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TOXICITY ASSESSMENT OF *KEDROSTIS AFRICANA* COGN: A MEDICINAL PLANT USED IN THE MANAGEMENT OF OBESITY IN SOUTH AFRICA USING BRINE SHRIMP (*ARTEMIA SALINA* LINN.) ASSAY

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ABSTRACT: Baboon's Cucumber (*Kedrostis africana* (Linnaeus) Cogn) is a monoecious caudiciform plant that belongs to Cucurbitaceae family. Baboon's Cucumber is used traditionally for the management of syphilis and obesity in South Africa. In the present study, we examined the hatchability and lethality of Baboon's Cucumber bulb extracts against brine shrimps. The tested samples were aqueous extract, acetone extract, and ethanol extract. Cytotoxicity was screened using Brine Shrimp Lethality Test (BSLT). The hatching success was in the order: ethanol extracts (49.2%) > aqueous extract (45.4%) > acetone extract (45.2%). All the extracts hatching success were significantly higher than the positive control (potassium dichromate) ($p < 0.05$). Based on Clarkson's toxicity index, $LC_{50} > 1$ mg/mL were considered non-toxic for acetone extract while the aqueous and ethanolic extracts were considered to be moderately toxic (LC_{50} 100-500 μ g/mL) with LC_{50} of 0.298 and 0.489 mg/mL respectively. In conclusion, since the aqueous and ethanolic bulb extracts of *K. africana* exhibits potent cytotoxic property comparable to that of standard drug. Therefore, this might be utilized for the development of novel anticancer drug leads and the nontoxic acetonic extracts could further be exploited for the development of plant-based pharmaceuticals.

INTRODUCTION: The healing potentials in plants are an antique concept. The World Health Organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the management or treatment of various ailments. This discovery has brought about the search for novel drugs which are plants-derived and possess the potency to combat the menace of drug resistance pathogenic microorganism, antitumor, anti-obesity and anticancer agents^{1,2}.

Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide, resulting into a significant increase in mortality and morbidity related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke and cancers³⁻⁵. Recently, there has been difficulty in the treatment of obesity because some of the drugs in the market have side effects, including increased blood pressure, dry mouth, constipation, headache, and insomnia^{6,7}.

Therefore it has become imperative to explore natural products for treating obesity as this may be an excellent alternative strategy for developing future effective, safe anti-obesity drugs which would also be affordable^{8,9}. *Kedrostis africana* (Linnaeus) Cogn. is among the plants used by traditional healer for the management of obesity in the Eastern Cape of South Africa by a survey conducted by Afolayan and Mbaebie¹⁰.

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Kedrostis africana is a monoecious caudiciform plant, commonly known as "Baboon's Cucumber" with lots and lots of herbaceous climbing or creeping vines growing rapidly from the swollen base and looking like English ivy with a tuber. The shoots emerge from a massive underground tuberous rootstock (or caudex). This tuber is a water-storage organ so it is very resistant to drought¹¹.

Kedrostis africana is found in Namibia and South Africa (Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, and Western Cape). According to vanWyk¹², *Kedrostis africana* tuber is used as an emetic, purgative, diuretic; dropsy, syphilis. Also, a decoction from crushed fresh bulb is used for the management of obesity^{10, 13}. Brine shrimp also known as sea monkey is now being used for toxicity assay because this assay involves the killing thus there have being a reasonable controversy over use of animal for such purposes and some people have ethical or religious objection to the killing of even lower organism¹⁴.

Brine shrimp (*Artemia salina*) assay is now being employed because it gives a valid method of evaluating the cytotoxic of plant extracts¹⁵⁻¹⁷. Some publications have suggested a good correlation between the toxic activity in the brine shrimp assay and the cytotoxicity against tumor cell lines¹⁸ and hepatotoxic activity¹⁹. Brine shrimp tests are normally conducted to draw inference on the safety of the plant extracts and further to depict trends of their biological activities. Although the ethno-medicinal purpose has been explored, there is a need to investigate its toxicity level. The aim of the present work is to *Kedrostis africana* for its cytotoxic effect on *Artemia salina* and correlate toxicity results with its known ethno pharmacological activity.

MATERIALS AND METHODS: The bulb of *K. africana* was used for this study were collected August 2015 near baddford farm in Fort beaufort which is in Amathole District Municipality, Eastern Cape, South Africa. This area lies at Latitude 32°43'28.66" and Longitude 26°34'5.88". The plant was authenticated by Mr. Tony Dold of Selmar Schonland Herbarium, Rhodes University, South Africa, and a voucher specimen (Unuofin

Med, 2015/2) was prepared and deposited in the Giffen Herbarium, University of Fort Hare. The bulb was rinsed with deionised water and gently blotted with paper towel to remove the water, chopped into smaller bits and subsequently oven-dried (LABOTEC, South Africa) at 55 °C for 72 hours until constant weight was achieved, then ground into powder (Polymix® PX-MFC 90D Switzerland). The ground sample was put into separate conical flasks containing acetone, ethanol and water and shaken in an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 48 hours.

The crude extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper. The acetone and ethanol extracts were further concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator (Strike 202 Steroglass, Italy) while the aqueous filtrate obtained was concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY).

Preparation of the Assay: The method described by Otang et al.,²⁰ was employed with little modifications. Five petri dishes containing 30 mL of the extracts were prepared in filtered sea water by first dissolving them in minute amount of the parent solvents to yield a two-fold dilution series of concentrations (1, 0.5, 0.25, 0.125 and 0.0625 mg/mL). A positive control was also prepared by dissolving potassium dichromate in seawater in the same concentrations as the plant extracts. Sea water only served as the negative control. The setup was allowed to stand for 30 minutes to allow the solvents to evaporate.

A. salina Hatching Assay: This assay was evaluated as described by Otang et al.,²⁰. A density of ten (10) *A. salina* cysts was stocked in each of the petri dishes containing 30 mL of the prepared two-fold concentrations (1 to 0.0625 mg/mL) of the plant fractions and positive control. The petri dishes were partly covered, incubated at 30 °C and under constant illumination for 72 hours. The number of free nauplii in each petri dish was counted after every 24 hours till end of 72 hours. The percentage of hatchability was assessed by comparing the number of hatched nauplii with the total number of cysts stocked.

A. salina Lethality Assay: *A. salina* cysts were hatched in sea water and 10 nauplii were pipetted into each petri dish containing the two-fold concentrations of the extracts and control as in the hatchability above. The petri dishes were then examined and the number of living nauplii (that exhibited movement during several seconds of observation) was counted after every 24 hours and the set up was allowed to stand for 72 hours under constant illumination. The percentage of mortality (M %) was calculated as: $\text{Mortality (\%)} = (\text{Total nauplii} - \text{Alive nauplii}) \times 100 / \text{Total nauplii}$.

Data Analysis: The percentage hatchability success and mortality data obtained from the 5 different concentrations of each fraction and control experiments were used to construct the dose-response curves. These were used to determine their corresponding LC₅₀ values. The LC₅₀ was taken as the concentration required for producing 50% mortality of the nauplii. LC₅₀ values were determined from the best-fit line obtained by regression analysis of the percentage hatchability and lethality versus the concentration. The statistical analysis was done on MINITAB version 17 for windows.

One-way analysis of variance (ANOVA) followed by Fischer's Least Significant Different (for means separation) was used to test the effect of concentration and time of exposure of the plant extracts on the hatchability success of the cysts and mortality of and larvae respectively.

RESULTS:

Brine Shrimp Hatchability Assay: The hatching success of *A. salina* incubated with different plant extracts and control is as shown in **Fig. 1** with the sea water having a significantly higher hatching success (71%) than the solvent extracts including the positive control (potassium dichromate) (5.4%) ($p < 0.05$). The hatching success of the cysts in the acetone (45.2%), aqueous (45.4%) and ethanol (49.2%) extracts showed no significant difference from each other ($p < 0.05$). The effect of different solvent concentrations on the hatching success of the cyst was also evaluated and the result is depicted in **Fig. 2**.

Fig. 2 shows the activities of the different plant extracts/positive control at varying concentrations

to the hatching success of the cysts. The hatching success of *A. salina* cysts significantly decreased with increasing concentrations of the ethanolic extract and the positive control (potassium dichromate) while acetone and aqueous extracts had the same pattern of hatching with the highest hatching potential being observed at 0.125 mg/mL (**Fig. 2**). The percentage hatching success of cysts incubated with the ethanol extract showed significant differences at varying concentrations. The lowest concentration (0.0625 mg/mL) had the highest hatching percentage (82%) and it was not significantly different from the cysts incubated at 0.125 mg/mL and 0.25 mg/mL with a hatching success of 67% and 60% respectively. There was zero percent (0%) hatchability observed from 0.25 mg/mL -1 mg/mL for potassium dichromate.

The acetone, aqueous and ethanol extracts had significant higher hatching percentage of the cysts at 0.125 mg/mL (58%, 58% and 67% respectively). There was no significant difference in percentage hatching at 0.0625, 0.125, 0.25 and 1 mg/mL, while in the aqueous extract; also there was no significant difference in percentage hatching at 0.125, 0.25 and 1 mg/mL in the acetic extract. In addition, no significant difference was observed in percentage hatching at 0.0625, 0.125, 0.25 and 0.5 mg/mL in the ethanolic extract and lastly there was no significant difference in percentage hatching at 0.0625, 0.125 and 0.25 mg/mL for the positive control (potassium dichromate) ($p < 0.05$).

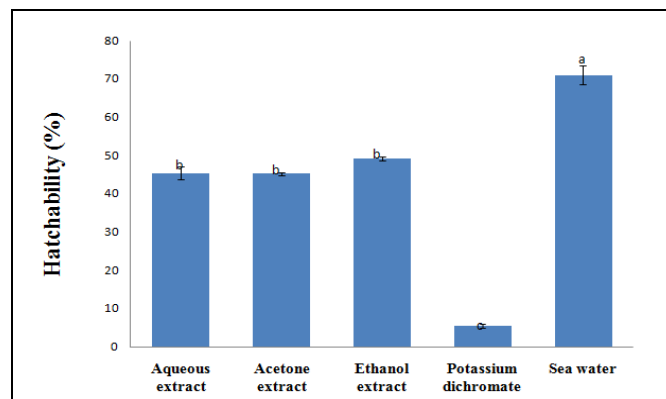


FIG. 1: PERCENTAGE HATCHING SUCCESS OF *A. SALINA* CYSTS INCUBATED IN DIFFERENT SOLVENT EXTRACTS AND CONTROLS. THE VALUES ARE MEANS OF FIVE CONCENTRATIONS FOR EACH PLANT EXTRACT/CONTROL \pm SD OF THREE REPLICATES. BARS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$)

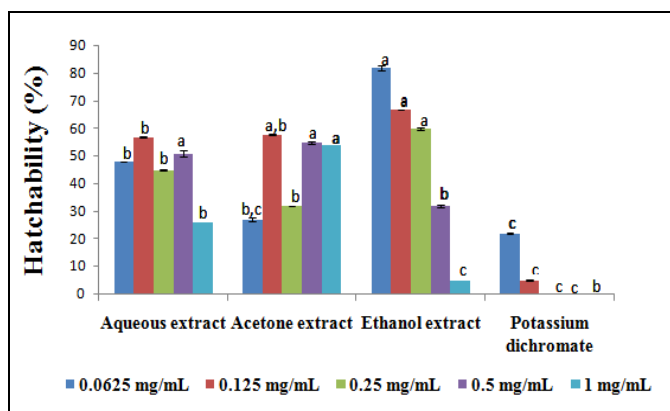


FIG. 2: PERCENTAGE HATCHING SUCCESS OF A. SALINA CYSTS INCUBATED IN DIFFERENT CONCENTRATIONS OF THE PLANT EXTRACTS AND CONTROL. THE VALUES ARE MEANS OF THE REPLICATES (AT DIFFERENT HOURS) FOR THE CONCENTRATIONS FOR EACH PLANT EXTRACT/CONTROL ± SD OF THREE REPLICATES. SET OF BARS WITH DIFFERENT LETTERS IS SIGNIFICANTLY DIFFERENT (P < 0.05)

The effect of exposure time on the hatching success on *A. Salina* is shown in **Fig. 3**. The same trend was observed in the acetone extract and sea water as there was significant differences in hatching success from 24 h to 72 h ($p > 0.05$). The lowest hatching success was observed at 24 hour in all the extracts and controls.

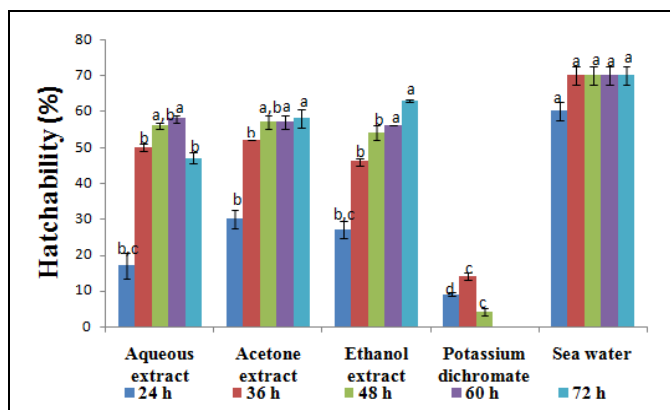


FIG. 3: PERCENTAGE HATCHING SUCCESS OF A. SALINA CYSTS INCUBATED AT DIFFERENT DURATIONS IN THE PLANT EXTRACTS/CONTROLS. THE VALUES ARE MEANS OF REPLICATES (OF ALL THE CONCENTRATIONS) FOR EACH PLANT EXTRACT/CONTROL ± SD OF THREE REPLICATES. SET OF BARS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P < 0.05)

The results from this study also showed that after 60 hours of exposure, hatching success of the cysts incubated in acetone and ethanol extracts only significantly increased by 1 and 1.1-fold,

respectively while with aqueous decreased significantly by 1.2-fold (**Fig. 3**). Potassium dichromate decreased significantly by 3.5-fold after 36 hours ($p < 0.05$), followed by no hatching of cyst after 48 hours.

Brine Shrimp Lethality Assay (BSLA): The percentage lethality/mortality of *A. salina* larvae (nauplii) incubated in different solvent extracts of *K. africana* and controls are shown in **Fig. 4**. There was a significantly higher mortality percentage (99%) of the nauplii incubated with potassium dichromate than the extracts and sea water ($p < 0.05$). Although there was no significant difference ($p < 0.05$) between the aqueous and ethanolic extracts. The acetone extract had mortality of 18.20% while the sea water had the least mortality of 0%.

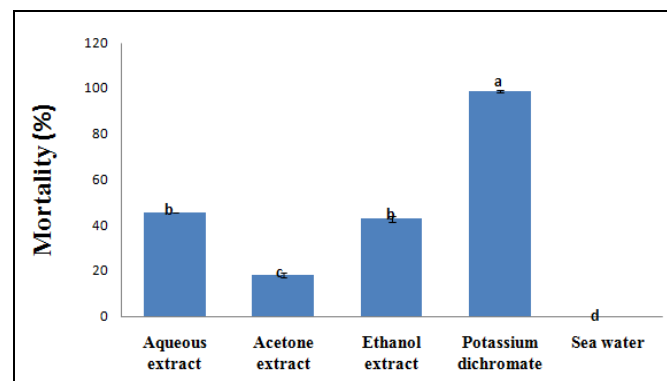


FIG. 4: PERCENTAGE MORTALITY OF A. SALINA NAUPLII INCUBATED IN DIFFERENT PLANT EXTRACTS AND CONTROLS. MEANS ARE VALUES OF FIVE CONCENTRATIONS FOR EACH PLANT FRACTION/CONTROL ± SD OF THREE REPLICATES. BARS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P < 0.05)

The effect of varying concentrations of the plant fractions on the mortality of larvae is shown in **Fig. 5**. The degree of mortality of nauplii was in a concentration-dependent fashion. The highest mortality was observed in all the extracts at 1 mg/mL while the control had a maximum mortality (100%) at 0.125 mg/mL. There was no significant in percentage mortality of the nauplii at concentrations of 0.0625 - 1 mg/mL ($p < 0.05$) in the aqueous extract and potassium dichromate. There was also no significant in percentage mortality at concentrations of 0.125 - 1 mg/mL in the acetone extract while the ethanolic extract had no significant at 0.0625, 0.125 and 0.5 mg/mL ($p < 0.05$).

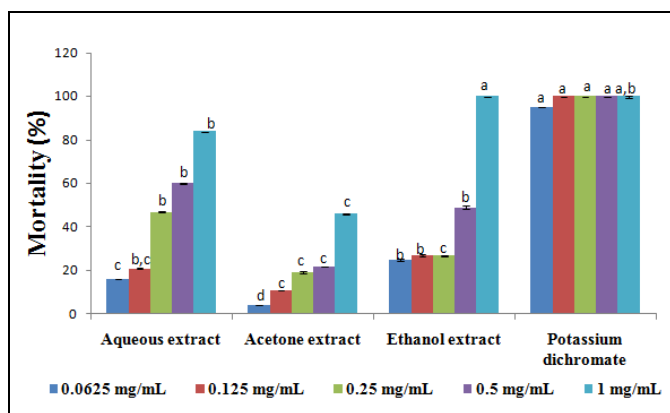


FIG. 5: PERCENTAGE MORTALITY OF A. SALINA CYSTS INCUBATED IN DIFFERENT CONC. OF THE PLANT EXTRACTS AND CONTROL. THE VALUES ARE MEANS OF THE REPLICATES (AT DIFFERENT HOURS) FOR THE CONCENTRATIONS FOR EACH PLANT EXTRACT/ CONTROL ± SD OF THREE REPLICATES. SET OF BARS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P < 0.05)

The results also show that the mortality of nauplii incubated in these plant extracts increased with time (Fig. 6). After 36, 48, 60 and 72 hours of incubation in acetone extract, the mortality significantly increased by 1.1, 1.1, 1.2 and 1.9-fold, respectively. With aqueous extract, the mortality significantly increased by 1.22, 1.5, 1.6 and 1.1 times more after 36, 48, 60 and 72 hours, respectively (p<0.05). The nauplii death rate also increased by 1.13, 1.0, 1.8 and 1.1-fold after

incubation in ethanol extract over the same period of time, respectively.

The increase in mortality of the positive control at all duration times are 1.01, 1, 1 and 1-fold (p<0.05). The sea water was observed to 0% mortality through the duration of the experiment. In overall, the mortality of nauplii was significantly similar when incubated with the ethanol, acetone extract and the aqueous which was significantly higher than in sea water (p<0.05) (Fig. 6).

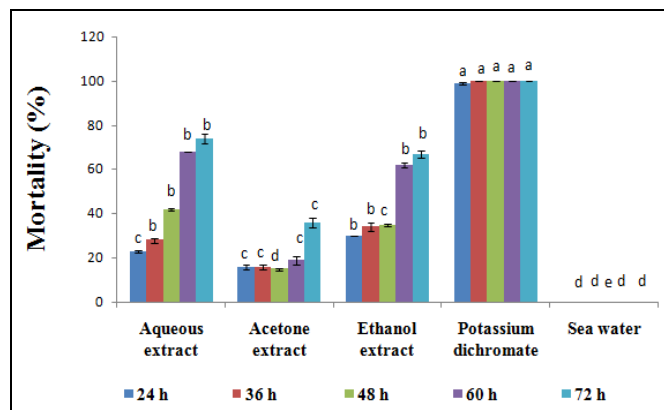


FIG. 6: PERCENTAGE MORTALITY OF A. SALINA CYSTS INCUBATED IN DIFFERENT TIME DURATIONS IN THE PLANT EXTRACTS/ CONTROLS. THE VALUES ARE MEANS OF REPLICATES (OF ALL THE CONCENTRATIONS) FOR EACH PLANT EXTRACT/CONTROL ± SD. SET OF BARS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (P < 0.05)

TABLE 1: THE ESTIMATED LD₅₀ RESULTS WERE 1.11, 0.4498 AND 0.298 mg/ml FOR THE ACETONE AND AQUEOUS EXTRACTS, RESPECTIVELY

Sample	Regression equation	LD ₅₀ (mg/mL)	Toxicity status	R ² (%)
Aqueous extract	Y = 25.247ln(x) + 80.6	0.298	Medium toxic	96.76
Acetone extract	y = 41.032x + 4.5	1.109	Non-toxic	96.21
Ethanol extract	Y = 102.24x	0.489	Medium toxic	86.3
Potassium dichromate	Y= 1.4427247ln(x) + 101	<0.100	Highly toxic	50

The toxicity regression equation used to LD₅₀ (mg/mL) values of acetone extract, aqueous extract, ethanol extract and potassium dichromate (positive control) lethality test in brine shrimp test. R² (%) denotes the coefficient of determination of the regression equation.

DISCUSSION: In the last three decades, *A. Salina* nauplii have been used as preliminary evaluation for general toxicity of herbal remedies. This present study evaluated the toxicity of *K. africana* using hatching success of cysts and mortality of the nauplii in different concentrations of plant extracts and controls. The hatching of *A. salina* cysts was

highest overall in sea water (71%), while the ethanolic extract had the highest (49.2%) hatching success among the extracts used.

The ethanolic extract at 0.0625 mg/mL had the overall best hatching success. The hatching success significantly decreased with increasing concentrations of the plant extracts in a dose-dependent manner with potassium dichromate eliciting 100% hatching inhibition at 0.25-1 mg/mL. This could be attributed to the high toxicity of potassium dichromate even at very low concentrations²¹. *A. Salina* is known to have a resistant cyst stage that can for bear a varied range

of pH stretching from freshwater to saturated saline²⁰ and as such, if the dormancy is not broken hatching will not occur, therefore at 0.125 mg/mL, the plant extracts exhibited an optimum breaking of dormancy of the cyst and further decrease in the concentration revealed an inhibitory action on the cyst.

Evaluation of the hatching success of the cysts in response to exposure time revealed that the aqueous and acetone extracts had no significant hatching success after 36-48 hours which is known to be the best hatching time for brine shrimp according to Meyer et al.,¹⁵ whereas, cysts incubated in the ethanolic extract continued to hatch until the end of 72 hours. A moderate hatching success of the cysts was observed in all the extracts except for those incubated in potassium dichromate which had a hatching success as low as 4 % at 48 hours and hatching thereafter. The poor hatching success observed in potassium dichromate could be attributed to its toxic nature which could probably result to resistance of the eggs to hatching in response to chemical toxins.

The brine shrimp lethality results in this study were interpreted in accordance to Meyer's toxicity index, $LC_{50} < 1000 \mu\text{g/mL}$ (ppm) is toxic, while $LC_{50} > 1000 \mu\text{g/mL}$ is nontoxic. Additionally, a more detailed criterion given by Clarkson as follows: $LC_{50} > 1000 \mu\text{g/mL}$ is nontoxic, LC_{50} 500-1000 $\mu\text{g/mL}$ is low toxic, LC_{50} 100-500 $\mu\text{g/mL}$ is medium toxic, and LC_{50} 0-100 $\mu\text{g/mL}$ is highly toxic²² was used.

The results indicate that the aqueous and ethanolic solvent extracts of *K. africana* bulb exhibited moderate toxicity with LC_{50} of 298 and 489 $\mu\text{g/mL}$ respectively; whereas the acetone extract were not toxic $LC_{50} > 1000 \mu\text{g/mL}$. Hence, this extract may be considered safe for consumption as an herbal medicine. On the other hand, its non-toxic nature discourages its use as an alternative for the treatment and management of cancer whereas the aqueous and ethanolic extracts could serve in that regards²³.

Comparing the relationship between increase in concentration and lethality of the nauplii, we observed that the degree of mortality increased in a concentration dependent manner which peaked at 1 mg/mL. Only the acetonic extract was less toxic at

1 mg/mL with a mortality of 46% whereas other fractions exhibited a mortality ranging from 84 - 100% at that same concentration. The mortality of nauplii incubated in these plant fractions increased exponentially with time, with the highest mortality observed at 72 hours for the plant extracts while in the case of potassium dichromate, maximum mortality was observed at 36 hours.

The essence of exposing the nauplii to plant extracts over a long period of time was to determine their threshold of withstanding toxic metabolites/chemical compounds present in the various fractions. According to Carballo et al.,²⁴, maximum sensitivity of nauplii to test compounds is achieved at the second and third instar stage and it is interpreted to be after 48 hours of incubation. However, in this study it was not the case as maximum sensitivity was reached after 72 hours of exposure. This could be due to presence of some nutritive metabolites that may have acted as food rather than toxic chemicals.

CONCLUSION: The results of this study indicated that aqueous, acetonic and ethanolic extracts of *Kedrostis africana* bulbs supported hatching of cysts in the Brine Shrimp Assay. The acetonic extract was not toxic ($LC_{50} > 1 \text{ mg/mL}$) whereas the both the aqueous and ethanolic displayed signs of moderate toxicity suggesting the need for further *in vivo* and *in vitro* toxicological studies. Cancer cell lines toxicity tests and isolation of cytotoxic compounds is necessary to ascertain if it has anticancer potentials. Based on the possible relationship between brine shrimp lethality and plant bioactivity, this work could serve for further pharmacological and phytochemical research.

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