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# Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays

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## Abstract

**Background:** Various assays are used to evaluate the cytotoxic effect of chemicals on cultured cells. The sulforhodamine B (SRB) colorimetric assay is based on the ability of the SRB dye to bind basic amino acid residues on proteins. In contrast, the MTT (dimethylthiazol-diphenyltetrazolium bromide) colorimetric assay is based on mitochondrial uptake and succinate dehydrogenase reduction of soluble, yellow, MTT tetrazolium salt to an insoluble blue MTT formazan product. The aim of this study was to evaluate the cytotoxicity of a Thai herb by comparing MTT and SRB assay results.

**Methods:** Mouse fibroblast (L929) cells were exposed to 0.01, 0.1, 0.25, and 0.5% (*w/v*) of a Thai herb in a 96-cluster well culture plate for 24 h. Cell viability after exposure to the Thai herb was determined by MTT and SRB assays in separate tissue culture plates. The two assays were compared using intra-class correlation coefficient (ICC) analysis.

**Results:** There were no significant differences between the two cytotoxicity assays ($p > 0.05$). The ICC values showing the agreement of the two assays in the negative and positive control groups and Thai herb concentrations of 0.01, 0.1, 0.25, and 0.5% were 0.93 and 0.99 and 0.53, 0.51, 0.95, and 0.98, respectively.

**Conclusions:** In general, the MTT and SRB assays performed similarly, exhibiting moderate to excellent correlation in the evaluation of the cytotoxicity of a Thai herb.

## Background

The MTT (dimethylthiazol-diphenyltetrazolium bromide) colorimetric assay determines the functional state of mitochondria, indicating cell viability. A mitochondrial dehydrogenase enzyme in living cells reduces yellow tetrazolium MTT salt to blue MTT formazan, which is precipitated in uninjured cells (Edmondson et al. 1998).

The MTT assay is the most widely used cell viability assay, and several modifications of the original method have been described (Mosmann 1983). However, the amount of MTT is not linear with cell number at high cell densities (Ruben and Neubauer 1987; Plumb et al. 1989). Cell lines under a number of conditions have large intra-assay and inter-assay variations. Intra-assay variations refer to variations in results within a dataset obtained from one experiment, while inter-assay variations refer to the precision of results among different assays (Park et al. 1987). The sulforhodamine B (SRB) protein stain is used for in vitro chemosensitivity testing. The SRB assay appears to be more sensitive than the MTT assay, with a better linearity with cell number and higher reproducibility (Skehan et al. 1990; Rubenstein et al. 1990). These assays are relevant to medical devices and materials used in dentistry, as pre-clinical evaluations are necessary to establish the biocompatibility of all devices and materials. The International Organization for Standardization (ISO) 7405 (International Organization for Standardization; ISO 7405 2008) recommends that high priority be given to minimizing the use of animals in the biological testing of materials. As scientific knowledge advances the understanding of basic mechanisms, an in vitro model that simulates an in vivo test or clinical use, which may yield equally relevant information, is advocated. A cell culture assay is one method of choice for toxicity screening. MTT, SRB, and neutral red uptake (NRU) assays are widely used for cytotoxic screening evaluation of dental agents and materials. MTT was used in both

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