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Research Articles

The potential of ethanol extracts 96% mature Papaya fruit seeds (*Carica papaya L*) as anti-diarrhea medicine

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ABSTRACT

Mature papaya fruit seeds (*Carica papaya L.)* reduce diarrhea because of phytochemical compounds. This study aimed to determine the potential of 96% ethanol extract of mature papaya fruit seeds as antidiarrhea. This study used experimental laboratory research with pre and post-test with control group design. The research subjects were 25 mice divided into 5 groups, KI group (negative control), KII (positive control) loperamide, and KIII-KIV-KV given treatment with a dose (200 mg; 400 mg; 800 mg). A decrease in diarrhea is known by observing the consistency of feces, stool weight, and stool diameter. Data were analyzed by the Kruskal Wallis test and the Mann Whitney Post Hoc test. The results of the consistency of feces in group K(I) were compared with groups of K(II), K(III), K(IV), K(V) p=0.046, 0.083, 0.014, and 0.014. In stool weight K(I) compared to K(II), K(III), K(IV), K(V) with p=0.053, 0.102, 0.016, and 0.053. In the observation of absorption diameter K(-) compared to K(+), KI(200), KII(400), KIII(800) the p=0.121, 0.0197, 0.051 and 0.21. In conclusion, the mature extract papaya fruit seeds can reduce diarrhea seen from the stool consistency in the group extract 400 and 800 mg/Kilograms body weight and fecal weight in the 400mg/Kilograms body weight extract group.

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INTRODUCTION

Diarrhea is still a global disease that occurs in developing and developed countries. Diarrhea increases the expenditure of feces with a softer or more fluid consistency. It can even be water and occurs at least three times in 24 hours (Anggreli *et al*., 2015). Approximately 1.8 billion people die every year worldwide due to diarrhea with complications such as malnutrition and impaired immunity (Kemenkes RI, 2012). Of all child deaths due to diarrhea, 78% occur in Africa and Southeast Asia (Farthing *et al*., 2012). The prevalence of diarrhea in Indonesia is still fluctuating. There are five provinces with the highest incidence of diarrhea; Papua (6.3% and 14.7%), South Sulawesi (5.2% and 10.2%), Aceh (5, 0% and 9.3%), West Sulawesi (4.7% and 10.1%), and Central Sulawesi (4.4% and 8.8%) (Candrasari *et al*., 2011.).

Bacterial and viral infections are the leading causes besides parasites and fungi. The most common bacteria are *Escherichia coli* and *Salmonella* sp. while for the virus most often found in cases of acute diarrhea is Rotavirus (Nurhalimah *et al*., 2014). Based on the results of Bonkoungou's study in Ouagadougou, Burkina Faso with subjects under five years of age found *Escherichia coli* bacteria ranked second in diarrhea at 24% after *Rotavirus* by 30%, *Salmonella* sp. at 9%, *Shigella* sp. at 6%, *Adenovirus* by 5 % *and Campylobacter* by 2% (Marcdante*, et al.,* 2014). Acute diarrhea is caused by an inflammatory process in the intestine or infection that directly affects the secretion of enterocytes and absorption function (Riskesdas*,* 2013).

Salmonella sp. bacteria adaptive hosts in animals and infections in humans in the gastrointestinal tract, including the stomach, small intestine, and large intestine. Eight to forty-eight hours after food contaminated by *Salmonella* sp.*,* sudden pain arises with soft or runny diarrhea, sometimes accompanied by mucus, blood, nausea, vomiting, fever with a temperature of 38 to 39 degrees Celsius (Siti *et al*., 2015).

Some previous studies explained that some medicinal plants effectively treat diarrhea because of the content of tannins, phenols, saponins, essential oils, alkaloids, and flavonoids such as guava leaves (Bonkoungou *et al*., 2013). Other medicinal plants still not utilized by the community are papaya seeds. The number of papaya production from year to year is increasing. This is in line with the increasing number of papaya seeds produced by the community. Papaya seeds have many benefits as an antibacterial effective against the bacteria *Escherichia colli*, *Salmonella*, and *Staphylococcus* (Irianto, 2013).

The administration of *beluntas* leaf extract contains tannins in male mice induced by *Salmonella typhimurium* bacteria to reduce the antidiarrheal effect (Nurhalimah et al., 2015). In addition, *Carica pubescens* seeds had been proven effective in lowering the diarrheal frequency in mice. Previous research by Purwaningdyah. *et al*. (2015) using papaya seeds can provide antidiarrheal effects. The study showed that papaya seeds with plant compounds could reduce the best dose 800mg / Kilograms body weight. Based on this, researchers are interested in testing the potential of mature ethanol extract 96% papaya seeds as an antidiarrheal drug in mice. The difference from the previous research was the production of 10^7 CFU / ml suspension with 0.2 ml of feed, using mature papaya fruit, and using 96% ethanol. Therefore this study aims to determine the potential of papaya fruit seeds (*Carica papaya L*) mature as an antidiarrheal drug and to know the potential of using dried papaya seeds as a diarrhea drug with the correct dose of use to reduce the use of chemical drugs for the treatment of diarrhea.

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METHOD

This research used experimental research design pretest and post-test with control group design methods. This study was conducted in the Pharmacology laboratory of the Faculty of Medicine, Muhammadiyah University, Surakarta. This study has passed ethical clearance No: 1204 / A-1 / KEPK-FKUMS / V / 2018. This research used the mature papaya fruit seeds (*Carica papaya L*) from the Mojosongo area, Boyolali. This study used 25 Swiss strains of mice using the Federer formula (Pangesti *et al*., 2013).

Making ripe papaya seed extract

The mature papaya fruit seeds (*Carica papaya L*) were washed, weighed, and dried. After washing and drying, the extraction process was carried out on papaya seeds. The process of extracting the dried papaya (*Carica papaya L*) fruit seeds was mashed and immersed in 96% ethanol. It stirred evenly and left to settle filtered with filter paper. Then rotatory evaporator was carried out at 60° C and evaporated with a water bath at 60 ° C until an extract was obtained thick.

Determination of dosage and treatment in test animals

Mice were divided into five groups consisting of 5 mice each group. They were weighed before treatment and then given a suspension of *Salmonella typhimurium* bacteria with a dose of 10^7cfu / ml as much as 0.2 ml (Marcdante, et al., 2014). The mice were observed to have diarrhea. After that, the consistency of feces, stool weight, and feces absorption diameters were observed.

The positive control group was a group of mice treated with loperamide. The recommended dose for diuretic effects is 4 mg/day. Thus, the dose of loperamid for mice was 0.01 mg / 20 grams body weight of mice. Loperamide 0.01 mg is then dissolved in 2 ml of water and given orally.

The negative control group was a group of mice given oral treatment with aqua dest. Group III, IV, and V groups of mice given 96% ethanol extract of papaya seeds ripened at a dose of 200 mg/kilograms body weight, a dose of 400 mg/kilograms body weight, and a dose of 800 mg/kilograms body weight were dissolved in 2 ml of aqua dest orally. Then observe the mice until the mice do not experience diarrhea for 10 hours.

RESULTS

The yield is 11.91% because it gets 123 grams of thick extract of ripe papaya fruit seeds from 1033 grams of Simplicia. Stool weight observations were made from the beginning before the diarrhea was 0.1 to 0.4. At the time of administration with *Salmonella* bacteria, the mice were diarrhea on filter paper. The feces of mice weighed with an average of 1.03grams, the positive control group averaged 1.04 grams, ripe papaya seed extract 200 mg/kilograms body weight averaged 1.025 grams, papaya fruit extract 400 mg/Kilograms body weight mean stool weight of 1.042gram and ripe papaya fruit seed extract 800 mg/Kilograms body weight with an average of 1.033 grams. Observation of feces consistency and feces absorption diameters on filter paper showed stool consistency in *Salmonella*-induced mice, which was soft and runny after administration of extract papaya fruit seeds did not get stool consistency and diameters of feces uptake in mice, or the result is zero.

Chi-square test results on the addition of feces consistency p-value = 0.005 and expected value of less than five there is 100% number of cells. The results of stool consistency did not meet the chi-square requirements, so an alternative test with the Kruskal Wallis test results p-value = 0.007. It showed a relationship between fecal consistency in the treatment group followed by the post hoc test.

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Shapiro Wilk test results on feces weight value p = 0.000 and feces absorption diameter p = 0.266. After extracting, the drug obtained stool weight p = 0.000 and absorption diameter p = 0.000. The distribution of feces weight data after extract and drug treatment obtained a value of p <0.05, indicating that the data distribution is not normal.

Based on Table 5, the Kruskal Wallis test of each group by observing the consistency of feces, feces weight, and diameter of feces absorption showed no statistically significant differences. In Table 6, there is a significant difference because mice do not experience diarrhea. This shows that mature papaya fruit extract (*Carica papaya L*) has the potential to reduce diarrhea by observing fecal consistency, absorption diameter, and stool weight.

Table 1. Results of Kruskal Wallis Test After Giving Medication and Extract

Antidiarrheal observation	P-Value	Interpretation
Stool consistency after administration of extracts and drugs	0.007 *	There are differences
Stool weight after administration of extracts and drug	0.008 *	There is a difference
Diameter of fecal absorption after administration of extracts and drugs	0.008 *	There are differences

* Different meaningful (p <0.05)

Table 2. Results of the Mann Whitney Antidiare Test on Stool Consistency Observations

Group	P-Value	Interpretation
Negative controls - Positive controls	0.046 *	There are differences
Negative control - 200 mg extract	0.083	There is no difference
Negative control - Extract 400 mg	0.014 *	There is a difference
Negative control - Extract 800 mg	0.046 *	There are differences
Positive control - Extract 200 mg	1000	There is no difference
Positive control - Extract 400 mg	1000	There is no difference
Positive control - 800 mg extract	1000	There is no difference
200 mg extract - Extract 400 mg	1000	There is no difference
200 mg extract - 800 mg extract	1000	There is no difference
400 mg extract - 800 mg	1000	extract There is no difference

* Different meaningful (p <0.05)

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Table 3. Mann Whitney Antidiare Test Results in Observation of Absorption Diameter

Group	P-Value	Interpretation
Negative control - Positive control	0.121	There is no difference
Negative control - 200 mg extract	0.197	There is no difference
Negative control - Extract 400 mg	0.051	There is no difference
Negative control - 800 mg extract	0.121	There is no difference
Positive control - Extract 200 mg	1000	There is no difference
Positive control - Extract 400 mg	1000	There is no difference
Positive control - 800 mg extract	1000	There is no difference
200 mg extract - Extract 400 mg	1000	There is no difference
200 mg extract - 800 mg extract	1000	There is no difference
400 mg extract - 800 mg extract	1000	There is no difference

* Different meaningful ($p < 0.05$)

Table 4. Mann Whitney Antidiare Test Results in Stool Weight Observation

Group	P-Value	Interpretation
Negative control - Positive control	0.053	There is no difference
Negative control - 200 mg extract	0.102	There is no difference
Negative control - Extract 400 mg	0.016 *	There are differences
Negative control - 800 mg extract	0.053	There is no difference
Positive control - Extract 200 mg	1000	There is no difference
Positive control - Extract 400 mg	1000	There is no difference
Positive control - 800 mg extract	1000	There is no difference
200 mg extract - Extract 400 mg	1000	There is no difference
200 mg extract - 800 mg extract	1000	There is no difference
400 mg extract - 800 mg extract	1000	There is no difference

* Different meaningful ($p < 0.05$)

To find out the differences between before and after extracts administration can be seen in Tables 5 and 6 below:

Table 5. Results of Kruskal Wallis Test Before Giving Medication and Extracts

Antidiarrheal Observation	P-Value	Interpretation
Stool consistency before administration of extracts and drugs	0.499	There is no difference
Stool weight before administration of drugs and drugs	0.889	There is no difference
Diameter of fecal absorption before administration of extracts and drugs	0.873	There was no difference

* Different meaningful ($p < 0.05$)

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Table 6. Results of the Kruskal Wallis Test After Giving Medication and Extracts

Antidiarrheal Observation	P-Value	Interpretation
Stool consistency after administration of extracts and drugs	0.499	There is no difference
Stool weight after administration of adhesives and drugs	0.889	There is no difference
Diameter of fecal absorption after administration of extracts and drugs	0.873	There was no difference

* Different meaningful ($p < 0.05$)

DISCUSSION

This study observed stool consistency of diarrhea mice. The parameters of this diarrhea are marked with stool along with the diameter of feces absorption on filter paper. This study found that feces consistency in Salmonella-induced mice was soft and runny due to bacterial infection, which caused intestinal hyper peristaltic and released a lot of fluid (Nurhalimah, 2014). In the chi-square test, fecal consistency was obtained $p = 0.005$, and the expected value of less than five there were 100% of cells. The results of stool consistency did not meet the chi-square requirements, so an alternative test with the Kruskal Wallis was obtained $p = 0.007$, which showed a relationship between fecal consistency in the treatment group then followed by the post hoc test.

The Mann-Whitney Post Hoc test showed a value of $p < 0.05$ found in the ratio between negative controls with positive controls, negative controls with extracts of 400 mg / Kilograms body weight, and 800 mg/ Kilograms body weight. These findings indicate significant differences in the observation of stool consistency. This is in line with the research of Purwaningdyah *et al.* (2015), where there was a decrease in stool consistency after administering papaya fruit extract.

Mice experience diarrhea after administration of *Salmonella* bacteria. The *Salmonella typhimurium* bacteria invaded the small intestinal mucosa, expelling the toxin to stimulate the intestinal epithelium. This stimulation can increase the intestinal peristaltic and secretes a lot of fluid. In the Kruskal Wallis test, the stool weight between diarrhea and after administration of extracts of loperamide and drug showed significant differences. These results are in line with Purwaningdyah. *et al*. (2015). The study showed decreased fecal weight after administering extracts and loperamide (Wijayanti *et al*., 2017). The extracts and loperamide slow down the movement of intraluminal fluid and allow more absorption and reduced motility (Purwaningdyah *et al*., 2015).

The Mann-Whitney Test aimed to compare between groups of fecal weight treatment, namely the negative control group-group III extract of 400 mg/Kilograms body weight obtained $p = 0.016$ ($p < 0.05$). It means that there was a difference in the weight of stool without treatment and given papaya fruit extract ripe at a dose of 400 mg/Kilograms body weight. This result is different from the previous study. The most effective dose to reduce fecal weight is papaya seed extract at a dose of 800 mg/ Kilograms body weight (Wijayanti *et al*., 2017).

The Mann-Whitney test was also used to compare the positive control group of extracts of papaya fruit (*Carica papaya L*) ripe. There is no difference between the groups. These results are similar to the research conducted by Fristia

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(2011). The study tested the antidiarrhea effect with ethanol of papaya leaves in mice. The study showed that papaya leaves did not have antidiarrheal effects by not reducing the weight of feces.

From the results of this study in the positive control group (extracts of papaya fruit seeds with doses of 200 mg, 400 mg, and 800 mg), the stool production was zero. Loperamide drugs and extracts can slow gastrointestinal motility through the circular and longitudinal muscles of the intestine. Tannin from papaya fruit seeds extracts functions as an astringent that can bond and form a membrane on the microbial reaction. So that condensed oil can be used for treatment in a mixture of less lost liquid from the digestive tract. Tannin binds to the tannate protein to facilitate the amino acid to be absorbed by the body, then attaches to the malein through the body to reduce the secretion of liquid and nasal mucus, which results in constipation (Riddle *et al*., 2016).

The p-value = 0.08 was obtained at the stool weight after drug administration and papaya seed extract in the Kruskal Wallis test. Statistically, it shows a significant difference from diarrhea until after the extract and loperamide drug was given. This research aligns with previous research conducted by Nurhalimah *et al.* (2015). This shows that ripe papaya seeds contain tannins which can suppress intestinal motility so that fluid discharge decreases (Enda, 2017). This study is also in line with Nurhalimah's research *et al*. (2015), which decreases fecal diameter after administration of *beluntas* leaf extract (Riddle *et al*., 2016).

Antidiarrheal studies can be shown to reduce diarrhea compared to the negative control group by observing feces weight and diameter of feces uptake. While the statistical test of the ethanol extract group of ripe papaya fruit seeds at doses of 400 mg/Kilograms body weight and 800 mg/Kilograms body weight can reduce the effects of diarrhea by decreasing fecal weight and stool consistency.

Papaya seeds contain alkaloids as antibacterial. Papaya seeds contain alkaloids, steroids, phenols, tannins, essential oils, terpenoids, and saponins (Irianto, 2013). The mechanism of action of phenols as an antibacterial is by denaturing cell proteins. Hydrogen bonds formed between phenols and proteins cause protein structures to become damaged (Zarghami *et al.*, 2017). The mechanism of action of alkaloids as an antibacterial disrupts the constituent components of peptidoglycan in bacterial cells. The cell wall layers are not formed intact and cause cell death. The mechanism of action of flavonoids as an antibacterial can be divided into three: inhibiting the synthesis of nucleic acids, inhibiting the function of cell membranes, and inhibiting energy metabolism. The mechanism of action of saponins as an antibacterial is by causing leakage of proteins and enzymes in cells, and cell death occur (Enda, 2017).

A limitation in this study is that no quantitative phytochemical tests were carried out to find out what percentage of tannin was obtained from the extraction results and observations when diarrhea occurred in each test animal.

CONCLUSION

The mature extract papaya fruit seeds have the potential to reduce diarrhea. This study showed that feces weight and diameter of feces uptake reduced compared to the negative control group in animal tests. In addition, the statistical test of the ethanol extract group of ripe papaya fruit seeds at doses of 400 mg/Kilograms body weight and 800 mg/Kilograms body weight can reduce the effects of diarrhea by decreasing feces weight and stool consistency.

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