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IN VITRO ANTICANCER ACTIVITY OF THE ROOTS OF PASPALUM PANICULATUM

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Abstract:

Cancer is a life-threatening disease associated with oxidative stress–induced cellular damage. Free radicals generated during metabolic processes contribute to carcinogenesis, while antioxidants neutralize these reactive species and reduce cellular injury. Paspalum paniculatum contains bioactive phytochemicals such as alkaloids, flavonoids, saponins, and triterpenoids with potential antioxidant and cytotoxic properties. This study evaluated the in-vitro antioxidant and cytotoxic activity of root extracts of Paspalum paniculatum. Aqueous, ethanolic, and chloroform extracts were assessed using DPPH radical scavenging, nitric oxide scavenging, reducing power assays, and the brine shrimp lethality bioassay. All extracts exhibited dose-dependent antioxidant activity. The DPPH IC_{50} values for chloroform, ethanolic, and aqueous extracts were 171.19, 99.09, and 125.73 µg/mL, respectively, compared to ascorbic acid (49.37 µg/mL). Cytotoxicity studies revealed LD_{50} values of 732.66, 398.21, and 598.56 µg/mL, respectively. These findings indicate that Paspalum paniculatum roots possess significant antioxidant and cytotoxic potential, supporting their possible role as a natural source of anticancer agents.

Keywords: Paspalum paniculatum, Antioxidant activity, Cytotoxicity, DPPH assay, Brine shrimp lethality, Medicinal plants.

1. INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide and is characterized by uncontrolled cell growth, tissue invasion, and metastasis. The development of cancer is a multistep process involving genetic mutations and epigenetic alterations that disrupt normal cellular regulatory mechanisms governing cell proliferation, differentiation, and apoptosis. Environmental factors, lifestyle choices, and exposure to chemical carcinogens contribute significantly to these genetic alterations, ultimately leading to malignant transformation of cells. [1]

A major contributing factor in cancer initiation and progression is oxidative stress. Under normal physiological conditions, cellular metabolic processes generate reactive oxygen and nitrogen species, commonly referred to as free radicals. These highly reactive molecules can interact with vital cellular components such as DNA, proteins, and lipids, causing structural damage and functional impairment. When the production of free radicals exceeds the capacity of endogenous antioxidant

defense systems, oxidative stress occurs, which plays a crucial role in carcinogenesis, tumor progression, and resistance to therapy. [2]

Antioxidants are substances capable of neutralizing free radicals by donating electrons, thereby preventing oxidative damage and maintaining cellular homeostasis. Natural antioxidants derived from plants have attracted significant interest due to their ability to modulate oxidative stress, inhibit tumor initiation, and enhance the effectiveness of conventional anticancer therapies. In addition to antioxidant properties, several plant-derived compounds exhibit cytotoxic effects against cancer cells, making them valuable candidates for the development of novel anticancer agents. [3]

Medicinal plants are rich sources of diverse bioactive phytochemicals, including alkaloids, flavonoids, saponins, tannins, and triterpenoids, many of which have demonstrated antioxidant, antiproliferative, and cytotoxic activities. *Paspalum paniculatum* is a traditionally used medicinal plant reported to contain several of these phytoconstituents. Despite its ethnomedicinal relevance, scientific evidence supporting its antioxidant and anticancer potential remains limited. [4]

Therefore, the present study was undertaken to evaluate the in-vitro antioxidant and cytotoxic activities of aqueous, ethanolic, and chloroform extracts of *Paspalum paniculatum* roots using established experimental models. This investigation aims to provide a scientific basis for the traditional use of the plant and to explore its potential as a source of natural antioxidant and anticancer agents.

2. MATERIAL AND METHODS:

Chemicals and reagents:

1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Methanol, Sodium nitroprusside, Phosphate buffer (pH 7.4), Sulphanilamide, O-phosphoric acid, N-(1- naphthyl) ethylenediamine dihydro - chloride, Sodium chloride, Artemiasalina leach (brine shrimp eggs), Potassium ferricyanide, Trichloroacetic acid, Phosphate buffer pH 7.4

Collection of plant material:

The roots of plant were collected within the college campus and near places of JAYAMUKHI COLLEGE OF PHARMACY at Narsampet, Warangal district. The preliminary evaluation was done, then the roots are thoroughly cleaned under tap water and the moisture was removed by pressing them in the tissue paper. Drying of crude drugs after collection and before extraction is necessary to avoid microbial growth, prevent chemical degradation, and determine the final weight of raw material. The time and choice of drying method depend on physical and chemical nature of the crude drug. Shade

drying is the most acceptable form of drying which involves less exposure to heat, and there are less chances of chemical alteration. After the complete drying of the roots smash and grind them with the help of grinder. Now the powder is used for the extraction process.

Preparation of extracts:

The parts of plant material/s subjected for successive extraction by using different solvents. The powder of plant materials was initially extracted with petroleum ether (60-80°C) followed by chloroform, ethanol and water by using a Soxhlet extractor for 72 hrs at a temp not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper (No. 1) and then concentrated in vacuum and dried for solvent removal, and the extracts were kept in sterile bottles under refrigerated conditions until use**.**

In-Vitro Antioxidant Activity- DPPH Radical Scavenging Assay

The antioxidant activity of aqueous, ethanolic, and chloroform root extracts of *Paspalum paniculatum* was evaluated using the DPPH free radical scavenging assay. A methanolic DPPH solution (0.0033% w/v) was prepared by dissolving 33 mg of DPPH in one liter of methanol and stored in the dark until use. Stock solutions of plant extracts (1000 µg/mL) were prepared by dissolving 10 mg of each extract in the respective solvent and adjusting the volume to 10 mL. Working concentrations ranging from 25 to 200 µg/mL were prepared from the stock solution. Ascorbic acid was used as the reference standard, with a stock solution of 1000 µg/mL prepared in ethanol and diluted to obtain the same concentration range. For the assay, 1 mL of each concentration of extract or standard was mixed with 5 mL of DPPH solution and incubated at 37 °C for 20 minutes in the dark. The absorbance was measured at 516 nm using a UV–Visible spectrophotometer, with methanol serving as the blank. The DPPH solution without extract was used as the control. [5]

The percentage of radical scavenging activity was calculated using the following equation:

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of } Control} \times 100$$

The IC_{50} value was determined from the plot of percentage inhibition versus concentration.

In-Vitro Cytotoxic Activity-Brine Shrimp Lethality Bioassay

The cytotoxic potential of the extracts was evaluated using the brine shrimp lethality bioassay. Artificial seawater was prepared by dissolving 38 g of non-iodized sea salt in one liter of distilled water and filtered to obtain a clear solution. A stock solution of each extract (1000 µg/mL) was prepared by

dissolving 10 mg of extract in a minimal quantity of PEG-400 and making up the volume to 10 mL with seawater. Serial dilutions were prepared to obtain concentrations of 25, 50, 100, 250, and 500 µg/mL. Brine shrimp eggs (*Artemia salina*) were hatched in seawater under continuous aeration for 48 hours at room temperature. The hatched nauplii were collected from the illuminated side of the tank due to phototactic behavior. Ten active nauplii were transferred into each test vial containing different concentrations of the extracts. Each concentration was tested in triplicate. After 24 hours of incubation, the number of surviving nauplii was counted using a magnifying lens, and the percentage mortality was calculated. The median lethal concentration (LD_{50}) was determined from the dose–response curve. [6]

Statistical Analysis:

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). IC_{50} and LD_{50} values were calculated using regression analysis from dose–response curves.

3. RESULTS:

In-Vitro Antioxidant Activity- DPPH Radical Scavenging Assay

The antioxidant potential of root extracts of *Paspalum paniculatum* prepared in chloroform, ethanol, and water was evaluated using the DPPH free radical scavenging assay. All extracts exhibited a concentration-dependent increase in percentage inhibition, indicating effective radical scavenging activity (Table 1). Among the tested extracts, the ethanolic extract demonstrated the highest antioxidant activity, followed by the aqueous and chloroform extracts. The IC_{50} values of chloroform, ethanolic, and aqueous extracts were found to be 171.19, 99.09, and 125.73 µg/mL, respectively, whereas the standard ascorbic acid exhibited an IC_{50} value of 49.37 µg/mL. These results suggest that the ethanolic extract possesses comparatively stronger antioxidant potential.

Table 1. DPPH Radical Scavenging Activity of *Paspalum paniculatum* Root Extracts

Concentration (µg/mL)	% Inhibition			
	Ascorbic Acid	Chloroform Extract	Ethanolic Extract	Aqueous Extract
25	23.12	8.09	14.18	11.51
50	41.28	15.32	30.28	16.25
75	58.26	22.32	41.13	25.46
100	68.11	29.22	48.45	38.14

125	72.25	37.27	56.76	44.14
150	88.55	41.14	68.13	58.76
175	92.18	48.17	71.89	71.87
200	98.79	51.21	82.18	78.85
IC_{50} (µg/mL)	49.37	171.19	99.09	125.73

In-Vitro Anticancer Activity-Brine Shrimp Lethality Bioassay

The cytotoxic activity of *Paspalum paniculatum* root extracts was evaluated using the brine shrimp lethality bioassay. All extracts exhibited a concentration-dependent increase in percentage mortality of brine shrimp nauplii (Table 2). Among the tested extracts, the ethanolic extract showed the highest cytotoxic activity, with an LD_{50} value of 398.21 µg/mL, followed by the aqueous extract (598.56 µg/mL) and chloroform extract (732.66 µg/mL). The results indicate moderate cytotoxic potential, with the ethanolic extract demonstrating comparatively higher anticancer activity.

Table 2. Brine Shrimp Lethality Bioassay of *Paspalum paniculatum* Root Extracts

Extract	Concentration (µg/mL)	% Mortality	LD_{50} (µg/mL)
Chloroform Extract	25	5.67	732.66
	50	11.27	
	100	17.56	
	250	24.00	
	500	33.67	
Ethanolic Extract	25	9.33	398.21
	50	19.33	
	100	24.10	
	250	32.40	
	500	55.47	
Aqueous Extract	25	7.23	598.56
	50	13.27	
	100	21.13	
	250	28.27	
	500	38.00	

4. DISCUSSION

Oxidative stress plays a pivotal role in the initiation and progression of cancer through the generation of reactive oxygen species that damage cellular macromolecules such as DNA, proteins, and lipids. Antioxidants counteract these effects by neutralizing free radicals, thereby reducing oxidative damage and suppressing carcinogenic processes [7]. The present study evaluated the in-vitro antioxidant and cytotoxic potential of root extracts of *Paspalum paniculatum* using established experimental models, providing scientific support for its traditional medicinal use.

The DPPH radical scavenging assay revealed that all tested extracts exhibited concentration-dependent antioxidant activity, indicating the presence of bioactive compounds capable of donating electrons or hydrogen atoms to neutralize free radicals [7]. Among the extracts, the ethanolic extract demonstrated the strongest antioxidant potential, as evidenced by its lower IC_{50} value compared to chloroform and aqueous extracts. This enhanced activity may be attributed to the ability of ethanol to extract a wide range of polar and moderately polar phytochemicals, particularly phenolic compounds and flavonoids, which are well known for their antioxidant properties. The comparatively lower activity of the chloroform extract suggests that non-polar constituents contribute less significantly to the antioxidant activity of *Paspalum paniculatum*. The moderate activity observed in the aqueous extract indicates that water-soluble phytochemicals also play a role, although their concentration and efficacy may be lower than those extracted with ethanol. These findings align with previous reports highlighting ethanol as an effective solvent for extracting antioxidant compounds from medicinal plants.

The brine shrimp lethality bioassay served as a preliminary screening method for assessing the cytotoxic and potential anticancer properties of the plant extracts. All extracts exhibited dose-dependent cytotoxicity against *Artemia salina* nauplii, indicating the presence of bioactive compounds with growth-inhibitory or toxic effects. Among the extracts, the ethanolic extract displayed the highest cytotoxic activity, as reflected by the lowest LD_{50} value, followed by the aqueous and chloroform extracts. This pattern parallels the antioxidant findings, suggesting a possible correlation between antioxidant capacity and cytotoxic potential [9]. The cytotoxic effects observed in the brine shrimp assay may be attributed to phytoconstituents such as alkaloids, flavonoids, saponins, and triterpenoids, which have been reported to induce cell membrane disruption, inhibit mitochondrial function, and trigger apoptotic pathways in cancer cells. Although the brine shrimp assay does not directly assess anticancer mechanisms in human cell lines, it provides a reliable and cost-effective preliminary indication of cytotoxic potential and guides further in-depth investigations.

The observed relationship between antioxidant activity and cytotoxicity suggests that the bioactive compounds in *Paspalum paniculatum* may exert dual protective and therapeutic effects by reducing oxidative stress and inhibiting abnormal cell proliferation. However, the cytotoxicity observed was moderate, indicating that the extracts may be relatively safe at lower concentrations while still possessing pharmacological potential.

Overall, the findings of this study demonstrate that *Paspalum paniculatum* root extracts, particularly the ethanolic extract, possess significant in-vitro antioxidant and cytotoxic activities. These results support the ethnomedicinal use of the plant and highlight its potential as a source of natural antioxidant and anticancer agents. Further studies involving isolation of active constituents, mechanistic evaluation, and in-vitro studies on specific cancer cell lines are warranted to validate and expand upon these findings.

5. CONCLUSION

Paspalum paniculatum root extracts, especially the ethanolic extract, exhibited notable in-vitro antioxidant and cytotoxic activities, indicating their potential as a source of natural anticancer agents.

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