

ASSESSMENT OF CYTOTOXIC ACTIVITY OF *CITRUS DEPRESSA* FRUIT EXTRACT.**B. Maria Regina Femi* & **Dr. T. Renisheya Joy Jeba Malar**

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KEYWORDS	Abstract
<i>Citrus depressa</i>, aqueous fruit extract, cytotoxicity, human dermal fibroblasts, MTT assay, cell viability.	<i>Citrus depressa</i> Hayata is a <i>Citrus</i> species recognized for its distinctive phytochemical composition and potential biological activities. <i>Citrus</i> fruits contain several classes of bioactive compounds, including flavonoids, phenolic compounds, carotenoids, and other secondary metabolites that may influence cellular functions. The present study evaluated the cytotoxic effects of <i>Citrus depressa</i> fruit extract on human dermal fibroblast (HDF) cells using the MTT cell viability assay. The toxin-treated group exhibited 47.74% cell viability, whereas untreated cells showed 100% viability. The extract-maintained cell viability above 70% at concentrations up to 25 µg/mL, while a substantial reduction in viability was observed at 50 and 100 µg/mL. Based on a 70% cell-viability criterion, 25 µg/mL was the highest tested concentration maintaining viability above this threshold. These findings indicate a concentration-associated reduction in HDF cell viability following treatment with the extract.
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INTRODUCTION

Citrus, which consists of several important commercial fruits such as oranges, tangerines, mandarins, grapefruit, lemons, and limes, are the most consumed fruit and became

the third most important fruit crop grown in tropical and subtropical regions of the world. Citruses are a fruit with the highest yield crop worldwide. *Citrus depressa* Hayata, also known as shiikuwasha, is a popular citrus fruit in

Okinawa - Japan, and Taiwan. It has small yellowish-green fruits, a very sour taste, and a strong, unique aroma. (Fakayode and Abobi, 2018; Saini *et al.*, 2019; Uckoo *et al.*, 2015; Victor *et al.*, 2021; Zhang *et al.*, 2019). In some countries, *Citrus* peels have been utilized as a traditional medicine for stomach ache, cough, skin inflammation, muscle pain, ringworm infections, and lowering blood pressure (Li *et al.*, 2009). *Citrus depressa* Hayata, also known as shiikuwasha, is a popular *Citrus* fruit in Okinawa - Japan, and Taiwan. It has small yellowish-green fruits, a very sour taste, and a strong, unique aroma. (Asikin *et al.*, 2012; Shiu *et al.*, 2016). Shiikuwasha (*Citrus depressa* Hayata), also known as Hiram lemon or thin-skinned flat lemon, is a citrus cultivar that grows naturally in the southernmost prefecture of Japan, Okinawa. This small *Citrus* fruit is commonly harvested from its unripe to ripe stages from September to December and is rich in beneficial phytochemical substances, such as phenolics, flavanones, and polymethoxylated flavones (Asikin *et al.*, 2012; Asikin *et al.*, 2016). The MTT Assay (Mosmann, 1983) is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product that is

insoluble in water. Viable cells are able to reduce the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue formation which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas cells being dead following a toxic damage, cannot transform MTT. This formation production is proportionate to the viable cell number and inversely proportional to the degree of Cytotoxicity. The reaction is mediated by dehydrogenases enzymes associated with the endoplasmic reticulum and the mitochondria (Fotakis and Timbrell, 2006). Although the MTT assay is widely applied, its results must be interpreted carefully because metabolic activity does not always correspond directly to cell number or cell death. Experimental factors, including cell density, incubation time, reagent concentration, culture conditions, and potential interference from the tested extract, can influence the measurements (Ghasemi *et al.*, 2021).

MATERIALS AND METHODS

• Collection and preparation of plant material

Fresh fruits of *Citrus depressa* were collected from Alamparai, Nagercoil, Kanyakumari District. The fruits were cleaned thoroughly to remove adhering dirt and other foreign materials. The whole fruits, including the fruit tissues used in the experiment, were processed according to the laboratory's established procedure. The prepared material

was used for aqueous extraction.

- **Preparation of *Citrus Depressa* Fruit Powder Extract**

The prepared whole-fruit material of *C. depressa* was extracted using Distilled water as the solvent. The extraction was carried out using the laboratory's established extraction procedure. Following extraction, the extract was separated from the residual plant material and concentrated using an appropriate solvent-removal procedure. The resulting aqueous extract was stored under suitable conditions until further analysis. Before the cytotoxicity experiment, the extract was prepared at the required concentrations using a compatible vehicle and culture medium, as appropriate.

- **MTT Assay –Cytotoxicity Assay**

The human dermal fibroblast (HDF) cell line was purchased from HiMedia Laboratories, Mumbai, India. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with high glucose, 10% fetal bovine serum (FBS), and 1% antibiotic–antimycotic solution. The cells were cultured in a CO₂ incubator at 37°C under an atmosphere containing 5% CO₂ and 18–20% O₂. The culture medium was renewed as required, and the cells were sub cultured every two days. HDF cells at passage number 09 were used for the present study. The cytotoxic activity of the test sample was evaluated using the MTT assay. Subsequently, different concentrations of the test sample, prepared by dilution in culture

medium according to the experimental concentrations, were added to the respective wells. The plate was then incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO₂.

After incubation, the MTT reagent was removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The resulting solution was then used for absorbance measurement to determine cell viability and evaluate the cytotoxic effects of the test sample.

The percentage of cell viability was calculated using the mean absorbance values obtained from the MTT assay.

Cell Viability (%)

$$= \frac{\text{Mean absorbance of treated cells}}{\text{Mean absorbance of untreated cells}} \times 100$$

RESULT AND DISCUSSION

- **Cell Viability Assay of *Citrus depressa* (CD) Against Human Dermal Fibroblast (HDF) Cells**

The cytotoxic activity of *Citrus depressa* fruit extract was evaluated using the MTT assay in human dermal fibroblast (HDF) cells. The untreated control exhibited 100% cell viability, whereas the toxin control showed 47.74% cell viability. The *Citrus depressa* extract exhibited cell viability values of 95.49%, 90.92%, and 85.70% at concentrations of 6.25, 12.5, and 25 µg/mL, respectively. At higher concentrations of 50 and 100 µg/mL, cell viability decreased to 83.75% and 80.72%,

respectively.

The results indicate that cell viability decreased with increasing extract concentration.

Based on the provided data, 100 µg/mL was

designated as the maximum safer concentration for further evaluation, although this designation should be confirmed using predefined safety criteria and appropriate statistical analysis.

Table: 1 Effect of *Citrus depressa* Fruit Extract on Cell Viability of Human Dermal Fibroblast (HDF) Cells Using the MTT Assay

S. No.	Treatment	Concentration	Cell viability (%)	Max safer conc (ug/ml)
1	Untreated control	—	100.00	100 µg
2	Toxin control	1 µg*	47.74	
3	CD extract	6.25 µg/mL	95.49	
4	CD extract	12.5 µg/mL	90.92	
5	CD extract	25 µg/mL	85.70	
6	CD extract	50 µg/mL	83.75	
7	CD extract	100 µg/mL	80.72	

Figure.1 Effect of Different Concentrations of *Citrus depressa* Extract on HDF Cell Viability

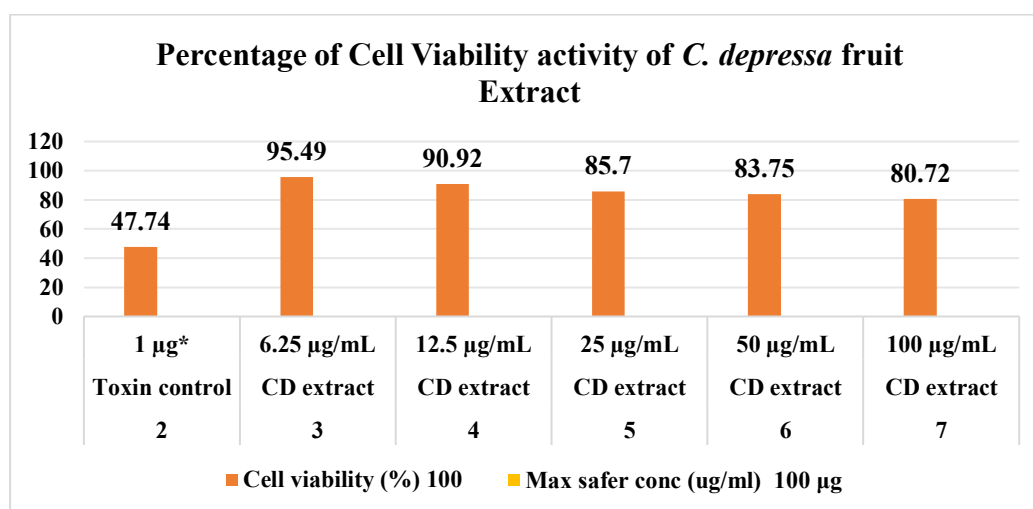
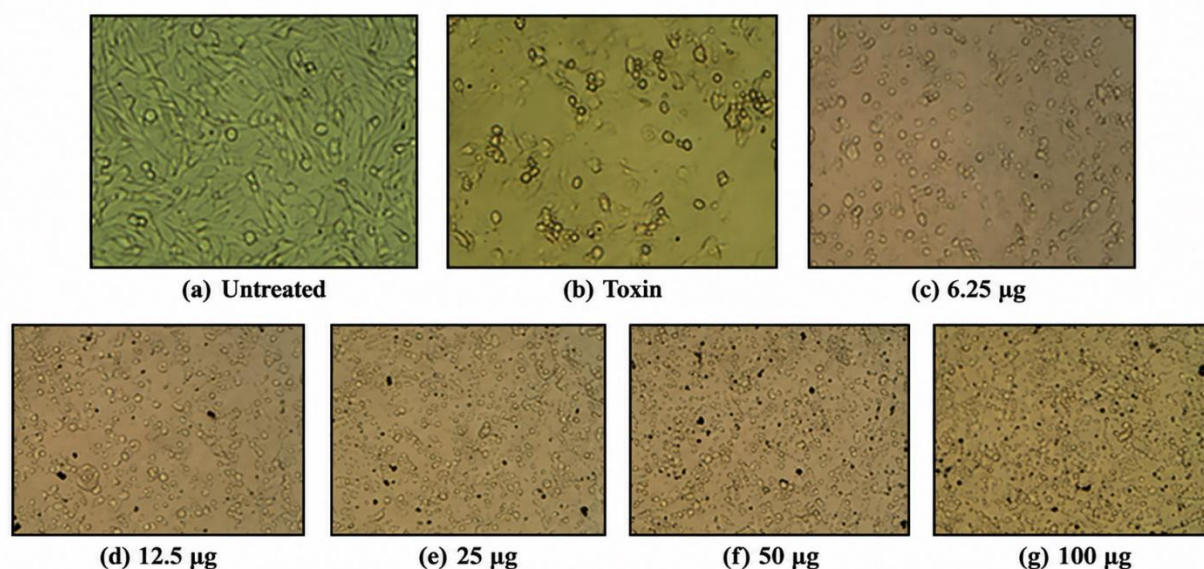


Plate: 1. Cell Viability Study of Aqueous *Citrus depressa* (Cd) Fruit Extract on Human Dermal Fibroblast (HDF) Cells



Discussion

The present study evaluated the effects of *Citrus depressa* whole-fruit extract on HDF cell viability using the MTT assay. The reported results demonstrate an overall concentration-dependent reduction in cell viability across the tested extract concentrations. At 6.25 µg/mL, cell viability was 95.49%, indicating a 14.51% reduction relative to the untreated control. Increasing the concentration to 12.5 µg/mL reduced viability to 90.92%. At 25 µg/mL, viability remained at 85.70%, while higher concentrations of 50 and 100 µg/mL produced viability values of 83.75% and 80.72%, respectively. These observations suggest that the higher concentrations of the extract had a

greater effect on HDF cellular metabolic activity under the reported assay conditions. Nevertheless, the MTT assay measures metabolic activity rather than directly measuring cell death. Changes in mitochondrial and other cellular reductive processes, along with possible chemical interference from plant extracts, can influence the measured response. These limitations should be considered when interpreting MTT results (Mosmann, 1983; Ghasemi *et al.*, 2021).

The viability value of 95.49% to 80.72% for 6.25 to 100 µg/mL concentration of the Aqueous *C. depressa* fruit extract identifies this concentration as the highest tested dose maintaining more than 80% viability in the

supplied dataset. This may support its selection as a candidate concentration for subsequent experiments, subject to confirmation of reproducibility and the suitability of the threshold for the intended application. It does not establish that the concentration is safe for all cell types or biological applications. Because the study used HDF cells, its findings relate to the response of the tested dermal fibroblasts. They do not demonstrate anticancer activity, since no cancer cell line was identified or tested in the supplied data.

Conclusion

Despite the concentration-dependent reduction, the extract preserved high cell viability retaining over 80% survival even at the maximum tested concentration of 100 µg/mL. In standard *in vitro* cytotoxicity frameworks, test substances that allow cell viability to remain above 80% are generally classified as non-cytotoxic or having high biocompatibility. This favourable safety profile indicates that the extract does not pose a severe toxic threat to normal dermal cells within this therapeutic window. Additional studies could investigate effects on other cell types, assess cell membrane integrity and apoptosis, and examine potential extract interference with the MTT reaction. The results provide preliminary evidence that *Citrus depressa* whole-fruit extract affects HDF cell viability in a concentration-dependent manner. Further validation is required before drawing

conclusions about cytotoxic potency, selectivity, skin compatibility, or therapeutic potential.

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