



ANTHELMINTIC EFFICACY OF SOME INDIGENOUS PLANTS AGAINST THE PARAMPHISTOME *COTYLOPHORON COTYLOPHORUM*

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ABSTRACT

Paramphistomosis is one of the parasitic diseases which affect the small ruminants especially goat and sheep. Heavy infection causes loss of appetite, diarrhoea subsequently leads to the death of the host animals. Chemical anthelmintics viz., albendazole, mebendazole, praziquantel, ivermectin and levamisole are used to treat parasitic infection in sheep and goat. But continuous use of these drugs for decades resulted in the development of resistance. Hence presently plant derived compounds are used to combat parasitic infections. The present study was undertaken to elucidate the anthelmintic efficacy of some medicinal plants viz., *Albizia lebbeck*, *Amomum subulatum*, *Carica papaya*, *Psidium guajava*, and *Tinospora cardifolia* against *Cotylophoron cotylophorum*. Parasites were exposed to aqueous, hexane, chloroform, ethyl acetate and ethanol extracts of plant materials. It was found that ethanol extract of bark of *A. lebbeck* possessed remarkable anthelmintic activity against *C. cotylophorum*.

INTRODUCTION

Livestock plays an important role in Indian economy and it is an important subsector of Indian agriculture. They play a major role in the rural economy, especially for the small and marginal farmers. Among the livestock population sheep (65.06 million) and goat (135.1 million) plays a major role in the Indian economy accounting 39.11% of total livestock population.^[1] Helminth infections remain one of the major constraints to small ruminant production in tropical regions.^[2] Infection with ruminant parasites is regarded as one of the major factors affects the production of livestock. Paramphistomosis has a wide geographical distribution in subtropical and tropical regions, where the infection leads to mortality and low productivity. It is considered to the most pathogenic disease of domestic ruminants in

India.^[3] Paramphistomosis is caused by a number of closely related amphistome species of various genera. Among several species, *Cotylophoron cotylophorum* is more prevalent in Tamil Nadu.^[4-5] It is a digenetic trematode that parasitizes the rumen and reticulum of livestock. Chemical anthelmintics viz. Niclosamide, benzimidazole, oxclozanide, triclabendazole and albendazole are commonly used to treat helminth parasitic infections. The efficiency of chemical anthelmintics against paramphistomosis in particularly *C. cotylophorum* infections was reported by several investigators.^[4,6] The high incidence of resistance to anthelmintic drugs in small ruminants, in addition to the relative toxicity and side effects of many of these drugs, urge the necessity of finding alternative safe and eco-friendly agents against helminth infections.

This applies to plant-based anthelmintics that have been used to destroy and expel the parasites from the gastrointestinal tract. Today several vulnerable compounds derived from plants are used in many countries. Many of the drugs were used today are simple synthetic modifications or copies of the naturally obtained substances.^[7] A vast number of natural, plant-based extracts and chemicals are purported to have beneficial effects. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants offer a new source of antibacterial, antifungal and antiviral activities.^[8] To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way.^[9] Hence, the present research work has been proposed to assess the efficacy of *Albizia lebeck*, *Amomum subulatum*, *Psidium guajava*, *Tinospora cordifolia* and *Carica papaya* against the paramphistome. *Albizia lebeck* belongs to the family Mimosaceae and commonly called as Vaagai in Tamil. Previous phytochemical investigation of *A. lebeck* revealed the presence of glycosides^[10], alkaloids^[11], terpenoids, steroids, saponins^[12], anthroquinones and phenols.^[13] Moreover, saponins of *A. lebeck* have been used to treat Alzheimer's and parkinson's diseases.^[14] Leaves of *A. lebeck* exhibit biological activities; it is used as anticonvulsant, antipyretic, analgesic and anti-inflammatory drug.^[15-19]

Amomum subulatum is the second largest genus and comes under the family Zingiberaceae. Methanol and acetone extracts of *A. subulatum* possess carbohydrates, flavonoids, amino acids, steroids, triterpenoids glycosides, tannins and phenols.^[20-21] *A. subulatum* possess biological activities such as anti-thrombotic, anti-atherosclerotic, hypolipidemic, hypoglycemic, hypotensive, anti-inflammatory, antiulcer, anti-arthritis and anti-diabetic activities^[22-24]. *Psidium guajava* belongs to the family Myrtaceae has been used traditionally against gastrointestinal problems and respiratory ailments. Leaves of *P. guajava* possessed tannins, phenols, flavonoids, terpenoids and glycosides.^[25] Pharmacological

investigations of *P. guajava* indicated that its bark, fruit and leaves exhibit antibacterial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic and CNS depressant activities.^[26] *Tinospora cordifolia* is extensively used herb in ayurvedic medicine. It belongs to the family Menispermaceae. The drugs are well known Indian bitter and prescribed in fevers, diseases, dyspepsia, Jaundice, urinary problems, skin diseases and chronic diarrhoea, dysentery, heart disease, leprosy and helminthiasis.^[27-28] Biologically active phytoconstituents isolated from different parts of *T. cordifolia* possessed alkaloids, tannins, glycosides, flavanoids, saponins, coumarins and steroids.^[28-31] *T. cordifolia* exhibit antidiabetic, anticancer, immunomodulatory, antioxidant and antimicrobial activities.^[28,30,32] *Carica papaya* is well known for its nutritional and medicinal properties used throughout the world.^[33] Papaya belongs to caricaceae family.^[34] Papaya seeds are used to treat psoriasis, liver cirrhosis, bleeding piles. Seeds of *C. papaya* possessed anthelmintic activity against nematodes.^[35] From the foregoing account it is evident that these plants could also be used to treat paramphistomosis in livestock. Hence, the present study was undertaken to elucidate the anthelmintic efficacy of bark of *Albizia lebeck*, seeds of *Amomum subulatum*, leaves of *Psidium guajava*, leaves of *Tinospora cordifolia* and seeds of *Carica papaya* against *Cotylophoron cotylophorum* in vitro.

2. MATERIALS AND METHODS

Adult live *Cotylophoron cotylophorum* (Fig. 1) were collected from abomasum of freshly euthanized sheep, in Perambur slaughter house, Chennai. The flukes were washed thoroughly in physiological saline and maintained in Hedon-Fleig solution (pH 7.0) for further studies.

2.1 Preparation of Hedon-Fleig solution: Hedon-Fleig solution (pH 7.0) is the best medium for *in vitro* maintenance of *C. cotylophorum*.^[4] It is prepared by dissolving 7 g of sodium chloride, 0.1 g of calcium chloride, 1.5 g of sodium bicarbonate, 0.5 g of disodium hydrogen phosphate, 0.3 g of potassium chloride, 0.3 g of magnesium

sulphate and 1 g of glucose in 1000 ml of distilled water.

2.2 Collection of plant materials: The bark of *Albizialebbeck*, seeds of *Amomum subulatum*, leaves of *Psidium guajava*, and *Tinosporacordifolia* and seeds of *Carica papaya* (Figs. 2-6) were purchased from local store, Chennai. The plant materials were identified and authenticated by a botanist in the Department of Botany, Pachaiyappa's college, Chennai - 600 030 and Department of Botany, Bharathiyar University, Coimbatore - 641 046. The vouchered specimens are deposited at Unit of Parasitology, Pachaiyappa's College, Chennai-30.

2.3 Preparation of plant extracts: Plant materials were shade dried, cleaned and coarsely powdered. Successive solvent extraction was done by cold percolation method (Harbone)^[36] by soaking in hexane, chloroform, ethyl acetate and ethanol in increasing polarity for 48 hours. Simultaneously aqueous extract was also prepared. After 48 h, the plant extracts were filtered using Whatmann filter paper no. 1 and concentrated using rotary evaporator (EQUITIRON). Extracts were dried using freeze drier (Lyodel Freeze Dryer) to remove the last traces of solvent.

2.4 Gross visual observation on the motility of the *C. cotylophorum* incubated in various plant extracts: Twenty-five millilitres of Hedon-Fleig solution containing various concentrations (1, 3 and 5 mg/ml) of the aqueous and solvent extracts were individually distributed in airtight sterile containers. Live and active flukes were incubated in each container. The activity of the flukes was checked at various intervals (5, 15, 30 min, 1, 2, 4, 6, 8, 12, and 24 h) of exposure. Simultaneously, control was also maintained in Hedon-Fleig solution devoid of the plant extracts. Based on the motility of the flukes, the observations were categorized as very active (++++), moderately active (+++), slightly active (++) , sluggish (+) and dead (-).

3. RESULTS AND DISCUSSION

Motility is one of the essential features that validate the endurance stature of all living organisms. The anthelmintic efficacy of *Albizialebbeck*, *Amomum subulatum*, *Psidium guajava*, *Tinosporacardifolia*, and *Carica papaya* on *Cotylophoroncotylophorum* was assessed based on the visual observation on the motility of the drug-treated parasites. The control parasites were highly flux and active throughout the experimental period, whereas the motility of the drug-treated parasites was impaired. Ethanol extract of bark of *A. lebbeck* was highly effective at 5mg/ml concentration after 1h of exposure. Whereas ethanol extracts of *A. subulatum* and *P. guajava* were moderately effective at 5mg/ml concentration after 4h of exposure. Chloroform extract of *T. cordifolia* was slightly effective at 5mg/ml concentration and ethanol extract of *C. papaya* showed minimum effectiveness at 5 mg/ml concentration after 8h of exposure (Table 1). The most important parameter used to gauge the anthelmintic property of a plant product is the motility response of the parasite *in vitro* condition.^[37] Assessment of drug efficacy based on the expectation that motility would decline faster when the flukes were exposed to an effective agent.^[38] Drug treated flukes exhibited loss of movement at varying intervals, depending on efficacy the plant extracts. Hindrance of motility could be indebted to the satiation of parasite surface receptors at maximum dose of bio active constituents. Satiation of binding receptors leads to hyper polarization of membrane confining excitation and impulse transmission, thus by beginning flaccid paralysis of the muscles of the parasite.^[39-40] Jain *etal.*^[41] opined that the phytoconstituents may act by inhibition of tubulin polymerization and blocking the glucose uptake. Damage to the mucopolysacchride membrane of parasite will reveal the outer layer confining their movement which finally may cause paralysis and eventually death of the parasites.^[42]



Fig. 1 *Cotylophoron cotylophorum* Fig. 2 *Albizia lebbbeck*



Fig. 3 *Amomum subulatum* Fig. 4 *Psidium guajava*



Fig. 5 *Tinosporacardifolia* Fig. 6 *Carica papaya*

Table 1 Gross visual observation on the motility of *C. cotylophorum* treated with various extracts

<i>Albizialebbbeck</i>											
Extracts	Conc. (%)	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
A/HE	1	++++	++++	++++	++++	++++	++++	+++	+++	++	-
	3	++++	++++	++++	++++	++++	++++	+++	++	+	-
	5	++++	++++	++++	++++	++++	+++	++	+	-	-
A/CE	1	++++	++++	++++	++++	++++	++++	+++	+++	++	-
	3	++++	++++	++++	++++	++++	++++	+++	++	-	-
	5	++++	++++	++++	++++	++++	+++	++	-	-	-
A/EaE	1	++++	++++	++++	++++	++++	+++	++	+	-	-
	3	++++	++++	++++	+++	++	++	+	-	-	-
	5	++++	++++	+++	++	++	+	-	-	-	-
A/EE	1	++++	++++	++++	+++	++	+	-	-	-	-
	3	++++	++++	+++	++	+	-	-	-	-	-

	5	++++	+++	++	+	-	-	-	-	-	-
AIAE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
	3	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++
	5	++++	++++	++++	++++	++++	++++	+++	+++	++	++
<i>Amomum subulatum</i>											
Extracts	Conc. (%)	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
AsHE	1	++++	++++	++++	++++	++++	+++	++	+	+	-
	3	++++	++++	++++	++++	+++	++	++	+	-	-
	5	++++	++++	++++	+++	++	+	-	-	-	-
AsCE	1	++++	++++	++++	++++	+++	+++	++	+	-	-
	3	++++	++++	++++	++++	+++	++	++	-	-	-
	5	++++	++++	++++	+++	+++	++	+	-	-	-
AsEaE	1	++++	++++	++++	++++	++++	+++	++	++	-	-
	3	++++	++++	++++	++++	+++	++	++	-	-	-
	5	++++	++++	++++	+++	++	+	-	-	-	-
AsEE	1	++++	++++	++++	++++	+++	++	+	-	-	-
	3	++++	++++	++++	+++	++	-	-	-	-	-
	5	++++	++++	+++	++	+	-	-	-	-	-
AsAE	1	++++	++++	++++	++++	++++	++++	++++	+++	++	-
	3	++++	++++	++++	++++	++++	++++	+++	++	+	-
	5	++++	++++	++++	++++	++++	+++	++	+	-	-
<i>Psidium guajava</i>											
Extracts	Conc. (%)	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
PgHE	1	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
	3	++++	++++	++++	++++	++++	++++	+++	+++	++	+
	5	++++	++++	++++	++++	+++	+++	++	++	+	-
PgCE	1	++++	++++	++++	++++	+++	+++	++	+	+	-
	3	++++	++++	++++	++++	+++	++	+	+	-	-
	5	++++	++++	++++	+++	++	+	-	-	-	-
PgEaE	1	++++	++++	++++	++++	++++	+++	++	+	-	-
	3	++++	++++	++++	++++	+++	++	++	-	-	-
	5	++++	++++	++++	+++	+++	++	+	-	-	-
PgEE	1	++++	++++	++++	++++	+++	++	-	-	-	-
	3	++++	++++	++++	+++	++	-	-	-	-	-
	5	++++	++++	+++	++	+	-	-	-	-	-
PgAE	1	++++	++++	++++	++++	++++	++++	++++	+++	++	-
	3	++++	++++	++++	++++	++++	++++	+++	++	+	-
	5	++++	++++	++++	++++	++++	+++	++	+	-	-
<i>Tinosporacardifolia</i>											
Extracts	Conc. (%)	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
TcHE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
TcCE	1	++++	++++	++++	++++	++++	++++	++++	++++	+++	+

	3	++++	++++	++++	++++	++++	++++	+++	+++	++	-
	5	++++	++++	++++	++++	++++	+++	+++	++	-	-
TcEaE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++	++++	++++	+++	+++	+++
TcEE	1	++++	++++	++++	++++	++++	++++	++++	++++	+++	+
	3	++++	++++	++++	++++	++++	++++	++++	++++	+++	-
	5	++++	++++	++++	++++	++++	++++	++++	+++	++	-
TcAE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Carica papaya											
Extracts	Conc. (%)	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
CpHE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
CpCE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++	++++	++++	+++	++	+
CpEaE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
	3	++++	++++	++++	++++	++++	++++	+++	+++	+++	-
	5	++++	++++	++++	++++	++++	++++	+++	+++	++	-
CpEE	1	++++	++++	++++	++++	++++	++++	++++	++++	+++	+
	3	++++	++++	++++	++++	++++	++++	++++	+++	++	-
	5	++++	++++	++++	++++	++++	++++	+++	++	+	-
CpAE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
	5	++++	++++	++++	++++	++++	++++	++++	+++	+++	++

++++ Very active; +++ moderately active; ++ slightly active; + Sluggish; - Dead.

AlHE - *Albizialebbeck* hexane extract; *AlCE* - *Albizialebbeck* chloroform extract; *AlEaE* - *Albizialebbeck* ethyl acetate extract; *AlEE* - *Albizialebbeck* ethanol extract; *AlAE* - *Albizialebbeck* aqueous extract.

AsHE - *Amomum subulatum* hexane extract; *AsCE* - *Amomum subulatum* chloroform extract; *AsEaE* - *Amomum subulatum* ethyl acetate extract; *AsEE* - *Amomum subulatum* ethanol extract; *AsAE* - *Amomum subulatum* aqueous extract.

PgHE - *Psidium guajava* hexane extract; *PgCE* - *Psidium guajava* chloroform extract; *PgEaE* - *Psidium guajava* ethyl acetate extract; *PgEE* - *Psidium guajava* ethanol extract; *PgAE* - *Psidium guajava* aqueous extract.

TcHE - *Tinosporacardifolia* hexane extract; *TcCE* - *Tinosporacardifolia* chloroform extract; *TcEaE* - *Tinosporacardifolia* ethyl acetate extract; *TcEE* - *Tinosporacardifolia* ethanol extract; *TcAE* - *Tinosporacardifolia* aqueous extract.

CpHE - *Carica papaya* hexane extract; *CpCE* - *Carica papaya* chloroform extract; *CpEaE* - *Carica papaya* ethyl acetate extract; *CpEE* - *Carica papaya* ethanol extract; *CpAE* - *Carica papaya* aqueous extract.

Our study strongly suggests that ethanol extract of *A. lebbeck* could be used to develop a potential anthelmintic drug as it exhibited remarkable anthelmintic activity against the paramphistome *C. cotylophorum*.

4. CONCLUSION

Motility is one of the essential features that validate the endurance stature of all living organisms. The present study elucidated the anthelmintic efficacy of ethanol extract of bark of *A. lebbeck* against *C. cotylophorum*. This study will help us to formulate potential anthelmintic drug of natural origin from the bark of *A. lebbeck*. However, it is mandatory to isolate the effective phytoconstituents from the plant extracts and to elucidate its mechanism which will be very useful for the discovery of natural anthelmintics.

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