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Anthelmintic Activity of Capsicum Annuum var. Longum (Siling-Haba) Placental Extracts Against Gastrointestinal Parasites in Broiler Chicken Stool

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Abstract: Soil-transmitted helminth infection is considered one of the Neglected Tropical Diseases (NTDs) worldwide. The Philippines is one of the countries in Asia with the highest prevalence of parasite infection. Adding to this problem is the resistance of pathogenic nematodes to commercial drugs in livestock hosts. Capsicum species exhibit antiparasitic activity against a wide range of endoparasites as fed to chicken. This study assessed the anthelmintic activity of the Capsicum annuum var. longum against gastrointestinal parasites found in broiler chicken stool and to identify the secondary metabolites from the placental region of the chili's fruit. Phytochemical screening test indicated the presence of alkaloid, flavonoid, and tannins. The ethanolic extracts of 1%, 10%, 50%, and 100% concentrations were administered to a total of 15 broiler chickens. The anthelmintic efficacy of the plant extract was evaluated through Fecal Egg Count Method. All treatments displayed very high effectiveness on reducing egg parasite count, which was comparable to the positive control. The result indicated that the Capsicum annuum var. longum (Siling-Haba) extracts were very effective against gastrointestinal parasites of the broiler chicken. This potency may be attributed to the high level of capsaicinoid content in the fruit. The findings may lead to a new organic anthelmintic drug alternative that is also environmentally sustainable.

Keywords: *Capsicum annuum var. longum, Anthelmintic activity, phytochemical screening, placental extracts, gastrointestinal parasites*

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I. INTRODUCTION

The Philippines is one of the countries in Asia with the highest prevalence of parasite infection [1, 2]. During a forum on Philippine Society of Parasitology, it was mentioned that there was an alarming escalation of soil-transmitted helminth infections in the country for the past years and predicted to triple for the next years [2]. It was found out that poor hygienic practices, walking barefoot and backyard poultry raisings were the primary cause of the increased rate of infections [1, 3, 4]. Soil-transmitted helminth infection was cited as one of the NTDs, causing substantial illness on more than two billion people of the global population [5]. Adding to this problem is the resistance of endoparasites to commercial anthelminthic drugs [5].

Helminthic parasites have continuously been one

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of the main constraints of fowl production which affected the growth, egg production, and overall health of a chicken. In severity, blockage in the intestines of the chicken may occur and cause death [6]. The most common parasitic nematodes that infect chicken are the Ascaridia galli and the Heterakis gallinarum [7].

Consequently, the Department of Health (DOH) promoted the use of plants as an alternative and organic means in combating the said nematodes [8] which could be readily available in the backyard gardens or as ornamental floras such as the chillis. There are five common domestic species of Genus Capsicum, from the family Solanaceae, namely: Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens, and Capsicum pubescens [9]. One of the varieties of Capsicum annuum is the Capsicum annuum var. longum which is locally known as 'Siling-Haba", commonly used for spicing numerous Filipino dishes for its strong purgency [10]. The fruits contain a high level of capsaicinoids which displayed antibiotic property and antiparasitic activity [11, 12]. In this paper, the researchers assessed the anthelmintic activity of the placental extracts from Capsicum annuum var. longum (Siling-Haba) against gastrointestinal parasites found in Broiler chicken stool.

II. MATERIALS AND METHOD

A. *Preparation and Extraction of Plant Material*

Species of Capsicum annuum var. longum were collected and purchased from different parts of Davao City from August to September 2018. They were packed in polyethylene bag placed inside an HDPE Plastic and was stored in a refrigerator before extraction [13].

The placental region with the seeds was separated from the whole chili. It was then chopped up into small pieces of approximately 2x2 mm. The selection of ethanolic concentration is crucial for the extraction of secondary metabolites because it is soluble in ethanol aqueous. The extraction efficiency in fresh peppers got a high yield at 50% aqueous ethanol. The optimal conditions for extraction of secondary metabolites from fresh peppers were the following: the ratio of solvent to material 4mL/g, the temperature of 90^{o}and extractive time in 2.0 hours. Afterward, thirty grams of the Capsicum annuum var. longum placental region along with the seeds were placed in a 250 ml glass flask with 90 mL of 50% aqueous ethanol and was left untouched for 30 min to optimize the extraction. It was then refluxed at 90^{o}C at 1200 rpm in 2 hours, filtered and was placed in a bottle for storage [13, 14, 15].

B. *Phytochemical Analysis of Extracts*

The specific reaction for each test confirms the positive result [16, 17].

1) Detection of alkaloids: 5 ml of the extract was dissolved in diluted Hydrochloric acid and filtered. Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Alkaloids are confirmed present if there is a formation of a yellow colored precipitate.

2) Detection of flavonoids: Alkaline Reagent Test: 5ml of the Extract was treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of diluted acid, indicates the presence of flavonoids.

3) Detection of saponins: . Foam Test: 0.5 ml of extract was shaken with 2 ml of water. If the foam is produced and persists for ten minutes, it indicates the presence of saponins.

4) Detection of tannins: Ferric Chloride Test –5 ml of the extract was dissolved in 5 ml of distilled water. To this, a few drops of 5% Ferric chloride were added. Dark green color indicates the presence of tannins.

C. *Administration of Treatment*

Extracts were mixed in distilled water to attain concentrations of 1%, 10%, and 50%. Approximately 0.2ml of the crude extract was diluted in 19.8 ml of distilled water in a bottle to attain 1% concentration and was labeled as T1. About 2 ml of the crude extract was diluted in 18 ml of distilled water in a bottle to achieve 10% concentration and was labeled as T2. Furthermore, 10 ml of the crude extract was diluted in 10 ml in a bottle to attain 50% concentration and was labeled as T3. For the 100% concentration, 20 ml of the crude extract was placed in a bottle and was labeled as T4. Treatments were orally administered to the chicken, 1 ml twice a day for three (3) days. Levamisole, a commercially available nematicide, was used as a positive control.

D. *Fecal Analysis*

The methods employed for the evaluation of anthelmintic efficacy of Capsicum placental extracts against gastrointestinal parasites in chicken stool was adapted from the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP). The standard protocols for primary fecal analysis and egg counting techniques in Parasitology were also followed. Proper collection and handling of the fecal samples were strictly implemented.

Collection of fecal materials for the initial count and identification of egg parasites were done a day prior to the administration of treatments. Succeeding collec-

tions were done for three consecutive days after the anthelmintic treatment.

Each chicken was placed in separate boxes to ensure the reliability of the sample collected. The fresh and newly voided droppings were collected, placed separately in a clean stool cup and labeled. These samples were then transported immediately to the Parasitology Laboratory at University of Mindanao, Davao City. Modified McMaster Method was followed in the egg counting. Three (3) g of each fecal sample was placed in a strainer. Then, 45 ml of water with a saturated salt solution was poured slowly to the sample. A container was placed below the filter to collect the filtrate. The collected solution was homogenized by shaking in a 250 ml beaker and was transferred in another beaker. This process was repeated for ten times. The homogenized solution was the one used for egg counting.

The counting chamber was improvised following Henriksen and Korshom model of the chamber, (1984). Using a medicine dropper, the sample from the homogenized solution was placed in the chamber and was examined under an electric compound microscope at X200 magnification. The number of eggs was counted and was multiplied by 26 representing the eggs per gram [18,19]. The given scale was used to interpret the presence of egg parasites in the feces: (-) = No infection/<100; (+) = 100-700 Light Infection; (++) = 800 - 1100 Moderate Infection; (+++) = 1200 - above Heavy Infection [18, 19].

E. *Identification of Parasites*

The Modified Wisconsin Sugar Flotation Technique was followed in the identification of parasitic eggs. Observation of the morphological characteristics was initially done and were photodocumented for further identification. The photos were sent to the Department of Agriculture (Region XI), Philippines for confirmation.

F. *Statistical Treatment of Data*

The statistical tool used to compare the level of efficacy of the different concentrations of treatment was the ANOVA test [20]. Results with $p < 0.5$ were considered to be statistically significant.

III. RESULTS AND DISCUSSION

A. *Secondary Metabolites from the Placental Extract of Capsicum Annuum var. Longum*

The phytochemical screening of the presence of different secondary metabolites was carried out in the Biology Laboratory in the University of Mindanao, Matina Campus, Davao City in September 2018. The presence of three secondary metabolites which were the alkaloids, flavonoids, and tannins in the plant extracts obtained from the Capsicum annuum var. longum were positively confirmed as shown in Table 1. A distinct alkaloid that can only found in chili, Capsaicinoids, was highly indicated in the seeds and placental region of Capsicum spp. [21]. However, saponins were not present in the extracts obtained.

TABLE 1
PHYTOCHEMICAL ANALYSIS OF CAPSICUM ANNUUM VAR. LONGUM PLACENTAL EXTRACTS

Secondary Metabolites	Qualitative Test	Indication
Alkaloids	Wagner's Test	+
Flavonoids	Alkaline Reagent Test	+
Saponins	Foam Test	–
Tannins	Ferric Chloride Test	+

B. *Gastrointestinal Parasite in the feces of Broiler Chicken*

Using Modified Wisconsin's Technique, the parasitic eggs were identified as Ascaridia galli (Fig 1a) and Heterakis gallinarum (Fig 1b). The eggs of Ascaridia galli were morphologically described as transparent with a large nucleus in the center. The granule-containing cytoplasm is enclosed in an oval-shaped shell that is a bit tapered at the end. Meanwhile, the eggs of Heterakis gallinarum were ovoid, having a thick membrane. They are very similar to the eggs of A. galli but are smaller in size. The number of A. galli egg parasites were found to be insignificant. H. gallinarum eggs, on the other hand, were consistently abundant in all samples.

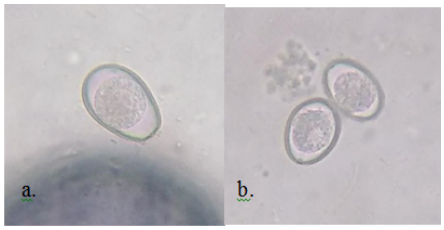

Fig. 1. Parasitic eggs found in the stool of the subjects: a. Ascaridia galli; b. Heterakis gallinarum

C. Anthelmintic Activity

Table 2 shows the presence and the number of egg in the specimen from the initial count (Day 0) to the final day (Day 3) of administration. The number of eggs indicates the level of infection. Initial egg count showed that the fowls have above heavy parasitic infection. The T4 (100%) significantly reduced the number of eggs from heavy to light infection on the first day of administration. On Day 2, T2 (10%) concentrations declined the number of parasitic eggs to light infection. The T1 (1%) concentration showed a decreasing trend of efficacy.

TABLE 2
PRESENCE OF EGG PARASITES OF HETERAKIS GALLINARUM IN THE FECES FROM DAY 0 TO DAY 3

Treatment	Day 0	Day 1	Day 2	Day 3
T0 - Control	+++	+	+	-
T1 - 1%	+++	+++	++	+
T2 - 10%	+++	+++	+	-
T3 - 50%	+++	+++	+++	+
T4 - 100%	+++	++	+	-

(-) = No infection/<100; (+) = 100-700 Light Infection; (++) = 800 - 1100 Moderate Infection; (+++) = 1200 - above Heavy Infection

The difference on the effect of the placental extracts of Capsicum annuum var. longum in different concentrations on the first day was significant and occurred by chance with a probability of less than 0.05, rejecting the null hypothesis. On the second day, all treatment demonstrated a decreasing number of parasitic eggs. In T1, T2, T3, and T4, the quantities of egg parasites were 99.26%, 99.93%, 99.87%, and 99.71%, respectively. The result was also comparable to the positive control group having 99.82% less than the initial count. The difference in the effect in different concentrations was insignificant and occurred by chance with a probability of greater than 0.1, thus accepting the null hypothesis. On the third day, the final egg parasites count were 99.94% lesser than the initial count in T2 and T3; 99.98% smaller than the initial count in T4. The anthelmintic efficacy in different concentrations is significant at 95% on the first day. However, the difference in the efficacy between the concentrations and the positive control used in this study has been insignificant on the second and third day.

TABLE 3
SIGNIFICANT DIFFERENCE ON THE EFFECT OF THE PLACENTAL EXTRACTS FROM CAPSICUM ANNUUM VAR. LONGUM AND THE POSITIVE CONTROL AGAINST HETERAKIS GALLINARUM FOUND IN BROILER CHICKEN STOOL IN TERMS OF THE NUMBER OF EGGS

Day	Concentration	*N*	Mean	SD	*F*	*p*-value
1	1%	3	92.40%	5.40	4.18	0.03*
	10%	3	97.59%	1.02		
	50%	3	97.63%	0.91		
	100%	3	99.22%	0.17		
	+Control	3	99.90%	0.06		
2	1%	3	99.26%	0.63	2.43	0.11ns
	10%	3	99.93%	0.03		
	50%	3	99.87%	0.05		
	100%	3	99.71%	0.09		
	+Control	3	99.82%	0.18		
3	1%	3	99.94%	0.03	1.53	0.27ns
	10%	3	99.97%	0.02		
	50%	3	99.97%	0.01		
	100%	3	99.98%	0.02		
	+Control	3	99.91%	0.08		

*Significant at 95% ns Not Significant

IV. CONCLUSION

The aqueous ethanolic extract of Capsicum annum var. longum had a positive indication of alkaloid, flavonoids, and tannins when phytochemically analyzed. Heterakis gallinarum and Ascaridia galli were identified and confirmed in the chicken's stool. The H. gallinarum were found to be significantly abundant. The T4 had the most effective anthelminthic activity among all treatments which was comparable to the positive control, a commercially synthetic parasiticide. The results demonstrated high level of anthelmintic properties against H. gallinarum. The capsicum has the potential to be an organic anthelmintic remedy for parasite infections. Thus, researchers should conduct further study on the isolation of secondary metabolites from Capsicum annuum var. longum (Siling-Haba) using other plant parts. In addition, other species under capsicum taxon may have a higher efficacy level of anthelmintic activity that can be used as a treatment for wider range of parasitic infection.

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