The Best Ways to Extract Papaya Seeds for Their Deworming Effects on *Ascaridia galli*

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ABSTRACT

Previous research has shown that papaya seeds have an anthelmintic effect on *Ascaridia galli*, but this effect was observed using traditional methods, such as boiling the seeds in water. To determine the most effective extraction method, various techniques were tested, including maceration, soxhlet extraction, and ultrasonic digestion. The study used both wet and dry papaya seeds, with solvents such as water, 70% ethanol, and n-hexane. The anthelmintic activity was measured by the percentage of worm death, LD_{50}, and relative potential compared to pyrantel pamoate, a positive control. The results indicated that the maceration method using 70% ethanol as the solvent for wet papaya seeds was the most effective, killing 60% of the worms with an LD_{50} of 9.36 mg and a relative potential of 0.66 times that of pyrantel pamoate.

Keywords: Papaya seed, Extract, Anthelmintic.

INTRODUCTION

Papaya seeds, often discarded as waste, have been found to possess anthelmintic properties, meaning they can kill intestinal worms. Ardana *et al*. (2021) demonstrated the effectiveness of papaya seed powder in eliminating Ascaris suum worms in pigs, while Pattianakotta *et al*. (2019) confirmed the efficacy of ethanol extract of papaya seeds against *Ascaridia galli* worms. Papaya seeds can also be used to treat worms in children. Additionally, papaya seeds are known for their antioxidant effects (Zou *et al*., 2021). To ensure ease of use, it is recommended that papaya seed extract be researched and formulated into a stable, appealing dosage form for children, with pleasant color, aroma, and taste.

This study aims to identify the most effective extraction methods and solvents for harnessing the anthelmintic properties of papaya seeds. The research design involves testing two types of papaya seed simplicia: wet seeds and dried seeds. Each type of simplicia is subjected to three different extraction methods: maceration, ultrasonic digestion, and soxhlet extraction. The solvents used in the extraction process include water, 70% ethanol, and n-hexane. This study builds upon the research conducted by Solhah (2019) and Himawan *et al*. (2020).

At this stage, the research has not yet made a significant impact, as it primarily provides an initial overview of the benefits of papaya seed extract. If successful in developing effective, safe, and acceptable dosage forms for pediatric patients, the new dosage form can be standardized for human use in herbal medicine (Gunawan *et al*., 2017). The success of this research phase will serve as the foundation for future research roadmaps aimed at identifying and extracting active compounds from papaya seed extract. These active compounds will be used as markers for the extract. This research aims to create a phytopharmaceutical dosage for both humans and animals (Kusumaharja, 2022).

TOOLS AND MATERIALS

The tools used in this study are macerators, soxhlet, petri dish, rotary evaporator, and ultrasonic digest.

The materials used in this study were papaya seeds, aquadest, 70% ethanol, and n-hexane.

METHODS

Simplicia of wet papaya seeds and dried papaya seeds was extracted with 3 methods, namely maceration, soxhletation, and ultrasonic digestion. In each method 3 types of solvents were used, namely aquadest, 70% ethanol and n-hexane. The extraction results were concentrated using a rotary evaporator until it became a thick extract with certain parameters.

In Vitro Test of Anthelmintics

Prepared petri dishes filled with each of 10 *Ascardia galli* worms. In the petri dish, normal saline (0.9% NaCl) was given which contained papaya seed extract with a level of 0.75; 1.5; 5.0; 7.5; 15; and 30%. Left for 15 minutes. Furthermore, it was observed the number of worm deaths in each petri dish, calculated as percentage of worm deaths.

RESULTS

The results of this research can be seen in table 1 and table 2.

Table 1. Results of Qualitative Test of Extract

No	Materials	Methods	Solvents	Results				
				Alk	Flav	Terp	Ster	Sap
1	Wet Papaya Seeds	Macerations	Aquadest	+	+	-	-	-
			70% Ethanol	+	+	+	+	-
			n-Hexane	-	+	+	+	+
		Soxhletation	Aquadest	+	+	-	-	-
			70% Ethanol	+	+	+	+	+
			n-Hexane	-	-	+	+	+
		Ultrasonic Digest	Aquadest	+	+	-	-	+
			70% Ethanol	+	+	+	+	+
			n-Hexane	-	+	+	+	+
2	Dried Papaya Seeds	Macerations	Aquadest	+	+	-	-	-
			70% Ethanol	+	+	+	+	-
			n-Hexane	-	+	+	+	-
		Soxhletation	Aquadest	+	+	-	-	-
			70% Ethanol	+	+	+	+	-
			n-Hexane	-	-	+	+	-
		Ultrasonic Digest	Aquadest	+	+	-	-	-
			70% Ethanol	+	+	+	+	-
			n-Hexane	-	+	+	+	-

Note: Alk=Alkaloids; Flav=Flavonoids; Terp=Terpenoids; Ster=Steroids; Sap=Saponin

Table 2. Result of Test of Extract on *Ascardia galli*

No	Materials	Methods	Solvents	Anthelmintic effect		
				% Death	LD_{50} (mg)	Rel Pot
1	Wet Papaya Seeds	Macerations	Aquadest	27	65.65	0.09
			70% Ethanol	**60**	9.36	**0.66**
			n-Hexane	43	18.29	0.34
		Soxhletation	Aquadest	26	66.65	0.90
			70% Ethanol	53	12.10	0.52
			n-Hexane	37	28.98	0.22
		Ultrasonic Digest	Aquadest	23	70.56	0.08
			70% Ethanol	43	19.34	0.32
			n-Hexane	30	55.67	0.11
2	Dried Papaya Seeds	Macerations	Aquadest	23	78.65	0.08
			70% Ethanol	53	11.40	0.55
			n-Hexane	37	27.55	0.23
		Soxhletation	Aquadest	26	70.74	0.09
			70% Ethanol	53	12.22	0.51
			n-Hexane	37	30.32	0.21
		Ultrasonic Digest	Aquadest	17	129.45	0.04
			70% Ethanol	43	20.01	0.31
			n-Hexane	30	50.18	0.12

DISCUSSION

The evaluation of papaya seed extract included various parameters such as yield, organoleptic properties, flavonoid content, terpenoids, drying losses, and ash residue. The highest yield was obtained by using ultrasonic extraction on wet papaya seeds with 70% ethanol solvent, resulting in a 33% yield. Ultrasonic extraction is highly effective for obtaining substances from simplicia due to the efficient dissolution process facilitated by ultrasonic vibrations. Conversely, the lowest yield was observed in the extract of wet seeds using the n-hexane solvent soxhlet extraction method. This is because n-hexane is a nonpolar solvent, while wet seeds are polar, and soxhlet extraction involves a heat method with relatively short immersion times, despite solvent circulation in this method.

The organoleptic test results revealed that all extracts had a brownish color and a bitter taste. The odor was not distinctive, as the smell of the solvents was more pronounced. However, the solvent distilled water had a papaya-like smell. In the phytochemical tests, which included alkaloids, flavonoids, terpenoids, and steroids, all extracts tested positive for alkaloids and flavonoids. The n-Hexane extract tested positive for all components, while the water extract was negative for terpenoids and steroids.

For the loss on drying test because it was used as a standard, all extracts were thickened to water content (drying losses) not less than 6% not more than 8%. And all extracts were in the range of 6-8% LOD. For the highest levels of ash (residual spawning) was the ethanol extract with ultrasonic and the lowest level of n-hexane extract with the soxhletation method.

The anthelmintic tests on *Ascaridia galli* worms yielded results as presented in Table 2. The findings indicated that the highest worm mortality rate was achieved with the wet seed extract using the 70% ethanol maceration method. The average worm mortality rate in the wet seed extract was significantly higher compared to the dry seed extract. This suggests that the anthelmintic active ingredient in papaya seeds diminishes when the seeds are dried. Additionally, among the extraction methods, the maceration method demonstrated a higher average killing power compared to other methods, such as the ultrasonic method. Despite the ultrasonic method yielding the highest extract, its worm mortality rate was low. This is likely due to the presence of numerous other substances in the extract that do not possess anthelmintic properties, thereby masking or reducing the effectiveness of the anthelmintic compounds.

Then to compare the type of solvent to the killing power, it can be seen from the results that the worm death of the extract with 70% ethanol gives a higher average worm death compared to other solvents. In theory, semi-polar solvents make it possible to attract all efficacious substances from simplicia, in contrast to polar solvents which tend to attract only polar compounds, and nonpolar solvents only attract nonpolar compounds. Thus, it is assumed that the anthelmintic properties of papaya seeds are semi-polar compounds.

The results indicated that the lowest concentration yielded the highest LD50, meaning that the highest worm mortality rate corresponded to the lowest LD50. Therefore, it can be concluded that the extract of wet papaya seeds with 70% ethanol as the solvent provides the best LD50.

When compared to pyrantel pamoate, the anthelmintic effect of the extract is considered adequate, suggesting that papaya seed extract could potentially be used as an anthelmintic. For ease of administration, it can be formulated into an appropriate dosage form.

CONCLUSION

The study found that the most effective extract for killing *Ascaridia galli* worms was obtained from wet papaya seeds using the maceration extraction method with 70% ethanol. This extract was able to kill an average of 60% of the worms when used in a 15% solution. The LD_{50} value was determined to be 9.36, with a relative potential of 0.66.

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