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Effects of Pomegranate Peel (*Punica granatum* L.) Extract as an Anthelmintic

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Abstract

Helminths infections caused by Soil-Transmitted Helminths (STH) are found in many people living in developing countries, especially in rural areas. People often use pomegranate as an anthelmintic. The aim of this research is to find out the effects of pomegranate peel (Punica granatum L.) as an anthelmintic to female Ascaris suum in vitro. The research on the effects of pomegranate peel extract has been conducted on 900 female Ascaris suum in vitro. Ascaris suum are divided into 5 groups, group I: pomegranate peel extract of dose 25%, group II: pomegranate peel extract of 50%, group III: pomegranate peel extract of 75%, group IV: 0.9% NaCl as a negative control, and group V is given mebendazole 0.5% as a positive control. The mean percentage of dead worms in group I is 39%, in group II 61%, while in group III 82%, but its potential is lower than mebendazole, which kills 100%. The treatment using pomegranate peel extract of 25%, 50% and 75% respectively has significant differences with $p < 0.05$ against negative control (NC) using a 0.9% NaCl. The research concludes that the pomegranate peel extract has an anthelmintic effect against Ascaris suum females in vitro.

***Keywords**: pomegranate peel extract, anthelmintic effect, in vitro*

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Khasiat Ekstrak Kulit Buah Delima (*Punica granatum* L.) Sebagai Antelmintik

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Abstrak

Infeksi cacing yang disebabkan oleh *Soil-Transmitted Helminths* (STH) banyak ditemukan pada orang yang tinggal di negara-negara berkembang terutama di daerah pedesaan. Herbal buah delima sering digunakan sebagai obat cacing. Tujuan dari penelitian ini adalah untuk mengetahui khasiat kulit buah delima (*Punica granatum* L.) sebagai obat cacing terhadap *Ascaris suum* betina *in vitro*. Penelitian tentang pengaruh ekstrak kulit buah delima telah dilakukan pada 900 *Ascaris suum* betina *in vitro*. *Ascaris suum* dibagi menjadi 5 kelompok, kelompok I diberikan ekstrak kulit buah delima dosis 25%, kelompok kedua diberi ekstrak kulit buah delima dosis 50%, kelompok ketiga diberi ekstrak kulit buah delima dosis 75%, kelompok keempat diberi 0,9% NaCl sebagai kontrol negatif, dan kelompok V diberi mebendazole 0,5% sebagai kontrol positif. Persentase rata-rata cacing mati dalam kelompok I 39%, kelompok II 61%, kelompok III 82%, namun potensinya lebih rendah dibandingkan mebendazole yang membunuh 100%. Pengobatan menggunakan ekstrak kulit buah delima 25%, 50%, dan 75% masing-masing memiliki perbedaan yang signifikan dengan $p < 0,05$ terhadap kontrol negatif (NC) menggunakan 0,9% NaCl. Penelitian ini menunjukkan bahwa ekstrak kulit buah delima memiliki khasiat anthelmintik terhadap *Ascaris suum* betina in vitro.

Kata kunci: ekstrak kulit buah delima, khasiat anthelmintik, in vitro

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Introduction

Helminths infections caused by Soil-Transmitted Helminths (STH) are found in many people living in developing countries, especially in rural areas. The worm in the group of STH is a worm that in completing its life cycle requires suitable soil to develop into an infective form. Four types of STH that are most commonly found are whipworm (*Trichuris trichiura*), roundworm (*Ascaris lumbricoides*), hookworm (*Ancylostoma duodenale* and *Necator americanus*).[1] Worm disease is a health problem in Indonesia, one of the researches on the prevalence of worms ever do is a research in Bali with a cross-sectional study design ever conducted in four villages. The first village is Belumbang village, 25 km from Denpasar, with an altitude of 0-500 meters above sea level, the second village is Baturiti village, 50 km from Denpasar with an altitude of 500-1000 above sea level, the third village is the village Kubutambahan 100 km of Denpasar with altitude of 0-500 km above sea level, and the fourth is the village of South Batur village, 70 km from Denpasar with an altitude of 1,000 meters above sea level. The results of research in four villages show the prevalence of helminth infections *Strongyloides stercoralis*, hookworms, *Trichuris trichiura* and *Ascaris lumbricoides* are respectively 1.6%, 24.5%, 62.6%, 73.7%.[2] Diseases caused by worms are mostly chronic diseases which often lead to increased morbidity. Anthelmintic efficacy of some drugs can be reduced by the mutation. The use of worm medicine should be based on the region where the worm infection and drug use can be tolerated by humans.[3] There is an indication that there has been resistance in worms to some anthelmintic on the market which has encouraged the use of medicinal plants as an anthelmintic. Pomegranate tree can grow to a height of 12-16 feet, and has many branches, and can live up to 200 years old. The lanceshaped leaf and the trunk turn into gray at the age of three years. The flowers are large, red, white or varied, and has a tubular calyx which produces fruit. Pomegranate peel can reach five inches thick. The fruit has many seeds, each of which is surrounded by a red pulp. Pomegranate tree are found in India, and in many places in Southeast Asia, East Indian, and African-tropical climates. The tree is also available in the park of California, and Arizona[4] People often use pomegranate as an anthelmintic. The parts of pomegranade used as anthelmintic herbs are the bark, root bark, and fruit peel. The aim of this research is to discover the effects of pomegranate peel (*Punica granatum* L.) as an anthelmintic to female *Ascaris suum* in vitro.

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Methods

The research materials are 0.9% of NaCl solution, pomegranate peel extract, distilled water, and Mebendazole suspension of 0.5%; while the research tools are gloves, measuring cup, glass beaker, water bath, sieve, 20 pieces of plastic containers, storage boxes *Ascaris*, tweezers, clinical thermometers, incubators, and equipment extraction.

The subjects are 900 female *Ascaris suum* obtained from one of the abattoirs in Bandung. The laboratory experimental research is designed with a completely randomized design (CRD). Anthelmintic effect is tested in vitro. The data measured is the number of live and dead worms after being incubated for 3 hours at the temperature of 37ºC.

Variable treatment includes pomegranate peel extract with concentration of 25%, 50% and 75%, 0.9% NaCl solution as a negative control, mebendazole of 0.5% as a positive control. The variable response shows by the number of paralyzed and dead worms after being incubated at 37°C for 3 hours. A live worm is a worm that still moves when lifted and handled with the rod of the container treatment. Paralyzed worm is a worm that does not move when lifted and handled with a rod of container treatment and move back when dyed and handled back in 50°C distilled water. A dead worm is a worm that does not move at all despite having been dyed and put back in 50°C distilled water. Worms that die from pomegranate peel extract have the characteristics of the bodies that are soft or mushy to the touch, pale white body color and more transparent when compared with live worms in the control group.

Pomegranates are picked when they are ripe. After that, dry sorting is done to separate foreign materials from crude drug materials (eg, soil, gravel, stems, or leaves). Pomegranate is washed with clean water. Meat fruit and pomegranate seeds are separated from the peel, then the peel is cut and sliced thinly. Pieces of dried pomegranate peel are put in direct sunlight to dry. Before being used in the manufacture of the extract, pomegranate rind is dry-blended until smooth to a certain degree. Dried pomegranate rind powder is considered necessary.

After the raw material has been refined, it is incorporated into the extraction bag. The extraction bag is poured into a saucepan and added some water at a ratio of 1: 4. The water is heated on the stove to boil, timed for 1 hour of boiling and then poured into a water bath. New water is added to the pan and repeated until the cooking water is colorless (usually 6-7 boilings). The water bath is heated until the solution becomes viscous, and the extract is then transferred to a drying tray and put in the oven at the temperature of 50-60 degrees until dry. After being dried, the extract is blendered until smooth. The extract is then used at a dose of 25%, 50%, and 75% (pomegranate peel

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extract 25% = 125 grams plus 500 ml water; pomegranate peel extract of 50% = 250 grams plus 500 ml water; pomegranate peel extract of 75% = 375 grams plus 500 ml of water).

The animals used are obtained from a slaughterhouse in Bandung. *Ascaris suum* females and males are separated by seeing the tip/tail. Ascaris worm males have a tail that curves like a hook, while the the size of the female worm is not larger than the males. In this experiment, only the female *Ascaris suum* is used.

Five pieces of plastic containers are prepared. Container I, II, III, IV, and V, each contains 25%, 50%, 75% of pomegranate peel extract, NaCl 0.9% and 0.5% mebendazole suspension. *Ascaris suum* females as many as 30 individuals are put into the container which has already had the extract solution. All solutions are incubated at 37° C for 3 hours. Observations are carried out in a way to disturb the worms with a stirring bar. The worm is said to live if they can move and can be distinguished from a worm that does not move, or paralysed. The worms are then soaked in distilled water at 50° C. After being soaked, the worms are then taken out to see if the worms are alive, paralysed or dead. The worms are declared dead if there is no movement at all. The number of live, paralysed and dead worms are calculated, recorded and tabulated. The experiments are conducted with 1-6 points with 6x repetitions.

The data analysis methods measured are the number of worms which becomes paralysed and dead after being incubated for 3 hours at 37° C. The data of the number of paralysed and dead worms are analyzed using Kruskal-Wallis nonparametric test with $\alpha = 0.05$. If there is a difference, it will be followed by Mann-Whitney test. The significance is determined and it is based on the value of $p < 0.05$ processing data done by computer software.

Result

The research on the effects of pomegranate peel extract has been conducted on 900 female *Ascaris suum* in vitro. *Ascaris suum* are divided into 5 groups. Group I is given pomegranate peel extract of 25%, group II 50%, group III 75%, group IV 0.9% of NaCl as a negative control, and Group V 0.5% of mebendazole as a positive control. The worms are incubated for 3 hours and then they are observed if the worms are dead or alive. The data is statistically tested by non-parametric test of Kruskal-Wallis. The mean percentage of dead worms after treatment can be seen in Table 1.

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Table 1 Average Percentage of Worms Died After Treatment

Repetition to	Treatment group				
	I	**II**	**III**	**IV**	**V**
1	30	60	80	0	100
2	43	63	80	0	100
3	47	60	90	0	100
4	43	77	87	0	100
5	33	53	73	0	100
6	40	50	83	0	100
Average (%)	39	61	82	0	100

Notes:
I Pomegranate peel extract dose 25%
II Pomegranate peel extract dose 50%
III Pomegranate peel extract dose 75%
IV NC: Negative control (0.9% NaCl)
V PC: Positive control (Mebendazole 0.5%)

The test results of non-parametric Kruskal-Wallis get a value of p <0.05, and a further test is then performed by using the Mann-Whitney, and its results can be seen in table 2.

Table 2 Mann-Whitney Test Results in the Treatment Group

	I PPE 25%	II PPE 50%	III PPE 75%	IV KN	V KP
I PPE 25%		NS = 0,065	**	**	**
II PPE 50%			**	**	**
III PPE 75%				**	NS = 0,180
IV NC					**
V PC					

Notes:

I	PPE 25% : Pomegranate Peel Extract dose 25%
II	PPE 50% : Pomegranate Peel Extract dose 50%
III	PPE 75% : Pomegranate Peel Extract dose 75%
IV NC	: Negative control (0.9% NaCl)
V PC	: Positive control (Mebendazole 0.5%)
**	: p < 0,05
NS	: Non-Significant

Discussion

The mean percentage of dead worms in Group I is given pomegranate peel extract of 25% is 39%. The mean percentage of dead worms in Group II pomegranate peel extract of 50% is 61%, while the average percentage of dead worms in Group III pomegranate peel extract of 75% is 82% and it has the highest percentage.

Mann-Whitney test results show that the treatment using pomegranate peel extract of 25%, 50% and 75% respectively has significant differences with p = 0.002 against negative control (NC)

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using a 0.9% NaCl. This indicates that the pomegranate peel extract has an anthelmintic effect on *Ascaris suum* females.

In the test, the results are obtained from the treatment using pomegranate peel extract of 25% and 50% pomegranate peel extract significant difference ($p = 0.002$) with a positive control (PC) which uses mebendazole 0.5%, while the treatment using pomegranate peel extract of 75% in treatment group with the highest dose has no significant difference ($p = 0.180$) with the positive control. This shows that pomegranate peel extract of 25% and 50% anthelmintic effect is no better than mebendazole, while pomegranate peel extract of 75% has an anthelmintic effect equivalent to mebendazole.

The constituents of pomegranate are gallic acid; phenolic punicalagins; and other fatty acids; catechin, EGCG; rutin, quercetin, and other flavonols; flavonones, flavones; tannins (punicalin and punicafolin), anthocyanidins; and flavone glycosides including apigenin and luteolin, piperidine. Constituents of pomegranate that have anthelmintic effects are alkaloids and tannin.[5,6] Worm disease that affects humans and animals causes a growth of disturbance. Medications used to treat worm diseases should be toxic to the parasite, but safe for humans. Mechanism of action of drugs for diseases of the worm can be neuromuscular paralysis in worms. Some medications such as pyrantel and levamisole have agonist effects on acetylcholine receptors in the muscles of worms and causes spastic paralysis. Other drugs have a working mechanism of the receptor GABA (gamma amino-butyric acid) as an agonist effect on worm muscle and causes flaccid paralysis.[7]

Recently, the drugs used to combat the worm seems to be resistant. Therefore, it is necessary to develop alternative dewormed drugs as was done in this study. Similar studies that have been done to address worm diseases with medicinal plants are to use the fruit of *Mallotus philippinensis*, family: Euphorbiaceae, commonly called kamela, whose results have anthelmintic effects and safe for human digestion.[8]

Conclusion

The research concludes that pomegranate peel extract has an anthelmintic effect on female *Ascaris suum* in vitro.

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