Anthelmintic properties of panyawan (*Tinospora rumphii* Boerl.) crude aqueous stem extracts against gastrointestinal helminths of naturally infected upgraded goats (*Capra hircus*)

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Abstract. Panyawan extract (PE) was evaluated against goat's internal parasite for their anthelmintic effects. The aqueous stem extracts were chemically evaluated using modified and standard protocols and were found to contain tannins, saponins, flavonoids, alkaloids, steroids and phenols. The experiment was carried out in 5-cages with 10 goats in each treatment group and each goat served as a replicate using different concentrations of Albendazole-Benalvit, 1 mL PE/10 kg BW, 1.5 mL PE/10 kg BW, 2.5 mL PE/10 kg BW, 3.5 mL PE/10 kg BW. Distilled water was used as negative control. All freshly excreted feces were collected and placed in plastic cups and were immediately examined through Modified McMaster test to evaluate the anthelmintic activity of the designated treatments against parasitic gastrointestinal nematodes. Efficacies of different anthelmintics were examined at Regional Animal Disease Diagnostic Laboratory (RADDL), Brgy. Taguibo, Butuan City, Caraga-XIII. The evaluation criteria were based on the frequency counts of egg parasites (EPG) under the different treatments. Results were analyzed using the analysis of variance (ANOVA) of a Completely Randomized Design (CRD). Tukey's test or HSD was used to compare treatment means showing significant differences. The anthelmintic efficacy of the PE preparations was monitored through faecal egg count reduction (FECR), at regular intervals for a period of 10 and 14 days post-treatment in no-choice laboratory test. The highest reduction (EPG) of expelled egg parasite was 62.80 and 78.11% when PE was given at a dose of 3.5 mL/10 kgBW after 10 and 14 days of treatment, respectively. Although the FECR of goats treated with this dosage level was higher than those groups treated with 1.5 and 2.5 mL/10 kgBW of PE, the difference were not statistically significant. In the FECR of positive control, albendazole reduced the faecal egg count by 83.01% and 92.80% at 10 and 14 days respectively after treatment. PE had the highest number of nematode eggs expelled when given at 3.5 mL/10 kgBW and exposed within 10 and 14 days with 3,385 and 4,210 fecal egg count reduction (EPG), respectively.

Key Words: internal parasites, helminthiasis, panyawan extracts, plant secondary metabolites (PSM), fecal egg count.

Introduction. Panyawan (*Tinospora rumphii* Boerl.) is a vine found throughout the Philippines. It contains a bitter principle, colombine (2.22%), traces of alkaloids, and a glucoside. The two alkaloids present are tinosporine and tinosporidine. It also contains an amorphous bitter principle, picroretine and traces of berberine. It is considered febrifuge, vulnerary, tonic, antimalarial, parasiticide, and insecticidal (Stuart 2014). In terms of constraints that hamper productivity and full development of goat subsector, high mortality rate at weaning, development of anthelmintic resistance, and endoparasitism were identified as the top three concerns that should be addressed (PCARRD 2004). High mortality at weaning is strongly seen in kids considering that this species is always prone to stress which more often result to lowered immunity.

Internal parasitism is one of the biggest problems in the small ruminant industry. Internal parasite infection of herds can cause major health issues, which have a major effect on animal's performance and cause great economic loss to the producer. In fact, most of the economic losses caused by internal parasites are actually not due to mortality but production loss (Waller 2004). Internal parasites of goat have been a problem ever since. For this reason, new technologies are introduced to livestock raisers to solve the internal parasites infection.

Today, the primary control of gastrointestinal parasites is based on the over-the-counter anthelmintics. The function of anthelmintic drugs is to cause paralysis of worms so that they are expelled in the feces of man and animals. Although numerous advances were made in understanding the mode of transmission and the treatment of these parasites during the past decades there are still no efficient products to control certain internal parasites and the indiscriminate use of some drugs has led to several cases of resistance (Nunomura et al 2006).

Commercial anthelmintics have been used for some decades throughout the world to minimize the losses caused by helminth infections (Waller 1997). The threats of anthelmintic resistance, risk of residue, availability and high cost, especially to farmers of low income in developing countries, have led to the notion that sustainable helminth control cannot be achieved with commercial anthelmintics alone. Other alternative options like biological control, vaccine and traditional medicinal plants are being examined in different corners of the world (Bain 1999; Chandrawathani et al 2003; Githiori 2004; Waller & Thamsborg 2004).

In the Philippines, farmers practice indigenous knowledge which forms a valid basis for popular adoption in the farm. Despite the availability of commercial dewormers, smallhold livestock farmers still use herbal medicine because it is cheaper, easily available, and has been a traditional knowledge handed down for generations. As part of the organic agriculture program for livestock production in the Philippines (Bureau of Agricultural Statistics 2014) from 2011 to 2013, the goat subsector is one of the most numbered in terms of the inventory by classification.

Plants constitute major part of traditional veterinary practices and have been found to be a rich source of botanical anthelmintic in animals for centuries (Jabbar et al 2006). Evaluation of the activities of medicinal plants claimed for anthelmintic property had a great interest over the years (Gathuma et al 2004; Githiori 2004). The role of plants in extending the use and increasing the efficacy of existing anthelmintics, should be explored especially those that might help in reversing resistance of some preparations in the market. Despite ample evidence of antiparasitic properties of several plants or its products, there is still a need to provide validated experimental data of biological meaningful reductions in infection levels to support the view that these may play a direct role in the sustainable control of helminth infections under farming situation. At present, no scientific studies has yet been done on the anthelmintic properties of panyawan or makabuhay against goat internal parasites. Hence, this study was conducted.

Material and Method. This study was conducted at the Caraga State University (CarSu) Sheep and Goat Project, Main Campus, Ampayon, Butuan City, Agusan del Norte, Philippines from July 23 to August 15, 2016. Fresh stems of panyawan plant including its leaves were gathered from different places in the Municipality of Talacogon, Loreto and San Luis, Agusan del Sur. The same variety of panyawan, the *Tinospora rumphii* Boerl was used in this study. This was presented to plant systematics or botanist faculty for identification and certification of species. Experimental goats from the small ruminant project of the school were used. These were raised free ranged on a pasture area to ensure that the goats could acquire endoparasites. Moreover, they were examined if they were positive to gastrointestinal helminth parasites through fecal flotation. Animals that were found positive were placed in pens three days before the administration of panyawan extract and the other treatments. The experimental animals were orally administered with panyawan extract using a calibrated drench gun. The dose for every animal consisted of 1.5 mL, 2.5 mL and 3.5 mL panyawan extract (PE)/10 kg body weight (BW) were properly administered to determine appropriate dose since underdosing can reduce the efficacy of treatment. Animals were properly observed after the administration of panyawan extract.

Preparation of crude stem aqueous extract. The stems were washed clean with running water. The cleaned collection were finely cut/chopped into smaller pieces and allowed to air-dry in the room with good air ventilation. Two chopped collections of panyawan stem, each collection weighing 150 grams were prepared. The collection was immersed in 1500 mL distilled water to obtain crude aqueous extracts, in a lidded glass jar or beaker for one week. The beakers were refluxed in low temperature, below boiling point of water, cooled and filtered using Whatman filter paper to remove filtrate. Liquid extracts were used in the phytochemical screening and filtrate was discarded.

Collection of faecal pellets. Fresh feces were collected from the goats' rectum through the use of disposable surgical gloves, placed and sealed in plastic cups and was immediately examined and analyzed through modified Mcmaster test (RADDL-CARAGA 2016).

EPG (eggs per gram) count. At day 0, a fecal sample was taken from the rectum. Presence of eggs was determined based on 4 g of faeces using the modified McMaster method with a sensitivity of 50 eggs per gram (EPG) of faeces. EPG count was done on 10th and 14th day post treatment.

Fecal egg count reduction test (FECRT). The percentage reduction in fecal egg counts was based on the formula: % Reduction = 100(1-[TM/CM]) where TM is the mean egg count of the treated group and CM is the mean egg count of the untreated group. Approximately degree of infection was evaluated based on the guideline to the interpretation of faecal egg counts in small ruminants. Rating scale was: Light (100 to 500 e.p.g.), Moderate (500 to 2,000 e.p.g.), and Heavy (2,000+ e.p.g.) (RADDL-CARAGA 2016).

Experimental animals, design and treatments. A total of 50 upgraded goats of varying sexes and weights were randomly assigned into five treatments, with 10 goats in each treatment group, and each goat served as a replicate. The study was laid out in a Completely Randomized Design (CRD) with the experimental treatments as follows:
T1 = distilled water (negative control);
T2 = Albendazole, 15% (positive control);
T3 = 1.5 mL of panyawan extract/10 kgBW;
T4 = 2.5 mL of panyawan extract/10 kgBW;
T5 = 3.5 mL of panyawan extract/10 kgBW.

Statistical analysis. All the data gathered were organized and analyzed statistically using the analysis of variance (ANOVA) of a Completely Randomized Design (CRD). Tukeys or HSD test was used to compare treatment means showing significant differences.

Results and Discussion

Phytochemical analysis. The stem extracts were analyzed for the presence of the following secondary metabolite phyto-constituents such as flavonoids, alkaloids, steroids, glycosides, tannins, saponins, and phenols using modified and standard protocols. The aqueous stem extracts were found to contain tannins, saponins, flavonoids, alkaloids, steroids and phenols (Table 1). Of the six active constituents tested, all of these have anthelmintic activities. Hossain et al (2011) said that phytochemicals such as steroids, carbohydrates, tannins, triterpenoids, flavonoids and cumarines were responsible for the potent anthelmintic activity. Similarly, Aswar et al (2008) stressed that secondary metabolites of plant derived extracts are responsible for anthelmintic activity. The major role played by the phyto-constituents is the alterations in the membrane permeability of which the possible mechanism of action is that the constituents bind the glycoprotein of the cuticle of the parasite (Kumar et al 2011; Thompson & Geary 1995) thereby causing death.

This medicinally active portions of the tested plant is believed to have anthelmintic activities because of the presence of various phyto-constituents with varying functions. Cho et al (2003) as cited by Jagadeesan et al (2014) suggested that better therapeutic effects of plant extracts may be obtained from a combination of active principles in each plant instead of single isolated compounds.

Table 1

Phytochemical profile by various tests of crude aqueous extracts (CAEs) of panyawan (*Tinosphora rumphii*, Boerl)

Phytochemicals	*Detection test**	*Reaction*
Alkaloids	Mayer's	+
	Dragendroff's	+
	Wagner's	+
	Hager's	+
Flavonoids	Alkaline reagent	+
	Lead acetate	+
Steroids	Liebermann Burchard	+
Glycosides	Modified Borntrager's	-
Tannins	Gelatin	+
Saponins	Froth	+
Phenols	Ferric chloride	+

Legend: (+) present, (-) absent (Source: CarSU, CAS-DNS, Division of Chemistry). *Each test was chemically examined as per the standard methods for phytochemical screening and extraction (Tiwari et al 2011).

Nematode egg counts of experimental goats before treatment. The predominant endoparasites of experimental goats prior to the treatment are shown on Table 2, roundworm eggs and coccidia oocysts were identified in feces of experimental goats. During the fecal examination, nematode eggs were seen in all fecal samples of the treatment groups. Only two (4%) goats were infected with coccidia, no helminth larvae were found in feces of goats. Bureau of Animal Industry (2009) stressed that identification of gastro-intestinal parasites diagnosed by classification revealed a percentage high of 52% for Nematodes, followed by Protozoa with 30%, Trematodes with 16%, and the lowest is Cestodes with only 2%.

Table 2

Identified endoparasitic species in fecal samples of experimental goats before Panyawan administration

Type of parasites	*Sign if it is positive or negative (+, -)*			*Number of infected goats*
	Eggs	*Larvae*	*Adults*	*50 heads (%)*
Roundworms	+++++	-	-	48 (96%)
Coccidia oocysts	+++++	-	-	2 (4%)

Legend: (+++++) too many, (-) absent.

Mean EPG count of parasite eggs at pre-treatment, 10 and 14 days post-treatment. The feces of the experimental goats were examined before the treatment was administered to ascertain that the goats used are really infected with gastrointestinal parasites. Figure 1 shows that the eggs per gram (EPG) feces of all treatment groups before dosing with PE, including those in the control group (T1 and T2), were within light to heavy infected (> 150-2,000+ EPG) by gastrointestinal parasites. The approximate degree of infection based on the pre-treatment EPG count revealed that the test animals were severely infected, the mean EPG count before treatment was 900, 5,975, 560, 4,140 and 5,390 in the goats of treatment group 1, 2, 3, 4 and 5 respectively, and this shows statistical significant ($p < 0.05$). Occurrence of internal parasite eggs was heavy just because the experimental animals did not undergo deworming, hence the goats used in this study has fulfilled the criteria regarding parasitic egg excretion at the time of pre-treatment which equal to or above 150 EPG feces.

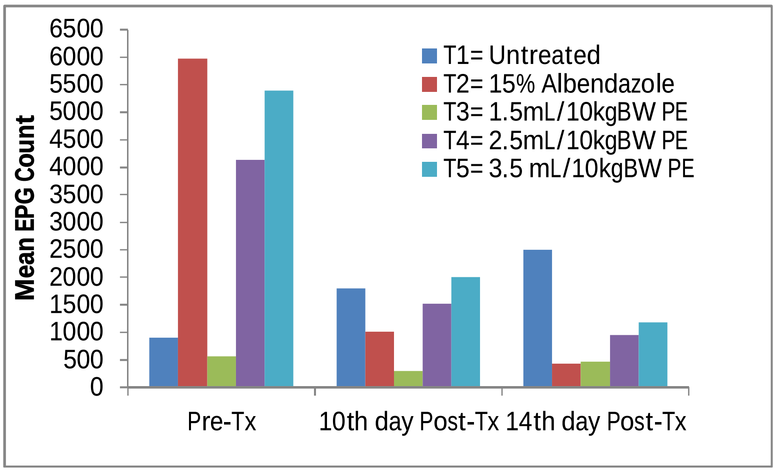

Figure 1. Mean EPG count of goats treated with three dosage levels of CAEs of panyawan at days 10 and 14 post-treatment.

On the number of eggs per gram (EPG) feces at day 10 post-treatment, goats treated with 3.5 mL PE/10kg BW obtained the highest total of 2005 EPG, this was followed by the goats in T4 with 1525 EPG, next is in T 2 wit 1015 EPG, while goats in T3 excreted the lowest number of nematode eggs with 300 EPG. The result of the reducing number of eggs expelled is due to the administration of PE and albendazole. It is most likely due to the bioactive compounds contained in PE which were responsible for the potent anthelmintic activity. Muller-Harvey & Mcallan (1992) further stressed that plant secondary metabolites (PSM) such as saponins, alkaloids, non-protein amino acids, tannins and other polyphenols, lignins, glycosides are all active compounds of plants which have been considered to have antiparasitic effects. T1 had increased EPG count with a total of 1,800 EPG. This could have been caused by the reduced of anthelmintic activity, and consequently faecal concentration of parasite eggs in these groups of untreated animals. Though observed differences were seen but these do not reach statistical significance.

Mean EPG counts of internal parasite eggs after fourteen days of administration showed that T2 obtained the lowest number of eggs expelled with a total of 430 EPG followed by T3, with a total of 460 EPG, then T4 and T5, with 960 and 1180 EPG, respectively. Apparently however, goats in T1, distilled water had EPG count higher than the other treatments with 2505 eggs. This result indicates that one effect of albendazole and PE is melting the eggs of the parasites because they were no longer visible during the fecal examination as compared with the untreated control group. Adediran & Uwalaka (2015) said that albendazole is still an effective drug in treating helminths in goats. Albendazole (ABZ) belongs to the benzimidazole (BZD) anthelmintic drug group. The mode of action of BZDs is by interference with polymerization of microtubules (Harder 2002). These drugs bind to the protein tubulin of the parasite, therefore causing death by starvation (Roos 1997). However, result showed no significant differences among treatment means.

Moreover, treatments given with higher dosage level of PE have indicated the highest reduction as compared to the goats dosed with distilled water. Again, this result could be due to the bioactive compounds in the PE. Suffice it to say that the bioactive compounds present in PE that are extracted by water are having anthelmintic properties. These compounds could be alkaloids, tannins, saponins, flavonoids, steroids and phenols. However, as to what specific component from these compounds is having a lethal effect against eggs of parasites is something that should be looked into in the future study. Paolini et al (2003) said that tannins is shown to have an anthelmintic activity. It has been demonstrated that tannins adversely affected the integrity of the cuticle of larvae and adult nematodes (Karim 2006; Sujon et al 2008). On the other hand, in the study on the anthelmintic activity of tannins against *Haemonchus contortus* and *Trichostrongylus colubriformis*, it was shown that tannins have a direct effect on the fecundity of the nematodes (Paolini et al 2003). Likewise, tannins stimulated the local immune response of treated cattle, thereby increasing the inflammatory cells of host in the fundus of abomasum. These inflammatory cells are capable of neutralizing either stages of the nematode. Thus, tannins in appropriate amount could be used as a dewormer without an adverse effect on the animals being treated.

FECR (%) of parasite eggs at 10 and 14 days post-treatment. Comparison of the % fecal egg count reduction (% FECR) on goats allocated to T1 (untreated control), between T2 (Albendazole, 15%) and T3-T5 at day 10 post-treatment showed that, there was a reduction of fecal egg count in all treatment groups at 10 and 14 days post-treatment, except in group treated with 2.5 mL/10kg BW (T4) of PE at day 10 post-treatment in which the FECR was fluctuating (Figure 2). The fecal egg count reduction could be attributed to the bioactive compounds contained in PE. However, no significant effect on %FECR was observed in goats treated with three preparations of PE (T3-T5) at 10 and 14 days post-treatment. Although the %FECR of groups treated with these varying dosage level was higher than the untreated control group (T1) at days 10 and 14 fecalysis, these were lower than groups treated with Albendazole, 15% (T2). The over-all percentage of FECR which used the frequency counts of parasite eggs 10-14 days after the administration of PE showed an average of .8764% and 16.4607%, respectively for 10 and 14 days.

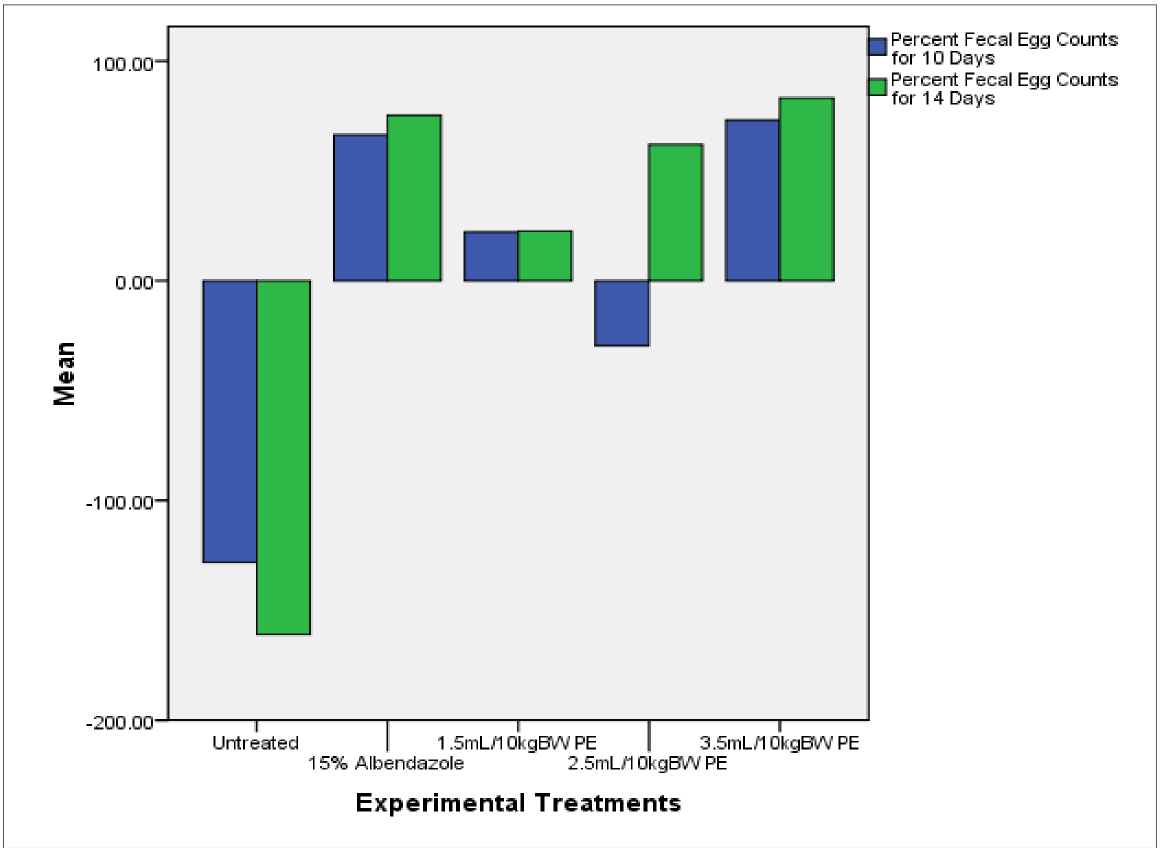

Figure 2. Percentage of fecal egg count reduction (%FECR) of goats treated with three dosage levels of CAEs of panyawan at days 10 and 14 post-treatment (EPG).

On the other hand, comparison of the %FECR between goats of T2 and in goats treated with 2.5 mL/10 kg BW PE (T4) and 3.5 mL PE/10 kg BW (T5) at days 10 and 14 fecalysis was not statistically significant. Goats from these treated groups, resulted in FECR ranging from -29.5-83.2%, however none of the reductions were significant. Comparing goats treated with various concentrations of PE and albendazole and distilled water, shows that efficacy of 3.5 mL PE/10 kg BW and albendazole were equally effective among the different treatments at 10 and 14 days post-treatment. This shows that dosage level of 3.5 mL PE/10 kg BW could be potentially used against gastrointestinal helminth infection in goats. Goats metabolize anthelmintic drugs much more rapidly than other livestock and require a higher dosage to achieve proper efficacy (Hennessy 1994). Consequently, it is recommended that goats be given a dose 1.5-2 times higher than for sheep or cattle. In other words, the higher concentrations of PE were effective in controlling internal parasites of goats.

Conclusions. The aqueous stem extracts were found to contain tannins, saponins, flavonoids, alkaloids, steroids and phenols. The highest reduction (EPG) of expelled egg parasite was 73.20 and 83.20 % observed when PE was given at a dose of 3.5 mL/10 kg BW after 10 and 14 days of exposure, respectively. Although the FECRT of goats treated with commercial dewormers was higher than those groups treated with 1.5 and 2.5 mL/10 kgBW of PE, this was not statistically significant ($p > 0.05$). In the FECRT of positive control, albendazole reduced the faecal egg count by 83.01 and 92.80% respectively for 10-14 days after treatment. PE had the highest number of parasites expelled when given at 3.5 mL/10 kg BW and exposed within 10-14 days with 3,385 and 4,210 fecal egg count reduction, respectively. Comparing goats treated with various concentrations of PE and albendazole and distilled water, % FECRT shows that efficacy of 3.5 mL PE/10 kg BW and albendazole were effective among the different treatments at 10 and 14 days post-treatment. This observation shows that the anthelmintic activity of goats treated with increasing amount of PE is having similar response between goats treated with albendazole. Indicating that the two concentrations were equally effective in controlling gastrointernal helminths of goats.

Acknowledgements. We deeply thank Regional Animal Disease Diagnostic Laboratory – Regional Field Unit (RADDL-RFU) Caraga-XIII, for the help in handling the samples, advice and significant discussions on some of the parasitological techniques and fecal examinations. We also acknowledged Professors in the Graduate School of Central Mindanao University who give worthwhile comments, shared views and patience during the conduct of this experiment and for their enormous interest to the research.

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Received: 28 November 2017. Accepted: 31 January 2018. Published online: 25 February 2018.
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How to cite this article:
Ramada J. M. S., Soriano M. L. L., Abella J. A. C., 2018 Anthelmintic properties of panyawan (*Tinospora rumphii* Boerl.) crude aqueous stem extracts against gastrointestinal helminths of naturally infected upgraded goats (*Capra hircus*). ABAH Bioflux 10(1):9-17.