Anticancer Potential of Wheatgrass (Triticum aestivum) in Breast Cancer: Molecular Pathways and Translational Insights

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Abstract:

Background: Breast cancer remains the second most common malignancy worldwide, with approximately 2.3 million new cases diagnosed in 2022, underscoring the urgent need for effective, low-toxicity adjunct therapies. Conventional treatments such as chemotherapy often induce severe myelotoxicity and resistance, prompting exploration of natural agents like wheatgrass (Triticum aestivum), a nutrient-dense young grass rich in chlorophyll, flavonoids, and antioxidants.

Objectives: This review synthesises evidence on the anticancer potential of wheatgrass against breast cancer, focusing on molecular pathways (e.g., apoptosis induction, cell cycle arrest) and translational insights from preclinical to clinical studies.

Key Findings: In vitro studies demonstrate wheatgrass extracts' cytotoxicity against breast cancer cell lines (MCF-7, MDA-MB-231), enhancing under simulated microgravity via unique bioactives like apigenin and tocopherol, which downregulate PI3K/AKT, NF-κB, and DNA repair genes while upregulating pro-apoptotic factors. Phytochemicals such as flavonoids inhibit proliferation, angiogenesis (VEGF suppression), and metastasis. In vivo models show tumour reduction and immune modulation (e.g., TH1 cytokine upregulation). Clinically, pilot trials indicate wheatgrass juice reduces chemotherapy-induced neutropenia and anaemia in breast cancer patients, with no reported adverse events, though large-scale RCTs are lacking.

Implications: Wheatgrass holds promise as a safe adjunct to mitigate treatment toxicities and enhance efficacy through targeted pathways, warranting further pharmacokinetic optimization and phase II trials for personalised integration in breast oncology.

Keywords: Wheatgrass; Triticum aestivum; Breast cancer; Anticancer activity; Apoptosis; Molecular pathways; Translational medicine

1. Introduction:

Breast cancer continues to pose a formidable global health challenge, ranking as the second most prevalent cancer and the leading cause of cancer-related mortality among women. In 2022 alone, an estimated 2.3 million new cases were reported worldwide, with over 685,000 deaths, disproportionately affecting low- and middle-income regions due to limited access to early detection and advanced therapies. [1]The disease's heterogeneity—spanning hormone receptor-positive (ER/PR+), HER2-enriched, and triple-negative subtypes—complicates management, while hallmarks such as uncontrolled proliferation, evasion of apoptosis, sustained angiogenesis, and metastatic dissemination drive progression. Standard interventions, including surgery, radiation, endocrine therapy, and chemotherapy (e.g., anthracyclines, taxanes), have improved survival rates to over 90% for localised disease.[2] However, these modalities are marred by significant drawbacks: chemotherapy-induced myelosuppression leads to life-threatening neutropenia in up to 50% of patients, anaemia requiring transfusions, and increased infection risks, often necessitating costly supportive agents like granulocyte colony-stimulating factors (G-CSF) and erythropoietin. Moreover, tumour heterogeneity fosters chemoresistance via upregulated survival pathways like PI3K/AKT/mTOR and NF-κB, resulting in recurrence rates of 20–30% in advanced stages. These limitations highlight the imperative for

adjunctive strategies that enhance efficacy, mitigate toxicities, and target molecular vulnerabilities with minimal side effects.[3]

Enter natural products, which have garnered renewed scientific scrutiny for their pleiotropic bioactivities and historical use in traditional medicine. Among these, wheatgrass (Triticum aestivum L.), the juvenile shoots of the common wheat plant, emerges as a compelling candidate. Cultivated for over 5,000 years in ancient Egyptian and Ayurvedic traditions, wheatgrass has been revered as a "green blood" tonic for its regenerative properties, purportedly aiding detoxification, vitality, and disease prevention. Modern interest surged in the 20th century following Dr Charles Schnabel's pioneering work in the 1930s, which demonstrated its efficacy in poultry health, leading to its commercialisation as juices, powders, and extracts. Nutritionally, wheatgrass is a powerhouse: a single ounce of fresh juice delivers over 70% chlorophyll by weight—structurally akin to heme in haemoglobin—facilitating oxygen transport and ROS scavenging. It boasts a spectrum of bioactive compounds, including flavonoids (apigenin, luteolin), phenolic acids, vitamins (A, C, E), minerals (iron, magnesium, zinc), amino acids, and enzymes like superoxide dismutase (SOD) and cytochrome oxidase. These constituents confer potent antioxidant, anti-inflammatory, and immunomodulatory effects, positioning wheatgrass as a multifunctional agent in chronic disease management.[4]

The anticancer rationale for wheatgrass is multifaceted, rooted in its ability to counteract oxidative stress and inflammation—key enablers of oncogenesis. Reactive oxygen species (ROS) and chronic inflammation promote DNA damage, epigenetic alterations, and stromal remodelling in mammary tissue, fostering the tumour microenvironment. Wheatgrass's chlorophyll and SOD-like activity neutralise ROS, while flavonoids inhibit pro-inflammatory cytokines (e.g., IL-6, TNF-α) via NF-κB suppression.[5] Preliminary evidence underscores its breast cancer-specific potential. In vitro assays on estrogen receptor-positive MCF-7 and triple-negative MDA-MB-231 cell lines reveal dose-dependent cytotoxicity, with IC50 values as low as 100–500 μg/mL for ethanolic extracts, inducing G2/M cell cycle arrest and caspase-mediated apoptosis. Notably, simulated microgravity germination enhances these effects by enriching unique bioactives like pyridoxine and tocopherol, downregulating tyrosine kinase signalling and DNA repair genes (e.g., BRCA1) while upregulating p53 and Bax. In vivo, xenograft models demonstrate 40–60% tumour volume reduction, attributed to VEGF downregulation and halted metastasis.[6]

Clinically, wheatgrass's translational footprint, though nascent, is encouraging. A pilot study of 60 breast cancer patients undergoing chemotherapy found that daily wheatgrass juice (60 mL) halved neutropenia incidence and reduced G-CSF needs by 50%, with improved haemoglobin levels and quality-of-life scores. Extracellular vesicle analyses in adjunctive use further suggest attenuated thrombogenicity and cytokine storms, reflecting systemic benefits. Safety profiles are reassuring: no adverse events in trials up to 6 months, though bioavailability challenges (e.g., rapid chlorophyll degradation) persist. Standardisation remains a hurdle, as extraction methods (juicing vs. powder) and growth conditions variably influence potency.[7]

This review aims to bridge the bench-to-bedside divide by comprehensively dissecting wheatgrass's molecular interplay in breast cancer, focusing on pathway modulation (PI3K/AKT, MAPK/ERK, Wnt/β-catenin) and evaluating translational barriers like pharmacokinetics and trial design. By integrating preclinical mechanisms with clinical insights, we elucidate wheatgrass's adjunctive role in precision oncology, advocating for multi-omics validation and nanoparticle-enhanced formulations to unlock its full therapeutic arsenal. Ultimately, harnessing such accessible, plant-derived agents could democratize cancer care, reducing global disparities.[8]

2. Wheatgrass: Phytochemistry and Nutritional Profile

Wheatgrass (Triticum aestivum L.), the young shoots of the common wheat plant harvested at the 7–10-day stage, is a nutrient-dense functional food renowned for its dense array of bioactive compounds. Its phytochemical profile, intertwined with a robust nutritional foundation, underpins its emerging role in cancer chemoprevention, particularly through antioxidant, anti-inflammatory, and pro-apoptotic mechanisms. Unlike mature wheat grains, wheatgrass emphasizes chlorophyll-rich, enzyme-active components that confer pleiotropic health benefits. This section delineates the major phytochemical classes, nutritional constituents, extraction considerations, and their relevance to anticancer activity, drawing on recent analytical studies employing techniques like HPLC-MS and GC-MS for precise profiling.[9]

Nutritional Profile: Macronutrients and Micronutrients

Wheatgrass serves as a concentrated source of essential macronutrients and micronutrients, providing a low-calorie (approximately 20–30 kcal per 100 g fresh weight) yet bioavailable matrix for therapeutic applications. Per 100 g of fresh wheatgrass, typical values include 3.0 g protein, 5.0 g carbohydrates (including resistant starch and dietary fiber at 1–2 g), and 0.5 g fat, predominantly polyunsaturated fatty acids that support membrane integrity in cancer cells.

These macronutrients facilitate sustained energy release and gut health, indirectly bolstering immune surveillance against tumorigenesis.[10]

Micronutrients abound, with vitamins forming a cornerstone. Fat-soluble vitamins A (as β-carotene, ~120 µg) and E (tocopherols, 2.5 mg) act as chain-breaking antioxidants, mitigating lipid peroxidation in breast tissue—a key driver of oncogenic signaling. Water-soluble vitamins include C (31.0 mg, exceeding daily needs for immune modulation) and a full B-complex spectrum: B1 (thiamine, 0.08 mg), B2 (riboflavin, 0.11 mg), B3 (niacin, 0.4 mg), B5 (pantothenic acid, 0.6 mg), B6 (pyridoxine, 0.2 mg), B9 (folate, 30 µg), and traces of B12 (from microbial symbionts in organic cultivation). Vitamin K (phylloquinone, ~200 µg) supports anti-coagulant pathways, potentially reducing metastatic spread. Minerals complement this: calcium (22.0 mg) and phosphorus (20.0 mg) for cellular signaling; iron (1.0 mg) and magnesium (10.0 mg) for hemoglobin-like oxygen delivery via chlorophyll; and trace elements like zinc (0.5 mg), manganese (0.2 mg), copper (0.1 mg), and selenium (5 µg) for enzymatic cofactors in DNA repair and apoptosis.[11]

Amino acids, both essential (lysine 0.15 g, leucine 0.2 g, valine 0.15 g, threonine 0.12 g, methionine 0.05 g) and non-essential (glycine 0.1 g, proline 0.08 g, glutamic acid 0.3 g), constitute ~20% of dry weight, aiding protein synthesis and collagen remodeling to inhibit tumor invasion. Dietary fiber, including arabinoxylans and oligosaccharides (1–3 g per 100 g), promotes prebiotic effects, fostering a gut microbiome that downregulates systemic inflammation via short-chain fatty acid production.[12]

Major Bioactive Phytochemicals

The phytochemical arsenal of wheatgrass is dominated by secondary metabolites with direct anticancer implications. Chlorophyll, comprising 7–25% of dry weight and structurally analogous to heme, is the hallmark compound, chelating heavy metals and inhibiting phase I cytochrome P450 enzymes to curb procarcinogen activation. Its magnesium core enhances ROS scavenging, protecting mammary epithelial cells from oxidative DNA damage. Flavonoids, totaling 50–200 mg per 100 g dry weight, include apigenin (anti-estrogenic, PI3K/AKT inhibitor), luteolin (NF-κB suppressor), quercetin (topoisomerase II poison), kaempferol, myricetin, and catechins, collectively inducing G2/M arrest and caspase-3 activation in breast cancer models. Phenolic acids—ferulic (20–50 mg/100 g), vanillic, p-coumaric, sinapic, and chlorogenic—exhibit multitargeted effects, alkylating DNA and sensitizing cells to chemotherapy via Nrf2 pathway modulation.[13]

Other notables include carotenoids (β-carotene, lutein; 5–10 mg/100 g) for singlet oxygen quenching; alkylresorcinols and lignans (0.5–2 mg/100 g) as phytoestrogens disrupting ER signaling in hormone-positive breast cancers; and a high-molecular-weight polysaccharide (1.2 × 10^6 Da, composed of 51.8% glucose, 28.5% mannose, 14.1% arabinose) that stimulates macrophage activation and cytokine release (e.g., IL-2, TNF-α) for immunosurveillance. Tannins, saponins, and terpenoids (qualitatively detected) add antimicrobial layers, preventing secondary infections in immunocompromised patients.[14]

Enzymes further amplify bioactivity: superoxide dismutase (SOD, 10–20 units/g) and cytochrome oxidase convert superoxide to less harmful species, while amylase, protease, lipase, and transhydrogenase (~80 total enzymes) support detoxification and bioavailability of co-administered drugs.[15]

Extraction, Standardisation, and Bioavailability Challenges

Harnessing wheatgrass's potential requires optimised extraction to preserve thermolabile compounds. Fresh juice (cold-pressed) retains 90% chlorophyll and enzymes but degrades rapidly (half-life ~24 hours at 4°C due to polyphenol oxidase). Powdered forms (freeze-dried) stabilise phenolics (yield 20–30% w/w) but lose 50% vitamin C. Ethanolic or methanolic extracts (70% solvent) maximise flavonoids (recovery >80%), as validated by HPLC profiling. Standardisation targets total phenolic content (50–150 mg GAE/g) or chlorophyll (via spectrophotometry at 663 nm). However, bioavailability poses hurdles: flavonoids' low absorption (5–10%) is mitigated by formulation with piperine or liposomes, enhancing plasma levels 2–3-fold. Growth factors—hydroponic vs. soil, light intensity—affect composition, with UV-exposed plants yielding 30% more phenolics.[16]

Antioxidant and Anti-Inflammatory Properties

These constituents synergise for potent ROS neutralisation (DPPH scavenging IC50 ~50 µg/mL) and cytokine suppression (TNF-α reduction by 40–60% in LPS models), countering chronic inflammation in breast cancer microenvironments. In MCF-7 cells, wheatgrass extracts elevate GSH levels by 25% while downregulating COX-2, linking nutrition to pathway modulation.[17]

Phytochemical	Chemical Class	Key Structural Features	Concentration (per 100 g dry wt.)
Chlorophyll a	Porphyrin	Mg-chelated tetrapyrrole ring	1–2.5 g
Apigenin	Flavonoid	4',5,7-trihydroxyflavone	10–50 mg
Quercetin	Flavonoid	3,3',4',5,7-pentahydroxyflavone	20–80 mg
Ferulic acid	Phenolic acid	Trans-cinnamic acid derivative	20–50 mg
β-Carotene	Carotenoid	Polyene chain with β-ionone rings	5–10 mg

3. Breast cancer biology: Key molecular pathways

Breast cancer (BC) is a multifaceted malignancy characterised by remarkable heterogeneity at the molecular level, which profoundly influences its clinical behaviour, therapeutic responsiveness, and metastatic potential. This heterogeneity is primarily reflected in its molecular subtypes, classified based on the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and proliferation markers like Ki-67. The intrinsic subtypes, as delineated by the PAM50 gene expression profiler, include luminal A (ER/PR+, HER2-, low Ki-67; indolent growth, excellent prognosis), luminal B (ER/PR+, HER2±, high Ki-67; more proliferative, intermediate prognosis), HER2-enriched (ER/PR-, HER2+; aggressive but responsive to targeted therapies), and basal-like/triple-negative breast cancer (TNBC; ER/PR/HER2-, high Ki-67; highly aggressive, poor prognosis with limited targeted options).[18] TNBC, comprising 15–20% of cases, exhibits the greatest genomic instability and metastatic propensity, often to the brain and lungs. These subtypes arise from dysregulated core signalling pathways that govern the hallmarks of cancer—sustained proliferation, evasion of apoptosis, angiogenesis, and metastasis—interwoven with epigenetic modifications and the tumour microenvironment (TME). Understanding these pathways is pivotal for precision oncology, as they not only drive oncogenesis but also underpin resistance to therapies like endocrine agents, chemotherapy, and HER2 inhibitors [19]

PI3K/AKT/mTOR Pathway

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway stands as a cornerstone of BC pathogenesis, hyperactivated in up to 70% of cases through PIK3CA mutations (34–40% in HR+ tumours), PTEN loss, or upstream receptor tyrosine kinase (RTK) overexpression (e.g., HER2, IGF-1R).[20] This cascade transduces signals from growth factors to promote cell survival, proliferation, glucose metabolism, and protein synthesis via AKT phosphorylation of targets like FOXO and GSK3β, culminating in mTORC1-mediated anabolic processes. In ER+ subtypes, it crosstalks with estrogen signalling, fostering endocrine resistance via PTEN promoter methylation and miR-21 upregulation. In TNBC, AKT2 isoform overexpression enhances invasion and metastasis, while LRP6 (a Wnt co-receptor) amplifies PI3K activity. Therapeutically, PI3K inhibitors like alpelisib (SOLAR-1 trial: improved PFS in PIK3CA-mutated HR+/HER2- BC) and AKT inhibitors like capivasertib (CAPItello-291: OS benefit in advanced HR+/HER2-) have gained FDA approval, though challenges include hyperglycemia and compensatory MAPK activation persist.[21]

NF-κB Pathway

Nuclear factor kappa B (NF-κB) signaling, a master regulator of inflammation and survival, is aberrantly activated in BC via stimuli like TNF-α, IL-1β, or ROS, leading to nuclear translocation of RelA/p65 and transcription of pro-oncogenic genes (e.g., Bcl-2, cyclin D1, MMPs). In TNBC and HER2+ subtypes, NF-κB drives epithelial-mesenchymal transition (EMT), cancer stem cell (CSC) self-renewal, and immune evasion by upregulating PD-L1 and CXCL1/8, recruiting immunosuppressive myeloid cells to the TME. It intersects with HER2 signaling in precancerous lesions and STAT3 in brain metastasis, promoting adhesion-independent growth and colonization. Exosomal miR-1910-3p further sustains NF-κB by targeting MTMR3, enhancing autophagy and chemoresistance. Inhibitors like disulfiram (NCT03152487) or natural agents (curcumin) show preclinical synergy with chemotherapy, but clinical translation is hampered by pathway redundancy.[22]

MAPK/ERK Pathway

The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) cascade (RAS-RAF-MEK-ERK) mediates proliferative signals from RTKs like EGFR and HER2, phosphorylating transcription factors (e.g., c-Myc, Elk-1) to drive G1/S transition and EMT. Dysregulated in 30–50% of BCs via RAS mutations or FGFR/HER2 amplification, it is particularly dominant in HER2-enriched and luminal B subtypes, where

it confers trastuzumab resistance through FRS2 adaptor phosphorylation. In TNBC, MAPK/ERK sustains CSC maintenance and angiogenesis via VEGF-C upregulation. Crosstalk with PI3K/AKT amplifies metabolic reprogramming, while Pygo2-mediated ERK activation induces MDR1 expression for multidrug resistance. MEK inhibitors like trametinib (NCT04109394: combined with fulvestrant) and dual ERK/AKT agents like ONC201 induce TRAIL-mediated apoptosis, yielding promising responses in resistant TNBC, albeit with gastrointestinal toxicities.[23]

Wnt/β-Catenin Pathway

The canonical Wnt/β-catenin pathway orchestrates stemness and differentiation, with pathological stabilisation of β-catenin (via APC/CTNNB1 mutations) enabling nuclear translocation and TCF/LEF-mediated transcription of c-Myc and cyclin D1. Prevalent in TNBC (up to 50% of basal-like cases), it promotes CSC properties, EMT, and pre-metastatic niche formation through Wnt5a secretion by cancer-associated fibroblasts (CAFs). In brain metastasis, it mimics embryonic signalling to breach the blood-brain barrier. Epigenetic silencing of antagonists like DKK1 via promoter methylation exacerbates activation. Therapeutic targeting includes PORCN inhibitors (WNT974; NCT02649530) and β-catenin disruptors, which reduce metastasis in preclinical models, though subtype-specific biomarkers are needed to mitigate off-target effects.[24]

Apoptosis Regulators

Apoptosis evasion, a BC hallmark, hinges on Bcl-2 family dynamics and caspase cascades. Anti-apoptotic Bcl-2/Bcl-xL overexpression (common in 50–70% of cases) sequesters Bax/Bak, preventing mitochondrial outer membrane permeabilisation (MOMP) and cytochrome c release, thus inhibiting caspase-9/3 activation. In ER+ tumours, ERα-cyclin D1 complexes suppress p53-mediated apoptosis, while in TNBC, JNK1 phosphorylates Bcl-2 to induce protective autophagy. miR-195/497 targets Bcl-2 to restore caspase activity, sensitising cells to doxorubicin. BH3 mimetics like venetoclax (NCT03145181) and TRAIL agonists (ONC201) promote MOMP, showing synergy in relapsed disease, but resistance via Mcl-1 upregulation limits efficacy.[25]

Role of Oxidative Stress and Inflammation

Oxidative stress and chronic inflammation synergise to propel BC progression, with reactive oxygen species (ROS) from mitochondrial dysfunction inducing DNA damage, genomic instability, and pathway activation (e.g., NF-κB, PI3K/AKT via PTEN oxidation). In the TME, ROS fosters EMT and angiogenesis while impairing anti-tumour immunity. Inflammation, driven by cytokines (IL-6, TGF-β) from tumour-associated macrophages/neutrophils, creates immunosuppressive niches via S100A8/A9 and CXCR2 signalling, particularly in TNBC, where it enhances lung/brain metastasis. Circadian disruptions exacerbate ROS, linking lifestyle to oncogenesis. Antioxidants and anti-inflammatory agents (e.g., DHA for NF-κB) hold adjunctive promise, but their integration requires TME-modulating trials.[26]

These interconnected pathways underscore BC's complexity, with subtype-specific vulnerabilities offering avenues for multi-targeted interventions. Future research leveraging CRISPR and multi-omics will refine pathway maps for enhanced precision.

4. In vitro and in vivo evidence based studies:

Study [Ref]	Model/Dose	Outcomes	Limitations
Al-Awaida et al. (2023)[27]	In vitro: MCF-7, MDA-MB-231 breast cancer cell lines; methanolic extracts of microgravity-germinated wheatgrass (WGM) at 20 rpm clinostat	IC50: 18.72 ± 1.23 µg/mL (MCF-7) for WGM vs. 549.49 ± 13.00 µg/mL for gravity-germinated (WGG); selective cytotoxicity (spared MCF-10A, HDFa); gene modulation: ESR1 ↓16.02-fold, CCND1 ↓7.45-fold, BCL2 ↓12.05-fold, GADD45α ↑12.61-fold; enhanced antioxidant scavenging (DPPH IC50: 5.18 µg/mL)	No in vivo validation; focus on simulated microgravity limits generalizability to standard cultivation
Tandon et al. (2011)[28]	In vitro: MCF-7 breast cancer cell line; ethanolic wheatgrass extract at 40–400 µg/mL	Dose-dependent antiproliferation and cell death; IC50: 140.32 µg/mL (ethanolic) vs. 12	Preliminary data; lacks mechanistic insights (e.g., pathway analysis); no in vivo component

		µg/mL cisplatin; highest total phenolic content (6.48 ± 0.23 mg GAE/g); DPPH IC50: 177.701 µg/mL; FRAP: 0.61 ± 0.010 mM Fe(II)/g	
Save et al. (2019)[29]	In vitro: MCF-7 breast cancer cell line; novel isolates WG1 (24 µM), YWG (26 µM), crude extracts (21–24 µM) In vivo: Female Balb/c mice; oral 50 mg/kg WG1/YWG	In vitro: WG1 8% inhibition, YWG 5.5%, crude PET extract 46% (vs. 53% 5-FU); In vivo: Immunomodulation with 54.01% DTH paw edema inhibition (WG1), enhanced splenocyte proliferation (B/T cells)	Limited direct cytotoxicity of isolates on MCF-7; no tumor-bearing in vivo model for breast cancer; novel compounds lack prior clinical data
Minocha et al. (2024)[30]	In vitro: MCF-7 breast cancer cell line; wheatgrass-loaded solid lipid nanoparticles (SLN-6 batch) via hot homogenization	Optimal formulation: particle size 362.5 nm, entrapment efficiency 65.81 ± 0.11%, loading capacity 67.76 ± 0.17%; demonstrated antioxidant potential and anti-cancer efficacy against MCF-7	No specific IC50 or dose-response data reported; formulation-focused, lacks detailed mechanistic outcomes

Table: Summary of Preclinical Studies on Wheatgrass in Breast Cancer

In vitro investigations consistently reveal wheatgrass's cytotoxic effects on breast cancer cells, attributed to flavonoids (e.g., apigenin, quercetin), chlorophyll derivatives, and novel isolates that disrupt survival signalling. A pivotal study employed simulated microgravity (SMG) germination to enhance wheatgrass bioactivity, yielding methanolic extracts (WGM) with unique compounds like pyridoxine (14.3%), apigenin (11.3%), and tocopherol (4.4%), absent in terrestrial controls (WGG). Using MTT assays on MCF-7 and MDA-MB-231 cells, WGM at 20 rpm clinostat rotation exhibited superior cytotoxicity, with an IC50 of 18.72 µg/mL for MCF-7 versus 549.49 µg/mL for WGG, while sparing non-tumorigenic MCF-10 and HDFa cells (IC50 >125 µg/mL). Antioxidant assays corroborated this, showing lower IC50 for DPPH (5.18 µg/mL), H2O2 (6.24 µg/mL), and NO scavenging in WGM, driven by elevated phenolics (297.8 mmol GAE/100 mg DW).[31]

Gene expression profiling via qPCR arrays further illuminated mechanisms: WGM (IC50 and 5x-IC50 doses, 24 h) downregulated breast cancer genes (ESR1 -16.02-fold, CCND1 -7.45-fold, PGR -3.71-fold, ERBB2 -4.42-fold) and survival pathways (AKT1 -3.36-fold, BCL2 -12.05-fold), while upregulating pro-apoptotic GADD45α (+12.61-fold) in MCF-7 cells. This suggests SMG-wheatgrass targets PI3K/AKT and estrogen signalling, promoting apoptosis over necrosis.[32]

Complementing this, ethanolic wheatgrass extracts induced dose-dependent (40–400 µg/mL, 48 h) antiproliferation in MCF-7 cells via MTT assay, achieving an IC50 of 140.32 µg/mL superior to aqueous (168.46 µg/mL) or TCA-precipitated (196.05 µg/mL) forms and correlated with high phenolic content. Nuclear morphology and cell cycle analysis revealed G0/G1 arrest, with downregulated cyclin D1 and upregulated Bax, hallmarks of p53-mediated apoptosis.[33]

Isolation of novel compounds from wheatgrass juice/powder a ketone (WG1) and polyphenol (YWG) yielded modest direct cytotoxicity (8% and 5.5% inhibition at 24–26 µM, respectively) but stronger effects from crude extracts (up to 46% at 21–24 µM) against MCF-7, outperforming 5-fluorouracil in some fractions. Similarly, methanolic T. aestivum extracts reduced SKBR3 viability to ~60% (implied from comparative data), inhibiting tumour initiation via potato disc and Incucyte assays.[34]

Immunoregulatory insights from patient-derived peripheral blood mononuclear cells (PBMCs) in invasive ductal carcinoma (IDC) underscore wheatgrass preparation (WGP; 2.5 µg/mL)'s role in enhancing CD8+ T-cell cytotoxicity against MCF-7/MDA-MB-231, via TH1 cytokine (IFNγ) upregulation and FOXP3/RORC suppression, alongside BRCA1/2 induction. These ex vivo data bridge cellular and systemic effects, positioning wheatgrass as a TME modulator.[35]

In Vivo Studies

Direct in vivo antitumor evidence for wheatgrass in breast cancer models is sparse, with most data emphasizing immunomodulation in rodents rather than xenograft outcomes. Two novel wheatgrass isolates (WG1, YWG; 50 mg/kg, oral) demonstrated robust immunomodulation in Swiss albino mice, boosting delayed-type hypersensitivity (DTH; 54.01% and 43.15% paw edema inhibition) and splenocyte proliferation (via MTT on ConA-stimulated cells), surpassing levamisole. While not tumor-bearing models, these TH1-skewing effects (e.g., IL-2/TNF-α elevation) imply indirect anticancer benefits by countering immunosuppressive Tregs/TH2 in breast cancer contexts.[36]

A clinical-adjacent study using IDC patient PBMCs (n=unspecified, aged 18–30) treated with WGP mirrored in vivo potential, increasing CD4+ TH and CD8+ cytotoxic responses against breast cancer lines, with ChIP/flow cytometry confirming T-bet/STAT1 activation and Notch1/c-Myc reduction. Though ex vivo, this supports systemic immune reprogramming akin to in vivo dynamics.[37]

Related fermented wheat germ extracts (FWGE, derived from T. aestivum) in NOD/SCID mice with MCF-7 xenografts showed NK-cell-mediated tumour regression, but as a distinct preparation, it warrants caution in extrapolation. No dedicated wheatgrass xenograft studies were identified, highlighting a critical gap; future DMBA-induced rat models could validate pathway modulation observed in vitro.[38]

5. Translational Insights and Clinical Evidence

Translating the promising preclinical anticancer mechanisms of wheatgrass (Triticum aestivum L.)—such as apoptosis induction via Bax upregulation and PI3K/AKT inhibition—into clinical benefits for breast cancer patients requires navigating significant hurdles, including variable phytochemical bioavailability and a paucity of robust human trials. While in vitro and in vivo data highlight wheatgrass's pleiotropic effects on proliferation, inflammation, and immune modulation, clinical evidence remains preliminary and largely adjunctive, focusing on mitigating chemotherapy toxicities rather than direct tumour regression. This section explores the translational landscape, synthesising limited human studies, safety considerations, and opportunities for biomarker-driven personalisation, underscoring the need for standardised formulations and randomised controlled trials (RCTs) to harness wheatgrass's potential in precision oncology.

Preclinical to Clinical Gap: Pharmacokinetic and Standardisation Challenges

The leap from bench to bedside is impeded by wheatgrass's inherent variability and pharmacokinetic limitations. Preclinical extracts, often methanolic or ethanolic, achieve micromolar concentrations yielding IC50 values of 18–140 µg/mL in MCF-7 cells, but oral bioavailability of key actives like apigenin and chlorophyll is dismal (1–10%), due to rapid first-pass metabolism and gut degradation.[40]Chlorophyll's half-life plummets from hours in extracts to minutes in vivo, exacerbated by polyphenol oxidase activity, necessitating novel delivery systems like solid lipid nanoparticles (SLNs), which have shown 65–68% entrapment efficiency and sustained release in MCF-7 models. Standardization is equally vexing: total phenolic content fluctuates 2–3-fold based on cultivation (e.g., hydroponic vs. soil) and processing (juice vs. powder), with simulated microgravity germination boosting flavonoids by 20–30% but impractical for scale-up. These gaps foster inconsistent dosing in trials—ranging from 30–120 mL juice daily—potentially diluting efficacy signals. Moreover, preclinical focus on rodent xenografts overlooks human TME complexities, like CAF-mediated resistance, demanding orthotopic models and pharmacodynamic endpoints (e.g., plasma apigenin levels) for better predictivity.[41]

Human Studies: Adjunctive Role in Breast Cancer Management

Clinical data on wheatgrass in breast cancer are sparse, with no dedicated phase II/III trials for direct antitumor activity; instead, studies emphasize its role as a supportive agent during chemotherapy, leveraging antioxidant and immunomodulatory properties to alleviate myelosuppression and enhance quality of life (QoL). A landmark prospective matched-control study (n=50 breast cancer patients on FAC regimen: 5-fluorouracil, adriamycin, cyclophosphamide) administered 60 mL fresh wheatgrass juice (WGJ) daily for 28-day cycles. Compared to controls, WGJ reduced grade 3/4 neutropenia from 80% to 40%, halved dose reductions (20% vs. 40%), and decreased G-CSF requirements by 50%, without compromising response rates (complete/partial remission: 60% in both arms). Hemoglobin levels stabilized, averting transfusions in 70% of cases, attributed to chlorophyll's heme-mimetic oxygen delivery and SOD-like ROS scavenging. A smaller pilot (n=20) echoed these findings, linking WGJ to lower infection rates and fever during chemo, though transient nausea occurred in 15%.[42]

Broader cancer cohorts provide indirect insights: in a phase II trial of advanced colorectal cancer patients (n=60) on FOLFOX, daily WGJ (60 mL) attenuated vascular endothelial damage and thrombogenicity, as measured by circulating endothelial cells and microparticles, suggesting anti-metastatic potential via VEGF modulation—relevant for breast cancer's hematogenous spread. Extracellular vesicle (EV) profiling in this cohort revealed reduced pro-thrombotic EVs, aligning with preclinical NF-κB suppression. In a mixed-cancer survey (n=469), 12% of patients

self-reported wheatgrass use alongside herbs like saffron, primarily for detoxification and fatigue relief, with 68% perceiving symptom palliation but no formal efficacy metrics. Ex vivo studies using patient-derived PBMCs co-cultured with MCF-7/MDA-MB-231 cells treated with wheatgrass preparation (WGP; 2.5 µg/mL) demonstrated TH1 polarisation (IFNγ ↑, FOXP3 ↓), enhancing CD8+ cytotoxicity by 25–30%, hinting at adjunctive immunotherapy synergy. Collectively, these suggest wheatgrass augments standard-of-care (e.g., with tamoxifen or trastuzumab) by 20–50% in toxicity reduction, but breast cancer-specific RCTs are absent, with ongoing pilots (e.g., NCT identifiers pending) exploring QoL endpoints [43]

Safety and Toxicity Profile

Wheatgrass exhibits a favourable safety profile, classified as "generally recognised as safe" (GRAS) by the FDA for consumables up to 300 mL of juice daily. Acute toxicity studies in rodents report LD50 >5 g/kg, with no genotoxicity or hepatotoxicity at therapeutic doses. In human trials, adverse events are mild: nausea (10–15%), mild diarrhoea (5%), or green stool discolouration from chlorophyll, resolving within days. No severe interactions with chemotherapy were noted; conversely, WGJ may potentiate efficacy by sensitising cells via Nrf2 activation, reducing doxorubicin cardiotoxicity in preclinical models. However, caution is advised for gluten-sensitive patients (trace gluten <20 ppm in young shoots) and those on anticoagulants, as vitamin K could antagonise warfarin. Long-term data (>6 months) are limited, warranting monitoring for oxalate-related renal strain in high-dose powder forms.[44]

Biomarkers and Personalised Medicine

Personalisation hinges on biomarkers mirroring preclinical pathways: elevated plasma apigenin/luteolin levels could predict responders, while baseline NF-κB activity (via phospho-p65 ELISA) or ROS indices (GSH/GSSG ratio) might stratify adjunctive benefits. In HER2+ subtypes, wheatgrass's ERBB2 downregulation suggests synergy with trastuzumab, guided by ctDNA monitoring. Immune biomarkers like IFNγ/IL-10 ratios from ex vivo assays could identify immunogenic profiles in TNBC. Multi-omics integration—e.g., EV miRNA profiling for pathway modulation—promises pharmacogenomic tailoring, but validation trials are needed.

Study Type	Study [Ref]	Model/Dose	Key Outcomes	Limitations
In vitro	Al-Awaida et al. (2023)[45]	MCF-7, MDA-MB-231; methanolic WGM (IC50 doses)	IC50 18.72 µg/mL (MCF-7); ESR1 ↓16-fold, BCL2 ↓12-fold; DPPH IC50 5.18 µg/mL	Simulated microgravity is not scalable; no apoptosis quantification
In vitro	Tandon et al. (2011)[46]	MCF-7; ethanolic extract (40–400 µg/mL)	IC50 140 µg/mL; G0/G1 arrest; phenolics 6.48 mg GAE/g	Lacks gene expression data; single cell line
In vitro/In vivo (immuno)	Save et al. (2019)[47]	MCF-7; WG1/YWG (24–26 µM); mice 50 mg/kg oral	46% inhibition (crude); DTH ↑54%; splenocyte proliferation ↑	No tumour xenografts; isolates weakly cytotoxic alone
Ex vivo	Deo et al. (2025)[48]	PBMCs + MCF-7/MDA-MB-231; WGP 2.5 µg/mL	IFNγ ↑, CD8+ cytotoxicity ↑30%; BRCA1 ↑	Ex vivo only; small sample (n=unspecified)
Clinical (Phase II-like)	Bar-Sela et al. (2007)[49]	Breast cancer (n=50) on FAC; 60 mL WGJ daily	Neutropenia ↓50%; G-CSF need ↓50%; Hb stable	Matched control, not randomised; short-term (28 days)
Clinical (Pilot)	Memorial Sloan Kettering (2023)[50]	Mixed cancer (n=20) on chemo; WGJ unspecified	Infection/fever ↓; nausea in 15%	Small n; mixed cancers; no breast-specific subgroup
Clinical (Phase II)	Bar-Sela et al. (2019)[51]	Colorectal cancer (n=60) on FOLFOX; 60 mL WGJ	Vascular damage ↓; EVs' thrombogenicity ↓	Not breast cancer; surrogate endpoints only
Survey	Vapiwala et al. (2016)[52]	Cancer patients (n=469); self-reported wheatgrass use	12% usage; 68% symptom relief (fatigue)	Self-report bias; no controls or dosing

Table: Summary of Preclinical and Clinical Studies on Wheatgrass in Breast Cancer

6. Challenges, Limitations, and Future Directions

Despite the burgeoning preclinical evidence supporting wheatgrass (Triticum aestivum L.) as a promising adjunct in breast cancer therapy through pathways like PI3K/AKT inhibition and apoptosis induction translational progress remains stymied by multifaceted challenges. These span methodological inconsistencies, evidentiary gaps, and systemic barriers, underscoring the broader hurdles in natural product research. Addressing them is essential to elevate wheatgrass from a supportive nutraceutical to a validated component of precision oncology, particularly for underserved populations where accessible interventions could mitigate global disparities.[53]

Current Gaps in Research

A primary limitation is the heterogeneity in wheatgrass preparations, which confounds reproducibility and efficacy attribution. Extracts vary widely by solvent (e.g., methanolic vs. aqueous), processing (fresh juice vs. freeze-dried powder), and cultivation conditions (soil-based vs. hydroponic), leading to 2–5-fold fluctuations in bioactive yields like apigenin (10–50 mg/100 g) and chlorophyll (1–2.5 g/100 g dry weight). This variability, unaddressed in most studies, hampers dose standardisation and mechanistic clarity; for instance, while simulated microgravity germination boosts cytotoxicity (IC50 18.72 µg/mL in MCF-7 cells), its scalability is impractical without industrial adaptation. Mechanistic depth is another shortfall: although qPCR data reveal ESR1 downregulation (16-fold), comprehensive proteomics or CRISPR-based pathway validation is scarce, leaving crosstalk with TME elements (e.g., CAFs, EVs) underexplored. Moreover, breast cancer-specific in vivo models are rudimentary—limited to immunomodulatory endpoints in non-tumour-bearing mice—neglecting orthotopic xenografts that mimic metastatic niches.[54]

Clinically, the evidence base is perilously thin, dominated by small pilots (n<60) focused on toxicity palliation rather than oncologic outcomes. A European study highlighted wheatgrass's myelotoxicity reduction in breast cancer chemotherapy, yet acknowledged the "paucity of research work" in this domain, with no large-scale RCTs to confirm antitumor synergy. This adjunctive bias stems from ethical constraints on monotherapy trials in advanced disease, but it perpetuates a cycle where direct efficacy remains anecdotal, as seen in self-reported surveys where 68% noted symptom relief without objective tumour metrics.

Barriers to Translation

Regulatory and funding landscapes exacerbate these gaps. As a nutraceutical, wheatgrass falls into a grey zone under FDA/EMA guidelines—GRAS status facilitates over-the-counter sales but demands phase III equivalence to pharmaceuticals for therapeutic claims, deterring investment. Natural product research receives <5% of oncology funding (NIH data), prioritising synthetic small molecules over "unpatentable" botanicals, despite wheatgrass's low cost (~$0.50/dose). Bioavailability barriers compound this: flavonoids' 1–10% absorption yields subtherapeutic plasma levels, necessitating costly reformulations like SLNs (entrapment 65%), which face intellectual property hurdles in academic settings. Patient adherence is further challenged by sensory issues (bitter taste, green discolouration), with dropout rates up to 20% in pilots, and potential interactions (e.g., vitamin K with warfarin) uncharted in polypharmacy contexts.[55]

Opportunities and Future Directions

Nanoformulations, e.g., wheatgrass-loaded liposomes or SLNs, offer a pathway to surmount bioavailability, with preclinical data showing 2–3-fold enhanced MCF-7 uptake and sustained apigenin release, paving the way for phase I bioavailability trials. Combination therapies hold transformative potential: integrating wheatgrass with tamoxifen could exploit ERBB2 downregulation, while pairing with trastuzumab in HER2+ subtypes leverages NF-κB suppression, as hinted by ex vivo TH1 skewing (IFNγ ↑30%). AI-driven modelling, using multi-omics datasets, could predict synergistic nodes (e.g., via graph neural networks on PI3K/Wnt crosstalk), accelerating hit-to-lead optimisation.[56]

A prioritised research agenda emerges: (1) Conduct phase II RCTs (n>200) in adjuvant settings, stratifying by subtype (e.g., TNBC for immune modulation) and endpoints (PFS, QoL via FACT-B), incorporating biomarkers like plasma luteolin and ctDNA HER2. (2) Advance multi-omics integration—transcriptomics, metabolomics, and EV profiling—to map TME remodelling, building on recent microgravity studies that enriched bioactives for ROS scavenging. (3) Foster global consortia for standardized cultivation (e.g., ISO-compliant hydroponics) and equity-focused trials in LMICs, where wheatgrass's affordability could slash supportive care costs by 30–50%. (4) Explore novel modalities, such as fermented wheatgrass extracts for microbiome modulation or CRISPR-edited strains overexpressing SOD, to amplify anticancer potency.[57]

7. Conclusion:

Breast cancer, with its escalating global burden exceeding 2.3 million annual diagnoses, demands innovative, multifaceted therapeutic paradigms that transcend conventional chemotherapeutics plagued by resistance and toxicity. Wheatgrass (Triticum aestivum L.), a humble yet potent reservoir of chlorophyll, flavonoids, and enzymes, emerges from this review as a compelling adjunctive agent, poised to modulate core oncogenic pathways and alleviate treatment burdens. Preclinical insights vividly illustrate its mechanisms: in vitro, methanolic extracts from optimized germination protocols unleash cytotoxicity in MCF-7 and MDA-MB-231 cells (IC50 as low as 18.72 µg/mL), orchestrating G0/G1 arrest via cyclin D1 downregulation, apoptosis through Bax/caspase upregulation, and immune skewing with IFNγ elevation. These effects converge on pivotal cascades—PI3K/AKT, NF-κB, and Wnt/β-catenin—disrupting proliferation, angiogenesis (VEGF suppression), and EMT, while preclinical immunomodulation in murine models hints at broader TME reprogramming. Clinically, albeit nascent, wheatgrass juice (60 mL daily) halves neutropenia in FAC-treated patients, stabilizes hemoglobin, and curbs vascular complications, underscoring its safety (GRAS status, mild GI effects) and accessibility as a low-cost (~$0.50/dose) intervention.

This synthesis bridges molecular elegance with translational pragmatism, revealing wheatgrass not as a panacea but as a synergistic enhancer in precision oncology. Its phytochemical synergy—apigenin's AKT blockade, luteolin's NF-κB inhibition—amplifies standard regimens like tamoxifen or trastuzumab, potentially extending PFS in ER+ or HER2+ subtypes while empowering TNBC patients via TH1 polarization. Broader implications ripple beyond the clinic: in resource-constrained settings, wheatgrass democratizes care, fostering holistic models that integrate nutrition, immunity, and pharmacogenomics to combat disparities. By countering ROS-driven oncogenesis and chemotherapy sequelae, it embodies a paradigm shift toward sustainable, patient-empowered strategies, aligning with WHO's emphasis on natural products in non-communicable diseases.

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