

In-vitro Efficacy of Various Fruit Extracts of *Duranta erecta* and *Piper longum* Against Adult Amphistomes

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ABSTRACT

Gastrointestinal infections among livestock are gaining much importance not merely due to its occurrence, but also due to wide spread anthelmintic resistance. Phytochemicals are ideal alternatives to combat the infection and resistance. The present study was aimed to assess the *in vitro* adulticidal activity of methanolic extract and its n-hexane, chloroform, n-butanol and aqueous fractions from fruits of *Duranta erecta* and *Piper longum* against adult amphistomes. Fresh fruits were collected, identified, shade dried, pulverised and extracted with methanol and the extracts were successively fractionated. The qualitative phytochemical analysis was done to detect phytoconstituents. The extracts and fractions were diluted serially in distilled water/tween 80 to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 mg/mL. Thiabendazole at 10 µg/mL acted as positive control and the solvents as negative control. The adulticidal activity and histopathology of treated amphistomes were performed. IC₅₀ was calculated to identify the potent extract/fraction. The fruits of both plants were identified and accession numbers 005 (*D. erecta*) and 006 (*P. longum*) were obtained. The phytochemical analysis revealed the presence of flavonoids in all the extracts and fractions while tannins, glycosides and diterpenes were absent in hexane fraction of *D. erecta*. Saponins were absent in *P. longum* extract and fractions. Amphistomes were highly sensitive for chloroform fraction of *D. erecta* and methanolic extract of *P. longum* with IC₅₀ of 1.354 mg/mL and 5.493 mg/mL respectively while all other extract/ fractions exhibited moderate anthelmintic activity. The histopathology revealed morphological changes in tegument, syncytium and parenchyma. From the present study it could be concluded that fruits of both plants possessed activity against amphistomes and further isolation of the active compounds from potent extract/fraction can provide a base for the development of a novel, safe anthelmintic which may have a novel mechanism of action.

Keywords: Adulticidal activity, *Duranta erecta*, Histopathology, *Piper longum*, Thiabendazole.

INTRODUCTION

Gastrointestinal nematode infections are one of the major constraint in profitable livestock farming. These infections not only affect animal health, but also indirectly affect global and national economic development¹. Control of these helminth infections is mainly achieved by the use of synthetic anthelmintic drugs. Unfortunately, due to indiscriminate usage, resistance to the drugs is emerging². In this scenario, phytotherapy has gained much importance as an alternative to treat helminth infections and to combat resistance. These herbal remedies are cheaper, reliable and less toxic as compared to the synthetic chemicals³. Hence in the present study, the methanolic extract and its fractions from fruits of *Duranta erecta* and *Piper longum* were evaluated for their effect on the adult amphistomes.

MATERIALS AND METHODS

Plant Materials

The fruits of *Duranta erecta* collected from premises of Veterinary College, Mannuthy while *Piper longum* procured from local vendors in Thrissur, were identified and authenticated by a Botanist at St. Thomas College Thrissur and were dried under shade and pulverized. Thimbles were made and extracted using methanol in soxhlet extraction apparatus, dried in a rotary vacuum evaporator and stored at 4°C. The extracts were further fractionated using n-hexane, chloroform, n-butanol and water. All the extracts were stored under refrigeration after drying.

Phytochemical Analysis

The qualitative phytochemical analysis was done for all extract and fractions⁴.

Assessment of Amphistomicidal activity

Collection of Amphistomes

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Table 1: Qualitative phytochemical analysis of extracts and fractions of *Duranta erecta* and *Piper longum*.

Phyto-constituents	<i>Duranta erecta</i>					<i>Piper longum</i>		
	Methanolic	n-Hexane Fraction	Chloroform Fraction	n-butanol Fraction	Aqueous fraction	Methanolic extract	n-hexane Fraction	Chloroform Fraction
Steroids	+	-	+	+	+	+	+	-
Alkaloids	+	+	+	+	-	+	-	+
Tannins	+	-	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Glycosides	+	-	+	+	+	+	+	+
Cardiac Glycosides	+	-	+	+	+	+	+	+
Phenolic compounds	+	-	+	-	+	+	+	+
Diterpenes	+	-	+	+	+	+	+	+
Triterpenes	+	-	-	+	+	+	+	-
Saponins	+	+	-	+	+	-	-	-

Table 2: Minimum concentration and time producing 50 per cent and 100 per cent mortality of amphistomes, (mg/mL and minutes).

Extract/ Fraction	50 % Mortality		100 % Mortality	
	Concentration (mg/mL)	Time (minutes)	Concentration (mg/mL)	Time (minutes)
Methanolic <i>D. erecta</i>	7.81	115	31.25	105
n-hexane <i>D. erecta</i>	31.25	95	62.5	95
Chloroform <i>D. erecta</i>	3.91	55	1.95	65
n-butanol <i>D. erecta</i>	3.91	115	7.81	120
Aqueous <i>D. erecta</i>	1.95	120	7.81	105
Methanolic <i>P. longum</i>	1.95	105	7.81	85
n-hexane <i>P. longum</i>	15.63	115	31.25	120
Chloroform <i>P. longum</i>	7.81	105	15.63	110
Thiabendazole Control	10 µg/mL	30	10 µg/mL	45
Tyrodes Control	-	120	-	150
Tween 80 Control (6.25 % in Tyrodes)	-	120	-	150

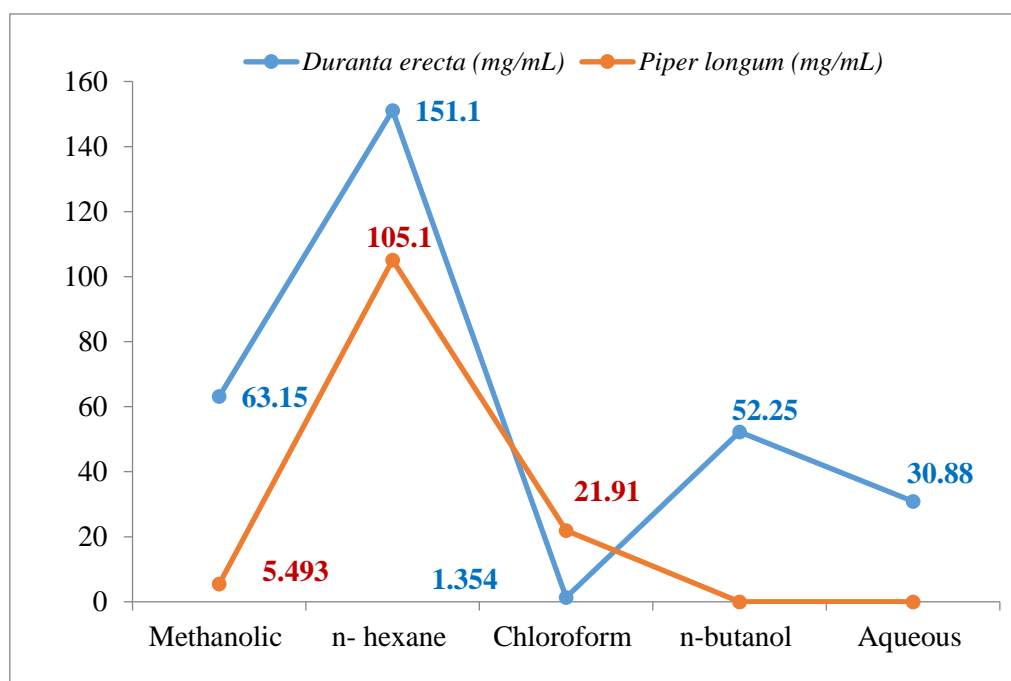


Figure 1: Inhibitory concentration-50 based on Adulticidal Assay (mg/mL.)

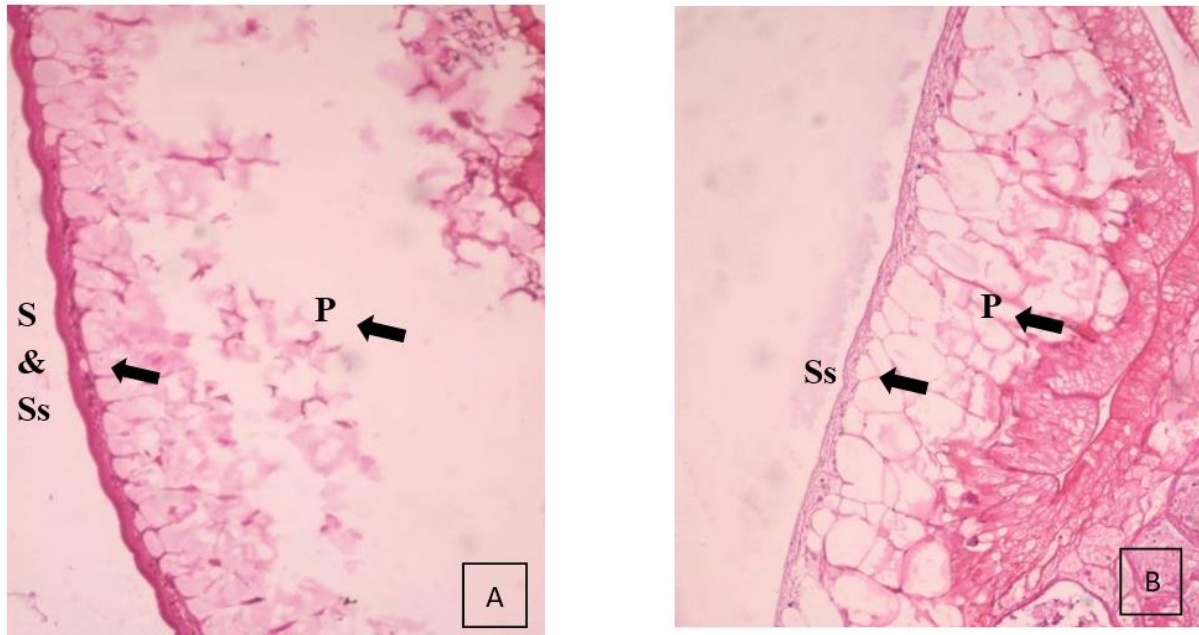


Figure 2: Plate 1. Histopathology of adult amphistomes exposed to potent extract / fractions (H&E, 40X)

- A. Chloroform fraction of *D. erecta* exposed adult amphistomes showing thinning of syncytium (S) and sub-syncytium (Ss) and complete vacuolisation of parenchyma (P)
- B. Methanolic extract of *P. longum* exposed adult amphistomes showing loss of tegument, thinning of syncytial (S) and sub-syncytial (Ss) layers and mild vacuolisation of parenchyma (P)

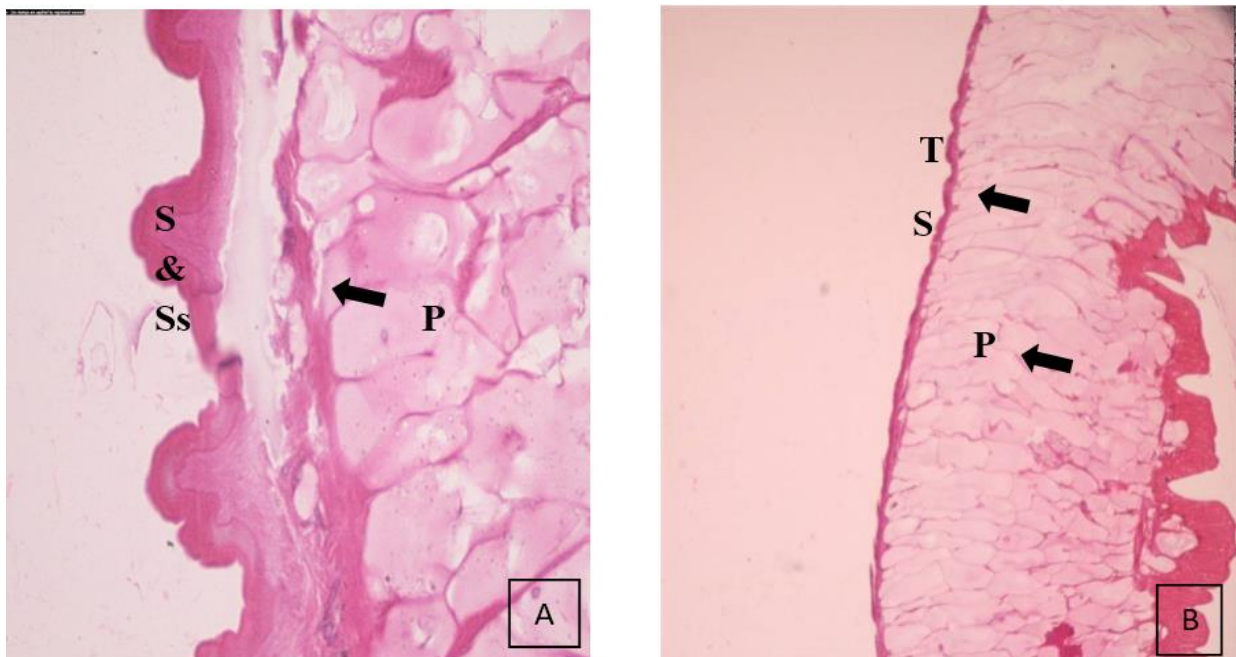


Figure 3: Plate 2. Histopathology of adult amphistomes exposed to potent extract / fractions (H&E, 40X)

- A. Chloroform fraction of *P. longum* exposed adult amphistomes showing detachment of syncytial (S) and sub-syncytial (Ss) layers from parenchyma (P) – bleb formation
- Thiabendazole (10 µg/mL) exposed adult amphistomes showing thinning of tegument (T) & syncytium (S), with muscular degeneration & parenchymal vacuolisation (P)

Fresh amphistomes were recovered manually from the rumen of slaughtered cattle and transferred into tyrodes solution. Care was taken during the collection of the amphistomes to have the sucker intact. The amphistomes

were washed in tyrodes solution and transferred to petriplates containing extracts⁵.

Test drug preparation

Extracts were diluted in tyrodes solution at 500, 250, 125, 62.5, 31.3, 15.63, 7.8, 3.91 and 1.95 mg/mL concentrations in petriplates to get a total volume of 20 ml. Tyrodes solution served as negative control while thiabendazole at 10 µg/mL acted as positive control⁵.

Test procedure

Amphistomocidal activity was done as per 12 with minor modifications. Six amphistomes were placed in the extract containing petriplates and their motility/ wriggling movements were noted every fifteen minutes. Cessation of movements even on stimulation were considered as the end point of observation. The experiments were done in triplicates and the average value was taken. The time and concentration at which 50 and 100 per mortality observed were noted⁶.

Histopathology

The dead amphistomes were fixed in Bouins solution for 12 hours initially and then followed by two changes of 10% formalin for routine haematoxylin and eosin staining procedure. The tissues were made into sections, stained and examined under microscope for finding out the morphological alterations⁶.

RESULTS

Phytochemical Analysis

Effect of various extracts on adult amphistomes

The lowest concentration and the time taken by methanolic extract and its fractions from fruits of *D. erecta* and *P. longum* to induce 50 and 100 per cent mortality are given in Table no. 2.

The chloroform fraction of *D. erecta* was highly effective to produced 50 and 100 per cent mortality at 55th and 65th minutes respectively even at lowest concentrations. The methanolic extract of *P. longum* was effective to produce complete mortality of adult amphistomes at 85th minute at 7.81 mg/mL.

Inhibitory concentration-50 (IC₅₀)

The IC₅₀ value obtained for all the extracts and fractions at 60 minutes of study is represented in Table no. 3 and Figure 4. The IC₅₀ data obtained in the study suggested that the chloroform fraction of methanolic extract of *D. erecta* was highly potent (IC₅₀ = 1.354 mg/mL) followed by methanolic extract of *P. longum* fruit (IC₅₀ = 5.493). The n-hexane fraction of *D. erecta* was least effective among all extracts and fractions assayed in the study (IC₅₀ = 151.1 mg/mL)

Histopathology of extract/fraction exposed amphistomes

The longitudinal sections of treated amphistomes revealed mild to severe morphological alterations in tegument, syncytium and parenchyma (Plates 1 & 2). The potent adulticidal chloroform fraction of *D. erecta* (IC₅₀ = 1.354 mg/mL) affected all the layers except outer tegument with complete degeneration of sub-syncytial zone including circular and longitudinal muscles and severe vacuolisation in parenchymal area was observed (Plate No. 1, A). The methanolic extract of *Piper longum* (IC₅₀ = 5.493 mg/mL) caused thinning of tegumental layers and severe vacuolisation in parenchymal area and mild degeneration of muscular layer (Plate No. 1, B). Detachment of syncytial layers from sub-syncytium with bleb formation was

induced by the chloroform fraction of *P. longum* (Plate No. 2, A) and aqueous fraction of *D. erecta* with mild loss of muscular integrity.

Histopathology of thiabendazole exposed flukes showed thinning of all layers with mild vacuolisation of the parenchyma (Plate No. 2, B). No morphological changes were observed in negative control (tyrodes solution)

DISCUSSION

Gastrointestinal nematode infections are common among ruminants and are mainly controlled by synthetic anthelmintic drugs. Occurrence of resistance against these chemical agents has made gastrointestinal nematodiosis a major threat to profitable livestock farming¹. Anthelmintic activity of various plant extracts against ova and larvae has been reported earlier⁷⁻¹⁰. Hence the present study aimed at adulticidal activity of various extracts and fractions of fruits of *D. erecta* and *P. longum* against amphistomes.

The qualitative phytochemical analysis showed varied distribution of steroids, alkaloids, tannins, glycosides, phenolics, terpenes. Saponins were absent in extract and fractions of *P. longum*. All the extracts and fractions assayed in the study were effective against amphistomes. The chloroform fraction of *D. erecta* and methanolic extract of *P. longum* possessed potent adulticidal activity with IC₅₀ of 1.354 and 5.493 mg/mL respectively. The histopathology of potent extract exposed amphistomes revealed mild to severe morphological changes in tegument, syncytium and parenchyma.

The adulticidal activity of extracts and fractions can be attributed to the presence of phytochemicals which effects the vital functions of the helminth viz. neuromuscular transmission, damage to cuticle leading to osmotic imbalance and ion exchange etc¹¹⁻¹². The polyphenolic tannins bind to the proline rich glycoprotein in the cuticle and uncouple the oxidative phosphorylation leading to death of the helminth due to lack of energy generation¹³⁻¹⁴. Terpenes and saponins interact with the collagen of cuticle and damage it leading to electrolyte imbalances¹⁴⁻¹⁵. The phytochemicals penetrate through these damaged surfaces and effect the muscular layers in parenchyma leading to the loss of motility and ultimately cause death of the helminth. The observations of histopathology showed morphological alterations in various layers of the helminth which was similar to those reported in earlier studies⁶⁻⁷.

Thus, from the present study it can be concluded that the chloroform fraction of fruits of *D. erecta* and methanolic extract of *P. longum* fruits possess potent adulticidal activity and can be considered for further research to develop them as promising plant derived chemical to control helminthic infections and to combating the resistance.

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