

Anti-Inflammatory Activity of White Radish Tuber Ethanol Extract (*Raphanus sativus* L.) Induced by Carrageenan

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ABSTRACT

Indonesia possesses abundant natural resources with significant potential for the development of nutraceutical foods and traditional medicines. However, many local plants remain insufficiently studied regarding their efficacy and safety. One promising plant is the white radish tuber (*Raphanus sativus* L.), which offers various health benefits and potential as a functional food ingredient. This study aims to evaluate the anti-inflammatory effect of the ethanol extract of white radish tubers on paw edema in male Wistar rats (*Rattus norvegicus*) induced with 1% carrageenan. The research employed a laboratory experimental design, producing ethanol extracts using three solvent concentrations—50%, 70%, and 96%—followed by phytochemical screening. Anti-inflammatory activity was tested using five treatment groups: a negative control (1% CMC-Na), a positive control (diclofenac sodium 2.25 mg/kgBW), and three extract groups administered at 300 mg/kgBW according to the respective ethanol concentrations. The primary parameter measured was the volume of edema formed in the rats' paws. Phytochemical screening revealed that the ethanol extract contained secondary metabolites such as alkaloids, flavonoids, saponins, and tannins, all known to possess anti-inflammatory properties. The extracts produced edema inhibition rates of 31.02% (50%), 21.80% (70%), and 18.44% (96%), comparable to diclofenac sodium at 18.63%. Statistical analysis using one-way ANOVA indicated an overall difference among the treatment groups. However, post-hoc Tamhane T2 analysis demonstrated that the anti-inflammatory activities of the 50%, 70%, and 96% ethanol extracts were not significantly different ($p > 0.05$), although all extract groups exhibited anti-inflammatory activity comparable to diclofenac sodium.

Keywords: Anti-inflammatory, white radish, solvent concentration

Introduction

White radish tubers (*Raphanus sativus* L.) have long been recognised as medicinal plants because they contain various bioactive compounds with potential therapeutic properties. In traditional medicine, white radish tubers have been used to treat a variety of health conditions, including inflammatory disorders, owing to their anti-inflammatory, antimicrobial, and antioxidant activities (Auzia *et al.*, 2017).

Inflammation is a fundamental biological response of the immune system to harmful stimuli, such as pathogens, tissue injury, or chemical irritants. Its primary function is to eliminate the causative agents of tissue damage and initiate the healing process. The classical clinical manifestations of inflammation include redness (*rubor*),

heat (*calor*), swelling (*tumor*), pain (*dolor*), and impaired tissue function (*functio laesa*) (Saputri *et al.*, 2016). Although inflammation serves as a protective mechanism, excessive or prolonged inflammatory responses may contribute to tissue damage and the development of various chronic diseases.

Inflammation can be classified into acute and chronic forms. Acute inflammation is characterised by a rapid onset and increased blood flow to the affected tissue due to vasodilation, resulting in erythema, oedema, and pain. In contrast, chronic inflammation develops more gradually and may persist for months or even years, leading to continuous tissue injury and impaired organ function (Emelda *et al.*., 2023). One of the major biochemical pathways involved in inflammation is the cyclooxygenase (COX) pathway, which catalyses the synthesis of prostaglandins, prostacyclin, and thromboxane. These inflammatory mediators contribute to vasodilation, pain sensation, platelet aggregation, and the overall inflammatory response.

Conventional anti-inflammatory drugs, including non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are widely used to manage inflammatory conditions. However, prolonged use of these medications is frequently associated with adverse effects, including gastrointestinal ulceration, renal impairment, cardiovascular complications, and immunosuppression. Consequently, there is growing interest in identifying safer anti-inflammatory agents derived from natural products.

According to the World Health Organization (WHO), medicinal plants remain an important source of traditional healthcare because they generally exhibit fewer adverse effects and are more accessible than synthetic medicines when used appropriately (Latief *et al.*, 2021). Among these medicinal plants, white radish tubers (*Raphanus sativus* L.) contain several phytochemical constituents, including flavonoids, saponins, and raphanin, which have been reported to possess anti-inflammatory and antimicrobial properties. Previous studies have demonstrated that extracts of white radish tubers inhibit the growth of both Gram-positive and Gram-negative bacteria and exhibit potential as natural anti-inflammatory agents for topical applications (Susanti *et al.*, 2015). Although previous studies have demonstrated anti-inflammatory activity of *Raphanus sativus*, limited evidence is available regarding the influence of extraction solvent polarity on anti-inflammatory efficacy. Different ethanol concentrations may selectively extract bioactive flavonoids and phenolic compounds, thereby influencing pharmacological activity.

Methodology

The study was an experimental laboratory research conducted to evaluate the anti-inflammatory activity of ethanol extract of white radish tubers (*Raphanus sativus* L.) on carrageenan-induced edema in the hind paws of male Wistar rats (*Rattus norvegicus*). The instruments used in this study included an analytical balance, oven, dark glass bottles, glass jar, stirring rod, racks, test tubes, glass funnel, blender, No. 44 sieve, aluminum foil, water bath, flannel cloth, filter paper, watch glass, beaker glass, volumetric flask, porcelain cup, plethysmometer, and oral gavage needle.

The research was conducted through several systematic stages. Fresh white radish tubers (*Raphanus sativus* L.) were collected from Giwangan Market, Yogyakarta, then processed by blending and sieving to obtain a fine powder. The powdered material was extracted using ethanol in a 1:6 ratio with varying solvent concentrations, followed by maceration for three days. The first macerate was filtered using flannel cloth and subsequently subjected to a 24-hour remaceration process. The combined macerates were then evaporated using a rotary evaporator at 40 °C to prevent thermal degradation of bioactive constituents, as elevated temperatures may decompose important compounds such as tannins and flavonoids. These compounds are highly sensitive to heat, and exposure to temperatures above 50 °C may induce structural changes that reduce their concentration and biological activity; therefore, maintaining low evaporation temperatures is essential. Following rotary evaporation, the extract was further concentrated using a water bath at 50 °C until a thick, viscous extract was obtained (Tunas *et al.*, 2019).

The next stage involved identifying the chemical constituents present in the ethanol extract of white radish tubers. Flavonoids were detected using hydrochloric acid and magnesium powder, which produce red or orange complex compounds upon reduction. Saponins were identified by adding hydrochloric acid, followed by warm distilled water, resulting in characteristic foam formation. Tannins were confirmed through the addition of 1% FeCl₃, which generates a greenish-black coloration. Alkaloids were determined using Dragendorff's and Mayer's reagents, both of which induce precipitation reactions indicative of alkaloid presence.

Subsequently, a 1% carrageenan solution was prepared to induce inflammation in the experimental animals. The inflammatory agent was made by dissolving 100 mg of carrageenan in 0.9% physiological NaCl to a final volume of 10 mL, yielding a 1% (w/v) concentration. A dosage of 0.1 mL of this 1% carrageenan solution was administered into the hind paws of the rats to induce edema.

The experimental animals used in this study were 30 male Wistar rats (*Rattus norvegicus*), which were divided into several treatment groups. All treatment procedures, observations, and edema measurements were performed consistently using rats as the experimental model.

Making the negative control solution

Na-CMC is made by adding 1 gram of Na-CMC little by little to 100 ml of hot distilled water at a temperature of 70°C while stirring until a homogeneous colloidal solution is formed (Rahman *et al.*, 2017).

Making the positive control solution (Sodium diclofenac)

The diclofenac sodium used is generic diclofenac sodium tablets (Novell®). The dose of diclofenac sodium as a positive control was 2.25 mg/kg rat body weight. To make a diclofenac sodium solution, an equal weight test is carried out, then the diclofenac sodium tablets are crushed and then weighed according to the equivalent powder and dissolved in the Na-CMC suspension solution, then stirred until homogeneous (Suryandari *et al.*, 2021).

Treatment of test animals

The test animals used in this research were 30 male Wistar rats, which were divided into 5 groups with 6 rats in each group. The test animals were adapted to the laboratory environment for 1 week in cages that were kept clean. Healthy test animals are characterized by agile movements. Test animals were first fasted for $\pm$ 18 hours and still given water before treatment (Rahmawati *et al.* , 2012) .

Anti-inflammatory testing

Before being examined, thirty male white rats were given drinking water and fasted for eighteen hours. Shared became 5 groups every 6 rats. Every rat weighed heavily, its body and feet. The rear right is marked above the measuring ankle volume, beginning with the foot of the rats with a plethysmometer (V_0) up to the mark limit that has been given. Group control negative given Na.CMC 1%. Group control positive given suspension sodium diclofenac. Group treatment, each rat was given white radish tuber ethanol extract suspension with varying solvent concentrations of 50%, 70%, and 96% with an optimal dose of 300 mg/kgBW, 30 minutes later, all groups of animals that had received treatment were injected with 1% carrageenin in 0.9% NaCl solution on the soles of the rats' right feet. Measurements of volume edema are done every 30 minutes after administration of carrageenin using a plethysmometer (V_t) (Sentat, 2016) .

Furthermore, anti-inflammatory activity was measured by measuring the volume of rats' leg edema using a plethysmometer for six hours, starting from 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 minutes after being induced by 1% carrageenan. After that, the data obtained on the volume of the rats' paws was then processed by calculating the percentage of edema inhibition

Result and Discussion

The extraction of white radish tubers (*Raphanus sativus* L.) was performed using the maceration method to isolate bioactive compounds while minimising thermal degradation. Maceration was selected because it is a simple extraction technique that does not require heating, thereby preserving thermolabile phytochemicals. Three ethanol concentrations (50%, 70%, and 96%) were used as extraction solvents to evaluate the effect of solvent polarity on extraction yield. Ethanol polarity varies according to its water content; lower ethanol concentrations contain more water and are therefore more polar, whereas higher ethanol concentrations are less polar. The extraction yields obtained from each solvent concentration are presented in **Table 1**.

Table 1. Results yield EEULP of the variance concentration

	Simplicia powder (grams)	Solvent (ml)	Extract weight (grams)	Extract Yield (%)
50%			255,066	63,766%
70%	400	2.400	74,476	18,619%

96%	169,35	42,337%
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The extraction yields obtained using 50%, 70%, and 96% ethanol were 63.77%, 18.62%, and 42.34%, respectively. According to Karlina (2022), an extract yield exceeding 10% is generally considered satisfactory, indicating that all extraction conditions produced acceptable yields. The highest extraction yield was obtained using 50% ethanol, suggesting that this solvent was more effective in extracting polar constituents from white radish tubers. Differences in extraction yield among solvent concentrations are closely associated with solvent polarity, which influences the solubility of phytochemical compounds, including flavonoids, phenolics, and saponins (Alara et al., 2021).

Percentage of Inflammation

The percentage of inflammation represents the degree of paw oedema observed after carrageenan induction. This parameter reflects the severity of the inflammatory response. Therefore, a higher percentage of inflammation indicates a greater inflammatory response and consequently a lower anti-inflammatory effect of the tested extract. Conversely, a lower percentage of inflammation indicates that the extract is more effective in suppressing oedema formation.

Percentage of Edema Inhibition

In contrast, the percentage of edema inhibition represents the ability of the extract to suppress paw swelling relative to the negative control. This parameter is inversely related to the percentage of inflammation. A higher percentage of edema inhibition indicates stronger anti-inflammatory activity, whereas a lower percentage indicates weaker anti-inflammatory efficacy. Therefore, the anti-inflammatory potential of white radish tuber extract should primarily be interpreted based on the percentage of edema inhibition rather than the percentage of inflammation.

To avoid misinterpretation, these two parameters should be reported and discussed separately because they describe different aspects of the inflammatory response and have opposite interpretations. The percentage of inflammation reflects the remaining inflammatory condition, whereas the percentage of edema inhibition reflects the therapeutic effectiveness of the tested extract. Phytochemical screening was carried out to determine secondary metabolites present in the ethanol extract of turnip white. Results from screening phytochemicals in the study are shown in Table 2.

Table 2. Phytochemical screening results of white radish tuber ethanol extracts

Phytochemical	Observation	50% Ethanol	70% Ethanol	96% Ethanol
Alkaloids (Dragendorff)	Orange precipitate	+	+	+
Alkaloids (Mayer)	Cream-white precipitate	+	+	+
Flavonoids	Orange-red colour	+	+	+
Saponins	Stable foam	+	+	+

Tannins	Greenish-black colour	+	+	+
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Note: (+) = detected; (–) = not detected.

The phytochemical screening results demonstrated that white radish tuber (*Raphanus sativus* L.) ethanol extracts prepared using 50%, 70%, and 96% ethanol all contained several major classes of secondary metabolites. As presented in **Table 2**, alkaloids were detected by the formation of an orange precipitate with Dragendorff reagent and a cream-white precipitate with Mayer's reagent. Flavonoids were identified by the appearance of an orange-red colour following the addition of magnesium powder and concentrated hydrochloric acid. Saponins were confirmed by the formation of stable, persistent foam, whereas tannins were indicated by a greenish-black colour after the addition of ferric chloride reagent. These findings suggest that the extraction process successfully recovered bioactive phytochemicals from white radish tubers regardless of the ethanol concentration used, although differences in solvent polarity may influence the abundance of each metabolite class.

In the flavonoid test, concentrated magnesium and HCl are used to decrease the benzopyrone nucleus in the flavonoid structure, resulting in the formation of red or orange flavonoid salts. Compounds having two aromatic rings and multiple hydroxyl groups are known as flavonoids (Abu-Ria et al., 2023). White radish tubers also tested positive for tannin because the color changed to greenish black after adding FeCl₃ 1%. Testing tannin shows that the tannins contained in the ethanol extract are a condensation tannin because it forms a greenish-black color (Zongo et al., 2025). The results obtained were the same as those of research conducted by Keyata et al., (2021), which stated that white radish extract contains alkaloids, flavonoids, saponins, and tannins (Fitriya, 2024).

The next induction of inflammation with carrageenan to produce edema was carried out on the soles of the rats' feet by inducing 0.1 ml of 1% carrageenan solution subplantarily. A plethysmometer was used to measure the edema volume 30 to 360 minutes after it was produced, and the average percentage of edema volume was obtained as shown in Table 3.

Table 3. Average Volume of Edema on the Feet of Rats in Each Treatment Group

Group	0°	30°	60°	90°	120°	150°	180°	210°	240°	270°	300°	330°	360°
K(+)	0,10 ±0,01	0,06 ±0,00	0,05 ±0,00	0,05 ±0,00	0,04 ±0,00	0,04 ±0,00	0,04 ±0,00	0,04 ±0,00	0,03 ±0,00	0,04 ±0,00	0,04 ±0,00	0,04 ±0,01	0,04 ±0,00
K(-)	0,09 ±0,01	0,08 ±0,00	0,08 ±0,01	0,08 ±0,01	0,07 ±0,01	0,08 ±0,00	0,07 ±0,01	0,07 ±0,00	0,07 ±0,00	0,07 ±0,00	0,07 ±0,01	0,06 ±0,01	0,05 ±0,00
KEULP 50%	0,09 ±0,01	0,07 ±0,00	0,06 ±0,01	0,05 ±0,01	0,04 ±0,00	0,05 ±0,00	0,05 ±0,01	0,05 ±0,01	0,05 ±0,00	0,05 ±0,00	0,05 ±0,01	0,05 ±0,01	0,05 ±0,00
KEULP 70%	0,09 ±0,00	0,07 ±0,01	0,06 ±0,00	0,05 ±0,01	0,05 ±0,01	0,05 ±0,00	0,05 ±0,00	0,04 ±0,00	0,05 ±0,00	0,05 ±0,00	0,04 ±0,00	0,05 ±0,01	0,05 ±0,00
KEULP 96%	0,07 ±0,01	0,06 ±0,00	0,05 ±0,01	0,05 ±0,01	0,04 ±0,00	0,05 ±0,00	0,05 ±0,00	0,05 ±0,00	0,04 ±0,00	0,04 ±0,00	0,04 ±0,00	0,03 ±0,00	0,03 ±0,00

Based on the average volume of edema in Table 3, it can be seen that after inducing 1% carrageenan, there was an increase in the average volume of edema on the soles of the rats' feet in each treatment group. All treatment groups experienced a decrease in the volume of edema every hour, including the negative control group. There was a decrease in the volume of edema, but it was not as significant as in other treatment groups, and the edema still persisted until the 6th hour. This indicates that the edema formed by carrageenan can last for 6 hours. The preparation given to the negative control group was Na-CMC 1%. The decrease in the percentage of edema volume that occurred in the negative control group was due to the animal's own immunity and not due to the administration of 1% Na-CMC.

Table 4. Average results (%) of edema inhibition

Group	Mean percentage of inhibition ($\bar{X} \pm SD$)
K(+)	35.14 $\pm$ 3.40
K(-)	11.40 $\pm$ 11.02
KEEULP 50%	19.67 $\pm$ 11.65
KEEULP 70%	27.81 $\pm$ 4.62
KEEULP 96%	28.94 $\pm$ 7.75

From the data in table 4, the % edema inhibition is determined, namely to see which treatment has the best effectiveness. Table 4 shows that the positive control with KEEULP 70% and 96% has effectiveness that is not much different, with KEEULP 50% it has anti-inflammatory effectiveness but not as good as KEEULP 70% and 96%, while the negative group has anti-inflammatory activity but its effectiveness is low.

The preparation given to the positive control group was diclofenac sodium. Diclofenac sodium was used as a positive control because it is a drug that has anti-inflammatory effects and has the same mechanism of action as the main compound in the ethanol extract of white radish tubers, namely flavonoid compounds, both of which work by inhibiting the metabolism of arachidonic acid, so diclofenac sodium was used as a comparison. The primary substances with anti-inflammatory properties in the ethanol extract of white radish tubers are flavonoid compounds. Flavonoids have the potential to inhibit enzymes involved in arachidonic acid metabolism, reducing the release of inflammatory mediators, such as inhibiting the biosynthesis of prostaglandins, thromboxane, leukotrienes, and COX (Cyclooxygenase) (Lutfiah *et al*, 2023).

Table 5. Data Normality Test Results

Group (n=30)	Normality Results (%Inhibition/%Inhibition) with Shapiro-Wilk Test	
	Average Value (df=6)	p-value
Positive Control	18.63 $\pm$ 6.99	0.963 ^(N)
	35.14 $\pm$ 3.40	0.315 ^(N)
Negative Control	57.19 $\pm$ 33.23	0.805 ^(N)
	11.40 $\pm$ 11.02	0.378 ^(N)

KEEULP 50%	31.02±26.62	0.063 ^(N)
	19.67±11.65	0.088 ^(N)
KEEULP 70%	21.80±9.99	0.419 ^(N)
	27.81±4.62	0.325 ^(N)
KEEULP 96%	18.44±7.61	0.558 ^(N)
	28.94±7.75	0.488 ^(N)

Table 6. Tamhane *t*2 % Trade test results

Treatment group		p value
Positive control	Negative control	0.303 ^(BTB)
	KEEULP 50%	0.977 ^(BTB)
	KEEULP 70%	1,000 ^(BTB)
	KEEULP 96%	1,000 ^(BTB)
Negative control	Positive control	0.303 ^(BTB)
	KEEULP 50%	0.834 ^(BTB)
	KEEULP 70%	0.384 ^(BTB)
	KEEULP 96%	0.298 ^(BTB)
KEEULP 50%	Positive control	0.977 ^(BTB)
	Negative control	0.834 ^(BTB)
	KEEULP 70%	0.998 ^(BTB)
	KEEULP 96%	0.975 ^(BTB)
KEEULP 70%	Positive control	1,000 ^(BTB)
	Negative control	0.384 ^(BTB)
	KEEULP 50%	0.998 ^(BTB)
	KEEULP 96%	0.999 ^(BTB)
KEEULP 96%	Positive control	1,000 ^(BTB)
	Negative control	0.298 ^(BTB)
	KEEULP 50%	0.975 ^(BTB)
	KEEULP 70%	0.999 ^(BTB)

Information :

BTB = Not significantly different ($p > 0.050$)

BB = Significantly different ($p < 0.050$)

Table 7. Tamhane *t*2 test results % edema inhibition

Treatment group		p value
Positive control	Negative control	0.024 ^(BB)
	KEEULP 50%	0.194 ^(BTB)
	KEEULP 70%	0.113 ^(BTB)
	KEEULP 96%	0.711 ^(BTB)
Negative control	Positive control	0.024 ^(BB)
	KEEULP 50%	0.932 ^(BTB)
	KEEULP 70%	0.121 ^(BTB)
	KEEULP 96%	0.105 ^(BTB)
KEEULP 50%	Positive control	0.194 ^(BTB)
	Negative control	0.932 ^(BTB)
	KEEULP 70%	0.823 ^(BTB)

	KEEULP 96%	0.780 (BTB)
KEEULP 70%	Positive control	0.113 (BTB)
	Negative control	0.121 (BTB)
	KEEULP 50%	0.823 (BTB)
KEEULP 96%	KEEULP 96%	1,000 (BTB)
	Positive control	0.711 (BTB)
	Negative control	0.105 (BTB)
	KEEULP 50%	0.780 (BTB)
	KEEULP 70%	1,000 (BTB)

Information :

BTB = Not significantly different ($p > 0.050$)

BB = Significantly different ($p < 0.050$)

The Shapiro-Wilk test for data normality and the Levene test for homogeneity were used in SPSS data analysis after the edema volume was recorded for six hours. The homogeneity test yielded a significance value of less than 0.050, indicating that the data is not homogeneous, while the normality test yielded a significance value greater than 0.050, indicating that the data is normally distributed. One-way ANOVA can be used for a parametric test once it is established that the data are not homogeneous and are normally distributed. The results of *the one-way ANOVA* data analysis on % inflammation and % edema inhibition had a significance value of <0.050 , which means that the mean % inflammation and % edema inhibition in two or more groups in this study had an influence. For the results of data analysis that show an influence between groups, followed by the *Tamhane T2 test*, the aim of analyzing the *Tamhane T2 test data* is to see which groups have differences.

The analysis of percentage inflammation using the post-hoc Tamhane T2 test showed that comparisons between the positive and negative control groups resulted in a significance value of >0.050 , indicating no statistically significant differences between these groups. Similarly, comparisons between the positive control group and KEEULP at concentrations of 50%, 70%, and 96% also produced significance values of >0.050 , suggesting no meaningful differences. Further analysis demonstrated that the negative control group, compared with the positive control and all KEEULP concentrations (50%, 70%, and 96%), consistently yielded significance values above 0.050, indicating that any observed differences were not statistically significant. Comparisons between KEEULP 50% and both positive and negative controls also showed non-significant differences ($p > 0.050$). Additionally, comparisons among KEEULP treatment groups (50%, 70%, and 96%) revealed no significant differences, as all significance values exceeded 0.050. KEEULP 70% with positive control, negative control, KEEULP 50%, and KEEULP 96% have a significance value of >0.050 , which means there is a difference between the groups that is not significant. In KEEULP 96% with positive control, negative control, KEEULP 50%, and KEEULP 70% have a significance value of >0.050 , which means there is a difference between the groups that is not significant.

The results of data analysis of % inhibition using the *Tamhane t2 post-hoc* test between the positive control group and the negative control group had a significance

value of <0.050 , which means there was a significant difference between the groups. Meanwhile, the positive control group with KEEULP 50%, KEEULP 70%, and KEEULP 96% had a significance value of >0.050 , which means there was a difference between the groups that was not significant. In the KEEULP 50% group with positive control, negative control, KEEULP 70%, and KEEULP 96% have a significance value is >0.050 , which means there is a difference between the groups that is not significant. In the KEEULP 70% group with positive control, negative control, KEEULP 50%, and KEEULP 96% have a significance value is >0.050 , which means there is a difference between the groups that is not significant. In the KEEULP 96% group with positive control, negative control, KEEULP 50%, and KEEULP 70% have a significance value is >0.050 , which means there is a difference between the groups that is not significant, as can be seen in Figure 1, the edema vs. time curve.

Conclusion

The ethanol extracts of white radish tubers (*Raphanus sativus* L.) prepared using 50%, 70%, and 96% ethanol demonstrated anti-inflammatory activity against carrageenan-induced paw edema in male Wistar rats. All extract concentrations contained alkaloids, flavonoids, saponins, and tannins, which are known to contribute to anti-inflammatory effects. Statistical analysis showed an overall difference among treatment groups; however, post-hoc Tamhane T2 analysis indicated that there were no statistically significant differences in anti-inflammatory activity among the three ethanol concentrations ($p > 0.05$). Therefore, varying the ethanol concentration from 50% to 96% did not significantly influence the anti-inflammatory efficacy of the white radish extracts under the experimental conditions used in this study.

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