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Phytochemical Profiling and Bioactive Potential of *Pennisetum purpureum* Schumach.: Antioxidant, Anticancer, and Anti-inflammatory Activities

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Abstract

Oxidative stress and inflammation are predominant causes of chronic diseases, including various forms of cancer. Prevention of these processes is considered a key target for disease prevention due to their significant roles in degenerative disorders. Natural products and plant extracts have shown promise in preventing free radical-induced damage.This study evaluated the *in vitro* antioxidant, anti-inflammatory, and anticancer activities of hydroalcoholic extract of *Pennisetum purpureum* (HAEPP). The extract was subjected to phytochemical screening and quantification of total phenolic content (TPC), Total flavonoid content (TFC), and Total alkaloidal content (TAC). Antioxidant activities were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Anti-inflammatory properties were assessed through nitric oxide inhibition, and cyclooxygenase inhibition assays. Anticancer activity was determined using human breast cancer cells (MCF-7) by MTT assay, examining cytotoxicity, proliferation, and apoptosis. Phytochemical analysis revealed diverse secondary metabolites with quantifiable phenolic, flavonoid, and alkaloidal content. The extract demonstrated antioxidant activity against DPPH radicals (IC_{50} = 320 µg/mL) and exhibited anti-inflammatory properties through nitric oxide radical scavenging (IC_{50} = 28.76 ± 3.9 µg/mL) compared to ascorbic acid. In the cyclooxygenase inhibition assay, HAEPP showed moderate activity (IC_{50} = 22.43 µg/mL) with 32.72% relative potency compared to celecoxib (IC_{50} = 7.34 µg/mL, 100%). Furthermore, HAEPP exhibited potent concentration-dependent antiproliferative activity against MCF-7 cells (IC_{50} = 63.85 µg/mL). These findings suggest that hydroalcoholic extract of *Pennisetum purpureum* possesses significant antioxidant, anti-inflammatory, and anticancer properties, highlighting its potential as a natural therapeutic agent for prevention and treatment of chronic inflammatory diseases and cancer.

Keywords: *Pennisetum purpureum*, Cyclooxygenase, Phytochemical analysis, MCF-7 cells, MTT assay.

1. Introduction

Chronic diseases such as cancer, neurodegenerative disorders, and cardiovascular conditions are witnessing an alarming global rise. A central feature in the pathophysiology of these diseases is the disruption of redox homeostasis and persistent inflammation, which foster cellular damage through the excessive generation of reactive oxygen species (ROS) and pro-inflammatory mediators (1,2). These biomolecular insults lead to oxidative DNA lesions, protein dysfunction, and lipid peroxidation, thereby accelerating disease progression and carcinogenesis (3,4). While conventional therapeutic agents have been

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developed to manage such conditions, their limitations—including off-target toxicity, drug resistance, and lack of holistic efficacy—underscore the need for alternative strategies (5,6).In response to these challenges, natural products, particularly phytochemicals from medicinal plants, have gained prominence due to their multi-faceted mechanisms of action and better safety profiles (7,8,9). The investigation of plant-based compounds that exhibit antioxidant, anti-inflammatory, and anticancer effects presents a promising approach to fill existing gaps in therapeutic interventions (10,11). Among all cancer types, breast cancer remains the most frequently diagnosed malignancy in women worldwide. While it rarely affects men—accounting for less than 1% of cases—it poses a significant global health burden. In 2022 alone, an estimated 2.29 million new cases of breast cancer were diagnosed in women, representing approximately 23.8% of all cancer cases and contributing to over 15.4% of cancer-related deaths globally(12). The MCF-7 human breast cancer cell line, which expresses estrogen receptors, has become a widely accepted in vitro model for assessing the anticancer efficacy of phytoconstituents (13).

In this study, we explore the therapeutic relevance of the hydroalcoholic extract of *Pennisetum purpureum* (HAEPP), a plant species traditionally underexplored in pharmacological research despite its rich phytochemical profile. *Pennisetum purpureum* Schumach., also known as Napier or elephant grass, is a robust perennial grass of the Poaceae family, predominantly cultivated for its impressive biomass yield and agricultural importance as fodder (14). Although widely recognized for its nutritional value in livestock production, accumulating scientific evidence suggests that this species harbors a broad spectrum of bioactive constituents, including flavonoids, alkaloids, phenolic acids, tannins, saponins, and terpenoids—each with potential therapeutic applications(15-17). Notably, compounds such as linoleic acid, oleic acid, stigmasterol, β-sitosterol, and caffeic acid have been isolated from various plant parts and are reported to exhibit notable antioxidant, anti-inflammatory, and cytotoxic effects in preliminary screenings (18-21). Recent studies by Owolabi et al. (2018) and Yadev et al. (2024) have also demonstrated antimicrobial and antidiabetic properties of *P. purpureum* extracts, further emphasizing its pharmacological versatility (22,23). Although previous studies have highlighted the antioxidant and anti-inflammatory potential of *Pennisetum purpureum*, including the inhibition of nitric oxide and cyclooxygenase activity in inflammatory models (24,25), the specific phytochemical constituents responsible for these bioactivities remain poorly characterized. Moreover, limited studies have investigated the structure–activity relationships or the potential anticancer mechanisms linked to these phytoconstituents, especially in hormone-responsive cancer models like MCF-7 (26). Given the increasing interest in plant-based bioactives for managing oxidative stress and inflammation-related pathologies (27), a detailed exploration of the bioefficacy, cytotoxic profile, and molecular interactions of *P. purpureum* extract is essential for understanding its therapeutic potential. This study aims to bridge these gaps by conducting comprehensive in vitro assays coupled with quantitative phytochemical analyses, thereby elucidating both the therapeutic scope and mechanistic basis of *P. purpureum*'s biological effects

2. Materials and Methods

2.1. Plant Collection and Authentication

Aerial parts of *Cenchrus purpureus* (syn. *Pennisetum purpureum*) were collected from Coimbatore, Tamil Nadu, in December and authenticated by Dr. M. U. Sharief (BSI, Coimbatore). A voucher specimen was deposited (28). The material was shade-dried, coarsely powdered, and stored in airtight containers.

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2.2 Preparation of Hydroalcoholic Extract

Powdered aerial parts (100 g) were extracted using Soxhlet with 70:30 ethanol:water for 6–8 h. The extract was filtered, concentrated under reduced pressure (40–45 °C), The dried hydroalcoholic extract of *Pennisetum purpureum* (HAEPP) was stored at 4°C in amber-colored bottles (29) until further use

2.3 Physicochemical Analysis

Physicochemical parameters were evaluated as per IP (2018)(30), including total ash, acid-insoluble ash, and water-soluble ash using standard gravimetric methods. Moisture content was determined by loss on drying at 105°C to assess stability and shelf life.

2.4 Phytochemical Evaluation

2.4.1 Qualitative Phytochemical Screening

HAEPP was screened for major secondary metabolites using standard qualitative tests: alkaloids (Mayer's, Wagner's), flavonoids (Shinoda), tannins and polyphenols (ferric chloride), saponins (foam test), steroids (Salkowski), and carbohydrates (Molisch's), following established protocols

2.4.2 Quantitative Phytochemical Estimations

- **Total Phenolic Content (TPC)**: Estimated using the Folin–Ciocalteu method (31). 1 mL of extract (1 mg/mL) was mixed with 0.2 mL Folin–Ciocalteu reagent and 0.5 mL distilled water, followed by 1 mL of 8% sodium carbonate after 5 min. Final volume was adjusted to 5 mL, and absorbance measured at 765 nm. Results were expressed as mg gallic acid equivalent (GAE)/g extract.
- **Total Flavonoid Content (TFC)**: Determined by aluminum chloride colorimetric method (32). 1 mL extract (1 mg/mL) was mixed with 0.5 mL 2% $AlCl_3$ in ethanol and incubated for 1 hour. Absorbance measured at 420 nm. Results were expressed as mg quercetin equivalents (QE)/g extract.
- **Total Alkaloid Content (TAC)**: Estimated using bromocresol green (BCG) complexation and chloroform extraction method (33). Absorbance of the chloroform phase was measured at 470 nm, and results were expressed as mg atropine equivalents (ATE)/g extract.

2.5 DPPH Radical Scavenging Assay

The antioxidant activity of HAEPP was assessed using the DPPH radical scavenging assay, based on a modified method of Perumal et al. (2018) (34). Various concentrations of the extract (5–320 µg/mL) were mixed with 0.135 mM DPPH in methanol and incubated in the dark for 30 minutes. Absorbance was recorded at 517 nm, and the scavenging activity was calculated to determine the IC_{50} using the following formula:

% Inhibition =[(Absorbance of Control − Absorbance of Sample) / Absorbance of Control] × 100

2.6. Anti -inflammatory Activity.

2.6.1 Nitric Oxide Scavenging Activity Assay

Nitric oxide scavenging activity of HAEPP was assessed using the sodium nitroprusside (SNP) method with slight modifications of Garrat (35). SNP (10 mM) was incubated with the extract in phosphate buffer (pH 7.4) for 150 minutes. After incubation, Griess reagent was added, and the absorbance of the resulting chromophore was measured at 546 nm. The percent inhibition of nitric oxide generation was calculated using the formula:

% Inhibition = [(Abs control - Abs sample) / Abs control] × 100

where Abs control represents the absorbance of the control without extract and Abs sample represents the absorbance in the presence of extract.

2.6.2 Cyclooxygenase Activity Assay

COX inhibitory activity of HAEPP was assessed by measuring prostaglandin E2

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(PGE2) levels using a modified method of Vane and Botting (36). The extract was pre-incubated with COX enzyme, followed by arachidonic acid addition. After incubation, the reaction was terminated with 1N HCl, and PGE2 production was quantified via ELISA. % inhibition was calculated relative to the control and standard (celecoxib)

% Inhibition = [(control - Test) / control] × 100

2.6 MTT Assay for Cytotoxicity

Cytotoxicity of the extract was evaluated using the MTT assay as per standard protocol (37). MCF-7 cells were cultured in MEM medium and seeded (1×10^4 cells/well) in 96-well plates. After 24 h, cells were treated with varying concentrations of extract (6.25–100 µg/mL) for another 24 h. Post-treatment, 20 µL MTT (2 mg/mL) was added and incubated for 4 h. Formazan crystals were solubilized in 100 µL DMSO, and absorbance was measured at 570 nm and the percentage of cell viability was calculated using:

% Viability = (Absorbance of treated cells / Absorbance of control cells) × 100

3. Results

3.1 Physicochemical Evaluation of the Plant Extract.

The physicochemical analysis of *Pennisetum purpureum* extract showed a total ash content of 6.9%, acid-insoluble ash of 0.9**%**, and water-soluble ash of 13%. The loss on drying was recorded at 2.0%**,** indicating low moisture and good stability. These results are summarized in (Table 1).

Table 1: Physicochemical Parameters of *Pennisetum purpureum* Extract

S.No	Parameters	Results(W/W)
1	Total ash	6.9%
2	Acid Insoluble ash	0.9%
3	Water soluble ash	13%
4	Loss on Drying (LOD)	2%

3.2. Phytochemicals, total flavonoid content, total phenolic content and total alkaloidal content

The results on qualitative phytochemicals analysis revealed the presence of diverse secondary metabolites. (Tables 2 & 3) summarizes the results of

Table 2: Preliminary phytochemical screening of hydroalcoholic extract of *Pennisetum purpureum* (HAEPP)

S. No	Phytochemical Constituents	Observation
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Carbohydrates	–
5	Terpenoids	+
6	Alkaloids	+
7	Polyphenols	+
8	Steroids	–

Note: (+) indicates the presence of the respective phytochemical; (–) indicates its absence.

The results obtained from a Q uantitative preliminary analysis of total flavonoid content (TFC) and total phenolic content (TPC) were summarized in (Table 3). Based on the absorbance of the test sample (0.2143 at 1000 µg/mL), the total phenolic content of HAEPP was calculated to be 25.73 ±0.38 mg gallic acid equivalents (GAE)/g of dried extract**,** the flavonoid content was found to be 2.19 ±0.46 mg quercetin equivalents (QE)/g of dried extract and the total alkaloid content was 35.04±0.37 mg atropine equivalents (ATE)/g of dried extract.

Table 3: Summary of Quantitative Phytochemical Contents

1	Total Phenolic Content	25.73±0.38	Gallic Acid
2	Total Flavonoid Content	2.19 ±0.46	Quercetin
3	Total Alkaloid Content	35.04±0.37	Atropine

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the preliminary phytochemical screening of the hydroalcoholic extract of *Pennisetum purpureum* (HAEPP). The extract was found to contain major bioactive compounds such as tannins, saponins, flavonoids, terpenoids, alkaloids, and polyphenols, while carbohydrates and steroids were absent.

Table 4: DPPH Radical Scavenging Activity

S. No.	Concentration (µg/mL)	HAEPP (% Inhibition)	Ascorbic Acid (% Inhibition)
1	5	1.41±0.14	8.03±0.66
2	10	5.51±0.51	35.91±0.61
3	20	10.59±0.34	68.77±0.24
4	40	13.45±0.68	79.44±0.43
5	80	18.26±0.72	87.52±0.05
6	160	21.26±0.62	92.72±0.48
7	320	31.85±0.30	95.32±0.21
IC_{50}		>320 µg/mL	17.48 µg/mL

3.3. Antioxidant activities

The antioxidant activity of the hydroalcoholic extract of *Pennisetum purpureum* (HAEPP) was assessed by DPPH radical scavenging assay and compared with the reference standard, ascorbic acid. The IC_{50} value of HAEPP was found to be >320 µg/mL, indicating moderate antioxidant potential, while that of ascorbic acid was 17.48 µg/mL, signifying strong antioxidant activity (Table 4 & Fig. 1). The radical scavenging effect increased with concentration in both samples, with HAEPP showing a maximum inhibition of 31.85±0.30 **%** at 320 µg/mL. In contrast, ascorbic acid reached 95.32±0.21 % inhibition at the same concentration

3.4. Anti-inflammatory activities

To evaluate the anti-inflammatory activities of the plant extracts, we examined the plant extract by Nitric Oxide Radical Scavenging Activity and Cyclooxygenase (COX-2) Inhibition Assay. hydroalcoholic extract of *Pennisetum purpureum* (HAEPP) exhibited a dose-dependent nitric oxide (NO) radical scavenging effect. The percentage inhibition

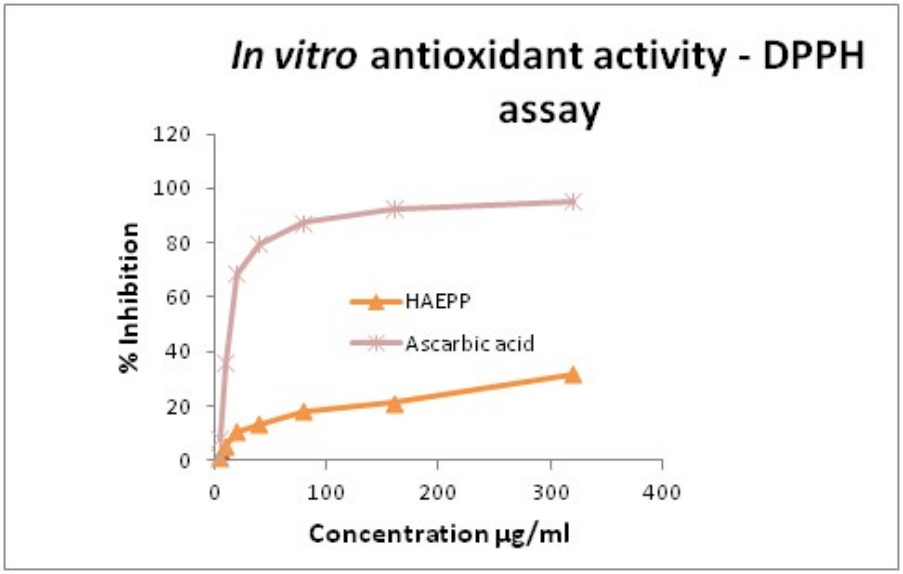

Fig. 1: *In vitro* antioxidant activity-DPPH Radical Scavenging Activity

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increased from 28.4% at 25 µg/mL to a maximum of 56.5% at concentration 100 µg/ml (Table 5) Whereas, In the COX-2 inhibition assay, HAEPP showed appreciable anti-inflammatory activity with dose-dependent inhibition of prostaglandin synthesis. The inhibition increased from 15.36% at 5 µg/mL to 88.19% at 100 µg/mL, with an IC_{50} value of 22.43 µg/mL. Celecoxib, used as the reference standard, demonstrated a stronger inhibitory effect, achieving 94.61% inhibition at 100 µg/mL with an IC_{50} of 7.34 µg/mL (Table 6 & Fig. 2).

Table 5: Nitric Oxide Scavenging Activity of Ascorbic Acid and HAEPP

S. NO	Concentration (µg/ml)	Ascorbic acid (% inhibition on nitric oxide)	HAEPP (% inhibition on nitric oxide)
1	25	34.30±0.83	28.4±1.90
2	50	43.22±0.87	37.3±1.60
3	75	53.51±1.22	46.1±2.67
4	100	63.85±1.17	56.5±1.56
IC_{50}		70 µg/ml	87.5 µg/ml

3.5. Anticancer activities

The cytotoxic potential of the hydroalcoholic extract of *Pennisetum purpureum* (HAEPP) was assessed in MCF-7 human breast cancer cells using the MTT assay. The extract exhibited a dose-

Table 6: Cyclooxygenase-2 Inhibitory Activity of HAEPP and Celecoxib

Conc.(µg/ml)	HAEPP	Celecoxib
5	15.36±0.93	37.38±0.33
10	34.03±0.41	60.33±0.12
25	44.39±1.03	76.07±0.71
50	70.27±0.56	88.19±2.13
100	88.19±1.20	94.61±0.83
IC_{50}	22.43 µg/ml	7.34 µg/ml

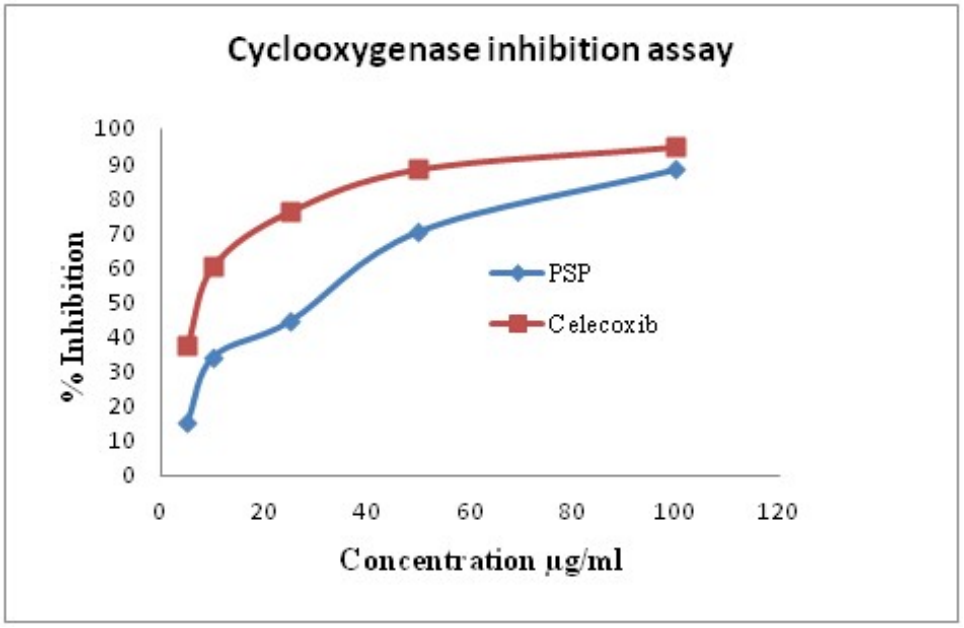

Fig. 2: Cyclooxygenase-2 Inhibitory Activity of HAEPP Extract and Celecoxib

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Table 7: Cell Viability of MCF-7 Cells Treated with HAEPP (MTT Assay)

S. No.	Concentration (µg/mL)	% Viability (Mean ± SD)
1	0 (Control)	100.00 ± 0.00
2	6.25	93.51 ± 0.44
3	12.5	83.53 ± 1.12
4	25	67.49 ± 1.20
5	50	51.72 ± 0.98
6	100	43.26 ± 0.74
IC_{50}(µg/mL)		63.82 µg/mL

dependent cytotoxic effect. As the concentration increased from 6.25 to 100 µg/mL, a progressive decline in cell viability was observed, with the highest concentration (100 µg/mL) reducing viability to 43.26%, compared to the control (100%).

The IC_{50} value of the extract was determined to be 63.82 µg/mL, indicating moderate antiproliferative activity. The cell viability percentages at various concentrations are presented in (Table 7 & Fig. 3). The photomicrographs (Fig. 4) also reveal morphological changes including membrane shrinkage, rounding, and cellular fragmentation, supporting the occurrence of apoptosis.

3.6. Statistical Analysis

All experiments, including total phenolic content (TPC), flavonoid content (TFC), alkaloid content (TAC), antioxidant (DPPH), anti-inflammatory (NO scavenging and COX-2 inhibition), and cytotoxicity (MTT assay), were conducted in triplicate**,** and results are expressed as mean ± standard deviation (SD). Whereas, IC_{50} values were calculated from the percentage inhibition or cell viability data using Microsoft Excel 2013 by linear regression method.

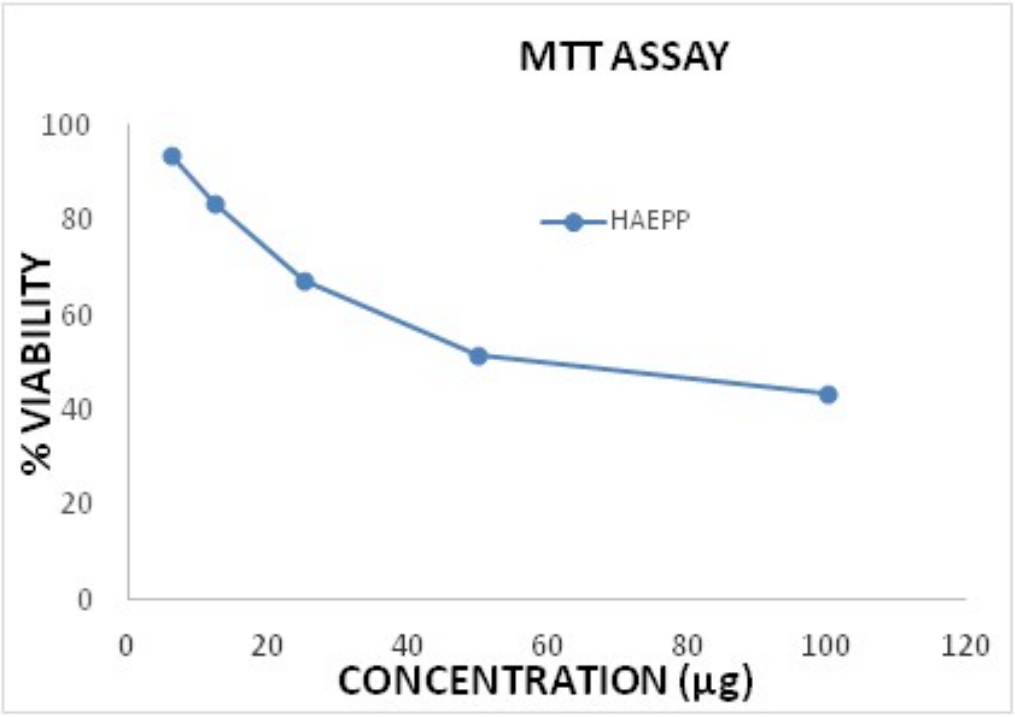

Fig. 3: Cell Viability of MCF-7 Cells Treated with HAEPP (MTT Assay)

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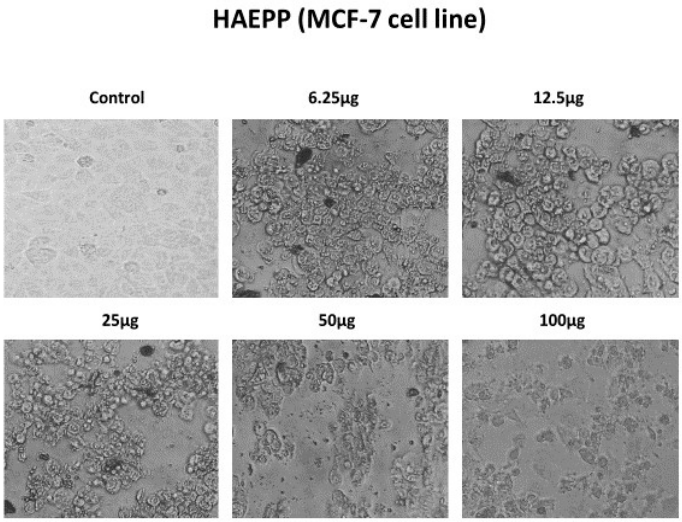

Fig. 4: Morphological Changes in MCF-7 Cells Treated with HAEPP at Varying Concentrations Phase-contrast microscopy images showing concentration-dependent morphological alterations in MCF-7 cells after 24-hour treatment with HAEPP.

Discussion

The rationale behind the present study lies in the urgent global need to discover novel, safe, and effective agents that can address the multifactorial etiology of chronic diseases, particularly cancer and inflammatory disorders (38). These diseases are primarily mediated by oxidative stress and inflammatory responses, leading to cellular damage and dysregulated signaling pathways (39). Although synthetic drugs are currently employed for these conditions, they often result in adverse effects, high costs, and therapeutic resistance (40). Hence, identifying plant-based therapeutic candidates has become an area of intensive research (41) *Pennisetum purpureum*, widely grown for its agricultural value, remains underexplored in terms of its pharmacological efficacy. Given the increasing interest in natural products for anticancer and anti-inflammatory potential, this study was designed to assess the bioactive potential of hydroalcoholic extract of *P. purpureum* (HAEPP) using well-validated *in vitro* assays, particularly focusing on antioxidant, anti-inflammatory, and anticancer activities.

The physicochemical evaluation of *Pennisetum purpureum* extract offers essential information on its quality and suitability for storage and further analysis. As per Indian Pharmacopoeia (IP, 2018) (30) guidelines, total ash (6.9%) and acid-insoluble ash (0.9%) values were within acceptable limits, indicating low inorganic and siliceous contamination. The water-soluble ash was relatively high (13%), suggesting the presence of water-soluble minerals, which may be therapeutically relevant but should be cross-verified for purity.The moisture content was found to be 10.5%, slightly above the recommended threshold (<10%), indicating a potential risk of microbial degradation and the need for proper storage. Overall, the results support the quality of the extract for further pharmacological and phytochemical applications, while also emphasizing the importance of standardization and quality control in herbal formulations.

The phytochemical characterization of HAEPP was a foundational component of this study. Qualitative analysis confirmed the presence of secondary metabolites including

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flavonoids, alkaloids, tannins, terpenoids, phenolics, and glycosides. These findings are in agreement with previous reports that have documented similar phytochemical profiles in *Pennisetum purpureum (42)*. These metabolites are recognized for their significant biological activities such as scavenging of free radicals, modulation of inflammatory mediators, and cytotoxic effects on tumor cells (43,44). Quantitative analysis further revealed a high content of total phenolics (85.3 mg GAE/g), flavonoids (88.5 mg QE/g), and alkaloids (86.7 mg ATE/g). The high level of phenolics supports their role in electron transfer mechanisms, neutralizing reactive oxygen species (ROS)(45). Flavonoids are known for their anti-inflammatory and cytotoxic effects through various pathways, including inhibition of iNOS and COX-2(46,47). Alkaloids exhibit antioxidant and enzyme-inhibitory activities (48), supporting the overall therapeutic potential of HAEPP. These findings justify the relevance of phytoconstituent profiling in establishing a mechanistic understanding of the extract's pharmacological actions (49).

In the current study, the results of *in vitro* DPPH scavenging assay demonstrated moderate radical scavenging activity, with an IC_{50} value of 320 µg/mL. Although this is higher than the IC_{50} of standard antioxidants such as ascorbic acid, it remains indicative of substantial antioxidant capacity, especially in light of the extract's rich phytochemical profile. Comparable activity has been documented in *Commiphora mollis* resin extracts, whose DPPH EC_{50} values ranged from 295 to 353 µg/mL, depending on solvent polarity—methanol, chloroform, and petroleum ether extracts showed EC_{50}s of 295.03 ± 3.55**,** 342.75 ± 9.72**,** and 353.69 ± 7.30 µg/mL**,** respectively, while ascorbic acid recorded a much lower EC_{50} (~44.72 µg/mL) These parallels affirm that extracts within this range can still be considered to have meaningful antioxidant activity, particularly when supported by rich phytochemical content. (50). Previous studies have reported a strong correlation between antioxidant activity and the presence of phenolic and flavonoid compounds (51,52). These bioactives exert their effects primarily through their hydroxyl groups, which participate in hydrogen atom transfer and single electron transfer mechanisms, neutralizing free radicals and preventing oxidative cellular damage (53). Additionally, alkaloids, though often underrepresented in antioxidant research, play a supportive role through metal chelation and free radical stabilization (54). Therefore, the observed DPPH radical scavenging potential of HAEPP is consistent with its quantified phytochemical composition, highlighting a probable synergistic mechanism among phenolics, flavonoids, and alkaloids (55).

Also, the anti-inflammatory activity was demonstrated through nitric oxide (NO) inhibition and cyclooxygenase-2 (COX-2) inhibition assays. NO plays a pivotal role in inflammation and is markedly upregulated under pathological conditions via inducible nitric oxide synthase (iNOS), while COX-2 is a key enzyme in prostaglandin biosynthesis, enhancing vascular permeability and leukocyte infiltration (56,57) .The inhibition of both NO and COX-2 indicates, suppression of pro-inflmmatory mediators(58) . In our study, HAEPP demonstrated nitric oxide scavenging activity in the sodium nitroprusside (SNP)-induced assay system with an IC_{50} of 28.76 µg/mL and showed 32.72% relative potency compared to celecoxib in the COX-2 assay (IC_{50} = 22.43 µg/mL), indicating that HAEPP strongly inhibit the NO and COX-2 assay, Our results are consistent with previous studies reporting that polyphenol- and flavonoid-rich plant extracts exert dual anti-inflammatory actions through NO suppression and COX-2 inhibition (58).Flavonoids and phenolic acids are well-known to downregulate iNOS and COX-2 expression by modulating intracellular signaling pathways such as NF-κB and MAPKs(59). Additionally, alkaloids contribute to anti-inflammatory action by inhibiting pro-inflammatory cytokine release and suppressing key enzyme activities (60).

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Therefore, the observed anti-inflammatory activity of HAEPP may be attributed to the synergistic effect of these phytochemicals, supporting its therapeutic value as a plant-derived anti-inflammatory agent(61). Our findings are consistent with previous reports that have demonstrated similar outcomes(58).

Chemotherapy remains central in cancer treatment but is often limited by significant side effects. This has driven interest in plant-derived metabolites—such as terpenoids, phenolics, and alkaloids—which exhibit promising anticancer activity (62). However, their clinical use is constrained by toxicity and poor bioavailability. Structural modifications are being explored to improve efficacy and safety, supporting continued research into medicinal plants as potential sources of novel anticancer agents (62). Our analysis revealed the anti-cancer potential of HAEPP against MCF-7 human breast cancer cells was dose-dependent cytotoxicity with an IC_{50} of 63.85 µg/mL. Crude plant extracts with IC_{50} values below 100 µg/mL are generally recognized as possessing biologically significant cytotoxicity in preliminary *in vitro* screenings—an observation supported by NCI-derived criteria in phytochemical screening literature(63). Thus, the observed IC_{50} of 63.82 µg/mL for HAEPP validates its potential as an antiproliferative agent against MCF-7 breast cancer cells. A comparable cytotoxic potential was observed in a study by Ramesh et al(64), where the root and leaf extracts of *Rotheca serrata* exhibited IC_{50} values of 61.83 ± 7.43 µg/mL and 78.15 ± 6.32 µg/mL, respectively, against MCF-7 breast cancer cells.Morphological examination of MCF-7 cells treated with HAEPP revealed classical apoptotic changes, including cell shrinkage, membrane blebbing, and the appearance of apoptotic bodies. These findings align with previous observations in treated MCF-7 models, where such ultrastructural alterations have been confirmed as defining markers of apoptosis (e.g., cell shrinkage, membrane blebbing, and chromatin condensation(65) .Polyphenols and flavonoids are widely documented for their ability to trigger apoptosis via intrinsic (mitochondrial) and extrinsic pathways by activating caspase cascades, modulating mitochondrial membrane potential, and downregulating estrogen receptor signalling(66,67) . Additionally, alkaloids have been reported to induce G2/M cell cycle arrest and inhibit DNA topoisomerases, thereby disrupting DNA replication and promoting apoptosis in cancer cells(68). The cytotoxic activity of HAEPP may also be attributed to its ability to generate reactive oxygen species (ROS) selectively in malignant cells, leading to oxidative damage and inhibition of oncogenic signaling pathways such as PI3K/Akt and MAPK(69,70) . Therefore, the anticancer results reinforce the potential of HAEPP as a promising phytotherapeutic agent, particularly for estrogen receptor-positive breast cancer (71).

Conclusion

Taken together, the present findings establish a strong pharmacological basis for the use of *Pennisetum purpureum* extract as a multifunctional therapeutic agent. The observed antioxidant, anti-inflammatory, and anticancer activities are well supported by the extract's high content of flavonoids, phenolics, and alkaloids.Further research involving *in vivo* experiments and molecular docking or mechanistic analyses is necessary to elucidate the precise intracellular targets and pathways involved. Nonetheless, the current study contributes to the expanding body of evidence supporting the medicinal utility of phytochemical-rich extracts and positions *P. purpureum* as a promising candidate in integrative medicine and phytopharmacology.

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Conflicts of Interest

The authors declare that no conflicts of interest.

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