

# Effect Of Loquat Leaves Methanol Extract On Morphology Of Benzo(A)Pyrene Induced Rats

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**Abstract:** Cancer is a disease caused by the growth of body cells develop uncontrollably. One type of cancer that has a fairly high mortality rate in the world is breast cancer. An effort to fight against cancer is the use of chemopreventive compounds derived from plants. Loquat (*Eriobotrya japonica* Lindl.) is one of plants believed to have the potential to be anti-cancer. This study used a laboratory experimental method with a completely randomized design (CRD) with five treatments and six replications. The treatments were K + (without any treatment), K- (rats induced by Benzo  $\alpha$  Pyrene). The treatment group was the supplementation of loquat leaf extract into BAP with various dosage concentrations starting from 200 (P1), 300 (P2) and 400 (P3) mg / kg body weight. The results showed that there were no significant differences between treatment groups either for rat weight, tumor weight, tumor diameter and tumor volume.

**Index Terms:** Benzo(a)Pyrene, Breast Cancer, Loquat

## 1. INTRODUCTION

Cancer is a disease caused by the growth of body cells that develop uncontrollably. The growth of these cells can cause disruption in tissue and physiological functions that can be fatal to human health and survival. Cancer is one of the deadliest diseases in the world and categorized as the cause of most deaths in the world. The growth of this disease increase from year to year rapidly<sup>1</sup>. Based on the results of a census conducted in 2012, there were reported to be 14.1 million cases of cancer in the world and 8.2 million of them died. Among the high mortality rates for this disease, almost 57% were experienced by developing countries[1]. One of the most common types of cancer is breast cancer. Breast cancer is the most common cancer among women and is the highest cause of death after lung cancer[2]. Research that has been done shows that in the United States, breast cancer sufferers reached 232,340 reported cases with a death rate of 39,620 people during 2013[3]. In 2015, still in the United States the number of deaths due to breast cancer increased up to 4,290 cases[4]. Meanwhile in Asia, as many as 639,824 cases of breast cancer were recorded in 2012[5]. A Research reported that five countries in Asia with the highest mortality rates were India, China, Indonesia, Pakistan and Japan. Indonesia in 2012 had 48,998 cases of breast cancer with a mortality rate reaching 19,750 patients[5]. There are several cancer treatments available such as surgery, chemotherapy, and radiotherapy. All of these treatments are commonly received by cancer sufferers. However, the aforementioned treatments have long-term effects and induce severe side effects in

patients. Therefore, other alternatives are developed to reduce the effects of cancer. One of them is developing chemopreventive compounds derived from plants[6]. Chemopreventive compounds are chemical compounds derived from plants that have anti-cancer potential as a new effective method by utilizing compounds from nature. One of the plants that is believed to be potentially anti-cancer is the loquat plant (*Eriobotrya japonica* Lindl.)[7]. Loquat (*Eriobotrya japonica* Lindl.) as known as loquat are native to China which grow widely over the world. Loquat has many health benefits. In their country, loquat plants have been used for herbal medicine. The leaves of loquat contain several phytochemical compounds such as triterpenes, sesquiterpenes, flavonoids, and tannins[8]. The content of these phytochemical compounds makes loquat plants very potential for treatment. Various studies have been conducted to test the effectiveness of loquat plants in treating diseases. Loquat plants have the potential for anti-inflammatory activity, as an antioxidant, anti-diabetes, treatment of acute bronchitis, anti-virus, anti-mutagen and anti-tumor[9]. Based on the description above, this research was conducted to find out the effectiveness of loquat extract on the growth of cancer cells and determine the accuracy of the dosage to inhibit the growth of cancer cells. It is hoped that through this research, further benefits can be revealed from the use of loquat extract in inhibiting the growth of cancer cells as well as new alternatives in the treatment of cancer

## 2. MATERIALS and METHODS

This research was conducted from 2018 to November 2019 and was located at the Animal Structure and Development Laboratory of the Faculty of Mathematics and Natural Sciences, the Natural Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences and the Anatomy Pathology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara.

### 2.1 Preparation of Extraction

Loquat leaves were collected from community plantations in Brastagi area of Karo Regency, North Sumatra. After being collected from the field, the cleaned loquat leaves were weighed and dried using an oven at 40°C to fit the standard of simplicia moisture content. Dried simplicia then sifted and sieved using a B30 sieve. Loquat leaf powder was extracted

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by maceration method using methanol. The liquid extract obtained was then thickened using a rotary evaporator.

## 2.2 Animal Preparation

Animals used for experiment have been divided into 5 groups and underwent an adaptation process for 2 weeks by equalizing the feeding and drinking water. Then the test animals in both positive control group and the treatment group were injected BAP with a single dose of 50 mg / kg body weight in the area around the mammary gland. After BAP injection, the development of lumps on experimental animals were monitored every day by performing palpation. After 1 cm of cancer tissue was formed, loquat extract then given to the rats orally according to the treatment dose of 200, 300, 400 mg / kg body weight for 30 days. On the 31st day, all test animals were killed by cervical dislocation in an anesthetized state and the mammary glands were taken, the tumors were weighed, the diameter of the tumors were measured, and the volume of the tumors were calculated.

## 2.3 Data Analysis

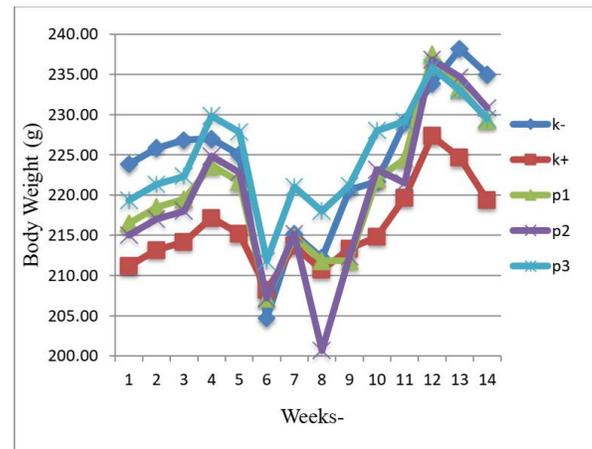
The data from various tests were arranged in tables. Homogeneity and statistical distribution were analyzed using SPSS software. The homogeneous and normally distributed data then tested using ANOVA at significance level of 5%. If the data were significantly different ( $p < 0.05$ ) they will be proceed into Post Hoc-Bonferroni test. However, if the data are not normally distributed and / or the variance is not homogeneous, then the data will be transformed. If the data still does not have a normal distribution and / or the variance of the data is not homogeneous, then the data will be analyzed using a non-parametric test, the Kruskal Wallis test. As for Post-Hoc Test, Duncan's Multiple Range test (DMRT) will be performed to measure specific differences between two treatments.

## 3. RESULTS AND DISCUSSION

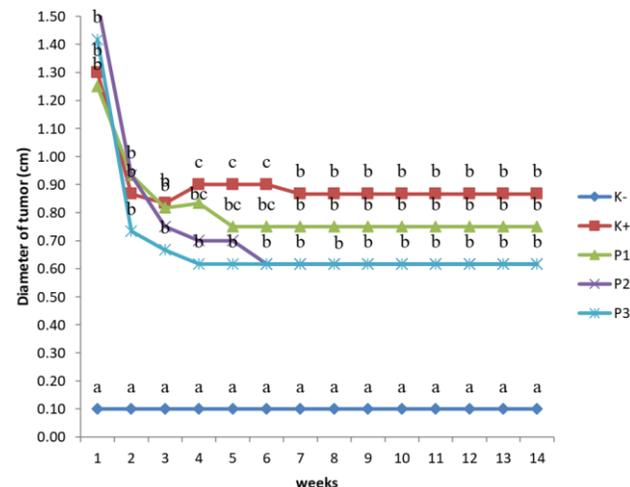
### 3.1 Body Weight

The results of this research show that the rats with the highest body weight were found in K-group with a weight of 224.20 g, while the lowest body weight was found in K + group with a weight of 215.89 g. Furthermore, one-way ANOVA test showed that there were no significant difference ( $p > 0.05$ ) in changes of rat's body weight. However, there was a tendency of decrease or increase in body weight between treatments (Figure 1). Fluctuations in body weight of rats are shown in Figure 1. The rat's body weight began to drop dramatically from the 5th week to the 6th week, then rose in the 7th week before experiencing a decrease in the 8th week. At week 9 to week 12 the body weight gradually increases and went down at week 13 and week 14. The causative factor for uninfluenced loquat leaf extract on the body weight of rats was considered to be due to the lack of time in dosing. However, treatment groups P1, P2, and P3 had a higher body weight compared to K+ treatment group. Loquat leaf methanol extract contains high antioxidant compounds used to inhibit oxidation reactions. Antioxidant binds free radicals and highly reactive molecules, resulting in cell damage inhibition[10]. The results showed the antioxidants contained in the ethanol extract of loquat leaves were classified as strong antioxidants and had the potential to be developed to treat various diseases[11]. Phytochemical screening results state that the loquat ethanol

extract contains secondary metabolites of flavonoids, glycosides, tannins and triterpenoids / steroids.



**Fig. 1.** Effect of Loquat Leaves Methanol Extract on Body Weight of Rats. K- = Negative Control; K+ = Positive Control (BAP); P1, P2 and P3= BAP + Loquat Leaves Methanol Extract (200, 300 and 400 mg/kg) ; in gram (g).



**Fig. 2.** Effect of Loquat Leaves Methanol Extract on Tumor Diameter of Rats. K- = Negative Control (without treatment); K+ = Positive Control (BAP); P1, P2 and P3 = BAP + Loquat Leaves Methanol Extract (200 mg/kg, 300 mg/kg and 400 mg/kg) ; in centimetre (cm)

### 3.2 Tumor Diameter

The results of this research indicate the treatment groups K +, P1, P2, and P3 have an increase in tumor diameter after the induction of benzo(a)pyrene. Measurement of initial diameter of the tumor showed the same results for almost all treatments. At the time of the induction of benzo(a)pyrene, the solution injected into the breast of rats has the same volume of 50mg / Kg BB of rat. Statistical analysis showed there were differences between the K-treatment groups and the K +, P1, P2 and P3 treatment groups. The diameter of the tumors in the treatment groups P1, P2 and P3 began to decrease in the week 2 and remained at week 7 until the week 14. Tumor diameter in treatment group K + increased at weeks 4, 5, and

6 until finally stabilized at week 7 to week 14. The administration of loquat leaf extracts at week 13 and week 14 does not affect the tumor diameter. It is presumably the short time of loquat extract administration to rats and the miscalculation of the dosage affects the reduction in tumor diameter (Figure 2). In the first week after the injection of benzo (a) pyrene, the rats' breasts in K +, P1, P2 and P3 treatment groups were inflamed marked by swelling and redness of the skin around the breast. Inflammation is a normal response caused by damage to the tissue caused by damaging chemicals, microbiological substances, and physical trauma[14]. Tumors begin at the site of chronic inflammation. The inflammatory process can also cause a proliferation process in cells, then the affected tissue will experience cell death (necrotic)[19]. Necrotic cells will emit a pro-inflammatory signal to the surrounding tissue. This signal will secrete bioactive to stimulate the surrounding cells to proliferate[15].

### 3.3 Tumor Weight

The results of statistical analysis for tumor weight (Table 1) shows a significant difference ( $P < 0.05$ ) between treatments. The difference was seen in the K- and K + while P1, P2 and P3 did not show any significant difference when compared to the K- and K + treatments. The data in Table 1 shows the reduction in tumor weight of the K + treatment from 0.84 gr to 0.51, 0.55 and 0.55 gr in the treatments P1, P2 and P3. This decrease indicates that loquat extract has the ability to reduce tumor weight. These results are in accordance with previous studies that provided extracts of *Eriobotrya fragrans* in rats induced by A549 cells subcutaneously on the legs for 2 weeks of treatment. Although positive control (without treatment) and treatment group (given the extract of *Eriobotrya fragrans*) experienced a decrease in tumor weight, both groups were not significantly different[12]. The reduction in tumor weight might be caused by the secondary metabolites contained in methanol extract of loquat leaves which is believed to be potential as an anti-tumor. In addition, loquat leaf methanol extract has the ability to inhibit cell proliferation and accelerate cell death. Tumor weight loss that occurs in test animals is the effect of Ursolic Acid (UA) derived from loquat plants that have the ability to suppress tumor growth[13].

### 3.4 Volume of Tumor

The statistical analysis of tumor volume (Table 2) shows a significant difference ( $P < 0.05$ ) between treatments K- with K +, P1, P2 and P3. Statistically, P1, P2 and P3 were not significantly different compared to K +. However, there was a tendency of decrease in tumor volume in treatments P1, P2 and P3 by 0.83, 0.85 and 0.85 ml respectively when compared to the K + with a volume of 1.08 ml. The level of reduction in tumor volume is presented in Table 3. The results in this study are in accordance with the results of previous studies which stated the volume of tumors in test animals that have been supplemented by loquat extract were lower compared to positive control treatments (rats without given loquat extract)[16]. The results in this study are in accordance with the results of previous studies which stated the volume of tumors in test animals that have been given loquat extract decreased when compared with positive control treatments without given loquat extract<sup>16</sup>. The same results were reported in test animals given ursolic acid from wild loquat plants showed a reduction in the volume of tumors that formed when compared with the group of test animals that were not given

ursolic acid[12]. Ursolic acid is a group of triterpenoid compounds found in loquat extracts used as an antitumor. One mechanism in inhibiting tumor cells is to dispel hypoxic signals in tumor cells[13]. Hypoxia is a condition which cells lack oxygen supply. Hypoxia stimulates a complex cell signaling network in cancer cell causing hypoxia inducible factor (HIF). HIF has an important role in regulating genes involved in invasion and metastatic events in breast cancer, therefore hypoxia signals occur in tumor cells must be driven to prevent the spread of cancer to other tissues[17]. Not only expressing HIF, hypoxia also stimulates the formation of complex signals in cancer cells including signals for the Phosphoinositide 3 Kinase (PI3K) pathway, Mitogen Activated Protein Kinase (MAPK), and Nuclear Factor Kappa B (NFkB). These pathways have a big impact on the cancer cell growth[18].

**Table 1**

*Weight of breast tumor in rats after supplementation of different dose of loquat extract*

Treatments	Tumor Weight (g) x ± SD
K- <sup>(1)</sup>	0.00 ± 0.00 <sup>a</sup>
K+	0.84 ± 0.66 <sup>b</sup>
P1	0.51 ± 0.33 <sup>ab</sup>
P2	0.55 ± 0.35 <sup>ab</sup>
P3	0.55 ± 0.60 <sup>ab</sup>

Note: Similar letter are not significantly different based on duncan's MRT ( $p < 0.05$ )

**Table 2**

*Tumor Volume of Rats by different dose of loquat extract*

Treatments	Tumor Volume (ml) x ± SD
K- <sup>(1)</sup>	0.00 ± 0.00 <sup>a</sup>
K+	1.08 ± 0.49 <sup>b</sup>
P1	0.83 ± 0.25 <sup>b</sup>
P2	0.85 ± 0.27 <sup>b</sup>
P3	0.85 ± 0.44 <sup>b</sup>

Note: Similar letter are not significantly different based on duncan's MRT ( $p < 0.05$ )

## 4. CONCLUSION

The treatment of loquat leaves extract had not shown a significant effect on the body weight in rats, weight of tumor, volume and diameter of tumor mammary tissue after induced cancer by Benzo(a)Pyrene but there is a reduction in the size of cancer its mean the methanol extract of loquat leaves has a potential to anticancer activity. The bioactive compound that found in loquat leaves extract is believed to have its role as anticancer substance.

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