Journal of Molecular Pharmaceutics & Organic Process Research

Research Article Open Access

Comparative In Vitro Anthelmintic Activity of Mentha piperita and Cassia Tora

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Abstract

Mentha piperita and Cassia Tara's anthelmintic activities will be distinguished in this study's overall analysis. A well-liked herbal tonic all throughout the world is Mentha piperita. As a flavouring agent, it is typically utilised. For more than 200 years, India has used it as a common at-home treatment for digestive disorders. The Caesalpiniaceous family plant Cassia Tora L. is a weed that spreads throughout most of India. The leaves and seeds, according to Ayurveda, are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardio tonic, and expectorant. The seeds and leaves can be used to treat cardiac diseases, bronchitis, cough, colic, dyspepsia, leprosy, ringworm, and flatulence. As a result of the extracts' effectiveness against the study's worms, the traditional usage of M.piperta and C. Tora leaf as an anthelmintic has been proven.

Keywords: Mentha piperita; Cassia Tora; Flavouring agent; Cardio tonic; Flatulence

Introduction

Helminthiasis

Growing interest has promoted researcher to screen scientifically various claims regarding properties and use of medicinal plant materials. Presently both common consumers and health care professional seek updated, authorities' information towards safety and efficacy of recommended medicinal plant as drug to its use [1]. Parasitic infection including, Helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide. Helminths produce serious problem in human and other animals around the world specifically to the third world countries [2].The WHO estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worm also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor [3].

Gastro intestinal parasites create a serious threat to the production of livestock in developing nations [4]. Anthelminthic are those agents that expel parasitic worms (helminths) from the body, by either stunning or killing them. Helminths parasite infections are global problems with severe social and economic repercussions in the third world countries [5]. The diseases affect the health status of a large fraction of human population as well as animals. Some type of dangerous helminths infections like filariasis has only a few therapeutic modalities at present [6]. Helminths infections are commonly found in community and being recognized as cause of much acute as well as cattle's use of herbs could be one of the major options to control these pathologies [7]. The plant was investigated and was found out to possess abortifacient, anti-bacterial, anti-cholesterol emic, anti-inflammatory, anti-spasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic and tonic properties [8] and (Figure 1).

Mentha piperita (Figure 2)

Botanical name: *Mentha x piperita*

Family: *Lamiaceae*

Order: Lamiales

Genus: Mentha

Mentha piperita is a popular herb tonic worldwide. It is generally used as a flavouring agent. It has been a popular home remedy for digestive ailments for two centuries in India. It is a perennial, Comparative In vitro Anthelmintic Activity of Mentha piperita and Cassia Tora glabrous, strong scented herb from the family Menispermaceae. The volatile oil obtained from this plant, known as mint or peppermint oil, is used as antiseptic, stimulant, carminative and for allaying nausea and vomiting and also has got commercial value [9]. The major components of this oil are menthofuran, [10-11] menthol, methyl acetate, neomenthol, Menthone and isomenthone [12]. The plant has been used for anti-nociceptive, anti-inflammatory, [13] antimicrobial and antioxidant activities [14]. The flavonoids namely eriocitrin, narirutin, hesperidin, luteolin-7-O-rutinoside, isorhoifolin, diosmin, rosmarinic acid, and 5, 7-dihydroxycromone-7-O-rutinoside isolated from the plant showed anti-allergic effects. Menthone is also a major constituent of the plant [15].

Cassia Tora (Figure 3)

Botanical name: *Senna Tora*

Family: *Fabaceae*

Order: Fabales

Genus: Senna

Cassia Tora L., Caesalpiniaceous, is a wild crop and grows in most parts of India as a weed. According to Ayurveda the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardio tonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders [16,17]. An ethanolic extract of seeds was

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Received: 31-May-2022, Manuscript No: JMPOPR-22-65513, **Editor assigned:** 02-Jun-2022, PreQC No: JMPOPR-22-65513(PQ), **Reviewed:** 16-Jun-2022, QC No: JMPOPR-22-65513, **Revised:** 21-Jun-2022, Manuscript No: JMPOPR-22-65513(R), **Published:** 01-Jul-2022, DOI: 10.4172/2329-9053.1000141

Citation: Gawali A (2022) Comparative In Vitro Anthelmintic Activity of Mentha piperita and Cassia Tora. J Mol Pharm Org Process Res 10: 141.

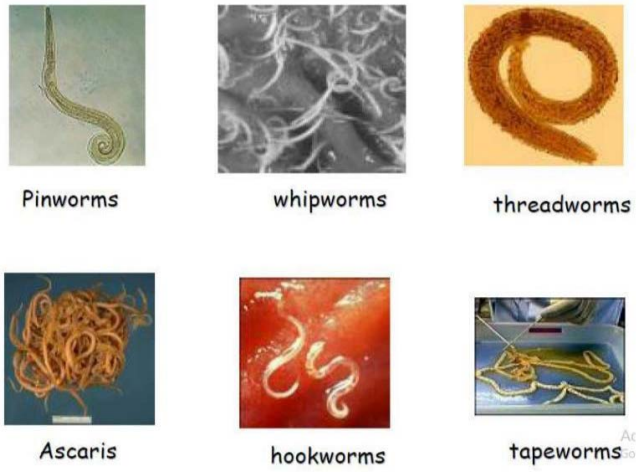

Figure 1: Images of different types of helminths.

Figure 2: Mentha piperita.

Figure 3: Cassia Tora.

evaluated by Parietal for its hypolipidemic activity on triton induced hyperlipidaemia profile18.Some synthetic phenolic anthelmintic e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation19.Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [18]. In literature survey anthelmintic activity of Cassia Tora against Pheretima Posthuma and Ascardia galli is reported [19]. The present study involves comparative investigation of phytochemicals followed by the anthelmintic activity of various extracts of seed and seed cover against Eisenia foetida using saline as control with a view to justify the use of the plant in the treatment of helminths.

Review of Literature

- Haque Rabiu, Mondal Subhasish (2011)

The aqueous extract of Azadirachta Indica Leaves was investigated for anthelmintic activity using earthworms (Pheretima Posthuma), tapeworms (Raillietina Spiralis) and roundworms (Ascardia Galli). Various concentrations (10-70 mg/ml) of plant extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at the concentration of 40 mg/ml. The result shows that aqueous extract possesses vermicide activity and found to be effective as an anthelmintic. Therefore, the anthelmintic activity of the aqueous extract of Azadirachta Indica Leaves has been reported.

- Sarabjot Kaur, Bimlesh Kumar (2010)

The aim of the present study was to evaluate the anthelmintic potential of ethanolic and aqueous extract of bark of Holoptelea integrifolia using Eisenia foetida. Various concentrations (10, 25, 50 and 100 mg/ml) of ethanolic and aqueous extract were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine Citrate (10 mg/ml) was included as standard. The results indicated that the ethanolic and aqueous extract significantly demonstrated paralysis and also caused death of worms especially at higher concentration as compared to standard references. In conclusion, the use of bark of Holoptelea integrifolia as an anthelmintic have been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

- Prashant Tiwari, Bimlesh Kumar (2010)

The present study aimed at the in-vitro evaluation of anthelmintic activity of aqueous and ethanolic extracts of stem of Tinospora cord folia using Eisenia foetida at four different concentrations (10, 25, 50 and 100 mg/ml) respectively. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. At the concentration of 100 mg/ml both the ethanolic and the aqueous extracts exhibited very significant activities as compared to the standard drug piperazine citrate (10 mg/ml). The time of paralysis and death was recorded as 9 ± 0.57 and 15.83 ± 0.60 in case of aqueous extract, on the other hand it was recorded as 4.16 ± 0.30 and 10.83 ± 0.60 in case of ethanolic extracts. In conclusion, the use of stem of Tinospora cordifolia as an anthelmintic have been confirmed and further studies are suggested to isolate the active principles responsible for the activity

- Rasika Kumarasingha, Sarah Preston (2016)

Parasitic roundworms (nematodes) cause substantial morbidity and mortality in livestock animals globally, and considerable productivity losses to farmers. The control of these nematodes has relied largely on the use of a limited number of anthelmintic. However, resistance too many of these anthelmintic is now widespread, and, therefore, there is a need to find new drugs to ensure sustained and effective treatment and control into the future. Recently, we developed a screening assay to test natural, plant extracts with known inhibitory effects against the free-living worm Caenorhabditis elegant. Using this assay, we assessed here the effects of the extracts on motility and development of parasitic larval stages of Haemonchus contortus, one of the most important nematodes of small ruminants worldwide. The study showed that two of five extracts from Picria fel-terrae Lour. Have a significant inhibitory

effect (at concentrations of 3–5 mg/ml) on the motility and development of H. contortus larvae. Although the two extracts originated from the same plant, they displayed different levels of inhibition on motility and development, which might relate to the presence of various active constituents in these extracts, or the same constituents at different concentrations in distinct parts of the plant. These results suggest that extracts from P. fel-terrae Lour. Have promising anthelmintic activity and that more broadly; plant extracts are a potential rich source of anthelmintic to combat helminthic diseases.

- Avani V Patel,Dr. Amit Vihabhai Patel (2011)

Various species of genus Tephrosea have been extensively studied for their pharmacological activity. Tephrosea purpurea is expected to possess many constituents among them triterpenoids are also major constituents. So deu to presence of triterpenoids Tephrosea purpurea may possess Anthelmintic activity. In the present work ethanolic and aqueous extract of leaves of Tephrosea purpurea were evaluated for its Anthelmintic activity against earthworm and tapeworm at three different concentrations (25, 50, 100 mg/ml). The Anthelmintic activity of both extracts was comparable with standard drug (Piperazine citrate, 50 mg/ml) having same concentration. Animal treated with distilled water was taken as a normal control. Standard drug solution was prepared in distilled water. Activity was evaluated by noting the time required for paralysis and death of worms by extracts. The data shows that ethanolic extract (100 mg/ml concentration) possess comparable anthelmintic activity with standard drug. The results show that the plant has the potential to be used as Anthelmintic.

- Myriam Esteban Ballesteros, Jaime Sanchis (2019)

The present study was conducted to evaluate the in vitro anthelmintic activity and safety of methanolic and aqueous extracts of 9 plants against the ovine GIN Teladorsagia circumcincta. Initially the ovicidal efficacy of all extracts was tested at 50 mg/ml and at 1%, this last concentration for the methanolic extract of Elettaria cardamom, by the Egg Hatch Assay. In those extracts with efficacy higher than 95%, the effective concentration required to inhibit 50% of egg hatching (EC50) and their cytotoxicity, as the lethal dose 50 (LC50), was also measured. The aqueous extracts of Aesculus hippocastanum, Isatis tinctoria, Chelidonium majus, E. cardamomum and Sisymbrium irio, with EC50 values ranging 1.30–2.88 mg/ml, and the aqueous extract of Jasminum polyanthum with a value of 6.41 mg/ml, showed the highest activities. The aqueous extract of J. polyanthum was the safest extract, followed by methanolic extract of E. cardamomum and aqueous extract of S. irio, all of them with a Selective Index higher than 1. According to our results, there is no correlation between the amount of total phenols or total tannins with the anthelmintic activity of the plants tested. Although results need to be interpreted with caution, as in vitro activity may not automatically translate into in vivo efficacy, those extracts with SI equal or higher than 1 and EC50 equal or lower than 6 mg/ml, could be taken into account for being used subsequently as feed or food additives in infected sheep.

- Lakshmipathy R, Ganesh Pandu (2012)

The development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintic. Traditional medicines act as a source of easily available effective anthelmintic agent. In the current study, In vitro experiments were conducted on Indian adult earthworms (Pheretima Posthuma) to determine the possible anthelmintic activity of crude methanol extracts of Gymnema sylvestre and Acalypha fruticosa forssk. Methanol extract of the two plants at different concentration (25, 50, 100, 200mg/ml) showed dose-dependent vermicidal activities and results were expressed in terms of time for death and time for paralysis of worms. Piperazine citrate was used as a reference standard at a concentration of 10 mg/ml. The present study revealed that the two plants possess potent anthelmintic activity when compared to Piperazine.

- Lipi Purwal, Vinamra Shrivastava (2009)

Helminthic infections are now being recognized as cause of chronic ill health and sluggishness amongst the tropical people. The aim of present study was to evaluate anthelmintic prospective of crude aqueous extracts of some saponins containing medicinal plants. The dried roots and leaves of W. somnifera and dried flowers and leaves of C. officinalis were used for the preparation of the extracts. Four concentrations (2, 4, 6 and 8 mg/ml) of aqueous extracts were investigated for in vitro anthelmintic activity employing Indian adult earthworms (Pheretima Posthuma), which involved determination of time paralysis (P) and the time of death (D) of the

Worms. Albendazole (10 mg/ml) was included as standard reference and distilled water as control. All the four aqueous extracts of roots and leaves of W. somnifera (L.) and dried flowers and leaves of C. officinalis (L.) showed significant anthelmintic activity. All the investigated extracts showed significant difference ($p<0.001$) against negative control. The study confirmed the anthelmintic potential of these extracts and further studies are suggested to isolate the saponins and other active principles responsible for the activity.

- A. Shruthi, K.P. Latha (2010)

Development of anthelmintic resistance and high cost of convectional anthelmintic drugs led to the evaluation of medicinal plants has an alternative source of anthelmintic. The aim of the present study was to determine the anthelmintic activity of crude petroleum ether and chloroform extracts of leaves of Wrightia tinctoria using Pheretima Posthuma. Three concentrations (2.5, 5.0, 7.5mg/ml) of each extracts were studied in the activity, which involved the determination of time of paralysis and time of death of the worms. Piperazine citrate is used as standard reference and normal saline as control. The present study proves the potential usefulness of leaves of Wrightia tinctoria as comparable anthelmintic agent.

- Archana Mehta, Shruti Shukla (2009)

Methanolic extract and its ethyl acetate fraction of Cassia Tora L. leaves were evaluated for anthelmintic property using the Indian adult earthworm (Pheretima Posthuma) as a model. Among the earthworms the ethyl acetate fraction was potent. The results were compared with a standard drug, Albendazole. The phytochemical analysis of both extracts showed the presence of phenolic like flavonoids and tannins as well as anthraquinones, which may be the active principle. The present study confirms the ethno-medicinal report of the plant as an anthelmintic drug.

- Musheerul Hassan, MIR RA (2019)

Objective: The present study was carried out to assess the in vitro anthelmintic activity of Abutilon theophrasti stem extracts. Methods: Simple maceration was employed for extraction. Solvents such as methanol, water, and hexane were used. Egg hatch test (EHT) and larval motility test were employed to check the anthelmintic activity of crude extracts. Concentrations of 500, 250, 125, 62.5, and 31.25 mg/ml were made. Levamisole and distilled water served as control, respectively. Results: All selected extracts displayed concentration-dependent

inhibition except aqueous extracts. At higher concentration (500 mg/ml), stem extracts (methanol, aqueous, and ethanol) showed 74.39%, 72.5 and 70.03% of efficacy in EHT, respectively ($p \leq 0.05$). Meanwhile, inhibition of larval motility was seen higher with inhibition percentage of 79.79, 75.65, and 71.28 by methanolic, aqueous, and hexane extracts, respectively ($p \leq 0.05$). Conclusion: The presents study suggests active principles having anthelmintic efficacy in Abutilon theopharsti stem.

- P. Karthikeyan, Vinayak Suresh (2011)

Aim: The aim of present study was to evaluate anthelmintic potential of methanol and aqueous extract of bark of Sesbania grand flora (L.) Poir. Using Pheretima Posthuma as test worm. Method: Various concentrations (10, 20, 30, 40 and 50 mg/ml) of methanol and aqueous extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate (10, 20, 30, 40 and 50 mg/ml) was included as standard reference and normal saline as control. Result: The results of present study indicated that the methanol and aqueous extract significantly demonstrated paralysis, and also caused death of worms especially at higher concentration as compared to standard reference Piperazine citrate. Conclusion: In conclusion, the use of the bark of the plant Sesbania grand flora (L.) Poir. As an anthelmintic have been confirmed and further studies are suggested to isolate the active principle/s responsible for the activity.

- Gururaja Mp, Joshi Himanshu (2011)

The objective of the present work was to evaluate the anthelmintic property of ethanolic extract of Tecona grand is fruits using Indian earthworm Pheretima Posthuma as test worm. Various concentrations of ethanolic extract were tested in bioassay which involved determination of time of paralysis (P) and time of death (D) of the worm. Piperazine citrate (10mg/ml) was used as reference standard. The result of present study indicates that the crude ethanolic extract significantly demonstrated paralysis, and also caused death of worms especially at higher concentration (50 mg/ml) compared to standard reference Piperazine citrate. To conclude, the use of the fruits of Tectonic grand is as anthelmintic has been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

- A.J. Dhembare, S.L. Kakad (2015) in the present investigation leaf extract of Ipomoea carnea Jacq was subjected to evaluation of anthelmintic activity. The leaf extracts of Chloroform, ethanol, methanol, and petroleum ether were tested on earthworm Pheretima Posthuma. The trend of anthelmintic activity was in order as chloroform > methanol > ethanol > petroleum ether. Among the screened different extracts, chloroform extract 25 mg/ml showed efficient anthelmintic activity with paralysis time (8.2 min) and death time (15.5 min). The chloroform extract showed highest anthelmintic activity than other extract, compared with Piperazine citrate. But all the extracts were less potent when compared with reference drug Piperazine citrate. The test of't' and 'f' were performed with in group and between the groups were significant at 0.05% level.

- Sucheta A Gaikwad, Milind S.Thakare (2017).

Parasitic infection including Helminthiasis is a critical serious problem in the tropical region. Helminths produce serious problem in human and other animals around the world specifically to the third world countries. As per WHO, only few drugs are frequently used in the treatment of these parasite infections. Albendazole, Piperazine citrate are the commercial anthelmintic drugs available. The drug is poorly absorbed and efficacy depends on transit time in the gastrointestinal tract. The toxicity is extremely low, but the drug has not been studied in children under two years of age. There is increasing demand of natural anthelmintic. Hence present study ethyl acetate, acetone, methanol and aqueous extracts of Cassia Tora, seed and seed cover were investigated for their phytochemical followed anthelmintic activity against Eisenia Foetida. Three concentrations (1, 2.5,5 mg/ml) of each extract were studied it involves the determination of time of paralysis and time of death of the worms. The gradual increased in a dose exhibited, a gradual increase in the activity. Phytochemical analysis revealed presence of flavonoids as one of the chemical constituent. It was observed that all extracts exhibit positive response to certain degree of anthelmintic activity in dose dependent manner. Aqueous and methanol extracts displayed significant anthelmintic activity at highest concentration of 5mg/ml which is significant in case of seed extracts compare to seed cover. It was concluded from the experimental details that the plant revealed noteworthy anthelmintic activity. The data were verified as statistically significant by using two ways ANOVA at 1% level of significance.

Research Envisaged and Plan of Work

Rational

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.

The Mentha piperita and Cassia Tora leaves powder formulation used for the treatment of Helminthiasis. The objective of study will be comparative in vitro screening for anthelmintic activity of individual herbs Mentha piperita and Cassia leaves powder.

Aim and objectives

Aim of the study: To study Comparative In vitro Anthelmintic Activity of Mentha piperita and Cassia Tora.

Objective of the study:

- To evaluate the anthelmintic potential of methanolic extraction of individual herbs of Mentha piperita & cassia Tora leaves powder using earthworm as in vitro animal model.
- To compare the anthelmintic potential of methanolic extraction of individual herbs of Mentha piperita and Cassia Tora leaves powder.
- To evaluate and improve upon (if required) the pre-formation parameter of most effective constituent.
- To develop bioassay of this constituent.
- To characteristic of Mentha piperita and Cassia tora leaves powder.

Materials and Methods

Plant Materials

The Mentha piperita and Cassia Tora Plant leaves are collected freshly botanical garden of Dattakala college of Pharmacy, Swami Chincholi bhigwan Pune India. . The leaves were air-dried and grinded to fine powder.

Chemicals

Chemicals and reagents used in this study include Menthol, 5% DMF Dimethylformamide, and normal saline water. All chemicals used were of analytical grade.

Preparation of extracts

The Menthol extraction process, The Mentha piperita and Cassia tora leaves powder 50gm of fine leaves powder was macerated with 250 ml methanol for 5 days at room temperature. The mixture was filtered using Whatman No. 1 filter paper. The filtrate extracts was first dried in hot air oven at room temperature (25°c) and cooled. The Mentha piperita and cassia Tora powder were reconstituted in saline water for each experiment.

Collection of animals

India adult earthworms, which were collected from moist soil of swami-Chincholi and washed with normal saline to remove all faecal matter, were used for anthelmintic study. The earthworms (Pheretima Posthuma) of 3–5 cm in length and 0.1–0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

Preliminary phytochemical screening

Various phytochemical tests were carried out on the Mentha piperita and Cassia Tora leaf extracts to detect the presence or absences of carbohydrates, tannins, glycosides, terpenes, steroids, and other compounds [20].

Test organism

Indian adult earthworms (Pheretima Posthuma) collected from moist soil of the botanical garden were washed with normal saline and used for the anthelmintic study. The earthworms (4-6 cm in length and 0.1-0.2 cm in width) were used for all the experimental protocols due to their anatomical and physiological resemblance with the intestinal round-worm parasites of human beings [21].

Anthelmintic bioassays

The anthelmintic study was carried following [22]. Both methanolic extract of Mentha piperita and Cassia tora were dissolved in normal saline containing 5% DMF and diluted to get concentrations of 20, 30 and 50 mg/ml. All drug and extract solutions were freshly prepared before starting the experiment. Two groups, with three earth-worms each group, were placed into 30 ml of desired formulations as following: vehicle (normal saline containing 5%DMF) and two sets of three different groups were treated with extracts of respective concentrations.

Observations were made for the time taken until the paralysis and death of an individual worm. The paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors [23].

Result

The results of the current investigation indicate that among the menthanolic extracts of Mentha piperita and Cassia tora, the methanolic extracts Mentha piperita is the most potent one and requires less time to the paralysis and death of the worms as compared to the methanolic extract Cassia tora. Both extracts showed a concentration depended anthelmintic property (Table 1). The preliminary phytochemical analysis of the extracts has shown the presence of phenolic, like tannins and flavonoids as well as anthraquinones. The functions of the anthelmintic drugs, like a DMF, are known to cause paralysis of the worms so that they are expelled in the faces of man and animals. The extracts not only demonstrated this property, but they also caused death of the worms.

Table 1: Comparative Anthelmintic activity of Mentha piperita and Cassia tora leaves.

Sr no	Extracts	Concentration	Time required to paralyze(min)	Time required to death
		(mg/ml)		(min)
1	Mentha piperita.	20	8±1.48	25±2.40
2	Mentha piperita	30	6±2.05	18±1.40
3	Mentha piperita	50	4±1.04	10±1.06
4	Cassia tora	20	22±2.50	55±1.56
5	Cassia tora	30	14±1.54	32±2.50
6	Cassia tora	50	7±1.76	15±2.04

Synthetic phenolic anthelmintics, like niclosamide, interfere with the energy generation in the helminths parasites by uncoupling the oxidative phosphorylation [24]. Another possible mechanism of action is that they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and by this cause death. Tannins have also been shown to produce anthelmintic activities [25]. There are reports for anthelmintic property of phenolic present in different plant extracts like Baliospermum montanum Muell. Roots [26] and (Figure 4).

Figure 4: Image Comparative Anthelmintic activity Of Mentha piperita and Cassia tora.

Discussion and Conclusion

The methanolic extracts of Mentha piperita is the more potent and requires less time to the paralysis and death of the worms as compared to the methanolic extract of Cassia tora. In conclusion, the traditional claim of leaf of M.piperta and C. Tora as an anthelmintic have been confirmed as the extracts displayed activity against the worms used in the study.

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