Cytotoxic potential of different fractions of methanolic extract of *Barringtonia asiatica* (L.) Kurz. seed kernel

P Ragutharan*, S Ekanayake

Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda

Email: *pragutharan@yahoo.com

Currently, there is an increasing awareness globally of the potential of unexploited natural products. Identification of active compounds with cytotoxic potential is important in the development of new anticancer drugs. The crushed *Barringtonia asiatica* (Mudilla) plant seed kernel was used as fish poison by Sri Lankans and preliminary investigations have proven the cytotoxic potential. This study aims to investigate different techniques for better fractionation of the crude methanolic extract of *B. asiatica* seed kernel and study the toxicity of different fractions, thus obtained and identify the class of compounds in fractions with toxicity as shown by brine shrimp assay (BSA).

The seed kernel was freeze dried and powdered (0.15 mm). The crude methanolic extract (15 g powder/40 mL MeOH; room temp., 24 hrs; dried at 45 °C) was separated by medium pressure liquid chromatography (MPLC). A solvent gradient was used to obtain different fractions (hexane, petroleum ether, ethyl acetate, ethanol and methanol). The cold methanolic extract was also separated using column chromatography (silica gel, 0.13-0.25 mm) with a gradient of solvents comprising of dichloromethane and methanol. The seed kernel powder was sequentially extracted in a Soxhlet (50 g/ 300 mL of solvent; petroleum ether, dichloromethane, ethyl acetate and methanol). Toxicity of all fractions were obtained by different extraction and separation methods were determined by BSA. Phytochemical screening was carried out with standard assays. BSA was conducted with a) methanolic crude extracts (50 ppm, 100 ppm), b) two MPLC fractions by pooling individual fractions (both fractions 50 ppm, 100 ppm), c) two fractions from silica gel column (both fractions 50 ppm, 100 ppm) and d) four fractions obtained from the Soxhlet extraction. All TLC separations were carried out with chloroform: acetic acid: methanol: water (64:32:12:08) solvent system.

The crude cold methanolic extract produced two distinct spots on TLC with R_f values of 0.43 and 0.25. Phytochemical screening of the MPLC fractions indicated steroids in the 1st pooled fraction with no spot being visible on TLC, 2nd pooled fraction had saponins, flavonoids, phenolic compounds, terpenoids and triterpenoids and showed two spots (R_f values 0.43 and 0.25), and the 3rd pooled fraction (R_f value 0.43) had saponins, terpenoids and triterpenoids. Out of the silica gel column, 1st fraction contained saponin (R_f value of 0.43) and 2nd fraction had saponin, phenols and triterpenoids and yielded two spots (R_f values 0.43 and 0.25). The methanol extract of the Soxhlet had saponin, phenols and triterpenoids and showed two spots (Rf values 0.43 and 0.25). Other extracts (petroleum ether and ethyl acetate) contained terpenoids, and dichloromethane extract had steroids and terpenoids despite not indicating any spots with TLC when visualized with *p*-anisaldehyde.

The crude cold methanolic extract (30 ppm) showed a LD_{50} value within 24 hours on BSA. Only the 2nd and 3rd MPLC fractions at 50 ppm and 100 ppm showed cytotoxic potential. 1st and 2nd fractions of the silica gel column (50 ppm, 100 ppm) and methanolic Soxhlet extracts (100 ppm, 150 ppm) showed positive results with BSA. The other Soxhlet extracts were not positive for BSA. Fractions 2 and 3 of MPLC, and fraction 2 of silica gel column and methanolic Soxhlet extract showed high cytotoxic activity. The difference in the cytotoxicity of cold methnolic extract (30 ppm) and the hot methanolic extract ($\geq$100 ppm) could be due to the heat degradation of the active components during Soxhlet extraction.

Cold methanolic extract, 2nd fraction of MPLC, hot methanolic extract and 2nd fraction of silica column indicated two spots with the same R_f values (0.43 and 0.25). MPLC 3rd fraction and 1st fraction of silica gel column indicated only one spot on TLC (R_f value 0.43).

The cold methanolic extract (30 ppm) showed a LD_{50} value within 24 hours on BSA. The MPLC resulted in better separation of active fraction. Heating may be detrimental to the active cytotoxic ingredient. Fractions that indicated toxicity contained saponins and triterpenoids.