ANTHELMINTIC EFFICACY OF SOME INDIGENOUS PLANTS USED IN THE TRADITIONAL REMEDIES OF NAGA TRIBES

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ABSTRACT

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SUBMITTED

IN

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Abstract

The present work incorporates a study on ascertaining the anthelmintic activity of seven medicinal plants that are commonly used in the folklore medicine system of Naga tribes in Nagaland to cure helminthic parasitic infections. The objectives of the study were:

1. to test the anthelmintic efficacy of some folklore medicinal plants used in the traditional medicine system of Naga tribes.
2. to compare the anthelmintic efficacy of these plants with broad-spectrum anthelmintic drugs.
3. to investigate the acute toxicity of plants in experimental animals.
4. to investigate the effects of these plant extracts on surface fine topography of parasites.

To evaluate the anthelmintic activity of folklore medicinal plants, seven plant species, namely - *Centella asiatica* L. (Apiaceae), *Clerodendrum colebrookianum* Walp. (Verbenaceae), *Curcuma longa* L. (Zingiberaceae), *Gynura angulosa* DC. (Asteraceae), *Houttuynia cordata* Thunb. (Piperaceae), *Lasia spinosa* L. (Araceae) and *Psidium guajava* L. (Myrtaceae) were included based upon the information collected about their use as deworming agents from the traditional practitioner and local people in the Nagaland state. The various usable plant parts were extracted in methanol and the crude extracts were

tested at different concentrations *in vitro* and *in vivo* against several helminth parasites. For *in vitro* study, *Raillietina echinobothrida*, *Hymenolepis diminuta*, *Gasthrothylax crumenifer*, *Ascaridia galli* and *Trichinella spiralis* served as the test parasites. While *Hymenolepis diminuta* - rat and *Trichinella spiralis* - mice animal models were involved to evaluate the *in vivo* anthelmintic efficacy of plant extracts.

In the *in vitro* studies the test parasites were exposed to 5, 10, 20 and 40 mg/ml concentrations of plant extracts and mortality of worms served as the anthelmintic criterion. In each case the parasites were also exposed to corresponding concentrations of a standard anthelmintic drug to compare the efficacy of plant extract. Out of the seven plant extract tested, *P. guajava*, *H. cordata*, *L. spinosa* (stalk and leaf), *G. angulosa* and *C. colebrookianum* revealed significant anthelmintic efficacy. However, a moderate level of anthelmintic efficacy was observed for *L. spinosa* (stem), *C. asiatica* and *C. longa*. With respect to various helminthic groups, the study revealed that the leaf extract of *P. guajava*, *H. cordata* and stalk of *L. spinosa* possess profound efficacy against the cestode parasite, *R. echinobothrida*. The leaf extract of *P. guajava*, *L. spinosa* and *G. angulosa* manifested appreciable anticestodal efficacy against *H. diminuta*. Of different plant extracts tested against *G. crumenifer*, leaf extracts of *L. spinosa*, *C. colebrookianum* and *H. cordata* showed good flukicidal efficacy. In case of roundworm *A. galli*, only the leaf extract of *L. spinosa* was found to possess promising anthelmintic activity. Lastly, against the adult *T. spiralis* worms leaf extracts of *G. angulosa*, *L.*

spinosa, *C. colebrookianum*, *H. cordata* and *P. guajava* revealed significant activity. The individual plant extracts showing significant efficacy were further tested in combination with other extracts to investigate whether they could have any synergistic effects on mortality of parasites. No substantial increase in the anthelmintic efficacy of extracts was observed in such investigations.

The present study revealed that *P. guajava* leaf extract possess significant level of efficacy against *R. echinobothrida*, *H. diminuta* and *T. spiralis*. In case of its efficacy against *R. echinobothrida*, both its 20 and 40 mg/ml concentrations revealed the mortality of parasites in 1.00 h. Against *H. diminuta*, the extract showed mortality of worms in 2.34 h at 40 mg/ml concentration. Mortality of *T. spiralis* in its 40 mg/ml concentration was observed to be in as early as in 0.92 h. The *H. cordata* extract showed significant *in vitro* anthelmintic efficacy against *R. echinobothrida*, *G. crumenifer* and *T. spiralis*. *R. echinobothrida* treated with the 40 mg/ml concentration of *H. cordata* extract showed mortality of worms within 2.00 h. The efficacy of extract was recorded to be slightly lower against *G. crumenifer*, wherein it caused mortality of worms in 3.00 h. Against *T. spiralis*, the 40 mg/ml concentration of extract showed mortality of worms in as early as in 0.89 h.

In the present study the *L. spinosa* leaf extract showed profound anthelmintic efficacy against *H. diminuta*, *G. crumenifer*, *A. galli* and *T. spiralis*. The *H. diminuta* worms showed mortality within 2.50 h at its 40 mg/ml

concentration. The amphistome, *G. crumenifer* exposed to 40 mg/ml concentration of extract revealed the mortality of worms in 2.09 h which was almost comparable to Praziquantel (PZQ), the reference drug. The stalk extract of *L. spinosa* was also evaluated for anthelmintic efficacy in the present study and showed good efficacy only against *R. echinobothrida*. Whereas the stem extract of *L. spinosa* was not found be as effective as leaf and stalk extract.

The present investigation revealed that *G. angulosa* possesses prominent anthelmintic activity only against *H. diminuta* and *T. spiralis*. The plant extract at 40 mg/ml concentration showed mortality of *H. diminuta* worms in 2.92 h compared to PZQ which showed mortality of parasites in 0.60 h at the same concentration. Similarly, for *T. spiralis* also the plant extract showed almost comparable efficacy with that of reference drug, Mebendazole (MBZ).

C. colebrookianum extract showed significant level of efficacy against *G. crumenifer* and *T. spiralis*. At 40 mg/ml concentration the efficacy of *C. colebrookianum* extract and reference drug was almost similar. The mortality time of parasites at this concentration was recorded to be 2.50 h and 2.10 h, respectively. The *C. colebrookianum* extract, however did not show notable efficacy against *R. echinobothrida*, *H. diminuta* and *A. galli*.

In the present study *C. asiatica* leaf extract showed moderate level of efficacy against *R. echinobothrida*, *G. crumenifer* and *A. galli* and rather

insignificant efficacy against *H. diminuta* and *T. spiralis*. Unlike other tested plant extracts, *C. longa* extract did not show anthelmintic efficacy worth pursuing further.

The parasites' tegument/cuticle has been implicated among one of several target sites by which natural anthelmintic products or synthetic drugs act. In the present study effects of selected plant extracts were studied on parasite body surface with the help of scanning electron microscopy (SEM) so as to provide some clues regarding their plausible mode of action. The study revealed that barring *H. cordata* other extracts, namely *L. spinosa*, *G. angulosa*, *P. guajava* and *C. colebrookianum* exhibited such morphological changes and damage to the parasite's body surface. The tegument of *H. diminuta* and *G. crumenifer* showed destruction in the form of erosion on all over the general topography of the body. In case of *Hymenolepis* the scolex also showed apparent damage. Similarly, for *A. galli* the SEM of extract treated worms revealed wrinkles and cracks on lips and body cuticle.

To further substantiate the efficacy of plant extracts, the *in vitro* studies were supplemented with *in vivo* studies wherein the plant extracts were also tested for their anthelmintic efficacy in *H. diminuta* - rat and *T. spiralis* - mouse experimental models. In *Hymenolepis* - rat model the extracts were administered at three different stages of parasites; the larval, immature and adults. Efficacy was adjudged by counting the eggs per gram of faeces (EPG),

worm reduction and host clearance rate. In all experiments Praziquantel, a broad spectrum anthelmintic drug was tested at 5 and 10 mg/kg, p.o. doses as a reference drug. The results indicated that there were significant changes in all these parameters in the treated groups of animals as compared to untreated control. With respect to efficacy of extracts against larval stages, more prominent effects were recorded for *P. guajava*, *L. spinosa* (leaf), *H. cordata*, *C. longa* and *G. angulosa* extracts. The treatment of rats with 1600 mg/kg doses of the above plant extracts on days 2-6 p.i. resulted in elimination of 66.50, 66.66, 62.50, 62.50 and 58.25% of adult worms, respectively. Administration of extract on days 21-25 p.i. to investigate the efficacy against the adult stage showed percentage worm reduction between 87.50 to 91.50% for the tested plant extracts. The extract treated group of animals also showed substantial decrease in EPG values. The acute toxicity study in the experimental rats showed that barring mortality of few animals as noticed in the *C. longa* and *L. spinosa* stem extract-treated groups no other plant extracts cause any mortality or any changes in behaviour of animals with regard to food and water intake.

The efficacy of extracts in *T. spiralis* - mouse model was investigated against the adult, migrating and encysted stages; percentage reduction in adult worms at necropsy or larvae encysted in tissue constituted the study parameters. In general, barring *C. asiatica*, all other plant extracts tested in this study showed moderate to high efficacy against the adult *Trichinella* worms and more or less similar was the case against their efficacy against the migrating

larvae. In contrast, barring *G. angulosa* the rest of the plant extracts showed either medium or very low level of efficacy against the encysted larvae. The leaf extract of *G. angulosa* showed up to 86.22% of adult worm reduction and 72.36% encysted larvae reduction. The efficacy when compared to 25 mg/kg dose of Mebendazole, the reference drug was noted to be 94.70 and 90.52%, respectively against these stages. The acute toxicity studies of extracts in mice showed maximum mortality of animals for *C. longa* extract, followed by *C. colebrookianum* and *H. cordata* extracts. However, the rest of the plant extracts neither caused any mortality nor any visible signs of toxicity in experimental animals.

This study thus validates the presence of appreciable anthelmintic property in many of the folk medicinal plants used by Naga tribes which may have therapeutic benefits in humans encountering helminthic infections. Further investigation on isolated chemical constituents of these plants should be pursued against different helminth parasite species.

Two photographic plates of seven plants, seven photographic plates of twenty five scanning electron micrograph pictures, twenty graphic figures, two life cycle diagrammatic figures and thirty nine tables support the study observations carried out in the present work. Altogether (176) citations are given in the references.

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Dedicated
To
Mom & Dad

NORTH-EASTERN HILL UNIVERSITY

SHILLONG – 793 022, INDIA

14th July, 2008

DECLARATION

I, Temjenmongla, hereby declare that the subject matter of this thesis is the record of the work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and the thesis has not been submitted by me for any research degree in any other University / Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

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Contents

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(Temjenmongla)

Preface

Infection with helminth parasites is a wide-spread problem in the developing world. The prevalence and severity of infection is especially high in developing countries where health and sanitation facilities are unable to cope with the needs of increasing population. It is in this context that the people, particularly of tropical countries follow the practice of using several indigenous methods of getting rid of worm infection. In many parts of India, especially those inhabited by tribal populations, there persists a rich folklore regarding the vermicidal and vermifugal properties of many foods of plant origin. And it is for these reasons that the people often consume several plants or plant derived preparations as common therapeutic interventions for worm infections.

In recent times, plant/herbal based medicines have become indispensable and are forming an integral part of the primary health care system in many nations, including India. Elsewhere in the world, studies reveal that owing to complexities and cost of controlling the helminth diseases, treatment from plant sources has been an alternative approach in several societies. Although until recently the majority of the evidence on the anthelmintic activity of folklore medicinal plants was anecdotal and lacked scientific validity, there is currently an increasing number of experimental studies that aim to verify quantify such plant activity. There are indeed a large number of plants whose anthelmintic activity has been demonstrated under controlled experimentation. However, contrary to traditional expectation, there are also a great number of

plants with purported antiparasitic properties, which have not been reproduced under experimental conditions.

Nagaland, a northeastern state of India, is inhabited by 16 major tribes and the people belonging to various tribes have a good faith in their indigenous system of medicine and follow a practice of using a number of plants/plant-supplemented preparations as common de-worming agents. However, no systematic study so far has been undertaken to investigate and scientifically validate the acclaimed anthelmintic efficacies of such folklore medicinal plants.

On the basis of above background information the proposed study was executed with the following objectives:

1. to test the anthelmintic efficacy of some folklore medicinal plants used in the traditional medicine system of Naga tribes.
2. to compare the anthelmintic efficacy of these plants with broad-spectrum anthelmintic drugs and monitor the acute toxicity of plant extracts.
3. to investigate the effects of plant extracts on surface fine topography of parasites.

The results of the present study provide a scientific base justifying the folkloric use of these medicinal plants which are used in the traditional medicine practice of indigenous Naga tribes in Nagaland (India). It also provides evidence that barring few most of these plants could be further exploited for the development of new anthelmintic agents.

Abbreviations/Symbols used

ABZ	-	Albendazole
b.p.	-	boiling point
°C	-	degree celsius
DMSO	-	Dimethylsulphoxide
d.p.i.	-	days post infection
EPG	-	Eggs per gram of faeces
Ft	-	feet
g	-	gram
h	-	hour
kg	-	kilogram
LD_{50}	-	Median Lethal Dose
MBZ	-	Mebendazole
mg	-	milligram
min	-	minute
ml	-	millilitre
mm	-	millimetre
PBS	-	Phosphate Buffered Saline
p.i.	-	post-infection / post inoculation
p.o.	-	Orally or by mouth
PZQ	-	Praziquantel
sq km	-	square kilometre
wt.	-	weight
w/w	-	weight by weight
w/v	-	weight by volume

CHAPTER 1

In vitro anthelmintic efficacy of folklore medicinal plants

Introduction

Intestinal helminthic infections such as, ascariasis, trichuriasis, hookworm and tapeworm infections continue to be a cause of major concern to human and animal health in several parts of the globe including India, causing malabsorbtion, diarrhoea, anaemia and other states of poor health. The cost of harboring these parasites in terms of human misery and economic loss is incalculable (Savioli *et al.*, 1992). While majority of intestinal parasitic infections transmits through ingestion of contaminated food or water as a result of poor sanitation and hygiene others such as, tapeworm infections results due to consumption of infected pork, beef or fish. In India, helminthic parasitic infections are considered to be among the common public health problems, particularly in hilly regions of India. In context of north-eastern region of India, the culinary habits of the people provide many opportunities to eat undercooked meat leading thereby to a considerably high prevalence of tapeworm infections.

Theoretically, most intestinal parasitic infections can be effectively prevented by proper sanitation, but in practice this is a process which may take decades and require comprehensive social, economic and educational development in order to be successful. In a tactical approach to control the intestinal helminthes, the World Health Organization (WHO) in its Tropical

Diseases Control Programme has provided a special emphasis on the use of traditional medicines to combat the menace of parasitic diseases globally (Savioli *et al.*, 1992). Case studies from several countries reveal that owing to complexities and cost of controlling the helminth diseases, treatment from plant sources has been an alternative approach in several societies (Akerele, 1990; Satyavati, 1990). The WHO has recently proclaimed that 80% of the population of developing countries relies on traditional medicine, mostly plant drugs, for their primary health care needs.

In many ancient civilizations plant infusions and decoctions have been used as popular anthelmintics (Athanasiadou *et al.*, 2007). The origin of many effective drugs is found in the traditional treatment practices and in view of this several workers have undertaken studies pertaining to testing of a large number of traditionally used medicinal plants for their proclaimed anthelmintic efficacy (Hukkeri *et al.*, 1993; Asuzu and Njoku, 1996; Hammond *et al.*, 1997; Njoku and Asuzu, 1998; Pal and Tandon, 1998a, b; Roy and Tandon, 1999; Sukul *et al.*, 1999). The tuber extract of *Flemingia vestita*, a traditional anthelmintic plant of Khasi tribe in Meghalaya was reported to possess significant efficacy against *Ascaris suum* (Yadav *et al.*, 1992). Crude extracts of *Cannabis sativa* and *Alpinia nigra* were reported to possess significant flukicidal activity against *Fasciolopsis buski* (Roy and Tandon, 1997; 1999). An *in vitro* study on fruit extract of Indian mulberry revealed highest anthelmintic activity against *Haemonchus contortus*, a nematode parasite of ruminants (Hildasari, 1998).

Sangwan and Sangwan (1998) reported the presence of anthelmintic efficacy in *Melia azedarach*. Purified condensed tannins from Danish legumes were reported to kill nematode larvae *in vitro* (Kahiya *et al.*, 1999). The leaf extract of *Spilanthes oleracea* was reported to possess significant activity against *Orthocoelium dinniki* (Roy, 2000). The essential oil of *Ocimum sanctum* and eugenol, tested *in vitro*, showed potent anthelmintic activity in the *Caenorhabditis elegans* model (Asha *et al.*, 2001). Different solvent fractions of *Berlina grandiflora* and its major triterpenoid, betulinic acid showed anthelmintic activity against *C. elegans* (Enwerem *et al.*, 2001a). Mølgaard (2001) reported a number of Zimbabwean plants, *Acacia karroo, Cassia singueana, Ozoroa insignis, Vernonia amygdalina, Ximenia caffra* etc. to bear significant anthelmintic properties against *Hymenolepis diminuta*, a tapeworm of zoonotic importance. Young pine apple fruit juice and the whole extract of coleus leaves and croton twigs showed *in vitro* anthelmintic activity against *H. nana* and *Aspiculuris tetraptera* (Satrija *et al.*, 2001). The crude extracts of *Artemisia santonica, Albizzia lebbek* and *Inula helenium* showed promising anthelmintic efficacy against *A. lumbricoides* (El-garhy and Mahmoud, 2002). Singh and Nagaichi (2002) evaluated the antiparasitic effects of ethyl alchohol extract of *Ocimum sanctum* against *Ascaridia galli in vitro*. Dash *et al.* (2002) tested *in vitro* anthelmintic activity of *Evolvulus alsinoides* extract against earthworm, *Pheretima posthuma* and reported it to be better than piperazine citrate.

The essential oil of *Ocimum gratissimum*, a tropical plant well known for its ethnoveterinary use, showed strong anthelmintic activity *in vitro* against *H. contortus* (Pessoa *et al.*, 2002). Plants such as, *Adhatoda vasica*, *Nicotiana tabacum* and *Spigelia anthelmia* were reported to possess considerable anthelmintic activity against *H. contortus* (Lateef *et al.*, 2003; Raje *et al.*, 2003; Assis *et al.*, 2003). The crude aqueous and methanol extracts of *Artemisia brevifolia* exhibited profound activity against *H. contortus in vitro* (Iqbal *et al.*, 2004). The woody plants, *Rubus fructicosus*, *Quercus robur* and *Corylus* showed remarkable anthelmintic activity when tested on 3rd-stage larvae (L_3) and adult worms of *Teladorsagia circumcincta*, *H. contortus* and *Trichostrongylus colubriformis* (Paolini *et al.*, 2004). Hounzangbe-Adote *et al.* (2005b) reported the anthelmintic activity of *Zanthoxylum zanthoxyloides*, *Morinda lucida* and *Newbouldia* leaf extracts and *Carica papaya* seed extracts collected in Western Africa against different stages of *H. contortus*. In another study, *Z. zanthoxyloides*, *M. lucida*, *N. laevis* and *C. papaya* extracts induced a dose-dependent inhibition of egg hatching of *T. colubriformis*. These plant extracts also showed their effects against the infective larvae of *T. colubriformis*. In contrast, for adult worms, the effects were statistically significant only for *N. laevis* and C. *papaya* (Hounzangbe-Adote *et al.*, 2005a).

Fajimi and Taiwo (2005) reported that *Nauclea latifola* possesses high anthelmintic efficacy against strongyle nematodes of small ruminants. Based on the results of ethnomedical survey in Northern Cote d'Ivoire, Koné *et al.* (2005)

made a pilot study on 79 plant species for their anthelmintic efficacy using *H. contortus* as the test parasite and found *Sclerocarya birrea*, *Lannea kerstingii*, *Aframomum alboviolaceum*, *Pericopsis laxiflora*, *Pseudocedrela kotschyi*, *Securidaca longepedunculata*, *Alchornea cordifolia*, *Anthostema senegalense*, *Ficus vallis-choudae*, *Ampelocissus grantiil*, *Vitellaria paradoxa* and *Hibiscus asper* to possess either significant larvicidal or ovicidal activity. *Cardiospermum halicacabum* extract when tested *in vitro* for its efficacy against L_3 of *Strongyloides stercoralis* showed reduction in the viability of larvae (Boonmars *et al.*, 2005). In a study by H¨ordegen *et al.* (2006), Bromelain, the enzyme complex of the stem of *Ananas comosus* (Bromeliaceae), the ethanolic extracts of seeds of *Azadirachta indica* (Meliaceae), *Caesalpinia crista* (Caesalpiniaceae) and *Vernonia anthelmintica* (Asteraceae), and the ethanolic extracts of the whole plant of *Fumaria parviflora* (Papaveraceae) and of the fruit of *Embelia ribes* (Myrsinaceae) showed anthelmintic efficacy (up to 93%), relative to pyrantel tartrate against infective larvae of *H. contortus*. Yadav and Tangpu (2006b) studied the anthelmintic activity of a few selected plants *viz.*, *Strobilanthes discolor* (leaf), *Adhatoda vasica* (leaf), *Butea minor* (seeds), *Solanum myriacanthum* (fruits), *Trifolium repens* (shoots) and *Zanthoxylum rhetsa* (leaf) and reported them to be effective against *Hymenolepis diminuta*. The methanol extracts of *Mentha piperita* and *Lantana camara* (leaves, stems and roots) exhibited considerable anthelmintic activity against *P. posthuma* (Girme *et al.*, 2006). The anthelmintic activity of the drupe extracts of *Melia azedarach* growing in Argentina was tested against tapeworms, hookworms,

nodular worms and earthworms, and was reported to be better than the standards piperazine phosphate and hexylresorcinol against tapeworms and hookworms, respectively (Szewczuk *et al.*, 2006). *In vitro* anthelmintic activities of crude aqueous and hydro-alcoholic extracts of the seeds of *Croton macrostachyus* and *Ekebergia capensis* showed significant activity on the egg and adult of *H. contortus* (Eguale *et al.*, 2006). *Trachyspermum ammi* seeds used locally in Pakistan as anthelmintic for worm control in sheep were evaluated for their ovicidal activity against *H. contortus* eggs and were reported to possess some anthelmintic properties (Jabbar *et al.*, 2006). The anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils and their major constituents, anethole and thymol were determined by *in vitro* assays with the eggs and larvae of *H. contortus* (Camurça-Vasconcelos *et al.*, 2007). The essential oils and their constituents prevented more than 98% of the *H. contortus* eggs from hatching at a concentration of 1.25 mg/ml and inhibited more than 90% of *H. contortus* larval development at a concentration of 10 mg/ml. Eguale *et al.* (2007a) reported that hydro-alcoholic extract of *Hedera helix* possesses better *in vitro* anthelmintic activity against adult *H. contortus* compared to aqueous extract. Yet in another study by Eguale *et al.* (2007b), it was reported that the hydro-alcoholic extract of the seeds of *Coriandrum sativum* showed better *in vitro* activity against adult *H. contortus* than the aqueous one. The stem bark extract of *Acacia oxyphylla*, a traditional anthelmintic plant of Mizo tribes in north-east India, have been demonstrated to exhibit profound anthelmintic effects on fowl cestode, *Raillietina echinobothrida*

(Lalchhandama *et al.*, 2007). Nirmal *et al.* (2007) reported that the ethyl acetate and petroleum ether extracts of *Pongamia glabra* seeds exhibit significant anthelmintic activity when tested against Indian adult earthworm *P. posthuma*. Cysteine proteinases from papaya, pineapple and fig were reported to be substantially effective against three rodent gastrointestinal nematodes, *Heligmosomoides polygyrus*, *Trichuris muris* and *Protospirura muricola* (Stepek *et al.*, 2007). López-Aroche (2008) evaluated the anthelmintic activity of twenty plants from Mexico and found *Bursera copallifera*, *B. grandifolia*, *Lippia graveolens*, *Passiflora mexicana*, *Prosopis laevigata*, *Randia echinocarpa* and *Urtica dioica* to have anthelmintic properties against *H. contortus* unsheathed third stage infective larvae. The ethanolic extract from the root bark of *Millettia pachycarpa*, traditionally used as a remedy for gastrointestinal infections among the Mizo tribes of north-east India, was tested *in vitro* against *R. echinobothrida* and reported to be possessing significant anthelmintic property (Roy *et al.*, 2008). Khadatkar *et al.* (2008) reported noteworthy anthelmintic activity in *Clitoria tematea* extract against *P. posthuma.*

The helminth parasites' tegument/cuticle has been ascertained as one of the principal target site for mode of action of synthetic and natural anthelmintic products (Mehlhorn *et al.*, 1983; Alvarez *et al.*, 2006). In order to suggest the plausible mode of action of putative anthelmintic plants or drugs the *in vitro* testing studies on parasites have also been extended to investigate their effects on parasite's body surface with the help of scanning electron microscopy (SEM).

Tegumental alterations and severe vacuolization on exposure to flukicidal drugs have been observed in several species of trematodes (Gupta and Sharma 1973; Mehlhorn *et al.*, 1983; Schmahl and Mehlhorn 1985; Schmahl and Tarascdhewski 1987; Schmahl 1993). In another study, the appearance of narrow cracks in the cuticle and wrinkles on the lips of *A. suum*, treated *in vitro* with piperazine, has been reported by Grywacz (1980). Tegumental bubbles of different sizes were found to occur on the surface of *Opisthorchis viverrini* post-exposure to praziquantel (Sirisinha *et al.*, 1984). The same fluke when treated with another anthelmintic, amoscanate exhibited severe swelling and pit formation, leading to total disruption of the surface tegument (Sobhon *et al.*, 1986). Structural disruption of the tegumental integrity is one of the early morphological effects caused by praziquantel (Van den Bossche, 1985; Schepers *et al.*, 1988). Disruption of the cuticular interface and/or intestinal epithelium and degenerative changes even in the subcuticular region has been reported in several nematode species exposed to anthelmintics *in vitro* (Kaur and Sood, 1983; Xiao *et al.*, 1989; An, 1990; Strote *et al.*, 1990). Destruction of attachment organs (suckers and hooks) was noticed in the monogenean parasite, *Dactylogrus extensus* when treated with praziquantel (Schmahl and Mehlhorn, 1985). The tuber extract of *F. vestita* which was reported to bring about paralysis of *A. suum* under *in vitro* conditions showed wrinkles and cracks on lips and body cuticle following treatment with plant extract (Yadav *et al.*, 1992). Vacuolization and pit formation was also recorded in *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* when treated *in vitro* with root tuber peel

extract of *F. vestita* (Roy and Tandon, 1996). In another study by Tandon *et al.* (1997), exposure of *R. echinobothrida* to genistein, an active principle of *F. vestita*, caused spontaneous loss of movement of cestode parasite followed by structural alteration in its tegumental architecture. Roy and Tandon (1999) reported *in vitro* anthelmintic activity as well as marked surface tegumental alternations in *Fasciolopsis buski* when treated with extract of *Alpinia nigra*. The extract-treated flukes manifested deformed body contours, particularly at the anterior sucker, with a shrunken and wrinkled surface tegument. The ventral papillae which have a distinct size and shape also showed deformity accompanied by deep scar formation at the base of each papilla. Roy (2000) in another study on the morphology of *Orthocoelium dinniki* after treatment with *Spilanthes oleracea* extract revealed contraction and deformation of general tegumental surface and suckers with total disorganization and elongation of sensory papillae in the oral aperture region of parasite. *In vitro* treatment of *R. echinobothrida* to the extract of *Stephania glabra*, a folklore anthelmintic plant in Meghalaya, showed pronounced disruption of its body tegument (Tandon *et al.*, 2004). Tegumental damage and severe vacuolization following exposure to flukicidal drugs have been observed in several species of trematodes (Jiang and Xia, 1992; Schmahl, 1993; Stitt and Fairweather, 1993; Xu-Lin *et al.*, 1994; Anderson and Fairweather, 1995). Similar changes in the body surface of parasites have also been reported for cestode parasites (Delabre-Defayolle *et al.*, 1989; Perez *et al.*, 1994; Pal and Tandon, 1998b).

Nagaland, a northeastern state of India, is inhabited by 16 major tribes of Mongolian stock collectively known as the **'Nagas'**. The state lies between latitudes of 25° 60"N and 27° 40"N and longitudes of 93° 20"E and 95° 15"E, covering an area of 16,579 sq. km. and is bounded by Assam in the North and West, by Myanmar and Arunachal Pradesh in the East and by Manipur in the South. Naga tribes have an immense heritage of oral traditions and many of the oral traditions involve beliefs and practices associated with plants and animals. After a long period of trial they have learned and developed an indigenous knowledge system to utilize local plants harvested from the wilds to cure different ailments. Their beliefs and folk practices are based on experience with various diseases and their cures. The knowledge of using some medicinal plants is kept as a clandestine for the interest of the traditional medicine man and handed down from one generation to the next, while some plants are known by common people. In the past ethnobotanical studies on the use of folklore plants by Naga tribes for various ailments attracted the attention of few workers (Changkija, 1999; Jamir, 1991, 1997). However, no systematic study so far has been undertaken to investigate and scientifically validate the acclaimed efficacies of such plants.

Considering the therapeutic importance of these plants in day to day lives of Naga tribes, it was felt necessary to evaluate experimentally the anthelmintic activity of few such commonly used plants. The present study reports the *in vitro* anthelmintic efficacy of seven plants against representative groups of helminth

parasites. It is hoped that the present study would scientifically validate the belief of local people about the use of these plants in their traditional medicine system.

Materials and Methods

Plant Materials

The ethnomedicinal informations about the use of various folklore medicinal plants for curing intestinal helminthic infections was collected from traditional practitioners (*arasentsur*) and local people with a request to make use of knowledge for exploration to scientific world. On the basis of gathered information, the most commonly used plants were included in the study (Table 1.1) and various usable plant parts (leaf, rhizome, stem and stalk) were collected from various regions of Nagaland State. Subsequent upon collection, the herbarium sheets and photographs of each of the plants were prepared. The plants were taxonomically identified and authenticated by Dr. P. B. Gurung, Curator, Department of Botany, North-Eastern Hill University, Shillong and by Prof. Jamir, Department of Botany, Nagaland University, Nagaland. Plants were assigned respective voucher specimens (Table 1.1) and herbarium sheets of the plants were deposited in the Department of Zoology, North-Eastern Hill University, Shillong. The details about the plants included in this study are provided in Table 1.1.

Table 1.1: List of the ethnomedicinal plants included in the present study

Name	Family	Local name	Plant type	Plant part(s) tested	Voucher No.
1. *Centella asiatica* L.	Apiaceae	Longsokorok	Herb	Leaf	AKY-004
2. *Clerodendrum colebrookianum* Walp.	Verbenaceae	Orema	Small tree	Leaf	AKY-005
3. *Curcuma longa* L.	Zingiberaceae	Nakong	Herb	Rhizome	AKY-007
4. *Gynura angulosa* DC.	Asteraceae	Ensu	Herb	Leaf	AKY-006
5. *Houttuynia cordata* Thunb.	Piperaceae	Mokma	Herb	Leaf	AKY-003
6. *Lasia spinosa* L.	Araceae	Jurang	Herb	Leaf, stem & stalk	AKY-002
7. *Psidium guajava* L.	Myrtaceae	Modiram	Tree	Leaf	AKY-001

Folk-lore Medicinal Plants and their Known Medicinal Use

1.1. *Centella asiatica* Linn. Plate 1.1 (A)

Family: Apiaceae

Local Name: *Longsokorok*

Commonly known as Indian Pennywort, *C. asiatica* is a perennial rhizomatous, small trailing aromatic prostrate herb. It is very common in moist forest floors, shady household gardens at the edge of ponds or dried up riverbeds and is found growing in damp places in India and other Asian countries. The whole plant is consumed for the treatment of dysentery, diarrhoea and gastric problems, as a tonic for liver and spleen and as blood purifier, diuretic. Plant extract is also applied as hair tonic. Asiaticoside, asiatic acid, madecassic acid, ursane and oleanane-type triterpene oligoglycosides, centellasaponins B, C, and D, madecassoside and sceffoleoside A are some of the principal ingredients reported in this plant (Brinkhaus *et al.*, 2000; Matsuda *et al.*, 2001; Hong *et al.*, 2005). Studies on this plant also indicate that it possess scientifically proven neuronal dendritic growth stimulating property, wound-healing potential, antioxidant, anticancer and anti-gastric properties, antibacterial, antidepressant, anxiolytic and immunomodulating activity, (Veerendra *et al.*, 2002; Chen *et al.*, 2003; Jayashree *et al.*, 2003; Cheng *et al.*, 2004; Punturee *et al.*, 2005; Zaidan *et al.*, 2005; Gnanapragasam *et al.*, 2006 Mohandas *et al.*, 2006; Shetty *et al.*, 2006; Wijeweera *et al.*, 2006).

1.2. *Clerodendrum colebrookianum* Walp. Plate 1.1 (B)

Family: Verbenaceae

Local Name: *Orema*

It is about 15 ft height tree with a globose crown and disagreeable smell. It's leaves are cordate, flowers numerous, rose purple or white, fruit drupe, bluish green to deep green when fully ripe. *C. colebrookianum* is widely used for curing various diseases. Soup is drunk to cure irregular blood pressure, heart troubles and malarial fevers. Breast feeding mothers consume its soup if the child happens to be suffering from stomach disorders. Five steroids colebrin A-E (1-5) and colebroside A (1), a new diglucoside of fatty acid ester of glycerin, has been reported to occur in the aerial parts of *C. colebrookianum* along with nine known compounds (2-10) (Yang *et al.*, 2000a; Yang *et al.*, 2000b).

1.3. *Curcuma longa* Linn. Plate 1.1 (C)

Family: Zingiberaceae

Local Name: *Nakong*

C. longa is a stemless, rhizomatous perennial herb. The plant is cultivated extensively in Asia, India, China and other countries with a tropical climate. The rhizomes of the plant used in medicinal and food preparations. Rhizome is crushed and decoction is drunk as stimulant tonic, carminative and blood purifier. It is also applied in case of conjunctivitis. Rhizome paste is applied to bone fracture and sprains. Previous studies suggest that the principal ingredients of *C. longa* are the curcumin and volatile oils, of which the former is the main active chemical constituent of the plant (Xia *et al.*, 1999; Cui *et al.*,

2006). Alpha-curcumene is the major chemical constituent of the volatile oil from *C. longa* (Hu *et al.*, 1998). It has been reported that curcumin could exhibit anticarcinogenic, antioxidative hypocholesterolemic and antidepressant activities (Cui *et* al., 2006; Peschel *et al.*, 2006; Xia *et al.*, 2006).

1.4. *Gynura angulosa* DC. Plate 1.1 (D)

Family: Asteraceae
Local Name: *Ensu*

G. angulosa is a succulent and glabrous herb which is mostly found distributed in South-East Asia. The native Naga tribes in their folk medicine system commonly use the hot water decoction of young tender leaves of *G. angulosa* as a deworming remedy. So far no report on its biological activity or chemical constituents is available in any literature.

1.5. *Houttuynia cordata* Thunb. Plate 1.2 (A)

Family: Piperaceae
Local Name: *Mokma*

H. cordata is a perennial creeping herb with broadly ovate-acuminate leaves. Plant bears minute flowers and fruits are sub-globose. In local traditional medicine, the juice of the plant is drunk in the treatment of ulcer and blood purification. Crushed plants are also spread in chicken cages as insect repellents. *H. cordata* is chemically composed of essential oils and alkaloids (Tutupalli *et al.*, 1975; Kim *et al.*, 2001; Lu *et al.*, 2006) and it was shown to

possess antileukemic, antiviral, and antibacterial activity (Hayashi *et al.*, 1995; Chang *et al.*, 2001; Chiang *et al.*, 2003; Lu *et al.*, 2006).

1.6. *Lasia spinosa* Linn. Plate 1.2 (B)

Family: Araceae

Local Name: *Jurang*

L. spinosa is a stout spinous marshy perennial herb. It is mostly found distributed in South-east Asia. The young tender leaves of plant are consumed as traditional food in several communities of South-east Asia. However, in the folk medicine of Naga tribes the porridge (pudding) of young delicate leaves of *L. spinosa* is frequently used to treat the intestinal-worm infections. Paste made from the plant is also applied to cure wounds. There is only one study on chemical constituents of *L. spinosa* which mentions the presence of three benzaldehyde derivatives, 2-(4'- methoxyphenyl) ethanol and adenine in the plant (Hồng Vân *et al.*, 2005). There is no report available in the literature regarding its biological activity.

1.7. *Psidium guajava* Linn. Plate 1.2 (C)

Family: Myrtaceae

Local Name: *Modiram*

P. guajava is a common horticulture plant of tropical regions. It is reported to possess several medicinal uses: CNS depressant (Meckes, 1996), antidiarrhoeal (Lutterodt, 1989), anticough (Jairaj *et al.*, 1999), antiamebic, antispasmodic (Lozoya *et al.*, 2002; Tona *et al.*, 2000), antifilarial,

(Temjenmongla and Yadav, 2003) and antimicrobial activity (Qadan *et al.*, 2005). The previously known chemical constituents from the plant include, ascorbic acid (Nogueira *et al.*, 1978), fatty acids (Opute, 1978), tannins, phenols, triterpenes, essential oils, saponins (Cuellar *et al.*, 1984) carotenoids (Mercadante *et al.*, 1999) and lectins (Coutino-Rodriguez *et al.*, 2001). 3beta-p-E-coumaroyloxy-2alpha-methoxyurs-12-en-28-oic acid, which is a new pentacyclic triterpenoid guajanoic acid have been isolated from the leaves of *P. guajava* (Begum *et al.*, 2004).

PLATE 1.1

A. *Centella asiatica*; whole plant

B. C*lerodendrum colebrookianum*; whole plant

C. *Curcuma longa*; rhizome part

D. *Gynura angulosa*; whole plant

PLATE 1.2

A. *Houttuynia cordata*; whole plant

B. *Lasia spinosa*; whole plant

C. *Psidium guajava*; leaves

Preparation of Plant Extracts

The usable portions of the plants were washed thoroughly with tap water, air-dried in shade (15-26°C) and finely pulverized into powdered form with the help of an electric grinder. Known amount of the powdered materials were suspended in an organic solvent, methanol as extractant and engaged for refluxing using Soxhlet Fractional Distillation method (Yadav *et al.*, 1992). The resulting suspension was decanted, the solute was discarded and the solvent was then removed by distillation under reduced temperature using rotatory vacuum evaporator; the extract was concentrated *in vacuo* and the residue was dried over anhydrous calcium chloride inside a desiccator. The percentage yields (w/w) of the final crude extracts were 9.97% (*Centella asiatica*), 5.71% (*Clerodendrum colebrookianum*), 4.62% (*Curcuma longa*), 6.46% (*Gynura angulosa*), 22.99% (*Houttuynia cordata*), 6.55, 9.93 and 10.15% (*Lasia spinosa* - leaf, stem and stalk) and 5.10% (*Psidium guajava*). These plant extracts were stored in respective plastic vials at - 4°C in a refrigerator until further use.

Parasite Material

To evaluate the *in vitro* anthelmintic activity of plant extracts following five helminth species served as tested parasites:

1. *Raillietina echinobothrida* (Family: Davaineidae, tapeworm of poultry).
2. *Hymenolepis diminuta* (Family: Hymenolepididae, tapeworm of rodents as well as man).
3. *Gasthrothylax crumenifer* (Family: Paramphistomatidae, ruminant fluke).

4. *Ascaridia galli* (Family: Ascarididae, poultry roundworm).
5. *Trichinella spirallis* (Family: Trichinellidae, a nematode parasite of humans that infects pigs as well as rodents).

Live specimens of adult *A. galli* and *R. echinobothrida* were collected in 0.9% PBS from intestines of freshly necropsied domestic fowl (*Gallus gallus domesticus* L.) at local abattoirs in Shillong. Similar procedures were followed for collection of *G. crumenifer*, except that they the worms were collected from the rumen of freshly slaughtered cattle. Adults live specimens of *H. diminuta* and *T. spiralis* were collected in 0.9% PBS from the intestines of previously infected rats and mice, respectively that were maintained in the laboratory.

Scanning Electron Microscopy (SEM)

Immediately after mortality the parasites from plant and reference drug-treated groups were collected in 0.9% physiological saline. At the same time the specimens were also collected from control group. The specimens were washed several times with additional changes in 0.9% physiological saline in order to remove any mucus or debris. The specimens (for *H. diminuta* 5-10 mm long cut pieces of their scolex and mature segments and for *A. galli* 8-10 mm long pieces of their anterior and posterior ends were used) were fixed in Karnovsky's fixative at 4°C for 24 h. Following fixation the material was washed in 0.1M Sodium Cacodylate buffer. After few washes in 0.1M Sodium Cacodylate buffer, the specimens were dehydrated with ascending series of acetone (30%, 50%, 70%,

80%, 90%, 95% and 100%) with two changes of 15 minutes in each, followed by a change to pure dry acetone at 30 min. The specimens were then treated with Tetramethylsilane (b.p. 23.3°C, surface tension, 10.2 dynes/cm at 20°C) as described by Dey *et al.* (1989). Following treatment with Tetramethylsilane the specimens were dried in an incubator at about 26°C, mounted on to brass stubs with adhesive tape in required orientation and coated with a thin layer of gold vapour in a Fine Coat Ion Sputter. The gold-coated specimens were observed using JEOL (JSM – 6360) scanning electron microscope, at electron accelerating voltages ranging between 10-20 keV.

Reagents

I. 0.9% Phosphate Buffered Saline

i) $NaH_2PO_4.2H_2O$ 2.106 g

ii) Na_2HPO_4 8.733 g

iii) NaCl 4.500 g

iv) 500 ml distilled water, pH adjusted to 7.4.

II. Hank's Solution:

i) NaCl 8.00 g

ii) KCl 0.40 g

iii) Na_2HPO_4 0.04 g

iv) $Na_2HPO_4.2H2O$ 0.06 g

v) Glucose 1.00 g

vi) $NaHCO_3$ 0.35 g

vii) 1000 ml distilled water, pH adjusted to 7.8

III. 0.2 M Cacodylate Buffer

i)	Sodium Cacodylate [Na(Ch3)2AsO2(3H2O)]	42.80 g
ii)	N HCl	6.90 ml
iii)	Double-distilled water to make volume to 1000 ml	

IV. Karnovsky's Fixative

i)	Para formaldehyde	20.00 g
ii)	0.2M Sodium Cacodylate buffer [Na (Ch3)2AsO2 (3H2O)]	250.00 ml
iii)	1N NaOH	2-3 drops
iv)	25% Gutaraldehyde	20.00 ml
v)	Distilled water	480.00 ml

Drugs and Chemicals:

Praziquantel [(Distocide®) Shin Poong Pharm. Co., Ltd., Seoul, Korea], Albendazole (Ambalai Sarabhai Enterprises Ltd, Vadodara) and Mebendazole [MEDITAB Specialists Pvt. Ltd., Goa] were the standard reference drugs used in the study. All the plant extracts and the drug solutions were prepared fresh in Hank's solution before starting the experiment with respective test parasites.

Experimental Design

The protocol was followed with slight modifications from Yadav *et al.* (1992). A known number of parasites (n = 6) were maintained in separate petridishes containing Hank's solution at 37 ± 1°C inside an incubator. The required amount of the test extract was weighed out and dissolved in a few drops of 1% dimethylsulphoxide (DMSO), and different concentrations of the test extract (5, 10, 20 and 40 mg/ml in Hank's solution) were prepared in separate petridishes. To each extract concentration a known number of worms (6 for each conc.) were gently placed inside the petridishes containing extract. Simultaneously, corresponding concentrations of reference drugs were also tested so as to provide a comparison of anthelmintic efficacy of plant extracts. In each case, a set of worms maintained without plant extract or reference drug but having a few drops of 1% DMSO in Hank's solution served as controls.

The *in vitro* anthelmintic efficacy was adjudged in terms of motility and mortality of test parasites and was monitored at every half an hour time interval. In order to confirm the mortality of parasites, two grades of one warm and the other hot saline solutions were employed. Worms not showing any physical movement on gentle stimulation by a soft brush were picked up and transferred to this warm saline solution. When parasites showed no movement in this warm saline, they were later dipped to the warmer grade of saline to finally confirm the mortality of the parasites. The mortality of parasites was assumed to have

occurred when all signs of movements had ceased and accordingly, time of mortality was recorded for each set of experiments.

In order to investigate the effects of plant extracts on parasites' body wall, immediately after completion of experiment the parasites from control and plant extract-treated groups were collected and processed for scanning electron microscopic (SEM) studies. The SEM studies were made only in case of those plants showing significant anthelmintic efficacy and *H. diminuta*, *G. crumenifer* and *A. galli* served as the test parasites.

Statistical Analysis

The experimental data were analyzed statistically and are expressed as mean plus or minus standard error of the mean (Mean ± SEM). Significance was evaluated by the Student's *t*-test and probability less than 5% ($p < 0.05$) was accepted as statistically significant.

Observations and Results

A. *In vitro* anticestodal activity of plant extracts against *Raillietina echinobothrida*

The results of *in vitro* anticestodal efficacy of various plant extracts and reference drug against *R. echinobothrida* are presented in Figs. 1.1 to 1.4. Of different plant extracts tested, *P. guajava* showed profound anticestodal efficacy as revealed by the mean mortality time of parasites which varied between 1.00-2.00 h as compared to the standard drug PZQ where it varied between 0.84-2.34 h. The worms incubated in the control medium showed physical activity for 69.33 h (Fig. 1.1 A). The efficacy was also noteworthy in case of *H. cordata* and *L. spinosa* (stalk) extracts as the mean mortality time of parasites following exposure to plant extracts varied between 2.00-3.00 and 2.00-3.66 h, respectively as compared to PZQ where it varied between 0.82-2.40 and 0.92-2.38 h, respectively. The worms maintained in the control medium showed physical activity till 70.00 and 67.66 h, respectively (Figs. 1.1 B and C). *C. colebrookianum*, *L. spinosa* (leaf) and *C. asiatica* extracts showed a moderate level of efficacy as adjudged by the mean mortality time of parasites which ranged from 4.00-14.66 h. In this case the mean mortality time of parasites treated with PZQ was noted to be 0.85-2.12, 0.92-2.38 and 0.98-2.50 h, respectively. The parasites maintained in the control medium showed physical activity for 71.33, 68.83 and 71.73 h, respectively (Fig. 1.2 A-C). The efficacy was noted to be insignificant with respect to *C. longa*, *G. angulosa* and *L.*

spinosa (stem) extracts, as the mean mortality time of parasites following treatment with these plant extracts varied from 6.00-41.00 h (Fig. 1.3 A-C).

Further experiments were carried out to investigate the synergistic effects, if any, of plant extracts. The extracts were mixed in 1: 1 ratio and tested against *R. echinobothrida*. The mean mortality time of parasites following treatment with *P. guajava* + *H. cordata*, *L. spinosa* (stalk) + *H. cordata* and *L. spinosa* (stalk) + *P. guajava* extracts varied from 2.00-8.66, 2.00-10.00 and 3.00-11.66 h, respectively. PZQ in this case showed mean mortality time of parasites as 0.92-2.38, 0.80-2.34 and 0.98-2.50 h. The worms maintained in the control medium showed physical activity for 72.84, 68.90 and 71.50 h, respectively (Fig. 1.4 A-C).

B. *In vitro* anticestodal activity of plant extracts against *Hymenolepis diminuta*

The results of *in vitro* anticestodal efficacy of various plant extracts and reference drug against *H. diminuta* are presented in Figs. 1.5 to 1.8. Of all the plant extracts tested, *P. guajava*, *L. spinosa* (leaf) and *G. angulosa* showed significant efficacy where the mean mortality time of parasites ranged from 2.34-4.09, 2.50-4.34 and 2.92-4.92 h, respectively. The parasites maintained in the control medium survived for 21.45, 22.33 and 20.66 h, respectively (Fig. 1.5 A-C). In case of *C. longa*, *H. cordata*, *C. colebrookianum* and *L. spinosa* (stalk) extracts the efficacy was noteworthy only at their higher concentrations (40

mg/ml) (Figs. 1.6 A-C and 1.7 A). Mortality time of *H. diminuta* was recorded to be comparatively at higher sides following exposure to extracts of *L. spinosa* (stem) and *C. asiatica* extracts, and ranged between 7.17-11.42 and 10.67-14.75 h, respectively (Fig. 1.7 B-C).

In case of combination plant extract tests, the mortality time of parasites was recorded as 2.92-5.00 h for *P. guajava* + *G. angulosa*, 3.25-5.59 h for *L. spinosa* (leaf) + *G. angulosa* and 4.00-6.17 h for *P. guajava* + *L. spinosa* (leaf). The mortality time of parasites following treatment with PZQ for the above combination tests was recorded to be as 0.56-1.58, 0.58-1.60 and 0.58-1.60 h. Worms maintained in the control medium showed physical activity for 22.25, 18.66 and 21.67 h (Fig.1.8 A-C).

C. *In vitro* anthelmintic activity of plant extracts against *Gasthrothylax crumenifer*

The results of *in vitro* anthelmintic efficacy of various plant extracts and reference drug against G. *crumenifer* are presented in Figs. 1.9 to 1.12. At 40 mg/ml concentration, *L. spinosa* (leaf), *C. colebrookianum* and *H. cordata* extracts showed a mean mortality time of parasites as 2.09, 2.50 and 3.00 h, respectively which was almost comparable (2.00, 2.10 and 2.31 h, respectively) with that of similar concentration of PZQ. The worms maintained in the control medium showed physical activity till 49.17, 47.67 and 47.50 h, respectively (Fig. 1.9 A-C). The efficacy of *C. asiatica*, *L. spinosa* (stalk), *P. guajava* and *L.*

spinosa (stem) extract was also notable but only at their 40 mg/ml concentration, and ranged from 4.67, 5.25, 6.00 and 8.00 h, respectively. The worms incubated in the control medium showed physical activity for 50.33, 47.33, 50.50 and 51.25 h, respectively. The mean survival time of parasites for the PZQ-treatment (40 mg/ml) was recorded to be 1.98, 2.17, 2.23 and 2.25 h, respectively (Figs. 1.10 A-C and 1.11 A). *C. longa*, and *G. angulosa* extracts did not show appreciable efficacy as the mean mortality time of parasites for these plants was found to be 10.50-17.50 and 13.34-19.75 h, respectively (Fig. 1.11 B-C).

In combination plant extract tests all showed moderate level of efficacy, the mean mortality time of worms ranged from 3.09-7.42, 3.84-8.25, and 4.17-9.00 h for *L. spinosa* (leaf) + *C. colebrookianum*, *L. spinosa* (leaf) + *H. cordata* and *C. colebrookianum* + *H. cordata*, respectively. Control worms showed physical activity up to 50.84, 47.67 and 49.30 h, respectively for the above combination tests (Fig. 1.12 A-C).

D. *In vitro* anthelmintic activity of plant extracts against *Ascaridia galli*

The results of efficacy of plant extracts against *A. galli* are presented in Figs. 1.13 to 1.16. Of all the extracts tested, efficacy was found to be most significant for *L. spinosa* (leaf) extract, which resulted into mortality of worms in 14.00 h (40 mg/ml). This was comparable with that of reference drug, ABZ which showed mortality of worms in 8.84 h at the same concentration. The mean mortality time of worms maintained in the control medium was recorded to be

154.00 h (Fig. 1.13 A). The efficacy of *G. angulosa*, *C. colebrookianum*, *L. spinosa* (stem), *C. asiatica*, *H. cordata* and *L. spinosa* (stalk) extract (40 mg/ml) was noted to be 20.00, 21.00 23.00, 23.00, 24.09 and 26.50 h, respectively. The parasites in the control medium showed physical activity till 155.17, 152.67, 158.34, 157.34 153.75 and 150.25 h, respectively (Figs. 1.13 B-C; 1.14 A-C and 1.15 A). The mortality time of *A. galli* following treatment with *P. guajava* and *C. longa* was recorded to be at higher sides when compared with ABZ (Fig. 1.15 B-C).

In combination extract tests, *L. spinosa* (leaf) + *G. angulosa*, *L. spinosa* (leaf) + *C. colebrookianum* and *G. angulosa* + *C. colebrookianum* revealed comparable efficacy with that of reference drug only at their higher concentration (18.84, 22.00 and 23.34 h for the respective combined extract test and 9.02, 8.90 and 8.69 h for the reference drug, ABZ. The parasites in the control medium exhibited survival for 155.17, 153.67 and 157.25 h (Figs 1.16 A-C).

E. *In vitro* anthelmintic activity of plant extracts against *Trichinella spiralis*

The results pertaining to *in vitro* anthelmintic efficacy of plant extracts against *T. spiralis* are presented in Figs. 1.17 to 1.20 respectively. It emerged that the activity of *G. angulosa*, *L. spinosa* (leaf), *C. colebrookianum*, *H. cordata*, *P. guajava*, *C. longa* and *L. spinosa* (stem) extracts was noteworthy at 40 mg/ml as it was recorded to be almost comparable with that of MBZ at their corresponding concentrations. The worm mortality time recorded was 10.50,

13.84, 13.50, 10.00, 10.67, 11.34 and 13.84 h, respectively for the control (Figs. 1.17 A-C; 1.18 A-C and 1.19 A). The mean mortality time of *L. spinosa* (stalk) and *C. asiatica* extract treated parasites was recorded to be between 1.09-2.17 h as compared to 0.47-1.14 h for MBZ. The parasites in the control medium survived for 11.25 and 9.84 h, respectively (Fig. 1.19 B-C).

Profound efficacy was exhibited in the combination extract tests for *G. angulosa* + *L. spinosa* (leaf), *L. spinosa* (leaf) + *C. colebrookianum* and *G. angulosa* + *C. colebrookianum* extracts, where the mean mortality time of parasites was found to range between 0.75-1.42, 0.84-1.50 and 0.92-1.59 h, respectively. The worms incubated in the control medium showed physical activity for 11.67, 12.17 and 10.00 h, respectively (Fig. 1.20 A-C).

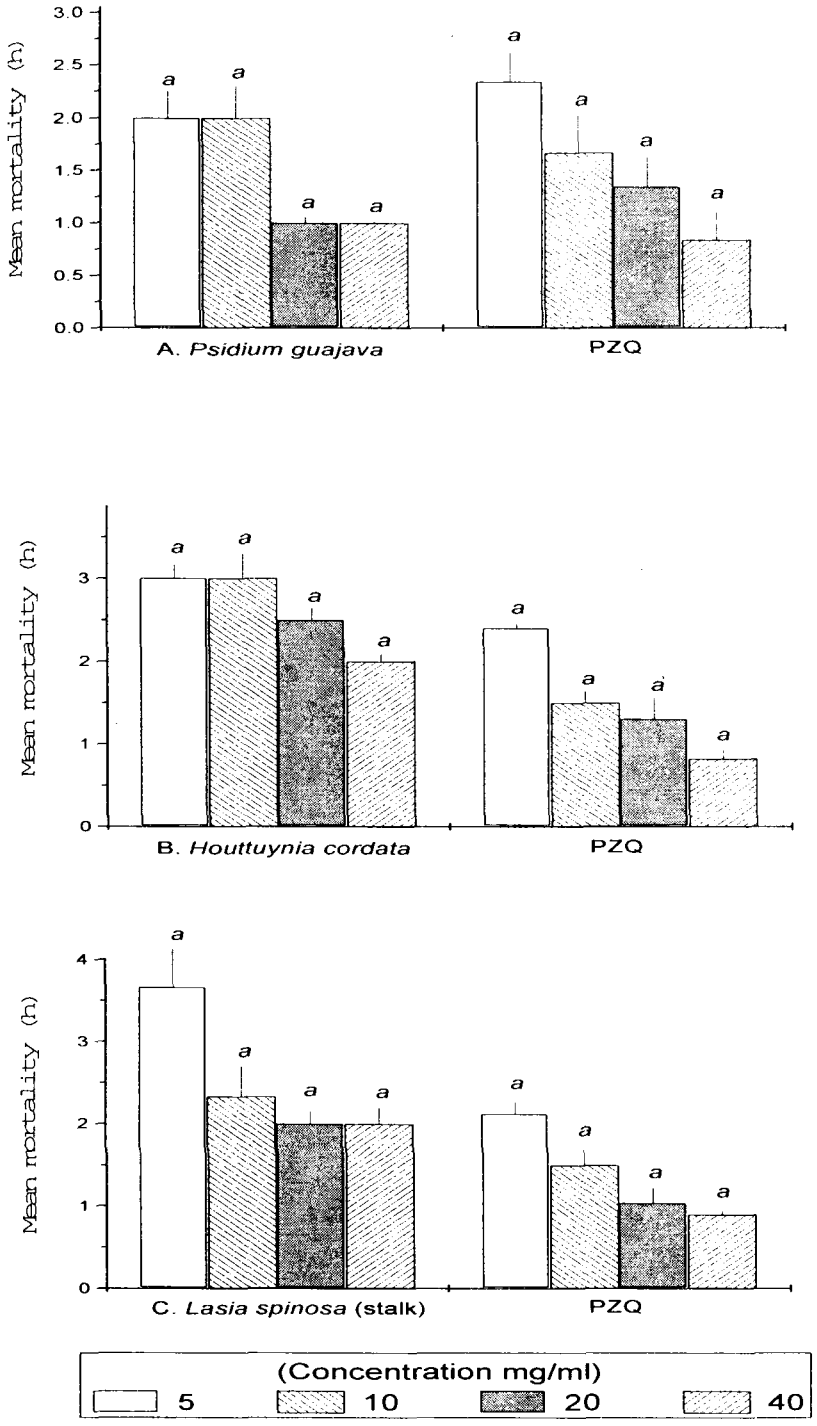


Figure 1.1 A-C: *In vitro* anticestodal activity of plant extracts against *Raillietina echinobothrida*

A. *P. guajava** **B.** *H. cordata*** **C.** *L. spinosa* (stalk)***
Worms incubated in control medium showed physical activity as follows:
*69.33 ± 3.15 **70.00 ± 4.16 ***67.66 ± 4.79 h
[a] $p < 0.001$ compared with control groups.

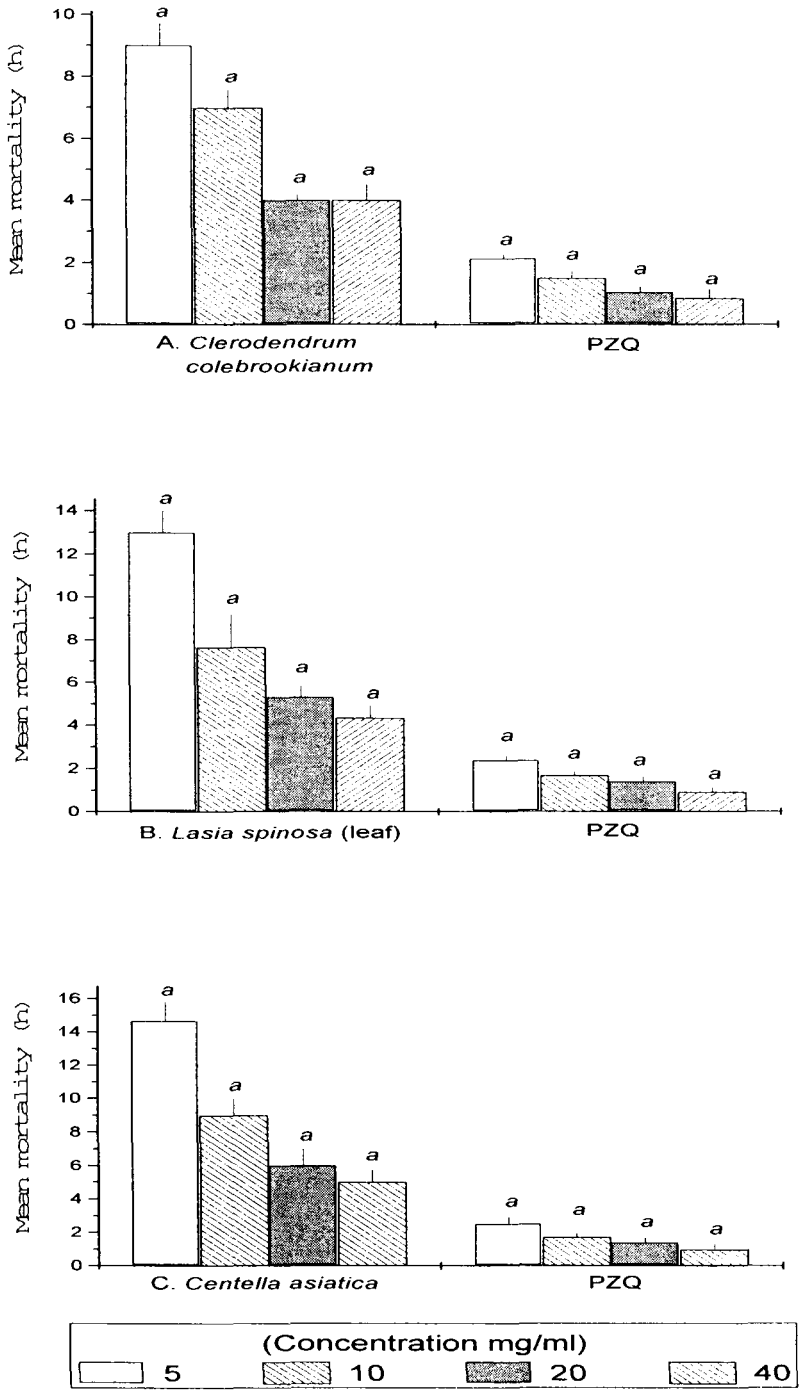


Figure 1.2 A-C: *In vitro* anticestodal activity of plant extracts against *Raillietina echinobothrida*

A. *C. colebrookianum** **B.** *L. spinosa* (leaf)**
C. *C. asiatica****
Worms incubated in control medium showed physical activity as follows:
*71.33 ± 3.66 **68.83 ± 4.90 ***71.73 ± 6.31 h
[a]$p < 0.001$ compared with control groups.

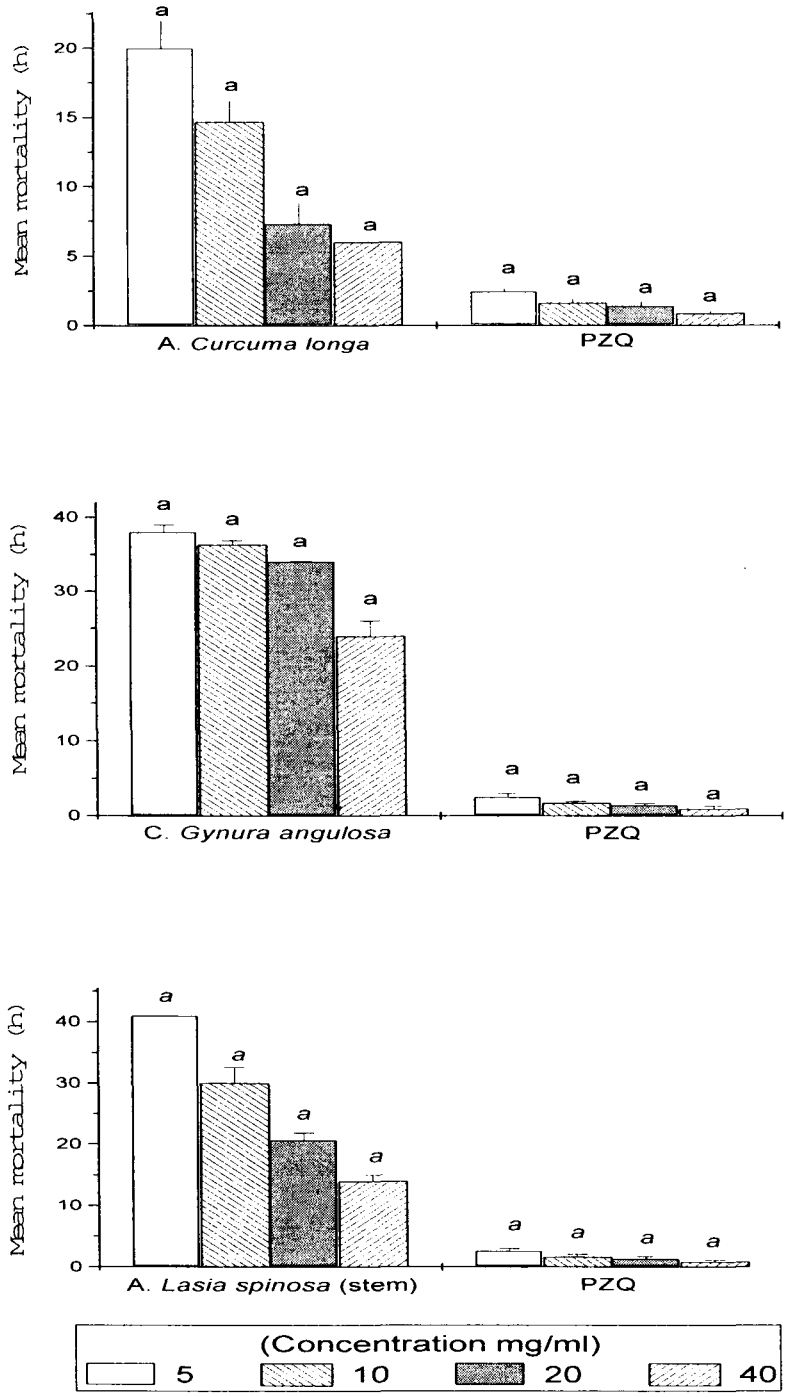


Figure 1.3 A-C: ***In vitro*** **anticestodal activity of plant extracts against** ***Raillietina echinobothrida***

A. *C. longa** **B.** *G. angulosa*** **C.** *L. spinosa* (stem)***
Worms incubated in control medium showed physical activity as follows:
*72.00 ± 5.47 **72.33 ± 3.31 ***68.79 ± 4.12 h
$^{a}p < 0.001$ compared with control groups.

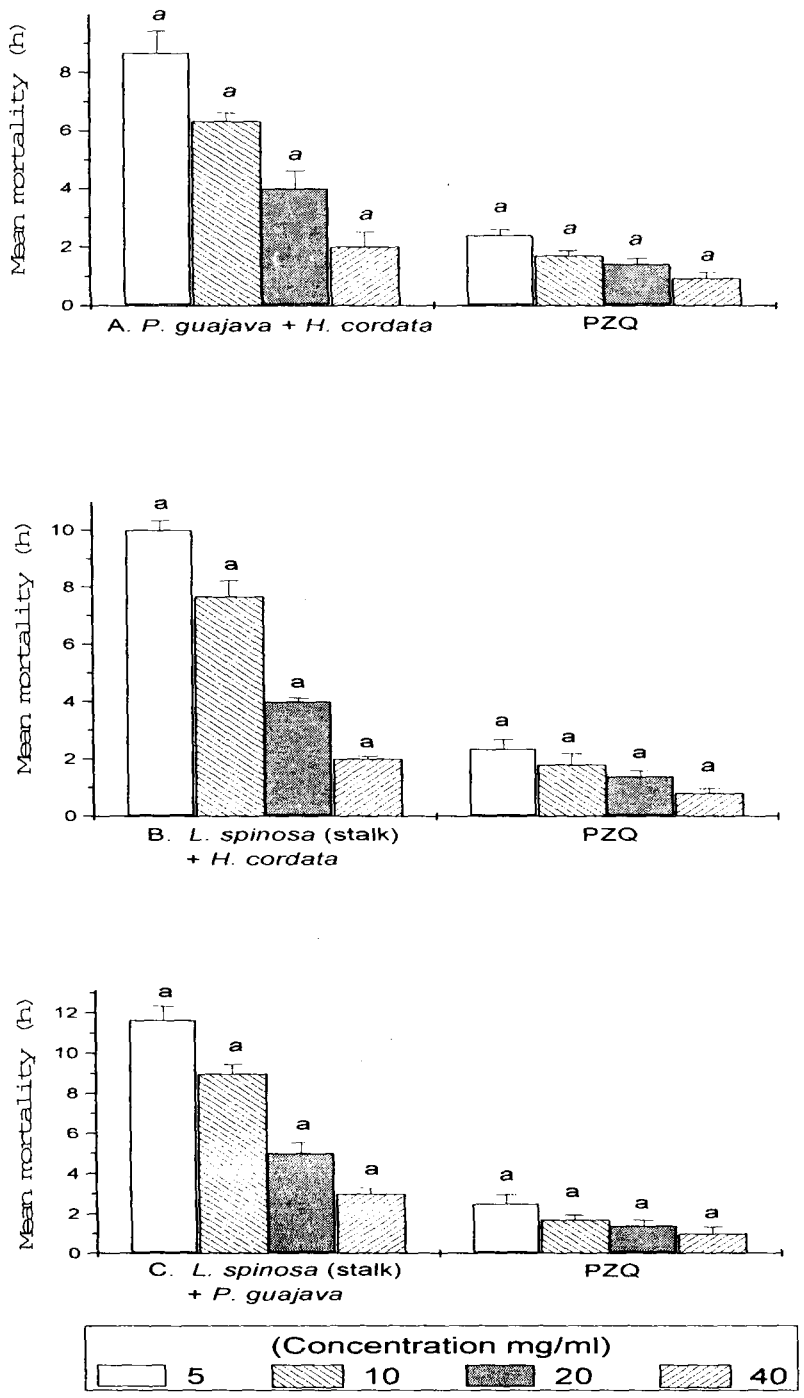


Figure 1.4 A-C: ***In vitro*** **anticestodal activity of combined plant extracts against** ***Raillietina echinobothrida***

A. *P. guajava* + *H. cordata** **B.** *L. spinosa* (stalk) + *H. cordata*** **C.** *L. spinosa* (stalk) + *P. guajava**** Worms incubated in control medium showed physical activity as follows:
*72.84 ± 5.59 **68.90 ± 4.99 ***71.50 ± 5.36 h
[a]$p < 0.001$ compared with control groups.

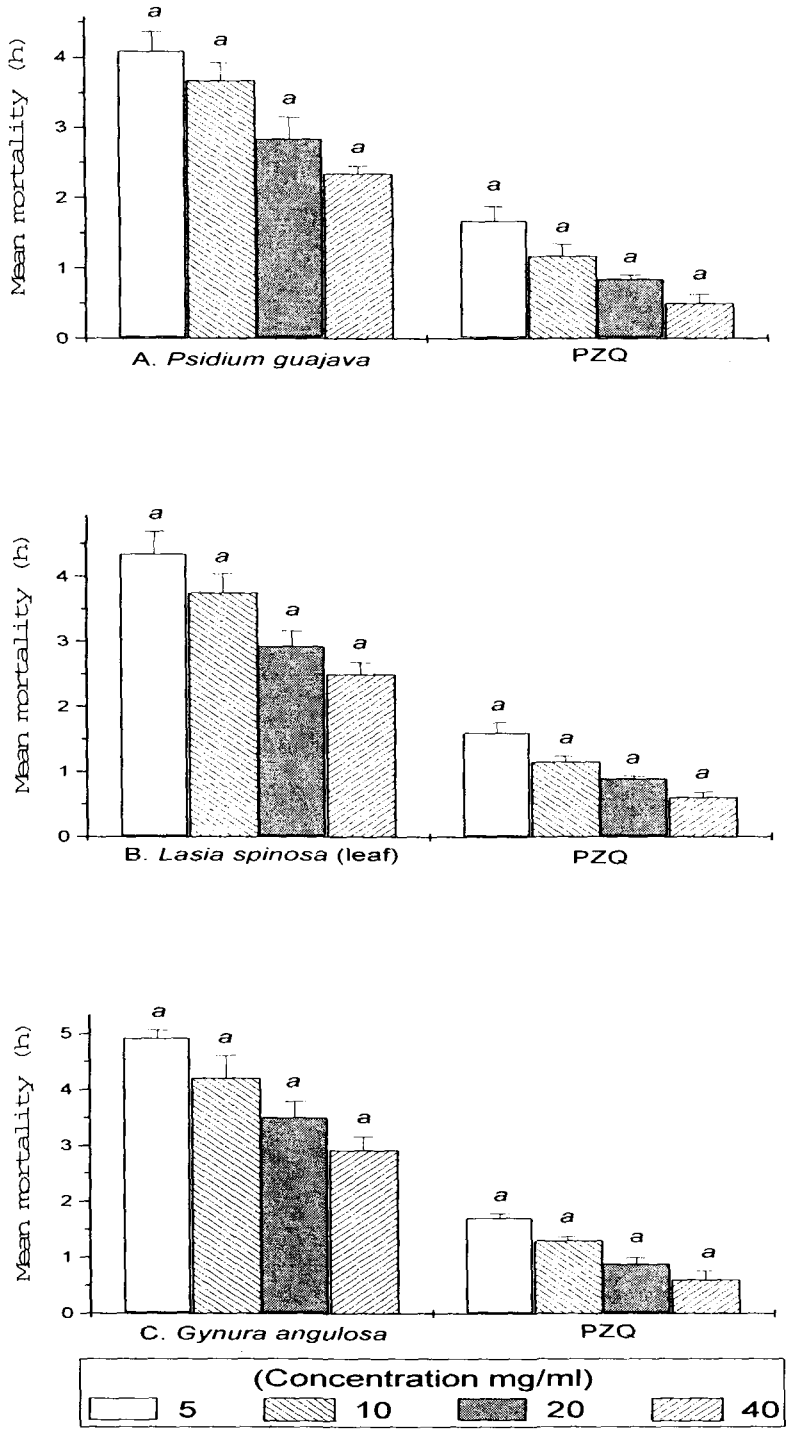


Figure 1.5 A-C: ***In vitro*** **anticestodal activity of plant extracts against** ***Hymenolepis diminuta***

A. *P. guajava** **B.** *L. spinosa* (leaf)** **C.** *G. angulosa****
Worms incubated in control medium showed
physical activity as follows:
*21.45 ± 1.63 **22.33 ± 2.73 ***20.66 ± 2.08 h
[a]$p < 0.001$ compared with control groups.

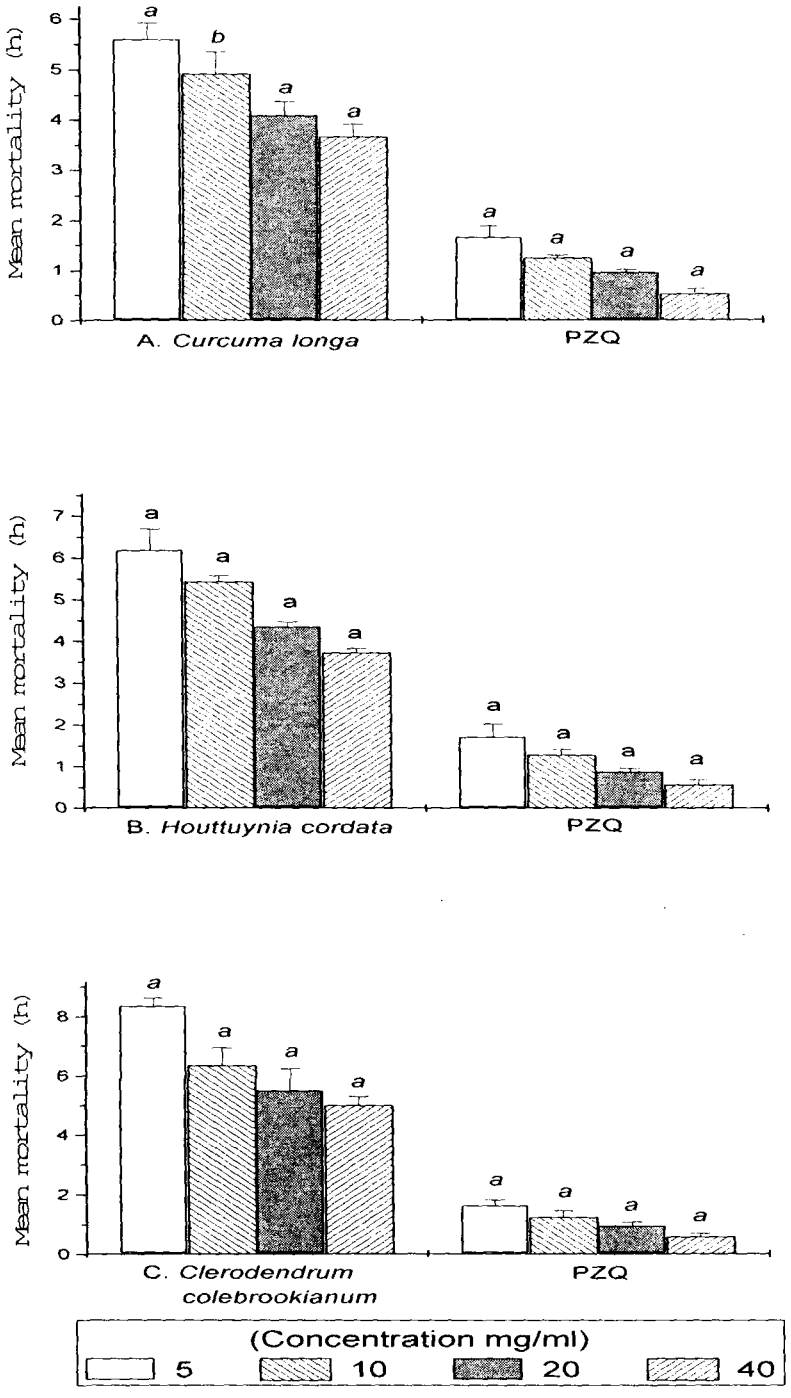


Figure 1.6 A-C: ***In vitro*** **anticestodal activity of plant extracts against** ***Hymenolepis diminuta***

A. *C. longa** **B.** *H. cordata*** **C.** *C. colebrookianum**** Worms incubated in control medium showed physical activity as follows:
*24.50 ± 2.46 **22.84 ± 2.75 ***19.33 ± 3.20 h
$^{a, b}p < 0.001$, and $p < 0.01$, respectively compared with control groups.

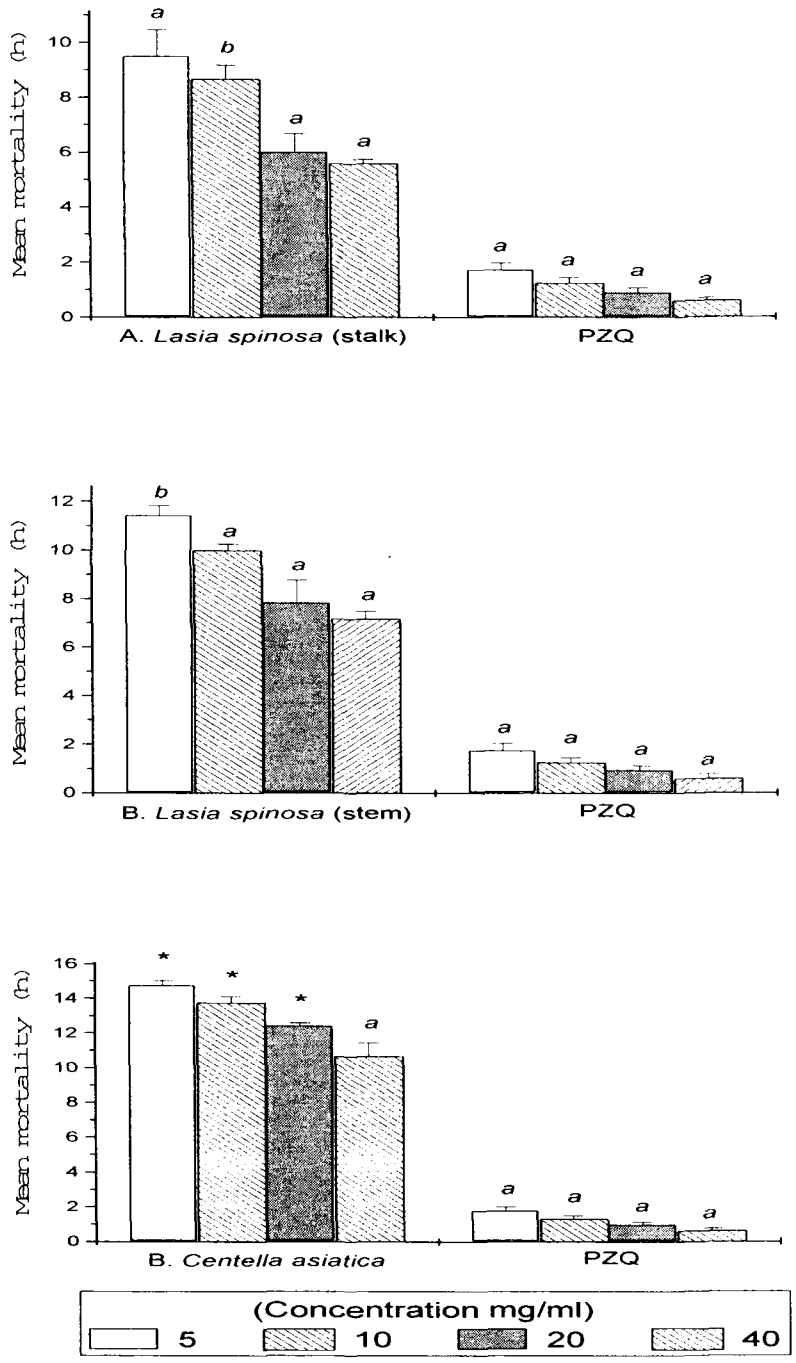


Figure 1.7 A-C: ***In vitro*** **anticestodal activity of plant extracts against** ***Hymenolepis diminuta***

A. *L. spinosa* (stalk)* **B.** *L. spinosa* (stem)**
C. *C. asiatica****
Worms incubated in control medium showed physical activity as follows:
*21.74 ± 2.04 **20.00 ± 2.86 ***22.50 ± 3.07 h
$^{a, b}p < 0.001$, and $p < 0.01$, respectively compared with control groups. *not significant

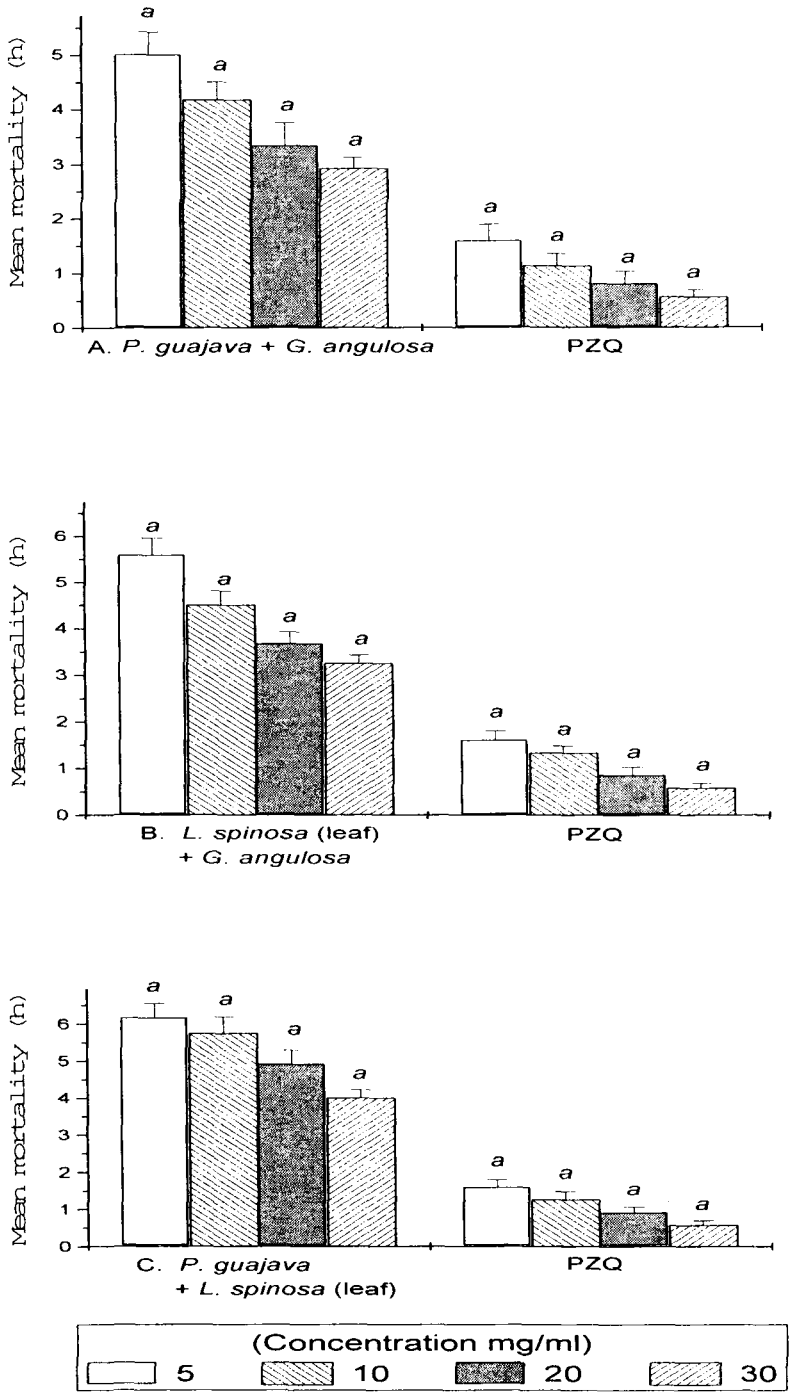


Figure 1.8 A-C: ***In vitro*** **anticestodal activity of combined plant extracts against** ***Hymenolepis diminuta***

A. *P. guajava* + *G. angulosa** **B.** *L. spinosa* (leaf) + *G. angulosa*** **C.** *P. guajava* + *L. spinosa* (leaf)***
Worms incubated in control medium showed physical activity as follows:
*22.25 ± 3.15 **18.66 ± 2.67 ***21.67 ± 2.85 h
[a]$p < 0.001$ compared with control groups.

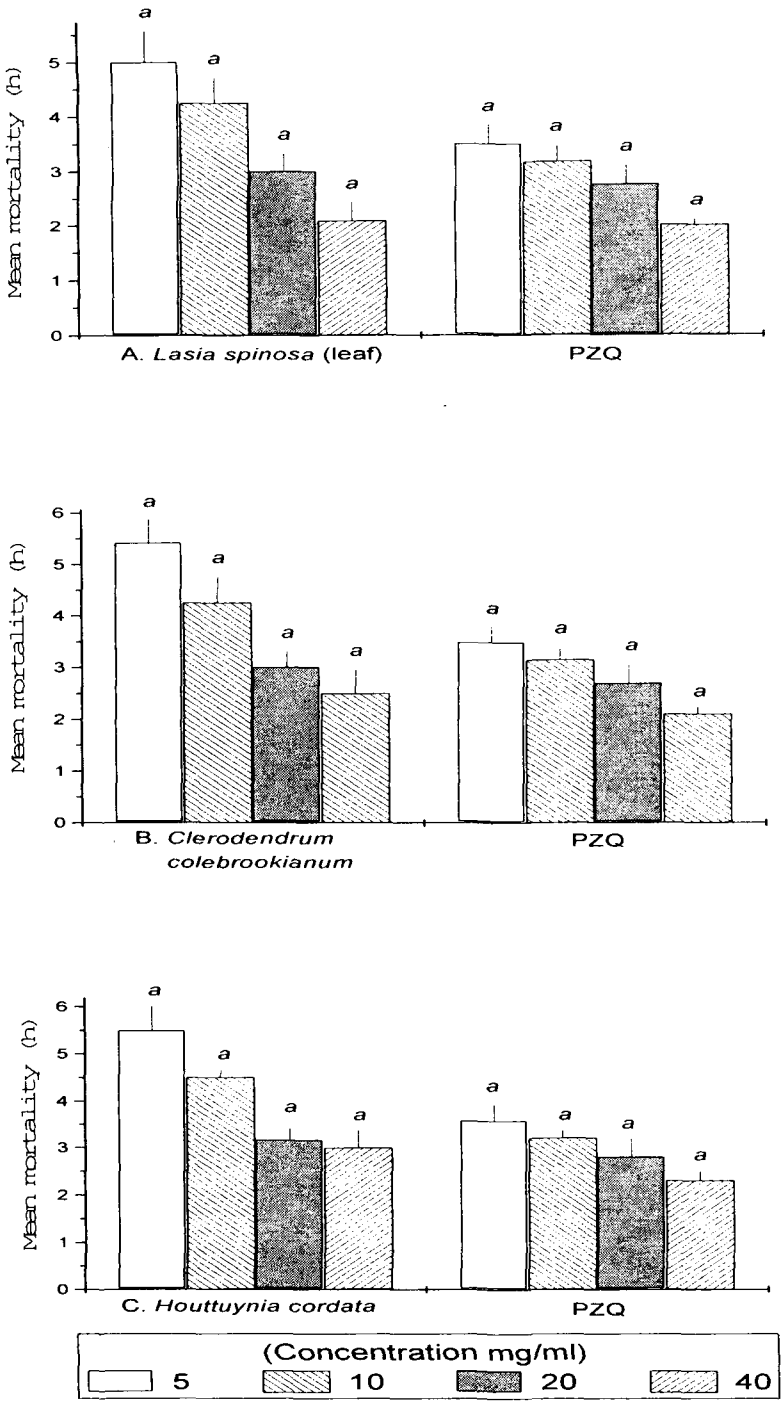


Figure 1.9 A-C: *In vitro* anthelmintic activity of plant extracts against *Gasthrothylax crumenifer*

A. *L. spinosa* (leaf)* **B.** *C. colebrookianum*** **C.** *H. cordata****

Worms incubated in control medium showed physical activity as follows:

*49.17 ± 2.23 **47.67 ± 3.09 ***47.50 ± 4.63 h

[a]$p < 0.001$ compared with control groups.

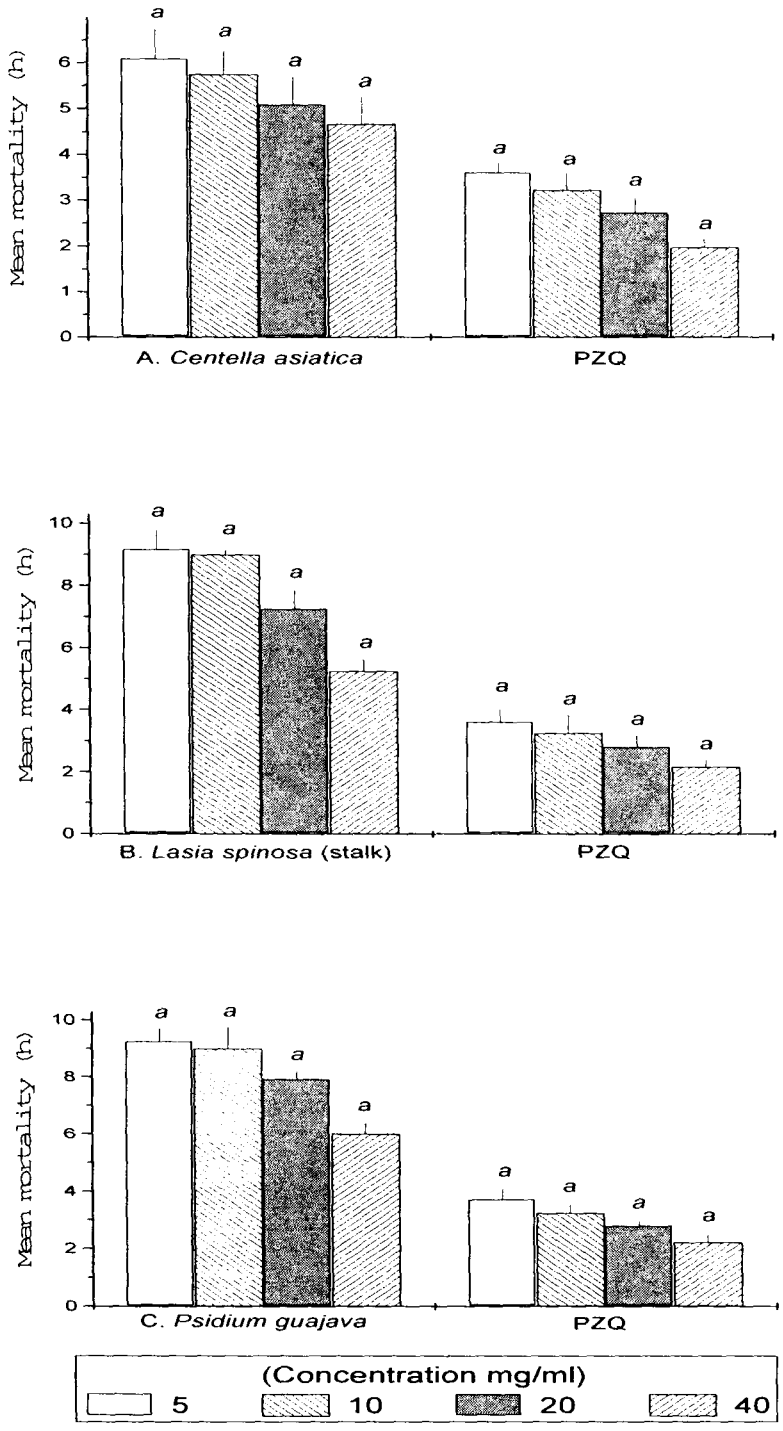


Figure 1.10 A-C: ***In vitro*** **anthelmintic activity of plant extracts against *Gasthrothylax crumenifer***

A. *C. asiatica** **B.** *L. spinosa* (stalk)** **C.** *P. guajava****
Worms incubated in control medium showed physical activity as follows:
*50.33 ± 5.36 **47.33 ± 2.87 ***50.50 ± 3.95 h
[a]$p < 0.001$ compared with control groups.

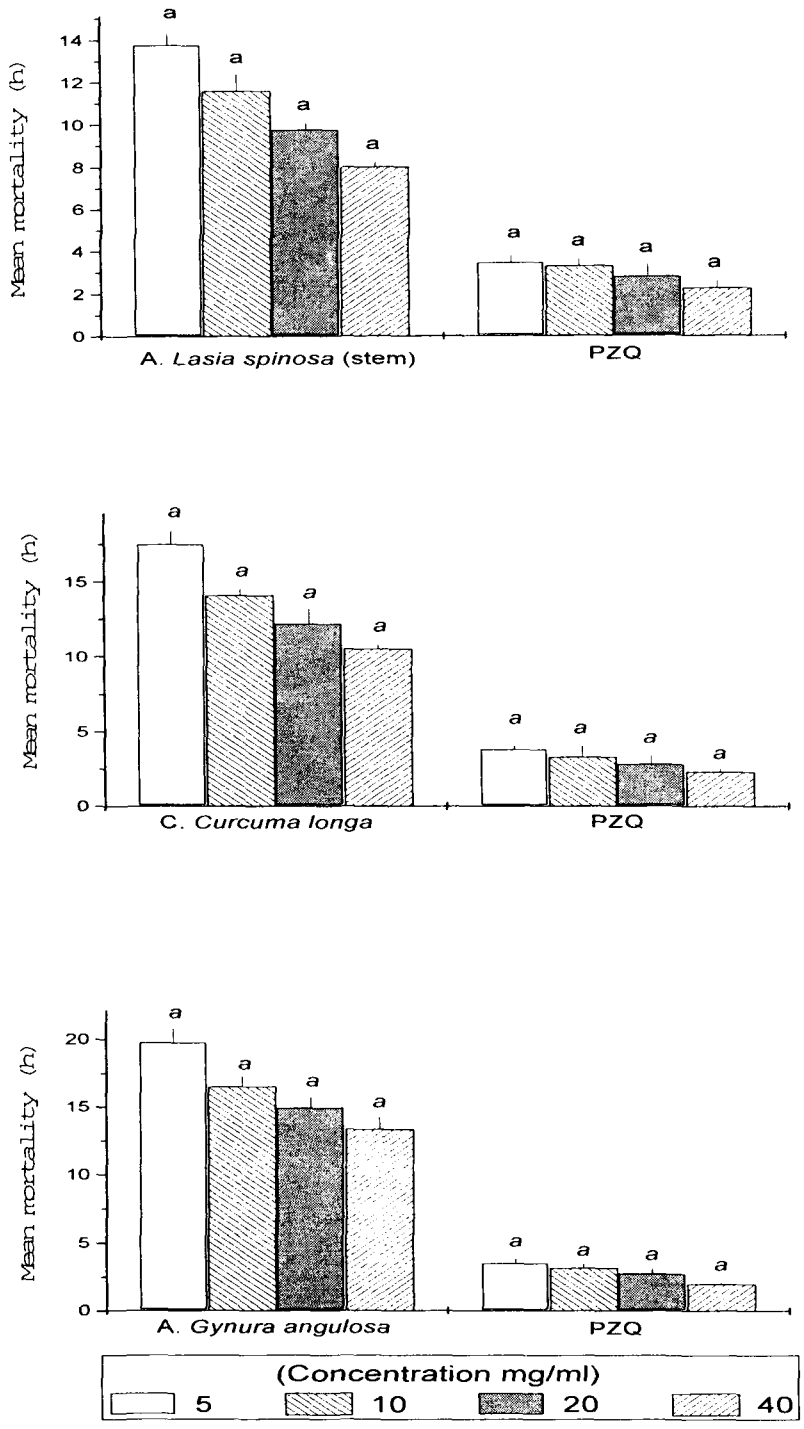


Figure 1.11 A-C: *In vitro* anthelmintic activity of plant extracts against *Gasthrothylax crumenifer*

A. *L. spinosa* (stem)* **B.** *C. longa*** **C.** *G. angulosa**** Worms incubated in control medium showed physical activity as follows:
*51.25 ± 4.38 **50.67 ± 4.17 ***48.00 ± 2.93 h
$^{a}p < 0.001$ compared with control groups.

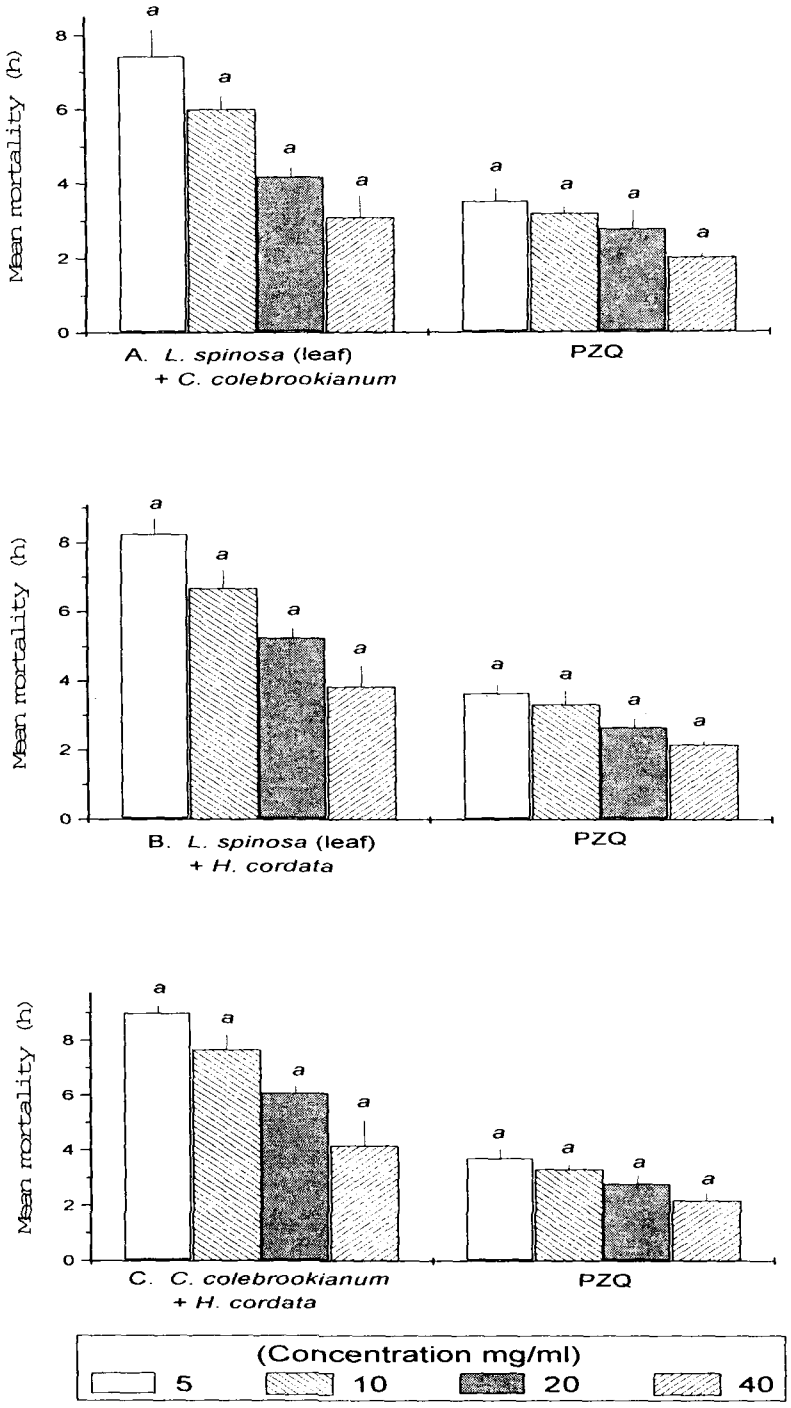


Figure 1.12 A-C: *In vitro* anthelmintic activity of combined plant extracts against *Gasthrothylax crumenifer*

A. *L. spinosa* (leaf) + *C. colebrookianum** **B.** *L. spinosa* (leaf) + *H. cordata*** **C.** *C. colebrookianum* + *H. cordata**** Worms incubated in control medium showed physical activity as follows:
*50.84 ± 5.04 **47.67 ± 3.38 ***49.30 ± 2.06 h
$^{a}p < 0.001$ compared with control groups.

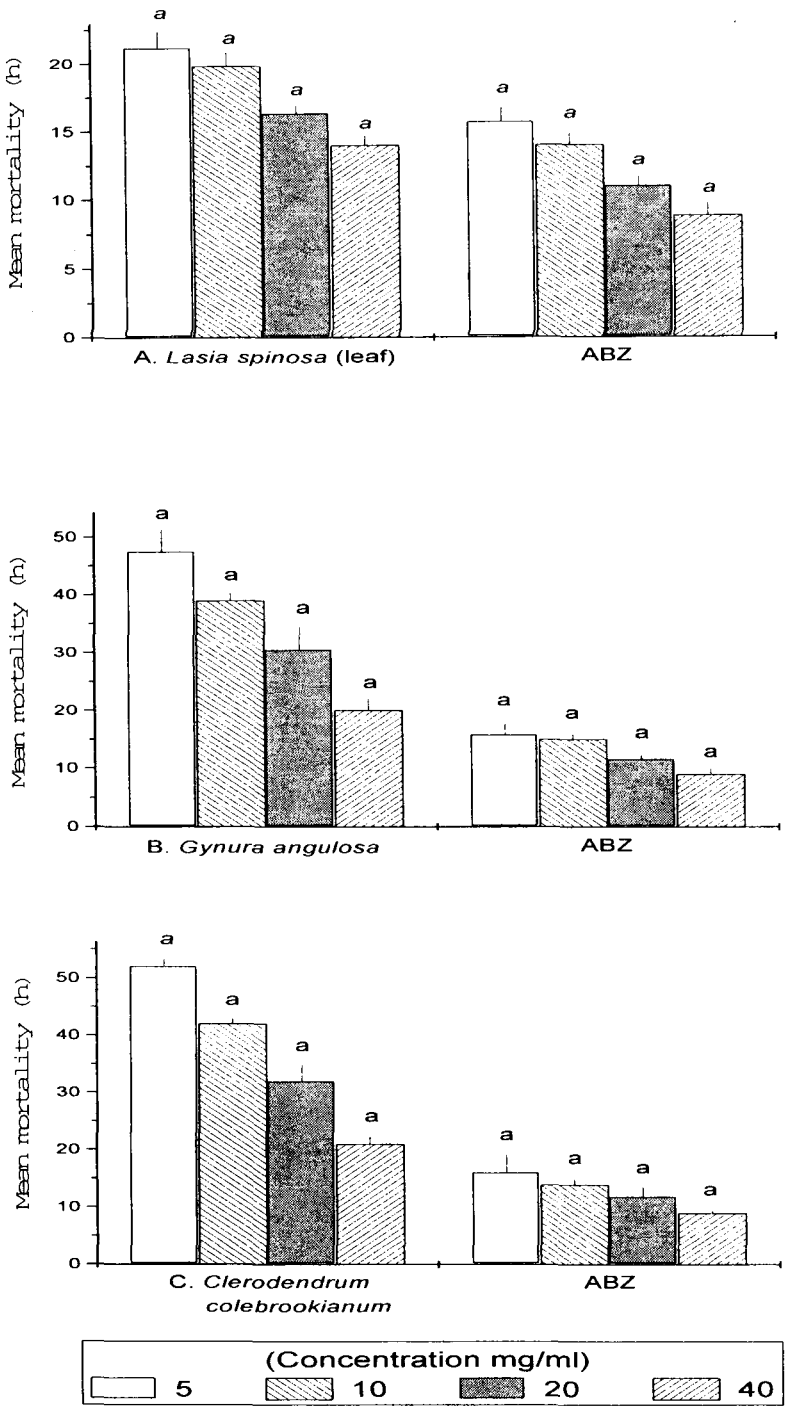


Figure 1.13 A-C: *In vitro* anthelmintic activity of plant extracts against *Ascaridia galli*

A. *L. spinosa* (leaf)* **B.** *G. angulosa*** **C.** *C. colebrookianum**** Worms incubated in control medium showed physical activity as follows: *154.00 ± 6.09 **155.17 ± 4.48 ***152.67 ± 5.21 h $^{a}p < 0.001$ compared with control groups.

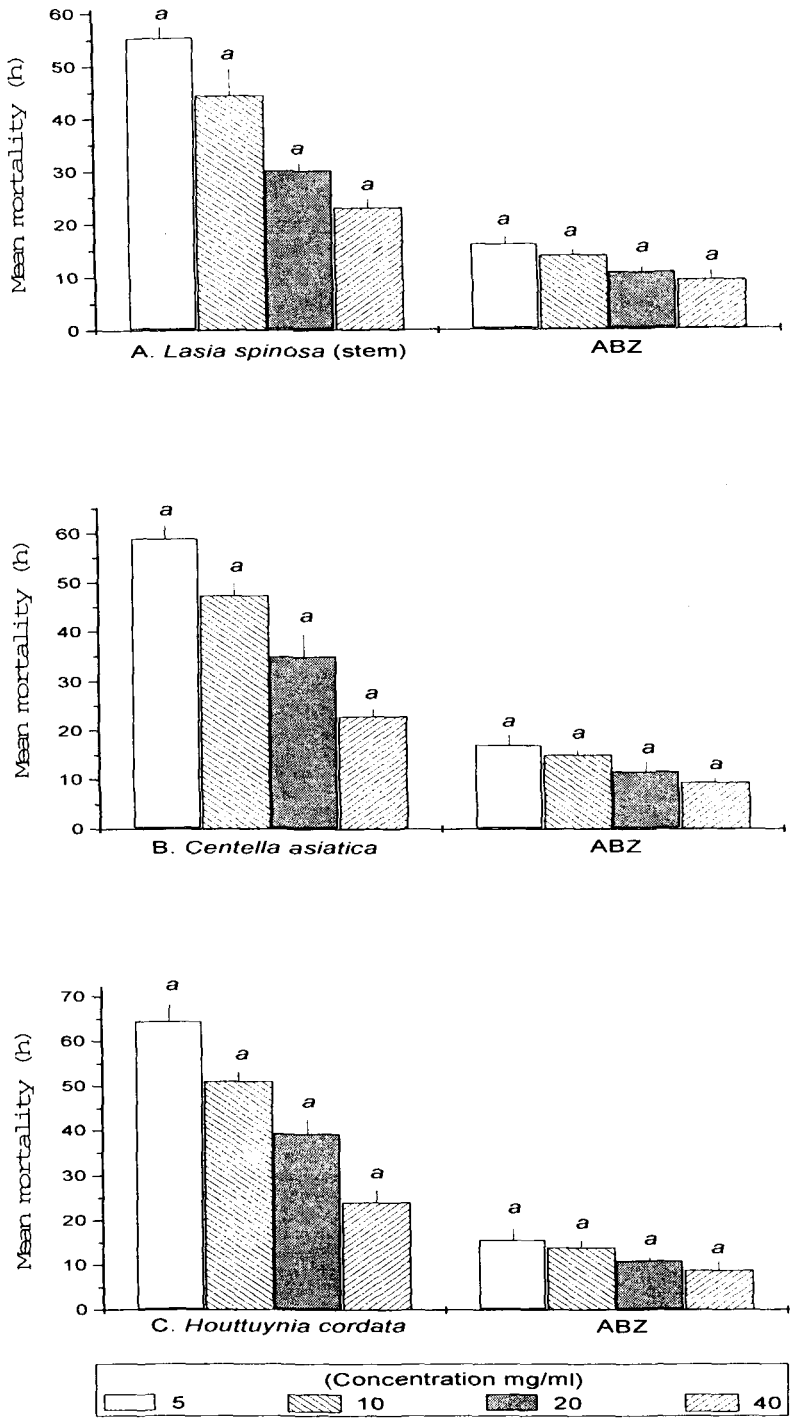


Figure 1.14 A-C: *In vitro* anthelmintic activity of plant extracts against *Ascaridia galli*

A. *L. spinosa* (stem)* **B.** *C. asiatica*** **C.** *H. cordata**** Worms incubated in control medium showed physical activity as follows:
*158.34 ± 2.27 **157.34 ± 6.86 ***153.75 ± 5.10 h
$^{a}p < 0.001$ compared with control groups.

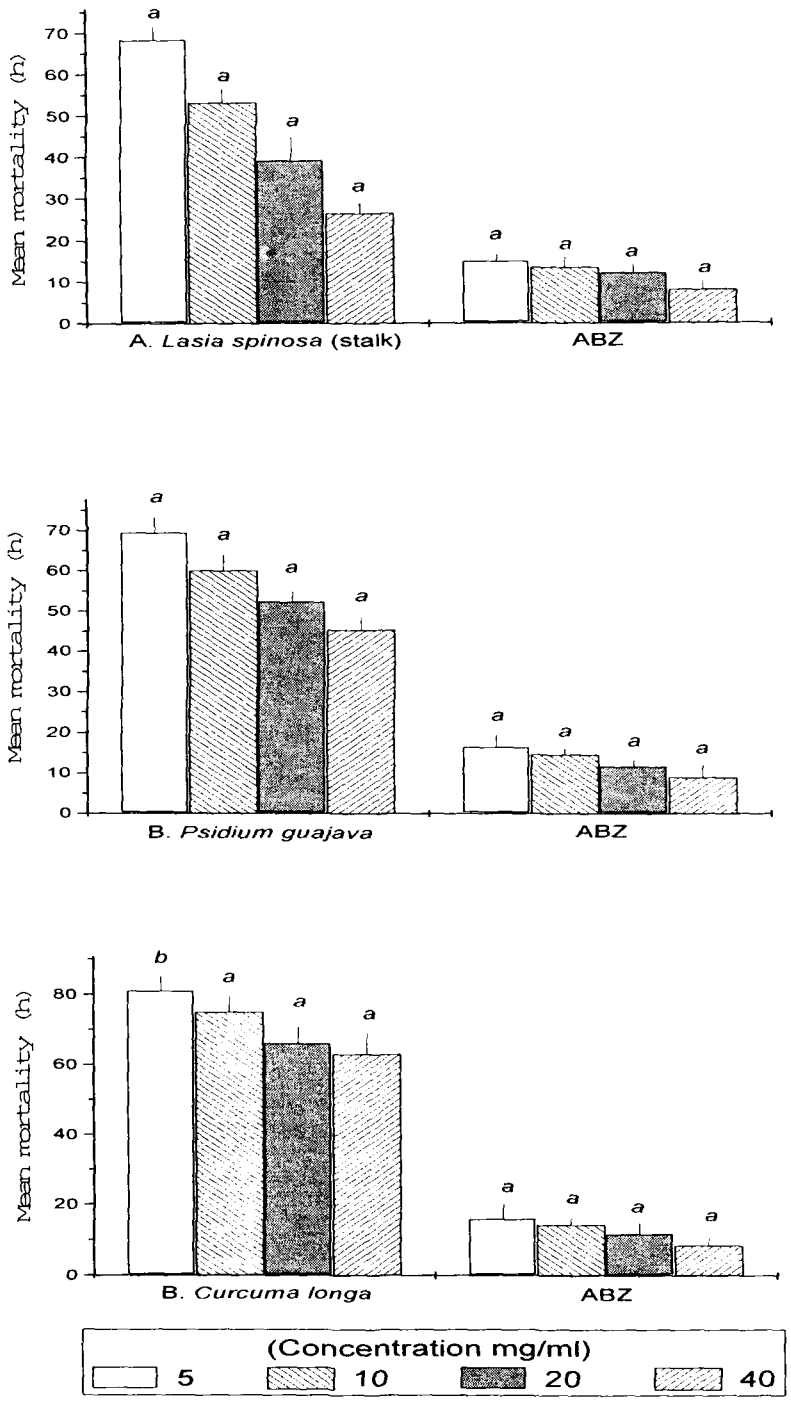


Figure 1.15 A-C: *In vitro* anthelmintic activity of plant extracts against *Ascaridia galli*

A. *L. spinosa* (stalk)* **B.** *P. guajava*** **C.** *C. longa**** Worms incubated in control medium showed physical activity as follows:
*150.25 ± 3.08 **155.50 ± 6.37 ***153.33 ± 6.86 h
$^{a,b}p < 0.001$, and $p < 0.05$ respectively compared with control groups.

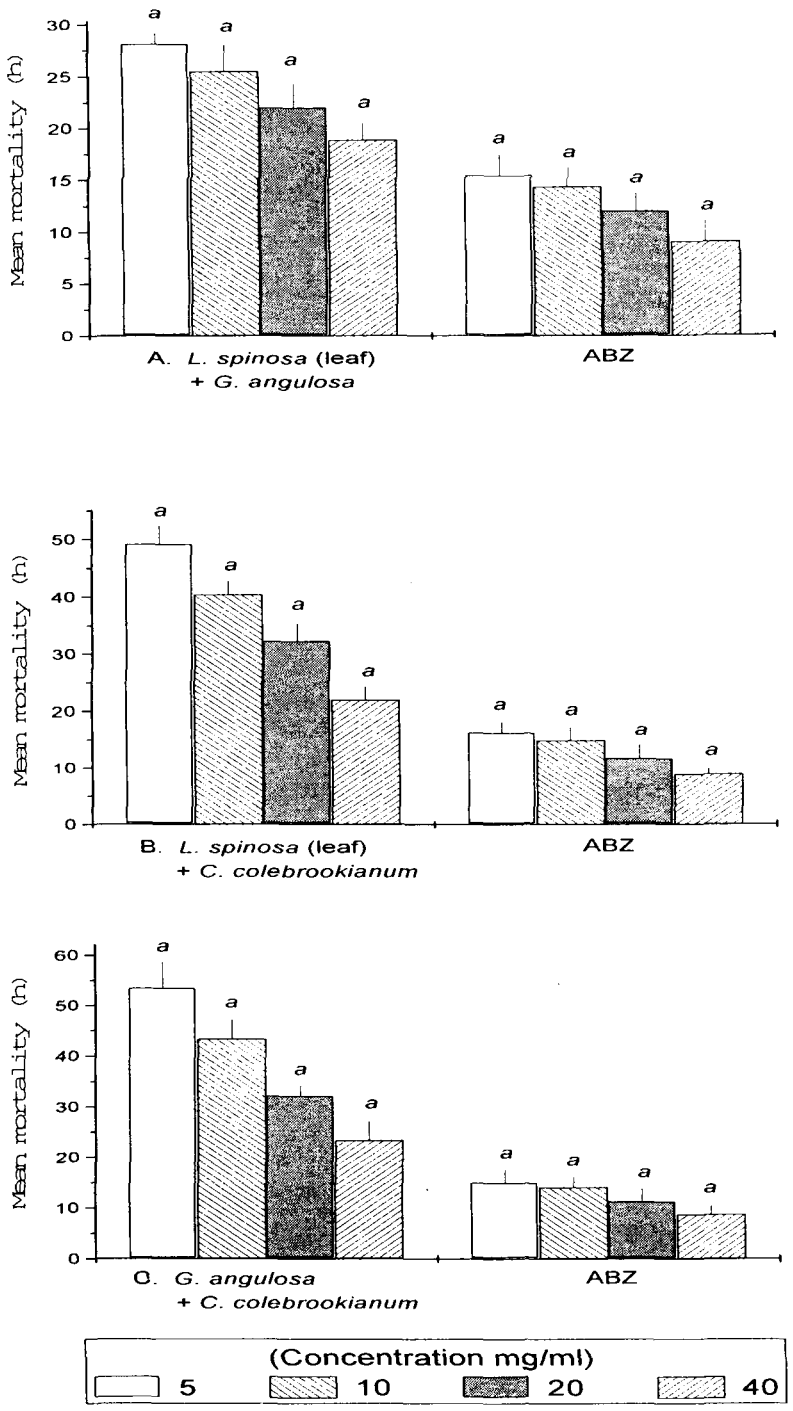


Figure 1.16 A-C: *In vitro* anthelmintic activity of combined plant extracts against *Ascaridia galli*

A. *L. spinosa* (stalk) + *G. angulosa** **B.** *L. spinosa* (stalk) + *C. colebrookianum*** **C.** *G. angulosa* + *C. colebrookianum**** Worms incubated in control medium showed physical activity as follows:
*155.17 ± 5.17 **153.67 ± 4.08 ***157.25 ± 4.46 h
$^{a}p < 0.001$ compared with control groups.

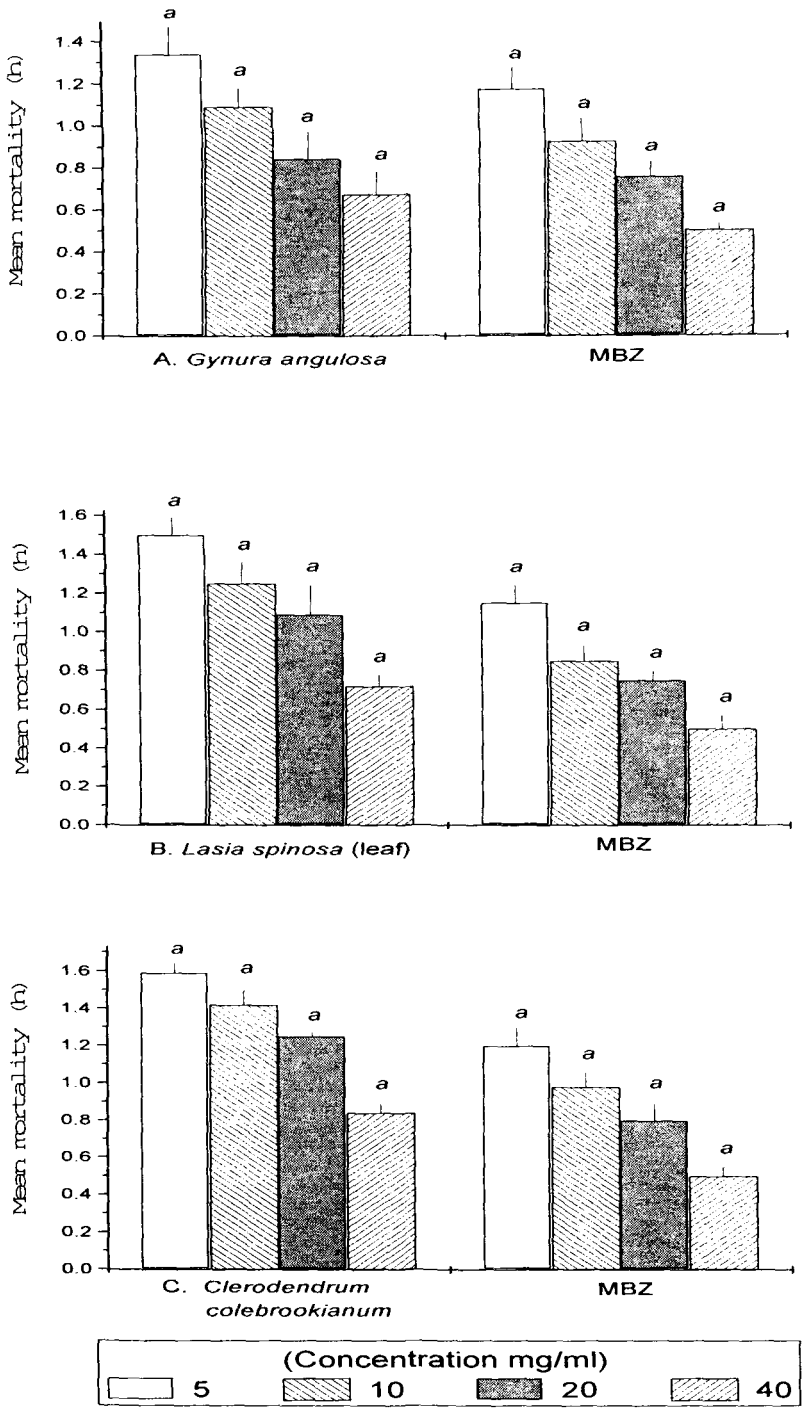


Figure 1.17 A-C: *In vitro* anthelmintic activity of plant extracts against *Trichinella spiralis*

A. *G. angulosa** **B.** *L. spinosa* (leaf)**
C. *C. colebrookianum****
Worms incubated in control medium showed physical activity as follows:
*10.50 ± 1.48 **13.84 ± 0.95 ***13.50 ± 1.05 h
[a]$p < 0.001$ compared with control groups.

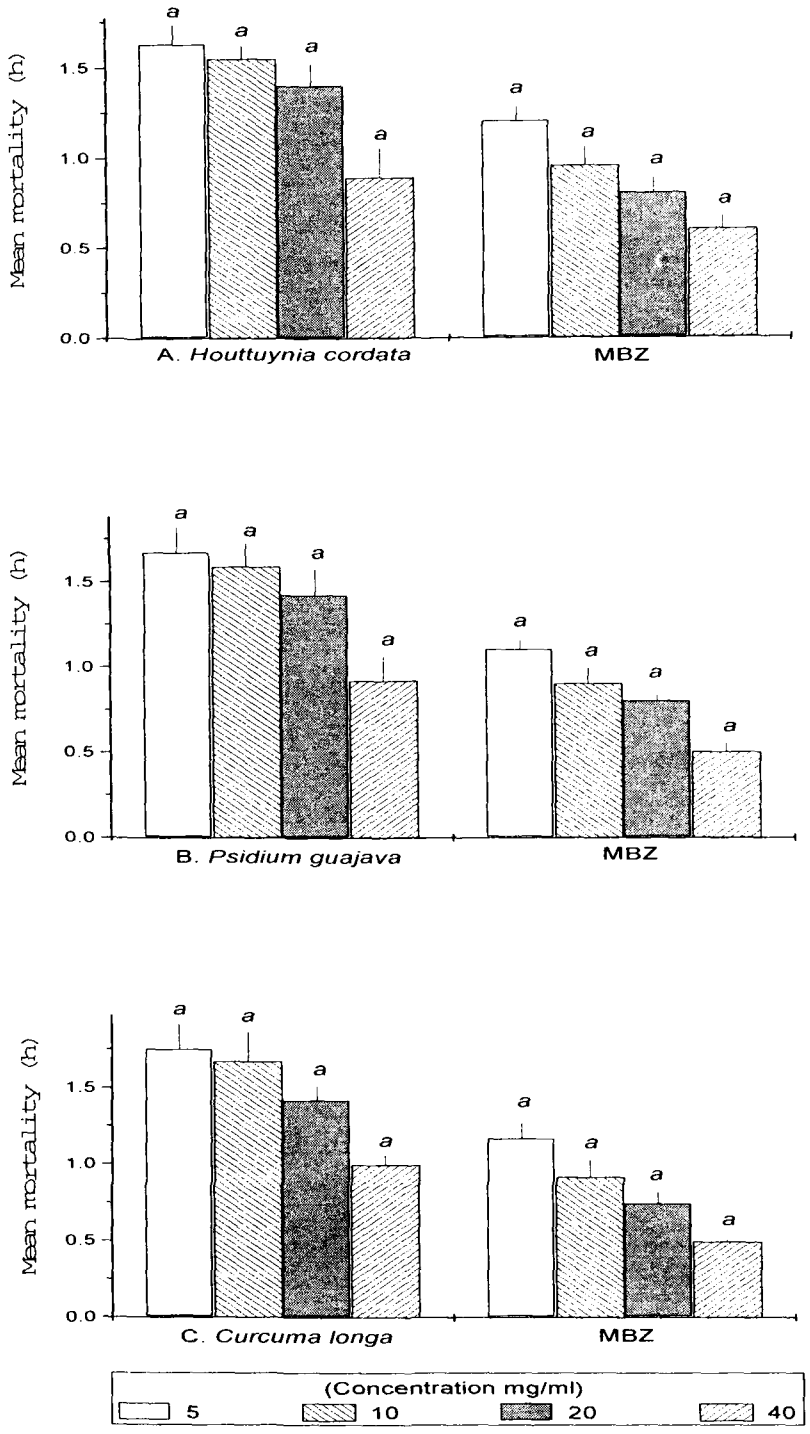


Figure 1.18 A-C: *In vitro* anthelmintic activity of plant extracts against *Trichinella spiralis*

A. *H. cordata** **B.** *P. guajava*** **C.** *C. longa**** Worms incubated in control medium showed physical activity as follows:
*10.00 ± 2.03 **10.67 ± 0.93 ***11.34 ± 1.65 h
[a]$p < 0.001$ compared with control groups.

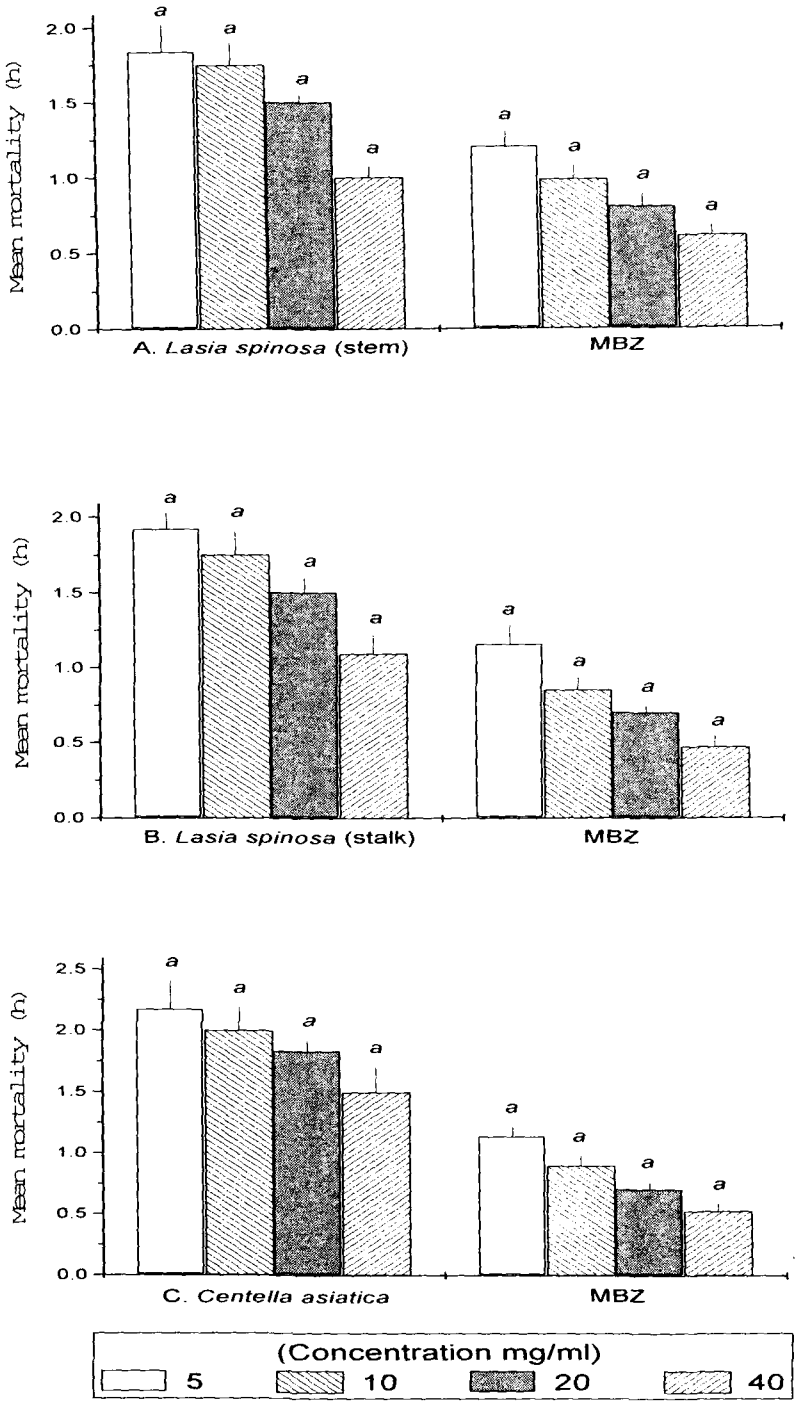


Figure 1.19 A-C: ***In vitro*** **anthelmintic activity of plant extracts against *Trichinella spiralis***

A. *L. spinosa* (stem)* **B.** *L. spinosa* (stalk)**
C. *C. asiatica****
Worms incubated in control medium showed physical activity as follows:
*13.84 ± 2.51 **11.25 ± 1.70 ***9.84 ± 0.88 h
$^{a}p < 0.001$ compared with control groups.

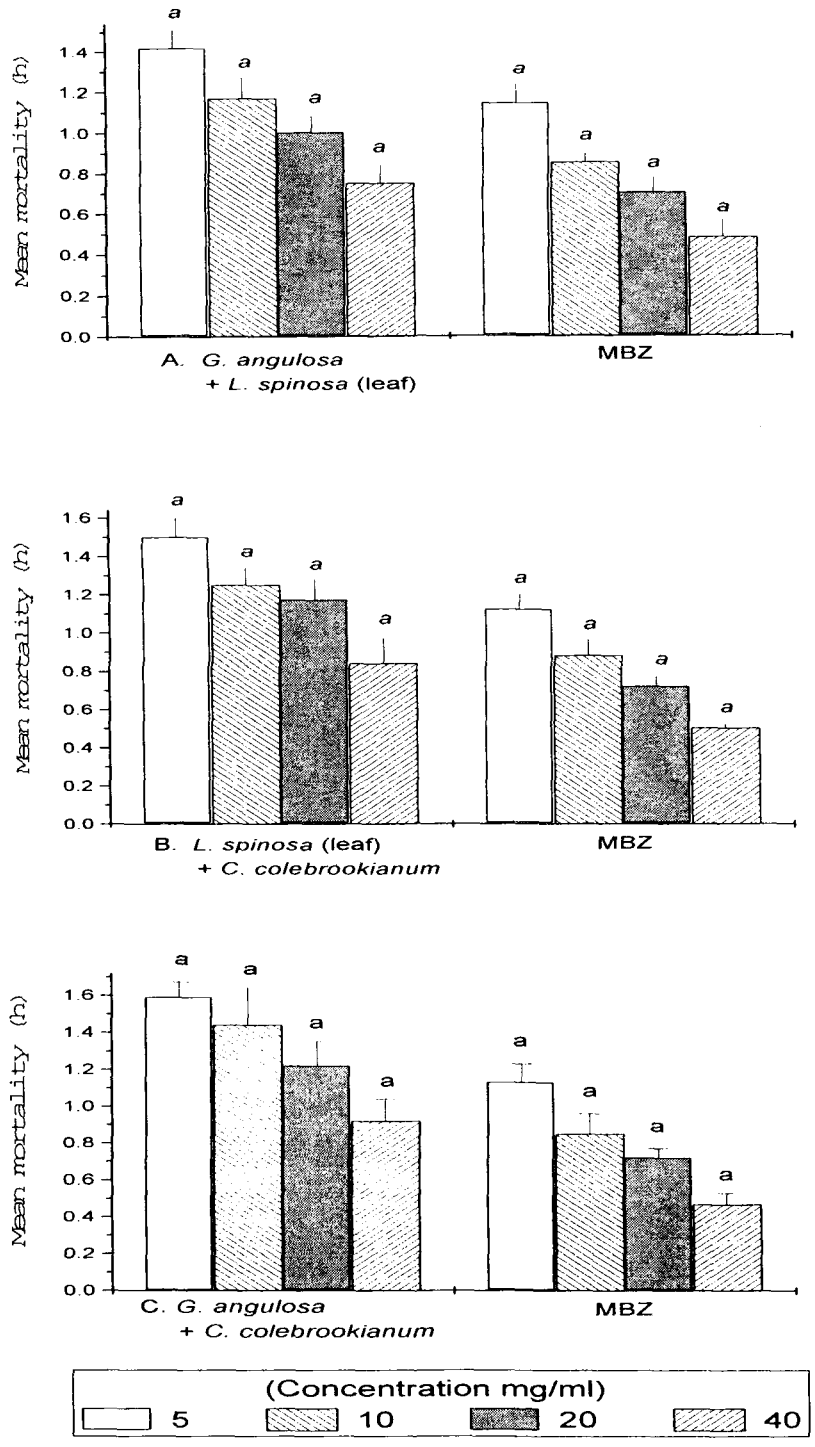


Figure 1.20 A-C: *In vitro* anthelmintic activity of combined plant extracts against *Trichinella spiralis*

A. *G. angulosa* + *L. spinosa* (leaf)* **B.** *L. spinosa* (leaf) + *C. colebrookianum*** **C.** *G. angulosa* + *C. colebrookianum**** Worms incubated in control medium showed physical activity as follows:
*11.67 ± 2.04 **12.17 ± 1.06 ***10.00 ± 1.38 h
$^{a}p < 0.001$ compared with control groups.

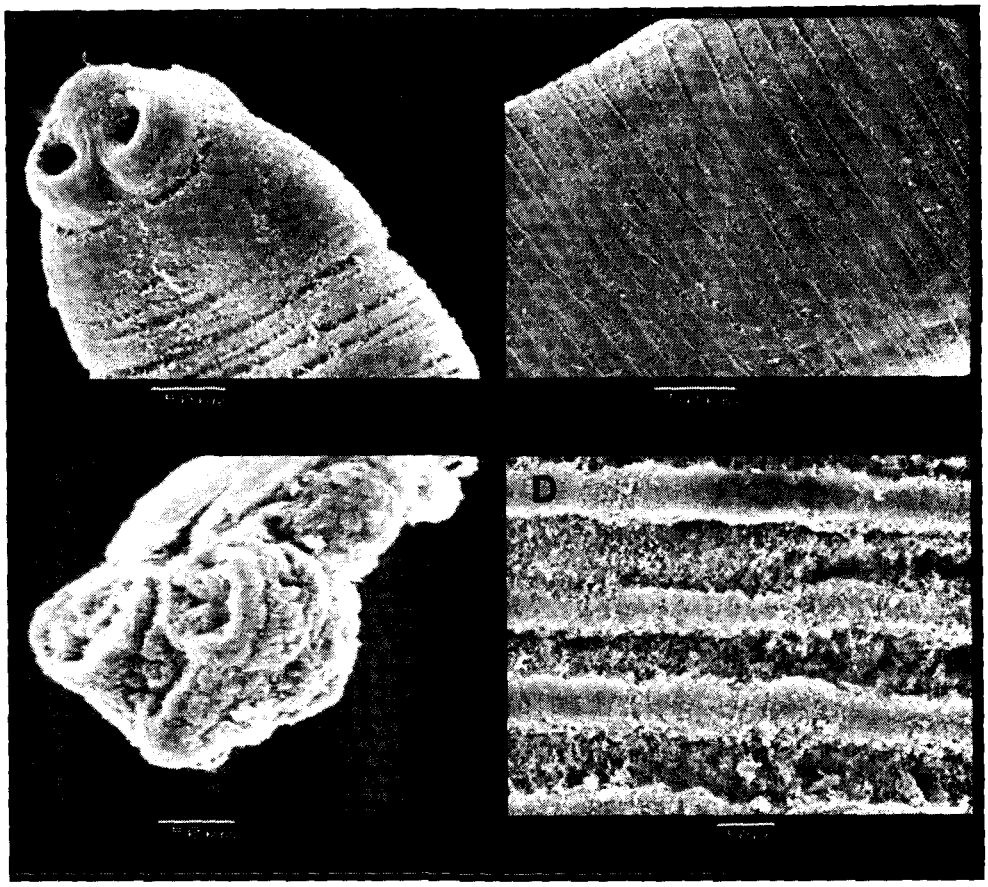
Observations on Surface Fine Topography

The effects of plant extracts were also studied on the surface fine topography of parasites with the help of scanning electron microscopy. The plant extracts chosen for this study were *L. spinosa* (leaf), *G. angulosa*, *P. guajava*, *H. cordata* and C. *colebrookianum* and the test parasites, *H. diminuta* and *G. crumenifer* and *A. galli.*

Scanning electron micrographs of the morphological organization of *H. diminuta* revealed a normal body contour on the control worm (Plate 1.3; Figs. A-B). In contrast, the cestode parasite treated with 40 mg/ml concentration of *P. guajava* extract showed irrevocable destruction over the general topography of the body (Plate 1.3; Figs. C-D). More or less similar kind of morphological changes were also observed in the worms treated with 40 mg/ml concentrations of *L. spinosa* leaf extract (Plate 1.4; Figs. A-B) and PZQ (Plate 1.4; Figs. C-D). The morphological organization of *G. crumenifer* worms belonging to control group showed normal morphology of tegument (Plate 1.5; Figs. A-B). However, the tegument of worms treated with *L. spinosa* leaf extract revealed extensive disorganization in its contour (Plate 1.5; Figs. C-D). The tegument of *C. colebrookianum* extracts treated worms showed moderate damage (Plate 1.6; Figs. A-B). In contrast, the tegument of *H. cordata* extract treated group of worms appeared to be normal when compared to control (Plate 1.6; Figs. C-D). The scanning electron microscopic observations on *A. galli* from control group

showed normal morphology of cuticle (Plate1.7; Figs. A-C). However, apparent damage and disorganization was noticed in the lips and cuticle of worms treated with *L. spinosa* and *G. angulosa* extracts (Plate 1.8; Figs. A-C and Plate1.9; Figs. A-C).

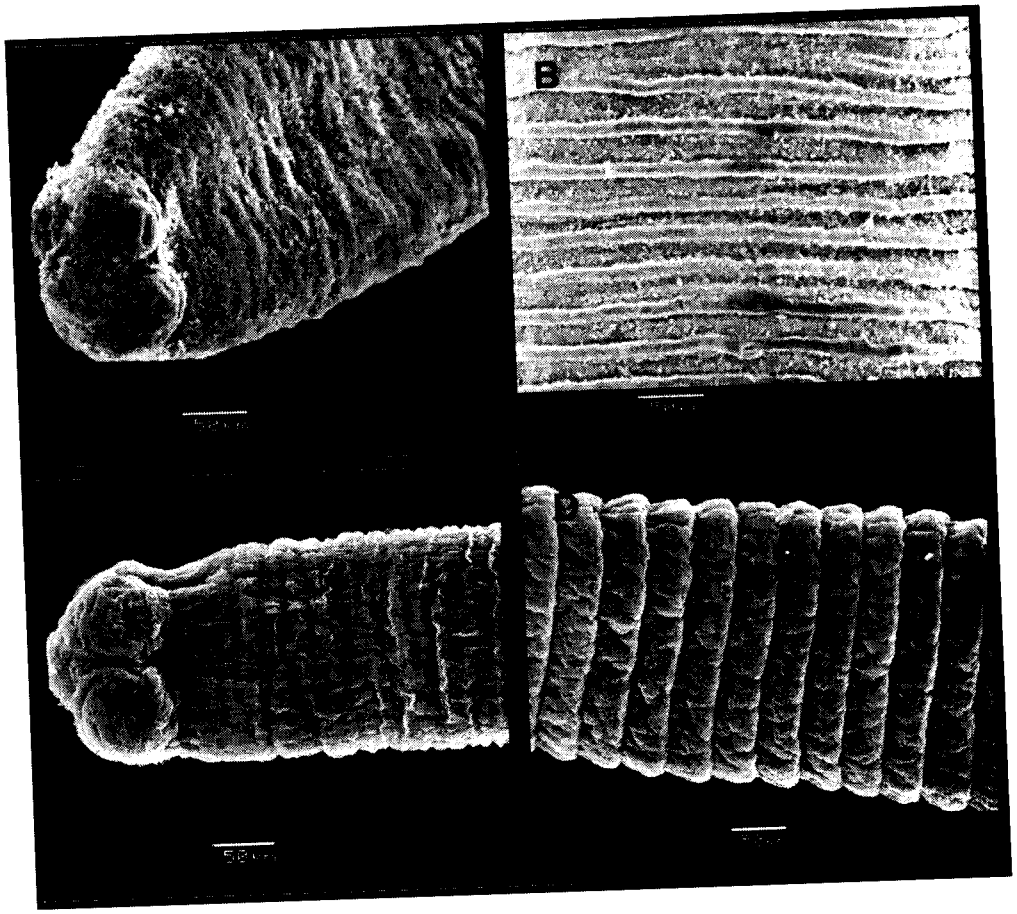
PLATE 1.3



Figs. A-D. Scanning electron micrographs of *Hymenolepis diminuta*: control/ plant extract treated (40 mg/ml) worms

Control:	A. Scolex	B. Tegumental surface of proglottid.
***P. guajava*:**	C. Scolex	D. Tegumental surface of proglottid.

PLATE 1.4



Figs. A-D. Scanning electron micrographs of *Hymenolepis diminuta*: PZQ/plant extract treated (40 mg/ml) worms

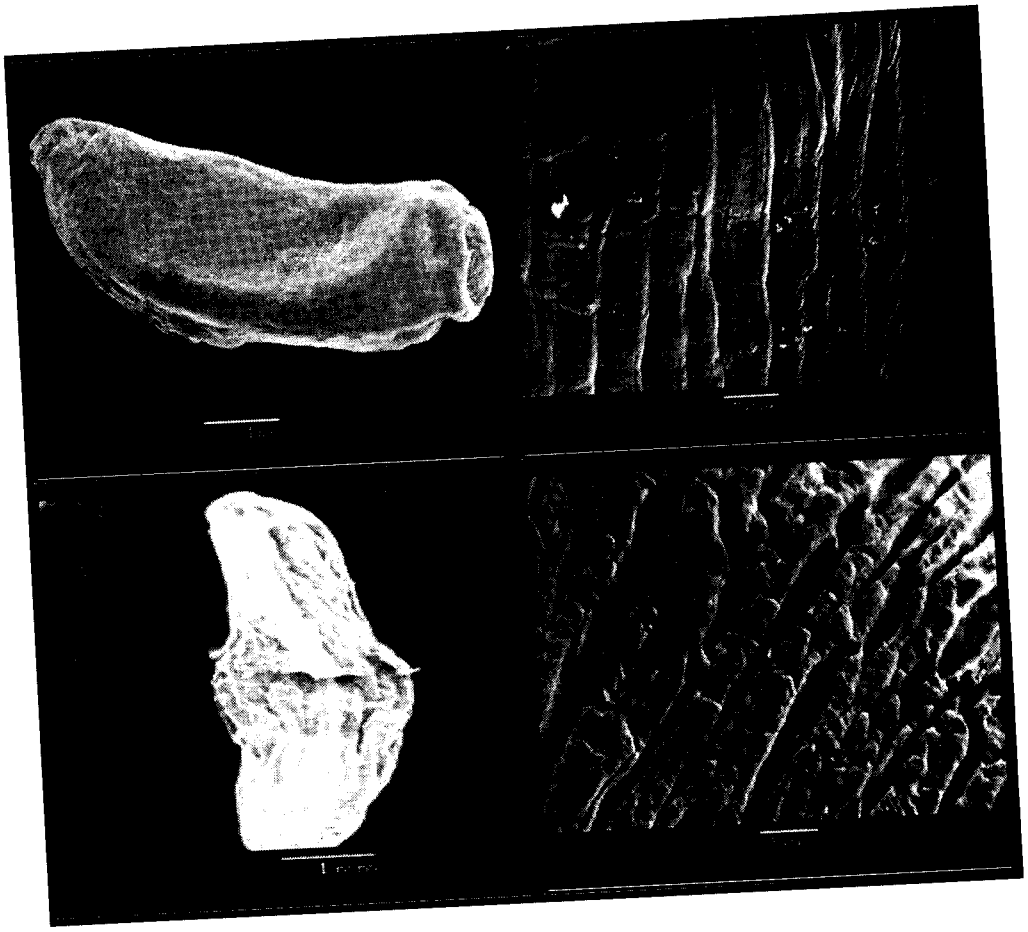
***L. spinosa* (leaf):**

A. Scolex.
B. Tegumental surface of proglottid.

PZQ:

C. Scolex.
D. Tegumental surface of proglottid.

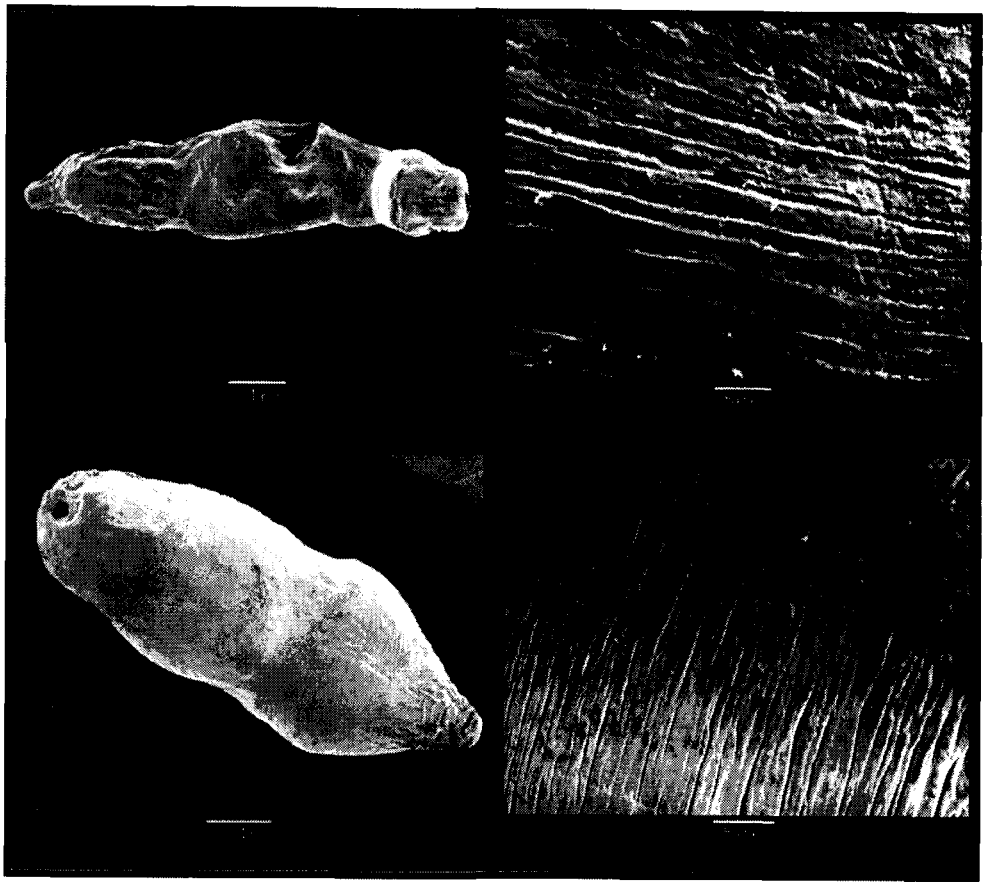
PLATE 1.5



Figs. A-D. Scanning electron micrographs of *Gastrothylax crumenifer*: control/plant extract treated worms (40 mg/ml)

Control:	A. Whole worm, ventral view. B. Body tegument, enlarged view.
***L. spinosa* (leaf):**	C. Whole worm, ventral view. D. Body tegument, enlarged view.

PLATE 1.6

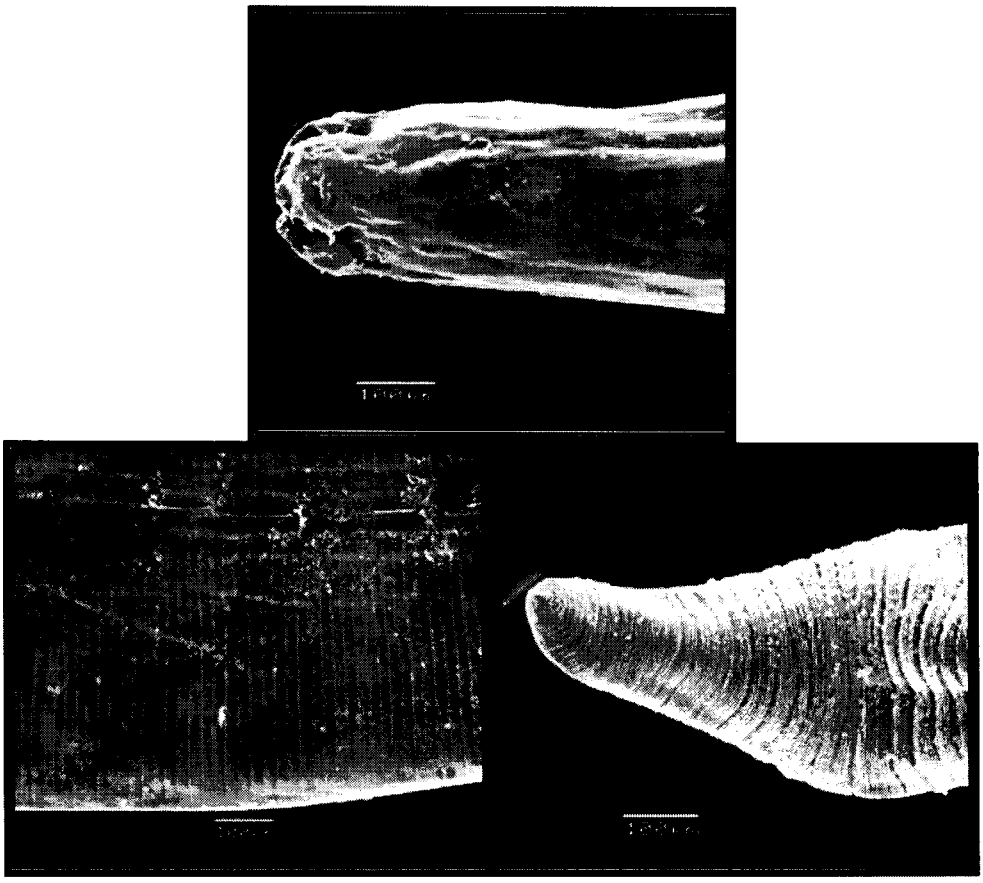


Figs. A-D. Scanning electron micrographs of *Gastrothylax crumenifer*: plant extract treated (40 mg/ml) worms

***C. colebrookianum*:** A. Whole worm, ventral view.
B. Body tegument, enlarged view.

***H. cordata*:** C. Whole worm, ventral view.
D. Body tegument, enlarged view.

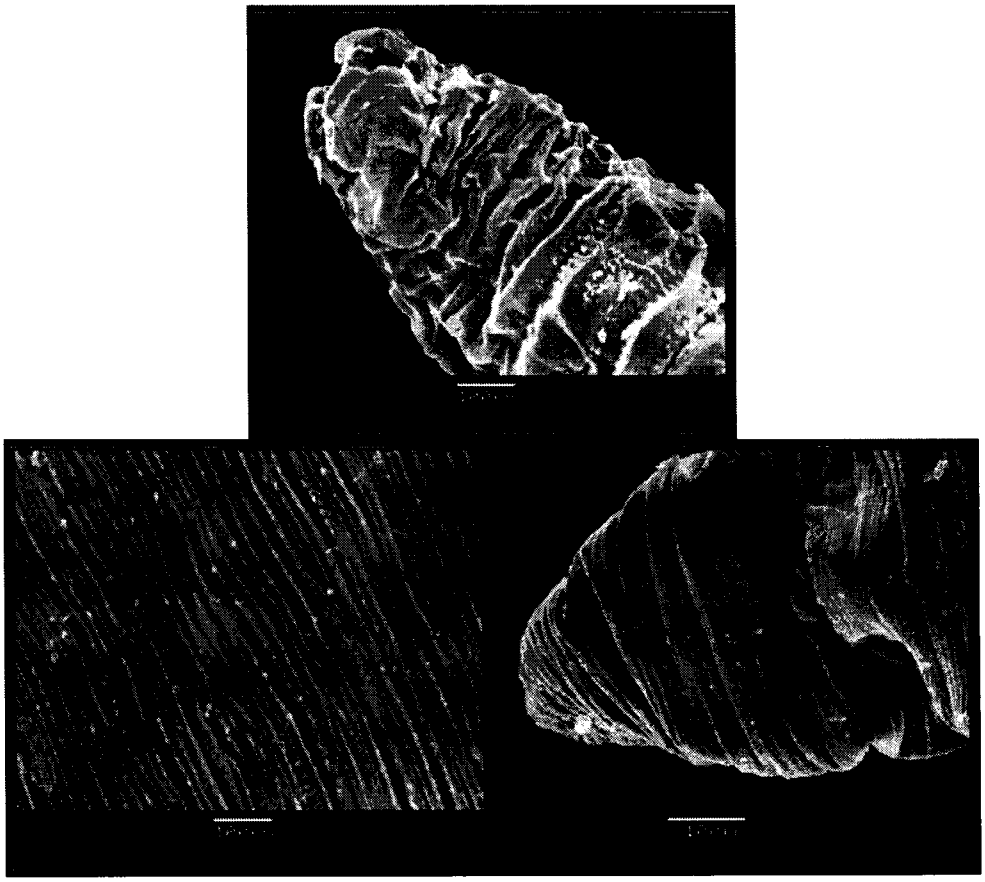
PLATE 1.7



Figs. A-C. Scanning electron micrographs of *Ascaridia galli*: worms maintained in control medium.

A. Anterior end of body.
B. Middle region of body.
C. Posterior end of body.

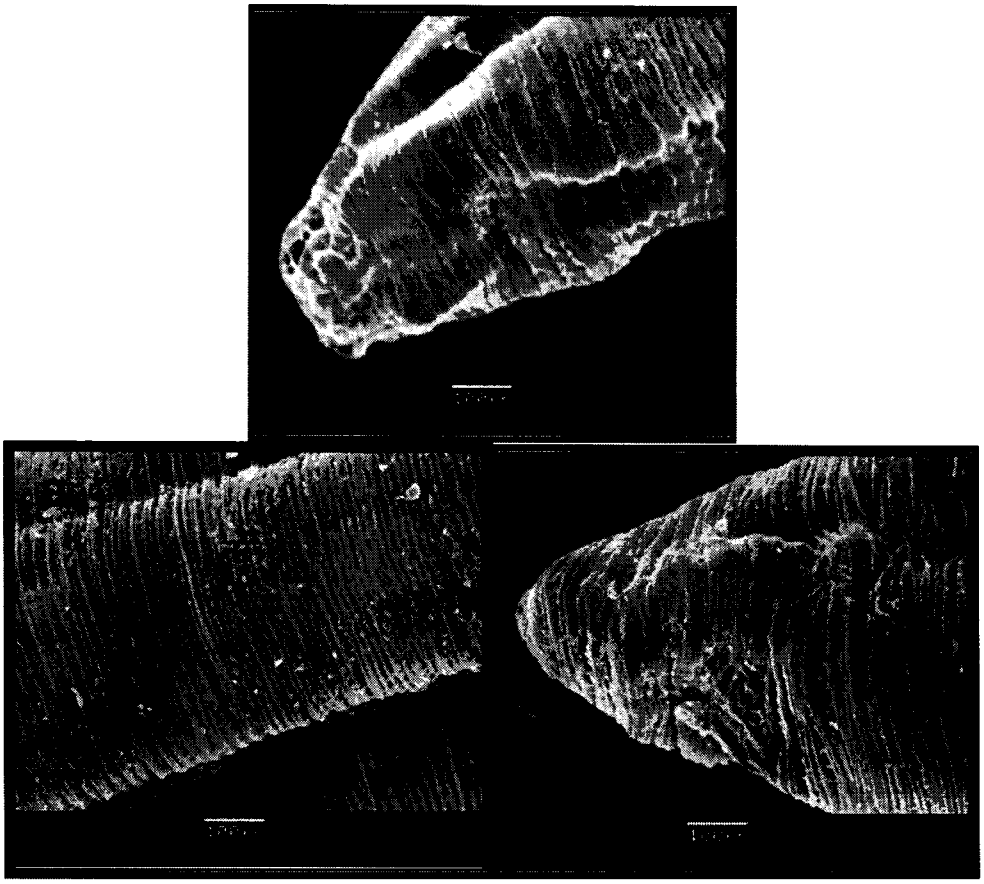
PLATE 1.8



Figs. A-C. Scanning electron micrographs of *Ascaridia galli*: *L. spinosa* (leaf) extract (40 mg/ml) treated worms

A. Anterior end of body.
B. Middle region of body.
C. Posterior end of body.

PLATE 1.9



Figs. A-C. Scanning electron micrographs of *Ascaridia galli*: *G. angulosa* extract (40 mg/ml) treated worms

A. Anterior end of body.
B. Middle region of body.
C. Posterior end of body.

Discussion

The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in tropical developing countries, including India. The rich floral diversity of India has provided traditional practitioners in the country with an impressive pool of 'natural pharmacy' from which plants are selected as ingredients to prepare herbal remedies to treat various diseases. The north-eastern region of India is inhabited by approximately 130 tribes, including the Naga tribes which reside in the Nagaland state. These tribes have a good faith in their traditional medicine system and thus they use many plant based medicines to cure various ailments, including intestinal helminthic infections. However, the purported efficacies of these plants, by and large, have not been scientifically evaluated. The present study was aimed at investigating the anthelmintic activity of few such commonly used plants in the Naga tribes using representative group of helminths as test parasites. It is hoped that this study would authenticate and scientifically valididate the anthelmintic activity of these plants, if any, as claimed by local people.

In the present study the anthelmintic efficacy of various plant extracts was adjudged by monitoring the mortality of the test parasites following exposure to serial concentrations of extracts. For comparison sake the test parasites were also exposed to corresponding concentrations of a reference drug in each case. Many previous workers have commonly used this criterion to base their studies

on anthelmintic efficacy of plant extracts (Tandon *et al.*, 1997; Tangpu and Yadav, 2003; Tandon *et al.*, 2004; Yadav and Tangpu, 2006b; Roy *et al.*, 2008). The present study revealed that not all seven plants/plant parts that were put to investigation possess significant anthelmintic efficacy. Further, the efficacy of plant extracts was also found to vary from one group of helminth parasite to another group. Of the seven plant extract tested, *P. guajava*, *H. cordata*, *L. spinosa* (stalk and leaf), *G. angulosa* and *C. colebrookianum* showed considerable anthelmintic efficacy. However, a moderate level of anthelmintic efficacy was observed for *L. spinosa* (stem), *C. asiatica* and *C. longa.* With respect to various helmintic groups, the study revealed that the leaf extract of *P. guajava*, *H. cordata* and stalk of *L. spinosa* possess profound efficacy against the cestode parasite, *R. echinobothrida.* The leaf extract of *P. guajava*, *L. spinosa* and *G. angulosa* manifested appreciable anticestodal efficacy against *H. diminuta*. Of different plant extracts tested against *G. crumenifer*, leaves of *L. spinosa*, *C. colebrookianum* and *H. cordata* showed good flukicidal efficacy. In case of roundworm *A. galli* only the leaf extract of *L. spinosa* was found to possess promising anthelmintic activity. Lastly, against *T. spiralis* worms the leaf extracts of *G. angulosa*, *L. spinosa*, *C. colebrookianum*, *H. cordata* and *P. guajava* revealed significant activity. The individual plant extracts showing significant efficacy were further tested in combination with other extracts to investigate whether they could have any synergistic effects on mortality of parasites. No substantial increase in the anthelmintic efficacy of extracts was observed in such investigations.

The present study revealed that *P. guajava* leaf extract possesses significant efficacy against *R. echinobothrida*, *H. diminuta* and *T. spiralis*. In case of its efficacy against *R. echinobothrida*, both 20 and 40 mg/ml concentrations of plant extract revealed the mortality of parasites in 1.00 h as compared to reference drug which at corresponding concentrations revealed the mortality of parasites at 1.34 and 0.84 h, respectively. The worms maintained in the control medium showed physical activity till 69.33 h. However, against *H. diminuta*, the 40 mg/kg dose of *P. guajava* extract showed mortality of worms in 2.34 h, whereas the reference drug showed the mortality as early as in 0.50 h. Of the different test parasites, the plant extract showed the maximum anthelmintic efficacy against *T. spiralis*. In this case the 40 mg/ml concentration of extract resulted into mortality of *T. spiralis* worms in as early as in 0.92 h, compared to the reference drug which at the same concentration showed mortality of parasites in 0.50 h. Unlike the previously mentioned test parasites, the *P. guajava* extract did not show significant level of anthelmintic efficacy against *G. crumenifer* and *A. galli*. *P. guajava* has been reported to possess several medicinal uses *viz*., antidiarrhoeal (Lutterodt, 1989), anticough (Jairaj *et al*., 1999), antiamebic, antispasmodic (Lozoya *et al*., 2002; Tona *et al*., 2000), and antimicrobial activity (Qadan *et al*., 2005). The previously known chemical constituents from the plant include, ascorbic acid (Nogueira *et al*., 1978), fatty acids (Opute, 1978), tannins, phenols, triterpenes, essential oils, saponins (Cuellar *et al*., 1984) carotenoids (Mercadante *et al*., 1999) and lectins (Coutino-Rodriguez *et al*., 2001). This study forms the first report of presence of *in vitro*

anthelmintic efficacy in the leaf extract of plant against *R. echinobothrida*, *H. diminuta* and *T. spiralis*.

The plant *H. cordata* has been reported to possess antiviral, antibacterial, and antileukemic activity (Hayashi *et al.*, 1995; Kim *et al.*, 2001; Chang *et al.*, 2001; Chiang *et al.*, 2003). In local traditional medicine of Naga tribes, besides its anthelmintic use the juice of the plant is also drunk in the treatment of ulcers. It is also used as an insect repellent. In the present study the *H. cordata* extract showed significant *in vitro* anthelmintic efficacy against *R. echinobothrida*, *G. crumenifer* and *T. spiralis*. The tapeworm, *R. echinobothrida* treated with the highest concentration (40 mg/ml) of *H. cordata* extract showed mortality of worms within 2.00 h, compared to the standard drug which at the same concentration showed mortality in 0.82 h. The efficacy of plant extract was recorded to be slightly lower against *G. crumenifer,* wherein its 40 mg/ml concentration caused mortality of worms in 3.00 h. The efficacy of extract was found to be most evident against *T. spiralis*, wherein the highest concentration of extract (40 mg/ml) showed mortality of worms in as early as in 0.89 h. The standard drug, MBZ in this case at its corresponding concentration revealed the mortality of parasites in 0.60 h. The anthelmintic efficacy of plant, however, was recorded to be moderate against *H. diminuta* and *A. galli*. Essential oils and alkaloids have been reported to be the main chemical constituents of *H. cordata* (Lu *et al.*, 2006; Tutupalli *et al.*, 1975; Kim *et al.*, 2001). The anthelmintic efficacy of plant may be due to the presence of these chemical constituents, as they

have been implicated to act as anthelmintic in other studies as well (Akhtar, 2000).

As for the *in vitro* anthelmintic efficacy of *L. spinosa* leaf extract is concerned, the results of the present study suggest that barring *R. echinobothrida* the extract showed profound anthelmintic efficacy against the rest four test parasites, *i.e.*, *H. diminuta*, *G. crumenifer*, *A. galli* and *T. spiralis*. Against *H. diminuta* the plant extract at its 20 and 40 mg/ml concentrations showed mortality of worms as early as in 2.92 and 2.50 h. The reference drug PZQ, however revealed rather an early mortality of parasites, 0.89 and 0.60 h, respectively. *G. crumenifer* exposed to 40 mg/ml concentration of plant extract revealed the mortality of worms in 2.09 h which was almost comparable to PZQ which showed mortality of flukes in 2.00 h at the same concentration. For *A. galli* the plant extract at 40 mg/ml concentration showed mortality of worms in 14.00 h. Worms exposed to the standard drug however showed considerably faster acquisition of mortality (8.84 h at 40 mg/ml concentration). *A. galli* worms maintained in the control medium showed physical activity till a period of 154.00 h. In case of *T. spiralis* at 40 mg/ml concentration the mortality of worms caused by the plant extract and reference drug was almost comparable and noted to be 0.72 and 0.50 h, respectively. *L. spinosa* which commonly grows surroundings ponds is consumed as traditional food in several communities of South-east Asia. The Naga tribes in their traditional medicine consume the porridge (pudding) of leaves plant to expel the intestinal-worms. Very scanty information

is available in the literature regarding chemical compounds of plant. Hồng Vân *et al.* (2005) reported three benzaldehyde derivatives, 2-(4'- methoxyphenyl) ethanol and adenine as chemical constituents of plant. The stalk extract of *L. spinosa* was also evaluated for anthelmintic efficacy in the present study and showed good efficacy only against *R. echinobothrida*. Exposure of tapewoms to the highest concentration of plant extract showed mortality of worms in as early as in 2.00 h, which was almost comparable (2.38 h) with that of the lowest concentration of the standard anticestodal drug. The stalk extract of *L. spinosa* however, showed moderate level of anthelmintic efficacy against *H. diminuta*, *G. crumenifer* and *A. galli*. At 20 and 40 mg/ml of extract, against *H. diminuta*, the mortality was recorded in 6.00 and 5.59 h. PZQ at the similar concentrations exhibited mortality time of 0.90 and 0.60, h respectively. Similar trends were observed for its anthelmintic efficacy against *G. crumenifer* and *A. galli.* The stem extract of *L. spinosa* was not found be as effective as leaf and stalk extract. The stem extract revealed only moderate efficacy against *G. crumenifer*, *A. galli* and *T. spiralis*. The extract, however was less effective against *R. echinobothrida* and *H. diminuta*.

In the present study the *G. angulosa* extract showed prominent anthelmintic activity only against *H. diminuta* and *T. spiralis*. The plant extract at 40 mg/ml concentration showed mortality of *H. diminuta* worms in 2.92 h compared to PZQ which showed mortality of parasites in 0.60 h at the same concentration. Similarly, for *T. spiralis* the plant extract showed almost

comparable efficacy with that of reference drug, MBZ. In this case the mortality of worms occurred at 0.67 h and 0.50 h at 40 mg/ml concentrations of plant extract and MBZ, respectively. Unlike, the two previous tested parasites, the extract showed moderate efficacy against *A. galli* and insignificant efficacy against *R. echinobothrida* and *G. crumenifer*. It may be mentioned here that the decoction of plant is used by Naga tribes as common deworming remedy. There is no scientific information available in the literature regarding chemical constituents or biological activity of the plant. Further studies thus seem desirable for the identification of active principles of plant.

The leaf decoction of *C. colebrookianum* is popularly consumed by Naga tribes in their traditional medicine with the belief that it works against worm infections and malarial fevers. In the present study, it showed significant level of efficacy against two test parasites, *i.e.*, *G. crumenifer* and *T. spiralis*. At the 40 mg/ml concentration the efficacy of both the plant extract and reference drug was comparable against *G. crumenifer*. The mortality time of parasites at this concentration was recorded to be 2.50 h and 2.10 h, respectively. The flukes maintained in the control medium showed physical activity till 47.67 h. With regard to the testing of plant extract against *T. spiralis*, the extract also showed an efficacy as good as shown by reference drug MBZ. The *C. colebrookianum* extract, however did not show notable efficacy against *R. echinobothrida*, *H. diminuta* and *A. galli*. Five steroids colebrin A-E, Colebroside and a new

diglucoside has been reported to occur in the aerial parts of plant (Yang *et al.*, 2000a; Yang *et al.*, 2000b).

In the present study *C. asiatica* leaf extract showed moderate level of efficacy against *R. echinobothrida*, *G. crumenifer* and *A. galli* and rather insignificant efficacy against *H. diminuta* and *T. spiralis*. *C. asiatica* has been reported to possess biologically active constituents, such as asiatic acid, madecassic acid, ursane and oleanane-type triterpene oligoglycosides, centellasaponins madecassoside and sceffoleoside (Brinkhaus *et al.*, 2000; Matsuda *et al.*, 2001; Hong *et al.*, 2005). The leaf extract of *C. asiatica* has been reported to possess wound healing (Shetty *et al.*, 2006), antibacterial (Zaidan *et al.*, 2005) and antioxidant properties (Gnanapragasam *et al.*, 2007).

Unlike other tested plant extracts, *C. longa* extract did not show anthelmintic efficacy worth pursuing further. The rhizome extract of *C. longa* showed somewhat moderate efficacy against *H. diminuta* and *T. spiralis* but no significant activity against *R. echinobothrida*, *G. crumenifer* and *A. galli.* Curcumin and volatile oils have been reported to be the principal ingredients of rhizomes of *C. longa* (Xia *et al.*, 1999; Cui *et al.*, 2006). Curcumin is well known to act as anticarcinogenic, antioxidative, hypocholesterolemic and antidepressant (Peschel *et al.*, 2007; Xia *et al.*, 2006).

The parasites' tegument/cuticle has been implicated among one of the several target sites by which natural anthelmintic products or synthetic drugs act (Tandon *et al.*, 1997; Martin *et al.*, 1997; Mehlhorn *et al.*, 1983; Alvarez *et al.*, 2006; Roy *et al.*, 2007). It has been reported that anthelmintic drugs enter inside the target parasites by oral ingestion or by diffusion through the external surface (Thompson *et al.*, 1993; Thompson and Geary, 1995). Studies made in past report the effect of natural/synthetic anthelmintics on parasites' body surface (Sirisinha *et al.*, 1984; Grzywacz, 1980; Roy and Tandon, 1996; Stitt and Fairweather, 1993; Xu-lin *et al.*, 1994; Anderson and Fairweather, 1995). In the present study effects of selected plant extracts were studied on parasite body surface so as to provide some clues regarding their plausible mode of action. In the present study, barring *H. cordata* the extracts of other plants, namely - *L. spinosa*, *G. angulosa*, *P. guajava* and *C. colebrookianum* showed such morphological changes and damage to the parasite body surface. The tegument of *H. diminuta* and *G. cruminefer* showed destruction in the form of erosion on all over the general topography of the body. In case of *Hymenolepis* the scolex also showed apparent damage. Similarly, for *A. galli* the SEM of extract treated worms revealed wrinkles and cracks on lips and body cuticle. Grzywacz (1980) also observed such changes in the cuticle of *A. suum* following treatment with piperazine. The tegument or cuticle of helminthes is considered to be metabolically active and morphologically specialized interface to perform selective absorption of nutrients. Therefore, the trans-cuticular or

transtegumental passive diffusion could be the mode of action of these plant extracts.

In conclusion, this study authenticates the presence of significant anthelmintic activity in many of the above mentioned investigated plants which are used in the traditional medicine system of Naga tribes as anthelmintics. Further investigations, however need to be carried out to isolate, identify and ascertain the constituent agents responsible for the anthelmintic efficacy of plants.

CHAPTER 2

In vivo anthelmintic efficacy of folklore medicinal plants

Introduction

As discussed in the previous chapter, gastrointestinal helminths represent a class of important parasites, some of which can cause serious diseases in humans and other mammalian hosts. For centuries, medicinal plants have been used to combat parasitism, and in many parts of the world are still used for this purpose (Giday *et al.*, 2003). However, as discussed previously most of these uses are in lack of scientific support or meaningful evidence. Therefore, in addition to *in vitro* testing of plant extracts (discussed in Chapter 1), from time to time workers have also used several host - parasite systems to investigate and/or further substantiate the anthelmintic efficacy of medicinal plants.

A review of literature reveals that great variety of animal models and methods exist to test the anthelmintic properties of plants. The hookworm, *Necator americanus* infections maintained in golden hamsters were used to evaluate the anthelmintic efficacy of 'Diospyrol' from *Diospyros mollis* (Sen *et al.*, 1974). Rats experimentally infected with Chinese liver fluke, *Clonorchis sinensis* has been used to monitor the therapeutic efficacy of praziquantel (Rim *et al.*, 1980). Maki and Yanagisawa (1983) employed *Hymenolepis nana* - mice model to evaluate the effects of alcoholic extract from *Diospyros mollis*, a shrub,

popularly known as Ma-Klua in Thailand. Ibrahim *et al.* (1984) studied 18 plants traditionally used for the treatment of animal and human helminthiasis in Nigeria for anthelmintic activity using the *Nlippostrongylus* - rat model. Comparative study of the anthelmintic effects of bithionol, paromomycin sulphate, febendazole and mebendazole on immature and mature *H. nana* in mice has been carried out by Maki and Yanagisawa (1985). The anthelmintic efficacy of *A. anthelmintica* and *A. lebbek* extracts was established following their testing in *H. diminuta* - rat model (Galal *et al.*, 1991a; Galal *et al.*, 1991b). *Zingiber officinale* extract tested against experimentally induced *Setaria cervi* infections in rats showed significant antifilarial activity (Ghosh *et al.*, 1992). Li *et al.* (1992) employed *T. spiralis* - rat model to evaluate the effects of mebendazole and albendazole against different developmental stages of parasite. Fan and Ito (1995) reported the minimum effective dose of PZQ using *H. diminuta* - rat experimental model. A high efficacy of papaya latex against experimental *Heligmosomoides polygyrus* infections has been reported by Satrija *et al.* (1995). Surin (1995) investigated the activity of 16 compounds against the immature larval *T. spiralis* infections in mice. Ghosh *et al.* (1996) reported the cestocidal efficacy of *Acacia auriculiformis* in *H. diminuta* - rat model. Bogh *et al.* (1996) reported the anthelmintic efficacy of extracts of *Embelia schimperi* against *Echinostoma caproni*, *H. polygyrus* and *H. microstoma* in mice and also against *H. diminuta* in rats. Lopez-Garcia *et al.* (1997) made a comparative study of albendazole and ricobendazole in a mouse model experimentally infected with *T. spiralis*. The stem bark extract of *Berlinia grandiflora* has been

reported to possess anthelmintic efficacy based on its testing against *N. brasiliensis* infections maintained in albino rats (Enwerem *et al.*, 2001b). The anthelmintic efficacy of *Leucana leucocephala* infusion has been ascertained using experimental *H. nana* infections in mice (Kustiawan, 2001). Githori *et al.* (2003a and 2003b) evaluated the anthelmintic properties of *Albizia anthelmintica* extracts against *H. polygrus* infections in mice. The anthelmintic properties of Vimang, an aqueous extract of *Mangifera indica* family stem bark and mangiferin, the major polyphenol present in Vimang, were investigated in the experimentally induced *T. spiralis* infections in mice (Garcia *et al.*, 2003). Bany *et al.* (2003) reported the effect of Alchinal, a complex preparation of three substances - *Echinacea purpurea* extract, *Allium sativum* extract, cocoa, on the development of *T. spiralis* in mice. Quinolines that exhibited good activity *in vitro* have been studied *in vivo* on *T. spiralis* in mice model (Martinez-Grueiro *et al.*, 2005).

H. diminuta - rat model has also been used to test the anticestodal properties of *Gladiolus gandavensis*, *Trifolium repens*, *Strobilanthes discolor* and *Butea minor* (Saha *et al.*, 1999; Tangpu *et al.*, 2004, 2006; Yadav and Tangpu, 2006a). Sukul *et al.* (2005) investigated the activity of potentized homeopathic drugs such as Cina 30, Santonium 30 and podophyllum mother tincture against the muscle phase of *T. spiralis* in mice. Kozan *et al.* (2006) reported the anthelmintic activity of some plants used in Turkish folk medicine in *Syphacia obvelata* and *Aspiculuris tetraptera* - mice models. Mice infected with adult *Trichuris muris*, a rodent gastrointestinal nematode, were used to examine

the anthelmintic efficacy of plant cysteine proteinases of *Carica papaya* (Stepek *et al.*, 2006). In another study, Stepek *et al.*, (2007) reported the effects of cysteine proteinases of *C. papaya* against *Protospirura muricola* in rodent model.

Among several *in vivo* models used for evaluating the anthelmintic efficacy of natural compounds/drugs, extensive laboratory investigations have been carried out on *Hymenolepis* species maintained in rodent hosts (Saha *et al.*, 1999; Tangpu *et al.*, 2004, 2006; Yadav and Tangpu, 2006a). This has been of special relevance with respect to the development of anticestodal chemotherapeutic agents, and also for studies on cestode metabolism with *H. diminuta* being the best - characterized system (Siles-Lucas and Hemphill, 2002). *H. diminuta* (Family: Hymenolepididae), a cosmopolitan parasite of rats, completes its life cycle (Fig. 2.1) involving beetles (*Tribolium* spp.) acting as intermediate hosts. The parasite eggs ingested by beetles develop into cysticercoid larvae. Rodents acquire the infection by ingesting the arthropods, containing the cysticercoids larvae. Humans, usually children can also be accidentally infected through the same mechanism.

Trichinella is a widely spread zoonosis acquired by ingestion of undercooked meat containing larvae of the parasite (Cheng, 1986). Due to its typical life cycle (Fig. 2.2) which passes through all phases of development (adult, migratory and encysted stage) in a single host, and due to its capacity to infect a wide variety of mammalian hosts, *T. spiralis* (Family: Trichinellidae) has

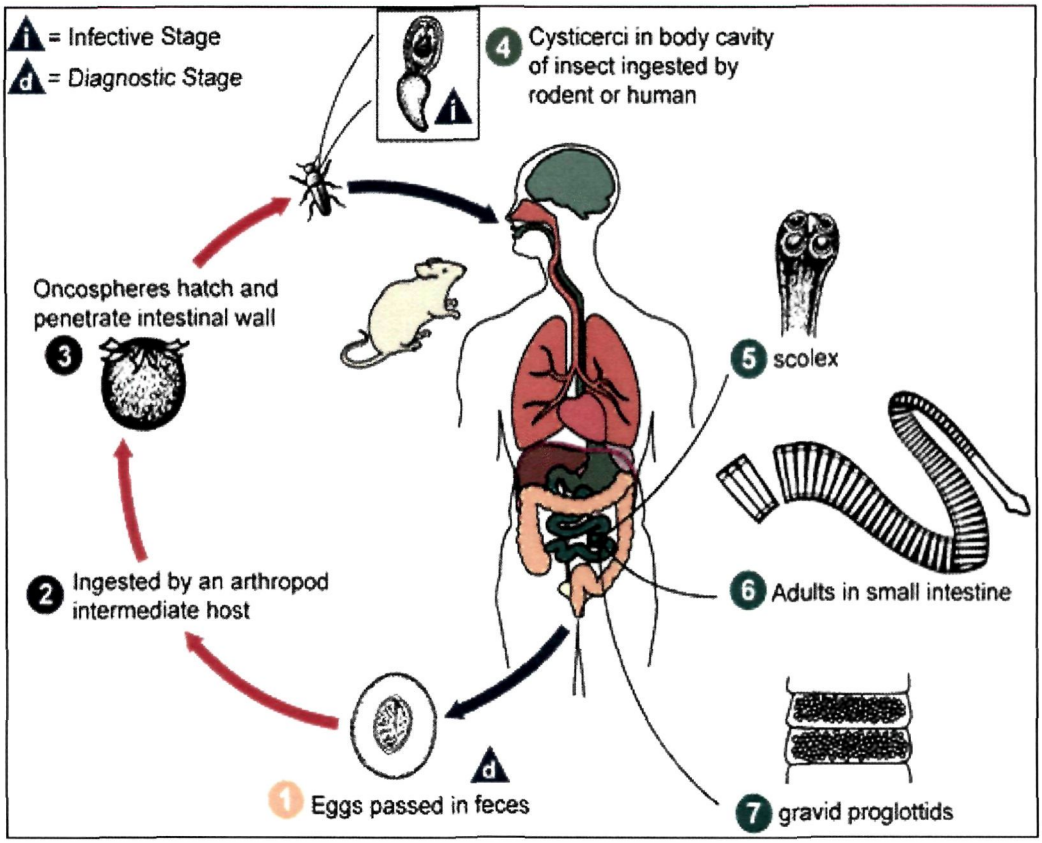


Fig. 2.1

Diagrammatic life cycle of *Hymenolepis diminuta* showing that two hosts are required to complete its development. The eggs when ingested by the beetles (intermediate host) develop into cysticercoids, (infective larval stage). The larvae when picked up by the definitive host, a rat (man is an accidental host), develop into adult parasites, and the gravid adults eliminate eggs through faeces of the host (Arai, 1980).

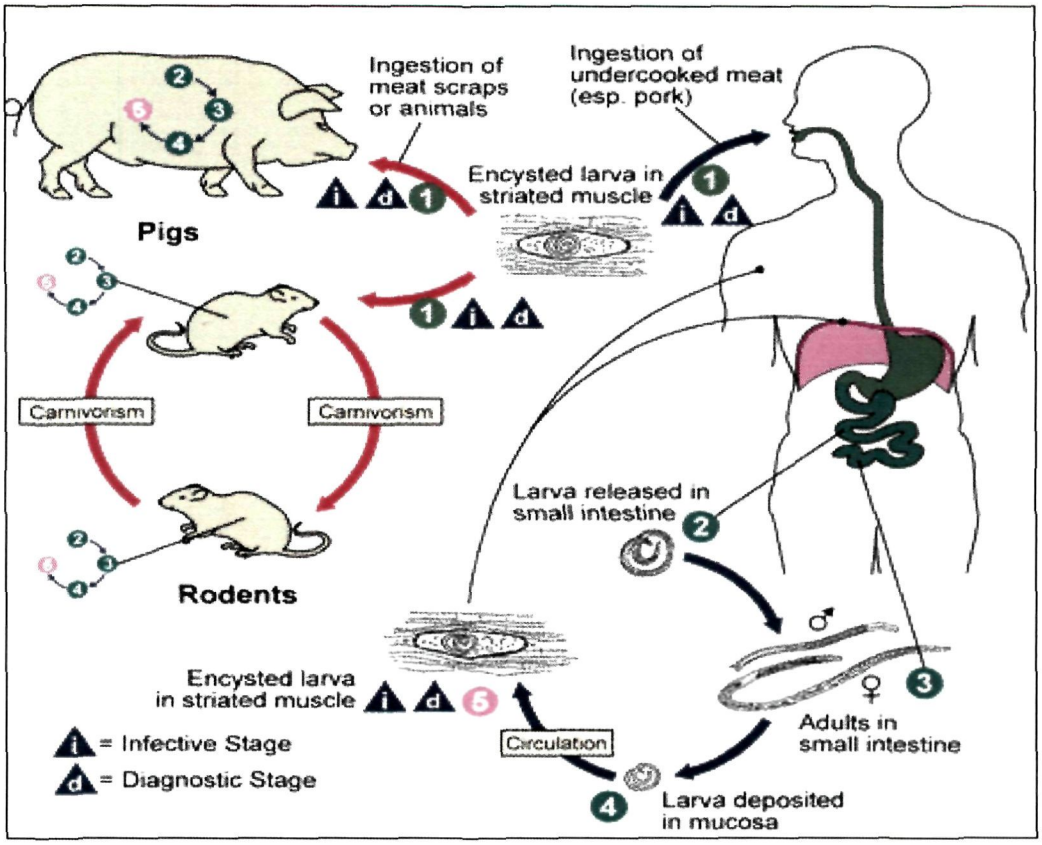


Fig. 2.2

Diagrammatic life cycle of *Trichinella spiralis* showing that it completes its larval and adult life in the same host which can be rodents, pigs or even humans. Trichinellosis is acquired by ingesting meat containing L_1. Inside the host the larvae are released from the cysts and invade the bowel mucosa where they develop into adults. The females release larvae which migrate to the striated muscles where they encyst. (Despommier, 1983).

also been employed as a suitable experimental model to evaluate the efficacy of several anthelmintic agents (Surin, 1995; Lopez-Garcia *et al.*, 1998; Bany *et al.*, 2003; Sukul *et al.*, 2005).

The investigations reported in the previous chapter dealt with evaluating the *in vitro* anthelmintic efficacy of seven plants (Table 1.1) that are used in the traditional medicine system of Naga tribes. The aim of the present study was to evaluate the *in vivo* anthelmintic efficacy of these plants in *H. diminuta* - rat and *T. spiralis* - mouse experimental models. It is hoped that testing of these plant extracts in respective animal models would provide additional information about the anthelmintic potentials of these medicinal plants.

Materials and Methods

Drugs and Chemicals:

Praziquantel [(Distocide®) Shin Poong Pharm. Co., Ltd., Seoul, Korea] and Mebendazole [MEDITAB Specialists Pvt. Ltd., Goa] were the standard references drugs used in the study. Trichlorfon (Accustandard, Inc., USA), Atropine Sulphate (Regain Labs, India), Pepsin, Chloroform and HCL (S.D. Fine-Chemicals Limited, India) were also employed in the study. Plant extracts and the drug solutions were prepared fresh in PBS before the start of *in vivo* experiments.

Reagents

I. **0.9% Phosphate Buffered Saline**

i) $NaH_2PO_4.2H_2O$ 2.106 g

ii) Na_2HPO_4 8.733 g

iii) NaCl 4.500 g

iv) 500 ml distilled water, pH adjusted to 7.4.

II. **Hank's Solution:**

i) NaCl 8.00 g

ii) KCl 0.40 g

iii) Na_2HPO_4 0.04 g

iv) $Na_2HPO_4.2H2O$ 0.06 g

v) Glucose 1.00 g

vi) $NaHCO_3$ 0.35 g

vii) 1000 ml distilled water, pH adjusted to 7.8

III. **Ringer's Solution**

i) NaCl 6.00 g

ii) KCL 0.075 g

iii) $CaCl_2$ 0.10 g

iv) $NaHCO_3$ 0.10 g

Plant Materials

The details about different plants/plant parts that were tested for their *in vivo* anthelmintic efficacy are provided in Chapter 1 (Table 1.1).

Experimental Animals

Wistar rats (90-100 g) and Swiss albino mice (25-30 g) of either sex were employed in the study. The stock was inbred in the animal house for several years. They were kept at standard environmental conditions and fed with standard rodent diet (Pranov Agro Industries Ltd., Delhi) and water *ad libitum*. It was ensured that the animals were free from any helminthic infections prior to experimentation. Adequate care was taken to protect the welfare of the experimental animals.

Maintenance of Infection

H. diminuta

The infection of *H. diminuta* was procured from Department of Zoology, Visva-Bharati, Santiniketan and has been maintained in the Parasitology laboratory, NEHU for past 7 years by alternating the hosts as described by Dixon and Arai (1991). Gravid segments of *H. diminuta* were scratched smoothly onto filter papers in Petri dishes and mixed with flour powder. *Tribolium confusum* Jacquelin du Val (Tenebrionidae), the intermediate host, were allowed to feed on flour mixed with the eggs of *H. diminuta* for 72 h and later had free access to flour and kept at 25°C for 14 days or until dissected. On dissecting the beetles, cysticercoids were collected and suspended in normal saline and inoculated to uninfected rats. Each experimental rat was inoculated with four cysticercoids using a feeding tube and later maintained in separate cages. After 18 days, parasite eggs could be observed in the faeces of rats,

which were mixed with flour powder and fed to the beetles and the life cycle continued in the laboratory throughout the study period.

T. spiralis

All experiments used *T. spiralis* strain which is marked by the ***Code ISS 1597*** by the International Trichinella Reference Centre, Itlay. The infection was procured from Department of Zoology, Visva-Bharati, Santiniketan and has been maintained in the Parasitology laboratory, NEHU by periodical passage through mice (Uno *et al.*, 1993). The parasitological procedures used for isolation, preparation of inocula, and administration of infective larvae were basically those of Campbell (1967). Larvae were freshly harvested from the skeletal musculature of mouse with infections of at least 5 weeks' duration by digestion at 37°C for 3 to 4 h in Kreb's Ringer saline containing commercial pepsin (1%, w/v) and concentrated HCL (1% by volume). Mice were infected by oral inoculation with 200 larvae suspended in 0.4 ml of suspension of Ringer saline through an 18-gauge feeding needle attached to an automatic Cornwall syringe.

Experimental Design

H. diminuta

Treatment against larval stage

Thirteen groups of animals comprising of six animals in each group (n = 6) were employed. All animals were inoculated with 4 cysticercoids each by a

blunt feeding tube and maintained in separate cages. Group 1 animals served as infected, untreated controls and were given 1.0 ml of saline per day for 5 days. Groups 2 to 9 animals were treated with single and double doses of the extract (200, 400, 800 and 1600 mg/kg) for 5 days. Groups 10 to 13 animals were administered with single and double doses of praziquantel (5 and 10 mg/kg) as a standard anticestodal drug for 5 days. From day 18 post-infection, fresh faeces was collected from each cage of the treated as well control rats for eggs per gram (EPG) counts (Anonymous, 1977) for 3 days (days 18-20). Follow-up examination of EPG was done on days 28-30 following a week EPG count. On completion of EPG monitoring, autopsy was performed by chloroform anesthesia killing of animals on day 31, and surviving worms in the intestine were recovered. Accordingly, the percentage worm reduction rate and host clearance rate was calculated as described by Rim *et al.* (1980).

Treatment against immature stage

The same experimental protocol was followed as given in the above experiment for the treatment against immature stage, except that the extract and PZQ were given on day 8-12 p.i. of cysticercoids.

Treatment against adult stage

Similar experimental protocol as described for the treatment against larval stage was followed, except for the administration of extract and PZQ, and days of EPG count and autopsy. Plant extract and PZQ were given on day 21-

25 post inoculation of cysticercoids. Faecal examination was undertaken for 3 days pre-treatment (day 18-20), 3 days post-treatment (day 26-28) and a follow-up examination of EPG count was done again for another 3 days (day 36-38), *i.e.*, after 1 week of the previous EPG count. Autopsy was carried out on day 39 and worm recovery rate (%) and host clearance (%) was calculated as described by Rim *et al.* (1980).

T. spiralis

Treatment against adult stage

Seven group of animals comprising of six animals in each group (n = 6) were employed. The animals were inoculated with 200 larvae of *T. spiralis* each by a blunt feeding tube and maintained in separate cages. Group 1 served as infected, untreated controls and was given 0.4 ml of saline per day for 2 days (3-4 days p.i.). Groups 2 to 5 animals were treated with single doses of plant extracts (200, 400, 800 and 1600 mg/kg) for 2 days. Groups 6 to 7 animals were administered with single doses of MBZ (25 and 50 mg/kg) as a standard anticestodal drug for 2 days. The animals were autopsied on day 10 and the number of surviving adult worms in the intestine was counted. Taking the worm burden of infected control animals as the reference, the percentage worm reduction was determined to compare the plant extract efficacy (Campbell and Denham, 1983).

Treatment against migrating stage

To treat the migrating larvae it was necessary first to remove the adults remaining in the intestine without affecting the migrating new born larvae (Denham and Martinez, 1970). This was achieved by treating both the controls and experimental groups, on day 7 p.i. with trichlorfon at 100 mg/kg administered orally plus one intramuscular injection of atropine sulphate. Later on the treatment was given for 3 consecutive days beginning on day 8 p.i., the animals were then sacrificed and muscle larvae was counted on day 30 p.i. as described by Blair (1983). The efficacy of extracts was determined as given in the above experiment.

Treatment against encysted stage

Similar experimental protocol as described for the treatment against migrating stage was followed, except for the administration of plant extracts and MBZ, and day of autopsy. Plant extracts and MBZ were given on day 34-40 p.i. of larvae. Sacrificing of animals and larval counts were carried out on day 48 p.i. and the efficacy of plant extracts was calculated out as described in first experiment.

Acute Toxicity Test

Determination of Mean Lethal Dose (LD_{50})

The plant extracts were administered orally at doses of 3200 and 6400 mg/kg, body wt. to six animals in each group. The general signs and symptoms

of toxicity, intake of food and water, and mortality rates were observed for 72 h post-administration of extract. From these observations, the median lethal dose (LD_{50}) was calculated using SPSS software (SPSS Inc. Chicago, IL, USA).

Statistical Analysis

The experimental data were analyzed statistically and are expressed as mean plus or minus standard error of the mean (Mean ± SEM). Significance was evaluated by the Student's *t*-test and probability less than 5% ($p < 0.05$) was accepted as statistically significant.

Observations and Results

A. Anthelmintic activity of plant extracts in *H. diminuta* - rat model

The anticestodal efficacy of *P. guajava* leaf extract against the larval, immature and adult stages of *H. diminuta* infections is presented in Tables 2.1a-2.1c. Against the larval stage, the 1600 mg/kg body wt. double dose of extract given for 5 days showed 68.66% reduction in EPG count and 66.50% reduction in worm count. The results were comparable with 10 mg/kg double dose of PZQ which revealed 90.13% reduction in EPG count and 91.75% reduction in adult worm count. More or less similar kind of results were obtained with regard to efficacy of extract against immature stages, where the EPG reduction and worm count reduction was noted to be 82.89 and 87.50%, respectively. Similar dose of

extract tested against the adult worms showed 95.34 and 87.50% reduction in EPG and worm count, respectively.

Tables 2.2a-2.2c show the efficacy of *L. spinosa* leaf extract against *H. diminuta* infections in rats. Against the larval stage, the 1600 mg/kg double dose of extract for 5 days showed 68.25% reduction in EPG count and 66.66% reduction in worm count. PZQ at 10 mg/kg double dose showed slightly better results where the reduction in EPG count and worm count was 90.80% and 91.50%, respectively. Against immature stages at the same treatment plan the extract and PZQ showed reduction in EPG count by 80.85 and 100% and worm reduction by 83.25 and 100%, respectively. Against the adult worm also the similar treatment showed a reduction in EPG count by 94.87 and 100% by extract and PZQ, respectively. In this case the worm reduction was 91.50 by plant extract and 100% by PZQ. The 1600 mg/kg dose of extract showed 83.34% host clearance rate.

The efficacy of *H. cordata* extract against the three stages of *H. diminuta* infections in rats is shown in Tables 2.3a-2.3c. The 1600 mg/kg double dose administered for 5 days revealed 65.58 and 62.50% reduction in EPG and worm count, respectively. The 10 mg/kg double dose of PZQ for the same duration revealed slightly better efficacy where the EPG reduction was noted to be 88.89% and worm reduction, 95.75%. In case of efficacy against immature stage, the same dose regime showed a 78.06% worm reduction compared to

PZQ which showed 100% worm reduction. The efficacy was recorded to be maximum against adult worms, where the same dose of plant extract showed 87.50% worm reduction compared to 100% worm reduction by PZQ.

The efficacy of *C. longa* rhizome extract against *H. diminuta* is shown in Tables 2.4a-2.4c. At 1600 mg/kg double dose for 5 days the extract revealed maximum efficacy against adult worms. The same dose of extract could result in to 62.50% worm reduction against the larval stage and 79.00% worm reduction against the immature stage.

Tables 2.5a-2.5c. show the efficacy of *G. angulosa* leaf extract different stages of *H. diminuta* infections in rats. The extract showed moderate efficacy against larval stage where the 1600 mg/kg double dose of extract administered for 5 days resulted into 58.25% worm reduction compared to PZQ (10 mg/kg dose) which showed 95.75% worm reduction. A worm reduction of 79.00% against immature stage and 87.50% against adult stage was recorded by the same dose of plant extract.

The findings related to the efficacy of *L. spinosa* (stalk) extract are summarized in Tables 2.6a-2.6c and that of *L. spinosa* (stem) extract in Tables 2.7a-2.7c. The efficacy of both its stalk and stem extract was recorded to be lower as compared to the efficacy of its leaf extract. However, not much difference was noted in the overall efficacy of its stalk and stem extract. Both,

the stalk as well as stem extract showed maximum efficacy against the adult stage. Against the adult stage, the 1600 mg/kg double dose of stalk and stem extract showed 83.25% worm reduction.

Tables 2.8a-2.8c summarizes the anthelmintic efficacy of *C. colebrookianum* extract against larval, immature and adult stages of *H. diminuta* infections in rats. At 1600 mg/kg double dose the extract showed comparatively less significant activity against the larval stage. In this case the reduction in EPG count was recorded as 55.56% and worm reduction as 50.00%. The same dose however revealed better efficacy against immature and adult worms where the EPG reduction ranged between 64.52 to 77.86% and worm reduction ranged between 75.00-79.00%.

The efficacy of *C. asiatica* extract against different stages of *H. diminuta* infections in rats is presented in Tables 2.9a-2.9c. For this plant also the extract at its 1600 mg/kg double dose (x 5 days) showed better efficacy against immature and mature stage as compared to larval stage. In case of larval stage the worm reduction was noted to be only 50.00%, whereas for immature and adult stages the worm reduction was recorded as 70.75 and 79.00%, respectively.

Acute Toxicity Effect of Plant Extracts in Experimental Rats

Median Lethal Dose (LD_{50}): The lethal effect to the experimental animals caused by oral treatment of different plant extracts at 3200 and 6400 mg/kg, body wt. dose within 72 h post-treatment observation is presented in Table 2.10. The study revealed that barring *C. longa* and *L. spinosa* (stem) extracts which showed mortality of few animals the other plant extracts did not cause any mortality or any changes in behaviour with regard to food and water intake. The LD_{50} values of *C. longa* (rhizome) and *L. spinosa* (stem) extracts were recorded to be 7487 and 7678, respectively.

Table 2.1a: Effect of *Psidium guajava* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17444 ± 325	17222 ± 325	-1.27	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	15700 ± 298[d]	6911 ± 301[c]	-55.98	2.67 ± 0.21[d]	33.25	0.00
2 x 200 x 5	14155 ± 663[c]	6400 ± 503[c]	-57.01	2.17 ± 0.17[c]	45.75	0.00
1 x 400 x 5	14000 ± 379[c]	5455 ± 360[c]	-58.03	2.00 ± 0.00[c]	50.00	0.00
2 x 400 x 5	10000 ± 705[c]	4755 ± 260[c]	-61.02	1.84 ± 0.17[c]	54.00	0.00
1 x 800 x 5	8111 ± 263[c]	3022 ± 156[c]	-62.23	1.67 ± 0.21[c]	58.25	0.00
2 x 800 x 5	7577 ± 399[c]	2800 ± 199[c]	-65.05	1.50 ± 0.22[c]	62.50	0.00
1 x 1600 x 5	5566 ± 214[c]	2122 ± 203[c]	-66.72	1.50 ± 0.43[c]	62.50	33.34
2 x 1600 x 5	5100 ± 236[c]	1400 ± 114[c]	-68.66	1.34 ± 0.21[c]	66.50	33.34
Praziquantel						
1 x 5 x 5	4050 ± 256[c]	1200 ± 98[d]	-70.33	1.17 ± 0.17[c]	70.75	16.67
2 x 5 x 5	2111 ± 174[c]	488 ± 40[c]	-76.84	1.00 ± 0.37[c]	75.00	50.00
1 x 10 x 5	1977 ± 130[c]	455 ± 31[c]	-76.97	0.84 ± 0.31[c]	79.00	50.00
2 x 10 x 5	1800 ± 217[c]	177 ± 22[c]	-90.13	0.34 ± 0.21[c]	91.75	66.67

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4).
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.1b: Effect of *Psidium guajava* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	15844 ± 161	15800 ± 136	-0.28	3.67 ± 0.21	8.25	0.00
Plant Extract						
1 x 200 x 5	12288 ± 188[c]	4533 ± 369[c]	-63.11	2.34 ± 0.34[d]	41.50	0.00
2 x 200 x 5	11177 ± 308[c]	3800 ± 403[c]	-66.00	1.67 ± 0.21[c]	58.25	0.00
1 x 400 x 5	10822 ± 285[c]	3522 ± 201[c]	-67.45	1.34 ± 0.34[c]	66.50	0.00
2 x 400 x 5	8600 ± 177[c]	2600 ± 138[c]	-69.77	1.17 ± 0.17[c]	70.75	0.00
1 x 800 x 5	7733 ± 222[c]	2222 ± 211[c]	-71.26	1.00 ± 0.37[c]	75.00	0.00
2 x 800 x 5	5155 ± 143[c]	1400 ± 95[c]	-72.84	0.84 ± 0.17[c]	79.00	16.67
1 x 1600 x 5	5400 ± 221[c]	1266 ± 111[c]	-76.54	0.67 ± 0.21[c]	83.25	33.34
2 x 1600 x 5	4155 ± 127[c]	711 ± 80[c]	-82.89	0.50 ± 0.34[c]	87.50	33.34
Praziquantel						
1 x 5 x 5	3555 ± 83[c]	644 ± 37[c]	-84.49	0.50 ± 0.22[c]	87.50	33.34
2 x 5 x 5	1822 ± 85[c]	177 ± 32[c]	-90.24	0.17 ± 0.17[c]	95.75	83.34
1 x 10 x 5	1488 ± 47[c]	111 ± 28[c]	-92.54	0.17 ± 0.17[c]	95.75	83.34
2 x 10 x 5	800 ± 64[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.1c: Effect of *Psidium guajava* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	16866 ± 269	16822 ± 283	16822 ± 422	-0.26	-0.26	4.00 ± 0.00	0.00	0.00
Plant Extract								
1 x 200 x 5	17200 ± 1488	12355 ± 978[d]	5000 ± 983[c]	-28.17	-70.93	2.34 ± 0.34[e]	41.50	0.00
2 x 200 x 5	18288 ± 197	10822 ± 155[c]	4200 ± 301[c]	-40.82	-77.03	1.17 ± 0.17[e]	70.75	0.00
1 x 400 x 5	17133 ± 133	11222 ± 1371[c]	4200 ± 203[c]	-34.50	-75.49	1.34 ± 0.21[e]	66.50	0.00
2 x 400 x 5	17444 ± 211	7977 ± 155[c]	2844 ± 155[c]	-54.27	-83.70	1.00 ± 0.00[e]	75.00	0.00
1 x 800 x 5	17133 ± 901	7600 ± 443[c]	2533 ± 501[c]	-55.64	-85.21	1.00 ± 0.26[e]	75.00	0.00
2 x 800 x 5	17377 ± 449	4177 ± 336[c]	1000 ± 153[c]	-75.96	-94.25	0.67 ± 0.34[e]	83.25	0.00
1 x 1600 x 5	17044 ± 270	4911 ± 160[c]	1000 ± 115[c]	-71.19	-94.13	0.67 ± 0.21[e]	83.25	0.00
2 x 1600 x 5	17155 ± 262	2466 ± 176[c]	800 ± 153[c]	-85.63	-95.34	0.50 ± 0.22[e]	87.50	16.67
Praziquantel								
1 x 5 x 5	17022 ± 699	3000 ± 66[c]	600 ± 155[c]	-82.38	-96.48	0.50 ± 0.34[e]	87.50	33.34
2 x 5 x 5	15955 ± 816	1000 ± 101[c]	0[c]	-93.73	-100.00	0[e]	100.00	100.00
1 x 10 x 5	17000 ± 542	800 ± 76[c]	0[c]	-95.29	-100.00	0[e]	100.00	100.00
2 x 10 x 5	16866 ± 926	0[c]	0[c]	-100.00	-100.00	0[e]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. pre-treatment value, Student's *t*-test.
[e]$p < 0.001$ vs. control value, Students *t*-test.

Table 2.2a: Effect of *Lasia spinosa* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17600 ± 586	17555 ± 466	-1.27	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	13966 ± 483[c]	6400 ± 190[c]	-54.18	2.84 ± 0.17[d]	29.00	0.00
2 x 200 x 5	13711 ± 390[c]	6000 ± 158[c]	-56.24	2.34 ± 0.21[d]	41.50	0.00
1 x 400 x 5	11000 ± 274[c]	4722 ± 286[c]	-57.07	2.17 ± 0.31[c]	45.75	0.00
2 x 400 x 5	10500 ± 394[c]	4166 ± 327[c]	-60.32	1.67 ± 0.21[c]	58.25	0.00
1 x 800 x 5	7588 ± 221[c]	2900 ± 142[c]	-61.79	1.67 ± 0.34[c]	58.25	0.00
2 x 800 x 5	4266 ± 324[c]	1522 ± 116[c]	-64.32	1.50 ± 0.22[c]	37.50	0.00
1 x 1600 x 5	3800 ± 170[c]	1311 ± 139[c]	-65.50	1.50 ± 0.21[c]	37.50	0.00
2 x 1600 x 5	2800 ± 215[c]	888 ± 70[c]	-68.25	1.33 ± 0.00[c]	66.66	0.00
Praziquantel						
1 x5 x 5	3088 ± 134[c]	922 ± 60[c]	-70.14	1.16 ± 0.17[c]	71.00	16.67
2 x5 x 5	3200 ± 147[c]	800 ± 51[c]	-75.00	0.84 ± 0.17[c]	79.00	50.00
1 x 10 x 5	2000 ± 199[c]	466 ± 29[c]	-76.67	0.67 ± 0.21[c]	83.25	50.00
2 x 10 x 5	1811 ± 153[c]	166 ± 14[c]	-90.80	0.34 ± 0.21[c]	91.50	66.67

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.2b: Effect of *Lasia spinosa* leaf extract[a] on immature stages of *Hymenolepis diminuta* infecions in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17166 ± 401	16611 ± 309	-3.24	3.84 ± 0.20	4.00	0.00
Plant Extract						
1 x 200 x 5	13311 ± 177[c]	4866 ± 492[c]	-63.44	2.34 ± 0.22[c]	41.50	0.00
2 x 200 x 5	11222 ± 297[c]	3822 ± 173[c]	-65.94	1.84 ± 0.31[c]	50.00	0.00
1 x 400 x 5	11733 ± 296[c]	3911 ± 360[c]	-66.67	1.34 ± 0.21[c]	66.50	0.00
2 x 400 x 5	8622 ± 176[c]	2588 ± 110[c]	-69.97	1.17 ± 0.40[c]	66.50	0.00
1 x 800 x 5	8388 ± 215[c]	2600 ± 127[c]	-69.00	1.17 ± 0.17[c]	66.50	16.67
2 x 800 x 5	5177 ± 131[c]	1377 ± 116[c]	-70.39	1.00 ± 0.26[c]	75.00	33.34
1 x 1600 x 5	5844 ± 210[c]	1533 ± 91[c]	-73.76	0.83 ± 0.31[c]	83.25	66.67
2 x 1600 x 5	2088 ± 58[c]	800 ± 64[c]	-80.85	0.67 ± 0.34[c]	83.25	66.67
Praziquantel						
1 x 5 x 5	3844 ± 76[c]	644 ± 88[c]	-83.24	0.67 ± 0.21[c]	83.25	50.00
2 x 5 x 5	1822 ± 85[c]	200 ± 100[c]	-89.02	0.34 ± 0.21[c]	91.50	83.34
1 x 10 x 5	1622 ± 47[c]	155 ± 56[c]	-90.14	0.34 ± 0.34[c]	100.00	100.00
2 x 10 x 5	800 ± 64[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.2c: Effect of *Lasia spinosa* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	16955 ± 669	16911 ± 345	16800 ± 336	-0.26	-0.91	3.70 ± 0.21	8.20	8.25
Plant Extract								
1 x 200 x 5	15933 ± 1699	11777 ± 954[d]	4777 ± 150[c]	-26.08	-70.02	2.34 ± 0.34[f]	41.50	0.00
2 x 200 x 5	17388 ± 669	10711 ± 498[c]	4044 ± 293[c]	-38.40	-76.74	1.34 ± 0.34[e]	66.50	0.00
1 x 400 x 5	15444 ± 631	9644 ± 859[c]	3688 ± 163[d]	-37.56	-76.12	1.67 ± 0.34[e]	58.25	0.00
2 x 400 x 5	17000 ± 178	7733 ± 232[c]	2755 ± 150[c]	-54.51	-83.79	1.00 ± 0.37[e]	75.00	0.00
1 x 800 x 5	16977 ± 791	7600 ± 205[c]	2666 ± 157[c]	-55.23	-84.30	1.00 ± 0.26[e]	75.00	0.00
2 x 800 x 5	17466 ± 376	4622 ± 214[c]	1200 ± 153[c]	-73.54	-93.13	0.67 ± 0.42[e]	83.25	0.00
1 x 1600 x 5	17311 ± 442	5177 ± 237[c]	1711 ± 156[c]	-70.09	-90.12	0.84 ± 0.29[e]	79.25	66.67
2 x 1600 x 5	16888 ± 425	3222 ± 385[c]	866 ± 110[c]	-80.92	-94.87	0.50 ± 0.22[e]	91.50	83.34
Praziquantel								
1 x 5 x 5	16333 ± 823	3088 ± 312[c]	800 ± 112[c]	-81.09	-95.10	0.67 ± 0.24[e]	83.25	50.00
2 x 5 x 5	16822 ± 190	600 ± 92[c]	222 ± 58[c]	-96.43	-98.68	0.34 ± 8.50[e]	91.50	83.34
1 x 10 x 5	16200 ± 795	600 ± 59[c]	0[c]	-96.30	-100.00	0[e]	100.00	100.00
2 x 10 x 5	16977 ± 187	200 ± 100[c]	0[c]	-98.82	-100.00	0[e]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.02$ vs. pre-treatment value, Student's *t*-test.
[e, f]$p < 0.001$ and $p < 0.01$ and vs. control value, Student's *t*-test.

Table 2.3a: Effect of *Houttuynia cordata* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	18888 ± 379	18600 ± 394	-1.53	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	16333 ± 358[c]	8000 ± 557[c]	-51.02	3.00 ± 0.26[e]	25.00	25.00
2 x 200 x 5	14155 ± 663[c]	6600 ± 453[c]	-53.38	2.50 ± 0.34[d]	37.50	37.50
1 x 400 x 5	14000 ± 379[c]	6588 ± 392[c]	-52.94	2.34 ± 0.21[c]	41.50	41.50
2 x 400 x 5	10000 ± 705[c]	4433 ± 246[c]	-55.67	2.17 ± 0.31[c]	45.75	45.75
1 x 800 x 5	8111 ± 263[c]	3400 ± 304[c]	-58.08	2.00 ± 0.26[c]	50.00	50.00
2 x 800 x 5	7577 ± 399[c]	2844 ± 227[c]	-62.46	1.84 ± 0.17[c]	54.00	54.00
1 x 1600 x 5	5566 ± 214[c]	2022 ± 124[c]	-63.67	1.67 ± 0.21[c]	58.25	58.25
2 x 1600 x 5	5100 ± 236[c]	1755 ± 129[c]	-65.58	1.50 ± 0.22[c]	62.50	62.50
Praziquantel						
1 x 5 x 5	3900 ± 243[c]	1055 ± 49[c]	-72.93	1.34 ± 0.21[c]	66.50	66.50
2 x 5 x 5	2800 ± 190[c]	700 ± 37[c]	-75.00	0.84 ± 0.17[c]	79.00	79.00
1 x 10 x 5	2244 ± 126[c]	533 ± 34[c]	-76.24	0.67 ± 0.21[c]	83.25	83.25
2 x 10 x 5	1000 ± 54[c]	111 ± 28[c]	-88.89	0.17 ± 0.17[c]	95.75	95.75

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e]$p < 0.001$, $p < 0.01$ and $p < 0.05$ vs. control value, Student's *t*-test.

Table 2.3b: Effect of *Houttuynia cordata* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17988 ± 425	17888 ± 410	-0.56	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x5	13800 ± 217[c]	5488 ± 159[c]	-60.23	2.50 ± 0.34[d]	37.50	0.00
2 x 200 x5	11755 ± 96[c]	4233 ± 226[c]	-63.99	1.84 ± 0.17[c]	54.00	0.00
1 x 400 x5	12377 ± 81[c]	4322 ± 305[c]	-65.08	1.50 ± 0.22[c]	62.50	0.00
2 x 400 x5	10066 ± 187[c]	3355 ± 202[c]	-66.67	1.34 ± 0.34[c]	66.50	16.67
1 x 800 x5	8555 ± 58[c]	2800 ± 160[c]	-67.27	1.34 ± 0.21[c]	66.50	0.00
2 x 800 x5	7088 ± 88[c]	2177 ± 134[c]	-69.28	1.00 ± 0.37[c]	75.00	33.34
1 x 1600 x 5	7244 ± 369[c]	2088 ± 196[c]	-71.17	1.00 ± 0.00[c]	75.00	0.00
2 x 1600 x 5	4000 ± 78[c]	877 ± 93[c]	-78.06	0.84 ± 0.17[c]	79.00	16.67
Praziquantel						
1 x 5 x 5	4200 ± 114[c]	3688 ± 76[c]	-83.60	0.75 ± 0.25[c]	81.25	50.00
2 x 5 x 5	2733 ± 141[c]	200 ± 73[c]	-92.68	0.17 ± 0.17[c]	95.75	83.34
1 x 10 x 5	1377 ± 101[c]	100 ± 22[c]	-92.74	0.17 ± 0.17[c]	95.75	83.34
2 x 10 x 5	911 ± 90[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.3c: Effect of *Houttuynia cordata* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	17777 ± 313	17711 ± 281	17622 ± 251	-0.37	-0.87	3.67 ± 0.21	8.25	0.00
Plant Extract								
1 x 200 x 5	15155 ± 557	10955 ± 848[d]	4933 ± 174[c]	-27.71	-67.45	2.50 ± 0.22[f]	37.50	0.00
2 x 200 x 5	16977 ± 228	10955 ± 170[c]	4311 ± 271[c]	-35.47	-74.61	1.34 ± 0.34[e]	66.50	0.00
1 x 400 x 5	17600 ± 290	11311 ± 241[c]	4444 ± 369[c]	-35.73	-74.75	1.34 ± 0.21[e]	66.50	0.00
2 x 400 x 5	16844 ± 178	8333 ± 233[c]	3288 ± 164[c]	-50.53	-80.48	1.17 ± 0.31[e]	70.75	0.00
1 x 800 x 5	17400 ± 517	7466 ± 385[c]	2688 ± 298[c]	-57.09	-84.55	1.00 ± 0.37[e]	75.00	0.00
2 x 800 x 5	17533 ± 1256	5222 ± 625[c]	1355 ± 136[c]	-70.22	-92.27	0.67 ± 0.21[e]	83.25	33.34
1 x 1600 x 5	16644 ± 686	5177 ± 325[c]	1800 ± 282[c]	-68.90	-89.19	0.84 ± 0.17[e]	79.00	50.00
2 x 1600 x 5	15622 ± 184	2800 ± 92[a]	588 ± 93[c]	-82.08	-96.24	0.50 ± 0.22[c]	87.50	83.34
Praziquantel								
1 x 5 x 5	18111 ± 316	3555 ± 243[c]	577 ± 141[c]	-80.37	-96.81	0.50 ± 0.22[e]	87.50	50.00
2 x 5 x 5	15977 ± 161	755 ± 166[c]	0[c]	-95.27	-100.00	0[e]	100.00	100.00
1 x 10 x 5	15588 ± 169	600 ± 75[c]	0[c]	-96.15	-100.00	0[e]	100.00	100.00
2 x 10 x 5	16511 ± 247	0[c]	0[c]	-100.00	-100.00	0[e]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.02$ vs. pre-treatment value, Student's *t*-test.
[e, f]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.4a: Effect of *Curcuma longa* rhizome extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	19500 ± 422	18600 ± 394	-0.06	4.00 ± 0.00	0.00	0.00
Plant Extract						
1 x 200 x 5	17300 ± 399[d]	8600 ± 354[c]	-50.29	3.00 ± 0.00*	25.00	0.00
2 x 200 x 5	16355 ± 497[c]	7800 ± 327[c]	-52.31	2.50 ± 0.50[e]	37.50	0.00
1 x 400 x 5	13800 ± 345[c]	6400 ± 186[c]	-53.62	2.34 ± 0.34[c]	41.50	0.00
2 x 400 x 5	14400 ± 531[c]	6400 ± 313[c]	-55.56	2.17 ± 0.17[c]	45.75	0.00
1 x 800 x 5	8088 ± 321[c]	3400 ± 288[c]	-57.97	2.17 ± 0.40[c]	45.75	0.00
2 x 800 x 5	8000 ± 356[c]	3200 ± 190[c]	-60.00	1.84 ± 0.31[c]	54.00	0.00
1 x 1600 x 5	3933 ± 452[c]	1533 ± 100[c]	-61.02	1.67 ± 0.34[c]	58.25	0.00
2 x 1600 x 5	4700 ± 137[c]	1722 ± 137[c]	-63.36	1.50 ± 0.34[c]	62.50	0.00
Praziquantel						
1 x 5 x 5	3677 ± 40[c]	1055 ± 40[c]	-71.30	1.34 ± 0.21[c]	66.50	0.00
2 x 5 x 5	3033 ± 43[c]	722 ± 43[c]	-76.19	1.00 ± 0.00[c]	75.00	16.67
1 x 10 x 5	2000 ± 31[c]	477 ± 31[c]	-76.12	0.67 ± 0.21[c]	83.25	33.34
2 x 10 x 5	1411 ± 17[c]	133 ± 17[c]	-90.55	0.34 ± 0.21[c]	91.50	66.67

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4).
[b]No. of animals in each group, n = 6.
[c, d, e] $p < 0.001$, $p < 0.01$ and $p < 0.02$ vs. control value, Student's *t*-test.
*not significant

Table 2.4b: Effect of *Curcuma longa* rhizome extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17111 ± 470	16977 ± 371	-0.78	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	14000 ± 164[c]	5800 ± 150[c]	-58.57	2.50 ± 0.43[d]	37.50	0.00
2 x 200 x 5	13488 ± 366[c]	5000 ± 149[c]	-62.93	2.00 ± 0.26[c]	50.00	0.00
1 x 400 x 5	12200 ± 193[c]	4388 ± 201[c]	-64.03	1.67 ± 0.21[c]	59.00	0.00
2 x 400 x 5	10000 ± 206[c]	6000 ± 212[c]	-66.78	1.59 ± 0.21[c]	60.25	0.00
1 x 800 x 5	10288 ± 300[c]	3222 ± 334[c]	-68.68	1.34 ± 0.21[c]	66.50	0.00
2 x 800 x 5	7988 ± 297[c]	3322 ± 202[c]	-69.17	1.00 ± 0.37[c]	75.00	16.67
1 x 1600 x 5	6000 ± 284[c]	1800 ± 132[c]	-70.00	1.00 ± 0.26[c]	75.00	16.67
2 x 1600 x 5	4000 ± 194[c]	1233 ± 145[c]	-75.19	0.84 ± 0.17[c]	79.00	16.67
Praziquantel						
1 x 5 x 5	3788 ± 166[c]	522 ± 55[c]	-86.22	0.50 ± 0.22[c]	87.50	50.00
2 x 5 x 5	3000 ± 383[c]	744 ± 91[c]	-92.00	0.17 ± 0.17[c]	95.75	83.34
1 x 10 x 5	2400 ± 106[c]	111 ± 50[c]	-95.37	0.17 ± 0.17[c]	95.75	83.34
2 x 10 x 5	1811 ± 147[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.02$ vs. control value, Student's *t*-test.

Table 2.4c: Effect of *Curcuma longa* rhizome extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	18800 ± 488	19000 ± 527	18855 ± 253	+1.05	-0.29	4.00 ± 0.00	0.00	0.00
Plant Extract								
1 x 200 x 5	18500 ± 350	13000 ± 514[c]	6388 ± 207[c]	-29.73	-65.47	2.50 ± 0.43[c]	37.50	0.00
2 x 200 x 5	17900 ± 345	11400 ± 148[c]	5011 ± 211[c]	-36.31	-72.01	1.67 ± 0.21[d]	59.00	0.00
1 x 400 x 5	18233 ± 422	12311 ± 686[c]	4944 ± 208[c]	-32.48	-72.88	1.50 ± 0.22[d]	62.50	0.00
2 x 400 x 5	17855 ± 275	8600 ± 229[c]	3888 ± 209[c]	-51.84	-78.22	1.17 ± 0.31[d]	70.75	0.00
1 x 800 x 5	17844 ± 415	8466 ± 389[c]	3555 ± 145[c]	-52.55	-80.08	1.17 ± 0.17[d]	74.75	0.00
2 x 800 x 5	17811 ± 572	5622 ± 177[c]	1422 ± 247[c]	-68.43	-92.02	0.84 ± 0.31[d]	79.00	50.00
1 x 1600 x 5	17800 ± 262	5777 ± 167[c]	1611 ± 123[c]	-67.54	-90.95	1.00 ± 0.26[d]	75.00	33.34
2 x 1600 x 5	17788 ± 213	3544 ± 156[c]	988 ± 71[c]	-80.07	-94.45	0.50 ± 0.34[d]	87.50	16.67
Praziquantel								
1 x 5 x 5	17800 ± 106	2800 ± 164[c]	600 ± 76[c]	-84.27	-96.63	0.67 ± 0.34[d]	83.25	66.67
2 x 5 x 5	17600 ± 559	733 ± 128[c]	0[c]	-95.83	-100.00	0[d]	83.25	50.00
1 x 10 x 5	16622 ± 640	488 ± 56[c]	0[c]	-97.06	-100.00	0[d]	100.00	100.00
2 x 10 x 5	17200 ± 223	200 ± 38[c]	0[c]	-98.84	-100.00	0[d]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, vs. pre-treatment value, Students *t*-test.
[d,e]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.5a: Effect of *Gynura angulosa* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	16000 ± 836	15788 ± 537	-1.32	3.67 ± 0.34	8.25	0.00
Plant Extract						
1 x 200 x 5	13800 ± 305[f]	7088 ± 207[c]	-48.63	3.00 ± 0.45[*]	25.00	0.00
2 x 200 x 5	14622 ± 363[*]	7311 ± 177[c]	-50.00	2.67 ± 0.34[*]	33.25	0.00
1 x 400 x 5	10244 ± 314[c]	4833 ± 216[c]	-52.82	2.50 ± 0.22[e]	37.50	0.00
2 x 400 x 5	11733 ± 325[c]	5388 ± 236[c]	-54.07	2.34 ± 0.21[d]	41.50	0.00
1 x 800 x 5	8000 ± 494[c]	3444 ± 214[c]	-56.95	2.17 ± 0.17[d]	45.75	0.00
2 x 800 x 5	6955 ± 237[c]	2822 ± 171[c]	-59.42	2.00 ± 0.26[d]	50.00	0.00
1 x 1600 x 5	5755 ± 326[c]	2400 ± 154[c]	-58.30	1.84 ± 0.17[c]	54.00	0.00
2 x 1600 x 5	3888 ± 238[c]	1533 ± 98[c]	-60.57	1.67 ± 0.21[c]	58.25	0.00
Praziquantel						
1 x 5 x 5	3300 ± 182[c]	933 ± 45[c]	-71.72	1.34 ± 0.42[d]	66.50	0.00
2 x 5 x 5	3000 ± 197[c]	711 ± 32[c]	-76.30	1.00 ± 0.26[d]	75.00	33.34
1 x 10 x 5	2133 ± 145[c]	488 ± 32[c]	-77.08	0.84 ± 0.17[d]	79.00	16.67
2 x 10 x 5	1400 ± 78[c]	155 ± 14[c]	-88.89	0.17 ± 0.17[d]	95.75	66.67

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e, f]$p < 0.001$, $p < 0.01$, $p < 0.02$ and $p < 0.05$ vs. control value, Student's *t*-test.
*not significant

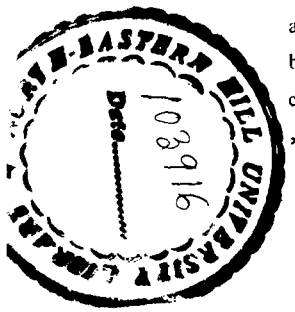


Table 2.5b: Effect of *Gynura angulosa* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	18388 ± 201	18200 ± 165	-1.03	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	13777 ± 232[c]	5800 ± 266[c]	-57.90	2.50 ± 0.56[e]	37.50	0.00
2 x 200 x 5	13000 ± 260[c]	5200 ± 172[c]	-60.00	2.00 ± 0.45[d]	50.50	0.00
1 x 400 x 5	12111 ± 261[c]	4555 ± 220[c]	-62.39	1.67 ± 0.42[c]	59.00	0.00
2 x 400 x 5	12433 ± 251[c]	4400 ± 191[c]	-64.61	1.50 ± 0.43[c]	60.25	0.00
1 x 800 x 5	10033 ± 188[c]	3500 ± 189[c]	-65.12	1.50 ± 0.22[c]	66.50	0.00
2 x 800 x 5	8000 ± 215[c]	2600 ± 205[c]	-67.50	1.17 ± 0.17[c]	75.00	0.00
1 x 1600 x 5	6077 ± 201[c]	1888 ± 167[c]	-68.92	1.17 ± 0.31[c]	75.00	0.00
2 x 1600 x 5	4600 ± 227[c]	1300 ± 129[c]	-71.74	0.84 ± 0.31[c]	79.00	33.34
Praziquantel						
1 x 5x 5	4000 ± 258[c]	611 ± 73[c]	-84.73	0.50 ± 0.22[c]	87.50	50.00
2 x 5x 5	3111 ± 118[c]	144 ± 40[c]	-95.35	0.17 ± 0.17[c]	95.75	83.34
1 x 10 x 5	983 ± 50[c]	100 ± 22[c]	-94.92	0.17 ± 0.17[c]	95.75	83.34
2 x 10 x 5	180 ± 134[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e]$p < 0.001$, $p < 0.01$ and $p < 0.05$ vs. control value, Student's *t*-test.

Table 2.5c: Effect of *Gynura angulosa* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	17700 ± 537	17455 ± 266	17522 ± 316	-1.38	-1.01	3.84 ± 0.1	70.00	0.00
Plant Extract								
1 x 200 x 5	17800 ± 404	12888 ± 211[c]	6555 ± 388[c]	-27.60	-63.17	2.50 ± 0.22[d]	37.50	0.00
2 x 200 x 5	16833 ± 259	10933 ± 239[c]	5000 ± 202[c]	-35.05	-70.30	1.67 ± 0.21[d]	59.00	0.00
1 x 400 x 5	17600 ± 154	12000 ± 328[c]	5077 ± 164[c]	-31.82	-71.15	1.50 ± 0.22[d]	62.50	0.00
2 x 400 x 5	17411 ± 260	8711 ± 479[c]	3822 ± 168[c]	-49.97	-78.05	1.34 ± 0.34[d]	66.50	16.67
1 x 800 x 5	17500 ± 348	8433 ± 255[c]	3744 ± 183[c]	-51.81	-78.61	1.17 ± 0.31[d]	70.75	0.00
2 x 800 x 5	17966 ± 263	6277 ± 256[c]	2000 ± 134[c]	-65.06	-88.87	0.84 ± 0.31[d]	79.00	33.34
1 x 1600 x 5	18000 ± 371	6133 ± 292[c]	1800 ± 94[c]	-65.93	-90.00	1.00 ± 0.00[d]	75.00	0.00
2 x 1600 x 5	17000 ±190	3555 ± 122[c]	1355 ± 78[c]	-79.09	-92.03	0.50 ± 0.22[d]	87.50	50.00
Praziquantel								
1 x 5 x 5	17166 ± 433	3400 ± 305[c]	611 ± 79[c]	-80.19	-96.44	0.50 ± 0.22[d]	87.50	50.00
2 x 5 x 5	17200 ± 405	333 ± 48[c]	0[c]	-98.06	-100.00	0[d]	100.00	100.00
1 x 10 x 5	17200 ± 486	788 ± 79[c]	0[c]	-95.42	-100.00	0[d]	100.00	100.00
2 x 10 x 5	17222 ± 285	166 ± 56[c]	0[c]	-99.04	-100.00	0[d]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat ($n = 4$)
[b]No. of animals in each group, $n = 6$.
[c]$p < 0.001$ vs. pre-treatment value, Student's *t*-test.
[d]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.6a: Effect of *Lasia spinosa* stalk extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17633 ± 312	17600 ± 445	-0.19	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	14300 ± 459[c]	7400 ± 283[c]	-48.25	3.00 ± 0.37*	25.00	0.00
2 x 200 x 5	12900 ± 192[c]	6577 ± 275[c]	-49.01	2.67 ± 0.21[d]	33.25	0.00
1 x 400 x 5	11611 ± 320[c]	5800 ± 523[c]	-50.05	2.50 ± 0.34[d]	37.50	0.00
2 x 400 x 5	10000 ± 237[c]	4800 ± 234[c]	-52.00	2.34 ± 0.56[e]	41.50	0.00
1 x 800 x 5	8000 ± 257[c]	3733 ± 275[c]	-53.34	2.34 ± 0.42[d]	41.50	0.00
2 x 800 x 5	6955 ± 197[c]	3122 ± 169[c]	-55.11	2.17 ± 0.17[c]	45.75	0.00
1 x 1600 x 5	4866 ± 151[c]	2144 ± 88[c]	-55.94	2.00 ± 0.26[c]	50.00	0.00
2 x 1600 x 5	4000 ± 325[c]	1655 ± 128[c]	-58.62	1.84 ± 0.17[c]	54.00	0.00
Praziquantel						
1 x 5 x 5	3600 ± 208[c]	1077 ± 55[c]	-70.06	1.17 ± 0.17[c]	70.75	0.00
2 x 5 x 5	2611 ± 205[c]	644 ± 22[c]	-75.32	0.84 ± 0.31[c]	79.00	33.34
1 x 10 x 5	2400 ± 94[c]	588 ± 31[c]	-75.46	0.84 ± 0.17[c]	79.00	16.67
2 x 10 x 5	1866 ± 112[c]	177 ± 14[c]	-90.48	0.34 ± 0.21[c]	91.50	66.67

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e] $p < 0.001$, $p < 0.01$ and $p < 0.05$ vs. control value, Student's *t*-test.
*not significant

Table 2.6b: Effect of *Lasia spinosa* stalk extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	16800 ± 175	16766 ± 251	-0.20	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	12277 ± 331[c]	5200 ± 200[c]	-57.65	2.67 ± 0.21[d]	33.25	0.00
2 x 200 x 5	12000 ± 204[c]	4888 ± 202[c]	-59.26	2.00 ± 0.45[d]	50.00	0.00
1 x 400 x 5	10555 ± 369[c]	4200 ± 209[c]	-60.21	1.84 ± 0.31[c]	54.00	0.00
2 x 400 x 5	10200 ± 217[c]	3866 ± 137[c]	-62.09	1.67 ± 0.21[c]	59.00	0.00
1 x 800 x 5	6000 ± 179[c]	2311 ±154[c]	-61.48	1.67 ± 0.42[c]	59.00	16.67
2 x 800 x 5	6177 ± 142[c]	2155 ± 147[c]	-65.11	1.17 ± 0.40[c]	70.75	0.00
1 x 1600 x 5	4333 ± 172[c]	1377 ± 102[c]	-67.37	1.17 ± 0.17[c]	70.75	0.00
2 x 1600 x 5	4500 ± 176[c]	1355 ± 132[c]	-69.88	1.00 ± 0.00[c]	75.00	0.00
Praziquantel						
1 x 5 x 5	3600 ± 226[c]	533 ± 70[c]	-85.19	0.50 ± 0.34[c]	87.50	66.67
2 x 5 x 5	2944 ± 96[c]	233 ± 24[c]	-88.68	0.34 ± 0.21[c]	91.50	50.00
1 x 10 x 5	2211 ± 159[c]	211± 26[c]	-90.45	0.34 ± 0.21[c]	91.50	66.67
2 x 10 x 5	2188 ± 130[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.6c: Effect of *Lasia spinosa* stalk extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	18400 ± 245	18600 ± 273	18377 ± 274	-1.09	-0.13	4.00 ± 0.00	0.00	0.00
Plant Extract								
1 x 200 x 5	19777 ± 250	14111 ± 212[c]	7888 ± 196[c]	-28.65	-60.12	2.67 ± 0.42[e]	33.25	0.00
2 x 200 x 5	19200 ± 384	12800 ± 212[c]	6400 ± 303[c]	-33.34	-66.67	1.84 ± 0.31[d]	54.00	0.00
1 x 400 x 5	19000 ± 267	12333 ± 140[c]	6000 ± 219[c]	-35.09	-68.42	1.67 ± 0.21[d]	59.00	0.00
2 x 400 x 5	18811 ± 291	9800 ± 218[c]	4800 ± 260[c]	-47.90	-74.48	1.50 ± 0.34[d]	62.50	0.00
1 x 800 x 5	17411 ± 210	9100 ± 142[c]	4200 ± 164[c]	-47.73	-75.88	1.34 ± 0.21[d]	66.50	0.00
2 x 800 x 5	18800 ± 275	7000 ± 205[c]	2800 ± 140[c]	-62.77	-85.11	1.00 ± 0.26[d]	75.00	0.00
1 x 1600 x 5	17133 ± 261	4200 ± 235[c]	2388 ± 129[c]	-75.49	-86.06	1.00 ± 0.37[d]	75.00	33.34
2 x 1600 x 5	18433 ± 279	4755 ± 258[c]	2000 ± 146[c]	-74.20	-89.15	0.67 ± 0.21[d]	83.25	66.67
Praziquantel								
1 x 5 x 5	17000 ± 203	3600 ± 156[c]	1111 ± 53[c]	-78.82	-93.46	0.67 ± 0.21[d]	83.25	66.67
2 x 5 x 5	18300 ± 458	433 ± 56[c]	0[c]	-97.63	-100.00	0[d]	100.00	100.00
1 x 10 x 5	16988 ± 217	600 ± 142[c]	0[c]	-96.47	-100.00	0[d]	100.00	100.00
2 x 10 x 5	17800 ± 334	800 ± 95[c]	0[c]	-95.51	-100.00	0[d]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. pre-treatment value, Student's *t*-test.
[d, e]$p < 0.001$ and $p < 0.02$ vs. control value, Student's *t*-test.

Table 2.7a: Effect of *Lasia spinosa* stem extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17955 ± 245	17400 ± 486	-3.09	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	12911 ± 496[c]	7000 ± 255[c]	-45.78	3.17 ± 0.17[e]	0.75	0.00
2 x 200 x 5	12600 ± 336[c]	6600 ± 200	-47.62	3.00 ± 0.27[f]	25.00	0.00
1 x 400 x 5	9977 ± 217[c]	5088 ± 244[c]	-49.00	2.84 ± 0.17[d]	29.00	0.00
2 x 400 x 5	8800 ± 382[c]	4355 ± 211[c]	-50.51	2.50 ± 0.22[c]	37.50	0.00
1 x 800 x 5	5133 ± 215[c]	2544 ± 248[c]	-50.43	2.50 ± 0.22[e]	37.50	0.00
2 x 800 x 5	5100 ± 162[c]	2388 ± 203[c]	-53.16	2.17 ± 0.17[c]	45.75	0.00
1 x 1600 x 5	4000 ± 216[c]	1822 ± 120[c]	-54.45	2.00 ± 0.00[c]	50.00	0.00
2 x 1600 x 5	3600 ± 131[c]	1500 ± 59[c]	-58.34	1.84 ± 0.17[c]	54.00	0.00
Praziquantel						
1 x 5 x 5	2844 ± 144[c]	777 ± 40[c]	-72.66	1.34 ± 0.21[c]	66.50	16.67
2 x 5 x 5	1900 ± 107[c]	466 ± 24[c]	-75.44	0.84 ± 0.17[c]	79.00	33.34
1 x 10 x 5	1600 ± 78[c]	377 ± 22[c]	-76.39	0.84 ± 0.31[c]	79.00	50.00
2 x 10 x 5	33 ± 47[c]	66 ± 17[c]	-92.00	0.50 ± 0.22[c]	87.50	100.00

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e, f]$p < 0.001$, $p < 0.01$, $p < 0.02$ and $p < 0.05$ vs. control value, Student's *t*-test.

Table 2.7b: Effect of *Lasia spinosa* stem extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17700 ± 196	17400 ± 266	-1.69	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	13800 ± 375[c]	6400 ± 259[c]	-53.62	2.67 ± 0.34[d]	33.25	0.00
2 x 200 x 5	14377 ± 185[c]	6333 ± 208[c]	-55.95	2.17 ± 0.17[c]	45.75	0.00
1 x 400 x 5	10388 ± 191[c]	4255 ± 215[c]	-59.04	2.00 ± 0.26[c]	50.00	0.00
2 x 400 x 5	10400 ± 361[c]	3855 ± 274[c]	-62.93	1.84 ± 0.17[c]	54.00	0.00
1 x 800 x 5	7200 ± 267[c]	2577 ± 163[c]	-64.20	1.67 ± 0.34[c]	59.00	0.00
2 x 800 x 5	6955 ± 226[c]	2400 ± 150[c]	-65.50	1.34 ± 0.42[c]	66.50	0.00
1 x 1600 x 5	5000 ± 150[c]	1722 ± 151[c]	-65.56	1.34 ± 0.34[c]	66.50	16.67
2 x 1600 x 5	4200 ± 135[c]	1366 ± 122[c]	-67.46	1.00 ± 0.45[c]	75.00	50.00
Praziquantel						
1 x 5 x 5	4011 ± 140[c]	700 ± 50[c]	-82.55	0.50 ± 0.34[c]	87.00	66.67
2 x 5 x 5	4000 ± 91[c]	344 ± 26[c]	-91.39	0.34 ± 0.34[c]	91.50	83.34
1 x 10 x 5	2000 ± 186[c]	166 ± 22[c]	-91.67	0.34 ± 0.21[c]	91.50	66.67
2 x 10 x 5	1933 ± 107[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)

[b]No. of animals in each group, n = 6

[c, d]$p < 0.001$ and $p < 0.02$ vs. control value, Student's *t*-test.

Table 2.7c: Effect of *Lasia spinosa* stem extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	19144 ± 231	19000 ± 652	18800 ± 254	-0.75	-1.80	4.00 ± 0.00	0.00	0.00
Plant Extract								
1 x 200 x 5	18822 ± 280	14111 ± 252[c]	8400 ± 293[c]	-25.03	-55.37	2.67 ± 0.34[d]	33.25	0.00
2 x 200 x 5	18800 ± 207	12500 ± 192[c]	7000 ± 156[c]	-33.51	-62.77	1.84 ± 0.17[d]	54.00	0.00
1 x 400 x 5	18600 ± 183	12600 ± 288[c]	6633 ± 349[c]	-32.26	-64.34	1.67 ± 0.21[d]	59.00	0.00
2 x 400 x 5	18400 ± 259	10000 ± 542[c]	5400 ± 155[c]	-45.65	-70.65	1.50 ± 0.34[d]	62.50	0.00
1 x 800 x 5	18333 ± 317	9800 ± 285[c]	5000 ± 282[c]	-46.54	-72.73	1.34 ± 0.42[d]	66.50	33.34
2 x 800 x 5	18277 ± 326	7600 ± 252[c]	3655 ± 152[c]	-58.42	-80.00	1.17 ± 0.48[d]	70.75	33.34
1 x 1600 x 5	18000 ± 201	7377 ± 300[c]	3555 ± 242[c]	-59.02	-80.25	1.17 ± 0.31[d]	70.75	16.67
2 x 1600 x 5	17800 ± 322	5222 ± 390[c]	3000 ± 119[c]	-70.66	-83.15	0.67 ± 0.34[d]	83.25	50.00
Praziquantel								
1 x 5 x 5	18000 ± 303	3611 ± 130[c]	394 ± 33[c]	-79.94	-97.82	0.50 ± 0.22[d]	87.50	50.00
2 x 5 x 5	17788 ± 144	400 ± 48[c]	0[c]	-97.75	-100.00	0[d]	100.00	100.00
1 x 10 x 5	17666 ± 251	344 ± 75[c]	0[c]	-98.05	-100.00	0[d]	100.00	100.00
2 x 10 x 5	17000 ± 219	0[c]	0[c]	-100.00	-100.00	0[d]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. pre-treatment value, Student's *t*-test.
[d]$p < 0.001$ vs. control value, Students *t*-test.

Table 2.8a: Effect of *Clerodendrum colebrookianum* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	16066 ± 465	15800 ± 607	-1.66	3.50 ± 0.34	12.50	0.00
Plant Extract						
1 x 200 x 5	12000 ± 347[c]	6711 ± 333[c]	-44.07	3.17 ± 0.17*	20.75	0.00
2 x 200 x 5	11400 ± 187[c]	6100 ±218[c]	-46.49	3.17 ± 0.17*	20.75	0.00
1 x 400 x 5	9977 ± 388[c]	5200 ± 242[c]	-47.88	3.00 ± 0.26*	25.00	0.00
2 x 400 x 5	8800 ± 305[c]	4411 ± 93[c]	-49.87	2.67 ± 0.21*	33.25	0.00
1 x 800 x 5	6511 ± 260[c]	3200 ± 217[c]	-50.85	2.50 ± 0.22[f]	37.50	0.00
2 x 800 x 5	4000 ± 145[c]	1866 ± 121[c]	-53.34	2.34 ± 0.21[e]	41.50	0.00
1 x 1600 x 5	3900 ± 66[c]	1777 ± 83[c]	-54.42	2.17 ± 0.17[d]	45.75	0.00
2 x 1600 x 5	3600 ± 153[c]	1600 ± 116[c]	-55.56	2.00 ± 0.26[e]	50.00	0.00
Praziquantel						
1 x 5 x 5	2688 ± 243[c]	1055 ± 49[c]	-70.25	1.17 ± 0.17[c]	70.75	0.00
2 x 5 x 5	1977 ± 92[c]	700 ± 37[c]	-76.40	1.00 ± 0.26[c]	75.00	16.67
1 x 10 x 5	1800 ± 51[c]	533 ± 34[c]	-77.78	1.00 ± 0.00[c]	75.00	33.34
2 x 10 x 5	833 ± 59[c]	111 ± 28[c]	-92.00	0.50 ± 0.22[c]	87.50	50.00

[a] dministration of extract on days 2-6 post-inoculation with four cysticercoids per rat ($n = 4$)
[b] No. of animals in each group, $n = 6$.
[c, d, e, f] $p < 0.001$, $p < 0.01$, $p < 0.02$ and $p < 0.05$ vs. control value, Student's t-test.
*not significant

Table 2.8b: Effect of *Clerodendrum colebrookianum* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17000 ± 435	16777 ± 402	-1.31	3.84 ± 0.17	4.00	0.00
Plant Extract						
1 x 200 x 5	14000 ± 221[c]	7000 ± 156[c]	-50.00	2.84 ± 0.17[d]	29.00	0.00
2 x 200 x 5	13411 ± 431[c]	6177 ± 123[c]	-53.94	2.34 ± 0.21[c]	41.50	0.00
1 x 400 x 5	11000 ± 168[c]	4611 ± 160[c]	-58.08	2.00 ± 0.37[c]	50.00	0.00
2 x 400 x 5	11655 ± 281[c]	4722 ± 388[c]	-59.49	2.00 ± 0.26[c]	50.00	0.00
1 x 800 x 5	6000 ± 273[c]	2400 ± 141[c]	-60.00	1.84 ± 0.48[c]	54.00	0.00
2 x 800 x 5	5500 ± 144[c]	2077 ± 138[c]	-62.22	1.34 ± 0.21[c]	66.50	0.00
1 x 1600 x 5	3888 ± 267[c]	1488 ± 93[c]	-61.71	1.34 ± 0.42[c]	66.50	16.67
2 x 1600 x 5	3100 ± 189[c]	1100 ± 59[c]	-64.52	1.00 ± 0.37[c]	75.00	33.34
Praziquantel						
1 x 5 x 5	3200 ± 124[c]	511 ± 37[c]	-84.03	0.67 ± 0.21[c]	83.25	33.34
2 x 5 x 5	3433 ± 192[c]	255 ± 31[c]	-92.56	0.17 ± 0.17[c]	95.75	83.34
1 x 10 x 5	1400 ± 54[c]	88 ± 22[c]	-93.65	0.17 ± 0.17[c]	95.75	83.34
2 x 10 x 5	1600 ± 82[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d]$p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.8c: Effect of *Clerodendrum colebrookianum* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	17844 ± 310	17833 ± 303	17800 ± 219	-0.25	-0.29	4.00 ± 0.17	0.00	0.00
Plant Extract								
1 x 200 x 5	17822 ± 390	13733 ± 431[c]	8222 ± 249[c]	-22.94	-53.87	2.84 ± 0.17[d]	29.00	0.00
2 x 200 x 5	17811 ± 206	12800 ± 236[c]	7800 ± 258[c]	-28.13	-56.21	2.00 ± 0.26[d]	50.00	0.00
1 x 400 x 5	17788 ± 203	12200 ± 220[c]	7600 ± 151[c]	-31.41	-57.28	1.84 ± 0.17[d]	54.00	0.00
2 x 400 x 5	17700 ± 196	10400 ± 172[c]	6100 ± 198[c]	-41.24	-65.54	1.67 ± 0.21[d]	59.00	0.00
1 x 800 x 5	17766 ± 395	10000 ± 275[c]	5611 ± 311[c]	-43.71	-68.42	1.50 ± 0.22[d]	62.50	0.00
2 x 800 x 5	17444 ± 334	8133 ± 202[c]	4800 ± 155[c]	-53.38	-72.48	1.50 ± 0.43[d]	62.50	16.67
1 x 1600 x 5	17600 ± 215	8777 ± 278[c]	4600 ± 189[c]	-50.13	-73.86	1.34 ± 0.49[d]	66.50	33.34
2 x 1600 x 5	17311 ± 487	6400 ± 408[c]	3833 ± 171[c]	-63.03	-77.86	0.84 ± 0.31[d]	79.00	0.00
Praziquantel								
1 x 5 x 5	17200 ± 336	3200 ± 221[c]	833 ± 90[c]	-81.40	-95.16	0.50 ± 0.34[d]	87.00	66.67
2 x 5 x 5	16600 ± 262	833 ± 70[c]	0[c]	-94.98	-100.00	0[d]	100.00	100.00
1 x 10 x 5	17166 ± 361	800 ± 100[c]	0[c]	-95.34	-100.00	0[d]	100.00	100.00
2 x 10 x 5	16555 ± 395	155 ± 56[c]	0[c]	-99.06	-100.00	0[d]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. pre-treatment value, Student's *t*-test.
[d]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.9a: Effect of *Centella asiatica* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	18377 ± 251	17800 ± 303	-3.14	4.00 ± 0.00	0.00	0.00
Plant Extract						
1 x 200 x 5	17000 ± 236[d]	9600 ± 145[c]	-43.53	3.34 ± 0.21[c]	16.50	0.00
2 x 200 x 5	16377 ± 370[d]	9000 ± 207[c]	-45.05	3.17 ± 0.17[c]	20.75	0.00
1 x 400 x 5	13466 ± 388[c]	7377 ± 235[c]	-45.21	3.00 ± 0.37[f]	25.00	0.00
2 x 400 x 5	12911 ± 256[c]	6688 ± 287[c]	-48.19	2.67 ± 0.21[c]	33.25	0.00
1 x 800 x 5	10044 ± 371[c]	5244 ± 49[c]	-47.79	2.67 ± 0.42[e]	33.25	0.00
2 x 800 x 5	7900 ± 159[c]	3944 ± 133[c]	-50.07	2.34 ± 0.21[c]	41.50	0.00
1 x 1600 x 5	5633 ± 276[c]	2711 ± 115[c]	-51.87	2.17 ± 0.17[c]	45.75	0.00
2 x 1600 x 5	5000 ± 232[c]	2300 ± 165[c]	-54.00	2.00 ± 0.26[c]	50.00	0.00
Praziquantel						
1 x 5 x 5	3333 ± 197[c]	1000 ± 45[c]	-70.00	1.17 ± 0.17[c]	70.75	0.00
2 x 5 x 5	2000 ± 173[c]	500 ± 28[c]	-75.00	0.84 ± 0.31[c]	79.00	33.34
1 x 10 x 5	2022 ± 122[c]	488 ± 22[c]	-75.82	0.84 ± 0.17[c]	79.00	16.67
2 x 10 x 5	1733 ± 64[c]	144 ± 11[c]	-91.67	0.34 ± 0.21[c]	91.50	66.67

[a]Administration of extract on days 2-6 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e, f]$p < 0.001$, $p < 0.01$, $p < 0.02$ and $p < 0.05$ vs. control value, Student's *t*-test.

Table 2.9b: Effect of *Centella asiatica* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (mean ± SEM)		Percentage difference in EPG between A and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
	Day 18-20 (A)	Day 28-30 (B)				
Control	17488 ± 245	17222 ± 378	-1.31	3.84 ± 0.17	96.00	0.00
Plant Extract						
1 x 200 x 5	12600 ± 270[c]	6200 ± 465[c]	-50.00	2.84 ± 0.40[e]	29.00	0.00
2 x 200 x 5	12633 ± 195[c]	5822 ± 63[c]	-53.94	2.34 ± 0.34[d]	41.50	0.00
1 x 400 x 5	11388 ± 183[c]	4933 ± 101[c]	-58.08	2.17 ± 0.17[c]	45.75	0.00
2 x 400 x 5	10244 ± 269[c]	4211 ± 113[c]	-59.49	2.00 ± 0.45[d]	50.00	16.67
1 x 800 x 5	6000 ± 197[c]	2400 ± 82[c]	-60.00	1.84 ± 0.17[c]	54.00	0.00
2 x 800 x 5	5600 ± 307[c]	2044 ± 151[c]	-62.22	1.50 ± 0.22[c]	62.50	0.00
1 x 1600 x 5	4900 ± 118[c]	1800 ± 57[c]	-61.71	1.34 ± 0.42[c]	66.50	33.34
2 x 1600 x 5	4455 ± 201[c]	1544 ± 121[c]	-64.52	1.17 ± 0.17[c]	70.75	0.00
Praziquantel						
1 x 5 x 5	3266 ± 350[c]	466 ± 34[c]	-84.03	0.50 ± 0.22[c]	87.50	50.00
2 x 5 x 5	2800 ± 151[c]	166 ± 22[c]	-92.56	0.17 ± 0.17[c]	95.75	83.34
1 x 10 x 5	1800 ± 115[c]	122 ± 31[c]	-93.65	0.17 ± 0.17[c]	95.75	83.34
2 x 10 x 5	711 ± 80[c]	0[c]	-100.00	0[c]	100.00	100.00

[a]Administration of extract on days 8-12 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d, e] $p < 0.001$ $p < 0.01$ and $p < 0.05$ vs. control value, Student's *t*-test.

Table 2.9c: Effect of *Centella asiatica* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm reduction rate and host clearance.

Group (Dose x mg/kg x days)	EPG (Mean ± SEM) Pre-treatment (Z)	Post-treatment (A)	Follow up (B)	Percentage difference in EPG between Z and A	Z and B	No. of worms recovered/rat (Mean ± SEM)	Percentage worm reduction	Percentage host clearance
Control	19800 ± 333	19600 ± 598	19666 ± 274	-1.01	-0.68	4.00 ± 0.17	0.00	0.00
Plant Extract								
1 x 200 x 5	19700 ± 216	14400 ± 311[c]	9200 ± 294[c]	-26.90	-53.30	2.84 ± 0.17[e]	29.00	0.00
2 x 200 x 5	19400 ± 1211	13555 ± 424[d]	8111 ± 393[c]	-30.13	-58.19	2.00 ± 0.37[e]	50.00	0.00
1 x 400 x 5	19600 ± 248	13200 ± 322[c]	8200 ± 125[c]	-32.65	-58.16	2.00 ± 0.52[f]	50.00	0.00
2 x 400 x 5	19111 ± 592	10800 ± 229[c]	6400 ± 404[c]	-43.49	-66.51	1.67 ± 0.34[e]	59.00	0.00
1 x 800 x 5	18800 ± 576	10377 ± 255[c]	6166 ± 245[c]	-44.80	-67.20	1.50 ± 0.43[e]	62.50	16.67
2 x 800 x 5	18655 ± 376	9322 ± 516[c]	5000 ± 261[c]	-50.03	-73.20	1.50 ± 0.22[e]	62.50	0.00
1 x 1600 x 5	18222 ± 216	9477 ± 254[c]	4933 ± 166[c]	-47.99	-72.93	1.34 ± 0.61[e]	66.50	50.00
2 x 1600 x 5	18400 ± 352	7200 ± 328[c]	4600 ± 232[c]	-60.87	-75.00	0.84 ± 0.31[e]	79.00	0.00
Praziquantel								
1 x 5 x 5	18000 ± 125	2400 ± 259[c]	400 ± 51[c]	-86.67	-97.78	0.50 ± 0.22[e]	87.50	50.00
2 x 5 x 5	18200 ± 259	166 ± 56[c]	0[c]	-99.09	-100.00	0[e]	100.00	100.00
1 x 10 x 5	18000 ± 383	366 ± 47[c]	0[c]	-97.97	-100.00	0[e]	100.00	100.00
2 x 10 x 5	17800 ± 683	0[c]	0[c]	-100.00	-100.00	0[e]	100.00	100.00

[a]Administration of extract on days 21-25 post-inoculation with four cysticercoids per rat (n = 4)
[b]No. of animals in each group, n = 6.
[c, d] $p < 0.001$ and $p < 0.01$ vs. pre-treatment value, Student's *t*-test.
[e, f] $p < 0.001$ and $p < 0.01$ vs. control value, Student's *t*-test.

Table 2.10: Lethal effects of plant extracts to the experimental rats by monitoring median lethal dose (LD_{50} value).

Group*	LD_{50} value (oral; mg/kg; rat)
Plant extract	
1. *L. spinosa* (stem)	7678
2. *C. longa*	7487
3. *C. asiatica*	NM **
4. *C. colebrookianum*	NM **
5. *L. spinosa* (leaf)	NM **
6. *L. spinosa* (stalk)	NM **
7. *G. angulosa*	NM **
8. *H. cordata*	NM **
9. *P. guajava*	NM **

*Oral administration of extracts given at doses of 3200 and 6400 mg/kg to groups of rats, n = 6.

**No mortality of animals was noticed for the extract when treatment was administered up to 6400 mg/kg dose, and observed for 72 h post treatment.

B. Anthelmintic activity of plant extracts in *T. spiralis* - mouse model

The efficacy of *G. angulosa* leaf extract on adult, migrating and encysted stages of *T. spiralis* infections in mice is presented in Table 2.11. Of all the plant extracts tested, the 1600 mg/kg dose of *G. angulosa* extract showed the maximum reduction (72.36%) of encysted larvae in the host. In this case, the reduction by 50 mg/kg dose of MBZ was recorded to be 92.93%. The same dose of extract also showed the efficacy at higher sides against the adult worms and migrating larvae. The percentage worm reduction by extract was noted to be 86.22% for adult worms and 78.53% for migrating larvae. The reference drug (50 mg/kg) however showed 100% adult worm reduction.

Table 2.12 summarizes the efficacy of *C. colebrookianum* leaf extract against different stages of *T. spiralis* infections in mice. At 1600 mg/kg dose the leaf extract showed significant efficacy against the adult and migrating stage. In this case the percentage adult worm reduction was noted to be 85.39% and percentage migrating larvae reduction by 75.84. Unlike, *G. angulosa* the C. *colebrookianum* extract showed only 55.06% reduction of encysted larvae.

The efficacy of *L. spinosa* leaf extract can be seen from the results presented in Table 2.13. The extract showed most profound efficacy against the adult stage where the percentage worm reduction was recorded to be 89.39% compared to 100% by MBZ (50 mg/kg). It however, showed rather weak efficacy against the encysted stage where the percentage reduction in the number of

encysted larvae was recorded to be 52.21% compared to MBZ which showed 78.45% larvae reduction.

Table 2.14 summarizes the anthelmintic efficacy of *P. guajava* leaf extract against *T. spiralis*. The extract (1600 mg/kg) showed more or less same profile as shown by *L. spinosa* leaf extract with adult worm reduction of 86.37%, migrating larvae reduction of 67.14% and encysted larvae reduction of 48.23%.

Table 2.15 represents the efficacy *H. cordata* leaf extract against the adult, migrating and encysted stages of *T. spiralis* infections in mice. This plant showed slightly low efficacy as compared to previous plant extracts. The 1600 mg/kg dose of *H. cordata* extract revealed 79.13% reduction in adult worms, 62.15% reduction in migrating larvae and only 45.48% reduction in encysted larvae.

The efficacy of *C. longa* rhizome extract was recorded to be more or less same as *P. guajava* extract (Table 2.16). The extract at 1600 mg/kg dose revealed 77.27% reduction in adult worms compared to 100% by MBZ (50 mg/kg). The same dose of extract showed only 44.39% reduction in encysted larvae.

The efficacy of *L. spinosa* stalk and *L. spinosa* stem extract is presented in Tables 2.17 and 2.18. Both the extracts showed almost similar kind of efficacy

against the 3 stages of infection. Further, the efficacy of stalk and stem extract of *L. spinosa* was recorded to be on lower sides when compared to the efficacy of its leaf extract. The 1600 mg/kg dose of stem extract revealed maximum reduction of 77.36% adult worms. The extract could reduce only 35.34% encysted larvae.

Of all the plant extracts tested in this study, *C. asiatica* leaf extract showed the weakest efficacy against different stages of *Trichinella* (Table 2.19). The 1600 mg/kg dose reduced the numbers of adult worms by 52.57%, number of migrating larvae by 47.59% and encysted larvae only by 26.68%.

Acute Toxicity Effect of Plant Extracts in Experimental Mice

Median Lethal Dose (LD_{50}): The lethal effect to the experimental animals caused by oral treatment of different plant extracts at 3200 and 6400 mg/kg, body wt. dose within 72 h post-treatment observation is presented in Table 2.20. The maximum mortality of animals was observed for *C. longa* extract at 6400 mg/kg dose (3 out of 6 animals died), followed by *C. colebrookianum* extract (2 out of 6 animals died) and *H. cordata* extracts (1 out of 6 animals died). Accordingly, LD_{50} (Oral; mg/kg) value was calculated to be 5622 for *C. longa*, 7487 for *C. colebrookianum* and 11558 for *H. cordata*. However, the rest of the plant extracts neither caused any mortality to animals nor any changes with regard to food and water intake up to 6400 mg/kg dose.

Table 2.11: Anthelmintic efficacy of *Gynura angulosa* leaf extract[a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	94.34 ± 6.36	-	31200 ± 117	-	29000 ± 716	-
Plant extract						
200 mg/kg	37.66 ± 3.50[c]	60.08	12400 ± 1140[c]	60.26	12466 ± 493[c]	57.01
400 mg/kg	31.50 ± 3.18[c]	66.61	11000 ± 700[c]	64.74	10800 ± 989[c]	62.76
800 mg/kg	21.84 ± 1.99[c]	76.85	8400 ± 113[c]	73.08	10050 ± 482[c]	65.34
1600 mg/kg	13.00 ± 1.72[c]	86.22	6700 ± 473[c]	78.53	8016 ± 506[c]	72.36
Mebendazole						
25 mg/kg	5.00 ± 1.29[c]	94.70	1883 ± 172[c]	93.96	2750 ± 374[c]	90.52
50 mg/kg		100.00	1166 ± 201[c]	96.26	2050 ± 298[c]	92.93

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.12: Anthelmintic efficacy of *Clerodendrum colebrookianum* leaf extract [a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	105.00 ± 8.08	-	36000 ± 519	-	35600 ± 378	-
Plant extract						
200 mg/kg	59.34 ± 2.96[c]	43.49	16300 ± 351[c]	54.68	20000 ± 360[c]	43.82
400 mg/kg	48.67 ± 2.96[c]	53.65	15200 ± 416[c]	57.78	19000 ± 115[c]	46.63
800 mg/kg	30.67 ± 2.34[c]	70.79	11200 ± 458[c]	68.86	17800 ± 435[c]	50.00
1600 mg/kg	15.34 ± 1.86[c]	85.39	8700 ± 173[c]	75.84	16000 ± 416[c]	55.06
Mebendazole						
25 mg/kg	4.67 ± 0.67[c]	95.55	3500 ± 172[c]	90.27	8600 ± 264[c]	75.84
50 mg/kg	0.34 ± 0.03[c]	99.68	1500 ± 201[c]	95.83	7000 ± 58[c]	80.34

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.13: Anthelmintic efficacy of *Lasia spinosa* leaf extract [a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	116.34 ± 2.96	-	35800 ± 907	-	36200 ± 642	-
Plant extract						
200 mg/kg	50.00 ± 3.61[c]	57.02	15900 ± 321[c]	55.59	21000 ± 435[c]	41.99
400 mg/kg	41.00 ± 1.53[c]	64.76	13800 ± 404[c]	61.45	20000 ± 305[c]	44.75
800 mg/kg	31.67 ± 3.18[c]	72.78	11900 ± 378[c]	66.76	18900 ± 577[c]	47.79
1600 mg/kg	12.34 ± 2.34[c]	89.39	9600 ± 635[c]	73.18	17300 ± 264[c]	52.21
Mebendazole						
25 mg/kg	7.00 ± 0.58[c]	93.98	3200 ± 115[c]	91.06	10000 ± 230[c]	72.38
50 mg/kg		100.00	1900 ± 230[c]	94.69	7800 ± 152[c]	78.45

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.14: Anthelmintic efficacy of *Psidium guajava* leaf extract[a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	115.00 ± 2.31	-	35300 ± 404	-	36700 ± 264	-
Plant extract						
200 mg/kg	50.00 ± 1.73[c]	56.52	18700 ± 602[c]	47.03	24000 ± 665[c]	34.60
400 mg/kg	42.00 ± 3.51[c]	63.48	16100 ± 173[c]	54.39	22000 ± 351[c]	40.05
800 mg/kg	31.00 ± 1.99[c]	73.04	13900 ± 115[c]	60.62	20500 ± 458[c]	44.14
1600 mg/kg	15.67 ± 1.00[c]	86.37	11600 ± 404[c]	67.14	19000 ± 529[c]	48.23
Mebendazole						
25 mg/kg	6.00 ± 0.58[c]	94.78	3500 ± 57[c]	90.08	10900 ± 115[c]	70.30
50 mg/kg		100.00	1700 ± 10[c]	95.18	8400 ± 152[c]	77.11

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.15: Anthelmintic efficacy of *Houttuynia cordata* leaf extract[a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	123.00 ± 4.04	-	36000 ± 536	-	37600 ± 472	-
Plant extract						
200 mg/kg	63.67 ± 4.37[c]	48.00	20800 ± 360[c]	41.68	26200 ± 435[c]	30.32
400 mg/kg	49.00 ± 3.11[c]	60.16	16600 ± 115[c]	53.46	24000 ± 378[c]	36.17
800 mg/kg	41.34 ± 2.31[c]	66.40	14900 ± 550[c]	58.22	22000 ± 550[c]	41.49
1600 mg/kg	25.67 ± 2.85[c]	79.13	13500 ± 200[c]	62.15	20500 ± 550[c]	45.48
Mebendazole						
25 mg/kg	8.34 ± 1.20[c]	93.22	4000 ± 200[c]	88.79	9400 ± 435[c]	75.00
50 mg/kg		100.00	1800 ± 152[c]	94.95	7000 ± 152[c]	81.38

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.16: Anthelmintic efficacy of *Curcuma longa* rhizome extract[a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	117.34 ± 2.34	-	36100 ± 550	-	39200 ± 585	-
Plant extract						
200 mg/kg	64.67 ± 2.03[c]	44.89	19700 ± 404[c]	45.43	27500 ± 288[c]	29.34
400 mg/kg	45.00 ± 2.89[c]	61.65	17800 ± 152[c]	50.69	24000 ± 321[c]	35.71
800 mg/kg	40.34 ± 2.96[c]	65.62	15200 ± 513[c]	57.89	21600 ± 305[c]	39.80
1600 mg/kg	23.00 ± 3.06[c]	77.27	12500 ± 208[c]	65.37	20300 ± 416[c]	44.39
Mebendazole						
25 mg/kg	8.34 ± 0.34[c]	92.90	3400 ± 152[c]	90.58	6900 ± 351[c]	71.94
50 mg/kg		100.00	1100 ± 115[c]	96.95	4600 ± 346[c]	80.36

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.17: Anthelmintic efficacy of *Lasia spinosa* stalk extract[a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	111.34 ± 5.24	-	36600 ± 611	-	36900 ± 173	-
Plant extract						
200 mg/kg	63.34 ± 2.85[c]	43.11	22000 ± 550[c]	39.89	29500 ± 642[c]	20.05
400 mg/kg	54.00 ± 0.58[c]	51.50	21100 ± 642[c]	42.35	28400 ± 378[c]	23.04
800 mg/kg	47.34 ± 2.73[c]	57.48	18000 ± 404[c]	50.82	25900 ± 550[c]	29.81
1600 mg/kg	40.34 ± 0.67[c]	63.77	15900 ± 458[c]	56.56	23400 ± 264[c]	36.59
Mebendazole						
25 mg/kg	6.67 ± 0.88[c]	94.01	4000 ± 300[c]	89.07	10000 ± 321[c]	72.90
50 mg/kg		100.00	2100 ± 115[c]	94.26	7000 ± 360[c]	81.03

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.18: Anthelmintic efficacy of *Lasia spinosa* stem extract [a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	116.34 ± 2.03	-	35600 ± 556	-	36500 ± 305	-
Plant extract						
200 mg/kg	64.00 ± 2.00[c]	44.99	21900 ± 503[c]	38.48	29600 ± 529[c]	18.90
400 mg/kg	44.67 ± 1.45[c]	61.60	19700 ± 152[c]	44.66	27500 ± 611[c]	24.66
800 mg/kg	40.00 ± 1.53[c]	65.62	16800 ± 208[c]	52.81	25500 ± 513[c]	30.14
1600 mg/kg	26.34 ± 4.84[c]	77.36	15400 ± 346[c]	56.74	23600 ± 378[c]	35.34
Mebendazole						
25 mg/kg	8.34 ± 0.88[c]	92.83	3400 ± 435[c]	90.45	9400 ± 288[c]	74.25
50 mg/kg		100.00	2000 ± 321[c]	94.38	6100 ± 200[c]	83.29

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.19: Anthelmintic efficacy of *Centella asiatica* leaf extract [a] against adult, migrating and encysted stages of *T. spiralis* infections in mice[b].

Groups	Adult stage		Migrating stage		Encysted stage	
	No. of worms/mouse (mean ± SEM)	Percentage worm reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction	No. of larvae/mouse (mean ± SEM)	Percentage larvae reduction
Control	116.67 ± 3.88	-	35300 ± 529	-	37100 ± 152	-
Plant extract						
200 mg/kg	80.67 ± 2.67[c]	30.86	23400 ± 360[c]	33.71	31300 ± 230[c]	15.54
400 mg/kg	72.67 ± 5.46[c]	37.71	22000 ± 264[c]	37.68	30700 ± 384[c]	17.25
800 mg/kg	60.34 ± 1.76[c]	48.29	20300 ± 230[c]	42.49	28400 ± 264[c]	23.45
1600 mg/kg	55.34 ± 1.45[c]	52.57	18500 ± 208[c]	47.59	27200 ± 100[c]	26.68
Mebendazole						
25 mg/kg	9.34 ± 1.20[c]	92.00	4200 ± 305[c]	88.10	9600 ± 360[c]	74.12
50 mg/kg		100.00	1700 ± 115[c]	95.18	7000 ± 208[c]	81.13

[a]Administration of extract on days 3-4, 8-10, and 34-40 against adult, migrating and encysted stage, respectively post-inoculation with 200 larvae per mouse.
[b]No. of animals in each group, n = 6.
[c]$p < 0.001$ vs. control value, Student's *t*-test.

Table 2.20: Lethal effect of plant extracts to the experimental mice by monitoring median lethal dose (LD_{50} value).

Group*	LD_{50} value (oral; mg/kg; mice)
Plant extract	
1. *H. cordata*	11558
2. *C. colebrookianum*	7487
3. *C. longa*	5622
4. *L. spinosa* (leaf)	NM **
5. *L. spinosa* (stalk)	NM **
6. *L. spinosa* (stem)	NM **
7. *C. asiatica*	NM **
8. *G. angulosa*	NM **
9. *P. guajava*	NM **

*Oral administration of extracts given at doses of 3200 and 6400 mg/kg to groups of mice, n = 6.

**No mortality of animals was noticed for the extract when treatment was administered up to 6400 mg/kg dose, and observed for 72 h post treatment.

Discussion

In vivo trials have also been conducted in small laboratory animal models for the evaluation of anthelmintic activity of various substances of plant origin. These include expulsion of worms from their hosts (Kalesaraj and Kurup, 1968; Lawrence, 1990; Philips, 1990; Pradhan *et al.*, 1992; Asuzu and Onu, 1994; Desta, 1995) or reduction in the number of eggs per gram of feces passed by the infected hosts compared with standard anthelmintic treated animals (Akhtar, 1988).

In the present study the plant extracts investigated for their *in vitro* anthelmintic efficacy previously were further subjected to *in vivo* studies in two animal models *viz.*, *H. diminuta* infections in rats and *T. spiralis* infections in mice. A review of literature reveals that various workers have demonstrated the anthelmintic activities of traditionally used folklore plants such as, *Albizzia anthelmintica*, *A. lebbek*, *Acacia auriculiformis*, *Gladiolus gandavensis*, *Trifolium repens*, *Strobilanthes discolor*, *Butea minor*, *Allium sativum*, *Echinacea purpurea*, *Mangifera indica* etc., where the efficacy of plant extract was adjudged on the basis of its effects on larval, immature and adult stages of *H. diminuta* and against adult, migrating and encysted phases of *T. spiralis*. The extracts of these plants have been shown to cause significant reductions in the average egg counts and number of adult *H. diminuta* worms at necropsy and noteworthy adult worm reduction as well as reduction in the number of encysted

larvae in the muscle for *T. spiralis* (Galal *et al.*, 1991b; Ghosh *et al.*, 1996; Saha *et al.*, 1999; Bany *et al.*, 2003; Garcia *et al.*, 2003; Tangpu *et al.*, 2004 and 2006; Yadav and Tangpu, 2006a). In the present study assessment of plant anthelmintic efficacy in *H. diminuta* - rat model was undertaken against three developmental stages *viz.*, larval, immature and adult stages. The judgment of efficacy was made on the basis of monitoring the eggs per gram of faeces (EPG) count, percentage reduction in adult worms and host clearance rate. While in *T. spiralis* - mice model the efficacy was evaluated against adult, migrating and encysted larvae. In this case, firstly by taking the worm burden of infected control animals as the reference, the percentage worm reduction was determined. Further the reduction in the number of migrating and encysted larvae also constituted as other parameters. The aforesaid parameters for assessing the anthelmintic efficacy of various traditional plants against different stages of *H. diminuta* and *T. spiralis* have also been employed by a number of earlier workers (Galal *et al.*, 1991b; Ghosh *et al.*, 1996; Saha *et al.*, 1999; Bany *et al.*, 2003; Garcia *et al.*, 2003; Tangpu *et al.*, 2004 and 2006; Yadav and Tangpu, 2006a).

In order to observe the effects of plant extracts on larval stages of *H. diminuta*, treatment was given on days 2-6 post inoculation of cysticercoids. On inoculation into rats, the cysticercoids are expected to undergo excystation on reaching the lumen of the host's small intestine. It is at this time, that the excystation and establishment of the parasite takes place in the lumen of the

host. In the study on effects of leaf extract against the immature stages the treatment was done at 8-12 days post-inoculation of cysticercoids. Of all the plant extracts tested against *H. diminuta*, efficacy was noticed to be most profound for *P. guajava* leaf extract. Against the immature stage the *P. guajava* extract treatment showed the maximum reduction of EPG count by 82.89%. The percentage worm reduction was also noted to be almost comparable with that of PZQ. In case of extract efficacy against adult stage, it showed reduction in EPG count by 95.34% and percentage worm reduction was comparable with that of PZQ. The reduction in EPG count noted in the present study may be attributed due to removal of worms and/or process of destrobilation by the effects of extract (Dixon and Arai, 1991). It has been reported that the process of destrobilation in cestodes generally initiates if they are exposed to anthelmintic drugs (Hopkins, 1973).

The 1600 mg/kg double dose of *L. spinosa* leaf extract when administered to rats harbouring immature *H. diminuta* infections revealed up to 80.85% reduction in EPG count and a percentage worm reduction equal to PZQ. Treatment against adult stage indicated dose-dependent declining trend both in EPG count and worm count. The highest dose of extract showed 91.50% worm reduction and 94.87% EPG count reduction. This finding is in agreement with other similar kind of studies on effects of leaf extract of *A. auriculiformis*, *G. gandavensis* and *T. repens* on *H. diminuta* infections in rats (Ghosh *et al.*, 1996; Saha *et al.*, 1999; Tangpu *et al.*, 2004) where the extract-

treated animals showed marked reduction both in EPG and worm count. The reduction in EPG count and worm count in extract-treated rats provide support that *L. spinosa* leaf extract possesses significant anticestodal property. The stalk and stem extract of *L. spinosa* also showed appreciable anticestodal efficacy. At its highest dose administered against the adult stages, an uniform worm reduction of 83.25% was noted in both the cases which was almost comparable to 5 mg/kg dose of PZQ which showed 83.25 and 87.50% adult worm reduction, respectively.

H. cordata extract showed comparatively better efficacy against adult *H. diminuta* worms than larval or immature stages. The efficacy when compared to 5 mg/kg dose of PZQ was little less for larval and immature stages and comparable against adult worms. At 1600 mg/kg double dose though the EPG count of different groups at pre-treatment (days 18-20) accounted for uniformity, significant reductions in the EPG count were recorded in the extract-treated groups during post treatment period. The above results authenticate the presence of appreciable anthelmintic efficacy in *H. cordata* leaf extract.

The present study revealed presence of moderate level of anticestodal efficacy in *C. longa* rhizome extract. Both the EPG and worm reduction in the extract-treated animals was observed to be low as compared to 5 mg/kg dose of PZQ. Further, the efficacy of extract was found to be almost of similar degree

against immature and adult worms. The extract when administered during larval phase of infection failed to clear the host from worm infections.

The *G. angulosa* leaf extract showed comparatively better efficacy against the adult worms than larval or immature stage. The host clearance rate was better when the extract was administered during adult phase. Against the immature stage the extract showed only 79.00% worm reduction, whereas the same was noted to be 87.50% against the adult stage. It may be mentioned here that *G. angulosa* extract also exhibited significant *in vitro* anthelmintic activity against *H. diminuta.* The extract of *G. angulosa* thus seems to be effective *in vitro* as well as *in vivo* against *H. diminuta.*

The *C. colebrookianum* leaf extract exhibited moderate efficacy against *H. diminuta* infections in rats. The reduction in EPG and worm count was rather low as compared to standard drug, PZQ. Further, the extract showed comparatively better efficacy against immature and adult stages than the larval stage. None of the extract dose could clear the infection in host when it was administered during larval phase. The *C. asiatica* leaf extract also showed more or less similar level of anticestodal efficacy as *C. colebrookianum* leaf extract.

The current investigation on acute toxicity effect of the plant extracts by determining LD_{50} (Oral; mg/kg) reveals that only *C. longa* rhizome extract, and *L. spinosa* stem extract caused some toxicity to rats as evident by mortality of

animals. However, there was no mortality or visible signs of toxicity or differences in food and water uptake in the animals up to 6400 mg/kg dose of plant extracts, which indicate towards their safety profile.

The larvae of *T. spiralis* develop into adults in the intestine within 28 to 36 h after oral ingestion (Despommier, 1983) and therefore in the present study the animals were treated with plant extracts on day 3 and 4 to assess the efficacy against the adult stage. To observe the efficacy of plant extracts on migratory and encysted stages, it was necessary first to remove the adult worms remaining in the intestine without affecting the migrating new larvae (Denham and Martinez, 1970). This was achieved by treating both the controls and experimental groups, on 7 d.p.i. with trichlorfon plus one intramuscular injection of atropine sulphate. Trichlorfon is virtually 100% effective against adult *Trichinella* but appears to have no effect on developing muscle larvae (Denham and Martinez, 1970). For migrating stage of infection the treatment was given for 3 consecutive days beginning on 8 d.p.i. and for encysted stage treatment was given day 34-40 post inoculation of larvae.

In general, barring *C. asiatica*, all other plant extracts tested in this study showed moderate to high efficacy against the adult *Trichinella* worms and more or less similar was the case against their efficacy against the migrating larvae. In contrast, barring *G. angulosa* the rest of the plant extracts showed either medium or very low level of efficacy against the encysted muscle larvae. This

may in part be explained by the fact that once the larvae have become encysted therapeutic intervention is generally less feasible (Blair, 1983). This might also be due to the fact that as the cyst matures its susceptibility to chemotherapeutic agents diminishes with duration of infection (Campbell and Blair, 1974). The leaf extract of *G. angulosa* showed up to 86.22% adult worm reduction and 72.36% encysted larvae reduction. The efficacy at 25 mg/kg dose of MBZ against these stages was noted to be 94.70% and 90.52%, respectively.

The efficacy of C. *colebrookianum* leaf extract against the adult and migrating stage was almost of similar level as *G. angulosa*, however it failed to show similar kind of activity against the encysted larvae, where the reduction was noted up to only 55.06%.

The *L. spinosa* leaf extract showed the maximum efficacy against the adult *Trichinella* worms where it reduced the worm burden by 89.39%. The efficacy of extract however was recorded to be just 52.21% against the encysted stage. The efficacy of stalk and stem extracts of *L. spinosa* extract was noted to be below the limits of comparison than the efficacy of its leaf extract. The reduction in worm count was noted to be 63.77 and 77.36%, respectively for these extracts. Both the extracts could reduce only 36.59% and 35.34% of encysted larvae.

Like its significant efficacy in *Hymenolepis* model, the *P. guajava* leaf extract also showed appreciable efficacy against adult and migrating stage of *Trichinella* infections in mice. At 1600 mg/kg dose the extract showed 86.37% worm reduction compared to 94.78% by 5 mg/kg dose of MBZ. Again at the same dose the extract showed up to 67.14% reduction in migrating larvae compared to 90.08% reduction by the same dose of MBZ.

The *H. cordata* leaf extract revealed 79.13% elimination of worms in the intestinal phase whereas, 93.22% of worms were reduced by MBZ at 25 mg/kg dose. In this case also the plant extract showed poor efficacy against the encysted larvae.

The *C. longa* rhizome extract also showed more or less similar kind of efficacy as *H. cordata* extract. A reduction of 77.27% worms was recorded at the highest dose (1600 mg/kg) compared to 100% by 50 mg/kg dose of MBZ. The *C. asiatica* leaf extract failed to show appreciable efficacy against any of the stages of *Trichinella* infections in mice.

The current investigation on acute toxicity effect of the plant extracts by determining LD_{50} (Oral; mg/kg) reveals that only *C. longa* rhizome extract, *C. colebrookianum* and *H. cordata* extract caused slight toxicity to mice as evident by mortality of few animals. However, there was no mortality or visible signs of

toxicity or differences in food and water uptake in the animals up to 6400 mg/kg dose of plant extracts, which indicate towards their safety profile.

It may be concluded from the *in vivo* studies on *H. diminuta* - rat model that *P. guajava*, *L. spinosa* (leaf), *H. cordata* and *G. angulosa* extracts possess most profound anthelmintic efficacy. These plants also showed good *in vitro* anthelmintic efficacy in previous investigations. With regard to efficacy of plant extracts in *T. spiralis* - mouse model, it may be concluded that by and large all tested plant extracts possess promising efficacy against adult worms. However, barring *G. angulosa*, none of the plant extract show appreciable efficacy against the encysted muscle larvae of *Trichinella*.

This study thus validates the presence of appreciable anthelmintic property in many of the folk medicinal plants used by Naga tribes which may have therapeutic benefits in humans encountering helminthic infections. Further investigation on isolated chemical constituents of these plants should be pursued against different helminth parasite species.

Summary

The present work incorporates a study on ascertaining the anthelmintic activity of seven medicinal plants that are commonly used in the folklore medicine system of Naga tribes in Nagaland to cure helminthic parasitic infections. The objectives of the study were:

1. to test the anthelmintic efficacy of some folklore medicinal plants used in the traditional medicine system of Naga tribes.
2. to compare the anthelmintic efficacy of these plants with broad-spectrum anthelmintic drugs.
3. to investigate the acute toxicity of plants in experimental animals.
4. to investigate the effects of these plant extracts on surface fine topography of parasites.

To evaluate the anthelmintic activity of folklore medicinal plants, seven plant species, namely - *Centella asiatica* L. (Apiaceae), *Clerodendrum colebrookianum* Walp. (Verbenaceae), *Curcuma longa* L. (Zingiberaceae), *Gynura angulosa* DC. (Asteraceae), *Houttuynia cordata* Thunb. (Piperaceae), *Lasia spinosa* L. (Araceae) and *Psidium guajava* L. (Myrtaceae) were included based upon the information collected about their use as deworming agents from the traditional practitioner and local people in the Nagaland state. The various usable plant parts were extracted in methanol and the crude extracts were

tested at different concentrations *in vitro* and *in vivo* against several helminth parasites. For *in vitro* study, *Raillietina echinobothrida*, *Hymenolepis diminuta*, *Gasthrothylax crumenifer*, *Ascaridia galli* and *Trichinella spiralis* served as the test parasites. While *Hymenolepis diminuta* - rat and *Trichinella spiralis* - mice animal models were involved to evaluate the *in vivo* anthelmintic efficacy of plant extracts.

In the *in vitro* studies the test parasites were exposed to 5, 10, 20 and 40 mg/ml concentrations of plant extracts and mortality of worms served as the anthelmintic criterion. In each case the parasites were also exposed to corresponding concentrations of a standard anthelmintic drug to compare the efficacy of plant extract. Out of the seven plant extract tested, *P. guajava*, *H. cordata*, *L. spinosa* (stalk and leaf), *G. angulosa* and *C. colebrookianum* revealed significant anthelmintic efficacy. However, a moderate level of anthelmintic efficacy was observed for *L. spinosa* (stem), *C. asiatica* and *C. longa*. With respect to various helminthic groups, the study revealed that the leaf extract of *P. guajava*, *H. cordata* and stalk of *L. spinosa* possess profound efficacy against the cestode parasite, *R. echinobothrida*. The leaf extract of *P. guajava*, *L. spinosa* and *G. angulosa* manifested appreciable anticestodal efficacy against *H. diminuta*. Of different plant extracts tested against *G. crumenifer*, leaf extracts of *L. spinosa*, *C. colebrookianum* and *H. cordata* showed good flukicidal efficacy. In case of roundworm *A. galli*, only the leaf extract of *L. spinosa* was found to possess promising anthelmintic activity. Lastly, against the adult *T. spiralis* worms leaf extracts of *G. angulosa*, *L.*

spinosa, *C. colebrookianum*, *H. cordata* and *P. guajava* revealed significant activity. The individual plant extracts showing significant efficacy were further tested in combination with other extracts to investigate whether they could have any synergistic effects on mortality of parasites. No substantial increase in the anthelmintic efficacy of extracts was observed in such investigations.

The present study revealed that *P. guajava* leaf extract possess significant level of efficacy against *R. echinobothrida*, *H. diminuta* and *T. spiralis*. In case of its efficacy against *R. echinobothrida*, both its 20 and 40 mg/ml concentrations revealed the mortality of parasites in 1.00 h. Against *H. diminuta*, the extract showed mortality of worms in 2.34 h at 40 mg/ml concentration. Mortality of *T. spiralis* in its 40 mg/ml concentration was observed to be in as early as in 0.92 h. The *H. cordata* extract showed significant *in vitro* anthelmintic efficacy against *R. echinobothrida*, *G. crumenifer* and *T. spiralis*. *R. echinobothrida* treated with the 40 mg/ml concentration of *H. cordata* extract showed mortality of worms within 2.00 h. The efficacy of extract was recorded to be slightly lower against *G. crumenifer*, wherein it caused mortality of worms in 3.00 h. Against *T. spiralis*, the 40 mg/ml concentration of extract showed mortality of worms in as early as in 0.89 h.

In the present study the *L. spinosa* leaf extract showed profound anthelmintic efficacy against *H. diminuta*, *G. crumenifer*, *A. galli* and *T. spiralis*. The *H. diminuta* worms showed mortality within 2.50 h at its 40 mg/ml

concentration. The amphistome, *G. crumenifer* exposed to 40 mg/ml concentration of extract revealed the mortality of worms in 2.09 h which was almost comparable to Praziquantel (PZQ), the reference drug. The stalk extract of *L. spinosa* was also evaluated for anthelmintic efficacy in the present study and showed good efficacy only against *R. echinobothrida*. Whereas the stem extract of *L. spinosa* was not found be as effective as leaf and stalk extract.

The present investigation revealed that *G. angulosa* possesses prominent anthelmintic activity only against *H. diminuta* and *T. spiralis*. The plant extract at 40 mg/ml concentration showed mortality of *H. diminuta* worms in 2.92 h compared to PZQ which showed mortality of parasites in 0.60 h at the same concentration. Similarly, for *T. spiralis* also the plant extract showed almost comparable efficacy with that of reference drug, Mebandazole (MBZ).

C. colebrookianum extract showed significant level of efficacy against *G. crumenifer* and *T. spiralis*. At 40 mg/ml concentration the efficacy of *C. colebrookianum* extract and reference drug was almost similar. The mortality time of parasites at this concentration was recorded to be 2.50 h and 2.10 h, respectively. The *C. colebrookianum* extract, however did not show notable efficacy against *R. echinobothrida*, *H. diminuta* and *A. galli*.

In the present study *C. asiatica* leaf extract showed moderate level of efficacy against *R. echinobothrida*, *G. crumenifer* and *A. galli* and rather

insignificant efficacy against *H. diminuta* and *T. spiralis*. Unlike other tested plant extracts, *C. longa* extract did not show anthelmintic efficacy worth pursuing further.

The parasites' tegument/cuticle has been implicated among one of several target sites by which natural anthelmintic products or synthetic drugs act. In the present study effects of selected plant extracts were studied on parasite body surface with the help of scanning electron microscopy (SEM) so as to provide some clues regarding their plausible mode of action. The study revealed that barring *H. cordata* other extracts, namely *L. spinosa*, *G. angulosa*, *P. guajava* and *C. colebrookianum* exhibited such morphological changes and damage to the parasite's body surface. The tegument of *H. diminuta* and *G. crumenifer* showed destruction in the form of erosion on all over the general topography of the body. In case of *Hymenolepis* the scolex also showed apparent damage. Similarly, for *A. galli* the SEM of extract treated worms revealed wrinkles and cracks on lips and body cuticle.

To further substantiate the efficacy of plant extracts, the *in vitro* studies were supplemented with *in vivo* studies wherein the plant extracts were also tested for their anthelmintic efficacy in *H. diminuta* - rat and *T. spiralis* - mouse experimental models. In *Hymenolepis* - rat model the extracts were administered at three different stages of parasites; the larval, immature and adults. Efficacy was adjudged by counting the eggs per gram of faeces (EPG),

worm reduction and host clearance rate. In all experiments Praziquantel, a broad spectrum anthelmintic drug was tested at 5 and 10 mg/kg, p.o. doses as a reference drug. The results indicated that there were significant changes in all these parameters in the treated groups of animals as compared to untreated control. With respect to efficacy of extracts against larval stages, more prominent effects were recorded for *P. guajava*, *L. spinosa* (leaf), *H. cordata*, *C. longa* and *G. angulosa* extracts. The treatment of rats with 1600 mg/kg doses of the above plant extracts on days 2-6 p.i. resulted in elimination of 66.50, 66.66, 62.50, 62.50 and 58.25% of adult worms, respectively. Administration of extract on days 21-25 p.i. to investigate the efficacy against the adult stage showed percentage worm reduction between 87.50 to 91.50% for the tested plant extracts. The extract treated group of animals also showed substantial decrease in EPG values. The acute toxicity study in the experimental rats showed that barring mortality of few animals as noticed in the *C. longa* and *L. spinosa* stem extract-treated groups no other plant extracts cause any mortality or any changes in behaviour of animals with regard to food and water intake.

The efficacy of extracts in *T. spiralis* - mouse model was investigated against the adult, migrating and encysted stages; percentage reduction in adult worms at necropsy or larvae encysted in tissue constituted the study parameters. In general, barring *C. asiatica*, all other plant extracts tested in this study showed moderate to high efficacy against the adult *Trichinella* worms and more or less similar was the case against their efficacy against the migrating

larvae. In contrast, barring *G. angulosa* the rest of the plant extracts showed either medium or very low level of efficacy against the encysted larvae. The leaf extract of *G. angulosa* showed up to 86.22% of adult worm reduction and 72.36% encysted larvae reduction. The efficacy when compared to 25 mg/kg dose of Mebendazole, the reference drug was noted to be 94.70 and 90.52%, respectively against these stages. The acute toxicity studies of extracts in mice showed maximum mortality of animals for *C. longa* extract, followed by *C. colebrookianum* and *H. cordata* extracts. However, the rest of the plant extracts neither caused any mortality nor any visible signs of toxicity in experimental animals.

This study thus validates the presence of appreciable anthelmintic property in many of the folk medicinal plants used by Naga tribes which may have therapeutic benefits in humans encountering helminthic infections. Further investigation on isolated chemical constituents of these plants should be pursued against different helminth parasite species.

Two photographic plates of seven plants, seven photographic plates of twenty five scanning electron micrograph pictures, twenty graphic figures, two life cycle diagrammatic figures and thirty nine tables support the study observations carried out in the present work. Altogether (176) citations are given in the references.

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3. *International Conference on Promotion and Development of Botanicals*. Kolkata. February 25-26, 2005.

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6. *Regional Symposium on Research Thrust in Animal Sciences: Interface with End Use Researchers and Stake Holders*. NEHU, Shillong, Meghalaya. March 15-16, 2007.

7. *National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology*. NEHU, Shillong, Meghalaya. March 3-14, 2008.