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Research Article

Anthelmintic Activity of Acacia (*Samanea Saman*) Leaves Extract Against *Ascaris Lumbricoides*

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About Article

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ABSTRACT

Over the years, researchers have come up to the discovery of anthelmintic drugs, albendazole, which can eliminate the presence of a soil-transmitted worm named *Ascaris lumbricoides* in our body. However, reports of its reduced-efficacy and side effects have come to existence which leads to the detection of many researchers of the potential anthelmintic compounds of medicinal plants. Species of Acacia such as *catechu* and *farnesiana*, and *nilotica* and *auriculiformis* were reported to be effective against *Pheritima postuma* and *Trichuris trichiura* respectively. *Samanea saman*, acacia specie, is a medicinal plant that can be found locally. The tree has components such as tannins, saponin, alkaloids, steroids, terpenoids, and flavonoids in its different parts. The study therefore determined the components of the plant's leaves extracted with ethanol and tested its anthelmintic activity against *A. lumbricoides*. This research can serve as a basis in the development of anthelmintic drugs as well as a new source for the upcoming studies. The testing was done at the Cagayan Valley Herbal Processing Plant utilizing an experimental design. The phytochemical screening revealed that *S. saman*'s leaf is positive in the constituents namely flavonoids, saponins, and tannins. Results of the anthelmintic activity reavealed that the *S. saman* leaves extract is effective in all of its concentration (50, 100, and 200 mg/mL). Furthermore, the one-way ANOVA also revealed that 50, 100, and 200 mg/mL of the *S. saman* leaves extract have no significant difference even when compared to the positive treatment 50 mg/mL albendazole. Therefore, the study reveals that using *S. saman* leaves extract leads to comparable anthelmintic activity with the drug albendazole.

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1. INTRODUCTION

Over the years, soil-transmitted helminths have caused worldwide health problems which are known to be serious (Patil *et al.*, 2013). According to the World Health Organization (2019), for about 1.5 billion or 24% of people around the world carry these intestinal helminthic parasites. Among these, *A. Lumbricoides* ranked as the highest with the prevalence of infecting 800 million people.

Ascaris lumbricoides is a well-known human helminth which causes major diseases such as ascariasis, schistosomiasis and, physical deficits which include anemia, malnutrition, and mental disabilities (Ross *et al.*, 2017). The presence of *A. lumbricoides* is usually asymptomatic but every year it had caused 3,000 to 60,000 of deaths, commonly with children, as a result of intestinal blockage (Abdellatif *et al.*, 2013). In the Philippines, 20.1% of indigenous children in a school at Davao del Norte were infected with *A. lumbricoides* (Belizario *et al.*, 2011). In addition to this, there is a positive prevalence of *A. Lumbricoides* in Tuguegarao City particularly with the students of Cataggaman National High School (Zamora, 2016).

The discovery of the drug albendazole, which has anthelmintic activity, was said to have side effects including abdominal pain, vomiting, and liver injury. In fact, there is a reduction with the efficacy of the drug (Krücken *et al.*, 2017). In addition to this, the incidence of having a resistant-drug *A. Lumbricoides* is increasing which leads to the detection of many researchers of the potential anthelmintic compounds of medicinal plants.

Acacia is a large tree with a rough bark and bi-pinnate leaves which is distributed throughout continents such as Asia and Latin America. Species of Acacia such as *catechu* and *farnesiana*, and *nilotica* and *auriculiformis* were reported to be effective against *Pheritima postuma* and Trichuris trichiura respectively. *Samanea saman*, acacia specie, is a medicinal plant that can be found locally. The tree has components such as tannins, saponin, alkaloids, steroids, terpenoids, and flavonoids in its different parts. Other than that, the bark of the tree showed good anthelminthic activity (Velan *et al.*, 2010). Moreover, it was recommended that more studies need to be conducted to identify and confirm the reported effects in its biological activities (Sowjanya, 2014).

This study will be carried out to test the *Samanea saman* leaf ethanolic extract's anthelmintic activity against a soil-transmitted helminth, *Ascaris lumbricoides*. Furthermore, this study will be observing the time of paralysis and mortality of the parasite using the treatments: 50, 100, 200 mg/mL ethanolic extract, albendazole and distilled water as the positive and negative treatments respectively; and will compare the concentrated extracts to albendazole as a parameter.

2. LITERATURE REVIEW

2.1. *Samanea saman* plant

Samanea saman (Merr) is a tree growing 20 meters in height, with 1.5 meter diameter trunk and with a huge canopy which gives shade because it has the structure comparable to umbrella. Its branches are expansive with a bark that is rough and crinkled (Velan *et al.*, 2010). *Samanea saman* is from the Fabaceae family and is known as Acacia worldwide.It is commonly referred to as "rain tree" or "monkey pod tree" that is native to the tropics of America (Obasi Nnamdi, 2010). The tree grows in a humid and warm environment. Additionally, this tree can serve as a source of fuel and food (Vinodhini, 2018).

2.1.1. Metabolites Found in *Samanea saman*

A study conducted by Velan *et al.* (2010) showed that the leaves, bark, stems and seeds of the *Samanea saman* are rich in alkaloids such as $C_8H_{17}ON$ and $C_{17}H_{36}ON_3$ pithecolobine. It was also revealed that the stems and leaves possess components such as saponin and tannin. The bark contains gum as well. In addition to this, it was reported by Obasi Nnamdi (2010) that the plant has the components like terpenoids, steroids, and cardiac glycosides distributed in its different parts. These compounds are revealed as few of the essential components that can have anthelmintic activity.

2.1.2. Folkloric Use

Samanea saman is used as a traditional medicine for diarrhea, colds, intestinal ailments, stomachache, and headache in Venezuela. Moreover, its roots are used for cancers in the stomach. Additionally, WestIndies uses its leaf and seed to cure tuberculosis and sore throat respectively (Velan *et al.*, 2010). It was also reported by Vinodhini (2018) that in the country of Venezuela the bark that has boiled is formed into bandage for constipation.

2.1.3. Medicinal Use

According to Arulpriya *et al.* (2010), *Samanea saman* is used and proven to cure and treat several diseases namely acute bacillary dysentery, enteritis, diarrhea, colds, sore throat, and headache. Furthermore, extracts of the bark and leaves were used to treat eczema, anaphylactic dermatitis, and skin pruritus. Additionally, various studies shows that S.saman contains plenty of bioactive chemicals which can be used in antibacterial, analgesic, anti-ulcer, antifungal, insecticidal, cytotoxic, anti-diabetic, and anti-oxidant activities (Vinodhini, 2018).

2.2. Extracts of *samanea saman*

Ethanolic extracts of *S. saman*'s barkwere found to possess different bioactive components such as resins and glycosides, terpenoids, and tannins even at a low concentration compared to aqueous extracts which is confirmed by Jacob and David (2016) to have terpenoids and steroids in a minimal percentage. These compounds were reported by Suyog *et al.* (2013) to be responsible in exhibiting significant effects in the death and paralysis of parasite. In addition to this, the *S. saman* extracted with ethanol also shows extreme percent of inhibition on bacteria, both gram positive and negative Jacob and David (2016). Sowjanya *et al.* (2014) also stated that it possesses antifungal activity since it demonstrated high inhibitory effect against fungal species. Additionally, according to Rathour (2017) the chevon roll with ethanolic extracts of aloe vera and cinnamon bark extract can be preserved for 28 days without any significant loss of physico-chemical, microbiological and sensory properties under refrigerated storage while also maintaining odor, color and overall acceptability of chevon rolls The methanolic extracts of *S. saman*'s podsmainly possess great amount of tannins in which in other extracts, this component

was not present (Obasi Nnamdi, 2010). The presence of tannins made the extract a better antimicrobial agent compared to others. In addition to this, Sowjanya *et al.* (2014) confirmed that extracting using methanolic extract is the most suitable decocting process in terms of antibacterial property even at a low concentration.

The analysis of the aqueous extract of *S. saman*'s barkexibits the presence of cardiac glycosides, flavonoids, steroids, saponins, tannins, and terpenoids (Vinodhini, 2018). In addition to this, it was reported by Velan *et al.* (2010) that aqueous extracts from the *Samanea saman*'s barks were proven to have anthelmintic activity against an earthworm, Pheretima posthuma.

2.3. Parts of *samanea saman*

The leaves are arranged alternately with the size of 1-2cm broad and 2-4cm elongated. Its shape has a blade-like appearance and more pinnately complex. Due to the tree's semi-deciduous characteristics, the tree remains leafless during the summer period. However, it quickly regains the leaves once enough moisture is attained which then will give an evergreen color. The leaves were also reported to possess bio-constituents namely as alkaloids, saponins, carbohydrates, tannins, reducing sugar, glycosides, steroids, and flavonoids making it a good antioxidant agent (Vinodhini, 2018). In the study conducted by Badar *et al.* (2011), it states that using the leaves of Acacia nilotica, which is in the same family with *S. saman*, shows a better effect in the in vitro anthelmintic activity against Haemonchus contortus.

The bark of a mature *Samanea saman* tree has a grey color and a rough texture unlike the younger which has pale grey to brownish color and smooth texture (Vinodhini, 2018). The bark is commonly used in several biological activities, one of which is the anthelmintic activity.

The pod is 10-20cm long and 15-19 cm wide with a curved quadrilateral shape and dark brown color (Vinodhini, 2018). The study of the phytochemical components and antimicrobial activity suggest the pods of *S. saman* can be used as a source of basic needs such as food and several raw materials for biodiesel industries (Jacob & David, 2016).

2.4. Anthelmintic activity of *samanea saman* plant

According to Velan, Suresh, Kumar, Bright, and Karthikeyan (2010), the presence of the phytochemical compounds alkaloids, flavonoids, gums, tannin, saponin and terpenoids in the bark of *Samanea saman* helps the plant exhibit a good anthelmintic property. In addition to this, it was also stated in the study that the polyphenolic compound, tannin, is responsible for producing the most significant anthelmintic activities. Moreover, the extracts of the *Samanea saman*'s bark, both ethanolic and aqueous, are said to be comparable to the reference drug, albendazole.

2.5. Biological effects of *samanea saman* plant

Diabetes mellitus is a non-contagious disease which causes various defects in the human body including insulin action and secretion. It was reported that in both in vitro and in vivo studies, the methanolic leaf extracts of *S. saman* exhibited anti-diabetic activity (Vinodhini, 2018).

Inflammation is the effect of tissue injury commonly brought by pathogenic microorganisms, physical trauma, and toxic chemicals. It can be evident through pain, reddish appearance, and swelling. Additionally, it was reported that the methanolic extract of *Samanea saman* possesses anti-inflammatory activity by showing higher inhibition of hemolysis compared to a commercial drug, diclofenac sodium. It was concluded by Vinodhini (2018) that the phytochemical compounds of *S. saman* such as tannins and phenolic, flavonoids, and glycosides are responsible for such activity and can be used for other possible biological effects.

Microbes such as bacteria and fungi contribute harmful diseases to human. The discovery of the plant *S. saman* has contributed significant effects in solving the problem. A study conducted by Vinodhini (2018) demonstrated that the aqueous extract of S.saman's leaves is a highly significant antimicrobial plant when used against Escherichia coli, Staph¬ylococcus aureus, and Candida albicans. *S. Saman* pods' ethanolic and aqueous extracts were said to be higher supply of natural but narrow-spectrum antimicrobial (Jacob & David, 2016). It was also reported in the study that the *S. saman* also confirmed inhibitory activity in opposition to all of the tested organisms. Tannins that can be found in *S. saman*'s pod extract are known antimicrobial agents that are proven to inhibit the growth of microorganisms by means of precipitating out the nutritional protein and for that reason depriving their growth and development. It was known that different types of extracts of *Samanea saman* varied in its antifungal and antibacterial activity (Arulpriya *et al.*, 2010). Additionally, the same findings about *Samanea saman* extracts were shown in a study conducted by Obasi Nnamdi *et al.* (2010).

Anti-oxidants play a vital role in the health of human beings. It can be grouped in terms of their action like the free radical terminators and chelators of metal ions which are involved in catalyzing lipid oxygen scavengers that react with oxygen closed system. Over the years, anti-oxidant activities have been observed among several medicinal plants. In the experiment conducted by Vinodhini (2018), such components were found to be evident in the analysis of the alcoholic extract of *S. saman*. Ethyl acetate, chloroform, aqueous and HCl, and petroleum ether were said to be the phytochemical components of *S. saman* which are responsible for its greater anti-oxidant mechanism (Arulpriya *et al*, 2010).

An ulcer is a result of an imbalance of aggressive gastrointestinal fluids like pepsin and defensive factors such as prostaglandins, and innate cell mucus resistance which is considered a rampant disease worldwide. According to Vinodhini (2018), *S. saman* has comparable anti-ulcer activity with the reference drug sucralfate.

Disease-carrier mosquitos such as Aedes aegypti have caused numerous deaths to the human race. Such organisms came from larvae and had undergone metamorphosis. The screening of the phytochemical components of *S. saman*'s bark gives compounds such as tannin, saponin, alkaloids, and flavonoids. These compounds are reported to be responsible for a high toxicity for larvicidal activity against Aedes aegypti by Velan *et al.* (2010).

2.6. *Ascaris lumbricoides*

scaris lumbricoides is a well-known human helminth which causes major diseases such as ascariasis, schistosomiasis and, physical deficits which includes anemia, malnutrition, and mental disabilities (Ross *et al.*, 2017). One-third of the human world population is being infected by diseases caused by these helminths which mainly affect continents such as Asia, Africa and, Latin America (Ross *et al.*, 2017). The presence of *A. lumbricoides* is usually asymptomatic but every year it had caused 3,000 to 60,000 of deaths, commonly with children, as a result of intestinal blockage (Abdellatif *et al.*, 2013). In the Philippines, 20.1% of indigenous children in a school at Davao del Norte were infected with *A. lumbricoides* (Belizario *et al.*, 2011). In addition to this, there is a positive prevalence of *A. Lumbricoides* in Tuguegarao City particularly with the students of Cataggaman National High School (Zamora, 2016).

2.6.1. Characteristics, Growth, and Survival of *Ascaris lumbricoides*

The worms were round and pinkish in shading. Adult males were estimated 15 to 30 cm long and 2 to 4 mm in diameter and their back end was bent with 2 spicules. Adult females were estimated 20 to 40 cm long and 3 to 6 mm in diameter and their back ends was straight. These worms were checked as *A. lumbricoides* from their visible appearance, size and egg shape (Abdellatif *et al.*, 2013). The *A. lumbricoides* is genetically similar to the A. suum present in the intestines of pigs (Monteiro *et al.*, 2019). Manifesting *A. lumbricoides* is by the mode of ingesting eggs in water, raw vegetables, or soil-contaminated hands, and in the intestine is where the fertilized eggs hatch (Abdellatif *et al.*, 2013). Small intestine particularly in the lumen is where adult worms of *A. lumbricoides* lives, this is also where unembryonated eggs are being discharged. In children the mode of transmission is through soiled fingernails and toys (Nisha *et al.*, 2019).

2.6.2. Resistance of *Ascaris lumbricoides*

According to Velan, Suresh, Kumar, Bright, and Karthikeyan (2010) as of today, problems about resistant drug helminths are constantly increasing. As parasite resistance is an acquired characteristic, after every generation the percentage of parasites that can survive after drug treatment might increase (Gárcia *et al.*, 2016). This resistance is a disturbing situation in some parts around the world. It was suggested by the same study that inventing new tools is needed to be developed to sustain the control of parasites.

2.7. Albendazole

Albendzole is a medical drug that is currently used by people to eliminate parasitic helminths in the body such as *A. lumbricoides*, hookworms, and whipworm (*T. trichiura*). It was stated by Krücken (2017) that deworming through this drug showed inefficacy in terms of reducing and eliminating *A. lumbricoides*. The study also reported that taking albendazole as a treatment is considered ineffective because of a great proportion of the worm's survival. The study stated that the need for other ways of deworming practice is highlighted.

2.7.1. Side Effects of Albendazole

According to Moon Sy *et al.* (2019), the drug albendazole was observed to have side effects which include nausea, vomiting, abdominal pain, dizziness, headache, alopecia, and increased liver enzyme. This study also instigated a case of liver injury caused by the use or administration of albendazole.

3. METHODOLOGY

3.1. Research design

The study used the Completely Randomized Design (CRD) under the experimental design to determine the anthelmintic activity of *Samanea saman* by observing the *Ascaris lumbricoides*' time of paralysis and death within 7 hours. The experimental treatments include 50, 100, and 200 mg/mL concentrations which have three replicates of the worm in each treatment. The reference drug, albendazole, in a 50 mg/mL concentration was used as a positive control, and 25 mL distilled water as a negative control. All treatments were compared in order to determine their significant differences. The table below shows the randomization of different treatments and replicates in the experiment.

Table 1. Different treatments and replicates

Date	Worms	Treatments and Replicates
January 16, 2020	1	T1R3
	2	T1R1
	3	-
	4	T1R2
January 21, 2020	1	T2R2
	2	T2R1
	3	T2R3
	4	-
February 08, 2020	1	T4R1
	2	T3R2
	3	-
	4	T5R3
	5	T4R2
	6	T3R1
	7	-
	8	-
	9	T4R3
	10	T3R3
	11	T5R2
	12	-
	13	T5R1

3.2. Locale of the study

The ethanolic extraction method was done at the University of Saint Louis Tuguegarao, Chemistry laboratory, Senior High School Department. The phytochemical screening was done at Saint Louis University, Baguio City. The collection of *Ascaris lumbricoides* was done in a slaughterhouse at Capatan, Tuguegarao City. The anthelmintic activity test was done at Cagayan Valley Herbal Processing Plant, Carig Sur, Tuguegarao City.

3.2.1. Materials

i. *Samanea saman* plant leaves
ii. Electric grinder
iii. 95% Ethyl Alcohol (4500 mL)
iv. Laboratory Water Bath
v. Whatman Filter Paper 1
vi. NH3 Solution (5 mL)
vii. Distilled water (25 mL)
viii. FeCl3 (5 mL)
ix. *Ascaris lumbricoides*
x. Plastic Containers
xi. 200 mg Albendazole
xii. Timer
xiii. Funnel
xvi. Erlenmeyer Flask

3.3. Data collection procedure

3.3.1. Collection and preparation of *samanea saman* leaves extract (Husori *et al.*, 2016)

i. *S. saman* leaves with a total of approximately 1 kilogram were collected within Tuguegarao City particularly at University of Saint Louis and Magallanes Street, Centro 5.

ii. The plant specimen was identified in the Department of Environment and Natural Resources, Carig Sur, Tuguegarao City.

iii. The leaves were cleaned with water, cut through scissors and, air dried for at least 3 hours, well enough for the leaves to be fully dried.

iv. The dried leaves were powdered using an electric grinder.

v. The ethanolic extract was prepared by percolating the dried leaves: 75 g, 150 g, and 300 g, representing the experimental group, in 95% ethanol (1500 mL per treatment).

vi. The solvent had undergone evaporation through the use of laboratory water bath to get the crude extract of *S. saman.*

vii. The extracts were refrigerated when not in use.

3.4. Phytochemical screening

The extracted leaves of the *S. saman* were submitted to the laboratory of Saint Louis University, Baguio City for the screening of its phytochemical constituents namely flavonoids, saponins and tannins.

i. Test for Flavonoids. (Guevara, 2005 cited in De la Cruz *et al.*, 2016)

• One percent NH_3 solution was added to 5 mL of each aqueous extracts.

• Flavonoids will be said to be present if there will be a formation of yellow coloration or turbidity.

ii. Test for Saponins. (Guevara, 2005 cited in De la Cruz *et al.*, 2016)

• For about 2 g of the powdered sample was boiled in 20 mL distilled water and then, it will be filtered.

• Ten milliliter of aqueous filtrate was mixed in 5 mL distilled water and will be shaken to form froth.

• The froth that was made was mixed with 3 drops of olive oil and will be shaken again.

• The emulsions will then determine the presence of saponins in the extracts.

iii. Test for Tannins. (Guevara, 2005 cited in De la Cruz *et al.*, 2016)

• The powdered sample of *S. saman* (0.5 g) was boiled in a 20 mL of distilled water using a test tube and was filtered.

• The FeCl3, in 0.1% concentration, was added to the filtrate and tannins will be determined present when its characteristic yields to a blue or blue-black color.

3.5. Collection of *ascaris lumbricoides* (Husori *et al.*, 2016)

i. The experiment involved a roundworm, *Ascaris lumbricoides.*

ii. The worms were isolated by one of the staff from a slaughterhouse at Capatan, Tuguegarao City, Cagayan throughout the experiment.

iii. The isolation was done by removing the roundworms including the pigs' feces and transferring them to a clean container to be transported at Cagayan Valley Herbal Processing Plant.

iv. The roundworms should measure approximately 10-30 cm in length.

v. After transporting, the worms were cleansed and stored with a normal saline solution just before the experiment.

vi. The worms were identified in the Cagayan Valley Herbal Processing Plant.

vii.The process was repeated thrice until all the worms were completed.

3.6. Testing of the anthelmintic activity of *samanea saman* (Husori *et al.*, 2016)

The anthelmintic activity of *Samanea saman* was tested according to a method conducted by Das *et al.* (2011) and Pillai *et al.* (2011)

i. The collected *A. lumbricoides* was placed on a plastic container with 25 mL of test solutions per treatment. Each treatment has containers, containing a single *Ascaris lumbricoides* each representing the replicates.

ii. The containers of the experimental group contained the dosages 50, 100, and 200 mg/mL acacia leaves extract, and 50 mg/mL of albendazole and 25 mL distilled water as positive and negative treatments respectively.

iii. The anthelmintic activity of the plants' extract was determined by observing the time of paralysis and death of the worms within 7 hours with the help of the Cagayan Valley Herbal Processing Plant staffs.

iv. If no movements are observed, the paralysis of the worms will be declared except when it will be moving vigorously when soaked with 50˚C water.

v. If the worm did not do any movements even when soaked in 50˚C water, the time of death will be recorded.

iv. The anthelmintic activity was done thrice. The 1stbatch using Treatment 1, 2nd batch using Treatment 2, and 3rd batch used Treatment 3, Treatment 4 and Treatment 5.

3.7. Data analysis

The data was analyzed through the use of one-way Analysis of Variance (ANOVA) to determine if there is a significant difference between the experimental group and the control groups. Additionally, the same statistical tool was used in determining if there is a significant difference among the treatments of the experimental group. The p values was under 0.01 to determine the significant difference of the treatments.

3.8 Waste disposal

All of the materials used were disposed of properly through segregation. The hazardous materials were deposited safely, the extracts were poured in a sink, and the plastic containers were cleansed thoroughly.

4. RESULTS AND DISCUSSION

Table 1. Phytochemical screening conducted on *samanea saman* leaves extract

Plant	Flavonoids	Saponins	Tannins
Samanea saman Leaves Extract	positive	positive	positive

Table 1 shows that the *Samanea saman* leaves extract proved positive with all the chemical constituents mentioned above namely the flavonoids, saponins, and tannins.

Table 2. Time of paralysis of *ascaris lumbricoides* among treatments

No.	Treatment	Time of Paralysis (minutes)			
		R1	R2	R3	Mean
1	50 mg/mL *S. saman*	11.33	7.42	13.43	10.7267
2	100 mg/mL *S. saman*	9.07	18.12	13.60	13.5967
3	200 mg/mL *S. saman*	14.07	5.92	12.12	10.7033
4	50 mg/mL Albendazole	82.02	32.40	54.63	56.3500
5	Distilled Water	123.25	204.25	130.12	152.5400

Table 2 shows that treatment 3 is observed to have the fastest time of paralysis of *Ascaris lumbricoides* while treatment 5 is observed to have the slowest among the experimental treatments and also the control group.

Table 3. Time of death of *ascaris lumbricoides* among treatments

No.	Treatment	Time of Death (minutes)			
		R1	R2	R3	Mean
1	50 mg/mL *S. saman*	12.28	8.17	15.63	12.0267
2	100 mg/mL *S. saman*	11.07	19.17	17.22	15.8200
3	200 mg/mL *S. saman*	16.05	6.70	13.17	11.9733
4	50 mg/mL Albendazole	84.50	35.25	55.38	58.3767
5	Distilled Water	124.48	204.72	131.00	153.4000

Table 3 shows that treatment 3 is observed to have the fastest time of death of *Ascaris lumbricoides* while treatment 5 is observed to have the slowest among the experimental treatments and also the control group.

Table 4. Test of significant difference in the time of paralysis of the *ascaris lumbricoides* between experimental treatments and the positive control

Treatment Pairs	F-value	Mean Difference	p-value	Decision
50 mg/mL *S. saman* leaves extract	20.909	-	.287	Accept H_o Reject H_a
100mg/mL *S. saman*leaves extract			.342	Accept H_o Reject H_a
200mg/mL *S. saman*leaves extract			.286	Accept H_o Reject H_a
Distilled Water		-96.19000	.008	Accept H_a Reject H_o

Table 4. shows that the different concentration of the *S. saman* leaves extracts have comparable effects to the positive control in terms of the time of paralysis of the *A. lumbricoides*. Moreover, negative control yielded longer amount of time of paralysis than the positive control as seen on their mean difference.

Table 5. Test of significant difference in the time of death of the *ascaris lumbricoides* between experimental treatments and the positive control

Treatment Pairs	F-value	Mean Difference	p-value	Decision
50 mg/mL *S. saman* leaves extract	20.938	-	.269	Accept H_o Reject H_a
100mg/mL *S. saman* leaves extract			.341	Accept H_o Reject H_a
200mg/mL *S. saman* leaves extract			.269	Accept H_o Reject H_a
Distilled Water		-95.02333	.008	Accept H_a Reject H_o

Table 5 shows that the different concentration of the *S. saman* leaves extracts have comparable effects to the positive control in terms of the time of death of the *A. lumbricoides*. Moreover, negative control yielded longer amount of time of death than the positive control as seen on their mean difference.

Table 6. Test of Significant Difference in the Time of Paralysis of the *Ascaris Lumbricoides* Between Experimental Treatments and the Negative Control

Treatment Pairs	F-value	Mean Difference	p-value	Decision
50 mg/mL *S. saman* leaves extract	20.909	141.81333	.000	Accept H_a Reject H_o
100 mg/mL *S. saman* leaves extract		138.94333	.000	Accept H_a Reject H_o
200 mg/mL *S. saman* leaves extract		141.83667	.000	Accept H_a Reject H_o

Table 6 shows that the experimental treatments do not have the same effect as the negative control in terms of the time of paralysis of the *A. lumbricoides*. The mean difference shows that the 200 mg/mL of *S. saman* leaves extract is the most effective among the experimental group, followed by the 50 mg/mL then the 100 mg/mL *S. saman* leaves extract.

Table 7. Test of significant difference in the time of death of the *ascaris lumbricoides* between experimental treatments and the negative control

Treatment Pairs	F-value	Mean Difference	p-value	Decision
50 mg/mL *S. saman* leaves extract	20.938	141.37333	.000	Accept H_a Reject H_o
100 mg/mL *S. saman* leaves extract		137.58000	.001	Accept H_a Reject H_o
200 mg/mL *S. saman* leaves extract		141.42667	.000	Accept H_a Reject H_o

Table 7 shows that the experimental treatments do not have the same effect as the negative control in terms of the time of death of the *A. lumbricoides*. The mean difference shows that the 200 mg/mL of *S. saman* leaves extract is the most effective among the experimental group, followed by the 50 mg/mL then the 100 mg/mL *S. saman* leaves extract.

Table 8. Test of significant difference in the time of paralysis of the different treatment groups

Treatment group	F-value	p-value	Decision
50mg/mL *S. saman* leaves extract 100mg/mL *S. saman*leaves extract 200mg/mL *S. saman*leaves extract	.520	.619	Accept H_a Reject H_o

Table 8 shows that, across treatment groups, the results proved non-significant which means that the same effects will be get from the varying concentrations: 50 mg/mL, 100 mg/mL and 200 mg/mL *S. saman* leaves extract.

Table 9. Test of significant difference in the time of death of the different treatment groups

Treatment group	F-value	p-value	Decision
50 mg/mL *S. saman* leaves extract 100 mg/mL *S. saman* leaves extract 200 mg/mL *S. saman* leaves extract	.800	.492	Accept H_a Reject H_o

The primary purpose of this study is to determine the phytochemical constituents as well as the anthelmintic activity of *Samanea saman* leaves in ethanolic extraction against *Ascaris lumbricoides*. It was said that the different species of Acacia such as *catechu*, *farnesiana*, and *nilotica* are widely use against different helminths. Some studies have suggested confirming the phytochemical constituents of *Samanea saman*, acacia specie, with its different parts. *Samanea saman*'s bark in ethanolic extractions was found to have bioactive components including resins and glycosides, terpenoids, and tannins (Jacob and David 2016). Leaves and stems have saponin and tannin; gum is present in the trunk. Additionally, steroids, cardiac glycosides, terpenoids are also present in the tree. The reports have relevance in the study since the phytochemical screening of the *Samanea saman* ethanolic leaves extract showed that the plant was proven to be positive in components namely carbohydrates, flavonoids, glycosides, saponins, and tannins. These compounds were revealed as few of the essential components that can have anthelmintic activity (Suyog *et al.,* 2010). The components were reported to show significant effects in the paralysis and death of parasites. Additionally, the compounds mentioned were also said to be responsible for different biological activities such as anti-diabetic, anti-inflammatory, antimicrobial, and antioxidant (Vinodhini, 2018). In addition, it was confirmed by Velan *et al.* (2010) that tannin is possible in showing its effect in anthelmintic activities. Suyog *et al.* (2013) also added that flavonoids are also responsible for the potent wormicidal activity. Moreover, saponins, tannins, and flavonoids of the plant extract revealed its anthelmintic activity in the conducted experiment. All of the said components of *Samanea saman* leaves ethanolic extracts leads to paralysis and death of *Ascaris lumbricoides*.

In the study conducted by Husori *et al.* (2016), the ethanolic and aqueous extracts of A. fistulosum leaves yielded anthelmintic activity at the concentration of 50, 100, and 200 mg/ml. The ethanolic and aqueous extracts both yielded paralysis and followed by the death of *Ascaris lumbricoides*. At the concentration of 200 mg/ml, both types of extracts of the plant were at its fastest time of paralysis and death of worm compared with the commercial drug. In this study, it was revealed that *Samanea saman* leaves extracts in 50, 100, and 200 mg/mL concentrations, containing similar constituents with the parts mentioned above, were proven to possess anthelmintic activity against a roundworm, *Ascaris lumbricoides*. Additionally, in terms of the positive control, albendazole, the results of the anthelmintic activity showed a non-significant difference when it is compared to the different concentrations of the extracts. In the study of Velan *et al.* (2010), it was stated that the alcoholic extract of *Samanea saman* (Merr) demonstrated time of paralysis and death that is comparable to the reference drug which is the same with the study being undertaken. Hence, it connotes that the same effects will be get using either the albendazole or the *Samanea saman* leaves extract. Moreover, when the different concentrations will be compared to the negative control, distilled water, it shows that there is a significant difference. It means that using the distilled water in eliminating helminths such as the *Ascaris lumbricoides* will give minimal effect in terms of the time of death and paralysis of the worms.

The test of the anthelmintic activity comparing the time of paralysis and death of the *Ascaris lumbricoides* using the different dosages have a minimal gap in this study. However, it was stated by Husori *et al.* (2016) that the time of paralysis and time of death of the *Ascaris lumbricoides* decreases as dosages of the plant extracts will be increased. In relation to this study, the highest dosage of the *Samanea saman* (200 mg/mL) yielded the fastest time of both the paralysis and death of the worms. Moreover, other worms such as Pheretima posthuma had the fastest time of paralysis and death in 60 mg/mL, which is also the plant's highest dosage, among the two other concentrations (Velan *et al.*, 2010).

Lastly, among the different treatments, fastest anthelmintic activity concerning the time of paralysis and death of the *Ascaris lumbricoides* has been observed in Treatment 3, the 200 mg/mL *Samanea saman* leaves extract. It was followed by Treatment 1 (50 mg/mL) and then Treatment 2 (100 mg/mL). However, the extracts have no variations in their effect. Moreover, in the study of Velan *et al.* (2010), it was also revealed that there's no significant difference across the different dosages of the experimental treatments.

5. CONCLUSIONS

Based on the data obtained and the analysis of these data, the researchers arrived with the following conclusions:

i. The phytochemical constituents flavonoids, saponins, and tannins were proven positive in the *Samanea saman* leaves extract.

ii. The Acacia (*Samanea saman*) leaves extract was proven to be effective in terms of anthelmintic activity against *Ascaris lumbricoides*. Additionally, among the different concentrations, Treatment 3 (200 mg/mL) yielded the fastest time of paralysis and death.

iii. The different concentrations of Acacia (*Samanea saman*) leaves extract are comparable to albendazole in terms of the time of paralysis and death of the *Ascaris lumbricoides*.

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