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CYTOTOXICITY OF SELECTED MEDICINAL PLANTS USED IN MT. FRERE DISTRICT, SOUTH AFRICA

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Abstract

Background: In South African traditional medicine, some are plants known to combat pediatric diseases and are commonly used by traditional healers. The aim was to evaluate cytotoxicity effects of plants.
Materials and methods: The ground plant material was exhaustively extracted using methanol, acetone and water separately for 72 hrs. These organic solvents were removed from filtrates using a rotavapour. Stock solutions were prepared at 40 mg/ml Dimethyl sulfoxide (DMSO) and test solutions were transferred into vials and 10 brine shrimps introduced in each. The number of dead shrimps was counted to ascertain toxicity. Ten *A. salina* nauplii (larva) were transferred into each sample vial and filtered brine solution was added to make 5 ml. The nauplii were counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension was added as food to each vial. Probit analysis was used to determine the concentration at which lethality to brine shrimp represents 50 % (LC_{50}).
Results: All the tested extracts showed that the concentration is directly proportional to death of brine shrimps. Fifty percent lethality (LC_{50}) of the tested crude extract ranged between 4.1 and 4.6 µg/ml with methanol extract of *B. abyssinica* being the lowest and *T. acutiloba* the highest.
Conclusion: This study revealed that 100% of plant crude extracts screened for activity against *Artemisia salina* larvae showed strong cytotoxicity below 10 µg/ml and plant species with LC_{50} values < 1000 µg/ml may not make good paediatric remedies due to their inherent toxicity.

Key words: Medicinal plants, traditional pediatrics, cytotoxicity

Introduction

Considering the vast use of folk medicine by local communities worldwide, the need for the evaluation of intrinsic toxicity of plant extracts arises. This is necessary for safe treatment and ascertaining the effects of acute overdose of these extracts (Nguta et al., 2012). Toxicity of medicinal plants is related to combination of active compounds (terpenes, alkaloids, saponins etc) interacting with other herbs, contaminants, adulterants, or their inherent toxicity (Saad et al., 2006). This toxicity and adverse effects of medicinal plants are generally herb-specific and their pharmaceutical properties are affected by global change/climate change (Saad et al., 2006). Malaria is one of the primary causes of mortality in children under the age of five while some children are treated with malaria medications in biomedical facilities, as the World Health Organization recommends, others obtain home-care or treatment from traditional healers (Foster and Vilendrer, 2009).

Two plants (*Tulbaghia acutiloba* and *Bulbine abyssinica*) were frequently mentioned by local villagers of Mount Frere to treat many pediatric diseases. The leaves of *T. acutiloba* are reported to be used to control colic (umoya in vernacular Xhosa) in children. It is indigenous to the Eastern Cape region of South Africa (Dyson, 1998) and is widely used as a herbal remedy for various ailments and its leaves and bulbs are the most commonly used (Olorunnisola et al., 2011). Both leaves and roots of *B. abyssinica* are believed to have potential healing properties such as the treatment of sickness in children (Plate) and to combat heart diseases in adults. These plants have been screened for their antimicrobial properties but little or no information is available on the screening of the leaves and roots of *T. acutiloba* and *B. abyssinica* respectively.

This study aimed at screening the crude extracts of *T. acutiloba* leaves and *B. abyssinica* roots, using the Brine shrimp lethality model. These plants are perceived to have healing properties and considered to be harmless by folks in the Mount Frere District of the Eastern Cape.

Materials and Methods

Study area

The ethnobotanical information was gathered around villages of Mount Frere District of the Eastern Cape Province of South Africa. The study area falls within the following co-ordinates: Mount Frere (30°55′S 28°59′E).

Plant Materials

The plant samples were collected in August 2011 from Mt Frere district in South Africa (30°55′S 28°59′E) based on interviews of ethnopharmacological uses within local communities and traditional health practitioners. The collected species were *T. acutiloba* and *B. abyssinica.* Information gathered included part of the plant used and the method of preparation of the herbal anti-malarial remedies. The plants were identified by taxonomists at the Walter Sisulu University Herbarium where voucher specimens (Mnengi 2011/1 and Mnengi 2011/2) were deposited. Species nomenclature followed the international code of botanical nomenclature. The plant parts were chopped into small pieces; air dried at room temperature (25^{o}C) under shade and pulverized using a laboratory mill (Christy & Nor-ris Ltd., England). The ground plant material was exhaustively extracted

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using methanol, acetone and water separately for 72 hrs. The organic solvents were removed from filtrates using a rotavapour and extracts dried in a fume cupboard. The aqueous extract was filtered using Whatman No 1 filter paper and thereafter, freeze-dried. The resultant dried filtrate was kept in an air-tight container until when needed.

Preparation of test concentrations

Stock solutions were prepared from each plant at 40 mg/ml Dimethyl sulfoxide (DMSO). Test solutions at appropriate amounts (4 µl, 40 µl, and 400 µl for 10 µg/ml, 100 µg/ml, and 1000 µg/ml respectively) were transferred into vials and 10 brine shrimps introduced in each (3 vials for each dose and 1 negative control). The vials were left under illumination for 48 hours. Thereafter, the number of dead shrimps was counted to ascertain toxicity.

Bioassay of *A. Salina*

For toxicity tests, ten *A. salina* nauplii (larva) were transferred into each sample vial using 230 mm disposable glass Pasteur pipettes (Ref. D812) (Poulten & Graf Ltd, Barking, UK) and filtered brine solution was added to make 5 ml. The nauplii were counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension (Red star) (3 mg in 5 ml artificial sea water) was added as food to each vial. All the vials were maintained under illumination. The surviving nauplii were counted with the aid of a 3x magnifying glass, after 48 hours, and the percentage of deaths at the three dose levels and control were determined. In cases where control deaths occurred, the data was corrected using Abbott's formula (Pelka et al., 2000) as follows: % deaths = [(Test-control)/control x 100].

LC_{50} Determinations

Probit analysis by Finney (1971) was used to determine the concentration at which lethality to brine shrimp represents 50 % (LC_{50}). LC_{50} (effective dose needed to kill 50% of shrimp larvae) values less than 100 ppm (or 100 µg/mL) were considered significant as described by Peter and Yu 2010.

Results and discussion

All the tested extracts showed that the concentration was directly correlated to the death of brine shrimps. The death of brine shrimp nauplii initially showed increase with concentration and reached its optimal capacity which means the number of brine shrimp dying begins to decrease at high concentrations (Fig 1 & 5). This implies that methanol and water extracts of *B. abyssinica* and *T. acutiloba* can be used at high concentrations. However, Brine shrimp lethality gradually increases with concentration in water and acetone extracts of *B. abyssinica* and in methanol extract of *T. acutiloba*, which demonstrates that higher concentrations of these extracts are harmful (Figure 2, 3, and 4). Fifty percent lethality (LC_{50}) of the tested crude extracts ranged between 4.1 and 4.6µg/ml with *B. abyssinica* methanol extract being the lowest and *T. acutiloba* the highest. This indicates that 4.1µg/ml of methanol extract of *B. abyssinica* was required to kill fifty percentage of the brine shrimp nauplii (*Artemisia salina* L.). According to Nguta et al., 2012; Peteros and Yu, 2010; the agent is regarded as cytotoxic if its probit value is less than 10 000 µg/ml. The findings of this study showed that 100% of plant crude extracts screened against *Artemisia salina* larvae showed strong cytotoxicity because they demonstrated activity below 10µg/ml. The variation in BSLA results may be due to the difference in the amounts and kinds of cytotoxic substances present in the crude extracts. Generally, these results correspond with the findings by Nguta et al., 2012; Peteros and Yu (2010), which revealed that medicinal plants' toxicity are concentration dependent (Figures 1-5).

The roots of *B. abyssinica* and *T. acutiloba* have been screened for their safety and effectiveness but no published information is available on screening of leaves which are used for various diseases especially in infants. Olorunnisola et al, (2011) screened essential oils of rhizome of *T. acutiloba* and their findings revealed that the oil of this plant had cytotoxic properties which were also concentration dependent. Though herbal remedies play a major role as alternative medication for various ailments there is still lack of appropriate dosage and safety in prolonged usage. It has been mentioned by Saad et al, (2006) that though medicinal plants can have pharmaceutical activity, they are also responsible for unexpected toxicity. It is, therefore, of paramount importance to evaluate cytotoxic and toxicological properties of plants used for traditional pediatrics because the immune system of infants is still immature and under-developed.

The results of this study revealed that traditional healers in Mt Frere prescribe the oral administration of infusions and decoctions to infants with no upper limit of the dosage at a given time. Moreover the medication is administered until the ailment is perceived to be treated. The use of herbal estimates and prolonged intake of the medication every time the patient feels pain could lead to cell damages. There is folk belief that anything natural is harmless; confusing plant nomenclature, identification and lack of quality control are important concerns in the application of herbal remedies to humans, especially children (Saad et al., 2006). Olajuyigbe and Afolayan, 2012 have cited that naming of medicinal plants differs with community hence; *T. acutiloba* and *T. violacea* are one and the same plant.

The brine shrimp nauplii was used as cells in the experiment and as the concentration increased the death of organism also increased in all extracts. Lethality test by brine shrimp bioassay is not only used to ascertain dangerous properties of the plants to human cells but it also help in the development of antitumor and pesticidal agents (Peter and Yu, 2010).According to Manilal et al, (2009) cytotoxic properties of plant materials might be due to the presence of antitumor compounds. Extracts from natural product sources have served (and are still serving) as a valuable source in many drug discovery programs. Our findings suggest that future applications from *T. acutiloba* and *B. abyssinica* crude extracts could serve as potent pesticides and antimicrobial agents. Considering the global climate change, effects on our livelihoods and rapid increase in disease spreading as well as use of herbal remedies the evaluation of medicinal plants safety and quality assurance should be an immediate response by researchers. Results from this study indicate that while plant species with LC_{50} values < 1000 µg/ml may not make good paediatric remedies due to their inherent toxicity, this study calls for further work aimed at isolating the cytotoxic compounds responsible for the observed activity. These compounds could serve as novel scaffolds in the search for new drugs against various diseases that affect infants. Further investigations into the *in vivo* assays and toxicological profile of these crude extracts are recommended.

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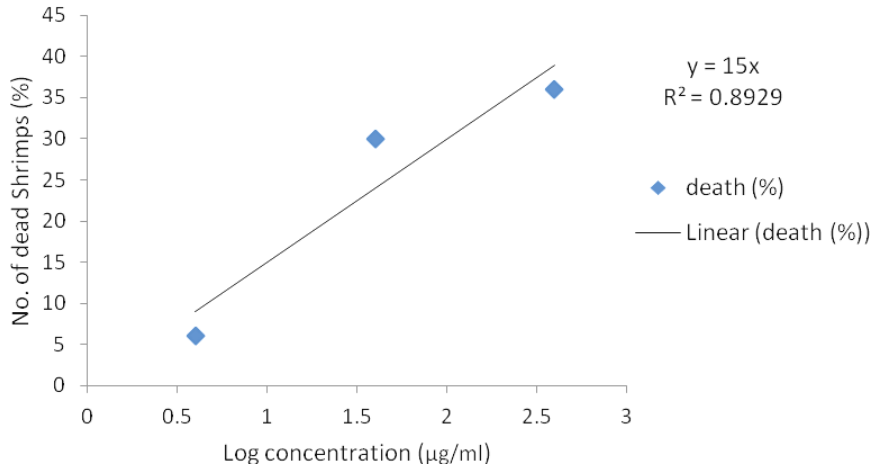

Figure 1: Brine Shrimp Lethality on methanol extract of *B. abyssinica*

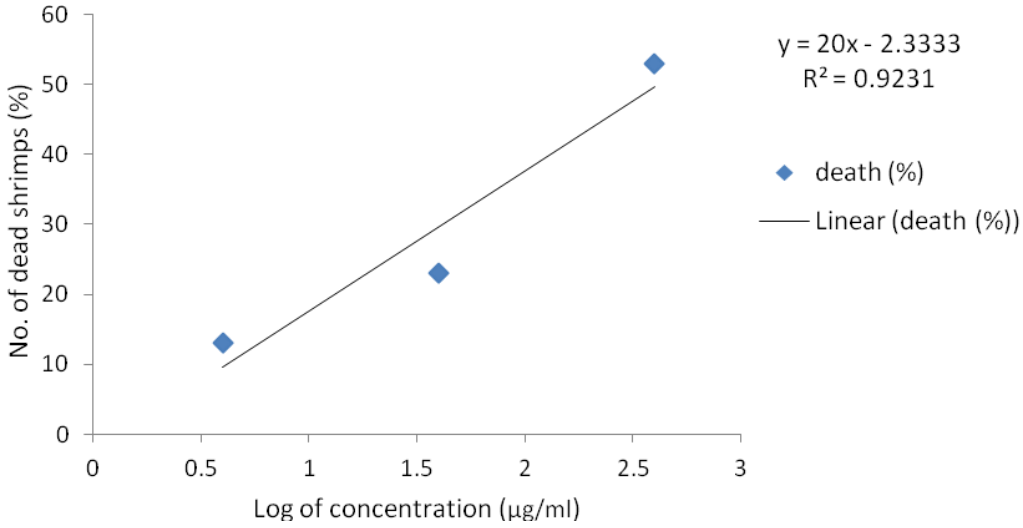

Figure 2: Brine Shrimp Lethality on water extract of *B. abyssinica*

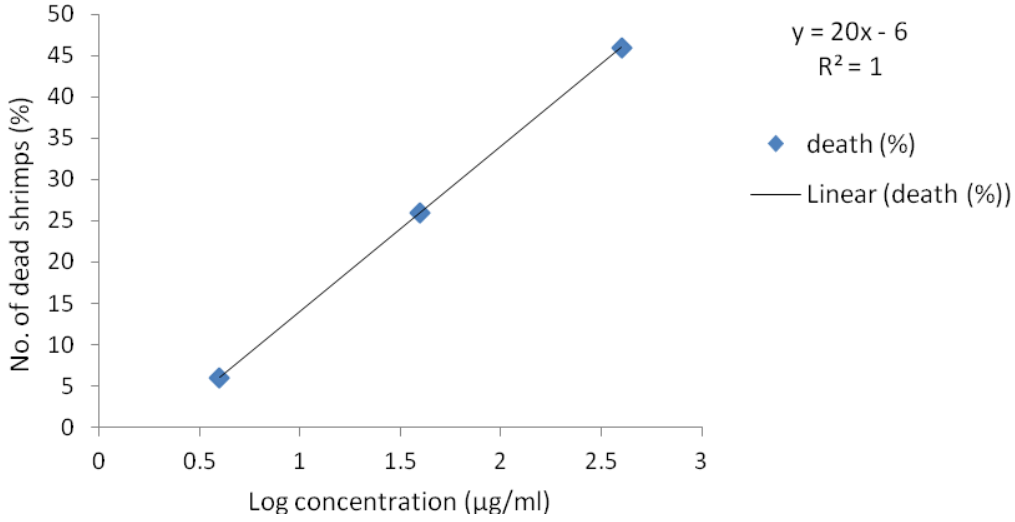

Figure 3: Brine Shrimp Lethality on acetone extract of *B. abyssinica*

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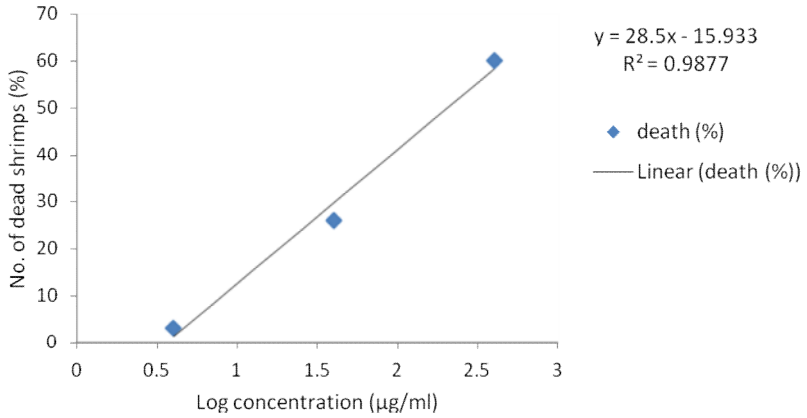

Figure 4: Brine Shrimp Lethality on methanol extract of *T. acutiloba*

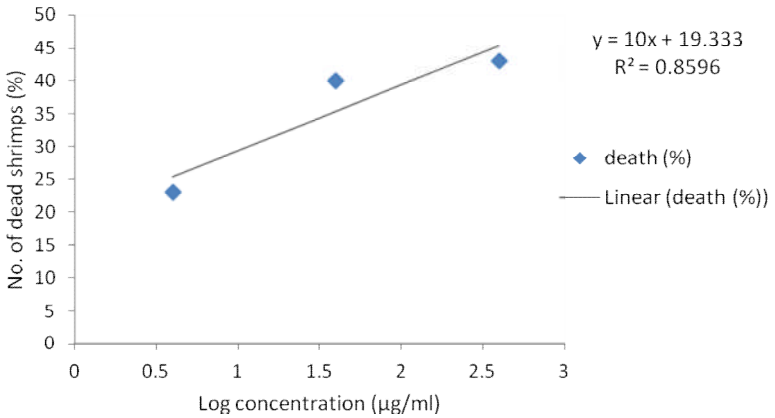

Figure 5: Brine Shrimp Lethality on water extract of *T. acutiloba*

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References

1. Carballo J.L., Hernandez-Inda Z.L., Perez, P., and Garcia-Gravalos, M.D. (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotech. **2**:17.
2. Dyson, A. (1998). Discovering Indigenous Healing plants of the herb and fragrance Gardens at Kirstenbosch (National Botanical Garden).
3. Foster, D., and Vilendrer, S. (2009): Two treatments one disease: childhood malaria management in Tanga, Tanzania. Malaria Jour. **8**:240.
4. Manilal, A., Sujith, S., Kiran, G.S., Selvin, J., and Shakir, C. (2009). Cytotoxicity potentials of Red algae, *Laurencia brandenii* collected from the Indian coast. Global Journal of Pharmacol. **3**:90-94.
5. Finney, D.J. (1971). *"Probit Analysis"* (3th Ed.). Cambridge University Press: Cambridge, pp.76-80.
6. Meyer, B.N., Ferrigni, N.R., Putman, J.E., Jacobson, L.B., Nichols, D.E., and McLaughlin, J.L.(1982). Brine shrimp: a convenient general bioassay for active plant constituents. Plant Med.**45**: 31-4.
7. Nguta, J.M., Mbaria, J.M., Dakuya, D.W., Gathumbi, P.K., Kubasa, J.D., and Kiama, S.G. (2012). Evaluation of acute toxicity of crude plant extract from Kenyan Biodiversity using Brine Shrimp, *Artemisia salina* L. (Artemiidae). The open conference Proceedings Journ **3**:30-34.
8. Olajuyigbe, O.O., and Afolayan, O. (2012). Ethno botanical survey on medicinal plants used in treatment of gastrointestinal disorders in the Eastern Cape Province, South Africa. Journal of Medicinal Plant Res. **6**(18):3415-3424.
9. Olorunnisola, O,S., Bradley, G., and Afolayan, A.J. (2011). Chemical composition, antioxidant activity and toxicity evaluation of essential oil of *Tulbaghia violacea* Harv. Journal of Medicinal Plant Res **6:**2340-2347.
10. Peteros, N.P., and UY, M.M. (2010). Antioxidant and cytotoxicity activities and phytochemical of four Philippine medicinal plants. Journal of Medicinal Plants Res. **4**: 407-414.
11. Pelka, M., Danzl, C., Distler, W., and Petschelt, A. (2000). A new screening test for toxicity testing of dental materials. J. Dent. 28, 341-345.
12. Saad, B., Azaizeh, H., Abu-Hijleh, G., and Said, O. (2006): Safety of traditional Arab Herbal Medicines. eCAM **3**:433-439.