

## DECOCTION OF POMEGRANATE (*PUNICA GRANATUM* L.) PEEL AS AN ANTHELMINTIC AGAINST *TAENIA SAGINATA*

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Received: 08 Aug 2021, Revised and Accepted: 15 Aug 2021

### ABSTRACT

**Objective:** The purpose of this study was to investigate the anthelmintic activity of pomegranate (*Punica granatum* L.) peel decoction against *Taenia saginata*.

**Methods:** The *in vitro* assay was conducted by observing the motility of *T. saginata*, which is isolated from cattle's gastrointestinal tract, in various concentrations of pomegranate peel decoction with albendazole as a positive control.

**Results:** The results showed that the anthelmintic activity was dependent on decoction concentration and the duration of contact between decoction and nematode. Decoction at moderate concentration causes paralysis, while high concentration causes death. The 75% and 100% pomegranate peel decoction started to cause death at 240 min and 150 min after contact with *T. saginata*.

**Conclusion:** It was concluded that pomegranate peel decoction has anthelmintic activity.

**Keywords:** Albendazole, Motility assay, Dose-dependent, Isolated *Taenia saginata*

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DOI: <https://dx.doi.org/10.22159/ijap.2021.v13s4.43831> Journal homepage: <https://innovareacademics.in/journals/index.php/ijap>

### INTRODUCTION

Human helminthic infections affect a large proportion of the world's population. In developing countries, they contribute to the prevalence of anaemia, malnutrition, eosinophilia and pneumonia [1]. These infections, mainly due to lymphatic filariasis, soil-transmitted helminths, and schistosomiasis. It belongs to the class of neglected tropical diseases and are major targets of global elimination programs [2]. In 1982, there was the first approved anthelmintic for use in humans, i.e. albendazole (methyl N-(6-propylsulfanyl-1H-benzimidazol-2-yl) carbamate) [3, 4]. Albendazole is contraindicated for pregnant women and exhibited embryo toxicity in animals [4].

About 80% of the world's population in developing countries, including Indonesia, use herbal medicine for their primary health [5]. Plant extracts, as an alternative treatment for parasite nematodes, also play an important role in traditional medicinal practices, especially in the tropics [6]. The alternative anthelmintics derived from plants has been growing interest due to the resistance, side effects, and high price of anthelmintics [5]. Toxicity studies with albendazole doses above 30-40 mg/kg/kg/day for 4-90 d induce weight gain retardation, leukopenia, proteinuria, anemia, and hypercholesterolemia in rats [4]. So, it is important to explore the use of herbal medicine from the Indonesian people to find anthelmintics as an alternative to overcome these problems.

Pomegranate (*Punica granatum* L., Punicaceae) is described as nature's power fruit. This plant is used in folkloric medicine for the treatment of various diseases [7]. Indonesian people use pomegranate as anthelmintic. Empirically, Indonesian people use an infusion of pomegranate root and temu giring (*Curcuma heyneana*) rhizome, once a day, repeated for 4 d [8]. Therefore, due to the medicinal properties of the pomegranate, this *in vitro* study aimed to investigate the anthelmintic activity of pomegranate peel decoction against *Taenia saginata*, which isolated from cattle's gastrointestinal tract from the slaughterhouse in West Java, Indonesia. This study used pomegranate peel decoction to increase the amount of extracted secondary metabolites.

### MATERIALS AND METHODS

#### Materials

Pomegranates were collected from Cikajang area, Garut district, West Java, Indonesia, in September 2019. Pomegranates were selected with

a perfect orange peel. Plants were identified in the Plant Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia, with No. 167/HB/09/2019.

*T. saginata* was obtained from a slaughterhouse in Bandung city, West Java, Indonesia. *T. saginata* was isolated from the cattle's gastrointestinal tract, selected 8-10 cm in length, then identified in the Animal Taxonomy Laboratory Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia with No. 169/HB/08/2019.

#### Methods

##### Samples preparation

Pomegranate peels were sliced and weighed 25, 50, 75, and 100 g, then extracted with 100 ml distilled water for 30 min at 90 °C. Decoction was filtered and diluted to the mark with distilled water in a 100 ml volumetric flask [9]. The method described by Tiwari *et al.* [10] was used for phytochemical screening of the secondary metabolites in simplicia and decoction. The specific reagents were used to detect flavonoids, polyphenols, tannins, alkaloids, saponins, monoterpenoids, terpenoids, and steroids.

##### Anthelmintic activity test

Nematode motility assay was conducted according to the method described by Fikri *et al.* [11] with some modification. This method was applied to investigate the anthelmintic activity of pomegranate peel decoction against *T. saginata*, which is isolated from the cattle's gastrointestinal tract. Motility scale was scored as 0 = dead, 1 = paralysed, 2 = slow-moving, 3 = active. *T. saginata* for each concentration were scored individually. The scores were converted to a numerical value and expressed as a percentage of the maximum score. *T. saginata* was placed in a petri dish containing 10 ml of sterile saline (normal control), 0.4% albendazole (positive control), pomegranate peel decoction at various concentrations (25, 50, 75, and 100%). All petri dishes were incubated at 27 °C for 8 h. The motility of *T. saginata* was recorded for every 30 min and stirred with a stirring rod. Three replicates were run for each concentration, a normal and positive control. If *T. saginata* were motionless, then it transferred to warm water at 50 °C. If *T. saginata* remained motionless, then *T. saginata* were dead, but if *T. saginata* moved, then *T. saginata* were only paralyzed [11].

### Statistical analysis

Data were presented as mean±standard deviation. One-way ANOVA followed by Newman-Keuls was used for data analysis between groups. Values were considered statistically significant at  $p < 0.05$ .

### RESULTS

The color of pomegranate peel decoction was pale to dark orange, which depended on the concentration (25, 50, 75, and 100%). The results of phytochemical screening showed that pomegranate peel and decoctions contained alkaloids, flavonoids, tannins, saponins, and quinones.

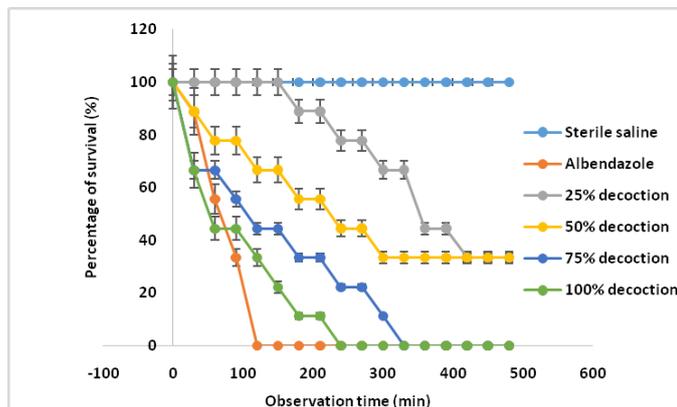


Fig. 1: Nematode motility assay of decoction against *T. saginata* at different concentrations

### DISCUSSION

Decoction was chosen as the extraction method, as an approach to the use of infusion in Indonesian people [8]. Decoction is an extraction method by heating at 90 °C for 30 min with water as the solvent. Preparation of infusion and decoction using the same solvent but different in extraction time. Decoction needs 30 min, while infusion only 15 min [9]. This is expected to increase the concentration of extracted secondary metabolites, thereby increasing their anthelmintic activity. Hot extraction indicates that the secondary metabolites, which are predicted to have anthelmintic activity, are thermostable.

The results of phytochemical screening were similar to Fikri *et al.* [11], which identified alkaloids, flavonoids, tannins, and saponins. In this study, quinones were identified, while Fikri *et al.* [11] were identified as triterpenoids and steroids. This difference occurs due to differences in the extraction method. Extraction method in this study was carried out at 90 °C for 30 min, while in Fikri *et al.* [11] was carried out at 50 °C for 24 h. This showed that quinones were soluble in hot water [12, 13], because of the glycosidic bonds, making it more polar.

This study uses fruit peels, not roots, because if using roots, then the pomegranate plant must be cut down so that it cannot produce fruit again and its benefits as a plant are lost. In addition, a study by Dkhil [14] proved that pomegranate peels have anthelmintic activity.

A total of 25, 50, 75, and 100 g of sliced pomegranate peels were decocted with 100 ml of distilled water to produce 92, 90, 88 and 87 ml of decoction. The results showed that the more pomegranate peels used, the less decoction was produced. This was because more distilled water was absorbed into the pomegranate peels, so it cannot be filtered, even though it has been squeezed.

Albendazole was chosen as a positive control because its activity preferentially affects parasites rather than the host [4]. Albendazole will bind to intracellular microtubules and prevent their elongation [3]. This activity preferentially affects parasites rather than the host [4].

Pomegranate peel decoction at 25, 50, 75, and 100% started to cause paralysis at 360 min, 180 min, 120 min, and 90 min after contact with *T. saginata*, respectively (fig. 1). The 25% and 50% pomegranate peel decoction did not cause death after contact with *T. saginata* for 8 h. The 75% and 100% pomegranate peel decoction started to cause death at 240 min and 150 min after contact with *T. saginata*, respectively (fig. 1). Albendazole causes paralysis at 90 min and death at 120 min (fig. 1). The 100% pomegranate peel decoction has the same strength as 0.4%

albendazole, which causes paralysis after 90 min. The 100% pomegranate peel decoction was weaker to cause death because decoction causes death after 150 min, while 0.4% albendazole after 120 min. Statistical analysis showed there was a significant difference in all test groups ( $p = 9.44 \times 10^{-20}$ ).

Fig. 1 and statistical analysis showed that the anthelmintic activity of pomegranate peel decoction depends on the concentration and duration of contact with *T. saginata*. The *in vitro* results showed the ability of pomegranate peel decoction as the candidate of anthelmintic for alternative treatment of *T. saginata* infection. These results were in accordance with Dkhil [14], although with different types of nematodes. Pomegranate peel extract at 100, 200, and 300 mg/ml had anthelmintic activity on live adult *Allolobophora caliginosa* in terms of paralysis and death [14].

In herbal medicines, antibacterial, antiprotozoal, anticestodal, and antinematodal activity is caused by antioxidant activity [15]. Pomegranate has anti-inflammatory and antioxidant properties [7]. The synergistic activity of secondary metabolites in pomegranate results in anthelmintic activity. The phytochemical screening results showed the presence of secondary metabolites affecting *T. saginata* motility. Pomegranate phytoconstituents that have been identified were gallotannins, ellagitannins, anthocyanins, gallagyl esters, hydroxybenzoic acids, hydroxycinnamic acids, and dihydroflavonols [16, 17]. These compounds have been shown to provide antinematodal activity [18-21].

The secondary metabolites, such as alkaloids, tannins, polyphenols, saponins, glycosides, and lignins, are responsible for the plant antiparasitic activity [22-24]. These Phyto-constituents showed dose-dependent antiparasitic properties [24]. The antioxidant activity of flavonoids and polyphenols in helminths is caused by uncoupling oxidative phosphorylation and hindering energy production [25]. The anthelmintic activity of tannins is caused by its capacity to bind onto some proteins of metabolism or the nematode's organs and muscles, thereby changing their function and causing paralysis or death [26]. The anthelmintic activity of saponins is caused by changing cell membrane permeability and pore formation, resulting in disintegration of the parasite teguments [26]. The alkaloid that has been identified in pomegranate peel is pelletierine. Pelletierine alkaloids cause muscle paralysis of nematodes, even causing death at large doses [27]. Anthelmintic activity of pomegranate peel could ease the economic burden on anthelmintic therapy. The treatment cost of helminth infections is saved by using pomegranate peel which is proven to have anthelmintic activity.

**CONCLUSION**

Pomegranate peel decoction showed an anthelmintic activity dependent on dose and duration of contact with *Tania saginata*. The 75% and 100% pomegranate peel decoction started to cause death at 240 min and 150 min after contact with *T. saginata*.

**ACKNOWLEDGEMENT**

The authors would like to thank Dytha Andri Deswati and Irvan Nugraha for technical assistance.

**FUNDING**

Nil

**AUTHORS CONTRIBUTIONS**

All the authors contributed equally.

**CONFLICT OF INTERESTS**

Declared none

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