeks to identify bioactive compounds, assess their pharmacological properties, and explore their molecular interactions with key metabolic pathways involved in diabetes management.

Materials and Methods

Collection and authentication of seeds

The seed sample was authenticated at the Central Ayurveda Research Institute, Bengaluru (Ministry of AYUSH, Government of India) and identified as *E. angustifolius* Blume (Family: *Elaeocarpaceae*) assigned Herbarium No. RRCB1-mus207 and Authentication No. SMPU/CARI/BNG/2024-25. Seeds of *Elaeocarpus angustifolius* were sourced from a farm in Shivamogga district, Karnataka. They were thoroughly cleaned, shade-dried, and ground into a coarse powder using a grinding stone.

Chemical reagents

All chemicals and reagents utilized for analytical procedures were of laboratory grade and procured from KLE College of Pharmacy, Bengaluru.

Solvent extraction

The powdered material of *E. angustifolius* seed was exposed to sequential solvent extraction using petroleum ether, chloroform, acetone, ethanol, methanol, and water in order of increasing polarity. A total of 250 g of the powdered material was loaded into a Soxhlet apparatus, and each solvent was used for approximately 20 hours. The respective extracts were then concentrated by rota evaporator to obtain solvent-free crude extracts, weighed and stored at 5-6 degrees Celsius (15-19).

Microscopic staining reagents

Various staining reagents were employed for microscopic evaluation, including chloral hydrate and concentrated hydrochloric acid, crystal violet, methylene blue, Kovac's reagent, Sudan red, iodine and nigrosine.

Physicochemical parameter analysis

To assess the physicochemical properties of *E. angustifolius* seeds, standard tests were conducted, including loss on drying (moisture content), total ash and acid-insoluble ash following established Standard Operating Procedures (SOPs).

Loss on drying (moisture content): The sample's moisture and volatile content were assessed using this assay. A pre-weighed weighing bottle was filled with two to six grams of the powdered sample, which was then precisely weighed and dried for 5 to 6 hours at 105 °C. After that, the sample was chilled in a silica gel desiccator until its weight remained constant. The weight difference before and after drying was used to determine the drying percentage loss.

Total ash content: Two to four grams of the sample were heated in a crucible that had been previously weighed to 550 °C for around four hours in order to calculate the total ash, which is the inorganic residue that remains after combustion. The sample was then cooled in a desiccator and weighed to determine the percentage of total ash.

Acid-insoluble ash: 25 mL of diluted hydrochloric acid was added to the whole amount of ash residue and heated for five minutes in order to identify the acid-insoluble ash. A quantitative filter paper was used to filter the mixture,

and hot water was used to completely wash away any remaining residue. To determine the percentage of acid-insoluble ash, the filter paper was dried and then burned. For quantitative filter paper, use a different term (20,21).

Phytochemical screening

Petroleum ether, chloroform, acetone, ethanol, methanol and water extracts of *E. angustifolius* seeds were subjected to preliminary phytochemical screening in order to identify the presence of several phytoconstituents. Alkaloids, sugars, glycosides, sterols, phenolics, flavonoids, and saponins were identified using standard qualitative assays.

Test for alkaloids: On a water bath, 20 mg of the ethanolic extract was agitated for five minutes with 6 mL of diluted HCl. Three equal portions of these filtrates were separated, and the extract is screened using the subsequent reagent.

Dragendroff's test: A potassium bismuth iodide solution (Dragendroff's reagent) was added to a portion of the filtrate, and the presence of an orange-red precipitate was monitored.

Mayer's test: A solution of potassium mercuric iodide (Mayer's reagent) was added to the filtrate part, and the presence of a cream-colored precipitate was monitored.

Wagner's test: Iodine and potassium iodide were mixed in distilled water, and the mixture was then diluted to 100 ml. The aforesaid solution was applied in little drops to the remaining filtrate, and the existence of a brown precipitate was monitored (21).

Tests for steroids and terpenoids

Salkowski test: Concentrated H2SO4 was added along the test tube's side after the crude extract was agitated separately with chloroform. The presence of steroids was detected by looking for a reddish-brown tint.

Liebermann-Burchard test: This test involved combining a portion of the extract with chloroform in a test tube, adding a few drops of acetic anhydride, boiling the mixture in a water bath, and quickly cooling it in ice water. Along the test tube's inner wall, concentrated sulfuric acid was gradually introduced. The test tube was examined for the presence of a brown ring where two layers met; the presence of steroids was indicated by the upper layer turning green, and the presence of terpenoids was indicated by the deep red colour (22).

Tests for carbohydrate

Molisch's test: A fraction of the material that had been dissolved in distilled water was mixed with a few drops of Molisch's reagent. Along the test tube's inner wall, concentrated sulfuric acid was gradually introduced. After two to three minutes, the mixture was let to stand, and the interphase of the two layers was checked for the development of a red or muted violet colour.

Benedict's test: Equal amounts of Benedict's reagent were added to the extract after it had been dissolved in distilled water. On a water bath, the mixture was cooked for five to ten minutes before being checked for red, yellow, or green hues (23).

Tests for protein

Million test: Five millilitres of Million's reagent were combined with around 3 mL of test solutions, and the mixture was watched for a white precipitate that turned brick red when heated.

Ninhydrin test: In a test tube set in a boiling water bath for one to two minutes, 2 mL of filtrate were treated with a few drops of Ninhydrin solution, and the production of a purple colour was monitored.

Tannin test: After mixing the extract with distilled water, it was filtered. After adding a few drops of 5% ferric chloride, the mixture was checked for the presence of any precipitate or black or blue-green colouring (24).

Test for saponins

Foam test: 10 mL of distilled water was mixed with 0.5 g of extract and shaken forcefully. The presence of saponins was shown by the foaming that formed and persisted after five minutes of warming in a water bath.

Precipitation test: A 1% lead acetate solution was added to one ml of extract. The presence of saponins was revealed by the formation of a white precipitate (24).

Tests for glycosides

Borntrager's test: 5% H_2SO_4 was added to the extract solution, and the mixture was heated in a water bath before being filtered. After collecting the filtrate, it was combined with an equal amount of chloroform and left for five minutes. Half of the volume of the bottom layer of chloroform was combined with diluted ammonia, and the ammoniacal layer was watched for the development of a rose pink to red hue, which signified the presence of anthraquinone glycosides.

Keller-Killiani test: Distilled water was added to the extract. A few drops of ferric chloride were added to this solution together with glacial acetic acid, and then a few drops of H2SO4 were put down the test tube's side. A brown ring was shown to form at the interface of this test mixture, which is a positive sign for cardiac glycoside (a violet ring may emerge below the brown ring) (25).

Tests for flavonoids

Alkaline reagent test: A few drops of a 20% sodium hydroxide solution were added to a small amount of extract that had been dissolved in distilled water. Flavonoids were detected in this test mixture by the production of an intense yellow colour that turned colourless when diluted hydrochloric acid was added (25).

Test for phenols

Ferric chloride test: A tiny portion of each extract was

exposed to 5% aqueous ferric chloride, and the creation of a deep blue or black colour was monitored (25).

Thin-layer chromatography (TLC) analysis

TLC analysis was conducted to detect and confirm the presence of quercetin in the sample. A quercetin standard solution (1 mg/mL) and the test sample were prepared by dissolving them in methanol and filtering to remove any insoluble particles. A silica gel TLC plate (10 × 5 cm) was used, with a baseline drawn 1 cm from the bottom. The standard and sample solutions (1–2 µL) were spotted onto the plate using a capillary tube and allowed to air-dry. Toluene, ethyl acetate, and formic acid were combined in a 7:2:1 ratio to generate the mobile phase. The TLC chamber was saturated with this solvent system for 15–20 minutes before development. Making sure the spots were above the solvent level; the plate was carefully positioned within the chamber. The mobile phase was allowed to migrate up to approximately 8 cm, after which the plate was removed and air-dried.

The TLC plate was viewed using UV light at 254 and 366 nm for visibility. Quercetin exhibited a bright yellow fluorescent spot under 366 nm UV light, indicating its presence. The retention factor (Rf) was computed using the formula that follows:

Rf = distance that the solute travelled from the origin line divided by the distance that the solvent travelled.

Network pharmacology analysis

Utilizing a network pharmacology method, the possible anti-diabetic effects of *E. angustifolius* by identifying key bioactive compounds, predicting their molecular targets, and analyzing their involvement in diabetes-related pathways.

Identification of bioactive compounds

Key bioactive compounds, including quercetin, kaempferol, ellagic acid, flavonoids, and alkaloids, were identified through phytochemical screening and an extensive literature review. With an emphasis on their function in diabetes-related pathways, the putative protein targets of these bioactive substances were obtained from the Swiss Target Prediction, STRING, and PubChem databases, while disease-associated genes relevant to diabetes were collected from the Gene Cards database. The STRING database was used to create a network of protein-protein interactions (PPIs), applying a confidence score threshold of ≥0.7 to identify key regulatory nodes within the diabetes-related network.

Common gene identification using Venny 2.1

To ascertain how targets connected to phytocompounds interact with genes linked to diabetes, the Venny 2.1 online tool was employed to generate a Venn diagram. The common genes shared between these datasets were extracted, representing potential molecular targets influenced by *E. angustifolius* in diabetes management. These overlapping genes were further analyzed to determine their biological significance in diabetes-related pathways.

Gene ontology (GO) and KEGG pathway analysis

GO analysis was performed to classify target genes based on their molecular functions, biological processes, and cellular components. KEGG pathway enrichment analysis identified key metabolic pathways associated with diabetes, including PI3K-Akt, AMPK, insulin, and TNF signalling, which are essential for glucose homeostasis and insulin sensitivity. Additionally, a compound-target-pathway network was constructed using Cytoscape to visualize molecular interactions and identify hub genes with significant roles in diabetes regulation.

By integrating these computational approaches, the study provided mechanistic insights into how *E. angustifolius* seed bioactives might exert anti-diabetic effects through multi-target interactions, offering a foundation for future in vivo and clinical studies.

Statistical analysis

Statistical analysis was performed to validate the significance of the identified targets and pathways. Using predictions from the STRING database, KEGG and GO enrichment studies were performed, with statistical significance determined primarily by the false discovery rate (FDR). In some cases, *P* values were reported alongside FDR values. Enrichment scores were calculated using a hypergeometric test, and pathways with an adjusted FDR < 0.05 were considered statistically significant. Using Cytoscape, network topology analysis was performed to determine hub genes according to degree centrality, betweenness centrality, and closeness centrality. The significance of PPIs was assessed using STRING, applying a confidence score threshold of ≥0.7. All statistical analyses were performed using R and SPSS software to ensure the reliability of the identified pathways and targets.

Results

Powder microscopy analysis

The powder microscopy of *E. angustifolius* seed revealed key diagnostic features critical for its pharmacognostic identification. Lignified xylem vessels exhibited elongated structures, while thick-walled phloem fibres and sclereids contributed to mechanical support and seed hardness. Wooden elements appeared as brownish, irregular fragments, while prismatic structures, likely representing mineral deposits or crystalline inclusions, were also observed (26) as shown in Figure 1. These findings further support its botanical authentication.

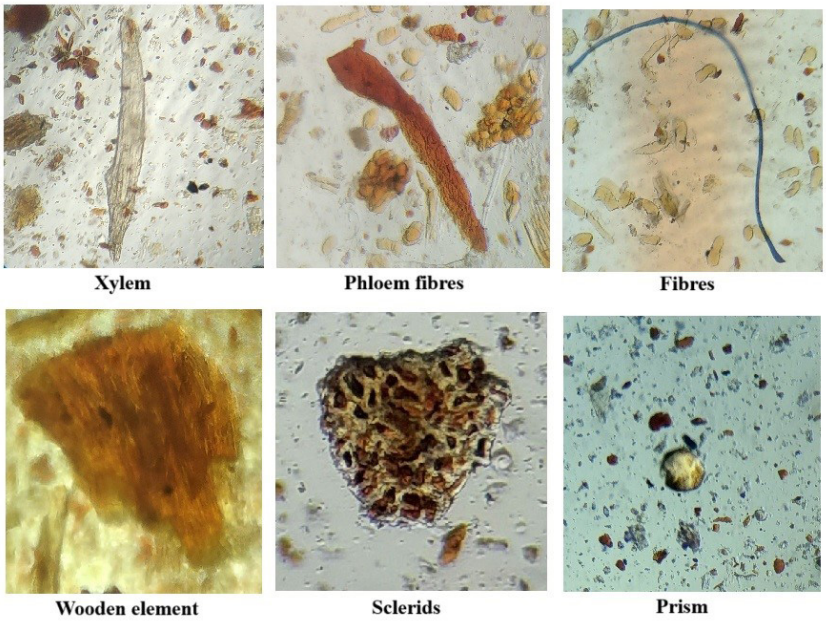

Figure 1. Powder microscopy of *Elaeocarpus angustifolius* seeds.

Physicochemical parameter analysis

Physicochemical analysis was performed to assess the quality and stability of *E. angustifolius* seed powder. The results indicated that the moisture content ranged between 5–8%, suggesting low susceptibility to microbial contamination and enhanced shelf life. Total ash content varied from 3% to 7%, indicating the presence of minerals with minimal extraneous matter. Acid-insoluble ash values ranged between 0.5% to 2%, confirming low contamination from soil or sand, thereby ensuring purity and quality compliance.

Microscopical analysis

Microscopic analysis of *E. angustifolius* was conducted using various staining agents to enhance cellular structure visibility, as shown in Figure 2. Each stain provided a distinct contrast, aiding in anatomical identification and authentication. Carbonyl fuchsin-stained lignified tissues are reddish-brown, confirming their structural reinforcement. Crystal violet produced a deep purple stain, effectively highlighting nucleic acids and cell wall components for nuclear differentiation. Methylene blue provided a blue-green contrast, efficiently staining acidic components such as nuclei and cytoplasm. Kovac's reagent yielded a yellow-brown stain, indicating the presence of specialized metabolites. Safranin highlighted lignified and secondary wall structures in red-pink, aiding in the identification of sclerenchyma and xylem. Nigrosin created a negative staining effect, enhancing overall cellular morphology without interfering with internal structures. The combination of chloral hydrate and HCl cleared the sample, improving the visualization of embedded prism crystals and oil glands.

The phytochemical analysis demonstrated that *E. angustifolius* seed, as shown in Table 1, contained a broad spectrum of bioactive compounds with potential medicinal benefits. The variation in solubility across different solvents underscores the significance of choosing the right solvent for phytochemical research and extraction techniques. These findings support the plant's traditional use in herbal medicine and warrant further investigation into its pharmacological applications.

TLC analysis

The presence of quercetin in the sample was verified by TLC. The quercetin standard exhibited a bright yellow fluorescent spot under UV light at 366 nm, with a distinct migration pattern. The test sample displayed a fluorescent spot at the same retention factor (Rf), indicating the presence of quercetin. The calculated Rf value for both the standard and sample was 0.53, confirming successful identification. Quercetin was successfully separated and seen using the solvent system of toluene, ethyl acetate, and formic acid (7:2:1).

Network pharmacology analysis of bioactive compounds in metabolic disorders

Network pharmacology analysis revealed that the bioactive compounds in *E. angustifolius* interacted with multiple target proteins involved in metabolic regulation. Quercetin, kaempferol, ellagic acid, flavonoids, and myricetin were identified as key bioactive compounds that modulated pathways linked to diabetes and metabolic disorders.

Table 2 presents the network pharmacology analysis of bioactive compounds from *E. angustifolius*, identified

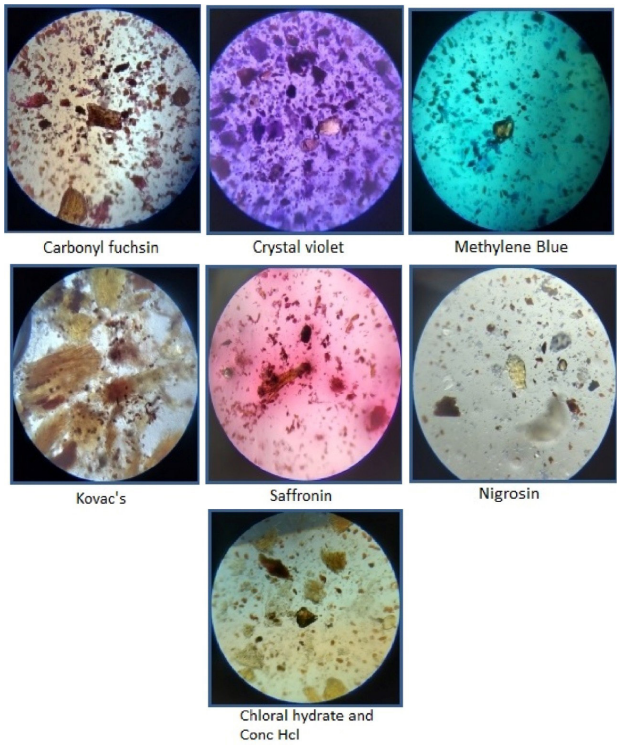

Figure 2. Microscopic analysis of *Elaeocarpus angustifolius* seeds stained with various agents.

through a literature survey (27). These compounds interact with multiple target proteins involved in metabolic regulation, influencing pathways related to diabetes and associated disorders. The statistical significance of pathway enrichment was indicated by *P* values, with lower values signifying stronger associations.

Quercetin ($P = 0.003$, 0.007, 0.012, 0.015) enhanced insulin sensitivity, glucose metabolism, and reduced inflammation via PI3K-Akt, AMPK, insulin, and TNF signalling. Kaempferol ($P = 0.007$) supported lipid metabolism and β-cell function through FoxO, cAMP, VEGF, and AMPK pathways. Ellagic acid ($P = 0.012$) regulated adipogenesis and insulin sensitivity via PPAR, adipocytokine, and insulin signalling. Flavonoids ($P = 0.003$, 0.007, 0.012) improved glucose uptake and reduced insulin resistance by targeting PI3K-Akt, AMPK, and insulin pathways. Myricetin ($P = 0.015$) exerted anti-inflammatory effects through T-cell receptor, TNF, and FoxO signalling as depicted in Figure 3.

Table 3 presents the results of pathway enrichment analysis, identifying key metabolic and inflammatory pathways modulated by bioactive compounds from *E. angustifolius*. The FDR values indicated the statistical significance of pathway enrichment compared to background gene expression, with lower FDR values suggesting stronger associations with diabetes-related mechanisms.

The most significantly enriched pathways included PI3K-Akt, AMPK, and insulin signalling pathways (FDR < 0.0001), which demonstrated a strong association with glucose uptake and metabolic regulation. Additionally, inflammatory pathways, such as TNF signalling (FDR = 7.35×10^{-5}) and IL-17 signalling (FDR = 2.75×10^{-5}) were identified, highlighting the potential anti-inflammatory effects of these compounds. These findings suggested that the bioactive compounds from *E. angustifolius* might contribute to both metabolic homeostasis and inflammation suppression, playing a crucial role in

Table 1. Phytochemical analysis of *Elaeocarpus angustifolius* across various solvent extracts

Test	Phytochemical test	Petroleum ether	Chloroform	Acetone	Ethanol	Methanol	Water
Test for alkaloids	Drangendroff's test	Positive	Positive	Positive	Positive	Positive	Positive
	Hager's test	Positive	Positive	Positive	Positive	Positive	Positive
	Mayers test	Positive	Negative	Positive	Positive	Positive	Positive
	Wagner's test	Positive	Positive	Positive	Positive	Positive	Positive
Test for steroids and terpenoids	Salkowski test	Positive	Negative	Positive	Positive	Positive	Negative
	Liebermann-Burchard test	Positive	Negative	Positive	Positive	Positive	Negative
Test for carbohydrate	Molisch's test	Positive	Positive	Negative	Positive	Positive	Positive
	Benedict's test	Positive	Positive	Negative	Positive	Negative	Positive
	Fehling's test	Negative	Positive	Negative	Positive	Negative	Positive
	Barfoed's test	Negative	Negative	Positive	Negative	Negative	Negative
Test for proteins	Million test	Negative	Negative	Negative	Negative	Negative	Negative
	Ninhydrin test	Negative	Negative	Negative	Negative	Negative	Negative
Test for tannins	Dilute nitric acid test	Positive	Positive	Positive	Positive	Positive	Negative
Test for saponins	Precipitation test	Positive	Positive	Positive	Positive	Positive	Positive
Test for glycosides	Borntrager's test	Positive	Negative	Positive	Positive	Positive	Negative
	Keller-Killiani test	Positive	Positive	Negative	Positive	Positive	Positive
Test for Flavonoids	Shinoda test	Negative	Negative	Negative	Positive	Positive	Negative
Test for phenols	Ferric chloride test	Positive	Negative	Negative	Positive	Positive	Negative

Table 2. Bioactive compounds of *Elaeocarpus angustifolius* seed and their target protein

Bioactive compound	Target proteins	Impacted pathways and p value	Relevance to metabolic disorders
Quercetin	MAPK1, PIK3R2, NFKB1, MTOR, GSK3B, PRKACA	PI3K-Akt (P = 0.003), AMPK (P = 0.007), Insulin (P = 0.012), TNF (P = 0.015)	Enhances insulin signaling, glucose metabolism and reduces inflammation.
Kaempferol	PIK3CD, ACACA, PIK3CB, MAPK1, NFKB1	FoxO, cAMP, VEGF, AMPK (P = 0.007)	Improves lipid metabolism and supports β-cell function.
Ellagic acid	NFKB1, STAT3, MTOR, SLC2A1, PTPN11	PPAR, Adipocytokine, Insulin (P = 0.012)	Regulates adipogenesis and insulin sensitivity.
Flavonoids	IRS1, INSR, PRKACA, GSK3B, MTOR	Insulin (P = 0.012), PI3K-Akt (P = 0.003), AMPK (P = 0.007)	Modulates glucose uptake, reduces insulin resistance.
Myricetin	MAPK1, PIK3R2, MAP2K2, PRKACA, GSK3B	T cell receptor, TNF (P = 0.015), FoxO	Anti-inflammatory effects, regulates immune response.

diabetes management.

Table 4 presents the disease-gene associations modulated by bioactive compounds from *E. angustifolius*. The FDR values indicated the statistical significance of gene enrichment, with lower values reflecting stronger associations.

Significant links were observed with T2DM (FDR = 2.31×10^{-12}), insulin resistance (FDR = 4.12×10^{-10}), and diabetic nephropathy (FDR = 1.02×10^{-8}). Key genes such as MAPK1, PIK3R2, NFKB1, MTOR, GSK3B, PRKACA, PIK3CD, ACACA, PIK3CB, IRS1, INSR, and TLR4 influenced insulin signalling, glucose uptake, lipid metabolism, and inflammation. These results highlighted the therapeutic potential of these compounds in managing metabolic and inflammatory complications of diabetes.

Pathway enrichment analysis revealed significant involvement of the PI3K/AKT signalling pathway (FDR: 1.0E-30) and PIP3-activated AKT signalling (FDR: 1.0E-25), both critical for insulin response, glucose uptake, and cell survival. Interleukin signalling (FDR: 1.0E-20) was enriched, linking cytokine activity to diabetes-related inflammation, while SCF-KIT signalling (FDR: 1.0E-18) suggested a role in β-cell regeneration.

Cellular component enrichment identified the plasma membrane region (FDR: 1.0E-10), receptor complexes (FDR: 1.0E-09), cell surface (FDR: 1.0E-08) and protein kinase complexes (FDR: 1.0E-07) as key components in insulin signalling and metabolic regulation.

Tissue expression analysis indicated the highest gene expression in the liver (FDR: 1.0E-12), followed by the placenta (FDR: 1.0E-10), leukemia cells (FDR: 1.0E-08) and bone marrow cells (FDR: 1.0E-07), suggesting a

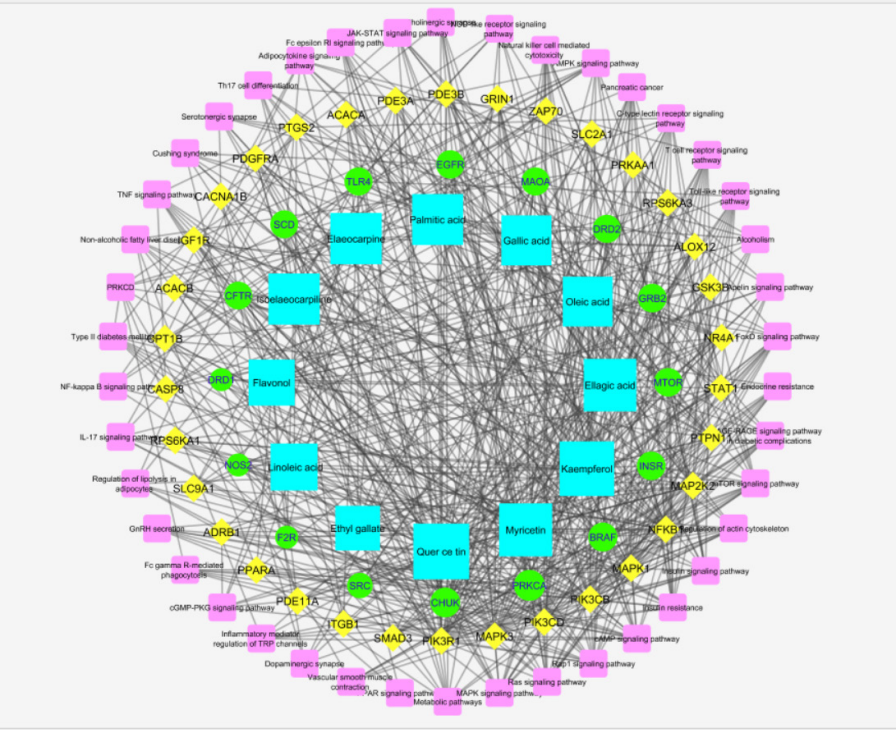

Figure 3: Network representation of molecular pathways and protein targets modulated by the phytocompounds from *Elaeocarpus angustifolia* seeds.

Table 3. Pathway enrichment analysis of bioactive compounds from *Elaeocarpus angustifolius* seeds in diabetes-related pathways

KEGG ID	Pathway name	Gene count	Background gene count	Strength (Compared to control)	FDR or adjusted *P* value	Key genes	Relevance to diabetes
hsa04151	PI3K-Akt signaling	9	349	0.95	1.77×10^{-7}	MAPK1, PIK3R2, NFKB1, MTOR, GSK3B, PRKACA, PIK3CD, ACACA, PIK3CB	Regulates glucose uptake, insulin sensitivity and β-cell function.
hsa04152	AMPK signaling	6	120	1.01	1.1×10^{-4}	CFTR, PIK3R2, MTOR, PIK3CD, ACACA, PIK3CB	Controls energy balance, lipid oxidation and insulin sensitivity.
hsa04910	Insulin signaling	9	132	1.15	1.77×10^{-7}	MAPK1, PIK3R2, MAP2K2, PRKACA, GSK3B, MTOR, PIK3CD, ACACA, PIK3CB	Enhances insulin response and glucose metabolism.
hsa04660	T cell receptor signalling	9	100	1.27	2.32×10^{-8}	MAPK1, PIK3R2, NFKB1, MAP2K2, GSK3B, PIK3CD, ITK, PAK4, PIK3CB	Modulates immune responses that contribute to insulin resistance.
hsa04668	TNF signalling	6	111	1.05	7.35×10^{-5}	MAPK1, PIK3R2, NFKB1, CASP8, PIK3CD, PIK3CB	Reduces inflammation and prevents insulin resistance.
hsa04657	IL-17 signalling	6	91	1.13	2.75×10^{-5}	MAPK1, NFKB1, MMP1, GSK3B, CASP8, HSP90AB1	Controls inflammatory responses linked to type 2 diabetes.
hsa04920	Adipocytokine signalling	5	68	1.18	9.03×10^{-5}	NFKB1, STAT3, MTOR, SLC2A1, PTPN11	Modulates fat metabolism and insulin action.
hsa04068	FoxO signalling	7	126	1.06	1.44×10^{-5}	MAPK1, PIK3R2, MAP2K2, STAT3, CDK2, PIK3CD, PIK3CB	Regulates oxidative stress, β-cell survival and glucose metabolism.
hsa04024	cAMP signalling	9	207	0.95	4.45×10^{-6}	CFTR, MAPK1, PIK3R2, NFKB1, MAP2K2, PRKACA, PIK3CD, ROCK1, PIK3CB	Enhances glucose regulation, promotes energy metabolism.
hsa04370	VEGF signalling	7	56	1.41	1.45×10^{-7}	MAPK1, PIK3R2, MAP2K2, NOS3, PIK3CD, PRKCA, PIK3CB	Supports vascular health and insulin delivery to tissues.

FDR: False discovery rate.

Table 4. Gene-disease associations of bioactive compounds from *Elaeocarpus angustifolius* seeds

Diabetes-related condition	Gene count	FDR or adjusted *P* value	Key genes	Implications
T2DM	12	2.31×10^{-12}	MAPK1, PIK3R2, NFKB1, MTOR, GSK3B, PRKACA, PIK3CD, ACACA, PIK3CB, IRS1, INSR, TLR4	Modulates insulin signaling and β-cell function, improving insulin sensitivity.
Insulin resistance	10	4.12×10^{-10}	PIK3R2, IRS1, INSR, PRKACA, GSK3B, MTOR, TLR4, PIK3CB, PIK3CD, ACACA	Reduces inflammation, enhances insulin receptor activity, and promotes glucose uptake.
Obesity and metabolic syndrome	9	6.78×10^{-9}	STAT3, MTOR, SLC2A1, ACACA, PIK3CB, NFKB1, PPARA, PPARG, PIK3CD	Regulates lipid metabolism, promotes fat breakdown, prevents weight gain.
Diabetic nephropathy	8	1.02×10^{-8}	MAPK1, NFKB1, TLR4, GSK3B, PIK3CB, PRKACA, PIK3CD, ACACA	Protects renal cells, reduces kidney damage from oxidative stress.
Diabetic neuropathy	7	2.33×10^{-8}	MAPK1, NFKB1, TNF, PIK3R2, GSK3B, PRKACA, PIK3CB	Prevents nerve damage, reduces oxidative stress and inflammation.
Inflammation-induced insulin resistance	6	4.75×10^{-8}	NFKB1, TNF, TLR4, STAT3, PIK3CB, PIK3CD	Suppresses chronic inflammation, improving insulin action.
Glucose uptake dysfunction	6	7.94×10^{-8}	INSR, IRS1, PIK3CB, SLC2A1, PIK3CD, PRKACA	Enhances insulin-stimulated glucose transport.
Lipid dysregulation and fat accumulation	5	1.15×10^{-7}	ACACA, PPARA, PPARG, MTOR, PRKACA	Promotes fatty acid oxidation and lipid metabolism.
β-Cell dysfunction and insulin secretion defects	5	2.38×10^{-7}	MAPK1, GSK3B, PIK3CB, PRKACA, MTOR	Maintains pancreatic β-cell health and insulin secretion.

FDR: False discovery rate; T2DM: Type 2 diabetes mellitus.

potential metabolic-immune system link.

Disease-gene associations showed strong links to diabetes and metabolic disorders (FDR: 1.0E-15), cardiovascular diseases (FDR: 1.0E-12), cancer via the PI3K/AKT pathway (FDR: 1.0E-10), and immune-related diseases (FDR: 1.0E-09).

Molecular function analysis highlighted protein tyrosine kinase activity (FDR: 1.0E-30), phosphotransferase activity (FDR: 1.0E-25), and transmembrane receptor activity (FDR: 1.0E-20), all crucial for insulin signalling and metabolic regulation.

Biological processes were enriched in protein autophosphorylation (FDR: 1.0E-40), a response to reactive oxygen species (FDR: 1.0E-35) and protein phosphorylation (FDR: 1.0E-30), indicating their essential roles in insulin receptor activation, oxidative stress regulation, and metabolic control as depicted in Figure 4.

Discussion

The pharmacognostic evaluation of *E. angustifolius* seeds provided key insights into their medicinal significance. Microscopic examination revealed the presence of xylem vessels, which contribute to water conduction and plant rigidity, and phloem fibres that support nutrient transport. These structural components suggest the seed's resilience to environmental stress and mechanical damage, contributing to its protective role (28). Sclereids, known for their thickened walls, offer additional protection to the seed coat. The presence of prismatic structures highlights the seed's ability to store essential compounds, contributing to its physiological stability (29).

Physicochemical testing confirmed the seed's quality by demonstrating low moisture content, enhancing stability and reducing microbial contamination. Ash content analysis further supported the seed's purity (30). Histological analysis with various staining agents provided detailed insights into the plant's structural features, allowing accurate identification and quality assessment. Safranin and carbonyl fuchsin highlighted lignified tissues, while methylene blue and crystal violet facilitated nuclear differentiation. These techniques reinforced the importance of employing diverse methods in the quality

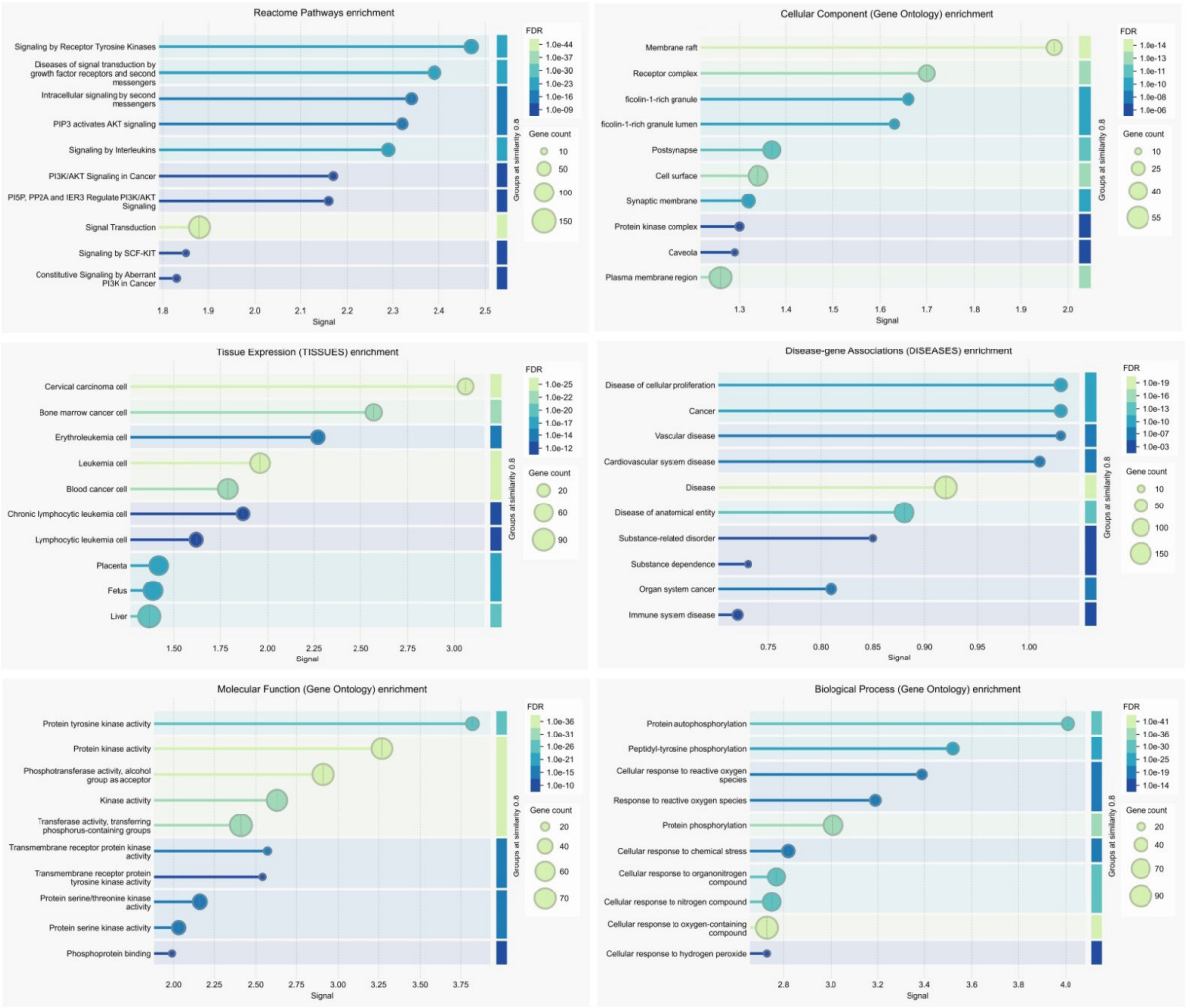

Figure 4. Gene ontology enrichment analysis of the protein targets hit by phytocompounds from *Elaeocarpus angustifolia* seed.

control of medicinal plants (31).

Phytochemical screening identified key bioactive compounds, including alkaloids, flavonoids, and terpenoids. The absence of proteins in all extracts indicated a focus on secondary metabolites, which have therapeutic potential, particularly for managing metabolic disorders (32). TLC analysis confirmed the presence of quercetin, a flavonoid known for its antioxidant and anti-inflammatory effects (33).

Network pharmacology analysis revealed that the bioactive compounds in *E. angustifolius* influenced vital metabolic pathways, including PI3K/Akt, AMPK, insulin, and TNF signalling. These pathways are critical in regulating glucose metabolism, insulin sensitivity, and inflammation (34). Enrichment analysis validated the compounds' involvement in glucose homeostasis, lipid metabolism, and β-cell protection. The activation of TNF and IL-17 signalling pathways suggests that the plant may help mitigate inflammation-induced insulin resistance (35).

Gene-disease association analysis identified critical genes, such as MAPK1, PIK3R2, NFKB1, and GSK3B, which are linked to type 2 diabetes, insulin resistance, and diabetic nephropathy (36). Additionally, genes like SLC2A1 and PRKACA, associated with glucose uptake and hyperglycemia prevention, highlight potential therapeutic targets (37). The study highlighted the importance of the PI3K/AKT signalling pathway (FDR: 1.0E-30) in insulin response and glucose uptake, reinforcing its role in maintaining insulin sensitivity (38).

The study also uncovered the role of interleukin signalling (FDR: 1.0E-20) in the interplay between chronic inflammation and metabolic disorders, while SCF-KIT signalling (FDR: 1.0E-18) suggested potential applications in β-cell regeneration (39). The findings underscore the critical role of cellular structures, such as plasma membrane complexes, in regulating insulin signalling and metabolic functions. Disruptions in these structures could lead to metabolic dysfunctions and impaired insulin responsiveness (40).

Furthermore, tissue expression patterns highlighted the liver's central role in glucose homeostasis and the placenta's role in metabolic adaptations during fetal development (41). The gene expression in leukemia and bone marrow cells suggested an emerging link between immune regulation and metabolic disorders (42). The PI3K/AKT pathway's involvement in both metabolic control and cancer progression offers potential for dual therapeutic strategies that balance metabolic benefits with cancer risks (43).

Finally, oxidative stress, a known contributor to insulin resistance and diabetic complications, was closely associated with the pathways analyzed. Therapeutic interventions targeting oxidative damage could potentially enhance insulin sensitivity and prevent the progression of metabolic disorders (44).

Overall, this research offers a thorough comprehension of the complex interplay between metabolic, inflammatory, and signalling pathways that regulate glucose metabolism and insulin function. Targeting these interconnected networks may lead to novel therapeutic strategies for diabetes management and metabolic disease prevention.

Conclusion

The detailed microscopic, physicochemical, and chromatographic analyses establish key diagnostic features and bioactive constituents that support its traditional use in herbal medicine. Through network pharmacology, we elucidated the molecular interactions of its phytocompounds, highlighting their relevance in metabolic disorders, particularly diabetes. These results add to the increasing amount of data regarding treatments generated from plants and emphasize the need for further pharmacological validation. This study is new since it takes an integrated approach, integrating contemporary computer analysis with traditional pharmacognostic methodologies to identify possible mechanisms of action. Future research should focus on in vivo studies and clinical trials to validate these therapeutic properties and explore formulation strategies for pharmaceutical applications.

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Conflict of interests

The authors declared no competing interests.

Ethical considerations

All authors observed ethical issues (including plagiarism, violations, falsification of data, falsification of double publication or submission, redundancy).

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