Chapter 7
Anthelmenthic Activity
Introduction

People of developing and under developing countries, often suffer from helminth infections, which more often physically impair their hosts than kill them. Majority of infections due to worms are generally limited to tropical regions. As an important component of complementary and alternative medicine, traditional Ayurvedic medicinal plants may be useful model for the discovery and development of new chemical substances for helminth control, which are generally considered to be very important sources of bioactive substances (Deore and Khadabadi, 2010). Helminthiasis or infection with parasitic worms, affects over two billion people worldwide. Human beings can spread these pathogens to previously uninvolved population through travel, migration, and military operations.

Helminthic infections continue to be major health hazard of people. Current estimates suggest that, over half of the world population is infected with intestinal helminths, such as *Ascaris*, hookworms, *Trichuris*, *Enterobius*, *Strongyloides*, and tapeworms. Most of these infected people live in remote rural areas of the developing countries (De Silva *et al.*, 2003; Horton, 2003).

Pathogenic worms of human beings are belongs to metazoa, classified into roundworms (nematodes) and two types of flatworms, viz., tapeworms (cestodes) and flukes (trematodes). These biologically diverse eukaryotes vary with respect to life cycle, bodily structure, development, physiology, localization within the host and susceptibility to chemotherapy. The immature forms invade human beings via skin or gastrointestinal
tract and evolve into well-differentiated adult worms, which have characteristic tissue distributions. The extent of exposure to these parasites dictates the severity of infection, and reduction in the number of adult organisms by chemotherapy is sustained unless reinfection occurs. The prevalence of parasitic helminths, typically displays a negative binomial distribution within an infected population, such that, relatively few persons carry heavy parasite burdens. Without treatment, those individuals are most likely to become ill and to perpetuate infection within their community (Goodman and Gillman, 2001). Anthelminthics are those agents that expel parasitic worms from the body, by either stunning or killing of them. Helminth infections are commonly found in community and being recognized as cause of much acute as well as chronic illness, among the various human being as well as cattle’s. More than half of the population of the world suffering from various types of infections and majority of cattle’s suffered from worm infection (Chandrashekar et al., 2010).

The plant based medicines have become indispensable and forming an integral part of the primary health care system in the world. According to many reports around the world, the medicinal plants that have been found to possess significant activity against helminth parasites (Akhtar et al., 2000; Mali and Mehta, 2008). Few of such studies were based on traditional method of treatment by using crude extracts of herbal plants, while in others the active ingredients responsible for the activity have also been identified and characterized to establish their mode of action.

Recently the majority of the evidence on the anthelmintic activity of folklore medicinal plants lacked scientific validity. Currently an increasing number of experimental studies, that aims to verify and quantify such plant activity. There are indeed a large number of plants, whose anthelmintic activity has been demonstrated
under controlled experimentation. However, contrary to traditional expectation, there are also a great number of plants with purported antiparasitic properties, which have not been reproduced under experimental conditions. Hence, an attempt has been made to study the anthelminthic activity of *Epiprinus mallotiformis*.

**Review of Literature**

In context of India, which is endowed with vast resources of medicinal plants, there is a strong tradition of using plant-based medicines in alternate system of medicine, among native societies (Prakash and Mehrotra, 1987; Akhtar *et al.*, 2000). Origin of many effective drugs is found in the traditional treatment practices. In the view of this, several workers have undertaken studies pertaining to testing of a large number of traditionally used medicinal plants for their proclaimed anthelminthic efficacy (Hammond *et al.*, 1997; Githiori *et al.*, 2003).

The literature collected pertaining to the study of anthelminthic activities of *Epiprinus mallotiformis* has interpreted meaning fully in this review part, which are as follows:

Dubey and Gupta (1968) investigated the anthelminthic activity by used hookworms, *Haemonchus contortus* and tapeworms and/or *Ascaris lumbricoides* for the different plant extracts. Some other researchers conducting in vitro studies have used an alteration of the larval development assay (LDA) or larval motility tests which are commonly used for testing of resistance of parasites to anthelmintics (Al-Qarawi *et al.*, 2001; Alawa *et al.*, 2003). These *in vitro* screening are important as they basis for further *in vivo* studies.
Plants have been used from prehistoric times to cure diseases of man and animals. This system of therapy is commonly referred as ‘unani, folk, eastern or indigenous’ medicine. There are a many plants, which have been reported in literature for their medicinal importance in anthelmintic screening importance. For example *Caesalpinia crista*, *Melia azedarach*, *Saussurea lappa*, *Morringa oleifera*, *Trachelospermum jasminoides*, *Butea frondosa* etc., have been quite commonly used.

In 1956, Chopra investigated the powdered seeds and *Peganum harmala* extractes by different organic solvent and it have been evaluated for narcotic, analgesic, antispasmodic in colic and as a remedy against tapeworm infection in man and animals. Garg and Atal (1963) isolated the proteolytic enzyme by the latex of *Calotropis procera* and investigated the anthelminthic activity, *Chebulic myrobalans*, *Belleric myrobalans* and *Emblic myrobalans* possess good anthelmintic activity (Gaind et al., 1964).

Chopra et al. (1958) reported that Seeds of *Butea superba* are extensively used as sedative and anthelmintic in the indigenous system of medicine.

Neogi et al. (1964) investigated the anthelmintic activity of some indigenous drugs. *Embellia ribes*, *Macuna prurita* and *Melia azedarach* have been found to bear significant activity against *Taenia canina* and *Paramphistomum cervi*.

Dhar et al. (1965) studied on anthelmintic activity of Carica papaya seeds. The aqueous extracts of Seeds of *Carica papaya* significant anthelmintic property against *Ascaris lumbricoides* and *Ascaridia galli*.

Shrivastava and Singh (1967) studied on anthelmintic activity of *Cucurbita maxima* seeds. The aqueous extracts of *Cucurbita mexicana* seeds have exhibited significant anthelmintic activity against *Moniezia expansa*, *Fasciolopsis buski*, *Ascaris lumbricoides* and *Hymenolepis diminuta* when compare to alcoholic extract.
Chapter 7

Anthelmintic Activity

Sharma et al. (1971) evaluated in vitro anthelmintic screening of indigenous medicinal plants against *Haemonchus contortus*. Extracts of *Cucurbita pepo*, *Calotropis gigantean*, *Juglans regia*, *Momordica charantia*, *Musa paradisaca* and *Scindapsus officinalis* have been found to show significant anthelmintic activity on *Haemonchus contortus* of goat origin.

In 1974, Kalesaraj has investigated the anthelmintic activity against human *Ascaris lumbricoides*, using alcoholic extracts of stem of *Helleborus niger*, rhizomes of *Zingiber officinale* (Zingiberaceae), seeds of *Carum copticum*, *Agati gratifola* (Leguminosae) and *Mangifera indica*. The extracts of different plants showed significant results which was appreciable.

Dixit and Varma et al. (1975) have evaluated anthelmintic activity of *Albizzia lebbek*, the bulb of *Allium sativum*, rhizomes of *Alpinia calcarata*, rind of *Citrus acida*, *Citrus aromatic*, *Punica granatu*, *Citrus medica*, rhizomes of *Cucuruma aromatic* using piperazine phosphate as a standard, in their study reported that the oils of the rhizomes of *Hedychium coronarium* and *H. spicatum* possess better than against earthworms and tapeworms.

The fruit of *Mallotus philippinensis* has been used as an anthelmintic, cathartic, aphrodisiac, lithotropic and styptic. It has also been used in external applications for the control of parasitic infections of the skin, as an antiseptic for ears and systemically for urinary disorders (Satyavati et al., 1987).

Raje and Jangde (2003) and Assis et al. (2003) were conducted anthelmintic activity against *H. contortus* and it shows the considerable activity against the extracts of *Adhatoda vasica*, *Nicotiana tabacum* and *Spigelia anthelmia*.

240
Hördegen et al. (2006) Bromelain, the enzyme complex of the stem of Ananas comosus, the ethanolic extracts of seeds of Azadirachta indica, Caesalpinia crista, Vernonia anthelmintica, Fumaria parviflora and of the fruit of Embelia ribes showed anthelmintic efficacy (upto 93%), relative to pyrantel tartrate against infective larvae of H. contortus.

López-Aroche et al. (2008) evaluated the anthelmintic activity of twenty plants from Mexico and found Bursera copallifera, B. grandifolia, Lippia graveolens, Passiflora mexicana, Prosopis laevigata, Randia echinocarpa and Urtica dioica to have anthelmintic properties against H. contortus unsheathed third stage infective larvae.

In 2011, Swati and Sagar evaluated anthelmintic potential of Stem Bark extract of cassia tora against earthworm, the significant anthelmintic activity found at higher concentration of 100 mg/ml.

Luffa cylindrica were evaluated for anthelmintic activity against Indian earthworm Pheritima posthuma and shows significant activity in 50 and 100 mg/ml concentration (Partap et al., 2012).

Swapna et al. (2012) reported that Garcinia indica have anthelmintic activity in different solvent extracts against Pheritima posthuma, in this study Methanol extract shows 90% activity when compared with Albendazole. Kirtiman, 2012 have evaluated Anthelmintic activity using Withania sominifera and Ocimum sanctum against earthworms Pheritima posthuma from this results shows that dose of 40 mg/ml possesses more wormicidal activity, out of the two, Ocimum sanctum has better anthelmintic activity.
Ahmed et al. (2013) studied on in vitro anthelmintic activity of crude extracts of selected medicinal plants against Haemonchus contortus from sheep. Ethanol extracts of 25 plant species were screened for anthelmintic effects against Haemonchus contortus. Ananas comosus, Aloe ferox, Allium sativum, Lespedeza cuneata and Warburgia salutaris shows high efficacies of anthelmintic activity upto 70% mortality.

The focus of this study is to evaluate the anthelmintic activity of Epiprinus mallotiformis which is used as traditional medicinal plant to curing gastro-intestinal infections in different regions of the south India, with particular reference to Karnataka.

**Materials and Methods**

*In-vitro anthelmintic activity*

Indian adult earthworms (Pheretima posthuma) were selected for in vitro anthelmintic assay. It has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. The Earthworms (Pheretima posthuma) were procured from the local supplier at Shimoga and were identified from the Department of Applied Zoology, Kuvempu University, Shankaraghatta. The worms were washed with water to remove adhering materials and shorted out for uniform size and length.

The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. The worms were kept in 6% dextrose solution for acclimatization. The worms with normal motility were selected for the experiment. The petridish of equal size were selected and in each petridish, different concentration i.e., 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml of petroleum ether, chloroform, methanol extracts of leaf and bark extracts and isolated pure compound, 13-labdone-2,3,8,15-tetrol of
Epiprinus mallotiformis were prepared in 0.1% tween 80 suspension used to evaluate the anthelmintic activity. As reference, standard piperazine citrate in 0.1% tween 80 suspensions was used. The volume of the solution in each petridish was made up to 25 ml, with an aqueous solution of 6% dextrose solution. In each petridish, 6 worms were placed; the time taken by each worm for paralysis was noted. The paralysis of worms was tested by placing the worms in water maintained at 50°C which was taken as death time.

Results

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTP in minutes (Mean and SD)</td>
<td>TTD in minutes (Mean and SD)</td>
<td>TTP in minutes (Mean and SD)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (10mg/ml)</td>
<td>21.98±0.84</td>
<td>59.92±0.80</td>
<td>21.98±0.84</td>
</tr>
<tr>
<td>20</td>
<td>46.79±0.98</td>
<td>93.51±1.67</td>
<td>45.3±1.09</td>
</tr>
<tr>
<td>40</td>
<td>45.60±1.33</td>
<td>91.83±0.82</td>
<td>44.04±0.68</td>
</tr>
<tr>
<td>60</td>
<td>44.81±1.10</td>
<td>92.00±1.59</td>
<td>40.25±0.63</td>
</tr>
<tr>
<td>80</td>
<td>43.25±1.45</td>
<td>85.93±2.06</td>
<td>39.28±1.07</td>
</tr>
<tr>
<td>100</td>
<td>40.34±1.01</td>
<td>83.51±2.04</td>
<td>36.78±0.90</td>
</tr>
</tbody>
</table>

TTP = Time taken for paralysis and TTD = Time taken for death
Table 7.2. Results of anthelmintic activity of bark extracts of *Epiprinus mallotiformis*

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTP in minutes (Mean and SD)</td>
<td>TTD in minutes (Mean and SD)</td>
<td>TTP in minutes (Mean and SD)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (10mg/ml)</td>
<td>21.98±0.84</td>
<td>59.92±0.80</td>
<td>21.98±0.84</td>
</tr>
<tr>
<td>20</td>
<td>52.25±0.62</td>
<td>104.71±1.08</td>
<td>52.98±0.31</td>
</tr>
<tr>
<td>40</td>
<td>49.43±1.31</td>
<td>102.36±0.86</td>
<td>51.58±0.89</td>
</tr>
<tr>
<td>60</td>
<td>47.19±1.30</td>
<td>99.59±0.59</td>
<td>48.88±0.70</td>
</tr>
<tr>
<td>80</td>
<td>45.63±0.56</td>
<td>98.19±0.53</td>
<td>46.44±0.60</td>
</tr>
<tr>
<td>100</td>
<td>43.35±0.53</td>
<td>95.9±0.87</td>
<td>45.31±0.93</td>
</tr>
</tbody>
</table>

TTP = Time taken for paralysis and TTD = Time taken for death

The results of anthelmintic activity of the plant *Epiprinus mallotiformis* was studied using different concentration such as 20, 40, 60, 80 and 100 mg/ml of solvent extracts of leaf and bark and isolated pure compound, 13-labdene-2,3,8,15-tetrol were compared with standard drug piperazine citrate of 10 mg/ml concentration. The results of anthelmintic activity of solvent extracts of leaf and bark were shown in Table 7.1 and 7.2 respectively. It was observed that, even at higher concentration of extract at 100 mg/ml produced paralytic effect much later and the time taken for death was longer in treated
When compared to standard drug-treated worms at 10 mg/ml concentration, the methanolic leaf extract of *Epiprinus mallotiformis* showed moderately significant anthelmintic activity at 100 mg/ml concentration followed by petroleum ether and chloroform extracts.

In petroleum ether extract-treated earthworms, low paralysis and death time was observed at 100 mg/ml concentration (paralysis 40.34±1.01 and death time 83.51±2.04), the very least paralysis and prolonged death time was recorded in 20 mg/ml (paralysis 46.79±0.98 and death time 93.51±1.67). Similarly, in chloroform extracts at 100 mg/ml concentration showed paralysis time of about 36.78±0.90 and death time of about 91±0.73. The increased degree of paralysis and decreased death time was observed in the methanol extract at 100 mg/ml concentration (paralysis 25.98±0.69, death time 65.11±1.04), when compared with petroleum ether and chloroform extracts. The standard drug piperazine citrate at 10 mg/ml showed increased anthelmintic activity (paralysis 21.98±0.84 and death time 59.92±0.80).

The bark extract of *E. mallotiformis* also showed considerable anthelmintic activity when compared to leaf extracts. Here also, methanolic bark extract showed considerable anthelmintic activity followed by the petroleum ether and chloroform extracts. In petroleum ether extract at 100 mg/ml concentration showed the least paralysis and increased death time (paralysis 52.25±0.62 and death time 104.71±1.08).

Similarly, chloroform bark extract showed moderate helminthic activity at 100 mg/ml concentration (paralysis 45.31±0.93 and death time 89.55±0.80) and considerable anthelmintic activity (paralysis 32.91±0.90 and death time 77.89±1.67) was exhibited by methanolic bark extract at 100 mg/ml concentration.
Table 7.3. Results of anthelmintic activity of 13-labdene-2,3,8,15-tetrol of *E. mallotiformis*

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Pure compound, 13-labdene-2,3,8,15-tetrol of <em>E. mallotiformis</em></th>
<th>TTP in minutes (Mean and SD)</th>
<th>TTD in minutes (Mean and SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (10mg/ml)</td>
<td></td>
<td>21.98±0.84</td>
<td>59.92±0.80</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>37.17±0.98</td>
<td>71.51±1.67</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>34.60±1.33</td>
<td>70.83±0.82</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>33.81±1.10</td>
<td>68.95±1.59</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>29.25±0.66</td>
<td>65.93±2.06</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>25.34±1.01</td>
<td>64.92±2.04</td>
</tr>
</tbody>
</table>

TTP = Time taken for Paralysis and TTD = Time taken for Death

The isolated pure compound, 13-labdene-2,3,8,15-tetrol, showed considerable anthelmintic activity over the standard drug. The least paralysis and prolonged death time was recorded in 20 mg/ml concentration (paralysis 37.17±0.98 and death time 71.51±1.67) and the maximum paralysis and decreased death time was observed at 100 mg/ml concentration (paralysis 25.34±1.01 and death time 64.92±2.04).

Overall study, the methanol leaf extract and isolated pure compound, 13-labdene-2,3,8,15-tetrol of *E. mallotiformis*, exhibited considerable anthelmintic activity in dose-dependent manner at par with the standard drug at higher concentration (100 mg/ml).
Discussion

Evaluation of medicinal plants, claimed for possessing anthelmintic properties is getting the attention of researches in these days. Screening and proper evaluation of the medicinal plants, could offer possible alternatives to search for effective plant based anthelmintic compounds (Partap et al., 2012).

In the present study, preliminary phytochemical screening of different solvent extracts of leaf and bark of *E. mallotiformis* revealed the presence of flavonoids, tannins, saponins, steroids and glycosides. The leaf methanolic extract demonstrated the significant anthelmintic activity at 100 mg/ml concentration (paralysis time 25.98±0.69 and death time 65.11±1.04 respectively). The least anthelmintic activity was found in petroleum ether extract at 100 mg/ml concentration (paralysis time 40.34±1.01 and death time 83.51±2.04) whereas, in methanolic bark extract exhibited low anthelmintic activity, when compare to leaf extract. The pure compound isolated from methanolic leaf extract, 13-labdone-2,3,8,15-tetrol, showed significant anthelmintic activity (paralysis 25.34±1.01 and death time 64.92±2.04) similar to leaf extracts. These results agree with the findings of Praveen *et al.* (2013), Haque Rabiu *et al.* (2011) and Pal (2011) respectively.

In general, the methanolic leaf extract and isolated pure compound, 13-labdone-2,3,8,15-tetrol showed considerable anthelmintic activity at higher concentration i.e., at 100 mg/ml, over the standard drug, piperazine citrate at 10 mg/ml concentration.
Plate - 7.1

Photographs showing anthelminthic activity of methanol extract of leaf of *Epiprinus mallotiformis* in different concentration.
Plate 7.1

Control  20 mg/ml

40 mg/ml  60 mg/ml

80 mg/ml  100 mg/ml
References


