

# ANTHELMINTIC PROPERTY OF SEMI-PURIFIED TANNINS FROM THE *Anacardium occidentale* (KASUY) LEAVES

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**Abstract:** Helminthiasis is one of the most common infectious diseases of the developing countries including the Philippines <sup>[1]</sup>. *Anacardium occidentale* is originated in Central and South America and now being cultivated in tropical trees like South East Asia <sup>[2]</sup>. The anthelmintic activity of the semi-purified tannins from the *Anacardium occidentale* (Kasuy) leaves was evaluated using *Ascaris suum* from the intestine of Pigs. In the assay, Pyrantel embonate was used as the positive control, normal saline solution as the negative control, and different concentration of the tannin extract (125mg/5mL, 250mg/5mL, and 500mg/5mL) and placed the worms on separated petri dishes. The time of paralysis and death was recorded and when no movement was observed, the worms were subjected to different motility tests through prick method, dipping the worm in 50°C distilled water, and shaking the petri dish. In conclusion, the *Anacardium occidentale* leaves have anthelmintic property in the dose of 500mg/5mL.

**Keywords:** Anthelmintic, *Ascaris suum*, Helminthiasis, Pyrantel embonate, *Anacardium occidentale* leaves, Tannins.

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## I. INTRODUCTION

Helminthiasis is one of the most common infectious diseases of the developing countries including the Philippines <sup>[1]</sup>. Soil transmitted helminth (STH) infections with *Ascaris lumbricoides*, *Trichuris trichiuria*, and *Ancylostoma duodenale* are prevalent in most of the provinces of the Philippines <sup>[3]</sup>.

Cashew is scientifically known as *Anacardium occidentale* from the family *Anacardiaceae* originated in Central and South America and now being cultivated in tropical areas like South East Asia <sup>[2]</sup>. Kasuy is also known for its different medicinal uses <sup>[2]</sup>. This time, the anthelmintic property of the *Anacardium occidentale* is to be determined. This study aimed to make an alternative solution for treating Ascariasis by producing cheaper anthelmintic preparation with the use of common medicinal plants.

## II. MATERIALS AND METHODS

### A. Plant Material

The leaves of *Anacardium occidentale* used in this investigation were collected from Amadeo, Cavite, Philippines during the month of June and July 2017. The sample was authenticated and identified at the Bureau of Plant and Industry. The plant sample was identified to be *Anacardium occidentale* Family *Anacardiaceae*.

### B. Collection and Preparation of Plant Material

#### Plant Extract

The collected plant sample leaves were washed to remove the dirt and other impurities and then air dried. The dried leaves were cut into small pieces and were pulverized using blender. The leaves were macerated using distilled water for 5 days. After the maceration, it was filtered using muslin cloth and further filtered using filter paper. The filtrate was collected

and washed using a diethyl ether. The lower aqueous layer containing the tannins was subjected to steam bath to incipient dryness and placed in desiccator to further remove moisture. The collected tannins were cooled and weighed using a mettler balance and a tarred porcelain evaporating dish. The percentage yield from the 500 grams sample of *Anacardium occidentale* leaves was determined.

#### Animals

About 50 pieces of *Ascaris suum* was gathered. It was placed immediately in a Bunge's solution right after obtaining them from the stomach of pigs.

#### C. Physical Evaluation of the Plant Sample

The color, odor, and physical appearance were observed from the *Anacardium occidentale* leaves extract. The solubility of the *Anacardium occidentale* leaves extract was determined using acetone, 80% ethanol, chloroform, ether, distilled water.

#### D. Phytochemical Screening of the Plant Sample

To determine the presence of tannins in *Anacardium occidentale* leaves, bromine water test, ferric chloride test, iodine test, lime water test, and 10% gelatin test were conducted.

#### E. Instrumental Evaluation of the Plant Sample

Fourier Transform Infrared (FTIR) was used to determine the presence of polyphenolic compounds and the purity of the tannins extracted from the *Anacardium occidentale* leaves.

#### F. Evaluation of Anthelmintic Activity

Each worm was placed in each disposable petri dish and conducted 3 trials for each test concentration. For the positive control, the worms received 125mg/5mL of Pyrantel embonate and normal saline for the negative control. For the test groups, each received 125mg/5mL, 250mg/5mL, and 500mg/5mL of the tannin extract dissolved in normal saline solution. The time of paralysis and death was recorded and when no movement was observed, the worms were subjected to motility test through prick method, dipping the worm in 50°C distilled water, and shaking the petri dish.

#### G. Statistical Treatment of Data

The mean was used to sum up the results of the anthelmintic activity of the *Anacardium occidentale* leaves in terms of the time of paralysis and death. Analysis of Variance (ANOVA) was used to test the non-significance difference among several groups.

### III. RESULTS AND DISCUSSION

#### A. Determination of the Percentage Yield

The percentage yield of the collected tannin extract from the leaves of *Anacardium occidentale* was 9.95%.

#### B. Physical Evaluation of the Tannin Extract

Based on the organoleptic evaluation, the tannin extract of the leaves of *Anacardium occidentale* was reddish-brown in color and has sweet odor and thick consistency.

**Table I: Results of Solubility Test of the Tannin Extract from the Leaves of *Anacardium occidentale***

Solvent	Actual Results
Acetone	Practically insoluble
Chloroform	Practically insoluble
Diethyl Ether	Practically insoluble
Distilled Water	Freely soluble
80% Ethanol	Soluble

**Table I.** It shows that the tannin extracts from the *Anacardium occidentale* leaves was practically insoluble in acetone, chloroform, and diethyl ether. The tannin extract was freely soluble in distilled water and soluble in 80% ethanol.

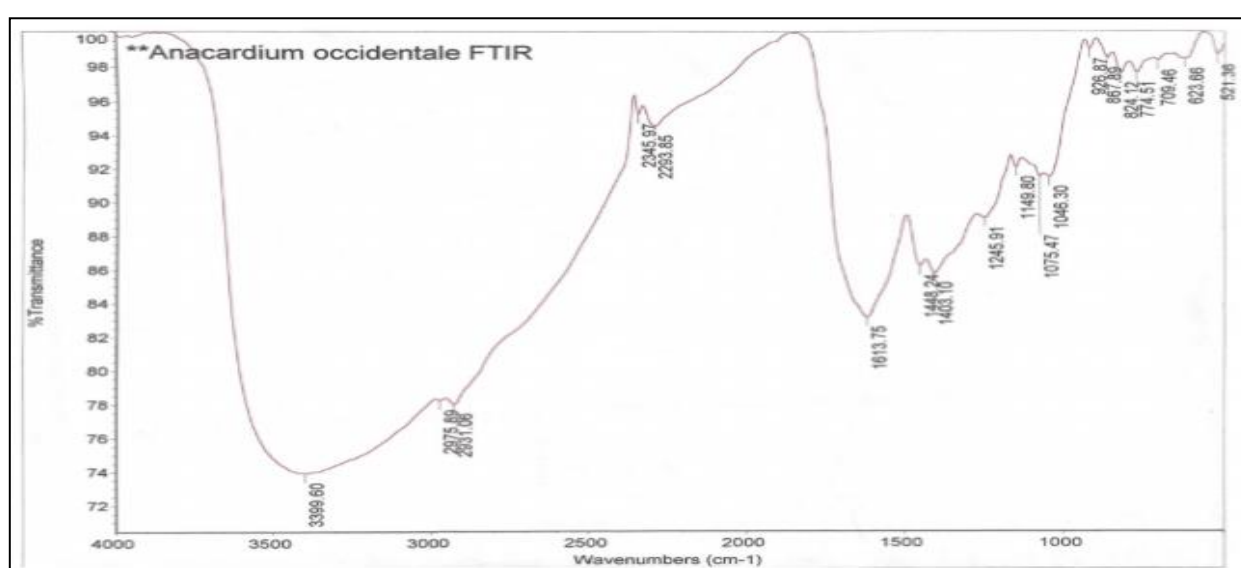
**Table II: Chemical Evaluation Result of the Tannin Extract from the Leaves of *Anacardium occidentale***

Test	Actual Result
Bromine Water Test	Negative
Ferric Chloride Test	Positive
Iodine Test	Positive
Calcium Hydroxide Test	Positive
10% Gelatin Test	Positive

**Table II.** It shows the presence of tannins from the *Anacardium occidentale* leaves extract. The tannin extract showed positive extract in Ferric Chloride test, Iodine test, Calcium Hydroxide test and 10% Gelatin test. The tannin extract produced a bluish-black precipitate in Ferric Chloride test indicating the presence of hydrolysable tannins.

**Figure 1**

**Results of the Instrumental Test of the Tannin Extract from the Leaves of *Anacardium occidentale* using Fourier Transform Infrared (FTIR)**



**Fig. 1.** It shows the results obtained in Fourier Transform Infrared (FTIR) from the tannins of *Anacardium occidentale* leaves. -OH (Hydroxyl) stretch is highly absorbed between the wave number 3200 to 3600 including 3399.60  $\text{cm}^{-1}$  indicating the presence of the phenolic group.

**Table III: Results of the Time of Paralysis and Death of the Worms**

Control		Time of Paralysis	Time of Death
Negative	Mean	360	360
Control	N	5	5
(Normal Saline Solution)	Standard Deviation	0	0
Positive	Mean	9.933	149.9900
Control	N	5	5
(Pyrantel embonate)	Standard Deviation	3.5247	70.26886
Tannin	Mean	360.000	360.000
Extract	N	5	5
(125mg/5mL)	Standard Deviation	0	0
Tannin	Mean	360.000	360.000
Extract	N	5	5
(250mg/5mL)	Standard Deviation	0	0
Tannin	Mean	97.8233	215.4300
Extract	N	5	5
(500mg/5mL)	Standard Deviation	8.82046	26.74328

**Table III.** It shows that the positive control (Pyrantel embonate) and the different dose of the tannins (125mg/5mL, 250mg/5mL, and 500mg/5mL) are not comparable in terms of paralysis. The test dose 500mg/5mL is comparable to the positive control in terms of death. This indicates that the tannin extract from the leaves of *Anacardium occidentale* exerts an anthelmintic property against *Ascaris suum* in the dose of 500mg/5mL.

**Table IV: Comparison of the Anthelmintic Activity of the Tannins Extracted from the Leaves of *Anacardium occidentale* with the Positive Control in Terms of Paralysis**

Dose	Mean	SD	F-Value	Significance	Remarks
Negative Control (NSS)	360.00	0			Negative to Positive
Positive Control (Pyrantel embonate)	9.93	3.525			Negative to Positive
			F = 4834.429	P = 0.000 < 0.01	
Tannin Extract (125mg/5mL)	360.00	0			Positive to 125mg/5ml
Tannin Extract (250mg/5mL)	360.00	0			Positive to 250 mg/5ml
Tannin Extract (500mg/5mL)	97.82	8.820			Positive to 500 mg/5ml

**Table IV.** It shows that there is a significant difference between the positive control (Pyrantel embonate) and the three test dose of the tannin extract (125mg/5mL, 250mg/5mL, and 500mg/5mL) in terms of paralysis. This indicates that time of paralysis in the test concentrations varies from the positive control.

**Table V: Comparison of Anthelmintic Activity of the Tannins Extracted from the Leaves of *Anacardium occidentale* with the Positive Control in Terms of Death.**

Dose	Mean	SD	F-Value	Significance	Remarks
Negative Control (NSS)	360.00	.000			Negative to Positive
Positive Control (Pyrantel embonate)	149.99	70.269			Negative to Positive
Tannin Extract (125mg/5mL)	360.00	.000	F = 26.442	P = 0.000 < 0.01	Positive to 125mg/5ml
Tannin Extract (250mg/5mL)	360.00	.000			Positive to 250 mg/5ml
Tannin Extract (500mg/5mL)	215.43	26.743			Positive to 500 mg/5ml

**Table V.** It shows that there is significant difference between the positive control and the two test dose of the tannin extract (125mg/5mL and 250mg/5mL) in terms of death indicating no anthelmintic activity exerted against *Ascaris suum*. The test dose 500mg/5mL showed no significant difference with the positive control indicating anthelmintic activity.

#### **IV. CONCLUSION**

Based on the results obtained, the semi-purified tannin extract of 125mg/5mL and 250mg/mL in *Anacardium occidentale* leaves has no potential anthelmintic effect in terms of their mean and standard deviation in time of paralysis and death. Therefore, the semi-purified tannin extract of 500mg/5mL showed a no significant difference with the Positive Control (Pyrantel embonate) and it has a potential of having an anthelmintic effects against *Ascaris suum*. Further studies are required to purify the tannins from *Anacardium occidentale* leaves. It is however, suggested to conduct utilization of other methods of biological assay in determining the time of death and paralysis of helminthes and also to perform the toxicity testing from the tannins obtained.

#### **REFERENCES**

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