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CYTOTOXICITY STUDY OF CYPERUS ROTUNDUS RHIZOME EXTRACT ON THE BRINE SHRIMP LETHALITY ASSAY

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ABSTRACT

Aim of the study: This test was carried out to assess the cytotoxicity bioassay of Cyperus Rotundus rhizome extract. Cyperus Rotundus rhizome Bach were subjected to the Brineshrimp Lethality Test. Three dilutions of th methanol extract was used three (3) tubes per dilution. 30 naupli were introduced per tube and mortality evaluated after 24hrs. Mortality data was analysed using the probit method of Finney Computor Programme. The programme uses the number of dose level, the number of brine shrimp for every concentration, percent mortality for every concentration and dose level to calculate lethal concentration (LC50) and its 95 % confidence interval. **Results:** the aqueous extract had an LC50 equal to or higher than 1000µg/ml which is considered non cytotoxic. The extracts showing a low LC50 (< 1000) are likely candidates for cytotoxic or anticancer drugs and can be investigated further. The extracts showing a high LC50 (> 1000) can be used as non-cytotoxic drugs and hence further investigations would be necessary. **Conclusion:** The bioactivity results in this study validate the use of the plants as herbal remedies by Sudan Traditional healers.

KEYWORDS: Brine shrimp lethality, Cyperus Rotundus, cytotoxic, Sudan.

INTRODUCTION

cyperus rotundus L. is a crop used worldwide in traditional medicine fo C. rotundus, were mentioned to contain phenols, flavonoids, alkaloids, saponins, glycosides, and tannins. Phenoic compounds, one of the largest groups of plant phytochemi-cals, have amazing effects based on their toxicity to foreign organism cells (Saxena, M., et al, 2013).[9] This group of compounds has been found to have a wide range of biological functions, in addition to their antioxidant role (Soto-Hernández, et al, 2017).[10] Cytotoxicity Study using *Brine Shrimp Lethality* Test (BSLT) In the current study, Brine Shrimp was used as testing organism to evaluate the cytotoxic effect of the plant extracts.

Scientific name: *Artemiasalina*, Common name: Brine shrimp Scientific Classification Kingdom: Animalia Phylum: Arthropoda Subphylum: Crustacea Class: Branchiopoda Order: Anostraca Family: Artemiidae Genus: *Artemia* This assay was developed by (Meyer *et al*.,1982).[5] is widely used as a simple, reliable and cheaper prescreens method to determine the cytotoxicity of crude plant extract and pure natural compounds, especially antitumor compounds from the natural source (Hullatti and Murthy, 2010).[3] Bioactive compounds are often toxic to shrimp larvae (*Artemiasalina*); therefore, Brine Shrimp Lethality Assay is in use to monitor different chemicals' in vivo lethality to shrimp larvae (Harwig and Scott, 1971; Solis *et al*., 1993; Lewan*et al*., 1992).[2] The general toxic activity was considered weak when the LC_{50} values of crude extracts and pure substances were between 500 and 1000 µg/ml, moderate when the LC_{50} was between 100and 500 µg/ml, and designated as strong when the LC_{50} ranged from 0 to 100 µg/ml but those with <20 µg/ml were considered to be very active (Padmaja*et al*., 2002). While, (Meyer, 1982)[4] considered the LC_{50} values > 1000 µg/ml as non-toxic. There is previous study for brine shrimp cytotoxicity of cyperus rotundus L. whereas, cytotoxicity using brine shrimp of methanolic,ethanoic extracts of *cyperus rotundus* was studied by (Ravikumar et al. 2014).[11]

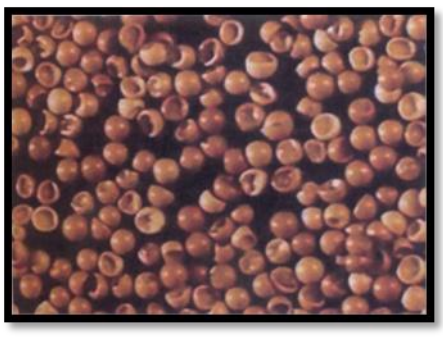

Fig (1): Cysts of *artemia.*

Fig (2): Hatched *artemianauplius.*

Fig (3): Adult *artemiasalina.*

MATERIALS AND METHODS

MATERIALS

Plant materials

Seeds of *cyperus rotundus* L. family *Cyperaceae* were collected from Kosti, White Nile Province, identified by Dr. Hayder Adbalgader and herbarium sheet was deposit at the herbarium of Medicinal and Aromatic Plants Research Institute. (MAPRI).

METHODS

Successive Extraction of the Roots

100 g of the one parts of the plant was with methanol using Soxhlet Apparatus. Extraction was carried out for about two hours for methanol. Extract were then dishes and left under for to dryness. Yield percentage of each extract was calculated as follow.

Weight of extract X100/ weight of the crude powder plant.

Cytotoxicity Study

Biological Assay of Brine shrimp

The brine shrimp lethality assay was performed following the reported procedure (Meyer*et al*, 1982 and McLaughin*et al.*, 1991).[4]

Hatching of Brine Shrimp Technique

Brine shrimp (*Artemiasalina* leach) eggs were purchased from fish pet shops. The eggs were then hatched in a shallow rectangular plastic dish, filled with artificial seawater prepared by dissolving 38 g of sea salt in 1litre of distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment that got darkened while the minor compartment was opened to ordinary light. Two days later, nauplii/larvae were collected by a pipette from the lighter side negative control (McLaughin*et al.*, 1991).[4]

Test Procedure

Three vials were prepared at each concentration (20mg of crude extract and 20mg of volatile oils dissolved in 2ml of the respective solvent). For speed the dissolving samples were sonicated, then 500 µL, 50 µL and 5 µL of this solution was transferred to vials corresponding to 1000, 100 and 10 µg/mL, respectively. The solvent was evaporated overnight. After two days of hatching, 10 Nauplii /larvae were placed into each vial and the volume was adjusted with sea water to 5ml per vial, and incubated at 27 °C for 24 hours under illumination. Then, the number of survivors were counted and recorded. Reference cytotoxic drug (Etoposide) served as positive control. Evaluation of the test results, using the values on died individuals in given concentrations determine the percent of mortality. The data were processed using a Finney computer programme (McLaughin, 1991).[4] and LD_{50} values were obtained at 95 % confidence intervals (Finney, 1971).[1]

Statistical analysis

Statistical Package for Social Science (SPSS) was used for the analysis of the data. The significance of differences between means was compared among the groups using Independent-sample T-test (Snedecor and Cochran, 1989). For brine shrimp test data was analyzed with Finney computer program to determine LD_{50} values with 95% confidence intervals.

RESULT AND DISCUSSION

Cytotoxicity results using Brine Shrimp Lethality Assay (BSLT) C. rotundus rhizome bioactive products have been extracted using methanol solvent (MRCr). Cytotoxic efficacy of C. rotundus extract was studied using Brine shrimp (Artemiasalina leach) eggs All these cell lines have been responding by apoptosis. The values of IC50 of tested cell lines were different with a minimum value of (25± 19) and a maximum value of (25± 28). Cyperus rotundus extract showed minor cytotoxic effect on the Brine shrimp (Artemiasalina leach) eggs This study sug-gested that the C. rotundus extract has apoptosis anti-proliferative activity that need more studies to investigate and characterize the purioed components against cancerous cells using the rhizome of C. rotundus.

Table (1): Cytotoxicity Results of *Cyperus rotundus* methanol extract.

Concentration	No of shrimps	survived	Dead	St.drug.µg/ml
10	30	11	19	(Etoposide 7.4625 µg/ml)
100	30	5	25	
1000	30	2	28	

*LD_{50}=77.2849 µg/ml

The extract of C. rotundus .showed LD50 values of > 1000µg/ml, which denotes that these extracts were not toxic. Cytotoxic activity of these extracts was tested using artemia Salina Brine Shrimp Lethality Bioassay. The extracts of C. Rotundus L. showed the LD5077.2849µg/ml these denote the cytotoxic effects of these extract.

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