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Brine Shrimp Lethality Assay of Selected Medicinal Plants in Tanzania

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Authors' contributions

This work was carried out in collaboration between both authors. Author BAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MK managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this study was to determine the level of cytotoxicity activity of *Mentha piperita* (L), *Tragia involucrata* (L) and *Urtica massaica (*Mildbr) used as medicinal plants in Tanzania.
Study Design: Experimental study was carried out by using Brine Shrimp lethality bioassay.
Place and Duration of Study: This study was conducted at the University of Dodoma, Tanzania, between July and October 2017.
Methodology: The plant materials were subjected to extraction using chloroform, ethyl acetate, and methanol for 48 hours. The potential cytotoxicity effect of the extracts was determined using brine shrimp toxicity assay.
Results: The leaf extracts of *M. piperita* ethyl acetate leaf and *M. piperita* methanol exhibited strong cytotoxicity effect against brine shrimp larvae with LC_{50} values of 32.65 and 41.58 µg/mL respectively while leaf extracts of *M. piperita* chloroform and *T. involucrate* ethyl acetate had weak cytotoxicity activity with LC_{50} values above 100 µg/mL.

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Conclusion: Majority of the extracts confirmed to be toxic and thus possess anticancer activity. Further study on the isolation of bioactive compounds which are responsible for the activity is recommended.

Keywords: Medicinal plants; brine shrimp; cytotoxicity; LC_{50} values.

1. INTRODUCTION

The use of medicinal plants has received significant attention in the world as an alternative to conventional drugs and the demand for these remedies has recently increased [1]. According to World Health Organization, 80% of the populations in developing countries rely on traditional medicines for their healthcare [2]. In Tanzania, about 60% of the population fulfills their medical needs from medicinal plants [3]. Some of the plants commonly used for the treatment of human diseases include *Mentha piperita, Tragia involucrata* (L) and *Urtica massaica.*

The *M. piperita* (Lamiaceae) is a perennial plant and grows to an average height of 50–90 cm high [4]. The plant is usually branched stems with purplish or tinged violet but sometimes they are gray in color [5]. The dark or light green leaves are short-petioled, oblong-ovate and serrate with their margins finely toothed [6]. The flowers are purple or pinkish having spikes with numerous inconspicuous bracts and rarely bear seeds [7]. The plant grows in a sunny side and prefers acid, neutral and basic, light, medium soils but can also grow in heavy clay soil [8]. This plant has been widely used by local practitioners particularly in Turkey for health purposes [9].

Similarly, *T. involucrate* is a perennial evergreen plant belonging to the family Euphorbiaceae [10]. Leaves are 2.5-10 cm long, oblong-lanceolate to broadly ovate, acuminate, serrate, hairy, base rounded or cordate while flowers are small, without petals, shortly pedicellate, in the terminal, axillary and leaf-opposed usually hairy racemes, 2.5-5 cm long [11]. The plant species are widespread across North and South America, Africa, the Arabian, Indian Subcontinent, Northern Australia and to various islands in the Caribbean and in the Indian Ocean [12]. This plant is well known by many communities worldwide for the management of various ailments such as diarrhea, abdominal pain, hemolytic uremic syndrome infections, headache, eczema, cancer, and wounds [13].

As regard *U. massaica* (Urticaceae), is an erect, herbaceous, perennial plant forming loose clumps of few-branched stems from a creeping rhizome [14]. The stem of the plant grows up to 2 meters long and are covered with fiercely stinging hairs. The plant grows well in gaps, on disturbed ground in montane forests, near human habitation, around cattle enclosures, in abandoned fields, and in secondary bush lands after clearing the forests in high-altitude areas, at elevations from 1,500 - 3,200 meters [15]. In Africa, the plant is native in Congo, Burundi, Rwanda, Kenya, Uganda and Tanzania where many communities reportedly use it as a remedy for stomachache, fractures, rheumatism, urethral leak and hepatic diseases [16].

In view of the foregoing information on the contribution of *M. piperita, T. involucrate* and *U. massaica* in the treatment of human diseases, it is therefore justifiable that these plants are amongst the medicinal plants which are well known for many remedial applications [17]. Despite of this, there is lack of scientific studies regarding the prevalence of the toxicological profile of the plants. This study therefore reports cytotoxicity activity of these three herbal plants which are commonly used in Tanzania.

2. MATERIALS AND METHODS

2.1 Acquisition of Materials

Methanol and chloroform were purchased from Avantor Perfomance Materials Limited, Gujarat, India. Dimethyl sulphoxide (DMSO) and ethyl acetate were bought from RFCL Limited, Haryana, India and Cyclophosphamide was bought from Khandelwa Laboratories Pvt Ltd, Mumbai, India. Brine shrimps eggs were obtained from the Aquaculture innovations (Grahamstown 6140, South Africa) and sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam coast, Tanzania.

2.2 Preparation of Plant Extracts and Extraction

The plant materials which included leaves were collected from different parts of northern Tanzania. These included King'ori, Leguruki,

Mulala, Kimundo and Kikatiti villages. Plant species were identified by Mr. Emmanuel Mboya, a botanist from Tropical Pesticide Research Institute (TPRI) and voucher specimens coded MP-01, TI-02 and UM-03 for *M. piperita, T. involucrate* and *U. massaica* are kept at the University of Dodoma (UDOM). Each plant materials were air dried separately under shade and pulverized into fine particles using electric blender. Pulverized materials (500 g) were successively macerated in chloroform, ethyl acetate and methanol for 48 hours. The respective extracts were filtered through Whatman No. 1 filter paper on a plug of glass wool in a glass column and solvents were evaporated through the vacuum using a rotary evaporator and stored in a deep freezer at -20°C.

2.3 Brine Shrimp Lethality Test

Brine shrimp (*Artemia salina*) larvae were used as indicator animals for preliminary cytotoxicity assay of the extracts as reported by [18] Artificial sea water was prepared by dissolving sea salt (3.8 g) in 1 L distilled water. The salt solution was poured into a glass container and the shrimp eggs were spread and a lamp was illuminated from one side in order to attract hatched shrimps. The hatched shrimps (mature nauplii) were collected after 36 and 48 h of hatching. Stock solution of each extract was prepared by dissolving 40 mg/mL in DMSO. Different levels of concentrations (240, 120, 80, 40, 24 and 8 µg/mL) were prepared by drawing different volumes from the stock solutions and then added in a 10 mL universal bottle containing 10 brine shrimps larvae. The volume was then adjusted to 5 mL with artificial sea water prepared by dissolving 3.8 g of sea salt in 1 L of distilled water. Each level of concentration was tested in duplicate. Cyclophosphamide was used as standard positive control drug whereas DMSO and artificial sea water as negative control. The number of surviving larvae was determined after 24hrs and the percentage mortality was determined by comparing the mean surviving larvae of the tests and the control.

2.4 Data Analysis

The mean results of the percentage mortality were plotted against the logarithm of concentrations using Fig P computer program. Regression equation obtained from the graph was used to calculate LC_{50}, LC_{16} and LC_{84}. The results were used to document safety and cytotoxicity activity of plant extracts. The LC_{50} greater than 100 µg/mL was considered non-toxic and below it as toxic [18].

3. RESULTS

The cytotoxicity activity of *M. piperita, T. involucrata* and *U. massaica* extracts were evaluated against brine shrimp larvae and results are summarized in Table 1. It can be noted from the Table that out of nine assessed extracts, seven extracts representing 77% of all extracts had LC_{50} values < 100 µg/mL. The level of cytotoxicity between plant species varied with *M. piperita* ethyl acetate leaf (MPEL) extract being the most toxic with LC_{50} value of 32.65 µg/mL followed by *M. piperita* methanol leaf (MPML) extract which had LC_{50} value of 41.58 µg/mL.

The *T. involucrate* methanol leaf (TIML) and *U. massaica* chloroform leaf (UMCL) extracts exhibited cytotoxicity activity against brine shrimp larvae with LC_{50} value of 49.22 µg/mL followed by *U. massaica* methanol leaf (UMML) and *U. massaica* ethyl acetate leaf (UMEL) extract with LC_{50} values of 50.99 and 71.14 µg/mL respectively as shown in Table 1. In this study however, the *M. piperita* chloroform leaf (MPCL) and *T. involucrate* ethyl acetate leaf (TIEL) extracts exhibited lower cytotoxicity activity with LC_{50} values above 100 µg/mL against brine shrimp larvae and therefore considered as non-toxic.

4. DISCUSSION

Brine shrimp lethality bioassay (BST) is the rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates with cytotoxic and antitumor properties [19]. Findings from this study revealed that *M. piperita* ethyl acetate leaf (MPEL) and *M. piperita* methanol leaf (MPML) extracts were the most toxic against brine shrimp larvae which is evidenced by LC_{50} range of 32.65 - 41.58 µg/mL. The highest susceptibility shown by brine shrimp larvae towards *M. piperita* ethyl acetate and methanol leaf extracts suggests that these extracts are potential antitumor agents. These results collaborate with the previous cytotoxicity investigation study of the same plant growing in China, which found to be active against four human cancer cells namely lung carcinoma SPC-A1 cells, human gastric cancer SGC-7901 cells, human leukemia K562 cells and hepatocellular carcinoma BEL-7402 cells [20].

Table 1. Brine shrimp lethality test of leaf extracts from *M. piperita, T. involucrate* and *U. massaica*

Plant extract	Regression equation	LC_{50} (µg/mL)	95% Confidence interval	Regression coefficient (r)
MPCL	Y = 46.64 log x - 52.08	154.4	96.99 - 245.79	0.75
MPEL	Y = 59.46 log x - 40.02	32.65	22.67 - 47.02	0.93
MPML	Y = 63.39 log x - 52.63	41.58	29.54 - 58.55	0.99
TICL	Y = 73.26 log x - 95.05	95.49	71.02 - 128.39	0.9
TIEL	Y = 86.98 log x - 139.43	150.63	117.39 - 193.29	0.81
TIML	Y = 52.13 log x - 38.22	49.22	32.47 - 74.61	0.95
UMCL	Y = 52.13 log x - 8.22	49.22	32.47 - 74.61	0.95
UMEL	Y = 58.59 log x - 58.52	71.14	49.13 - 102.99	0.89
UMML	Y = 50.21 log x - 35.73	50.99	33.1 - 78.53	0.95
Cyclophosphamide	Y = 69.97 log x - 34.94	16.37	12.01 - 22.31	0.99

Key: MPCL= M. piperita chloroform leaf extract, MPEL= M. piperita ethyl acetate leaf extract, MPML= M. piperita methanol leaf extract, TICL= T. involucrate chloroform leaf extract, TIEL= T. involucrate ethyl acetate leaf extract, TIML= T. involucrate methanol leaf extract, UMCL= U. massaica chloroform leaf extract, UMEL= U. massaica ethyl acetate leaf extract, UMML = U. massaica methanol leaf extract

The *T. involucrate* methanol leaf (TIML) and *U. massaica* chloroform leaf (UMCL) extracts demonstrated a potential source of antitumor agent as evidenced by LC_{50} value of 49.22 µg/mL. The sensitivity shown by brine shrimp larvae against methanol and chloroform leaf extracts provides a circumstantial evidence that secondary metabolites in leaves of *T. involucrate* and *U. massaica* might be a good source of anticancer compounds. According to Godfrey [21] utilization of leaves is highly recommended for sustainability of plants as the use of roots and stems increases risk of plants extinction. These results are in agreement with the traditional use of *T. involucrate* leaves by people of different ethnic groups in India for treatment of cancer [22].

5. CONCLUSION

Based on the results it can be concluded that the extracts of *M. piperita, T. involucrata* and *U. massaica* exhibit varying degrees of cytotoxicity activities against brine shrimp larvae. However, a majority of the extracts confirmed to be toxic and thus possess anticancer activity. Further studies would be required to isolate the bioactive compounds responsible for these activities and determine their underlying molecular mechanism action to find out novel lead candidates.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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