In-Vitro Anticancer and Antioxidant Evaluation of Ethanolic *Allium cepa* Seed Extract against MCF-7 Breast Cancer Cells with In-Silico ADME-Toxicological Profiling of Principal Bioactive Constituents

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Abstract

Background: Breast cancer remains the most frequently diagnosed malignancy in women globally, with current therapeutic strategies limited by systemic toxicity, drug resistance, and high treatment costs. Natural products, particularly those derived from underexplored plant parts such as seeds, represent promising sources of novel anticancer agents. *Allium cepa* (onion) seeds, though phytochemically rich, remain significantly underinvestigated for anticancer potential.

Objective: This study aimed to evaluate the in-vitro anticancer and antioxidant activities of ethanolic onion (*Allium cepa*) seed extract (OSE) against the MCF-7 human breast cancer cell line, and to computationally profile the absorption, distribution, metabolism, excretion (ADME), and toxicological properties of its principal bioactive constituents.

Methods: Ethanolic OSE was prepared by Soxhlet extraction and subjected to physicochemical standardization, qualitative and quantitative phytochemical screening, and HPTLC fingerprinting. Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays. Cytotoxicity against MCF-7 cells was evaluated by the MTT assay, with morphological assessment by inverted microscopy. Computational ADME profiling was performed using SwissADME and pkCSM, while toxicological prediction was conducted using ProTox-II.

Results: The extract yield was 8.62% w/w, with total phenolic content of 84.32 ± 2.76 mg GAE/g and total flavonoid content of 52.14 ± 1.93 mg QE/g. OSE demonstrated concentration-dependent antioxidant activity (DPPH IC50: 148.5 ± 3.21 µg/mL; ABTS IC50: 132.4 ± 2.87 µg/mL; FRAP: 215.8 ± 8.43 µM Fe^{2+} eq/g). Significant cytotoxicity against MCF-7 cells was observed with an IC50 of 178.3 ± 4.87 µg/mL at 48 hours, accompanied by apoptotic morphological changes. In-silico analysis confirmed that quercetin, kaempferol, and thymoquinone satisfied Lipinski's Rule of Five. Thymoquinone exhibited the most

favorableADME profile, while all three compounds showed absence of predicted hepatotoxicity, carcinogenicity, and mutagenicity.

Conclusion: Ethanolic onion seed extract demonstrates significant in-vitro anticancer and antioxidant activities, with its principal bioactive constituents exhibiting favorable drug-likeness and safety profiles. These findings establish a scientific basis for further preclinical development of onion seed-derived formulations for breast cancer management.

Keywords:*Allium cepa*; onion seeds; breast cancer; MCF-7; antioxidant; MTT assay; ADME; in-silico; quercetin; thymoquinone

1. Introduction

Breast cancer represents the most commonly diagnosed malignancy among women worldwide and constitutes the leading cause of cancer-related mortality in females across both developed and developing nations [1]. According to data from the Global Cancer Observatory, breast cancer accounted for approximately 2.3 million new cases and over 670,000 deaths globally in 2022 alone [1]. In India, the incidence has been rising steadily, with estimates indicating over 192,000 new cases annually, a figure projected to increase significantly in coming decades owing to shifting demographic profiles, urbanization, and changing lifestyle patterns [2]. The molecular heterogeneity of breast cancer, classified into subtypes such as luminal A, luminal B, HER2-enriched, and triple-negative breast cancer, necessitates individualized therapeutic approaches that are complex, resource-intensive, and not uniformly accessible across all healthcare settings [3].

Contemporary breast cancer management relies on a multimodal approach integrating surgery, radiation therapy, cytotoxic chemotherapy, hormonal therapy, targeted biological therapy, and immunotherapy [4]. While these modalities have demonstrably extended survival rates, each is associated with a distinct spectrum of adverse effects and limitations. Cytotoxic chemotherapeutic agents such as doxorubicin, paclitaxel, and cyclophosphamide are associated with systemic toxicities including cardiotoxicity, myelosuppression, peripheral neuropathy, and hepatotoxicity [4]. Prolonged chemotherapy is frequently associated with the emergence of drug resistance, mediated through mechanisms such as overexpression of efflux transporters, apoptosis evasion pathways, and alterations in drug metabolism [4]. These limitations necessitate the discovery of novel therapeutic candidates that are effective, well-tolerated, economically viable, and mechanistically distinct from existing agents.

Oxidative stress, defined as an imbalance between reactive oxygen species (ROS) production and cellular antioxidant defense capacity, plays a central role in the oncogenesis of breast cancer [5]. ROS promote carcinogenesis through DNA oxidative modification, lipid peroxidation, activation of pro-survival signaling pathways including NF-κB and PI3K/AKT/mTOR, and suppression of apoptotic pathways [5]. Given this critical involvement, compounds with potent antioxidant activity represent promising candidates for breast cancer chemoprevention and adjunct therapy by attenuating ROS-mediated oncogenic signaling [5].

The pharmacognostic heritage of medicinal plants represents an invaluable source of novel bioactive molecules. Of the approximately 1,200 anticancer agents developed between 1981 and 2019, nearly 49% were derived from natural products or their semi-synthetic analogs [6]. Polyphenolic compounds, including flavonoids, phenolic acids, and tannins, have been extensively investigated for their antioxidant and anticancer properties. Flavonoids exert anticancer effects through diverse mechanisms including cell cycle arrest, induction of apoptosis, inhibition of angiogenesis, and modulation of signaling kinases [6].

Allium cepa L. (common onion) is a biennial herb belonging to the family Amaryllidaceae, with a history of cultivation spanning more than 5,000 years [7]. While the onion bulb has been extensively studied, the seeds of *Allium cepa* remain significantly underexplored despite containing a unique and concentrated array of bioactive molecules [7]. The seeds harbor a distinctive phytochemical composition including fixed oils, flavonoids (quercetin, kaempferol, isorhamnetin), phenolic compounds (ferulic acid, caffeic acid), and sulfur-containing compounds such as thymoquinone [8]. Quercetin, the predominant flavonoid, has demonstrated anticancer activity against MCF-7, HeLa, HepG2, and A549 cancer cell lines [9]. However, systematic studies evaluating the in-vitro anticancer potential of ethanolic onion seed extracts against breast cancer cell lines, accompanied by pharmacokinetic profiling, remain scarce.

The present study was designed to bridge this critical knowledge gap by conducting a systematic evaluation of ethanolic onion seed extract: an in-vitro biological efficacy assessment using the MCF-7 breast cancer cell line, and a computational in-silico ADME and toxicological profiling of its key bioactive constituents. This integrated methodological framework represents the contemporary paradigm in early-phase drug discovery, enabling simultaneous evaluation of biological activity and pharmacokinetic feasibility [10].

2. Materials and Methods

2.1 Collection, Authentication, and Extraction

Onion seeds (*Allium cepa* L.) were procured from a certified agricultural seed supplier and authenticated by a botanist at the Department of Botany, Career Point University, Kota (Voucher specimen number: CPU/BOT/2024/OSE-01). Authentication was performed based on macroscopic features (angular, black, rugose seeds approximately 3–4 mm in size), microscopic examination, and cross-referencing with published botanical monographs. Seeds were cleaned, washed with distilled water, and shade-dried at 30–35°C for 10–14 days. Dried seeds were powdered using a mechanical grinder and passed through a 40-mesh sieve.

Ethanolic onion seed extract (OSE) was prepared by Soxhlet extraction. Accurately weighed onion seed powder (100 g) was packed into the thimble, and extraction was performed using 500 mL of 95% ethanol at 60–65°C for 18 hours. The extract was filtered, concentrated under reduced pressure using a rotary evaporator at 45°C, and further dried in a desiccator to constant weight. Percentage yield was calculated as: % yield = (weight of extract / weight of seed powder) × 100.

2.2 Physicochemical Standardization

The prepared extract was subjected to physicochemical standardization including determination of moisture content, total ash value, acid-insoluble ash, water-soluble extractive value, ethanol-soluble extractive value, and pH of 1% w/v aqueous solution, following WHO and Indian Pharmacopoeia guidelines.

2.3 Phytochemical Screening

Qualitative phytochemical screening was performed according to standard procedures [11] to detect alkaloids (Mayer's and Dragendorff's tests), carbohydrates (Molisch's and Fehling's tests), steroids and triterpenoids (Liebermann-Burchard and Salkowski tests), phenols and tannins (ferric chloride and lead acetate tests), flavonoids (Shinoda's and alkaline reagent tests), saponins (froth test), and proteins/amino acids (ninhydrin test).

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method with gallic acid as standard, expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g). Total flavonoid content (TFC) was quantified by the aluminum chloride colorimetric method with quercetin as standard, expressed as mg quercetin equivalents per gram of dry extract (mg QE/g).

2.4 In-Vitro Antioxidant Activity Assays

DPPH Radical Scavenging Assay: The assay was performed based on the method of Brand-Williams et al. [12] with modifications. Stock solution of DPPH (0.1 mM in methanol) was prepared fresh. OSE solutions (25–1000 µg/mL) were mixed with DPPH solution in a 96-well plate. After 30 minutes incubation at room temperature in the dark, absorbance was measured at 517 nm. Ascorbic acid was used as reference standard. Percent inhibition was calculated and IC50 determined from the dose-response curve.

ABTS Radical Scavenging Assay: ABTS radical cation was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate, standing in the dark for 16 hours. The solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. OSE at various concentrations was mixed with ABTS•+ solution, incubated for 6 minutes, and absorbance measured at 734 nm. Results were expressed as Trolox Equivalent Antioxidant Capacity.

FRAP Assay: FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and FeCl3·6H2O (20 mM) in a 10:1:1 ratio. OSE solutions were mixed with FRAP reagent, incubated at 37°C for 30 minutes, and absorbance measured at 593 nm. Results were expressed as µM Fe^{2+} equivalents per gram of extract.

2.5 In-Vitro Anticancer Activity

Cell Culture: MCF-7 human breast adenocarcinoma cells (ATCC HTB-22) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin, in a humidified 5% CO_2 incubator at 37°C.

MTT Assay: Cells were seeded in 96-well plates at 5×10^3 cells/well and allowed to adhere for 24 hours. OSE (12.5–800 µg/mL) prepared in DMEM (DMSO ≤ 0.1%) was added in triplicate. Doxorubicin (0.1–10 µg/mL) served as positive control. After 48 hours, 20 µL of MTT solution (5 mg/mL in PBS) was added per well and incubated at 37°C for 4 hours [13]. Formazan crystals were dissolved in 150 µL DMSO, and absorbance was measured at 570 nm. Cell viability (%) was calculated, and IC50 values were determined by non-linear regression analysis using GraphPad Prism 9.0.

2.6 In-Silico ADME and Toxicity Prediction

The SMILES notations for selected bioactive compounds (quercetin, kaempferol, thymoquinone) were retrieved from PubChem. ADME properties were predicted using SwissADME [10] and pkCSM. Lipinski's Rule of Five compliance was assessed. Toxicological profiling was performed using ProTox-II [14] for prediction of oral LD50, GHS toxicity class, and endpoint toxicities including hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity.

2.7 Statistical Analysis

All experiments were performed in triplicate (n = 3) and results expressed as mean ± standard deviation. IC50 values were determined by non-linear regression analysis. One-way ANOVA with post-hoc Tukey's test was used for group comparisons. Pearson's correlation coefficient was calculated to assess correlations between antioxidant activity and phenolic/flavonoid content. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Extraction Yield and Physicochemical Parameters

Soxhlet extraction of 100 g onion seed powder with 95% ethanol yielded a dark brown semi-solid extract with a percentage yield of 8.62 ± 0.43% w/w. The physicochemical parameters were: moisture content 5.12 ± 0.21%, total ash 4.87 ± 0.31%, acid-insoluble ash 0.92 ± 0.07%, water-soluble extractive value 14.3 ± 0.58%, ethanol-soluble extractive value 8.62 ± 0.43%, and pH (1% w/v aqueous solution) 5.8 ± 0.12. All parameters were within acceptable pharmacopoeial limits, confirming extract quality.

3.2 Phytochemical Screening

Qualitative screening revealed the presence of flavonoids (+++), phenols/tannins (+++), carbohydrates (+++), fixed oils (+++), saponins (++), proteins/amino acids (++), steroids (+), and triterpenoids (+). Alkaloids were absent by both Mayer's and Dragendorff's tests.

3.3 Quantitative Phytochemical Analysis

Total phenolic content was 84.32 ± 2.76 mg GAE/g dry extract, and total flavonoid content was 52.14 ± 1.93 mg QE/g dry extract, indicating a rich polyphenolic composition.

3.4 Antioxidant Activity

OSE demonstrated concentration-dependent antioxidant activity across all three assays (Table 1). The DPPH IC50 was 148.5 ± 3.21 µg/mL (ascorbic acid: 24.8 ± 1.12 µg/mL, $p < 0.001$). The ABTS IC50 was 132.4 ± 2.87 µg/mL (ascorbic acid: 19.3 ± 0.94 µg/mL, $p < 0.001$). FRAP antioxidant capacity was 215.8 ± 8.43 µM Fe^{2+} eq/g extract. Pearson correlation analysis revealed a strong positive correlation between TPC and DPPH activity ($r = 0.912$, $p < 0.01$), and between TFC and ABTS activity ($r = 0.887$, $p < 0.01$).

Table 1: In-Vitro Antioxidant Activity of Ethanolic Onion Seed Extract

Assay	*OSE IC50 (µg/mL)*	*Standard IC50 (µg/mL)*	*Standard Used*	*p-value*
DPPH	148.5 ± 3.21	24.8 ± 1.12	Ascorbic acid	< 0.001
ABTS	132.4 ± 2.87	19.3 ± 0.94	Ascorbic acid	< 0.001
FRAP (µM Fe^{2+} eq/g)	215.8 ± 8.43	380.2 ± 10.11	Ascorbic acid	< 0.01

3.5 Anticancer Activity

OSE demonstrated concentration-dependent cytotoxicity against MCF-7 cells (Table 2). Cell viability decreased from 91.4 ± 2.31% at 12.5 µg/mL to 11.4 ± 1.87% at 800 µg/mL. The IC50 value was 178.3 ± 4.87 µg/mL (95% CI: 169.8–186.9, $R^2 = 0.982$), compared to doxorubicin IC50 of 1.24 ± 0.09 µg/mL ($R^2 = 0.996$). Morphological examination at IC50 concentration revealed cell shrinkage, membrane blebbing, loss of adherence, and nuclear condensation, consistent with apoptotic cell death.

Table 2: Effect of Ethanolic Onion Seed Extract on MCF-7 Cell Viability (MTT Assay, 48 h)

Concentration (µg/mL)	*% Cell Viability (mean ± SD)*	*% Inhibition (mean ± SD)*
Control (0.1% DMSO)	100.00 ± 0.00	0.00 ± 0.00
12.5	91.4 ± 2.31	8.6 ± 2.31
25	82.7 ± 2.87	17.3 ± 2.87
50	70.3 ± 3.12	29.7 ± 3.12
100	55.8 ± 2.64*	44.2 ± 2.64
200	38.2 ± 3.08**	61.8 ± 3.08
400	22.1 ± 2.43***	77.9 ± 2.43

800	11.4 ± 1.87***	88.6 ± 1.87

*p < 0.05, **p < 0.01, ***p < 0.001 vs. control

3.6 In-Silico ADME Profiling

All three principal compounds satisfied Lipinski's Rule of Five with zero violations (Table 3). Thymoquinone exhibited the most favorable profile: highest intestinal absorption (93.82%), high GI absorption, and minimal CYP inhibition. Quercetin showed low predicted GI absorption and bioavailability score (0.17), consistent with published reports of poor oral bioavailability due to extensive first-pass metabolism [15].

Table 3: Lipinski's Rule of Five Parameters — SwissADME Predictions

Parameter	***Quercetin***	***Kaempferol***	***Thymoquinone***	***Lipinski Limit***
Molecular Weight (Da)	302.24	286.24	164.20	≤ 500
LogP (iLOGP)	1.54	1.58	1.78	≤ 5
H-bond Donors	5	4	0	≤ 5
H-bond Acceptors	7	6	2	≤ 10
TPSA (Å²)	131.36	107.22	34.14	≤ 140
Bioavailability Score	0.17	0.55	0.55	—
GI Absorption	Low	High	High	—
Lipinski Violations	0	0	0	≤ 1 (pass)

3.7 Toxicological Prediction

ProTox-II predictions revealed favorable safety profiles (Table 4). Kaempferol demonstrated the highest predicted LD50 (3919 mg/kg, GHS Class V). Thymoquinone showed LD50 of 794 mg/kg (Class IV) with no predicted carcinogenicity, mutagenicity, or hepatotoxicity. Quercetin was predicted as GHS Class III (LD50 = 159 mg/kg). Importantly, no compound showed predicted hepatotoxicity or carcinogenicity.

Table 4: In-Silico Toxicity Predictions (ProTox-II)

Toxicity Parameter	***Quercetin***	***Kaempferol***	***Thymoquinone***
Predicted LD50 (mg/kg, oral, rat)	159	3919	794
GHS Toxicity Class	III (Harmful)	V (May be harmful)	IV (Harmful)
Hepatotoxicity	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Inactive	Inactive

Mutagenicity	Inactive	Inactive	Inactive
Cytotoxicity	Active	Inactive	Inactive
Immunotoxicity	Active	Inactive	Inactive

4. Discussion

This study represents the first systematic and integrated evaluation of ethanolic *Allium cepa* seed extract for in-vitro anticancer and antioxidant activities against breast cancer, combined with computational ADME-toxicological profiling of its principal bioactive constituents. The percentage yield of 8.62% w/w is consistent with values reported for ethanolic extracts of other *Allium* species, which typically range from 5–12% depending on solvent system and extraction conditions [16].

The physicochemical parameters were all within pharmacopoeially acceptable ranges, confirming the quality and suitability of the prepared extract. The relatively low moisture content (5.12%) indicates adequate drying, minimizing the risk of microbial contamination and hydrolytic degradation of active constituents. The absence of alkaloids differentiates onion seeds from other *Allium* species such as *Nigella sativa*, while the predominance of polyphenolic constituents supports the rationale for evaluating anticancer and antioxidant activities [8].

The quantitative analysis revealed TPC of 84.32 mg GAE/g and TFC of 52.14 mg QE/g. These values are considerably higher than those reported for onion skin water extracts (approximately 15–40 mg GAE/g dry extract) [9], which may reflect the differential phytochemical distribution between seed and skin, as well as the superior extraction efficiency of ethanol for flavonoids due to its intermediate polarity [9].

The concentration-dependent antioxidant activity of OSE in DPPH, ABTS, and FRAP assays confirms the antioxidant potential attributed to its polyphenolic content. The DPPH IC50 of 148.5 µg/mL is within the range reported for crude plant extracts from *Allium* species and other polyphenol-rich botanicals [9]. The strong correlation between TPC and DPPH activity ($r = 0.912$) is consistent with extensive published data demonstrating phenolic compounds as principal contributors to free radical scavenging in *Allium cepa* preparations [17]. The ABTS IC50 (132.4 µg/mL) was marginally lower than the DPPH IC50, consistent with the general observation that ABTS assay detects both hydrophilic and lipophilic antioxidants [18].

The MTT assay results demonstrate significant concentration-dependent cytotoxic activity of OSE against MCF-7 cells, with an IC50 of 178.3 µg/mL at 48 hours. According to widely applied criteria in natural product anticancer drug screening, crude plant extracts with IC50 < 250 µg/mL against human cancer cell lines are considered significantly cytotoxic [19]. The observed IC50 value thus meets this criterion, validating OSE as a candidate for further anticancer investigation. The morphological changes observed at IC50 concentration are

hallmarks of apoptotic cell death, consistent with the reported mechanisms of quercetin-induced cytotoxicity in MCF-7 cells [20].

The higher IC50 observed for the crude extract compared to isolated quercetin is expected, as the extract contains a complex mixture of constituents including non-active components that dilute the overall cytotoxic potency [20]. Previous studies have reported IC50 values of approximately 42–78 µg/mL for quercetin isolated from various plant sources against MCF-7 cells, with evidence of G1-phase arrest and caspase-mediated apoptosis [20]. The cytotoxic activity of OSE is thus attributable primarily to its quercetin and flavonoid content, acting through disruption of cell cycle progression, induction of mitochondrial apoptosis, and modulation of redox-sensitive signaling pathways [9].

Computational ADME profiling revealed that all three principal compounds satisfy Lipinski's Rule of Five without violations, establishing their theoretical oral drug-likeness [10]. However, quercetin's low predicted GI absorption and bioavailability score (0.17) are consistent with published reports documenting its poor oral bioavailability in humans, attributed to extensive glycoside hydrolysis, conjugation in the intestinal wall, and rapid metabolic clearance [15]. These findings rationalize the extensive efforts toward nanoformulation of quercetin to improve bioavailability [21]. Thymoquinone demonstrated the most favorable ADME profile, with high intestinal absorption and minimal CYP inhibition, suggesting good oral bioavailability and low potential for drug-drug interactions [22].

The toxicological predictions revealed a generally favorable safety profile. The absence of predicted hepatotoxicity across all three compounds is particularly significant, given that hepatotoxicity is among the most common causes of drug attrition in later development stages [14]. Kaempferol's excellent safety profile is consistent with its GRAS (Generally Recognized As Safe) status as a natural dietary flavonoid [23]. The predicted cytotoxicity and immunotoxicity of quercetin should be interpreted cautiously, as these endpoint models may overestimate in-vivo toxicity due to the absence of metabolic biotransformation and tissue distribution dynamics in the computational model [14].

5. Conclusion

This study systematically evaluated the in-vitro anticancer and antioxidant activities of ethanolic *Allium cepa* seed extract and computationally profiled its principal bioactive constituents. The key findings are: (1) Ethanolic onion seed extract was successfully prepared with a yield of 8.62% w/w meeting all pharmacopoeial quality standards; (2) The extract demonstrated significant concentration-dependent antioxidant activity strongly correlated with phenolic and flavonoid content; (3) OSE exhibited significant cytotoxic activity against MCF-7 cells with an IC50 of 178.3 µg/mL, meeting NCI criteria for significant cytotoxicity in crude plant extracts, with apoptotic morphological characteristics; (4) In-silico ADME predictions confirmed oral drug-likeness of quercetin, kaempferol, and thymoquinone, with

thymoquinone showing the most favorable profile; (5) ProTox-II predictions revealed no hepatotoxicity, carcinogenicity, or mutagenicity for any compound.

Allium cepa seeds represent a significant and underexplored botanical resource with demonstrated in-vitro anticancer and antioxidant potential, mechanistically attributable to their rich flavonoid and phenolic content. This study establishes a robust scientific foundation for further preclinical development, including bioactive fraction isolation, mechanistic apoptosis studies, nanoformulation development, and in-vivo anticancer evaluation.

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