Anthelmintic Potential of Tamarind (*Tamarindus indica*) Seeds

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Abstract: *Ascaris suum* is known to be the prevalent internal parasites affecting swine. Swine raisers are beset with poor average daily gain in weight of swine, once this parasite competes with the nutritional requirement of the animal. The seeds of *Tamarindus indic*a are commonly thrown after consumption of the fruit pericarp, without realizing its potential anthelmintic use. This study determined the mortality of A. suum exposure to different concentrations of Tamarind seed extract at 36 hours observation. Experimental research method with 4 treatments and 10 samples per treatment was used to gather pertinent data in this study. T- had 10 *A. suum* exopose to Goodwins solution, T+ had 10 *A. suum* exposure to Goodwins and Albendazole, T1 had 10 *A. suum,* Goodwins solution and 40% Tamarind seed extract (TSE) and T2 had 10 A. *suum*, Goodwins solution and 80% TSE. Data were analyzed by Analysis of Variance and Fisher Least Significant Difference test was employed to determine what treatment is significant over the other.. Result of the study revealed that T2 (80% TSE) killed the *A. suum* as early as 12 hours of exposure while *A. suum* in T1 (40% TSE) had 90% mortality at 24 hours of exposure. This implies that TSE is potent at 12 hours of exposure at 80% concentration and can be used as anthelmintics from herbal source.

Key words: *Tamarindus indica*, *Ascaris suum.*

1. Introduction

Ascaris lumbricoides of humans and *Ascaris suum* of swine are said to be two ascarids that were probably recognized by humans since prehistory, due to their abundance, adult size, symptoms and distribution. Because of their remarkable similarity, several hypotheses have been proposed to explain their origin in their respective hosts [1]. *Ascaris suum* has a high prevalence in pigs or swines in both developing and developed country resulting in significant economic penalties for pig farmers. Diseases brought about by this, can be cured by deworming the patient and the animal and one of the possible method is through the use of the natural extracts of plants as anthelmintics that has been practiced by many indigenous cultures for centuries. Small holder farmers in many developing countries often do not have access to expensive anthelmintic drugs and, in developed countries, many organic and low-input farms are not able to prophylactically treat animals with synthetic drugs. Therefore, there is an urgent need to investigate alternative and/or complementary options for the control of these parasites [2]. The use of natural plant extracts as anthelmintics has been practiced in many indigenous cultures for centuries. Indeed, in many developing countries ethnomedicine is still the primary treatment option for many parasitic diseases [3]. In the Philippines, the “Sampaloc” or commonly known as “Tamarind” is a neutral fruit that grows in any kind

of season. It is commonly used in countries like India, Cambodia, Mauritius, Madagascar, East Sudan, etc for medicinal purposes. The whole Sampaloc fruit, leaves, bark and roots has many uses for creating alternative medicines for diseases. The Sampaloc is used as a sweet sour condiment. In the Philippines, the unripe fruit and the young leaf bud is used as main ingredient for the sour soup known as "sinigang" and the ripe fruit is sold as sweetened candies. It is also a source of vitamin B and vitamin C. The seeds, surrounded by a brownish pulp, tamarind are made into balls from which jams, sweets and drinks are made. The pulp is often eaten outright with or without salt and it is also an ingredient in Indian curries. It was observed that after consumption of the fruit the seeds are just thrown away, without realizing that it can be used as source of herbal dewormer. The "Sampaloc" has a property of tannin which is good for the treatment of helminthes which in the present time has a few drugs for treating it. An analysis of tamarind pulp yielded: citric acid, 9.40; tartaric acid, 1.55; malic acid, 0.45; bitartrate of potash, 3.25; sugar, 12.5, gum, 4.7; vegetable jelly (pectin), 6.25; parenchyma, 34.35; and water, 27.55. Seed yielded tannin, a fixed oil, and insoluble matter. Analysis showed albuminoids, fat, carbohydrates, 63.22; fiber; and ash containing phosphorus and nitrogen [4]. Literature survey revealed that there is no available record regarding the Anthelmintic potential of *Tamarindus indica* Linn seeds against *Ascaris suum*. The aim of this study is to provide baseline information on getting rid of *Ascaris suum* with the use of Tamarind Seed Extract (TSE). It seeks to determine the percentage of mortality of *A. suum* in each treatment expose in TSE for 36 hours with interval of 12 hours observation.

2. Methodology

2.1. Preparation of Goodwins Solution

The components of Goodwin's Physiological solution, was patterned from the anthelmintic study [5] using herbal plant. It contains; 0.20 grams for calcium chloride, 5 grams for glucose, 0.10 grams for magnesium chloride, 0.2 grams potassium chloride, 0.15 grams sodium bicarbonate, 8 grams of sodium chloride and 0.5 grams for sodium hydrogen phosphate all these ingredients was dissolved in a 1 litter of distilled water. The Calcium chloride was later added after dissolving the ingredients with salt to avoid precipitation

2.2. Preparation of *Tamarindus indica* Seeds Herbal Extract

The Tamarind seeds were collected from Caloocan Philippines from a household who process and sell Tamarind candies. After collecting the Tamarind seeds, it was peeled and washed thoroughly to remove excess pericarp (Fig. 1). It was air dried (Fig. 2) and oven dried in °C for 45 minutes [6]. After oven drying, it was pounded using mortar and pestle. Tamarind seeds was then subjected and turned into smaller particles in the mechanical grinder. The seeds were macerated in 95% ethanol (Fig.3) at 1:1.5 for 3 days until content agitate [7]. It was filtered using cheesecloth and Whatman filter papers no. 1 (Fig. 4) and the filtrate was subjected to the Rotary Evaporator at 40°C (Figure 4). Afterwards, it was contained in a colored bottle (Fig. 5) that was closed tightly and placed in the refrigerator until needed

Fig. 1. Tamarind seeds.

Fig. 2. Air drying of Tamarind seeds.

Fig. 3.Maceration.

Fig. 4. Rotary evaporation.

Fig. 5. Tamrind seeds ethanol extract.

2.3. Collection and Authentication of *Ascaris suum*

Adult *Ascaris suum*, a gastrointestinal parasite of swine, was collected live from a slaughter house from Labudahon Abattoir at 24 Dalsol street, Project 8, Quezon City Philippines. All the adult *Ascaris suum* were placed and contained in a glass jar with Goodwin's solution and were assigned in different treatments as shown in the experimental layout (Fig. 6). Samples of A *suum* from the same group were authenticated by a parasitologist from University of the Philippines, Manila Philippines.

2.4. Mortality Inhibition Assay

Forty (40) 300 mL glass bottles was assigned into four major groups Ten *A. suum* were assigned individually in 10 bottles per treatment following the pattern from anthelmintic studies with slight modification [8]. For the control groups; T-, is composed of 10 *A. suum* + 1000 mL Goodwin's solution; T+ had 10 *A. suum* + 900 mL of Goodwin's solution + 100 grams of Albendazole. For the experimental groups; T1 had 10 *A. suum* + 600 mL of Goodwin's solution + 40 mL of Tamarind seed extract. T2 had 10 *A. suum* + 200 mL of Goodwin's solution +80 mL of Tamarind seed extract.

2.5. Management of Experimental Animals

To maintain the temperature of 37°C, experimental parasites in bottles were placed inside the improvised incubator (Fig. 7) [9]. Bottles with experimental animals were arranged according to the experimental lay-out.

Control		Experimental	
T-S1	T+S1	T1S1	T2S1
T-S2	T+S2	T1S2	T2S2
T-S3	T+S3	T1S3	T2S3
T-S4	T+S4	T1S4	T2S4
T-S5	T+S5	T1S5	T2S5
T-S6	T+S6	T1S6	T2S6
T-S7	T+S7	T1S7	T2S7
T-S8	T+S8	T1S8	T2S8
T-S9	T+S9	T1S9	T2S9
T-S10	T+S10	T1S10	T2S10

Fig. 6. Experimental lay-out.

Fig. 7. Experimental parasites inside the improvised incubator.

3. Results and Discussion

3.1. Weight (Grams) of *A. suum* on the Start of the Study

Table 1 a. shows the weight of *A. suum* on the start of the study. The highest mean weight was obtained from T1 and T2 (3.1 g), followed by T- (2.8 grams), and the least mean weight was obtained from T+ (2.7 g). Analysis of Variance (ANOVA-Table 1.b), revealed that there is no significant difference among the treatments which shows that the weight of *A. suum* were treated equal to avoid bias.

3.2. Mortality of *A. suum* after 12 Hours of Observation

Mortality after 12 hours of immersion in different concentration of Tamarind seeds extract is displayed in Table 2 a. The highest mortality was obtained from T2 (100%), followed by T1 (60%). No mortality was observed from T- and T+ (albendazole). ANOVA (Table 2 b) revealed that there were significant differences among treatments. LSD was used to identify what treatment is significant over the other. No significant difference between T- and T+ as shown in the same superscript. Significant differences were obtained from the comparisons of means in the following pairs; T- vs T1, T- vs T2, T+ vs T1, T+ vs T2 and lastly, T1 vs T2, as shown in the different superscripts. The result further implied that using concentration of 40% and 80% as early as 12 hours will kill *A. suum*. Number of studies conducted with nematode parasites of ruminant livestock have demonstrated direct anthelmintic effects of extracts from tannin-containing plants in in vitro assays and in vivo verification of these results [10]. Similar findings was obtained from the study on using *T. riparia* with tannin wherein *A. suum* died at 12 hours after exposure [11] .

3.3. Mortality of *A. suum* after 24 and 36 Hours of Observation

Ascaris suum were immersed in Tamarind seeds extract. Mortality rate after 24 and 36 hours, is displayed in Table 3.b. It shows that 100% mortality in T2 and 90% for T1. No mortality rate shown in both T- and T+

(Albendazole). Significant difference among treatments is revealed in ANOVA (Table 3.b). LSD is used to identify treatments that are highly significant over the other. According to the table, T- vs T+ and T1 vs T2 showed no significant difference. Pairs of means with significant difference are; T-vs T1, T- vs T2, T+ vs T1, and T+ vs T2 show significant difference among them as observed in the superscript. The result further implied that using concentration of 20% and 80% as early as 24 hours and same potency after 36 hours of exposure to TSE. Phytochemical analysis of juice of *Tamarindus indica* Linn revealed the presence of tannins.The concentration of *Tamarindus indica* linn leaves will kill *A.suum* [12]. Tannins were shown to produce anthelmintic activities [13].

Table 1 a. Weight of *A. suum* on Start of the Study

Sample	T-	T+	T1 (40% TSE)	T2 (80% TSE)
1	3	5	1	3
2	4	5	3	2
3	5	4	2	3
4	5	4	5	4
5	4	4	4	3
6	2	1	4	3
7	1	1	3	3
8	1	1	3	3
9	2	1	3	3
10	1	1	3	4
Total	28	27	31	31
Mean	2.8	2.7	3.1	3.1

Table 1 b. ANOVA on Weight of *A. suum*

s.v	SS	df	MS	F	p-value	f crit
Between groups	1.275	3	.425	.227$^{n.s}$	.877	2.85
Within groups	67.5	36	1.875			
Total	68.775	39				

Legend: n.s not significant at 5% level of significance

Table 2 a. Mortality after 12 Hours

Sample	T-	T+	T1 (40% TSE)	T2 (80% TSE)
1	0	0	0	1
2	0	0	0	1
3	0	0	1	1
4	0	0	1	1
5	0	0	0	1
6	0	0	0	1
7	0	0	1	1
8	0	0	1	1
9	0	0	1	1
10	0	0	1	1
Total	0	0	6	10
Mean	0	0	.6	1

Table 2 b. ANOVA on Mortality after 12 Hours

s.v	SS	df	MS	F	pvalue
Between groups	7.2	3	24	36*	6.22
Within groups	2.4	36	.67		
Total	9.6	39			

Legend: *significant at 5% level of significance

Table 3 a. Mortality after24 and 36 Hours

Sample	T-	T+	T1 (40% TSE)	T2 (80% TSE)
1	0	0	0	1
2	0	0	0	1
3	0	0	1	1

4	0	0	1	1
5	0	0	0	1
6	0	0	0	1
7	0	0	1	1
8	0	0	1	1
9	0	0	1	1
10	0	0	1	1
Total	0	0	6	10
Mean	0	0	.6	1

Table 3 b. ANOVA on Mortality after 24 and 36 Hours

s.v	SS	df	MS	F	P value	F crit
Between groups	.675	3	.225	3.86*	.018	2.87
Within groups	21	36	.584			
Total	21.675	39				

Legend: *significant at 5% level of significance

Table 4. Summary of Mortality per Treatment

HOURS	T-	T+	T1 (40% TSE)	T2 (80% TSE)
1st 12 hours	0	0	6	10
2nd 12 hours	0	0	3	0
3rd 12 hours	0	0	.0	0
TOTAL	0	0	9	10
Mean	0	0	3	3.33
% Mortality	0	0	90%	100%

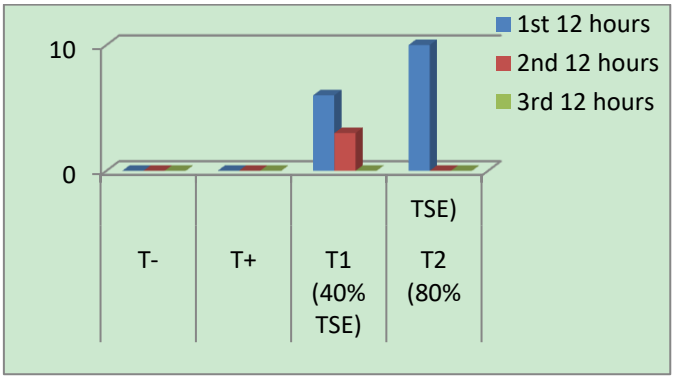

Fig. 7. Mortality of treatment.

3.5. Summary of Mortality per Treatment

Table 4 and Fig. 7 posited the summary of mortality per treatment. It revealed that the highest mortality was found in Treatment 2; followed by Treatment 1, T(+), and T(-) after 24 hours. It implies that percentage of mortality increases as the concentration of Tamarind seed extract in the solution increases, thus attesting to its potential as source of herbal anthelmintics. Traditional uses of tamarind are not yet popular but studies show that, it is not only a good commercial use but it is also a good use as herbal plant. Many countries used Tamarind traditionally in abdominal pain, diarrhea and dysentery, helminthes infections, wound healing, malaria and fever, constipation, inflammation, cell cytotoxicity, gonorrhea and eye diseases [14].

4. Conclusion

Ethnobotanical studies on anthelmintics is gaining popular in congruence with sustainable agriculture. The use of herbal extracts that has no use for human consumption is keeping at pace with sustainable agriculture and creating environmentally friendly farm practices. The seeds of Tamarind can now be

explored as source of anthelmintics particularly for swine. The result of this study showed that *Tamarindus indica* seeds extract have antihelmintic potential against *Ascaris suum* and the effect was found to be effective as early as 12 hours of exposure.

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References

[1] Leles, D., *et al*. (2012). Are *Ascaris lumbricoides* and *Ascaris suum* a single species? *Parasites & Vectors. 5(42)*, 1-5.

[2] Williams, A., *et al*. (2014). Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum. Plos One, 9(6)*, 1-16.

[3] Stangeland, T., Dhillion, S. S., & Reksten, H. (2008) Recognition and development of traditional medicine in Tanzania*. Journal of Ethnopharmacology, 117*, 290–299.

[4] Stuart, G. (2016) Sampaloc. Stuartxchange. *Stuart Medicinal Plant*.

[5] Donahue, M. J., *et al*. (1981). Glycogen metabolizing enzymes during starvation and feeding of *A. suum* maintained in a perfusion chamber. *Journal of Parasitology, 67(4)*, 505-510

[6] Komutarin, T., *et al*. (2004) Extract of the seed coat of *Tamarindus indica* inhibits nitric oxide production by murine macrophages in vitro and vivo. *Food and Chemical Toxicology*, *42(1)*, 649-658.

[7] Acero, L. (2018). Cashew (*Anacardium occidentale*) nut shell extract in the control of cockroach (*Periplenata americana*). *Science International (Lahore*), *30(3)*, 427-429.

[8] Nalule, A. S., *et al*. (2013) In vitro anthelmintic potential of Vernonia amygdalina and Secamone Africana on gastrointestinal nematodes. *Agriculture and Biology Journal of North America, 4(1)*, 54-66.

[9] Lagu, L., & Kayanja, F. (2013). The in vitro Antihelmintic efficacy of *Erhthrina abyssinica* extracts on *Ascaridia galli. Insights from Veterinary Medicine*. *Intech Open,* 2-15.

[10] Sargison, N. (201). Pharmaceutical treatments of gastrointestinal nematode infections of sheep-future of anthelmintic drugs. *Veterinary Parasitology*, *189(1)*, 79-84.

[11] Wasswa, P., & Deogracious, O. (2006) The *In-vitro* ascaricidal activity of selected indigenous medicinal plants used in ethno veterinary practices in Uganda. *African Journal of Traditional, Complementary and Alternative Medicines*, *3(1)*, 94-103

[12] Mute, V., Sampat, V., Patel, K., Sanghavi, K., Mirchandani, D., & Barbaria, P. (2013). Anthelmintic effect of *Tamarind indica linn* leaves juice extract on *Pheretima pohuma. International Journal of Pharmaceutical Research and Development, 2(4),*

[13] Bhupendra, S., Hema, B., Dilly, B., & Sharan, P. (2009). Anthelmintic and Antimicrobial activity of the chloroform extract of pergularia daemia forsk leaves. *Advance. Pharmacology Toxicology*, *10(1)*, 13-16.

[14] Raul, S. K., Padhy, G., Dhavala, Mr., & Shravani, B. (2012). Comparative study of anthelmintic activity of vitex negundo, *Moringa oleifera, Tamaindus indica* on Indian earthworm *Phertima postuma*. *Drug Invention Today*, *4(7)*, 407-408.

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