Research Article

IN VITRO ANTHELMINTHIC ACTIVITY OF HERBAL FORMULATION AGAINST *EISENIA FETIDA*

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ABSTRACT:

The present study was undertaken to evaluate anthelmintic activity of Herbal formulation of leaves of *Platyclatus orientalis*, *Momordica charantia* and *Punica granatum* against *Eisenia fetida.* For this study used various concentrations (25-100 mg/ml) herbal formulation were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Albendazole was used as standard anthelmintic drug and distilled water was used as control. The results of present study indicated that the herbal formulation significantly exhibited paralysis (P<0.01) in worms in higher dose 100 mg/ml and also caused death of worms especially at higher concentration of 100 mg/ml, as compared to standard drug.

Key Words: Albendazole, Anthelmintic activity, Herbal formulation, *Eudrilids eugenia.*

1. INTRODUCTION:

Helminthic infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminths become resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminths diseases. Hence there is an increasing demand towards natural anthelmintics[1-3].

Platycladus orientalis Linn. (Synonymous: *Thuja orientalis*) is belong to family Cupressaceae, it is a coniferous shrub with evergreen and scale-like leaves; It is used in traditional medicine in the treatment of bronchitis, cystitis, enuresis, psoriasis, amenorrhea, uterine carcinomas and rheumatism[4]. The essential oils obtained from *Platycladus orientalis* contains β-thujone and β-thujone which showed toxic effects on ruminant animals[5]. However, the ethanol extract of *Platycladus orientalis* is nontoxic at low concentration (≤ 0.6 mg/ml) and it suppressed the growth of human lung cancer cell line [6].

Momordica charantia locally called as 'Karela' is famous for its fruit and eaten as vegetable. The fruits are considered as tonic, stomachic, carminative agents and have been used for diabetes. The fruits are also used in rheumatism, gout and diseases of liver and spleen and are febrifuge. The seeds and leaves are used as anthelmintic. The leaves are also reported to be useful in piles, leprosy and jaundice[7] .The leaves, fruits and roots have been used as laxative and antipyretic agents[8] . It has been used to treat anaemia, cholera, bronchitis and ulcers. The fruit juice is used for the treatment of colic, wounds, sores and worms. The seeds have been reported to have antiviral, antiulcer and anti-leukemia properties[9] . MAP 30, a protein from *Momordica charantia* has been reported to have anti-HIV and antitumor properties [10] . Keeping in view the medicinal importance of *L. cylindrica* and *M. charantia*, the current study was carried out to find the antibacterial, antifungal and phytotoxic activities of these two plants.

Punica granatum L. known as 'Annar' in Urdu and 'Pomegranate' in English is the famous

edible fruit. In traditional medicine it has been used for the treatment of various diseases in America, Europe, Africa and Asia. In addition to past uses, P. granatum is used in several medicines for a variety of ailments [11]. Different parts of P. granatum contain a variety of chemicals. Tannin and alkaloid are present in both bark and roots. Antimicrobial activity of tannins, flavonoids and polyphenols is well studied [12-14]. Tannin-containing beverages consumption such as tea could be helpful in curing or prevention of several illnesses (Cowan, 1999). Different parts of P. granatum such as roots, peels and fruits have been used generally in herbal therapies by local therapists in many states. Peels of P. granatum have been used traditionally for treatment of dysentery and diarrhea [15-18]. The crude extracts of P. granatum peels were successfully used against Agrobacterium tumefaciens, causative agent of plant tumor [18]. One of the known pharmacological property of tannins is astringency (Cowan, 1999). The seed consist of steroids while, fruit pulp contains vitamins, minerals and macromolecules like fats, proteins and carbohydrates [19].

Punica granatum L. is a shrub belonging to the unigeneric family Punicaceae, a native of semitropical Asia. The different parts commonly used are leaf, flower, fruit,fruit rind, seed, dried bark of stem, and root[20]. The root bark shows activity against tapeworms. Astringent properties of the fruit rind and fruit juice explain the antidiarrheal activity. The bark and seeds are useful in bronchitis. The flowers are used in epistaxis. The unripe fruit is a good appetizer, and it is useful in nausea and vomiting. The ripe fruit is tonic, astringent to the bowels, and relieves burning sensation of the body. The rind of the fruit is very useful in diarrhea and dysentery.The fresh juice is used in cooling and refrigerant mixtures of some medicines for dyspepsia. The root bark has been used as an anthelmintic.

2. MATERIALS AND METHODS:

2.1: Plant Collection and authentication:

The plant leaves of *Platyclatus orientalis*, *Momordica charantia* and *Punica granatum* were collected from Latur, Dist-Latur (Maharashtra); and authenticated by Dr.Wadkar G.S, Dept. of Pharmacognosy, Kasegaon, (Maharashtra).

2.2: Preparation of Herbal Formulation:

The fresh leaves of *Platyclatus orientalis*, *Momordica charantia* and *Punica granatum* collected from local area and obtained fresh juice with the help of an electric grinder. After that filter with muslin cloth and collected the filtrate of fresh juice.

2.3: Worms Collection and authentication:

The African species of earthworms *Eudrilus eugeniae* were collected from the water-logged areas of soil worms were obtained from freshly slaughtered fowls.

2.4: Preparation of test sample:

The samples for in-vitro study were prepared by dissolving and suspending 2.5 g of each herbal formulation in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 125, 250 and 500 mg/ml.

2.5: Anthelmintic assay:

The anthelmintic assay was carried out as per the method of Ajayieoba et al with minor modifications[21]. The assay was performed on adult African species of earthworm *Eudrilus eugeniae*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[22]. *Eudrilus eugeniae* worms are easily available and used as a suitable model for screening of anthelmintic drug [23-25]. The 50 ml formulations containing four different concentrations of each herbal formulation (125,250 and 500 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 ^{o}c [26,27]. Albendazole (50,75 and 100 mg/ml) was used as reference standard while distilled water as the control.

3. RESULTS AND DISCUSSION:

As shown in Table.1 & Graph.1 & 2 showed aqueous and ethanolic extract of whole plant of *Ficus glomerata* exhibited anthelmintic activity using *Eudrilus eugeniae* worms in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 500 mg/ml concentration. The herbal formulation caused paralysis at 9.23 ± 0.1050 min. and time of death of 16.07 ± 0.2823 min. respectively against the earthworm *Eudrilus eugeniae*. The standard drug Albendazole showed the same paralysis at 5.23± 0.1020 and time of death of 9.03 ± 0.2751 minutes.

Herbal Formulation

Albendazole by increasing chloride ion conductance of worm muscle membrane produced hyperpolarization and reduced excitability that led to muscle relaxation and flaccid paralysis[28]. The herbal formulation not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 500 mg/ml, in shorter time as

compared to standard drug Albendazole. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents. Due the presence of tannins the anthelmintic activity has been shown singnificantaly. It is possible that tannins contained in the herbal formulations produced similar effects. The anthelmintic effect shown because of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death[29,30].

Tables:

Drug	Concentration in mg/ml	Paralysis Time (min)	Death Time (min)
Albendazole (Standard Drug)	50	8.35± 0.1056	13.20± 0.1275
	75	7.25± 0.1140	12.26± 0.1033
	100	5.23± 0.1020	9.03 ± 0.2751
Herbal Formulation	125	17.27± 0.1506	51.38 ± 0.3228
	250	13.25± 0.0875*	36.29 ± 0.1105*
	500	9.23 ± 0.1050**	16.07 ± 0.2823**

Table 1: Anthelmintic activity of Herbal Formulation.

Figures:

Fig.1: Anthelmintic assay of Herbal formulation

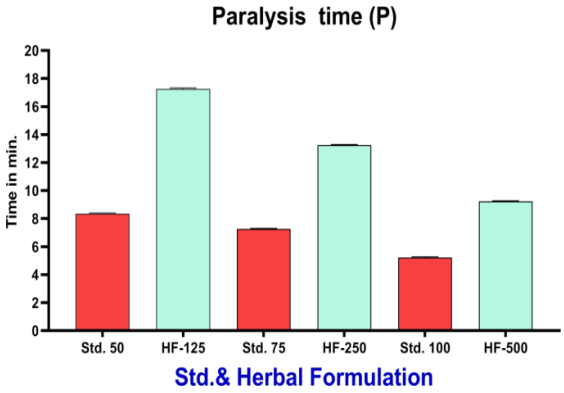
Fig.2: Anthelmintic assay of Std.Drug

Values are expressed as MEAN±SEM

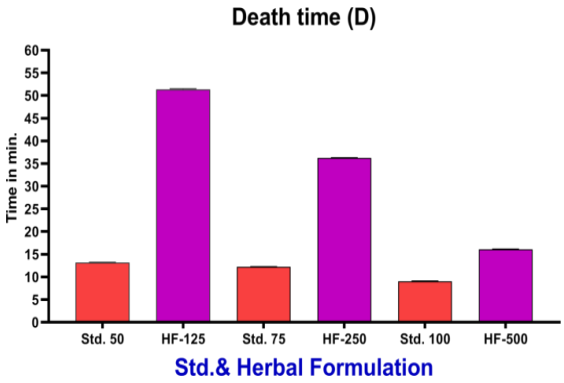
One way ANOVA followed by Dunnett's 't' test.

Note: n=6 in each group. *P<0.05, **P<0.01, ***P<0.001

Graphs:



Graph.1. Paralysis time of Std. & Herbal Formulation



Graph.2. Death time of Std. & Herbal Formulation

4. CONCLUSION:

The results of this study indicate that the herbal formulation of *Platyclatus orientalis*, *Momordica charantia* and *Punica granatum* exhibited a significant anthelmintic activity. The herbal formulation was the same as albendazole in terms of good paralysis time and death time at concentrations of 500 mg/mL. The herbal formulation showed good activity.

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