Topical Anti-Inflammatory Test of Purple Cabbage (Brassica Oleracea) Extract and Fraction As Topical Anti-Inflammatory Test on Rats

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Abstract: Purple cabbage (Brassica oleracea L) has been used for traditional medicine, because it has antioxidant, anti-inflammatory and antibacterial properties that can be used to treat inflammation. This study aims to prove the ethyl acetate fraction and extract of purple cabbage can be efficacious as an anti-inflammatory against rats with -carrageenan induction. Extraction and fractionation research of purple cabbage (Brassica oleracea L. var. capitata f.rubra) extract has been carried out. The test was carried out by injecting 1% carrageenan in physiological NaCl injected intraplantar as much as 0.2 mL into the paws of male rats Sprague-Dawley strain. Purple cabbage extract was obtained by maceration using ethyl acetate solvent and tested for anti-inflammatory effects using the pow edema method using a plethismometer. Maintenance and preparation of experimental animals (acclimatization) for 1 week by being fed standard pellets and drinking moderately. The number of samples was 75 rats selected at random and divided into 15 treatment groups with each group consisting of 5 rats. The number of each group is determined by the Federer Formula.Test result Aquadest-methanol fraction of purple cabbageprovides an anti-inflammatory effect, a dose of 1 (0.625%) mg/kg body weight gives the same anti-inflammatory effect as hydrocortisone acetate 1%and is able to relieve inflammation of the feet of mice. This result shows that Aquadest-methanol fraction of purple cabbage with a dose of 1 (0.625%) mg/kgd body weight gave significant results with hydrocortisone acetate 1%after ANOVA test with 95% confidence level.

Keywords: purple cabbage, topical anti-inflammatory, extract, fraction

INTRODUCTION

Indonesia is a country known for its rich biodiversity with various plants that have been used empirically for treatment for health problems. One type of plant that is widely used in efforts to deal with health problems is purple cabbage (Brassica oleracea). The content in purple cabbage includes vitamins A, B, C and E, minerals, potassium, calcium, phosphorus, sodium and iron, and contains anthocyanins. The purple color of purple cabbage comes from the anthocyanin content (Marwati, 2011). The use of purple cabbage in traditional medicine is as an antioxidant, anti-inflammatory and antibacterial, especially in the treatment of symptoms associated with gastric indigestion and duodenal ulcers (Chandrasenan, et al, 2016). Research related to purple cabbage as an anti-inflammatory has been carried out including testing the anti-inflammatory effect of purple cabbage ethyl acetate extract on rats orally which gave the results of its anti-inflammatory ability of 125 mg/kg BW and 150 mg/kg BW which is equivalent to sodium diclofenac 4,5 mg. In addition, the results of testing on the inhibition of swelling in the feet of mice showed a decrease in swelling or inflammation of 83, 35% and 84.11% (Fatiah, 2017).

In the field of nursing, cabbage is used to treat breast swelling in nursing mothers. One effort to deal with cases of breast swelling is symptomatic therapy using cabbage leaves (Marmi, 2012). Various non-pharmacological studies have been carried out to determine the effectiveness of non-medical interventions for the treatment of swelling. One of the non-medical interventions is treatment with leaf vegetable cabbage (cabbage). This one vegetable is increasingly popular for consumption, but not many know the true benefits of purple cabbage for the body. Cabbage has become increasingly popular cheap, easy to obtain and is a natural remedy found by several studies. Cabbage leaves have been found to be effective in the treatment of swelling due to the action of drawing cabbage leaves in human tissue. (Caplan, 1999). Quasi-experimental research on the difference in effectiveness between cold and room temperature cabbage leaves, concluded that there was no difference in effectiveness if the leaves were cooled before use. (Roberts, 1995). Cabbage has antibiotic and anti-inflammatory properties due to the content of sinigrin (Allylisothiocyanate), rapine, mustard oil, magnesium, and sulfur which can help widen the capillaries,

thereby increasing blood flow in and out of the area, and allowing the body to reabsorb fluids. contained in the breast (Mars Theory, 2014). This study aimed to obtain the anti-inflammatory effect of purple cabbage extract in pre-clinical trials on rats.

RESEARCH METHODOLOGY

Materials and ingredients

Purple cabbage simplicia(Brassica oleracea L,) obtained from Balitro Bogor, aquadest, 70% ethanol, methanol, ethyl acetate, n-hexane, 5% FeCl3, magnesium powder, hydrochloric acid, amyl alcohol, 10% ammonia, chloroform, Meyer's reagent, Liebermann's reagent -Burchard, carrageenin, Biocream®, 2.5% hydrocortisone acetate cream, 0.9% NaCl solution,carbopol 940, triethanolamine, %, propylene glycos, methyl paraben.

The tools used in this study were analytical balance (vibra), animal balance (GW-1500), mouse cage, Digital plethismometer (Ugo Basile cat No.7140), mortar and stamper, 10 ml measuring cup (pyrex), beaker 100 ml glass (pyrex), 10 ml volumetric flask (pyrex), 100 ml volumetric flask (pyrex), 500 ml round bottom flask (pyrex), cup, watch glass, stopwatch, dropper dropper, spatula, oral sonde , rats, syringes, spatulas, parchment paper, and stir sticks. and Sprague-Dawley rats aged 2-3 months weighing 200-250 grams

The methodology used in the anti-inflammatory test of purple cabbage ethyl acetate extract (using a digital plethismometer (Ugo Basile Cat No.7140) with the principle of measurement based on Archimedes' law. Inflammation was chemically induced using 1% (w/v) -carrageenan solution on the palm hind legs of rats, each injected intraplantar into the soles of the rats' paws as much as 0.05 ml.

Selection and Provision of Test Animals

The test animals used were white male rats, with the following inclusion criteria:

Sprague-Dawley male rats obtained from the Faculty of Animal Husbandry IPB, Bogor, aged: 2-3 months and weight: 200-250 g, normal behavior and activity conditions, and no visible anatomical abnormalities. Mice that met the inclusion criteria were used as experimental animals. Mice were adapted in a laboratory environment for 1 week by being fed standard pellets and drinking enough water to get used to living in a new environment. During acclimatization, the rats still received drinking water and pelleted feed containing water, crude protein, crude fat, crude fiber, calcium and phosphorus. Mice were placed in cages with room temperature 22° ± 3° C, with relative humidity of 30–70%, and 12 hours of light and 12 hours of darkness.

On the day of testing, each rat was weighed and marked on the back paw of the rat on the left
Ways of working:

1. 75 rats were randomly selected and divided into 15 treatment groups with each group consisting of 5 rats.
2. The volume of the rat's paws was measured at 0 hours. Then 1% carrageenan was injected.
   0.2 mL and measured for edema every 30 minutes for 6 hours. Cream was applied immediately after the injection of carrageenan. The test group consists of:

   a) Group 1 negative control only carrageenan was injected.
   b) Group 2 positive control was injected with carrageenan and applied hydrocortisone cream.
   c) Group 3 was the control base that was injected with carrageenan and smeared with cream base.
   d) Groups 4-6 treatments were injected with carrageenan and smeared with extract cream in 3 different doses.
   e) Groups 7-9 treatments were injected with carrageenan and smeared with N-Hexane fraction 3 in various doses.
   f) Groups 10-12 treatments were injected with carrageenan and smeared with ethyl acetate fraction in 3 different doses.
   g) Groups 13-15 treatments were injected with carrageenan and smeared with aqua + methanol fraction in 3 different doses.

The number of samples from each treatment group will be calculated using the Federer formula. There are 4 sample groups with 3 doses each so that the total sample group is 12, the base group, the positive control group, and the negative control group are 1 group each, so a total of 15 groups. treatment.

The research data were analyzed using the SPSS 16 program and the research results were determined for homogeneity and normality to determine the statistical analysis used. Data were analyzed using ANOVA test to determine the average difference between groups. If there was a difference, it was continued with Duncan's test to see whether there was a significant difference between treatments.

RESULTS AND DISCUSSION

Simpliasi making

Purple cabbage (Brassica oleracea L) that has been collected, cleaned of impurities, washed under running water several times until clean, then drained and spread on newspapers until the water is absorbed, then the cabbage is sliced into small pieces (chopped) and dried in the cupboard. dryer, during drying the purple cabbage is inverted. Then the dried samples were powdered with a blender

Extract Making

Dried simplicia powder from purple cabbage (Brassica Oleracea Var. Capitata Alba) was obtained from the Research Institute for Medicinal and Aromatic Plants (Balitro) Bogor. The extract was made using the maceration method using 70% ethanol as a solvent. The purple cabbage simplicia powder was weighed as much as 1000 g, then added 75 parts of 7500 ml of 70% ethanol extract. The simplicia is soaked for 6 hours, stirring occasionally, then allowed to stand for up to 5 days. The maserate was filtered using a flannel cloth and collected into a brown bottle. The simplicia dregs was added again with the type and amount of solvent as much as 25 parts of 2500 ml, stirred and then allowed to stand for 2 days then the macerate was filtered. The obtained maserate is then concentrated using a rotary evaporator to obtain a thick extract.

$$Rendemen = \frac{Bobot\ ekstrak\ yang\ diperoleh}{Bobot\ simplisia\ yang\ ditimbang} x\ 100\%$$

Fractionation process

Fractionation of purple cabbage extract was carried out by partitioning using a separating funnel. The purple cabbage extract was weighed as much as 100 g dissolved in 100 mL of water:methanol (1:1) until completely dissolved. Then the extract was added to 100 mL of ethyl acetate solution in a separating funnel. The mixture was shaken to form 2 layers and separated the formed phases. The remaining aqueous phase: methanol is added with 100 mL of n-hexane and shaken until separation occurs again. Do the same way until the phase of the solution
Fractionation Results

The fractionation yield calculation is calculated as follows:

$$Rendemen = \frac{Bobot\ fraksi\ yang\ diperoleh}{Bobot\ ekstrak\ yang\ ditimbang} x\ 100\%$$

In this study, the extract was fractionated with 3 different solvents, namely, water-methanol, ethyl acetate, and n-hexane.

Table. Yield result

Fraction	% Yield
Water - methanol	86.2
Ethyl acetate	11.2
n-Hexane	0.3

Furthermore, the extract and fraction of purple cabbage was carried out by phytochemical screening to determine the class of compounds or secondary metabolites contained in it
Fractionation Results

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In this study, the extract was fractionated with 3 different solvents, namely, water/aquadest -methanol, ethyl acetate, and n-hexane.

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Furthermore, the extract and fraction of purple cabbage were carried out by phytochemical screening to determine the class of compounds or secondary metabolites contained in it. The results of phytochemical screening of extracts and fractions of purple cabbage are as follows:

Table. Results of phytochemical screening of purple cabbage extract and fraction

Group of compounds	Extract	Water-methanol fraction	Ethyl acetate fraction	n-Hexane Fraction
Phenol	+	+	+	-
Flavonoids	+	+	+	+
Tannins	+	+	+	-
Alkaloids	-	-	-	-
Steroids	-	-	-	-
triterpenoids	+	+	+	+
Saponins	+	-	-	-

Making Purple Cabbage Extract

1000 grams of dried purple cabbage simplicia soaked in 10 liters of 96% ethanol for 3 days at room temperature with occasional stirring. The dregs and the filtrate are separated, resulting in filtrate 1. then the pulp is macerated again for 2 days and filtrate 2. filtrate 1 and 2 are combined and evaporated using a rotary evaporator at 400 C and followed by a water bath at 40-500 C until a thick extract is obtained. . Furthermore, the extract was tightly closed and stored in the refrigerator.

Identification of steroids and terpenoids

1 gram of the extract was added with 2 mL of chloroform in a test tube, then dropped into a drip plate, and allowed to dry. After that, 1 drop of Liebermann-Burchard reagent was added. The formation of a red color indicates the presence of triterpenoid compounds and the formation of a blue or purple color indicates the presence of steroid compounds (Harborne, 2006).

Identification of saponins

1 gram of extract was added with 20 mL of distilled water, then heated for 5 minutes. The solution is poured into a hot test tube. The solution was taken as much as 10 mL, then shaken vigorously vertically for 10 seconds. The presence of saponins is indicated by the formation of a stable foam as high as 1-10 cm for 10 minutes and does not disappear when added with one drop of 2 N HCl.

Phytochemical screening

a. Phenol Identification
   The extract was put into a test tube and then into a test tube 2 drops of 5% FeCl3 were added with a strong green, red-purple, blue or black color.
b. Identification of Flavonoids
   The extract was added with magnesium powder and 2mL of hydrochloric acid, then shaken with 10 ml of amyl alcohol. A positive reaction is indicated by the formation of an orange, yellow, or red color on the amyl alcohol layer.
c. Identification of Tannins
   The extract was put into a test tube and reacted with a 1% solution of iron (III) chloride if a blue-black color was formed indicating the presence of tannins.
d. Identification of alkaloids
   1-gram extract was added with 3 drops of 10% ammonia and 1.5 mL of chloroform, then shaken. The chloroform layer was taken and then dissolved in 1 mL of 2 N sulfuric acid, then shaken. After that, the extract was added with Meyer's reagent. The formation of a white precipitate indicates the presence of alkaloid compounds.

Inflammation testing procedure in test animals.

Before the test, the rats were fasted for 18 hours while still being given adequate drinking water. Rats were grouped into 7 groups, the solvent control group was given 0.5% sodium carboxy methyl cellulose suspension, the test material group for 5 doses was given a suspension of purple cabbage ethyl acetate extract, and the positive control group was given diclofenac sodium suspension.

On the day of testing, each animal was weighed and marked on the back paw of the rat on the left, then the left leg of the rat was inserted into a cell containing a reservoir solution that had been prepared previously until the liquid rose to the upper limit line, the pedal was then held down, the number was recorded on the monitor as initial volume (V0) is the volume of the leg before being given the drug and induced with -carrageenan solution.

Each rat according to its group was orally given 1% of the rat body weight suspension of 0.5% sodium carboxy methyl cellulose (solvent control group), purple cabbage ethyl acetate extract suspension at a dose of 50 mg/kg bw, 75 mg/kg bw, 100 mg/kg bw, 125mg/kg bw and 150 mg/kg bw (test material group) and 0.1 ml of diclofenac sodium suspension at a dose of 4.5 mg (positive control group) after 60 minutes of treatment, each animal was induced with 0.05 ml of 1% -carrageenan solution was administered intraplantarly on the soles of the rats' feet. After 30 minutes, measurements were taken by dipping the mouse's paw into a plethysmometer cell filled with a special liquid until the solution reached the upper limit line and the pedal was held and the numbers were recorded on the monitor. Changes in fluid volume that occur are recorded as the volume of the rat's paws (Vt). Inflammation of the rat's feet was measured every 30 minutes for 360 minutes. Each time the measurement of the cell solution is still sufficient.

The mechanism is that 75 rats were randomly selected and divided into 15 treatment groups with each group consisting of 5 mice. The volume of the rat's paws was measured at 0 hours. Then 0.2 mL of 1% carrageenan was injected and the edema was measured every 30 minutes for 6 hours. Cream was applied immediately after the injection of carrageenan. The test group consisted of: Group 1 negative control only carrageenan was injected. Group 2 positive control was injected with carrageenan and applied hydrocortisone cream. Group 3 was the control base that was injected with carrageenan and smeared with cream base. Groups 4-6 treatments were injected with carrageenan and smeared with extract cream in 3 different doses. Groups 7-9 treatments were injected with carrageenan and smeared with N-Hexane fraction 3 in various doses. Groups 10-12 treatments were injected with carrageenan and smeared with ethyl acetate fraction in 3 different doses. Groups 13-15 treatments were injected with carrageenan and smeared with aqua + methanol fraction in 3 different doses. The number of samples from each treatment group will be calculated using the Federer formula. There are 4 sample groups with 3 doses each so that the total sample group is 12, the base group, the positive control group, and the negative control group are 1 group each, so a total of 15 groups. treatment.

Federer formula:

(n-1) (t-1) 15 ; with
t = number of groups = 15
n = number of samples
(n-1) (15-1) 15
14 (n-1) 15
n 2.07

Based on these calculations, the minimum number of samples required is three. In this study, it was decided to use 5 samples/test animals per group. So the total sample required is 5 x 15 = 75 test animals.

The analysis was carried out by measuring the volume of the rat's feet as measured by a plethysmometer.
The value of the difference in edema per hour was measured and the total AUC value for each treatment was calculated:

$$AUC_{0-6} = \sum_{0}^{6} \left[\frac{(y_{n-1} + y_n)(x_n + x_{n-1})}{2} \right]$$

Information:

AUC_{0-6} = area under the curve from 0th hour to 6th hour (cm2.hour)
Y_{n-1} = area of pigmentation area at (n-1) hour (cm2)
Y_n = area of pigmentation at the nth hour (cm2)
X_n = nth hour (hour)
Xn-1 = hour-(n-1) (hour)

Then calculated the percentage of inflammation inhibition

$$Penghambatan\ inflamasi\ (\%) = \frac{(AUC0-6)0 - (AUC0-6)n}{(AUC0-6)0}$$

(AUC0-6)0 = AUC_{0-6}negative control mean (mm.hour)
(AUC0-6)n= AUC $_{0-6}$each rat in the group that was given the test compound with a concentration of n (mm.hour)

The results of the test of the Aquadest-methanol fraction of purple cabbage that were given topically to the spangue-dawley strain rats in the 1st group (0.625%) had a more optimal anti-inflammatory effect than the 2nd

dose (1.25%). The dose group 1 (0.625%) had an anti-inflammatory effect but was not as good as the positive control (hydrocortisone acetate 1%).

Test resultAquadest-methanol fraction of purple cabbageprovides an anti-inflammatory effect, a dose of 1 (0.625%) mg/kg body weight gives the same anti-inflammatory effect ashydrocortisone acetate 1%and is able to relieve inflammation of the feet of mice. This result shows thatAquadest-methanol fraction of purple cabbagewith a dose of 1 (0.625%) mg/kgd body weight gave significant results withhydrocortisone acetate 1%.after ANOVA test with 95% confidence level.

The results of the test of the N-Hexane Fraction of Purple Cabbage and Purple Cabbage when administered topically to rats. The spangue-dawley strain in the dose group 2 (1.25%) had a more optimal anti-inflammatory effect than dose 1 (0.625%) and dose 3 (2.5%) . The dose group 2 (1.25%) had an anti-inflammatory effect but was not as good as the positive control (hydrocortisone acetate 1%).

The results of the sample test. The ethyl acetate fraction of purple cabbage that was given topically to rats of the spangue-dawley strain in the group 3 (2.5%) had a more optimal anti-inflammatory effect than the dose 1 (0.625%) and dose 2 (1.25%). The dose group 3 (2.5%) had an anti-inflammatory effect but was not as good as the positive control (hydrocortisone acetate 1%).

The results of testing samples of purple cabbage setat extract which were administered topically to the spangue-dawley strain rats in the dose group 2 (1.25%) had a more optimal anti-inflammatory effect than dose 1 (0.625%) and dose 2 (1.25%). The dose group 3 (2.5%) had an anti-inflammatory effect but was not as good as the positive control (hydrocortisone acetate 1%).

Thus, it can be stated that the results of the Aquadest-methanol fraction of purple cabbage showed the most optimal results and would be used as the basis for the formulation of purple cabbage cream for anti-irritation testing.

CONCLUSIONS AND SUGGESTIONS

Conclusion

Based on the discussion and results on the research that has been done can be concluded

The Aquadest-methanol fraction of purple cabbage with a dose of 1 0.625%) had a more optimal anti-inflammatory effect.

Suggestion

It is recommended to use water and oil based ingredients for the manufacture of purple cabbage fraction cream.

BIBLIOGRAPHY

1. Hardiani E. 2012. Potential of Indonesian medicinal plants.
2. Ministry of Agriculture, extension agency and development of agricultural human resources
3. Mardiana L. 2012. Magic Leaf Kills Disease. Self-Help Spreader. Jakarta
4. Lutfiana. 2013. Anti-inflammatory activity test on Moringa leaves (Moringa oleifera Lam) with the red blood cell membrane stabilization method (Thesis) UINSharif Hidayatullah. Jakarta
5. Müller WA. 2002. Leukocyte-endothelial cell interactions in the inflammatory response. LabInvestigation
6. Neal MJ. 2005. At a Glace of Medical Pharmacology 5th edition
7. Mitsui T. 1997. New cosmetics science. Elsivier, Amsterdam
8. Arthur H Kibbe. 2000. Handbook of pharmaceutical excipients 3rd edition. Pharmaceutical Press. London
9. Pure. 2013. Anti-inflammatory effectiveness test of methanol extract of congkok rhizome (CurculigoorchioidesGeartn.). Secubisah College of Pharmacy, Makassar
10. Iswanana R. 2009. In vitro determination of penetration power and physical stability test of creams, ointments and gels containing curcumin from turmeric (Curcuma Longa L.). Essay. Undergraduate

Program in the Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Indonesia. Jakarta
11. Lachman L, et al., 1994. The theory and practice of industrial pharmacy. Translated by Suyatmi S et al. UI Press. Jakarta
12. Voight R. 1995. Lehburch der pharmazeutishentechnologie, translated by Soewandhi SN et al., Pharmaceutical Technology, UI Press, Jakarta
13. Mariani R, et al. 2010. Anti-inflammatory activity of the dominant compound of the god's crown fruit (Phaleria macrocarpa Scheff Boerl). Faculty of Mathematics and Natural Sciences Department of Pharmacy. Garut University