FORMULATION AND ACTIVITY TESTING OF A PREPARATION OF CHERRY FRUIT (*Muntingia calabura* L.) SYRUP AS ACUTE ANTIDIARRHEA IN MICE

Widya Suci Musfirah[1], Abdul Rahim*[1], Homely Fachreza[1], Karmelita Ani[1], dan Syifa Syahira[1]

[1.] Departement of Pharmacy, Faculty of Pharmacy, Mulawarman University, Samarinda, Indonesia, 75242, 081907390033

Email: abdulrahim@farmasi.unmul.ac.id

Abstract

The problem of diarrhea is an important problem to overcome, especially in the Samarinda area, East Kalimantan Province. The number of diarrhea cases is increasing every year. In 2021, there were 34,436 cases nationally and 4,706 cases in Samarinda. Viruses and bacteria can cause diarrhea. The cherry plant has antibacterial and antidiarrheal properties because of its Alkaloid, flavonoid and tannin content. This research focuses on investigating the potential of syrup from the secondary metabolite juice of cherry fruit syrup in curing acute diarrhea in male Wistar rats. The research method used was to compare 5 groups with varying concentrations of cherry syrup. Measurements include assessing when the diarrhea started, how often the diarrhea occurs, its texture, severity, and how long the diarrhea lasts. Research shows that cherry syrup has a positive effect in reducing diarrhea symptoms such as bowel movement frequency, consistency, weight and duration. Based on the results of research conducted, it shows that cherry fruit syrup (*Muntigia calabura* L.) can treat acute diarrhea.

Keywords: formulation, syrup, cherry fruit, diarrhea

Background

One of the diseases that is a major problem in Indonesia is diarrhea. Diarrhea is a disease characterized by changes in the shape and consistency of stool as well as excessive frequency of defecation (more than 3 times in one day) (Prawati and Haqi, 2019). Diarrhea is divided into acute diarrhea and chronic diarrhea, where diarrhea that lasts less than 2 weeks is known as acute diarrhea. If diarrhea has lasted for 2 weeks or more, it is classified as chronic diarrhea. Symptoms that arise from diarrhea include nausea, vomiting, abdominal pain, heartburn, tenesmus, fever, and signs of dehydration. In addition, the feces excreted may be accompanied by or without mucus and blood (Amin, 2015).

One alternative treatment for diarrheal disease at a fairly affordable cost can be done by utilizing the potential of the cherry plant (Muntingia calabura L.). The cherry plant (Muntingia calabura L.) contains secondary metabolite compounds in the form of flavonoids and tannins which act as antibacterial and antidiarrheal (Jaya & Eliza, 2022). These compounds can be used to treat diarrhea. Based on the results of research conducted by Prasetyo (2015), cherry leaf extract has antibacterial activity at concentrations of 25%, 12.5%, 6.25% and 3.125% which can inhibit the growth of Bacillus subtilis and Shigella dysenteriae bacteria. Prasetyo's research (2015) also showed the antibacterial activity of cherry leaf extract at concentrations of 25%, 50%, 75% and 100% which effectively inhibited the growth of Escherichia coli and Shigella dysenteriae bacteria. Other research from Azzahra (2022) shows that the ethanol fraction of cherry leaves can provide antidiarrheal activity in vivo using male Wistar white rats induced by Salmonella typhi bacteria with an effective dose (ED50) of 63.548 mg/kgBW. The aim of the research is to determine the effect of cherry fruit (Muntingia calabura L.) syrup preparations in curing acute diarrhea in male Wistar mice induced by diarrhea using the in vivo method. The results of the research that has been carried out show that the use of the cherry plant as an antidiarrhea has so far still used the extract form and only the leaves, so the latest innovation is needed by using the cherry fruit which is processed into a pharmaceutical product in the form of a syrup with the properties of being able to cure acute diarrhea and its consumers can enjoy it without tasting bitter like medicine, and at a fairly affordable price. This is the basis for the need for further research in vivo in the form of cherry fruit syrup in curing diarrhea in mice induced by acute diarrhea.

Materials and Methods

Material

Cherry fruit syrup with a weight of 30 mL prepared from medicinal preparation. The cherry syrup preparation formula consists of cherry juice as the active ingredient, Na CMC, sucrose, methyl paraben, aquadest and vanilla essentials.

Preparation of the sample

Samples of cherry fruit (Muntingia calabura L.) were from Samarinda. Cherry fruit is sorted to separate high-quality fruit. The cherry fruit that will be processed is ripe and tender, producing a lot of juice and having a pleasant aroma. Each syrup formulation requires 1 kilogram of cherry fruit.

Phytochemical screening

Phytochemical screening was carried out to determine the compound content contained in cherry juice (Muntingia calabura L.). Phytochemical screening tests on alkaloid, flavonoid, tannin and saponin compounds. The tests include:

1. Flavonoid Test

A total of 7 mL of cherry juice as a sample was put into 3 test tubes, then in tube 1, add 5 mL of concentrated H2SO4. A red color change indicates positive flavonoids. Very red: +++, medium red: ++, slightly: +. In tube 2, 5 mL of concentrated HCl was added, and a little Mg powder was added. The color change to red indicates positive flavonoids. Next, add 5 mL of NaOH to tube 3 of cherry juice samples, if the color changes to yellow, it shows that it is positive for flavonoids.

2. Alkaloid Test

Alkaloid test, 5 mL of cherry juice was taken as a sample then added with Mayer's reagent. Color changes and the formation of precipitates indicate a positive test for alkaloids. Alkaloid testing can be done using the Mayer and Dragendorff methods. The amount of sediment is large: +++, medium: ++, small: +

3. Tannin Test

A total of 5 mL of cherry juice sample, then FeCl3 was added. A change in color to green, blue-green or blue-black, or the presence of sediment indicates a positive tannin. The amount of sediment is large: +++, medium: ++, small: +

4. Saponin Test

Mix 5 mL of cherry juice with distilled water, shake vigorously then let sit for 30 seconds. Observe the foam that forms. A positive result shows that the saponin test is a foam height of 1 cm within 30 seconds. A lot of foam: +++, medium: ++, little: +.

Cherry fruit syrup making

The tools used are measuring cups, test tubes, glass funnels, chocolate bottles, blenders, beakers, Erlenmeyers, filter paper, cups, hot plates, filter paper, analytical scales, injection probes, oral probes, individual vessels (mouse containers). Meanwhile, the ingredients used are cherry fruit, vanilla essential, Diapet syrup for children, oleum ricini, distilled water, sucrose, methyl paraben. Meanwhile, the test animals used were male white mice with an average weight of 22 grams which had been adapted and given standard food and drink.

Table 1. Cherry Fruit Syrup Preparation Formula (Pratiwi, 2021; Ameliya, 2018)

Quantity	Function	Components
Cherry juice	Active ingredients	30 %
Vanilla Essentials	Taste	1 %
Na CMC	Thickener	2 %
Sucrose	Sweetener	65 %
Methyl paraben	Preservative	2 %
Aquadest	Solvent	Ad 30 mL

Cherry juice syrup is made by dispersing Na-CMC in distilled water then adding methyl paraben and grinding it homogeneously (mixture a). Dissolve cherry juice in sufficient distilled water while stirring to dissolve completely (mixture b). After that, mixture b is added to mixtures a and b, stirred until homogeneous. Next, sucrose is added and then put into a brown container/bottle until the mark is reached (Syakri et al., 2017). Bottles used to package syrup products are sterilized first by boiling at 100°C for 30 minutes.

Antidiarrheal effect of cherry fruit syrup testing

Mice were fasted for 18 hours before testing. Each group of mice had their body weight weighed before being given treatment. Mice were induced orally by oleum ricini with an administration volume of 0.5 mL to create diarrhea. After 1 hour, each mouse was given each test substance, including: P1 = Control Group (K-), test animals were only given syrup without active ingredients; P2 = Control Group (K+) test animals were only given 90 mg Children's Diapet syrup (contains active herbal substances in the form of guava leaf extract, turmeric, mojokeling

time of onset of diarrhea, frequency of diarrhea, stool consistency, stool weight, and duration of diarrhea.

Result and Discussion

The secondary metabolites from cherry juice include tannins, alkaloids and flavonoids. These results are in accordance with research from (Alouw et al., 2022; D. A. Putri & Fatmawati, 2019) which shows that there are saponin, tannin and flavonoid compounds in cherry juice. Certain groups of compounds obtained from cherry fruit juice can be seen in **Table 1**, where where +: little, ++: moderate, +++: much, -: none.

Table 2. Results of phytochemical testing of cherry fruit juice

No	*Compound*	*Reagent*	*Colour*	*Precipitate*	*Result*
1.	Alkaloid	*Mayer*	Brownish white to yellowish	++	++
		Dragendorff	-	+++	+++
2.	Flavonoid	HCl + Mg	Red	-	++
		H2SO4	Reddish	-	++
		NaOH	Brownish yellow	-	+++
3.	Saponin	H2O	-	-	-
4.	*Tannin*	*FeCl3*	*Blackish blue*	+++	+++

Positive results of the alkaloid test using the Dragendorff method are characterized by the formation of a light brown to yellow precipitate. This precipitate is potassium alkaloid. In making Dragendorff's reagent, bismuth nitrate is dissolved in HCl to prevent a hydrolysis reaction because bismuth salts are easily hydrolyzed. forms bismuth ions (BiO+). In the alkaloid test with Dragendorff reagent, nitrogen is used to form coordinate covalent bonds with K+ which is a metal ion (Hadi & Permatasari, 2019). The formation of a precipitate in the Mayer test means that the cherry leaf juice contains alkaloids. Samples of cherry fruit that were reacted with Mayer's reagent showed a precipitate and a color change from brownish white to yellowish in the alkaloid test. The presence of proteins that precipitate upon the addition of reagents containing heavy metals (Mayer's reagent) can provide a positive reaction to cherry juice compounds (Fajrin & Susila, 2019).

Flavonoid test of cherry fruit samples, obtained positive results. In the flavonoid test, 3 reagents were given each, namely H2SO4, NaOH, and HCl. When the sample was reacted with H2SO4, a positive result was obtained. The proof was stated because before being reacted with H2SO4 the cherry fruit sample was brownish white. After being reacted with the reagent, the cherry fruit sample changed color to reddish and at the base of the test tube it became more concentrated. This shows that cherry fruit contains a lot of flavonoids (Hadi & Permatasari, 2019). The sample was reacted with NaOH, positive results were also obtained from cherry fruit. The result of the reaction between the sample and NaOH is a color change that occurs. The initial color is brownish white from the cherry fruit sample, changing to a slightly darker color, like yellow. The sample was reacted with HCl and Mg, positive results were obtained. By showing a color change from brownish white to red.

Tannin tests on cherry fruit samples obtained positive results. The presence of tannins will precipitate gelatin proteins. Tannin reacts with gelatin to form a stable copolymer that is insoluble in water. When 3 mL of the sample was reacted with the addition of 2 drops of FeCl3 and 3 drops, the color changed, from brownish white to blackish blue. This reaction is more sensitive with the

addition of NaCl to increase the salinity of tannins and precipitate gelatin proteins (Hadi & Permatasari, 2019). In the saponin test, negative results were obtained on cherry fruit samples. Positive results are obtained if after stirring vigorously, the sample produces froth or foam as high as 1 cm for 30 seconds. The absence of foam in the Forth test indicates the absence of glycosides which have the ability to form foam in water which is hydrolyzed into glucose and other compounds (Hadi & Permatasari, 2019).

The results of the antidiarrheal activity test of cherry fruit syrup with syrup concentrations of 10%, 15%, 30% (various concentrations based on the results of research conducted by Prasetyo (2015), negative control and positive control of Children's Diapet syrup (contains active herbal substances in the form of guava leaf extract, turmeric, mojokeling fruit and pomegranate peel) on male mice induced by oleum raccini were observed including the onset of diarrhea, fecal weight, frequency of diarrhea and duration of diarrhea data as follows in **table 3**.

Table 3. Antidiarrheal activity of cherry fruit syrup in mice

Antidiarrheal Activity Test Table						
30 minutes		Syrup 10% (gram)	Syrup 15% (gram)	Syrup 30% (gram)	Control - (gram)	Control + (gram)
I		3.67	3.67	3.64	4.15	3.29
II		3.47	3.41	3.61	3.79	3.25
III		3.4	3.6	3.5	3.87	3.08
IV		3.44	3.41	3.6	3.79	3.1
V		3.46	3.41	3.56	3.76	3.08
VI		3.45	3.45	3.61	3.69	3.08
VII		3.38	3.4	3.59	3.63	3.08
VIII		3.38	3.4	3.54	3.62	3.1
Initial paper weight		3.38	3.38	3.38	3.38	3.06
Feces weight during diarrhea		3.9	3.98	3.97	4.27	3.55
Time (minute)	Analysis result	Syrup 10% (gram)	Syrup 15% (gram)	Syrup 30% (gram)	Control- (gram)	Control + (gram)
30	3.61	0.29	0.29	0.26	0.77	0.23
60	3.38	0.52	0.03	0.23	0.41	0.19
90	3.88	0.02	0.22	0.12	0.49	0.02
120	3.84	0.06	0.03	0.22	0.41	0.04
150	3.82	0.08	0.03	0.18	0.38	0.02
180	3.83	0.07	0.07	0.23	0.31	0.02
210	3.9	0	0.02	0.21	0.25	0.02
240	3.9	0	0.02	0.16	0.24	0.04
Total Diarrhea		**1.04**	**0.71**	**1.61**	**3.26**	**0.58**
Feces weight during diarrhea		3.9	3.98	3.97	4.27	3.55

Testing the antidiarrheal effect of cherry fruit syrup on male mice showed that the syrup concentration of 10% showed antidiarrheal results with a total diarrhea weight of 1.04 grams. Cherry fruit syrup with a concentration of 15% showed antidiarrheal activity with a total diarrhea weight of 0.71 grams. Cherry fruit syrup with a concentration of 30% had a total diarrhea weight

of 1.61 grams during 4 hours of observation. Cherry fruit syrup successfully demonstrated antidiarrheal activity by reducing the weight of diarrhea at concentrations of 10% and 15%. Compared with the positive control, 15% concentration of cherry fruit syrup was effective in treating diarrhea in mice compared to other concentrations of cherry fruit syrup.

Conclusion

Cherry fruit syrup successfully demonstrated antidiarrheal activity by reducing the weight of diarrhea at concentrations of 10% and 15%. Compared with the positive control, 15% concentration of cherry fruit syrup was effective in treating diarrhea in mice compared to other concentrations of cherry fruit syrup.

References

Alouw, G., Fatimawali, F., & Lebang, J. S. (2022) 'Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kersen (*Muntingia calabura* L.) Terhadap Bakteri *Staphylococcus aureus* Dan *Pseudomonas aeruginosa* Dengan Metode Difusi Sumuran'. *Pharmacy Medical Journal*, 5(1), p.36. https://doi.org/10.35799/pmj.v5i1.41430

Amin, L. Z. (2015) 'Tatalaksana Diare Akut'. *Cermin Dunia Kedokteran*, 42(7), p.504-508. https://dx.doi.org/10.55175/cdk.v42i7.986

Azzahra, N. (2022) 'Aktivitas Antidiare Fraksi Etanol Daun Kersen (*Muntingia calabura* L.) Pada Tikus Putih Jantan Galur Wistar yang Diinduksi Bakteri Salmonella typhi'. *Tesis.* Fakultas Matematika dan Ilmu Pengetahuan Alam, Sriwijaya University.

Hadi, K., dan Permatasari, I. (2019) 'Uji Fitokimia Kersen (*Muntingia calabura L.)* dan Pemanfaatannya Sebagai Alternatif Penyembuhan Luka'. *Prossiding Sains Tenaga Kesehatan 1* (1). Available at: https://ejurnal.umri.ac.id/index.php/Semnasmipakes/article/view/1579

Fajrin, F. I., & Susila, I. (2019) 'Uji Fitokimia Ekstrak Kulit Petai Menggunakan Metode Maserasi'. *e- Prosiding SNasTekS*, 1(1), 455-462. Available at: https://journal.unusida.ac.id/index.php/snts/article/view/116

Jaya, N. R., Herlina, H., & Eliza, E. (2022) 'Uji Aktivitas Antidiare Fraksi Etil Asetat Daun Kersen (*Muntingia Calabura* L.) Pada Tikus Putih Jantan Galur Wistar Yang Diinduksi Bakteri *Escherichia Coli*'. *Doctoral dissertation*, Sriwijaya University.

Prawati, D. D., & Haqi, D. N. (2019) 'Faktor yang mempengaruhi kejadian diare di Tambak Sari, Kota Surabaya'. *Jurnal Promkes*, 7(1), p.34-45. https://doi.org/10.20473/jpk.V7.I1.2019.34-45

Syakri, Syamsuri, & Dandi Nuari Pigiutra, (2017) 'Formulasi Dan Uji AktivitasSyrup Sari Buah Sawo Manila Terhadap Beberapa Mikroba Penyebab Diare'. *JF FIK UINAM*, 5(2). https://doi.org/10.24252/jurfar.v5i2.3267