



Comparative *In Vitro* Anthelmintic Activity of a Medicinal Plant *Amaranthus cruentus*

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Abstract : *Amaranthus cruentus* is a medicinal plant commonly found as a leafy vegetable. It belongs to family Amaranthaceae and distributed all over the world. *A. cruentus* has high nutritional value. Medicinally it is very important. It is used for young children and lactating mothers, for treating constipation, anemia, kidney complaints, roots are boiled with honey as a laxative for infants, its water extract is used to treat pains in the limbs, as a tape worm expellant, wound dressing and tumours; has antioxidant properties. Amaranth seeds, seed oil and leaves are used for health benefits such as to reduce blood pressure, cholesterol and weight, increase immunity, treat anemia, gastro intestinal tract disorders, antioxidant properties and anti inflammatory properties. Lunasin, a peptide in amaranth seeds is considered to exert anti cancer properties. The consumption of *A. cruentus* products is advised for patients with celiac disease, therefore for diabetic persons. Taking this into consideration, anthelmintic study of different extracts is carried out. The data was verified as statistically significant by using two ways ANOVA at 1% level of significance (P-value < 0.01). The anthelmintic activity of all the extracts may be due to the presence of polyphenolic compounds. The experimental evidence obtained in the laboratory model could provide data for being used this plant as an anthelmintic. The significant wormicidal activity of the aqueous extract against earthworms suggests that it could be effective against parasitic infections of humans than synthetic drugs.

Key Words: -*Amaranthus cruentus*, Amaranthaceae, Anthelmintic Activity, *Eiciniafeotida*, Albendazole.

Introduction: -

The current choices of drugs like Mebendazole, Albendazole, Piperazine etc. for worm infections are common in Indian sub- continent¹. The gastro – intestinal helminthes becomes resistant to currently available anthelmintic drugs²⁻⁴. Synthetically prepared drugs have been reported to have several side effects. Some anthelmintic drugs are contraindicated for certain groups of patients like pregnant and lactating women. These drugs have also to be used with caution in hepatitis patients and in children below two years of age⁵.

Development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintic. Hence there is an increasing demand towards natural anthelmintics²⁻⁴. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effects⁶. The use of natural products with therapeutic properties for a long time,

mineral, plant and animal products were the main sources of drugs serve as an important therapeutic agents as well as important raw material for the manufacture of traditional and modern medicines⁷. In spite of modern development of sophisticated pharmaceutical chemicals to treat illnesses, medicinal plants remain an important tool for treating illness⁸.

Amaranthus cruentus is used mainly as a leaf vegetable. The powdered dry leaves are used in sauces during the dry season⁹. In some countries they are grown as ornamentals, for fodder, and for making dye. In Benin Republic, the dried plant is burnt for the preparation of potash. Medicinally it is very important. It is used for young children and lactating mothers, for treating constipation, anemia, kidney complaints, roots are boiled with honey as a laxative for infants, its water extract is used to treat pains in the limbs, as a tape worm expellant, wound dressing and tumors; has antioxidant properties⁹⁻¹².

Materials and Methods: -

Plant material:–

Fresh plant material was collected from Pune, Maharashtra, India. The plant material was taxonomically identified and authenticated by the Botanical survey of India, Pune. Its authentication number is BSI/ WRC/ Cert/2015/AV02.

Extraction Method: -

Air shade dried and powdered aerial part (10 g) was extracted with solvents (50 ml) of different polarity by keeping it for 24 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude extract. Four extracts C1 (Chloroform extract), C2 (Ethyl Acetate), C3 (Ethanol Extract) and C4 (aqueous extract) were prepared by this method.

Air shade dried and powdered aerial part (10 g) was refluxed with acetone(50ml) for eighteen hours. Solvent was recovered under reduced pressure to obtain crude extract. This acetone extract H1 along with C1, C2, C3and C4 was used for the assessment of anthelmintic activity.

Animals: -

The assay was performed on adult Indian Earthworm *Eicinia feotida* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being^{13,14}. Easy availability of earthworms prompts their extensive use for preliminary *invitro* evaluation of anthelmintic compounds¹⁵. Earthworms were provided and identified by Dept. of Entomology, College of Agriculture, Pune, Maharashtra, India. The earth worms of ten cm in length were used for the experimental protocol. These earth worms were washed with normal saline to remove all faecal matters.

Drug: -

Albendazole was used as standard for the assay. It was purchased from G.S.K. Pharma Pvt Ltd, Mumbai along with saline NA 0.91 %NaCl. All the chemical and solvents used were of analytical grade for the experimental protocol.

Anthelmintic Activity: -

The anthelmintic assay was carried out as per the method of Nargund¹⁶ with minor modifications. The assay was performed on adult Indian Earthworm *Eicinia feotida* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being^{18,19}. These earth worms were washed with normal saline to remove all faecal matters. The earth worms of ten cm in length were used for the experimental protocol. All the test solution and standard solution were prepared freshly before starting experiments. Different dilutions of Albendazole with normal saline were used as standard, same dilutions of extracts in normal saline solution were used for the assay where normal saline served as control. The earthworms were divided into different groups, each group containing six worms. Two different concentrations of each extract (0.1mg / ml and 0.25 mg / ml) were prepared. External stimuli were applied to ascertain the paralysis time. The time taken

by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm followed by fading away of their body color.

Results and Discussion: -

The experiment was performed with various concentrations of *A.cruentus* (0.1 and 0.25 mg/ml). Each extract was compared with standard albendazole under the same set of conditions and concentrations. The extracts causes paralysis followed by death of the worms at all tested levels. The potency of the test samples were found to be inversely proportional to the time taken for paralytic effect and death of worms. The activity confirms the dose dependent nature of the test samples. The worms selected for the anthelmintic activity shows activity for all five extracts but they were most sensitive to the aqueous extract. Aqueous extract shows significant activity than that of standard albendazole at 0.25 mg/ml concentration. Chloroform extract also shows comparable activity with that standard at 0.25 mg/ml concentration. Results are expressed as mean ± SEM of 6 observations (Fig 1).

Statistical Analysis: -

The data were verified as statistically significant by using two ways ANOVA (Table 1) at 1% level of significance (P-value < 0.01)

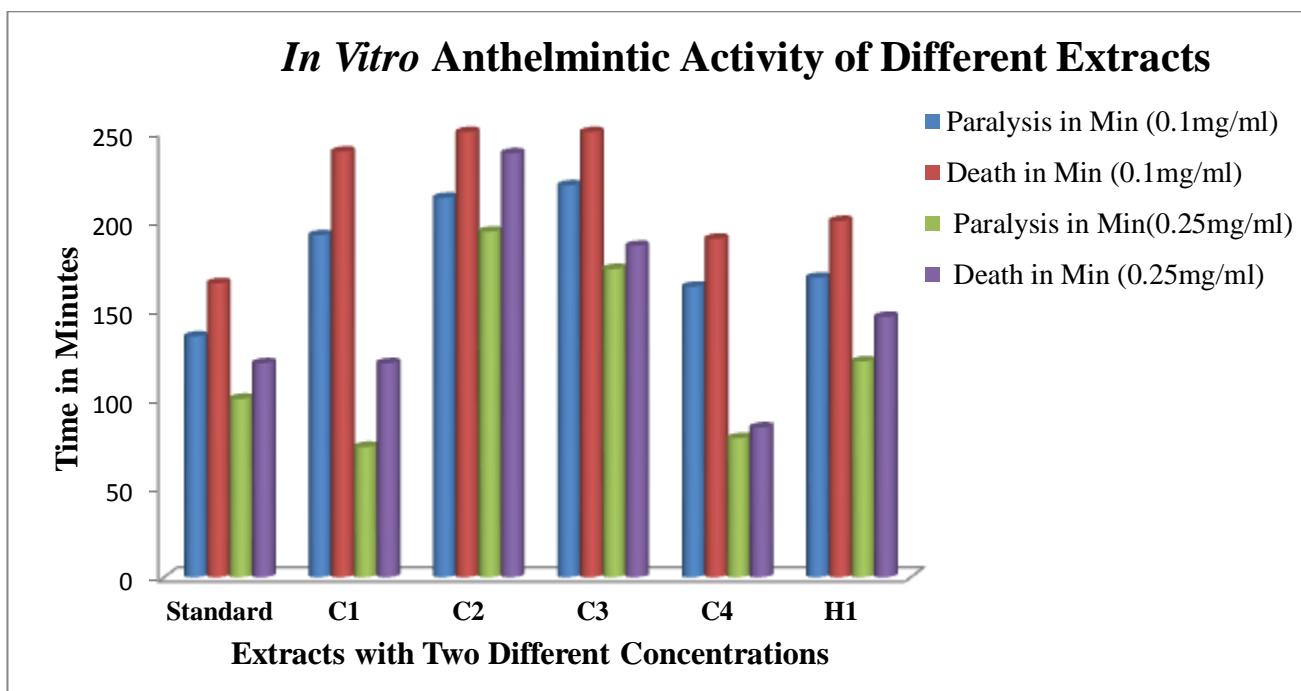


Fig 1: In Vitro Anthelmintic Activity of *Amaranthus cruentus* Extracts

Table1: Statistical Variance of Analysis (ANOVA) on Anthelmintic Activity of *Amaranthus cruentus* Extracts.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	12561.13	1	12561.13	59.12277	0.004571	34.11622
Within Groups	11347.38	3	3782.458	17.80329	0.020485	29.4567
Total	23908.51	4				

Conclusion: -

There is considerable evidence that all extracts of *Amaranthus cruentus* have the potential to be developed into agents that can be used as preventive or treatment therapies for helminthes. The aqueous extractisfound to be more potent than the standard albendazole. In future it can be employed as anthelmintic drug. This study is certainly useful to do further biological study.

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Conflict of Interest Statement: -

The authors report no conflict of interest.

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