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Anthelmintic activity of different extracts of *Anacyclus pyrethrum* along with Powder microscopy of roots

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ABSTRACT

Medicinal herbs as potential source of therapeutics have marked a crucial role in health system globally for both humans and animals. These are not only beneficial in the diseased condition but also used as potential material for maintaining proper health. The present study highlights the pharmacognostic studies on roots like powder microscopy and anthelmintic activity of different extracts on Indian adult earthworms. Different concentrations (50-150 mg/ml) of plant extract were tested in this study. Albendazole (20 mg/ml) was used as reference standard drug whereas CMC as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant vermicide activity in dose dependent manner. The result shows that methanolic extract possesses anthelmintic activity and found to be effective as an anthelmintic.

Keywords: Roots, Microscopy, In-vitro, Anthelmintic, Spanish pellitory, Fetida

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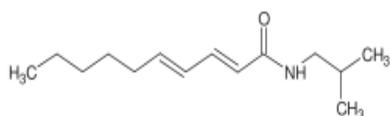
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INTRODUCTION

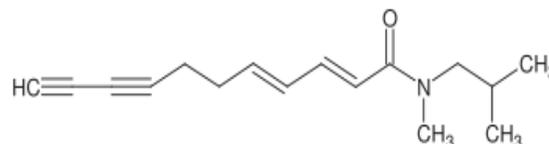
Majority of the infections with helminth took place due to unhygienic conditions and are commonly restricted to the tropical regions. In 31th summit of World Health Organization (WHO) has recommended administration of medicinal herbs, because of their standards, advantages and robustness. (Gurib-Fakim *et al.*, 2006)¹. Medicinal plants were the potent source of many pharmacological activities.

Anacyclus pyrethrum from Asteraceae family and Anacyclus genus is a native plant of India and Arabic countries and its root has therapeutic effects (Naderi *et al.*, 2012)². It is distributed in North Africa, Arabia, Syria, Algeria, in the mediterranean region and in some place of India such as Jammu and Kashmir, Bengal. *Anacyclus pyrethrum* is a mild aromatic herb and fosters its uses in ayurvedic, unani and homeopathy system of medicine. *A. pyrethrum* is customarily known as pellitory roots in English and as Akarkara in Hindi. Akarkara is used as pain-killer and more commonly for the treating male sexual weakness or debility. (Sharma V, *et al.*, 2012)³. Pellitory root in Indian traditional medicine has been widely used as an aphrodisiac and as a medicine for rejuvenation and vitality (Singh J, *et al.*, 1995)⁴. It also posses positive effect on the immune system regulating (Sharma *et al.*, 2010)⁵. Also, root has a pungent efficacy in promoting a free flow of saliva, in relieving toothache, in alleviating chronic catarrh and acne (Rassayana PH, *et al.*, 2003)⁶ and local stimulant in palsy of the tongue or throat.

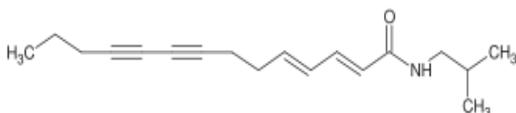
The root of Pellitory contains Anacylin (Boonen J, *et al.*, 2012)⁷ as the major alkylamides (13 Alkylamides are known which are based on isobutylamide structure), *N*-isobutyldienedynamide Pellitorine or Pyrethrine (Sharma V, *et al.*, 2012)³, Hydrocarolin, Sesamin, inulin, Polysaccharides (Bendjeddou D, *et al.*, 2003)^{8,13} and a trace of essential oil. Flavonoids and coumarins are also present (Hanane *et al.*, 2014)⁹. Roots contain acid resinous substance, pyrethrine



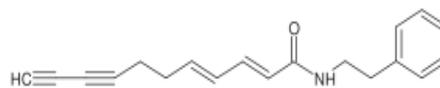
Pellitorine (Deca-2E,4E-dienoic acid IBA)



Undeca-2E,4E-diene-8,10-diynoic acid N-me IBA



Anacyline (Tetradeca-2E,4E-diene-8,10-diynoic acid IBA)



Undeca-2E,4E-diene-8,10-diynoic acid 2-PEA

MATERIALS AND METHOD

Drug and chemicals

Methanol, Potassium dichromate (Fisher scientific), CMC (CDC), Distilled water and Albendazole was purchased from Apollo pharmacy Limited, Amritsar (India).

Plant material

Dried roots of *Anacyclus pyrethrum* (500 gms) were purchased from a local vendor of Hall bazaar, Amritsar, Punjab, India and is further authenticated by an expert Taxonomist of Botany Department, Khalsa college, Amritsar (India) using relevant microscopic and manual procedures, where a voucher specimen was kept in the herbarium of the Department of Botany, Khalsa College, Amritsar (India).

Experimental worms

Indian adult earthworms (*Eisenia fetida*) were used for the anthelmintic activity of *Anacyclus pyrethrum* extract. They were collected from moist soil and washed with distilled water to remove all faecal matter and waste surrounding its body. *E.fetida* worms were authenticated by Dr. Jasjit Kaur Randhawa, Head Department of Zoology, Khalsa College, Amritsar. All the experiments are carried out on Indian adult earthworms (*E. fetida*) because they possess anatomical resemblance to intestinal roundworm parasites of human beings.(Pillai, *et al.*, 2011)¹⁰

Preparation of Methanolic Extract

The roots of *Anacyclus pyrethrum* are dried in shade and crushed in an electric blender to obtain coarse powder. The hot extraction was carried out by method described by Islam *et al.*, in 2015¹². by using Soxhlet extractor. About 175 grams of coarse root powder was packed in extractor and extracted by methanol as solvent. The extracts are concentrated by rotary evaporator and used for testing anthelmintic activity.

Preparation of test sample and standard

The suspension of Methanolic extract of roots of *Anacyclus pyrethrum* of different concentration (50,100,150 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent and final volume is made up to 10 ml for respective concentration. Albendazole was used as standard for anthelmintic activity. Albendazole (20 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent. Groups of approximately equal size of worms are released in each petri dish containing 10 ml of desired concentration of extract and drug.

Anthelmintic study

The anthelmintic activity was performed according to the method with minor modifications to the

method described by Ghosh (T Ghosh *et al.*, 2009)¹¹. On adult Indian earthworm *E. fetida* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Worms were placed in petri dish containing different concentrations (50,100,150 mg/ml) of methanolic extract of roots of *Anacyclus pyrethrum*. Each petri dish was placed with 4 worms and observed for paralysis or death of the worms. Mean time for paralysis (movement was absent) was noted is observed, except when the worm was shaken vigorously; the death time of worm (in minutes) was recorded after ascertaining that worms neither moved nor shaken when given external stimuli. The test results were compared with reference compound Albendazole (20 mg/ml).

RESULTS AND DISCUSSION

Powder Microscopic studies

The results of microscopic characters of root powder of *Anacyclus pyrethrum* clearly meet the characters of Asteraceae family. Powder microscopy is one of the simplest and cheapest methods for establishing the correct identity of the source materials and helps in proper identification of crude drugs. In this study, fine powder of the roots when visualized under Olympus microscope revealed the presence of vessels, pigmented cells, fibres, stone cells, cork cells, secretory cells and prismatic crystals.

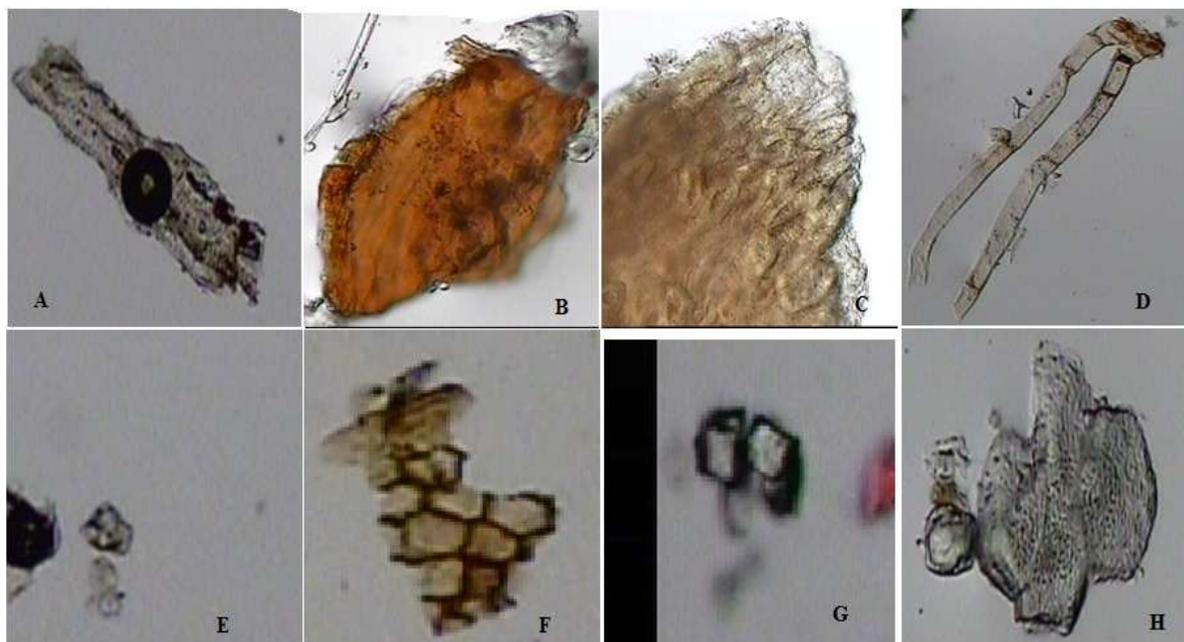


Fig. Powder microscopic characteristics of Akarkara roots

A- Perforated Vessels

B- Pigmented cells

C- Stone cells

D- Elongated fibres

E- Starch grains

F- Cork cells

G- Prismatic crystals

H- Secretory cells

Figure 1: Powder microscopic characters of roots of *Anacyclus pyrethrum*

Anthelmintic Activity

While studying the literature review of plant it was found that methanolic extract of plant possess various pharmacological activities and most of its chemical constituents are soluble in methanol. So, the methanolic extract of *Anacyclus pyrethrum* has been prepared and percentage yield of that extract was found to be 18.6%. Further the prepared methanol extract was evaluated by anthelmintic activity against *Fetida* worms (diseases/ infections caused by helmenthes) at three concentrations (50,100,150 mg/ml).The study showed that methanolic extract of *Anacyclus pyrethrum* was effective as Anthelmentic. Results interpret dose dependent reduced motility of worms with reference to the standard (Albendazole). Therefore, current investigation leads to conclusion that the roots of *Anacyclus pyrethrum*h have potent anthelmintic activity when compared with the conventionally used drugs. and hence can be treatment of Helmenthes infections.

Table 1: Anthelmintic potency of Methanolic extract of *Anacyclus pyrethrum*

Extract	Concentration (mg/ml)	<i>Fetida</i> Paralysis time	Death time
Control (0.5% CMC)	-	-	-
Standard (Albendazole)	20mg/ml	132 min	160 min
Methanolic extract	50mg/mg	58 min	104 min
	100mg/ml	44 min	83 min
	150mg/ml	28 min	58 min

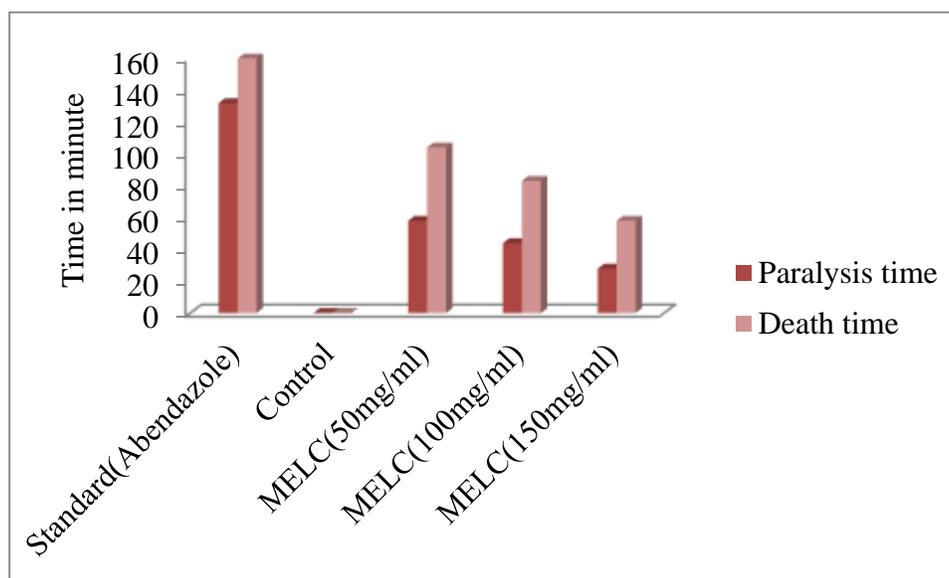


Figure 2: Effect of different concentrations of methanol extract of *Anacyclus pyrethrum* roots on *E. fetida*

CONCLUSION

These findings suggest that methanolic extract from *Anacyclus pyrethrum* have promising anthelmintic effect. We propose that future work should focus on attempting to fractionate extract in order to identify and characterize the constituents that are active against worms followed by their mechanism of action using *in vivo* models. Still further plant drugs are needed to be explored to reduce the price of synthetic anthelmintic drugs as well as high side effects when compared to natural drugs. Hence, further research must be carried out to explore effective plant drugs.

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