ABSTRACT
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Introduction: Cancer is the second leading cause of death worldwide. Conventional treatment modalities pose several adverse effects and usage of phytotherapy looks promising in treatment. *Cyperus rotundus* (L.) is a perennial herb, having tremendous medicinal properties and it has been used in various formulations in Indian system of medicine.

Aim of the study: To study the *in vitro* and *in vivo* antineoplastic effects of aqueous and ethanol extracts of *Cyperus rotundus* (L.).

Objectives: 1) To evaluate the antioxidant potential and phytoconstituents of aqueous and ethanol extracts of *C. rotundus*. 2) To study the *in vitro* antiproliferative activity of aqueous and ethanol extracts of *C. rotundus* against Ehrlichs Ascites Carcinoma (EAC) and Breast cancer cell lines. 3) To study the *in vivo* anticancer activity of the aqueous and ethanol extracts of *C. rotundus* in EAC induced in Swiss albino mice.

Methodology: Fresh rhizomes of *C. rotundus* were collected, shade dried and coarsely powdered. Aqueous and ethanol extracts of rhizomes were prepared. The extracts were subjected to preliminary phytochemical screening and *in vitro* antioxidant studies. Antiproliferative activities were evaluated using SRB assay and trypan blue assay on MCF-7 and EAC cells. Apoptotic activity was studied by nuclear staining and DNA fragmentation assays. *In vivo* anticancer activities of the extracts were studied by assessing percentage increase in mean life span, hematological alterations, antioxidant status and histopathological changes in EAC induced mice. Cellular alterations induced by the extracts were assessed in ascitic fluid from different treatment groups.

Results: Microscopic analysis revealed the presence of wiry fibrous materials on the roots. Physicochemical screening of rhizomes showed parameters like loss on drying at 105°C was 12.65%, alcohol soluble extractive 1.9% and water soluble extractive 5.2%.
Preliminary phytochemical screening indicated the presence of coumarins, carbohydrates, steroids, phenols and saponins. In ethanol extract, terpenoids and tannins were present in addition. HPTLC profile of ethanol and aqueous extracts at UV 254nm revealed the presence of 15 and 9 peaks respectively. Total phenolic content of aqueous extract was 74.85% and ethanol extract was 61.12%. GC-MS analysis revealed the presence of fifty low polar constituents in the ethanol extract, phenolic hydrocarbons and sterols being the major ones. Results of DPPH free radical scavenging assay revealed the IC₅₀ values of aqueous and ethanol extracts as 90.6 µg/ml and 85.03µg/ml respectively. Reducing power of the extracts increased with increase in concentration. In trypan blue assay, IC₅₀ values for aqueous and ethanol extracts on MCF-7 cells were 4187.117 and 65.081µg/ml respectively. In EAC cells, IC₅₀ value was found to be 2158.653 and 160.190 µg/ml for aqueous and ethanol extracts respectively. The results of Hoechst 33342 and AO-EB staining assay revealed that treatment with ethanol extract induced early/late apoptotic cells, with fragmented chromatin and apoptotic bodies. DNA fragmentation assay revealed that ethanol extract treatment resulted in DNA ladder formation in tumor cells. Tumor volume was significantly decreased in all the treatment groups except aqueous extract at 500 mg/kg dosage. Ethanol extract administered at 250 & 500 mg/kg and aqueous extract at 500 mg/kg dose showed significant reduction in lipid peroxidation when compared to EAC control group. Ethanol and aqueous extracts increased the glutathione peroxidase activity. Ethanol extract at both dosage levels and aqueous extract at 500 mg/kg dose increased catalase activity significantly. Ascitic smears from ethanol extract treated groups showed marked signs of apoptosis. Histopathological analysis of liver and kidney sections from extract treated animals did not reveal any toxicity.

Discussion: Both ethanol and aqueous extracts of *C. rotundus* showed significantly higher cytotoxic activity on EAC and MCF-7 cells, with the ethanol extract being more effective. Reason for higher cytotoxic efficacy of the ethanol extract could be due to its higher solubility, stability and antioxidant activity. This was further supported by the percentage increase in mean life span of EAC induced mice, comparable to that of standard drug cisplatin. Decrease in tumor volume and improvement in hematological
parameters was also documented. GC-MS analysis revealed that phenolic hydrocarbons and sterols were predominant in *C. rotundus* extract, which are known to possess cytotoxic activities.

**Conclusion:** Ethanol extract of *C. rotundus* displayed potential antioxidant and anticancer activities, which prompt the possibility of developing the extract into a therapeutic formulation.

**Keywords:** *Cyperus rotundus*, anticancer activity, MCF-7 cells, Ehrlich’s ascites carcinoma