Reference

(1) Monnier, F.,Taillefer, M., **2008**, *Angew. Chem. Int. Ed.*, 47, 3096-3099.

(2) Asif, M. **2014**, *American Journal of Medicine Studies*, 2 (1), pp 24-32.

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Activity of *Barringtonia asiatica* extracts on MCF-7 breast cancer cells

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Currently cancer has become a critical burden to the world. Patients have to undergo surgeries, radiotherapy and chemotherapy while chemotherapy provides a worthwhile increase in both relapse- free interval and survival. Its effect is relatively modest and there is a pressing need to identify new drugs with greater efficiency against these tumours. Organ specific *in vitro* assays are imperative in large scale screening of agents with useful clinical activity. Among many such assays, sulforhodamine B (SRB), assay employs a protein binding aminoxanthene dye, to provide a quantitative analysis of viable cells in a culture following the introduction of potential extracts or isolates of extracts. Preliminary investigations indicated that the crude methanolic extract of *Barringtonia asiatica* seed kernel and a fraction obtained with medium pressure liquid chromatography (MPLC) of crude extract were positive for brine shrimp assay (unpublished data).

Thus this study aims to investigate the toxicity of the crude methanolic extract (15 g powder / 40 mL MeOH) and the brine shrimp active fraction obtained by MPLC by SRB assay with the breast cancer cell line MRF-7. The cells were cultured, trypsinized and cell density was measured. Cells were suspended in appropriate growth medium (5×10^3 cells in the well). The cell suspension (200 l) in Dulbecco's Modified Eagle's Medium (DMEM) was aliquot in to 96 wells plate. After incubation cells were treated with different concentrations of methanolic crude extract and brine shrimp active fraction of *B. asiatica* (12.5, 25, 50, 100 and 200 ppm) extract. After incubation (24 hours), medium was removed and washed with phosphate buffered Saline to remove cell debris. The wells were then fixed with 25 l of ice-cold 50% trichloroacetic acid solution and the plate was incubated and wells were rinsed. SRB solution (0.4%; 50 l stain/ well) was added to each well. Acetic acid (1%) was used to remove unbound dye. The bound SRB dye was solubilized by adding of tris-base solution (200 l/well), and plates were placed on a shaker (1 hour) at room temperature. Plates were read at OD 540 nm, using a micro plate reader. Half maximal inhibitory concentration (IC_{50}) values were determined by analyzing sigmoid dose-response inhibition curves using Graph Pad Prism software (version 5.0).

After adding of SRB dye to crude methanolic extract at low concentrations (12.5 and 25 ppm) a pink colour was observed but with concentrated solutions (100 and 200 ppm) clear wells were observed. The fractionated Brine shrimp assay active fraction, above 25 ppm (50, 100 and 200 ppm) showed clear wells indicating that no viable cells were present. The crude methanolic extract showed an IC_{50} value 34.13 ppm on SRB assay within 24 hours and brine shrimp active fraction showed an IC_{50} value of 11.32 ppm. Crude methanolic extract and brine shrimp active fraction have shown high cytotoxic activity on SRB assay. Further cytotoxic assays and purification and structure elucidation of brine shrimp active fraction is being carried out.

References

1. K Haselsberger, DC Peterson,DGT Thomas and JL Darling (1996) Assay of anti cancer drugs in tissue culture: comparison of a tertrazolium- based assay and a protein binding dye assay in short-term cultures derived from human malignant glioma: pp 331-338
2. Akalanka H.M.K and Ekanayake S(2013) Cytotoxicity study of *Barringtonia asiatica* kurz extracts,3rd Young Scientists forum symposium : Page 5-7
3. KT Papazisis, GD Geromichalos, KA Dimitriadis and AH Kortsaris (1997) Optimization of the sulforhodamine B colorimetric assay. 151-158.

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