APPENDIX I

TRYPAN BLUE EXCLUSION METHOD
(Gupta and Bhattacharya, 1983)

Principle
Viable cells exclude the dye trypan blue and unstained while nonviable cells take up dye and stained blue in colour.

Reagents
Trypan blue 0.1 per cent in phosphate buffered saline (PBS) pH 7.0,
KCl - 40 mg
KH₂PO₄ - 20 mg
Na₂HPO₄·2H₂O - 57.5 mg
NaCl - 900 mg
Distilled water - 100 ml

Procedure
The Ehrlich ascite tumor cells propagated in the peritoneal cavity of mice were taken and washed with saline thrice by centrifuging at low speed. 0.1 ml containing 1 x 10⁶ cells were used for the in vitro assay. 1 x 10⁶ cells were suspended in 0.9 ml of phosphate buffered saline (pH 7.0) in the presence and absence of various concentrations of extracts of Cynodon dactylon in respective vehicles were incubated at 37°C for 3 hours. At the end of the incubation
period 0.1 ml of Trypan blue solution was added and layered the cells on the hemocytometer for counting. The dead cells were blue in colour were counted and the percentage of dead cells (per cent cytotoxicity) was calculated using the formula.

$$\text{Per cent cytotoxicity} = \frac{\text{Dead cell count}}{\text{Viable cell count} + \text{dead cell count}} \times 100$$