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Research article

BRINE SHRIMP LETHALITY BIOASSAY OF SOME SELECTED ZIMBABWEAN TRADITIONAL MEDICINAL PLANTS

Tafadzwa Munodawafa[1*], Sylvester Moyo[2], Batsirai Chipurura[3], Lameck Chagonda[1]

[1]School of Pharmacy, College of Health Sciences, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe.
[2]School of Health and Applied Sciences at Polytechnic, Windhoek, Namibia.
[3]Institute of Food, Nutrition and Family Sciences, University of Zimbabwe, P.O. Box MP 167, Mt Pleasant, Harare.

ABSTRACT

Brine shrimp bioassay is a simple method for natural product research. The test provides a useful tool for preliminary assessment of toxicity. Twenty methanol extracts were prepared from various plant parts of twelve medicinal plants that are endangered and commonly used by communities and traditional medical practitioners in 5 districts of Zimbabwe. The extracts were tested for bioactivity using Brine Shrimp (*Artemia salina*) Lethality Test (BSLT). Twelve (60 %) of the extracts had $LC_{50} \geq 1000$ µg/ml and were considered as relatively safe to use. The top three LC_{50} margins were recorded for *Erythrina abyssinica* roots (5 444.4 µg/ml), *Ziziphus mucronata* leaves (4 556.9 µg/ml) and *Dicoma anomala* tuber (3 034.6 µg/ml). Eight (40 %) extracts had $LC_{50} < 1000$ µg/ml and were considered moderately safe to use. None of the tested extracts were more toxic than the reference/ positive control *Nerium oleander* (LC_{50} = 141.7 µg/ml). The mortality rate of brine shrimp was found to be increased with the increased concentrations of sample, and the root extracts showed weaker lethality to the brine shrimps. The front line screening test showed that most of the extracts are relatively safe to use and this is justified by the fact that the plants are already being used by traditional medical practitioners.

Key words: Medicinal plants, toxicity, brine shrimps, Zimbabwe.

Corresponding Author **Tafadzwa Munodawafa** Email: tafadzwamunodawafa@yahoo.co.uk

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (Hassan *et al*., 2009). Bioactive compounds are almost always toxic in high doses (McLaughlin, 1982). Like any therapeutic agent, when overdosed or incorrectly used medicinal plants have the potential to induce adverse effects. The historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public. Despite plant advantages, several studies have established that some species are potentially toxic to

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humans and animals (Hassan *et al*., 2009), (Khoo *et al*., 2001). Some traditional herbs are relatively unpalatable and their digestibility may be limited hence toxic. Plants that are found to be toxic can be used as poisons, pesticides and in cancer treatment.

Microscale bioassay techniques provide a frontline screen and are attractive because of simplicity, rapidity, cost-effectiveness and reasonable reliability, therefore are used extensively for the screening of biological activity of plant materials. The brine-shrimp bioassay, introduced by Meyer, is an *in vivo* lethality test technique for the prediction of cytotoxicity (Mclaughlin *et al*., 1991) and pesticidal activity. This bioassay technique has been adopted in several publications to explain the anticancer property of several plant materials (Bajracharya and Tuladhar 2009), (Sreeshma and Bindu 2014). Although the brine-shrimp bioassay alone is inadequate to evaluate the anticarcinogenic property, however, it is reasonably reliable to screen the bioactivity of natural product extracts, fractions or pure compounds (Asheem *et al*., 2011)

Since most traditional medicinal plants are commonly consumed in Zimbabwe, screening of the plants needs to be carried out especially to determine the bioactivity levels.

In this context the present study was conducted to evaluate for the first time the brine shrimp lethality of 12 medicinal plants which are extensively used in the treatment of various ailments in Zimbabwe.

MATERIALS AND METHODS

Plant Materials

12 plants were selected for this study using ethno-botanical surveys from five districts in Zimbabwe (Bulilima, Chimanimani, Chipinge, Mangwe and Matobo). The collected plants were identified and authenticated by the National Herbarium and Botanic Gardens, Harare, where voucher specimen numbers were recorded i.e. *Annona stenophylla* Engl. & Diels (Annonaceae)(45570), *Clausena anisata* (Willd.) Hook.f.ex. Benth. (Rutaceae)(5982), *Dicoma anomala* Sond. (Asteraceae)(6903), *Erythrina abyssinica* (Lam.) ex. DC. *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don. *Lannea edulis* Sond. (Anacardiaceae)(4918), *Peltophorum africanum* Sond. (Fabaceae)(3078), *Pterocarpus angolensis* DC. (Fabaceae)(1604), *Turrea nilotica* (Meliaceae)(45532), *Vangueria infausta* Burch. *Ximenia caffra* Sond. (Olacaceae)(210) *and Ziziphus mucronata* Willd. (Rhamnaceae)(288).

Preparation of extracts for bioactive tests

Plant roots, leaves and bark were dried, finely ground and stored. Powdered plant material (30 g) was macerated in methanol (200 ml) (Merwe and Eloff 2007), filtered and the filtrate evaporated off under reduced pressure, freeze dried and stored at -20 ^{0}C. Part of the lyophilised extracts were dissolved in DMSO (10 mg/ml), filtered under aseptic conditions and stored for further bioactive use at -20 ^{0}C.

Brine shrimp lethality assay

The brine shrimp (*Artemia salina*) toxicity bioassay test was conducted according to McLaughlin 1982. Briefly artificial seawater was prepared by dissolving sea salt (38.0 g/L of distilled water, pH 7.4). The two compartments of the plastic chamber with several holes on the divider were used for hatching the brine shrimp eggs. The brine shrimp eggs were sprinkled into one compartment which was darkened, while the other compartment was illuminated. After 12 hours of incubation at room temperature (25-28 ºC), nauplii (larvae) were collected by a pipette from the lighted side. Different concentrations of plant extracts were prepared, using brine in triplicates (1000, 500, 100, 50 and 10 µg/ml). Nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml. of the plant extract was added to 4.5 ml of brine solution and maintained at room temperature for 12 h under the light and surviving larvae were counted and recorded. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} (95 % confidence intervals values) were obtained from the line of best-fit plotted as concentration versus percentage lethality. *Nerium oleander* was used as a positive control in the bioassay (Krishnaraju *et al*., 2006).

RESULTS AND DISCUSSION

Several studies have shown that brine shrimp assay has been an excellent method for preliminary investigations of toxicity, to screen medicinal plants popularly used for several purposes and for monitoring in isolation a great variety of biologically active compounds (Apu *et al*., 2012). Most of the plants in this study were found to be either safe or moderately safe to use. These preliminary observations give an early indication of safety when plants are used in traditional medicinal practice.

The use of TMPs is often plagued with issues of safety and or toxicity. 60 % of the plant extracts had $LC_{50} \geq 1000$ µg/ml and were considered as safe to use, whilst 40 % of the extracts had $250 < LC_{50} < 1000$ µg/ml and were considered moderately safe to use. Only the positive control *Nerium*

oleander had LC_{50} value of 141.7 µg/ml a value which was less than 250 µg/ml the lower limit for the moderately safe margins and therefore was considered toxic. Test samples generally showed different mortality rates at different concentrations. The mortality rates of brine shrimps were found to increase with the increase in concentration of the samples.

Extracts of *Annona stenophylla, Ziziphus mucronata, Dicoma anomala, Erythrina abyssinica, Turrea nilotica and Horlarrhena pubescens* ($LC_{50} \geq$ 1000µg/ml)were found to be safe to use and there are no reports of other studies about their toxicity and this justifies the reason why the plants are currently being used to treat various ailments (Breyer-Brandwijk and Watt 1962). In the current study *Peltophorus africanum* (882±106 µg/ml), *Clausena anisata* (533±37.0 µg/ml) and *Lannea edulis* (971±86.0 µg/ml) were found to be moderately safe to use but are reported to be toxic (Bizimenyera *et al*., 2005), (Fafioye, 2005), (Sohni *et al*., 1995) respectively. The slight difference in these results could be because low concentrations of 10mg/ml were used in this study and exposure period was up to 12 hours whilst in the other studies concentrations of more than 10mg/ml were used and exposure periods ranged from 4 to 48 hours. The plants are currently being used and further tests can be done to ascertain their safety. *Pterocarpus angolensis* root extract was shown to be safe to use whilst the bark was shown to be moderately safe. Reports from Merwe and Eloff 2007 have also shown that the bark of *Pterocarpus angolensis* is toxic to brine shrimps. The plant concentration used was much concentrated (0-5 mg/ml) compared to current studies (5-500 µg/ml) and this may have led to the slight variation but suggestions of care in use is highlighted in both cases. In this study more roots were shown to be safer to use compared to leaves. This could possibly be because plants take in different constituents' through the roots and these can accumulate and concentrate in the leaves making them more toxic. The toxicity of a plant varies during different stages of maturity or may be influenced by the environmental conditions (Orech *et al*., 2005).

Ximenia caffra root (LC50 1 590±752 µg/ml) and the leaf extract (1020 ± 52.7 µg/ml) as shown in table 1 showed that the plant is safe to use. In a report by Moshi *et al*., 2003, toxicity on brine shrimps LC50= 11.3 µg/ml was reported and it was concluded that the plant is potentially toxic. The difference in the results could be due to the different environments of where the plants were collected and exposure periods to the brine shrimps.

The leaf and the root extracts of *Vangueria infausta* did show moderate safe levels of lethality. LC_{50} values were close to toxic levels and if Déciga-campos criterion for toxicity had been used the extract would have fallen under the toxic ranges as it stretches up to 500 µg/ml. Caution must therefore be exercised when using the extract. Previous work has shown low levels of toxicity and that the extract of *Vangueria infausta* supported the relatively low antiplasmodial activity and this result is consistent with reports from (Ayuko *et al*., 2010).

There is a real need for reliable, general bioassays which can detect a broad spectrum of pharmacological activities and more sophisticated bioassays could later then be employed (Meyer *et al*., 1982).The toxicity results should be used to create awareness as to which plants are safe for consumption as food and medicines (Orech *et al*., 2005), (Wu, 2014). Further studies should be done *in-vivo* and identification of bioactive constituent to human cell line culture for cytotoxic effect.

Table 1. Brine Shrimp Lethality Test results (LC 50 µg/ml)

No	Plant name	Plant part	LC50 µg/ml	Status
1	Annona stenophylla Muroro (Sh), Ububese (Nd)	leaves	1 190 ± 212	S
		roots	2 300 ± 276	S
2	***Clausena anisate,*** Muvengahonye (Sh)	leaves	533± 37.0	MS
3	***Dicoma anomala*** Chifumuro (Sh),Ukhalimela (Nd)	tuber	3 040 ± 1060	S
4	***Erythrina abyssinica*** Munhimbiti (Sh), Umgqogqogqo (Nd)	roots	5 440 ± 0	S
5	***Holarrhena pubescens*** Muhatsu (Sh), Umhatsu (Nd)	leaves	2 260 ± 484	S
		roots	2 260± 930	S

6	***Lannea edulis*** Mutsombori (Sh),Intakubomvu (Nd)	leaves	971 ± 86	MS
7	***Peltophorum africanum*** Muzeze (Sh), Umsehla (Nd)	leaves	913 ± 7.32	MS
		bark	882 ± 106	MS
		roots	1 060 ± 106	S
8	***Pterocarpus angolensis*** Mubvamaropa (Sh), Umvagazi (Nd)	bark	478 ± 29.7	MS
		roots	1 320 ± 266	S
9	***Turrea nilotica,*** Mukandanyoka (Sh)	roots	701 ± 25.6	MS
10	***Vangueria infausta*** Mutsviru (Sh), Umthofu (Nd)	leaves	338 ± 23.4	MS
		roots	416± 28.3	MS
11	***Ximenia caffra*** Munhengeni (Sh), Umthunduluka (Nd)	leaves	1 020 ± 52.7	S
		roots	1 590 ± 752	S
12	***Ziziphus mucronata*** Muchecheni (Sh), Umphafa (Nd)	leaves	4 560± 1540	S
		roots	1 180 ± 144	S
13	***Nerium oleander*** (positive control)	leaves	142± 68.2	T

Key: S = safe = LC50 ≥ 1000 µg/ml, MS = moderately safe= values between 250 < LC50 < 1000 µg/ml, T =toxic = LC50 ≤ 250µg/ml

CONCLUSION

In this study the Brine Shrimp Lethality test provided a simple platform to be able to quickly screen extracts for potential toxicity. Most of the extracts were shown as being safe to use and this is justified by the fact

that most of the plants are already being used by traditional medical practitioners and some are used as vegetables and herbivores feed on their leaves. Some follow up work is worth for some of the extracts like *Clausena anisata*, *Lannea edulis*, *Peltophorum africanum*, *Turrea nilotica and Vangueria infausta* as they are moderately safe to use and should be used with caution. Some *in vivo* tests at different concentrations could be done to ascertain safety of use of the plants.

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