

## THE EFFECT OF SOURSOP (*ANNONA MURICATA L.*) ESSENTIAL OILS ON VIABILITY CELLS: AN IN-VITRO STUDY

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### ABSTRACT

**Background:** Development of new preventive agents for dental caries is needed. One of the candidates for preventive agents from natural products is Soursop leaf. The present study aimed to determine the effect of Soursop leaf oil on the cultured epithelial and fibroblast cells.

**Methods:** In this experimental study, Soursop leaf essential oils were provided, and their effect was discovered on epithelial and fibroblast cells line using MTT assay. The MTT assay was conducted to measure the activity of enzymes that reduce MTT and switch it to formazan dye creating a purple colour. Using a microplate reader, the optical density was measured at 550 nm and the absorbance value directly represented relative cell numbers.

**Results:** Data compilation and analysis were done using one-way analysis of variance. Soursop leaf essential oils exhibited variable noxious effects on cultured cells. The present study shows that epithelial cell death was less than 30% at the concentration 2.5 µl/ml while the percentage of fibroblast cell death was less than 30% at smaller concentrations of 1.25 µl/ml. Through an increase in the concentration of Soursop leaf essential oils, the toxicity of these materials substantially increased ( $p < 0.05$ )

**Conclusion:** Soursop leaf essential oils at certain concentrations may cause epithelial and fibroblast cell death.

### INTRODUCTION

The most common global oral health problems are dental caries and dental plaque. Worldwide, the incidence of caries among adults is high, affecting almost 100% of the population in the majority of countries<sup>1</sup>. In Indonesia, the most prevalent infectious oral disease is dental caries. According to

the Basic Health Survey project in 2013, the mean DMFT index was categorized high by the World Health Organization<sup>2</sup>.

Dental caries is initiated by acids from bacterial products diffusing into hard tissues and dissolving the minerals<sup>3</sup>. Irreversible cavitation occurs by demineralization of the organic substance of the

tooth, then causing the destruction of surrounding tissues to become inflamed and infected, discoloured tooth, and ultimately there is tooth loss<sup>4,5</sup>.

With the growing incidence of caries prevalence, the global demand for development of prevention and treatment methods which are effective, safe and cost-effective has expanded. Oral health maintenance can be gained, essentially, by either a mechanical or chemical agent<sup>6</sup>. Among chemical agents, antiseptic mouthwash as personal oral hygiene care has been widely used to inhibit dental plaque<sup>7</sup>. On the other hand, alcohol-based mouthwash has adverse effects on the oral cavity such as xerostomia and sore or burning sensations<sup>8</sup>.

Nowadays, new pharmacologically active aggregates from natural products have been broadly studied as alternative mouthwash ingredients<sup>9</sup>. The natural products have less adverse effects and provide economical alternatives to pharmaceutical drugs<sup>10</sup>. Traditionally, many countries in the world use the leaves of Soursop for treatment of various diseases<sup>11</sup>. Pharmacologically active constituents in Soursop leaf are flavonoids, alkaloids, saponins, tannins<sup>12,13</sup> and essential oils<sup>14,15</sup>. Previous studies indicated that Soursop leaf may be a natural agent for use in preventing dental caries as an antimicrobial on oral pathogens<sup>12,16-18</sup>. There are many studies have been reported on the cytotoxicity action of the Soursop leaf on cancer cells<sup>19</sup>, but none of them have yet reported the effect of Soursop leaf essential oils for mouthwash ingredients. The oral epithelium is a part of the oral cavity that will be exposed directly to everything consumed or gargled in the oral cavity. Considering the research for new therapeutic mouthwash, the aim of this study was to determine the effects of

Soursop leaf on epithelial and fibroblast viability cells.

## METHODS

This research is a preliminary study to examine the viability of epithelial and fibroblast on Soursop essential oils. This research was conducted based on approval from the Research Ethics Commission of the Faculty of Dentistry, Gadjah Mada University with number 1542/KKEP/FKG-UGM/EC/2018.

Essential oils isolation: In July 2018, Soursop fresh leaves were collected from Pati, Central Java District, Indonesia. A sample of Soursop fresh leaves (2000 grams) were washed and boiled in a Clevenger-type apparatus for 4 hours. The amount of essential oils obtained after each steam distillation was small. Anhydrous sodium sulfate was used to trap the oils. Prior to analysis, the essential oils were stored under refrigeration in a vial.

Cell culture: Vero fibroblast cells were cultured in M199 culture medium (Sigma) while Kb epithelial cells were planted in DMEM medium (Gibco), then added with 10% fetal bovine serum (Gibco), 0.5% fungizone (Gibco), 2% penicillin and streptomycin (Gibco), and Hepes. The confluent culture was harvested and plated into 96-well plate cells then incubated at 37°C under 5% CO<sub>2</sub> for overnight. Various concentrations of each Soursop leaf essential oil was prepared in cell culture medium then added to the cells followed by 24 hours incubation at 37°C. Untreated cells were used as the control group. The control groups included Vero and Kb cells in proper culture media. Using MTT assay, the cells were measured for cytotoxicity.

Cytocompatibility assay: The MTT assay was conducted to measure the activity of enzymes that reduce MTT and switch it to formazan dye creating a purple colour. MTT (200 µl) were added to each

well on the 96-well microplate upon 24 hours exposure to various concentrations of Soursop leaf essential oils, and then it was incubated at 37°C for 4 hours. To solubilize the formazan crystal Sodium Dodecyl Sulfate-Hydrochloride Acid (SDS-HCl) was added to each well and the well plate was incubated overnight at 37°C. Using a microplate reader, the optical density was measured at 550 nm. The assay was performed in triplicate and the absorbance value directly represented relative cell numbers. According to the following equation, the percentage of cell viability was calculated.

$$\% \text{ viable cells} = \frac{\text{Absorbance (sample-blank)}}{\text{Absorbance (control-blank)}} \times 100$$

Statistical analysis: The data were expressed as the mean  $\pm$  standard deviation (SD) values. Data

normality and homogeneity of variance were measured by the Shapiro-Wilk and the Levene's tests. The differences between means of the experimental variables were evaluated by one-way Analysis of variance (ANOVA). Values of  $p < 0.05$  were considered as statistically significant. Resulting data were statistically analysed by SPSS package program for Windows.

## RESULTS

The results obtained in this study are shown in Figure. 1. The data were normally distributed according to Shapiro-Wilk test. The results of ANOVA tests demonstrated a significant difference in optical density after exposure to Soursop leaf essential oils ( $p < 0.05$ ). Figure. 2 shows that the group treated with lowest concentration has the highest percentage of viable cells.

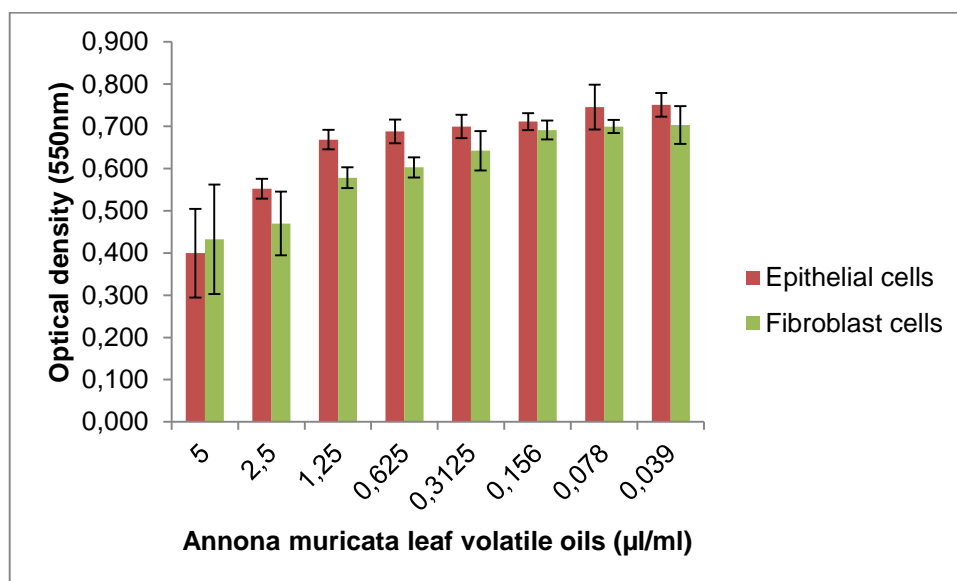
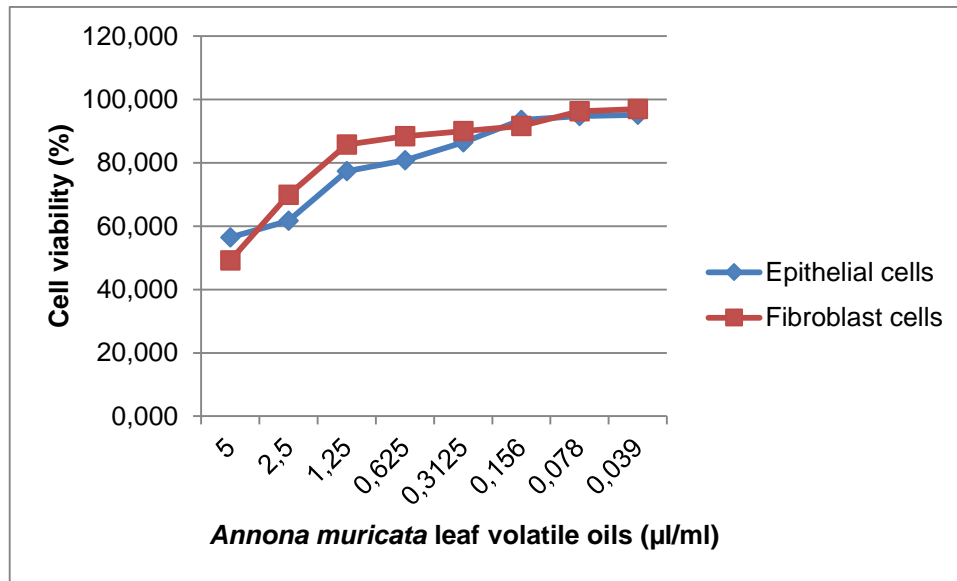


Figure 1. The absorbance of MTT assay



**Figure 2.** Effect of *Annona muricata* leaf essential oils on cell viability

## DISCUSSION

This investigation confirms the effects of Soursop leaf on epithelial and fibroblast viability cells. The development of natural remedy to prevent caries has become growing over time since its effectivity, safety and economic reason. Soursop leaf contains a wide range of biological substances such as antimicrobial, anti-inflammatory, anti-protozoan, antioxidant and insecticide agents. Such effects have been attributed to the presence of acetogenins, alkaloids, phenols, and other compounds. The main plant organs studied were the leaves, probably because they are the most traditionally used<sup>20</sup>.

This study was using Kb cell line as a model of oral epithelium, although the cell line is different from the normal oral epithelium but several studies report that the reaction from treatments given to normal cell line or oral epithelium shows the same results<sup>21,22</sup>. This study was using Vero cell line which has been widely used and published in cytotoxicity testing relating to materials used in dentistry<sup>23-25</sup>. Freimoser

et al.<sup>26</sup> claimed MTT assay is a cytotoxicity assay that measures the mitochondrial activity of live cells.

Material can be categorized as non-toxic if the percentage of cell deaths is less than 30%<sup>27</sup>. The present study shows that epithelial cell death was less than 30% at the concentration 2.5 µl/ml while the percentage of fibroblast cell death was less than 30% at smaller concentrations of 1.25 µl/ml. These results show that Soursop leaf essential oils have cytotoxic effects on the epithelial and fibroblast cells in certain concentrations. Epithelial and fibroblast cell death mechanism induced by Soursop leaf essential oils in this study has not been clarified. Soursop leaf essential oils are composed largely of the sesquiterpene derivatives. Cytotoxicity activity of Soursop leaf essential oils could probably have been due to the disruption of mitochondrial membrane that arrested cells in the G0/G1 phase, and the induction of apoptosis<sup>20</sup>. The study has the following limitations, the following is an in-vitro study and the recommended dose and response in oral humans may vary. This research was done to see if Soursop

leaf was worth pursuing, hence, studies with higher evidence can be pursued to confirm these results. Further evaluations should be done in future experiments to confirm these results.

## CONCLUSION

From our study it is concluded that *in vitro* application of Soursop leaf essential oils at certain concentrations may cause epithelial and fibroblast cell death.

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