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Brine shrimp lethality and phytochemical determination of aqueous extracts of *Senna singueana, Musa paradisiaca, and Ziziphus mucronata* in Malawi

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Abstract: Traditional medicine is still practiced in different parts of the world; with traditional healers as powerful and important members of the society. *Senna singueana, Musa paradisiaca, and Ziziphus mucronata* plants are commonly used by traditional healers to treat different ailments in Malawi. There is need to conduct a scientific lethality evaluation of these plants to assess their fitness for human consumption as traditional medicine. The aqueous extraction of leaves and roots of *Senna singuenna*, *Musa paradisiaca*, and *Ziziphus mucronata* mimicking similar environment as provided by traditional healers, afforded dry extracts. Brine shrimp toxicity testing was done following a modified procedure to determine in-vitro cytotoxicity for the herbal extracts. The findings suggest that the leaves and roots tested exhibited concentration dependent toxicity against brine shrimps with the exception of *Ziziphus mucronata* roots which showed some toxicity to brine shrimps after 48 hours of incubation. Brine shrimp results suggest that the plant extracts were virtually non-toxic on the shrimps save *Senna singueana* leaves, *Ziziphus mucronata* roots, which exhibited low toxicity on brine shrimps after 48 hours of incubation. The observed toxic activity for *Ziziphus mucronata* roots may be due to the presence of well documented cytotoxic compounds such as Swertish and Apigenine glucoside.

INTRODUCTION

Traditional medicine is widespread in different parts of the world, (Sofowora, 1996; Adam and Abdull Rasad, 2015; Majali, et al., 2015). Traditional healers are said to be the most accessible care providers in many parts of Africa, (King & Homsy, 1997). Traditional medicine also known as complementary medicine is considered either the mainstay of health care delivery or its complement in most parts of the world (WHO, 2013). It is the oldest form of healthcare used by humans. An herb is any plant with leaves, seeds, or flowers valued for its medicinal, aromatic, or savoury qualities. Historically, early humans depended on herbal medicine as a source of primary health care, and distinguished toxic from non-toxic plants through trial and error approach (reviewed in (Kunle, Egharevba, & Ahmadu, 2012)).
In Malawi, medicinal plants and products of animal origin are considered to have intrinsic powers, and thus thought to possess inherent curing properties. Medical herbalism is widely practiced in Malawi such that some people in rural areas of Malawi have knowledge of a wide variety of herbs that can be used to treat common ailments, (Morris, 2011). This study investigates the toxicity of three plants (*Senna Singueana*; *Musa Paradisiaca;* and *Ziziphus Mucronata* commonly used by traditional healers in Malawi.

Senna singueana (local name: Mpatsachokolo) is a shrub belonging to Fabaceae family. Commonly known as winter cassia, sticky pod or scrambled egg. It has many medicinal uses throughout Africa and different plant parts are used, (Abate, 1989). Both leaves and bark of *Senna Singueana* are used by traditional healers in Malawi and other parts of Africa to treat different ailments. Information

sourced from the traditional healers showed that the plant is used for skin cancer, fresh bark chewed for stomach spasm. The leaves are used to treat fever, Malaria, Leprosy, sexual transmitted infections such as Syphilis, Conjunctivitis, abdominal cramps and many more, (Mebrahtom Gebrelibanos, 2012). The fruit pulp soaked in water and cooked with a staple food is considered lactogenic, (Kareru P. G., Gachanja A. N., Keriko J M., 2008).

Musa Paradisiaca (local name: Nthochi) is a monoherbacious plant belonging to Musaceae family; commonly known as plantain. Banana is the common name for herbaceous plants of the genus *Musa* and for the fruit they produce. Traditionally the plant *Musa paradisiaca* was used to treat abscess, alopecia (female), diabetes, diarrhoea, snake bite, hypertension, shingles, smallpox, syphilis, among others, (Sanjeev Kumar, Chanchal Kumar Mishra, Anil Ahuja, 2012). Studies showed that banana powder treatment strengthens mucosal resistance against ulcerogens as well as promotes healing by inducing cellular proliferation, (Shodehinde & Oboh, 2013).

Ziziphus mucronata (local name: Kankhambe), belongs to Rhamnaceae family and it is known as Cape Thorn or Buffalo Thorn in English. The fruits and leaves of *Ziziphus mucronata* can be applied as powder on boils. Root extracts are drunk as treatment for abdominal pains and infertility in women whereas the root powder is applied on wounds, (Olajuyigbe & Afolayan, 2011; Olivier & van Wyk, 2013). The plant is used to heal illnesses like dysentery, swellings, chest pains, toothache, eye diseases, swollen and open wounds, (Kwape & Chaturvedi, 2012).

A number of laboratory based studies have already demonstrated that medicinal plants used in folkloric treatment of infectious diseases possess potent antimicrobial activity, (Perianayagam, Sharma, Pillai, Pandurangan, & Kesavan, 2012). Brine shrimp toxicity assay is an excellent method for preliminary evaluation of toxicity of medicinal plant products and other chemicals, and for monitoring the isolation of a variety of biologically active compounds. The technique is easy, cheap, and utilizes small amount of test material, (Bastos et al., 2009). Previous studies reported a positive correlation between the lethality to brine shrimp and the corresponding oral lethal dose in mice, (LAGARTOPARRA, 2001). During the time of this study, there are few studies in Malawi that have been conducted to assess the efficacy and safety of medicinal plants. The aim of this study was to analyse the phytochemical components present in the three plants; and evaluate their toxicity properties using brine shrimp lethality assay.

METHODS AND MATERIALS

Plants collection and Extraction

The roots and leaves of *Senna singueana* were collected and processed; whereas roots of *Musa paradisiaca*, and *Ziziphus mucronata* were collected and processed as well. All plants were collected on Zomba plateau, and Blantyre in Southern Malawi. Freshly collected plant materials were thoroughly cleaned using water and air-dried at room temperature. Dried plant roots or leaves were pulverised in a blender. The coarsely ground plant materials were macerated in distilled water for 72 hours at room temperature and collected in a beaker. The materials were filtered using Whatmann filter paper No. 2. The filtrate was left to dry through evaporation in an open space for a period between 72 and 144 hours. The evaporation process was enhanced by the use of an electrical fan which was left to blow over the plant extracts. The dry crude extracts were scrapped from the beakers and the powder was immediately used for toxicity experiment according to the study protocol. Water, ethanol, acetone and methanol are some of the commonly used solvents for plant extractions, (Sahu, Vermaand, & Harris, 2014).

In vitro toxicity assay of test extracts

The presence or absence of toxicity in herbal extracts was predicted by performing Brine shrimp lethality test, (Meyer et al., 1982). Brine shrimp (*Artemia salina*) eggs were obtained from Physiology Department, University of KwaZulu-Natal in Durban, South Africa. The eggs were hatched into *nauplii* following a modified procedure as described below; Artificial sea water was made by dissolving 38g of Sodium chloride (NaCl) into 1 litre of water and adjusting the pH to 8.3 using Sodium bicarbonate ($NaHCO_3$). The improvised hatching tank was made from used a 2 litre plastic Orange squash bottle. A perforated partition (single hole) divided the chamber into two. One half of the chamber was painted black and was wrapped with a black plastic paper while the other half was left clear. 100mg of dry shrimp eggs were incubated in 100ml of brine solution, placed on the darkened side and covered with a perforated lid. The hatching chamber was then carefully put in the water bath at a tilting angle of about 15^o. The clear half was constantly subjected to bright light from a 40W tungsten bulb placed approximately 15cm from the water bath. The hatching chamber was kept in a water bath, and temperatures were monitored that ranged between 29^oC to 30^oC with good aeration.

The *nauplii* were left to hatch for 48 hours. Live and active *nauplii* freely passed through the perforated hole from the dark side of the hatching chamber to the bright side.

Powdered test extracts were dissolved in 1% dimethyl sulfoxide (DMSO) (serially diluted with 3.8% brine solution) to yield graded concentrations of 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml and 15.6µg/ml. Cytotoxicity of each dose of test extract towards *nauplii* was assessed in 6 replicates. Using a Pasteur pipette 10 larvae were transferred to test tubes and exposed to graded doses of test extracts for 24 hours and 48 hours. The control set up contained 10 *nauplii* exposed to 1 % DMSO without plant extracts. The test tubes containing the *nauplii* were maintained under illumination by a 40W bulb during incubation. The number of *nauplii* that died (non-motile) after 24 hours were counted and the survival rate (active *nauplii*) was determined.

Phytochemical analysis:

Flavonoids

In order to determine the presence or absence of flavonoids from the extracts, 0.5 ml of 0.2% aluminium chloride (AlCl3) was added to 0.5 ml of extract solution and the mixture allowed to stand at room temperature for 60 minutes. The absorbance was read at 420 nm in order to determine the concentration of flavonoids in the reaction mixture. Thin Layer Chromatography (TLC) Kiesel gel 60 F254 (0.2mm, Merck, Silica Gel plate, silica gel Merk) was used, whereby one gram of powdered test samples was extracted with 10ml methanol on water bath (60^oC for 5 min). The filtrate was condensed by evaporation. A mixture of Water and Ethyl acetate (EtOAc) (10:1 ml) was prepared and mixed thoroughly. The EtOAc phase was retained and used for chromatography. Flavonoid spots were separated using Chloroform and Methanol (19:1) solvent mixture. The colour and homologous restriction factor (hRf) values of the separated spots were recorded under ultraviolet (UV254nm) light.

Alkaloids

A drop of extract was spotted on a small piece of percolated TLC plate. The plate was sprayed with Dragendorff's reagent. The extract tested positive with an orange spot. Phytochemical analysis done on *Ziziphus*
mucronata involved alkaloids as a major class of compounds. They are primarily cyclopeptide alkaloids, collectively known as mucronines and abyssenines, together with polar isoquinolinealkaloid (Nmethylcoclaurine).

Saponins

Two grams of powdered test samples were extracted with 10 ml 70% ethanol (EtOH) by refluxing for 10 minutes. Filtrate was condensed, enriched with saturated butanol (n-BuOH), and mixed thoroughly. Butanol was retained, condensed and used for chromatography. Saponins were separated using Chloroform, Glacial acetic acid, Methanol and water (64:34:12:8) solvent mixture. The chromatogram was exposed to the iodine vapours. The colour (yellow) and hRf values of the spots were recorded by exposing chromatogram to the iodine vapours.

RESULTS AND DISCUSSION

In-vitro toxicity assay

After incubating the shrimp eggs in brine solution for 24 hours, they could not hatch; however, they started hatching after 32 hours. By the end of 48 hours, nearly 500 nauplii hatched. It was suggested that the eggs could not hatch properly after 24 hours probably because of electricity blackouts at night which caused the temperatures to drop to 23^oC as recorded the following morning. The toxicity testing done on serially diluted extracts yielded the following results indicated in figure 1 below after 24 and 48 hours. The toxicity testing was repeated once, and the presented results are the average of the two repeated experiments.

The findings suggest that *Senna singuena* leaves and roots; and *Musa Paradisiaca* roots did not show significant toxicity after 24 hours. *Senna singuena* leaves showed some mortality (5 nauplii died) at the concentration of 62.5 µg/1ml after 48 hours. When the concentration was diluted to 250 µg/1ml, 2 nauplii died after 24 hours and a total of 5 nauplii died after 48 hours. Both concentrations gave a 50% nauplii mortality rate after 48 hours. The roots of *Senna singuena* showed nearly 20% nauplii mortality (2 nauplii died) at 125 µg/1ml and 31.25 µg/1ml after 24 and 48 hours. The same mortality rate was observed at 250 µg/1ml and 62.5 µg/1ml after 48 hours. A maximum of 4 nauplii died with *Musa Paradisiaca* roots at the concentration of 31.25 µg/1ml. *Ziziphus mucronata* roots showed great mortality for plant extract concentrations with the highest mortality (8 nauplii died) observed at the concentration of 15.6 µg/1ml after 48 hours. The findings could not establish if the nauplii mortality observed in some plant extracts was due to plant toxicity or the hatched nauplii were weak due to low temperatures caused by intermittent power supply.

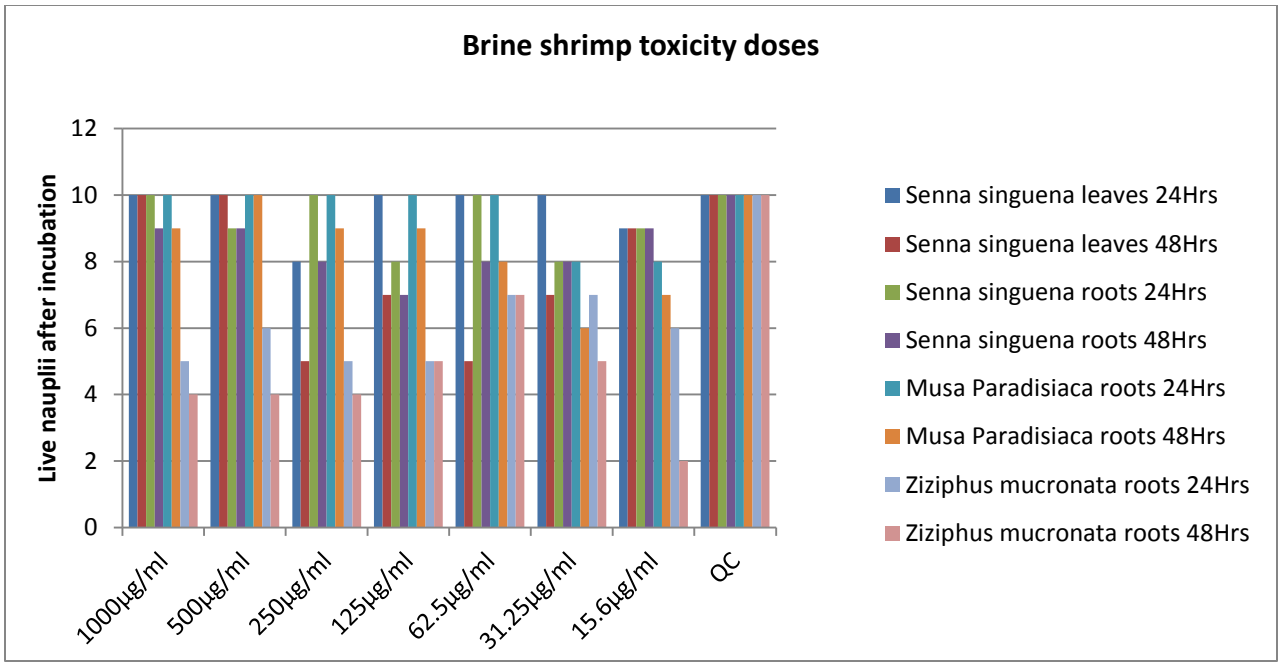


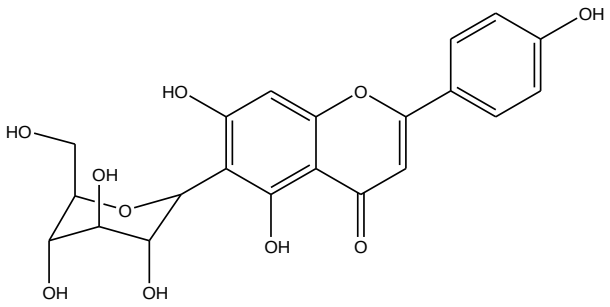
Figure 1 shows the number of live and active nauplii which were subjected to different serial dilutions of plant extracts

Phytochemistry

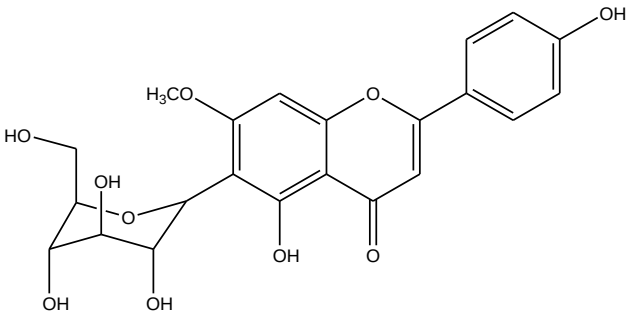
The common flavonoids isolated from Ziziphus species are Swertish and Apigenine glucoside (Cheng et al., 2000). Chem Draw Ultra 8.0 software was used to produce the structures.

For the tested Alkaloids, Mucronines AH was isolated from the roots and was characterized by the presence of a styrylamine group in a 15-membered cyclopeptide alkaloid (13 membered in mucronine D) (Barboni, Gariboldi, Torregiani, & Verotta, 1994; Tripathi et al., 2001). Mucronine D was reported as the most abundant alkaloid in the roots of the three plants, together with two other structurally related alkaloids.

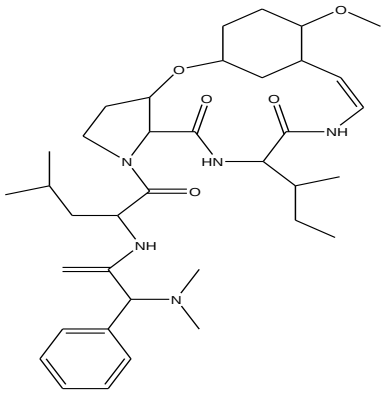
The TLC method managed the presence of Saponins. The oleanane skeleton was the most common skeleton present in *Senna singueana* and the dammarene saponin type were reported to be most common in *Ziziphus mucronata*.



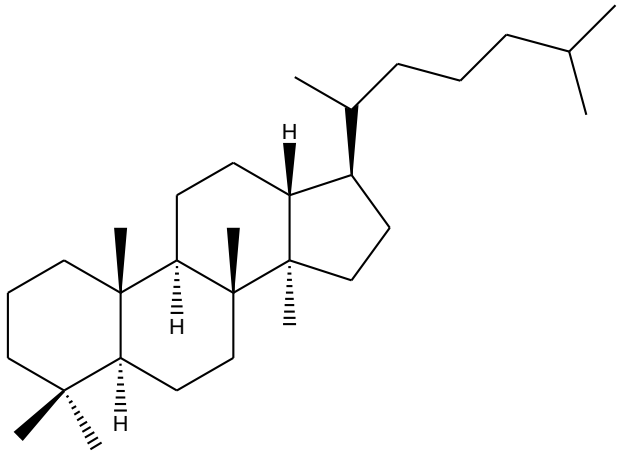
Apigenine glucoside



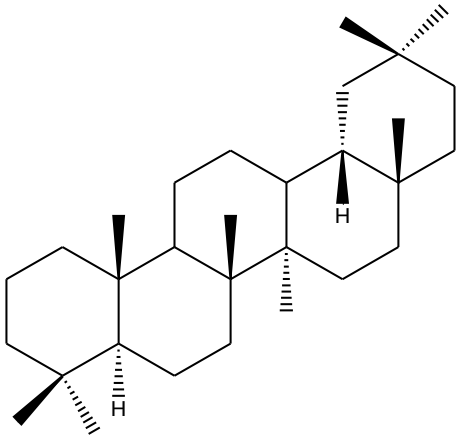
Swertish



Mucronine D



Dammarene



Oleananes

Traditional healers form integral part of the society in Central and Eastern parts of Africa, with villages having at least one traditional healer who is easily accessible, (Brouwer, Boeree, Kager, Varkevisser, & Harries, 1998). Traditional herbal formulations are usually prepared from water or alcohol solvents, this study used water for extraction, as opposed to 80% methanol which was used in Gebrelibanos's study, (M Gebrelibanos, 2012). With the exception of *Senna singuena* leaves extracts, and *Ziziphus mucronata* roots, of different concentrations, all the other extracts of stem bark were not active against the tested species of microorganisms. The test controls for all extracts extracts had live nauplii, which indicated that the brine solution had no lethal elements. The best activity of *Ziziphus mucronata* roots were observed at 15.6µg/ml where 8 out of 10 nauplii died. The observed low toxicity for *Ziziphus mucronata* roots after 48 hours of incubation suggest that the roots extracts could be more toxic. The aqueous extracts which are commonly used by traditional healers in Malawi for the three tested plants showed less lethal activity against the tested shrimp nauplii. There is lack of published evidence to support the use of the aforementioned herbal aqueous extracts by traditional healers in Malawi. Biological and pharmacological activities of such extracts require vigorous investigations to ascertain the presence of absence of active ingredients that treat different ailments. Despite the usage of the three tested herbal extracts by herbalists to treat different ailments in Malawi, this study may speculate that the aqueous extracts do not pose any threat to humans.

CONCLUSION

The aqueous extracts of the leaves and roots of *Senna singueana*, and *Ziziphus mucronata* exhibited varied levels of lethality activity on brine shrimps. The findings support the traditional medicinal use of the three tested plants without any significant toxicity to humans. However, the observed toxic activity for

Ziziphus mucronata roots may be due to the presence of well documented cytotoxic compounds such as Swertish and Apigenine glucoside. Further experiments to isolate active compounds in *Ziziphus mucronata* would be recommended to ascertain its toxic effects.

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