

Anthelmintics Activity of Red Pomegranate (*Punica granatum*) Peel Extract and Red Pomegranate (*Punica granatum*) Juice in Rabbit Experimental Animals

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Received: January 12, 2023

Revised: April 23, 2023

Accepted: April 29, 2023

Published: April 30, 2023

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DOI: [10.29303/jppipa.v9i4.2901](https://doi.org/10.29303/jppipa.v9i4.2901)

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Abstract: Traditional medicines from plants (herbs) are less effective due to inappropriate doses for treatment, but are relatively safe from side effects. The purpose of this study was to determine the anthelmintic activity of red pomegranate peel extract and red pomegranate juice in rabbits as experimental animals. This research is experimental research using *Completely Randomized Design (CRD)* and *Post Test Only Control Design* approach. The population for this study were rabbits (*Orytolagus cuniculus*), the sample for this study were 9 rabbits aged 4-5 months with a body weight of 1.5 kg - 2 kg which were divided into two treatment groups and one control group. The results of anthelmintic activity of red pomegranate peel extract (*Punica granatum L*) with a dose of 100 mg/ml an average activity of 43.01%, a dose of 150 mg/ml an average activity of 100% and a dose of 200 mg/ml an average activity of 50, 9%. The results of the anthelmintic activity of red pomegranate juice (*Punica granatum L*) with a dose of 1 ml / kg/BW an average activity of 100%, a dose of 5 ml / kg/BW an average activity of 91.67% and a dose of 10 ml /kg/ BW average activity 58.69%.

Keywords: Anthelmintics; Red pomegranate; Rabbits

Introduction

More than 1.5 billion people or 24% of the world's population are infected with soil-transmitted worms (*soil-transmitted helminths*). Infection is widespread in tropical and subtropical regions, with the greatest numbers occurring in Africa, America, China and East Asia. More than 267 million preschool-age children and more than 568 million school-age children live in areas where this helminthic parasite is intensively transmitted, requiring treatment and preventive interventions (Fong and Chan, 2022).

Worms are an infectious disease that is still a public health problem in Indonesia because it is widespread in most parts of Indonesia and can lead to a decline in health, nutrition, intelligence and productivity. The target of the Deworming Program is to reduce worms in 2019 with an indicator of reducing the prevalence of

worms below 10% in each district/city (Kemenkes RI, 2017)

The results of a survey by the West Nusa Tenggara Provincial Health Office showed that the prevalence of roundworms (*Ascaris lumbricoides*) was 63.57%, whipworms (*Trichuris trichiura*) were 33.98%, and hookworms were 7.71%. The results of research in the Batu Dawa Environment, South Ampenan Village, found a prevalence of 32%, obtained *Soil Transmitted Helminth (Ascaris lumbricoides, Trichuris trichiura and hookworms)* and *Cestoda (Hymenelopsis nana)* (Reshanleksmana et al., 2011).

Infestation of worm parasites in the host's body can affect the host's immunity against other antigens. Infection with intestinal worms can prevent allergic and autoimmune diseases, but can also make the host susceptible to infection by intracellular microorganisms, such as *Mycobacterium tuberculosis, Plasmodium sp* and

How to Cite:

Kristinawati, E., Tatontos, E. Y., Fikri, Z., & Urip, U. (2023). Anthelmintics Activity of Red Pomegranate (*Punica granatum*) Peel Extract and Red Pomegranate (*Punica granatum*) Juice in Rabbit Experimental Animals. *Jurnal Penelitian Pendidikan IPA*, 9(4), 2276-2281. <https://doi.org/10.29303/jppipa.v9i4.2901>

Human Immunodeficiency Virus (HIV) (McSorley and Maizels, 2012; Méndez-Samperio, 2012; Mutiara *et al.*, 2015)

The results research showed that the prevalence of worms in the mild category for *Trichurin trichura* was 10%, and overall worm infection was 12% while the average eosinophil count for the normal category was between 50-300 cells/mm². The results of the statistical analysis were not significant (*Asymp. Sig. 0.059*), which meant that there was no effect of the immune response on the number of eosinophils on the level of worm infection in the working area of the Tanjung Karang Health Center, Mataram City (Gunarti *et al* 2018)

Modern synthetic drugs are effectively used to fight diseases, however, they often cause many side effects. Traditional medicine from plants (herbs) is less effective in terms of the right dose for treatment, but is relatively safe from side effects. Herbal remedies are used to combat intestinal worms. These traditional medicines are still widely used for the treatment of parasitic diseases. India and China have enormous medicinal plant resources. The use of herbal medicines for the treatment of diseases in India dates back to 3500 BC (Bizhani, 2015)

In Indonesia, there are many plants that contain anti-worms, including temu giring (*Curcuma heyneana val*) used by people in Wonosobo district as an anthelmintic for pre-school-aged children (Nursiyah, 2013).

The results of research on papaya leaf extract are effective as vermifugal and ovicidal in direct contact against *Ascaris suum* worms in vitro (Murni *et al.*, 2015). One of the plants that is thought to be used as an anthelmintic is the pomegranate plant (*Punica granatum L*) (Yones *et al.*, 2016). Tests for anthelmintic activity were carried out on pork roundworm (*Ascaris suum Goeze*) and their eggs. The results showed that all concentrations of the test extracts used in the test had anthelmintic activity by causing spastic paralysis to death in worms and had ovicidal activity against worm eggs with the strongest concentration of 20% (Apriliani *et al.*, 2022).

The results of research on the methanol extract of Pomegranate peel doses of 50, 100, 150 mg/ml significantly showed anti-worm activity for all doses. The highest dose of 150 mg/ml in 13 minutes causes convulsions and death of the *Pheretima posthuma* worm (earthworm) in 23 minutes (Y. Swarnakar *et al.*, 2013)

Pomegranate is easier to consume in the form of fresh fruit or made into juice, but has never been tested for worms. So far, research has been carried out using Pomegranate peel extract. Therefore, the purpose of this study was to determine the anthelmintic activity of red pomegranate peel extract and red pomegranate juice in rabbits as experimental animals.

Method

This research is an experimental research using *Completely Randomized Design (CRD)* and *Post Test Only Control Group Design* approach. The population in this study were rabbits (*Oryctolagus cuniculus*) and the samples were rabbits (experimental animals) aged 4-5 months with a body weight of 1.5 kg-2 kg. The sample in this study used 9 rabbits (*Oryctolagus cuniculus*) aged 4-5 months with a Body Weight (BW) of 1.5 kg-2 kg. Prior to the experimental animal treatment, the rabbit (*Oryctolagus cuniculus*) was acclimatized in the laboratory for seven days. Furthermore, worm infection was carried out on rabbit (*Oryctolagus cuniculus*) experimental animals.

Samples of rabbits (*Oryctolagus cuniculus*) infected with worms were divided into three treatment groups. Group 1 was given ethanol extract of red pomegranate peel (*Punica granatum L.*) doses of 100 mg/ml, 150 mg/ml and 200 mg/ml. Group 2 was given red pomegranate juice (*Punica granatum L.*) doses of 1 ml, 5 ml and 10 ml per kg/BW. Group 3 was given the worm medicine Albendazole suspension 10 mg/ml.

The ethanol extract of red pomegranate peel (*Punica granatum L*) was prepared by maceration method. Red Pomegranate juice (*Punica granatum L.*) is made by grinding the pieces of fruit using a blender. Comparative data analysis of anthelmintic activity with the ethanol extract of red pomegranate rind (*Punica granatum L*) and red pomegranate juice (*Punica granatum L*) is by using the one-way ANOVA test.

Result and Discussion

The anthelmintics activity test of red pomegranate peel extract (*Punica granatum L*) in rabbits was used in three doses, namely 100 mg/ml, 150 mg/ml and 200 mg/ml with the results as shown in Table 1.

Table 1. Test results of anthelmintic activity of red pomegranate peel extract (*Punica grenade L*) in rabbit experimental animals

Dose (mg/ml)	Number of worm eggs (grain)	Anthelmintic activity of red pomegranate peel extract							Average Anthelmintic activity (%)
		AmountWorm eggs day-1 (grain)	Anthelmintic activity Day -1 (%)	AmountWorm eggs day-2 (grain)	Anthelmintic activity Day -2 (%)	AmountWorm eggs day-3 (grain)	Anthelmintic activity Day -3 (%)		
100	8	3	62.50	3	0.00	1	66.70	43.03	
150	1	0	100.00	0	100.00	0	100.00	100.00	
200	300	96	68.00	51	46.90	37	37.80	50.90	
Average anthelmintic activity (%)									64.64

Test results of anthelmintic activity of red pomegranate peel extract (*Punica granatum L*) in experimental animals of rabbits for doses of 100 mg/ml average activity of 43.01%, doses of 150 mg/ml average activity of 100% and doses of 200 mg/ml average activity of 50.9%.

To test the anthelmintic activity of red pomegranate juice (*Punica granatum L*) in experimental animals, rabbits used doses of 1 ml per kg/BW, 5 ml per kg/BB and 10 ml/kg/BW with the results shown in Table 2.

Table 2. Test results for the anthelmintic activity of red pomegranate juice (*Punica granatum L*) in rabbit experimental animals

Dose (ml/kg/ BW)	Number of worm eggs (grain)	Anthelmintic activity of red pomegranate juice							Average Anthelmintic activity (%)
		AmountWorm eggs day-1 (grain)	Anthelmintic activity Day -1 (%)	AmountWorm eggs day-2 (grain)	Anthelmintic activity Day -2 (%)	AmountWorm eggs day-3 (grain)	Anthelmintic activity Day -3 (%)		
1	2	0	100.00	0	100	0	100.00	100	
5	4	1	75.00	0	100	0	100.00	91.67	
10	28	20	28.57	8	60	1	87.50	58.69	
Average anthelmintic activity (%)									83.45

Test results of the anthelmintic activity of red pomegranate juice (*Punica granatum L*) in experimental animals of rabbits with a concentration of 1 ml / kg/BW with an average activity of 100%, a concentration of 5 ml

/kg/BW with an average activity of 91.67% and a concentration of 10 ml / kg/BW average activity 58.69%. The anthelmintic activity test using Albendazole as a control with a dose according to the rules of use, namely 10 mg/ml can be seen in Table 3.

Table 3. Test results for the anthelmintic activity of Albendazole at a dose of 10 mg/ml rabbit experiment

Dose (mg/ml)	Number of worm eggs (grain)	Anthelmintic activity of Albendazole							Average Anthelmintic activity (%)
		AmountWorm eggs day-1 (grain)	Anthelmintic activity Day -1 (%)	AmountWorm eggs day-2 (grain)	Anthelmintic activity Day -2 (%)	AmountWorm eggs day-3 (grain)	Anthelmintic activity Day -3 (%)		
10	5	3	40	1	67	0	100	69.00	
10	1	0	100	0	100	0	100	100.00	
10	4	1	75	0	100	0	100	91.70	
Average anthelmintic activity (%)									86.90

The average percentage of Albendazole anthelmintic activity test results in rabbits was 86.9%. Anthelmintic activity of red pomegranate peel extract (*Punica granatum L*) at doses of 100 mg/ml, 150 mg/ml and 200 mg/ml compared to red pomegranate juice

(*Punica granatum L*) at doses of 1 ml, 5 ml and 10 ml respectively per kg/body weight in experimental rabbits and administration of Albendazole as a control can be seen in Figure 1.

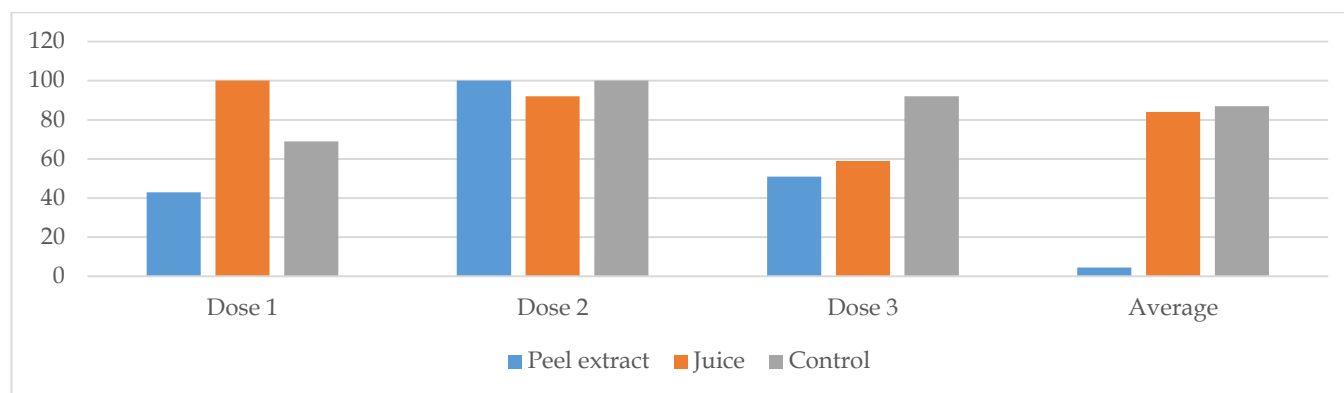


Figure 1: Anthelmintic activity of red pomegranate peel extract (*Punica granatum L*) compared to red pomegranate juice (*Punica granatum L*) in experimental rabbits with Albendazole as a contro

Statistical analysis of the anthelmintic activity test of the ethanol extract of red pomegranate rind (*Punica granatum L*) and red pomegranate juice (*Punica granatum L*) in rabbit experimental animals with three dose groups using the *Kruskall Wallis* test because they are not normally distributed.

The results of the normality test with the *Shapiro Wilk* test in the concentration group 2, namely the test concentration of 150 mg/ml red pomegranate peel extract, 5 ml / kg/BW of pomegranate juice and 10 mg/ml Albendazole as a control, are not normally distributed Sig 0.01 Sig. (<0.05) except for red pomegranate juice with normal distribution Sig. 0.86 (> 0.05). Furthermore, the *Kruskal Wallis* test was carried out because it was not normally distributed to determine the effect of anthelmintic activity in the three dose groups. The results of the *Kruskal Wallis* test at dose 2 in the anthelmintic activity of red pomegranate had no effect on rabbits with $p = 0.594$ (<0.05) compared to group 3 $p = 0.011$ (<0.55).

Pomegranate (*Punica granatum*) is considered as "A pharmacy unto itself" in Ayurvedic medicine. It's antioxidant, anticancer, anti inflammatory, antimicrobial, anti atherosclerotic, antidiabetic actions and many more (Jacob et al., 2019). The chemical characters particularly total soluble solids, total sugars, ascorbic acid and anthocyanin contents play a major role in crop due to their essentiality in fruit quality (Mir et al., 2012).

The medical application of pomegranate and its skin has attracted human attention. Research has been carried out on the chemical analysis of *Punica granatum* fruit peels and their biological properties in vitro and in vivo. The results of the study included among the solvent extracts tested, the ethyl acetate extract of fruit peel showed a broad spectrum of antimicrobial activity. *Ethyl acetate* extract supplemented by *C.elegans* worms showed inhibition of lipid accumulation similar to acarbose showing good hypoglycemic activity (Barathikannan et al., 2016).

A urinary metabolite of ellagic acid (Urolithin-A glucuronide), was not significantly different with the three interventions (Pomegranate Juice, a Pomegranate Polyphenol Liquid extract and a Pomegranate Polyphenol Powder extract), reaching levels of approximately 1,000 ng/mL. Ellagitannin metabolites, delivered from pomegranate fruits, as PJ, POMxl, and POMxp, reach equivalent levels with a delay in time of maximum concentration of POMxp compared to PJ and POMxl (Seeram et al., 2008). Pomegranate polyphenols, when present in a beverage but not in a supplement, can reduce the postprandial glycemic response of bread, whereas microbial metabolites from pomegranate polyphenols exhibit the potential to further modulate sugar metabolism much later in the postprandial period (Kerimi et al., 2017).

The results of this study showed an average anthelmintic activity of red pomegranate peel extract (*Punica granatum L*) and 83.45% in rabbit (*Orytolagus cuniculus*) experimental animals. Albendazole's anthelmintic activity average was 86.9%. *Punica granatum Linn* (Pomegranate) commonly known as Anar is an ancient fruit with great medicinal importance related to the family *Punicaceae*. This study was conducted to evaluate the anthelmintic activity of methanol extract of Pomegranate peel against *Pheretima posthuma* (earthworm). Various concentrations (50, 100 & 150mg/ml) of methanol extract were evaluated in bioassays involving the determination of the time of paralysis (P) and time of death (D) of worms. Albendazole is used as a standard anthelmintic drug. The results of this study indicated that the methanol extract of Pomegranate peel exhibited significant dose-dependent anthelmintic activity (Swarnakar et al, 2013).

Research Pomegranate extract methanol and ethanol were tested in-vitro for the potency of anthelmintic by determining the time required for paralysis and death of earthworms. Bark extracts showed dose-dependent activity. Short times to

paralysis and death were observed for the methanol extract at a concentration of 150 mg/ml. From the above observations it was concluded that the methanol extract exhibited a dose-dependent maximum response for antimicrobial and anthelmintic activity (Leena et al., 2016).

Albendazole 400 mg single dose is the WHO recommended anthelmintic for combating STH. However, these drugs are only effective for eradicating *Ascaris lumbricoides* and not effective against trichuriasis, so the dose needs to be increased (Kartika, 2016). Triple dose albendazole is found to be efficacious against *Ascaris lumbricoides* and hookworm infections but has moderate curative effect with high ERR (Egg Reduction Rate) against *Trichuris trichiura* (Horton, 2000; Tee et al., 2022).

There are several kinds of drugs that have few side effects that are used to treat intestinal worms, such as albendazole, mebendazole, ivermectin, nitazoxanide, pyrantel pamoate and levamisole. Research (Indriyati et al, 2017) showed that Albendazole mass treatment was less effective because the post-treatment worm prevalence was 82.9%. This happens due to community disobedience in consuming the drugs distributed, high prevalence of helminthiasis, low healthy behavior and poor environmental sanitation (Tumiwa MJ et al, 2021).

The risk of mass anthelmintics drug administration leading to resistance, that existing drugs are not ideal for all human helminth infections, has led to new focus on the need to develop new anthelmintics (Partridge et al., 2020).

The results of statistical analysis stated that the anthelmintic activity of the test concentration group, namely 150 mg/ml in the ethanol extract of red pomegranate peel (*Punica granatum*) and 5 ml /kg/BW of red pomegranate juice (*Punica granatum*) did not have a significant effect. In the highest concentration group, namely 200 mg/ml of red pomegranate (*Punica granatum*) ethanol extract and 10 ml / kg/BW of red pomegranate juice (*Punica granatum*) had a significant effect as an anthelmintic in rabbits.

The results of the study by Swarnakar et al, 2013 the antihelminth activity of the methanol extract of Pomegranate peel at a dose of 150 mg/ml in 13 minutes caused seizures and death of the *Pheretima posthuma* worm (earthworm) in 23 minutes. The results of this study were different because the treatment was given through experimental animals infected with *Soil Transmitted Helminth* worms especially *Ascaris lumbricoides* and antihelminth activity was measured by the number of worm eggs before and after treatment, so the results were significant at a concentration of 200 mg/ml.

Conclusion

The anti-helminth activity of Pomegranate peel extract (*Punica granatum* L) was significant at a concentration of 200 mg/ml and the anti-helminth activity of Pomegranate fruit juice (*Punica granatum* L) was significant at a concentration of 10 ml /kg/BW.

Acknowledgments

We would like to take this opportunity to express our deepest gratitude to the Director of the Mataram Health Polytechnic and his staff who have provided financial support and a research location (Parasitology Laboratory) to the researchers so that this research can be carried out

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