

## PHYTOCHEMICALS ANALYSIS AND CYTOTOXIC ACTIVITY OF *Lansium domesticum* Corr EXTRACT-CISPLATIN COMBINATION AGAINST PANC-1 CELL LINE

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### ABSTRACT

The use of chemotherapy such as cisplatin in treating cancer alone can cause many problems, including side effects and resistance. Therefore, currently, efforts are being made to combine cisplatin with natural ingredients such as ethanol extract from duku leaves. Then extracted using ethanol as a solvent. The secondary metabolites of the extract were identified using LC-MS/MS with mobile phase A (0.1 percent formic acid in water) and phase B (0.1 percent formic acid in acetonitrile). The extract and cisplatin were determined by their IC<sub>50</sub> values as the basis for carrying out the combination test using the MTT method. All data obtained were processed with Microsoft Excel and analyzed with SPSS 23. The ethanol extract of duku leaves contains about 15 compounds that cannot be clearly identified because they are not listed in the LC-MS/MS library used. Interestingly, there were 5 compounds that gave a sufficiently large absorption each at a retention time of 1.08; 5.18; 9.45; 9.57; 10.70; and 10.82. Cisplatin has stronger cytotoxic properties than the extract with IC<sub>50</sub> values of 3.02±0.05 µg/mL and 28.61±0.13 µg/mL, respectively. The combination of these two substances is synergistic with a combination index <1. the combination of extract and cisplatin at doses of 1.75:0.75 and 1.75:0.375 had very strong synergy with a combination index of 0.01 (p>0.05). However, the mechanism of action of this combination is not yet known clearly, so further research is needed on it.

**Keywords:** Duku Leaf, Cisplatin, MTT Assay, Combination Index, PANC-1 Cell.

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### INTRODUCTION

Pancreatic cancer is still a deadly disease that is expected to overtake lung cancer as the second biggest cause of cancer mortality in the United States in the next twenty to thirty years.<sup>1</sup> According to GLOBOCAN 2018 estimates, it is the seventh greatest cause of cancer mortality in both men and women globally, accounting for around 459.000 new cases and 432.000 fatalities.<sup>2</sup> Pancreatic cancer is now being treated using traditional cancer therapy approaches such as chemotherapy. Cisplatin is a very potent anti-tumor chemical that is widely used in clinics to treat a variety of human solid tumors through numerous mechanisms.<sup>3</sup> Cisplatin's anti-tumor action is due to its capacity to cause DNA damage in tumor cells and interfere with their repair mechanisms. Cisplatin-induced DNA damage activates a number of signaling pathways in cancer cells, which can lead to apoptosis.<sup>4</sup> Cisplatin has been linked to serious adverse effects including nephrotoxicity, ototoxicity, neurotoxicity, nausea, and vomiting, despite its effectiveness against malignant cancers.<sup>5,6</sup> The development of combination therapy is a significant new cancer treatment trend.<sup>7</sup> In addition to reducing the side effects of chemical drug administration, combination chemotherapy can also reduce resistance.<sup>8</sup> Herbal chemicals have also been proven to work in tandem with antitumoral medications to improve therapeutic efficacy and reduce side effects.<sup>9</sup> Duku (*Lansium domesticum* Corr.) is a plant that is easy to find, especially in Southeast Asia, especially Indonesia.<sup>10</sup> Several studies reported that duku leaves have cytotoxic activity against several cancer cells so that they can be developed as anticancer

agents.<sup>11</sup> Some parts of duku that have reported cytotoxic activity are fruit, fruit peel, and seeds.<sup>12-14</sup> Meanwhile, the leaf part of this plant is not widely known for its cytotoxic activity. Several studies using duku leaves are testing their antioxidant activity with the DPPH method. Duku leaves water extract was reported to have very strong antioxidant activity with an IC<sub>50</sub> value of 5.40±1.23 µg/ML.<sup>15</sup> The aim of this study is to look into the anticancer potential and synergistic impact of duku leaves extract in conjunction with cisplatin to see if it may be used as an alternative treatment.

## EXPERIMENTAL

### Material and Methods

The materials used in this study were distilled water, formic acid, acetonitrile, PANC-1 cell, cisplatin, duku leaf samples, and ethanol. Phytochemical analysis of the samples was carried out using the LC-MS tool, and testing for cytotoxic activity on PANC-1 cells was carried out using the MTT assay method, judging from the IC<sub>50</sub> value obtained.

### General Procedure

Duku leaves were extracted by the maceration method using ethanol as a solvent. The extract was identified for its phytochemical content by the LC-MS method using 0.1% formic acid in water as the mobile phase and 0.1% formic acid in acetonitrile. Cytotoxic testing was carried out on PANC-1 cells using the MTT assay method, where the extract concentration was varied to 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm, and 7.8125 ppm. In this test, cisplatin was used as a positive comparator.

### Preparation of Extract

The air-dried and powdered leaves of duku (1000 g) were macerated with ethanol 96% (3 x 3 d, 7.5 L). The filtrate was evaporated to give a viscous extract.<sup>16</sup>

### Phytochemicals Constituent Analysis

The gradient technique was used to analyze phytochemicals from extract using TSQ Exactive (Thermo) (LIPI, Indonesia) with mobile phase A (0.1 percent formic acid in water) and phase B (0.1 percent formic acid in acetonitrile). The Hypersil GOLD aQ column had a flow rate of 40 L/min, a diameter of 50 x 1 mm x 1.9 m, and an analysis duration of 70 minutes. The data were evaluated with mzCloud and the Compound Discoverer program.<sup>17</sup>

### Cytotoxic Activity

PANC-1 cell line (1x10<sup>4</sup> cells) was grown in DMEM complete medium. After 24 hours of incubation, the medium was replaced and treated with the extract. The further procedure was followed as previously described.<sup>18</sup> Based on the IC<sub>50</sub> value, the combined concentrations of the extract and Cisplatin were determined, respectively, as 1/2, 1/4, 1/8, and 1/16 of the IC<sub>50</sub>. The calculation of the index combination is determined by the following formula.<sup>19</sup>

$$CI = (D)_1 / (D_x)_1 + (D)_2 / (D_x)_2$$

D<sub>1</sub> and D<sub>2</sub> represent the concentrations used in combinational treatment, while D<sub>x1</sub> and D<sub>x2</sub> are single-treatment concentrations that give the same response as D<sub>1</sub> and D<sub>2</sub> respectively. CI value represents the potency of extract in combination treatment with cisplatin.

### Data Analysis

Each experiment was repeated 3 times. The data obtained were processed with the help of Microsoft Excel and SPSS 23 using the ANOVA test.

## RESULTS AND DISCUSSION

### Phytochemicals Analysis of Duku Leaves Ethanol Extract

Identification of secondary metabolites from extracts is important to do. Identification will provide an overview of the compounds contained in the extract that may provide anticancer activity. In this experiment, the identification of secondary metabolites from the ethanolic extract of duku leaves was carried out using LC-MS/MS. The identification results can be seen in Fig.-1. Based on Fig.-1, there are 15 peaks that provide absorption at a retention time of 0-16 minutes with mobile phase A (0.1 percent formic acid in water) and phase B (0.1 percent formic acid in acetonitrile). Of the 15 peaks, only 6 peaks gave a sufficiently large

absorption, each at a retention time (RT) of 1.08; 5.18; 9.45; 9.57; 10.70; and 10.82. The 6 peaks whose molecular weights and possible molecular formulas have been identified can be seen in Table-1. The limited data in the library causes these compounds to be unknown. This is an opportunity for other researchers to be able to identify what compounds are contained in the extract. Based on previous research, it was reported that duku leaves contain cardiac glycosides, namely honghelin<sup>20</sup>, and triterpenoid derivatives, namely Lansium Acid XI and Lansium Acid XIII.<sup>21</sup> Triterpenoid compounds were also detected in seeds and fruit, namely Langsatides A<sup>22</sup> and Lansioside C<sup>23</sup>, respectively.

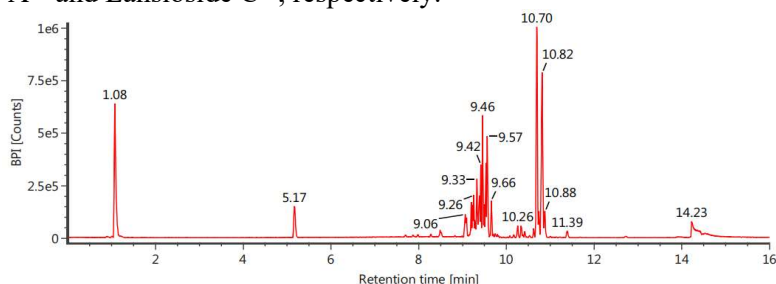


Fig.-1: The Phytochemical Content of Extract Using LC-MS/MS

Table-1: Candidate Mass and Molecular Weights of Sample Identified

No	Component name	Observed m/z	Neutral mass (Da)	Observed RT (min)	Detector counts
1	C32H42O10	609.2722	586.27780	9.45	518880
2	C45H84O15	887.5689	864.58102	10.70	1348419
3	C45H84O14	871.5744	848.58611	10.82	2008374
4	C6H11NO3	146.0807	145.07389	1.08	348608
5	C21H27N3O2	354.2184	353.21033	5.18	236384
6	C26H48O14	607.2926	584.30441	9.57	499223

### Cytotoxic Activity of Duku Leaves Ethanol Extract

Determination of the cytotoxic activity of duku leaves extract was carried out using the MTT assay method on PANC-1 cells with cisplatin as a comparison. Extracts and cisplatin were tested at various concentrations, namely 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm, and 7.8125 ppm. Cytotoxic test results were obtained in the form of absorbance which is converted into %viability (Fig.-2).

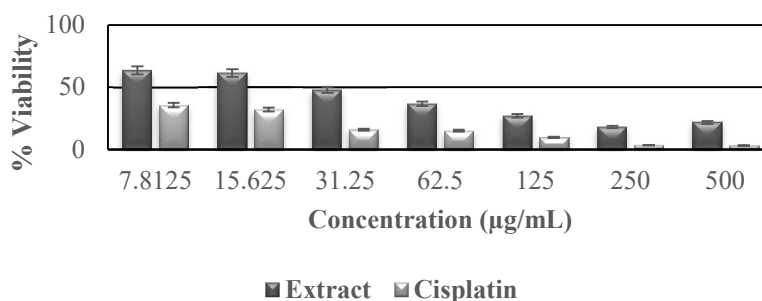


Fig.-2: Inhibition of PANC-1 Cell Population Growth by Duku Leaves Extracts,  $p < 0.05$  (mean  $\pm$  SE,  $n = 3$ )

The cytotoxic effect is an effect that is dependent on the material concentration level, the higher the material concentration, the higher the cytotoxic impact levels.<sup>24</sup> At the same dose, it was seen that cisplatin had stronger cytotoxic properties than the extract. For example, at a concentration of 500 ppm, cisplatin caused a very low PANC-1 cell viability of 3.2%, while the extract was 21.79%. The strength of the cytotoxic properties of the extract will be better illustrated by determining the  $IC_{50}$  value. Based on the calculation results, the  $IC_{50}$  of the extract and cisplatin can be seen in Table-2.

Based on the calculation results, the  $IC_{50}$  values of the extract and cisplatin were respectively  $28.61 \pm 0.13$   $\mu\text{g/mL}$  and  $3.02 \pm 0.05$   $\mu\text{g/mL}$ . If we compare the extract with cisplatin from its  $IC_{50}$  value, it is concluded that cisplatin is superior to the extract. With such an  $IC_{50}$  value, cisplatin is categorized as having very strong anticancer properties, while the extract has strong anticancer properties against PANC-1 cells.<sup>25</sup> Cytotoxic results obtained from the ethanolic extract of duku leaves on PANC-1 cells showed better activity

than extracts obtained from other plant parts, such as fruit and seeds. Cytotoxic test performed on HepG2 cells with chloroform extract samples of young fruit obtained  $IC_{50}$  of  $934.00 \pm 46.20 \mu\text{g/mL}$ .<sup>26</sup> Meanwhile, the methanol extract tested on HT-29 cells obtained an  $IC_{50}$  of  $50.00 \pm 0.02 \mu\text{g/mL}$  after being incubated for 2 days.<sup>13</sup> Referring to this, it can be stated that the ethanolic extract of duku leaves has the potential to be developed as an anticancer agent.

Table-2:  $IC_{50}$  Value of Ethanol Extract of Duku Leaves and Cisplatin

Extract	$IC_{50}$ ( $\mu\text{g/mL}$ )
Ethanol	$28.61 \pm 0.13$
Cisplatin	$3.02 \pm 0.05$

### Combination Activity of Extract-Cisplatin Against PANC-1 cell

This combination test was carried out using the MTT method. The concentration of the test substance was referenced based on the  $IC_{50}$  value obtained from a single test of extract and cisplatin. From the  $IC_{50}$  value, adjustments were made for the combination test concentration with values of 1/2, 1/4, 1/8, and 1/16 of the  $IC_{50}$ . The test results obtained are in the form of a combination index (CI) value which can be seen in Fig.-3.

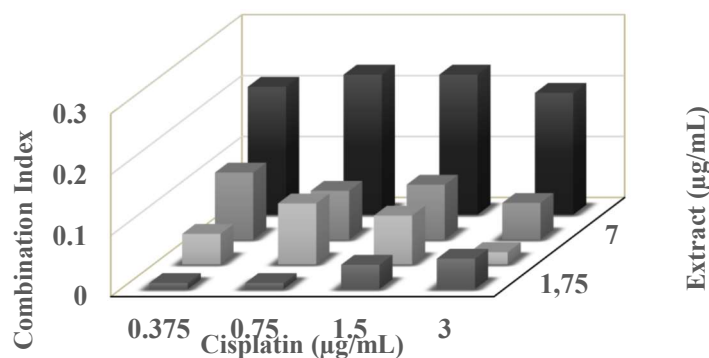


Fig.-3: Combination Index of Extract-Cisplatin Against PANC-1 cell,  $p < 0.05$  (mean  $\pm$  SE,  $n = 3$ )

Combination treatment of extract and cisplatin gave a synergistic effect at all extract concentrations (14, 7, 3.5, and 1.75  $\mu\text{g/mL}$ ) and all cisplatin concentrations (3, 1.5, 0.75 and 0.375  $\mu\text{g/mL}$ ) with CI values 0.01-0.29 (Fig.-1). The smaller the CI value, the greater the synergy between the two active substances.<sup>27</sup> Based on the above results, the combination of extract and cisplatin at doses of 1.75:0.75 and 1.75:0.375 had very strong synergy with a CI value of 0.01 ( $p > 0.05$ ).<sup>28,29</sup> Judging from these results, the use of a reduced dose of cisplatin will still have a maximum cytotoxic effect against PANC-1 cells when combined with ethanol extract of duku leaves. This is certainly very beneficial, because the side effect of cisplatin can be reduced, and chemotherapy resistance can also be avoided.<sup>30</sup> Currently, the mechanism of the combination of these active substances has not been known. Further molecular testing is needed to explain the synergistic action mechanism between the extract and cisplatin.

### CONCLUSION

Duku leaves are a plant that is easy to find in Southeast Asia, especially Indonesia. This plant contains secondary metabolites that have the potential as anticancer. To reduce the risk of side effects and resistance of cisplatin in the treatment of pancreatic cancer, it is necessary to do a combination therapy, thereby reducing the dose of cisplatin but still maintaining its activity. Duku leaf ethanol extract with cisplatin was proven to be synergistic in inhibiting the growth of PANC-1 cells. However, the mechanism of action of this combination is not yet known clearly, so further research is needed on it.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

## AUTHOR CONTRIBUTIONS

Muhammad Fauzan Lubis and Poppy Anjelina Z Hasibuan carried out research activities, data acquisition, data interpretation, and manuscript preparation. Vera Estefania Kaban and Ririn Astyka prepared the script. All authors read and agree to the published version of the manuscript. The research profile of the faculty authors can be verified from their ORCID ids, given below:

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