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The Best Extraction Methods of Wet and Dry Papaja (*Carica papaja* L.) Seed as Anthelmintics Effect on *Ascardia galli*

Inding Gusmayadi[1*], Priyanto[1], and Dwitiyanti[1]

[1]Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta Timur, 14360.

*indinggusmayadi@uhamka.ac.id

ABSTRACT

Papaya seed in previous research is known has an effect of anthelmintic on *Ascardia galli*, but the effect of papaya seed just by traditional method i.e. boiling in water. To get the effective extraction methods, it still needs to be done many kinds of extraction methods of papaya seed, to know which one is the best. Methods of extraction in this research were maceration, soxhletation, and ultrasonic digestion. We used two kinds of papaya seed: wet and dry, using solvent: water, 70% ethanol, and n-hexane. Data of anthelmintic activity were percentage of worm death, LD_{50}, and relative potential to pyrantel pamoate as positive control. The result showed that the best extraction method of papaya seed which had highest in vitro effect on *Ascardia galli* worm was maceration method with 70% ethanol as solvent of wet papaja seed. It killed 60% of worm with LD_{50} 9.36 mg and its relative potential is 0.66 times pyrantel pamoate.

Keywords: Papaja seed, extract, anthelmintic

1. INTRODUCTION

Papaya seeds are one of the wastes that are not used by people, but in fact the results of preliminary studies show that papaya seeds are efficacious as anthelmintics (can kill worms in the intestine). Ardana et al. [1] showed the effectiveness of papaya seed powder in killing *Ascaris suum* worms in pigs, while Pattianakotta *et al*. [2] proved the effectiveness of ethanol extract of papaya seeds on *Ascardia galii* worms. Means papaya seeds can actually be used for the treatment of worms in children. In general, papaya seeds have been known to have antioxidant effects [3]. In order to be able to use it comfortably and easily, it is necessary to research and formulate this papaya seed extract in a stable and attractive modern drug dosage form for children, which has color and attractive aroma and good taste.

In this study we will look for extraction methods and solvents which will provide the most effective anthelmintic effect of papaya seeds. The design made was to try 2 types of simplicia papaya seeds, wet seeds and dried seeds. Each simplicia was then extracted using 3 kinds of extraction methods, maceration, ultrasonic digestion, and soxhletation. The solvents used are water, 70% ethanol and n-hexane. This design follows the research conducted by Himawan *et al*. [5] and Solhah [6].

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At this stage the research has not yet made an over-visible impact because basically it only gives an initial picture of the benefits of papaya seed extract, if it is successful in getting good preparations, efficacious and acceptable dosage forms for pediatric patients, then new dosage form can be applied to humans in standardized herbal forms [7]. The success of this research phase will be the basis for further research road maps, which are the basis for finding and obtaining active compounds from papaya seed extract. This active compound will be used as an extract marker. And the gain of this research is expected to get a dosage form. This preparation can be used for humans as well as for animals in veterinary dosage forms [8].

2. METHODOLOGY

Materials

Material used in this work is papaya seeds, aquadest, 70% ethanol, and n-hexane. Animal test used in this work is *Ascardia galli* worms. Tools used in this work is macerators, soxhlet, petri dish, rotary evaporator, and ultrasonic digest.

Methods

Preparation of extract

Simplicia of wet papaya seeds and dried papaya seeds was extracted with maceration, soxhletation, and ultrasonic digestion. In each method solvents were used aquadest, 70% ethanol and n-hexane solvents. 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.

3. RESULT AND DISCUSSION

Evaluation of papaya seed extract obtained included rendemen, organoleptic, flavonoid test, terpenoids, drying losses and ash residual. The highest extract yield was obtained by extracting with ultrasonic on wet papaya seeds with 70% ethanol solvent which was obtained 33% of rendemen. This extraction by ultrasonic is indeed the most effective way to get substances from simplicia because it occurs with a very effective dissolution process with ultrasonic vibrations. While the lowest yield was in the extract of wet seeds with n-hexane solvent soxhletation method. This happens because n-hexane is a nonpolar solvent, while wet seeds with polar conditions and soxhletation are heat methods with not too long immersion, even though solvent circulation occurs in this method.

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The organoleptic test results showed that all extracts were brownish in color, bitter taste, odor was not distinctive, because the smell of solvents was more pronounced, whereas for the solvent distilled water smelled of papaya. In phytochemical tests which include alkaloids, flavonoids, terpenoids, and steroids. All positive extracts of alkaloids and flavonoids. While the n-hexane extract was positive all tested, negative terpenoid and steroid water extracts.

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.

Table 1. Results of Qualitative Test of Extract

Materials	Methods	Solvents	Results				
			Alk	**Flav**	**Terp**	**Ster**	**Sap**
Wet Papaya Seeds	Macerations	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	-
		n-Hexane	-	+	+	+	+
	Soxhletation	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	+
		n-Hexane	-	-	+	+	+
	Ultrasonic Digest	Aquadest	+	+	-	-	+
		70% Ethanol	+	+	+	+	+
		n-Hexane	-	+	+	+	+
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

The results of anthelmintic test on *Ascardia galli* worms were obtained as shown in table 2. The results showed that the highest worm death was in wet seed extract with 70% ethanol maceration method. The average worm death in wet seed extract was relatively higher than the average worm death of dry seed extract. This shows that the anthelmintic active ingredient contained in papaya seeds will decrease if the seeds are dried. Furthermore, among the extraction methods it was seen that the killing power of the extract with the maceration method was on average higher than the worm death of the extract produced by another method, the ultrasonic method even though the yield was highest, but the worm death was

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low. This is presumably because there are too many other substances that do not have extracted anthelmintic properties that mask or reduce the effectiveness of the power to kill substances that have anthelmintic properties.

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.

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

Materials	Methods	Solvents	Anthelmintic effect		
			% Death	LD_{50} (mg)	Rel Pot
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
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

For the LD_{50}, the result showed that the lowest concentration was the highest for LD_{50}. Therefore, the highest of worm death gave the lowest of LD_{50}. It could be concluded that extract of wet papaya seed with 70% ethanol as solven give the best of LD_{50}.

Compare with pyrantel pamoate, the anthelmintic effect of extract could be said that it had adequate anthelmintic effect. So it is possible the papaja seed extract use as anthelmintic. For easier and practice on administration it can be made in the appropriate dosage form.

4. CONCLUSION

From the overall results of the test in this study obtained the best extract of its worm death on *Ascardia galli* worm was wet papaya seed extract with maceration extraction method using 70% ethanol.

The worm death of the extract was able to kill an average of 60% of worms with the extract content in 15% solution. The LD_{50} obtained 9.36 with relative potential 0.66.

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