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# Cytotoxicity Assessment of the Aerial Parts of *Macrotyloma uniflorum* Linn.

[1]S.M.A. Kawsar, [2]E. Huq and [3]N. Nahar
[1]Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong-4331, Bangladesh
[2]Department of Chemistry, Faculty of Engineering,
Bangladesh University of Engineering and Technology, Dhaka-1000, Bangladesh
[3]Department of Chemistry, Faculty of Science, University of Dhaka, Dhaka-1000, Bangladesh

**Abstract:** The fractionated crude extracts dichloromethane ($CH_2Cl_2$), ethyl acetate (EtOAc), 1-butanol (1-BuOH) and aqueous ($H_2O$) from aerial parts of *Macrotyloma uniflorum* were screened for cytotoxicity using the brine shrimp lethality bioassay technique. Most of the extracts were found to be non-toxic and this indicates that the ethnobotanical use (oral applications) of the experimental plant are justified.

**Key words:** *Macrotyloma uniflorum*, brine shrimp, cytotoxicity, mortality, Fabaceae, extracts

## INTRODUCTION

The plant kingdom comprises many species of plants containing substances of medicinal value, which are yet to be explored. A large number of plants are constantly being screened for their possible medicinal value. For thousands of years, people have relied and survived on the bounty of plants and natural grasslands and continue to do so through today to maintain a healthy diet. The importance of medicinal plants and traditional health systems in solving the health care problems is gaining increasing global attention and because of this resurgence of interest, research on plants of medicinal importance is rapidly increasing at the international level. However, this is occurring while natural habitats in countries of origin are being lost. Plants are the natural reservoir of many antimicrobial (Cowan, 1999), antimalarial (Schwikkard and Heerden, 2002), anticancer (Kintzios, 2006) and drug (Rates, 2001) agents. The medicinal use of plants in the Indian subcontinent is found in the Rig Veda (4500-1600 BC) and the Indo-Aryans used the soma-plant (*Amanita muscaria*) (L.) as a medicinal agent (Ghani, 2003). Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the treatment of various diseases. However, scientific studies have been conducted only to a limited extent with few medicinal plants (Rahman *et al*., 2002; Haque *et al*., 2000; Nutan *et al*., 1997). Some of the medicinal plant exhibit moderate (Sohrab *et al*., 2004; Asha *et al*., 2003) to good (Rahman *et al*., 2007) cytotoxicity.

*Macrotyloma uniflorum* Linn. (Family: Fabaceae) is found in Rajshahi and Dinajpur districts in Bangladesh (Kirtikar and Basu, 1998). Local name of this plant is kurti kalai and English name is horse gram plant. It has annual branches sub-erect or twining, downy or glabrescent while stipules are oblong and basifixed. Leaflets are 2.5-5 cm, broadly lanceolate or oblong, membranous, downy; stipules subulate. Different parts of the plants are used for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary discharges and for treatment of kidney stones (Ghani, 2003). On the basis of the folkloric use, this plant was selected for pharmacological testing with focus on cytotoxicity determined by the brine shrimp (*Artemia nauplii*) lethality bioassay (Meyer *et al*., 1982).

The assay is considered as an useful tool for preliminary assessment of toxicity and it has been used for the detection of plant extract toxicity (McLaughlin *et al*., 1998), heavy metals (Martinez *et al*., 1999), pesticides (Barahona and Sanchez-Fortun, 1999) and cytotoxicity testing of dental materials (Pelka *et al*., 2000). It is also a useful tool for the isolation of bioactive compounds from plant extracts (Sam, 2007). The method is attractive because it is simple, inexpensive and low toxin amounts are sufficient to perform the test on the microwell scale. As a part of our investigations on the medicinal plants of Bangladesh, we investigated *M. uniflorum* and isolated Kaempferol-3-O-β-D-glucoside, β-sitosterol and stigmasterol (Kawsar *et al*., 2003). In the present study, we report herein on the cytotoxicity studies of crude extracts of the aerial part of *Macrotyloma uniflorum* plant.

**Corresponding Author:** Sarkar M.A. Kawsar, Yokohama City University, Yokohama-Shi, Hodogaya-Ku, Kamisugeta Chuo 1500, Sasayama Apartment 30-401, P.O. Box 240-0051, Japan
Tel: +81 45 382 7823, +81 45 9085128671

## MATERIALS AND METHODS

**Plant material:** *Macrotyloma uniflorum* was collected from the village, Susunda of Muradnagar, Comilla, Bangladesh in March 2002. The botanical identification was made by Prof. Salar Khan (University of Dhaka) and voucher specimen was deposited at the Bangladesh National Herbarium (BNH) (DACB Accession No. 28264). The whole plants were cleaned, dried in air under shade, ground to a fine powder and kept at 20°C in air-tight wide-mouth bottles.

**Preparation of extracts:** Plant powder (3.5 Kg) was successively extracted with aqueous 80% ethanol (18 L×3 times, 24 h) at room temperature. The extract was filtered and the filtrate was evaporated to dryness at 40°C under vacuum and finally freeze-dried to have crude ethanolic extract of 484 g (13.82%) as solid material. The ethanol extract (484 g) was suspended in water (~2000 mL) and the suspension was transferred into a separating funnel. The aqueous suspension was successively partitioned with dichloromethane ($CH_2Cl_2$, 2000 mL ×3), ethyl acetate (EtOAc, 1500 mL ×3) and 1-butanol (1-BuOH, 1500 mL ×3). All extracts were evaporated separately and freeze-dried. The extracts of $CH_2Cl_2$, EtOAc, 1-BuOH and aqueous extracts were 40 g (1.14%), 48 g (1.37%), 110 g (3.14%) and 58.5 g (1.67%) respectively.

**Hatching of shrimp:** Sea salt (USA) was dissolved in distilled water (1000 mL) and then filtered off. A small amount of seawater kept in a small tray (40×20 cm) having a partition (1:4) with a 1 cm gap at the bottom. Some brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) were sprinkled on the longer side of the tray covered with aluminium foil. The smaller compartment of the tray was kept open under a desk lamp. The hatched shrimps swam from the large compartment into the small compartment within 24 h. Living shrimps (age 48 h) were used to determine the toxicity of the extracts.

**Brine shrimp bioassay and mortality counted:** The assay was performed as described by Meyer *et al.* (1982) and McLaughlin (1991). Dichloromethane, ethyl acetate, 1-butanol and aqueous extracts (50 mg of each extract) were taken in 4 different vials and 5 mL seawater was added in each vial to prepare the main sample solution. Appropriate concentrations of this main solution were than prepared as 5, 25, 50, 250 and 500 μL to give concentrations of 10, 50, 100, 500 and 1000 μg $mL^{-1}$ in vial type-1, 2, 3, 4 and 5, respectively. Solvents were evaporated from each type of vial using a vacuum evaporator. In each type of vial 5 mL of sea salt water was added and ultrasonicated (5 min). Afterwards 30 living shrimps drawn by Pasteur pipette were put into each vial and a few drops yeast solution were added to each the vials. Control experiments were prepared using only sea water and triplicates were prepared for each dose level. Survivors were counted with the stereomicroscope after 24 and 48 h and the death at each level and control were determined. No deaths were found in the controls.

## RESULTS AND DISCUSSION

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extract bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties (McLaughlin *et al.*, 1993). The cytotoxicity of plant material is considered to be due to the presence of antitumour compounds. Many scientists have reported cytotoxicity of plants using brine shrimp as a zoological specimen (Desmarchelier *et al.*, 1996; Gurkan *et al.*, 1995).

The brine shrimp lethality test was conducted on dichloromethane, ethyl acetate, 1-butanol and aqueous extracts of *M. uniflorum* at 10, 50, 100, 500 and 1000 μL $mg^{-1}$ concentration.. Although there was no mortality in the controls, the test samples showed different mortality rates at different concentrations of the sample. The mortality rate of brine shrimp nauplii was found to increase with the increase in concentration of the samples. The 1-butanol extract was showed 26.64 and 33.30% death at 100 and 1000 μL $mg^{-1}$, respectively (Table 1). Relatively high levels of toxicity were also displayed by ethyl acetate extract which had 26.64% shrimp death at the highest concentration tested (Table 2). Other extracts displayed some or little toxicity at concentrations of 100 and 1000 μL $mg^{-1}$ and included

Table 1: Brine shrimp lethality assay for the 1-butanol extract

| Sample code (vial type) | Dosage (μg $mL^{-1}$) | No. of mortality | | Percentage mortality (mean %) of extract[a] |
|---|---|---|---|---|
| | | After 24 h | After 48 h | |
| Type 1 | 10 | 1 | 1 | 3.33 |
| Type 2 | 50 | 2 | 4 | 10.00 |
| Type 3 | 100 | 6 | 8 | 23.31 |
| Type 4 | 500 | 7 | 9 | 26.64 |
| Type 5 | 1000 | 9 | 11 | 33.30 |
| Control | -- | 0 | 0 | 0.00 |

[a]Values are mean of three replicates

Table 2: Brine shrimp lethality assay for the ethyl acetate extract

| Sample code (vial type) | Dosage (μg $mL^{-1}$) | No. of mortality | | Percentage mortality (mean %) of extract[a] |
|---|---|---|---|---|
| | | After 24 h | After 48 h | |
| Type 1 | 10 | 0 | 1 | 0.00 |
| Type 2 | 50 | 2 | 2 | 6.66 |
| Type 3 | 100 | 4 | 6 | 16.65 |
| Type 4 | 500 | 4 | 8 | 20.00 |
| Type 5 | 1000 | 5 | 11 | 26.64 |
| Control | -- | 0 | 0 | 0.00 |

[a]Values are mean of three replicates

Table 3: Brine shrimp lethality assay for the Dichloromethane extract

| Sample code (vial type) | Dosage ($\mu g\ mL^{-1}$) | No. of mortality | | Percentage mortality (mean %) of extract[a] |
|---|---|---|---|---|
| | | After 24 h | After 48 h | |
| Type 1 | 10 | 0 | 0 | 0.00 |
| Type 2 | 50 | 1 | 1 | 3.33 |
| Type 3 | 100 | 2 | 4 | 10.00 |
| Type 4 | 500 | 3 | 5 | 13.32 |
| Type 5 | 1000 | 4 | 8 | 20.00 |
| Control | -- | 0 | 0 | 0.00 |

[a]Values are mean of three replicates

Table 4: Brine shrimp lethality assay for the aqueous extract

| Sample code (vial type) | Dosage ($\mu g\ mL^{-1}$) | No. of mortality | | Percentage mortality (mean %) of extract[a] |
|---|---|---|---|---|
| | | After 24 h | After 48 h | |
| Type 1 | 10 | 0 | 0 | 0.00 |
| Type 2 | 50 | 0 | 0 | 0.00 |
| Type 3 | 100 | 1 | 2 | 5.00 |
| Type 4 | 500 | 4 | 5 | 15.00 |
| Type 5 | 1000 | 6 | 8 | 23.31 |
| Control | -- | 0 | 0 | 0.00 |

[a]Values are mean of three replicates

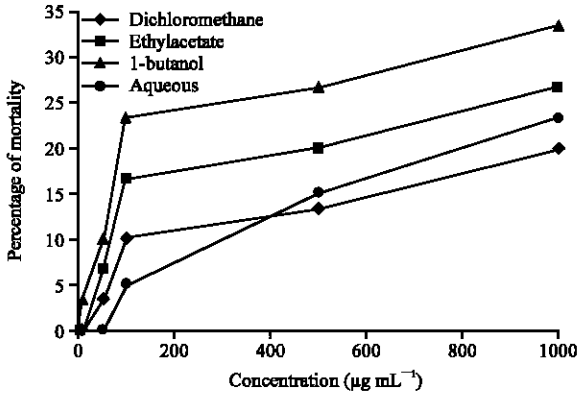


Fig. 1: Plot of concentration ($\mu g\ mL^{-1}$) versus shrimp mortality (%) of different extracts of *M. uniflorum*

dichloromethane extracts (20.0%) (Table 3) and aqueous extracts (23.31%) (Table 4). Cytotoxic activity is also displayed in Fig. 1 as concentration versus percentage of mortality and indicated that the highest percent of death occurred from 1-butanol extract. According to the literature in order for a test compound to be considered highly toxic it needs to show shrimp death of 50% or less. None of the extracts in this assay showed extreme levels of toxicity with all of the extracts showing shrimp survival of greater than 50% at the highest concentration tested (1000 $\mu L\ mg^{-1}$). However the extracts did show certain levels of toxicity.

These results suggest that the extracts of *M. uniflorum* were not very toxic. *M. uniflorum* plant is being used traditionally for the treatment of various diseases. Traditional healers normally apply the folk medicine orally and the non-toxic effects of this plant are also claimed by traditional practitioners.

## CONCLUSIONS

In this study, the brine shrimp lethality assay was proven to be a convenient tool for screening the biological activities of plant species that are used in Bangladesh traditional medicine. The above results suggest that *M. uniflorum* may be useful for the treatment of various diseases due to its low cytotoxicity. But further acute toxicity and other pharmacological tests are necessary to utilize the extracts as a potential therapeutic agent.

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## REFERENCES

Asha, K.N., R. Chowdhury, C.M. Hasan and M.A. Rashid, 2003. Antibacterial activity and cytotoxicity of extractives from *Uvaria hamiltonii* stem bark. Fitoterapia, 74: 159-163.

Barahona, M.V. and S. Sanchez-Fortun, 1999. Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. Environ. Pollut., 104: 469-476.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Desmarchelier, C., E. Mongelli, J. Coussio and G. Ciccia, 1996. Studies on the cytotoxicity, antimicrobial and DNA-binding activities of plants used by the Ese/ejas. J. Ethnopharmacol., 50: 91-96.

Ghani, A., 2003. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 2nd Edn. Asiatic Society of Bangladesh, Dhaka, ISBN: 9-845123481, pp: 5-16.

Gurkan, E., O.T. Tuzun and F. Hirlak, 1995. Cytotoxicity assay of some papaver alkaloids using *Artemia salina* (*Brine shrimp*). Fitoterapia, 66: 544-545.

Haque, N., S.A.R. Choudhury, M.T.H. Nutan, G.D.S. Rahman and M.A. Rashid, 2000. Evaluation of antitumor activity of some medicinal plants of Bangladesh by potato disk bioassay. Fitoterapia, 71: 547-552.

Kawsar, S.M.A., M.R. Rahman, E. Huq, M. Mosihuzzaman, N. Nahar and M.I.R. Mamun, 2003. Studies of different extractives of *Macrotyloma uniflorum*. Dhaka Univ. J. Pharm. Sci., 2: 81-84.

Kintzios, S.E., 2006. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Crit. Rev. Plant Sci., 25: 79-113.

Kirtikar, K.R. and B.D. Basu, 1998. Indian Medicinal Plants. 2nd Edn., International Book Distributors, Dehradun, India, ISBN: B-0007B1B64, pp: 804-806.

Martinez, M., J.D. Ramo, A. Torreblanca and J. Diaz-Mayans, 1999. Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia parthenogenetica*. Aquaculture, 172: 315-325.

McLaughlin, J.L., 1991. Crown-Gall Tumours in Potato Discs and Brine Shrimp Lethality: Two Simple Bioassays for Higher Plant Screening and Fractionation. In: Methods in Plant Biochemistry: Assays for Bioactivity, Hostettmann, K. (Ed.). Academic Press, London, ISBN: 0-124610161, pp: 1-31.

McLaughlin, J.L., C.J. Chang and D.L. Smith, 1993. Simple Bench-Top Bioassays (Brine Shrimp and Potato Discs) for the Discovery of Plant Antitumour Compounds: Review of Recent Progress. In: Human Medicinal Agents from Plants, Kinghorn, A.D. and M.F. Balandrin (Eds.). American Chemical Society Publication, Washington D.C., ISBN: 0-841227055, pp: 112-137.

McLaughlin, J.L., L.L. Rogers and J.E. Anderson, 1998. The use of biological assays to evaluate botanicals. Drug Inform. J., 32: 513-524.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, J.E. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine Shrimp: A convenient general bioassay for active plants constituents. J. Med. Plant Res., 45: 31-34.

Nutan, M.T.H., A. Hasnat, M.A. Rashid and S. Rahman, 1997. Cytotoxic and antiproliferative medicinal plants of Bangladesh a review. Bang. J. Life Sci., 9: 61-67.

Pelka, M., C. Danzl, W. Distler and A. Petschelt, 2000. A new screening test of dental materials. J. Dentol., 28: 341-345.

Rahman, M.M., A.H.M.K. Alam, G. Sadik, M.R. Islam, P. Khondkar, M.A. Hossain and M.A. Rashid, 2007. Antimicrobial and cytotoxicity activities of *Achyranthes ferruginea*. Fitoterapia, 78: 260-262.

Rahman, S., C.M. Hasan, M.A. Rashid and M. Ilias, 2002. Pharmacological evaluation of Bangladeshi medicinal plants: A review. Pharm. Biol., 39: 1-6.

Rates, S.M.K., 2001. Plants as source of drugs. Toxicon, 39: 603-613.

Sam, T.W., 2007. Toxicity Testing Using the Brine Shrimp: *Artemia salina*. In: Bioactive Natural Products Detection, Isolation and Structural Determination, Colegate, S.M. and R.J. Molyneux (Eds.). CRC Press, Boca Raton, FL, ISBN: 0-849372585, pp: 442-456.

Schwikkard, S. and F.R. van Heerden, 2002. Antimalarial activity of plant metabolites. Nat. Prod. Rep., 19: 675-692.

Sohrab, M.H., R. Chowdhury, K.M. Rahman, C.M. Hasan and M.A. Rashid, 2004. Antibacterial activity and cytotoxicity of extractives from *Ravenia spectabilis*. Fitoterapia, 75: 510-513.