# Herbal formulation for anthelmintic activity: Design and Evaluation

A THESIS
SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
**MASTER OF PHARMACY**

**IN**

**Pharmaceutics**

**By**

**Deepak Ghai**

(Reg.No.11512544)

**Under the guidance of**

Supervisor:
**Dr. Monica Gulati**
Professor & Sr. Dean

Co-supervisor
**Dr. Sachin Kumar Singh**
Associate professor


School of Pharmaceutical Sciences

Lovely Professional University

Punjab 144411

May, 2017

INDIA'S LARGEST UNIVERSITY

LOVELY PROFESSIONAL UNIVERSITY

*Transforming Education, Transforming India*

**TOPIC APPROVAL PERFORMA**

LIT (Pharmacy)/Department of Pharmaceutical Sciences

**Program :** P570-NN1::M.Pharm. (Pharmaceutics)

**COURSE CODE :** PHS514 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** PHRRGD0013

**Supervisor Name :** Dr. Monica Gulati **UID :** 11045 **Designation :** Sr. Dean

**Qualification :** _____ **Research Experience :** _____

| SR.NO. | NAME OF STUDENT | REGISTRATION NO | BATCH | SECTION | CONTACT NUMBER |
|---|---|---|---|---|---|
| 1 | Deepak Ghai | 11512544 | 2015 | Y1507 | 8146838244 |

**SPECIALIZATION AREA :** Pharmaceutics **Supervisor Signature:** _____

**PROPOSED TOPIC :** Polyherbal formulation for anthelmintic activity:Design and evaluation

| Qualitative Assessment of Proposed Topic by PAC | | |
|---|---|---|
| **Sr.No.** | **Parameter** | **Rating (out of 10)** |
| 1 | Project Novelty: Potential of the project to create new knowledge | 8.00 |
| 2 | Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students. | 8.00 |
| 3 | Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program. | 8.50 |
| 4 | Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills. | 9.50 |
| 5 | Social Applicability: Project work intends to solve a practical problem. | 8.50 |
| 6 | Future Scope: Project has potential to become basis of future research work, publication or patent. | 8.50 |

| PAC Committee Members | | |
|---|---|---|
| PAC Member 1 Name: Dr. Amit Mittal | UID: 13145 | Recommended (Y/N): NA |
| PAC Member 2 Name: Saurabh Singh | UID: 12208 | Recommended (Y/N): Yes |
| PAC Member 3 Name: Dr. S. Tamilvanan | UID: 16391 | Recommended (Y/N): NA |
| PAC Member 4 Name: Dr. Navneet Khurana | UID: 18252 | Recommended (Y/N): NA |
| DAA Nominee Name: Dr. Sazal Patyar | UID: 17050 | Recommended (Y/N): Yes |

**Final Topic Approved by PAC:** **Polyherbal formulation for anthelmintic activity:Design and evaluation**

**Overall Remarks:** Approved

**PAC CHAIRPERSON Name:** 11045::Dr. Monica Gulati **Approval Date:** 29 Nov 2016

5/11/2017 1:19:39 PM

# Statement by the candidate

This is to submit that this written submission in my thesis entitled **"Herbal formulation for anthelmintic activity: design and evaluation"** represents original ideas in my own words and where other ideas and words have been included; I have adequately cited and referenced the original sources. I also declare that i have struck to all the principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which thus have not been properly cited or from whom proper permission have not been taken when required.

Patents related to API, process, product, method and equipments, if any, have been examined to ensure non infringing approach to the existing patents.

This thesis encompasses the information generated by me based on experimental work carried out in the institute. I assure and hold full responsibility for its genuineness.

**Deepak Ghai**

Forwarded Through

**1. Dr. Monica Gulati**
Sr. Dean
Domain: Pharmaceutics
School of Pharmaceutical sciences
LFAMS, LPU

**2. Dr. Sachin Kumar Singh**
Associate professor
Domain: Pharmaceutical chemistry

# Certificate by Supervisor

The work described in this thesis entitled **"Herbal formulation for anthelmintic activity: Design and evaluation"** has been carried out by **Mr. Deepak Ghai** under my supervision. We certify that this is his bonafide work. The work described is original and has not been submitted before for purpose of getting degree in any university. The candidate has performed the work with his dedication to the best of my satisfaction.

**Date:**

**Place:**

**Dr. Monica Gulati**
Sr. Dean & Professor
School of pharmaceutical sciences
Lovely Professional University, Punjab

**Dr. Sachin Kumar Singh**
(Associate Professor)
Domain of pharmaceutical chemistry,
School of pharmaceutical sciences
Lovely Professional University, Punjab
Research Co- Supervisor.

# Certificate by School

This is to certify that the project dissertation entitled, **"Herbal formulation for anthelmintic activity: Design and evaluation"** submitted by **Mr. Deepak Ghai** under the guidance of **Dr. Monica Gulati and Dr. Sachin Kumar Singh** being duly certified and approved by the guide, and accepted for submission to lovely Professional University in partial fulfilment of the requirements for the award of the degree of Masters in pharmacy.

**Dr. S. Tamilvanan**
Professor
Head of domain
School of Pharmaceutical Sciences
Lovely professional university, Punjab

**Dr. Monica Gulati**
(Senior Dean)
Head of School
School of Pharmaceutical Sciences
Lovely professional university, Punjab

# ACKNOWLEDGEMENT

*Research is an arduous task, which despite of hard labour also requires patience and courage. This could only be made possible with the benediction and grace of the almighty. Towards the completion of my thesis it is a pleasurable aspect that I have now break, to communicate my thankfulness to all those who have been influential in the completion of this task.*

*The person whose picture come first in mind is that of esteemed guide* ***Dr. Monica Gulati****, Sr. Dean, Lovely School of Pharmaceutical Sciences, Lovely Professional University &* ***Dr. Sachin Kumar Singh****, Associate professor, Domain of pharmaceutical chemistry, Lovely Professional university, Punjab. I am extremely thankful to them for their valuable warm encouragement, thoughtful guidance, gracious attention and motivation for completing my project work. I appreciate all their contribution of time and ideas to make my M.pharmacy experience productive and stimulating. It gives me immense pleasure to submit my dissertation work as their student.*

*I am very obliged to* ***Dr. S. Tamilvanan,*** *Head of department of pharmaceutics, Lovely School of Pharmaceutical Sciences , Lovely Professional University for giving his support to proceed my project work. Acknowledgment can't be completed without expressing gratitude to our worthy chancellor* ***Mr. Ashok Mittal*** *who provided the facilities, equipment, and faculty without which, I would not have been able to reach my destination.*

*I am extremely thankful to* ***Dr. Jagdish Prashad Singh****, CEO, and* ***Dr. Ravi Singh****, Director, for provided me the gift samples of drugs and their support.*

*I cannot forget to owe my heartiest thanks to* ***Mr. Amit Sharma****, Head of department of research, Shree Dhanwantri Herbal Pvt Ltd for his support and valuable suggestion during my research.*

*I am very thankful to* ***Dr. K. Gowthamarajan*** *professor and head, Department of pharmaceutics, JSS college of pharmacy, Ooty for his support in my sample characterization process.*

*I am very thankful to* ***Dr. Subheet Kumar Jain****, professor and head, Department of pharmaceutics, Guru Nanak Dev University for his support in my sample characterization process.*

*I am extremly thankful and pay my gratitude to **Mr. Bimlesh Rathore,** Associate professor, Domain of pharmacology, Lovely Professional University for helping me in understanding the statistical analysis of tha data.*

*I would like to thank my parents **Mr. Naresh Ghai** and **Mrs Neeru Ghai** who have always been with me. It was due to my parent's dream, ambition, and sacrifice that I get so much ability to face all challenges during my research work today with misty eyes and folded hands.*

*I owe my loving thanks to my sister **Poonam Ghai** and my brother **Nitin Ghai** who were always there to help me fulfil my desires and for their inseparable support.*

*I also acknowledge with a deep sense of reverence, my gratitude towards **Mr. Gopal Krishan** and **Mr. Satish Tiwari** who timely provided me all the materials and resources required. **Mr. Hansraj, Mr. Manawar, Mr. Vijay Kumar, Mr. Anil Kumar** and **Mr. Prithavi** were very helpful during my project work.*

*I would like to express my heartiest gratitude to my friends **Harish Rathee, Adil Hussain Malik, Palak Bawa, Parth Sharma, Yadav Sarvi Rajesh, Paras Famta, Yusuf Nawaz Khan, Pushpak Mehriwala, Rhythm Bassi**, **Swati Patial, Anshul Attari and Nuni Sagar** as without their love and support it would not have been possible to complete the task.*

*At last but not least gratitude goes to all my friends who directly or indirectly helped me to complete this project report, as well as expressing my apology that I could not mention personally one by one.*

.

*Date:* *Deepak Ghai*

*Place:*

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURE

## LIST OF SYMBOLS AND ABBREVIATIONS

| Symbol/Abbreviations | Full form |
|---|---|
| WHO | World Health Organisation |
| LF | Lymphatic filariasis |
| Fig. | Figure |
| STH | Soil transmitted helminthiasis |
| g | Gram |
| h | Hour |
| MDA | Mass drug administration |
| i.e. | That is |
| DEC | Diethylcarbamazine |
| GIT | Gastrointestinal tract |
| mm | Millimetre |
| mL | Millilitre |
| mg | Milligram |
| min. | Minute |
| µm | Micrometre |
| Cm | centimetre |
| EPG | Egg per gram |
| % | Percentage |
| Rpm | Rotations per minute |
| CAP | Cellulose acetate phthalate |
| CAT | Cellulose acetate trimellitate |
| PVAP | Polyvinyl acetate phthalate |
| HCl | Hydrochloric acid |
| S.D | Standard deviation |
| Kg | Kilogram |
| XRD | X-ray diffraction |
| SEM | Scanning electron microscope |
| HPMCP | Hydroxypropyl methylcellulose phthalate |
| kV | Kilovolt |
| USP | United States Pharmacopeia |
| *et al* | And co-workers |
| ˚ADMFH | Aqueous decoction of mixture of fourteen herbs |

## ABSTRACT

Helminthiasis is the most common cause of the intestinal infestation. The World Health Organization revealed that over two billion people are suffering from parasitic worm infestation. Use of Conventional anthelmintic drugs like praziquantel, albendazole etc. is associated with number of side effects and resistance against the helminths. Thus it is necessary to look for more effective anthelmintic drugs with lesser side effects. Anthelmintic from natural sources may play a key role in the treatment of parasitic infestation. Krimihar™ syrup is marketed formulation used for the treatment of helminthiasis. The objective of study was to evaluate the anthelmintic activity of a marketed polyherbal formulation, Krimihar™ syrup. Interestingly the anthelmintic activity of one of the constituent i.e. *Centratherum anthelminticum* was found to much higher than the multicomponent formulation. The activity got further enhanced by conversion into its spray dried powder. This spray dried powder that showed excellent flow properties was compressed into tablets which were given an enteric coating. The enteric coated tablets were subjected to dissolution testing wherefrom it could be concluded that the formulation would be able to show its parasiticidal effect in approximately 2 h after reaching the intestine



Keywords: Helminthiasis, Anthelmintic, *Centratherum anthelminticum*, *Eisenia foetida*

# CHAPTER 1

# INTRODUCTION

## 1.1 Helminthiasis

The word Helminth is derived from the Greek word “helmins” which means worms. Unlike other parasites, the body of worms is multicellular and they have a complex life cycle. Humans and animals are more prone to helminth infestation than to any other parasites. Helminths fulfil their nutritional requirement by feeding on the host’s nutrient supply, which results in the malnutrition like condition in the host (Jain et al., 2013). Helminths infestations such as ascariasis, ancylostomiasis and schistosomiasis constitute the bulk of the thirteen diseases classified as neglected tropical diseases by the WHO (Jain et al., 2013). Helminthiasis is the most common infestation, which affects the human body parts like liver, gastrointestinal tract and other organs. It may cause malnutrition, loss of appetite, anemia, pneumonia and eosinophilia. There are two clinically important types of worm infestation, one in which the worms live in the host alimentary canal and the other in which worms live in other tissue of the host’s body (Manke et al., 2015). Helminths infestation in gut and tissue constitute a major cause of death in the developing countries (Abbas and Newsholme, 2011). Mainly, the helminths are divided into two phyla; nematodes and platyhelminths. Nematodes (commonly known as roundworms) consist of the major intestinal worms (also known as soil-transmitted helminths) and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis. Platyhelminths (commonly known as flatworms) consist of the flukes (also known as trematodes) and the tapeworms (also known as cestodes) (Hotez et al., 2008). Among the helminths, soil-transmitted helminths (STHs) are the most common intestinal parasites. Common soil transmitted helminths comprise are *Ascaris lumbricoides*, *Trichuris trichiura* and *Ancylostoma duodenale* (Pullan et al., 2014).


Fig 1.1 Images of different types of helminths

**1.2 Epidemiology**

Helminthiasis is the most common type of the intestinal infestation. Almost one-third of the world population is suffering from these intestinal parasites. The disease gains much attention as it affects both the nutrition and immune status of the infected individual, particularly those living in the tropical and sub-tropical regions (Hotez et al., 2008). This infestation is highly spread in the regions of South Asia, China, Central Africa and in the coastal regions of west Africa. There is the widespread presence of Trichuris infestations in Central Africa, Southeast Asia and, southern India. Hookworm infestations, however, are common throughout much of sub-Saharan Africa, in addition to South China and Southeast Asia. Recent investigation indicates that infestation caused by ascariasis is common with 1.2 billion infestations worldwide. Out of these, 50% of infestation is limited to China, which still has the maximum pervasiveness. Trichuris and hookworms infest about 700–800 million people every year (De Silva et al., 2003). .

Table 1.1 The main human helminthiasis and their worldwide pervasiveness and distribution (Hotez et al., 2008)

| Disease | Parasite | Worldwide pervasiveness | Region of maximum pervasiveness |
|---|---|---|---|
| **Nematodes** | | | |
| Ascariasis | Ascariasis lumbricoides | 807 million | Africa, Asiaand Latin America |
| Trichuriasis | Trichuris trichuira | 604 million | Africa, Asia and Latin America |
| Hookworms | Necator americanus | 576 million | Africa, Asia and Latin America |
| LF | Brugia malayi | 120 million | India, sub-Saharan Africa and South east Asia |
| **Trematodes** | | | |
| Schistosomiasis | Schistosoma haematobium | 207 million | Sub-Saharan Africa |
| | Schistosoma mansoni | | Sub-Saharan Africa |
| **Cestodes** | | | |
| Cysticercosis | Taenia solium | 0.4 million | Latin America, Asia and Sub-Saharan Africa |

Hookworm infestation influences approximately 500 million populations, accompanied by 5.1 billion people at risk for receiving this infestation all over the world. (Bartsch et al., 2016). In India, the pervasiveness of hookworm infestations was found to be unpredictable (De Silva et al., 2003).

### 1.3 Economic burden of helminthiasis:

#### 1.3.1 Indian scenario:

The economic liability of helminthiasis can be evaluated by adding the direct costs and indirect costs including the expenditure on prevention and treatment, as well as productive labour cost for the reason of morbidity and mortality (Conteh et al., 2010). In total, it has been projected as an average of $1 billion per year from India only (Chu et al., 2010). The struggles on treatment and control have resulted in protection of nearly 22 million populations from above-mentioned disease with an estimation of $24.2 billion savings (Global alliance 2010). As per certain estimations, the patients suffering from chronic lymphatic filariasis in India drop as much as 11 years of productivity, which is about $50 lost per year or equal to 15% of an individual income (Ramaiah et al., 2006). The treatment cost of Cysticercosis affected by *Taenia solium* was assessed at $15.27 million (Conteh et al., 2010). On the other hand, 11.78 million people suffered from Hookworm infestation caused by *Ancylostoma duodenale and Necator americanus* and treatment cost sum up as $471 million (Bartsch et al., 2016).

### 1.3.2 Worldwide scenario:

In humans, infestations caused by Helminths are highest in comparison to other infestations (Kosalge and Fursule, 2009). The World Health Organization carried out eight-year program to eliminate Lymphatic Filariasis by which direct economic benefit of $21.8 billion would be gained by treating and considering 31.4 million patients (Chu et al., 2010). In China, each person invests $1 in the treatment of lymphatic filariasis (Molyneux et al., 2002). The cost for treatment of Cysticercosis caused by *Taenia solium* was estimated at $28.3 million in Central America and $16.6 million in the Eastern Cape (Conteh et al., 2010). In sub-Saharan Africa, soil-transmitted helminths infect about 90 million from child population, but these children can be treated with single dose of anthelmintic which is the practiced school-based approach of treating children at an estimation cost of $5-$7.6 million (Brooker et al., 2006). A broad range of deworming program was conducted at Vietnam that involved around 2.7 million children which costed $0.03 per student (Montresor et al., 2007). Based on the future scenario, controlling soil-transmitted helminths in the Caribbean and Latin America would cost approximately $41 million (Bitran et al., 2009). The treatment of hookworm infestation cost $20.9 billion worldwide. In china, the presence of 35.91 million hookworm patients associated with health outcomes leads to $6.7 billion in productivity losses. After the STHs, the second biggest cause of parasite infestation is schistosomiasis (Fenwick et al., 2009). In order to control the Schistosomiasis, some programs were started. In Burkina Faso, the mass drug administration (MDA) of praziquantel and albendazole to children by the community and school attain more than 90% coverage at a rate of $0.32 per child (Gabrielli et al., 2006). The total cost of the program was reported to be $1.07 million, from which about two third was spent on medications (Marano, 2012).

## 1.4 Etiology

In the transmission of the infestation, sanitation plays a major role. Unpurified drinking water and improperly cooked meat from infected animals are the most common source of the infestation. Also, the infestation may be spread by insect bites, swimming in polluted water and contact between the wound and polluted soil. These worms mainly infect the humans. They reproduce inside the host, producing eggs and larvae, which pass from the primary host to the secondary host spreading the infestation. The presence of eggs or larvae in the host may lead to cysticercosis. This condition is characterized by encysted larvae in the muscles, viscera and more critically in the eye or the brain (Manke et al., 2015).

## 1.5 Pathology

### 1.5.1 Direct damage caused by the helminths

Blockage of internal organs physically or by pressure exerted by worms is considered as direct damage. Large nematodes (*Ascaris*) or tapeworms (*Taenia*) physically block the intestines and blood flow to the liver leading to a pathological condition. Cysts of the tapeworm (*Echinococcus multilocularis*) develop in the liver, brain, lungs or other parts of body cavities and can lead to unusual enlargement, organ metastasis and may cause necrosis due to pressure exerted by cysts (Manke et al., 2015).

### 1.5.2 Indirect damage by the helminths

Schistosome infestations, especially with *Schistosoma mansoni*, are one of the examples of indirect damage. Hypersensitivity-based, formation of granuloma produces blockage of liver sinusoids impeding blood flow that further leads to liver diseases. Inflammatory changes based on hypersensitive reaction may also lead to the lymphatic blockage related with filarial infestations (Manke et al., 2015).

## 1.6 Treatment of helminthiasis

### 1.6.1 Conventional treatment:

Parasitic helminth infestations are reported to increase the mortality and morbidity rate worldwide (Yadav and Singh, 2011). Anthelmintic drugs act locally to throw out worms from the gastrointestinal track (GIT) or systemically eliminate adult helminths or/and development forms that invade tissues & organs (Tiwari et al., 2011)**.** Almost all types of helminth infestations can be treated with the use of five anthelmintic drugs i.e. praziquantel, albendazole, ivermectin, mebendazole, and diethylcarbamazine (DEC) (Stephenson et al., 2000).

Albendazole and mebendazole are broad spectrum oral anthelmintics. Albendazole acts by inhibiting the microtubule synthesis in roundworms. Mebendazole irreversibly blocks glucose uptake, which in turn, leads to paralysis and death of roundworm and tapeworm (Jain et al., 2013). Standard doses of albendazole 400 mg and mebendazole 500 mg are used for the treatment of patients above the age of one year. These treatments may not eliminate heavy infestation, but efficiently reduce the morbidity and worm burden (Lain Stephenson et al., 2000). Albendazole is reported to be better tolerated and slightly more efficacious than mebendazole (Abbas and Newsholme, 2011).

Ivermectin is most effective semisynthetic macrocyclic lactone with a broad spectrum. The drug shows activity against a wide range of the helminthic parasites. Ivermectin is effective against a number of human infestations including lymphatic filariasis and Ascaris onchocerciasis (Stephenson et al., 2000).

Ivermectin causes ion channel mediated helminth muscle paralysis while praziquantel causes muscle paralysis of helminths only. The other drugs include piperazine, niclosamide, and levamisole which expel the worms from GIT (Abbas and Newsholme, 2011).

Concomitant administration of two or three drugs is effective treatment option and serves two purposes i.e. to increase the efficacy of the drug against parasites such as *Trichuris trichiura* which are very difficult to treat with a single drug and to reduce the drug administration frequency, especially in mass treatment programs. Pyrantel and mebendazole are effectively used for the treatment of trichuris infestation which is more effective than treatment with the single drug (Stephenson, 2001).

Recently, newer anthelmintic drugs have been reported. Tribendimidine is licensed in China since 2004 as an effective treatment of intestinal helminths and acts by binding to nicotinic acetylcholine receptor as an agonist. A single dose of tribendimidine is specifically effective against Ascaris lumbricoides and *Ancylostoma duodenale* but not against *Trichuris trichiura*. Another new drug is monepantel, an amino acetonitrile derivative that acts on nicotine acetylcholine receptor and leads to paralysis and death of worms (Blair and Diemert, 2015)

Table 1.2 Conventional drugs for the treatment of helminths

| S.No | Disease | Treatment | Dose | Reference |
|---|---|---|---|---|
| 1 | **Nematodes** | | | |
| 1.1 | Enterobiasis | Albendazole or mebendazole | 400 mg daily once a day<br>100 mg daily once a day | Jaogota SC et al.,1986,<br>Max J. Miller et al.,1974 |
| 1.2 | Trichuriasis | Albendazole or mebendazole | 400 mg daily once a day<br>100 mg daily twice a day | Ramalingam S et al.,1983<br>Max J. Miller et al., 1974 |
| 1.3 | Ancylostomiasis | Albendazole or mebendazole | 400 mg orally once a day<br>100 mg orally twice a day | Ramalingam S et al.,1983<br>Steinmann et al., 2011 |
| 1.4 | Trichinosis | Albendazole or mebendazole | 400 mg orally twice a day<br>200-400 mg orally twice a day | Gottstein et al., 2009 |
| 1.5 | Strongyloidiasis | Ivermectin | 200 µg/kg orally once a day<br>For 2 d | Annick Datry et al., 1994 |
| 2 | **Cestodes** | | | |
| 2.1 | Cysticercosis | Praziquantel | 5-10 mg/kg orally once a day | Garcia et al., 2002 |
| 2.2 | Echinococcosis | Albendazole | 400 mg orally twice a day | M. Keshmiri et al., 2001 |
| 3 | **Trematodes** | | | |
| 3.1 | Fascioliasis | Triclabendazole | 10mg/kg orally once or twice | Apt W et al., 1995 |

| | | | | |
|---|---|---|---|---|
| 3.2 | Schistosomiasis | Praziquantel | 20mg/kg orally twice a day | Gray et al., 2011 |

**1.6.1.1 Limitations of Conventional treatment:**

Conventional drugs have been reported to produce side effects and dose-related toxicity (Yadav and Singh, 2011). Albendazole, when used for short-term therapy of gastrointestinal helminths produces side effects like headache, nausea, dizziness, vomiting, rashes and edema. Dose-dependent side effects of Mebendazole include allergic reaction, alopecia, and hypothermia. (Kappagoda et al., 2011). All the anthelmintic drugs are unsafe to prescribe for pregnant women and young children. Praziquantel cause headache, diarrhoea, abdominal pain, seizures and mental changes. The side effects of Ivermectin include nausea, diarrhoea, hepatitis or dizziness (Jain et al., 2013). Drug resistance is the major limitation to the helminths which is threatening human health (Bauri et al., 2015)

**1.6.2 Herbal treatment:**

About 80% of world population, specially in developing countries rely on natural sources for primary health care (Yadav and Singh, 2011). Conventional anthelmintic drugs are less effective due to development of resistance in helminths. This has led to increased demand of screening medicinal plant for their anthelmintic activity (Iqbal et al., 2004). Plant-based medicines have shown great efficacy against a variety of parasites of medical and veterinary importance. Moreover, the chances of drug resistance against phytoanthelmintics are less than that for chemical anthelmintics (Bauri et al., 2015). Herbal compounds like artemisinin and quinine alkaloids are effective against schistosomiasis. Anthelmintic drug compounds are also reported to be obtained from plants including arecoline, pelletierine, filixic acid, aspidin, ascaridole and Curcumin (Wink, 2012). Curcumin extracted from turmeric is well known to exhibit anti-parasitic effect against Schistosoma (Bahmani et al., 2014). Ascaridole is an anthelmintic compound isolated from chenopodium plant. It is reported to be effective against hookworm infestation (Kliks, 1985). Aspidin and filixic acid isolated from *Dryopteris filixmas* show activity against intestinal cestodes. Pelletierine isolated from *Punica granatum* and arecoline from *Areca catechu*, which targets acetylcholine receptors show anthelmintic activity (Wink, 2012). Rottlerin and isorottlerin isolated from *Mallotus philippensis* or kamala tree also have shown strong anthelmintic activity. (Patel et al., 2009)

### 1.7 Centratherum anthelmenticum (kalijiri):

Drug consists of dried fruit part of *Centratherum anthelmenticum* or *vernonia anthelmentica*, family Asteraceae. The plant is widely distributed throughout India upto 5500 feet in the Himalayas and Khasia hills. It is commonly known as kalijiri in India. (Yadava et al., 1996)

Other names

Beng: Somaraj

Eng: Purple fleabane

Guj: Kalijiri

Hind: Somraj

Punj: Bukowski

Tam: Kattu-Shira gam

Tel: Adarijilakara

Parts used: Seed part (Tandon et al., 2010)

### 1.7.1 Chemical constituent:

The major chemical constituent present in *C. anthelminticum* is vernodalin, butein, daucosterol, vernolic acid, vernodalol, vernovan, stigmastadienol, lupeol and beta-sitosterol. Other chemical constituents are vernolic acid, linoleic acid, oleic and Palmitic acid, stearic acid, stigmasterol, vernosterol, avenasterol, D-lactose, L-sorbose, D-arabinose, protein, lipids and fats (Amir and Chin, 2011, Tandon et al., 2010, Bhatia et al., 2008b). The anthelmintic activity of *C. anthelminticum* is attributed to the presence of anthraquinone (Hordegen et al., 2003).

Table 1.4**.** Uses of *Centratherum anthelminticum extracts* (kalijiri)

| S.No | Solvent used for extraction | Activity | Reference |
|---|---|---|---|
| 1 | Ethanol | Hypotensive, laxative and antifungal activity | Singh et al., 2012 |
| 2 | Methanol | Larvicidal activity | Hellert et al., 2012 |
| 3 | Petroleum Ether | Anti-inflammatory activity | Ashok P et al., 2010 |
| 4 | Ethyl acetate & Acetone | Antifilarial activity | Amir and Chin, 2011 |
| 5 | Polyphenolic | Antihyperglycemic and antioxidant | Naidu, 2008 |
| 6 | Aqueous | Antidiabetic activity | Bhatia et al., 2008 |
| 7 | Chloroform | Antibacterial activity | Patel VP et al., 2012 |
| 8 | Not reported | Diarrhoea, cough, and fever | Amir and Chin, 2011 |
| 9 | Aqueous | Anthelmintic activity | Hordegen et al.,2003 |

Table 1.5 Marketed formulations used for treatment of Helminthiasis

| S.no | Anthelmintic drugs | Brand name | Marketed formulation | Unit dose | Company name |
|---|---|---|---|---|---|
| **1** | Albendazole | ABD | Tablet | 400 mg | Intas Pharmaceutical Limited |
| | | Gekare | Capsule | 400 mg | Glaxo Smithkline Pharmaceuticals Ltd |
| | | Emiben | Suspension | 10 mL/5mL | Plenteous Pharmaceuticalsltd |
| | | Encenil | Syrup | 10 mL/5mL | Ortin Laboratories Ltd |
| **2** | Mebendazole | Mex | Tablet | 100 mg | Kaleon Laboratory |
| | | Helmintol | Suspension | 5 mL | Medley Pharmaceuticals Pvt. Ltd. |
| | | Eben | Syrup | 5 Ml | Gufic Limited |
| **3** | Praziquantel | Distocide | Tablet | 600 mg | Chandra Bhagat Pharma Pvt. Ltd. |
| **4** | Ivermectin | Iverstar | Tablet | 12 mg | Santiago Life Sciences |
| | | Iverstar | Disintegrating tablet | 12 mg | Santiago Life Sciences |
| | | Ivercid | Suspension | 6 mg | East West Pharma |
| **5** | Levamisole | Carisnil | Tablet | 150 mg | Ortin Laboratories Ltd |
| | | Vizole | Capsule | 50 mg | Mission Mountain Laboratories |
| | | Vermisol | Syrup | 50 mg | Khandelwal Laboratories Pvt Ltd. |
| **6** | Piperazine | Avizine | Tablet | 500 mg | Taj Pharmaceuticals Ltd |
| | | Piperazine citrate | Syrup | 750 mg/5 mL | Glaxo Smithkline Pharmaceuticals Ltd. |
| **7** | Niclosamide | Niclosan | Tablet | 500 mg | Glaxo Smithkline Pharmaceuticals Ltd. |
| **8** | Nirgundyadi kashayam | NA | Liquid | 5-15 mL | VaidyaRatnam Oushadhasala |
| **9** | Vidamgarishtam | NA | Liquid | 12-24 mL | Sandu Pharmacuticals Ltd. |
| **10** | Khadirarishta | NA | Syrup | 12-24 mL | Bhardwaj Pharmaceutical Works |
| **11** | Wormicid plus | NA | Syrup | 5-10 mL | Prakruti Products Pvt. Ltd |
| **12** | Wormnil | NA | Capsule | 510 mg | Mukthi Pharma |

# CHAPTER 2
# REVIEW OF LITERATURE

## 2.1 Helminthiasis

Helminthic parasites are basically multicellular organisms having bilateral symmetry with three germ layers (Chatterjee, 2009). In developing countries, it is reported as the most common cause of morbidity that affects the gut (Abbas and Newsholme, 2011). Helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide (Deb et al., 2013). Although, helminth infestations are generally not serious and remain in asymptomatic state, but in heavily infected individuals, they may lead to severe morbidity (Hossain et al., 2012). Helminthiasis leads to increased rate of malnutrition, anaemia, eosinophilia, pneumonia and loss of appetite (De et al., 2016). Different type of helminths infect humans and animals, out of which intestinal round worms (*Ascardia sp.*) are most common (Tripathi, 2003). It is the single group of parasites which is responsible for highest morbidity rate in humans and animals (Amirmohammadi et al., 2014)

### 2.1.1 Classification of Helminths

There are two major classes of helminths which are nematodes and Platyhelminthes.

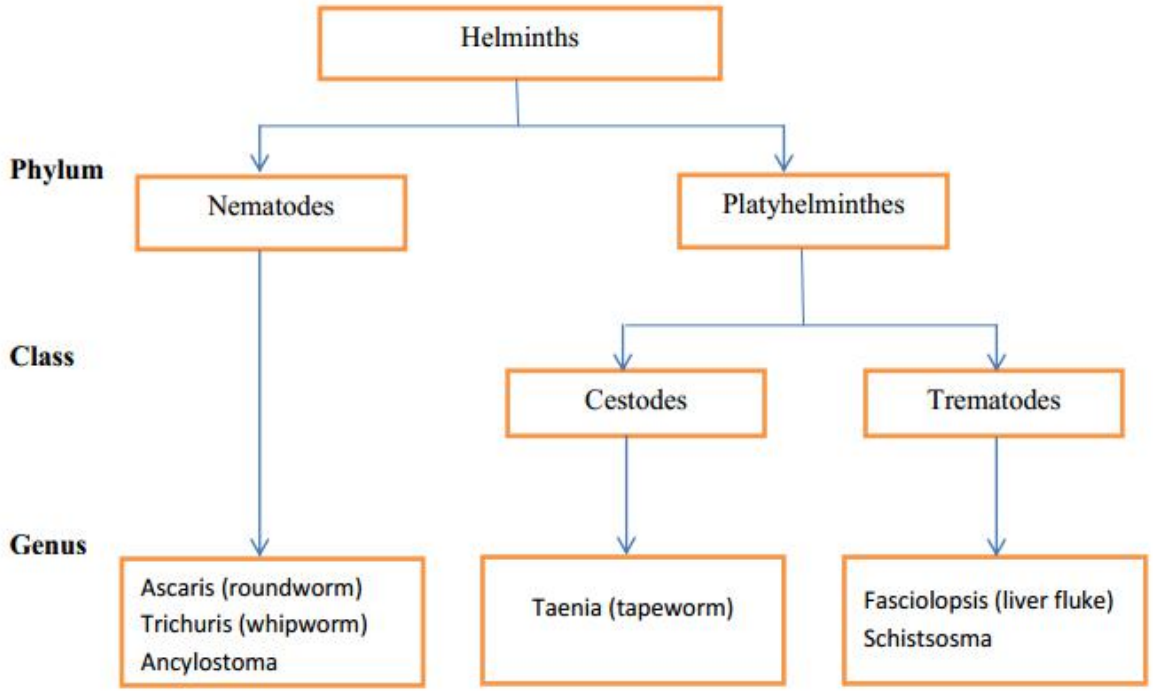



Fig1. Classification of Helminths

Nematodes include filarial worms and intestinal worms which cause onchocerciasis and lymphatic filariasis. While Platyhelminthes consist of flukes such as tape worms and schistosomes (Peter J et al., 2008).

**2.1.1.1. Nematodes:**

Nematodes are also called as roundworms. They are cylindrical, elongated having bilateral symmetry with pointed ends. They have fully developed body cavity consisting of various organs such as excretory system, digestive system, nervous system and genital system. The size of the nematodes varies from 5 mm to 1metre. Their infestation spreads either by ingesting infective eggs or through larvae which penetrate the body by invading the tissues. (Ochei et al., 2000 ).

Intestinal nematodes includes:

- Whipworm
- Thread worm
- Round worm
- Hookworm

**Whipworm: (Trichuris trichuira)**

Whipworm or trichuris infestation spreads mostly in moist and warm climates. Whipworms are found in caecum region of large intestine. (Ochei et al., 2000). Symptoms generally do not arise with light infestation but in case of heavy infestation, several symptoms appear like weight loss, diarrhoea with blood in the faeces, and abdominal pain (Allifia abbas et al., 2009). The shape of the worm resembles whip, the anterior three- fifth being thin, long and hair like while posterior two-fifth is stout, thick and small. The size of male worms is smaller as compared to that of female whipworm. Male whipworms are 3-4 cm in length whereas female whipworms are 4-5 cm in length. Both male and female whipworms consist of long esophagus tube which extends two third of body length (Chatterjee, 2009)

The infestation mainly spreads in the form of inflammation or inflammatory bowel disease. It also causes edema, bleeding and hyperaemia. Tropical countries with warm and humid climate have highest rates of these infestations (Arora et al., 2010).

**Thread worm:**

*Enterobius vermicularis*, commonly known as threadworm infects the people worldwide (Arora et al., 2010). Adult worms are white colored and spindle shaped. Length of female worms varies from 8-13 mm and they are characterized by the presence of wing like elongation of body wall at the anterior part. Distension of the body occurs due to the presence

of large number of eggs in the uterus region with a pointed tail. Male worms are 2-5 mm in length with curved tail (Chatterjee, 2009).

Pinworms are not highly infective as they cause little injury in the region of perianal and vaginal areas when eggs pass through these regions.

Heavy infestations can occur in children and the symptoms include sleeplessness, hyperactivity, abdominal pain, weight loss, grinding of teeth and vomiting. In case of female patients, the worms can get entrapped in the tissues that may lead to vaginitis. From there, they can also migrate to peritoneal cavity and urinary bladder. As far as geographical distribution is concerned, the worm is present worldwide (Arora et al., 2010).

**Round worm**

The roundworms are cylindrical in shape, elongated and enervated at both ends. Female worm is larger in size than male. A fully developed body cavity is present with digestive tract. Roundworms are free living also while a large number of species parasitize on plants, animals and humans. In larva stage, host is required for further growth. Human parasites include tissue penetrating and intestinal envading species (Ochei et al., 2000).

**Hookworm**

*Ancylostoma duodenale* and *Necator americanus* are hookworms. They are present in warm and moist areas. Hookworms are smaller in size and cylindrical in shape. The worms can be differentiated on the basis of morphological characters (Ochei et al., 2000).

Symptoms: Skin reaction at the site of penetration is the initial symptom of hookworm infestation which is known as ground itch. When the eggs of roundworm migrate to lungs and heart, they cause respiratory problems and eosinophilia. Heavy symptoms appear when worms are present in intestine. Iron deficiency anemia occurs with chronic infestation and abdominal pain, fatigue and diarrhoea occurs with acute infestation (Arora et al., 2010).

**2.1.1.2 Cestodes:**

Cestodes are commonly known as tapeworms. Infestation of *Taenia saginta* and *Taenia solium* spreads in humans by digesting the larvae in meat, either beef or pork which is undercooked or raw. Cyst develops in the abdomen and leads to mild abdominal pain, loss of appetite and weight loss symptom. It is also responsible for invading central nervous system (Yadav et al., 2011). The adult tapeworms are segmented, long, and tape like in structure. The size of the worms varies from few mm to several meters. Tapeworms are found in intestine of the animals and human. Sexes are not separate in tapeworms i.e. every worm is hermaphrodite. In tapeworms, alimentary canal is entirely absent but nervous system and

excretory system is present. Tapeworms have highly developed reproductive system in each segment (Chatterjee, 2009).

**2.1.1.3 Trematodes:**

They are also known as intestinal flukes. These are the species which spread infestation through direct contact with fresh water. Intestinal flukes are categorised in to two groups i.e. those which reside in intestine e.g. *Fasciolopsis buski* and others that reside in bile duct of liver e.g. *Fasciola hepatica* (Ochei et al., 2000).

*Fasciolopsis buski* is Asiatic trematode and is distributed mainly in Malaysia, India, Thailand and China. *Fasciolopsis buski* lives in the small intestine of pig and man. It is the largest trematode present in human with a length 2 to 7.5 cm (Chatterjee, 2009).

*Fasciola hepatica* lives in the biliary passage of liver. It is a large leaf shaped fluke having brown to pale grey colour. Symptoms of fascioliasis include vomiting, jaundice, diarrhoea and biliary colic (Chatterjee, 2009).

**2.1.2 Location of helminths**

Table 2.1 Distribution of helminths in human gastrointestinal tract (GIT) is given below (Arora et al., 2010)

| S.NO | Disease | Causative helminth | Location |
|---|---|---|---|
| 1 | **Nematodes** | | |
| 1.1 | Enterobiasis | *Enterobius vermicularis* | Small or large Intestine |
| 1.2 | Trichuriasis | *Trichuris trichiura* | Colon |
| 1.3 | Ancylostomiasis | *Ancylostoma duodenale & Necator americanus* | Small intestine |
| 1.4 | Trichinosis | *Trichinella spiralis* | Small intestine |
| 1.5 | Parasitic pneumonia | *Ascaris lumbricoides* | Small intestine |
| 2 | **Cestodes** | | |
| 2.1 | Cysticercosis | *Taenia solium* | Intestine |
| 2.2 | Taeniasis | *Taenia saginata* | Intestine |
| 2.3 | Echinococcosis | *Echinococcus Granulosus* | Intestine |
| 3 | **Trematodes** | | |
| 3.1 | Fasciolopsiasis | *Fasciolopsis buski* | Small Intestinal |
| 3.2 | Schistosomiasis | *Schistosoma japonicum* | Small intestine |
| 3.3 | Schistosomiasis | *Schistosoma mansoni* | Intestine |

**2.2 Pharmacological models of anthelmintic activity**

Selection of a suitable host to study particular parasitic infestation is quite challenging and is generally done by examining its physiological, metabolic, anatomical and immunological similarities that each possesses, since all these properties may influence results of

experiments. There are several models which have been established that include rodents, primates, fowl and pig (Boes and Helwigh, 2000). Models of nematodes include *Toxocara* spp, *Trichuris and Ancylostoma* (Beer et al., 1976, Schad et al., 1979). Models of cestodes include *Raillietina tetragona.* Earthworm is commonly used as an in-vitro model, which is reported to evaluate the antihelmintic activity of both herbal and synthetic drugs (Kundu et al., 2012, Tiwari et al., 2011).

*Ascaris suum* in the pig, *Ancylostoma caninum* in the dog and *Trichuris muris* in the mouse, are the infestations that are used as in-vivo models corresponding to human infestation (Stephenson et al., 1987, Tritten et al., 2011).

**2.2.1 Pig model**

*Oesophagostomum dentatum* is the most common infestation that exists in the large intestine of pigs. This infestation is of economic importance because it causes huge production losses. *O. dentatum* also serves as a model parasite because of its amiability to be cultured in the lab. It is considered as a good model for biological investigations because it is easy to maintain its several different life stages (Kim, 2016).

**2.2.2 Rodent model**

Rodent models are most popular and are frequently used for various forms of human diseases. Rats, rabbits, and mice are easy to carry, handle, less expensive and can reproduce easily in large numbers (Boes and Helwigh, 2000). *Trichuris muris* and *Ancylostoma ceylanicum* are well known parasitic models corresponding to human STH. *Ancylostoma ceylanicum* was developed in the laboratory using eggs from infected human or dog to another host, the golden hamster and serves as a model for hookworm infestation (Tritten et al., 2011). Mouse model *Trichuris muris* is well reported model for *Trichuris*. However, the rodent models shows limitations due to parasite size constraints, host physiology and short host life span (Boes and Helwigh, 2000).

**2.2.3 Fowl model**

Some worms like *Heterakis gallinarum and Ascardia galli* are easily available in large numbers in freshly slaughtered fowl. These worms are relevant for the study of antihelmintic drugs (Mali et al., 2008 ). Another worm *Raillietina tetragona* has been reported for the study of the antihelmintic activity of cassia plant and was obtained from the intestine of freshly slaughtered domestic fowl (Kundu et al., 2012).

**2.2.4 Earthworm model**

Earthworm is one of the most reported model for evaluation of in vitro anthelmintic activity. This may be attributed to its anatomical and physiological similarities with the intestinal

parasites. Moreover, its easy availability, maintenance and non-microscopic size make it convenient to be used in such studies.

Table 2.2 References for evaluation of in vitro anthelmintic activity of various plants using earthworm

| S.No | Name of plant | Earthworm used | References |
|---|---|---|---|
| 1 | *Thespesia lampas* | *Pheretima posthuma* | Kosalge and Fursule, 2009 |
| 2 | *Holoptelea integrifolia* | *Eisenia foetida* | Sarabjot et al., 2010 |
| 3 | *Clitoria ternatea* | *Eisenia foetida* | Salhan et al., 2011 |
| 4 | *Juglans regia* | *Pheretima posthuma* | Das et al., 2011 |
| 5 | *Leea asiatica* | *Pheretima posthuma* | Saiket et al., 2012 |
| 6 | *Luffa cylindrica* | *Pheretima posthuma* | Partap et al., 2012 |
| 7 | *Oxalis corniculata* | *Eisenia foetida* | Santosh et al., 2012 |
| 8 | *Oenothera rosea* | *Eisenia foetida* | Dahiya et al., 2012 |
| 9 | *Tinospora cordifolia* | *Eisenia foetida* | Pawar et al., 2014 |
| 10 | *Jasminum mesnyi* | *Eisenia foetida* | Vibhuti et al., 2014 |

Earthworms are composed of many segments. They lack bones and move by contracting and relaxing the body segments in sequence. Earthworms have the ability to move by ciliary movement. The outer layer of earthworm is made up of complex polysaccharides and is known as mucilaginous layer. It allows the earthworm to move freely. Any injury to the mucopolysaccharide membrane would expose the outer layer and this restricts its movement, leads to paralysis. It can also result to the death of the worm. (Pawar et al., 2014)

#### 2.2.4.1 *Eisenia foetida*

The anthelmintic activities have been reported by using adult earthworm *Eisenia foetida* by a number of references due to its anatomical and physiological resemblance with the intestinal parasite of human beings (Tiwari et al., 2011, Kumar et al., 2011, Deore et al., 2009, chatterjee, 1967). Its easy availability and maintenance makes it one of the most commonly used model (Salhan et al., 2011)

*Eisenia foetida*, lives mainly on dead plant material (Neuhauser et al., 1980). It is dark brown in colour with yellow colour in the tip of the tail. The adult worms are about 5-7 cm in length, 3-5 mm in diameter and 500-600 mg of weight. It can tolerate the temperature upto 29˚C and high level of moisture (Venter and Reinecke, 1988).

Fig 2.2 Image of *Eisenia foetida*

(http://www.happydranch.com/articles/images/clip image008 000.jpg Accessed on March 27, 2017 at 9:51 PM).

**2.3 Drug Profile:**

**2.3.1 *Centratherum anthelminticum*:**

**Taxonomy**

Biological source: Drug consists of fruits or seeds of *Centratherum anthelminticum.*

Family: Asteraceae.

The plant is widely distributed throughout India upto 5500 feet in the Himalayas and Khasia hills. It is commonly known as kalijiri in India (Yadava et al., 1996). This plant is robust, erect, leafy and highly branched herb distributed throughout the world.

**Synonyms**

*Vernonia anthelmintica*.

**Common names**

Kan. - Kadu-jirage

Punj. - Bukoki, Kakshama

Beng. -Somaraj, Kali-ziri

Guj. - Kalijiri

Tam. - kattu-shiragam

Eng. - Purple fleabane

Hind.- Somraj, Buckshi

Mar. - kalenjiri

Mal. - Kalajirakam, Kadujirain (Tandon et al., 2010)

Fig 2.3 Plant of *Centratherum anthelminticum*

(http://www.biosamen-und-pflanzenwelt.de/epages/62058757.sf/de_AT/?ObjectPath=/Shops/62058757/Products/165P Accessed on April 02, 2017 at 1:04 PM).

### 2.3.2 Description of seeds

The seeds of *Centratherum anthelminticum* have a hot sharp taste. It is reported to be used in the treatment of various diseases such as kidney disorder, cough, asthma and leucoderma. It also exhibits anthelmintic activity (Yadava et al., 1996)

Fig 2.4 Seeds of *Centratherum anthelminticum* (Thara et al., 2016)

### 2.3.3 Ethnobotanical data

As per Ayurveda, the seeds are termed to have hot sharp taste, acrid, cure ulcers, anthelmintic, used in fever, skin diseases and leucoderma.

Based upon the Unani system of medicine, the seeds are reported to have sharp bitter taste and show purgative, anthelmintic activity. They are also used in treatment of kidney ailments, asthma, inflammation, cough, swelling, sores and itching of eyes (Atmiya and Manvar, 2015). The seeds are well accepted as powerfully anthelmintic by Vaidyans. They are an important ingredient of compound formulation prescribed in snake bite. Furthermore, such seeds have diuretic, tonic and stomachic properties

The powdered seeds along with incorporation of castor oil have been used for removal of roundworms in half to one and half drachms doses and the result outcome showed the seeds to possess considerable anthelmintic properties (Kirtikar et al., 2004).

The seeds of *Centratherum anthelminticum* have been mentioned in Sanskrit Materia Medica as a medicine for white leprosy and other skin diseases. However, it has been also introduced as an anthelmintic and used in combination with a number of other medicaments.

In chronic skin diseases, seeds are taken either alone or in combination with other medicines. In the acute forms of skin diseases, such as psoriasis and leprosy, the medicine is recommended to be continued daily for one year or till a complete cure is said to be effected (Khandelwal et al., 2004).

**2.3.4 Pharmacological data of Centratherum anthelminticum:**

Traditionally in India, seeds of *Centratherum anthelminticum* are used as remedy to cure the helminthiasis. As the name indicates, seeds of *C. anthelminticum* exhibit a great potential to eradicate all type of worms. It is capable of removing intestinal parasites and has exhibited satisfactory results in deworming adults and small children. This activity of the plant is abundantly reported by number of scientific evaluations.

Anthelmintic activity of *C. anthelminticum* has been reported in combination with other plants; for example, its combination with *Carica papaya, and Butea monosperma* is reported against oxyurids of mice (Mehta & Parashra, 1966). It is also reported that *Embelia ribes* and *Centratherum anthelminticum* combination (as crude methanol extract) exhibit anthelmintic activity against gastrointestinal nematodes of goats. Post treatment days 3, 10 and 15, the administration of 0.5 g/kg mixture of powdered *Embelia ribes* and *C. anthelminticum* could not help to minimise the faecal egg per gram (EPG) counts. Egg per gram is laboratory test that determines the number of eggs per gram of faeces in patients suspected of parasitological infestation, such as schistosomiasis. However, on 15$^{th}$ day after administration of 1g/kg of the powder, a significant reduction in the EPG count was observed. The result shows that EPG

count was decreased from 526 ±72 to 251± 43. The percentage EPG reduction was 52 ± 4 %. The result was excellent with administration of 2 g/kg of powdered drug exert significant EPG reductions. The values reduced from 568 ± 80 to 255 ± 54 and 99 ± 41 on $10^{th}$ and $15^{th}$ days. The percentage of reduction was 55 ± 7 and 83 ± 2 % respectively (Javed & Akhtar et al., 1990).

Hördegen et al., 2003, illustrated, that a combination study of alcoholic extract of *C. anthelminticum* and *Embelia ribes* did not exert any anthelmintic activity against haemonchosis in sheep.

Singh et al., 1985, demonstrated an in vitro study where aqueous and alcoholic extract of seeds of *C. anthelminticum* were found to exert anthelmintic activity against *Ascaris lumbricoides, hymenolepis nana and Fasciolopsis buski.* Alcoholic extract of *C. anthelminticum* was found to exert *in vivo* anthelmintic activity against *Fasciolopsis buski*.

Iqbal et al., 2006, conducted a comparative study of in vitro and in vivo anthelmintic activities of *C. anthelminticum* seeds with levamisole as reference standard. In vitro studies of methanolic extract of *C. anthelminticum* show a stronger anthelmintic effect as compared to that of its aqueous extract against live *Haemonchus contortus*. For in vivo studies, seeds of *C. anthelminticum* were delivered as crude powder, methanolic extract, and ethanolic extract to sheep infected with intestinal nematodes. A dose of aqueous extract of *C. anthelminticum* at 3 g/kg body weight on day three post treatment was found to exert maximum effect to decrease the faecal egg per gram (73.9 %). But crude powder as 3 g/kg on day three post treatment showed only 55.6 % reduction in faecal egg per gram. However, methanol extract failed to show any anthelmintic activity.

A number of reports indicate that seeds of *C. anthelminticum* exhibit antifilarial activity.

Singhal et al., 1992, reported that alcoholic and aqueous extracts of *C. anthelminticum* seeds exert antifilarial activity against *Setaria cervi*.

Nisha et al., 2007, conducted an in vitro study of *C anthelminticum* seeds and it has reported to exert macrofilaricidal activity against *Setaria digitata* (filarial worm). Fruit extracts of *Centratherum anthelminticum* induced 65.64 % inhibition in formazan formation at a 2 mg/mL concentration when the incubation period was 4 h. However, 1 mg/ml concentration of test sample showed no significant inhibition (43.15 %) in formazan formation even at 4 h incubation period. Maximum percentage inhibition (97.46%) was noticed in formazan formation at increased concentration and longer incubation periods.

Mehta et al., 2010. reported the mechanism of action of aqueous and methanolic extracts of *Centratherum anthelminticum* against filariasis. Both extracts were reported to act by inhibiting instinctive motility of the *Setaria cervi*. In vitro study of aqueous and methanolic extracts of *C. anthelminticum* induced death of microfilariae with $LC_{50}$ and $LC_{90}$ values of 75 and 32.5 mg/mL respectively.

**2.4 Enteric coated dosage form**

An enteric coating acts as a barrier to control the release of orally administered drug in the upper part of digestive system. The word "enteric" symbolises the small intestine, enteric coating thereby meaning that it prevents the release of drug before the formulation reaches the small intestine (Islam et al., 2016).. Enteric coating polymers remain unionised at low pH, and therefore remain insoluble. But as they encounter an increased pH, the acidic functional group of coating ionise, and the polymer itself gets swollen and becomes soluble in the intestinal fluid (Hussan et al., 2012).

Enteric coating is generally carried out:

- To preserve the drug substance from the acidic environment of the stomach.
- To protect against gastric distress or nausea caused by the drugs which are irritant to gastric mucosa. (e.g. sodium salicylate).
- To confer a delayed-release component.
- In order to minimize the first pass metabolism of drugs (Pole et al., 2016).

Selection of the polymers and the thickness of the coated layer are the two most critical parameters in the preparation of the enteric coated dosage forms. The examples of drugs that cause gastric distress include aspirin, diclofenac and naproxen. These are available with enteric coatings (Islam et al., 2016). Omeprazole, which is a drug which stops the stomach from producing acid itself but gets degraded in the gastric is available as an enteric coated formulation. Sulfasalazine that is used for the treatment of Crohn's disease or is given with an enteric coating to exert its physiological effect at the site of pathology. However, when the same drug is administered for the treatment of arthritis through its systemic effect, it is used as an uncoated formulation (Bozdag et al., 1999)

Drug generally used for enteric coating are as given below:

- Which irritate the gastric mucosa or get degraded at gastric pH.
- The action of which is required only in the intestines such as anthelmintics and amoebicides.
- Absorption of which takes place from the intestines.

- Delayed action of drug is required (Pole et al., 2016).

### 2.4.1 Polymer used for enteric coating:

#### 2.4.1.1 Classification of Polymers used for the enteric coating

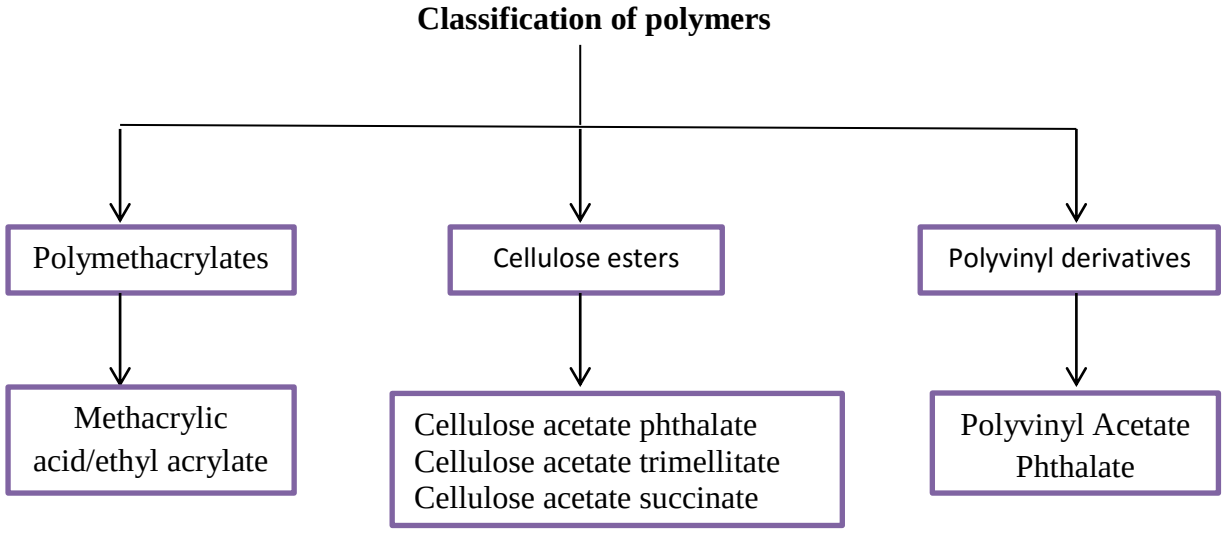



Fig 2.5 Classification of enteric coated polymers (Pole et al., 2016).

**1) Polymethacrylates**

(Methacrylic acid/ethyl acrylate)

Eudragit L and Eudragit S are two types of marketed enteric acrylic resins. Both resins form films that are resistant to gastric fluid. Eudragit L and Eudragit S are soluble in intestinal fluid at pH 6 to 7 respectively (Pole et al., 2016). In vitro dissolution study of sodium para amino salicylate pellets coated with Eudragit L30 D-55 is reported. Eudragit L30 D-55 (60%w/w) was found to exhibit satisfactory results against the gastric attack (Rahman et al., 2008 ). Colon targeted drug delivery of tegasterod maleate was tried using Eudragit L and Eudragit S Ratio 1:2 was found to yield the best results (Venkatesh et al., 2009). Colon targeted drug delivery of albendazole showed better results with Eudragit S 100 as compared to hydroxy propyl methyl cellulose phthalate (HPMC phthalate) and Ethyl cellulose **(**Reddy et al., 2013).

**2) Cellulose esters**

Cellulose esters are used as enteric polymer in the pharmaceutical industry. The major disadvantage of CAP is that it dissolved only above the pH 6, and delays the absorption of drugs. CAP polymer degrades as pH increases above 6.5 but CAT polymer degrades at pH 5.5 (Naresh et al., 2012). HPMCP-50, 55,55S is derived from Hydroxy propyl cellulose and

dissolves at pH (5 to 5.5) lower than that of CAP. It also exhibits more stability as compared to CAP because the labile acetyl group is absent (Pole et al., 2016).

**3) Polyvinyl Derivatives Polyvinyl acetate phthalate (PVAP)**

Polyvinyl acetate phthalate (PVAP) is synthesised by the esterification of a partially hydrolysed polyvinyl acetate with phthalic anhydride (Pole et al., 2016). Gastric juice is not able to permeate PVAP. Also it is more resistant to hydrolysis, and gets ionized at a lower pH. It gives a rapid release of actives in the duodenum (Mahdi B et al., 1991).

# CHAPTER 3

# RESEARCH ENVISAGED AND PLAN OF WORK

## 3.1 Rationale

Last few decades conventional drugs were used for the treatment of helminthiasis. These drugs are associated with a number of side effects e.g. nausea, headache, alopecia, hypothermia and dizziness etc. Also conventional drugs produce drug resistance against helminths that led to increased demand of screening medicinal plants for their anthelmintic activity.

Krimihar$^{TM}$ syrup is marketed polyherbal formulation consists of fourteen herbs used for the treatment of helminthiasis. The objective of the study will be the evaluation of Krimihar$^{TM}$ syrup as well as in vitro screening for anthelmintic activity of individual herbs and in combination. The most effective constituent/s would be formulated into enteric coated formulations to achieve the maximum physical interaction of the drug with the parasites. The developed tablet formulation will be evaluated for various quality control parameters.

## 3.2 Aim and objectives

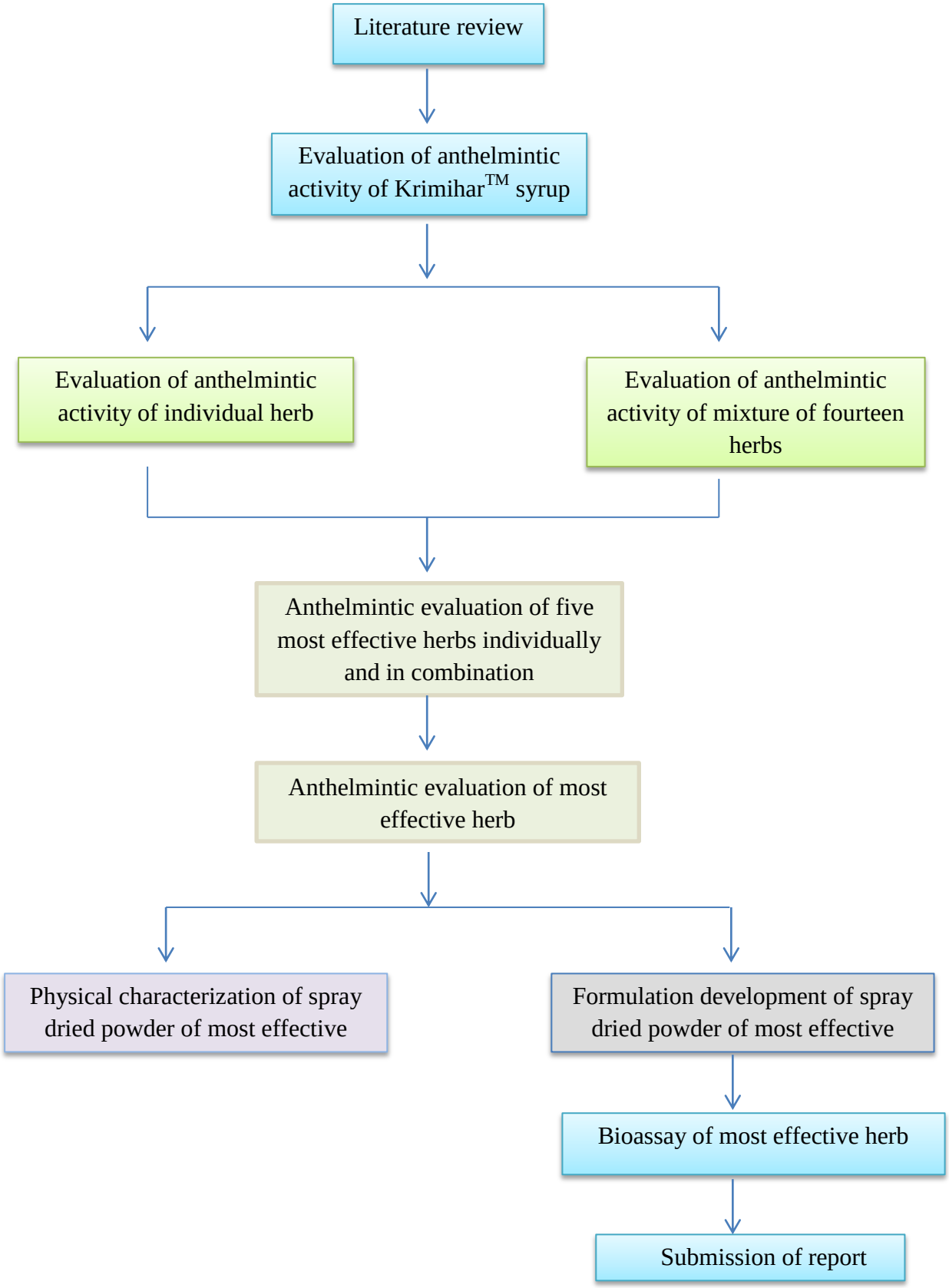
### 3.2.1 Aim of the study

The aim of the presented work is "Herbal formulation for anthelmintic activity: Design and Evaluation".

### 3.2.2 Objective of the study

- To evaluate the anthelmintic potential of aqueous decoction of individual herb present in Krimihar$^{TM}$ syrup using earthworm as in vitro animal model
- To compare the anthelmintic potential of aqueous decoction of individual active ingredient with aqueous decoction of mixture of component of krimihar$^{TM}$ syrup
- To evaluate and improve upon (if required) the pre-formulation parameters of the most effective constituent
- To formulate an enteric coated tablet of the most effective constituent
- To develop bioassay of this constituent
- Characterization of developed enteric coated tablets

### 3.3. Research Methodology



# CHAPTER 4

# MATERIALS AND EQUIPMENTS

## 4.1 List of materials and equipment used during the study

**Table 4.1**

List of materials used in study

| S.No | Materials | Manufacturer |
|---|---|---|
| 1. | Herbs | Shree Dhanwantri Herbals Pvt Ltd. Amritsar, India |
| 2. | Sodium hydroxide | LOBA Chemie Pvt. Ltd. Mumbai, India. |
| 3. | Potassium dihydrogen Orthophosphate | LOBA Chemie Pvt. Ltd. Mumbai, India. |
| 4. | Hydrochloric acid | Thermo fisher scientific Pvt Ltd, Mumbai, India |
| 5. | Potassium chloride | LOBA Chemie Pvt. Ltd. Mumbai, India |
| 6. | Methanol | LOBA Chemie Pvt. Ltd., Mumbai, India |
| 7. | Acetone | LOBA Chemie Pvt. Ltd. Mumbai, India |
| 8. | Petroleum ether | LOBA Chemie Pvt. Ltd. Mumbai, India |
| 9. | Piperazine citrate | Titan biotech Ltd. Rajasthan, India |
| 10. | Lactose | LOBA Chemie Pvt. Ltd. Mumbai, India |
| 11. | Magnesium stearate | CDH Pvt Ltd, New Delhi, India |
| 12. | Microcrystalline cellulose | Jackson Laboratories Pvt. Ltd., Amritsar, India. |
| 13. | Dicalcium phosphate | LOBA Chemie Pvt. Ltd. Mumbai, India |
| 14. | Sodium starch glycolate | LOBA Chemie Pvt. Ltd. Mumbai, India |
| 15 | Eudragit S 100 | Evonik Industries Pvt. Ltd. Germany |

**Table 4.2**

List of equipment used in study

| S. No | Machine | Model and manufacturer |
|---|---|---|
| 1. | Spray dryer | Spray mate, JISL, Mumbai, India. |
| 2. | Electronic balance | CY360, Shimadzu Co. Ltd., Japan. |
| 3. | Ultrasonication bath | LOBA LIFE, Loba Chemie, Mumbai, India |
| 4. | pH meter | Phan, LABINDIA, Thane West, Maharashtra, India |
| 5 | Hot air oven | Cadmach Drying Oven, Cadmach Machinary Ltd., Ahmadabad, India |
| 6. | Sieves | Sieve No. 60, Bhushan Engineering & Scientific Traders, Ambala, India |
| 7. | Heating mentle | Navyug India limited, New Delhi, India |
| 8. | Tablet Dissolution | DS 8000 (Manual) LABINDIA, Maharashtra, India |

| | | |
|---|---|---|
| | apparatus | |
| 9. | Tablet punching machine | Trover Pharmamec, Punjab, India |
| 10. | Electronic Grinder | Stovekraft Pvt. Ltd. Bangalore, India |
| 11. | Pan coater | Navyug India, Jalandhar, India |
| 12. | V-cone blender | Swastika Pvt Ltd, Ambala, India |
| 13. | Hardness tester | Monsanto hardness apparatus, Navyug India, Jalandhar, India |

# CHAPTER 5

# EXPERIMENTAL WORK

## 5.1 Evaluation of Krimihar™ syrup for its anthelmintic activity

In vitro anthelmintic assay was performed as per the method of Ghosh et al., (2005). Use of *Ascaris lumbricoides, Ascaridia galli* and *Eisenia foetida* has been reported for the screening of anthelmintic activity. But *Eisenia foetida* has been most commonly used as test worm to evaluate the anthelmintic activity of natural extracts because it possesses physiological and anatomical similarity with the intestinal parasites (Mali et al., 2005). *Eisenia foetida* was procured from Jeevan Organic Fertilizer and authenticated from the Zoology department. Worms having length of 2-4 cm and width 0.2-0.3 cm were used for study.

As the site of action of the anthelmintic syrup is intestine, the procedure for evaluation of its anthelmintic activity was designed as follows:

Krimihar™ syrup (15 mL) was transferred into beaker containing 200 mL of 0.1 N HCl solution and kept aside for 2 hours. After that, solution was diluted upto 900 mL using phosphate buffer (pH 6.8) in order to achieve concentration of 1.6% v/v. Aliquots (10 mL) of above solution were transferred into three petri plates. One earthworm (*Eisenia foetida*) was added to each petri plate. Paralysis time (PT) was noted as the time when no movement was observed except when the worms were shaken vigorously. Death time (DT) of worms was recorded as the time when the worms neither moved when shaken vigorously nor when dipped in warm water at 50˚C (Daksha et al., 2012, Mulla et al., 2010). Average paralysis time (APT) and average death time (ADT) were noted.

In the first phase, studies were conducted to rule out the effect of various additives like sweeteners, preservatives, and colorants etc. that are present in the marketed formulation. To rule out the effect of these additives, aqueous decoctions were prepared from raw drugs and further evaluated individually as well as in combination, for their anthelmintic activity.

## 5.2. Evaluation of aqueous decoction of various ingredients of Krimihar™ syrup

### 5.2.1 Preparation of aqueous decoction of individual herbs

Each ingredient was crushed using Electronic Grinder (Stovekraft Pvt. Ltd. Bangalore, India) and was passed through the sieve no. 60. Crushed powder was weighed individually (quantity as listed in Table 5.1) and transferred into 500 mL beaker containing 200 mL of distilled water individually. The solution was kept at 100˚C for 40 min. The solution was allowed to cool to room temperature. The cooled solution was filtered through muslin cloth and filtrate was collected (Lem et al., 2014, Washington et al., 2016). It is pertinent to add here that on

cooling the decoction became turbid and some of the turbidity persisted even after filtration through the muslin cloth.

Table 5.1 List of herbs present in Krimihar™ syrup

| Herbs | Quantity (g) in 200 mL | Actual concentration (mg/mL) | Final concentration of aqueous decoction of anthelmintic activity (mg/mL) |
|---|---|---|---|
| *Embelia ribes* (Vidang) | 8g | 40 | 0.66 |
| *Cyperus rotundus* (Nagarmotha) | 8g | 40 | 0..66 |
| *Gardenia gummifera (*Nadihingu) | 4g | 20 | 0.33 |
| *Carum roxburghianum* (Tukham ) | 4g | 20 | 0.33 |
| *Butea monosperma (*Palash Beej) | 4g | 20 | 0.33 |
| *Punica garnatum* (Naspal) | 4g | 20 | 0.33 |
| *Mollotus phillppiness ( *Kabila) | 4g | 20 | 0.33 |
| *Cassia angustifolia (*Sanai) | 4g | 20 | 0.33 |
| *Hyoscyamus niger* (Ajwain ) | 2g | 10 | 0.16 |
| *Holarrhena antidysnterica* (Inderyav) | 2g | 10 | 0.16 |
| *Artemisia absinthium* (Afasanteen ) | 2g | 10 | 0.16 |
| *Andrographis paniculata* (Kalmegh) | 2g | 10 | 0.16 |
| *Centratherum anthelminticum* (Kalijiri) | 2g | 10 | 0.16 |
| *Swertia chirata* (Chirata) | 2g | 10 | 0.16 |

### 5.2.2 Evaluation of anthelmintic activity of various ingredients of Krimihar™ syrup

Samples (15 mL) were withdrawn from above prepared dilution and transferred to 1000 mL separate beaker containing 200 mL of 0.1 N HCl and kept for 2 hours. After that, solution was diluted upto 900 mL using 6.8 phosphate buffer (0.2 M). The final concentration that has

been used for anthelmintic evaluation of various decoctions is shown in Table 5.1. From each of these, 10 mL aliquot was transferred to petri plates containing one earthworm each.

**5.2.3 Preparation of aqueous decoction of mixture of fourteen herbs (ADMFH)**

For preparation of ADMFH, all the ingredients were weighed accurately as listed in Table 4 and transferred into 500 mL beaker containing 300 mL of distilled water. The final concentration in the solution was calculated to be 173.3 mg/mL. The solution was kept at 100˚C for 40 min. and allowed to cool to room temperature. The cooled solution was filtered through muslin cloth. It is pertinent to add here that on cooling the decoction became turbid and some of the turbidity persisted even after filtration through the muslin cloth. The filtrate was then collected and kept for further study.

**5.2.4 Anthelmintic activity of ADMFH**

ADMFH (15 mL) was transferred to 1000 mL beaker containing 200 mL of 0.1 N HCl and kept aside for 2 h. This solution was then diluted upto 900 mL using phosphate buffer (pH 6.8). The final concentration in the solution was calculated to be 2.88 mg/mL. From these, 10 mL of diluted aqueous decoction was transferred to petri plate containing earthworm (*Eisenia foetida).* APT and ADT were recorded.

**5.2.5 Spray drying of ADMFH**

ADMFH was transferred in 1000 mL beaker and placed at the sample inlet of spray dryer. The operation parameters are of given below:

- Inlet temp 120˚C
- Outlet temp 60˚C
- Aspirator speed (rpm) 1400
- Feed pump speed (rpm) 16
- Automisation pressure ($Kg/cm^2$) 2
- Concentration (%) 10

After the drying process, the powder was collected and kept in a dessicator till further evaluation (Kaur et al., 2015).

**5.2.6 Anthelmintic evaluation of spray dried powder obtained from ADMFH**

Spray dried ADMFH powder was weighed in quantities of 2, 4, 6, 8, 10, 20, 30, 40, 50 mg. Weighed amounts were transferred to 10 mL volumetric flasks and volume was adjusted with phosphate buffer (pH 6.8) to get the concentration of 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5 mg/ mL. Prepared samples of different concentrations were transferred into petri plates. One

earthworm (*Eisenia foetida*) was added to each petri plate. Each concentration was evaluated in triplicate and mean data was recorded.

As a very large variation in death time was observed i.e. from 135 min to 980 min for individual drugs, it was decided to select the five most effective ingredients and check the effect of this combinations vis-à-vis the effect of the combination of 14 drugs. The short listed drugs included *Hyoscyamus niger, Centratherum anthelminticum, Mallotus philippensis, Embelia ribes and Butea monosperma.*

### 5.3 Anthelmintic evaluation of aqueous decoction of mixture of five most effective herbs (ADMFEH)

#### 5.3.1 Preparation of ADMFEH

Each ingredient was crushed properly using electronic grinder and passed through the sieve no. 60. Crushed powder was weighed individually (quantity as listed in Table 5.2) and transferred into 500 mL beaker containing 200 mL of distilled water. The final concentration in the solution was calculated to be 100 mg/mL. The solution was kept at 100˚C for 40 min. It is pertinent to add here that on cooling the decoction became turbid and some of the turbidity persisted even after filtration through the muslin cloth. The solution was allowed to cool to room temperature. The cooled solution was filtered through muslin cloth and filtrate was collected.

Table 5.2 List of five most effective herbs

| Herbs | Quantity (g) |
|---|---|
| *Embelia ribes* (Vidang) | 8g |
| *Butea monosperma (*Palash Beej) | 4g |
| *Mollotus phillppiness (* Kabila) | 4g |
| *Hyoscyamus niger* (Ajwain) | 2g |
| *Centratherum anthelminticum* (Kalijiri) | 2g |

#### 5.3.2 Anthelmintic activity of ADMFEH

ADMFSH (15 mL) was transferred to 1000 mL beaker containing 200 mL of 0.1 N HCl and kept it for 2 hours. After that solution was diluted upto 900 mL using phosphate buffer (pH 6.8). The final concentration in the solution was calculated to be 1.67 mg/mL. From this, 10 mL solution was transferred to petri plates containing one earthworm each. The study was conducted in triplicate and APT as well as ADT were recorded.

#### 5.3.3 Spray drying of ADMFEH

Aqueous decoction of five most effective herbs was spray dried keeping the parameters as described in section 5.2.5.

### 5.3.4 Anthelmintic evaluation of spray dried ADMFEH

Spray dried ADMFEH powder was weighed in quantities of 2, 4, 6, 8, 10, 20, 30, 40, 50 mg. Weighed amount was transferred in 10 mL volumetric flask and volume was adjusted with phosphate buffer (pH 6.8) to get the concentration of 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5 mg/ mL. Prepared samples of different concentrations were transferred into petri plate. One earthworm *(Eisenia foetida)* was added in each petri plate. The study was carried out in triplicate and mean data was recorded.

## 5.4 Preparation of spray dried powders of five most effective herbs individually

Aqueous decoction of five most effective herbs was prepared individually as mentioned in section 5.2.1 and dried using spray drier maintaining the conditions mentioned in section (5.2.5).

### 5.4.1 Anthelmintic evaluation of spray dried powders of five most effective herbs individually

Spray dried powders of individual herbs were weighed in quantities of 5, 10, 20, 30, 40, 50 mg. Weighed amounts were transferred to 10 mL volumetric flasks and volume was adjusted with phosphate buffer (pH 6.8) to get the concentration of 0.5, 1, 2, 3, 4, 5 mg/ mL. Anthelmintic activity was evaluated for all the solutions individually. ADT and APT were recorded.

Spray dried powders of individual herbs were evaluated at different concentration as mentioned above. Among these herbs, *C. anthelminticum* shows better activity as compared to other four herbs. Hence *C. anthelminticum* was selected for further studies.

## 5.5 Evaluation of anthelmintic activity of *Centratherum anthelminticum*:

Anthelmintic activity of *C. anthelminticum* was evaluated using *Eisenia foetida*. Different concentrations of spray dried aqueous decoction of *C. anthelminticum* were tested in the bioassay. APT and ADT were noted. Piperazine citrate is used as standard drug for investigation of biological activity (Mehta et al., 2012).

Spray dried powder of *C. anthelminticum* was weighed is quantities of 2, 4, 6, 8, 10, 20, 30, 40, 50 mg. Weighed amounts were transferred to 10 mL volumetric flask and volume was adjusted with distilled water to get the concentration of 0.1, 0.2, 0.3 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5 mg/ mL. The same concentration of standard drugs i.e. Piperazine citrate was also prepared. Distilled water was used as control. One earthworm was added to each petri plate

containing different concentration of test sample. Each study was conducted in triplicate. APT and ADT were noted as mentioned in section 5.1.

**5.6 Bioassay for *Centratherum anthelminticum***

**Preparation of standard plot**

To prepare standard plot, *C. anthelminticum* (100 mg) was accurately weighted and transferred to 100 mL volumetric flasks and volume was adjusted with distilled water to get the concentration of 1 mg/mL. Further dilutions were made from stock solution (1 mg/mL). Aliquots of 9, 8, 7, 6, 5, 4.5, 4, 3.5, 3, 2.5 and 2 mL were transferred from stock solution into 10 mL volumetric flask and volume was adjusted with distilled water to get the concentration of 0.9, 0.8, 0.7, 0.6, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25 and 0.2, mg/mL. Different weights i.e. 20, 30 and 40 mg were accurately weighed and transferred to 10 mL volumetric flasks and volume was adjusted with distilled water to get the concentration of 2, 3 and 4 mg/mL. Sample (10 mL) from each dilution was transferred to petri plate containing one earthworm each. The study was conducted in triplicate and ADT was noted. Logarithms of ADT were plotted against logarithms of concentration to find out linearity of response with respect to coefficient of regression.

**5.7 Formulation development**

**5.7.1 Calculation of percentage yield**

The product obtained by spray drying was weighed and percentage yield of spray dried product was calculated as per the given formula

$$\text{Percentage yield} = \frac{\text{practical yield}}{\text{theoretical yield}} \times 100 \qquad \text{Equation (1)}$$

**5.7.2 Pre-compression parameters of spray dried powder**

**5.7.2.1 Angle of repose**

The angle of repose of the spray dried powders was measured using fixed funnel and standing cone method as reported by Kaur et al., (2015). The angle of repose was calculated by using following formula.

$$\tan \alpha = 2h/D \qquad \text{Equation (2)}$$

where, "h'' is the height of the heap of powder and "D" is the diameter of the base of the heap of powder.

**5.7.2.2 Bulk density**

Bulk density was determined by transferring powder mixture into a measuring cylinder and gently tapping it two lines. The volume obtained was recorded as "$V_b$". The weight of the

powder was measured and recorded as "M". Bulk density was calculated by the equation below.

Bulk density = $M/V_b$ Equation (3)

**5.7.2.3 Tapped density**

Tapped density was determined by transferring powder mixture into a measuring cylinder and tapping it 100 times. The volume obtained was recorded as "$V_t$". The weight of the powder was measured and recorded as "M". Then tapped density was calculated by the equation,

Tapped density = $M/V_t$ Equation (4)

**5.7.2.4 Compressibility index (C.I.)**

It was also known as Carr's index which was calculated by the equation,

C.I. = (Bulk density–-Tapped density)/ Bulk density × 100 Equation (5)

**5.7.2.5 Hausner's ratio**

It was determined by the equation,

H.R. = Bulk volume/ Tapped volume Equation (6)

(Subrahmanyam et al., 2000)

**5.7.3 Preparation of enteric coated tablets of the spray dried extract of *Centratherum anthelminticum* (SDECA)**

**5.7.3.1 Preparation of core tablet**

Core tablets of SDECA were prepared by direct compression technique. The blend of drug and excipients was made using geometrical mixing in a 'V' cone blender (Swastika Pvt Ltd, Ambala, India). The blend was mixed for 20 min in order to get homogeneous mixing and passed through sieve no 30. Finally, the blend was compressed using Multipunch tablet compression machine (Trover, Pharmamach, Nakodar, India) with compression force of 7 KN. The unit formula composition is shown in Table 5.3

Table 5.3 Unit formula for prepared tablet

| S.No | Ingredients | B1 | B2 |
|---|---|---|---|
| 1 | *Centratherum anthelminticum* extract | 400 | 400 |
| 2 | Microcrystalline cellulose pH 102 | 100 | 100 |
| 3 | Lactose | 50 | 40 |
| 4 | Magnesium stearate | 10 | 10 |
| 5 | Sodium starch glycolate | 10 | 30 |
| 6 | Dicalcium phosphate | 30 | 20 |
| | Total weight | 600 | 600 |

**5.7.3.2. Preparation of coating solutions**

The coating solution was prepared by dissolving 5 g of Eudragit S100® in 100 mL of acetone-isopropyl alcohol mixture (50:50 % v/v). The prepared solution was stirred for 1 h on a magnetic stirrer to make a homogenous solution (Prudhviraj et al., 2015).

**5.7.3.3 Enteric coating of prepared *Centratherum anthelminticum* core tablet:**

Prepared tablets were coated using Eudragit S100® using pan coater (Navyug India, Jalandhar, India). For this, 100 tablets were taken, weighed and kept in the pan for prior heating. Then 5% w/v Eudragit S100® solution was sprayed on the tablets and coated tablets were kept for drying. The film thickness is expressed as the percentage of the weight gained relative to the weight of coated tablets.

$$\% \text{ weight gain} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100 \qquad \text{Equation (7)}$$

**5.8 Evaluation of the prepared tablets**

**5.8.1 Weight variation**

Twenty tablets of each formulation were weighed individually using digital weighing balance and their average weight was calculated. Then individual tablet weight was compared with average weight (Indian pharmacopeia, 2014).

**.5.8.2 Hardness test:**

Hardness of tablets was tested using Monsanto tester. The force required to crush the tablet was recorded as hardness in $Kg/cm^2$. Three tablets were subjected to hardness test and the crushing strength of each tablet was measured. Average hardness of the tablets was calculated and standard deviation was determined (Indian pharmacopeia, 2014).

**5.8.3 Thickness and Diameter**

Thickness and diameter was also measured for prepared tablets using Vernier caliper (Indian pharmacopeia, 2014).

**5.8.4 Friability**

Six tablets were weighed accurately and then placed in Roche-type friabilator which was rotated at 25 rpm for 4 min (i.e. 100 revolutions). Then tablets were taken out of the friabilator and again weighed after dedusting. The percent friability was calculated as follow:

$$\% \text{ Friability} = \frac{W_i - W_f}{W_i} \times 100 \qquad \text{Equation (8)}$$

Where, $W_i$ - initial weight of tablets; $W_f$ -final weight of tablets (Indian pharmacopeia, 2014)

**5.8.5 Disintegration test**

The disintegration test for prepared tablets was performed using USP Disintegration Test Apparatus. One tablet was placed in each of six disintegration tube. The apparatus was operated using 0.1 N HCl solution as medium maintained at 37±2˚C. After 2 hours, discs were added and the apparatus was operated using phosphate buffer pH 6.8 as medium maintained at 37±2˚C. (Indian pharmacopeia, 2014)

**5.8.6 Dissolution study of enteric coated tablet**

The dissolution testing of enteric coated formulation was carried out according to USP 27 NF 22 (711), by adopting Method B (USP convention INC., 2004) in 0.1N HCl and subsequently phosphate buffer (pH 6.8). The paddle was stirred at 50 rpm and a temperature of 37 ± 0.5°C. Prepared tablets were added to the vessels containing the described dissolution medium. For the first two hours, in vitro bioassay study was carried out in 200 mL, 0.1 N HCl. Samples (10 mL) were withdrawn at time interval of 30, 60, 90, 120 min and replaced with fresh media. The volume of dissolution media was then made up to 900 mL and its pH was adjusted to pH 6.8 by addition of phosphate buffer. The study was continued in this medium for 1 h. The analysis of the withdrawn samples was carried out using the method of bioassay as reported in section 5.6. A number of reports exist in literature where the analysis of dissolution samples has been carried out using bioassays (Liu et al., 2015, Long et al.., 2011). Samples were withdrawn at regular time interval of 125, 130, 135, 140, 145, 150, 160, 170 and 180 min and replaced with fresh media. (Bushra et al., 2010, Liya teklu et al., 2014). Each sample was transferred to petri plate containing one worm of *Eisenia foetida.* APT and ADT were calculated as reported in section 5.1. Concentrations of drug present in the samples were calculated from the standard plot of *C. anthelminticum*

**5.9 Physical characterization of Spray dried powders:**

**5.9.1 Powder X-ray diffraction (PXRD)**

The PXRD patterns of sample was recorded using high power powder X-ray diffractometer (Ru-200B, Pune, India) with Cu line as the source of radiation, having a voltage of 40- KV, 40 mA current at a scan speed of 4˚/min. The samples were analysed at 2θ angle range of 5-50˚. Step time was 0.5 s and time of acquisition was 1 h (Renuka et al., 2014).

**5.9.2 Scanning Electron Microscopy (SEM)**

The surface morphology of the sample was studied by SEM. Samples were fixed onto a metallic stub with double-sided conductive tape (diameter 12 mm, Oxon, Oxford 356 Instruments, UK). A Supra 35 VP (Oberkochen, Zeiss, Germany) Scanning electron 357

microscope was used with an acceleration voltage of 15 kV and a secondary detector (Shinde et al., 2015).

### 5.9.3 Particle size analysis

The particle size of unprocessed powder and spray dried powder of *C. anthelminticum* was measured using Malvern Zetasizer Version 7.11. Both the powders (each 5 mg) were dispersed in 10 mL water to get the concentration of 0.5 mg/mL. Three readings were taken for each sample. The mean values of particle size of both the powdered samples were recorded (Kaur et al., 2015).

## 5.10 Statistical analysis of data

All the experimental data are expressed as mean ± standard deviation (SD), respectively. Statistical analysis of obtained data was carried out either by analysis of variance or Tukey's multiple comparison test using GraphPad Prism version 7.0 (GraphPad Software Inc., CA, USA) . A value of $P < 0.05$ indicates significant difference in the obtained results

# CHAPTER 6

# RESULTS AND DISCUSSIONS

## 6.1 Evaluation of Krimihar™ syrup for its anthelmintic activity

APT and ADT for 1.6% v/v of Krimihar™ syrup were found to be 200±5.00 and 285±2.64 respectively. The result was shown in Table 6.1.

Table 6.1 Anthelmintic activity of Krimihar™ syrup

| S.No | Volume of syrup (mL) | Diluted with pH 6.8 buffer | Paralysis time (min) | Death time (min) |
|---|---|---|---|---|
| 1 | 15 | 900 | 200 | 284 |
| 2 | 15 | 900 | 195 | 283 |
| 3 | 15 | 900 | 205 | 288 |
| | | | Mean 200±5.00 | Mean 285±2.64 |

The results indicate that a physical contact time of more than 4 h in intestine is required for the marketed formulation to exert its anthelmintic activity.

## 6.2 Evaluation of anthelmintic activity for aqueous decoction of individual herbs of Krimihar™ syrup

Keeping in view the fact that the earlier reports indicate a strong anthelmintic activity of certain individual herbs but weaker activity of their combinations, we decided to explore the anthelmintic effect of each of the individual herbal constituent of the marketed formulation (Hordegen et al., 2003, Quereshi & Sabir 1979. Paydar et al., 2013)

The evaluation of aqueous decoction of each of the ingredients for anthelminthic activity was done to check the effectiveness of the individual herb. Effectiveness of the various herbs was checked on the basis of two parameters; paralysis time and death time which is recorded in Table 6.2 and Fig 6.1

Table 6.2 Anthelmintic activity of fourteen herbs present in Krimihar™ syrup

| S.No | Herbs (Amount) | APT (min) | ADT (min) |
|---|---|---|---|
| 1 | *Embelia ribes* (ER) | 410±2 | 450±4 |
| 2 | *Cyperus rotundus* (CR) | 540±3 | 615±5 |
| 3 | *Gardenia gummifera (GG*) | 435±5 | 480±2 |
| 4 | *Carum roxburghianum* (CRB ) | 780±2 | 980±4 |
| 5 | *Butea monosperma (*BM) | 240±5 | 340±6 |
| 6 | *Punica garnatum* (PG) | 600±8 | 630±3 |
| 7 | *Mollotus phillppiness (* MP) | 210±4 | 260±3 |
| 8 | *Cassia angustifolia (*CAG) | 450±6 | 530±6 |

| | | | |
|---|---|---|---|
| 9 | *Hyoscyamus niger* (HN ) | 410±3 | 430±5 |
| 10 | *Holarrhena antidysnterica* (HA) | 900±6 | 975±5 |
| 11 | *Artemisia absinthium* (AA ) | 600±7 | 690±2 |
| 12 | *Andrographis paniculata* (AP) | 720±5 | 780±3 |
| 13 | *Centratherum anthelminticum* (CA) | 120±4 | 195±4 |
| 14 | *Swertia chirata* (SC) | 540±5 | 560±5 |

Results are expressed as mean±SD of three observations.

From the obtained data it was concluded that while some of the component herbs were found to be quite effective, some exhibited very weak activity. Some of the individual extracts were found to be more effective than the combination used in the marketed formulation. Our results are in tune with some of the previous reports where the combinations of two drugs proved to be ineffective whereas the individual drugs had shown the effect (Hordegen et al., 2003). The most effective ingredients were found to be *Centratherum anthelminticum* with APT 120±4 min and ADT 195±4 min followed by *Mallotus phillppiness* with APT 210±4 min and ADT 260±3 min, *Butea monosperma* with APT 240±5 min and ADT 340±6 min, *Embelia ribes* with APT 410±2 min and ADT 450±4 min. On the other hand, the effect of *Holarrhena antidantidysnterica*, *Carum roxburghianum*, and *Cyperus rotundus* was found to be quite less.

The ADT was found in the following decreasing order.
CRB> HA> AP> AA> PG> CR> SC> CAG> GG> ER> HN>BM > MP>CA

Fig 6.1 Anthelmintic activities of individual herbs using Earth worm (*Eisenia foetida*)

### 6.3 Anthelmintic activity of the mixture of aqueous decoction of fourteen herbs present in Krimihar™ syrup

To attribute the less activity of the marketed formulation as compared to that of some of the individual constituents to the effect of formulation additives or antagonistic combination amongst the individual drugs, further study was designed.

APT and ADT for aqueous decoction of mixture having concentration of 2.88 mg/mL were found to be 190±1.00 min and 255±0.57 min. These values are found to be slightly lower than those observed for the commercial formulation. The slightly higher anthelmintic activity of the aqueous decoction may be attributed to the absence of excipient like colorants, sweeteners, flavours and preservatives present in formulation.

Slight improvement in the anthelmintic activity of the decoction of fourteen drug constituent over that of the marketed formulation may be attributed to the effect of excipients as has been reported in some earlier studies also (Hoegberg et al., 2003).

### 6.4 Comparison of aqueous decoction of individual herbs vs aqueous decoction of mixture

It is important to note that the APT and ADT of aqueous decoction of *Centratherum anthelminticum* and *Mallotus phillppiness* was 120±4 min and 135±4 min, 135±4 min and 180±3 min respectively. These were found to be significantly (P<0.001) better than that of the mixture of aqueous decoction of all the fourteen herbs. The APT and ADT of other twelve drugs were found significantly more (P<0.001) than that of aqueous decoction of the mixture. The results are shown in Table 6.3

Table 6.3 Statistical analysis of Comparison between decoctions of mixture and individual herbs

| S.No | Comparison between decoctions of mixture and individual herbs. | P value | Anthelmintic activity of individual herb as compared to mixture |
|---|---|---|---|
| 1 | Mixture vs CA | P<0.001 | ↑ |
| 2 | Mixture vs ER | P<0.001 | ↓ |
| 3 | Mixture vs CR | P<0.001 | ↓ |
| 4 | Mixture vs GG | P<0.001 | ↓ |
| 5 | Mixture vs CRB | P<0.001 | ↓ |
| 6 | Mixture vs BM | P<0.001 | ↓ |
| 7 | Mixture vs PG | P<0.001 | ↓ |
| 8 | Mixture vs MP | P<0.001 | ↑ |
| 9 | Mixture vs CAG | P<0.001 | ↓ |
| 10 | Mixture vs HN | P<0.001 | ↓ |
| 11 | Mixture vs HA | P<0.001 | ↓ |
| 12 | Mixture vs AA | P<0.001 | ↓ |
| 13 | Mixture vs AP | P<0.001 | ↓ |
| 14 | Mixture vs SC | P<0.001 | ↓ |

### 6.5 Anthelmintic evaluation of spray dried ADMFH

The study revealed marked increase in anthelmintic efficiency for the spray dried powder of ADMFH against *Eisenia foetida*. While APT of about 190±1.0 min and ADT of 255±0.57 min were observed at 2.88 mg/mL concentration of ADMFH. Similar effect could be achieved at 0.4 mg/mL of ADMFH after spray drying. As mentioned in section 5.2.1, the aqueous decoction was found to be slightly turbid indicating the presence of some constituent which are not soluble at room temperature. The procedure of spray drying of the decoction at high temperature renders this relatively less soluble fraction more available to dissolution by decreasing the particle size due to attrition as well as quick drying procedure along with amorphization. A 7.2 folds decrease in concentration of ADMFH for achieving the APT and ADT after spray drying could be attributed to reduction in particle size of ADMFH powder as compared to its unprocessed form. The increase in surface area of particles leading to increased penetration of solvent is expected to increase the solubility of active constituents. This could be the reason of enhancement of anthelmintic activity after the spray drying. The results are shown in Table 6.4.

Table 6.4 APT and ADT of spray dried ADMFH

| S.No | concentration (mg/mL) | APT (min) | ADT (min) |
|---|---|---|---|
| 1 | 5 | 60±4 | 105±2 |
| 2 | 4 | 75±3 | 125±1 |
| 3 | 3 | 95±1.4 | 165±5 |
| 4 | 2 | 120±1.3 | 180±2 |
| 5 | 1 | 136±1.23 | 200±1.7 |
| 6 | 0.8 | 160±1.1 | 224±1.5 |
| 7 | 0.6 | 180±1.0 | 240±1.4 |
| 8 | 0.4 | 190±1.0 | 265±1.3 |

### 6.6 Anthelmintic activity of ADMFEH and spray dried ADMFEH

*Hyoscyamus niger , Centratherum anthelminticum, Mallotus philippensis,. Embelia ribes and Butea monosperma* were found to be most effective. Hence anthelmintic activity was checked for these five herbs. While APT and ADT for ADMFEH having concentration of 1.67 mg/mL were found to be 50±1.00 min and 115±0.57 min, similar effect could be achieved for spray dried ADMFEH having concentration of 0.4 mg/mL (See table 6.5). As mentioned in section 5.2.1, the aqueous decoction was found to be slightly turbid indicating the presence of some constituent which are not soluble at room temperature. The procedure of spray drying of the decoction at high temperature renders this relatively less soluble fraction more available to dissolution by decreasing the particle size due to attrition as well as quick drying procedure

along with amorphization. The study revealed marked increase in anthelmintic efficiency for the spray dried powder of ADMFEH against *Eisenia foetida*. This 4.17 folds decrease in concentration of ADMFEH for achieving the APT and ADT after spray drying could be attributed to reduction in particle size of ADMFEH powder as compared to its unprocessed form. The increase in surface area of particles leading to increased penetration of water is expected to increase the solubility of active constituents. This could be the reason of enhancement of anthelmintic activity after the spray drying. APT and ADT of spray dried obtained from ADMFEH were noted and shown in Table 6.5.

Table 6.5 APT and ADT of spray dried ADMFEH

| S.No | concentration (mg/mL) | APT (min) | ADT (min) |
|---|---|---|---|
| 1 | 5 | 10±2 | 25±2 |
| 2 | 4 | 13±1 | 29±3 |
| 3 | 3 | 19±0.7 | 40±0.7 |
| 4 | 2 | 28±0.6 | 51±1.1 |
| 5 | 1 | 35±0.6 | 62±1.0 |
| 6 | 0.8 | 40±0.5 | 70±0.9 |
| 7 | 0.6 | 46±0.5 | 79±0.8 |
| 8 | 0.4 | 50±05 | 85±0.8 |


Fig 6.2 Anthelmintic activities of ADMFH and ADMFEH

**6.7 Anthelmintic evaluation of spray-dried powders of five most effective herbs individually:**

The anthelmintic activity of five most effective herbs was evaluated on the basis of APT and ADT. The activity was checked on various concentrations such as 0.5, 1, 2, 3, 4 and 5 mg/mL

for individual herb. The results revealed that *Centratherum anthelminticum* showed the lowest APT and ADT for *Eisenia foetida.* It was also observed that the APT and ADT for *Centratherum anthelminticum* were better than that of their mixture. The results are shown in Table 6.6 and Fig 6.3.

Table 6.6 Anthelmintic activity of spray dried powder of five most effective herb.

| S.No. | Spray dried herb | Concentration (mg/mL) | APT (min) | ADT (min) |
|---|---|---|---|---|
| 1 | *Mollotus phillppiness* | 0.5 | 145±2 | 205±4 |
| | | 1 | 136±5 | 190±3 |
| | | 2 | 120±4 | 170±1 |
| | | 3 | 110±5 | 155±5 |
| | | 4 | 90±4 | 140±3 |
| | | 5 | 80±3 | 120±3 |
| 2 | *Centratherum anthelminticum* | 0.5 | 26±2 | 65±2 |
| | | 1 | 20±2 | 42±2. |
| | | 2 | 15±3 | 30±2 |
| | | 3 | 11±1 | 20±2 |
| | | 4 | 9±1 | 17±2 |
| | | 5 | 7±1 | 15±2 |
| 3 | *Embelia ribes* | 0.5 | 280±4 | 405±5 |
| | | 1 | 260±3 | 380±6 |
| | | 2 | 245±4 | 345±5 |
| | | 3 | 200±3 | 310±6 |
| | | 4 | 180± 3 | 255±5 |
| | | 5 | 120± 2 | 190±6 |
| 4 | *Butea monosperma* | 0.5 | 230±4 | 300±5 |
| | | 1 | 210±5 | 280±6 |
| | | 2 | 195± 2 | 245±7 |
| | | 3 | 175± 3 | 215±3 |
| | | 4 | 135± 4 | 180±2 |
| | | 5 | 110±2 | 160±3 |
| 5 | *Hyoscyamus niger* | 0.5 | 250±2 | 330±2 |
| | | 1 | 240±3 | 310±3 |
| | | 2 | 210±4 | 290±6 |
| | | 3 | 220±5 | 267±4 |
| | | 4 | 170±4 | 240±3 |
| | | 5 | 140±2 | 215±5 |

.

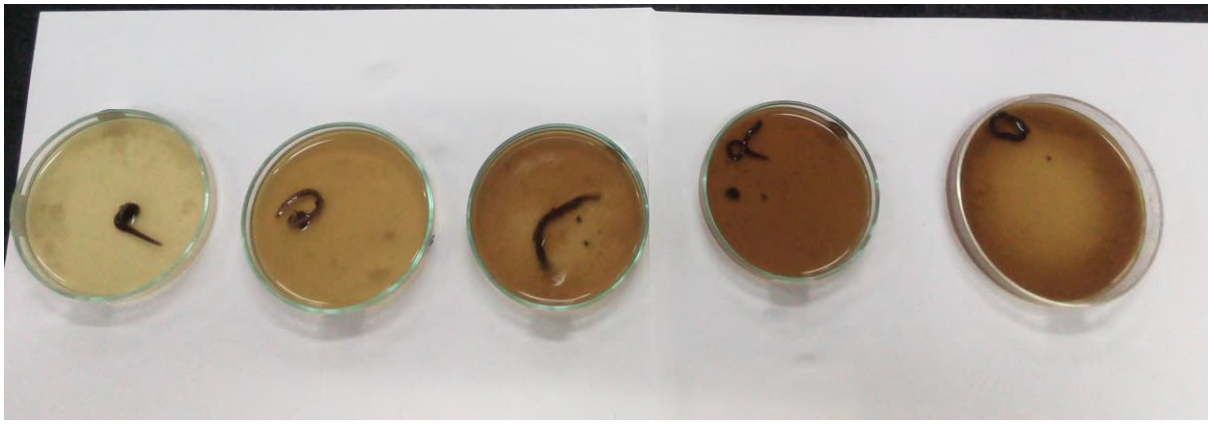


Fig 6.3 Anthelmintic activity of *Centratherum anthelmenticum* (kalijiri) using earthworm (*Eisenia foetida*)

### 6.8. Evaluation of anthelmintic activity of *Centratherum anthelminticum*

In order to evaluate the use of *C. anthelminticum* as a single ingredient vis-à-vis the poly herbal constituent, its activity was compared with that of Piperazine citrate. Piperazine citrate was taken as standard due to its well established anthelmintic potential in the available literature (Mehta et al., 2012). The anthelmintic activity was recorded for various concentrations of *C. anthelminticum* from 0.1-5 mg/mL. The results revealed that APT and ADT for *C. anthelminticum* were found lesser than those of Piperazine citrate. The results are tabulated below:

Table 6.7 Anthelmintic activity of *Centratherum anthelminticum* compared with Piperazine citrate**.**

| Groups | Concentration (mg/ml) | APT (min) | ADT (min) |
|---|---|---|---|
| | | *E. foetida* | |
| | | P | D |
| Control | 0 | 0 | 0 |
| Centratherum anthelminticum | 5 | 7±0.67 | 15±0.56 |
| | 4 | 9±0.41 | 17±0.38 |
| | 3 | 11±0.42 | 20±0.44 |
| | 2 | 15±0.49 | 30±0.41 |
| | 1 | 20±0.31 | 42±0.49 |
| | 0.8 | 20±0.40 | 50±0.32 |
| | 0.6 | 25±0.34 | 60±0.26 |
| | 0.4 | 36±0.36 | 75±0.51 |
| | 0.2 | 69±0.43 | 105±1.16 |
| | 0.1 | 75±0.53 | 120±1.23 |
| Piperazine citrate | 5 | 1±0.51 | 2±0.61 |
| | 4 | 3±0.31 | 5±0.50 |
| | 3 | 5±0.41 | 7±0.46 |
| | 2 | 10±0.40 | 15±0.41 |

| | | | |
|---|---|---|---|
| | 1 | 16±0.39 | 20±0.31 |
| | 0.8 | 25±0.42 | 37±0.43 |
| | 0.6 | 29±0.36 | 40±0.38 |
| | 0.4 | 32±0.63 | 45±0.73 |
| | 0.2 | 50±1.10 | 65±1.21 |
| | 0.1 | 59±1.10 | 80±1.21 |

Results are expressed as mean ±SEM of three observations, P- Paralysis and D- Death

### 6.9 Bioassay of *Centratherum anthelminticum*

A linear relationship between log of concentration and its log of ADT as shown in Fig 6.4. This could be understood from the plot which was found to be linear in the range of 0.2-4 mg/mL having coefficient of regression ($r^2$) of 0.9913. The obtained regression equation was Y = -0.6273x + 1.6282.

Table 6.8 Results for bioassay of *Centratherum anthelminticum*

| S.No | Concentration (mg/mL) | ADT (min) | Log concentration | Log ADT |
|---|---|---|---|---|
| 1 | 0.2 | 105±4.0 | -0.69 | 2.02 |
| 2 | 0.25 | 96±3.0 | -0.6 | 1.98 |
| 3 | 0.3 | 92±3.8 | -0.52 | 1.96 |
| 4 | 0.35 | 85±3.0 | -0.45 | 1.93 |
| 5 | 0.4 | 75±0.5 | -0.39 | 1.87 |
| 6 | 0.45 | 72±1.3 | -0.35 | 1.85 |
| 7 | 0.5 | 69±1.3 | -0.3 | 1.83 |
| 8 | 0.6 | 60±1.3 | -0.22 | 1.77 |
| 9 | 0.7 | 58±1.3 | -0.154 | 1.76 |
| 10 | 0.8 | 50±1.2 | -0.09 | 1.7 |
| 11 | 0.9 | 46±1.2 | -0.04 | 1.66 |
| 12 | 1 | 42±1.1 | 0 | 1.62 |
| 13 | 2 | 30±2.0 | 0.3 | 1.47 |
| 14 | 3 | 20±1.0 | 0.47 | 1.3 |
| 15 | 4 | 17±2.0 | 0.6 | 1.23 |

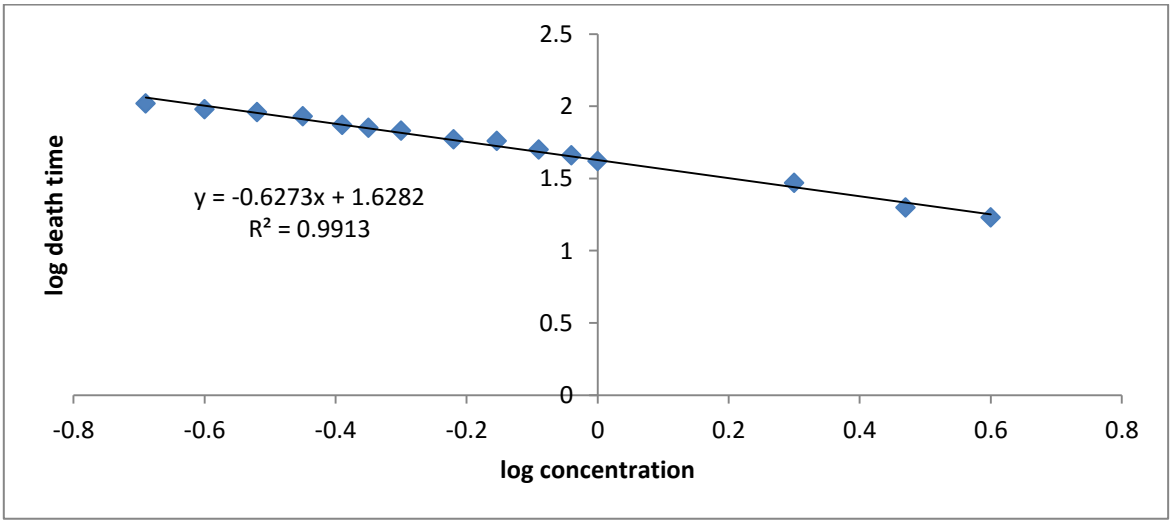


Fig 6.4. Standard plot of *Centratherum anthelminticum*

## 6.10 Formulation development

### 6.10.1 Calculation of percentage yield of spray dried herbs

Powder of most effective herbs was obtained by spray drying. Total amount of individual herb taken and its percentage yield is reported in Table 6.9

Table 6.9 Percentage yield of powders obtained from spray drying

| S.No | Spray dried herb | Amount taken (g) | Amount obtained (g) | % yield |
|---|---|---|---|---|
| 1 | *Mollotus phillppiness* | 100 | 2.00 | 2.00 |
| 2 | *Centratherum anthelminticum* | 60 | 2.59 | 4.31 |
| 3 | *Embelia ribes* | 60 | 2.96 | 4.90 |
| 4 | *Butea monosperma* | 45 | 2.21 | 4.91 |
| 5 | *Hyoscyamus niger* | 60 | 0.73 | 1.21 |

Percentage yield was found to vary between 1.21 to 4.91 % for various raw drugs. These may attributed to the water soluble constituents present in these raw drugs.

### 6.10.2 Pre-compression parameters of spray dried powder

The flow parameters used to evaluate the compression properties of spray dried powders revealed excellent powder behaviour as all these parameters were found within the pharmacopoeial limits (Indian pharmacopeia, 2014) as shown in Table 6.10

Table 6.10 Pre compression parameter of spray dried herbs

| S.No. | Spray dried herb | Tap density (g/mL) | Bulk density (g/mL) | Angle of repose | Carr's index | Hausner ratio |
|---|---|---|---|---|---|---|
| 1 | *Mollotus phillppiness* | 0.341±0.44 | 0.250±0.73 | 9.31±0.82 | 26.68±0.78 | 1.36±0.46 |
| 2 | *Centratherum anthelminticum* | 0.265±0.85 | 0.198±0.98 | 23.94±0.79 | 25.28±0.70 | 1.33±0.91 |
| 3 | *Embelia ribes* | 0.296±0.95 | 0.227±0.96 | 27.90±0.71 | 23.31±0.61 | 1.30±0.85 |
| 4 | *Butea monosperma* | 0.368±0.83 | 0.276±0.43 | 27.92±0.67 | 25.00±0.58 | 1.33±0.98 |
| 5 | *Hyoscyamus niger* | 0.243±0.86 | 0.182±0.57 | 19.44±0.80 | 25.24±0.91 | 1.33±0.95 |

### 6.10.3 Evaluation of prepared tablet

#### 6.10.3.1 Post compression parameters

The results of post compression studies of Batch B2 were found to be better in terms of friability, weight variation and disintegration time as shown in Table 6.11

Table 6.11 Results of post-compression parameters

| S.N | Batch | Thickness (mm) | Diameter (mm) | Friability (%) | Hardness ($Kg/cm^2$) | Weight variation (%) | Disintegration time 0.1 N HCl (min) | Disintegration time PB pH 6.8 (min) |
|---|---|---|---|---|---|---|---|---|
| 1 | B1 | 3.5 | 11 | 0.9±0.10 | 4.0±0.5 | 0.85±0.45 | 80 | NA |
| 2 | B2 | 4.0 | 12 | 0.3±0.09 | 3.5±0.5 | 0.58±0.37 | No disintegration in 120 min | 35 |

PB*- phosphate buffer

Based on the results of post compression parameters, B2 was selected for further studies

#### 6.10.3.2 Dissolution study of enteric coated tablet

In vitro dissolution study of enteric coated tablet of *C. anthelminticum* was performed using 0.1N HCl (200 mL). The samples were withdrawn at different time interval i.e. 30, 60, 90 and 120 min. At each time interval no paralysis and death could be observed as shown in Table 6.12. Hence it can be inferred that during this time period no perceptible release of active constituent was there. Finally the volume was adjusted upto 900 mL with phosphate buffer (pH 6.8). The samples were withdrawn at different time interval i.e. 125, 130, 135, 140, 145, 150, 160, 170 and 180. At these time intervals paralysis and death could be observed except in samples withdrawn at 125 and 130 min. The percentage drug release after 30 and 60 min were found to be 91.37 and 101.4 %. The results was shown in Table 6.13 and Fig 6.5

Table 6.12. In vitro Dissolution study of *Centratherum anthelminticum* tablet in 0.1N HCl

| S.No | Sample time (min) | PT (min) | DT (min) |
|---|---|---|---|
| 1 | 30 | no paralysis | no death |
| 2 | 60 | no paralysis | no death |
| 3 | 90 | no paralysis | no death |
| 4 | 120 | no paralysis | no death |

Table 6.13 In vitro drug release of *Centratherum anthelminticum* in phosphate buffer (pH 6.8)

| S.No | Time (min) | ADT (min) | Log death time | Log conc. | Anti log conc. | Amount/10 mL | Amount/900 mL | Cumulative drug release | %age drug release |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 125 | ND | ND | ND | ND | ND | ND | ND | ND |
| 2 | 130 | ND | ND | ND | ND | ND | ND | ND | ND |
| 3 | 135 | 96±2.0 | 1.98 | -0.5643 | 0.2727 | 2.727 | 245.43 | 245.430 | 61.35 |
| 4 | 140 | 89±1.6 | 1.94 | -0.5119 | 0.3076 | 3.076 | 276.84 | 279.567 | 69.89 |
| 5 | 145 | 80±1.5 | 1.90 | -0.4381 | 0.3646 | 3.646 | 328.14 | 333.943 | 83.48 |
| 6 | 150 | 76±3.0 | 1.88 | -0.4027 | 0.3956 | 3.956 | 356.04 | 365.489 | 91.37 |
| 7 | 160 | 73±1.0 | 1.86 | -0.3748 | 0.4218 | 4.218 | 379.62 | 393.025 | 98.25 |
| 8 | 170 | 73±1.0 | 1.86 | -0.3748 | 0.4218 | 4.218 | 379.62 | 401.556 | 100.38 |
| 9 | 180 | 72±1.0 | 1.85 | -0.3652 | 0.4313 | 4.313 | 388.17 | 405.793 | 101.44 |

ND*- no death

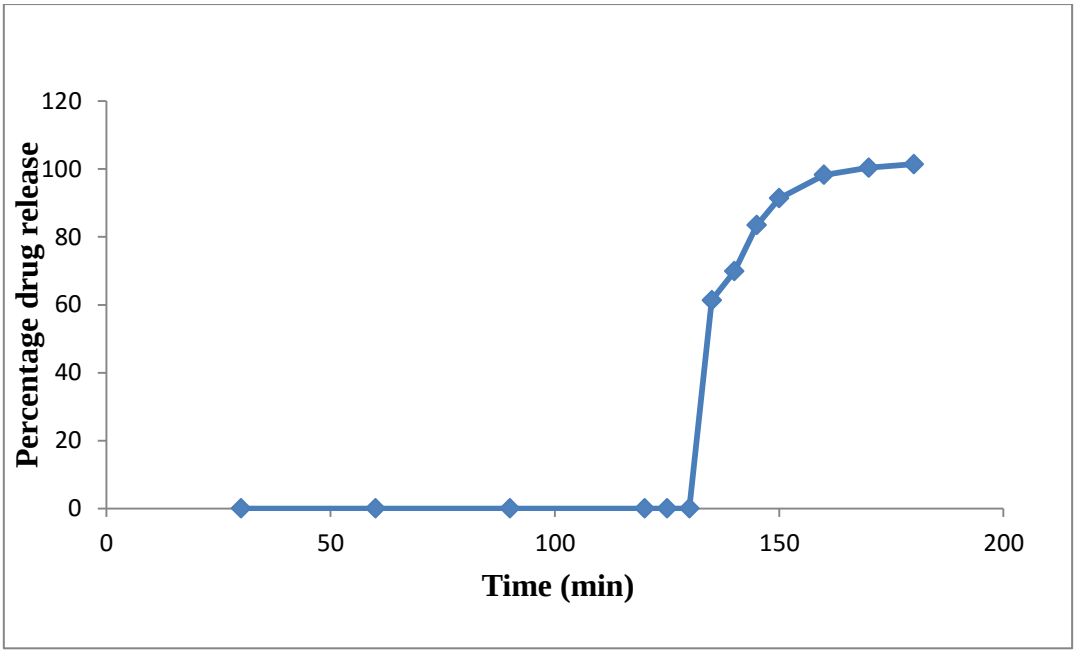



Fig 6.5. In vitro drug release of *Centratherum anthelminticum* in 0.1N HCl and phosphate buffer (pH 6.8)

## 6.11 Physical Characterization of unprocessed powder and spray dried powder of C. anthelminticum

### 6.11.1 Scanning Electron Microscopy (SEM)

The SEM images of unprocessed *C. anthelminticum* extract powder revealed long and flat crystals with reticulated habit as shown in Fig 6.6. This revealed the crystalline nature of powder. However, after spray drying, the powder revealed spherical, smooth unorganised mass with no discernible crystallinity. Hence, the spray dried powders were considered as amorphous form as shown in Fig 6.7. In order to obtain better understanding about crystal morphology, the samples were analysed for PXRD studies.

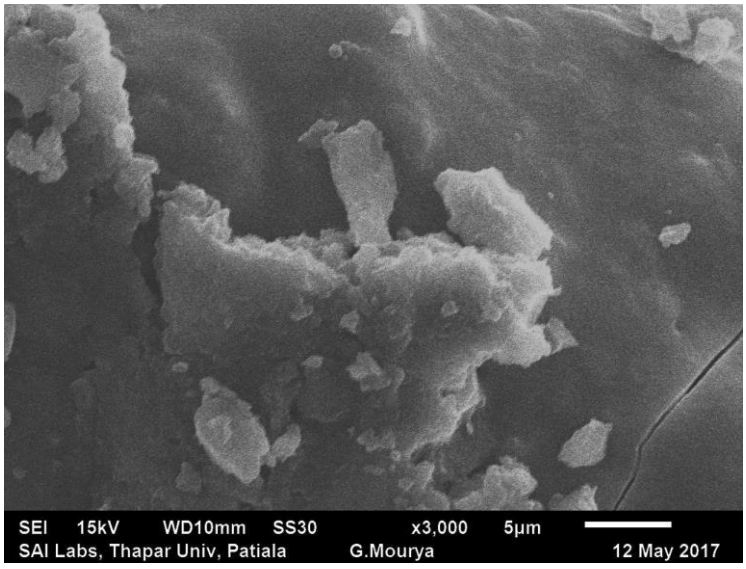


Fig 6.6 Scanning Electron Microscopy of unprocessed powder at 3000X

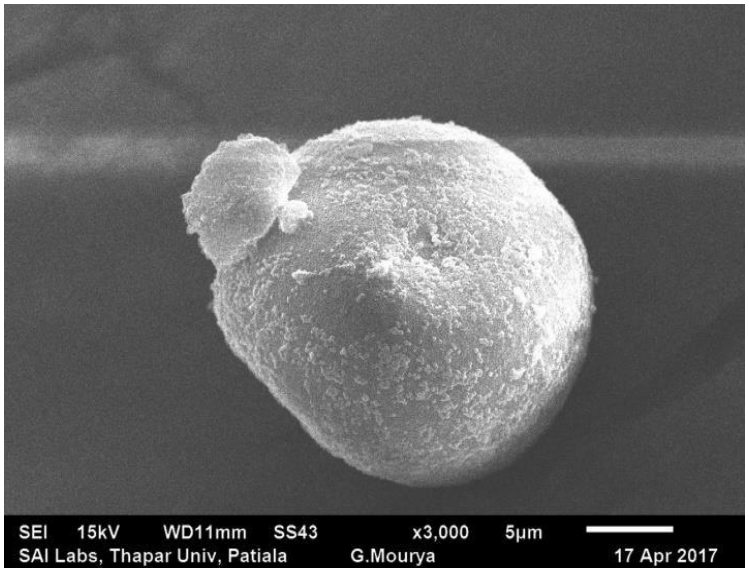



Fig 6.7. Scanning Electron Microscopy of spray dried product at 3000X

### 6.11.2 Powder X-ray diffraction (PXRD)

The PXRD patterns of unprocessed and spray dried *Centratherum anthelminticum* were recorded. The results are shown in Fig 6.8 and 6.9. Sharp peaks were observed at 18˚, 19˚, 24˚, 27˚, 33˚, 34˚, 36˚, 38˚, 41˚ and 55˚, indicating crystalline form of unprocessed *Centratherum anthelminticum* powder. Whereas for spray dried *Centratherum anthelminticum* powder, only two peaks were observed at 22˚ and 24˚, indicating decrease in powders crystallinity almost approaching an amorphous form. Hence, it was confirmed from XRD and SEM studies that after spray drying the drug got converted into amorphous form. Amorphous form does not possess any well defined arrangement and exhibits more Gibbs free energy as compared to crystalline forms. As a result, amorphous form leads to increase the solubility (Lobmann et al., 2014). This could be the reason of enhancement of anthelmintic activity after the spray drying along with the fact that the particle size achieved for the spray dried formulation was quite less as compared to the conventionally dried product.

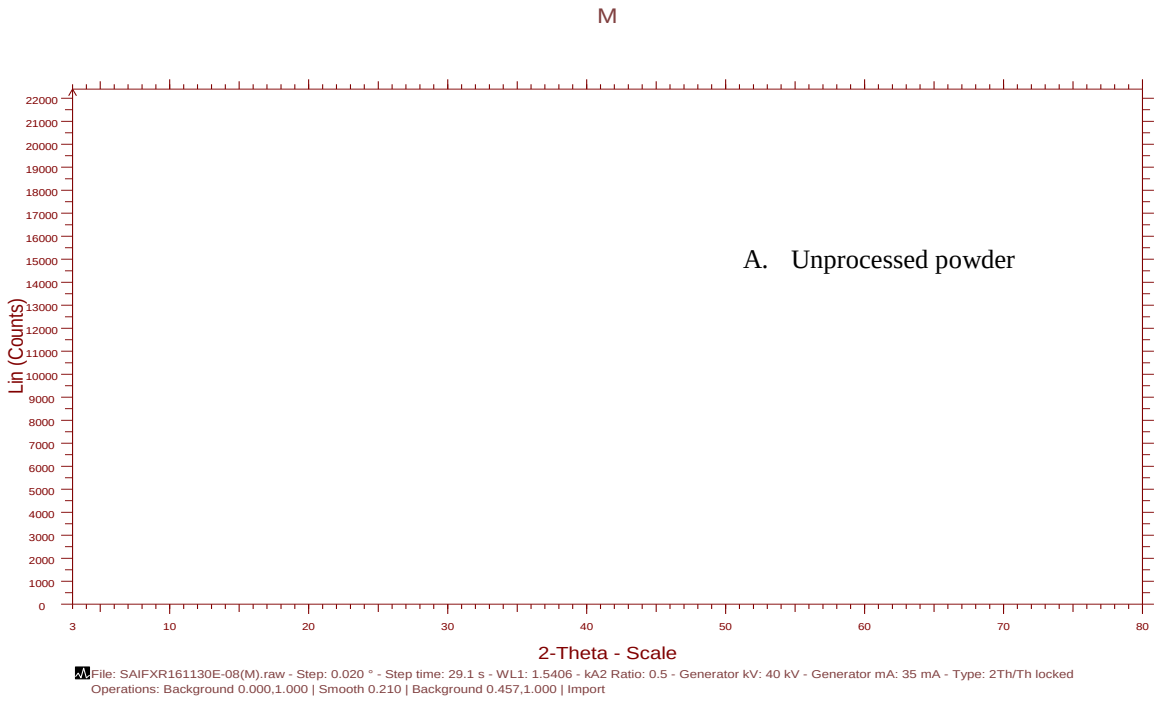



Fig 6.8. X- ray diffraction pattern of unprocessed powder

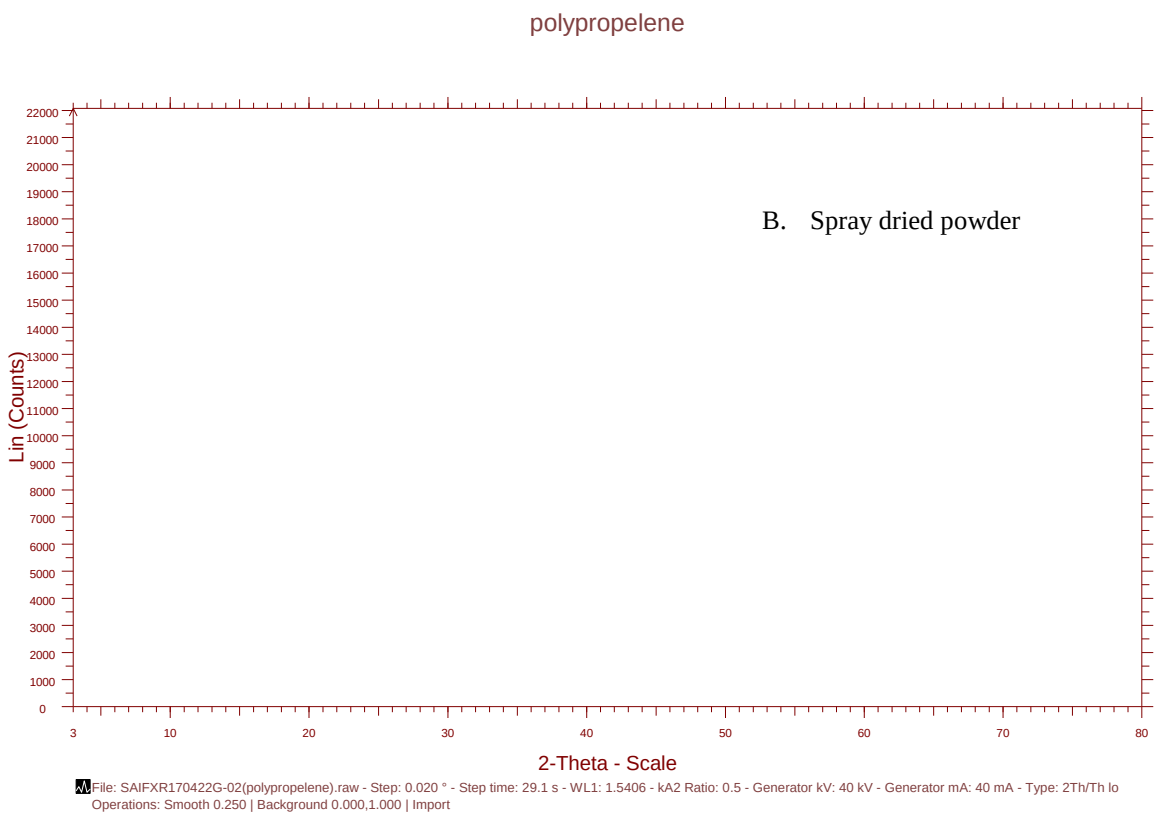



Fig 6.9. X- ray diffraction pattern of spray dried powder

### 6.11.3 Particle size analysis

The average particle size of unprocessed powder and spray dried powder of *C. anthelminticum* was found to be 834.6 nm and 220.2 nm respectively. The polydispersity index (PDI) of unprocessed powder and spray dried powder is shown in Fig. 6.10 and 6.11. The results revealed that the particle size of spray dried powder is about 3.79 times smaller than that of unprocessed powder of *Centratherum anthelminticum*. It is important to note that the PDI value of prepared spray dried powder of *Centratherum anthelminticum* (Fig. 6.11) is found to be 0.261 which shows excellent particle size distribution

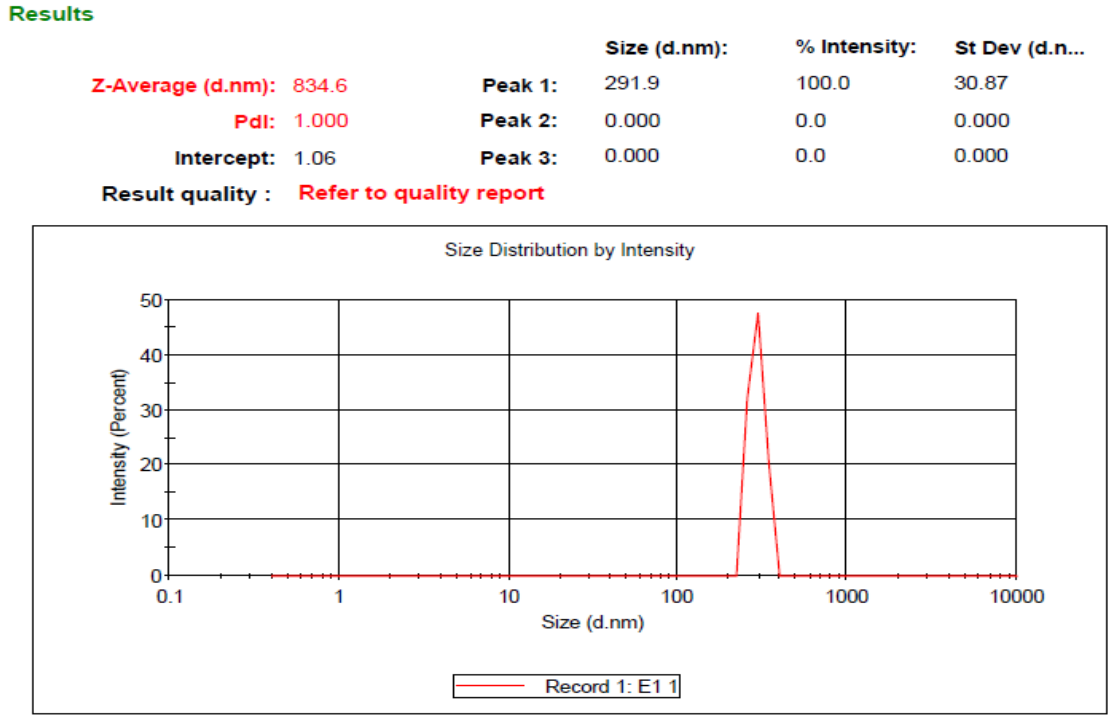



Fig 6.10 Particle size distribution of unprocessed powder of *Centratherum anthelminticum*

**Results**

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 220.2 | Peak 1: | 266.4 | 94.2 | 95.61 |
| PdI: 0.261 | Peak 2: | 56.07 | 5.8 | 10.94 |
| Intercept: 0.960 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Good

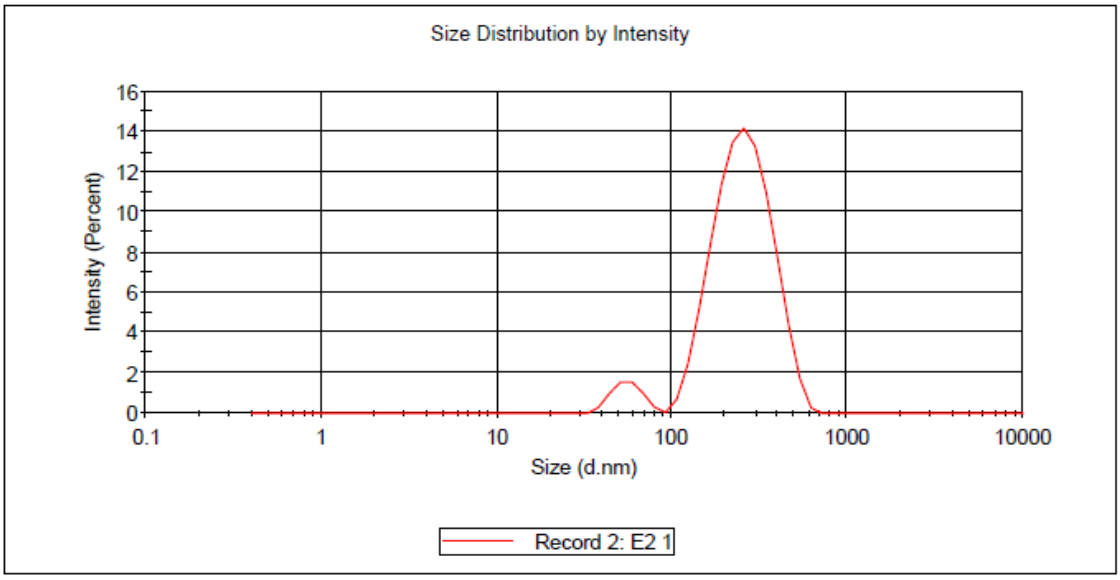



Fig 6.11 Particle size distribution of spray dried powder of *Centratherum anthelminticum*

# CHAPTER 7

# SUMMARY AND CONCLUSION

Based on the reports of limiting side effects and development of resistance to conventional anthelmintic therapy herbal formulation was selected. A polyherbal formulation, Krimihar™ syrup is in clinical use for its anthelmintic activity for last few decades. However, no systematic study on its therapeutic/pharmacological effect is reported. The current research work was under taken to evaluate the anthelmintic property of this marketed formulation. Another aim of the study was to attribute the pharmacological effects to individual constituent as the formulation is of polyherbal nature. Suitable modifications in the existing formula based on the inferences of the study may render it more effective.

On comparison of the anthelmintic activity of the marketed formulation, decoction of all constituents (prepared in our lab), decoction of five most effective constituents (prepared in our lab) and decoction of individual constituent following interesting observations were made.

1. Decoction of mixture of all constituents of the formulation is slightly more effective than the marketed formulation indicating some role of excipients like sweeteners, flavours, preservatives etc.

2. The anthelmintic activity of the decoction containing all constituent as well as that containing five most effective constituents is less than that of the decoction of *C. anthelminticum.*

Based on above results, we prepared the spray dried powder of the decoction of *C. anthelminticum*. Another interesting observation was made. Spray dried powder of decoction showed an enhanced anthelmintic activity indicating the role of amorphization and particle size reduction, particularly in light of the fact that the obtained decoctions were slightly turbid in nature.

The spray dried powder was formulated into enteric coated tablets. Dissolution testing of these tablets using the developed bioassay method indicated that a concentration of approximately 0.4 mg/mL could be achieved by the developed formulation in about 40 min after reaching the intestinal milieu. Which is able to give an ADT of approximately 75 min.

The study indicates that enteric coated tablet of a single constituent i.e. *C. anthelminticum* will offer an option which is more convenient, effective and cost effective as compared to the original formulation.

Further studies will be conducted to study the potential of resistance development of the designed formulation.

# CHAPTER 8

# REFERENCES

Abbas, A., Newsholme, W., 2011. Diagnosis and recommended treatment of helminth infections. Prescriber 22, 56–64.

Akhtar, M.S. and Riffat, S., 1985. Efficacy of *Punica granatum*, Linn. (Anar) fruit–rinds against naturally acquired nematodal and cestodal infections. J. Pharm. 6, 17–24

Akhtar, M.S. and Javed, I., 1985. Comparative efficacy of *Fumaria parviflora* and morantel tartrate against gastrointestinal nematode infection in sheep. Pakistan J. Pharmacol. 2, 31–35

Akhtar, M. S., Ahmad, I., 1990. Anthelmintic and Phytochemistryical studies on Hyoscyamus niger Linn. (Ajwain Khurasani) seeds and Morringa oleifera, Lam (Sohanjna) roots. J. Pharm. 3, 75–81.

Amir, F., Chin, K.Y., 2011. The chemical constituents and pharmacology of centratherum anthelminticum. Int. J. PharmTech Res. 3, 1772–1779.

Amirmohammadi, M., Khajoenia, S., Bahmani, M., Rafieian-Kopaei, M., Eftekhari, Z., Qorbani, M., 2014. In vivo evaluation of antiparasitic effects of Artemisia abrotanum and Salvia officinalis extracts on Syphacia obvelata, Aspiculoris tetrapetra and Hymenolepis nana parasites. Asian Pacific J. Trop. Dis. 4, 5–9.

Apt, W., Aguilera, X., Vega, F., Miranda, C., Zulantay, I., Perez, C., Gabor, M., Apt, P., 1995. Treatment of human chronic fascioliasis with triclabendazole: drug efficacy and serologic response. Am J Trop Med Hyg. 52, 532-535.

Arora, D. R., Arora, B. B., 2010. Medical parasitology, CBS publishers and distributors, third edition, 123-188.

Asha, M. K**.,** Prashanth, D., Murali, B., Padmaja, R and Amit, A., 2001. Anthelmintic activity of essential oil of *Ocimum sanctum* and eugenol. Fitoterapia, 72, 669–670.

Ashok, P., Koti, B. C., Thippeswamy A. H., Tikare V. P., Dabadi P., 2010. Evaluation of antiinflammatory activity of *Centratherum anthelminticum* (L) Kuntze seed. Indian J. Pharm. Sci. 72, 703

Atmiya, M. M., Manvar, M., 2012. Vernonia anthelmintica Willd: An Overview on Phytopharmacological Properties, Inventi J. 2012, 1-4

Bahmani, M., Rafieian-Kopaei, M., Hassanzadazar, H., Saki, K., Karamati, S.A., Delfan, B., 2014. A review on most important herbal and synthetic antihelmintic drugs. Asian Pac. J. Trop. Med. 7, S29–S33

Bartsch, S.M**.,** Hotez, P. J., Asti, L., Zapf, K. M., Bottazzi, E., Diemert, D.J., Lee, B.Y., 2016. The Global Economic and Health Burden of Human Hookworm Infection. PLoS Negl Trop Dis. 10, 1-17

Bauri, R.K., Tigga, M.N., Kullu, S.S., 2015. A review on use of medicinal plants to control parasites. Indian J. Nat. Prod. Resour. 6, 268–277

Beer, R. J., 1976. The relationship between *Trichuris trichiura* (Linnaeus 1758) of man and *Trichuris suis* (Schrank 1788) of the pig. Research in Veterinary Science**.** 20, 47-54.

Bhatia, D., Gupta, M.K., Bharadwaj, A., Pathak, M., Kathiwas, G., Singh, M., Road, M., Sciences, M., 2008. Pharmacologyonline. 3, 1–5.

Bhatia, D., Gupta, M.K., Gupta, A., Singh, M., Kaithwas, G., 2008b. Pharmacognostical. studies on seeds of *centratherum anthelminticum* Kuntze. Indian J. Nat. Prod. Resour. 7, 326–329

Bitran, R., Martorell, B., 2009. Controlling and Eliminating Neglected Diseases in Latin America and the Caribbean. Health Affairs. 28, 1707-1719.

Blair, P., Diemert, D., 2015. Update on Prevention and Treatment of Intestinal Helminth Infections. Curr. Infect. Dis. Rep. 17, 12

British Veterinary Codex, 1953. Pharmaceutical Press, London .189.

Brooker, S., Clements, A. C., Bundy, D. A., 2006. Global epidemiology, ecology and control of soil transmitted helminth infections. Adv Parasitol. 62, 221-261.

Bushra, R., Shoaib, M. H., Aslam, N., Mehmood, Z. A., Hashmat, D., 2010. Enteric coating of ibuprofen tablets (200 mg) using an aqueous dispersion system. Brazilian J. Pharm. Sci. 46, 99–107.

Chatterjee, K. D., 1967. Parasitology, Protozoology and Helminthology, sixth edition., Guha Ray Sree Saraswaty Press, 140-141

Chatterjee, K. D., 2009. Parasitology Protozoology and Helminthology in relation to clinical medicine, CBS publishers & distributors, thirteenth edition, 143-258.

Chu, B. K., Hooper, P. J., Bradley, M. H., 2010. The economic benefits resulting from the first 8 years of the Global Programme to Eliminate Lymphatic Filariasis. PLoS Negl Trop Dis. 4, 708-710

Conteh, L., Engels, T., Molyneux, D.H., 2010. Neglected Tropical Diseases 4 Socioeconomic aspects of neglected tropical diseases. Lancet 375, 239–247

Dahiya, S. S., Kaur, R., Sharma, S. K., 2012. Evaluation of in vitro anthelmintic activity of Oenothera rosea L'Hér. ex Aiton. stem and root, J. Nat. Prod. Plant Resour. 2, 534–539.

Daksha, G., Chandrashekar, Lobo, R., Nilesh, G., 2012. Anthelmintic activity of stem bark of Bauhinia purpurea Linn, Der Pharm. Lett. 4, 662–664

Das, R., Mehta, D. K., Gupta, A., 2011. In Vitro Anthelmintic Activity of Leaves of Juglans Regia L Against Pheretima Posthuma, Sci. Rev. Chem. Commun. 1, 78–82.

Datry, A., Hilmarsdottir, I., Sagastume, R. M., Lyagoubi, M., Gaxotte, P., Biligui, S., Chodakewitz, J., Neu, D., Danis, M., Gentilini., 1994. Treatment of Strongyloides stercoralis infection with ivermectin compared with albendazole: results of an open study of 60 cases Trans. R. Soc. Trop. Med. Hyg. 88, 344-345.

D'Cruz, J. L., Nimbkark, A. Y., Kokate, C. K., 1980. Evaluation of fruits of Piper longum Linn. and leaves of Adhatoda vesica for anthelmintic activity. Indian Drugs. 17, 99- 101

De, S., Das, D. C., Mandal, T., 2016. In-vitro anthelmintic activity of Cardanthera difformis. Druce whole plant methanolic extract in Indian adult earthworm, J. Pharmacogn. Phytochem. 5, 203–205

De Silva, N. R., Brooker, S., Hotez, P. J., Montresor, A., Engels, D., Savioli, L., 2003. Soil-transmitted helminth infections: Updating the global picture. Trends Parasitol. 19, 547–551.

Deb, P. K. R., Ghosh, R., Das, S., Bhakta, T., 2013. In-vitro anthelmintic activity of acorus calamus leaves. 6, 6–8.

Deore, S. L., Khadabadi, S. S., Kamdi, K. S., Ingle, V. P., Kawalkar, N. G., Sawarkar, P. S., Patil, U. A., Vyas, A. J., 2009. In vitro anthelmintic activity of Cassia tora. Int. J. ChemTech Res. 1, 177–179.

Dhar, R. N., Garg, L. C., Pathak, R. D., 1965. Anthelmintic activity of *Carica papya* seeds. Indian J. Pharm. 27, 335–336

Dighe, S. B., Kuchekar, B. S., Wankhede, S. B., 2012. Pharmacological Evaluation of *Oxalis corniculata* Linn for Anthelmintic Activity, RJPP. 4, 1-4.

Dullu, V., 2014. Anthelmintic activity of ethanolic leaf extract of Jasminum mesnyi Asian Pac. J. Trop. Dis. 4, S273-S275.

Edwards, C. A., and Boglen, P. J., 1996. Biology and Ecology of earthworms, third edition, Chapman and Hall publication, 2-6 Boundary Row London, UK, 202-217

El, G. M. F., Mahmoud, L. H., 2002. Anthelmintic efficacy of traditional herbs on Ascaris lumbricoides, J. Egyptian Soc. Parasitol. 32, 893–900.

Fenwick, A., Webster, J. P., Bosque, O. E., 2009. The Schistosomiasis Control Initiative (SCI): rationale, development and implementation from 2000-2008, J. Parasitol. 136, 17190-17193.

Fernandez, T. J., 1991. Local plants having anthelmintic activity. Asian J. Sci. Technol. Develop. 8, 115–119.

Gabrielli, A. F., Touré, S., Sellin, B., 2006. A combined school- and community-based campaign targeting all school-age children of Burkina Faso against schistosomiasis and soil-transmitted helminthiasis: performance, financial costs, and implications of sustainability, Acta. Trop. 99, L234-L242

Garcia, D., Escalante, M., Delgado, R., Ubeira, F. M., Leiro, J., 2003. Anthelminthic andantiallergic activities of Mangifera indica L. stem bark components vimang and mangiferin. Phytother. Res. 17, 1203–1208

Garcia, H. H., Evans, C. a W., Nash, T. E., Takayanagui, O. M., Jr, a C.W., Botero, D., Rajshekhar, V., Tsang, V.C.W., Schantz, P.M., Allan, J. C., Flisser, A., Correa, D., Sarti, E., Friedland, S., Martinez, S. M., Gonzalez, A. E., Gilman, R. H., Brutto, O. H. Del., García, H. H., White, a C., 2002. Current Consensus Guidelines for Treatment of Neurocysticercosis Current Consensus Guidelines for Treatment of Neurocysticercosis. Clin. Microbiol. Rev. 15, 747–756.

Girgune, J. B., Jain, N. K and Garg, B. D., 1979. Antimicrobial and anthelmintic activity of essential oil from *Gardenia lucida* Roxb. Indian Perfumer. 23, 213–215.

Global Alliance to Eliminate Lymphatic Filariasis. 2010. Half-Time in LF Elimination: Teaming Up with NTDs. Sixth Meeting of the Global Alliance to Eliminate Lymphatic Filariasis. 1- 3

Gottstein, B., Pozio, E., Nöckler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis, Clin. Microbiol. Rev. 22, 127–145.

Gowda, S. K., 1997. Biological effects of neem *(Azadirachta indica)* derivatives in animals. In: Ethnoveterinary medicine: alternatives for livestock development, Abst. Proc. Int. Conf. Puna. 2, 4-6

Gray, D. J., Ross, A. G., Li, Y. S., McManus, D. P., 2011. Diagnosis and management of Schistosomiasis, BMJ. 342, d2651

Hellert, A., Sharma, G., Kumar, K., Agrawal, V., 2012. Exploration of larvicidal activity of Vernonia anthelmintica ( L .) wild seed crude extracts in different solvents against malaria ( Anopheles stephensi ) and dengue ( Aedes aegypti ) vectors, Malar J. 11, 1–3

Hoegberg, L.C., Angelo, H.R., Christophersen, A.B, Christensen, H.R., 2003. The effect of food and ice cream on the adsorption capacity of paracetamol to high surface activated charcoal: in vitro studies, Pharmacol Toxicol. 93, 233-237.

Hördegen, P., Hertzberg, H., Heilmann, J., Langhans, W., Maurer, V., 2003. The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs, Vet. Parasitol. 117, 51–60.

Hossain, E., Chandra, G., Nandy, A. P., Mandal, S. C., Gupta, J. K., 2012. Anthelmintic effect of a methanol extract of leaves of dregea volubilis on paramphistomum explanatum, Parasitol. Res. 110, 809–814.

Hotez, P. J., Brindley, P. J., Bethony, J. M., King, C. H., Pearce, E. J., Jacobson, J., 2008. Helminth infections: the great neglected tropical diseases, J. Clin. Invest. 118, 1311–1321.

Hussan, S. D., Santanu, R., Verma, P., Bhandari, V., 2012. A review on recent advances of enteric coating, IOSR J. Pharm. 2, 5–11.

Indian pharmacopeia 2014, The indian pharmacopeia commission Ghaziabad, volume 2, 959-961

Islam, M. D. S., Khatun, F. M., Md, D., Bakra, A., Mehta, D. K., Bhandari, D. R., 2016. A Review on Biodegradable Polymers for Enteric Coating Material, Int. j. pharm. pharm. sci. 6, 141-159

Iqbal, Z., Lateef, M., Ashraf, M., Jabbar, A., 2004. Anthelmintic activity of *Artemisia brevifolia* in sheep, J. Ethnopharmacol. 93, 265-268.

Iqbal, Z., Lateef, M., Jabbar, A., Akhtar, M. S and Khan, M. N., 2006. Anthelmintic activity of vernonia anthelmintica. Seeds against Trichostrongylid Nematodes of Sheep, Pharm. Biol. 44, 563-567

Iqbal, Z., Nadeem, Q. K., Khan, M. N., Akhtar, M. S and Waraich, F. N., 2001. In vitro anthelmintic activity of Allium sativum, Zingiber officinale, Curcurbita mexicana and Ficus religiosa, Int. J. Agri. Biol. 3, 454–457

Jagota, S. C., 1986. Albendazole, a broad-spectrum anthelmintic, in the treatment of intestinal nematode and cestode infection: a multicenter study in 480 patients, Clin Ther. 8, 226-31.

Jain, P., Singh, S., Singh, S. K., Verma, S. K., Kharya, M. D., Solanki, S., 2013. Anthelmintic Potential of Herbal Drugs, Int. J. Res. Dev. Pharm. L. Sci. 2, 412–427.

Javed, I., Akhtar, M. S., 1990. Screening of Vernonia anthelmintica seeds and Embelia ribes fruit mixed in equal parts against gastrointestinal nematodes, Pakistan J. Pharm. Sci. 3, 69–74

Kailani, S. U. R., Akhtar, M. S and Ashraf, M., 1995. Antifasciolic efficacy of indigenous plant drugs: Kalonji, Shahterah and Karanjwa in buffaloes, Pakistan J. Pharm. Sci. 8, 17– 27.

Kalesaraj, R., 1974. Screening of some indigenous plants for anthelmintic action against human Ascaris lumbricoides, Indian J. Physiol. Pharmacol. 18, 129–131

Kaur, K., Kumar, B., Puri, S., Tiwari, P., Divakar, K., 2010. Comparative study Of Anthelmintic Activity of aqueous and ethanolic extract of bark of *Holoptelea Integrifolia,* Int. J. Drug Dev. Res. 2, 758-763

Kaur, P., Singh, S. K., Garg, V., Gulati, M., Vaidya, Y., 2015. Optimization of spray drying process for formulation of solid dispersion containing polypeptide-k powder through quality by design approach, Powder Technol. 284, 1-11

Keshmiri, M., Baharvahdat, H., Fattahi, S. H, Davachi, B., Dabiri, R. H., Baradaran, H., Rajabzadeh, F., 2001. Albendazole versus placebo in treatment of echinococcosis, Trans R Soc Trop Med Hyg. 95, 190-194.

Khandelwal, K. R., Practical Pharmacognosy, Nirali Prakashan, Pune, twelfth edition. 2004, 149-156.

Kim, H., 2016. A Study on the Utilization of the Earthworms Eisenia foetida and Eisenia andrei for the Disposal of Polymers, Int. J. Env. Sci. Dev. 7, 5–8.

Kirtikar, K. R and Basu, B. D., 2004. Indian Medicinal Plants published by Lalit Mohan Basu, Allahabad. 2, 1325-1326.

Kliks, M. M., 1985. Studies on the traditional herbal anthelmintic chenopodium ambrosioides L.: Ethnopharmacological evaluation and clinical field trials, Soc. Sci. Med. 21, 879–886.

Kosalge, S., Fursule, R. A., 2009. Investigation of in vitro anthelmintic activity of thespesia lampas (Cav.), Asian J. Pharm. Clin. Res. 2, 69–71.

Kumar, T., Alexander, A., Ajazuddin, D. D., Khan, J., Sharma, M., 2011. Investigation of in-vitro anthelmintic activity of Bauhinia racemosa linn, J. Appl. Pharm. Sci. 1, 73–75.

Kundu, S., Lyndem, L. M., 2013. In vitro screening for cestocidal activity of three species of cassia plants against against the tapeworm *Raillietina tetragona*, J. Helminthol. 87, 154-159.

Lem, M. F., Payne, V. K., Poné, J. W., Gertrude, M. T., Tchoumboue, J., 2014. In vivo Anthelmintic Activity of Terminalia glaucescens (Combretaceae) Extracts against Gastrointestinal Nematodes of Sheep, Br J. Pharm. Res. 4, 2136–2145.

Liu, Y., Wang, Z., Su, J., Liu, W., Hussain, D.K., Guo, Y., 2015. The Efficacy of Chinese Medicinal Herbs towards Grape Phylloxera (Daktulosphaira vitifoliae Fitch) (Hemiptera, Phylloxeridae), PLoS ONE. 10, e0128038.

Lobmann, K., Flouda, K., Qiu, D., Tsolakou, T., Wang, W., Rades, T., 2014. The influence of pressure on the intrinsic dissolution rate of amorphous indomethacin. Pharmaceutics. 6, 481–493.

Long, X., Jin, H., Xue, H., Yuan, Z., Hai, L. Y., Xiao, H. X., 2011. Bioassay-based dissolution test of Shuanghuanglian tablet, J. Chi pharmac sci. 20, 77-82

Mahdi, B., Fawzi, D. A., Kubert, K. S. Murthy Stabilization of enteric coated dosage form. Patent number US5068110A, 1991.

Mali, R. G., Mahajan, S., Patil, K. S., 2005. Anthelmintic activity of root bark of *Capparis spinosa*, Indian J Nat Product. 21, 50-51

Mali, R. G., Wadekar, R. R., 2008. In Vitro Anthelmintic Activity of Baliospermum montanum Muell. Arg roots, Indian J Pharm Sci. 70, 131–133.

Manke, M. B., Dhawale, S. C., Jamkhande, P. G., 2015. Helminthiasis and medicinal plants: A review. Asian Pacific J. Trop. Dis. 5, 175–180.

Marano, K., Norris, J., Adelman, C., Spantchak, Y., 2012. Social and Economic Impact Review on Neglected Tropical Diseases**.** 1-26

McGaw, L. J., Jager, A. K and Staden, J. V., 2000. Antibacterial, anthelmintic andantiamoebic activity in South African medicinal plants. J. Ethnopharmacol. 72, 247–263

Mehta, B. K., Mehta, D and Itoriya. A., 2010. Isolation and structure determination of acetylated triterpenoid saponins from the seeds of *centratherum anthelminticum*. Nat. Prod. Res. 24, 120-130.

Mehta, D.K., Das, R., Bhandari, A., 2012. In-vitro anthelmintic activity of seeds of Zanthoxylum armatum DC against Pheretima Posthuma, Int J Green Pharm. 6, 26-28

Mehta, R. K., Parashra, G. C., 1966. Effect of Butea frondosa, Vernonia anthelmintica and Carica papya against oxyurids in mice. Ind Vet J. 43, 744–748.

Miller, M. J., Krupp, I. M., Little, M. D., Santos, C., 1974. Mebendazole an effective anthelmintic for Trichuriasis and Enterobiasis, *J. Am. Med. Assoc.* 230, 1412-1414.

Molyneux, D. H., Zagaria, N., 2002. Lymphatic filariasis elimination: progress in global programme development. Ann Trop Med Parasitol. 96, S15–40.

Montresor, A., Cong, D. T, Le, A.T., Ehrhardt, A., Mondadori, E., Thi, TD, Le., Khanh, T., Albonico, M., Palmer, K.L., 2007. Cost containment in a school deworming program targeting over 2.7 million children in Vietnam. Trans R Soc Trop Med Hyg. 101, 461-464.

Mulla, W. A., Thorat, V. S., Patil, R. V., Burade, K. B., 2010. Anthelmintic activity of leaves of Alocasia indica Linn. Int. J. PharmTech Res. 2, 26–30.

Naidu, V. A. K. A., 2008. Antihyperglycemic activity of polyphenolic components of black bitter cumin *Centratherum anthelminticum* ( L .) Kuntze seeds, Eur Food Res Technol. 226, 897–903.

Naresh, A., Anil, B., Yadav, D., Garg, A., Khanna, S., 2012. A Review on Development of Hpmcp Based Aqueous Enteric Coating Polymer. Int. J. Res. Pharm. Chem. 2, 570–574.

Neogi, N. C., Baliga, P. A. C.and Srivastava, R. K., 1964. Anthelmintic activity of some indigenous drugs, Indian J. Pharmacol. 26, 37-39

Neuhauser, E. F., Kalpan, D. L., Malecki, M. R and Hartenstein, R., 1980. Materials supportive of weight gain by the earthworm *Eiseniafoetida* in waste conservation system. Agric.Wastes. 2, 43-60

Nisha, M., Kalyanasundram, M., Paily, K. P. A., Vanamail. P., Balaraman, K., 2007. In vitro screening of medicinal plant extracts for macrofilaricidal activity. Parasitol. Res. 100, 575-579.

Ochei, J., Kolhatkar, A., 2000. Medical Laboratory Science Theory and Practice, Tata mcgraw hill, 1015-1025

Partap, S., Kumar, S., Kumar, A., Sharma, N.K., Jha, K.K., 2012. In-Vitro Anthelmintic Activity of Luffa cylindrica Leaves in Indian Adult Earthworm, J. Pharmacogn. Phytochem. 1, 27–30.

Patel, V., Patel, M., Patel, R., 2009. Development and validation of a RP-HPLC method for quantification of rottlerin in Kamala (Mallotus philppinensis), Drug. invent. Today. 1, 116–118.

Patel, V. P., Hirpara, M., Suthar, M. P., 2012. In vitro screening for antibacterial activity of various extract of *Centratherum anthelminticum* seeds, AJPST. 2, 1-4.

Pawar, S. D., Patil, Y. B., Premchandani, L. A., Borse, S. L., Borse, L. B., Pawar, S. P., 2014. Study of anthelmintic activity of chloroform extract of tinospora cordifolia, World J. Pharm. Pharm. Sci. 3, 2253–2268.

Paydar, M., Moharam, B.A., Wong, Y.L., Looi, C.Y., Wong, W.F., Nyamathulla, S., Pandy, V., Kamalidehghan, B., Arya, A., 2013. Centratherum anthelminticum (L.) Kuntze a Potential Medicinal Plant with Pleiotropic Pharmacological and Biological Activities, Int. J. Pharmaco. 9, 211-226.

Perrett, S. and Whitfield, P. J., 1995. Atanine (3-Dimethylallyl-4-methoxy-2- quinolone), an alkaloid with anthelmintic activity from the Chinese medicinal plant, Evodia rutaecarpa. Planta Med, 61, 276–278.

Pole, S., Maurya, S., Hasnale, P., Rathod, N., Bendale, S., Khutle, N.M., 2016. A detail Understanding of Enteric Coated Tablet: Manufacturing and Evaluation. Eur. J. Pharm. Med. Res. 3, 135–144.

Prashanth, D., Asha, M.K., Amit. A and Padmaja, R., 2001. Anthelmintic activity of *Butea monosperma.* Fitoterapia. 72, 421–422.

Prudhviraj, G., Vaidya, Y., Singh, S. K., Yadav, A. K., Kaur, P., Gulati, M., Gowthamarajan, K., 2015. Effect of co-administration of probiotics with polysaccharide based colon targeted delivery systems to optimize site specific drug release. Eur J. Pharm. Biopharm. 97, 164-172.

Pullan, R. L., Smith, J. L., Jasrasaria, R., Brooker, S. J., 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010, Parasit. Vectors. 7, 37-38

Qureshi, M. A. and Sabir, M., 1979. Preliminary study on anthelmintic efficacy of Embellia seeds (Babarung) against tapeworms of poultry. Pakistan J. Sci. 31, 218–220.

Rahman, M. A., Ali, J., 2008. Development and evaluation of enteric coated multiparticulate system for resistant tuberculosis, Indian J, Pharma. 70, 477-481.

Ramaiah, K. D., Das, P. K., Michael, E., Guyatt, H., 2006. The economic burden of lymphatic filariasis in India, Parasitol Today. 16, 251-253.

Ramalingam, S., Sinniah, B., Krishnan, U., 1983. Albendazole, an effective single dose, broad spectrum anthelmintic drug. Am J Trop Med Hyg. 32, 984-989.

Reddy, K. S., Roa, J. V., 2013. Formulation and evaluation of matrix tablets of albendazole for Colon targeted drug delivery, Indo. Am. J. Pharm. Res. 1-7

Renuka, Singh, S.K., Gulati, M., Kaur, I., 2014. Characterization of solid state forms of 700 glipizide, Powder Technol. 264, 365-376.

Salhan, M., Kumar, B., Tiwari, P., Sharma, P., Sandhar, H.K., Gautam, M., 2011. The Netherlands Comparative Anthelmintic Activity of Aqueous and Ethanolic Leaf Extracts Of Clitoria Ternatea. Int. J. Drug Dev. Res. 3, 68–69.

Schad, G. A., 1979. *Ancylostoma duodenale* maintenance through six generations of helminth-naive pups, Exp. Parasitol. 47, 246-253

Sen, S., De, B., Devanna, N., Chakraborty, R, 2012. Anthelmintic and *in vitro* antioxidant evaluation of fractions of methanol extract of *Leea asiatica* leaves. Anc. Sci. Life. 31, 101-106

Shinde, G., Patel, M., Mehta, M., Kesarla, R., Bangale, G., 2015. Repaglinide Loaded Nanocrystal for Diabetes Therapy, Adv. Pharmac. 2015, 1-7

Shrivastava, M. C. and Singh, S. W., 1967. Anthehnintic activity of Cucurhita maxima seeds. Indian J. Med. Res., 55, 629–632.

Shuhua, X., Chollet, J., Weiss, N. A., Bergquist, R. N and Tanner, M., 2000. Preventive effect of artemether in experimental animals infected with *Schistosorna mansoni.* Parasitol. Int. 49, 19–24.

Singhal, K. C., Sharma, S., Mehta, B. K., 1992. Antifilarial activity of *Centratherum anthelminticum* seed extracts on Setaria cervi‖, Ind. J. Exp. Biol. 30, 546-548.

Singh, O., Ali, M., Husain, S.S., 2012. Phytochemical investigation and antifungal activity of the seeds of *centratherum anthelminticum* Kuntze, Acta. Pol. Pharm. 69, 1183–1187

Singh, S., Ansari, N. A., Srivastava, M. C., Sharma, M. K., Singh, S. N., 1985. Anthelmintic activity of Vernonia anthelmintica, Indian Drugs. 22, 508-511.

Steinmann, P., Utzinger, J., Du, Z. W., Jiang, J. Y., Chen, J. X., Hattendorf, J., Zhou, H., Zhou, X.N., 2011. Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and taenia spp: A randomized controlled trial, PLoS One. 6, e25003.

Stephenson, L.S., 2001. Optimising the benefits of anthelmintic treatment in children. Paediatr Drugs. 3, 495–508.

Stephenson, L. S., 1987. The design of nutrition parasite studies. In The Impact of Helminth Infections on Human Nutrition: Schistosomes and Soil-transmitted Helminths, Tay. Franc. 21-46.

Stephenson, L., Wiselka, M., 2000. Drug Treatment of Tropical Parasitic Infections. Recent development in tropical parasitic infections, Drugs. 60, 985-995

Subrahmanyam, C. V. S., 2000. Textbook of physical pharmaceutics, vallabh prakashan, second edition, 214-227.

Tandon, N., Sharma, M., 2010. Quality standard of Indian medicinal plants, Indian council of medical research. 8, 128-136.

Thara, K.M., Zuhara, K.F., 2016. Biochemical Analysis and Antiproliferation Action of *Centratherum Anthelminticum* (L) Kuntze Seed Extract on Cancer Cell Lines Like Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC). Indo. Am. J. Pharm. Res. 6, 3941-3948.

Tripathi, K. D., 2003. Essential of medical pharmacology, Jaypee brothers medical publishers, fifth edition, 759-766.

Tiwari, P., Kumar, B., Kumar, M., Kaur, M., Debnath, J., Sharma, P., 2011. Comparative anthelmintic activity of aqueous and ethanolic stem extract of Iinospora cordifolia, Int. J. Drug Dev. Res. 3, 70–83.

Tritten, L., Silbereisen, A., Keiser, J., 2011. In Vitro and In Vivo Efficacy of Monepantel ( AAD 1566 ) against Laboratory Models of Human Intestinal Nematode Infections, PLoS. Negl. Trop. Dis. 5, 1–7.

Venkatesh, D.N., Reddy, A.K., Samanta, M.K., Suresh, B., 2009. Development and in vitro evaluation of colonic drug delivery systems for tegaserod maleate, Asian J. Pharm. 50–53.

Venter, J. M and Reinecke, A ., 1988. Sublethal ecotoxicological studies with the earthworm *E. foetida* (Lumbricidae), in earthworms in Waste and Environmental Management, SPB Acad.Publ. 337-354

Washington, F., Borges, R., Cezar, L., Maurício, J., Pereira, J., Alberto, J., 2016. Anthelmintic activity of Cratyliamollis leaves against gastrointestinal nematodes in goats. 17, 753–762.

Wink, M., 2012. Medicinal plants: A source of anti-parasitic secondary metabolites, Molecules. 17, 12771–12791.

Xiao, P. G., and Lin, F. S., 1986. Traditional antiparasitic drugs in China. Parasitol. Today. 2, 353–355

Yadav, P., Singh, R., 2011. A review on anthelmintic drugs and their future scope. Int. J. Pharm. Pharm. Sci. 3, 17–21.

Yadava, R. N., Barsaiya, D., 1996. Analysis of carbohydrates from the seeds of Centratherum anthelminticum. Kuntz. Asian J. Chem. 8, 813-814

https://www.britannica.com/animal/pinworm

http://www.globalnetwork.org/neglected-tropical-diseases/fact-sheets

https://web.stanford.edu/group/parasites/ParaSites2006/Ascariasis/

http://www.simplydiscus.com/library/disease_medications/internal/tapeworms.shtml (Access on September 21, 2016 at 9:00 PM)

# CHAPTER 9

# APPENDIX

## LIST OF PRESENTATION

### Poster Presentation

- Phyto-anthelmintics: A pragmatic approach to conventional drugs, **Deepak Ghai**, Harish Rathee, Adil Hussain Malik, Palak Bawa, Parth Sharma, Sarvi Yadav, Sachin Kumar Singh, Monica Gulati in International Conference of Pharmacy held at Lovely Professional University (7-8$^{th}$ April, 2017)

# Phyto-anthelmintics: A pragmatic alternative to conventional drugs

Deepak Ghai[1], Harish Rathee[1], Adil Hussain Malik[1], Palak Bawa[1], Parth Sharma[1], Yadav Sarvi Rajesh[1], Monica Gulati[1], Sachin Kumar Singh[1], Amit Sharma[2]

[1]Lovely Professional University, Phagwara-144401, Punjab, India

[2]Shree Dhanwantri Herbals, 277, Amritsar-143006, Punjab, India

PCB123

deepakghai44@gmail.com

## Abstract

Helminth infestation is referred to as the most extensive infestation in the world distressing a large number of populations. Unlike other parasites, the body of particular worm is multicellular along with complex life cycle. Humans and animals have more threat from helminth infestation as compared to other parasites. Normally, such worms infest the gastrointestinal tract, liver and other organs. It has been observed that conventional anthelmintic drugs i.e. albendazole, mebendazole, niridazole, ivermectin, praziquantel and diethylcarbamazine (DEC) are generally used in treatment of almost all types of helminth infestations. However, these drugs are associated with serious side effects such as headache, nausea, dizziness, vomiting, rashes edema, alopecia, hypospermia and diarrhoea. Hence, it has become mandatory to look for alternative effective anthelmintic drugs that could offer a better treatment with minimum side effects. Plant based medicines have been reported to exhibit great efficacy against a varieties of parasites. Furthermore, the chance of drug resistance against phytoconstituents such as curcumin, artemisinin, arecoline, ginger and pelletierine is less as compared to that for chemical anthelmintics. In vivo study of Ginger and curcumin methanolic extract at dose of 100 mg/mL exhibit significant anthelmintic activity against *Ascaridia galli*. Herbal formulation such as wormicid plus and wormnil are effective against intestinal parasites. This review emphasises on helminthiasis and the significance of phytoconstituents in the treatment of helminthiasis.

## Introduction

The word Helminth is derived from the Greek word "helmins" which means worm. Unlike other parasites, the body of worms is multicellular along-with complex life cycle. Helminths infestations such as ascariasis, schistosomiasis and infestation by *Ancylostoma duodenale* constitute the bulk of the thirteen diseases classified as neglected tropical diseases by the WHO. It may cause malnutrition, loss of appetite, anaemia, pneumonia and eosinophilia. Helminth infestations are one of the most prevalent diseases in developing and developed countries. Globally, an estimated 2 billion people are infected by intestinal nematodes.

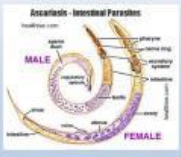



Fig 1. Ascariasis lumbricoides

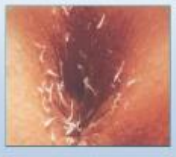


Fig 2. Pinworm


Fig 3. Schistosomiasis mansoni

## Conventional Treatment

| S.no | Disease | Treatment | Dose | Reference |
|---|---|---|---|---|
| 1 | **Nematodes** | | | |
| 1.1 | Enterobiasis | Albendazole or mebendazole | 400 mg daily once a day<br>100 mg daily once a day | Jaogota SC et al.,1986.<br>Max J. Miller et al.,1974 |
| 1.2 | Trichuriasis | Albendazole or mebendazole | 400 mg daily once a day<br>100 mg daily twice a day | Ramalingam S et al.,1983<br>Max J. Miller et al., 1974 |
| 1.3 | Ancylostomiasis | Albendazole or mebendazole | 400 mg orally once a day<br>100 mg orally twice a day | Ramalingam S et al.,1983<br>Peter Steinmann et al., 2011 |
| 1.4 | Trichinosis | Albendazole or mebendazole | 400 mg orally twice a day<br>200-400 mg orally twice a day | Bruno Gottstein et al., 2009 |
| 1.5 | Strongyloidiasis | Ivermectin | 200 µg/kg orally once a day For 2 d | Annick Dutry et al., 1994 |
| 2 | **Cestodes** | | | |
| 2.1 | Cysticercosis | Praziquantel | 5-10 mg/kg orally once a day | Hector H. Garcia et al., 2002 |
| 2.2 | Echinococcosis | Albendazole | 400 mg orally twice a day | M. Keshmiri et al., 2001 |
| 3 | **Trematodes** | | | |
| 3.1 | Fasciolasis | Triclabendazole | 10mg/kg orally once or twice | Apt W et al., 1995 |
| 3.2 | Schistosomiasis | Praziquantel | 20mg/kg orally twice a day | Darren J Gray et al., 2011 |

## Limitations of conventional treatment


## Herbal treatment

| S.no | Name of plant | Part used | Active ingredient | Parasite | Reference |
|---|---|---|---|---|---|
| 1 | Allium sativum | Bulb | Allicin | Roundworm | Iqbal et al., 2001 |
| 2 | Artemisia annua | Not reported | Artemisinin | Schistosoma mansoni | Shuhua et al., 2000 |
| 3 | Azadirachta indica | Leaves | Azadirachtin | H.Contortus | Gowada et al., 1997; Mostofa et al., 1996 |
| 4 | Butea monosperma | Seeds | Palasonin | Nematode | Prashanth et al., 2001carica |
| 5 | Calotropis procera | Latex | Calotropain | H.Contortus | Shivkar Y M et al., 2003 |
| 6 | Carica papaya | Seeds | Benzylisthiocynate | Ascaris lumbricoides | Dhar et al., 1965; Lal et al.,1976 |
| 7 | Cassia obtusifolia | | Santonin | Nematode | El Garhy & Mahmoud, 2002 |
| 8 | Carum copticum | Seeds | Thymol | Ascaris lumbricoides | Krantz & carr, 1967 ; uma et al., 1993 |
| 9 | Combretum macrocarpum | Leaf | Procyanidins | Nematode | Verma spiegler et al., 2015 |
| 10 | Curcubita maxima | Seeds | Cucurbitin | Ascaris lumbricoides | Shrivastva and singh,. 1967 |
| 11 | Curcurbita moschata | Seeds | Cucurbitin | Cestodes | Xiao & lin,. 1986 |
| 12 | Cymbogon martini | Whole Plant | Geraniol | Nematode | Mcgawet et al., 2000 |
| 14 | Embelia ribes | Seeds | Embelin | Tapeworm | Qureshi & sabir et al., 1979 |
| 15 | Evodia ruteacarpa | Fruit | Atanine | Nematode | Mcgawet et al., 2000 |
| 16 | Flemingia procumbens | Root | Genistein | Cestode, trematode | Tendon v et al., 2007 |
| 17 | Mallotus philippinesis | Fruit | Rottlerin | Tapeworm | British veterinary codex, 1993 |
| 18 | Mangifera indica | Stem | Mangiferin | Trichinella spiralis | Garcia D et al, 2000 |
| 19 | Nigella sativa | Seeds | Thimoquinone | Antifasciolic | Kadasi et al., 1995 |
| 20 | Ocimum sanctum | Leaves | Eugenol | Caenorhabditis elegans | Asha et al., 2001 |
| 21 | Piper longum | Fruit, Leaves | Piperine | Ascaris lumbricoides | D'-cruz JI, et al., 1980 |
| 22 | Zingiber officinale | Rhizomes | Zingiberene | Ascaris lumbricoides | Kalesaraj et al 1974, 1975 |

## Future prospective

Anthelmintic from the natural sources may play a key role in the treatment of these parasitic infestations. Plants have been used for medicinal purposes long before ancient time. In the last few decades, there has been an exponential growth in the field of herbal medicine. Owing to their natural origin and lesser side effects. Herbal formulation such as wormicid plus and wormnil are effective against intestinal parasites. Researches are being carried out now days on a large scale to discover the herbal medicines to treat helminthiasis. .

## References

Jain, P., Singh, S., Singh, S.K., Verma, S.K., Kharya, M.D., Solanki, S., 2013. Anthelmintic Potential of Herbal Drugs. Int. J. Res. Dev. Pharm. L. Sci. Int. J. Res. Dev. Pharm. Life Sci. 2, 412–427.

Yadav, P., Singh, R., 2011. A review on anthelmintic drugs and their future scope. Int. J. Pharm. Pharm. Sci. 3, 17–21.

**International Conference of Pharmacy (ICP-2017) on 7th and 8th April-2017**
**At: Lovely Professional University**