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CYTOTOXICITY EVALUATION OF *MORINGA OLEIFERA* (*M. OLEIFERA*) LAM. FLOWERS ON HUMAN LIVER CANCER CELL LINE AND *A. SALINA* (BRINE SHRIMP) MODELS

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Keywords:

Brine shrimp, Nauplii, Mortality, Cells, Cytotoxicity, *Moringa oliefera*

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ABSTRACT: *Moringa oleifera* Lam is a fast-growing deciduous tree widely cultivated in tropical and sub-tropical regions. All parts of the plant have widely acclaimed nutrition and health-promoting values, but little has been done regarding the toxicity. This study evaluated the safety of different extracts from Moringa flowers using brine shrimp and cell line models. The effect of different concentrations (0.0625-1 mg/mL) of *Moringa oleifera* flower extracts was assessed on the percentage hatching of brine shrimp cysts and Lethal Concentration (LC_{50}) mortality of nauplii, respectively. Successful hatching of cysts was in the order: Tea (boiled aqueous extract) >aqueous extract>methanol extract>ethanol extract>acetone extract, and mortality of nauplii was concentration-dependent. The highest mortality was observed for all the extracts at 1 mg/mL, with acetone extract exhibiting the highest toxicity (LC_{50} at 40µg/ml). Cytotoxicity activity of *Moringa oleifera* flower extract on human liver cancer HepG2 cells and computational validation of cell death was evaluated on HepG2/C3A cell lines treated with 12.5, 25, 50, 100, and 200 µg/mL of *Moringa oleifera* flower for 48 hours and analyzed using MTT assay. Only the acetone extract of *Moringa oleifera* flowers inhibited the growth and proliferation of liver cancer cells. These findings add to the growing evidence supporting the safety of aqueous extracts of *Moringa oleifera* flowers and the promising potential of its acetone extract as an anti-cancer agent.

INTRODUCTION: Toxicity can either be acute or chronic, based on the number/duration of exposures to poison and the time it takes for toxic symptoms to develop. Acute toxicity is due to short-term exposure, usually, a high dose, whereas chronic exposure is due to repeated or long-term exposure involving low doses [1].

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Cytotoxicity testing using cell cultures is a rapid, standardized, sensitive, and inexpensive means to determine whether a material contains significant quantities of biologically harmful extractable [2]. The high sensitivity of the tests is due to the isolation of the test cells in cultures and the absence of the protective mechanisms that assist cells within the body.

There are several *in-vitro* toxicity tests where different cell lines are used, each test using different indicators. Standard acute toxicity tests with aquatic macro-invertebrates have long played a major role in aquatic hazard and risk assessments, especially at a "screening" level of evaluation.

A number of alternative tests that have been proposed for rapid screening are freshwater rotifer (*Branchionus calyciflorus*), brine shrimp (*Artemia salina*), lettuce (*Luctuca saliva*), and mysid shrimp (*Mysidop sishahia*). These tests are useful in situations where their rapidity and relatively low cost make it practical to screen large numbers of samples for preliminary indications of toxicity [3]. The brine shrimp lethality assay is considered a useful tool for preliminary toxicity assessment. Brine shrimp, commonly known as sea monkeys, are crustaceans living in saline environments [4].

Their eggs (actually cysts), can be inexpensively purchased from pet stores, hatch quickly and the larvae, termed a nauplius (plural, nauplii) are sensitive to small doses of biologically active chemicals. The brine shrimp bioassay is rapid, inexpensive and simple, used for testing plant extract lethality, which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [5]. Most often, a desired biological response is not due to one component but rather due to a mixture of bioactive plant components. Therefore, crude extracts must be screened for biological activity. The brine shrimp lethality assay has been proved to be a convenient system for monitoring the biological activities of natural products [6].

The importance of medicinal plants and traditional health systems in solving health care problems is gaining increasing attention globally. Most developing countries have adopted the traditional medical practice as an integral part of their culture [7]. *Moringa oleifera* Lam, also known as the 'drumstick tree' or the 'horse radish tree', is the most widely distributed and cultivated member of the *Moringaceae* family. This highly valued plant is native to western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Bangladesh, Africa, and Arabia and has become naturalized in many tropical and subtropical areas [8, 9]. The various parts of *M. oleifera* are used extensively in traditional medicine in many regions of the world. In its native land of India, it is used in ayurvedic medicine and is believed to be able to prevent 300 diseases. It is used for varied functions such as cleansing the blood and liver, strengthening the heart, increasing fat metabolism to promote weight loss and even removing worms. In other regions of prevalent moringa cultivation, such as Southeast and South Asia, different parts of the moringa plant are thought to have anti-diabetic, antibacterial, anti-tumor, antipyretic, anti-epileptic, anti-inflammatory, anti-ulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, hepatoprotective, antibacterial and antifungal activities [10].

Worldwide, in South Africa inclusive, liver cancer is a public health problem and the second largest contributor to cancer mortality, with 810,000 deaths recorded in 2015 [11]. Liver cancer is one of the most common malignancies with high morbidity and mortality rates, with an annual incidence of nearly one million [12].

Chemotherapy is a process that uses chemical drugs to kill cancer cells. It is used in cases where surgical interventions cannot be implemented or the patient did not respond to other treatment methods such as radiotherapy. Given the disadvantages of chemotherapy, including the toxic side effects and development of resistance to chemicals, efforts to find drugs with natural origins that have less serious side effects are important and pertinent [13]. There has been extensive interest in exploring the anti-cancer activities of various parts of the *M. oleifera* tree [14, 15]. There is little information in literature about *Moringa oleifera* flowers hence this study evaluates the cytotoxicity of *Moringa oleifera* flowers using brine shrimp and liver cell lines assay models.

MATERIALS AND METHODS:

Collection of Plant Materials: The flowers of *Moringa oleifera* were purchased from Lefakong farm, located in Bosplaas North West Province, South Africa. The flowers were identified and validated by Professor Cupido of the Botany Department, University of Fort Hare, and a voucher specimen (MAP/004/2019) was deposited at the Giffen Herbarium.

Reagents and Materials: Human liver hepatocellular carcinoma C3A cells were purchased from Cellonex, South Africa. Foetal bovine serum (FBS), Eagles minimal essential medium (EMEM), and non-essential amino acids (NEAA) were purchased from GE Healthcare Life Sciences (Logan, UT, USA). PBS with and without

Ca^{2+} and Mg^{2+} and trypsin was purchased from Lonza (Wakersville, MD, USA). MTT was purchased from Sigma (St. Louis, MO, USA). The brine shrimps were purchased from Finny Flipper (pet store) in East London, 5241.

Preparation of Extracts: *Moringa oleifera* flowers were air-dried, then ground to a fine powder. 60 g of each ground plant material was extracted separately in distilled water, methanol, acetone, hot water (tea), and ethanol for 48 h on an orbital shaker. Extracts were filtered using a Buckner funnel and filter paper (Whatman No. 1).

A rotary evaporator was used to concentrate the acetone, ethanol, and methanol extracts to dryness under reduced pressure at 57°C. In contrast, the aqueous filtrates were concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY). Samples were stored at 4°C until required. Test extracts were reconstituted in dimethyl sulfoxide (DMSO) to give a final 100 mg/mL concentration. Samples were sonicated if solubility was a problem.

Hatching Assay: This assay was evaluated as described by Maposa *et al.*, [16]. Ten (10) *A. salina* cysts were stocked in each petri dishes containing 30 ml of the prepared extracts and positive control (potassium dichromate).

The petri dishes were partly covered and incubated at 30°C under constant illumination for 72 h. The number of nauplii in each petri dish was counted every 12 hours for 72 h. The percentage of hatched cysts was calculated by subtracting the number of nauplii from the total number of cysts stocked.

Lethality Assay: The assay was evaluated as described byIdris *et al.*, [17]. *A. salina* cysts were hatched in sea water, then 10 nauplii were pipetted into each petri dish containing the *Moringa oleifera* extracts and controls as described for the hatching assay.

The petri dishes were then examined by counting the number of living nauplii every 12 h for 72 h under constant illumination. The percentage mortality (M %) was calculated as:

Mortality (%) = (Total nauplii-living nauplii) / Total nauplii × 100

Cell Culture (MTT Assay): This study used human liver hepatocellular carcinoma cell line C3A cells, a clonal derivative of HepG2 cells, to assess cytotoxicity. Cells were maintained at 37°C in a humidified incubator with 5% CO_2 in 10 cm culture dishes. The complete growth medium consisted of EMEM supplemented with 10% FBS, 1x penicillin-streptomycin, and 1x NEAA for C3A cells.

Cytotoxicity Assessment: Cells were seeded into 96 well microtiter plates at a density of 4000 cells/well using a volume of 100 µl in each well. Cells were left overnight at 37°C, 5% CO_2, and 100% relative humidity to allow cell attachment. Cells were preserved in 12.5, 25, 50, 100, and 200 µg/mL of *Moringa oleifera* flower extract.

Melphalan was used as a positive control, and cells were treated with 2.5, 5, 10, 20, and 40 µM. 100 µL aliquots of the diluted extract in a fresh medium were used to treat cells. The treatment medium was aspirated from all wells after 48 hours. Treatment was replaced with a medium containing 0.5 mg/mL MTT and further incubated for 30 minutes at 37°C. MTT was removed, and 200 µL DMSO was added to each well to solubilize the formazan crystals. Absorbance was read at 540 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA).

Data Analysis: The hatching and mortality data obtained from the 5 different concentrations of each fraction and control experiments were used to construct the dose-response curves. From which the corresponding LC_{50} values were derived. The LC_{50} was taken as the concentration required for 50% death of the nauplii.

The statistical analysis was performed on MINITAB version 17 for windows. One-way analysis of variance (ANOVA) followed by Fischer's Least Significant Difference (for means separation) was used to test the effect of concentration and time of exposure of the plant extracts on the hatching of cysts and mortality of nauplii, respectively. Data were quantified using Microsoft Excel. Percentage viable cells were calculated as a percentage of the average untreated control cell population after 48 hours of treatment. Quantifying live and dead cells using the

ImageXpress Micro XLS Widefield Microscope (Molecular Devices) using a 10x Plan Fluor objective and DAPI and Texas Red filter cubes.

Nine image sites were acquired per well, representing roughly 75% of the surface area of the well. Acquired images were analyzed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module.

Acquired data were transferred to an EXCEL spreadsheet, and data was analyzed and processed. Dose-response analysis was performed using the statistical software GraphPad Prism, and IC_{50} values were calculated from the concentration-response data using a mathematical Hill function.

RESULTS: The hatching *of A. salina* cysts incubated with tea extract was highest when compared with other extracts of *M. oleifera* flowers, meaning that this is the least toxic extract. This explains why most traditional herbal medicines are prepared using water as a solvent because it is not or less toxic.

This could also suggest why the cysts showed more resistance to hatching in the acetone and ethanol extracts than in the aqueous extracts. This resistance could be attributed to the permeability barrier provided by the cysts[18].

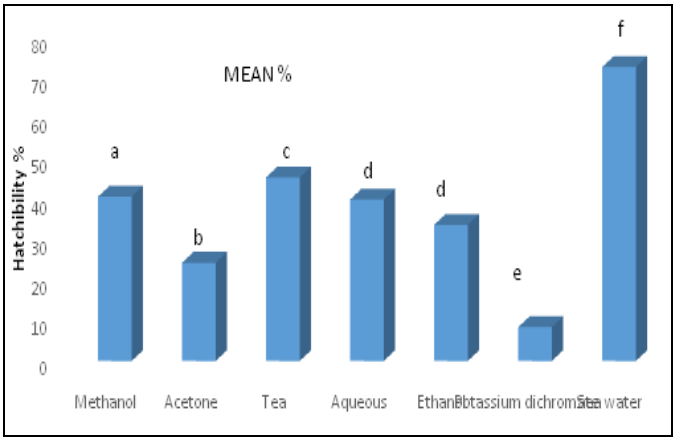

FIG. 1: EFFECT OF DIFFERENT SOLVENT EXTRACTS OF *M. OLEIFERA* FLOWERS AND CONTROLS ON HATCHING (%) OF *A. SALINA* CYSTS. Values are means ± SD, n=3. Bars with different superscript letters are significantly different at $p < 0.05$.

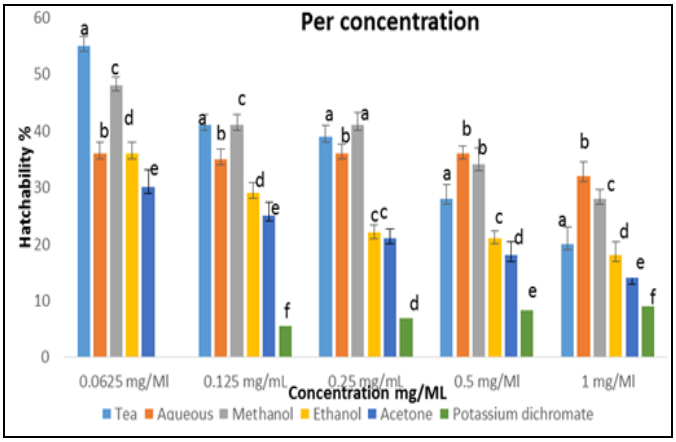

FIG. 2: EFFECT OF CONCENTRATION (MG/ML) ON HATCHING (%) OF *A. SALINA* CYSTS IN *M. OLEIFERA* FLOWERS. Values are Means ± SD of three replicates of the concentrations for each plant extract/control. Set of bars with different letters are significantly different at $p > 0.05$.

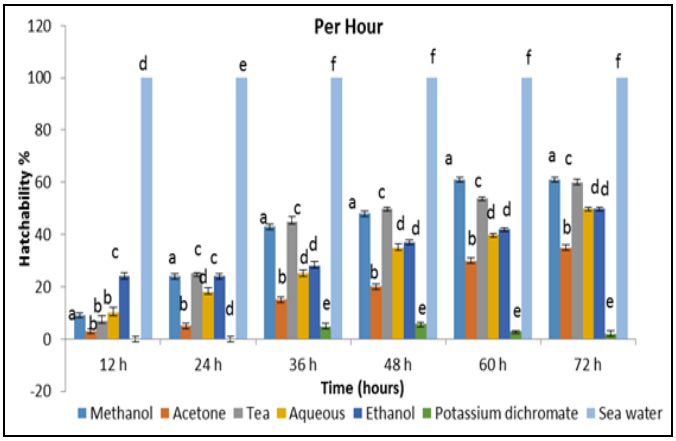

FIG. 3: EFFECT OF EXPOSURE TIME (H) OF *MORINGA OLEIFERA* FLOWER EXTRACTS/ CONTROLS ON HATCHED *A. SALINA* CYSTS. Values are means of three replicates ± SD (at different h). Set of bars with different letters are significantly different at $p < 0.05$.

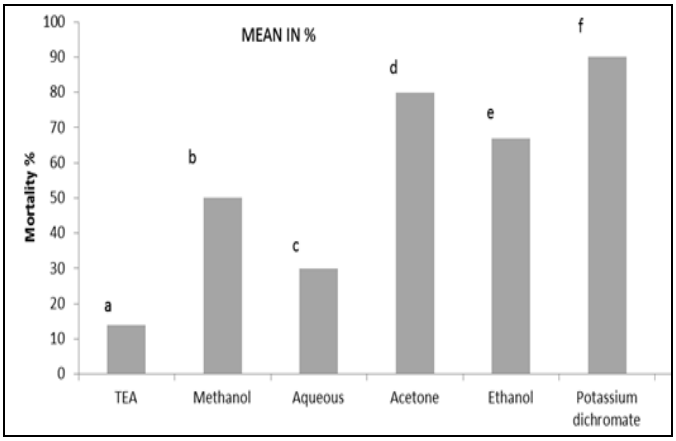

FIG. 4: EFFECT OF DIFFERENT SOLVENT EXTRACTS OF *M. OLEIFERA* FLOWERS ON MORTALITY (%) OF *A. SALINA* NAUPLII. Values are means ± SD, n=3. Bars with different superscript letters are significantly different at $p < 0.05$.

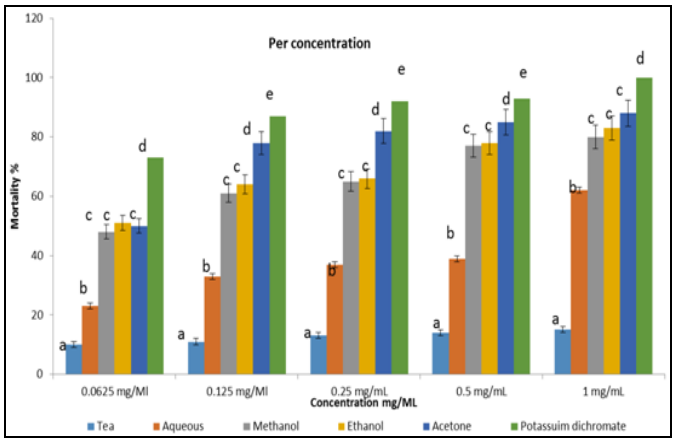

FIG. 5: EFFECT OF CONCENTRATION (MG/ML) ON MORTALITY (%) OF *A.SALINA* NAUPLII IN *M. OLEIFERA* FLOWERS. Values are Means ± SD of three replicates of the concentrations for each plant extract/control. Set of bars with different letters are significantly different at $p > 0.05$.

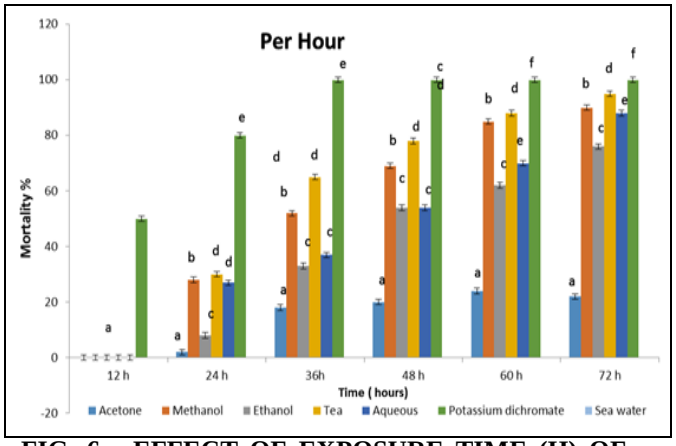

FIG. 6: EFFECT OF EXPOSURE TIME (H) OF *MORINGA OLEIFERA* FLOWER EXTRACTS/ CONTROLS ON MORTALITY (%) OF *A. SALINA* NAUPLII. Values are means of three replicates ± SD (at different h). Set of bars with different letters are significantly different at $p < 0.05$.

TABLE 1: LETHAL DOSE (LC_{50}) CONCENTRATION OF *MORINGA OLEIFERA* FLOWERS EXTRACTS AGAINST *A. SALINA* BRINE SHRIMP

Extracts	LC_{50} (mg/ml	Toxicity status
Methanol	1.74	non-toxic
Ethanol	0.74	medium toxic
Tea	5.17	non-toxic
Aqueous	1.466	non-toxic
Acetone	0.04	Highly toxic

Table 1 presents the half-minimal lethal dose (LC_{50}) concentration of *Moringa oleifera* flowers extracts against brine shrimp nauplii.

Cell-line cytotoxicity Assay:

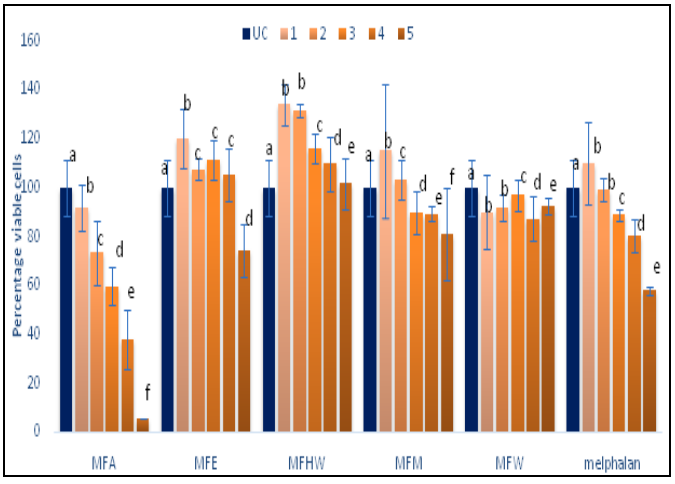

FIG. 7: CYTOTOXICITY OF *MORINGA OLEIFERA* FLOWERS AT VARYING CONCENTRATIONS AGAINST C3A CELLS AFTER 48 HOURS OF EXPOSURE. UC: UNTREATED CONTROL. ERROR BARS INDICATE STANDARD DEVIATION OF QUADRUPLICATE VALUES DONE AS A SINGLE EXPERIMENT

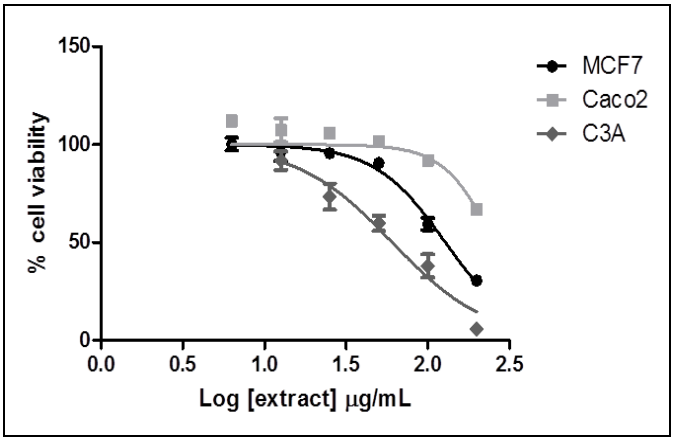

FIG. 8: DOSE-RESPONSE CURVES FOR ACETONE EXTRACT OF *MORINGA OLEIFERA* FLOWERS AGAINST MCF7, $CACO_2$ AND C3A CELL LINES. DATA REPRESENTS THE MEAN ± SD FOR REPLICATE WELLS AT EACH OF THE INDICATED CONCENTRATIONS

TABLE 2: INHIBITION CONCENTRATION (IC50) OF MORINGA OLEIFERA FLOWERS EXTRACTS AGAINSTMCF7, $CACO_2$ AND C3A CELL LINES

Cell lines	IC_{50} (µg/mL)
MCF7	128,2 ± 1,01
Caco2	256,0 ± 1,05
C3A	60,60 ± 1,02

DISCUSSION: The cysts' hatching percentage was also evaluated at different concentrations (**Fig. 2**. The tea extract exhibited maximum hatching at 0.0625 mg /mL (55%), while the methanol extract was next. However, the hatching of *A. salina* cysts decreased with an increase in the concentration of the acetone extract. The observed decrease in hatching success of *A. salina* cysts as the concentrations of extract increased could be due to the relative concentration of toxic metabolites present. In **Fig. 3**, the response of the cysts to different solvent extracts at a particular time of exposure is shown. The cysts exhibited higher hatching in tea extract at all levels of exposure; seawater showed maximum hatching of cysts at all-time intervals reported.

The acetone and ethanol extracts showed little difference from one another at all levels of exposure, while potassium dichromate exhibited the lowest hatching at all levels of exposure time. The highest percentage of hatched cysts was achieved within 72 h of exposure. In all extracts, hatching of the cyst was lower when compared with sea water at all the hours exposed. In all the extracts, the hatching of the cysts increased with exposure time. The lowest hatching was observed at 12 h for all the extracts and controls, while the highest hatching was at 72 h. This implies that *A. salina* was strongly sensitive to the extracts and hatching strongly depended on exposure time. These results are in agreement with[19], which stated that *A. salina* is extremely susceptible to toxins during its early stage of development.

Otang *et al*.,[20] reported that brine shrimp cysts have a high resistance to unfavorable environmental conditions, which makes the hatchability assay less reliable; therefore, the lethality assay is a more appropriate and inclusive test for the preliminary screening of herbal toxicity because it has the advantage of circumventing the toxin tolerance of the cyst stage as the nauplii are very sensitive when exposed to toxins. This assay is based on the ability to kill laboratory-cultured *Artemia* nauplii and is considered a useful tool for preliminary toxicity assessment [21]. One indicator of the toxicity of a substance is LD_{50}, which refers to the amount (*i.e.*, lethal dose or concentration) of a substance that kills 50% of the test organisms. Activities are considered significant if the LD_{50} is less than 30 µg/ml (0.03 mg/ml) [22]. In this bioassay, the mortality of brine shrimp incubated in the test solutions is recorded. Although the brine shrimp assay provides no information on the mechanism of action, it is still a very useful preliminary tool in assessing the toxicity of extracts [3]. The percentage mortality of *A. salina* larvae (nauplii) incubated in different solvent extracts of *Moringa oleifera* flowers and controls are shown in **Fig. 4**. There was high mortality of nauplii incubated in acetone and ethanol extracts, while the least mortality was in the tea extract. In a study by Usman et al., 23 methanolic extracts ofMoringa oleifera leaf gave the highest mortality, while Urmi *et al.,* [24] reported ethyl acetate extract of *Moringa oleifera* fruit exhibited the highest toxic activity with LC_{50} of 0.43 µg/ml. However, potassium dichromate showed the greatest mortality significantly when compared to all the extracts and seawater in this study.

The effect of varying concentrations of MO flower extracts on the mortality of nauplii is shown in Figure 5. The degree of mortality of nauplii was in a concentration-dependent manner. The highest mortality was observed for all the extracts at 1 mg/Ml, while the least mortality of nauplii was at concentrations of 0.0625 mg/mL in the tea and aqueous extracts. The administration of different concentrations of extracts was used to set a baseline between safe and lethal limits to prevent the effect of an acute overdose in future *in-vivo* trials.

The percentage of mortality due to exposure time is captured in **Fig. 6**. The result showed that mortality was time-dependent as the longer the nauplii were exposed to the plant extracts, the higher the mortality **Fig. 6**. This trend was also reported by [25] for all three parts of *Moringa oleifera* (seeds, bark, and leaves). This indicates that the degree of lethality is dependent on concentration and time, as mortality (%) increased as the number of hours increased. Potassium dichromate showed higher significant mortality of the nauplii at 12-60 h ($p<0.05$). All plant extracts screened for toxicity against the brine shrimp gave negative results in terms of mortality at 12 hours, an indication that the plant was not toxic at its early stage. Still, if consistently consumed and accumulated in the body for a number of days, it could result in toxic effects [25].

Over time, the effect of the flower extracts on the nauplii was evaluated to ascertain the maximum sensitivity of nauplii to the toxic metabolites/ chemical compounds present in the different solvent extracts. According to [20] toxic effects of the plant, toxins can be delayed, thus suggesting that a long exposure time is advisable for evaluating the toxicological effect of plant extracts with brine shrimp lethality assay. Clarkson's *et al.*,[26] toxicity criterion **Table 1** for the assessment of plant extracts classifies LC_{50} of 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[16, 27]. This study showed that the acetone extract with LC_{50} of (0.04) was considered very toxic compared to other extracts. The non-toxic result observed in tea, methanol, and aqueous extracts could mean that these extracts are safe for use in other conditions such as diabetes, obesity, inflammation, *etc*.

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (figure 7), is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which are largely impermeable to cell membranes, thus resulting in their accumulation within healthy cells [28]. The MTT cytotoxicity assay is considered a significant advancement in toxicity testing and is the most widely used *in-vitro* cytotoxicity assay. It is rapid, sensitive, versatile, quantitative, and highly reproducible. It is also adaptable to a large-scale screening relevant for most cells. MTT reduction correlates to indices of cellular protein and viable cell number [29].

In the present study, acetone extract of *Moringa oleifera* flowers inhibited the growth and proliferation of liver cancer cells in a concentration-dependent manner. Nejad *et al.,* [13] reported a methanolic extract of *Moringa oleifera* flower to have the same concentration-dependent effect on the Human hepatoma HepG2 cell line. The results indicated that the toxicity and anti-proliferative effect of the extract at some concentrations were similar to the melphalan used as a positive control. On the contrary, this study showed that the acetone extract of Moringa olifera flower decreased the survival of liver cancer cells, whereas [13] reported methanolic extracts to have decreased the survival of liver cancer. These results suggest that the acetone extract of *Moringa oleifera* flowers has potential in managing liver cancer, although *in-vivo* and clinical validations are needed. Acetone extract of *Moringa oleifera* flowers proved cytotoxic in initial screening; thus, IC_{50} determination was performed using the selected cell lines. The lowest IC_{50} value was evident against C3A cells (60.60 µg/mL), then MCF7 cells (128.2 µg/mL), and the highest IC_{50} value was determined against Caco2 cells (256.0 µg/mL). On the contrary, Elsayed et al., 30 reported oil of moringa seeds against Caco2 to obtain higher IC50 of 721.7 compared to the results presented for this study. Fernandes *et al.,* [31] exhibited a very significant level of toxicity against Mcf7 breast cancer cell line for aqueous extract of *Moringa oleifera* flowers.

CONCLUSION: In this study, the toxic effect of the extracts varied widely depending on the solvent used. Acetone extract of *Moringa oleifera* flowers may be a valuable therapeutic tool for use as part of therapy for the treatment and management of liver cancer. These findings add to the growing evidence supporting the promising role of *Moringa oleifera* flowers as an anti-cancer agent.

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CONFLICTS OF INTEREST: The authors declared no conflict of interest.

REFERENCES:

1. Annangi B, Rubio L, Alaraby M, Bach J, Marcos R and Hernández A: Acute and long-term *in vitro* effects of zinc oxide nanoparticles. Archives of Toxicology 2016; 90(9): 2201-13.

2. Liu X, Rodeheaver DP, White JC, Wright AM, Walker LM, Zhang F and Shannon S: A comparison of *in-vitro* cytotoxicity assays in medical device regulatory studies. Regulatory Toxicology and Pharmacology 2018; 1(97): 24-32.
3. Chan W, Shaughnessy AE, van den Berg CP, Garson MJ and Cheney KL: The validity of brine shrimp (*Artemia sp*.) toxicity assays to assess the ecological function of marine natural products. Journal of Chemical Ecology 2021; 47(10): 834-46.
4. Ogbole OO, Ndabai NC, Akinleye TE and Attah AF: Evaluation of peptide-rich root extracts of *Calliandria portoriscensis* (Jacq.) *Benth (Mimosaceae*) for *in-vitro* antimicrobial activity and brine shrimp lethality. BMC Complementary Medicine and Therapies 2020; 20(1): 1-7.
5. Mustafa GI, Abdalla AA, Ahmed KE, Al-magboul AZ and Fadul E: Cytotoxicity Potential of Essential Oils and Extracts of Oleo-Gum Resins from *Boswellia papyrifera* (*Tarak tarak*) Grown in Different Regions of the Sudan. Arabian Journal of Medicinal and Aromatic Plants 2021; 4:7(2): 215-26.
6. Yadav AR, Mohite SK. Toxicological Evaluation of *Psidium guajava* Leaf Extracts using Brine Shrimp (*Artemia salina* L.) Model. Research Journal of Pharmaceutical Dosage Forms and Technology 2020; 1:12(4):258-60.
7. Mothibe ME and Sibanda M: African traditional medicine: South African perspective. Traditional and Complementary Medicine 2019; 4: 1-27.
8. Otunola GA and Afolayan AJ: *In-vitro* antibacterial, antioxidant and toxicity profile of silver nanoparticles green-synthesized and characterized from aqueous extract of a spice blend formulation. Biotechnology & Biotechnological Equipment 2018; 32(3): 724-33.
9. Neergheen-Bhujun VS, Ruhomally ZB, Dunneram Y, Boojhawon R and Sun MC: Consumption patterns, determinants and barriers of the underutilised *Moringa oleifera* Lam in Mauritius. South African Journal of Botany 2020; 129: 91-9.
10. Okorie C, Ajibesin K, Sanyaolu A, Islam A, Lamech S, Mupepi K, Mupepi T, Oseni A, Oyeleke O and Abioye A: A review of the therapeutic benefits of *Moringa oleifera* in controlling high blood pressure (hypertension). Current Traditional Medicine 2019; 5(3): 232-45.
11. Mak D, Sengayi M, Chen WC, de Villiers CB, Singh E and Kramvis A: Liver cancer mortality trends in South Africa: 1999–2015. BMC Cancer 2018; 18(1): 1-9.
12. Miller KD, Goding Sauer A, Ortiz AP, Fedewa SA, Pinheiro PS, Tortolero-Luna G, Martinez-Tyson D and Jemal A, Siegel RL: Cancer statistics for hispanics/latinos, 2018. CA: a Cancer Journal for Clinicians 2018; 68(6): 425-45.
13. Nejad AS, Fotouhi F, Mehrbod P and Alikhani MY: Antitumor immunity enhancement through Newcastle viral oncolysate in mice model: A promising method to treat tumors. Saudi Journal of Biological Sciences 2021; 28(10): 5833-40.
14. Barhoi D, Upadhaya P, Barbhuiya SN, Giri A and Giri S: Aqueous extract of *Moringa oleifera* exhibit potential anti-cancer activity and can be used as a possible cancer therapeutic agent: a study involving *in-vitro* and *in-vivo* approach. Journal of the American College of Nutrition. 2021; 40(1): 70-85.
15. Mumtaz MZ, Kausar F, Hassan M, Javaid S and Malik A: Anti-cancer activities of phenolic compounds from *Moringa oleifera* leaves: *in-vitro* and *in-silico* mechanistic study. Beni-Suef University Journal of Basic and Applied Sciences 2021; 10(1): 1-1.
16. Maposa S, Afolayan AJ and Otunola GA: Toxicity Assessment of *Vachellia karro* (Hayne) Banfi and Galasso Pods using Brine Shrimp Assay. Pharmacognosy Journal 2020; 12(1).
17. Idris OA, Wintola OA and Afolayan AJ: Evaluation of the bioactivities of *Rumex crispus* L. leaves and root extracts using toxicity, antimicrobial and antiparasitic assays. Evidence-Based Complementary and Alternative Medicine 2019; 30: 2019.
18. Unuofin JO, Otunola GA and Afolayan AJ: 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. International Journal Pharmaceutical Science and Research 2017; 8(9): 3719-25.
19. Subhadra S, Kanacharalapalli VR, Ravindran VK, Parre SK, Chintala S and Thatipally R: Comparative toxicity assessment of three Tephrosia species on *Artemia salina* and animal cell lines. Journal of Natural Pharmaceuticals 2011; 2(3): 1.
20. Otang WM, Grierson DS and Ndip N: Assessment of potential toxicity of three South African medicinal plants using the brine shrimp (*Artemia salina*) assay. African Journal of Pharmacy and Pharmacology 2013; 7(20): 1272-9.
21. Suryawanshi V, Yadav A, Mohite S and Magdum CS: Toxicological Assessment using Brine Shrimp Lethality Assay and Antimicrobial activity of *Capparis grandis*. Journal of University of Shanghai for Science and Technology 2020; 22(11): 746-59.
22. Morris-Schaffer K and McCoy MJ: A review of the LD50 and its current role in hazard communication. ACS Chemical Health & Safety 2020; 28(1): 25-33.
23. Usman A, Kawu MU, Shittu M, Saleh A, Jolayemi KO, Ibrahim NB, Oyetunde JS and Okoronkwo MO: Comparative effects of methanol leaf extract of *Moringa oleifera* and ascorbic acid on haematological and histopathological changes induced by subchronic lead toxicity in male Wistar rats. Pharmacological Research-Modern Chinese Medicine 2022; 2: 100031.
24. Urmi KF, Masum NH, Zulfiker AH, Hossain MK and Hamid K: Comparative Anti-microbial activity and brine shrimp lethality bioassay of different parts of the plant *Moringa oleifera* lam. Journal of Applied Pharmaceutical Science 2012; 2(12): 85.
25. Obi OO: Phytochemical, Anti-nutritional and Toxicity Assessment of *Moringa oleifera* Seeds, Stem Bark and Leaves Using Brine Shrimp (*Artemia Salina*) Assay. Jordan Journal of Chemistry (JJC) 2018; 13(3): 171-8.
26. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P and Matsabisa MG: *In-vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J Ethnopharmacol 2004; 92(2-3): 177-91.
27. Adegbaju OD, Otunola GA and Afolayan AJ: Influence of plant maturity on antimicrobial properties and toxicity of Celosia argentea. Journal of Biological Sciences 2019; 19(4): 280-9.
28. Sujitha R and Sharmila R: Phytochemical analysis and *in-vitro* anti-cancer activity of *Tridax procumbens* Linn. World Journal Pharmaceutical Research 2018; 27(7): 867-78.
29. Braissant O, Astasov-Frauenhoffer M, Waltimo T and Bonkat G: A review of methods to determine viability, vitality, and metabolic rates in microbiology. Frontiers in Microbiology 2020; 2726.

30. Elsayed EA, Sharaf-Eldin MA, El-Enshasy HA and Wadaan M: *In-vitro* assessment of anti-cancer properties of *Moringa peregrina* essential seed oil on different cell lines. Pakistan Journal of Zoology 2016; 1: 48(3).

31. Fernandes EE, Pulwale AV, Patil GA and Moghe AS: Probing regenerative potential of *Moringa oleifera* aqueous extracts using *in-vitro* cellular assays. Pharmacognosy Research 2016; 8(4): 231.

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