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Integrated Antioxidant and Network Pharmacology Evaluation of Enriched *Ficus septica* Extracts against Oxidative Stress

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Abstract

Oxidative stress is closely associated with the development of various chronic diseases, including cancer and metabolic disorders. *Ficus septica* contains phenolic and flavonoid compounds with potential antioxidant activity. This study aimed to evaluate and compare the antioxidant activity of methanol and purified extracts of *F. septica* leaves collected from two sites in Southeast Sulawesi, Indonesia, and to identify the oxidative stress-related molecular targets and pathways associated with its bioactive compounds. Leaves of *F. septica* from Kendari City and Muna Regency were extracted with methanol and purified using n-hexane. Antioxidant activity was evaluated using DPPH and FRAP assays. Phenolic and flavonoid compounds were further analyzed using a network pharmacology approach to identify the main hub targets and their associations with oxidative stress-related diseases. Purified extracts showed stronger antioxidant activity than crude methanol extracts in both assays. The purified Muna extract exhibited the highest activity, with IC_{50} values of 28.168 µg/mL (DPPH) and 36.171 µg/mL (FRAP). Purification increased flavonoid content by approximately 43-44%. Network pharmacology analysis identified 185 overlapping targets between *F. septica* metabolites and oxidative stress-related genes. TP53, AKT1, HSP90AA1, SRC, and ESR1 were identified as the main hub targets. KEGG pathway enrichment analysis highlighted several oxidative stress-related pathways, particularly PI3K/AKT, p53, endocrine-related, and chemical carcinogenesis pathways. These findings suggest that purified *F. septica* extracts possess strong antioxidant activity and potential multi-target therapeutic effects against oxidative stress-related diseases.

Keywords: Antioxidant activity; *Ficus septica*; flavonoids; network pharmacology; oxidative stress; phenolic compounds.

Abbreviations: Abbreviations: absorption, distribution, metabolism, and excretion (ADME); AKT serine/threonine kinase 1 (AKT1); biological process (BP); cellular component (CC); estrogen receptor 1 (ESR1); false discovery rate (FDR); ferric reducing antioxidant power (FRAP); *Ficus septica* (*F. septica*); gallic acid (GA); gallic acid equivalent (GAE); Gene Ontology (GO); gastrointestinal (GI); half-maximal inhibitory concentration (IC_{50}); heat shock protein 90 alpha family class A member 1 (HSP90AA1); hydrogen atom transfer (HAT); Kyoto Encyclopedia of Genes and Genomes (KEGG); mitogen-activated protein kinase (MAPK); molecular function (MF); phosphoinositide 3-kinase/protein kinase B (PI3K/AKT); protein-protein interaction (PPI); quercetin equivalent (QE); reactive oxygen species (ROS); single electron transfer (SET); SRC proto-oncogene non-receptor tyrosine kinase (SRC); total flavonoid content (TFC); total phenolic content (TPC); tumor protein p53 (TP53); 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ); ultraviolet-visible (UV-Vis).

INTRODUCTION

Oxidative stress, defined as the imbalance between the overproduction of reactive oxygen species (ROS) and the biological capacity to neutralize them, has been established as a central pathomechanism underlying a broad spectrum of chronic non-communicable diseases, including cancer, type 2 diabetes mellitus, cardiovascular disease, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Pizzino et al., 2017; Sies & Jones, 2020). At the cellular level, excessive ROS induces oxidative damage to lipids, proteins, and DNA, activating redox-sensitive signaling cascades, particularly the PI3K/AKT, MAPK, and p53 pathways that drive inflammation, genomic instability, apoptotic dysregulation, and ultimately disease progression (Liu et al., 2025). The global burden of oxidative stress-related diseases has intensified the search for effective antioxidant agents, with particular interest directed toward plant-derived natural products as safer and potentially multi-target alternatives to synthetic antioxidants (Muscolo et al., 2024).

Phenolic and flavonoid compounds represent the most pharmacologically significant class of plant secondary metabolites with antioxidant activity (Tungmunnithum et al., 2018). Their structural features, particularly the

number and position of hydroxyl groups on aromatic rings confer potent free radical-scavenging capacity through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, as well as the ability to chelate pro-oxidant metal ions and modulate the expression of endogenous antioxidant enzymes (Fraga et al., 2019; Mutha et al., 2021). Beyond direct chemical scavenging, mounting evidence indicates that flavonoids such as genistein and phenolic acids such as vanillic acid can modulate key redox-sensitive proteins including TP53, AKT1, and Nrf2, thereby reinforcing cellular antioxidant defense networks at the molecular level (Shirvanian et al., 2024; Swislocka et al., 2025). This dual action, direct radical scavenging combined with molecular target modulation positions phenolic-rich plant extracts as compelling candidates for multi-target antioxidant therapeutics.

Ficus septica Burm.f. (Moraceae), known locally in Southeast Sulawesi as *libho*, is a tropical medicinal plant widely distributed across the Indonesian archipelago. Ethnobotanical records document its traditional use for the treatment of inflammatory conditions, febrile illnesses, and dermatological disorders (Hamzah et al., 2022; Tahoangako et al., 2023). Phytochemical investigations have identified a structurally diverse array of bioactive secondary metabolites in *F. septica*, including isoflavones (genistein, genistoside), phenolic acids (vanillic acid, aesculin, pungenin), alkaloids (ficuseptamine A-C, norruspoline), and unique arylnaphthalide lignans (ficusin A, ficusin B) (Hawari et al., 2021; Ueda et al., 2009). These compound classes have individually demonstrated antioxidant, anti-inflammatory, and cytotoxic activities in related Ficus species (Arfan et al., 2025; Hasnat et al., 2024), suggesting that *F. septica* possesses significant bioactive potential that remains incompletely characterized.

Despite the phytochemical profile of *F. septica* having been previously reported, important gaps remain in the current literature. In particular, the effect of purification on the antioxidant activity of *F. septica* extracts, especially in relation to changes in phenolic and flavonoid composition, has not been investigated. In addition, the molecular targets and signaling pathways underlying the antioxidant effects of *F. septica* compounds and their relevance to oxidative stress-related diseases remain unclear.

Network pharmacology provides a complementary approach to address these gaps by integrating bioinformatics and molecular target prediction to identify interactions between bioactive compounds, protein targets, and disease-related pathways (Li & Kar, 2025). This approach also enables the identification of hub proteins and enriched signaling pathways underlying the multi-compound and multi-target activities characteristic of plant extracts (Akinduko et al., 2026). Combined with in vitro validation, network pharmacology can provide both experimental evidence of antioxidant activity and computational insights into the underlying molecular mechanisms.

Accordingly, the present study was designed to evaluate and compare the antioxidant activity of methanol and purified extracts of *F. septica* leaves collected from two geographically distinct sites in Southeast Sulawesi, and to identify the key molecular targets and oxidative stress-related pathways associated with the phenolic and flavonoid compounds of *F. septica* using an integrated network pharmacology approach. Therefore, this study providing a scientific basis for their traditional use and potential development as phytopharmaceutical candidates for oxidative stress-related diseases.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Leaves of *Ficus septica* were collected from two locations in Southeast Sulawesi, Indonesia, namely Kadia District, Kendari City, and Batalaiworu District, Muna Regency (Figure 1). The plant material was taxonomically identified at the Biology Education Program, Faculty of Teacher Training and Education, Universitas Halu Oleo, under identification number 9/BIO/PB/VI/2024. Species identification was conducted using the key sequence 11a-2b-4b-5a-6a-7a-8b-9a-10b-11b-12b-13b-14b-15b, which corresponded to *Ficus septica*. The collected leaves were air-dried under controlled conditions at temperatures below 45°C for four days. The dried material was then ground into coarse powder and stored in sealed dry containers prior to the maceration process.

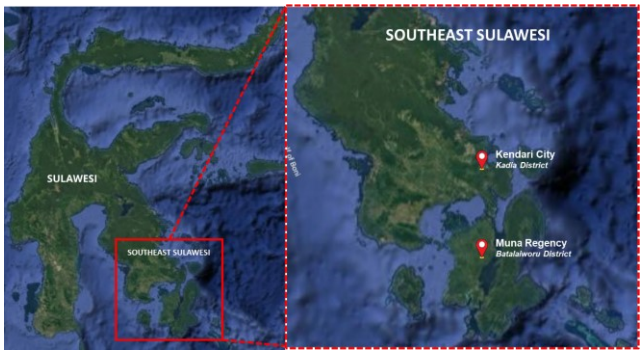


Figure 1. Sampling locations of *Ficus septica* leaves collected from Southeast Sulawesi, Indonesia.

Extraction and Purification

One kilogram of dried leaf powder was extracted using the maceration method with 10 L of methanol solvent over 3 × 24 hours. Maceration was selected as the extraction technique to prevent degradation of thermolabile bioactive compounds. Methanol was employed due to its broad polarity spectrum, enabling effective co-extraction of polar phenolic acids and less-polar flavonoid aglycones. The crude methanolic extract

was filtered and concentrated under reduced pressure using a rotary evaporator.

Purification of the crude extract was performed using liquid-liquid partitioning with n-hexane to remove non-polar ballast substances without eliminating polar active compounds. The purification endpoint was confirmed when the n-hexane layer appeared visually clear and colorless, indicating complete removal of lipophilic impurities. The purified extract was concentrated and stored in amber glass vials at 4°C until analysis.

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. Briefly, 0.1 mL of extract solution was mixed with 0.5 mL of Folin-Ciocalteu reagent and 1 mL of 10% Na_2CO_3, then incubated at room temperature for 30 min. Absorbance was measured at 765 nm. Gallic acid (GA) was used as the standard, and results were expressed as milligrams of gallic acid equivalents per grams of sample (mg GAE/g).

Total flavonoid content (TFC) was measured using the aluminum chloride ($AlCl_3$) colorimetric method. An aliquot of extract was reacted with 10% $AlCl_3$ in methanol, and absorbance was recorded at 415 nm after 15 min incubation. Quercetin was used as the reference standard, and results were expressed as milligrams of quercetin equivalents per grams of sample (mg QE/g). All measurements were performed in triplicate.

Antioxidant Activity Assays

DPPH Radical Scavenging Activity

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Extracts were dissolved in ethanol at concentrations ranging from 50 to 250 µg/mL. A DPPH solution (0.2 mmol/L) was prepared in ethanol. Equal volumes (3 mL each) of DPPH solution and extract were mixed and incubated for 30 min at room temperature in the dark. Absorbance was recorded at 517 nm using a UV–Vis spectrophotometer.

The Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was performed according to the method described by Sekhon Loodu and Rupasinghe (2019) with slight modifications. The FRAP reagent consisted of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), $FeCl_3$ solution, and acetate buffer (300 mM, pH 3.6). The mixture containing the extract and FRAP reagent was incubated at 37°C for 15 min. Absorbance was measured at 594 nm using a UV–Vis spectrophotometer. All assays were conducted in triplicate, and ascorbic acid was used as the positive control.

The percentage inhibition for each antioxidant activity assay was calculated using the following equation: % inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 represents the absorbance of the blank control and A_1 represents the absorbance of the sample. The IC_{50} value, defined as the concentration required to inhibit 50% of radical activity, was determined by linear regression analysis.

Network Pharmacology Analysis

Compound Retrieval and Pharmacokinetic Screening

The phenolic and flavonoid compounds present in *Ficus septica* were obtained from the KNApSAcK database (https://www.knapsackfamily.com/KNApSAcK/). The compounds were subsequently subjected to pharmacokinetic profile screening using SwissADME (https://www.swissadme.ch/) including several parameters such as molecular weight, solubility, gastrointestinal (GI) absorption, bioavailability, and Lipinski's rule of five violations. Compounds that met the screening criteria were further used for network pharmacology analysis related to oxidative stress-associated diseases.

Target Prediction and Disease Target Collection

Molecular targets of each bioactive compound were predicted using SwissTargetPrediction (www.swisstargetprediction.ch), filtering for *Homo sapiens* targets. Oxidative stress-related disease targets were independently retrieved from the GeneCards database (www.genecards.org) using 'oxidative stress' as the search term. Overlapping targets common to both the compound target set and the disease target set were identified using Venn diagram analysis and constituted the common target pool for downstream network analysis.

PPI Network Construction and Topology Analysis

The protein-protein interaction (PPI) network of common targets was constructed using the STRING database (https://string-db.org) with a high interaction confidence score of 0.700. The resulting network was imported into Cytoscape (v3.10) for visualization and topological analysis using CytoHubba. Three key topological parameters were calculated for each node: degree (number of direct interactions), betweenness centrality (frequency of occurrence on shortest paths between other nodes), and closeness centrality (inverse of the sum of shortest path distances to all other nodes). Hub genes were defined as targets appearing in the top rankings across all three parameters simultaneously.

Gene Ontology and KEGG Pathway Enrichment

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using ShinyGO (v0.80) with a false discovery rate (FDR) threshold of < 0.05. GO analysis encompassed biological processes (BP), cellular components (CC), and molecular functions (MF). KEGG pathway enrichment results were visualized as bar plots.

RESULTS AND DISCUSSION

Extraction Yield and Purification

Maceration of 1 kg of dried *F. septica* leaf powder with methanol yielded crude extracts of 60.32% (Kendari) and 60.55% (Muna). Following n-hexane purification, the yields decreased to 50.66% (Kendari) and 52.23% (Muna), respectively (Table 1). This reduction in yield is consistent with the removal of non-polar ballast compounds, such as lipids, chlorophylls, and waxes. The completion of the purification process was visually confirmed by the formation of a clear, colorless n-hexane layer, indicating the effective removal of lipophilic impurities. The purified extracts were consequently enriched in polar phenolic and flavonoid compounds, as further confirmed by TPC and TFC analyses.

Table 1. Yield of crude methanol and purified extracts of *F. septica* leaves collected from two locations in Southeast Sulawesi.

Extract type	Location	Weigh of samples (g)		
		Simplicia	Crude extract	Purified extract
Methanol extract	Kendari City	1000	603.2	305.5
Methanol extract	Muna Regency	1000	605.5	316.2

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC and TFC values of methanol and purified extracts from both locations are presented in Table 2. TPC values ranged from 10.772 to 11.8 mg GAE/g across all extracts, with Kendari methanol extract showing the highest phenolic content. Notably, purification did not markedly increase TPC, suggesting that phenolic acids were already well-represented in the crude extract.

In contrast, TFC values were substantially elevated in purified extracts compared to their methanol counterparts. Purified Kendari extract achieved 28.211 mg QE/g versus 19.637 mg QE/g in the crude extract, representing a 43.7% increase. Similarly, purified Muna extract exhibited 27.824 mg QE/g compared to 19.308 mg QE/g in the crude extract (44.1% increase). This marked enrichment of flavonoids following purification indicates that non-polar ballast substances in the crude methanol extract partially masked or diluted the flavonoid-rich fraction, and their removal by n-hexane partitioning effectively concentrated these bioactive constituents.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of *F. septica* leaves extracts from two locations in Southeast Sulawesi.

Location	Extract type	TPC (mg GAE/g sampel)	TFC (mg QE/g sampel)
Kendari City	Methanol	11.8 ± 0.603	19.637 ± 0.757
Muna Regency	Methanol	11.3 ± 0.273	19.308 ± 0.640
Kendari City	Purified	11.106 ± 0.701	28.211 ± 0.384
Muna Regency	Purified	10.772 ± 0.473	27.824 ± 0.313

Antioxidant Activity

The antioxidant activities of all extracts, assessed using the DPPH and FRAP assays, are summarized in Table 3. A consistent pattern was observed across both methods, in which the purified extracts exhibited significantly stronger antioxidant activity (lower IC_{50} values) than their corresponding crude methanol extracts, while the Muna-derived extracts showed the highest activity within each extract category.

Table 3. Antioxidant activity of crude methanol and purified extracts of *F. septica* leaves collected from two locations in Southeast Sulawesi.

Samples	Location	DPPH IC_{50} (µg/mL)	FRAP IC_{50} (µg/mL)
Methanol extract	Kendari City	46.602 ± 0.797	49.198 ± 0.451
Methanol extract	Muna Regency	40.681 ± 0.257	43.667 ± 0.575
Purified extract	Kendari City	35.921 ± 0.762	38.749 ± 0.838
Purified extract	Muna Regency	28.168 ± 0.747	36.171 ± 0.745
Ascorbic acid (std)	-	9.310 ± 0.078	8.557 ± 0.417

In the DPPH assay, the purified Muna extract exhibited the lowest IC_{50} value of 28.168 µg/mL, compared to 40.681 µg/mL for the corresponding crude extract, representing a 44.42% improvement. Meanwhile, the purified Kendari extract showed an IC_{50} value of 35.921 µg/mL, compared to 46.602 µg/mL for the crude extract, corresponding to a 29.73% improvement.

In the FRAP assay, the purified and crude extracts from Kendari yielded IC_{50} values of 38.749 µg/mL and 49.198 µg/mL, respectively, indicating a 26.96% improvement after purification. Similarly, the purified and crude extracts from Muna showed IC_{50} values of 36.171 µg/mL and 43.667 µg/mL, respectively, corresponding to a 20.72% improvement.

All extracts exhibited lower antioxidant activity than ascorbic acid (IC_{50} = 8.557-9.310 µg/mL across assays), which is expected because ascorbic acid is a pure reference compound rather than a complex plant extract. Nevertheless, the extracts can still be classified as having strong antioxidant activity, as all IC_{50} values were below 50 µg/mL (Itam et al., 2021). The consistent enhancement of antioxidant activity following n-hexane purification, observed across all assays, may be attributed to the enrichment of flavonoid compounds, as indicated by the 43-44% increase in TFC after the removal of non-polar ballast substances.

The higher antioxidant activity consistently observed in extracts from Muna compared to Kendari may reflect differences in the ecological microenvironments of the two collection sites. Environmental factors such as UV exposure, soil nutrient composition, and water availability are known to influence the biosynthesis of secondary metabolites in plants (Qaderi et al., 2023), potentially leading to variations in the accumulation of phenolic and flavonoid compounds among populations.

The consistent results obtained from both antioxidant assays, which involve different mechanisms of action (hydrogen atom transfer in DPPH and single-electron transfer in FRAP), further support the role of the enriched flavonoid fraction in enhancing the antioxidant activity of purified *F. septica* extracts.

Network Pharmacology Analysis

Pharmacokinetic Screening and Target Identification

A total of 12 phenolic and flavonoid compounds from *F. septica* were subjected to pharmacokinetic analysis to evaluate their suitability for subsequent analyses. The identified compounds from *F. septica* are presented in Figure 2, while the predicted pharmacokinetic profiles are summarized in Table 4.

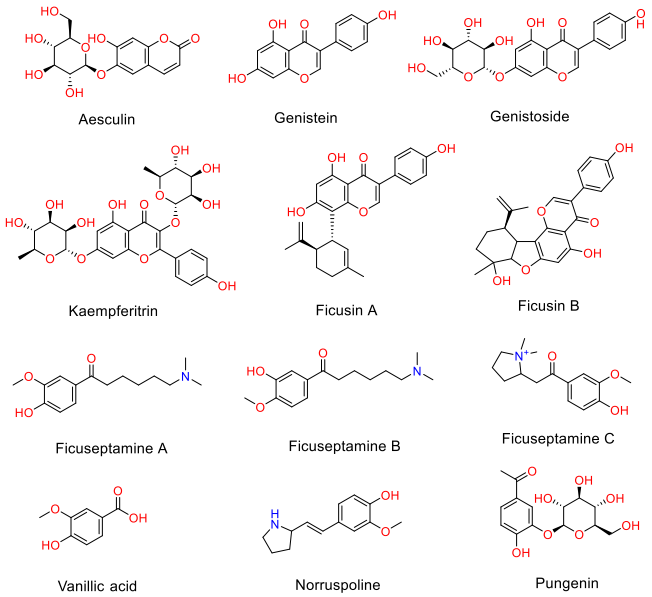


Figure 2. Phenolic and flavonoid compounds identified from *F. septica* collected from KNApSAcK database.

Table 4. Predicted pharmacokinetic profiles of phenolic and flavonoid compounds from *F. septica.*

Compounds	Formula	MW (g/mol)	Solubility (ESOL)	GI absorption	BA score	Ro5 violation
Aesculin	$C_{15}H_{16}O_9$	340.28	Very soluble	Low	0.55	0
Genistein	$C_{15}H_{10}O_5$	270.24	Soluble	High	0.55	0
Genistoside	$C_{21}H_{20}O_{10}$	432.38	Soluble	Low	0.55	1
Kaempferitrin*	$C_{27}H_{30}O_{14}$	578.52	Soluble	Low	0.17	3
Ficusin A	$C_{25}H_{24}O_5$	404.46	Poorly soluble	High	0.55	0
Ficusin B	$C_{25}H_{24}O_6$	420.45	Moderate soluble	High	0.55	0
Ficuseptamine A	$C_{15}H_{23}NO_3$	265.35	Soluble	High	0.55	0
Ficuseptamine B	$C_{15}H_{23}NO_3$	265.35	Soluble	High	0.55	0
Ficuseptamine C	$C_{15}H_{22}NO_3$	264.34	Soluble	High	0.55	0
Vanillic acid	$C_8H_8O_4$	168.15	Soluble	High	0.85	0
Norruspoline	$C_{13}H_{17}NO_2$	219.28	Soluble	High	0.55	0
Pungenin	$C_{14}H_{18}O_8$	314.29	Very soluble	Low	0.55	0

Note: **Excluded from downstream analysis due to low bioavailability (BA) score and ≥2 Lipinski's rule of five violations. MW, molecular weight; GI, gastrointestinal absorption; BA, bioavailability; Ro5, Lipinski's rule of five.*

Based on the analysis, kaempferitrin was excluded due to its low bioavailability and non-compliance with Lipinski's rule of five. Consequently, only 11 compounds were selected for target identification against oxidative stress-related diseases. Initially, 523 unique targets associated with *F. septica* metabolites were retrieved from SwissTargetPrediction. In parallel, 1,464 oxidative stress-related targets were collected from the GeneCards database. Venn analysis identified 185 overlapping targets, representing potential therapeutic intersections between *F. septica* metabolites and oxidative stress (Figure 3A).

These shared targets were subsequently used to construct a compound–target interaction network

consisting of 185 nodes and 479 edges (Figure 3B), indicating a complex multi-target interaction profile. This network highlights the polypharmacological potential of phenolic and flavonoid metabolites from *F. septica* in modulating oxidative stress-related targets.

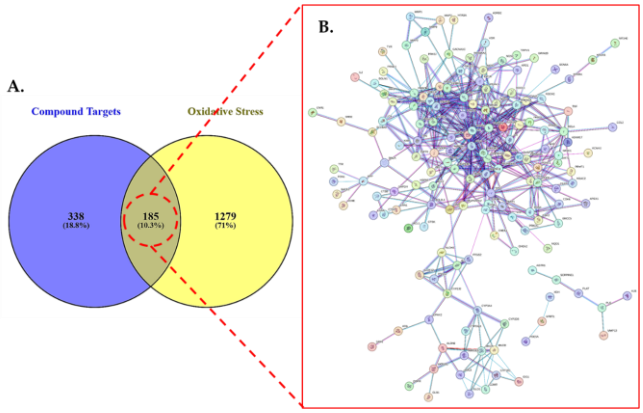


Figure 3. Predicted targets associated with *F. septica* metabolites and oxidative stress. (A) Venn diagram illustrating the overlap between oxidative stress-related targets and predicted targets of *F. septica* metabolites. (B) Protein-protein interaction (PPI) network of 185 shared targets associated with oxidative stress and *F. septica* metabolites.

PPI Network Construction and Hub Gene Identification

Network analysis of the 185 overlapping targets identified several proteins with prominent roles in oxidative stress-related diseases. Hub targets were determined based on three topological parameters, namely degree, closeness centrality, and betweenness centrality, which collectively reflect the biological importance and connectivity of proteins within the protein–protein interaction (PPI) network.

Among these parameters, degree value represents the number of predicted interactions between compounds and their associated target proteins. Proteins with higher degree values are considered to have greater influence within the network. Meanwhile, network topology was further evaluated using closeness centrality and betweenness centrality analyses. Closeness centrality measures how closely a protein is connected to other nodes in the network, whereas betweenness centrality identifies proteins that act as key intermediaries in network communication. The top 10 proteins obtained from each centrality analysis were subsequently compared using a Venn diagram (Figure 4A-C).

Based on this analysis, five proteins, namely tumor protein p53 (TP53), AKT serine/threonine kinase 1 (AKT1), heat shock protein 90 alpha family class A member 1 (HSP90AA1), SRC proto-oncogene non-receptor tyrosine kinase (SRC), and estrogen receptor 1 (ESR1), were consistently identified across all three parameters (Figure 4D). These proteins were therefore designated as major hub proteins due to their high interaction frequencies, suggesting their central roles in the oxidative stress-related network and their potential as therapeutic targets. Meanwhile, the degree, betweenness, and closeness values of the identified hub proteins are presented in Table 5.

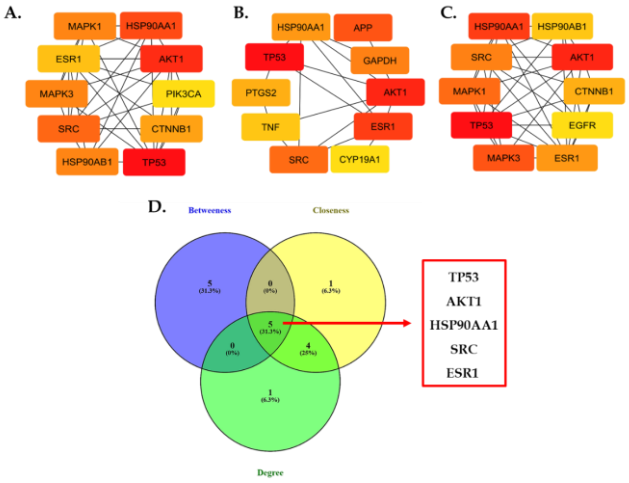


Figure 4. Top 10 potential target proteins ranked based on (A) degree centrality, (B) betweenness centrality, and (C) closeness centrality analyses. (D) Venn diagram showing five consensus targets shared across all three centrality parameters.

Table 5. Key hub proteins identified from the protein-protein interaction (PPI) network of shared targets between *F. septica* bioactive compounds and oxidative stress-related disease targets.

Gene symbol	Full name	Degree	Betweenness	Closeness	Pathway association
TP53	Tumor protein p53	44	4562.99	88.36	p53 signaling, apoptosis, ROS regulation
AKT1	AKT serine/threonine kinase 1	35	2901.29	85.31	PI3K/AKT signaling, oxidative stress
HSP90AA1	Heat shock protein 90 alpha class A member 1	31	1817.91	81.83	Protein folding under oxidative stress
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	28	2321.55	79.54	MAPK cascade, oxidative phosphorylation
ESR1	Estrogen receptor 1	23	2457.45	78.54	Estrogen signaling, redox regulation

Gene Ontology and KEGG Pathway Enrichment

GO biological process enrichment analysis revealed that the common targets are predominantly involved in the regulation of cell cycle arrest, response to reactive oxygen species, apoptotic signaling, transcriptional regulation of oxidative stress response genes, and protein

phosphorylation cascades (Figure 5A). GO cellular component analysis indicated localization of targets in the nucleus, cytoplasm, and membrane-associated signaling complexes (Figure 5B). GO molecular function terms were dominated by protein kinase activity, transcription factor binding, and ATP binding (Figure 5C).

KEGG pathway enrichment analysis identified several significantly enriched pathways (FDR < 0.05), particularly the PI3K/AKT, p53, endocrine/hormone-related, and MAPK signaling pathways, which are well known to be involved in oxidative stress responses and ROS-mediated disease progression (Figure 5D). These pathways are closely associated with oxidative stress-driven diseases, including cancer and metabolic disorders, suggesting a mechanistic link between the antioxidant compounds of *F. septica* and their potential therapeutic effects.

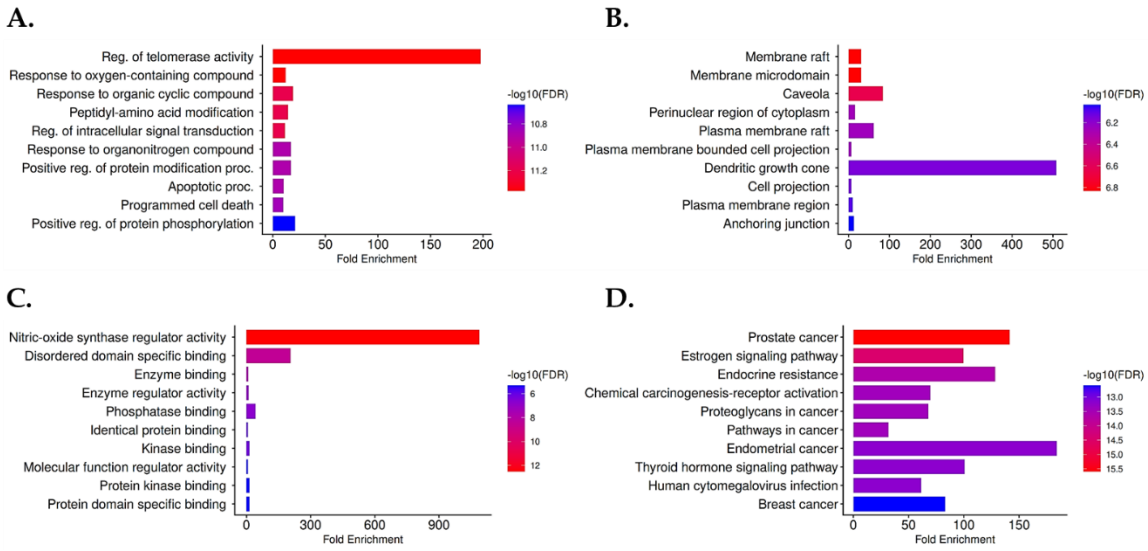


Figure 5. Gene Ontology and KEGG Pathway Enrichment.

In the context of cancer, oxidative stress promotes carcinogenesis through ROS-induced mutagenesis, activation of redox-sensitive oncogenic transcription factors (NF-κB and AP-1), and suppression of tumor suppressor functions (Henkler et al., 2010). The enrichment of the chemical carcinogenesis–reactive oxygen species and p53 signaling pathway, within the *F. septica* target network suggests that its phenolic and flavonoid constituents may exert protective effects against oxidative carcinogenesis by enhancing p53-mediated DNA damage surveillance and apoptotic elimination of ROS-damaged cells. This mechanism is consistent with the reported anticancer activities of genistein (Naponelli et al., 2025).

In metabolic disorders, the PI3K/AKT signaling cluster appears particularly relevant, as oxidative stress disrupts insulin signaling through inhibition of AKT1 phosphorylation in peripheral tissues, thereby contributing to insulin resistance and type 2 diabetes (Henriksen et al., 2011). The identification of AKT1 and PIK3CA as key hub targets, together with the enrichment of the PI3K-Akt signaling pathway, suggests that *F. septica* extracts may mitigate oxidative stress-induced insulin resistance through restoration of AKT1-mediated signaling.

Despite the promising findings, this study has several limitations. The antioxidant activity of *F. septica* extracts was evaluated only through in vitro assays, which may not fully reflect their biological effects under physiological conditions. In addition, the phytochemical analysis mainly focused on identified phenolic and flavonoid compounds, while other potentially bioactive metabolites present in *F. septica* were not comprehensively explored. These limitations should be addressed in future studies to further elucidate the therapeutic potential and molecular mechanisms of *F. septica*.

CONCLUSIONS

This study demonstrated that purified extracts of *F. septica* leaves exhibited stronger antioxidant activity than crude methanol extracts, with the Muna Regency-derived extract showing the highest activity in both DPPH and FRAP assays. The enhanced antioxidant activity following n-hexane purification was associated with the enrichment of phenolic and flavonoid compounds after the removal of non-polar constituents. Network pharmacology analysis of the phenolic and flavonoid compounds of *F. septica* further identified TP53, AKT1,

HSP90AA1, SRC, and ESR1 as key hub targets potentially involved in its antioxidant mechanisms. KEGG pathway enrichment analysis revealed the involvement of several oxidative stress-related pathways, particularly the PI3K/AKT, p53, endocrine-related, and chemical carcinogenesis pathways. These findings indicate that *F. septica* possesses antioxidant potential and may serve as a promising phytopharmaceutical candidate for oxidative stress-related diseases.

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Authors' Contributions: Arfan, Yamin, and Ruslin designed the study. Arfan conducted the computational analyses. Arfan and Muhammad Jefriyanto Budikafa contributed to validation, formal analysis, data curation, and wrote and revised the manuscript. Arfan, Sitti Aisyah Aprilia, Nur Rayani, Finantha Annisa, and Wa Ode Fitrawati Basrin contributed to the experimental work, antioxidant evaluation, and manuscript preparation. Yamin and Ruslin supervised the study and provided critical revisions. Ruslin acquired the funding. All authors reviewed and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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