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Original Article

Luteolin and Kaempferol from *Allium cepa* var. *aggregatum* and *Allium porrum*: Integrated Metabolomic and Network-Based Evidence for Antioxidant Activity in THP-1 Cells

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ABSTRACT

Background: Oxidative Stress (OS) is a crucial contributor to chronic diseases, such as cancer and metabolic disorders, through an excess of Reactive Oxygen Species (ROS), which leads to redox imbalance and causes molecular and cellular damage, mitochondrial dysfunction and chronic inflammation. Antioxidants derived from edible plant species are recognized as safer therapeutic alternatives due to their diverse bioactive metabolites. **Objectives:** The antioxidant potential of leaf extract of Shallot greens (*Allium cepa* var. *aggregatum*) and leek (*Allium porrum*) was evaluated by using *in vitro* assays, metabolite profiling and system pharmacology analysis. **Materials and Methods:** Total phenolic and flavonoid contents were determined and antioxidant potential was evaluated on the basis of DPPH, FRAP and radical scavenging assays. Cellular antioxidant activity in hydrogen peroxide induced oxidative stress in THP-1 monocyte cells was studied by MTT assay. **Results:** Shallot greens revealed higher antioxidant content than leek leave extract, with TPC of 98.2±0.5 mg GAE/g (ACA), 93.8±0.7 mg GAE/g (AP) and TFC of 144.1±2.3 mg rutin/g (ACA) 106.8±3.8 mg rutin/g (AP). In DPPH assay, showed 70.07±0.25% inhibition at 100 µg/mL (IC_{50} 57.01 µg/mL) compared with leek extract 65.15±0.34% (IC_{50} 70.88 µg/mL). LC-MS analysis identified 35 bioactive metabolites, mainly flavonoid and phenol including luteolin, kaempferol, morin, ferulic acid and p-coumaric acid. Network pharmacology results revealed AKT1, STAT3, HSP90AA1, EGFR, ESR1 and CASP3 as key hub proteins. **Conclusion:** These results provide mechanistic insights supporting the potential development of *Allium*-derived phytochemicals as therapeutic candidates for oxidative stress-related metabolic disorders.

Keywords: *Allium cepa* var. *aggregatum*, *Allium porrum*, LC-MS metabolite profiling, Luteolin, Oxidative stress, THP-1 monocyte cells.

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INTRODUCTION

During routine mitochondrial ATP production, Reactive Oxygen Species (ROS) are generated but are quickly removed by enzymatic and non-enzymatic antioxidants to maintain cellular homeostasis. Oxidative eustress maintains a subtle balance between pro-oxidant and the body's integrated antioxidant system, which is essential for proper cellular function and redox signaling (Avila *et al.*, 2020). However, when mitochondrial dysfunction or external triggers disturb this balance, it can results in the excessive accumulation of free radicals, which are unstable molecules with free electrons that readily react with essential biomolecules, forming reactive oxygen and nitrogen species. These excess reactive species results in oxidative stress. A condition that disrupts cellular homeostasis and inflicts structural damage to DNA, protein, and lipids. This oxidative overload creates a toxic cellular environment that boosts chronic inflammation and contributes to the progression of various degenerative diseases (Uremis and Uremis 2025).

Intake of greens, vegetables, seeds, nuts and fruits in our regular diet, which are rich in secondary metabolites and additionally the presence of minerals, vitamins and dietary fibers together increase the antioxidant levels and protect cells against oxidative stress and chronic diseases (Connell *et al.*, 2018). Thus, the study of natural antioxidants is a necessary cornerstone of emerging therapeutics. Moreover, the increasing public inclination towards herbal based

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therapies instead of synthetic ones throws light on the underlying concerns towards the adverse side effects of synthetic drugs, thus emphasizing the need to look towards natural sources (Othman *et al.*, 2019).

The genus *Allium* includes almost 800 species, and has a long history of culinary and medicinal use around the world. Common *Allium* species include garlic, leeks, shallots, onion, Welsh onion, wild garlic, French garlic and chives. Among its diverse varieties, species such as *Allium cepa* (onion) and *Allium sativum* (garlic) are predominantly cultivated with many nutritional profiles and untapped pharmacological activities against oxidative stress and diabetes with substantial global impact (Samtiya *et al.*, 2021).

Allium cepa var. *aggregatum* G. Don (Shallot greens) is a perennial herb, distinct from *Allium cepa* by its small cluster of bulbs, subtle flavor and strong odor (attributed to high sulfur content). Although cultivated primarily for its bulbs, the leaves of shallots, commonly referred to as spring onions, are widely used in culinary preparations. These leaves are rich in phytochemicals, including flavonoids, organosulfur compounds, vitamins, and sterols, which possess anti-inflammatory, diuretic, and cardioprotective properties (Marrelli *et al.*, 2019).

Similarly, *Allium porrum* L. (leeks) is characterized by a long, cylindrical stalk, yellowish-green leaves with a mild, sweet flavor that distinguishes it from others. Unlike onions, leeks do not form a solid bulb and can be consumed raw or cooked (Moldovan *et al.*, 2022). They contain essential amino acids, fatty acids, alliin (precursor of allicin), and other bioactive compounds that exhibit anti-inflammatory, anti-hypertensive, antioxidant, antifungal, and anticancer properties (Sadeghian *et al.*, 2020). While significant research has focused on the outer skin (peel) and bulbs of different Allium species (Xie *et al.*, 2023), there are still very few studies on their leaves, particularly regarding their bioactivity and bioavailability.

Therefore, the present study aims to investigate the antioxidant potential of the leaves of *Allium cepa* var. *aggregatum* G. Don and *Allium porrum* L., combined with systems pharmacology and molecular docking approaches. Furthermore, the study focuses on *in vitro* validation in THP-1 cell line to evaluate the pharmacological potential of the extracts.

MATERIALS AND METHODS

Reagents

The solvents and reagents of analytical grade were used for extraction and analysis purposes. Deionized (DI) water was used for the preparation of standards and aqueous extract. Ascorbic acid, gallic acid, rutin, DPPH, and other required chemicals for this work were procured from HIMEDIA Laboratories (P) Ltd., Mumbai, India.

Collection and Processing of Plant Samples

Fresh whole plants of shallots and leeks were purchased from local markets in and around Coimbatore, Tamil Nadu, India. Both species were authenticated for taxonomical identification at the Botanical Survey of India (BSI), Coimbatore. The identified plant specimen vouchers are BSI/SRC/5/23/2023/Tech-660 (*Allium cepa* var. *aggregatum* G.Don) and BSI/SRC/5/23/2024/Tech-207 (*Allium porrum* L.). The leaves of shallot greens (ACA) and leeks (AP) were washed with tap water to remove debris, drained, evenly chopped, and shade-dried for a period of 15 days. The dried leaves were pulverized into fine powder using a mixer grinder and stored in airtight containers.

Continuous Reflux Extraction Method

The powdered sample was placed in a thimble, which was assembled in a Soxhlet apparatus, using solvents of increasing polarity at a sample-to-solvent ratio of 1:10 (W/V). The extraction was carried out for 8 hr at 50-70°C to ensure an efficient vaporization and condensation process. The extracts were filtered through Whatman No. 1 filter paper. The filtrates of the aqueous extracts were lyophilized (Alpha 1-2 LD plus, Martin and Christ), while organic solvent extracts were concentrated using the traditional conventional method, and the crude extracts obtained were collected, weighed, and the yield percentage was calculated.

Estimation of *in vitro* Antioxidant Activity

To validate the antioxidant potential in the leaves of ACA and AP leaf extracts, their free radical scavenging activity was evaluated using DPPH (Mansor Hakiman and Mahmood Maziah, 2009), NO (Perez-Torres *et al.*, 2020), SO, and FRAP (Sethi *et al.*, 2020) assay.

Antioxidative effects against stress-induced THP1 cells by MTT assay

Initially, THP1 cells were cultured in RPMI-1640 medium containing 10% FBS, 100 U/mL Penicillin, 100 µg/mL Streptomycin, and 0.25 µg/mL Amphotericin-B at 37°C with 5% CO_2. To evaluate the antioxidant effects of ethanolic leaf extracts of ACA and AP, the cells were seeded at a density of $5x10^5$ cells/mL in a sterile 96-well microtiter plate. The cells were incubated for 24 hr, followed by the addition of 200 µM hydrogen peroxide in all the samples, including the control, except for the blank (Mrid *et al.*, 2022). The extracts (1:10 diluted with 1x PBS) were added at final concentrations of 2.5, 5 and 10 uL. The final volume in all the wells was made up to 150 uL with the medium and incubated for 24 hr. After incubation, MTT reagent was added and left in the dark for 4 hr. The formazan crystals thus formed were dissolved by adding the solubilization buffer containing 10% SDS in 0.01 N HCl and read colorimetrically at 570 nm.

Estimation of total phenols and flavonoids

Total phenolic content was estimated using Folin-Ciocalteu method, and total flavonoid content was determined by applying the aluminium chloride method, respectively, using a UV-visible spectrometer. The results were expressed as grams of gallic acid equivalent (mg GAE/g) for TPC and as grams of rutin equivalent (mg rutin/g) for TFC (Li *et al.*, 2007).

Identification of metabolites in Shallot greens and leek extracts

LC-MS analysis of the ethanol extracts of ACA and AP leaves was performed using an LC-MS-8030 triple quadrupole mass spectrometer (Shimadzu) equipped with an electrospray ionization source. Column used for separation is BEH C18 (1.7 µm, 2.1 × 100 mm; Acquity) using gradient elution. The mobile phase consisted of acetonitrile (Pump A) and 1% formic acid in deionized water (Pump B) at a flow rate of 0.8 mL/min. Mass spectra were recorded in positive and negative modes, and data acquisition was processed using LabSolutions software.

In silico System Pharmacology Validation

Target Data Collection

The active compounds from shallot greens and leek were obtained from the LC-MS database, and their 2D SMILES structures were retrieved from the "PubChem" database. Subsequently, all the metabolites were screened for oral bioavailability and toxicity using pkCSM followed by the prediction of potential targets using "Swiss Target Prediction" tool. Similarly, genes associated with oxidative stress were identified using GeneCards, and DrugBank was used to collect detailed information on genes related to oxidative stress (Bitew *et al.*, 2021).

Venn Analysis and Protein-Protein Interaction

To construct a Protein-Protein Interaction (PPI) network, target gene and disease-associated gene were analyzed using "Venny 2.1" to identify common intersecting genes, which were then used to establish a PPI network in "STRING". Further hub genes were retrieved from "Cytoscape software" (version 3.7.2) (Shiau *et al.*, 2022).

Integrated GO and KEGG Pathway Analysis

Gene ontology annotation and KEGG enrichment analysis were carried out in "ShinyGo". The intersected gene was analyzed to identify its significance in biological, molecular, and signaling pathways associated with the study targets. Enrichment significance was evaluated using a threshold of $p<0.05$. Furthermore, overlapping annotations were removed, and the most relevant functional clusters were prioritized to improve clarity and biological relevance of the enrichment results (Jannapureddy *et al.*, 2021).

In silico Molecular Docking

Molecular docking was used to assess the specific binding interaction of protein and ligand; the selected compounds, which belong to flavonoid and phenol, were docked using PyRX. The 3D structures of the ligands were docked against the targeted proteins. Resulting complexes were ranked based on binding energies (kcal/mol), and the minimal energy conformations were selected for further study. The protein-ligand stability was further analysed using Discovery Studio Visualizer to identify hydrogen bond interactions, binding residues, bond distances, and other non-covalent interactions such as π-π and hydrophobic interactions, which were visualized through 2D and 3D images (Rajagopal *et al.*, 2024).

Ethical Statement

We used the THP-1 human monocyte cell line for *in vitro* validation. Since no human participants or animal subjects were involved, ethical approval from an institutional review board was not required.

Statistical Analysis

All analyses were performed in triplicate for reproducibility and the results were expressed as mean±standard deviation using the "GraphPad Prism 10.5.0". One way ANOVA followed by Tukey's *post hoc* test for pairwise comparisons was used for comparison between extracts.

RESULTS

Total Yield of Extract

The leaves of *Allium cepa* var. *aggregatum* G.Don (ACA) and *Allium porrum* L. (AP) were extracted with five solvents of varying polarity. The highest yield was obtained from aqueous extracts, with ACA showing at 16.9% and AP at 15.8% (w/w). Followed by ethanol extracts, with the percentage yields of 15.2% and 13.6% (w/w) for ACA and AP, respectively.

Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents of shallot greens (ACA) and leek (AP) extracts were determined using Folin-Ciocalteu and aluminium chloride methods, respectively. Phenolic content (mg GAE/g) was found to be highest in aqueous extracts of shallot greens (98.2±0.5) than in leek (93.8±0.7), followed by ethanol extracts and acetone extracts. A similar trend was observed for flavonoids (mg rutin/g), with shallot greens showing higher levels than leek across all extracts. Ethanol extracts exhibited the highest flavonoid content (ACA- 144.1±2.3, AP-106.8±3.8), followed by aqueous extracts and acetone extracts. Overall, shallot extracts contained greater phenolic and flavonoid concentrations than leek extracts, and polar solvents demonstrated superior extraction efficiency, indicating stronger antioxidant potential in shallot.

Estimation of the *in vitro* antioxidant Enzymatic Assay

DPPH

DPPH is a highly reliable, abiding free radical, widely used to ascertain antioxidant activity. When the stable DPPH is reacted with the sample, it will either be reduced by Hydrogen Atom Transfer (HAT) or Electron Transfer (ET), which is visualised through the color change from deep purple to yellow. In Figure 1A, the percentage of inhibition for the standard and samples (ACA and AP) is shown. In both the samples, there was an increase in antioxidant activity in a dose-dependent manner, with the maximum percentage of DPPH scavenging activity at 100 µg/mL obtained at 70.07±0.25% (ACA) and 65.15±0.34% (AP). The standard ascorbic acid showed the lowest IC_{50} value of 28.18 µg/mL. It was reported that the half inhibitory concentration of less than 50% is referred to be a powerful antioxidant. The IC_{50} values for ACA and AP samples were 57.01 and 70.88 µg/mL.

FRAP

When the plant extract is treated with FRAP reagent, the phenolic content in the extracts can reduce Fe^{3+} to Fe^{2+}, which was indicated by the development of a blue-colored ferrous tripyridyltriazine complex, which was recorded against a blank at 593 nm using standard procedures (Sethi *et al.*, 2020). The FRAP assay showed the strongest antioxidant capacity in ACA and AP leaf extracts with values of 71.24±0.18 and 60.34±0.24% respectively. The standard ascorbic acid showed a greater reducing effect at the highest concentration, which was followed by ACA and AP leaf extracts and the results are given in Figure 1B.

Nitric Oxide Assay

Nitric oxide generation causes lipid peroxidation, breakdown of DNA strands, degradation of tyrosine, and ultimately results in cell death (Perez-Torres *et al.*, 2020). Ethanolic leaf extract of *Allium cepa* var. *aggregatum* can scavenge nitric oxide mainly because of the large quantity of flavonoids. The RSA% and IC_{50} values of ACA were 74.29±0.26% and 50.61 µg/mL, followed by AP were 71.92±1.1% and 58.48 µg/mL, and the consequence is shown in Figure 1C.

Superoxide Radical Scavenging Assay

In the state of oxidative stress, singlet oxygen and hydroxyl radicals are generated inside the living cells. These free radicals damage the cells, which are responsible for defense mechanisms (Ighodaro *et al.*, 2018). Shallot had the highest percentage of inhibition, which was closer to standard ascorbic acid, with a value of 80.22±0.53%. Whereas *Allium porrum* L. exposed a value of 75.46±0.22% with IC_{50} values of 31.20 and 37.47 µg/mL. Thus, the outcome (Figure 1D) was clear that these *Allium* varieties possess good scavenging activity.

Phytochemical characterization and ADME screening of shallot greens and leek

LC-MS/MS analysis revealed the presence of 35 compounds that were identified from both Indian cultivated Allium varieties, as shown in Table 1. A substantial proportion of phenolic and

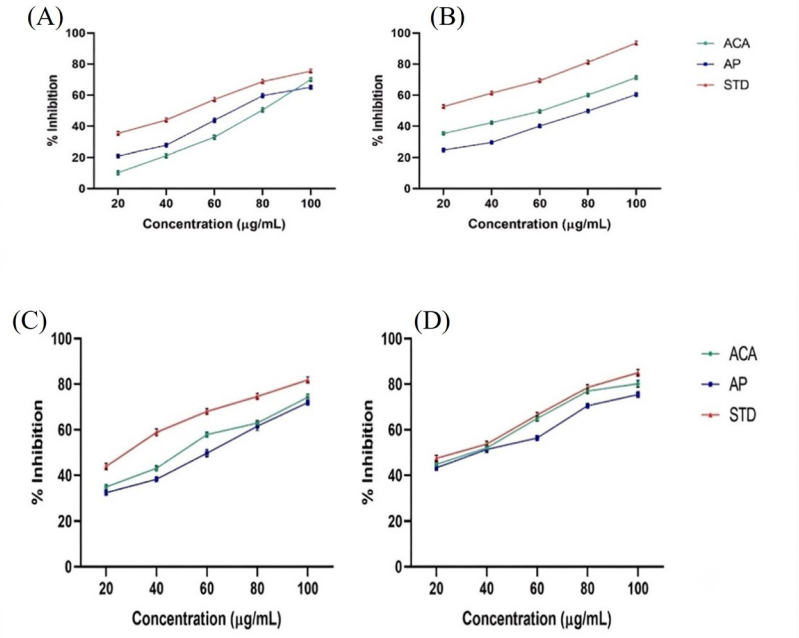

Figure 1: Determination of (A) DPPH (B) FRAP (C) NO and (D) SO assay activities in leaf extracts of *Allium cepa* var. *aggregatum* G. Don and *Allium porrum* L.

Table 1: Identified compounds in ACA and AP extracts: molecular properties and pharmacokinetic prediction.

RT	Fragments m/z	MW (g/mol)	Compounds	Bioavailability Score	Lipinski's rule of five violations
ACA (shallot greens)					
10.33	364, 603	302.24	Morin	0.55	0
18.96	323,555	478.4	Isorhamnetin 4'-O-glucoside	0.177	2
19.97	325,559	286.24	Luteolin	0.55	0
23.89	355,627,887	516.45	Dicaffeoylquinic acid	0.11	3
26.02	383	368.34	Feruloylquinic acid	0.11	0
AP (leek)					
8.67	313,520,608	610.52	Quercetin-3-O-rutinoside	0.17	3
13.68	149, 177, 325	194.18	Ferulic acid	0.85	0
13.33	149, 177	164.16	p-Coumaric acid	0.85	0
17.19	149, 223, 325	432.38	Kaempferol 3-rhamnoside	0.55	1
19.28	273, 455	184.15	O-methyl dihydroxybenzoic acid	0.56	0

*Data includes Retention Time (RT), base peak ions, and characteristic fragment ions used for structural elucidation.

flavonoid derivatives are the major compounds detected in the leaves of ACA and AP. In the case of ACA extract, flavonoids were predominantly characterized with morin and luteolin emerging as the most significant constituents. In contrast, AP extract is dominated by phenolic groups, specifically ferulic acid and p-coumaric acid. From the above Table 1, it is clear morin, luteolin and feruloylquinic acid showed druglikeness properties and successfully passed Lipinski's Rule of Five with zero violations with bioavailability score of 0.55. The phenolic acids in AP, such as ferulic acid and p- coumaric acid, demonstrated the most promising pharmacokinetic profiles with a superior bioavailability score of 0.85 and no Lipinski violations. On the other end, larger metabolites such as dicaffeoylquinic acid and quercetin-3-O-rutinoside underwent Lipinski violations of >1. These results suggest that even though they are bioactive rich compounds, they may require specialized nano-formulations to improve their oral absorption in drug development.

System Pharmacology Analysis

The therapeutic potential of the identified *Allium* metabolites was evaluated through a network pharmacology analysis. Initially, 10 compounds were filtered down to 3 (ACA) and 4 (AP) based specifically on the Lipinski Rule of Five to ensure drug-likeness. The potential phytochemical targets were then predicted using SWISS Target prediction tool, the targets obtained were consolidated and duplicates were removed. Finally, ACA (178) and AP (266) were predicted and annotated in UniProt KB. Similarly, the genes associated with oxidative stress were retrieved from the Gene Card and DisGeNET database.

To pinpoint the exact mechanism by which the promising candidates of *Allium* species contest oxidative stress, a comparative intersection was performed using Venn diagram (Figure 2). Out of 178 predicted phytochemical targets of shallot green, 122 common targets were found to overlap with genes associated with oxidative stress (8735). Similarly, in leek (AP), only 194 overlapping targets were identified at the intersection of the plant metabolites and oxidative stress pathways.

The PPI network ACA and AP provide distinct insights into their respective antioxidative mechanisms. The analysis revealed the number of overlapping nodes in AP (194 nodes and 728 edges) are more than ACA (122 nodes connected by 827 edges), which suggests that leek exerts more extensive, multi-target regulatory effects in the context of oxidative stress. However, ACA network exhibits significantly higher number of edges, indicating the synergistic interactions between the targeted compounds and key oxidative stress proteins. Moreover, its higher connectivity indicates may trigger the integrated biological response within its specific target pathways.

Identification of Hub Proteins

The CytoHubba was used for network analysis to identify hub genes, the top 20 hub genes were standardised based on Maximal Clique Centrality (MCC), which ranks proteins based on their function significance and relative importance of key regulatory nodes.

In ACA network, key hub proteins are AKT1, MMP9, CASP3, PARP1, ESR1, and EGFR. Major hub proteins identified in AP network included STAT3, SRC, EGFR, ESR1, and CCND1. Furthermore, using MCODE plugin in Cytoscape, 5 clusters were identified in ACA and 10 clusters in AP.

These clusters provide valuable insight into the biological pathways and molecular networks. In both ACA and AP networks, EGFR and ESR1 ranked among the top 10 hub proteins, emphasising

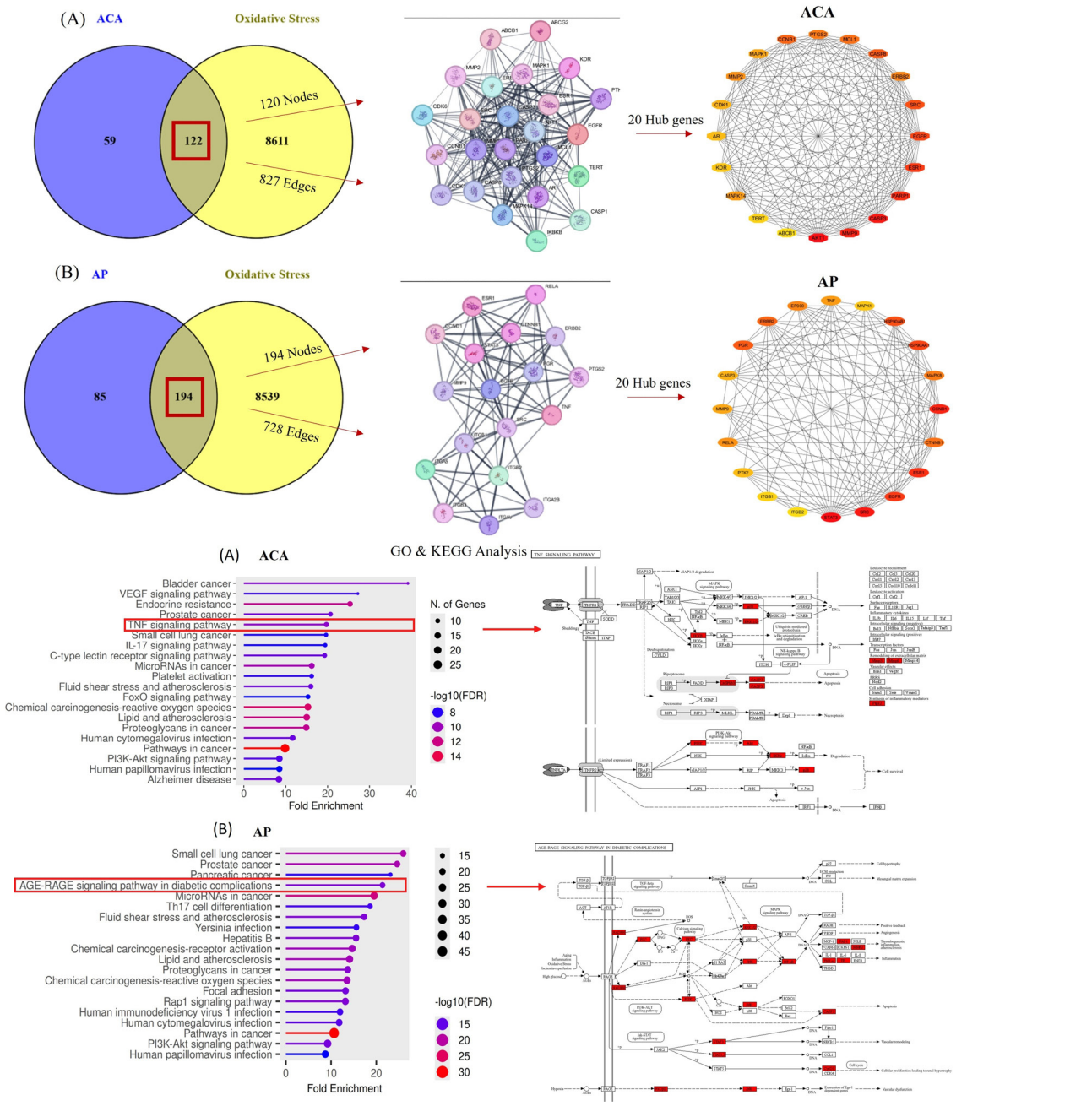

Figure 2: Network identification (Top panel): (A) ACA (Top) and (B) AP (Bottom) (left) Venn diagrams, (middle) Protein-Protein Interaction (PPI) interaction and (right) cytoHubba layouts showing common targets with top 20 hub genes (Node color reflects connectivity from high to low (yellow: low, orange: moderate, red: high).

their pivotal role in multicomponent antioxidant response. It also shared some of the core regulatory proteins, including SRC, MMP2, and CASP3.

GO and KEGG pathway analysis

KEGG pathway enrichment analysis shown in Figure 2, revealed that protein targets are significantly involved in oxidative stress and inflammation related pathways that are linked to cancer progression. In shallot greens (ACA), TNF and IL-17 signaling pathways are enriched, which highlights their critical roles in ROS mediated inflammatory responses. Similarly, fold enrichment analysis of leek (AP) showed enrichment in AGE-RAGE signaling pathway in diabetic complications, indicating a strong association with ROS generation and redox imbalance.

Figure 2, GO and KEGG pathway enrichment analysis (Bottom Panel). Dot plots show significantly enriched pathways based on fold enrichment, gene count, and FDR. (A) TNF signalling pathway and (B) AGE-RAGE signaling pathway in associated with oxidative stress are highlighted. On the right hub genes involved in the pathways were highlighted in red.

In addition, pathways such as lipid and atherosclerosis, fluid shear stress and atherosclerosis were enriched, which also involve oxidative stress mechanisms. Key regulatory pathways, including PI3K-Akt and FoxO signaling pathways, were also enriched, suggesting their contribution to antioxidant defence mechanism.

Molecular Docking

Molecular docking was performed to assess the binding affinity of phytoconstituents of shallot greens (ACA) and leek (AP) against the targeted proteins. As shown in Table 2, ACA derived compounds, luteolin exhibited the strongest binding affinity of -9.8 kcal/mol against AKT1 and -9.7 kcal/mol for MMP-9. Next to that, morin possesses high binding affinity with AKT1 (-9.0 kcal/mol) and MMP-9 (-8.9 kcal/mol). Feruloylquinic acid demonstrated comparatively moderate binding scores ranging from -9.2 to -6.4 kcal/mol.

The results of AP exhibited that kaempferol-3-rhamnoside had the highest binding affinity, with HSP90AA1 (-8.9 kcal/mol) and EGFR (-8.4 kcal/mol). Other ligands, including ferulic acid, p-coumaric acid, and O-methyl dihydroxybenzoic acid, displayed relatively lower binding affinities, with docking scores ranging from -5.2 to -6.9 kcal/mol.

Antioxidative Effects against Stress-Induced THP-1 Cells by MTT Assay

It was observed that increasing concentrations of ACA and AP ethanolic leaf extracts reduced hydrogen peroxide-induced oxidative stress in THP-1 cells. On comparison, ACA extract exhibited relatively more antioxidative effect than the AP extract as assessed by the MTT assay. The cells treated with hydrogen peroxide showed reduced cell viability by 77.61±3.52% relative to the blank (untreated cells). These findings suggest that phytochemical constituents in the extracts may contribute to cellular protection against oxidative stress.

The cytoprotective effect demonstrated a clear dose-dependent increase in cytoprotection. As the concentration increased from 25 µg/mL to 100 µg/mL, there was a progressive enhancement in the ability of the extracts to maintain cells. ACA proved to be more effective than AP at all concentrations. At the maximum concentration of 100 µg/mL, ACA reached its highest protective efficacy at 24%, whereas AP at 15% as shown in Figure 3.

DISCUSSION

This study offers an integrated experimental and computational assessment of the antioxidant potential of *Allium cepa* var. *aggregatum* (ACA) and *Allium porrum* (AP) leaves. The results show that both plant species contain strong antioxidant properties, but their antioxidant strength favours shallot greens because they demonstrate better free radical elimination capabilities (Monika *et al.*, 2018; Xie *et al.*, 2021). The extraction results indicated that polar solvents, particularly aqueous and ethanol extracts, yielded the highest percentage of extraction, suggesting that polar phytochemicals constitute the major bioactive components in both plant species (Mubarak *et al.*, 2025). The study conducted by Kim *et al.* (2025) found that phenolic compounds together with flavonoids (Royani *et al.*, 2025) act as hydrogen donors and acceptors, thereby acting against reactive oxygen species, protecting the cells from oxidative damage. Based on our findings, it can be concluded that shallot green extract contains relatively more polyphenols compared to leek extracts.

Table 2: Docking scores of the ACA (Shallot greens) and AP (Leek) with target proteins.

ACA (Shallot greens)				
Ligands/Proteins	**Morin**	**Luteolin**	**Feruloylquinic acid**	
AKT1	-9.0	-9.8	-9.2	
CAS3	-7.4	-7.5	-6.4	
MMP-9	-8.9	-9.7	-7.8	
PARP-1	-8.5	-8.8	-7.9	
ESR1	-8.3	-8.4	-6.4	
AP (Leek)				
Ligands/Proteins	**Ferulic acid**	**p-Coumaric acid**	**Kaempferol 3 rhamnoside**	**O-methyl dihydroxybenzoic acid**
STAT3	-5.5	-5.4	-7.2	-5.2
HSP90AA1	-6.8	-6.9	-8.9	-6.2
MAPK1	-5.7	-5.7	-7.5	-5.5
CTNNB1	-5.4	-5.3	-7.6	-5.4
EGFR	-6.8	-6.4	-8.4	-5.8

Docking scores (kcal/mol) of major compounds from ACA (shallot greens) and AP (leek) against key protein targets, showing their binding affinity.

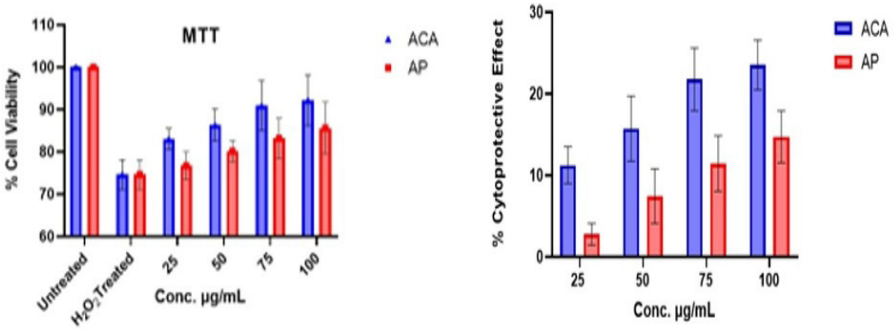

Figure 3: MTT assay showing cell viability and cytoprotective effect. Bar graphs represent the effect of ACA and AP extracts on cell viability (left) and cytoprotective activity (right) at different concentrations. Data are expressed as mean± SD.

The DPPH radical scavenging assay demonstrated dose-dependent activity in both extracts, with ACA showing stronger scavenging efficiency than AP (Aljuhaimi *et al.*, 2025). The FRAP test along with nitric oxide and superoxide radical scavenging assays showed identical patterns which proved these extracts could fight against various types of reactive oxygen species (Singh *et al.*, 2025). The research shows that multiple radical scavenging activities demonstrate that *Allium* varieties contain various antioxidants that protect plants from oxidation (Sutriana *et al.*, 2025). The MTT test confirmed that the plant extracts (ACA and AP) protect THP-1 cells from hydrogen peroxide damage, validating their ability to shield against oxidative stress. These results are in accordance with the work of Muscolo *et al.*, 2024, which shows that the antioxidant activity observed in biochemical assays can be translated into real protection at the cellular level.

Indeed, the MTT test validated that the plant extracts could protect the THP-1 cells against damage caused by hydrogen peroxide, which validated their capability to defend against oxidative stress. The antioxidant properties, which biochemical tests showed produces actual protection for cells based on the research conducted by Li *et al.* (2021).

The LC-MS analysis revealed multiple bioactive metabolites such as phenolic, flavonoid organosulfur compounds and terpenoids (Benke *et al.*, 2025). ACA analysis result showed that morin and luteolin were the most abundant compounds, while AP extracts mainly contained phenolic acids, which included ferulic acid and p-coumaric acid (Nile *et al.*, 2018; Morin *et al.*, 2017). Many researchers have established that these compounds contain antioxidant, anti-inflammatory, antidiabetic, and antiproliferative effects (Amari *et al.*, 2025). The pharmacokinetic screening process identified multiple metabolites that followed Lipinski's rule of five, thus demonstrating their potential as oral drugs with good drug-like characteristics (Amidon *et al.*, 2015). Network pharmacology provided more information on phytochemical targets that share numerous common pathways with oxidative stress-related genes which shows these metabolites can target multiple biological pathways. The protein-protein interaction networks revealed multiple hub proteins which include AKT1 and EGFR and STAT3 and ESR1 and CASP3 that control oxidative stress signalling and apoptosis and metabolic homeostasis (Bitew *et al.*, 2021; Shiau *et al.*, 2022). The identification of such hub proteins and functional enrichment analysis are evidence that phytochemicals from ACA and AP, identified many signaling pathways related to oxidative stress and cell survival at high significance levels (Marwah *et al.*, 2025). The TNF signalling pathway together with FoxO signalling pathway and PI3K-Akt signalling pathway emerged as the most important pathways for multitargeting drug delivery (Jannapureddy *et al.*, 2021). The FoxO transcription factors maintain essential control over antioxidant enzyme production (Rajagopal *et al.*, 2024), while the PI3K-Akt pathway enables cells to survive and controls their metabolic activities (Merecz-Sadowska *et al.*, 2020). The AGE-RAGE signalling pathway shows signs of enrichment which indicates these phytochemicals might help reduce oxidative stress related diabetic complications (Krafczyk and Klotz, 2022). *Allium* leaf extracts fight free radicals through two separate mechanisms which include direct antioxidant effects and indirect control of oxidative stress response systems (Yu and Cui, 2016). Results from the network analysis showed that plant phytochemicals exerted their biological effect through multi-target mechanisms rather than single target molecules.

The 2D interaction analysis in (Figure 4) demonstrated that ligand-protein stability depends on hydrogen bonds and hydrophobic interactions which occur between key active site residues. Luteolin was able to maintain constant interaction with all five target molecules including MMP-9, PARP-1, AKT1, CASP3, and ESR1 due to its capability of forming stronger hydrogen bonds. The research demonstrated that AKT1 and MMP-9 showed strong binding interactions with luteolin through their -9.8 kcal/mol and -9.7 kcal/mol values. The binding of luteolin to its target proteins involved polar residues Ser and Thr and Asp and

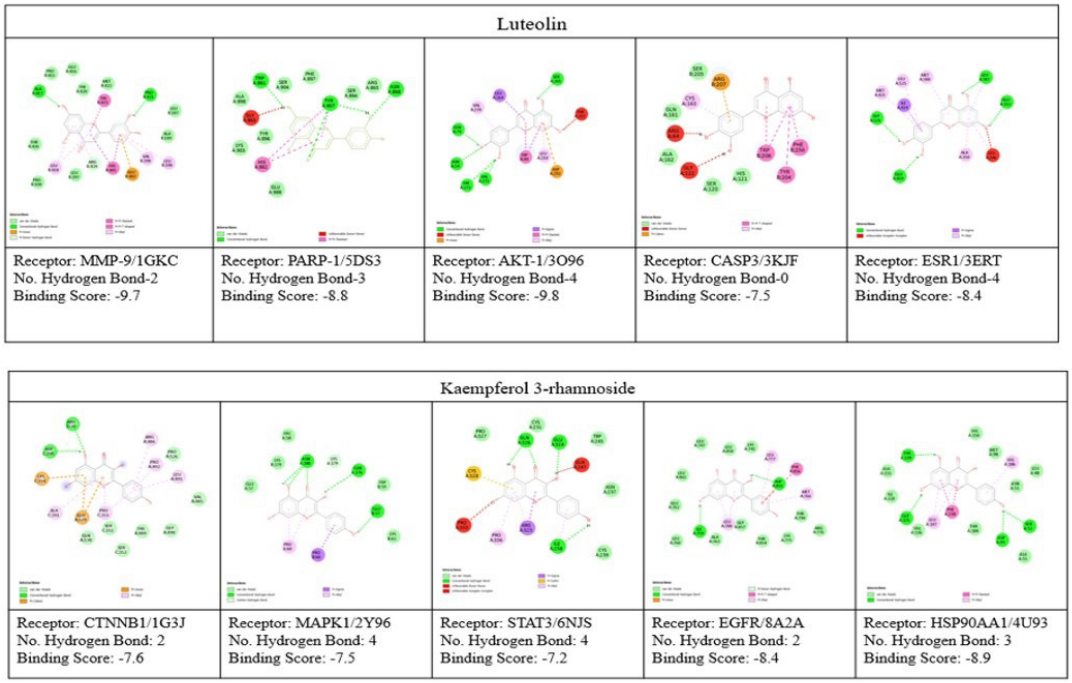

Figure 4: Molecular docking interaction profiles of selected phytochemical leads with key therapeutic targets. The 2D image illustrates the binding orientations, amino acid residue interactions, and specific bonding networks of Luteolin (top) and Kaempferol 3-rhamnoside (bottom) within the active sites of their respective protein receptors.

hydrophobic residues Leu and Val and Phe which created a stable environment for luteolin inside the binding pocket (Parveen *et al.*, 2021). A combined effect of these interactions results in more specificity and complex stability, 2D interaction maps show binding of luteolin and kaempferol 3-rhamnoside with target proteins, highlighting hydrogen bonds and key interactions along with their binding scores. Kaempferol-3-rhamnoside showed strong binding affinity with HSP90AA1 (-8.9 Kcal/mol) and EGFR (-8.4 Kcal/mol) and showed interaction with polar residues surrounded by hydrophobic residues. However, its bulky glycoside moiety may restrict optimal fitting, which leads to lower binding affinity for other targets.

CONCLUSION

The present study indicates that *Allium cepa* var. *aggregatum* and *Allium porrum* leaves are excellent sources of phenolic and flavonoid compounds with significant antioxidant activity. Luteolin and Kaempferol showed strong interactions with AKT1 and HSP90AA1 respectively, which effectively target TNF, FoxO and PI3K-Akt pathways, indicating the therapeutic potential of Allium extract against oxidative stress associated metabolic stress.

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ABBREVIATIONS

OS: Oxidative Stress; **ROS:** Reactive Oxygen Species; **ACA:** *Allium cepa* var. *aggregatum*; **AP:** *Allium porrum*; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **MTT:** 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide; **LC-MS:** Liquid Chromatography-Mass Spectrometry; **ATP:** Adenosine Triphosphate; **DNA:** Deoxyribonucleic Acid; **GAE:** Gallic Acid Equivalent; **HAT:** Hydrogen Atom Transfer; **ET:** Electron Transfer; **NO:** Nitric Oxide; **SO:** Superoxide Radical Scavenging; **RT:** Retention Time; **MCC:** Maximal Clique Centrality; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **AGE-RAGE:** Advanced Glycation End Products-Receptor for Advanced Glycation End Products; **PI3K/AKT:** Phosphoinositide 3-kinase/protein kinase B; **FBS:** Fetal Bovine Serum; **SMILES:** Simplified Molecular Input Line Entry System; **PPI:** Protein-Protein Interaction; **GO:** Gene Ontology; **ADME:** Absorption, Distribution, Metabolism, and Excretion.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Balasubramanian Ramesh: Final approval of the article, **Thilagarani Mani:** Research concept and design, data analysis and interpretation, writing the article, **Sakthivel Kunnathur**

Murugesan: Research concept and design, data analysis and interpretation, critical revision of the article, **Gowtham Kalidas:** Collection and/or assembly of data, **Nandhini Velappan Manickam:** Collection and/or assembly of data, writing the article.

SUMMARY

This study integrated experimental assays and network pharmacology to evaluate the antioxidant potential of shallot greens (*Allium cepa* var. *aggregatum*) and leeks (*Allium porrum*). LC-MS identified 35 metabolites, with luteolin and kaempferol showing high binding affinity to key proteins like AKT1 and HSP90AA1. The findings demonstrate that these Allium species provide cellular protection against oxidative stress by modulating inflammatory and survival signalling pathways.

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