STUDY ON ANTIDIARRHOEAL AND ANTICESTODAL EFFICACY OF SOME PLANTS USED IN FOLKLORE MEDICINE SYSTEM IN MANIPUR

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ABSTRACT

BY

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SUBMITTED

IN

FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

OF

NORTH-EASTERN HILL UNIVERSITY

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ABSTRACT

The present work incorporates a study on ascertaining the antidiarrhoeal and anticestodal potentials of some plants that are used in the folklore medicine system of tribal populations in Manipur, a northeastern state of India. The study aimed at 1) Evaluating the antidiarrhoeal efficacy of some folklore medicinal plants in experimentally induced diarrhoea in albino mice, 2) Ascertaining the anticestodal property of such traditional medicinal plants against cestode parasite, *Hymenolepis diminuta*, *in vitro* as well as *H. diminuta* – rat *in vivo* models, 3) Comparing their activities with respective reference antidiarrhoeal and anticestodal drugs, and 4) Studying their acute toxicity effects in these animal models by determining LD_{50} values of the plant extracts and also by assaying changes in some blood serum biochemical parameters.

To evaluate the antidiarrhoeal efficacy of folklore medicinal plants, nine plant species, namely – *Rhus javanica* L. (Anacardiaceae), *Galinsoga parviflora* Cav. (Asteraceae), *Bidens pilosa* L. (Asteraceae), *Swertia angustifolia* Buch.-Ham. ex D. Don. (Gentianaceae), *Lithocarpus dealbata* Rehder (Fagaceae), *Cymbopogon citratus* (DC) Stapf (Gramineae), *Zingiber cassumunar* Roxb. (Zingiberaceae), *Urena lobata* L. (Malvaceae) and *Potentilla fulgens* Wall. ex Hook. (Rosaceae) were included based upon a

questionnaire response conducted among native people in Manipur, where these plants emerged out to be the most commonly used in the traditional practice. The plant extracts were prepared in methanol and tested for their antidiarrhoeal activity against experimentally induced diarrhoea in albino mice. The activity was assessed by four different approaches: 1) Measurement of faecal output, 2) Castor oil-induced diarrhoea, 3) PGE_2-induced enteropooling, and 4) Gastrointestinal transit test.

The different plant extracts were administered to animals at four different doses: 100, 200, 400 and 800 mg/kg, p.o. The results show dose-dependent antidiarrhoeal effects in all the four study parameters for all nine plant extracts. The plant extracts' maximum doses could reduce the faecal output by 55.09% for *S. angustifolia*, 53.69% for *B. pilosa*, 53.57% for *R. javanica*, 53.44% for *C. citratus*, 47.57% for *L. dealbata*, 33.22% for *G. parviflora*, 26.37% for *P. fulgens*, 21.34% for *Z. cassumunar* and 19.48% for *U. lobata*. In the castor oil-induced diarrhoea study, there was a significant fall in the number of diarrhoeal episodes in all the treated animals, and the most interesting results emerged from treatment with extracts of *R. javanica, S. angustifolia, C. citratus* and *L. dealbata* where 66.67% of animals were protected from diarrhoea provoked by castor oil.

PGE_2 could increase the volume of small intestinal fluids accumulated per 100 g mouse from 1.35 ml in normal control to 3.21 ml in vehicle control

animals. The plant extracts significantly reduced the intestinal fluid accumulation from 18.47% (*Z. cassumunar* extract at 800 mg/kg dose) to 40.50% (*R. javanica* at 800 mg/kg dose). The distance travelled by the charcoal marker in the small intestines of the treated groups with different extracts showed significant difference from the control, and the best inhibition of the intestinal transit was exhibited by *U. lobata* extract (57.47% inhibition), followed by *C. citratus* extract (57.22%) and by *R. javanica* extract (55.84%).

In all the experiments, Loperamide was also tested as the reference drug at 5 and 10 mg/kg, p.o. doses. It emerged out that treatment with Loperamide showed reduction in faecal output by 33.74-57.31%, animals' protection from diarrhoea was 66.67-100%, and reduced intestinal fluids accumulation by 25.44-39.93% and showed inhibition in gastrointestinal transit by 50.40-58.57%. Simultaneously, two active components of plants; citral, an active essential oil component of *C. citratus* and quercetin, a major flavonoid component of *U. lobata* were tested with the same doses of the standard reference drug, and their efficacy was almost comparable with that of the reference drug, Loperamide.

The anticestodal efficacy of six plant extracts [namely, *Strobilanthes discolor* T. Anders (Family: Acanthaceae), *Adhatoda vasica* Nees. (Family: Acanthaceae), *Butea minor* Ham. in Wall (Family: Fabaceae; Papilionaceae), *Solanum myriacanthum* Dunal (Family: Solanaceae), *Trifolium repens* L.

(Family: Fabaceae; Papilionaceae) and *Zanthoxylum rhetsa* DC (Family: Rutaceae)] was ascertained by testing their extracts against *Hymenolepis diminuta* parasites both *in vitro* as well as *in vivo* models. The *in vitro* efficacy was found to be most significant for *S. discolor* extract treatment, where parasites showed paralysis at 0.92 h and death at 2.58 h post-incubation. While other five extracts also showed notable differences from the untreated control worms (parasites incubated in control medium showed paralysis at 29.17 ± 3.06 h and death at 29.75 ± 3.04 h).

In vivo testing of six plant extracts was carried out against *H. diminuta* infections in rats. The treatments were given at three different stages of parasites; the larval, immature and adults. Efficacy was adjudged by counting the eggs per gram of faeces (EPG), worm recovery and host clearance at necropsy. The results indicated that there were significant changes in all these parameters in the treated groups of animals as compared to control. However, the most remarkable effect was achieved by *S. discolor* and *Z. rhetsa* extracts where the treatment given at 800 mg/kg, p.o. doses on days 2-4 post-inoculation, totally eliminated *H. diminuta* infection from the experimental rats as evident by monitoring EPG and worm recovery rate. Throughout the experiments, praziquantel, a broad anticestodal drug, was also tested at 5 and 25 mg/kg, p.o. doses as a reference drug for comparing efficacy of the extracts. And the effects of most plant extracts were almost comparable with that of this reference agent.

Studies on acute toxicity effects of the fifteen plant extracts by determining LD_{50} revealed high values of lethal doses for nine plants. The LD_{50} (Oral; mg/kg; rat) values were tabulated as 2737.34, 3093.24, 3200.03, 3755.62 and 6993.18 for *Z. rhetsa*, *S. myriacanthum*, *B. minor*, *A. vasica* and *S. discolor* extracts, respectively. Whereas LD_{50} values (Oral; mg/kg; mouse) were charted as 3415.64, 3617.20, 4080.40 and 5355.97 for *B. pilosa*, *G. parviflora*, *Z. cassumunar* and *P. fulgens*, respectively. However, no mortality was observed for the other six plants, namely, *R. javanica*, *S. angustifolia*, *C. citratus*, *U. lobata*, *P. fulgens* and *T. repens* even when treatment was given upto 3200 mg/kg., p.o. and observed for 72 h post-treatment. Further toxicity analysis on some of the serum biochemical profiles yielded 135.33 U/L of SGOT, 118.00 U/L of SGPT, 134.33 mg/dL of cholesterol and 6.73 g/dL of total protein from the blood samples of untreated control mice, and it was 149 U/L of SGOT, 75.83 U/L of SGPT, 122.50 mg/dL of cholesterol and 6.88 g/dL of total protein from that of control rats. There were no major changes in these levels for the blood samples of treated animals with various plant extracts baring negligible exceptions for few plants. Therefore, it is secure to conclude from this acute toxicity study that the plant extracts having no or high LD_{50} values and no significant change in serum biochemistry are practically safe to use, as also native Naga people in Manipur use these plants' preparations without alleging any side effects.

The present investigation appears to provide a scientific base justifying the folkloric use of fifteen medicinal plants which are consumed in the traditional practice of indigenous tribal communities in Manipur, and the study further endows that these plants are safe to use without showing adverse effects.

Eight photographic plates of fifteen plants, eleven graphic figures and thirty-two tables support the study observations carried out in the present work. Total 203 citations are given in the references.

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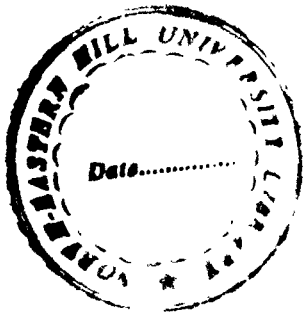

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DEDICATED TO MY LOVING PARENTS

(Sothing and Mahala)

NORTH-EASTERN HILL UNIVERSITY

SHILLONG – 793 022, INDIA

3rd October, 2006

DECLARATION

I, **Vareishang Tangpu**, 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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DR. ARUN K. YADAV
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Contents

Acknowledgement

When through the woods and forest glades I wander

And hear the birds sing sweetly in the trees;

When I look down from lofty mountain grandeur

And see the brook and feel the gentle breeze:

There is a road we all travel and it's called the road of life. Ups and downs are its nature, its course and its path: they call it a crazy road of life. Being a traveller in this path, searching for a shelter to a destiny, I find no one but a kind-hearted person who readily accommodates me to be his guest. He is no body other than my guide, my supervisor and my teacher, Dr. Arun Kumar Yadav, Reader, Department of Zoology, North-Eastern Hill University, Shillong, who has brought me right from the days of bewilderment to this stage of research career. He is always there for me in every moment of my need for consultation, instruction, organizing plan of work, coordinating methodology and supervising overall design for the progress of work. For his kind acts of untiring help and concern, I express my deepest and heartfelt gratitude to my respectable supervisor. His sincerity and devotion to research will always grow inside of me and I will carry with me such zealous qualities for the rest of my life and cherish.....

I would like to thank the present Head of the Department, Prof. SB Prasad, and former Heads of the department of Zoology, Prof. BBP Gupta and Prof. K Chatterjee (retd.) for providing me necessary laboratory facilities through out my research work. My personal thank goes out for Madam V Tandon, Dean, School of Life Science, who is always a helpful resource person in various academic activities, especially one who is our steer whenever we go out attending seminars and conferences in various parts of the country. I thank all the faculty members of the department for their high-quality suggestions and positive criticisms to attain this stage of my research journey.

My indebted gratefulness goes to Dr. PB Gurung, herbarium curator, and Dr. Y Kumar, Department of Botany, NEHU for rendering helping hands in identification and authentication of the plant materials. I would like to make a special acknowledgement to local herbalists; Sothing Tangpu, KPYO W Kapai and Ngaraipam Yangya who helped me in collection of folklore plant materials.

I thank my special team of friends – they are my thoughtful lab-mates: Among, Lalramniana, Somita and Mrs. Pde. All the possible types of their helps and considerate acts shown towards me throughout the stay are gratefully acknowledged. I can not miss out to mention names of my close friends – Kuheli, Shritapa, Zabha, Moitreyee, Bidhya, Promod, Larisha, Lungshi, Tia, Mhathung, Chumbekni, Proforma, Mary, Amenla, Sama, Sanjeev, Sanjib, Shy, Marky, Icydora, Rakshak who are readily available to come and help at time of my need. Occasional helps rendered by Guesstar, Bahdeng, Kong Dohling, Sitap, Prakash, Vanlal and Bahrit in photographic work, duplicating copies of research materials and looking after experimental animals are worth a mention here.

My parents are my root-treasure, a source of inspiration behind the scene, who unceasingly give moral, emotional, physical, mental, spiritual, prayer and financial supports right from my stage of toddler hood to adulthood and from ignorance to comprehension. Mom-Dad! This thesis is being dedicated to you two. Behind every successful man there is a woman, my better-half Atear (Glory) is behind all strive and hard work, thank you Atear. My sisters and my brothers are my shield in the time of mental and physical storm, and all my family members who bear along till date.

The award of JRF (NET) by joint CSIR-UGC, New Delhi, and thereafter a research fellowship awarded by CSIR, New Delhi for 5 years is thankfully acknowledged.

Above all, I thank the Almighty God for He is always who He is.
How Great Thou Art; How Great Thou art.

DATE: 03.10.2006

PLACE: **Shillong**

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Preface

Diarrhoeal diseases and tapeworm infections (cestodiasis) are among important public health problems of the world, and they play an important major cause of morbidity and mortality not only in under-developed areas but also in the most developed countries of the world. Cestodiasis in particular, is a significant contributor to malnutrition and poor health. It is estimated that diarrhoea produces more illness and causes more death than all diseases combined. Similarly, millions of people in the world are infected with intestinal worms; infection rate is higher in the developing countries including India.

The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. As a strategy to combat the problems of diarrhoea and tapeworm infections globally, the WHO also has provided a special emphasis on use of traditional folklore medicines, particularly in the context of developing countries, in the control and management of diarrhoea, as well as for curing various parasitic diseases including worm infections. Ethnomedicine is an integral part of the traditional practices in developing countries of the world, and medicinal plants play a provital role in traditional medicines. Over the past few years, the medicinal plants have gained a wide recognition due to an escalating faith of people in herbal medicine with in view of its lesser side effects compared to allopathic medicines. As to provide scientific base to the claims of putative traditional

plants, numerous studies pertaining to the treatment and control of diarrhoeal diseases as well as cestodiasis, have been made round the world.

In the northeast region of India, various tribal communities including Naga tribes in Manipur in particular, have a rich folklore regarding the use of several plants or plant-derived preparations for treating various ailments such as gastrointestinal disorders and intestinal worm infections, and the people have a good faith in their traditional medicine system. However, ethnomedicinal studies in the light of scientific justification of the traditional practice are very scarce in this part of Northeast India.

Keeping in mind the aforesaid account, it was felt necessary to carry out an investigation on the most commonly used or claimed folklore medicinal plants against experimental diarrhoea and cestode parasitic infections. Therefore, the proposed study has been designed to focus on:

- Evaluating the antidiarrhoeal efficacy of some folklore medicinal plants in experimentally induced diarrhoea in albino mice.
- Ascertaining the anticestodal property of such traditional medicinal plants against cestode parasite, *Hymenolepis diminuta*, *in vitro* as well as *H. diminuta*–rat *in vivo* models.
- Comparing the activities of plant extracts with respective reference antidiarrhoeal and anticestodal drugs.

- And studying their acute toxicity effects in these animal models by determining LD_{50} values of the plant extracts and also by assaying changes in some blood serum biochemical parameters.

The results of the present investigation provide a scientific base justifying the folkloric use of fifteen medicinal plants which are used in the traditional practice of indigenous tribal communities in Manipur, and the study further endows that barring few most of these plants are safe to use without showing any adverse effects.

Abbreviations

ad libitum	-	At will, at discretion (Latin: Ad, *according to* + Libēre, *to please*)
ALT	-	Alanine amino transferase
AST	-	Aspartate amino transferase
BSA	-	Bovine serum albumen
CTRL	-	Control
D	-	Death time
DDC	-	Diarrhoeal disease control programme
DMSO	-	Dimethylsulphoxide
EC	-	Enzyme code
EPG	-	Eggs per gram of faeces
et al.	-	et alii/alia (Latin: and others)
Fig.	-	Figure
ft.	-	feet
g	-	gram
g/dL	-	gram per decilitre
h	-	Hour
IACUC	-	Institutional Animal Care and Use Committee
IFCC	-	International Federation for clinical chemistry
LD_{50}	-	Median Lethal Dose
LDH	-	Lactate dehydrogenase
Lopax	-	Loperamide HCl
MDH	-	Malate dehydrogenase
Mg	-	Magnesium
mg	-	milligram
mg/dL	-	milligram per decilitre
mg/kg	-	milligram per kilogram body weight of animals
min	-	Minute
µg	-	microgram

µL	-	microlitre
µmol	-	Micromolar
µmmol	-	Micromillimole
MSL	-	Mean sea level
NAD	-	Nicotinamide Adenine dinucleotide (oxidized)
NADH	-	Nicotinamide Adenine dinucleotide (reduced)
NO	-	Nitric oxide
p	-	*Probability*
P	-	Paralysis time
PBS	-	Phosphate Buffer Saline
PGE_2	-	Prostaglandin of E_2 series (a diarrhoeagenic agent)
pi	-	Post-infection / post-inoculation
p.o.	-	Orally or by mouth (Latin: per os; per orum /p = by, o = mouth)
PO_4	-	Phosphate
PSM	-	Plant secondary metabolite
PZQ	-	Praziquantel
SEM	-	Standard error of the mean
SGOT	-	Serum glutamate oxaloacetate transaminase
SGPT	-	Serum glutamate pyruvate transaminase
SSHE	-	School sanitation and hygiene education programme
SO_4	-	Sulphate
SSPS	-	Statistical program for social science (software)
Syn.	-	Synonymous
TDC	-	Tropical disease control programme
WHO	-	World Health Organization
U/L	-	Unit per litre
UNICEF	-	United Nations Children's Fund
UV	-	Ultra-violet
w/w	-	weight by weight
WES	-	Water, Environment and Sanitation programme

CHAPTER 1

STUDY ON ANTIDIARRHOEAL EFFICACY OF MEDICINAL PLANTS

INTRODUCTION

Diarrhoea is recognized worldwide as one of the most important and most serious public health problems, especially in the developing countries of the world, due to its potential cause of morbidity and mortality especially in infants and children (Syder and Merson, 1982). It is estimated that global distribution of diarrhoea accounts for more than 5-8 million deaths each year in small children of less than 5 years old (Fauci *et al.*, 1998). According to World Health Organization (WHO), there were about 7.1 million deaths caused due to diarrhoea in a worldwide estimation for the year 1998 (Park, 2000). There is also a statistics that in under-developed areas of the world, diarrhoea alone produces more illness and causes more deaths of infants and children than all other diseases combined (Weber, 1976). In India alone, it is estimated that around 500 million episodes of diarrhoea occur every year with an incidence of 7.9 per child per year (Mata, 1983). This high prevalence has been attributed mainly to poor water quality and poor sanitation (Pegram *et al.*, 1998).

Diarrhoea may be defined as having symptoms of excessive faecal discharge, unusually frequent passage of loose or liquid or watery stools and

hypermotility due to peristaltic movement of the bowel (Yegnanarayan and Shrotri, 1982; Pegram *et al.*, 1998; Cabrera, 2000). The WHO defines diarrhoea as a situation in which an adult's daily stool exceeds 200 g and contains 60-95% water, three or more loose or watery stools discharged in a period of 24 h (Abdullahi *et al.*, 2001; Allen *et al.*, 2003). Diarrhoea may be classified as acute or chronic forms, acute being the most common form. Acute diarrhoea has an abrupt onset, which resolves within about 14 days and is usually caused by an infectious agent, although drugs, poisons including bacterial toxins, or acute inflammatory reactions can also contribute it (Thapar and Sanderson, 2004). Worldwide, rotavirus is the major cause of infectious diarrhoea, particularly among young children. However, other causative agents: viruses such as adenovirus, enterovirus and norovirus; bacteria such as *Escherichia coli, Salmonella, Shigella, Campylobacter* and *Vibrio cholerae*; and parasites such as *Cryptosporidium* and *Giardia,* are also considered as important pathogens of diarrhoea (Allen *et al.*, 2003). There are various possible types and causes of diarrhoea. Cabrera (2000) describes diarrhoea as having four major types, such as osmotic diarrhoea, secretory diarrhoea, exudative diarrhoea and diarrhoea of short transit time. Osmotic diarrhoea occurs when there is an excess of non-absorbable water-soluble substances present in the bowel leading to retention of water in the stool. Possible causes include lactose intolerance, ingestion of large amounts of sugars, excessive intake of vitamin C, over use of laxatives containing Mg, PO_4 or SO_4, general nutrient malabsorption and the use of certain antacids containing Mg. In this type of diarrhoea the extent and severity

is proportional to the amount of the offending substance ingested and the situation is alleviated by cessation of the intake of the substance (Cabrera, 2000).

Secretory diarrhoea occurs when the large intestine secretes rather than absorbs electrolytes and water. Possible causes include the presence of bacterial toxins, certain enteropathogenic viruses, unabsorbed dietary fats in liver or gall bladder diseases, excessive use of anthraquinone cathartics or laxatives, imbalances of certain hormones such as secretin, calcitonin or prostaglandins. Secretory diarrhoea is the most dangerous symptom of all gastrointestinal problems (Fontaine, 1988).

Exudative diarrhoea occurs when there is acute or chronic inflammation in the gastrointestinal tract leading to copious production of inflammatory exudates. Short transit time also causes diarrhoea because there is insufficient time for fluid absorption to occur. The most common causes of this are intestinal resection, which reduces the surface area of the intestines, and stress which speeds up peristalsis (Cabrera, 2000).

In order to combat the problems of diarrhoea globally especially in the developing countries of the world, the WHO has constituted a Diarrhoeal Disease Control (DDC) programme which has provided a special emphasis on the use of traditional folklore medicines in the control and management of

diarrhoea, together with the evaluation of health education and prevention approaches (Anonymous, 1979; Syder and Merson, 1982). Recently, in a joint statement with UNICEF, the WHO has recommended implementing the traditional preventive measures for cholera diarrhoea control such as a provision of safe water and adequate sanitation (Anonymous, 2005).

Ethnomedicine is an integral part of the traditional practices in developing countries of the world (Githiori *et al.*, 2005), and medicinal plants and plant-derived products play a dominant role in traditional medicines, as more than 50% of all modern drugs in clinical uses are derived from natural products (Huang *et al.,* 1992). In rural areas of the developing countries of the world, medicinal plants continue to be used as the primary source of medicine (Chitme *et al.*, 2003), and about 80% of people in developing countries, use traditional medicines for their health care (Kim, 2005). As per an estimate, in India of the 17,000 species of higher plants, 7,500 are known for medicinal uses (Shiva, 1996). This proportion of medicinal plants is the highest proportion of plants known for their medical purpose in any country of the world (Kala, 2006). Review of literature reveals that there is a growing focus of research on evaluating the medicinal plants that are traditionally used for treatment of various ailments by indigenous people of different communities in the world. Numerous studies have been made round the world on the basis of scientific explanation of folklore medicinal plants pertaining to treatments and control of diarrhoeal diseases, these putative plants include *Psidium guajava* (Lutterodt,

1992; Almeida *et al.*, 1995), *Euphorbia hirta* (Galvez *et al.*, 1993b), *Egletes viscosa* (Rao *et al.*, 1997), *Waltheria americana, Commelina coelestis, Alternanthera repens* (Zavala *et al.,* 1998), *Pentaclethra macrophylla* (Akah *et al.,* 1999), *Satureja hortensis* (Hajhashemi *et al.,* 2000), *Terminalia avicennoides* (Abdullahi *et al.*, 2001), *Cassia nigricans* (Nwafor and Okwuasaba, 2001), *Roureopsis obliquifoliolata*, *Epinetrum villosum* (Otshudi *et al.*, 2001), *Bridelia mecrantha, Eleutherina bulbosa* (Lin *et al.,* 2002), *Artemisia ludoviciana* (Zavala *et al.*, 2002), *Rumex maritimus* (Rouf *et al.*, 2003), *Baccharis teindalensis* (Vidari *et al.*, 2003), *Pycnocycla spinosa* (Sadraei *et al.*, 2003a), *Melissa officinalis* (Sadraei *et al.*, 2003b), *Sphaeranthus senegalensis* (Adzu *et al.*, 2004), *Alchornea cordifolia* (Agbor *et al.*, 2004), *Mentha mucrophylla, Conyza dioscoridis, C. linifolia, Zygophyllum album* (Atta and Mouneir, 2004), etc. Most recent studies in this regard reveal many other plants which have also been shown to have antidiarrhoeal activity in various rodent animal models, such as *Cynachum acutum* (Atta and Mouneir, 2005), *Casimiroa tetrameria* (Heinrich *et al.*, 2005), *Saccharomyces boulardii* (Girard *et al.*, 2005), *Loeselia mexicana* (Perez *et al.,* 2005), Jacquez grapes, *Vitis aestivalis* (Vitali *et al.*, 2005), *Byrsocarpus coccineus* (Akindele & Adeyemi, 2006), *Eremomastax speciosa* (Oben *et al.*, 2006), *Cylicodiscus gabunensis* (Mabeku *et al.,* 2006), to name a few. From India alone, several such studies that have been undertaken to assess the antidiarrhoeal potential of traditionally used medicinal plants of various native communities include that of *Punica granatum* (Pillai, 1992), *Nelumbo nucifera* (Mukherjee *et al.*, 1995; 1998), *Jatropha curcus* (Majumdar *et*

al., 2000), *Jussiaea suffruticosa* (Murugesan *et al.*, 2000), *Acorus calamus, Pongamia glabra, Aegle marmelos, Strychnos nux-vomica* (Shoba & Thomas, 2001), *Strychnos potatorum* (Biswas *et al.*, 2002), *Albizzia lebbeck* (Besra *et al.*, 2002), *Camellia sinensis* (Besra *et al.*, 2003), *Cissampelos pareira* (Amresh *et al.*, 2004), *Holarrhena antidysenterica* (Kavitha *et al.*, 2004), *Cyperus rotundus* (Uddin *et al.*, 2005), *Asparagus racemosus* (Venkatesan *et al.*, 2005), *Aegle marmelos* (Mazumder *et al.*, 2006), *Paederia foetida* (Afroz *et al.*, 2006) etc. In general, in these studies, the putative plant extracts have been tested for their efficacy by employing experimentally induced diarrhoea in various rodent models. Reductions in faecal discharge, in diarrhoeal droppings, in intestinal fluid accumulation and inhibition in the propulsive movement of small intestine, have been reported in these studies for most of the plants. However, studies in this regard from north-eastern region of India are very scarce.

The northeast region of India is densely settled by approximately 130 indigenous tribal communities, including the Naga tribes. Like any other tribal community, Nagas have a rich folklore regarding the use of several plants or plant-derived preparations for treating various ailments among indigenous Naga folks. While majority of Naga tribal population resides in Nagaland state, however the same is also distributed in parts of Manipur state, and adjacent areas of Assam and Arunachal Pradesh. The state of Manipur lies stretching between latitudes of 23.80^0N to 25.68^0N and longitudes of 93.03^0E to 94.78^0E, covering an area of 22,327 sq. kms that constitutes about 0.68% of the entire

country. It is encircled by nine hill ranges on all sides with a small Imphal valley at the centre. Various Naga communities occupying the hilly areas in Manipur, in particular, have a good faith in their traditional medicine system and thus they use many plant based medicines to cure various ailments, including diarrhoea.

Bestowing upon with thick vegetations around and with rich biodiversity in fauna and flora of this region, the Naga people have acquired lifestyles of depending upon the natural resources for their existence including wealth of medicinal plants for treatment of various diseases. Subsequently, they have also developed the knowledge and information about curing several ailments through their age long experiences and pass on the knowledge from one generation to another through oral tradition. At some point of time, the knowledge of using some medicinal plants was kept as a clandestine for the interest of the traditional medicinemen and some as a family heritage, while some plants are known by most villagers. To get the most correct and authentic informations about the uses of medicinal plants and their preparations which are claimed to have gastrointestinal remedy including remedy for diarrhoea in folklore medicine system, information was confirmed time and again from the local healers/medicinemen called *Haori-Khanong* (in Tangkhul dialect) with a request to make use of the knowledge for exploration to scientific world. These informations gathered from the local *Haori-khanings* were analyzed to select which plants would be included in this study. Further a baseline survey in the form of questionnaire based upon the use or disuse of suggested putative

medicinal plants was conducted among the local people in the villages of tribal populations in Manipur.

Based upon the questionnaire response (the questionnaire response is presented in Table 1.1), the most commonly used/claimed folklore plants, namely *Rhus javanica* L. (Anacardiaceae), *Galinsoga parviflora* Cav. (Asteraceae), *Bidens pilosa* L. (Asteraceae), *Swertia angustifolia* Buch.-Ham. ex D. Don. (Gentianaceae), *Lithocarpus dealbata* Rehder (Fagaceae), *Cymbopogon citratus* (DC) Stapf (Gramineae), *Zingiber cassumunar* Roxb. (Zingiberaceae), *Urena lobata* L. (Malvaceae) and *Potentilla fulgens* Wall. ex Hook. (Rosaceae) were included in the present study to evaluate their antidiarrhoeal properties in suitable experimental animal models. To supplement this study, an acute toxicity test of each plant extract was also undertaken to see if these extracts had any adverse side effects to the experimental animals, by way of determining the median lethal dose (LD_{50}) and also by assaying the changes in few of the serum biochemical parameters, i.e., serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total protein and cholesterol, after treatment with these extracts.

Table 1.1: Preliminary baseline survey of the ethnomedicinal plants used among various tribal communities in Manipur against diarrhoeal disorders.

Study parameters	Response (%)	*R. javanica*	*G. parviflora*	*B. pilosa*	*S. angustifolia*	*L. dealbata*	*C. citratus*	*Z. cassumunar*	*U. lobata*	*P. fulgens*
1. Do you use this plant as antidiarrhoeal remedy?	i) YES	73.33	35.0	45.0	50.0	66.5	67.5	70.83	60.0	50.0
	ii) NO	26.67	65.0	55.0	50.0	32.5	32.50	29.17	40.0	50.0
2. If YES, how often do you use this plant?	i) Frequently	72.73	0.0	22.22	0.0	41.99	55.56	70.59	22.23	10.0
	ii) Sometimes	22.73	61.9	66.67	66.67	43.11	24.70	14.11	66.67	67.0
	iii) Rarely	4.54	23.8	11.11	0.0	4.25	16.0	9.41	0.0	17.6
	iv) Used once	0.0	14.3	0.0	33.33	12.50	3.74	5.89	11.0	5.40
3. If YES, why do you use this plant?	i) As a myth/belief	9.09	9.52	14.81	0.0	9.87	4.93	18.82	8.33	0.0
	ii) Traditional practice	69.32	28.58	66.67	31.67	70.38	49.37	42.36	55.56	50.0
	iii) Prescribed by local medicinemen	21.59	61.90	18.52	68.33	19.75	45.70	38.82	36.11	50.0
4. If YES, how efficacious is this plant?	i) Very high	40.91	14.29	0.0	0.0	6.18	30.87	29.41	0.0	13.5
	ii) Medium	35.23	85.71	83.33	100.0	79.01	69.13	58.82	91.67	65.5
	iii) Very poor	23.86	0.0	16.67	0.0	14.81	0.0	11.77	8.33	21.0
5. If YES, what is your usage preference of this plant?	i) Only priority	31.82	11.9	14.81	41.67	11.12	20.99	23.52	11.11	16.22
	ii) First priority	60.23	88.1	85.19	35.0	69.13	79.01	60.0	72.22	44.50
	iii) Undecided	7.95	0.0	0.0	23.33	19.75	0.0	16.48	13.87	39.28
6. If NO, what is the reason that you do not use this plant?	i) It is a myth	15.63	15.39	0.0	10.0	7.70	23.0	0.0	8.33	0.0
	ii) Not efficacious	9.37	41.0	48.49	56.67	0.0	46.16	34.29	6.25	0.0
	iii) Ignorance about the use	75.0	56.41	51.51	33.33	92.3	30.84	65.71	85.42	100.0

Number of individuals participated in questionnaire response, N = 480.

Table 1.2: Details of the ethnomedicinal plants included in the present study.

Scientific Name	Voucher Specimen No.	Common Name	Local Name	Family	Usable parts	Distribution	Known uses
1. Rhus javanica	AKY-202	Chinese sumac	Comfuitei	Anacardiaceae	Ripen fruit	Subtropical & tropical EA	Anti-herpes simplex, anti-cytomegalus virus
2.Galinsoga parviflora	AKY-203	Quick weed	Japan khavu	Asteraceae or Compositae	Leaf	Tropical & subtropical	Insecticidal
3 Bidens pilosa	AKY-204	Beggar's tick	Napnar	Asteraceae or Compositae	Leaf	Temperate & tropical	Antimalarial, antiinflammatory, antibacterial
4. Swertia angustifolia	AKY-201	Hill chirata	Ramkuinin	Gentianaceae	Aerial shoot	Temperate areas of Asia	Cough, fever
5. Lithocarpus dealbata	AKY-205	Oak tree	Prungtei	Fagaceae	Seed	Temperate & Tropical	Antioxidant, antimicrobial, antiprotozoal
6.Cymbopogon citratus	AKY-206	Lemon grass	Harvosing	Poaceae or Gramineae	Stalk	China, Taiwan South East Asia	Larvicidal, anti-fungal, anticancer
7. Zingiber cassumunar	AKY-208	Cassumunar ginger	Hearue	Zingiberaceae	Rhizome	Tropical Asia	Antioxidant, antiinflammatory, anticancer
8. Urena lobata	AKY-218	Caesar's weed	Keashongrue	Malvaceae	Leaf	Tropical	Antibacterial, antioxidant
9. Potentilla fulgens	AKY-221	Yellow Cinquefoil	Ngarunri	Rosaceae	Root	Himalayan region	Antitumour, antidiabetic

MATERIALS AND METHODS

Collection of Plant Materials

From the questionnaire account (Table 1.1), the most commonly used plants were included in the study. Various usable plant parts (leaf, inflorescence, seed, fruit, stalk, rhizome, root, etc.) were collected from various places in Manipur. Herbarium sheets and photographs of each of the plant materials were prepared for identification and authentication done by Dr. PB Gurung, herbarium curator, and further confirmed by Dr. Y Kumar, Plant Taxonomist, Department of Botany, North-Eastern Hill University, Shillong. Respective voucher specimens (Table 1.2) along with herbarium sheets of the plants were deposited in the Department of Zoology, North-Eastern Hill University, Shillong.

Plant Materials

(Detail accounts of each of the plants are presented in Table 1.2)

1.1. *Rhus javanica* L. (Anacardiaceae): Plate 1.1 (A)

Syn. *R. semialata* Murray; *R. buckiamela* Roxb.

R. javanica L. (common name: Chinese sumac) is a small pretty tree with branchlets, petioles, leaves beneath and panicles clothed with a dense soft pubescence. Leaves are opposite, sessile and oblong. Flowers are small white or yellow-green, bark is rough, and seeds are compressed, red, orbicular and shining when ripe. The ripen fruits, locally called as *Comfuitei*,

are eaten with a good sour taste. These small acid drupes of 0.3 in. in diam. have a long history of folklore medicine use among the traditional healers (*Haori-Khanong*) of Naga tribal community to treat dysentery and diarrhoea as well as for other gastrointestinal disorders.

This tree is found in grassland area of Naga hills and in temperate range of the Himalayas at the altitude of 3 – 6,000 ft. Barring a few studies related to testing its efficacy against herpes simplex (Kurokawa *et al.,* 1999) and cytomegalus virus (Shiraki *et al.*, 1998), there is apparently no reference available in the literature regarding the antidiarrhoeal effects of this plant either in humans or in any animal models. Triterpenoids such as moronic acid and betulonic acid, gallotannins and anthocyanidin are some of the chemical ingredients reported in this plant (Kurokawa *et al.,* 1999; Taniguchi *et al.,* 2000). Three triterpenes – semialactone, isofouquierone peroxide and fouquierone have also been isolated from the stem bark of *R. javanica* (Lee *et al.*, 2001).

1.2. *Galinsoga parviflora* Cav. (Asteraceae; Compositae): Plate 1.1 (B)

G. parviflora Cav., locally known as *Japan-khavu,* is a weak weed, growing to a height of 2 ft. The upper parts of the plant have a slender, slightly hairy stem. Leaves are petioled, ovate, obtusely acuminate, 3-nerved, and subserated. This weed is found in cultivated and waste places in the Himalaya at the altitude of 4 – 8000 ft. The leaf and inflorescence are used in treating diarrhoea in the folklore medicine practice of Naga community in Manipur. Insecticidal activity of this plant has been reported (Macedo *et al.,*

1997). There is no other study on this species available in the literature of the biological activity and its active principles.

1.3. *Bidens pilosa* L. (Compositae; Asteraceae): Plate 1.2 (A)

B. pilosa L. locally known as *Napnar*, is a tall erect herb 2 – 4 ft. with opposite 3-foliolated leaves. Leaves are 5-7 white rays, easily recognized by its angular slender black crypseles (0.5" – 0.7" long) with 2 – 4 rigid awns with bristles by which they adhere to the clothes/garments or body. The leaf and inflorescence of this plant are comparatively lesser used to cure diarrhoea, wound healing etc. in the Tangkhul Naga traditional medicine system. It is found throughout India, ascending the Himalaya and other mountains to 6000 ft. and distributed in all other tropical countries.

B. pilosa has been reported to possess hepatoprotective (Chin *et al.*, 1996), antimalarial (Brandao *et al.*, 1997; Andrade-Neta *et al.*, 2004), immunosuppressive and anti-inflammatory (Pereira *et al.*, 1999), antihyperglycemic (Ubillas *et al.*, 2000), antiulcerogenic (Tan *et al.*, 2000), antimicrobial (Khan *et al.*, 2001a), antileukemic (Chang *et al.*, 2001), and also antihypertensive (Dimo *et al.*, 2002) properties. New compounds such as quercetin 3-O-rabinobioside, quercetin 3-O-rutinoside 4, 5-di-O-caffeoylquinic acid, jacein, centaurein etc. have also been reported to occur in *B. pilosa* (Chiang *et al.*, 2004). However, Ethyl caffeate, a natural phenolic compound, isolated from this plant has been elucidated for its anti-inflammatory functions (Chiang *et al.*, 2005).

1.4. *Swertia angustifolia* Buch.-Ham. ex D. Don. (Gentianaceae):

Plate 1.2 (B)

S. angustifolia Buch.-Ham. ex D. Don., is locally known as *Ramkuinin* in Paoyi. It is a once-flowering, erect herb, 1 – 3 ft. high. Leaves are narrowly lanceolate, sub-1-nerved narrow at the base; corolla-lobes are white or bluish oblong acute with one large orbicular gland near the base. Some workers prefer to give a common name of this plant as *Hill chirata*. It is found in subtropical Himalaya, alt. 1 – 6000 ft. The decoction of this plant is commonly consumed by the local people in treating high fever, cough as well as diarrhoea. Review of literature reveals studies on isolation of xanthones, xanthone O-glucosides and iridoid glycosides from this plant (Ghosal *et al.*, 1978; Luo and Nie, 1992). And mangiferin is the active chemical constituent reported from this plant (Duke, 1992).

1.5. *Cymbopogon citratus* (DC) Stapf (Poaceae; Gramineae): Plate 1.3 (A)

Syn. *Andropogon citratus* DC. ex Nees.

C. citratus DC Stapf is a tall aromatic grass that grows abundantly in the grassland near the cultivated land of the Naga hills. The grass shoot grows up to 6 ft. high. Leaves are usually narrow, base rarely surrounded or cordate. Inflorescence of solitary binate digitate is fascicled or panicled spikes. The whole stalk and the leaf are boiled and the decoction is drunk to relieve cough, stomachache and to treat diarrhoea. This lemongrass is called *Harvosing* in local dialect. In the literature there is no mention of any experimental study related to its antidiarrhoeal activity, although other studies on this plant indicate that it possesses scientifically proven antibacterial

(Sumita *et al.*, 2004; Wannissorn *et al.*, 2005), antifungal (Abe *et al.*, 2003; Bankole *et al.*, 2005), antimalarial (Tchoumbougnang *et al.*, 2005), anticancer (Dudai *et al.*, 2005) and larvicidal activity against *Aedes aegypti* (Cavalcanti *et al.*, 2004). The main chemical constituent of this plant is citral, which accounts for 75-80% of its oil's volume (Abe *et al.*, 2003; Rauber *et al.*, 2005).

1.6. *Lithocarpus dealbata* Rehder (Fagaceae): Plate 1.3 (B)

Syn. *Quercus dealbata* Hook. f. & Thomas.

L. dealbata Rehder is a lofty evergreen tree whose acorns or seeds in roasted and bark or bark juice are taken in treating dysentery and diarrhoea. This oak tree, local name *Hoktheithing/Machithing*; *Prungtei* is the name for the acorn, grows in the forests at higher elevation in the eastern Himalayas such as Naga hills at alt. 5800 ft., Khasia Mountains at alt. 3-6500 ft., China, Bhutan, Mayanmar, Thailand and Vietnam. There is no report of its biological activity so far in the literature, however other studies have shown that related species of *L. dealbata* possesses antioxidant, antimicrobial and anti-protozoal activities (Khan *et al.*, 2001b; Yang *et al.*, 2004; Subeki *et al.*, 2005).

1.7. *Zingiber cassumunar* Roxb. (Zingiberaceae): Plate 1.4 (A)

Z. cassumunar Roxb. commonly known as cassumunar ginger is a rhizomatous aromatic herb, which is widely cultivated in tropical Asia. Rootstock is biennial, bearing many sessile tubers. Leafy stem is about 3-4 ft. high. Leaves are 6-23 by 1", tapering gradually to the point. Spike 2-3 by 1"

diam., peduncle 0.5-1ft., very rarely flowers. Known as *Hearue* in local dialect, in the traditional medication, the fresh juice of this rhizome is drunk as to cure cough, stomach running, stomachache and other abdominal complaints. Review of literature reveals that its rhizome possesses anti-inflammatory (Ozaki *et al.*, 1991; Jeenapongsa *et al.*, 2003), anti-tumor (Vimala *et al.*, 1999) and antioxidant (Nakatani, 2000) activities. Its chemical constituents include isomers of phenylbutenoid glucosides that inhibit prostaglandin E_2 production (Han *et al.*, 2005) and two curcuminoids (Nagano *et al.*, 1997).

1.8. *Urena lobata* L. (Malvaceae): Plate 1.4 (B)

Syn. *Urena trilobata* Vell.

U. lobata L., commonly called as *Caesar's weed*, is a small shrub that grows quite abundantly on the roadsides in Naga hills. This mallow family plant is a perennial, flowering and seed bearing vascular shrub growing up to 1-2 ft. high with pink flower. The lobed leaves are covered in star-shaped plant hairs which give the leaves a grayish colour and raspy feel. Its distribution ranges from tropical to subtropical countries including South America, Florida (USA), Australia, Himalaya ranges including Naga hills.

In traditional practice, the leaf and offshoot of *U. lobata* (locally known as *Keashongrue)* are smashed to extract juice and the juice is drunk to cure gastric complaints, such as diarrhoea. Mazumder *et al.* (2001) have reported that its root extract have a broad-spectrum antibacterial activity against G (+) and G (-) microorganisms. *U. lobata* is also reported to possess antioxidant

activity and inhibitory action against nitric oxide (NO) release from macrophages (Choi and Hwang, 2005). In addition, its aerial parts contain two major chemical constituents, the mangiferin and quercetin (Ghosh, 2004).

1.9. *Potentilla fulgens* Wall. ex Hook. (Rosaceae): Plate 1.5 (A)

P. fulgens is a shrubby yellow cinquefoil herb of 1-2 ft. high, which is found growing at higher altitudes of 1500-2000 MSL, in the hilly northeast regions of India. In traditional practice of the tribal communities in Manipur, the fresh roots of this plant (vernacular name: *Ngarunri*) are either chewed or taken in decoction in treatment of diarrhoeal disorders. Its root extract has been reported to have hypoglycemic effects in normal and alloxan-induced diabetic mice (Syiem *et al.*, 2002) and antitumour activity against murine ascites Dalton's lymphoma (Rosangkima and Prasad, 2004).

PLATE 1.1

A. Rhus javanica; whole plant and ripen fruits

B. *Galinsoga parviflora*; whole plant and leaf & inflorescence

PLATE 1.2

A. ***Bidens pilosa***; whole plant and Leaves

B. ***Swertia angustifolia***; whole plant and leaf & inflorescence

PLATE 1.3

A. *Cymbopogon citratus*; whole plant and stalk part

B. *Lithocarpus dealbata*; whole plant and seeds

PLATE 1.4

A. *Zingiber cassumunar*, whole plant and rhizomes

B. *Urena lobata*; whole plant and leaves

PLATE 1.5

A. *Potentilla fulgens*; whole plant and flower

Preparation of Plant Extracts

The usable portions of the plants were air-dried under shade and pulverized into powder. Known amount of the powdered materials were suspended in an organic solvent, methanol as extractant and engaged for refluxing using a Soxhlet fractional distillation apparatus (Yadav *et al.*, 1992) at 40-50 ^{0}C for 4-6 h. The resulting suspension was decanted out discarding the remnants and the filtrate was further concentrated in a Rotatory Evaporator under reduced temperature and pressure for removal of the solvent. Further, the recovered semi-solid residue was placed over calcium chloride anhydrous for complete evaporation of the extractant from the crude extract.

The percentage yields (w/w) of the final crude extracts were 8.27% (*R. javanica*), 3.30% (*G. parviflora*), 2.54% (*B. pilosa*), 4.30% (*S. angustifolia*), 1.72% (*L. dealbata*), 3.72% (*C. citratus*), 10.60% (*Z. cassumunar*), 3.30% (*U. lobata*) and 5.15% (*P. fulgens*). These methanol extracts were stored in respective plastic vials at – 4 ^{0} C in a refrigerator until used for assaying their antidiarrhoeal efficacy.

Drugs and chemicals:

Loperamide (Lopax, Axar Pharmaceuticals, Baroda) as a standard reference antidiarrhoeal drug, Citral (Merck Schuchardt OHG, Germany) and Quercetin (HiMedia Laboratories Pvt. Ltd., Mumbai) as respective active components of two plants: *C. citratus* and *U. lobata*, Castor oil (S. D. Fine, Mumbai) and Prostaglandin E_2 (Sigma-Aldrich Chemical Pvt. Ltd., USA) as diarrhoea-

inducing agents, Activated Charcoal (E. Merck, India) as an intestinal transit marker, and Gum Acacia (S. D. Fine Chem, Boisar) and Tragacanth Powder (Central Drug House Pvt. Ltd., Bombay) as suspension agents, were used in this study.

Preparation of medium solutions

I. **0.9% Phosphate Buffer Saline (0.15 M 0.9% Sodium PBS)**

i) 2.106 g $NaH_2PO_4.2H_2O$

ii) 8.733 g Na_2HPO_4

iii) 4.500 g NaCl

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

II. **2% gum acacia suspension**

Fresh suspension of 2% gum acacia was prepared by dissolving 2 g of gum acacia in 100 ml of double distilled water.

III. **Charcoal meal suspension**

To 100 ml of 4% gum tragacanth suspension, 2 g charcoal powder was added making a charcoal marker suspension, used for assaying small intestine transit in mice.

Experimental Animals

Six to eight weeks old mice (20-30 g) were used. The animals were acclimatized for 15 days under the standard laboratory conditions following initial procurement from Pasteur's Institute, Polo, Shillong, and inbred colonies were raised in the laboratory. All the animal experiments were

carried out in accordance with the Rules and Regulations approved by the Institutional Animal Care and Use Committee (IACUC).

ANTIDIARRHOEAL ACTIVITY

Antidiarrhoeal activities were assessed by employing four different experimental parameters for each of the different plant extracts and also the active principles of two plants, *C. citratus* (citral) and *U. lobata* (quercetin). The following detailed protocol is applicable for each one of the plant extracts/active principles.

A. Measurement of faecal output

Faecal output was measured following the methods of Bass *et al.* (1972) and Pillai (1992) with modifications. Six groups of mice (n = 6) were housed singly in separate cages. Group I served as the control and received 2% gum acacia (0.5 ml); Groups II – V mice were treated respectively with 100, 200, 400 and 800 mg/kg of plant extract, while group VI mice received 0.5 ml of Loperamide at 5 mg/kg, p.o., the standard antidiarrhoeal drug. Respective active components, citral of *C. citratus* and quercetin of *U. lobata*, were also tested at 5 and 10 mg/kg doses each in comparison with the same doses of Loperamide. Following treatment, the faecal materials were collected for 8 h post treatment, were dried in an incubator and their weights measured. The percentage faecal output (%FOP) was calculated as follows:

$$\% \ FOP = \frac{f_t \times 100}{f_c}$$

where, f_t is the mean faecal weight of each treatment group, and f_c is that of control group (Akah *et al* .1999).

B. Castor oil model

The method was modified from Akah (1996), Jacoby *et al.* (2001) and Otshudi *et al.* (2001). Overnight-fasted mice were randomly divided into six groups (n = 6). Group I received 0.5 ml of 2% gum acacia suspension; groups II – V were treated with 100, 200, 400 and 800 mg/kg of plant extract; Group VI mice were given 0.5 ml of 5 mg/kg of Loperamide. While active components, citral and quercetin, were also tested at 5 and 10 mg/kg doses each in comparison with the same doses of Loperamide. 1 h later, diarrhoea was induced in all groups by inoculating castor oil (0.5 ml/mouse, p.o.). The numbers of diarrhoeal episodes were recorded for each time and cumulative values were calculated for 4 h post induction of diarrhoea, and the numbers of animals devoid of diarrhoeal droppings at 4 h were considered as a percentage protection from diarrhoea.

C. PGE_2-Enteropooling assay

The protocol adopted by Di Carlo *et al.* (1994) and Murugesan *et al.* (2000) was used with modifications. Overnight-fasted mice were randomized into seven groups (n = 6). The animals received a diarrhoeal agent (0.5 ml of 100 µg/kg PGE_2 in 5% ethanol in saline). Group I served as the control (0.5 ml; 2% gum acacia); Group II served as a vehicle control (100 µg/kg PGE_2 + 2% gum acacia); groups III – VI received 100, 200, 400 and 800 mg/kg of

plant extract, respectively; group VII received 5 mg/kg dose of Loperamide. Citral and quercetin were tested at 5 and 10 mg/kg doses each with respective plant extracts. All these treatments were done 1 h prior to prostaglandin-diarrhoeal induction. 30 min later, animals were sacrificed and their small intestines were ligated from pyloric sphincter to ileocaecal junction, and assessments of the accumulation of intestinal fluid secretion induced by PGE_2 were made. The percentage reduction in secretion was calculated as described by Robert *et al* (1976).

D. Gastrointestinal transit test

The animals were starved for 16 h prior to the experiment. The test extract (100, 200, 400 and 800 mg/kg) was given orally to groups II – V of mice (n = 6). Group I served as the control, group VI animals received 5 mg/kg Loperamide. 5 min later, 0.5 ml of charcoal meal was orally inoculated to each mouse. Citral and quercetin (5 and 10 mg/kg, p.o. doses each) were tested with respective plant extracts. All the mice were sacrificed 30 min later, their small intestines from pylorus to caecum cut out and distance travelled by the charcoal marker measured, and expressed as a percentage of the total length of small intestines. The percentage inhibition of the marker transit in the intestine was calculated as described by Akah & Offiah (1992).

ACUTE TOXICITY TEST

A. Determination of Median Lethal Dose (LD_{50})

For each of the plant extracts seven groups of 6 animals each were selected. Group I served as untreated control and groups II-VII were

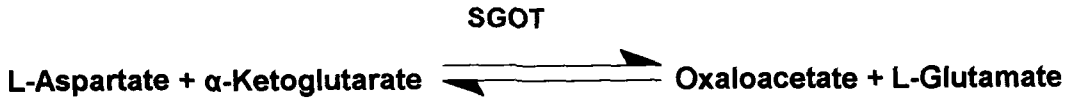
administered with test extract at the escalating doses of 100, 200, 400, 800, 1600 and 3200 mg/kg, p.o., respectively. The general signs and symptoms of toxicity, intake of food and water, and mortality rates were observed for 72 h post administration of extract. From these observations, median lethal dose (LD_{50}) was calculated using SPSS software.

B. Serum biochemical test

In another set of experiment, a serum biochemical profile was studied. The blood samples were collected by cardiac puncture from the animals 24 h post-treatment with 800 mg/kg, p.o. of each extract; the highest dose showing antidiarrhoeal activity in this study. The blood was kept for 30 min without disturbing and the clot was dispersed with glass rod and then centrifuged for 15-20 min at 2000 rpm to separate serum. From this processed serum, levels of serum glutamate oxaloacetate transaminase (SGOT; EC 2.6.1.1), serum glutamate pyruvate transaminase (SGPT; EC 2.6.1.2), cholesterol and total protein were estimated as per the methods of Stricklad *et al.* (1961), Allain *et al.* (1974) and Henry *et al.* (1974), using a semi-automated biochemical analyzer (Bayer).

Serum Glutamate Oxaloacetate Transaminase (SGOT/AST; EC 2.6.1.1)

The activity of SGOT was assayed following the kinetic (IFCC) method described by Henry *et al.* (1974). Oxaloacetate released by the action of SGOT from L-Aspartate is converted to L-Malate by the enzyme Malate dehydrogenase (MDH). In the reaction, NADH is oxidized to NAD. The measurement of absorbance is proportionate to SGOT activity in the sample.



The reagents required were:

Reagent 1 (Enzymes):

MDH	≥	600 U/L
NADH	-	0.20 mmol/L
α-Ketoglutarate	-	12 mmol/L

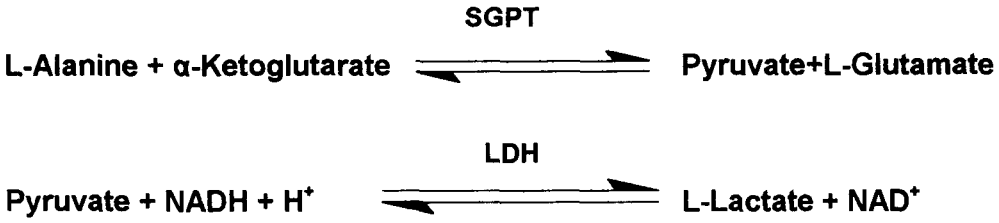
Reagent 2 (Buffer):

Tris buffer (pH 7.80)	-	88 mmol/L
L-Aspartate	-	260 mmol/L

The serum samples and reconstituted reagents were brought to room temperature at 37^0C prior to use. The reaction mixture contained 1 ml of constituted reagent and 100 µL of serum. After mixing them, the mixture was dispensed immediately into test tube and read absorbance at 340 nm in the semi-automated biochemical machine, and the enzyme activity was expressed in Units/Litre (U/L). One unit of SGOT activity was expressed as that amount of enzyme which catalyzed the oxidation of 1 µmol of NADH to NAD^+ per min at pH 7.8 and 37^0C.

Serum Glutamate Pyruvate Transaminase (SGPT/ALT; EC 2.6.1.2)

The activity of SGPT was assayed following the kinetic (IFCC) method by Henry *et al.* (1974). Pyruvate released by the action of SGPT from L-Alanine is converted to L-Lactate by the enzyme LDH. In the reaction, NADH is oxidized to NAD. The measurement of absorbance is proportionate to SGPT activity in the sample.



The reagents required were:

Reagent 1 (Enzymes):

LDH	≥	1200 U/L
NADH	-	0.20 mmol/L
α-Ketoglutarate	-	16 mmol/L

Reagent 2 (Buffer):

Tris buffer (pH 7.50)	-	110 mmol/L
L-Alanine	-	550 mmol/L

The serum samples and reconstituted reagents were brought to room temperature (37^0C) prior to use. The reaction mixture contained 1 ml of constituted reagent and 100 µL of serum. After mixing them, dispensed immediately into test tube and read absorbance at 340 nm in the semi-automated biochemical machine, and the enzyme activity was expressed in Units/Litre (U/L). One unit of SGPT activity was expressed as that amount of

enzyme which catalyzed the oxidation of 1 µmol of NADH to NAD^+ per min at pH 7.5 and 37^0C.

Total protein

Biuret method as described by Henry *et al.* (1974) and Stricklad *et al.* (1961) was followed for the estimation of total protein. Peptide bonds of protein form a blue-violet coloured complex with cupric ions in an alkaline medium. The intensity of the colour is proportional to the number of peptide bonds and the colour is read at 546 nm (530-570 nm).

Reagents required were:

Reagent 1 (Biuret reagent)

Sodium Hydroxide	-	3.8 mol/L
Potassium Sodium tartrate	-	0.1 mol/L
Cupric Sulphate	-	33 mmol/L
Potassium Iodide	-	30 mmol/L

Reagent 2 (Surfactant):

Surfactant	-	20 g/L

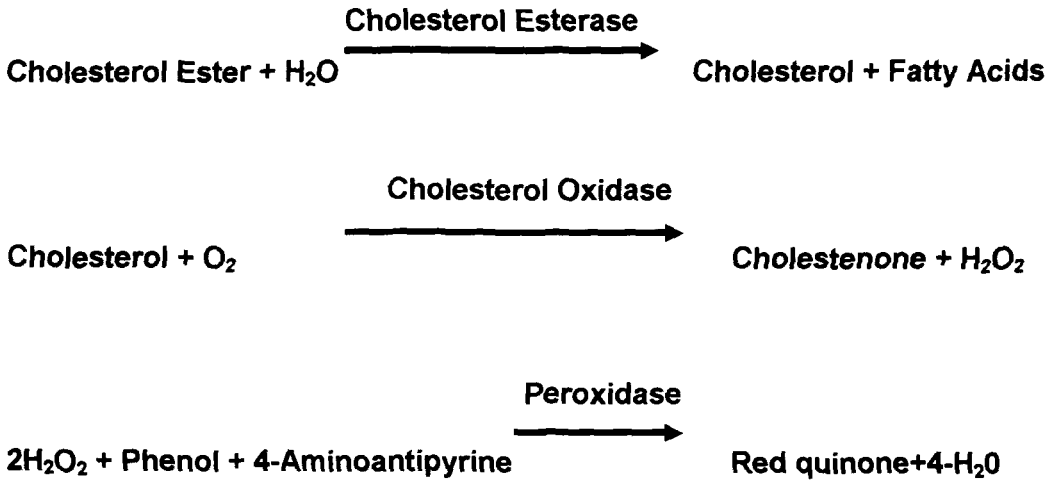
Standard (Total Protein 6 g/dL)

BSA	-	60g/L

The serum samples (10 µL) or standard (10 µL) and reconstituted reagent (1 mL) were kept at room temperature and incubated for 20 min. The reaction mixture was dispensed into test tube, and read endpoint colouration at 546 nm. The total protein was expressed as g/dL.

Cholesterol

Cholesterol was assayed by enzymatic method as described by Allain *et al.* (1974). Cholesterol released by the action of cholesterol esterase from the cholesterol ester is oxidized to cholestenone and hydrogen peroxide H_2O_2 by cholesterol oxidase. Hydrogen peroxide in reaction with phenol and 4-Aminoantipyrine by the action of peroxidase forms a Red Quinone. The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measured at 500 nm *(492-550 nm).*



Reagents used were:

Reagent 1 (Enzymes/Chromogen):

Cholesterol Esterase	≥	200 U/L
Cholesterol Oxidase	≥	250 U/L
Peroxidase	≥	1000 U/L
4-Aminoantipyrine	-	0.5 mmol/L

Reagent 2 (Buffer):

Pipes buffer, pH 6.90	-	50 mmol/L

Phenol	-	24 mmol/L
Sodium Cholate	-	0.5 mmol/L
Standard (Cholesterol 200 mg/dL):		
Cholesterol	-	2 g/L

The serum samples and reconstituted reagent were kept at room temperature prior to mixing. The reaction mixture (1 mL reagent and 10 µL serum or standard sample) was dispensed into test tubes, incubated for 5 min at 37^0C and read red colour complex at 500 nm. The intensity of red complex (Red Quinone) measured was direct indication of cholesterol concentration, which was expressed as mg/dL.

Statistical Analysis

The data were analyzed statistically and are represented as mean ± standard error of mean (SEM). The significance of the difference between the means was determined by the Student's *t*-test. And probability less than 5% (p values < 0.05) was accepted as statistically significant.

OBSERVATIONS AND RESULTS

ANTIDIARRHOEAL EFFICACIES OF THE PLANT EXTRACTS

1.1. *Rhus javanica* ripen fruit extract:

Treatments of *R. javanica* ripen fruit extract at 100, 200, 400 and 800 mg/kg, p.o. doses could reduce faecal production of the treated mice significantly in a dose-dependant manner. The reduction in faecal output was recorded to be 53.57% and 55.88% for the extract (800 mg/kg, p.o.) and Loperamide (5 mg/kg, p.o.), respectively as compared to the control. In the castor oil-induced diarrhoea study, there was a drastic fall in the number of diarrhoeal episodes in the treated animals, and upto 66.67% of animals were protected from diarrhoea provoked by castor oil, when animals were treated with 800 mg/kg dose of the extract and 83.33% protection of animals by the Loperamide at 5 mg/kg dose.

PGE_2 could increase the volume of small intestinal fluids accumulated per 100 g mouse from 1.35 ml in normal control (0.5 ml gum acacia) to 3.21 ml in vehicle control (gum acacia + 0.5 ml of 100 µg/kg PGE_2) animals. The plant extract could reduce the intestinal fluid accumulation from 26.48% (100 mg/kg) to 40.50% (800 mg/kg dose), whereas, it was 39.25% reduction in Loperamide treatment at 5 mg/kg dose, as evaluated from the vehicle control. The distance travelled by the charcoal marker in the small intestines of the treated mice showed significant difference from the control. The percentage inhibition of the intestinal transit was 55.84% for 800 mg/kg

treated group, whereas it was 58.57% for Loperamide. The results are shown in Fig. 1.1.

1.2. *G. parviflora* leaf and inflorescence extract:

The efficacy of *G. parviflora* leaf and inflorescence extract was lesser in comparison with that of *R. javanica* extract. Fig. 1.2 shows that the test extract reduced the faecal output ranging from 7.07 – 33.22% as compared to 38.63% in Loperamide treated group. Its highest dose (800 mg/kg, p.o.) could protect the animals to 33.33% after castor oil challenge. And reduction in the intestinal fluid production by the test extract was 21.26% when compared to the control. With regard to its ability to inhibit the small intestinal transit, the extract showed up to 33.68% inhibition and Loperamide by 55.81%, respectively.

1.3. *B. pilosa* leaf extract:

B. pilosa leaf extract could show a slightly higher reduction in faecal output up to 53.69% by its highest dose (800 mg/kg) than that of Loperamide where it was recorded to be 51.44%. Again, 66.67% animals were protected from diarrhoeal droppings after castor oil challenge by 800 mg/kg dose of the plant extract, whereas it was 83.33% protection by Loperamide. The extract inhibited production of intestinal fluid secretion up to 26.15%, as compared to 28.62% by Loperamide, when compared to the control. Further, motility in the small intestine was significantly inhibited by *B. pilosa* extract in a dose-dependant manner (15.76 – 54.26%), and Loperamide inhibited the transit by 57.42% (Fig. 1.3).

1.4. *Swertia angustifolia* aerial shoot extract:

Fig. 1.4 represents the antidiarrhoeal activity of *S. angustifolia* aerial shoot extract. The percentage reductions of the faecal output in 100, 200, 400 and 800 mg/kg doses were noted as 26.11%, 32.31%, 40.28% and 55.09%, respectively., whereas that in Loperamide treatment (5 mg/kg) was 57.31% when compared to the control. It was notable that diarrhoeal episodes induced by castor oil were delayed significantly in the treated groups of mice, and upto 66.67% mice were cured from diarrhoea, as compared to 83.33% by Loperamide.

It was observed that the extract could minimize the fluidity in the small intestine induced by PGE_2 upto 22.03%, and that in Loperamide was 27.12%. With respect to gastrointestinal motility, transit inhibition was 54.86% in 800 mg/kg dose of extract as compared to 55.37% in case of reference drug at its 5 mg/kg concentration.

1.5. *Cymbopogon citratus* stalk extract and its active component, citral:

It is interesting to observe that *C. citratus* stalk extract showed a high percentage of faecal reduction, 47.15% and 53.44% by 400 and 800 mg/kg doses respectively, as compared to 21.62% and 40.62% by citral, its active component at 5 and 10 mg/kg doses, and 42.99% and 57.01% by Loperamide at 5 and 10 mg/kg doses, respectively. Castor oil induced diarrhoeal episodes were minimized significantly by the test extract, whereas animals protected from diarrhoeal episodes were documented to be 50.00%

for the treatments of extract at 800 mg/kg and 5 mg/kg dose each of citral and Loperamide, whereas, 100% protection of animals from diarrhoea induced by castor was observed in 10 mg/kg dose each of citral and Loperamide.

PGE_2-induced enteropooling assay revealed that test extract reduce secretion of intestinal fluids by 22.82%, citral by 33.56% and Loperamide by 39.93%. However, *C. citratus* extract showed maximum inhibition in the transit assay upto 57.22%, as compared to 43.47% by citral and 55.83% by Loperamide (Fig. 1.5).

1.6. *Lithocarpus dealbata* seed extract:

The maximum activity of *L. dealbata* extract was observed in the animals protection from diarrhoea-induced by castor oil, where, the extract (800 mg/kg) could protect the animals from diarrhoea by 66.67%, as compared to 83.33% protection in Loperamide-treated group. Faecal output reduction was noted to be 47.57% in extract treatment group, which was comparable to 49.39% reduction by Loperamide. Prostaglandin induced diarrhoea test yielded 25.60% reduction in intestinal secretion in the extract treated group, whereas that in Loperamide treated group, reduced to 29.35%. And the distance travelled by the charcoal marker revealed 51.04% and 54.17% inhibitions of the transit in small intestine by the plant extract and Loperamide, respectively (Fig. 1.6).

1.7. *Zingiber cassumunar* rhizome extract:

The results of the *Z. cassumunar* extract showed that only the higher doses (400 and 800 mg/kg, p.o.) revealed significant differences from the control in all four parameters of study (Fig. 1.7). Faecal reduction was 21.34%, animal protection after castor oil challenge was 16.67% and intestinal fluid secretion was reduced by 18.47% by its maximum dose. Whereas, gastrointestinal transit was inhibited by 24.11%, as compared to 50.40% inhibition by Loperamide.

1.8. *Urena lobata* leaf extract & its active component, quercetin:

The results of the antidiarrhoeal activity of *U. lobata* extract and quercetin, its active component are shown in Fig. 1.8. It was observed that the extract in its maximum dose could reduce the faecal production by 19.48%, quercetin in its maximum dose (10 mg/kg) by 44.81% and Loperamide by 48.05%. Percentage animal protections from diarrhoeal droppings provoked by castor oil were tabulated as 50.00% by test extract, 33.33% and 100% by quercetin at 5 and 10 mg/kg doses, and 66.67% and 100% by Loperamide at 5 and 10 mg/kg doses respectively.

Small intestinal fluid accumulations were significantly reduced to 21.38%, 33.88% and 41.12% by test extract, quercetin and Loperamide, respectively in their maximum doses tested. While the small intestinal motility was inhibited upto 57.94% by the test extract, 45.83% by quercetin and 57.49% by Loperamide (Fig. 1.8).

1.9. *Potentilla fulgens* root extract:

Fig. 1.9 shows the effects of *P. fulgens* root extract against experimental diarrhoea in mice. It is notable that the test extract could reduce the faecal production up to 26.37%, and its 200, 400 and 800 mg/kg doses could protect 50.00% each of the animals from diarrhoeas evoked by castor oil. However, 29.27% and 30.31% are comparable reduction values of the PGE_2-induced intestinal secretion by the extract (800 mg/kg, p.o., dose) and Loperamide, respectively. There were significant differences in the distance travelled by the charcoal meal along the small intestines between the treated groups and control, although 23.18% inhibition by test extract was a low value as compared to 44.37% inhibition by the standard reference drug.

ACUTE TOXICITY EFFECTS OF PLANT EXTRACTS

(A) Median Lethal Dose (LD_{50}): Oral administration of plant extracts starting from 100 mg/kg to 3200 mg/kg caused mortality to some of the animal groups observed within 72 h post-treatment for some of the plant extracts. The number of animals showing mortality was observed in extract treatments of *B. pilosa, G. parviflora* and *Z. cassumunar*, where two mice exhibited mortality out of total six in each group at 3200 mg/kg dose. One mouse showed mortality for the *P. fulgens* extract treatment group at 3200 mg/kg dose. Using SPSS software, the median lethal dose was calculated and expressed as LD_{50} (Oral; mg/kg). The values of LD_{50} are 3415.64, 3617.20, 4080.40 and 5355.97 for *B. pilosa, G. parviflora, Z. cassumunar* and *P. fulgens* extracts, respectively. While no mortality was observed for the

other plant extracts, namely, *R. javanica, S. angustifolia, C. citratus, U. lobata* and *P. fulgens*, when treatment was given upto 3200 mg/kg., p.o. (Fig. 1.10).

(B) Serum biochemical profile: The serum collected from the normal control mice yielded 135.33 U/L of SGOT, 118.00 U/L of SGPT, 134.33 mg/dL of cholesterol and 6.73 g/dL of total protein (Fig. 1.11). There was no significant difference in the levels of these parameters of the serum samples collected from the mice 24 h post-treated with the plant extracts at their maximum dose (800 mg/kg, p.o.) when compared with that of the control values. However, there are few exceptions of significant rise in SGOT for *Z. cassumunar* extract (176.67 U/L), SGPT for *L. dealbata* (162.67 U/L) and *Z. cassumunar* (161.50 U/L) and cholesterol for *S. angustifolia* extract (186.33 mg/dL).

Rhus javanica

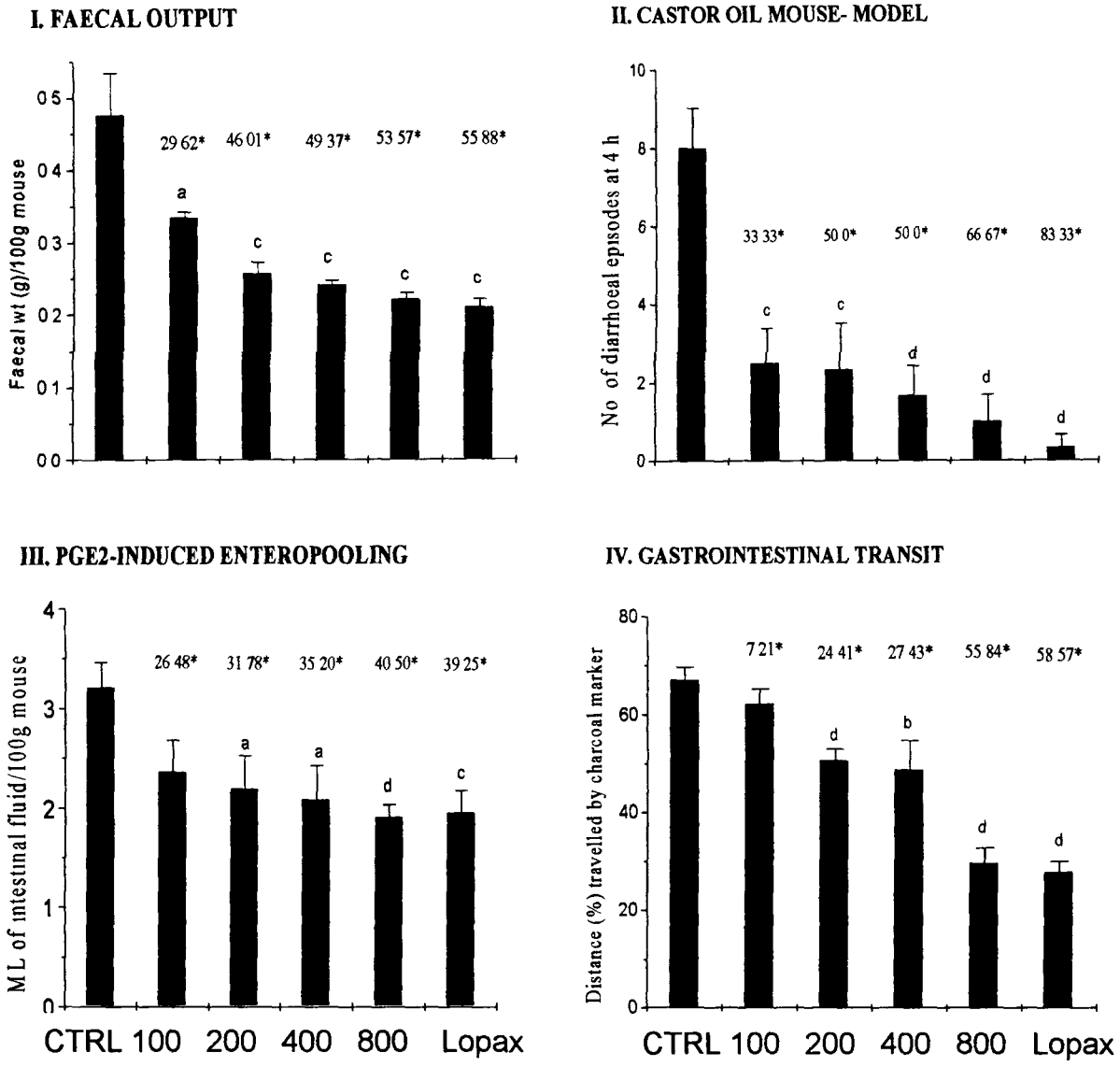


Fig. 1.1: Antidiarrhoeal efficacy of *Rhus javanica* ripen fruit extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

[a, b, c, d] $p < 0.05, 0.02, 0.01$ and 0.001, vs. control, Student's *t*-test.

Galinsoga parviflora

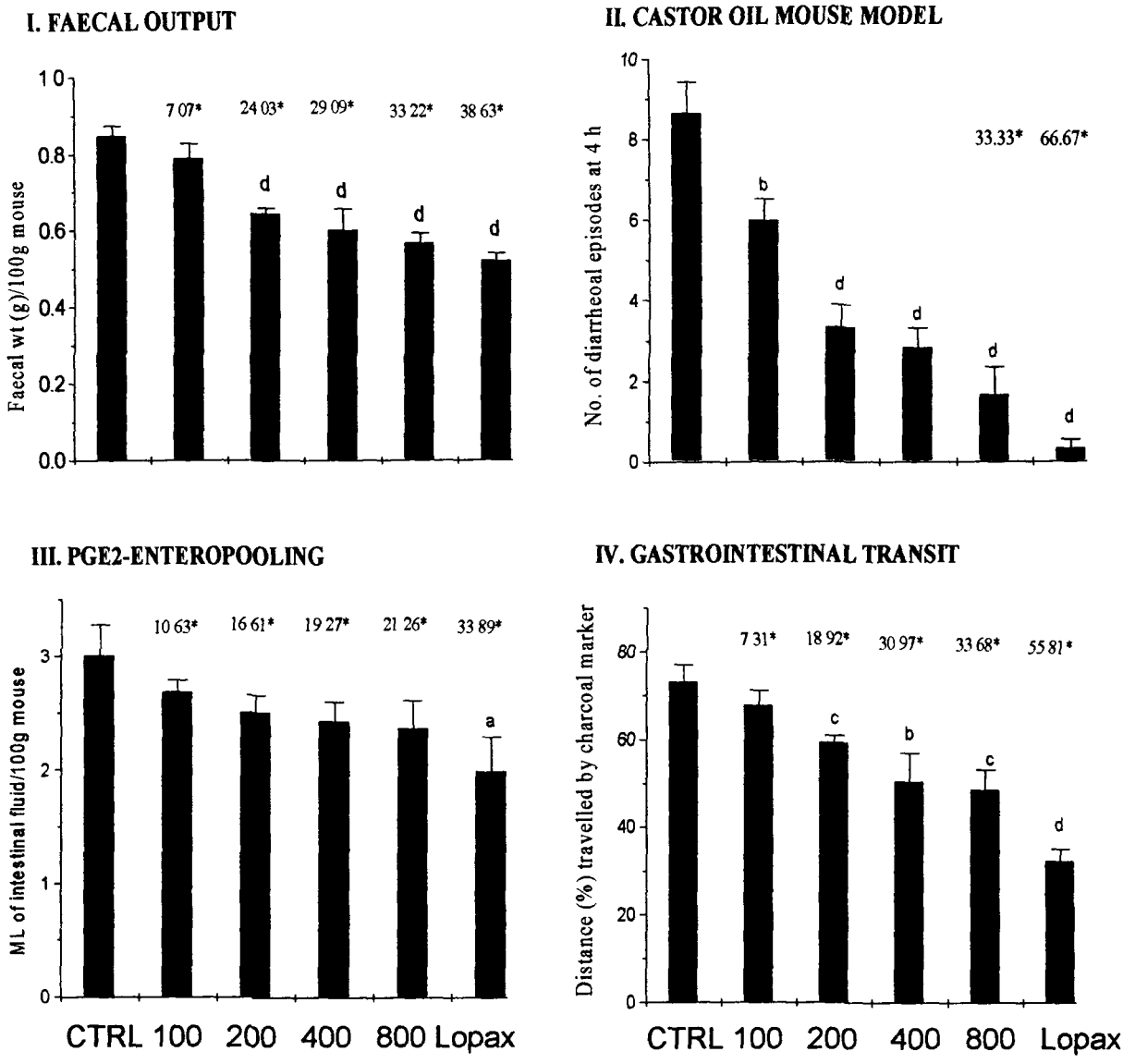


Fig. 1.2: Antidiarrhoeal efficacy of *Galinsoga parviflora* leaf and inflorescence extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

$^{a, b, c, d}$ $p < 0.05$, 0.02, 0.01 and 0.001, vs. control, Student's *t*-test.

Bidens pilosa

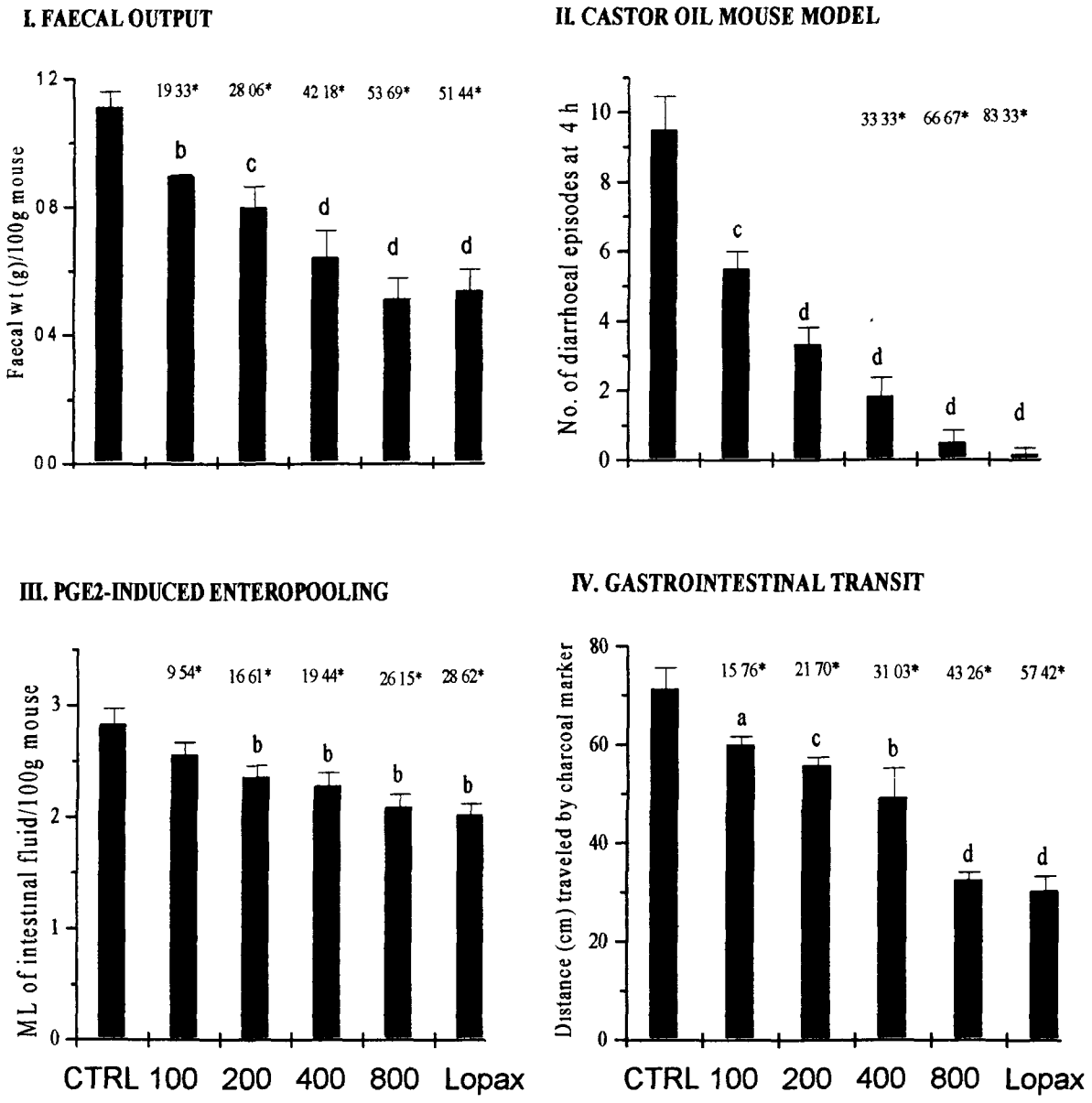


Fig. 1.3: Antidiarrhoeal efficacy of *Bidens pilosa* leaf extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

$^{a, b, c, d}$ $p < 0.05, 0.02, 0.01$ and 0.001, vs. control, Student's *t*-test.

Swertia angustifolia

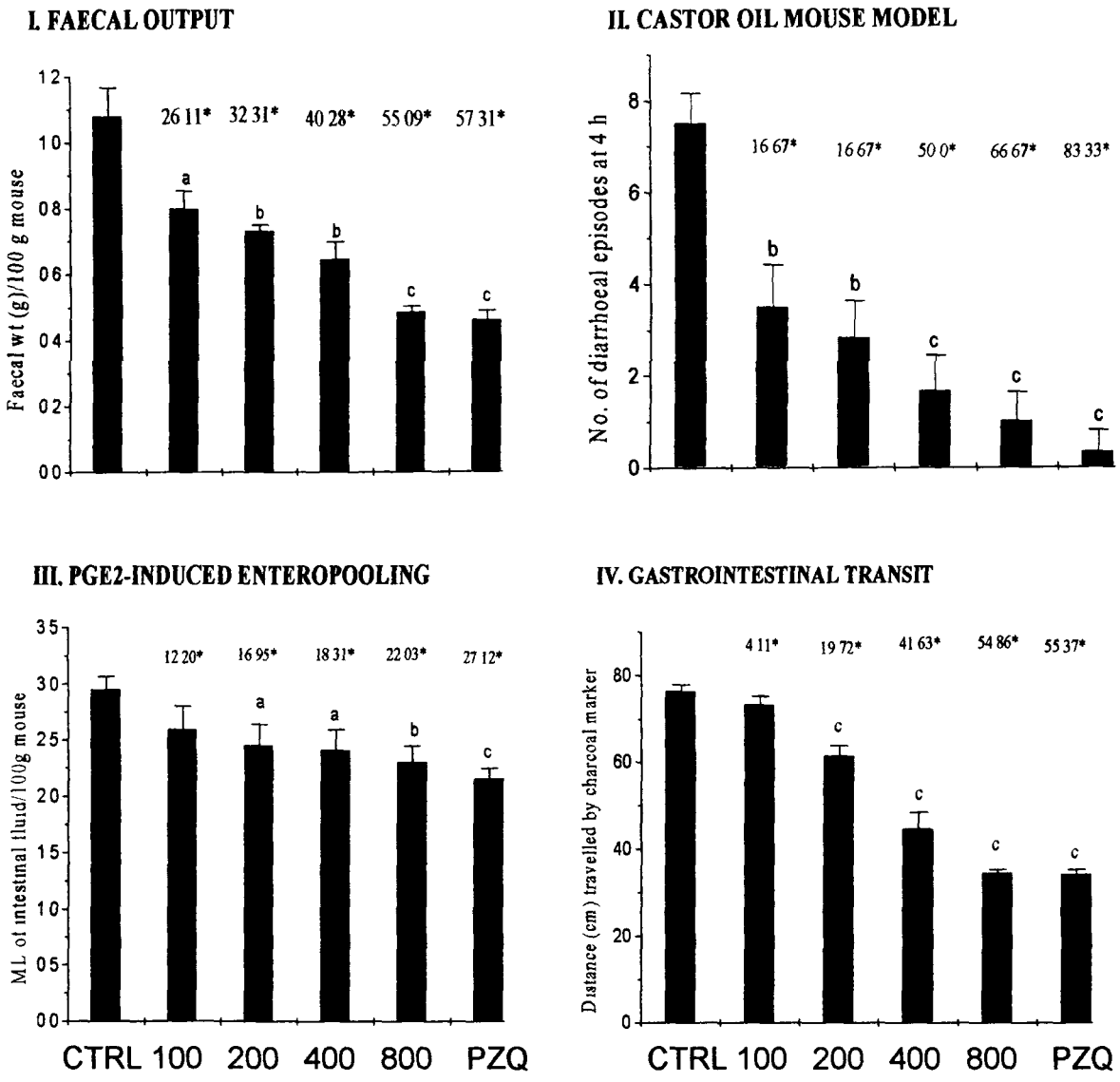


Fig. 1.4: Antidiarrhoeal efficacy of *Swertia angustifolia* aerial part extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

$^{a, b, c, d}$ $p < 0.05$, 0.02, 0.01 and 0.001, vs. control, Student's *t*-test.

Cymbopogon citratus & citral

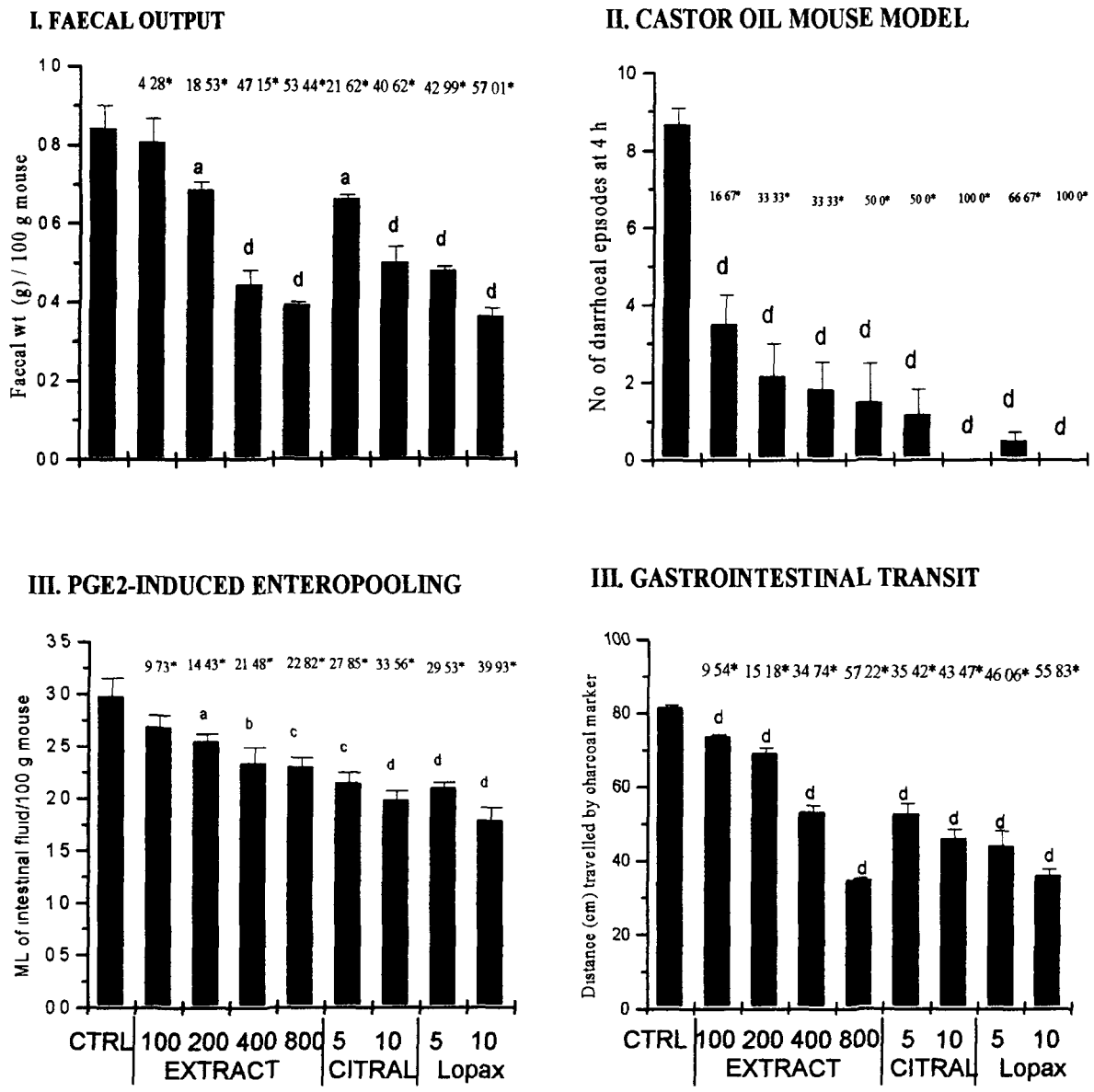


Fig. 1.5: Antidiarrhoeal efficacy of *Cymbopogon citratus* stalk extract and its active component, Citral, as represented by four different study parameters. Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

$^{a, b, c, d}$ $p < 0.05$, 0.02, 0.01 and 0.001, vs. control, Student's *t*-test.

Lithocarpus dealbata

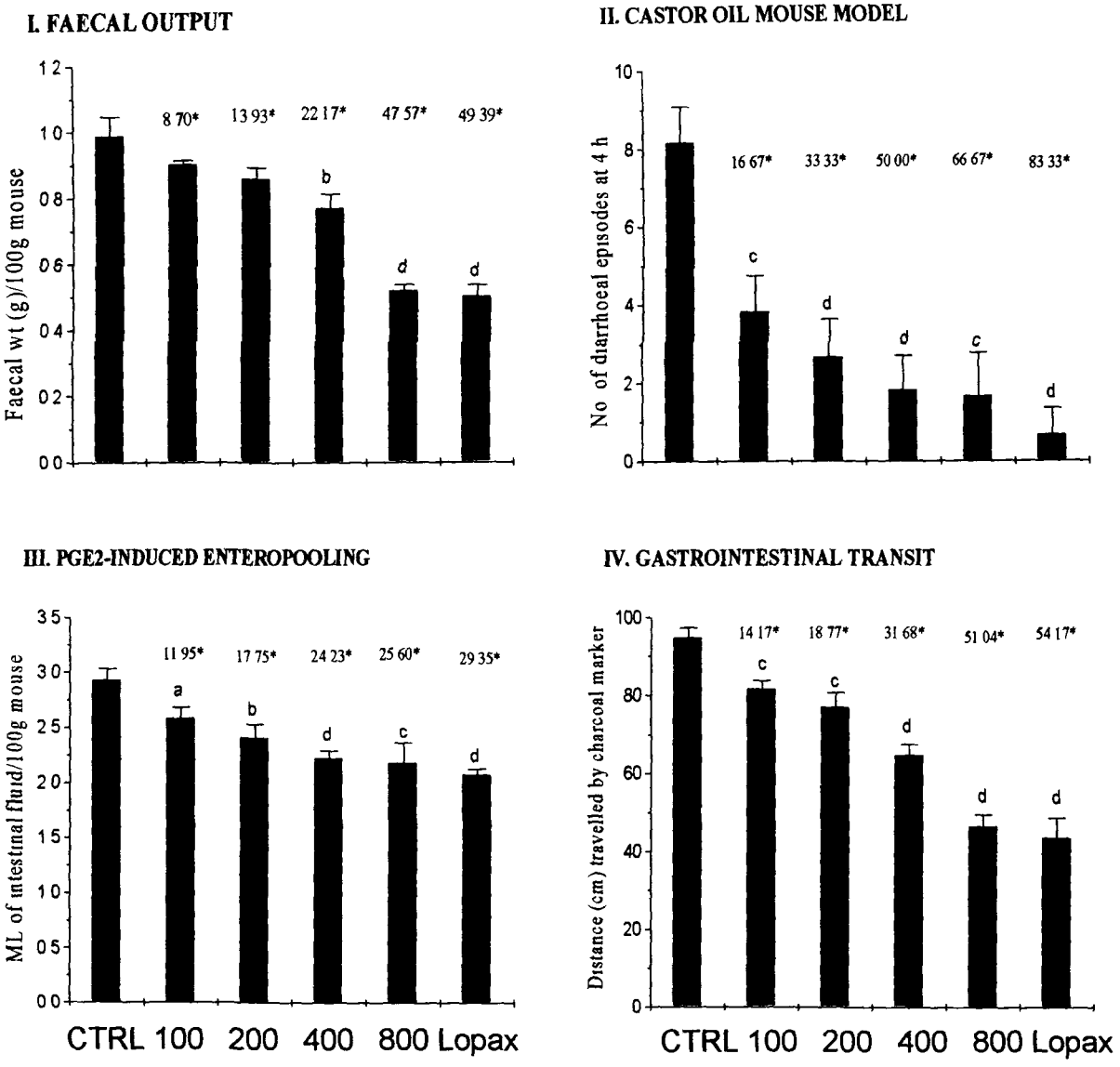


Fig. 1.6: Antidiarrhoeal efficacy of *Lithocarpus dealbata* seed extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

[a, b, c, d] $p < 0.05$, 0.02, 0.01 and 0.001, vs. control, Student's *t*-test.

Zingiber cassumunar

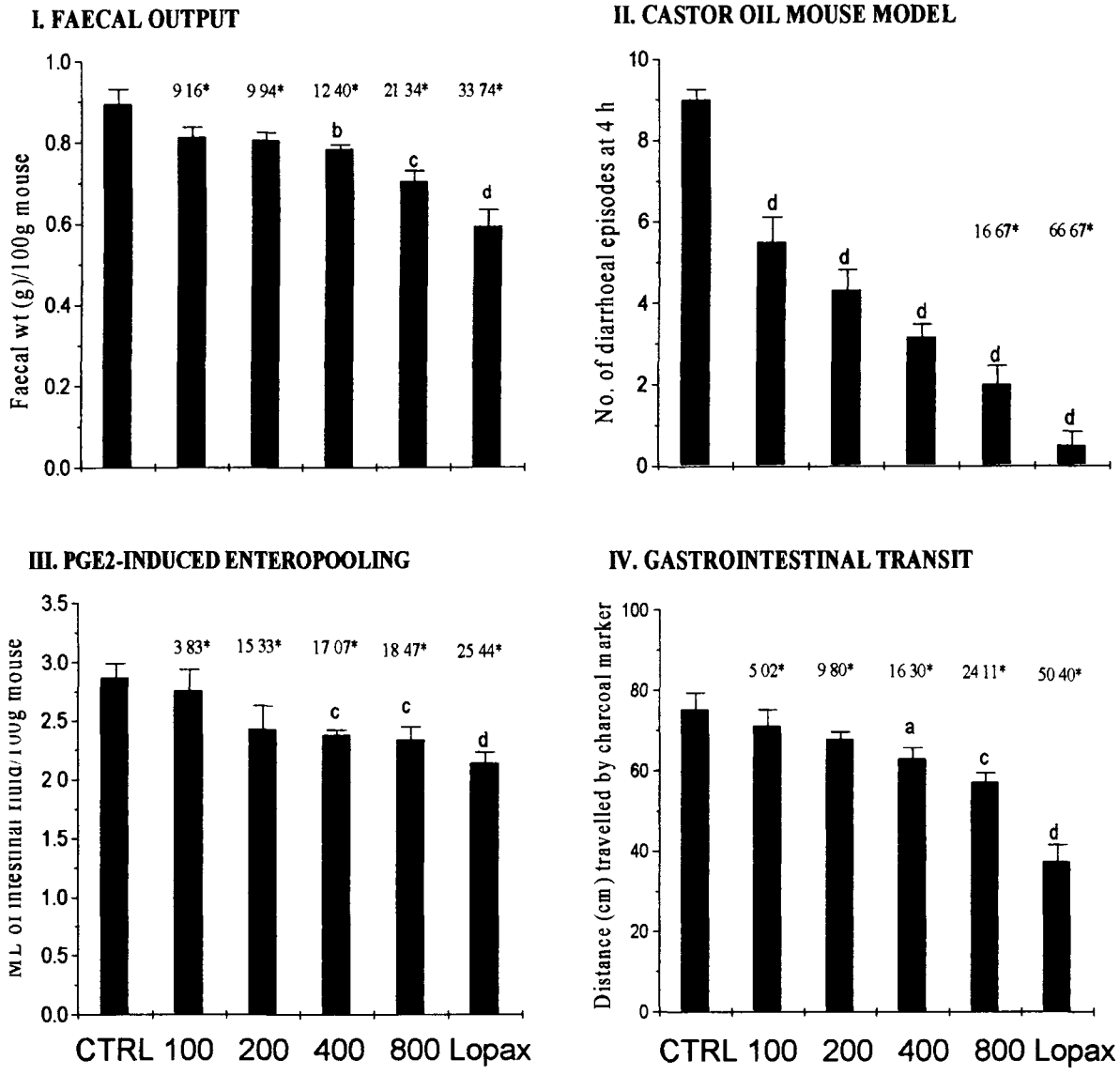


Fig. 1.7: Antidiarrhoeal efficacy of *Zingiber cassumunar* rhizome extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

$^{a, b, c, d}$ $p < 0.05$, 0.02, 0.01 and 0.001, vs. control, Student's *t*-test.

Urena lobata & Quercetin

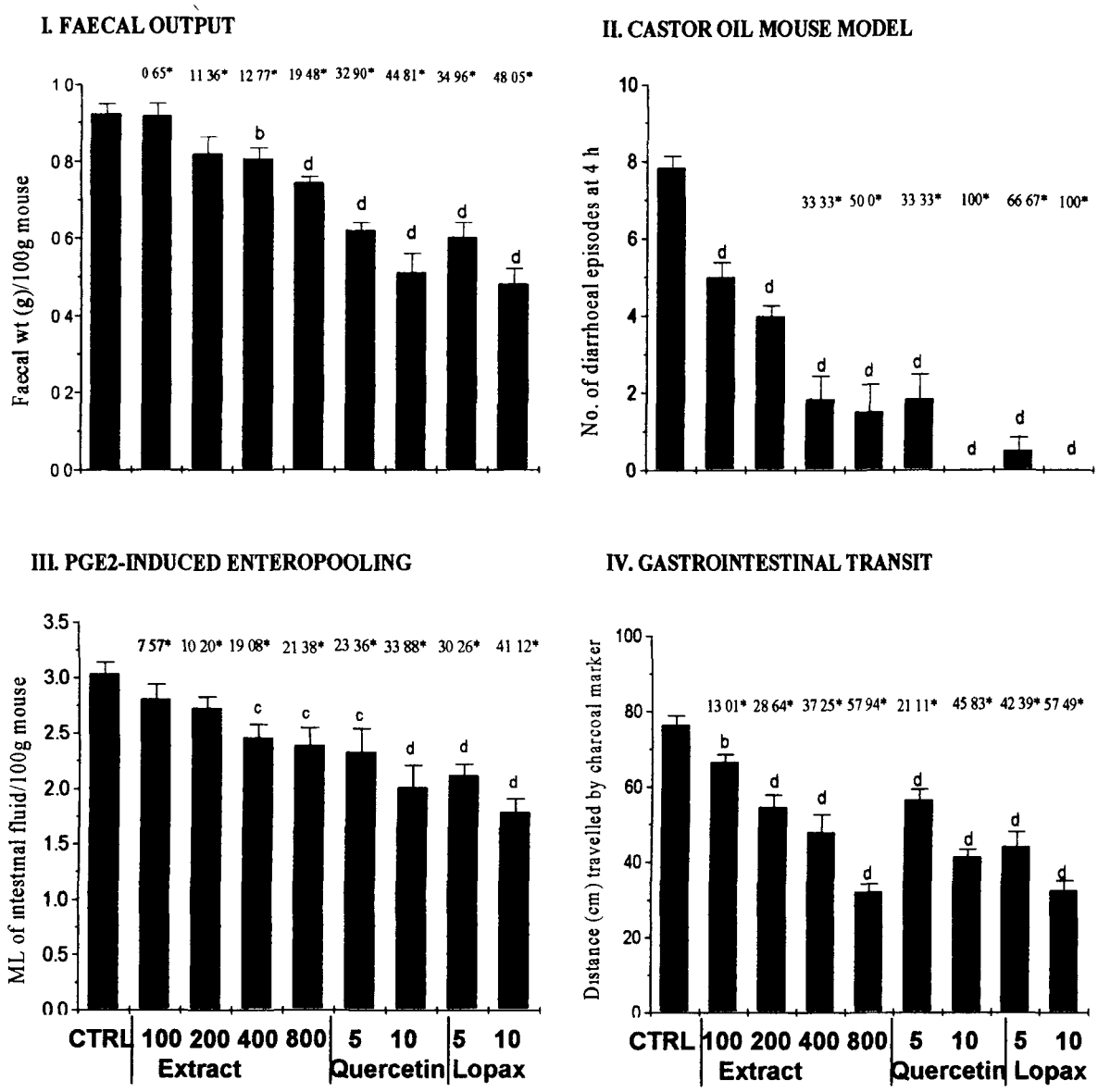


Fig. 1.8: Antidiarrhoeal efficacy of *Urena lobata* leaf extract, and its active component, quercetin, as represented by four different study parameters. Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

[a, b, c, d] $p < 0.05, 0.02, 0.01$ and 0.001, vs. control, Student's *t*-test.

Potentilla fulgens

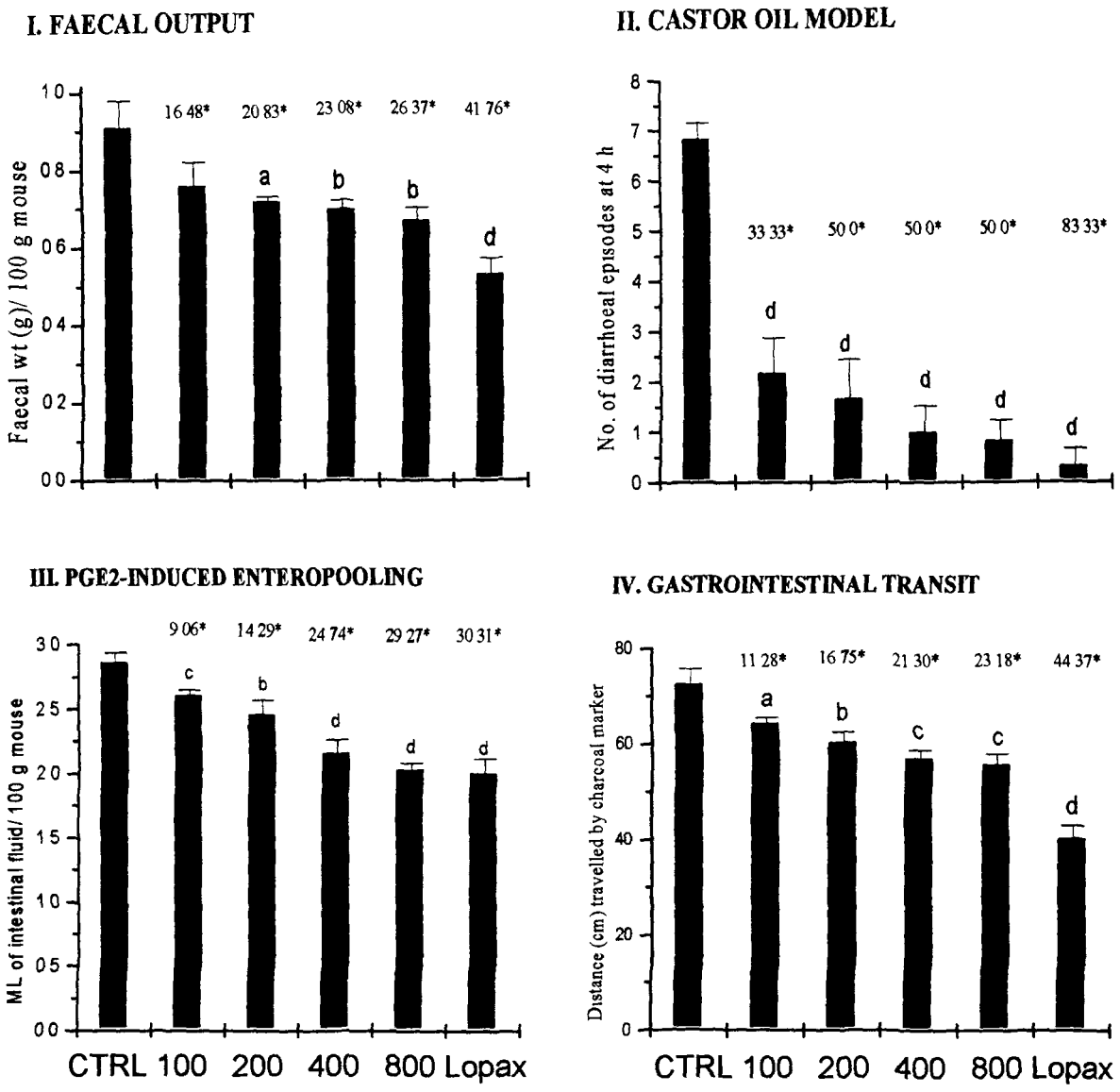


Fig. 1.9: Antidiarrhoeal efficacy of *Potentilla fulgens* root extract, as represented by four different study parameters.

Values are plotted as mean ± SEM (n = 6).

***% reduction or, % Inhibition or, % animal protection from diarrhoea.**

Treatment (mg/kg, p.o.): Extract at 100, 200, 400 & 800; Lopax at 5.

$^{a, b, c, d}$ $p < 0.05$, 0.02, 0.01 and 0.001, vs. control, Student's *t*-test.

Lethal effects of the plant extracts

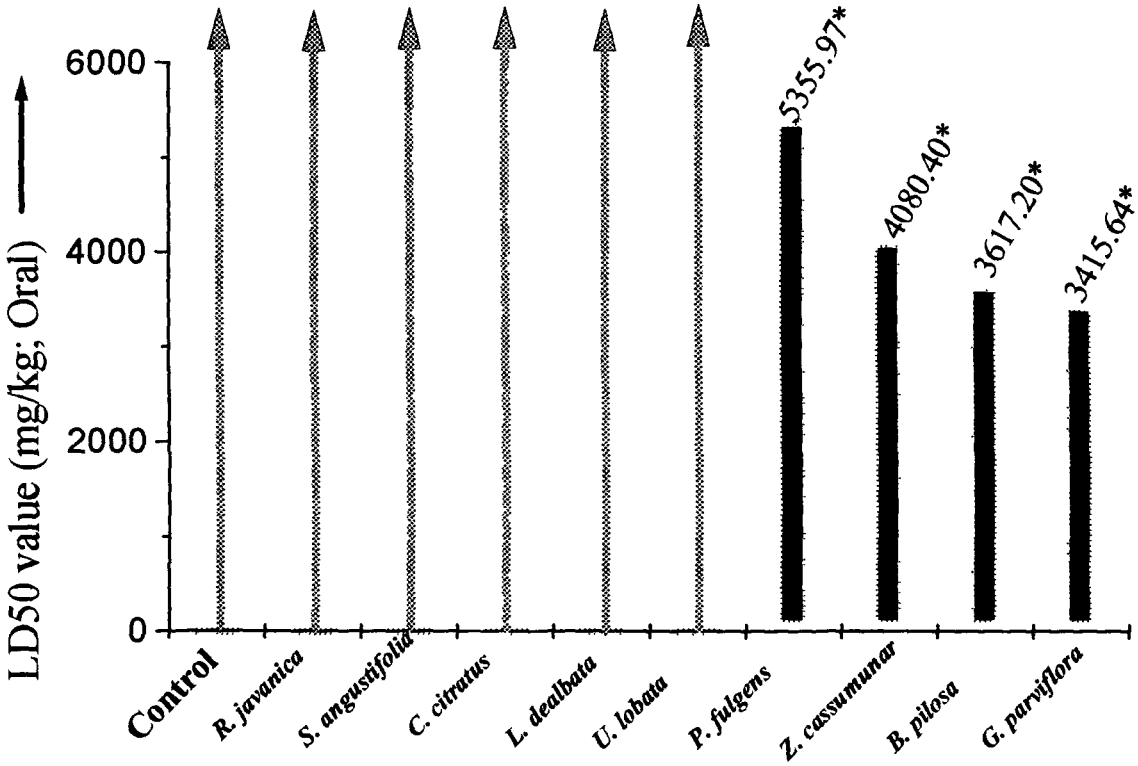


Fig. 1.10: Lethal effects of the different plant extracts to the experimental mice on the acute toxicity basis. LD_{50} values are calculated from the number of animals (n = 6) showing mortality in each group (using SPSS software). LD_{50} signifies that lower its value more toxic is the extract, higher its value less harmful is the extract. Arrow (↑) indicates no mortality caused by the extract. Lowest LD_{50} value is exhibited by *G. parviflora* extract.

Blood Serum Profile

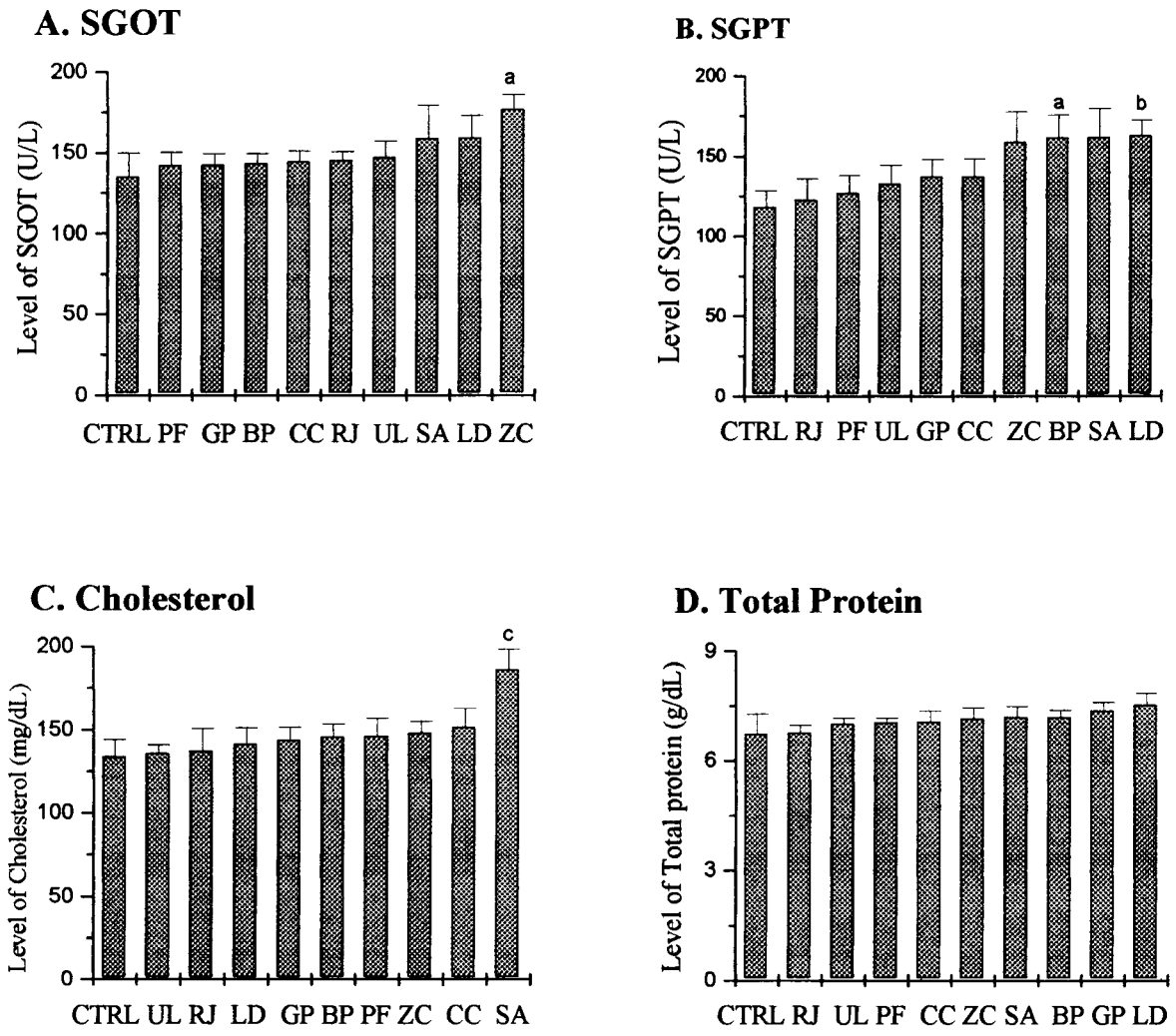


Fig. 1.11: Effects of the plant extracts on the levels of SGOT, SGPT, cholesterol and total protein of the blood serum collected from the mice 24 h post-treated with the different plant extracts at their maximum dose (800 mg/kg, p.o.). There was no significant difference between treated and control groups, with few exceptions.

Values are plotted as mean ± SEM (n = 6).

$^{a, b, c}$ $p < 0.05, 0.02, 0.01$ vs. control, Student's *t*-test.

Different plant extracts are RJ=*Rhus javanica*; UL=*Urena lobata*; PF=*Potentilla fulgens*; CC=*Cymbopogon citratus*; ZC=*Zingiber cassumunar*; SA=*Swertia angustifolia*; BP=*Bidens pilosa*; GP=*Galinsoga parviflora* and LD=*Lithocarpus dealbata*.

DISCUSSION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of developing countries, they continue to be used as the primary source of medicine (Chitme *et al.,* 2003). The natural products derived from medicinal plants have been proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead compounds for pharmaceuticals. With respect to northeastern region of India, which is richly inhabited by several native tribes there is an age old practice to use such medicinal plants for the treatment of numerous ailments. Diarrhoeal diseases are among common health problems of people in the northeastern region of India because of lack of potable water supply, particularly so in rural areas. It is in this regard, native tribes in Manipur state customarily use various traditional plants and plant derived preparations considering them to be efficacious against diarrhoeal disorders without any scientific base to explain their efficacy or mode of actions. These tribes, Naga tribes in particular, have a valuable heritage of herbal remedies and there is an excellent scope of research for the ethnopharmacological studies in this area. However, so far there is no report or any study in this regard, although there are several unique ways of treating diarrhoeal problems varying from tribe to tribe, from village to village and from one local medicineman to another, as prevalence of diarrhoeal disorders is among one of the common health problems in the rural areas of this part of India. From a list of plants claimed to have antidiarrhoeal

properties among the local people of area, the most commonly used plants or plant parts resulting from a preliminary questionnaire study (Table 1.1) were therefore included to experimentally investigate the antidiarrhoeal potentials in suitable diarrhoea-animal models in the present study.

The small intestine plays a vital major role in diarrhoeal diseases. The main feature of the small intestine is to absorb and to secrete biological materials. Diarrhoea results from an imbalance between the absorptive and secretive mechanisms in the intestinal tract, accompanied by intestinal hurry, which results in an excess loss of fluids through the faeces (Yegnanarayan and Shrotri, 1982). Clinically, the secretory component predominates in some cases of diarrhoea, while other diarrhoeas are characterized by hypermotility. Numerous studies have validated the traditional use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have been reported to have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption or reduce intraluminal fluid accumulation (Almeida *et al.*, 1995; Atta and Mouneir, 2005). Inhibition of experimental diarrhoea and reduction in faecal output by a substance are also considered as one of the basic parameters to judge the pharmacological credibility of an antidiarrhoeal agent (Akah, 1996). These experimental animal models were therefore employed for the evaluation of antidiarrhoeal efficacy of folklore medicinal plant extracts in the present study.

The results of the present study demonstrate that most of the individual plant extracts have the potentials to reduce more than 50% of the faecal productions in the experimental mice. These plants include *R. javanica, S. angustifolia, C. citratus,* and *L. dealbata.* This observation indicates towards the presence of either an antisecretory or proabsorptive properties in these plant extracts.

It is a well known established mechanism how castor oil acts inside the body system. Castor oil is first metabolized into its active component, ricinoleic acid in the lumen of the intestinal tract, which in turn irritates and causes inflammation in the intestinal mucosa, resulting in to release of various inflammatory mediators, such as prostaglandins, histamine, etc. (Luderer *et al.,* 1980). The prostaglandins thus released promote vasodilatation, smooth muscle contraction and mucus secretion in the small intestines (Pierce *et al.*, 1971; Robert, 1973). The prostaglandins of the E series are considered to be good diarrhoegenic agents in experimental animals as well as in human beings (Jaffe, 1979). The inhibitors of prostaglandins biosynthesis are therefore considered to delay the castor oil - induced diarrhoea (Pierce *et al.*, 1971).

The present results indicated that all the plant extracts significantly prevent droppings of diarrhoeal episodes after castor oil challenge, and extracts of *R. javanica, B. pilosa, S. angustifolia* and *L. dealbata*, in particular, showed a very high percentage of protection of animals from diarrhoea (66.67% protection). From these results it may be assumed that these plant

extracts either inhibit the biosynthesis of prostaglandin or alter its mechanism of action in the gastrointestinal gut.

With respect to the PGE_2–induced enteropooling, *R. javanica* extract emerges out to be the most promising agent showing the highest reduction of intestinal fluid accumulation after PGE_2–induced diarrhoea, among all the plant extracts tested including the standard drug, Loperamide. It is likely that the plant extract brings out their aforesaid action either through their proabsorptive property that promotes faster fluid absorption in the intestine or an antisecretory mechanism may also be the basis for their mechanisms of action. Our first speculation gains support from the fact that castor oil, which was used as a diarrhoea inducing agent in the experimental protocol, is regarded to induce diarrhoea by increasing the volume of intestinal content by prevention of the re-absorption of water. Therefore any agent which could allow or promote the absorption of water from intestine obviously would have an antidiarrhoeal potential.

On comparing the efficacy among the plant extracts under question with that of the reference antidiarrhoeal agent, *U. lobata* and *C. citratus* extracts appear to show the maximum inhibitions in the gastrointestinal motility of the charcoal meal marker, which is even slightly better than that of the Loperamide. Nevertheless, the extracts of *R. javanica, B. pilosa, S. angustifolia* and *L. dealbata* could also inhibit more than 50% of the transit, suggesting that these extracts contain absorptive or antimotility compounds. As the activated charcoal absorb drugs on the surface of charcoal particles

thereby preventing absorption in the small intestines (Levy, 1982); however, the results of the present study showing a very positive inhibition in the transit of the small intestines incline to attest that the extracts invigorate the absorption in the enterocytes of the small intestine.

Triterpenoids including gallotannins (Kurokawa *et al.,* 1999; Taniguchi *et al.,* 2000) and triterpenes (Lee *et al.*, 2001) have been isolated from *Rhus javanica*. The triterpenes are aliphatic compounds based on a skeleton with 30 carbon atoms, and they are present in all parts of many plants such as roots, pollen, fruit and seeds (Mahato *et al.,* 1988). Anti-inflammatory effects have also been reported as biological effects of triterpenes (Recio *et al.*, 1995; Ryu *et al.*, 2000; Kim *et al.*, 2005). A promising antidiarrhoeal activity of *Rhus javanica* extract revealed in the present study may be attributed due to presence of these compounds that may mainly act on inhibition of diarrhoeal symptoms when castor oil and PGE_2 were inoculated to induce diarrhoea.

Bidens pilosa is another plant which is known to possess anti-inflammatory property (Pereira *et al.,* 1999), antiulcerogenic (Tan *et al.,* 2000), antimicrobial (Khan *et al.,* 2001a), etc. Quercetin, a flavonoid compound, is one of the fractions isolated from this plant (Usami *et al.*, 2004), and has been reported to possess gastric antisecretory activity (Alvarez *et al.*, 1999). And Ethyl caffeate, a natural phenolic compound, isolated from this plant has also been elucidated for its anti-inflammatory functions (Chiang *et al.,* 2005). The present study also illustrates a highly positive antidiarrhoeal activity, whose efficacy may be attributed due to the

presence of an antiinflammatory molecule, ethyl caffeate as well as an antisecretory flavonoid, quercetin in this plant.

The plant, *S. angustifolia*, contains xanthones, glucosides (Ghosal *et al.,* 1978; Luo and Nie, 1992) and mangiferin as active chemical constituents (Duke, 1992). Mangiferin is well known to act against anti-inflammatory response by inhibiting the production and release of inflammatory molecules, such as prostaglandins, mast cells and histamine (Garcia *et al.*, 2003; Perrucci *et al.*, 2006; Rivera *et al.,* 2006; Sairam *et al.*, 2003). The antidiarrhoeal effects of this plant extract could be due to the action of these compounds in the gut system.

It becomes interesting to note here that *C. citratus* possesses essential oil, citral as its major active chemical component, which accounts for 75-80% citral content in its volatile oil (Abe *et al.,* 2003; Rauber *et al.*, 2005). It is advocated that the plants that have essential oils, are generally used traditionally for gastrointestinal disorders. In several studies on relaxant effects of essential oils, including citral it has been reported that the inhibition of contractile over-activity or reduction of inflammatory response of the ileum is their basis for the treatment of gastro-intestinal disorders such as, diarrhoea (Hajhashemi *et al.,* 2000; Sadraei *et al.*, 2003a; 2003b; Skocibusic´ and Bezic´, 2003). The present investigation of *C. citratus* extract and its active component, citral, provides an evidence of antidiarrhoeal property whose activity is comparable to that of the Loperamide, which indicates to emerge out the similar efficacy of the mentioned principle.

The seed of *L. dealbata* contains tannins, and the plants that have tannins in their composition can present an anti-diarrhoeic effect too (Mukherjee *et al.,* 1998; Agbor *et al.,* 2004; Atta & Mouneir, 2005), since these substances precipitate the proteins of enterocytes, reducing the peristaltic movements and intestinal secretion (Almeida *et al.*, 1995). A similar mechanism may explain the antidiarrhoeal activity of *L. dealbata* as observed in the present study.

Review of the literature reveals that rhizome of *Z. cassumunar* possesses anti-inflammatory (Ozaki *et al.*, 1991; Jeenapongsa *et al.*, 2003) and its chemical constituent, phenylbutenoid has been reported to inhibit prostaglandin E_2 production (Han *et al.,* 2005). It is therefore obvious that its antidiarrhoeal efficacy as revealed in the present study may be due to presence of chemical ingredients that in particular could inhibit prostaglandin biosynthesis in the gut. Though the mechanism of antidiarrhoeal activity of *P. fulgens* root extract cannot be explained at this stage, its efficacy observed in this study, particularly in protecting animals from castor oil-induced diarrhoea, may be attributed to presence of unknown chemicals which are responsible for prostaglandin inhibition in the gastrointestinal tract.

The phytochemical analysis of the aerial parts of *U. lobata* reveals that quercetin and mangiferin are the two major active constituents of the plant (Ghosh, 2004). Plants that have quercetin, a common dietary flavonoid in their composition, can present an antidiarrhoeal effect mainly due to their

antihistamine and anti-inflammatory activities (Izzo, 1994; Galvez *et al.*, 1993a). Like, in a lactose-induced diarrhoea study, Galvez *et al.* (1995) reported that the rats treated with quercetin had less diarrhoeal output as compared to the control. Similarly, in another study by Lutterodt (1989) it was found out that the *Psidium guajava* leaf extract brings out its antidiarrhoeal action through quercetin, this according to him and other workers opinion is mediated through by an inhibition of the gastrointestinal release of acetylcholine (Sweis *et al.*, 1984; Di Carlo *et al.*, 1994). Salah *et al.* (2002) also reported that quercetin is also able to inhibit the intestinal motility in experimental diarrhoeal study in rats. The role of quercetin as a promising antidiarrhoeal molecule has also been verified by many other workers (Galvez *et al.*, 1993b; 1995; Perrucci *et al.*, 2006). Similarly, several studies made in the past provide ample evidence to the fact that the mangiferin acts against antiinflammatory response by inhibiting the production and release of inflammatory molecules, such as prostaglandins, mast cells and histamine (Garcia *et al.*, 2003; Perrucci *et al.*, 2006; Rivera *et al.*, 2006). The large number of such anti-inflammatory molecules has the ability to relax the smooth muscles and thereby relieve gastro-intestinal tract (Katzung, 2004). In recent year, Choi and Hwang (2005) also found that *U. lobata* also plays an inhibitor role in nitric oxide (NO) production in lipopolysaccharides stimulated macrophages. NO plays an important role in intestinal fluid and electrolyte secretion in the intestine (Izzo *et al.*, 1998), and inhibitors of NO synthesis seem to block the laxative action of diarrhoeal agents (Izzo *et al.*, 1994). Based on the above facts, it seems reasonable to suggest that the antidiarrhoeal effects of *U. lobata* may be due to the inhibitory effect of its

active principle, quercetin on prostaglandins biosynthesis or an anti-inflammatory role played by mangiferin.

Loperamide is an opioid, which is the most useful pharmacological antidiarrhoeal agent and it acts by a combination of inhibition of intestinal transit, proabsorptive and antisecretory mechanisms (Jacoby *et al.*, 2001). The mode of action of Loperamide is believed due to its direct effect on the circular and longitudinal muscles of the intestinal wall. Further it is advocated that a decrease in the motility of gut muscles increases the amount of time substances stay in the intestine (Katzung, 2004). This allows for more water to be absorbed out of the intestinal secretion. In the present study, most of the plant extracts tested revealed almost comparable efficacy against experimental diarrhoea with that of the Loperamide. It may therefore be assumed that the antidiarrhoeal properties of the plant extracts follow the same mechanisms in the current animal models like that of the standard drug.

The Median Lethal Dose (LD_{50}) test involves the administration of a substance to a group of animals at increasing doses in order to determine the dose that kills 50 percent of the test subjects within a set time frame. Typically, administration of the test substance is via a tube inserted down the esophagus into the stomach. Other routes of administration include inhalation and applying the substance to the animals' skin. For determining the acute toxicity profile of any drug, LD_{50} test is one way to measure the short-term poisoning potential (acute toxicity) of a material (Lorke, 1983). In general,

smaller the LD_{50} value, the more toxic the chemical is and the opposite is also true: the larger the LD_{50} value, the lower the toxicity is.

In the present acute toxicity study, LD_{50} values were determined for *B. pilosa, G. parviflora, Z. cassumunar* and *P. fulgens*, extracts, however, the values were very high (3415.64–5355.97 mg/kg, p.o.), while no mortality was observed for the other five plants, namely, *R. javanica, S. angustifolia, C. citratus, U. lobata* and *L. dealbata*, even when treatment was given upto 3200 mg/kg., p.o. This study indicates that the plants are practically safe to use, as indigenous locales also take them without alleging any complaints of side effects.

Liver plays a very important role in metabolism of drugs in animals including human (Valame *et al.*, 1974). Drugs that have high levels of toxicity would alter liver functions in short span of time. Serum contains many different aminases, of which, the most frequently determined are SGOT and SGPT; both activities are reliable indicators of liver damage. Increase of SGOT and SGPT indicates mainly liver damage, and thereby release of dying and injured cells into body fluids. Decrease of these activities, on the other hand, indicates vitamin deficiency, liver congestion etc. Similarly, the determination of serum cholesterol is considered to be significant in various liver diseases; high level indicates severe damages in liver, low level is found in anemia, malnutrition and acute infections. And plasma protein concentration decreases in malnutrition and liver damages, however an increased level occurs in chronic infection and dehydration. In the present

study, the mice treated with 800 mg/kg dose of plant extracts did not show any significant change in the levels of SGOT, SGPT, cholesterol and total protein as compared to the appropriate controls for most of the plants tested however a significant difference was observed for *Z. cassumunar* in SGOT and SGPT levels, for *L. dealbata* in SGPT level, *C. citratus* and *S. angustifolia* in cholesterol. It is important to put remarks on non-toxicity of *U. lobata* leaf extract in the present study, in particular, which gain support from the findings of a similar study where the leaf extract of the *U. lobata* was also reported to be non-toxic on the basis of histopathology of liver and kidneys and serum biochemical profile in rats (Oladele and Abatan, 2004). In general, on the basis of these preliminary observations the study indicates that the plant extracts apparently have no harmful effects on experimental animals. However, a more comprehensive investigation is needed to comment further on the toxicity of these plant extracts in suitable animal models.

In conclusion, this experimental study validates the presence of antidiarrhoeal activity in these folk medicinal plants, which may have therapeutic benefits in humans encountering with diarrhoeal disorders. These plant extracts appeared to fulfill at least partially the requirements of WHO for the acceptance of a drug as an antidiarrhoeal agent (Akah, 1989), such requirements are (i) inhibition of the production of wet faeces in animals; (ii) inhibition of the production of watery stool or fluid evacuation and (iii) inhibition of gastrointestinal propulsive action. And the results of the study undertaken give a scientific base justifying the folkloric use of these plants in the traditional practice of indigenous tribal communities in Manipur. Further,

they are safe to use without showing adverse side effects. However, additional studies are necessary for the identification of the active principles of each of the plants and the detailed elucidation of their mechanism of action, and to prove their non-toxicity in detail.

CHAPTER 2

STUDY ON ANTICESTODAL EFFICACY OF MEDICINAL PLANTS

INTRODUCTION

Cestodes or tapeworms represent a class of important endoparasitic organisms, some of which can cause serious diseases in humans and other mammalian hosts. The sources and prevalence of various zoonotic tapeworm infections or clinically termed as cestodiasis, continue to be an important major cause of morbidity and mortality, not only in most underdeveloped countries but also in industrialized countries, particularly in rural areas or among immigrant groups from endemic areas (Raether and Hanel, 2003). These cestodes have a worldwide distribution but incidence is higher in developing countries. Infection rate is as low as 1 per 1000 in most developed countries and as high as 10% in the developing countries including India. Globally, over 3.5 billion people are infected with intestinal worms of which, children aged in between 5 – 15 years suffer the highest infection rate of about 400 million cases of worm burden that are attributed to poor sanitation and hygiene (Luong, 2003). These parasites consume nutrients from children they infect, thus retarding their physical development. They destroy tissues and organs, cause abdominal pain, diarrhoea, intestinal obstruction, anaemia, ulcers and other health problems. All of these consequences of infection can slow cognitive development and thus impair learning (Luong, 2003). Therefore, the helminth parasitic infection is a

fundamental cause of disease resulting in malnutrition, poor health and economic loss for the society besides gastrointestinal tract disturbances.

Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections with specific reference to the years 1999-2003 worldwide have been studied by Luong (2003) who reported that various zoonotic tapeworm infections were caused by adult and larval stages of the genera *Taenia, Echinococcus, Diphyllobothrium, Hymenolepis* and *Dipylidium*. In India, four species of tapeworms, namely *Taenia solium, T. saginata, Hymenolepis nana* and *H. diminuta* are known to cause infections in man (Gupta and Srivastava, 1994) playing a significant role in causing malnutrition and poor health among children and thereby resulting in immense economic loss for the country.

Human Taeniasis caused by the pork, *T. solium* which can lead to neurocysticercosis is declared as the most focused problem among cestode infections in regards to worldwide public-health importance, and that it is an eradicable parasitic disease worldwide (Ito *et al.*, 2003). However, there is no evidence yet that it is feasible and recommendable to envisage this within a reasonable time frame. A number of initiatives at international levels and opportunities currently exist in which a more pro-active attitude towards the control of *T. solium* cysticercosis can be integrated and promoted (Engels *et al.*, 2003).

For several years researchers have been studying medically and economically important members of cestodes, aiming to develop effective measures against infections and diseases mediated through the cestodes. In order to achieve these goals, both *in vitro* as well as *in vivo* models have been established to work in the laboratory system. Among several models developed so far, extensive laboratory investigations have been carried out on *Hymenolepis* species which is not necessarily justified on the basis of the medical and economic importance of this genus, but more importantly *Hymenolepis* has been extensively used as a versatile cestode parasite model. 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 is a cosmopolitan parasite of rats (a rodent tapeworm). In natural conditions, its life cycle (Fig. 2.1) is completed through arthropods acting as intermediate hosts. Eggs ingested by arthropods develop into cysticercoid larvae. Rodents become infected by ingesting the arthropods; humans, usually children, can accidentally be infected through the same mechanism. Rodents, particularly rats, are the definitive hosts and natural reservoirs of *H. diminuta*. Recently, Pappas and Barley (1999) reported a beetle-to-beetle transmission of *H. diminuta* occurring in natural environments and that eggs can be dispersed in the environment via beetle feces, thereby representing a source of additional infections and a mechanism of egg dispersal.

The World Health Organization (WHO) in its Tropical Diseases Control Programme has advocated the use of traditional medicines in order to combat the consequences of parasitic diseases globally such as tapeworm infections (Savioli *et al.*, 1992). Parasitic infections are among those infections that traditional healers confidently treat and against which an enormous variety of remedies exist (Githiori *et al.*, 2005).

Medicinal plants and plants-derived products form the essential components in traditional medicines. On the other hand, UNICEF has supported various governments to assist in the provision of water supply and sanitary facilities and intensive hygiene education in many schools through the Water, Environment and Sanitation (WES) programme, school sanitation and hygiene education (SSHE) programme, and other programmes which could effectively enhance behaviour change in children to break the routes of worm transmission and other waterborne diseases (Luong, 2003).

Recent trends in evaluation of traditional medicines show that several plants which are claimed to have the deworming or curing property of tapeworm infections in folklore practice have been studied by several workers. Few of such putative plants include *Artocarpus lakoocha, Artocarpus tonkinensis, Embelia shimperi, Acacia auriculiformis, Albizzia anthelmintica, Gladiolus gandavensis, Flemingia vestita, Psidium guajava* etc., which have been reported to possess anticestodal properties by several workers across the globe (Charoenlarp *et al.*, 1981; Rasfon, 1991; Galal *et*

al., 1991a; Bogh *et al.*, 1996; Ghosh *et al.*, 1996; Saha *et al.*, 1999; Tandon *et al.*, 2003; Temjenmongla *et al.*, 2006). Anthelmintic activity of several such plants has been studied by employing *in vitro* as well as *in vivo* models. The primary screening of putative plant extracts for their anticestodal efficacy has been undertaken against *H. diminuta* and *H. nana*, where the time taken for paralysis and/or mortality of worms was one of the main parameters. *Hymenolepis in vitro* models have become the most frequently used tools for evaluation of drug action or efficacy of plant extracts against parasites due to reduced costs and number of animals involved. Andrews and Thomas (1979) studied the contraction and paralysis of adult *H. diminuta* after exposure to broad spectrum anthelmintic, praziquantel and these effects were found to be reversible.

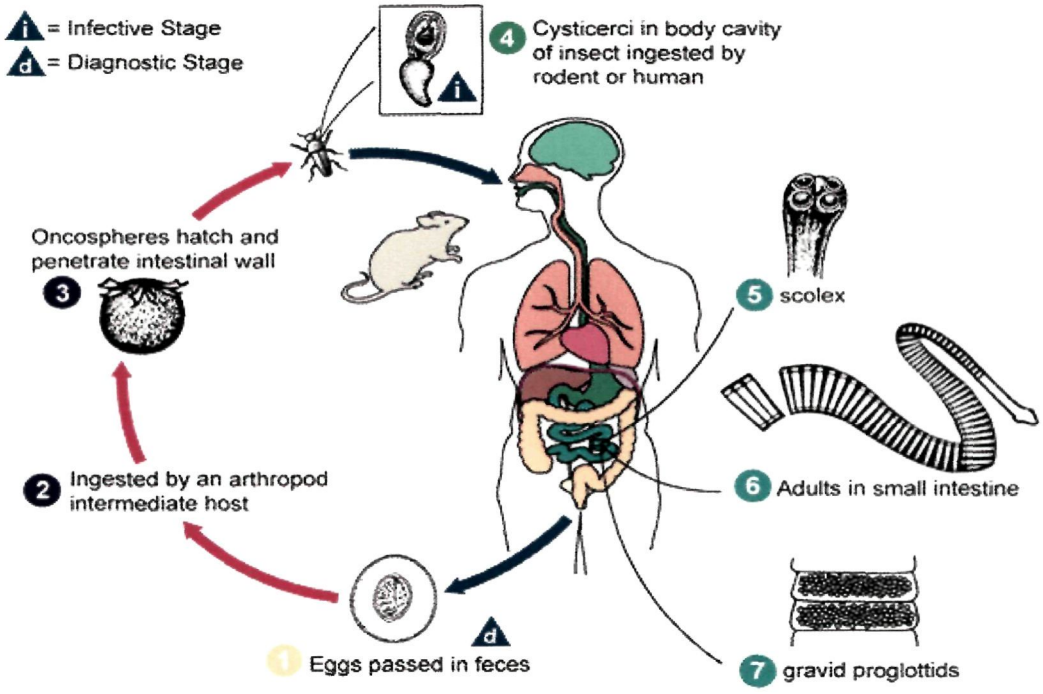


Fig. 2.1: Schematic representation of *Hymenolepis diminuta* life-cycle to show that two hosts are required to complete its development. Eggs are the diagnostic stage of infections, which are detected in the faeces by stool examination. These eggs when ingested by the beetles, the intermediate host, develop in to cysticercoids, the infective larval stage. These larvae when picked up by the definitive host, a rat (man is an accidental host), develop in to adult parasites, and the gravid adults eliminate eggs by the process of destrobilation through faeces of the host. (after Arai, 1980)

Other compounds which have also been tested against *Hymenolepis in vitro* treatment include bunamidine (Hart *et al.*, 1977), paromomycin sulphate (Aji *et al.,* 1983), trifluoperazine (Hipkiss *et al.*, 1995), tunicamycin (Hildreth *et al.,* 1997), etc; most of these studies assayed the action of the drugs on the tegument of adult worms. Similar *in vitro* drug activities were also assayed in isolated mitochondria of the parasite (Yorke and Turton, 1974) and parasite brush border (Hipkiss *et al.*, 1987). Other less common compounds from artificial fertilizers (Hamdy *et al.*, 1984), preparations from breadfruit (Rasfon, 1991), several Zimbabwean plants used in traditional medicine (Molgaard *et al.*, 2001) and some important medicinal plants of Naga Tribes (Temjenmongla and Yadav, 2005) have been demonstrated to exhibit *in vitro* activity against tapeworm eggs, cysticercoids, and adult worms.

In general, on the other hand, *in vivo* testing of plant extracts has been carried out using the *H. diminuta*-rat model and the efficacy has been adjudged in terms of a reduction in the parasite eggs per gram of feces (EPG) and/or expulsion or mortality of worms following administration of plant extracts (Maki *et al.*, 1983; Temjenmongla *et al.* 2006). Dixon and Arai (1991) performed a very indicative study regarding the real calculated drug efficacy of niclosamide and praziquantel against *H. diminuta* infections in rats and they suggested counting of worms in the small intestine at 8-10 days after the end of treatment, which allowed the most effective dosage to be calculated with greater confidence. Recently, Temjenmongla *et al.* (2006) reported another study on anticestodal efficacy of *Psidium guajava* against *H.*

diminuta infections in rats judging the efficacy on the basis of parasite eggs per gram of faeces (EPG count), direct count of surviving worms at autopsy and percentage host clearance of parasites. The activity of benzimidazole, cambendazole and thiabendazole against different stages following *Hymenolepis* infections has also been extensively studied using *in vivo* models employing both intermediate and final hosts (Evans *et al.*, 1980; Novak and Blackburn, 1985).

As mentioned in Chapter 1, several such folklore medicinal plants have been claimed by Naga communities in Manipur for treatment of tapeworm infections as well. The local medicinemen or *Haori-Khanong* uphold a list of herbal plants which are alleged to have deworming remedy in the folklore medicine system. These informations have been collected and a baseline questionnaire survey on suggested putative medicinal plants conducted among the local people in the villages reveals that the following plants are most commonly in use as deworming agents among several other plants (and the questionnaire response is presented in Table 2.1). Therefore, these plants were selected for investigation in the present study.

1. Leaf of *Strobilanthes discolor* T. Anders (Family: Acanthaceae)
2. Leaf of *Adhatoda vasica* Nees. (Family: Acanthaceae)
3. Seed of *Butea minor* Ham. in Wall (Family: Fabaceae; Papilionaceae)
4. Ripen fruit of *Solanum myriacanthum* Dunal (Family: Solanaceae)

5. Aerial part of *Trifolium repens* L. (Family: Fabaceae; Papilionaceae)
6. Leaf of *Zanthoxylum rhetsa* DC (Family: Rutaceae).

A review of literature on ethnobotanical study reveals that there are 36 plant species used by the Tangkhul Naga tribe of Ukhrul Manipur (Elangbam, *et al.*, 1989) which was simply a survey report about the various uses of the medicinal plants from the local people. Baring this report, there is no mention of any other study on ethnomedicinal plants or other biological activity of folklore medicinal plants from this area. With a view of these rich folklore medicinal plants used in the traditional remedial system, and keeping in mind the common prevalence of tapeworm infections among the aboriginal natives in this region, this study was undertaken on anticestodal efficacy of methanol extracts of *S. discolor, A. vasica, Z. rhetsa, T. repens, S. myriacanthum and B. minor* to scientifically validate their deworming remedial claim, by employing both *in vitro* as well as *in vivo Hymenolepis diminuta* – rat experimental models. To supplement their activities, an acute toxicity test of each plant extract was also undertaken to see if these extracts would have any adverse side effects to the experimental animals, by way of determining the median lethal dose (LD_{50}) and also by assaying the changes in few of the serum biochemical parameters, namely serum glutamate oxaloacetate tranaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), cholesterol and total protein after plant extract treatment.

Table 2.1: Preliminary baseline survey of ethnomedicinal plants used by Tribal community in Manipur to cure intestinal worm infections.

Parameter	Response (%) #	*S. discolor*	*A. vasica*	*T. repens*	*S. myria canthum*	*B. minor*	*Z. rhetsa*
1. Do you use this plant as deworming remedy?	i) YES ii) NO	56.67 43.33	86.67 13.33	33.33 66.67	58.33 41.67	70.83 29.17	30.00 70.00
2. If YES, how often do you use this plant?	i) Frequently ii) Sometimes iii) Rarely iv) Used once	23.52 63.33 4.41 8.84	26.92 56.73 7.69 8.66	10.00 47.50 25.00 17.50	20.00 41.42 25.71 12.87	78.82 14.13 7.05 0.00	11.11 47.22 0.00 41.67
3. If YES, why do you use this plant?	i) As a myth or belief ii) Prevailing traditional practice iii) Prescribed by local healers	16.17 69.11 14.72	3.86 72.11 24.04	20.00 30.00 50.00	7.14 57.14 35.72	0.00 84.71 15.29	13.87 66.67 19.44
4. If YES, how efficacious is this plant?	i) Very high ii) Medium iii) Very poor	19.11 69.11 11.79	28.84 68.27 2.89	0.00 62.50 37.50	10.00 82.86 7.14	29.41 70.59 0.00	0.00 55.56 44.44
5. If YES, what is your usage preference of this plant?	i) Only priority to the plant ii) First priority to plant, then to other drug iii)Undecided	27.94 35.30 36.76	23.08 54.81 22.11	12.50 50.00 37.50	17.15 28.57 54.28	20.00 75.29 4.71	0.00 27.78 79.22
6. If NO, what is the reason that you do not use this plant?	i) It is a myth ii) It is not efficacious iii) Ignorance about the use of the plant	0.00 70.58 29.42	0.00 18.75 81.25	0.00 16.25 83.75	36.00 0.00 64.00	0.00 0.00 100.0	9.52 33.33 57.15

No. of individuals of indigenous tribal community in Manipur participated in questionnaire study was 480 (N = 480).

MATERIALS AND METHODS

Collection of Plant Materials

From the preliminary questionnaire response, the most commonly used plants were included in the study. Usable plant parts (leaf, aerial shoot, seed, fruit, etc.) were collected from various villages in Manipur. Herbarium sheets and photographs of each of the plants were prepared and were duly identified and authenticated by Dr. PB Gurung, herbarium curator, and further confirmed by Dr. Y Kumar, a Plant Taxonomist at Department of Botany, North-Eastern Hill University, Shillong. Respective voucher specimens (Table 2.2) along with herbarium sheets of the plants were deposited in the Department of Zoology, North-Eastern Hill University, Shillong.

Plants Materials (Details in Table 2.2)

2.1. *Strobilanthes discolor* T. Anders (Acanthaceae): Plate 2.1 (A)

(Syn. *Goldfussia discolor* Nees)

Commonly called as *Lavender bell* and locally known as *Masupni*, *S. discolor* is a large gregarious shrub which leaves are elliptic cuspidate-acuminate glabrous, hairy calyx and 1.3" nearly glabrous corolla. It is found growing in the shady garden yard in sub-gregarious rows. Its distribution ranges from Himalayas range including Naga Hills, Bhutan, Assam and Khasia mountains. The leaf decoction of this shrub is consumed by the Tangkhul Nagas to get rid of gastrointestinal worms, to cure severe cough, and to treat backache.

2.2. *Adhatoda vasica* Nees, (Acanthaceae): Plate 2.1 (B)

A. vasica is a bushy dense shrub, 4–8 ft. high sometimes arborescent 20 ft. high with large minute pubescent elliptic leaves 8" by 3" long, flowers in Feb.-March, large flowers sub-sessile in the axils of opposite bracts of axillary and terminal spikes. It is commonly called as *Maiden hair*. It is distributed in Malaya and S. E. Asia, in India, it is found in Punjab and Assam to Ceylon and Singapore. The leaf decoction of this large shrub, locally known as *Sorukni*, is popularly used in eliminating intestinal worms of both the dogs and human in the folklore practice. The leaves of the plant contain two major quinazoline alkaloids, vasicine and vasicinone (Atal, 1980; Das *et al.*, 2005), and its root extract has glycosides besides alkaloids (Lateef *et al.*, 2003). Its leaf has been reported to have hepatoprotective (Bhattacharyya *et al.*, 2005), anti-inflammatory (Chakraborty and Brantner, 2001), antiphlogistic and antiallergic (Wagner, 1989), and antibacterial activities (Brantner and Chakraborty, 1998).

2.3. *Butea minor* Ham. in Wall (Fabaceae): Plate 2.2 (A)

B. minor is a medium small sub-erect shrub with crooked trunk; reaching to a height of 5 – 12 ft. Shoots are clothed with grey or brown silky pubescence. Leaves are 3-folioted and broad. Flowers are large, scarlet and orange. Pod is velvety brown, seed is oval compressed and brown. It is found distributed in the plains from Himalayas to Ceylon, ascending to 4000 ft. in the north-west. In this region of Northeast India, it is found in the grassland forming a gregarious forest. The seed's (*Kamkutei* in local name), fresh as

well as the roasted ones, has a legendry name in expelling gastrointestinal worm infections in children.

2.4. *Solanum myriacanthum* Dunal (Solanaceae): Plate 2.2 (B)

Vernacularly known as *Changranlotei* S. *myriacanthum* is a thorny shrub which fruits are used as shampoo in the folk cosmetics and paste of fruits is applied after warming to abdomen to relief stomachache suspected to be of gastrointestinal worm burden. It is found in the waste lands and also roadsides in the Naga Hills. The ripen berries extract has been reported to be efficacious against adult filarial parasites of cattle, *Setaria cervi* (Tangpu and Yadav, 2003). Solasodine and other steroidal alkaloids as well as sapogenins have been isolated from this plant by Weissenberg (2001).

2.5. *Trifolium repens* L. (Fabaceae): Plate 2.3 (A)

T. repens is a small perennial herb that is used as a common forage crop in the temperate and sub-tropical regions of the world. It is widely distributed in moist temperate zones, Mediterranean areas and some cool subtropical parts of the world, viz. Europe, North America, Southern Latin America, Australasia and Japan. It is glabrous perennial with trifoliate leaves; leaflets ovate or circular with minutely serrate margins and usually whitish leaf markings on the upper mid surface; stipules pale and translucent with a short point. Inflorescences are globular racemes, with 20-40 florets at the end of long peduncles originating from leaf axils on the stolons. Florets are white, often tinged pink, becoming deflexed with age. Seeds are heart-shaped with a smooth surface, coloured bright yellow to yellowish brown. In the folk

medicine practice of the Nagas, the hot decoction of the aerial shoots of the plant *T. repens* (locally known as *Anikatum*) is used as a deworming remedy. In the recent time, *in vitro* filaricidal activity of this plant extract has been reported against *S. cervi* (Tangpu and Yadav, 2003).

2.6. Zanthoxylum rhetsa DC (Rutaceae): Plate 2.3 (B)

(Syn. *Z. budrunga* Wall; *Fagara budrunga* Roxb.)

Z. rhetsa is commonly called prickly ash, Crocodile or Satin wood (*Mangangteini* is the local name). It is a moderate-sized deciduous tree with pale corky bark, covered with conical prickles on stems and branches. It is relatively a white, hard wood; carving usually have a very smooth finish – making them look a little like an ivory. Leaves are clustered towards the ends of the stout branchlets. Flowers are yellow and 4-merous in large terminal panicles with opposite branches. Seeds are blue-black, tasting of black pepper. It is commonly found in shaded moist localities, ascending to 1800 m. Phytochemical studies show the presence of Terpenoids (Mathur *et al.*, 1967) and 3,5-Dimethoxy-4-geranyloxycinnamyl alcohol, 8-methoxy-*N*-methylflindersine, xanthyletin and sesamin (Ahsan *et al.*, 2000). And its antinociceptive and antidiarrhoeal (Rahman *et al.*, 2002) and antifilarial activity (Tangpu and Yadav, 2003) have also been studied in the past.

Table 2.2: Details of the ethnomedicinal plants included in the present study.

Scientific Name	Voucher Specimen	Common Name	Local Name	Family	Usable parts	Distribution	Known uses
1. *Strobilanthes discolor* T. Anders	AKY–212	Lavender Bell	Masupni	Acanthaceae	Leaf	Tropical SE Asia	Astringent, Diuretic, Ague,
2. *Adhatoda vasica* Nees.	AKY–214	Maiden Hair	Sorukni	Acanthaceae	Leaf	Indian sub-continent	Anti-inflammatory, antiallergic, antidiarrhoeal
3. *Butea minor* Buch.-Ham. ex Wall	AKY–215	NONE	Kamkutei	Fabaceae	Acorn	Himalayas to Ceylon and Birla	NONE
4. *Solanum myriacanthum* Dun.	AKY–216	Himalayan Nightshade	Changranlotei	Solanaceae	fruit	All over the world	Antifilarial
5. *Trifolium repens* L.	AKY–211	White clover	Anikatum	Fabaceae	Aerial shoot	All over the world	Antifilarial
6. *Zanthoxylum rhetsa* DC	AKY–213	Prickly Ash	Mangangteini	Rutaceae	Leaf	Temperate & tropical	Antidiarrhoeal, antifilarial

PLATE 2.1

A. Strobilanthes discolor: whole plant and tender leaves

B. Adhatoda vasica: whole plant and leaves

PLATE 2.2

A. Butea minor: whole plant and seeds

B. *Solanum myriacanthum*: whole plant and ripen fruits

PLATE 2.3

A. *Trifolium repens:* whole plant and leaves

B. *Zanthoxylum rhetsa:* whole plant and leaves

Preparation of Plant Extracts

The usable portions of the plants were air-dried under shade and pulverized into powder. Known amount of the powdered materials were suspended in an organic solvent, methanol as extractant and engaged for refluxing using a Soxhlet fractional distillation apparatus (Yadav *et al.*, 1992) at 40-50^{0}C for 4-6 h. Then the resulting suspension was decanted out discarding the remnants and the filtrate was further concentrated in a Rotatory Evaporator under reduced temperature and pressure for removal of the solvent. Further the recovered semi-solid residue was placed over calcium chloride anhydrous for complete evaporation of the extractant from the crude extract. The percentage yields (w/w) of the final crude extracts were 3.40% (*S. discolor*), 1.67% (*A. vasica*), 2.56% (*Z. rhetsa*), 1.98% (*T. repens*), 8.11% (*B. minor*) and 7.56% (*S. myriacanthum*). These extracts were stored in respective plastic vials at –4^{0}C in a refrigerator until used for assaying their anticestodal efficacy.

Preparation of medium solutions

IV. 0.9% Phosphate Buffer Saline (0.15 M 0.9% Sodium PBS)

i) 2.106 g $NaH_2PO_4.2H_2O$

ii) 8.733 g Na_2HPO_4

iii) 4.500 g NaCl

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

V. Hank's Solution:

i) 8.00 g NaCl

ii) 0.40 g KCl

iii) 0.04 g Na_2HPO_4

iv) 0.06 g $Na_2HPO_4.2H_2O$

v) 1.00 g Glucose

vi) 0.35 g $NaHCO_3$

vii) 1000 ml distilled water, pH adjusted to 7.8.

Drugs

Praziquantel (PZQ; Distocide®), the standard reference drug used in the study was manufactured by Shin Poong Pharm. Co., Ltd., Seoul, Korea. Plant extract and PZQ solutions were prepared fresh in PBS before treatment with test parasites.

EXPERIMENTAL DESIGNS

IN VITRO ANTICESTODAL STUDIES

The protocol was followed with modifications from Yadav *et al.* (1992). Adults live specimens of *H. diminuta* were collected in 0.9% warm PBS (pH 7.4) from intestines of the previously infected rats which were maintained in laboratory. A known number of the parasites (n = 6 in each case) were maintained in separate Hank's solutions at 37 ± 2^{0}C in an incubator. A required amount of the test extract was weighed out and dissolved in a few drops of dimethylsulphoxide (DMSO), and different concentrations of the test extract (5, 10, 20, 40, 50 and 100 mg/ml in Hank's solution) were prepared in separate

petridishes. To each extract suspension a known number of worms (n = 6 for each conc.) were gently placed inside the petridishes containing extract. The above steps were followed for treatment of parasites with different concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 mg/ml in Hank's solution) of Praziquantel (PZQ), and one set of worms was maintained in control medium having few drops of DMSO but without extract or reference drug.

The anticestodal efficacy was adjudged by monitoring the time of paralysis and mortality of the test parasites which was observed at every half an hour time interval. Two grades of one warm and the other hot saline solutions were employed to confirm the paralysis and mortality of parasites. Worms not showing any physical movement on gentle stimulation by a soft brush were picked up and transferred to this warm saline solution. Consequently when parasites showed no movement in this warm saline, were dipped to the warmer grade of saline to confirm total mortality of the parasites. Accordingly, time of paralysis and mortality was recorded for each set of experiments. The data of recorded time (h) are presented as mean ± SEM (n = 6) in the result.

IN VIVO ANTICESTODAL STUDIES

2.1. Maintenance of *Hymenolepis diminuta* infections in experimental rats

Male and female albino rats (100–120 g) were employed in the study. The animals were acclimatized for 15 days in the laboratory prior to use for experiments. During this period the stool samples were continuously examined

in order to ensure that they do not have any intestinal helminthic infections. They were maintained under standard environmental conditions and fed with rodent diet (Pranov Agro Industries Ltd., Delhi) and water *ad libitum*. Proper care was taken to protect the welfare of the experimental animals and all the experiments were performed according to the rules laid down by the Institutional Animal Care and Use Committee (IACUC).

The infection of *H. diminuta* was maintained in the laboratory by alternating the hosts (Gupta and Srivastava, 1994). Adults of *H. diminuta* were collected from the intestine of previously infected rats. Gravid segments of *H. diminuta* were scratched smoothly onto filter papers in petridishes. Flour beetles, *Tribolium confusum* Jacquelin du Val (Tenebrionidae) were used as the intermediate host. These beetles were starved for 24 h prior to infection. They were allowed to feed on the eggs of *H. diminuta* for 72 h and had free access to flour and kept for 12-14 days at room temperature or until dissected. On dissecting upon these beetles, cysticercoids were collected and suspended in normal saline and known number of larvae inoculated to fresh rats using blunt feeding tube. After 18-20 days, eggs of *H. diminuta* could be observed in the faeces of the rats, which were mixed with flour powder and fed to the fresh 24 h fasted beetles. By alternating the infection to these host animals, the life cycle of *H. diminuta* continued in the laboratory conditions throughout the study periods.

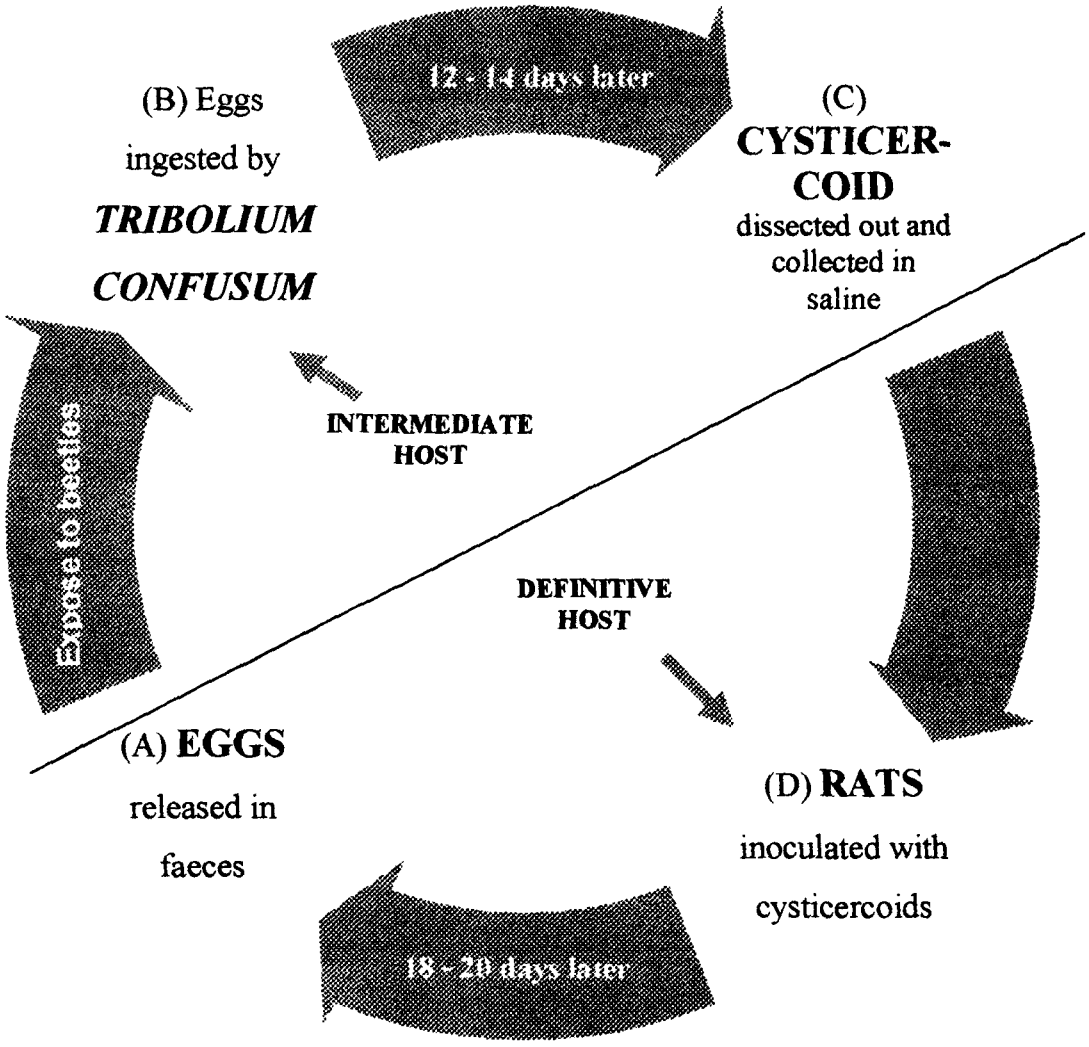


Fig. 2.2. Maintenance of infection of *H. diminuta* in rats. Eggs (A) released in faeces were ingested by (B) *Tribolium*; Cysticercoids (C) were dissected out, and (D) Inoculated to fresh rats.

2.2. ANTICESTODAL EFFICACY TEST BY EMPLOYING *H. DIMINUTA*-RAT *IN VIVO* MODEL

Three different approaches of treatment stages were designed to investigate the anticestodal activities of the plant extracts in the present study. Separate sets of experiment were carried out for each plant extract in comparison with the standard drug, praziquantel (PZQ) for each extract, and the following is shown as an experimental protocol for one set, which was repeated for each plant extract.

2.2.1. Treatment at larval stages

For each extract treatment, thirteen groups of animals (6 rats in each group) were used in this experiment. All animals were inoculated with 5 cysticercoids each of *H. diminuta* parasite using a blunt feeding tube and maintained them in separate cages. This day was assigned as day 1 to determine the remaining days of experimental assessments. Treatments at different single and double doses per day (100, 200, 400 and 800 mg/kg, p.o.) in groups II-IX, and with PZQ at single and double doses per day (5 and 25 mg/kg, p.o.) in groups X-XIII respectively, were administered to the rats for three consecutive days [days 2-4 post inoculation (pi) of cysticercoids]. The remaining group I served as the control with 1.0 ml of saline per day for the same three days. From day 18 post infection, fresh faeces was collected from each cage of the treated and control rats for counting the eggs per gram of faeces (EPG) using modified Mc Master method (Anonymous, 1977) for three days (days 18-

20 pi). Follow-up examination of EPG was done on days 28-30 pi following a week first EPG count. Finally an autopsy was performed by chloroform anaesthesia killing of the animals on day 31 to recover the surviving worms. Accordingly, the percentage worm recovery rate was determined from the number of worms recovered divided by number of cysticercoids inoculated, and percentage host clearance from the number of animals devoid of worms at autopsy.

2.2.2. Treatment at immature stages

Thirteen groups of animals (n = 6) were used in this experiment and treatment of plant extracts and standard anticestodal drug was given on days 8-10 post inoculation. Then EPG counts, worm recovery rate (%) and host clearance (%) at autopsy were carried out as given in the above experiment.

2.2.3. Treatment at adult stages

Animals were divided into twenty six groups (n = 6), each animal was inoculated with 5 cysticercoids each. Group I served as the control untreated. Groups II-XIII received single and double doses of a plant extract (100, 200, 400 and 800 mg/kg, p.o.) and PZQ (5 and 25 mg/kg, p.o.) respectively for 3 days (days 21-23 pi). And groups XV-XXVI received the same doses of the plant extract and PZQ for 5 days (21-25), while group XIV served as the second control group (1.0 ml saline for 5 days). EPG counts for each group (groups I-XXVI) were done for 3 days (days 18-20) before treatment, for 3 days after

treatment (days 24-26 for 3 day-treatment groups and days 26-28 for 5 day-treatment groups) and for another follow-up three days after one week post treatment (days 34-36 for former treatment groups and days 36-39 for the latter groups). Finally an autopsy was performed for all the groups on completion of experiments and accordingly worm recovery rates and host clearance were calculated from the surviving worms recovered at autopsy.

2.3. ACUTE TOXICITY TEST

2.2.1. Determination of Median Lethal Dose (LD_{50})

For each of the plant extracts seven groups of six animals each were selected. Group I served as untreated control and groups II-VII were administered with test extract at the escalating doses of 100, 200, 400, 800, 1600 and 3200 mg/kg, p.o. respectively. 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, median lethal dose (LD_{50}) was calculated using SPSS software.

2.2.2. Serum biochemical test

In another set of experiment, a serum biochemical profile was studied. The blood samples were collected from the animals 24 h post-treatment with 800 mg/kg, p.o. of each extract; the maximum dose showing anticestodal activity in this study, and levels of serum glutamate oxaloacetate transaminase (SGOT; EC 2.6.1.1), serum glutamate pyruvate transaminase (SGPT; EC 2.6.1.2),

cholesterol and total protein were estimated as per the methods of Stricklad *et al.*, (1961), Allain *et al.* (1974) and Henry *et al.* (1974), using a semi-automated biochemical analyzer (Bayer), as described in Chapter 1.

Statistical Analysis

The data were analyzed statistically and are represented as mean plus or minus standard error of the mean (Mean ± SEM). The significance of the difference between the means was determined by the Student's *t*-test. And probability less than 5% ($p < 0.05$) was accepted as statistically significant.

OBSERVATIONS AND RESULTS

IN VITRO EFFECTS OF PLANT EXTRACTS ON *H. DIMINUTA* PARASITES

The results of *in vitro* treatment of *H. diminuta* adult worms to different concentrations of plant extracts and praziquantel are given in Tables 2.3 and 2.4, respectively. The mean paralysis and mortality time of *H. diminuta* parasites maintained in the control medium was recorded to be 27.33 h and 29.58 h, respectively. There was no significant difference between paralysis and mortality time of the parasites in this control group. The plant extract treated groups showed significant differences in the paralysis as well as mortality time at $p < 0.001$, when compared to the control group. There was gradual decline in the paralysis and mortality time as the concentrations of the extracts increased. For *S. discolor* extract the parasites showed 0.92 – 3.00 h as paralysis time and 2.58 – 7.33 h as mortality time. Similarly, paralysis and mortality times recorded were observed to be 1.00 – 3.58 h and 3.75 – 10.00 h for *T. repens* extract, 1.17 – 3.25 h and 3.92 – 8.75 h for *Z. rhetsa* extract, 1.58 – 5.08 h and 4.33 – 11.00 h for *A. vasica* extract, 2.33 – 5.33 h and 4.00 – 14.33 h for *B. minor* extract, and 2.08 – 7.00 h and 4.08 -14.25 h for *S. myriacanthum* extract, respectively. With respect to the difference between the paralysis and mortality time for each concentration of the respective treated groups, there was an indication that paralysis of the parasites preceded the mortality time considerably. The treated worms which showed no physical movement revived back when picked up and dipped in slightly warmer saline and on gentle stimulation. However there was

slight decline in the level of significance in treatment groups at their highest concentration (100 mg/ml) for *S. discolor* ($p < 0.01$), *B. minor* ($p < 0.01$) and *S. myriacanthum* ($p < 0.02$) extracts, whereas all the rest showed significant difference at $p < 0.001$ when compared between paralysis time and mortality time (Table 2.3).

As for the exposure of the parasites to different concentrations of praziquantel (PZQ), the standard anticestodal drug, the mean paralysis time recoded (Table 2.4) was as low as 0.03 h for its highest concentration (50 mg/ml) and as high as 0.67 h for its lowest concentration (0.01 mg/ml), whereas mean mortality time ranged between 0.03 – 5.08 h. It is evident that there was drastic difference between the time of paralysis and mortality at the lower concentration (0.01, 0.05, 0.10 and 0.50 mg/ml). However at higher concentrations (1.0, 5.0, 10.0 and 50.0 mg/ml) there was no significant difference between the time of paralysis and mortality, indicating that paralysis did not precede the mortality in this case. Exposure of parasites to plant extracts at their highest concentration showed comparable effects with that at the lower concentrations of the standard anticestodal drug.

IN VIVO ANTICESTODAL EFFECTS

Effect of plant extracts against *H. diminuta* infections in rats

The *in vivo* anticestodal activities of the plant extracts and praziquantel are presented in Tables 2.5 – 2.28. The efficacy of the test extracts was based

on reduction in the EPG (Eggs per gram of feces) count, % worm recovery rate and % host clearance, by monitoring the effects of test extracts when treated at three different stages against *H. diminuta* parasites. EGP count for the control untreated group with five cysticercoids infections in each rat was recorded to be ranging from 28583 – 34978, while worm recovery rate was from 90 -100%.

The effects of leaf extract of *S. discolor* on larval, immature and adult stages of *H. diminuta* infections in rats, are shown in Tables 2.5 – 2.8. Table 2.5 shows the effect of the leaf extract on the larval stages as monitored by EPG count, worm recovery rate and host clearance at autopsy. The most profound observation was noticed in the treatment group of 800 mg/kg, p.o. twice daily for 3 days (days 2-4 pi), where there were no eggs present at EPG count or surviving worms recovered at autopsy. Significant results of reduction of worm burdens emerged from the rest treatment regimes in dose-dependant manner. With regard to the effects on the immature stages, though there was a significant reduction ($p < 0.01$ to 0.001) in EPG count and worm recovery as compared to the control, none of the animals were completely devoid of the infection (Table 2.6). Comparison between EPG values on days 18-20 and days 28-30 pi did not show much difference. Treatment of the leaf extract against the adult stages of the parasite (Tables 2.7 and 2.8) revealed gradual reduction in the EPG values, as evident from more EPG differences between pretreatment and follow-up treatment than that between pretreatment and post-treatment groups. Significant reductions in the EPG counts and worm recovery rates of the

treated groups were recorded when compared with control. EPG values of all the different groups at pretreatment showed almost a uniform trend (values ranging from 30000 to 34567). In all the extract treated groups, effects were enhanced by 5 days-treatment than that by 3 days-treatment both in terms of EPG counts as well as surviving worms recovered.

Tables 2.9 – 2.12 represent the effects generated by the treatment of leaf extract of *A. vasica* against the larvae, the immature and the adult stages of *H. diminuta* infection in rats. The results indicated that the treated groups were significantly different from the control in all three respects of study parameters in the different stage-treatment regimes. Faecal samples collected from infected untreated control animals were observed to contain 32717 eggs per gram which reduced to 9406 – 20267 eggs in the treated animals with different varying doses of the extract for three days i.e. days 2-4 pi. The worm recovery rate was reduced up to 66.60% and worm burden in host was cleared up to 16.67% at 800 mg/kg, p.o. (Table 2.9). The same dose yielded 5522 eggs as the effect of the extract on immature stage of the parasite, when compared to 30078 eggs recorded for the control. A profound result was observed when this plant extract was administered at 800 mg/kg twice daily for 3 days, which resulted into 80.0% reduction of surviving worms at autopsy (Table 2.10). With regards to effects of the extract on the adult worms, treatment at the highest dose given twice daily for three days could reduce the pretreatment EPG of 31589 to 10978 when counted on days 38-40 pi. However, the same treatment for five days resulted in

reduction of EPG from 33522 to 4206 (Tables 2.11 and 2.12). Though 33.33% of hosts were devoid of worm burden, the recovery of worm at autopsy signified reduction up to 83.40% at 800 mg/kg administered twice daily for five days.

Tables 2.13 – 2.16 show the effects of *B. minor* seed extract against different stages of *H. diminuta* infections in experimental rats. The effects on the larval and immature stages were noticed both in terms of EPG counts and surviving worms recovered; however no animal was totally cleared from the worm infection. Treatment against the adult stages showed more pronounced effects for this plant extract. EPG examination revealed significant reduction from pretreatment to post-treatment in the treated groups of animals, more significant at the higher doses. At 800 mg/kg dose given for 3 days, egg count of 32211 reduced to 4028 eggs after treatment, 80% worms reduced and 33.33% host completely cured from the infection. In this case, treatment for 5 days did not reveal enhanced effects than that of 3 days treatment.

The efficacy of *S. myriacanthum* ripen fruit extract can be seen from the results presented in Tables 2.17 – 2.20. The results indicate that the extract showed more activity on the larval stages of the test parasite than immature or adult stages. 76.60% reduction of worm and 33.33% host clearance were observed for the treatment group of its highest dose tested (Table 2.17). As effects on the immature stage, up to 50% parasites were recovered, however none of the rats were free from the infection when examined at necropsy (Table

2.18). Significant activity of the crude extract was observed only at the increasing doses treated against adult worms. Double doses for 5 days did not enhance the effects on EPG counts; however, two animals were cured from infection when treated for 5 days (Table 2.19) against one animal cured for 3 days treatment (Table 2.20).

Tables 2.21 – 2.24 present the effects of *T. repens* aerial part extract in *H. diminuta* infections in rats. Up to 70.00, 73.40 and 76.60% of the parasites were eliminated from the infected animals when *T. repens* extract was treated at larval, immature and adult stages, respectively. This trend indicated that the extract had more or less similar effects on all the stages of parasites. EPG counts revealed significant reductions of eggs in the faecal matters of the infected rats in a dose-dependant fashion in all treatment levels and with various doses of the extract. Although, no animal was noticed to be devoid of *H. diminuta* infections for the treatments against larvae and immature parasites, treatment for 5 days (800 mg/kg, p.o. twice daily) against adults resulted in 16.67% animals being cured from the worm burden.

As for the results of *Z. rhetsa* activity against *H. diminuta* infections in rats which are depicted in Tables 2.25 – 2.28, it was interesting to observe that 100% of the infected rats were devoid of worm load as a result of the treatment of the animals with this plant extract given orally at 800 mg/kg double doses on days 2-4 post-infection. Treatment of the same on days 8-10 and days 21-25

post-infection showed worm reduction rates of 66.60 and 88.00%, and 0.00 and 50.00% host clearance, respectively. Similar results emerged out on monitoring the EPG counts of the respective treatment regimes.

ACUTE TOXICITY EFFECT OF THE PLANT EXTRACTS

The lethal effect to the experimental rats caused by oral treatment of different test extracts from 100 mg/kg dose to 3200 mg/kg within 72 h post-treatment observation is presented in Table 2.29. The maximum number of mortality was observed in the *Z. rhetsa* extract at 3200 mg/kg dose (4 out of 6 animals died), followed by *B. minor* and *S. myriacanthum* extracts (3 out of 6 animals each died), *A. vasica* extract (2/6 each at 1600 & 3200 mg/kg) and *S. discolor* extract (1/6 at 3200 mg/kg). Accordingly, LD_{50} (Oral; mg/kg) value was calculated to be 2737.34 for *Z. rhetsa*, 3093.24 for *S. myriacanthum*, 3200.03 for *B. minor*, 3755.62 for *A. vasica* and 6993.18 for *S. discolor.* However, there was no mortality observed in the case of *T. repens* extract even up to 3200 mg/kg dose.

With respect to biochemical assays, the levels of SGOT, SGPT, cholesterol and total protein from blood samples of the control normal rats were recorded as 149.0 U/L, 75.83 U/L, 122.50 mg/dL and 6.88 g/dL, respectively (Table 2.30). Treatment of the test extracts at their maximum anticestodal-efficacious dose (800 mg/kg, p.o.) resulted in statistical significant changes in

the levels of SGOT and SGPT for *S. discolor, A. vasica* and *Z. rhetsa* extracts at $p < 0.05$ when compared with that of the control. Whereas there was no significant change in the levels of cholesterol and total protein for all the six plant-extracts tested from the control (Table 2.30).

Table 2.3: *In vitro* anticestodal activity of different plant extracts against adult *Hymenolepis diminuta*.

Treatment	Time (h) taken for Paralysis (P) and Death (D) of the adult worms post–incubation* at varying concentration (mg/ml)					
	5	10	20	40	50	100
***S. discolor* extract**						
P	3.00±0.73[a]	2.75±0.64[a]	2.42±0.27[a]	2.08±0.15[a]	1.92±0.15[a]	0.92±0.24[a]
D	7.33±0.53 [a, d]	7.00±0.34 [a, d]	6.83±0.42 [a, d]	6.50±0.34 [a, d]	6.33±0.28 [a, d]	2.58±0.44 [a, c]
***T. repens* extract**						
P	3.58±0.40 [a]	3.17±0.56 [a]	2.92±0.37 [a]	2.58±0.30 [a]	2.08±0.35 [a]	1.00±0.22 [a]
D	10.00±0.37 [a, d]	8.83±0.44 [a, d]	7.92±0.20 [a, d]	7.17±0.63 [a, d]	6.42±0.65 [a, d]	3.75±0.69 [a, d]
***Z. rhetsa* extract**						
P	3.25±0.53 [a]	3.00±0.43 [a]	2.58±0.35 [a]	2.42±0.40 [a]	2.00±0.18 [a]	1.17±0.25 [a]
D	8.75±0.54 [a, d]	8.33±0.57 [a, d]	7.83±0.21 [a, d]	7.42±0.42 [a, d]	6.92±0.30 [a, d]	3.92±0.44 [a, d]
***A. vasica* extract**						
P	5.08±0.33 [a]	4.17±0.42 [a]	3.50±0.34 [a]	3.17±0.25 [a]	2.90±0.42 [a]	1.58±0.24 [a]
D	11.00±0.85 [a, d]	9.17±0.54 [a, d]	8.50±0.22 [a, d]	8.25±0.22 [a, d]	7.00±0.26 [a, d]	4.33±0.28 [a, d]
***B. minor* extract**						
P	5.33±0.42 [a]	4.58±0.20 [a]	4.08±0.35 [a]	3.75±0.34 [a]	2.83±0.21 [a]	2.33±0.25 [a]
D	14.33±0.51[a, d]	8.42±0.52 [a, d]	7.00±0.34 [a, d]	6.75±0.42 [a, d]	6.33±0.64 [a, d]	4.00±0.32 [a, c]
***S. myriacanthum* extract**						
P	7.00±0.29 [a]	6.50±0.43 [a]	4.50±0.29 [a]	4.00±0.29 [a]	6.58±0.30 [a, d]	2.08±0.33 [a]
D	14.25±0.59 [a, d]	11.17±0.33 [a, d]	7.50±0.39 [a, d]	7.00±0.41 [a, d]	3.25±0.28 [a]	4.08±0.58 [a, b]

* Data represent Mean ± SEM, n = 6. [a] Statistically significant at $p < 0.001$, vs. control, Student's *t*–test. [b, c, d] $p<0.02$, 0.01, and 0.001, vs. respective paralysis time, Student's *t*-test. ** Not significant when compared with respective paralysis time.
Worms incubated in control medium: Paralysis at 29.17 ± 3.06 h, Death at 29.75 ± 3.04 h.

Table 2.4: *In vitro* anticestodal activity of praziquantel against adult *Hymenolepis diminuta*.

Treatment	Time (h) taken for Paralysis (P) and Death (D) of the parasite worms post–incubation* at varying concentration (mg/ml)								
	0.01	0.05	0.10	0.50	1.0	5.0	10.0	25.0	50.0
Praziquantel (PZQ):									
P	0.67±0.09[a]	0.53±0.10[a]	0.42±0.06[a]	0.39±0.04[a]	0.31±0.05[a]	0.18±0.03[a]	0.15±0.04[a]	0.10±0.05[a]	0.03±0.01[a]
D	5.08±0.57[a, c]	4.58±0.44[a, c]	4.00±0.37[a, b]	3.83±0.33[a, c]	0.92±0.33[a,N]	0.45±0.07[a, b]	0.26±0.06[a,N]	0.11±0.04[a,N]	0.03±0.01[a,N]

* Data represent Mean ± SEM, n = 6.

[a] Statistically significant at $p < 0.001$, vs. control, Student's *t*–test.

[b, c] $p < 0.01$, and 0.001, vs. respective paralysis time, Student's *t*-test.

[N] Not significant when compared with respective paralysis time.

Worms incubated in control medium: Paralysis at 29.17 ± 3.06 h, Death at 29.75 ± 3.04 h.

Table 2.5: Anticestodal effects of *Strobilanthes discolor* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	*Days 28-30* (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	31256 ± 2139	31817 ± 2098	+ 1.79	5.00 ± 0.00	100.0	0.00	0.00
Plant extract							
100x1x3	13539 ± 1602[c]	13856 ± 1449[c]	+ 2.34	2.17 ± 0.31[c]	43.40	56.60	0.00
100x2x3	10715 ± 1073[c]	10411 ± 1003[c]	- 2.84	1.67 ± 0.33[c]	33.40	66.60	0.00
200x1x3	11783 ± 1017[c]	10017 ± 938[c]	- 14.99	1.83 ± 0.40[c]	36.60	63.40	0.00
200x2x3	10517 ± 1002[c]	8455 ± 842[c]	- 19.61	1.50 ± 0.22[c]	30.00	70.00	0.00
400x1x3	6289 ± 1732[c]	3017 ± 1345[c]	- 52.03	0.67 ± 0.21[c]	13.40	86.60	33.33
400x2x3	3294 ± 1442[c]	1634 ± 595[c]	- 50.39	0.50 ± 0.22[c]	10.00	90.00	50.00
800x1x3	1939 ± 826[c]	945 ± 323[c]	- 51.26	0.50 ± 0.22[c]	10.00	90.00	50.00
800x2x3	0[c]	0[c]	0.00	0[c]	0.00	100.0	100.0
Praziquantel (PZQ)							
5x1x3	7667 ± 5265[c]	5528 ± 3672[c]	- 27.90	0.83 ± 0.48[c]	16.60	83.40	83.40
5x2x3	1406 ± 725[c]	661 ± 418[c]	- 52.99	0.33 ± 0.21[c]	6.60	93.40	93.40
25x1x3	0[c]	1889 ± 1205[c]	∞	0.33 ± 0.21[c]	6.60	93.40	93.40
25x2x3	0[c]	0[c]	0.00	0[c]	0.00	100.0	100.0

[a] Administration of the plant extract on days 2-4 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c] $p < 0.001$, vs. control value, Student's *t*-test.

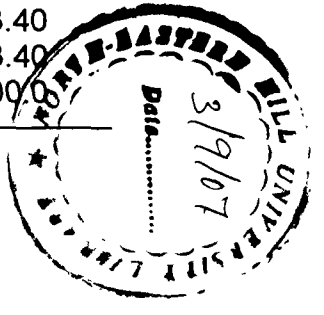


Table 2.6: Anticestodal effects of *Strobilanthes discolor* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
1. Control	30956 ± 1891	30500 ± 2043	- 1.47	5.00 ± 0.00	100.00	0.00	0.00
Plant extract							
100x1x3	19189 ± 2068 [c]	18378 ± 1943 [c]	- 4.23	2.67 ± 0.21 [d]	53.40	46.60	0.00
100x2x3	18161 ± 1191 [d]	18467 ± 1574 [d]	+ 1.68	2.50 ± 0.34 [d]	50.00	50.00	0.00
200x1x3	14906 ± 1313 [d]	16239 ± 844 [d]	+ 8.94	2.67 ± 0.21 [d]	53.40	46.60	0.00
200x2x3	12378 ± 1218 [d]	13278 ± 385 [d]	+ 7.27	2.17 ± 0.31 [d]	43.40	56.60	0.00
400x1x3	12139 ± 1082 [d]	12144 ± 1103 [d]	+ 0.04	2.00 ± 0.26 [d]	40.00	60.00	0.00
400x2x3	9617 ± 971 [d]	9811 ± 669 [d]	+ 2.02	1.67 ± 0.33 [d]	33.40	66.60	0.00
800x1x3	6456 ± 885 [d]	9945 ± 921 [d]	+ 54.04	1.83 ± 0.31 [d]	36.60	63.40	0.00
800x2x3	5811 ± 988 [d]	4728 ± 823 [d]	- 18.64	1.17 ± 0.17 [d]	23.40	76.60	0.00
Praziquantel (PZQ)							
5x1x3	4228 ± 1379 [d]	3894 ± 1337 [d]	- 7.90	0.67 ± 0.21 [d]	13.40	86.60	0.00
5x2x3	1717 ± 909 [d]	3628 ± 1741 [d]	+ 111.30	0.83 ± 0.40 [d]	16.60	83.40	0.00
25x1x3	2433 ± 1555 [d]	3395 ± 2151 [d]	+ 39.54	0.50 ± 0.34 [d]	10.00	90.00	0.00
25x2x3	0 [d]	0 [d]	0.00	0 [d]	0	100.0	100.0

[a] Administration of the plant extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d] $p < 0.01$ and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.7: Anticestodal effects of *Strobilanthes discolor* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 3 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	31417±779	32517±1632	29761±582	+3.50	-5.27	5.00±0.00	100.00	0.00	0.00
Plant extract									
100x1x3	30750±1473	25173±2383[c]	22439±2410[d]	-18.14	-27.03	3.50 ± 0.43[f]	70.00	30.00	0.00
100x2x3	32428±1182	15400±1248[e]	12995±899[e]	-52.02	-59.93	2.33 ± 0.21[g]	46.60	53.40	0.00
200x1x3	31250±1704	22945±604[e]	20433±85[e]	-26.58	-34.61	2.83 ± 0.31[g]	56.60	43.40	0.00
200x2x3	32011±1392	11839±1439[e]	9584±2352[e]	-63.02	-70.06	1.83 ± 0.40[g]	36.60	63.40	0.00
400x1x3	33028±3064	20133±1309[e]	16806±673[e]	-39.04	-49.12	2.33 ± 0.21[g]	46.60	53.40	0.00
400x2x3	31639±1169	9338±739[e]	5378±1172[e]	-70.49	-83.00	1.00 ± 0.26[g]	20.00	80.00	16.67
800x1x3	30000±1349	14472±650[e]	12872±765[e]	-51.72	-57.09	2.00 ± 0.00[g]	40.00	60.00	0.00
800x2x3	32833±1402	6156±1223[e]	3595±876[e]	-81.25	-89.05	0.50 ± 0.22[g]	10.00	90.00	50.00
Praziquantel (PZQ)									
5x1x3	32317±1619	12689±868[e]	9817±755[e]	-60.74	-69.62	2.00 ± 0.45[g]	40.00	60.00	0.00
5x2x3	32378±1133	2533±277[e]	833±408[e]	-92.18	-97.43	0.50 ± 0.34[g]	10.00	90.00	66.67
25x1x3	32539±937	7411±953[e]	3045±874[e]	-77.22	-90.64	0.83 ± 0.17[g]	16.60	83.40	50.00
25x2x3	32961±1145	256±125[e]	0[e]	-99.52	-100.0	0[g]	0.00	100.0	100.0

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[f, g] $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.8: Anticestodal effects of *Strobilanthes discolor* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 5 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	33811±1784	32799±1608	31472±1852	-2.99	-4.26	5.00±0.00	100.0	0.00	0.00
Plant extract									
100x1x5	33561±1483	15767±1657[c]	11033±1098[c]	-53.02	-67.13	2.17 ± 0.40[d]	43.40	45.60	0.00
100x2x5	31589±1990	11750±1467[c]	8489±1289[c]	-62.80	-73.13	1.83 ± 0.40[d]	36.60	63.40	0.00
200x1x5	32400±1813	13356±1204[c]	9589±768[c]	-58.78	-70.40	1.67 ± 0.33[d]	33.40	66.60	0.00
200x2x5	32472±624	13811±794[c]	6017±965[c]	-57.47	-81.47	1.00 ± 0.00[d]	20.00	80.00	0.00
400x1x5	32200±1142	10556±977[c]	8050±2020[c]	-67.22	-75.00	1.50 ± 0.34[d]	30.00	70.00	0.00
400x2x5	34567±1312	11034±1090[c]	3639±1168[c]	-68.08	-89.47	0.60 ± 0.22[d]	12.00	88.00	33.33
800x1x5	33222±1502	7483±1274[c]	1733±489[c]	-77.48	-94.78	0.83 ± 0.31[d]	16.60	83.40	33.33
800x2x5	32699±939	5760±1938[c]	3150±1735[c]	-82.39	-90.37	0.50 ± 0.34[d]	10.00	90.00	66.67
Praziquantel (PZQ)									
5x1x5	30895±1350	7650±2612[c]	4049±1653[c]	-75.25	-86.89	1.00 ± 0.45[d]	20.00	80.00	33.33
5x2x5	33561±1149	3106±1533[c]	1039±802[c]	-90.75	-96.90	0.33 ± 0.21[d]	6.60	93.40	66.67
25x1x5	31428±1842	3650±1678[c]	1061±671[c]	-88.39	-96.62	0.50 ± 0.34[d]	10.00	90.00	66.67
25x2x5	32034±1536	2111±1311[c]	0[c]	-93.41	-100.0	0[d]	0.00	100.0	100.0

[a] Administration of extract on days 21-25 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, 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 2.9: Anticestodal effects of *Adhatoda vasica* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B (%)	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
	Days 18-20 (A)	Days 28-30 (B)					
Control	32361 ± 1304	32717 ± 1359	+ 1.10	5.00 ± 0.00	100.00	0.00	0.00
Plant extract							
100x1x3	21217 ± 888 [c]	20267 ± 969 [c]	- 4.48	3.33 ± 0.33 [c]	66.60	33.40	0.00
100x2x3	17350 ± 945 [c]	16211 ± 694 [c]	- 6.57	2.17 ± 0.17 [c]	43.44	56.60	0.00
200x1x3	20317 ± 1306 [c]	17806 ± 707 [c]	- 12.36	3.00 ± 0.26 [c]	60.00	40.00	0.00
200x2x3	15439 ± 1381 [c]	13417 ± 1254 [c]	- 13.09	2.00 ± 0.00 [c]	40.00	60.00	0.00
400x1x3	17045 ± 985 [c]	16561 ± 1111 [c]	- 2.84	2.83 ± 0.31 [c]	56.60	43.40	0.00
400x2x3	14839 ± 2149 [c]	12133 ± 3407 [c]	- 18.24	1.83 ± 0.31 [c]	36.60	63.40	0.00
800x1x3	11334 ± 843 [c]	9589 ± 908 [c]	- 15.40	1.50 ± 0.22 [c]	30.00	70.00	0.00
800x2x3	13378 ± 2081 [c]	9406 ± 2761 [c]	- 29.69	1.67 ± 0.40 [c]	33.40	66.60	16.67
Praziquantel (PZQ)							
5x1x3	10006 ± 4448 [c]	6439 ± 3131 [c]	- 35.65	1.00 ± 0.45 [c]	20.00	80.00	33.33
5x2x3	7222 ± 3364 [c]	6217 ± 2880 [c]	- 13.92	0.83 ± 0.48 [c]	16.60	83.40	50.00
25x1x3	1428 ± 1428 [c]	1102 ± 1122 [c]	- 21.43	0.17 ± 0.17 [c]	3.40	96.60	83.33
25x2x3	0 [c]	0 [c]	0.00	0 [c]	0.00	100.0	100.0

[a] Administration of the plant extract on days 2-4 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c] $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.10: Anticestodal effects of *Adhatoda vasica* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat (Mean ± SEM)	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)		(%)	(%)	(%)
Control	30950 ± 1257	30078 ± 1159	- 2.82	5.00 ± 0.00	100.00	0.00	0.00
Plant extract							
100x1x3	20861 ± 1391 [c]	17361 ± 1361 [c]	- 16.78	3.00 ± 0.26 [c]	60.00	40.00	0.00
100x2x3	16856 ± 1231 [c]	14161 ± 1576 [c]	- 15.99	2.17 ± 0.40 [c]	43.40	56.60	0.00
200x1x3	19033 ± 914 [c]	18489 ± 1216 [c]	- 2.86	2.83 ± 0.40 [c]	56.60	43.40	0.00
200x2x3	12567 ± 621 [c]	10150 ± 1407 [c]	- 19.33	1.83 ± 0.40 [c]	36.60	63.40	0.00
400x1x3	16500 ± 1389 [c]	9922 ± 1293 [c]	- 39.87	2.17 ± 0.17 [c]	43.40	56.60	0.00
400x2x3	6928 ± 1779 [c]	6795 ± 2134 [c]	- 1.92	1.17 ± 0.40 [c]	23.40	76.60	16.67
800x1x3	11561 ± 1279 [c]	8522 ± 2053 [c]	- 26.29	2.00 ± 0.52 [c]	40.00	60.00	0.00
800x2x3	7711 ± 1709 [c]	5522 ± 1732 [c]	- 28.39	1.00 ± 0.26 [c]	20.00	80.00	16.67
Praziquantel (PZQ)							
5x1x3	4728 ± 1300 [c]	3567 ± 1449 [c]	- 24.56	0.83 ± 0.31 [c]	16.60	83.40	33.33
5x2x3	4767 ± 2200 [c]	4367 ± 2041 [c]	- 8.39	0.67 ± 0.33 [c]	13.40	86.60	50.00
25x1x3	2767 ± 1154 [c]	2450 ± 1114 [c]	- 4.56	0.50 ± 0.22 [c]	10.00	90.00	50.00
25x2x3	0 [c]	0 [c]	0.00	0 [c]	0	100.0	100.0

[a] Administration of the plant extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c] $p < 0.001$, vs. control value, Student's *t*-test.

Table 1.11: Anticestodal effects of *Adhatoda vasica* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 3 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	34183±1300	32550±1739	34789±1329	- 4.78	- 1.78	5.00 ± 0.00	100.00	0.00	0.00
Plant extract									
100x1x3	33267±1358	29011±1801	26661±1868[d]	- 12.79	- 19.86	3.83 ± 0.40 [g]	76.60	23.40	0.00
100x2x3	31961±1938	28667±1292	26828±1042[c]	- 10.31	- 16.06	3.67 ± 0.42 [g]	73.40	26.60	0.00
200x1x3	33067±2142	25722±1596[c]	22889±2713[d]	- 22.31	- 30.78	3.00 ± 0.52 [g]	60.00	40.00	0.00
200x2x3	32806±1802	25533±1759[d]	21789±1457[f]	- 22.17	- 33.58	2.83 ± 0.31 [g]	56.60	43.40	0.00
400x1x3	31583±1363	21044±2011[e]	17072±1674[f]	- 33.37	- 45.95	2.50 ± 0.22 [g]	50.00	50.00	0.00
400x2x3	31417±1746	19695±2174[e]	14311±2123[f]	- 37.31	- 54.45	2.17 ± 0.40 [g]	43.40	56.60	0.00
800x1x3	32667±1708	13989±1448[f]	11211±883[d]	- 57.18	- 65.68	2.17 ± 0.17 [g]	43.40	56.60	0.00
800x2x3	31589±1267	12583±1958[f]	10978±1578[f]	- 60.17	- 65.25	1.83 ± 0.31 [g]	36.60	63.40	0.00
Praziquantel (PZQ)									
5x1x3	31800±1764	13295±2396[f]	8189±1006[f]	- 58.19	- 74.25	2.00 ± 0.37 [g]	40.00	60.00	0.00
5x2x3	33022±1927	11911±1789[f]	5222±1674[f]	- 63.93	- 84.19	1.17 ± 0.31 [g]	23.40	76.60	16.67
25x1x3	32128±2153	9372±1366[f]	615±1267[f]	- 70.83	- 80.84	1.33 ± 0.21 [g]	26.60	73.40	0.00
25x2x3	31211±1889	4867±2114[f]	0[f]	- 84.41	-100.0	0[g]	0.00	100.0	100.0

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[g] $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.12: Anticestodal effects of *Adhatoda vasica* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 5 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	33387±1672	32433±1160	32800±1873	- 2.86	- 1.76	5.00±0.00	00.0	0.00	0.00
Plant extract									
100x1x5	30372±615	26872±1535[d]	20217±1358[d]	- 11.19	- 33.44	2.83 ± 0.31 [e]	56.60	43.40	0.00
100x2x5	31106±799	15311±1194[d]	13878±1890[d]	- 50.78	- 55.38	2.17 ± 0.40 [e]	43.40	56.60	0.00
200x1x5	31495±1447	19683±1641[d]	18717±1815[d]	- 37.50	- 40.57	2.83 ± 0.41 [e]	56.60	43.40	0.00
200x2x5	32600±1867	12672±3307[d]	12811±2162[d]	- 61.13	- 60.70	1.83 ± 0.31 [e]	36.60	63.40	0.00
400x1x5	31561±1711	17722±2685[c]	14089±1835[d]	- 43.85	- 55.36	2.50 ± 0.43 [e]	50.00	50.00	0.00
400x2x5	30983±1977	14317±2088[d]	8417±1641[d]	- 53.79	- 72.83	1.17 ± 0.48 [e]	23.40	76.60	0.00
800x1x5	30991±2032	13689±1784[d]	12967±1870[d]	- 55.83	- 58.16	1.83 ± 0.31 [e]	36.60	63.40	0.00
800x2x5	33522±730	6850±2041[d]	4206±1999[d]	- 79.57	- 87.45	0.83 ± 0.31 [e]	16.60	83.40	33.33
Praziquantel (PZQ)									
5x1x5	30194±2135	7928±1803[d]	5667±2158[d]	- 73.74	- 81.23	1.17 ± 0.31 [e]	23.40	76.60	16.67
5x2x5	31267±1429	2900±1869[d]	2672±1691[d]	- 90.73	- 91.45	0.33 ± 0.21 [e]	6.60	93.40	66.67
25x1x5	30894±1559	3739±2261[d]	0[d]	- 87.90	-100.0	0 [e]	0.00	100.0	100.0
25x2x5	31917±1482	2156±1373[d]	0[d]	- 93.25	-100.0	0 [e]	0.00	100.0	100.0

[a] Administration of extract on days 21-25 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d] $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[e] $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.13: Anticestodal effects of *Butea minor* seed extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	32117±1920	30700±1151	-4.41	4.67±0.21	93.40	6.60	0.00
Plant extract							
100x1x3	20295±1534 [d]	18639±1095 [d]	-8.16	3.17±0.30 [c]	63.40	36.60	0.00
100x2x3	16344±794 [d]	15545±635 [d]	-4.89	3.17±0.31 [c]	63.40	36.60	0.00
200x1x3	18545±683 [d]	17372±676 [d]	-6.34	3.00±0.26 [d]	60.00	40.00	0.00
200x2x3	15906±940 [d]	15445±1153 [d]	-2.90	2.83±0.17 [d]	56.60	43.40	0.00
400x1x3	14445±785 [d]	13306±780 [d]	-7.78	2.67±0.21 [d]	53.40	46.60	0.00
400x2x3	12906±812 [d]	11217±1404 [d]	-13.09	2.50±0.43 [c]	50.00	50.00	0.00
800x1x3	11267±1098 [d]	10022±1110 [d]	-11.05	2.50±0.22 [d]	50.00	50.00	0.00
800x2x3	10317±887 [d]	9256±735 [d]	-10.28	2.17±0.17 [d]	43.40	56.60	0.00
Praziquantel (PZQ)							
5x1x3	4583±2059 [d]	2039±1239 [d]	-55.51	0.50±0.34 [d]	10.00	90.00	66.67
5x2x3	783±365 [d]	461±224 [d]	-41.12	0.33±0.21 [d]	6.60	93.40	66.67
25x1x3	0 [d]	0 [d]	0.00	0 [d]	0	100.0	100.0
25x2x3	0 [d]	0 [d]	0.00	0 [d]	0	100.0	100.0

[a] Administration of extract on days 2-4 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d] $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.14: Anticestodal effects of *Butea minor* seed extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	28639±2188	28583±1773	-0.20	4.50±0.22	90.00	10.00	0.00
Plant extract							
100x1x3	24183±1228	22222±1089 [d]	-8.11	3.67±0.33	73.40	26.60	0.00
100x2x3	23339±870 [c]	22950±830 [d]	-1.67	3.67±0.21 [c]	73.40	26.60	0.00
200x1x3	22306±752 [c]	21745±842 [e]	-2.52	3.50±0.22 [e]	70.00	30.00	0.00
200x2x3	19878±1479 [e]	19511±1669 [e]	-1.85	3.00±0.37 [e]	60.00	40.00	0.00
400x1x3	22417±1545 [c]	21445±1591 [d]	-4.34	3.00±0.26 [e]	60.00	40.00	0.00
400x2x3	16545±972 [f]	15772±969 [f]	-4.67	2.17±0.17 [f]	43.40	56.60	0.00
800x1x3	17445±1389 [e]	16728±1104 [f]	-4.11	2.50±0.22 [f]	50.00	50.00	0.00
800x2x3	13300±223 [f]	12500±265 [f]	-6.02	2.00±0.00 [f]	40.00	60.00	0.00
Praziquantel (PZQ)							
5x1x3	6828±2320 [f]	5678±2207 [f]	-16.84	0.83±0.40 [f]	16.60	83.40	50.00
5x2x3	3345±1843 [f]	1906±1227 [f]	-43.20	0.67±0.42 [f]	13.40	86.60	66.67
25x1x3	0 [f]	0 [f]	0.00	0 [f]	0	100.0	100.0
25x2x3	0 [f]	0 [f]	0.00	0 [f]	0	100.0	100.0

[a] Administration of extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 215: Anticestodal effects of *Butea minor* seed extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 3 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	34667±1888	34978±1291	34639±1601	+0.90	-0.08	5.00±0.00	100.00	0.00	0.00
Plant extract									
100x1x3	33811±669	31150±1468	28128±2112	-7.87	-16.81	4.00±0.37 [g]	80.00	20.00	0.00
100x2x3	33172±1031	33517±1051	30317±810	+1.04	-8.61	4.17±0.17 [h]	83.40	16.60	0.00
200x1x3	33794±1312	30856±965	26406±1482[d]	-8.69	-21.86	3.67±0.37 [h]	73.40	26.60	0.00
200x2x3	33789±1072	30045±421[c]	24061±812 [f]	-11.08	-28.79	3.50±0.22 [i]	70.00	30.00	0.00
400x1x3	33217±887	28706±1651	25522±1699[d]	-13.58	-23.17	2.83±0.31 [i]	56.60	43.40	0.00
400x2x3	32900±811	28822±718[d]	22800±1853[e]	-12.40	-30.70	2.67±0.33 [i]	53.40	46.60	0.00
800x1x3	33317±877	22683±1237[f]	13856±2881[e]	-31.92	-58.41	1.67±0.33 [i]	33.40	66.60	16.67
800x2x3	32211±1684	8711±1708[f]	4028±954[f]	-72.96	-87.50	1.00±0.37 [i]	20.00	80.00	33.33
Praziquantel (PZQ)									
5x1x3	34234±1469	4150±1986[f]	1828±916[f]	-87.88	-94.66	0.83±0.40 [i]	16.60	83.40	50.00
5x2x3	33639±1005	695±457[f]	478±304[f]	-97.94	-98.58	0.33±0.21 [i]	6.60	93.40	66.67
25x1x3	34467±899	1295±682[f]	711±459[f]	-96.24	-97.94	0.33±0.21 [i]	6.60	93.40	66.67
25x2x3	33639±955	0 [f]	0 [f]	-100.0	-100.0	0 [i]	0	100.0	100.0

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[g, h, i] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.16: Anticestodal effects of *Butea minor* seed extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 5 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	33411±3259	32428±2918	29739±2878	-2.94	-10.99	4.83±0.17	96.60	3.40	0.00
Plant extract									
100x1x5	32472±1729	25445±1154[d]	18967±2008[e]	-21.64	-41.60	3.33±0.67	66.60	33.40	0.00
100x2x5	33494±2704	27133±1418	22011±3401[c]	-18.99	-34.28	3.17±0.60 [g]	63.40	36.60	0.00
200x1x5	32306±2644	21694±1786[c]	18356±2039[e]	-32.85	-43.18	3.17±0.40 [h]	64.40	36.60	0.00
200x2x5	32317±1752	20583±1903[e]	13922±1345[f]	-36.31	-56.92	2.83±0.43 [h]	56.60	43.40	0.00
400x1x5	33072±1479	20545±1584[e]	15806±3074[e]	-37.88	-52.21	2.00±0.58 [i]	40.00	60.00	16.67
400x2x5	31611±2201	20689±2775[c]	11300±1987[e]	-34.55	-64.25	1.83±0.48 [i]	36.60	63.40	16.67
800x1x5	31667±1085	20739±2225[d]	10400±3083[f]	-34.51	-67.16	1.83±0.65 [i]	36.60	63.40	33.33
800x2x5	32606±3005	20950±2514[c]	9890±1937[e]	-35.75	-69.67	1.50±0.56 [i]	30.00	70.00	33.33
Praziquantel									
5x1x5	31511±1993	19445±3222[c]	8028±3231[c]	-38.29	-74.52	1.00±0.52 [i]	20.00	80.00	50.00
5x2x5	31711±2568	16956±1906[c]	4611±2095[f]	-46.53	-85.46	0.50±0.34 [i]	10.00	90.00	66.67
25x1x5	31811±3381	18261±3210[c]	3361±2272[f]	-42.60	-88.49	0.33±0.33 [i]	6.60	93.40	83.33
25x2x5	32083±2562	3672±1271[f]	0 [f]	-88.56	-100.0	0 [i]	0	100.0	100.0

[a] Administration of extract on days 21-25 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[g, h, i] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.17: Anticestodal effects of *Solanum myriacanthum* ripen fruit extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	32406±616	33128±678	+2.23	5.00±0.00	100.0	0.00	0.00
Plant extract							
100x1x3	25411±1213 [e]	26206±1466 [d]	+3.13	4.00±0.37 [c]	80.00	20.00	0.00
100x2x3	21078±1615 [e]	20145±760 [e]	-4.43	3.67±0.21 [e]	73.40	26.60	0.00
200x1x3	22639±647 [e]	22772±793 [e]	+0.59	3.50±0.21 [e]	70.00	30.00	0.00
200x2x3	17556±379 [e]	16983±240 [e]	-3.26	3.00±0.00 [e]	60.00	40.00	0.00
400x1x3	23383±763 [e]	20494±598 [e]	-12.36	3.33±0.21 [e]	66.60	33.40	0.00
400x2x3	16689±1627 [e]	15611±1468 [e]	-6.46	2.83±0.31 [e]	56.60	43.40	0.00
800x1x3	16222±1350 [e]	15211±1235 [e]	-6.23	2.17±0.31 [e]	43.40	56.60	0.00
800x2x3	7067±2300 [e]	5283±1707 [e]	-25.24	1.17±0.57 [e]	23.40	76.60	33.33
Praziquantel (PZQ)							
5x1x3	3739±2365 [e]	2678±1716 [e]	-28.38	0.67±0.42 [e]	13.40	86.60	66.67
5x2x3	2278±2278 [e]	1972±1922 [e]	-13.43	0.33±0.33 [e]	6.60	93.40	83.33
25x1x3	228±228 [e]	211±211 [e]	-7.46	0.17±0.17 [e]	3.40	96.60	83.33
25x2x3	0 [e]	0 [e]	0.00	0 [e]	0	100.00	100.00

[a] Administration of the plant extract on days 2-4 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.18: Anticestodal effects of *Solanum myriacanthum* ripen fruit extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and percentage host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	31561±1060	31606±1231	+0.14	4.67±0.33	93.40	6.60	0.00
Plant extract							
100x1x3	18772±465 [f]	18061±882 [f]	-3.79	4.17±0.17	83.40	16.60	0.00
100x2x3	14983±1063 [f]	14890±1860 [f]	-1.22	3.50±0.22 [d]	70.00	30.00	0.00
200x1x3	16.783±696 [f]	18483±338 [f]	+9.20	4.00±0.00	80.00	20.00	0.00
200x2x3	12439±1169 [f]	12406±1461 [f]	-0.27	3.17±0.17 [e]	63.40	36.60	0.00
400x1x3	14228±1065 [f]	15328±1234 [f]	+7.73	3.50±0.34 [c]	70.00	30.00	0.00
400x2x3	7556±1190 [f]	7572±1134 [f]	+0.21	2.33±0.33 [f]	56.60	43.40	0.00
800x1x3	13300±1280 [f]	14400±911 [f]	+8.27	3.00±0.26 [e]	60.00	40.00	0.00
800x2x3	5600±1211 [f]	5922±1207 [f]	+5.75	2.50±0.22 [f]	50.00	50.00	0.00
Praziquantel (PZQ)							
5x1x3	6728±3025 [f]	6495±2914 [f]	-3.46	1.00±0.45 [f]	20.00	80.00	50.00
5x2x3	1711±1711 [f]	1056±1056 [f]	-38.28	0.17±0.17 [f]	3.40	96.60	83.33
25x1x3	0 [f]	0 [f]	0.00	0 [f]	0	100.0	100.0
25x2x3	0 [f]	0 [f]	0.00	0 [f]	0	100.0	100.0

[a] Administration of the plant extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.19: Anticestodal effects of *Solanum myriacanthum* ripen fruit extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 3 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	33111±1289	31678±1056	31706±641	-4.33	-4.24	5.00±0.00	100.00	0.00	0.00
Plant extract									
100x1x3	31083±1040	28572±1094	29272±1173	-8.08	-5.83	4.50±0.22 [g]	90.00	10.00	0.00
100x2x3	31533±942	27544±1063[c]	21806±2210[e]	-12.65	-30.85	3.83±0.31 [h]	76.60	23.40	0.00
200x1x3	32989±1191	24872±1283[e]	25239±1632[d]	-24.61	-23.49	4.00±0.26 [h]	80.00	20.00	0.00
200x2x3	31745±1517	18611±1711[e]	17950±878[f]	-41.37	-43.46	3.17±0.31 [i]	63.40	36.60	0.00
400x1x3	32111±1476	15856±1965[e]	14950±1639[f]	-50.62	-53.44	3.00±0.26 [i]	60.00	40.00	0.00
400x2x3	33767±982	18661±1833[f]	16128±1393[f]	-44.74	-52.24	2.83±0.60 [h]	56.60	43.40	0.00
800x1x3	32283±1378	12756±1380[f]	9672±1130[f]	-60.49	-70.04	2.17±0.31 [i]	43.40	56.60	0.00
800x2x3	31539±1330	13133±2673[e]	7567±1349[f]	-58.36	-67.01	1.50±0.43 [i]	30.00	70.00	16.67
Praziquantel									
5x1x3	30978±1781	14928±1492[f]	9139±2482[f]	-51.81	-70.50	1.50±0.56 [i]	30.00	70.00	33.33
5x2x3	30589±880	6239±1815[f]	3078±1745[f]	-79.60	89.94	1.00±0.52 [i]	20.00	80.00	50.00
25x1x3	30267±1212	5478±1648[f]	4694±2639[f]	-81.90	-94.40	1.00±0.52 [i]	20.00	80.00	50.00
25x2x3	32557±1291	4334±2125[f]	1928±1510[f]	-86.69	-94.08	0.33±0.33 [i]	6.60	93.40	83.33

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[g, h, i] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.20: Anticestodal effects of *Solanum myriacanthum* ripen fruit extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 5 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	33300±2213	34511±2161	29622±2420	+3.64	-11.64	4.83±0.17	96.60	3.40	0.00
Plant extract									
100x1x5	31150±2088	25794±1926	17878±665[e]	-17.19	-42.61	2.17±0.31	83.40	16.60	0.00
100x2x5	30461±2359	26411±1848	22806±1682[c]	-13.30	-28.41	3.33±0.41[h]	66.60	33.40	0.00
200x1x5	30767±2486	19372±1481[d]	17561±1367[e]	-37.04	-42.92	3.33±0.33[h]	66.60	33.40	0.00
200x2x5	30772±1514	23700±2184[c]	19006±1700[e]	-22.98	-38.24	2.50±0.34[i]	50.00	50.00	0.00
400x1x5	31878±2505	17206±2446[e]	15856±2871[e]	-46.03	-50.26	3.00±0.45[h]	60.00	40.00	0.00
400x2x5	30467±2580	24183±2559	13000±2794[e]	-20.63	-50.26	1.83±0.54[i]	36.60	63.40	16.67
800x1x5	31650±2591	13195±2067[e]	11795±2623[e]	-58.31	-62.73	1.50±0.50[i]	30.00	70.00	16.67
800x2x5	30280±2055	22506±1707[c]	11922±3713[e]	-25.67	-60.63	1.83±0.65[h]	36.60	63.40	33.33
Praziquantel									
5x1x5	30745±1761	13222±2089[e]	8572±1957[f]	-57.00	-72.12	0.83±0.40[i]	16.60	83.40	50.00
5x2x5	31789±2713	9456±3513[e]	4767±2983[e]	-70.25	-85.00	0.67±0.49[i]	13.40	86.60	66.67
25x1x5	31139±2299	14117±3240[e]	1544±699[f]	-.54.67	-95.04	0.33±0.21[i]	6.60	93.40	66.67
25x2x5	30800±2474	0[f]	0[f]	-100.0	-100.0	0[i]	0	100.0	100.0

[a] Administration of extract on days 21-25 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[g, h, i] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.21: Anticestodal effects of *Trifolium repens* aerial part extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	30945 ± 1301	31067 ± 1437	+ 0.39	5.00 ± 0.00	100.00	0.00	0.00
Plant extract							
100x1x3	28217 ± 3100	25506 ± 2378	- 9.61	3.83 ± 0.48 [e]	76.60	23.40	0.00
100x2x3	23189 ± 2353 [c]	23711 ± 1394 [d]	+ 2.25	3.50 ± 0.43 [d]	70.00	30.00	0.00
200x1x3	23783 ± 2128 [c]	18250 ± 3168 [d]	- 23.26	3.00 ± 0.52 [d]	60.00	40.00	0.00
200x2x3	17783 ± 1481 [e]	17833 ± 1848 [e]	+ 0.28	2.67 ± 0.33 [e]	53.40	46.60	0.00
400x1x3	19328 ± 2461 [d]	17472 ± 1815 [e]	- 9.60	2.67 ± 0.42 [e]	53.40	46.60	0.00
400x2x3	13839 ± 1344 [e]	13039 ± 1218 [e]	- 5.78	2.00 ± 0.26 [e]	40.00	60.00	0.00
800x1x3	17094 ± 3174 [d]	13999 ± 2121 [e]	- 18.11	2.17 ± 0.40 [e]	43.40	56.60	0.00
800x2x3	11200 ± 2400 [e]	10889 ± 2139 [e]	- 2.78	1.50 ± 0.22 [e]	30.00	70.00	0.00
Praziquantel (PZQ)							
5x1x3	10156 ± 1231 [e]	8672 ± 818 [e]	- 14.61	1.17 ± 0.17 [e]	23.40	76.60	0.00
5x2x3	4683 ± 2098 [e]	4322 ± 1936 [e]	- 7.71	0.50 ± 0.22 [e]	10.00	90.00	50.00
25x1x3	5728 ± 2070 [e]	4322 ± 1731 [e]	- 24.15	0.67 ± 0.21 [e]	13.40	86.60	33.33
25x2x3	0 [e]	0 [e]	0.00	0 [e]	0.00	100.0	100.0

[a] Administration of the plant extract on days 2-4 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e] $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.22: Anticestodal effects of *Trifolium repens* aerial part extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	32128 ± 980	32767 ± 1754	+ 1.99	5.00 ± 0.00	100.00	0.00	0.00
Plant extract							
100x1x3	26772 ± 1329 [e]	26684 ± 1446 [c]	+ 0.33	3.83 ± 0.31 [e]	76.60	23.40	0.00
100x2x3	24983 ± 2337 [d]	22761 ± 1931 [c]	- 8.89	3.17 ± 0.48 [e]	63.40	36.60	0.00
200x1x3	21139 ± 1644 [f]	20733 ± 2002 [e]	- 1.92	2.67 ± 0.33 [f]	53.40	46.60	0.00
200x2x3	17461 ± 2521 [f]	17728 ± 1721 [f]	+ 1.53	2.33 ± 0.42 [f]	46.60	53.40	0.00
400x1x3	15261 ± 1013 [f]	16039 ± 1932 [f]	+ 5.09	2.33 ± 0.33 [f]	46.60	53.40	0.00
400x2x3	15717 ± 2252 [f]	15250 ± 2925 [f]	- 2.97	2.00 ± 0.45 [f]	40.00	60.00	0.00
800x1x3	10311 ± 2457 [f]	7306 ± 2016 [f]	- 29.14	1.50 ± 0.34 [f]	30.00	70.00	0.00
800x2x3	11400 ± 2086 [f]	10533 ± 1546 [f]	- 7.61	1.33 ± 0.21 [f]	26.60	73.40	0.00
Praziquantel (PZQ)							
5x1x3	5861 ± 2443 [f]	4328 ± 2066 [f]	- 26.16	1.00 ± 0.37 [f]	20.00	80.00	33.33
5x2x3	4850 ± 3088 [f]	5683 ± 3595 [f]	+ 17.18	1.00 ± 0.63 [f]	20.00	80.00	66.67
25x1x3	4694 ± 3156 [f]	4995 ± 3162 [f]	+ 6.41	0.83 ± 0.54 [f]	16.60	83.40	66.67
25x2x3	0 [f]	0 [f]	0.00	0 [f]	0.00	100.0	100.0

[a] Administration of the plant extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e, f] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.23: Anticestodal effects of *Trifolium repens* aerial part extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 3 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	31161±1822	31361±2048	31733±1690	+ 0.64	+ 1.84	4.83 ± 0.17	96.60	3.40	0.00
Plant extract									
100x1x3	32061±1727	25533±1768[c]	23339±1513[d]	- 20.36	- 27.20	3.17 ± 0.31 [g]	63.40	36.60	0.00
100x2x3	31972±1264	20411±2027[e]	20947±2176[d]	- 36.16	- 34.48	2.83 ± 0.41 [f]	56.60	43.40	0.00
200x1x3	32217±2010	19917±2053[d]	19161±2374[d]	- 38.18	- 40.53	3.00 ± 0.52 [f]	60.00	40.00	0.00
200x2x3	30556±2198	16933±2155[d]	14750±2000[e]	- 44.58	- 51.73	2.00 ± 0.26 [g]	40.00	60.00	0.00
400x1x3	30189±2641	18067±2479[d]	16271±1828[d]	- 40.15	- 46.10	2.33 ± 0.42 [g]	46.60	53.40	0.00
400x2x3	31117±1329	14528±2169[e]	13607±2307[e]	- 53.31	- 56.27	1.83 ± 0.31 [g]	36.60	63.40	0.00
800x1x3	31356±1225	16389±2277[e]	15133±1836[e]	- 47.73	- 51.74	2.17 ± 0.48 [g]	43.40	56.60	0.00
800x2x3	31044±1510	12211±1617[e]	11100±1814[e]	- 60.67	- 64.24	1.67 ± 0.33 [g]	33.40	66.60	0.00
Praziquantel (PZQ)									
5x1x3	31800±1764	13295±2396[e]	8189±1006[e]	- 58.19	- 74.25	2.00 ± 0.37 [g]	40.00	60.00	0.00
5x2x3	32772±1845	11667±1845[e]	5067±1609[e]	- 64.40	- 84.54	1.00 ± 0.26 [g]	20.00	80.00	16.67
25x1x3	32128±2153	9372±1366[e]	6156±1267[e]	- 70.83	- 80.84	1.33 ± 0.21 [g]	26.60	73.40	0.00
25x2x3	31211±1889	4867±2114[e]	0 [e]	- 84.41	-100.0	0[g]	0.00	100.0	100.0

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[f, g] $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.24: Anticestodal effects of *Trifolium repens* aerial part extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 5 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	30095±1396	31372±1967	30083±2306	- 4.24	- 0.04	4.83 ± 0.17	96.60	3.40	0.00
Plant extract									
100x1x5	30856±1331	24222±2268[c]	22678±1792[d]	- 21.50	- 26.50	2.83 ± 0.31 [g]	56.60	43.40	0.00
100x2x5	31867±1670	16945±2237[e]	15806±1899[e]	- 46.83	- 50.40	2.67 ± 0.33 [g]	53.40	46.60	0.00
200x1x5	32717±1431	26311±2228[c]	22628±2284[d]	- 22.64	- 30.84	2.67 ± 0.49 [f]	53.40	53.40	0.00
200x2x5	32039±1501	15729±1159[e]	15611±983[e]	- 50.88	- 51.28	2.50 ± 0.34 [g]	50.00	50.00	0.00
400x1x5	31906±2255	21033±1833[d]	17917±1972[e]	- 34.08	- 43.84	2.00 ± 0.52 [g]	40.00	60.00	0.00
400x2x5	32761±1082	14617±1467[e]	12806±1490[e]	- 55.38	- 60.91	2.00 ± 0.26 [g]	40.00	60.00	0.00
800x1x5	31656±1278	14133±2183[e]	11672±1730[e]	- 55.35	- 63.13	1.50 ± 0.34 [g]	30.00	70.00	0.00
800x2x5	34478±1915	13178±2325[e]	8300±2079[e]	- 61.78	- 75.93	1.17 ± 0.31 [g]	23.40	76.60	16.67
Praziquantel (PZQ)									
5x1x5	30895±1350	7650±2612[e]	4049±1653[e]	- 75.24	- 86.89	1.00 ± 0.45 [g]	20.00	80.00	33.33
5x2x5	31867±1346	6667±3037[e]	3411±1902[e]	- 79.08	- 89.30	0.67 ± 0.33 [g]	13.40	86.60	50.00
25x1x5	31428±1842	3650±1678[e]	1061±671[e]	- 88.39	- 96.62	0.50 ± 0.34 [g]	10.00	90.00	66.67
25x2x5	31689±1353	5817±3841[e]	0[e]	- 81.64	- 100.0	0 [g]	0.00	100.0	100.0

[a] Administration of extract on days 21-25 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e] $p < 0.05$, $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[f, g] $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.25: Anticestodal effects of *Zanthoxylum rhetsa* leaf extract[a] on larval stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B (%)	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
	Days 18-20 (A)	Days 28-30 (B)					
Control	33161 ± 1383	32600 ± 1301	- 1.69	4.83 ± 0.17	96.60	3.40	0.00
Plant extract							
100x1x3	23389 ± 2372 [d]	22695 ± 1657 [d]	- 2.97	3.00 ± 0.45 [c]	60.00	40.00	0.00
100x2x3	21995 ± 2256 [d]	22672 ± 2429 [d]	+ 3.08	2.83 ± 0.40 [d]	56.60	43.40	0.00
200x1x3	19200 ± 2329 [d]	19633 ± 1405 [d]	+ 2.26	2.83 ± 0.40 [d]	56.60	43.40	0.00
200x2x3	16184 ± 5328 [c]	15533 ± 5164 [c]	- 4.02	2.17 ± 0.31 [d]	43.40	56.60	0.00
400x1x3	13461 ± 1678 [d]	14611 ± 1132 [d]	+ 8.54	2.17 ± 0.17 [d]	43.40	56.60	0.00
400x2x3	5772 ± 1809 [d]	3761 ± 1283 [d]	- 34.84	1.33 ± 0.42 [d]	26.60	73.40	16.67
800x1x3	0 [d]	3233 ± 1722 [d]	∞	0.67 ± 0.33 [d]	13.40	86.60	50.00
800x2x3	0 [d]	0 [d]	0.00	0 [d]	0.00	100.0	100.0
Praziquantel (PZQ)							
5x1x3	8050 ± 5033 [b]	5267 ± 4126 [d]	- 34.57	1.00 ± 0.52 [d]	20.00	80.00	50.00
5x2x3	6200 ± 5264 [b]	2450 ± 1715 [d]	- 60.48	0.67 ± 0.49 [d]	13.40	86.60	66.67
25x1x3	0 [d]	0 [d]	0.00	0 [d]	0.00	100.0	100.0
25x2x3	0 [d]	0 [d]	0.00	0 [d]	0.00	100.0	100.0

[a] Administration of the plant extract on days 2-4 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d] $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.26: Anticestodal effects of *Zanthoxylum rhetsa* leaf extract[a] on immature stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance.

Group (mg/kg x dose x days)	EPG (mean ± SEM)		Difference in EPG between A & B	No. of worms recovered/rat	Worm recovery	Worm reduction	Host clearance
	Days 18-20 (A)	Days 28-30 (B)	(%)	(Mean ± SEM)	(%)	(%)	(%)
Control	30461 ± 675	32383 ± 1337	+ 6.31	5.00 ± 0.00	100.00	0.00	0.00
Plant extract							
100x1x3	23934 ± 1863 [c]	23039 ± 1020 [e]	- 3.74	3.17 ± 0.31 [e]	63.40	36.60	0.00
100x2x3	21995 ± 2256 [d]	22672 ± 2429 [d]	+ 3.08	3.00 ± 0.37 [e]	60.00	40.00	0.00
200x1x3	21145 ± 1965 [d]	21506 ± 1582 [e]	+ 1.71	2.83 ± 0.31 [e]	56.60	43.40	0.00
200x2x3	19083 ± 723 [e]	19694 ± 954 [e]	+ 3.20	2.67 ± 0.31 [e]	53.40	46.60	0.00
400x1x3	19517 ± 136 [e]	19705 ± 1287 [e]	+ 0.96	2.67 ± 0.42 [e]	53.40	46.60	0.00
400x2x3	16184 ± 5328 [c]	15533 ± 5164 [c]	- 4.02	1.67 ± 0.62 [e]	33.40	66.60	33.33
800x1x3	15628 ± 1147 [e]	17272 ± 1313 [e]	+ 10.52	2.00 ± 0.26 [e]	40.00	60.00	0.00
800x2x3	12400 ± 3670 [e]	12150 ± 3203 [e]	- 2.02	1.67 ± 0.33 [e]	33.40	66.60	0.00
Praziquantel (PZQ)							
5x1x3	5350 ± 3148 [e]	4611 ± 2919 [e]	- 13.81	0.67 ± 0.42 [e]	13.40	86.60	66.67
5x2x3	5372 ± 2594 [e]	3745 ± 2149 [e]	- 30.64	0.67 ± 0.33 [e]	13.40	86.60	50.00
25x1x3	1789 ± 1193 [e]	1733 ± 1103 [e]	- 3.13	0.33 ± 0.21 [e]	6.60	93.40	66.67
25x2x3	0 [e]	0 [e]	0.00	0 [e]	0	100.0	100.0

[a] Administration of the plant extract on days 8-10 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d, e] $p < 0.02$, $p < 0.01$ and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.27: Anticestodal effects of *Zanthoxylum rhetsa* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 3 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	34845±3041	33311±1457	32345±1209	- 4.40	- 7.18	4.83 ± 0.17	96.60	3.40	0.00
Plant extract									
100x1x3	32867±455	27895±1267[c]	25500±718[d]	- 15.13	- 22.41	3.83 ± 0.75	76.60	23.40	0.00
100x2x3	35650±1877	17928±832[d]	13572±1127[d]	- 49.71	- 61.93	2.83 ± 0.31[e]	56.60	43.40	0.00
200x1x3	34578±763	27111±307[d]	18233±799[d]	- 21.59	- 47.27	3.33 ± 0.21[e]	66.60	33.40	0.00
200x2x3	33033±1194	14444±792[d]	5256±290[d]	- 56.27	- 84.09	2.17 ± 0.17[e]	43.40	56.60	0.00
400x1x3	33545±1554	25150±408[d]	15989±387[d]	- 25.02	- 52.33	3.17 ± 0.21[e]	63.40	36.60	0.00
400x2x3	30749±1887	11261±1312[d]	4200±785[d]	- 63.38	- 86.22	1.50 ± 0.53[e]	30.00	70.00	16.67
800x1x3	32944±702	16845±248[d]	11624±1157[d]	- 48.88	- 64.72	2.17 ± 0.17[e]	43.40	56.60	0.00
800x2x3	31500±1463	6756±945[d]	1528±98[d]	- 78.55	- 95.15	0.83 ± 0.17[e]	16.60	83.40	16.67
Praziquantel (PZQ)									
5x1x3	34278±1592	13722±973[d]	6733±356[d]	- 59.97	- 80.36	2.00 ± 0.26[e]	40.00	60.00	0.00
5x2x3	32378±1133	2533±277[d]	833±408[d]	- 92.18	- 97.43	0.50 ± 0.34[e]	10.00	90.00	66.67
25x1x3	32039±1234	8787±516[d]	989±362[d]	- 72.57	- 96.91	0.67 ± 0.42[e]	13.40	86.60	66.67
25x2x3	32961±1145	256±125[d]	0[d]	-99.52	-100.0	0[e]	0.00	100.0	100.0

[a] Administration of extract on days 21-23 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d] $p < 0.01$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[e] $p < 0.01$, and $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.28: Anticestodal effects of *Zanthoxylum rhetsa* leaf extract[a] on adult stages of *Hymenolepis diminuta* infections in albino rats[b] as monitored by EPG count, worm recovery rate and host clearance (Treatment for 5 days).

Group (mg/kg x dose x days)	EPG (mean ± SEM) Pre-treatment (A)	Post-treatment (B)	Follow-up (C)	Difference in EPG between (%) A&B	A&C	No. of worms recovered/rat (Mean ± SEM)	Worm recovery (%)	Worm reduction (%)	Host clearance (%)
Control	31894±2057	32056±1911	27878±609	-0.50	-12.59	5.00±0.00	100.0	0.00	0.00
Plant extract									
100x1x5	32056±763	27700±1649[c]	15533±1964[d]	- 13.59	- 51.54	3.33 ± 0.33[e]	67.60	33.40	0.00
100x2x5	31595±1201	8045±786[d]	4472±784[d]	- 74.54	- 85.85	2.00 ± 0.41[e]	40.00	60.00	0.00
200x1x5	33450±695	24667±1518[d]	14128±824[d]	- 26.26	- 57.76	3.00 ± 0.37[e]	60.00	40.00	0.00
200x2x5	31883±1097	6572±854[d]	4233±4233[d]	- 79.39	- 86.72	1.33 ± 0.42[e]	26.60	73.40	16.67
400x1x5	30700±527	20545±1168[d]	12678±618[d]	- 33.09	- 58.70	2.67 ± 0.33[e]	53.40	46.60	0.00
400x2x5	33828±1483	6266±1274[d]	1087±237[d]	- 81.48	- 96.78	1.17 ± 0.48[e]	23.40	76.60	33.33
800x1x5	34334±305	16656±582[d]	8483±406[d]	- 51.49	- 75.29	2.00 ± 0.26[a]	40.00	60.00	0.00
800x2x5	30283±2032	5384±1280[d]	487±136[d]	- 82.22	- 98.39	0.60 ± 0.25[e]	12.00	88.00	50.00
Praziquantel (PZQ)									
5x1x5	33617±566	11283±712[d]	6383±879[d]	- 66.44	- 81.01	1.00 ± 0.26[e]	20.00	80.00	16.67
5x2x5	32028±2794	172±66[d]	0[d]	- 99.46	-100.0	0[e]	0.00	100.0	100.0
25x1x5	35195±1872	1239±326[d]	0[d]	- 96.48	-100.0	0[e]	0.00	100.0	100.0
25x2x5	33461±1104	0[d]	0[d]	-100.00	-100.0	0[e]	0.00	100.0	100.0

[a] Administration of extract on days 21-25 post-inoculation with five cysticercoids per rat (n = 5).
[b] No. of animals in each group is six, n = 6.
[c, d] $p < 0.05$, and $p < 0.001$, vs. pre-treatment value, Student's *t*-test.
[e] $p < 0.001$, vs. control value, Student's *t*-test.

Table 2.29: Lethal effect of the different 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. S. discolor	6993.18
2. A. vasica	3755.62
3. *B. minor*	3200.03
4. *S. myriacanthum*	3093.24
5. *Z. rhetsa*	2737.34
6. *T. repens*	NM**

* Oral administration of the extracts was given in escalating doses from 100, 200, 400, 800, 1600 and 3200 mg/kg doses to groups of rats, n = 6.

** No mortality of animals was noticed for the *T. repens* extract when treatment was administered upto 3200 mg/kg dose, and observed for 72 h post-treatment.

Table 2.30: Effect of plant extracts on the serum biochemical levels of SGOT, SGPT, cholesterol and total protein in the blood samples of the rats 24 h post-treatment[a].

Group (mg/kg, p.o.)	**SGOT** (U/L)	**SGPT** (U/L)	**Cholesterol** (mg/dl)	**Total protein** (g/dl)
Control (saline)	149.00±4.34	75.83± 3.55	122.50±4.22	6.88±0.19
S. discolor 800	213.00±25.64[b]	114.83±14.15[b]	128.33±7.68	7.27±0.25
A. vasica 800	188.50±14.46[b]	106.50±12.84[b]	128.00±7.41	7.18±0.30
B. minor 800	155.17±5.53	77.50±2.17	124.67±3.87	6.95±0.20
S. myriacanthum 800	150.67±7.81	86.00±5.14	124.50±1.26	7.72±0.34
T. repens 800	183.67± 1.30[c]	94.83±14.83	132.17±11.20	6.92±0.21
Z. rhetsa 800	203.17±20.07[b]	106.33±12.99[b]	122.83±2.70	7.03±0.37

[a] Data represent mean ± SEM, n = 6.

[b, c, d, e] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, vs. control, Student's *t*-test.

DISCUSSION

In the quest of studying the ethnomedicinal plants of the indigenous Naga tribal communities in Manipur, a list of folklore plants has come into the notice, as claimed in traditional medicinal practice to have deworming remedy, particularly in eliminating the tapeworm parasites. A preliminary study in a questionnaire form (Table 2.1) amongst the local people in the villages reveals a positive response of six plants, which are in use to eliminate the intestinal worm infections in folklore practice. Therefore, these six plant extracts, viz., *S. discolor, A. vasica, B. minor, S. myriacanthum, T. repens* and *Z. rhetsa* were studied for their anticestodal efficacies in experimental cestode parasite, *H. diminuta*, both *in vitro* as well as *in vivo* models in the present study.

In vitro study of the present investigation illustrates that all the test extracts at varying concentrations had caused significant effects on the motility and mortality of *H. diminuta*, a cestode parasite, as compared to the control group. The worms maintained in the control medium had an insignificant difference between the motility and mortality time, which validates that there was no precession of paralysis to death. However, paralysis preceded mortality considerably in the case of treated worms with various concentrations of plant extracts. It may be suggested that paralysis of worms occurred for a very short interval following exposure of parasites to these plant extracts, and this inactiveness could also lead to elimination of worms in host body (Cox, 1982;

Tandon *et al.*, 1997). In a related study on *in vitro* effects of *Flemingia vestita* on *Ascaris suum*, Yadav *et al.* (1992) also reported that worms exposed to varying concentrations of plant extract become paralysed, though they do not show any mortality for sometime to follow. It may therefore be suggested that the plants extracts under the current study possibly exerts a reversible action on the neuromuscular system of the worms and in routine practice under the influence of plant extracts the paralysed worms are evacuated out by the host's gut movements.

In vitro screening for cestocidal drugs was devoted to understanding the mode of action of praziquantel in the adult-stage *Hymenolepis* species (Siles-Lucas and Hemphill, 2002). Contraction and paralysis of adult *H. diminuta* after exposure to this drug as well as the efflux of glucose from the parasite were studied by Andrews and Thomas (1979), who also demonstrated that these effects were reversible. Scanning and transmission electron microscopy studies revealed tegument impairment and vacuolization which were confined to the neck region of the parasite (Becker *et al.*, 1981; Tandon *et al.*, 1997), leading to disruption of syncytial layer of the organism. In the present study, *S. discolor* extract emerges out to be the most efficacious one, whose paralysis time, 0.92 h (at 100 mg/ml) is comparable with 0.67 h of the PZQ treated at 0.01 mg/ml concentration. The paralyzing effect of PZQ was much faster even at its lowest concentration; parasites lost motility within 1 h of incubation, however, mortality effects (3.83 – 5.08 h) caused by its 0.01 to 0.50 mg/ml concentrations were

comparable with those effects of the plant extracts at their highest concentration (100 mg/ml). It may therefore be suggested that the anticestodal activities of these plant-extracts on *H. diminuta* worms might be related to changes induced in the integument integrity of the parasite. However, care must be ensured when extrapolating results obtained from *in vitro* models, as some of the solvents required to solubilize the drugs, such as dimethylsulphoxide (DMSO) alone, could also induce the alteration on the tegumental brush border of *H. diminuta in vitro* (Forman and Oaks, 1992). Thus, while *in vitro* drug screening may only serve to reduce the cost and number of animals involved, at present *in vivo* laboratory models could not be fully replaced (Siles-Lucas and Hemphill, 2002).

In vivo studies were undertaken to evaluate the anticestodal efficacy of the plant extracts against *H. diminuta* infections in rats at three different developmental stages of the parasite (larval, immature and adult stage). Various workers have demonstrated the anticestodal activities of traditional plants, such as *Acacia auriculiformis, Gladiolus gandavensis, Albizzia anthelmintica, A. lebbek, Psidium guajava* etc, where efficacy was assessed on the basis of effects against the adult stages of *H. diminuta*. Extracts obtained from these plants have been shown to cause significant reductions in the average egg counts and number of worms recovered at necropsy (Galal *et al.,* 1991b; Ghosh *et al.*, 1996; Saha *et al.,* 1999; Temjenmongla *et al.,* 2006). In the present study, assessment of activity was extended to see if plant extracts would have effects

on the larval and immature stages as well, besides the adult worms of the parasite, and then accordingly, efficacy was judged by monitoring the eggs per gram of feces (EPG), worm recovery and host clearance at autopsy.

In order to observe the effects of plant extracts on larval stages, treatment was given on days 2 - 4 after inoculation of cysticercoids. On inoculation into the rats, 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 host. And by 8 – 10 days pi, the larval cestode is supposed to be growing to immature worms, until attaining the maturity from day 18 pi. It was at these three developmental stages of *H. diminuta* that treatment was done with different plant extracts for assessment of the anticestodal activities.

It was revealed that the leaf extract of *S. discolor* had most profound effects on either the establishment rate and/or larval stages of parasites. In this study, the 800 mg/kg double doses of extract given for 3 days showed a complete eradication of worms as evidenced by no recovery of parasite eggs in the faeces and also no recovery of worms at necropsy. The standard drug, Praziquantel at 25 mg/kg., p.o. dose also showed similar results. These findings suggest that either the test extract might have expelled the worms from the host lumen during the larval stage itself or retarded the worms so that they could not grow into maturation and therefore were eliminated before the time of autopsy

examination on day 31 pi. In the study on effects of leaf extract against the immature stages the mean EPG in the extract treated groups maintained uniformity during the entire post treatment period but the same showed a significant reduction as compared to control. The worm recovery rate showed a 100% recovery of worms in the control as compared to extract treated group which showed a dose dependent decrease in the worm recovery rates. In general, in both these studies the standard drug, Praziquantel, however showed better results as compared to leaf extract.

Further treatments of *S. discolor* extract against the mature stages of parasites indicated dose-dependent decline both in EPG counts and recovery of worms. The 800 mg/kg extract-treated group showed minimum recovery of worms as compared to control, where a 100% surviving worms were recovered. This finding is in agreement with other related studies on effects of leaf extract of *A. auriculiformis, G. gandavensis* and *P. guajava* on *H. diminuta* infections in rats (Ghosh *et al.*, 1996; Saha *et al.*, 1999 and Temjenmongla *et al.*, 2006). Ghosh *et al.* (1996) in their study reported a 100% cure rate of parasite burden and no recovery of eggs in the faeces of rats following treatment with funicles extract of *A. auriculiformis*. This could be due to expulsion of adult worms or destrobilation by the effects of extract. It has been reported that the process of destrobilation in cestodes may initiate if they are exposed to hostile physiological conditions, including exposure to anthelmintic drugs (Hopkins *et al.*, 1973). Dixon and Arai (1991) in a similar study regarding the real calculated drug

efficacy against *H. diminuta* suggested counting of worms in the small intestine at 8-10 days after the end of treatment. However in the present study, autopsy was performed two weeks after the end of treatment, and it was observed that EPG counts kept declining from post-treatment to one week follow-up examination. This indicates that the test extract could have effects on parasites requiring sufficient period of time after treatment was administered.

With regard to the effects of *A. vasica* extract, efficacy was most evident from treatment at the adult stages of the parasite, where number of eggs had dropped down drastically, and recovery of surviving worms at autopsy was reduced from 63.40% when treated for 3 days to 83.40% for 5 days for the maximum dose of the extract. This anticestodal activity emerged from this study may be attributed due to the presence of two major alkaloids, vasicine and vasicinone and glycosides from *A. vasica* (Atal, 1980; Das *et al.,* 2005; Lateef *et al.*, 2003). Several studies have reported that plant secondary metabolites (PSM), such as alkaloids, glycosides, saponins, tannins etc are the active compounds responsible for anti-parasitic effects of many plants (Taylor and Murant, 1966; Akhtar and Ahmad 1992; Mueller-Harvey and McAllan, 1992; Guarrera, 1999; Mohamed *et al.*, 2000; Athanasiadou *et al.*, 2000; Athanasiadou and Kyriazakis 2004; Paolini *et al.*, 2003). Akhtar and Ahmad (1992), in particular, reported of glycosides contained *Mallotus philippinensis*, which have shown to have anticestodal activity against the cestode infections in goats.

While there is no other study pertaining to phytochemical activity of *B. minor* available in the literature, the present study yields a satisfactory result in elimination of *H. diminuta* infections in experimental rats up to 56.60-80.00%. And *S. myriacanthum* extract yields 50.00-76.60% cure from the cestode infections. As mentioned above, this efficacy may be due to the PSMs, such as alkaloids which have been reported to be present in this plant (Weissenberg, 2001).

Isoflavones such as daidzein, formononetin, genistein and coumarine have been isolated from the white clover, *T. repens* (Francis and Killington, 1967; Nykanen-Kurki *et al.,* 1993). An anthelmintic efficacy of genistein, an isoflavone, has been well recognized against cestode, *Raillietina echinobothrida* in recent times (Tandon *et al.*, 1997; Tandon *et al.,* 2003), and antifilarial activity of this plant extract against *Setaria cervi* has also been studied (Tangpu and Yadav, 2003). With regard to the present study, the effect of *T. repens* aerial part extract in *H. diminuta* infections in rats was evident from elimination of 70.00, 73.40 and 76.60% of the parasites from the infected animals when *T. repens* extract was treated at larval, immature and adult stages, respectively. This trend indicates that the extract had more or less similar effects against all stages of the parasites. And the efficacy may be assumed to be due to the presence of isoflavones, genistein in particular.

The result of *Z. rhetsa* activity against *H. diminuta* infections in rats attests that 100% of the infected rats were devoid of worm load when treated at 800 mg/kg double doses of the plant extract on days 2-4 post-infection, though treatment of the same on days 8-10 and days 21-25 post-infection showed worm reduction rates of 66.60 and 88.00%, respectively. This plant extract has been shown to have effects against filarial worm, *S. cervi*, by Tangpu and Yadav (2003). A phytochemical study reveals the presence of terpenoids (Mathur *et al.*, 1967) and 3, 5-Dimethoxy-4-geranyloxycinnamyl alcohol, 8-methoxy-*N*-methylflindersine, xanthyletin and sesamin (Ahsan *et al.*, 2000) from this plant. Though it is not certain which compound is responsible for the efficacy at this stage, it is evident from the current study that *Z. rhetsa* is a potential candidate for to be adjudged as an agent to treat tapeworm infections.

The current investigation on acute toxicity effect of the plant extracts by determining LD_{50} (Oral; mg/kg) reveals high values ranging from 2737.34 for *Z. rhetsa*, 3093.24 for *S. myriacanthum*, 3200.03 for *B. minor*, 3755.62 for *A. vasica* to 6993.18 for *S. discolor*. However, there was no mortality observed in the case of *T. repens* extract even up to 3200 mg/kg dose treatment, so it is secure to conclude that using of *T. repens* would be almost harmless in case of humans, as well. This study indicates that the plant extracts having such high LD_{50} values are practically safe to use, as also native people in Manipur use these plants' preparations without alleging any complaints of side effects.

As discussed in Chapter 1, liver is the major organ playing an important role in metabolism of drugs in animals including human. Various serum aminases are the indicators of damages and injuries in this organ; releasing of dying and injured cells into body fluids. On assaying the four serum parameters, viz. SGOT, SGPT, cholesterol and total protein in the present study, the rats treated with 800 mg/kg dose of plant extracts did not show any significant change in the level of these parameters as compared to the appropriate controls for most of the plants tested. Exceptions to this observation are those of *S. discolor, A. vasica* and *Z. rhetsa* extracts showing an increased rise in the levels of SGOT and SGPT, as compared to that of the control. Moreover, there was no significant change in the levels of cholesterol and total protein for all the six plant-extracts tested from the control. Therefore, this preliminary study of acute toxicity indicates that the barring exceptions, other plant extracts are relatively harmless to the animals. However, a more comprehensive investigation would be required to remark further on the toxicity of these plant extracts in other suitable animal models as well.

There is a vast scope of research on the folklore medicinal plants in this part of northeast India, where a major portion of information still remains unexplored in the light of scientific justification. It is, with this current study taken up, a conclusion may be drawn that the experimental investigation authenticates the presence of anticestodal properties in these six folklore medicinal plants, which may have therapeutic advantages to apply in humans encountering with

tapeworm infections. These plants may have potentials for exploring their efficacy profile against other helminth parasites too. Further, they are safe to use without revealing adverse side effects. Identification and characterization of the active principles from the plants and the detailed elucidation of mechanism of their anticestodal action needs to be addressed in detail, and also further proof of their non-toxicity remains opened.

SUMMARY

The present work incorporates a study on ascertaining the antidiarrhoeal and anticestodal potentials of some plants that are used in the folklore medicine system of tribal populations in Manipur, a northeastern state of India. The study aimed at 1) Evaluating the antidiarrhoeal efficacy of some folklore medicinal plants *in experimentally induced diarrhoea in albino mice*, 2) Ascertaining the anticestodal property of such traditional medicinal plants against cestode parasite, *Hymenolepis diminuta*, *in vitro* as well as *H. diminuta* – rat *in vivo* models, 3) Comparing their activities with respective reference antidiarrhoeal and anticestodal drugs, and 4) Studying their acute toxicity effects in these animal models by determining LD_{50} values of the plant extracts and also by assaying changes in some blood serum biochemical parameters.

To evaluate the antidiarrhoeal efficacy of folklore medicinal plants, nine plant species, namely – *Rhus javanica* L. (Anacardiaceae), *Galinsoga parviflora* Cav. (Asteraceae), *Bidens pilosa* L. (Asteraceae), *Swertia angustifolia* Buch.-Ham. ex D. Don. (Gentianaceae), *Lithocarpus dealbata* Rehder (Fagaceae), *Cymbopogon citratus* (DC) Stapf (Gramineae), *Zingiber cassumunar* Roxb. (Zingiberaceae), *Urena lobata* L. (Malvaceae) and *Potentilla fulgens* Wall. ex Hook. (Rosaceae) were included based upon a questionnaire response conducted among native people in Manipur, where these plants emerged out to

be the most commonly used in the traditional practice. The plant extracts were prepared in methanol and tested for their antidiarrhoeal activity against experimentally induced diarrhoea in albino mice. The activity was assessed by four different approaches: 1) Measurement of faecal output, 2) Castor oil-induced diarrhoea, 3) PGE_2-induced enteropooling, and 4) Gastrointestinal transit test.

The different plant extracts were administered to animals at four different doses: 100, 200, 400 and 800 mg/kg, p.o. The results show dose-dependent antidiarrhoeal effects in all the four study parameters for all nine plant extracts. The plant extracts' maximum doses could reduce the faecal output by 55.09% for *S. angustifolia*, 53.69% for *B. pilosa*, 53.57% for *R. javanica*, 53.44% for *C. citratus*, 47.57% for *L. dealbata*, 33.22% for *G. parviflora*, 26.37% for *P. fulgens*, 21.34% for *Z. cassumunar* and 19.48% for *U. lobata*. In the castor oil-induced diarrhoea study, there was a significant fall in the number of diarrhoeal episodes in all the treated animals, and the most interesting results emerged from treatment with extracts of *R. javanica, S. angustifolia, C. citratus* and *L. dealbata* where 66.67% of animals were protected from diarrhoea provoked by castor oil.

PGE_2 could increase the volume of small intestinal fluids accumulated per 100 g mouse from 1.35 ml in normal control to 3.21 ml in vehicle control animals. The plant extracts significantly reduced the intestinal fluid accumulation from 18.47% (*Z. cassumunar* extract at 800 mg/kg dose) to 40.50% (*R. javanica*

at 800 mg/kg dose). The distance travelled by the charcoal marker in the small intestines of the treated groups with different extracts showed significant difference from the control, and the best inhibition of the intestinal transit was exhibited by *U. lobata* extract (57.47% inhibition), followed by *C. citratus* extract (57.22%) and by *R. javanica* extract (55.84%).

In all the experiments, Loperamide was also tested as the reference drug at 5 and 10 mg/kg, p.o. doses. It emerged out that treatment with Loperamide showed reduction in faecal output by 33.74-57.31%, animals' protection from diarrhoea was 66.67-100%, and reduced intestinal fluids accumulation by 25.44-39.93% and showed inhibition in gastrointestinal transit by 50.40-58.57%. Simultaneously, two active components of plants; citral, an active essential oil component of *C. citratus* and quercetin, a major flavonoid component of *U. lobata* were tested with the same doses of the standard reference drug, and their efficacy was almost comparable with that of the reference drug, Loperamide.

The anticestodal efficacy of six plant extracts [namely, *Strobilanthes discolor* T. Anders (Family: Acanthaceae), *Adhatoda vasica* Nees. (Family: Acanthaceae), *Butea minor* Ham. in Wall (Family: Fabaceae; Papilionaceae), *Solanum myriacanthum* Dunal (Family: Solanaceae), *Trifolium repens* L. (Family: Fabaceae; Papilionaceae) and *Zanthoxylum rhetsa* DC (Family: Rutaceae)] was ascertained by testing their extracts against *Hymenolepis*

diminuta parasites both *in vitro* as well as *in vivo* models. The *in vitro* efficacy was found to be most significant for *S. discolor* extract treatment, where parasites showed paralysis at 0.92 h and death at 2.58 h post-incubation. While other five extracts also showed notable differences from the untreated control worms (parasites incubated in control medium showed paralysis at 29.17 ± 3.06 h and death at 29.75 ± 3.04 h).

In vivo testing of six plant extracts was carried out against *H. diminuta* infections in rats. The treatments were given at three different stages of parasites; the larval, immature and adults. Efficacy was adjudged by counting the eggs per gram of faeces (EPG), worm recovery and host clearance at necropsy. The results indicated that there were significant changes in all these parameters in the treated groups of animals as compared to control. However, the most remarkable effect was achieved by *S. discolor* and *Z. rhetsa* extracts where the treatment given at 800 mg/kg, p.o. doses on days 2-4 post-inoculation, totally eliminated *H. diminuta* infection from the experimental rats as evident by monitoring EPG and worm recovery rate. Throughout the experiments, praziquantel, a broad anticestodal drug, was also tested at 5 and 25 mg/kg, p.o. doses as a reference drug for comparing efficacy of the extracts. And the effects of most plant extracts were almost comparable with that of this reference agent.

Studies on acute toxicity effects of the fifteen plant extracts by determining LD_{50} revealed high values of lethal doses for nine plants. The LD_{50} (Oral; mg/kg; rat) values were tabulated as 2737.34, 3093.24, 3200.03, 3755.62 and 6993.18 for *Z. rhetsa*, *S. myriacanthum*, *B. minor*, *A. vasica* and *S. discolor* extracts, respectively. Whereas LD_{50} values (Oral; mg/kg; mouse) were charted as 3415.64, 3617.20, 4080.40 and 5355.97 for *B. pilosa, G. parviflora, Z. cassumunar* and *P. fulgens*, respectively. However, no mortality was observed for the other six plants, namely, *R. javanica, S. angustifolia, C. citratus, U. lobata, P. fulgens* and *T. repens* even when treatment was given upto 3200 mg/kg., p.o. and observed for 72 h post-treatment. Further toxicity analysis on some of the serum biochemical profiles yielded 135.33 U/L of SGOT, 118.00 U/L of SGPT, 134.33 mg/dL of cholesterol and 6.73 g/dL of total protein from the blood samples of untreated control mice, and it was 149 U/L of SGOT, 75.83 U/L of SGPT, 122.50 mg/dL of cholesterol and 6.88 g/dL of total protein from that of control rats. There were no major changes in these levels for the blood samples of treated animals with various plant extracts baring negligible exceptions for few plants. Therefore, it is secure to conclude from this acute toxicity study that the plant extracts having no or high LD_{50} values and no significant change in serum biochemistry are practically safe to use, as also native Naga people in Manipur use these plants' preparations without alleging any side effects.

The present investigation appears to provide a scientific base justifying the folkloric use of fifteen medicinal plants which are consumed in the traditional practice of indigenous tribal communities in Manipur, and the study further endows that these plants are safe to use without showing adverse effects.

Eight photographic plates of fifteen plants, eleven graphic figures and thirty-two tables support the study observations carried out in the present work. Total 203 citations are given in the references.

REFERENCES

Abdullahi AL, Agho MO, Amos S, Gamaniel KS and Wambebe C (2001): Antidiarrhoeal activity of aqueous extract of *Termanalia avicennoides* roots. ***Phytotherapy Research 14***: 431-434.

Abe S, Sato Y, Inoue S, Ishibashi H, Maruyama N, Takizawa T, Oshima H and Yamaguchi H (2003): Anti-*Candida albicans* activity of essential oils including Lemongrass (*Cymbopogon citratus*) oils and its active component, citral. ***Nippon Ishinkin Gakkai Zasshi 44:*** 285-291.

Adzu B, Tarfa F, Amos S and Gamaniel KS (2004): The efficacy of *Sphaeranthus senegalensis* Vaill extract against diarrhoea in rats. ***Journal of Ethnopharmacology 95***: 173-176.

Afroz S, Alagir M, Khan MT, Jabbar S, Nahar N and Choudhuri MS (2006): Antidiarrhoeal activity of the ethanol extract of *Paederia foetida* Linn. (Rubiaceae). J***ournal of Ethnopharmacology 105***: 125-230.

Agbor GA, Leopold T and Jeanne NY (2004): The antidiarrhoeal activity of *Alchornea cordifolia* leaf extract. ***Phytotherapy Research 18***: 873-876.

Ahsan M, Tahmina AZ, Choudhury MH, Ito C and Nazrul Islam SK (2000): Constituents and cytotoxicity of *Zanthoxylum rhetsa* stem bark. ***Fitoterapia 71***: 697-700.

Aji T, Tongu Y, Itano K, Inatomi S, Harada M and Suguri S (1983): Ultrastructural changes of the tegument on *Diphyllobothrium erinacei* and

Hymenolepis nana treated with paromomycin sulfate *in vitro*. ***Japanese Journal of Antibiotics* 36**: 585-593.

Akah PA (1989): Purgative potentials of *Euphorbia heterophylla*. ***Fitoterapia* 60**: 45-48.

Akah PA (1996): Antidiarrhoeal activity of *Kigelia africana* in experimental animals. ***Journal of Herbs, Spices and Medicinal Plants* 4**: 31-38.

Akah PA and Offiah VN (1992): Gastrointestinal effects of *Allamanda cathartica* leaf extracts. ***International Journal of Pharmacognosy* 30**: 213-217.

Akah PA, Aguwa CN and Agu RU (1999): Studies on the antidiarrhoeal properties of *Pentaclethra macrophylla* leaf extracts. ***Phytotherapy Research* 13**: 292-295.

Akhtar MS and Ahmad I (1992): Comparative efficacy of *Mallotus philippinensis* fruit (Kamala) or Nilzan (R) drug against gastrointestinal cestodes in Beetal goats. ***Small Ruminant Research* 8**: 121-128.

Akindele AJ and Adeyemi OO (2006): Evaluation of antidiarrhoeal activity of *Byrsocarpus coccineus*. ***Journal of Ethnopharmacology* 18**: [Epub ahead of print] 16750338.

Allain CC, Poon LS, Chan CS, Richmond W and Fu PC (1974): Enzymatic determination of total cholesterol. ***Clinical Chemistry* 20**: 470-475.

Allen SJ, Okoko B, Martinez E, Gregorio G and Dans LF (2003): Probiotics for treating infectious diarrhoea. ***Cochrane Database of Systematic Reviews***

Issue 4: Art. No.: CD003048.pub2. DOI: 10.1002/14651858. CD003048.pub2.

Almeida CE, Kamikowski MG, Foleto R and Baldisserotto B (1995): Analysis of antidiarrhoeal effect of plants used in popular medicines. ***Review of Saude Publication 29***: 428-433.

Alvarez A, Pomar F Sevilla and Montero MJ (1999): Gastric antisecretory and antiulcer activities of an ethanolic extract of *Bidens pilosa* L. var. *radiata* Schult. Bip. ***Journal of Ethnopharmacology 67***: 333-340.

Amresh, Reddy GD, Rao CV and Shirwaikar A (2004): Ethnomedical value of *Cissampelos pareira* extract in experimentally induced diarrhoea. ***Acta Pharmacology 54***: 27-35.

Andrade-Neto VF, Brandao MG, Oliveira FQ, Casali VW, Njaine B, Zalis MG, Oliveira LA and Krettli AU (2004): Antimalarial activity of *Bidens pilosa* L. (Asteraceae) ethanol extracts from wild plants collected in various localities or cultivated in humus soil. ***Phytotherapy Research 18:*** 634-639.

Andrews P and Thomas H (1979): The effect of praziquantel on *Hymenolepis diminuta in vitro*. ***Tropenmedizin und Parasitologie* 30**: 391-400.

Anonymous 1977. Manual of Veterinary Parasitological Techniques. ***Technical Bulletin*** No. 18, London, H. M. S. O. pp. 1-57.

Anonymous (1979): Diarrhoeal Disease Control Programme. ***Weekly Epidemiological Record 16:*** 121.

Anonymous (2005): Cholera, 2004. ***Weekly Epidemiological Record 80***: 261-268.

Arai HP (1980): **Biology of the Tapeworm *Hymenolepis diminuta***. Academic Press, New York, pp 733.

Atal CK (1980): **Chemistry and Pharmacology of vasicine - A new oxytocic and abortifacient,** P.58, New Delhi.

Athanasiadou S, Kyriazakis I, Jackson F and Coop RL (2000): Consequences of long-term feeding with condensed tannins on sheep parasitized with *Trichostrongylus colubriformis*. ***International Journal of Parasitology 30***: 1025-1033.

Athanasiadou S and Kyriazakis I (2004): Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. ***Proceedings of Natural Society 63***: 631-639.

Atta AH and Mouneir SM (2004): Antidiarrhoeal activity of some Egyptian medicinal plant extracts. ***Journal of Ethnopharmacology 92***: 303-309.

Atta AH and Mouneir SM (2005): Evaluation of some medicinal plant extracts for antidiarrhoeal activity. ***Phytotherapy Research 19:*** 481-485.

Bankole SA, Joda AO and Ashidi JS (2005): The use of powder and essential oil of *Cymbopogon citratus* against mould deterioration and aflatoxin contamination of "egusi" melon seeds. ***Journal of Basic Microbiology 45***: 20-30.

Bass P, Kennedy JA and Wiley JN (1972): Measurement of fecal output in rats. ***American Journal of Digestive Diseases 17***: 925-928.

Becker B, Mehlhorn H, Andrews P and Thomas H (1981): Ultrastructural investigations on the effect of praziquantel on the tegument of five species of cestodes. ***Zeitschrift für Parasitenkunde 64***: 257-269.

Besra SE, Gomes A, Chaudhury L, Vedasiromini JR and Ganguly DK (2002): Antidiarrhoeal activity of seed extract of *Albizzia lebbeck* Benth. ***Phytotherapy Research 16***: 529-533.

Besra SE, Gomes A, Ganguly DK and Vedasiromini JR (2003): Antidiarrhoeal activity of hot water extract of black tea (*Camellia sinensis*). ***Phytotherapy Research 17***: 380-384.

Bhattacharyya D, Pandit S, Jana U, Sen S and Sur TK (2005): Hepatoprotective activity of *Adhatoda vasica* aqueous leaf extract on D-galactosamine-induced liver damage in rats. ***Fitoterapia 76***: 223-225.

Biswas S, Murugesan T, Sinha S, Maiti K, Gayen JR, Pal M and Saha BP (2002): Antidiarrhoeal activity of *Strychnos potatorum* seed extract in rats. ***Fitoterapia 73***: 43-47.

Bogh HO, Andreassen J and Lemmich J (1996): Anthelmintic usage of extracts of *Embelia shimperi* from Tanzania. ***Journal of Ethnopharmacology 50***: 35-42.

Brandao MG, Krettli AU, Soares LS, Nery CG and Marinuzzi HC (1997): Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. ***Journal of Ethnopharmacology 57***: 131-138.

Brantner AH and Chakraborty A (1998): In vitro antibacterial activity of alkaloids isolated from *Adhatoda vasica* NEES. ***Pharmaceutical and Pharmacology Letters 8***: 137-139.

Cabrera C (2000): Phytotherapeutic Approaches to Lower Bowel Diseases, Part II Constipation, Diarrhoea, IBS and Diverticular Disease. ***Medical Herbalism 11***: 1-8.

Cavalcanti ES, Morais SM, Lima MA and Santa EW (2004): Larvicidal activity of essential oils from Brazillian plants against *Aedes aegypti* L. ***Memórias Instituto Oswaldo Cruz 92***:: 541-544.

Chakraborty A and Brantner AH (2001): Study of alkaloids from *Adhatoda vasica* Nees on their anti-inflammatory activity. ***Phytotherapy Research* 15**: 532-534.

Chang JS, Chiang LC, Chen CC, Liu LT, Wang KC and Lin CC (2001): Antileukemic activity of *Bidens pilosa* L. var. minor (Blume) Sheriff and *Houttuynia cordata* Thunb. ***American Journal of Chinese Medicines 29***: 303-312.

Charoenlarp P, Radomyos P and Harinasuta, T (1981): Treatment of Taeniasis with Puag-Haad: A crude extract of *Artocarpus lakoocha* wood. ***Southeast Asian Journal of Tropical Medicine and Public Health* 12**: 568-570.

Chiang YM, Chuang DY, Wang SY, Kuo YH, Tsai PW and Shyur LF (2004): Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. ***Journal of Ethnopharmacology 95***: 409-419.

Chiang YM, Lo CP, Chen YP, Wang SY, Yang NS, Kuo YH and Lie FS (2005): Ethyl caffeate suppresses NF-κB activation and its downstream inflammatory mediators, iNOS, COX-2, and PGE_2 *in vitro* or in mouse skin. ***British Journal of Pharmacology 146***: 352-363.

Chin HW, Lin CC and Tang KS (1996): The hepatoprotective effects of Taiwan folk medicine ham-hong-chho in rats. ***American Journal of Chinese Medicines 24:*** 231-240.

Chitme HR, Chandra R and Kaushik S (2003): Studies on anti-diarrhoeal activity of *Calotropis gigantea* R. BR. in experimental animals. ***Journal of Pharmacy and Pharmaceutical Science 7***: 70-75.

Choi EM and Hwang JK (2005): Screening of Indonesian medicinal plants for inhibitor activity on nitric oxide production of RAW264.7 cells and antioxidant activity. ***Fitoterapia 76:*** 194-203.

Cox FEG (1982): ***Modern Parasitology***. Blackwell, Oxford London.

Das C, Poi R and Chowdhury A (2005): HPTLC determination of vasicine and vasicinone in *Adhatoda vasica*. ***Phytochemical Analysis* 16**: 90-92.

Di Carlo G, Mascolo N, Izzo A, Capasso F and Autore G (1994): Effect of quercetin on gastrointestinal tract in rats and mice. ***Phytotherapy Research 8***: 42-45.

Dimo T, Rakotonirina SV, Tan PV, Azay J, Dongo E and Cros G (2002): Leaf methanol extract of *Bidens pilosa* prevents and attenuates the hypertension induced by high-fructose diet in Wistar rats. ***Journal of Ethnopharmacology 83***: 183-191.

Dixon BR and Arai HP (1991): Anthelmintic-induced destrobilation and its influence on calculated drug efficacy in *Hymenolepis diminuta* infections in rats. ***Journal of Parasitology 77***: 769-774.

Dudai N, Weinstein Y, Krup M, Rabinski T and Ofir R (2005): Citral is a new inducer of caspase-3 tumor cell lines. ***Planta Medica 71***: 484-488.

Duke JA (1992): ***Handbook of phytochemical constituents of GRAS herbs and other economic plants***, Boca Raton FL. CRC Press.

Elangbam JS, Yadava PS and Thingbaijam BS (1989): Ethnobotanical study of the Tangkhul Naga tribe of Ukhrul Manipur. Journal of Economic and Taxonomic Botany 13: 11-16.

Engels D, Urbani C, Belotto A, Meslin F and Savioli L (2003): The control of human (neuro) cysticercosis: which way forward? ***Acta Tropica 87***: 177-182.

Evans WS, Hardy M and Novok M (1980): A comparison of the effect of albendazole, cambendazole, and thiabendazole on the larval development of three hymenolepidid cestodes. ***Journal of Parasitology 66***: 935-940.

Fauci AS, Bravnwold E, Isselpacher K, Wilson JD, Martin JB, Kasper DL, Hauser SL and Longo DL (1998): ***Harrison's Principles of Internal Medicine*** (New Yolk, McGraw Hill Company) pp. 236-242.

Fontaine O (1988): Diarrhoea and treatment. ***Lancet 28***: 1234-1235.

Forman LA and Oaks JA (1992): The effect of dimethylsulphoxide on the tegumental brush border of the cestode, *Hymenolepis diminuta*. ***Parasitology Research 78***: 66-73.

Francis CM and Millington AJ (1967): Varietal variation in the isoflavone content of subterranean clover: its estimation by a microtechnique. ***Australian Journal of Agricultural Research 16***: 557-564.

Galal M, Bashir AK, Salih AM and Adam SEI (1991a): Efficacy of aqueous and butanolic fractions of *Albizzia anthelmintica* against experimental *Hymenolepis diminuta* infestation in rats. ***Veterinary and Human Toxicology 33***: 537-539.

Galal M, Bashir AK, Salih AM and Adam SEI (1991b): Activity of water extracts of *Albizzia anthelmintica* and *A. lebbek* barks against experimental *Hymenolepis diminuta* infections in rats. ***Journal of Ethnopharmacology 31***: 333-337.

Galvez J, Crespo ME, Jimenez J, Suarez A and Zarzuelo (1993a): Anti-diarrhoeic activity of quercetin in mice and rats. ***Journal of Pharmacy and Pharmacology 45***: 157-159.

Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA and Jimenez J (1993b): Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. ***Planta Medica 59***: 333-336.

Galvez J, Sanchez de M, Jimenez J, Torres MI, Nunez MC, Rios A, Gil A and Zarzuelo A (1995): Effect of quercetin on lactose-induced diarrhoea in rats. ***Planta Medica 61***: 302-306.

Garcia D, Escalante M, Delgado R, Ubeira FM and Leiro J (2003): Anthelmintic and antiallergic activities of *Mangifera indica* L. stem bark components Vimang and mangiferin. ***Phytotherapy Research 17***: 1203-1208.

Ghosal S, Sharma PV and Jaiswal DK (1978): Chemical constituents of gentianaceae XXIII: Tetraoxygenated and pentaoxygenated xanthones and xanthone O-glucosides of *Swertia angustifolia* Buch.–Ham. ***Journal of Pharmaceutical Science 67***: 55-60.

Ghosh K (2004): A flurocoumarin, imperatonin isolated from *Urena lobata* L. ***Molbank M 382***.

Ghosh NK, Sinha Babu SP, Sukul NC and Ito A (1996): Cestocidal activity of *Acacia auriculiformis*. ***Journal of Helminthology 70***: 171-172.

Girard P, Pansart Y, Lorette I and Gillardin JM (2005): Dose-response relationship and mechanism of action of *Saccharomyces boulardii* in castor oil-induced diarrhea in rats. ***Digestive Diseases and Sciences 48***: 770-774.

Githiori JB, Hoglund J and Waller PJ (2005): Ethnoveterinary plant preparations as livestock dewormers: Practices, popular beliefs, pitfalls and prospects for the future. ***Animal Health Research Review 6***: 91-103.

Guarrera PM (1999): Traditional anthelmintic, antiparasitic and repellant uses of plants in central Italy. ***Journal of Ethnopharmacology 68***: 183-192.

Guha S, Ghosal S and Chattopadhya U (1996): Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. ***Chemotherapy 42***: 433-451.

Gupta S and Srivastava JK (1994): Test models for Enteric Helminthiasis. In: ***Cultivation of Parasites***. Proceedings of Workshop on Cultivation of

Parasites of Biomedical Importance. Central Drug Research Institute, Lucknow, pp. 74-94.

Hajhashemi V, Sadraei H, Ghannadi AR and Mohseni M (2000): Antispasmodic and anti-diarrhoeal effects of *Satureja hortensis* L. essential oil. ***Journal of Ethnopharmacology 71***: 187-192.

Hamdy EI, Ahmed TH, Amin FM, el-Matarawy OM and el-Rahimy HH (1984): Laboratory studies on the ovicidal and larvicidal effects some artificial fertilizers. ***Journal of the Egyptian Society for Parasitology* 14**: 131-136.

Han AR, Kim MS, Jeong YK, Lee SK and Seo EK (2005): Cyclooxygenase-2 inhibitory phenylbutenoids from the rhizomes of *Zingiber cassumunar*. ***Chemical and Pharmaceutical Bulletin (Tokyo) 53***: 1466-1468.

Hart RJ, Turner R and Wilson RG (1977): A biochemical and ultrastructural study of the mode of action of bunamidine against *Hymenolepis nana*. ***International Journal for Parasitology* 7**: 129-134.

Heinrich M, Heneka B, Ankli A, Rimpler H, Sticher O and Kostiza T (2005): Spasmolytic and antidiarrhoeal properties of the Yucatec Mayan medicinal plant *Casimiroa tetrameria*. ***Journal of Pharmacy and Pharmacology 57***: 1081-1085.

Henry RJ, Cannon DC and Winkelman JW (1974): ***Clinical Chemistry: Principles and Techniques***, New York, 2nd Edition, Harper & Row Publisher, pp. 1288.

Hildreth MB, Pappas PW and Oaks JA (1997): Effect of tunicamycin on the uptake and incorporation of galactose in *Hymenolepis diminuta*. ***Journal of Parasitology* 83**: 555-558.

Hipkiss JB, Skinner A and Brandford White CJ (1987): Biochemical and ultrastructural investigation of the effects of Stelazine (trifluoperazine) on *Hymenolepis diminuta* (Cestoda). ***Parasitology* 94**: 135-149.

Hipkiss JB, Brandford White CJ, Peters TJ and Whish WJ (1995): Influence of trifluoperazine on tegument membranes in *Hymenolepis diminuta*. ***Biochemical Society Transactions* 23**: 572S.

Hopkins CA, Grant PM and Stallard H (1973): The effect of oxyclozanide on *Hymenolepis microstoma* and *H. diminuta*. ***Parasitology* 66**: 355-365.

Huang Paul L, Huang Philip L, Huang P, Huang HI and Huang SL (1992): Developing drugs from traditional plants. ***Chemical Industry* 8**: 290-293.

Ito A, Nakao M and Wandra T (2003): Human Taeniasis and cysticercosis in Asia. ***Lancet* 362**: 1918-1920.

Izzo AA (1994): Effects of quercetin on gastrointestinal tract: further studies. ***Phytotherapy Research* 8**:179-185.

Izzo AA, Gaginella TS, Mascolo N and Capasso F (1994): Nitric oxide as mediator of the laxative action of magnesium sulphate. ***British Journal of Pharmacology* 113**: 228-232.

Izzo AA, Mascolo M and Capasso F (1998): Nitric oxide as a modulator of intestinal water and electrolyte transport. ***Digestive Diseases and Sciences* 43**: 1605-1620.

Jacoby HI, Moore G and Mnorowski G (2001): Inhibition of diarrhoea by immune egg: A castor oil mouse model. ***Journal of Nutraceuticals, functional and Medical Foods*** 3: 47-53.

Jaffe BM (1979): Prostaglandins and serotonins: Nonpeptide diarrhoegenic hormones. ***World Journal of Surgery 3:*** 565-578.

Jeenapongsa R, Yoovathaworn K, Sriwatanakul KM, Pongprayoon U and Sriwatanakul K (2003): Anti-inflammatory activity of (E)-1-(3-4-dimethoxyphenyl) butadiene from *Zingiber cassumunar* Roxb. ***Journal of Ethnopharmacology 87***: 143-148.

Kala CP, Dhyani PP and Sajwan BS (2006): Developing the medicinal plants sector in northern India: challenges and opportunities. ***Journal of Ethnobiology and Ethnomedicine 2***: 32.

Katzung BG (2004): ***Basic and clinical Pharmacology***. Boston, McGraw Hill, 1202 pp.

Kavitha D, Shilpa PN and Devaraj SN (2004): Antibacterial and antidiarrhoeal effects of alkaloids of *Holarrhena antidysenterica* WALL. ***Indian Journal of Experimental Biology 42***: 589-594.

Khan MR, Kihara M and Omoloso AD (2001a): Anti-microbial activity of *Bidens pilosa, Bischofia japonica, Elmerillia papuana* and *Sigesbekia orientalis*. **Fitoterapia 72**: 662-665.

Khan MR, Kihara M and Omoloso AD (2001b): Antimicrobial activity of *Lithocarpus celebicus*. ***Fitoterapia 72:*** 703-705.

Kim HS (2005): Do not put too much value on conventional medicines. ***Journal of Ethnopharmacology 100***: 37-39.

Kim SH, Park HH, Lee S, Jun CD, Choi BJ, Kim SY, Kim SH, Kim DK, Park JS, Chae BS and Shin TY (2005): The anti-anaphylactic effect of the gall of *Rhus javanica* is mediated through inhibition of histamine release and inflammatory cytokine secretion. ***International Immunopharmacology 5***: 1820-1829.

Kurokawa M, Basnet P, Ohsugi M, Hozumi T, Kadota S, Namba T, Kawana T and Shiraki K (1999): Anti-herpes simplex virus activity of moronic acid purified from *Rhus javanica in vitro* and *in vivo*. ***Journal of Pharmacology and Experimental Therapy 289***: 72-78.

Lateef M, Igbal Z, Khan MN, Akhtar MS and Jabbar A (2003): Anthelmintic activity of *Adhatoda vasica* roots. ***International Journal of Agricultural Biology 5***: 86-90.

Lee IS, Oh SR, Ahn KS and Lee HK (2001): Semialactone, isofouquierone peroxide and fouquierone, three new dammarane triterpenes from *Rhus javanica*. ***Chemical and Pharmaceutical Bulletin (Tokyo) 49***: 1024-1026.

Levy G (1982): Gastrointestinal clearance of drugs with activated charcoal. ***New English Journal of Medicine 307***: 676-678.

Lin J, Puckree T and Mvelase TP (2002): Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. ***Journal of Ethnopharmacology 79***: 53-56.

Lorke D (1983): A new approach to practical acute toxicity testing. ***Archives of Toxicology 54:***275-87.

Luderer JR, Dermers IM and Hayes AH Jr. ((1980): ***Advances in Prostaglandin and Thromboxane Research***. New Yolk, Raven Press, pp. 1633-1638.

Luo YH and Nie RL (1992): Studies on iridoid glycosides from *Swertia angustifolia*. ***Yao Xue Xue Bao 27***: 125-129.

Luong TV (2003): De-worming school children and hygiene intervention. ***International Journal of Environment and Health Research Supplementary* 1S**: 153-159.

Lutterodt GD (1992): Inhibition of Microlax-induced experimental diarrhoea with narcotic-like extracts of *Psidium guajava* leaf in rats. ***Journal of Ethnopharmacology 37***: 151-157.

Lutterodt GD (1989): Inhibition of gastrointestinal release of acetylcholine by quercetin as possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal diseases. ***Journal of Ethnopharmacology 25***: 235-247

Mabeku KLB, Beng PV, Kouam J, Ngadjui BT, Fomum ZT and Etoa FX (2006): Evaluation of antidiarrhoeal activity of the stem bark of *Cylicodiscus gabunensis* (mimosaceae). ***African Journal of Biotechnology 5***: 1062-1066.

Macedo ME, Consoli RAGB, Grandi TSM, Anjos AMGD, Oliveira ABD, Mendes NM, Queiroz RO and Zani CL (1997): Screening of Asteraceae

(Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis*. ***Memórias Instituto Oswaldo Cruz 92***: 565-570.

Mahato SB, Sarkar SK and Poddar G (1988): Triterpenoid saponins. ***Phytochemistry 27***:3037–3067.

Majumdar AM, Upadhye AS and Misar AV (2000): Studies on antidiarrhoeal activity of *Jatropha curcus* root extract in albino mice. ***Journal of Ethnopharmacology 70***: 183-187.

Maki J, Kondo A and Yanagisawa T (1983): Efficacy of alcoholic extract from Ma-Klua (*Diospyros mollis*) on adults and the larvae of the dwarf tapeworm, *H. nana* in mice and on the infectivity of the eggs. ***Parasitology 87***: 103-111.

Mata L (1983): Influence of growth parameters in children. In: ***Acute diarrhoea and its nutritional consequences in children***. Ballanti JA (Ed.). Nestle/Raven Press, New Yolk, pp. 85-94.

Mathur RK, Ramaswamy SK, Rao AS and Bhattarcharyya SC (1967): Terpenoids. 108. Isolation of an oxidodiol from *Zanthozylum rhetsa*. ***Tetrahedron 23***: 2495-2498.

Mazumder R, Bhattacharya S, Mazumder A, Pattnaik AK, Tiwary PM and Chaudhary S (2006): Antidiarrhoeal evaluation of *Aegle marmelos* (Correa) Linn. root extract. ***Phytotherapy Research 20***: 82-84.

Mazumder UK Gupta M, Manikandan L and Bhattacharya S (2001): Antibacterial activity of *Urena lobata* roots. ***Fitoterapia 72***: 927-929.

Mohamad ASA, Mori T, Islam SQ, Sato M and Yamasaki T (2000): Lethal activity of gallo- and condensed tannins against the free-living soil-inhabiting nematodes, *Caenorhabditis elegans*. ***Journal of pesticide and Science 25***: 410-415.

Molgaard P, Nielsen SB, Rasmussen DE, Drummond RB, Makaza N and Andreassen J (2001): Anthelmintic screening of Zimbabwean plants traditional used against schistosomiasis. ***Journal of Ethnopharmacology 74***: 257-264.

Mueller-Harvey I and McAllan AB (1992): Tannins: their biochemistry and nutritional properties. ***Advances in Plant Cell Biochemistry and Biotechnology 1***: 151-217.

Mukherjee PK, Das J, Balasubramanian R, Saha K, Pal M and Saha BP (1995): Antidiarrhoeal evaluation of *Nelumbo nucifera* rhizome extract. ***Indian Journal of Pharmacology 27***: 262-264.

Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M and Saha BP (1998): Screening of antidiarrhoeal profile of some plant extract of a specific region of West Bengal, India. ***Journal of Ethnopharmacology 60***: 85-89.

Murugesan T, Ghosh L, Mukherjee K, Das J, Pal M and Saha BP (2000): Evaluation of antidiarrhoeal profile of *Jussiaea suffruticosa* Linn. extract in rats. ***Phytotherapy Research 14***: 381-383.

Nagano T, Oyama Y, Kajita N, Chikahisa L, Nakata M, Okazaki E and Masuda T (1997): New curcuminoids isolated from *Zingiber cassumunar* protect cells

suffering from oxidative stress: a flow-cytometric study using rat thymocytes and H_2O_2. ***Japanese Journal of Pharmacology 75***: 363-370.

Nakatani N (2000): Phenolic antioxidants from herbs and spices. ***Biofactors 13***: 141-146.

Novak M and Blackburn BJ (1985): Comparison of the effects of imidazol [1, 2-a] pyridine-2-carbamates and benzimidazole-2-carbamates on the development of *Hymenolepis nana* in *Tribolium confusum*. ***Experimentia 41***: 687-689.

Nwafor PA and Okwuasaba FK (2001): Effect of methanolic extract of *Cassia nigricans* leaves on rat gastrointestinal tract. ***Fitoterapia 72***: 206-214.

Nykanen-Kurki P, Saloniemi H, Kallela K and Saastamoinen I (1993): Phyto-oestrogen content and oestrogenic effect of white clover. ***White clover in Europe: State of the art Series title: REU Technical Series – 29***.

Oben JE, Assi SE, Agbor GA and Musoro DF (2006): Effect of *Eremomastax speciosa* on experimental diarrhoea. ***African Journal of Traditional, Complementary and Alternative Medicines 3***: 95-100.

Oladele GM and Abatan MO (2004): Histopathological and serum biochemical changes following oral administration of aqueous crude extract of *Hypis suaveolena, Urena lobata* and *Cleome viscosa* in rats. ***Tropical Veterinary 22***: 9-15.

Otshudi AL, Vercruysse A and Forriers A (2001): Antidiarrhoeal activity of root extracts from *Roureopsis obliquifoliolata* and *Epinetrum villosum*. ***Fitoterapia 72:*** 291-294.

Ozaki Y, Kawahara N and Harada M (1991): Anti-inflammatory effects of *Zingiber cassumunar* Roxb. and its active principles. ***Chemical and Pharmaceutical Bulletin (Tokyo) 39:*** 2353-2356.

Paolini V, Bergeaud JP, Grisez C, Prevot F, Dorchies P and Hoste H (2003): Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. ***Veterinary Parasitology 113***: 253-261.

Pappas PW and Barley AJ (1999): Beetle-to-beetle transmission and dispersal of *Hymenolepis diminuta* (Cestoda) eggs via the feces of *Tenebrio molitor*. ***Journal of Parasitology 85***: 384-385.

Park K (2000): ***Park's Textbook of Preventive and Social Medicine.*** Jabalpur, India, Banarsidas Bharat Publishers, pp. 172-175.

Pegram GC, Rollins N and Espey Q (1998): Estimating the costs of diarrhoea and epidemic dysentery in KwaZulu-Natal and South Africa. ***Water South Africa 24***: 11-20.

Pereira RL, Ibrahim T, Lucchetti L, Da Silva AJ and Goncalves de Moraes VL (1999): Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. ***Immunopharmacology 43***: 31-37.

Perez GS, Perez GC and Zavala SMA (2005): A study of the antidiarrhoeal properties of *Loeselia mexicana* on mice and rats. ***Phytomedicine 12***: 670-674.

Perrucci S, Fichi G, Buggiani C, Rossi G and Flamini G (2006): Efficacy of mangiferin against *Cryptosporidium parvum* in a neonatal mouse model. ***Parasitology Research 99***: 184-188.

Pierce NF, Carpenter CC Jr., Elliot KL and Greenough WB (1971): Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. ***Gastroenterology 60***: 22-32.

Pillai NR (1992): Antidiarrhoeal Activity of *Punica granatum* in experimental animals. ***International Journal of Pharmacognosy 30***: 201-204.

Raether W and Hanel H (2003): Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections: an update. ***Parasitology Research*** 91: 412-438.

Rahman MT, Alimuzzaman M, Ahmad S and Chowdhury AA (2002): Antinociceptive and antidiarrhoeal activity of *Zanthoxylum rhetsa*. ***Fitoterapia 73***: 340-342.

Rao VRN, Santos FA, Sobreira TT, Souza MF, Melo CL and Silveira ER (1997): Investigations on the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from *Egletes viscosa*. ***Planta Medica 63***: 146-148.

Rasfon K (1991): The anticestodal activity of preparations made from the breadfruit. ***Meditsinskaia Parazitologiia i Parazitarnye Bolezni 5***: 49-52.

Rauber Cda S, Guterres SS and Schapoval EE (2005): LC determination of citral in *Cymbopogon citratus* volatile oil. ***Journal of Biomedicine Analysis 37***: 597-601.

Recio MC, Giner RM, Manez S, Gueho J, Julien HR, Hostettmann K and Rios JL (1995): Investigations on the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. ***Planta Medica 61***: 9–12.

Rivera DG, Balmaseda IH, Leon AA, Hernandez BC, Montiel LM, Garrido GG, Cuzzocrea S and Hernandez RD (2006): Anti-allergic properties of *Mangifera indica* L. extract (Vimang) and contribution of its glucosylxanthone mangiferin. ***Journal of Pharmacy and Pharmacology 58***: 385-392.

Robert A (1973): Prostaglandins and gastric secretion. ***Research in Prostaglandins 2***: 1-4.

Robert A, Nezamis JE, Lancaster C, Hanchar AJ and Klepper MS (1976): Enteropooling assay: a test for diarrhea produced by prostaglandins. ***Prostaglandins 11***: 809-828.

Rosangkima G and Prasad SB (2004): Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dalton's lymphoma. ***Indian Journal of Experimental Biology 42:*** 981-988.

Rouf AS, Islam MS and Rahman MT (2003): Evaluation of antidiarrhoeal activity *Rumex maritimus* root. ***Journal of Ethnopharmacology 84***: 307-310.

Ryu SY, Oak MH, Yoon SK, Cho DI, Yoo GS, Kim TS and Kim KM (2000): Anti-allergic and anti-inflammatory triterpenes from herb of *Prunella vulgaris*. ***Planta Medica 66***: 358–360.

Sadraei H, Asghari G and Naddafi A (2003a): Relaxant effect of essential oil and hydro-alcoholic extract of *Pycnocycla spinosa* Decne. ex Boiss. on ileum contractions. ***Phytotherapy Research 17***: 645-649.

Sadraei H, Ghannadi H and Malekshahi K (2003b): Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. ***Fitoterapia 74***: 445-452.

Saha A, Ghosh NK and Sinha Babu SP (1999): Cestocidal activity of *Gladiolus gandavensis*. ***Journal of Parasitic Diseases 23***: 135-136.

Sairam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Shankar M and Venkataraman S (2003): Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica*. ***Journal of Ethnopharmacology 84***: 11-15.

Salah AM, Gathumbi J and Vierling W (2002): Inhibition of intestinal motility by methanol extracts of *Hibiscus sabdariffa* L. (Malvaceae) in rats. ***Phytotherapy Research 16***: 283-285.

Savioli LD, Bundy DAP and Tompkins A (1992): Intestinal parasitic infections: a soluble public health problem. ***Transaction of the Royal Society of tropical Medicine and Hygiene 86***: 352-354.

Shiraki K, Yukawa T, Kurokawa M and Kageyama S (1998): Cytomegalovirus infection and its possible treatment with herbal medicines. ***Nippon Risho 56***: 156-160.

Shiva MP (1996): Inventory of Forestry Resources for Sustainable Management and Biodiversity Conservation, New Delhi, Indus Publishing Company. Indus Publishing Company; 1996.

Shoba FG and Thomas M (2001): Study of antidiarrhoeal activity of four medicinal plants in castor oil-induced diarrhoea. ***Journal of Ethnopharmacology 76***: 73-6.

Siles-Lucas M and Hemphill A (2002): Cestode Parasites: Application of *In Vivo* and *In Vitro* Models for Studies on the Host – Parasite Relationship. ***Advances in Parasitology* 51**: 134-230.

Skocibusic′ M and Bezic′ N (2003): Chemical composition and antidiarrhoeal activities of winter Savory (*Satureja Montana* L.) essential oil. ***Pharmaceutical Biology 41***:622-626.

Stricklad RD, Freeman ML and Gurule FF (1961): Copper Binding by Proteins in Alkaline Solution. ***Annals of Chemistry 33***.

Subeki, NS, Matzuura H, Yamasaki M, Yamata O, Maede Y, Katakura K, Suzuki M, Trimurningsih, Chairul and Yoshihara T (2005): Anti-babesial activity of some central Kalimantan plant extracts and active oligostilbenoids from *Shorea balangeran*. ***Planta Medica 71***: 420-423.

Sumita TC, Furlan MR, Jorge AO and Ueno M (2004): Antibacterial activity of essential oils on microorganisms isolated from urinary tract infection. ***Review of Saude Publications 38***: 326-328.

Sweis J, Robak J, Dabrowski L, Dunicz S, Michalska Z and Gryglenski RJ (1984): Antiaggregatory effects of flavonoids in vitro and their influence on lipozygenase and cyclooxygenase *in vitro*. ***Polish Journal of Pharmacology and Pharmacy 36***: 455-463.

Syder JD and Merson MH (1982): The magnitude of the global problem of acute diarrhoeal diseases: A review of active surveillance of data. ***Bulletin of World Health Organization 60***: 605-613.

Syiem D, Syngai G, Khup PZ, Khongwir BS, Kharbuli B and Kayang H (2002): Hypoglycemic effects of *Potentilla fulgens* L. in normal and alloxan-induced diabetic mice. ***Journal of Ethnopharmacology 83***: 55-61.

Tan PV, Dimo T and Dongo E (2000): Effects of methanol, cyclohexane and methylene chloride extracts of *Bidens pilosa* on various gastric ulcer models in rats. ***Journal of Ethnopharmacology 73***: 415-421.

Tandon V, Pal P Roy B, Rao HSP and Reddy KS (1997): *In vitro* anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. ***Parasitology Research 83***: 492-498.

Tandon V, Das B and Saha N (2003): Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): Effect of genistein on glucose metabolism in the cestode, *Raillietina echinobothrida* ***Parasitology International* 52**: 179-183.

Tangpu V and Yadav AK (2003): *In vitro* filaricidal activity of some folklore medicinal plants of Manipur, India. In: Gupta N, Gupta DK, eds., ***Parasites and Diseases***: Bareilly, Neeraj Publisher pp. 127–132.

Taniguchi S, Yazaki K, Yabuuchi R, Kawakami K, Ito H, Hatano T and Yoshida T (2000): Galloylglucoses and riccionidin A in *Rhus javanica* adventitious root cultures. ***Phytochemistry 53***: 357-363.

Taylor CE and Murant AF (1966): Nematicidal activity of aqueous extracts from raspberry canes and roots. ***Nematologica 12***: 488-494.

Tchoumbougnang F, Zollo PH, Dagne E and Mekonnen Y (2005): *In vivo* antimalarial activity of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* on mice infected with *Plasmodium berghei*. ***Planta Medica 71***: 20-23.

Temjenmongla and Yadav AK (2005): Anticestodal efficacy of folklore medicinal plants of Naga tribes in north-east India. ***African Journal of Traditional, Complementary and Alternative Medicines 2***: 129-133.

Temjenmongla, Tangpu V and Yadav AK (2006): Anticestodal efficacy of *Psidium guajava* against experimental *Hymenolepis diminuta* infection in rats. ***Indian Journal of Pharmacology 38***: 29–32.

Thapar N and Sanderson IR (2004): Diarrhoea in children: an interface between developing and developed countries. ***Lancet 363***: 641-653.

Ubillas RP, Mendez CD, Jolad SD, Luo J, King SR, Carlson TJ and Fort DM (2000): Antihyperglycemic acetylenic glucosides from *Bidens pilosa*. ***Planta Medica 66***: 82-83.

Uddin SJ, Mondal K, Shilpi JA and Rahman MT (2005): Antidiarrhoeal activity of *Cyperus rotundus*. ***Fitoterapia 77***: 134-136.

Usami E, Kusano G, Katayose T, Wachi H and Seyama Y (2004): Assessment of antioxidant activity of natural compound by water- and lipid-soluble antioxidant factor. ***Yakugaku Zasshi 124***: 847-850.

Valame SP, Tanksale OKG and Wet UK (1974): Liver function tests in rats – Effects of prolonged administration of some drugs used clinically. ***Indian Journal of Pharmacology 6***: 128-133.

Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Kumar SGV, Rajarajan T and Perianayagam JB (2005): Anti-diarrhoeal potential of *Asparagus racemosus* wild root extracts in laboratory animals. ***Journal of Pharmacology and Pharmaceutical science 8***: 39-45.

Vidari G, Vita Finzi P, Zarzuelo A, Galvez J, Zafra C, Chiriboga X, Berenguer B, La Casa C, Alarcon de la Lastra C, Motilva V and Martin MJ (2003): Antiulcer and antidiarrhoeic effect of *Baccharis teindalensis*. ***Pharmaceutical Biology 41***: 405-411.

Vimala S, Norhanom AW and Yadav M (1999): Anti-tumor promoter activity in Malaysian ginger rhizobia used in traditional medicine. ***British Journal of Cancer 80***: 110-116.

Vitali F, Bonina FP, Saija A, Tomaino A, Fonte G, Pennisi C and Tita B (2005): Studies on antidiarrhoeal activity of Jacquez grapes in mice. ***Phytotherapy Research 19***: 924-927.

Wagner H (1989): Search for new plant constituents with potential antiphlogistic and antiallergic activity. ***Planta Medica 55***: 235-241.

Wannissorn B, Jarikasem S, Siriwangchai T and Thubthimthed S (2005): Antibacterial properties of essential oils from Thai medicinal plants. ***Fitoterapia 76:*** 233-236.

Weber DM (1976): The diarrhoeal diseases and food-borne illness. In: ***Tropical Medicine***. Hunter GW, Swartzwelder JC and Clyde DF (Eds.), WB Saunders Co.: Philadelphia.

Weissenberg M (2001): Isolation of Solasodine and other alkaloids and sapogenins by direct hydrolysis-extraction of *Solanum* plants or glycosides there from. ***Phytochemistry* 58**: 501-508.

Yadav AK, Tandon V and Rao HSP (1992): *In vitro* anthelmintic efficacy of fresh tuber extract of *Flemingia vestita* against *Ascaris suum*. ***Fitoterapia* 63**: 395-398.

Yang YM, Liu JK, Qin XD, Wu WL and Chen ZH (2004): Antioxidant activities of three dihydrochalcone glucosides from leaves of *Lithocarpus pachyphyllus*. ***Z Naturforsch* 59**: 481-484.

Yegnanarayan R AND Shroti MDDS (1982): Comparison of antidiarrhoeal activity of some drugs in experimental diarrhoea. ***Indian Journal of Pharmacology* 14**: 293-299.

Yorke RE and Turton JA (1974): Effects of fasciolicidal and anti-cestode agents on the respiration of isolated *Hymenolepis diminuta* ***mitochondria.* *Zeitschrift für Parasitenkunde* 45**: 1-10.

Zavala MA, Perez S, Perez C, Vargas R and Perez RM (1998): Antidiarrhoeal activity of *Waltheria americana, Commelina coelestis* and *Alternanthera repens*. ***Journal of Ethnopharmacology* 61**: 41-47.

Zavala SMA, Perez SG, Perez CG, Sanchez DS and Arias LG (2002): Antidiarrhoeal activity of Nonanal, an aldehyde isolated from *Artemisia ludoviciana*. ***Pharmaceutical Biology* 40**: 263-268.

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MSc	2000	I	NEHU
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Research Publications:

1. **Tangpu V** and Yadav AK (2003): *In vitro* filaricidal activity of some folklore medicinal plants of Manipur, India. In: Gupta N, Gupta DK, eds., ***Parasites and Diseases***: Bareilly, Neeraj Publisher pp. 127-132.
2. **Tangpu V** and Yadav AK (2004): Antidiarrhoeal activity of *Rhus javanica* ripen fruit extract in albino mice. ***Fitoterapia 75***: 39-44.
3. **Tangpu V**, Temjenmongla and Yadav AK (2004): Anticestodal activity of *Trifolium repens* extract. ***Pharmaceutical Biology 42***: 656-658.
4. **Tangpu V** and Yadav AK (2005): Antidiarrhoeal efficacy of *Swertia angustifolia*. ***Proceedings – International Conference on Botanicals***, Kolkata, India pp. 418-421.
5. **Tangpu V**, Temjenmongla and Yadav AK (2006): Anticestodal efficacy of *Strobilanthes discolor*: An experimental study in *Hymenolepis diminuta*–rat model. ***Journal of Ethnopharmacology 105***: 459-463.
6. Temjenmongla, **Tangpu V** and Yadav AK (2006): Anticestodal efficacy of *Psidium guajava* against *Hymenolepis diminuta* infection in rats. ***Indian Journal of Pharmacology 38***: 29-32.
7. **Tangpu V**, Temjenmongla and Yadav AK (2004): Some Important Medicinal Plants used by Tangkhul Nagas of Ukhrul District, Manipur. ***Recent Progress in Medicinal Plants.*** Stadium Press LLC, Houston TX, USA, Volume 16 (In Press).
8. **Tangpu V** and Yadav AK (2006): Antidiarrhoeal activity of *Cymbopogon citratus* and its main constituent, Citral. ***Pharmacologyonline 2***: 290-298.
9. **Tangpu V** (2006): Documentation of Traditional Herbal Medicinal Plants of the Tangkhul Naga. Bi-monthly Journal ***THE LEGACY 4***: 27-34.
10. Yadav AK and **Tangpu V** (2006): Antidiarrhoeal Activity of *Lithocarpus dealbata* and *Urena lobata* Extracts: Therapeutic Implications. ***Pharmaceutical Biology 45*** (In Press).

Conference/Workshop attended and presented research findings: Seven (7)

1. **Tangpu V** and Yadav AK: *In vitro* filaricidal activity of some folklore medicinal plants of Manipur, India. In: ***XVI National Congress of Parasitology, Bareily*** on 31st Oct to 2nd Nov., 2002.
2. **Tangpu V**, Temjenmongla and Yadav AK: Anticestodal activity of *Trifolium repens* L. (Papilionaceae) in experimentally induced *Hymenolepis diminuta* infections in rats. In: ***3rd Global Meet on Parasitic Diseases, Bangalore*** on Jan 12-16, 2004.
3. **Tangpu V** and Yadav AK: Antidiarrhoeal efficacy of *Swertia angustifolia* in experimental diarrhoea in mice. In: ***International Conference on Promotion and Development of Botanicals, Kolkata*** on Feb 25-26, 2005.
4. **Tangpu V**, Temjenmongla and Yadav AK: Anticestodal efficacy of *Strobilanthes discolor*: An experimental study in *Hymenolepis diminuta*–rat model. In: ***17th National congress of Parasitology, Dibrugarh, Assam*** on Oct 24-26, 2005.
5. **Tangpu V** and Yadav AK: Antidiarrhoeal activity of *Cymbopogon citratus* stalk decoction used in the folklore medicine of Tangkhul Naga tribe. In: ***Regional Symposium, NEHU, Shillong*** on Mar 24-25, 2006.
6. **Tangpu V**, Temjenmongla and Yadav AK: Experimental Investigation on the efficacy of *Zanthoxylum rhetsa* against cestode parasite, *Hymenolepis diminuta*. In: ***National symposium on Biosafety perspectives, Allahabad, UP*** on Sept 25-26, 2006.
7. ***Young scientists workshop on Parasitic and Microbial taxa, Biodiversity and aquaculture implications, Allahabad, UP*** on Sept 23-28, 2006.