

CYTOTOXIC POTENTIAL OF THE ETHANOLIC LEAF EXTRACT OF *Pterospermum acerifolium* AGAINST HUMAN CANCER CELL LINES

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ABSTRACT

Pterospermum acerifolium (L.) Willd., commonly called Kanak Champa, is a folk medicine for inflammation, wounds, infections, ulcers, and tumours. The present study primarily evaluated the cytotoxic potential of the ethanolic leaf extract of *P. acerifolium* on human cancer cell lines, along with antioxidant and antimicrobial activities. The ethanolic extract was obtained using the Soxhlet extraction technique. The antimicrobial performance was tested against the bacteria *Bacillus subtilis* and *Escherichia coli* and the fungi *Aspergillus niger* and *Candida albicans*, employing the agar well diffusion technique. The extract demonstrated moderate activity against *Escherichia coli* and limited inhibitory activity towards *B. subtilis*. Only low antifungal activity was observed. DPPH assay was employed for assessment of radical scavenging potential, and the extract exhibited maximum inhibition of 57.40%. The MTT and brine shrimp lethality assays were used to measure the cytotoxic activity. Brine shrimp mortality reached 50.0% at 1000 µg/mL, indicating preliminary general cytotoxic potential. The extract produced a concentration-related reduction in cell viability, with IC₅₀ values of 67.50, 78.20, 67.84 and 68.80 µg/mL for MCF-7, Colo-205, HepG2 and A549 cells, respectively. These findings provide a basis for further phytochemical isolation, mechanistic investigation and evaluation of selectivity toward normal cells. The present work aligns with Good Health and Well-being (SDG-3) by supporting scientific investigation of plant-derived materials relevant to health-related research. It also aligns with the Special Issue theme by linking traditional medicinal knowledge with contemporary biological evaluation.

Keywords: *P. acerifolium*, Antioxidant, Antimicrobial, Brine Shrimp, MTT Assay

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INTRODUCTION

Nature has traditionally been a rich source of therapeutic medicines, and many beneficial medications have been derived from plants. In addition to offering medicinal substances, natural resources stimulate the development of novel structural leads and modern drug discovery. Plant-based medical systems are still crucial in many civilisations. The close relationship between traditional knowledge and contemporary pharmacology was highlighted by a survey carried out in nations hosting WHO-Traditional Medicine Centres, which found that a sizable percentage of pure plant-derived compounds are used as medications.¹ Cancer is a disease that remains a significant global health concern, accounting for many millions of people who succumb to mortality yearly. Despite developments in modern medicine, there's an immediate and continuing demand for new therapeutic approaches to both prevent and treat this life-threatening condition. Current treatment modalities, including chemotherapy, radiotherapy, and synthetic drugs, though effective, are often associated with severe toxicity and undesirable side effects that compromise patients' quality of life. Consequently, the scientific community has increasingly turned its focus toward naturally derived compounds, which are considered safer and less toxic alternatives to conventional chemotherapeutics.² Flavonoids are among the most widely distributed and biologically active compounds found in the plant kingdom. Their anticancer potential has been documented across a substantial body of preclinical work and, increasingly, in clinical settings as well. Interestingly, epidemiological data point in the same direction; populations consuming flavonoid-rich diets tend to show lower cancer incidence, lending real-world weight to laboratory findings.³ In traditional systems of medicine, *Pterospermum acerifolium* Linn. (Family: Malvaceae) had a remarkably broad therapeutic

footprint, used variously as a antitumor remedy and in the management of conditions ranging from diabetes and gastrointestinal complaints to leprosy, bronchitis, migraine, and smallpox, among others. Haemostatic and antimicrobial applications are also documented.⁴

Although the traditional medicinal relevance of *P. acerifolium* is well documented, comparative evaluation of its ethanolic leaf extract against different human cancer cell lines requires further investigation. The present study primarily evaluates the cytotoxic effect of the plant leaf extract on MCF-7, Colo-205, A549 and HepG2 human cancer cell lines using the MTT assay. Antioxidant, antimicrobial and brine shrimp lethality assays were additionally performed to provide complementary information on the biological profile of the extract. The work is also aligned with Good Health and Well-being (SDG 3). Furthermore, the work supports the theme of Indian Knowledge Systems for Chemical and Environmental Sustainability by scientifically evaluating traditionally relevant plant material using contemporary biological assays.

EXPERIMENTAL

Collection and Authentication of Plant Parts

Pterospermum acerifolium (L.) Wild plant parts were gathered from the Sirsi area of the Uttara Kannada district in Karnataka, India. A botanist, Ms. M. D. Pawar, from BSI, Western Regional Centre, Pune, recognised and verified the plant material. A sample voucher was placed for future use.

Soxhlet Extraction

For the extraction procedure, about 50 g of this powdered material is precisely weighed. The next step is sequential extraction using ethanol as a solvent. 300–500 mL of the chosen solvent is added to the powder in a Soxhlet device. To guarantee complete extraction of the target chemicals, the Soxhlet extraction process is run for four to eight hours. The extracted material is finally kept in amber-colored vials at 4°C.

Evaluation of Antimicrobial Activity

The antimicrobial potential was evaluated through the agar well diffusion technique. Wells (6 mm) were made in inoculated agar plates, and 100 µL of the test sample, standard (1 mg/mL), or DMSO (control) was added. After incubation for a period of 24 to 48 hours, the antimicrobial effect was measured by the diameter of inhibition zones in millimetres.⁵⁻⁷

Antioxidant Activity

The antioxidant potential of the extract was examined using the DPPH assay. Equal volumes (100 µL each) of the test sample or standard and freshly prepared DPPH solution were mixed in a microplate and kept in the dark at ambient temperature. for 30 minutes. Antioxidant potential was expressed as the percentage of radical-scavenging inhibition based on absorbance measured at 517 nm.⁸⁻¹⁰

Brine shrimp (*Artemia Salina*) assay

The cytotoxic potential of the extract was initially screened using the brine shrimp lethality bioassay. Freshly hatched *Artemia nauplii* were transferred to artificial seawater containing different concentrations of the test sample. Following 24 h of incubation at 25–28 °C, the surviving nauplii were enumerated, and mortality was calculated as a percentage.¹¹⁻¹²

Anti-cancer Activity using Cell line MCF-7, COLO-205, A549, HePG2 by MTT assay

In vitro anticancer effect of the test sample was evaluated on MCF-7, COLO-205, A549, and HepG2 cell lines using the MTT colourimetric assay. Cell lines were treated with different concentrations of extract, followed by MTT reagent addition. After incubation, insoluble formazan crystals generated by metabolically active cells were solubilised in dimethyl sulfoxide, and absorbance was noted at 570 nm. Cell viability and growth inhibition were determined from the corresponding absorbance measurements.¹³

RESULTS AND DISCUSSION

Ethanolic extract of *P. acerifolium* exhibited weak antibacterial effects against tested bacterial strains. As the concentration of the extract increased, activity increased. At 10 mg/mL, the extract produced larger zones of inhibition compared to 5 mg/mL. However, the activity was lower than that of the standard Streptomycin, which indicated strong inhibition against both organisms. The antibacterial effect of the

extract is a result of phytoconstituents such as flavonoids, phenolics, & tannins. These findings show that the ethanolic extract possesses moderate antibacterial potential. Table-1 summarises the results of the activity of the extract.

Table-1: Antibacterial Efficacy *P. acerifolium* Extract

S. No.	Sample	Concentration	Zone of Inhibition (mm)	
			<i>E. coli</i>	<i>Bacillus subtilis</i>
1.	Control		-	-
2.	Streptomycin	1 mg/ml	32	35
3.	Extract	5 mg/ml	03	01
		10 mg/ml	08	03

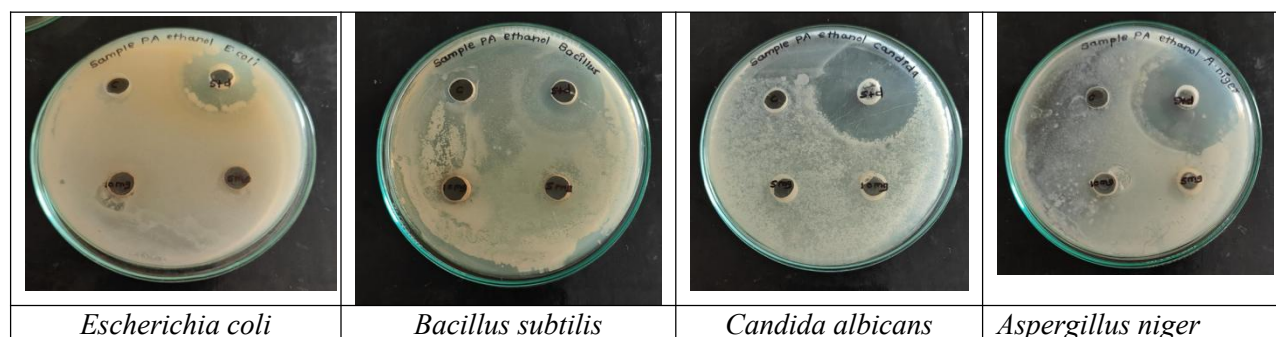
P. acerifolium ethanolic extract exhibited mild antifungal activity.

Table-2 summarises the antifungal activity of the ethanolic extract of *Pteroserium acerifolium*.

Table-2: Antifungal Efficacy *P. acerifolium* Extract

S. No.	Sample	Concentration	Zone of Inhibition (mm)	
			<i>Candida albicans</i>	<i>A. niger</i>
1.	Control		-	-
2.	Fluconazole	1 mg/ml	39	35
3.	Extract	5 mg/ml	01	01
		10 mg/ml	02	03

Higher zones of inhibition were formed by the extract at 10 mg/mL as compared to 5 mg/mL, suggesting concentration-dependent antifungal action (Fig.-1). However, the activity was considerably lower than Fluconazole, which produced noteworthy zones of inhibition against both fungal strains. These results indicate that the extract possesses moderate antifungal potential.

Fig.-1: Inhibition Zone of Extract of *P. acerifolium*Table-3: Antioxidant Activity of Ethanolic Extracts of *P. acerifolium*

S. No.	Sample	Concentration	Absorbance (OD)			Mean $\pm$ SD	% inhibition
1	Control		1.456	1.564	1.485	1.502 $\pm$ 0.056	
2	Ascorbic acid (Standard)	200	0.334	0.337	0.347	0.339 $\pm$ 0.007	77.40288568
		400	0.315	0.316	0.303	0.311 $\pm$ 0.007	79.26748058
		600	0.299	0.298	0.289	0.295 $\pm$ 0.005	80.33296337
		800	0.145	0.141	0.143	0.143 $\pm$ 0.002	90.4772475
		1000	0.089	0.088	0.082	0.086 $\pm$ 0.004	94.25083241
3	Extract	200	0.795	0.789	0.785	0.790 $\pm$ 0.005	47.41398446
		400	0.687	0.658	0.698	0.681 $\pm$ 0.021	54.65038846
		600	0.654	0.644	0.621	0.640 $\pm$ 0.017	57.40288568
		800	0.675	0.672	0.668	0.672 $\pm$ 0.004	55.27962716
		1000	0.698	0.699	0.697	0.698 $\pm$ 0.001	53.51831299

The extract exhibited 47.41% to 57.40% inhibition, lower than Ascorbic acid. In comparison, ascorbic acid produced markedly higher inhibition values ranging from 77.40% to 94.25% across the tested concentrations (Table-3). The antioxidant response indicates the presence of constituents capable of

interacting with DPPH radicals; however, the activity was lower than that of ascorbic acid under the tested conditions. The non-linear response at higher concentrations may reflect differences in the relative contribution or interaction of extract constituents.

Table-4: Cytotoxic Activity of Ethanolic Extract of *P. acerifolium* by Brine Shrimp

Treatment	Dose (µg/mL)	Total initial shrimps (n)	Survived (T1)	Survived (T2)	Survived (T3)	Total viable shrimps	% Mortality
Control	–	30	10	10	10	30	0.0
Extract	10	30	7	6	6	19	36.7
	100	30	6	6	7	19	36.7
	1000	30	5	5	5	15	50.0

The extract produced 36.7% mortality at both 10 and 100 µg/mL. Mortality increased to 50% at 1000 µg/mL (Table-4). The assay therefore provides preliminary evidence of general cytotoxicity and should not be considered a cancer-specific cytotoxicity assay.

Table-5: Cytotoxic Activity of 5-Fluorouracil on different Human Cancer Cell Lines by the MTT Assay

Cell Line	Control OD (Mean ± SD)	IC ₅₀ (µg/mL)	5-
MCF-7 (Human breast adenocarcinoma)	2.110 ± 0.002	52.56	
Colo-205 (Human colorectal adenocarcinoma)	2.236 ± 0.002	33.89	
HepG2 (Human liver carcinoma)	2.326 ± 0.002	46.73	
A549 (Human lung carcinoma)	2.110 ± 0.002	56.06	

Fluorouracil is used as a standard and exhibited strong cytotoxic activity towards all four tested cell lines, with the half-maximal inhibitory concentration. (IC₅₀) values of 52.56, 33.89, 46.73, and 56.06 µg/mL against MCF-7, Colo-205, HepG2, and A549 cells, respectively (Table-5). Among the tested cell lines, Colo-205 cells were the most sensitive to 5-Fluorouracil, whereas A549 cells showed comparatively lower sensitivity.

Table-6: Effect of the Ethanolic Leaf Extract of *P. acerifolium* on Cell Viability (%)

Concentration (µL/mL)	MCF-7 Cell Viability (%)	Colo-205 Cell Viability (%)	HepG2 Cell Viability (%)	A549 Cell Viability (%)
Control	100.00	100.00	100.00	100.00
20	85.94	78.40	78.49	87.45
40	77.98	73.20	65.59	83.13
60	67.46	66.06	59.59	69.02
80	39.99	44.00	36.28	46.20
100	35.42	38.18	33.41	40.07

Cell viability decreased progressively with increasing extract concentration. The response varied among the tested cell lines. Cell viability decreased from 85.94% to 35.42% in MCF-7, 78.40% to 38.18% in Colo-205, 78.49% to 33.41% in HepG2, and 87.45% to 40.07% in A549 cells over the tested concentration range (Table-6).

Table-7: IC₅₀ Values of the Ethanolic Leaf Extract of *P. acerifolium*

Cell Line	Control OD (Mean ± SD)	IC ₅₀
MCF-7 (Human breast adenocarcinoma)	2.110 ± 0.002	67.50 µg/mL
Colo-205 (Human colorectal adenocarcinoma)	2.236 ± 0.002	78.20 µg/mL
HepG2 (Human liver carcinoma)	2.236 ± 0.002	67.84 µg/mL
A549 (Human lung carcinoma)	2.110 ± 0.002	68.80 µg/mL

The corresponding IC₅₀ values were 67.50, 78.20, 67.84, and 68.80, respectively (Table-7), indicating moderate cytotoxic activity of the ethanolic extract against all four cancer cell lines. MCF-7 showed the greatest apparent sensitivity based on its lower IC₅₀ value, whereas Colo-205 showed comparatively lower sensitivity. The extract showed higher IC₅₀ values than 5-fluorouracil in all four cell lines, indicating lower apparent cytotoxic potency than the reference drug. The differences in response among the cell lines may reflect variations in their cellular characteristics and susceptibility to extract constituents. However, the present study used a crude extract, and the contribution of individual

phytoconstituents was not established. Further isolation and mechanistic studies are therefore required. These findings represent preliminary in vitro evidence and should not be interpreted as therapeutic efficacy. Evaluation of cytotoxic selectivity against normal cells would also be important for determining the biological relevance of the extract. The findings obtained from the different experiments provide a complementary biological profile of the ethanolic leaf extract of *P. acerifolium*. The extract showed limited antibacterial and antifungal activity compared with the respective reference drugs, while moderate DPPH radical-scavenging activity was observed. The brine shrimp assay demonstrated preliminary general cytotoxicity, although the response was not uniformly concentration-dependent. The MTT assay provided the main biological finding of the study. The extract reduced cell viability with increasing concentration in all four tested human cancer cell lines. The extract exhibited half-maximal inhibitory concentrations (IC_{50}) between 67.50 and 78.20 $\mu\text{g/mL}$ across the tested cell lines. MCF-7 showed the lowest IC_{50} and therefore the greatest apparent sensitivity, whereas Colo-205 showed the highest IC_{50} value. In comparison, 5-fluorouracil showed lower IC_{50} values in all four cell lines, indicating greater apparent cytotoxic potency under the experimental conditions. The observed cytotoxic response may be associated with the combined effects of multiple constituents present in the crude extract. Nevertheless, the present findings do not establish which phytoconstituents are responsible for the observed activity or identify a specific molecular mechanism. Therefore, the results should be regarded as preliminary evidence supporting further investigation rather than as evidence of therapeutic anticancer efficacy.

CONCLUSION

The ethanolic extract of *P. acerifolium* exhibited moderate antioxidant activity and mild antibacterial and antifungal activity compared with standard drugs. In brine shrimp lethality assessment, the extract also showed a moderate level of cytotoxicity. The presence of bioactive phytoconstituents with anticancer potential was indicated by the MTT assay's notable dose-dependent anticancer activity against all tested cell lines. Overall, the findings suggest that *P. acerifolium* is a promising biologically active source of phytoconstituents. The combination of traditional medicinal relevance and contemporary biological evaluation provides a scientific basis for further investigation of *P. acerifolium*. Bioassay-guided fractionation may facilitate the identification of active fractions, followed by isolation and structural elucidation of their major constituents. Further mechanistic investigation and assessment of cytotoxic selectivity toward normal cells and subsequent in vivo studies would help to establish the biological significance of the observed cytotoxicity. The study contributes to Good Health and Well-being (SDG 3) by supporting scientific investigation of plant-derived materials relevant to health-related research. It also demonstrates an approach for linking traditional medicinal knowledge with contemporary biological evaluation, consistent with the scope of the Special Issue.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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