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Research Article

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IN VITRO ANTHELMINTIC ACTIVITY OF *ZYZIPHUS OENOPLIA* (L.) MILL ROOT EXTRACTS – A PROMISING ETHNOMEDICINAL PLANT.

PULAK MAJUMDER* Dept. of Pharmacognosy, National College of Pharmacy, Balraj-Urs road,Shimoga-577201, Karnataka, India.

*Corresponding Author: pulak2007@gmail.com

ABSTRACT

The anthelmintic activity of the various extracts (Petroleum ether, chloroform, Ethanol, and Aqueous) of Zizyphus oenoplia (L.) Mill roots were evaluated on Indian adult earth worm Pheretima posthuma. All the extracts caused paralysis followed by death of the worms at all tested doses. Chloroform extract exhibited more potent anthelmintic activity compared to other extracts and also found to be potent as reference standard albendazole.

Key words: Zizyphus oenoplia, root extracts, Anthelmintic activity.

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INTRODUCTION

The World Heath Organization estimates that a staggering two billion people harbor parasitic worm infestation. In the developing world, the greatest impact of parasitic disease is indirect and potential productivity losses. According to World Health Organization, only few drugs are frequently used in the treatment of helminthes in human being. In this regards, the anthelmintics from natural sources may play a key role in the treatment of these parasitic infestation[1]. In view of this, an attempt has been made to study the anthelmintic activity of roots of Zyziphus oenoplia (L.) Mill.

In this study, ethanol, aqueous, chloroform, and petroleum ether extracts were used and studied for paralysis and death of earth worm.

The roots of *Zyziphus oenoplia,* belongs to the family "Rhamnaceae". It is commonly known as Jackal jujube. According to Ayurveda, the plant root is very useful in the treatment of fresh cuts and wounds, ulcer, ascaris infection, stomachalgia etc[2-3].

One of tribe called 'Chakmas' in Bangladesh were use the plant traditionally for gastrointestinal disorder[4]. Among the 'Munda' tribe also the fruit is used as an ingredient in the preparation of stomach ache pill[5]. The plant *Zyziphus oenoplia* is an erect, straggling or climbing shrub up to 3 m tall.

Zyziphus oenoplia is an important source of chemicals namely cyclopeptide alkaloids, zizyphine (A-G), abyssinine A&B and also butulnic acid. The plant root posses as an astringent, bitter, anthelmintic, digestive and antiseptic property[2-3].

The present study is intended to evaluate the anthelmintic activity of the root of *Zyziphus oenoplia* in a scientific manner.

EXPERIMENTAL

MATERIALS AND METHODS

Zyziphus oenoplia roots were collected in and around Shimoga district of Karnataka, India, in the month of June 2009. The plant material was authenticated by Dr. Krishna Swamy (Principal investigator of UGC major project), Dept. of Botany, Sahydri Science College, Shimoga.

The roots were washed under running tap water and cut into small pieces of 2-3cm, shade dried (30^0c, 45% relative humidity) for 15days and than grind the material to get the coarse powder. The powder was stored in air tight container for further extraction.

PREPARATION OF EXTRACTS

Aqueous extract (By cold maceration method).- 200gm coarse powder of root *Zyziphus oenoplia* was mixed with 700ml of distilled water and kept for 5 days with occasional shaking. The powder-water mixture was filtered by vacuum filtration. The extract was dried on water bath and the percentage yield was found to be 6.75%.

SOLVENT EXTRACTION-Various extracts like petroleum ether, chloroform and ethanol, were prepared from the dried powder of *Zyziphus oenoplia* roots by using successive extraction method. In this process 45gm of dried powder was extracted with 200ml of petroleum ether, chloroform and ethanol. Total 50 cycles were run to obtain the liquid extract, than evaporated on water bath and got the thick paste or powder. The percentage yield of petroleum ether, chloroform and ethanol was found to be 2.79%, 3.15% and 4.66% respectively

ANIMALS

Indian adult earth worms (*Pheretima posthuma)* were used to study anthelmintic activity. The earth worm were collected from moist soil and washed with normal saline to remove all fecal matter. Adult worms of 3-5cm in length and 0.1- 0.2 cm in width were used for experimental protocol. The worth worms resembles both anatomically and physiologically to the intestinal round worm parasites of human beings[6].

DRUGS AND CHEMICALS

Albendazole, Ethanol AR, Chloroform GR, Petroleum ether AR (60-80^{o}c), Gum acacia, Sodium chloride.

ANTHELMINTIC ACTIVITY

The activity was tested according to the method described by Kailashraj and Kurup (1962)[7].The animals were divided into six groups. Each group containing three animals all these extracts were dissolved in gum acacia and volume was adjusted with normal saline water

Each group was treated with one of the following; vehicle (1% gum acacia in normal saline, albendazole 20 mg/ml, extract (10, 20, 30 and 40 mg/ml) in normal saline with 1 % gum acacia.

Observations were made for the time to paralyze and death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility, followed with fading away of their body color.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed the presence of alkaloids, flavanoids, steroids, triterpenes, saponins, and tannins like phytoconstituents in the extract of *Zyziphus oenoplia* .Some of these phytoconstituents may be responsible to show a potent anthelmintic activity

From the observation made all the extract of root *Zyziphus oenoplia* was found to show the anthelmintic activity when compared to the standard drug. Aqueous extract showed anthelmintic activity at concentration of 10mg/ml, where as ethanol, chloroform and petroleum extract also showed paralysis and motility at similar concentration. Other test concentrations also showed anthelmintic effect.

The anthelmintic effect of chloroform extract at 20 mg/ml concentration is comparable with that of effect produced by the reference standard albendazole. The petroleum ether, ethanol and aqueous extract show effect that beyond 40 mg/ml concentration is comparable with reference standard.

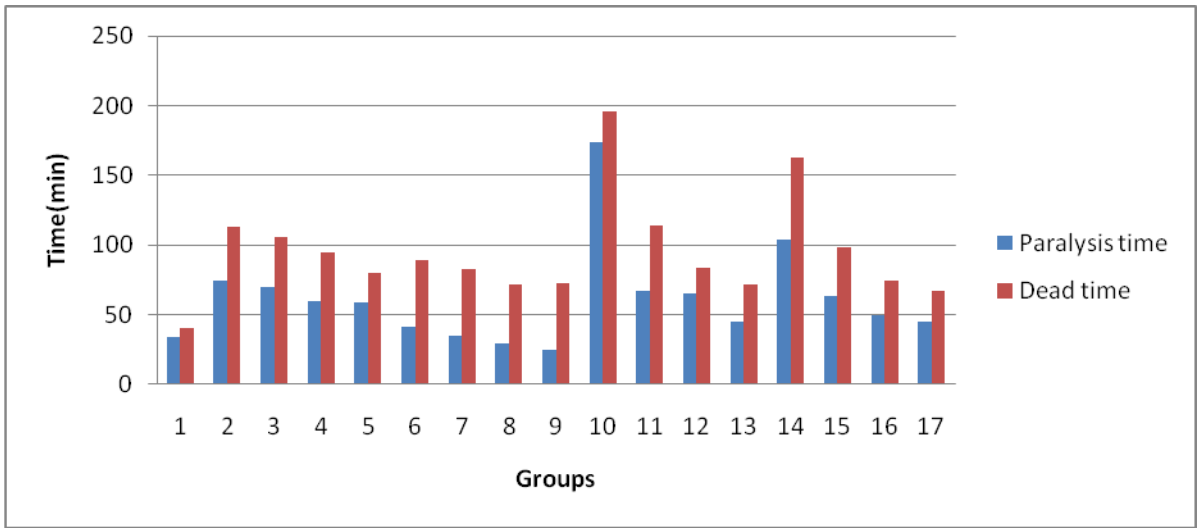
The present study, revels that the chloroform extract was more potent then ethanol, aqueous and petroleum ether extract, even through all these extracts were endowed with anthelmintic property. The activity reveals concentration dependent nature of different extracts. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity.

Sl no.	Treatment/ Dose (mg/ml)	Time taken for paralysis.(min) (X ± S.D.)	Time taken for death.(min) (X ± S.D.)
00	Vehicle (1% gum Acacia)	—	—
01	Albendazole (20 mg/ml)	34±1.15	40±2.08
	Petroleum ether extract;		
02	10 mg/ ml	74.00±2.89	113.33±2.33
03	20 mg/ ml	70.67±1.76	106.67±2.85
04	30 mg/ ml	60.00±3.51	95.00±3.21
05	40 mg/ ml	59.33±2.03	80.67±2.91
	Chloroform extract;		
06	10 mg/ml	41.00±2.65	89.00±3.06
07	20 mg/ ml	35.67±2.33	83.00±1.15
08	30 mg/ ml	29.33±2.03	72.00±3.06
09	40 mg/ ml	25.00±2.65	73.33±1.86
	Ethanol extract;		
10	10 mg/ ml	174.00±2.31	196.33±0.88
11	20 mg/ ml	67.00±2.08	114.00±2.00
12	30 mg/ ml	65.67±2.40	84.33±2.85
13	40 mg/ ml	45.00±2.52	72.67±1.45
	Aqueous Extract;		
14	10 mg/ ml	104.00±2.8	163.00±2.52
15	20 mg/ ml	63.67±2.03	98.00±1.73
16	30 mg/ ml	50.00±2.65	74.33±2.40
17	40 mg/ ml	45.00±1.53	67.00±2.65

Table1. Anthelmintic activity of various extracts of *Zyziphus oenoplia* (L.) Mill.

Each values is represented as mean ± standard deviation (n = 3). Standard error mean < 0.294.Data are found to be significant by testing through one way ANOVA at 5 % level of significance ($p < 0.05$).

Fig. 1 : Anthelmintic activities of various extracts of roots of plant *Zyziphus oenoplia* (L.)Mill. on Indian earthworm *Pheretima postuma*.



Each bar is represented as mean ± standard deviation (n = 3).

Group 1 - Standard (Albendazole). Group 2 to 5 – Petroleum ether extract (dose 10, 20, 30 and 40 mg/ml.). Group 6 to 9 – Chloroform extract (dose 10, 20, 30 and 40 mg/ml.). Group 10 to 13 – Ethanolic extract (dose 10, 20, 30 and 40 mg/ml.). Group 14 to 17 – Aqueous extract (dose 10, 20, 30 and 40 mg/ml) respectively.

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