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Original paper

In vitro anthelminthic activity of fringed spiderflower (*Cleome rutidosperma*) ethanolic leaf extract against *Fasciola* spp.

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ABSTRACT. Fasciolosis is considered as one of the leading causes of morbidity and mortality among ruminants in the Philippines. Though anthelmintic drugs are widely used to treat and control the condition, it is still worthwhile to search for alternative treatments especially when resistance to commonly used anthelmintic drugs has been reported. In this study, the ethanolic leaf extract of fringed spiderflower (*Cleome rutidosperma*) was evaluated for its *in vitro* anthelmintic activity against *Fasciola* spp. Specifically, the study compared the different concentrations of ethanolic leaf extract and the commonly used anthelmintic drug (albendazole) on the gross motility and histology of *Fasciola* spp. The study consisted of five treatments: treatment 1, 2, and 3 which contain 10%, 20%, and 40% leaf extract, respectively, treatment 4 with 10% albendazole as the positive control, and treatment 5 with nutrient broth as the negative control. The motility of the *Fasciola* spp in all treatments was visually analyzed based on the established criteria. In addition *Fasciola* spp. in different treatments were subjected to tissue processing and histological examination. Results showed that increasing concentrations of leaf extract resulted in a decreasing time for *Fasciola* spp. to have a motility score of zero. Specifically, 10%, 20%, and 40% leaf extract resulted in a cumulative time period of 55.00 ± 5.00 min, 26.67 ± 2.89 min, and 15.00 ± 0.00 min, respectively, for the *Fasciola* spp. to have a motility score of zero. On the other hand, albendazole resulted in a 240.00 min cumulative time before it can cause a motility score of zero. Histologic examination showed that the different concentrations of leaf extract affected the tegument and parenchyma of the *Fasciola* spp.

Keywords: albendazole, *Fasciola* spp., fringed spiderflower, *Cleome rutidosperma*

Introduction

The large ruminant industry has three components: buffalo, beef cattle, and dairy cattle. Backyard farmers use buffaloes for draft, milk, and meat purposes. Domesticated water buffalo is one of the major milk producing animal in several countries and contributes a large share in global milk production [1]. The demand for milk and meat is also increasing due to urbanization and growing population thus, strengthening the need for a viable number of efficient large ruminant productions [2].

Parasitic diseases are the major obstacles in the growth, development and health of animals. Bovine and bubaline fascioliosis is common in the Philippines [3]. *Fasciola* sp. infection is a serious animal health problem in many rural and urban areas of the world, causing significant financial losses due to a decrease in production and viscera condemnation in animals specifically in terms of reduction in milk and meat and high mortality in all ages [4].

The effective control of *Fasciola* sp. currently includes the strategic and tactile use of anthelmintic drugs and careful management of grazing lands, including control of stocking rates and appropriate rotation strategies. Because of the limited availability of drugs and their high costs, development of resistance, chemical residues in milk and meat, toxicity problems, and failed snail control measures, thus, majority of the world population depends on traditional remedies. It is estimated that some 20,000 species of higher plants are used medicinally throughout the world for controlling various diseases [5].

Cleome rutidosperma, commonly known as fringed spiderflower, is one of those plants enriched with medicinal properties [6]. It is a species of flowering plant in the genus *Cleome* of the family Cleomaceae, native to tropical Africa. Traditionally, the leaves, roots, and seeds of the plants of *Cleome* species are used as stimulants, antiscorbutic, anthelmintic, rubefacient, vesicant, and carminative [7]. Recent phytochemical analysis confirmed that *C. rutidosperma* also contains several types of phytochemicals which have anthelmintic activities against gastrointestinal nematodes [6]. Thus, this study was conducted to determine the effects of *C. rutidosperma* ethanolic leaf extract on *Fasciola* spp. by determining the concentration of leaf extract that produced highest efficacy based on restricting the gross motility of the adult *Fasciola* spp. and described the gross and histological changes in the morphology of *Fasciola* spp. after exposure to difference concentractions of the plant extact.

Materials and Methods

Collection of samples

Two liver samples were collected from large ruminants slaughtered at the abattoir of San Jose City, Nueva Ecija. The liver was cut in its bile duct part and morphologically identified *Fasciola* spp. found were collected using non-traumatic forceps, cleaned using phosphate-buffered saline (PBS) and transported to Veterinary Public Health Laboratory, Central Luzon State University, Science City of Muñoz, Nueva Ecija using a nutrient broth approximately one hour after extraction in the liver. Only the flukes that showed motility upon visual inspection with normal tegument upon gross morphologic examination were selected. The flukes were weighed using a digital weighing scale.

Preparation of fringed spiderflower leaf extract

Mature fringed spiderflower leaf samples were collected from Science City of Muñoz. The fresh mature leaves were cleaned and washed gently with tap water and subjected to sun-drying. The dried leaves were powdered using a blending machine. The 100 g of the powdered dried leaves were soaked with 500 ml of 70% ethanol for 48 h to obtain the concentrated extracts. The extract was double-filtered using a mesh and Whatman No.1 filter paper. The ethanol was removed through the use of a rotary evaporator machine (or Rotavap) to collect the crude plant extract. The crude extract was stored in an amber bottle stored at 4°C until further use.

Experimental design

The experimental design was based on the standard procedure [8]. Motile *Fasciola* spp. weighing 1 to 2 g were separated into five groups, each containing five *Fasciola* spp. Using a non-traumatic forceps, the flukes were carefully and individually placed on a digital weighing scale. Only those flukes that fall under the acceptable weight range were placed in the Petri dishes

Table 1. *In vitro* anthelminthic activity of ethanolic extract of fringed spiderflower (FS) and albendazole

Group	Concentration	Description	Cummulative time todeath (min)
T1	10% FS extract	2.5 ml FS/25ml nutrient broth	55.0 ± 5.0
T2	20% FS extract	5ml FS/25ml nutrient broth	26.7 ± 2.9
T3	40% FS extract	10ml FS/25ml nutrient broth	15.0 ± 0.0
T4 (Positive Control)	10% Albendazole	2.5 ml Albendazole/ 25 ml nutrient broth	240.0 ± 0.0
T5 (Negative Control)	Nutrient broth	25 ml nutrient broth	670.0 ± 17.3

containing nutrient broth and different crude plant extract concentrations. The experiment was set-up as shown in Table 1.

Motility and morphology examination

The motility of the *Fasciola* spp. were scored using the following criteria: Score 3 – moving whole body, Score 2 – moving 50% of the body parts, Score 1 – grossly immobile but microscopically alive and Score 0 – dead [9].

The elapsed time to cause death to the *Fasciola* spp. exposed to different treatments was observed and recorded. For *Fasciola* spp. with no movement in visual examination, they were placed in a slide and viewed and examined in a stereomicroscope to confirm any movement before subjecting to 10% formalin preservation.

After the death of the *Fasciola* spp., one fluke from each group was selected for carmine staining for histopathogical changes. *Fasciola* spp. from each group were fixed in 10% formaldehyde for 24 h, dehydrated with ascending series of ethanol and cleared with xylene. They were embedded in paraffin, then sectioned at 5 µm thickness, stained with FYG carmine dye solution and examined under light microscope and photographed for abnormalities particularly distraction of the tegument and vacuolation of the parenchyma [10].

Statistical analysis

Statistical analysis was done in all treatments using analysis of variance for completely randomized design (CRD) and factorial in CRD. Each treatment group is composed of 5 flukes with 3 replicates. Cumulative time period when flukes have a motility score of zero was presented as mean ± standard deviation and compared using analysis of variance (ANOVA) followed by Tukey's highly significant difference (HSD) as post hoc test. Statistical significance was set at a 95% confidence interval and $p<0.05$.

Results and Discussion

The effect of fringed spiderflower leaf extracts on the morphology and motility of the *Fasciola* spp. exposed to extracts was recorded. The mean motility scores of the different treatments were calculated and also served as the basis of the efficacy interpretation. Changes in the histological structure of the flukes' tegument were described in comparison with the normal structure using the representative samples of the different treatments.

Table 1 showed that the mean cumulative period for the *Fasciola* spp. to have a motility score of zero which means either total complete paralysis or death of the flukes. Sudden changes in the movement were observed upon exposure to the different concentrations of fringed spiderflower leaf extract. Gradually, the *Fasciola* spp. become paralyzed. The different concentrations of leaf extract (10%, 20%, and 40%) resulted in a cumulative time-period of 55.0±5.0 min, 26.7±2.9 min and 15.0±0.0 min, respectively, while the control treatment albendazole resulted in a cumulative time-period of 240.0±00.0 min. *Fasciola* spp. were observed to have become immobile and non-motile in 15 minutes to less than an hour after exposure in a dose-dependent FS extract. At 40% FS, it was observed that in 15 minutes the *Fasciola* spp. experienced immobility and non-motility when observed under the microscope. The cumulative time-period obtained from each of these groups was found to be statistically significant with each other ($p<0.05$), including the negative control nutrient broth which showed a cumulative time-period of 670.0±17.3 min.

Shahed and Hasan [6] reported that the aqueous leaf extract of fringed spiderflower is effective in a dose-dependent manner against *Haemonchus contortus* as compared to 15 mg/ml of albendazole. Besides, Morah and Apebende [11] also reported that 1%, 5%, and 10% petroleum extract of *C. rutidosperma* is effective against *Lumbricus terrestris* (earthworm) after 3 hours' exposure of the worms at room temperature. Moreover, it was demonstrated that the chloroform fraction of the ethanol extract of the aerial part of the same plants also effective against earthworms [11]. The eucalyptol and phytol present in *C. rutidosperma* may be responsible for the anthelminthic effects of the plant wherein phytol is a potent anthelmintic agent against schistosomosis [12] while eucalyptol is an effective substance against monogenean infections in fish [13]. However, no literature has suggested that *C. rutidosperma* extracts are effective against *Fasciola* spp. and other flukes besides *Schistosoma* spp. This is the first report of the anthelminthic effect of the plant against *Fasciola* spp. Thus, further study on the chemical constituents of *C. rutidosperma* is needed to identify the components responsible for its anthelminthic effects against flukes.

Grossly, all *Fasciola* spp. in the treatments

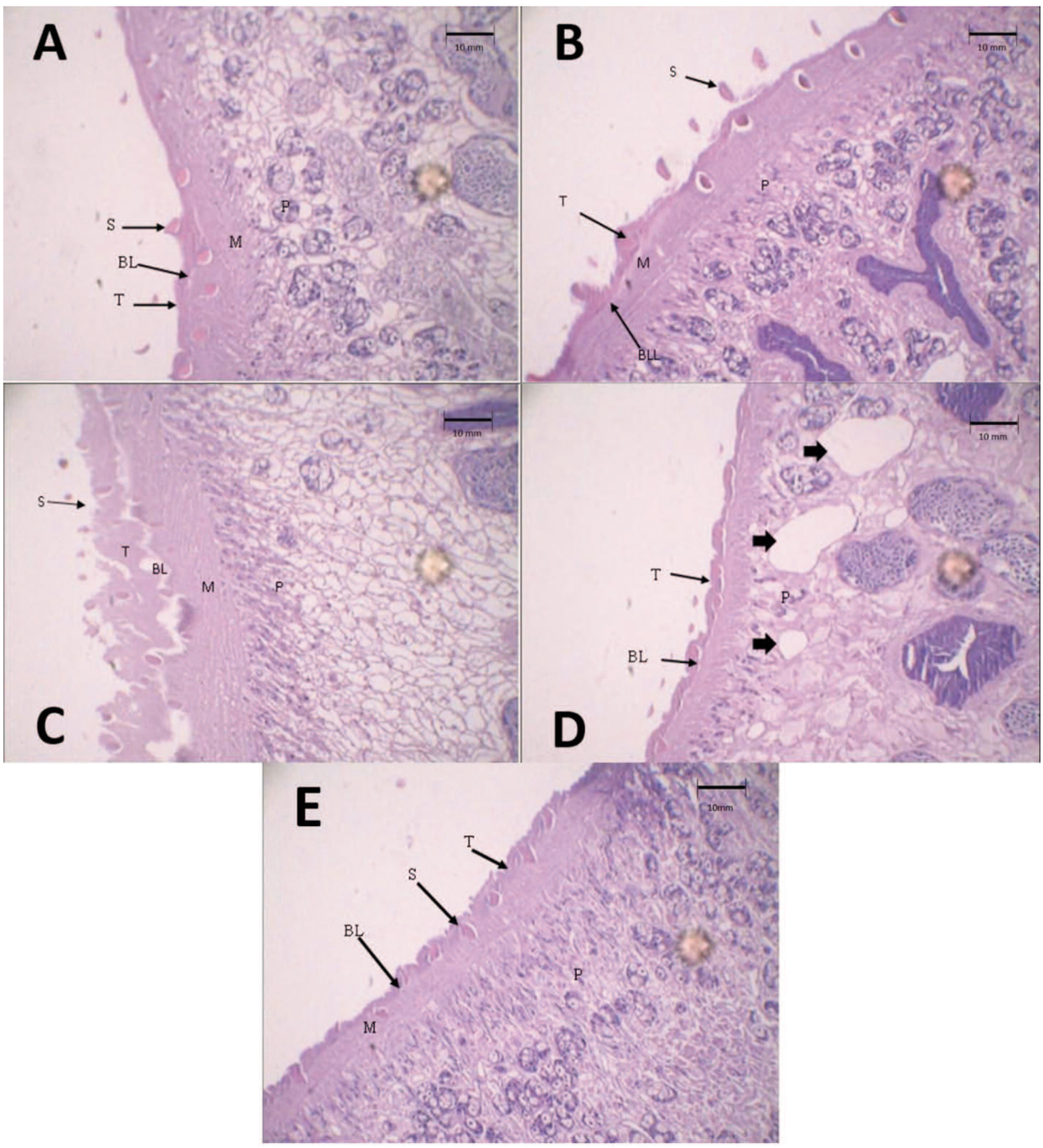

Figure 1**.** Carmine staining of *Fasciola* spp. from different treatment groups. **(A)** Horizontal section of the *Fasciola* spp. exposed to 10% FSE showing a slightly disruption of tegument (T) and separation of the spines (S) ; **(B)** Horizontal section of the *Fasciola* spp. exposed to 20% FSE showing slight blebbing of the tegument (T) and dislodging the spine (S); **(C)** Horizontal section of the *Fasciola* spp. exposed to 40% FSE showing massive desquamation of the tegument (T) from basal lamina (BL) of the fluke; **(D)** Horizontal section of the *Fasciola* spp. exposed to 10% albendazol with the slight swollen and desquamation of the tegument (T) and vacuolation of the parenchyma (P); **(E)** Horizontal section of the *Fasciola* spp. in nutrient broth showing no disruption of the teguments (T) and no vacuolation on the parenchyma (P). M = muscular layer

exposed to different concentrations of leaf extract displayed no significant changes but microscopically, desquamation, deformation and slight separation of the tegument from the basal lamina of the flukes were noticed. *Fasciola* spp. subjected at 10% fringed spiderflower leaf extract concentration showed slight disruption of the tegument and separation of spines (Fig. 1 A). At

20% leaf extract concentration, there was slight blebbing of the tegument and an evident dislodging of the spine from the *Fasciola* spp. (Fig. 1 B) while, there were massive separation or desquamation of the tegument from basal lamina of the flukes exposed to 40% concentration of fringed spiderflower leaf extract (Fig. 1 C).

Microscopically, *Fasciola* spp. exposed to 10% albendazole showed slight swelling and corrugation of the tegument and vacuolation of the parenchyma (Fig. 1 D). Albendazole has been recommended for use against fascioliosis, although its activity is restricted to mature flukes and it requires elevated dose rates [14].

Fasciola spp. in the negative control group remained active until 8 hours of exposure to the nutrient broth. The morphology of these flukes was not altered grossly; the tegument remained intact showing no disruption and desquamation; no vacuolation on the parenchyma of the flukes was observed microscopically (Fig. 1 E).

The tegument is one of the main absorptive surfaces for the uptake of drugs by the fluke. Consequently, it is also one of the tissues that are most immediately exposed to anthelmintic and are likely to represent a primary drug target. The tegument has several important roles: the synthesis and secretion of various substances, absorption of nutrients, osmoregulation, protection against the host's immune response, digestive enzymes and bile, and sensory perception [15]. Therefore, the integrity of the surface plasma membrane and the tegumental syncytium is essential for the viability of the fluke. Any drug-induced disruption to the tegument is likely to have serious consequences for the fluke because it would allow the drug to penetrate to deeper-lying tissues and (in the *in vivo* situation) the damage would be exacerbated by the surfactant action of bile and the immune response [16].

The fringed spiderflower leaf extract caused not only immobility and paralysis of *Fasciola* sp. as a subsequent effect on gross motility but also histopathological changes on the fluke's tegument as shown in the results of this study.

Based on the findings of the study, the following are concluded: The fringed spiderflower leaf extract showed high *in vitro* anthelmintic activities against *Fasciola* spp. and a dose-response relationship where a higher concentration of the leaf extract resulted to a shorter time for the liver flukes to be paralyzed and then eventually die. There was no significant changes on the gross morphology of the flukes in all treatments but histologically the flukes that subjected to different concentrations of the fringed spiderflower leaf extract showed severe separation and desquamation of the tegument compared to the positive and negative control groups. Hence, this herbal medicine can be used as an alternative drug to prevent excessive use of anthelmintics, which usually initiates drug resistance in some parasitic infections. Furthermore, studies should be made to assess the pharmacokinetics and pharmacodynamics especially the toxic effects of the plant extract prior to its extensive use in veterinary medicine.

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