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Analgesic Activity Test of Carrot (Daucus carota L.) Peel Extract on White Mice

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ABSTRACT
Pain is an unpleasant experience characterized by potential tissue damage. One plant that has the potential to inhibit pain through its chemical compounds, namely flavonoids and alkaloids, is carrot peel (Daucus carota L.). The purpose of this study was to determine the analgesic activity of carrot peel extract in male mice. Carrot peel was extracted using the maceration method with 96% solvent. This study used 15 mice as test animals, which were divided into 5 treatment groups, namely the negative control group (CMC-Na), the positive control group (Sodium Diclofenac), carrot peel extract 100 mg/kgBW, 150 mg/kgBW, and 200 mg/kgBW. The test was conducted using the tail flick method with an analgesia measuring device. The test was conducted using the tail flick method with an analgesia measuring device. The data obtained were then analyzed using a paired sample t-test. The results showed that the three doses of carrot peel ethanol extract, 100 mg/kgBW, 150 mg/kgBW, and 200 mg/kgBW, did not have analgesic activity in male mice.

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INTRODUCTION

Pain is a subjective sensory and emotional perception that is also a signal from the body regarding disturbances occurring within the body. Pain is an uncomfortable condition associated with actual tissue damage or the potential for damage to occur within the body within a certain period of time (Yusuf *et al.,* 2020). The mechanism of pain onset is based on multiple processes, namely non-ception, peripheral sensitization, phenotypic changes, central sensitization, ectopic excitability, structural reorganization, and decreased inhibition. Between tissue injury stimuli and the subjective experience of pain, there are four processes: transduction, transmission, modulation, and perception (Bahrudin, 2018).

Analgesics can be defined as drugs used to reduce pain without decreasing consciousness. Analgesics are drugs that are often used to help relieve pain (Wardoyo & Oktarlina., 2019). Analgesics can be divided into two groups, namely narcotic analgesics and non-narcotic analgesics. Narcotic analgesics are drugs that have properties similar to morphine and opium. Meanwhile, non-narcotic analgesics are drugs that can relieve or eliminate pain without affecting the central nervous system, so they do not

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reduceconsciousness. Non-narcotic and narcotic analgesics do not cause addiction when used (Mita & Husni., 2017). Long-term use of analgesics has side effects such as stomach disorders, liver damage, kidney damage, intestinal damage, and allergic reactions on the skin. Therefore, alternative treatments using natural ingredients are needed (Wardani et al., 2021).

Since ancient times, plants have been widely used by communities as alternative treatments to cure various diseases. Until now, treatment using traditional medicine is still preferred by the community because traditional medicine is easily accessible, economical, and has fewer side effects compared to modern medicine (Sumayyah & Salsabila., 2017). One plant that is commonly consumed is carrots (*Daucus carota* L.). Only the root of the carrot is often used because it is high in beta-carotene, vitamins, and minerals (Siregar, 2017). Carrot roots are rich in antioxidants that can neutralize free radicals, containing several antioxidant compounds, namely β-carotene, anthocyanin, and vitamin C. This plant also shows nutraceutical benefits as an antioxidant, anticancer, immunological, anti-inflammatory, analgesic, and antipyretic (D *et al,* 2022). Based on research by Neno Ariffah (2017), phytochemical screening of 96% ethanol extract of carrot tubers (*Daucus corata* L.) contains alkaloids, phenols, tannins, flavonoids, steroids, and triterpenoids. Due to the presence of flavonoids and alkaloids, they can inhibit pain in certain parts of the body (Desiana et al., 2018). Flavonoids and alkaloids play a role in analgesic activity because they function as inhibitors of an important phase in prostaglandin biosynthesis, namely in the cyclooxygenase pathway (Wemay *et al.,* 2013).

Based on the above description, it is known that no research has been conducted on the analgesic activity of carrot peel, so this study aims to determine the activity of carrot peel extract in male mice using the tail flick method.

METHODS

Type of Research

This study was conducted using an experimental method at the Pharmacology Laboratory and Natural Materials Laboratory of Muhammadiyah Sorong University in July-August 2025. The design used in this study was a *pretest-posttest* control group design with a tail-flick analgesy meter applied to the tails of white mice. The results obtained were analyzed using a *paired samples t-test.*

Population and Sample

The population in this study was carrot peel (*Daucus Carota* L.) obtained from Tugu Merah Jalan Gabus and Pasar Pagi, Sorong Regency. The samples used in this study were fresh orange-colored carrot peels.

Tools and Materials

The equipment used in this study included aluminum foil, an analgesic meter, porcelain dishes, desiccators, beakers, measuring cups, animal cages, cannulas, filter paper, measuring flasks, mortars, maceration bowls, test tube clamps, droppers, test tube rack, rotary evaporator, stopwatch, test tubes, analytical balance, and maceration container.

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The materials used in this study were carrot peel (*Daucus Carota* L.), 96% ethanol, 0.5% CMC-Na, 50 mg sodium diclofenac, distilled water, FeCl3, Dragendrof, lead II acetate, and Liberman buchard.

Preparation of Carrot Peel Extract

Carrot peel extract was prepared using the maceration method with 96% ethanol as the solvent. Three hundred grams of carrot peel powder was weighed and placed in a glass jar, then 1,500 ml of 96% ethanol was added at a ratio of 1:5 until the sample was completely submerged. The sample was then left for 5 days in a closed container while being stirred every 8 hours. After 5 days, filtration was carried out using filter paper to produce filtrate 1. The residue from the filtration process was then re-macerated with the same solvent (1,500 ml) and left for 2 days to obtain filtrate 2. Filtrate 1 and filtrate 2 were then evaporated using a water bath to obtain a concentrated extract.

Phytochemical Screening

a. Flavonoid Test
   A 2 ml sample was placed in a test tube, then 4 drops of Pb II Acetate were added. The presence of flavonoid compounds was indicated by the formation of a yellow precipitate (Yuda *et al.,* 2017).
b. Alkaloid Test
   1 ml of carrot peel extract is placed in a test tube, then 1-3 drops of Mayer's reagent are added to obtain a white/yellow precipitate, Burchardt's reagent to obtain a blackish brown precipitate, and Dragendrof's reagent to obtain an orange color (Riza Marjoni, 2016).
c. Tannin Test
   A sample of 2 ml was placed in a test tube, then 3-4 drops of 0.1% FeCl3 were added to the filtrate, and the reaction was observed. If the sample produced a brownish green or blackish blue color, the tannin test was considered positive (Yunita *et al.,* 2019).
d. Saponin Test
   1 ml of extract is placed in a test tube, then 1-3 drops of distilled water are added and shaken for 10 seconds. A positive result for saponin is indicated by the formation of foam 1-10 cm high (Astika *et al.,* 2022).
e. Steroid Test
   A sample of 2 ml is placed in a test tube and 2 ml of chloroform and concentrated H2SO4 and anhydrous acetate are added to the filtrate, and the color change is observed (Yunita *et al.,* 2019).
f. Phenol Test
   Add 1 ml of extract to 1-2 reagents of FeCl3. Phenol is detected positively if a blackish green color is formed (Riza Marjoni, 2016).

Analgesic Activity Test

Before testing, the test animals were fasted for 8 hours but were still given water. The test animals were then weighed and divided into five groups, each consisting of five mice. Before treatment, the mice were tested using an analgesy meter to record the time as T0. Each group was then given an oral dose and volume according to the calculation. After 30

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minutes, the mice were tested using an analgesy meter, and the time when the mice pulled or twitched their tails was recorded. This test was conducted at 30, 60, 90, and 120 minutes.

Data Analysis

Paired t-test

The data obtained before and after treatment will be tested using a t-test (paired sample t-test) with the help of statistical software (SPSS). According to (Reza Akbar et al, 2015), the paired sample t-test is a testing method used to test whether there is a difference in the average after treatment. The hypothesis used is:

H0 = There is no difference in the analgesic test results before and after treatment H1 = There is a difference in the analgesic test results before and after treatment

If t-count > t-table, then H0 is rejected.

If t calculated < t table, then H0 is accepted.

RESULTS AND DISCUSSION

Carrot Peel Yield Results (*Daucus Carota* L.)

Table 1. Carrot Peel Yield Results (*Daucus Carota* L.)

Simplisia	Weight Simplisia (g)	Weight Extract (g)	Sample Weight (kg)	Yield (%)
Carrot Peel	300 grams	81.5 grams	6 kg	27.1

Table 1 shows the weight of the crude drug used in this study, which was 300 grams, and the concentrated extract obtained was 81.5 grams, resulting in an ethanol extract yield of 27.1%.

Yield Results of Carrot Peel Extract (*Daucus Carota* L.)

Table 2. Yield Results of Carrot Peel Extract (*Daucus Carota* L.)

Compound Group	Reagent	Observation	Notes
Flavonoids	Pb II Acetate	Yellow precipitate formed	+
Alkaloid	Mayer	White/orange precipitate formed	+
	Buchardt	Brown/red precipitate formed	
Tannin	$FeCl_3$	Forms a blue-black color	+
Steroid	Liberman Buchard	No blue color	-
Saponin	Aquades	There is a stable foam deposit	+
Phenol	$FeCl_3$	Forms a blackish green color	+

Phytochemical screening tests were conducted on carrot peel extracts to determine the chemical content of the extracts. Based on the phytochemical screening results in Table 2, carrot peel was found to contain flavonoids, alkaloids, tannins, saponins, and phenols.

Average Time (Seconds) of Pain Resistance Response

Table 3. Average Time (Seconds) of Pain Resistance Response

	Average ± SD (seconds) tail flick response				
Group	T0	T30	T60	T90	T120
Na CMC	4.99±2.79	13.41±0.63	11.76±1.91	7.88±4.96	12.20±0.83
Sodium Diclofenac	5.74±3.11	10.17±0.75	7.57±3.05	11.19±0.27	13.12±0.50

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Group	Average ± SD (seconds) tail flick response				
	T0	T30	T60	T90	T120
Dose 100mg	7.80±1.91	11.06±4.19	10.46±3.15	11.26±1.57	11.35±0.43
Dose 150mg	8.14±0.42	7.49±2.55	7.60±2.90	8.67±1.90	10.72±0.37
Dose 200mg	6.36±0.07	9.95±1.47	10.54±0.58	10.85±1.53	12.45±3.01

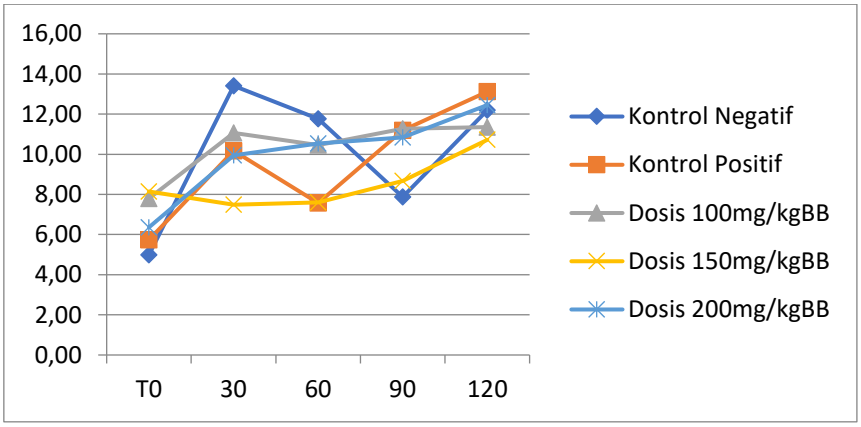

Figure 1. Average pain resistance response time (seconds)

Figure 1 shows the average pain resistance response time. The treatment group showed an increase in response time with a slight decrease at a certain point. However, the negative control group showed an increase at 30 minutes, but CMC Na as a raw material or thickener did not have an analgesic effect like the other controls. The positive control group (sodium diclofenac) showed an increase at 90-120 minutes.

Paired Sample T-test

Table 4. *Paired Sample T-test*

Group	T0-T30	T0-T60	T0-T90	T0-T120
Na-CMC	0.047	0.119	0.384	0.062
Sodium diclofenac	0.130	0.377	0.039	0.028
Dose 100mg/kgBB	0.203	0.324	0.226	0.076
Dose 150 mg/kgBB	0.652	0.801	0.953	0.421
Dose 200 mg/kgBB	0.187	0.091	0.119	0.154

In Table 4, the positive control group (sodium diclofenac) showed differences before and after treatment at 90 and 120 minutes, with values of 0.039 and 0.028, respectively. Meanwhile, the groups receiving ethanol extract of carrot peel at doses of 100mg, 150mg, and 200mg did not experience any significant difference before and after treatment, indicating no analgesic activity.

Discussion

In the production of carrot peel extract, carrot peel powder was extracted with 96% ethanol solvent because it is selective, easily obtained, non-toxic, has good absorption, and requires little heat for the concentration process. The extraction method used was the maceration method. The maceration resulted in a liquid extract, which was then evaporated to obtain a thick extract of 8.15 grams. From this extract, the yield of carrot peel ethanol

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extract (*Daucus carota* L.) was obtained. The yield of carrot peel ethanol extract obtained was 27.1%, as shown in Table 1. The purpose of calculating the yield was to determine the percentage or amount of substance dissolved in the solvent used (Novi et al., 2023). The yield is considered good if the value is more than 10%, and the yield obtained in this study can be considered good because it has a yield value above 10%, namely 27.1%.

Based on the results of phytochemical screening of ethanol extracts of carrot peel, the results show that carrot peel contains flavonoids, alkaloids, tannins, saponins, phenols, and sterols. In the examination of flavonoids, the reagent used was Pb II Acetate. The test results showed that carrot peel extract was positive for flavonoid compounds, as indicated by the formation of a yellow precipitate when Pb II Acetate reagent was used. This occurred because flavonoids have a benzene ring with a hydroxyl group, which can form a yellow precipitate (Maulidie *et al.,* 2019). In the alkaloid compound test, the reagents used were Mayer's reagent and Bouchardat's reagent. The test results showed that carrot peel extract contained alkaloid compounds, as evidenced by the presence of a white precipitate in Mayer's reagent and a black-brown precipitate in Bouchardat's reagent. The precipitate was produced due to the formation of a potassium-alkaloid complex. Alkaloids have free electron pairs on nitrogen atoms that will bond with K+ ions in alkaloid reagents (Oktavia & Sutoyo, 2021). The examination of tannin compounds showed that the addition of 2-3 drops of $FeCl_3$ to the extract produced a greenish-black color. Tannin compounds are polar because they have OH groups, which when reacting with $FeCl_3$ will change color to greenish-black. This color change occurs because tannin and $FeCl_3$ undergo hydrolysis, forming a greenish-black color (Halimu et *al.,* 2017). The saponin compound test showed positive results. This is because saponin has two (2) groups, namely a hydrophilic group, which is a group that is soluble in substances and is polar like water, while a lipophilic group is a group that is soluble in nonpolar substances such as fats/oils. The absorption of saponin molecules in the water surface causes a decrease in water surface tension, resulting in the formation of foam (Darma & Marpang, 2020). The phenol test showed positive results, indicated by a blackish green color. The blackish green color is caused by phenolic reacting with 1% $FeCl_3$ to form a dense red, purple, blue, or black color because $FeCl_3$ reacts with aromatic OH groups. The colored complex formed is suspected to be iron (III) hexaphenolate. The Fe^{3+} ion undergoes d^2 sp^2 orbital hybridization so that the Fe^{3+} ion ($4s^0$ $3d^5$) has 6 empty orbitals that are filled by electron pair donors, namely oxygen atoms in phenolic compounds that have free electron pairs (Ahmad Ikhwan Habibi *et al.,* 2018). In the steroid test, there was no green color when *Liberman-Burchard* was added. This is because steroids do not have double bonds in ring A of their structure because sterols such as cholesterol are derivatives of steroids that contain single bonds, and this causes no characteristic color change when tested with *Liberman-Burchard.* Steroids that do not have double bonds do not produce color complexes that can change to green or blue.

Based on Table 3, the average tail-flicking response shows that in the negative control group, Na-CMC increased the time mice flicked their tails, but Na CMC is a raw material that functions as a thickener in topical, oral, and parenteral preparations and as a binder and disintegrant in solid oral preparations. The reason for using Na CMC as a negative control is

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that it has no activity or effect on pain and inflammation (Veniartin *et al.,* 2024). In the positive control group administered sodium diclofenac, an increase was observed from minute 90 to minute 120, as sodium diclofenac has a half-life of 1–3 hours (Ministry of Health, 2016). The mechanism of sodium diclofenac is that it can inhibit COX-1 and COX-2, thereby inhibiting prostaglandin synthesis, which provides analgesic and anti-inflammatory effects (Oktaviana *et al.,* 2014). In carrot peel extract at 100 mg/kgBW, 150 mg/kgBW, and 200 mg/kgBW, there was an increase in tail-flicking time from minute 30 to minute 120. This is because carrot peel extract (*Daucus carota* L.) contains flavonoids and tannins that can inhibit the prostaglandin production pathway and physiological adaptation of mice to repeated pain stimuli, resulting in an increased pain threshold. Carrot peel extract contains flavonoids and tannins that can inhibit the activity of cyclooxygenase (COX) enzymes, which play a role in prostaglandin production and cause inflammation and pain (Sentat & Pangestu, 2017).

Testing of the analgesic activity of carrot peel ethanol extract on test animals divided into five groups, each given a different test substance, namely the negative control group (Na CMC), the positive control group (sodium diclofenac), and groups given carrot peel extract doses of 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW. The analgesic activity test aimed to determine the analgesic activity of carrot peel ethanol extract. Groups one to five were given oral suspensions of continuously, then an analgesic test was conducted using an analgesy meter until the mice pulled or flicked their tails.

The Paired Sample T-test in this study aimed to determine whether there was a statistically significant difference between the values before and after treatment. The condition for *the Paired Sample T-test is* that if the p-value is 0.05, then there is no significant difference between the pretest and treatment (Ardhana & Rahman, 2024). Table 4 shows that the negative control group (Na-CMC) experienced an increase at T0-T30 with a value of 0.047, while the positive control group (sodium diclofenac) experienced an increase at T0-T90 and T0-T120 with p-values of 0.039 and 0.028, respectively. Meanwhile, the carrot peel ethanol extract dose groups of 100mg, 150mg, and 200mg did not experience a significant increase, indicating no analgesic activity in carrot peel ethanol extract at doses of 100mg, 150mg, and 200mg. This indicates that carrot peel ethanol extract does contain active compounds that inhibit pain, but it was not able to produce a statistically significant analgesic effect at the tested doses.

CONCLUSION

Based on the research conducted, it can be concluded that: In the phytochemical screening test of carrot peel ethanol extract (Daucus carota L.), the following compounds were found: flavonoids, alkaloids, saponins, tannins, and phenols. Carrot peel (Daucus carota L.) ethanol extract does not have analgesic activity on male Swiss Webster strain mice. It is recommended that future researchers conduct further studies on the pharmacological activity of carrot peel extract (*Daucus carota* L.) to determine its other benefits besides analgesic activity, and it is also recommended that they continue developing formulations from ethanol extract of carrot peel (*Daucus carota* L.).

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