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ORIGINAL RESEARCH ARTICLE

Comparative In Vitro Anthelmintic Activity Of Chloroform And Acetone Extracts Of *Mentha Piperita*

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ABSTRACT

Peppermint is a hybrid mint, a cross between the water mint (*Mentha aquatica*) and spearmint (*Mentha spicata*). Different commercial preparations exhibit various activities. Peppermint oil and menthol have moderate antibacterial effects against both Gram-positive and Gram-negative bacteria. Chloroform and acetone extracts of the plant *Mentha Piperita* were investigated for their anthelmintic activity against *Pheritima posthuma*. Each extract was studied at 20 mg/ml, which involved determination of time of paralysis and time of death of the worms. Both the extracts of the plant exhibited considerable anthelmintic activities, and the order of sensitivity of the extracts to the worms was that chloroform extract of *M. piperita* showed the best anthelmintic activity when compared with acetone extract. Albendazole (20 mg/ml) and distilled water were included in the assay as standard reference drug and control, respectively.

Key words: *Mentha piperita, Pheritima posthuma, in vitro* anthelmintic activity.

INTRODUCTION

Peppermint (*Mentha* × *piperita*, also known as *M. balsamea* Wild[1]) is a hybrid mint, a cross between the water mint (*Mentha aquatica*) and spearmint (*Mentha spicata*)[2] Peppermint has a high menthol content, and is often used as tea and for flavouring ice cream, confectionery, chewing gum, and toothpaste. The oil also contains menthone and menthyl esters, particularly menthyl acetate[3] Dried peppermint typically has 0.3-0.4% of volatile oil containing menthol (29-48%), menthone (20-31%), menthyl acetate (3-10%), menthofuran (1-7%) and many trace consituents including limonene, pulegone, eucalyptol, and pinene[4] .Peppermint has promising radioprotective effects for cancer patients undergoing cancer treatment.[5]. It also has a high concentration of natural pesticides, mainly menthone[6]. Peppermint is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders, and liver complaints [7,8]. In Eastern and Western traditional medicine peppermint and its oil have been used as an antispasmodic, aromatic, antiseptic and also in the treatment of cancers, colds, cramps, indigestion, nausea, sore throat and toothaches [9].

Peppermint oil possesses antibacterial activity *in vitro*. Different commercial preparations exhibit various activities [10]. Peppermint oil and menthol have moderate antibacterial effects against both Gram-positive and Gram-negative bacteria [11]. Peppermint is also found to possess antiviral and fungicidal activities [12]. Aqueous extracts of the leaves demonstrated significant antiviral activity against Influenza A, Newcastle disease, *Herpes simplex*, *Vaccinia*, Semliki Forest and West Nile viruses in egg and cell culture system [13]. It was also found to reduce the incidence and multiplicity of benzo[α]pyrene-induced lung carcinogenicity and mutagenicity [14]. In clinical trials peppermint oil's role in irritable bowel syndrome affirms its effectiveness compared with a placebo with no serious constipation or diarrhea [15,16,17].

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MATERIALS AND METHODS:

Plant material:

Leaves of *Mentha piperita were* collected from Ranga Reddy district, Andhra Pradesh (India) and in January 2010 authenticated by Dr.N.Ravindra, Department of Medicinal chemistry, Chilkur Balaji College of Pharmacy, Ranga Reddy district, Andhra Pradesh, India.

Preparation of Extracts:

Dried and coarsely powdered leaves (50gm) of *Mentha piperita are* subjected to extraction in Soxhlet extractor using chloroform and acetone. The extracts of various solvents were concentrated by vacuum distillation and then dried in open air [18].

Animals:

Indian adult earthworms (*Pheretima posthuma*) collected from vermi compost of RTP, National Institute of Rural Development (NIRD), Rajendranagar, Hyderabad, are washed with normal saline to remove all the compost matter, were used for the anthelmintic study. The earthworms 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 in human beings [19,20].

Drugs and Chemicals:

The following drugs and chemicals were used.

Drugs: Albendazole (ZENTEL,Glaxo smithkline pharmaceuticals ltd, Himachal Pradesh).

- TEST DRUGS: ***Mentha piperita*** extracts
  - Chloroform -200 µg / ml.
  - Acetone -200 µg / ml.

Anti Helmenthic Activity:

All the extracts of *M. piperita* were dissolved in minimum amount of DMSO and the volume was adjusted to 10 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment.

In each case, five earthworms were released into 10 ml of desired formulations as follows; vehicles (5% DMSO in normal saline), Albendazole (20 mg/ml), or total chloroform and acetone extracts of leaves of *M. piperita* (20 mg/ml, each) in normal saline containing 5% DMSO.

Observations were made for the time taken to paralysis and death of individual worm. 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 [21].

RESULTS AND DISCUSSION:

It is evident from the experimental data that, the crude chloroform and acetone extracts of leaves of the *M. piperita* showed significant anthelmintic activity at 20 mg/ml. Results were comparable with the standard drugs, Albendazole, at same concentration.

(**Table 1**) reveals that chloroform extract of *M. piperita* showed the best anthelmintic activity. The chloroform extract required the least time for causing paralysis and death of the earthworms when compared with acetone extract. As shown in Table 1 chloroform extract of *M. piperita* displayed intrinsic anthelmintic properties with 20 mg/ml giving a shortest time of paralysis and death than the acetone extract.

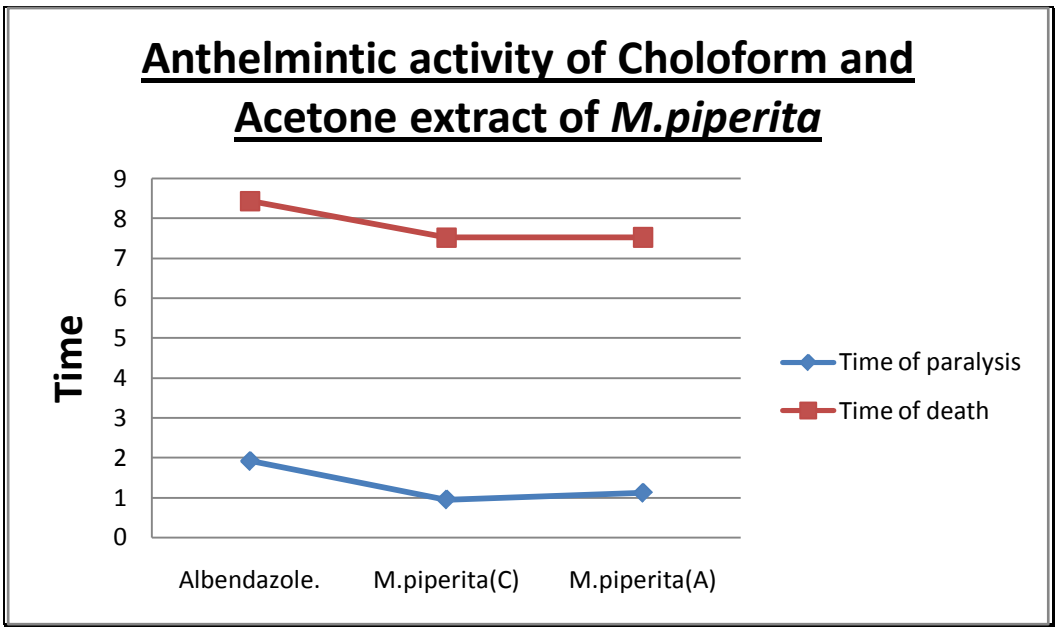
The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feaces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 20 mg/ml as compared with the Albendazole. In conclusion, these plants have been confirmed to display anthelminticactivities

Table I: Anthelmintic activity of Chloroform and Acetone extract of *M.piperita*

Treatment	Time Taken For Paralysis(Min)	Time taken for death.(min)
Albendazole.	1.918 ± 0.312	8.44±0.432
M.piperita(C)	0.948±0.214	7.529±1.464
M.piperita(A)	1.116±0.160	7.534±0.758
Control (in normal saline).	-	-

M.piperita (C) = Chloroform extract of *Mentha piperita*,
M.piperita (A) = Acetone extract of *Mentha piperita.*

Graph I: Anthelmintic activity of Chloroform and Acetone extract of *M.piperita*



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CONCLUSION:

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