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TOXICITY SCREENING OF SELECTED POACEAE SPECIES IN KANO, NORTHERN NIGERIA

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ABSTRACT

The study was aimed at searching for alternative sources of affordable food from the selected Poaceae species. This involved ten Poaceae species which were identified, percolated (whole plant) with methanol, filtered and evaporated to dryness in Rotary Evaporator (Yamamoto, Tokyo, Japan) at 40^0C. Cytotoxicity of the Poaceae species were determined using brine shrimp (*Artemia salina*) bioassay; the results: *Urelytrum muricatum* (566.607µg/ml), *Eleusine indica* (550.021µg/ml), *Pennisetum purporeum* (546.842µg/ml), *Panicum maximum* (542.620µg/ml), Andropogon gayanus (536.97µg/ml), *Imperata cylindrica* (527.257µg/ml), *Cynodon dactylon* (512.781µg/ml), *Leersia hexandra* (512.781µg/ml), *Dactyloctenium aegyptium* (507.272µg/ml), and *Eragrostis cilliaris* (507.272µg/ml). According to Clarkson's criterion for the toxicity assessment of plant extracts, this result revealed that the Poaceae species are low toxic. Since all the Poaceae species exhibited low toxicity, it can be concluded that the grasses can serve as alternative safe and affordable food and drug sources. Confirmation of the Brine shrimp lethality bioassay results with Acute Oral Toxicity Assay using laboratory animals are recommended.

KEYWORDS: brine shrimp lethality assay; LC_{50}; poaceae extracts; probit analysis; toxicity testing.

INTRODUCTION

Although many plants have valuable properties, some of them are known to carry toxicological properties as well. Studies indicate that although numerous plants are used as food sources, some of them may have mutagenic or genotoxic potential. The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant (Tülay and Özlem, 2007; Ugya, 2015; Ugya *et al*., 2015a, Ugya *et al*., 2015b; Ugya *et al*., 2015c).

A universal bioassay that appears capable of detecting a comprehensive spectrum of bioactivity present in plant crude extracts is the Brine Shrimp (*Artemia* sp.) Lethality Assay (BSLA) (Pisutthanan, *et al*. 2004). Brine Shrimp Lethality Assay (BSLA) has been applied as an alternative bioassay technique to screen the toxicity of plant extracts and also as a guide for the detection of antitumor and pesticidal compounds (Moshi *et al*. 2010; Sharma *et al*. 2013; Ugya and Umar, 2015; Ugya *et al*., 2015d; Ugya *et al*., 2016). *Artemiasalina* is most extensively studied of the *Artemia* species, estimated to represent over 90% of the studies in which *Artemia* is used as an experimental test organism (Mirzaei M. and Mirzaei, A. 2013; Veni and Pushpanathan, 2014). The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs makes BSLA a very useful bench top method (Ogugu *et al*. 2012; Sharma *et al*. 2013). This assay has been renowned as a useful device for the isolation of bioactive compounds from plant extracts (Konan *et al*. 2007). Additionally, several studies substantiated that there is a good correlation between the results for the lethal concentration that kills 50% of the exposed population (LC_{50}) obtained with the Brine Shrimp Lethality Assay using *A. salina* and the results of the Acute Oral Toxicity Assay in Mice (Parra *et al*. 2001; Arlsanyolu and Er-demgil, 2006). In this present study, methanolic extracts of the ten selected Poaceae species were tested *in vivo* for their cytotoxic effect against the brine shrimp phototrophic nauplii. Thus, the findings of this present work would give baseline information on the most promising Poaceae species that could be utilize as source of energy in cases of food crisis. As an important source of nutrition and substances which produce physiological action on the human body, plants are recommended for their therapeutic values which can be used in drug development and synthesis (Ramawat and Merillon 2008; Olowa and Nuñeza 2013; Ugya et al., 2017; Ugya and Imam, 2017). Koleva *et al*. (2000) showed that in developing countries, a huge number of people live in extreme poverty and some are suffering and dying for want of safe water, food, shelter and affordable alternatives to modern medicine. As grasses are low-

priced sources of materials for developing cost-effective products, their utilization can be of advantage in production of affordable alternative natural food. This research work therefore, is aimed at evaluating the cytotoxic properties of the methanolic extracts using brine shrimp bioassay

MATERIAL AND METHOD

Ten species of freshly growing Poaceae were collected at the Old Campus of Bayero University Kano, Northern Nigeria in September 2014. The species are *Urelytrum muricatum, Leersia hexandra, Imperata cylindrica,Andropogon gayanus, Dactyloctenium aegyptium, Pennisetum porpureumPanicum maximum, Eragrostis ciliaris,Eleusine indica* and *Cynodon dactylon.*

The species were identified and authenticated at the Department of Plant Biology, Faculty of Science, Bayero University, Kano. Each identified species of Poaceae were collected in mass, freshly chopped with knife and further crushed using blender to increase surface area during the percolation. One hundred grams of freshly crushed species were weighed accurately with analytical balance for percolation. The samples were dissolved in amber bottle containing (*Urelytrum muricatum*, 420ml; *Leersia hexandra*, 450ml; *Imperata cylindrica*, 440ml; *Andropogon gayanus*, 400ml; *Dactyloctenium aegyptium*, 600ml; *Pennisetum porpureum*, 550ml; *Panicum maximum*, 570ml, *Eragrostis ciliaris*, 500ml; *Eleusine indica*, 540ml, and *Cynodon dactylon* 600ml) methanol and stored in cupboard to avoid/ minimize light penetration for five days. The mixtures were shaken manually at least twice daily to facilitate the process. The mixture was then filtered through a Whatman® filter paper No. 1 (Sargent-Welch, USA) and evaporated to dryness in Rotary Evaporator (Yamamoto, Tokyo, Japan) at 40^0C. The methanolic extract concentrates were refrigerated at 4^0C for further analysis. The extracts were weighed and value of each noted in grams ranging from 0.5 to 3.2 as shown in Table 4.2. The extracts are greenish and gummy in nature.

Evaluation of Cytotoxic Properties of the Extracts

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts of ten Poaceae species. In the present study, the brine shrimp lethality of extracts of 10 Poaceae species was determined using the procedure of Moshi *et al*., 2010. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a 500 ml beaker with seawater to the level of 200 ml under constant aeration for 48 hours. After hatching, active nauplii free from egg shells were collected from the beaker and used for the assay. Ten milligrams of each extract was dissolved in 1000 ml of methanol from which serial dilutions of 1000 µg/ml, 500 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml were made in the vials. Each concentration was triplicated. The content of the vials was allowed to dry and called sample vials. One to two drops of Dimethyl sulphoxide (DMSO) was added to the dried extract in the sample vial and mixed thoroughly. 1ml of seawater was then added to the content of each sample vial. Ten phototrophic *Artemia* nauplii were drawn through a glass capillary pipette, placed in each sample vial andmaintained at room temperature for 24 hours under light and air. After 24 hours, the vials were inspected and the numbers of survivors were counted. The mortality was calculated for each concentration. The concentration-mortality data were analyzed statistically by SPSS 16.0. The LC_{50} for each extract was determined.The lethality was calculated from the mean survival larvae of sample vials (extract treated vials). LC_{50} values were obtained using probit analysis (SPSS 16, Chicago, IL, USA).

RESULTS AND DISCUSSION

Identification of the Poaceae Species

The species of grasses(Poaceae) were collected purposively at theBayero University Kano old campus and identified atDepartment Plant Biology of Bayero University, Kano are shown in table 1.

Table 1: The Scientific, Common, Hausa and Nupe Names of the Ten Poaceae Species.

Scientific names	Common names	Hausa names	Nupe Names
Andropogon gayanus	Gamba grass	Jimfi	Banngi
Cynodon dactylon	Bermuda grass	Buntun kuda/ Jaja maza	Kiri-kiri
Dactyloctenium aegyptium	Crowfoot grass	Gude-gude	Chinchere emi
Eleusine indica	Goose grass	Ciyawar tuji/Tauban daji	Chinchere lati
Eragrostis ciliaris	Love grass	Tsarkiyar Zomo	Egogi
Imperata cylindrical	Spear grass	Zarenshi	Etwo
Leersia hexandra	Cut grass	-	-
Panicum maximum	Guinea grass	Gatsaura/Gyero tsuntsaye	-
Pennisetum purporeum	Elephant grass	Kyambama/Kansuwa	Kasuwa
Urelytrum muricatum	Centipede grass	Jemma/Rumiya	-

***Andropogon gayanus* (Kunth)**

***Dactyloctenium aegyptium* (L.)**

***Cynodon dactylon*(L.)**

Imperata cylindrical (L.)

***Leersia hexandra* (Sw).**

***Panicum maximum* (Jacq)**

Figure 3: The Pictures of some Poaceae Species in their Natural Environment.

Extracts Yield of the sampled Poaceae species

The extracts, greenish gummy in nature were weighed and weight values in gram are shown below.

Table 2: TheExtracts' yield of the Poaceae species from 100 g of fresh samples.

S/N	Scientific Name	Weight(g)
1	*Andropogon gayanus*	2.0
2	*Cynodon dactylon*	0.8
3	*Dactyloctenium aegyptium*	1.7
4	*Eleusine indica*	2.6
5	*Eragrostis ciliaris*	1.4
6	*Imperata cylindrica*	0.8
7	*Leersia hexandra*	0.5
8	*Panicum maximum*	2.9
9	*Penisetum porpureum*	2.5
10	*Urelytrum muricatum*	2.9

Brine Shrimp Bioassay

The LC_{50} values of the brine shrimp obtained for extracts of these species are given in Table 4.1.5 below. As can be seen in the table *Urelytrum muricatum* has the highest value of 566.607 µg/mL followed by *Eleusine indica* with 550.021 µg/mL. The lowest value was observed in *Dactyloctenium aegyptium* and *Eragrostis ciliaris* both with 507.272 µg/mL.

Table 3: Result of Brine Shrimp Bioassay of the Poaceae Species Extracts.

S/NO.	Poaceae species	No. of Death Nauplii per concentration					
		1000	500	100	50	10	LC_{50} (µg/mL)
1	*Andropogon gayanus*	24	15	9	6	0	536.975
2	*Cynodon dactylon*	30	9	6	3	0	512.781
3	*Dactyloctenium aegyptium*	27	12	9	6	0	507.272
4	*Eleusine indica*	27	12	6	3	0	550.021
5	*Eragrostis ciliaris*	27	14	9	6	0	507.272
6	*Leersia hexandra*	30	11	6	3	0	512.781
7	*Imperata cylindrica*	30	14	9	6	0	527.257
8	*Panicum maximum*	27	12	7	3	0	542.620
9	*Pennisetun porpureum*	27	10	7	6	0	546.842
10	*Urelytrum muricatum*	27	9	6	6	0	566.607

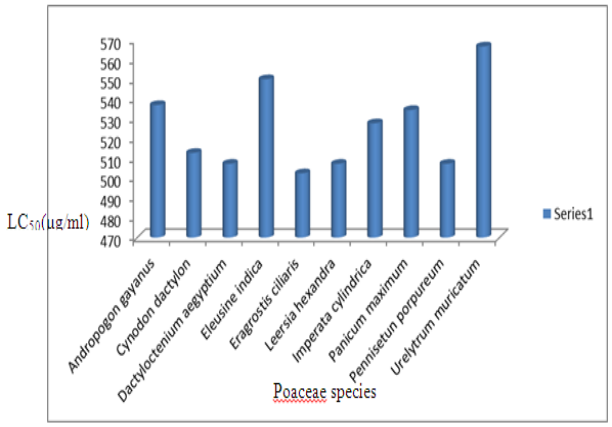

Fig 2: Chart representing the Brine Shrimp Test value of the ten Poaceae Species.

Toxicity Testing Criterion used.

Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC_{50} above 1000 µg/ml are non-toxic, LC_{50} of 500 - 1000 µg/ml are low toxic, extracts with $LC_{50}$of 100 - 500 µg/ml are medium toxic, while extracts with $LC_{50}$of 0 - 100 µg/ml are highly toxic (Clarkson *et al*. 2004).

Brine Shrimp Bioassay of the Poaceae

The LC_{50} represents the concentration of the extract that produces death in half of the test subjects after the exposure period of 24hours. The degree of lethality was found to be directly proportional to the concentration of the extracts. Maximum mortalities took place at a concentration of 1000 µg/ml while least mortalities at 10 µg/ml concentration which agrees with report of Olowa and Nuñeza (2013); observed that the brine shrimp lethality of the three plant extracts (*Chromolaena odorata, Lantana camara* and *Euphorbia hirta* were found to be concentration-dependent. The observed lethality of the ten Poaceae extracts to brine shrimps indicated the presence of potent cytotoxic and probably therapeutic components of these Poaceae species is in agreement with study of Konan *et al*. (2007); observed significant lethality in twenty five Sudanese medicinal plant extracts to brine shrimp, indicative of the presence of potent cytotoxic components. All the Poaceae species extracts displayed toxicities (LC_{50}< 1000µg/ml). However, according to Clarkson's toxicity index, the Poaceae species toxicities fall under low toxic (LC_{50} of 500 - 1000 µg/ml) (Clarkson *et al*. 2004). The results indicate that all grass extracts tested have LC_{50} values above 500 µg/ml which suggests that they are practically low toxic and probably have no obvious danger. The present finding on *Eleusine indica* supports Al-Zubairi *et al* (2011) which was also in agreement with Responte *et al* (2015) that ethanolic extract of *E. indica* possess cytotoxic behaviour, suggesting the presence of potential bioactive chemical components (509.73 ppm). The present finding on *C. dactylon* contradicts Alluri *et al*. (2006) that *C. dactylon* showed non-toxicity to the brine shrimp (>5,000 µg/ml)

CONCLUSIONS AND RECOMMENDATION

The Poaceae species being inexpensive and easily available can serve as potential food and nutraceutical resources, capable of offering significant nutritional dietary supplements and enhance health benefits. The Acute Oral Toxicity Assay using laboratory animal can be used to confirm this result and study the details of toxicity effects.

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