Current Pharma Research
ISSN: 2230-7842
CPR 2(3), 2012, 543-545.

Preliminary Phytochemical and Anthelmintic activity of *Zizyphus oenoplia (L) Mill.*

[*1]Suryakant A. Jadhav, [2]Deepali S. Chavan, [3]Prakash D. Jadhav.
[*1]indira Institute of Pharmacy, Devrukh, Ratnagiri, Maharashtra, India, [2]Rajarambapu College of Pharmacy, Kasegaon, Maharashtra, India, [3]Arvind Gavali College of Pharmacy, Jaitapur, Satara, Maharashtra, India.

Abstract
The aim of present study is to investigate the preliminary phytochemical constituent and anthelmintic activity of *Zizyphus oenoplia* against Pheretima postuma. Three concentrations (20, 50 & 100 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis & time of death of the worms. The total alcoholic extract & its ethanolic & aqueous fraction exhibited anthelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate in same concentration as that of extract was included as standard reference & normal saline water as control.

Key Words
Zizyphus oenoplia, anthelmintic activity, Pheretima postuma, Piperazine citrate.

Introduction
Helminthiasis or worm infestations, is one of the most prevalent disease & one of the most serious public health problem in the world. Hundreds of millions if not billions of human infection by helminthes exist worldwide & increased world travel & immigration from the developing countries.[1] The root, stem, bark of the plant *Zizyphus oenoplia* is an important source of chemicals namely cyclopeptide alkaloids zizyphine (A-G) and abyssinine A and B. Root, bark contains zizyphine A, zizyphineB and betulnic acid. The roots of the plant Z*izyphus oenoplia* possess astringent, bitter, anthelmintic, digestive and antiseptic. They are useful in hyperacidity, ascaris infection, stomachalgia and healing of wounds[2, 3].

Material and methods
Authentication, Collection and drying of plant material
Fresh roots of *Zizyphus oenoplia* was identified and authenticated by Dr. R. Manjunatha, Professor, and department of Botany, D.V.S College of Arts and Science, Shimoga. The collected roots were washed with water, dried in shade and powdered using hand grinder to make a coarse powder, sieved and packed in air tight container and stored in cool and dry place until further use.

***Corresponding Author:**
suryakant_1883@yahoo.com

Extraction of plant material
Alcoholic solvent extraction
The extraction of plant material with alcohol solvent was carried out in soxhlet extractor. The extraction vessel was made up of borosil glass which includes round bottomed flask, extractor and condenser. The plant material to be extracted was packed in the extractor and heated under reflux. 95% Alcohol was used as a solvent. Heat was supplied through a heating mantle. The extract was collected directly from round bottomed flask and solvent was evaporated using Rota flash evaporator.

Preparation of plant extract for biological screening[4, 5]
To detect active substance present in very small quantities in the extract a 'primary' fractionation of the total extract was carried out prior to pharmacological screening to separate polar from less-polar constituents by sequential use of solvent from high to low polarity. This provided better discrimination between fractions that exhibit a specific activity. This 'primary' fractionation scheme may also contain dereplication step to avoid re-isolation of known compound. Alcohol is the moderately polar solvent utilized to extract various type of functional group present in the crud drug. For extraction of more lipophilic compound chloroform is used. In this process non-polar solvent like Petroleum ether is used to remove chlorophyll.

Preliminary Phytochemical Screening[6, 7]

Preliminary phytochemical screening of extracts for the presence of secondary metabolites were performed using the following reagents & chemicals for alkaloids with Mayer's, Wagner's & dragendroff's reagents, for flavonoids with shinoda test, for tannins 7 phenolics with potassium permagnate, lead acetate & 5% ferric chloride.

Animals

Indian adult earth worm Pheretima postuma collected from moist soil & washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworm of 3-5 cm in length & 0.1-0.2 cm in width were used for all experimental protocol due to its anatomical & physiological resemblance with intestinal round worm parasite of human beings.[8]

Anthelmintic activity

Total alcoholic extract & its fractions from the root of Z*izyphus oenoplia* were investigated for their anthelmintic activity against Pheretima postuma; various concentration (20, 50,100 mg/ml) of total alcoholic extract & its ethanol & aqueous fraction were tested in bioassay, which involved determination of time of paralysis & time of death of worms. Piperazine citrate was included as standard reference & normal saline water as control. The anthelmintic assay was carried as per the method of [9]with minor modification. The assay was performed on adult Indian earthworms Pheretima postuma due to its resemblance with the intestinal round worm parasite of human being.[10,11] because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compound in vitro.[12,13] In the first set of experiment, six groups of six earthworms were released in to 50ml of solution of piperazine citrate, total alcoholic extract, ethanol fraction & aqueous fraction of root of *Zizyphus oenoplia* (25,50,100 mg/ml each) in distilled water. All drugs & extract solutions were freshly prepared before starting the experiment. Piperazine citrate was used as reference standard while saline water as control. Observations were made for the time taken to paralysis & death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms lost their motility followed with fading away of their body colours.

Results and Discussion

Preliminary phytochemical screening reveals that the presence of flavonoids, carbohydrates, tannins, steroids, triterpenes and phenols in *Zizyphus oenoplia*. According to phytochemical investigation alkaloids, carbohydrates, flavonoids and tannins are present in total alcoholic extract, where as flavonoids, tannins and phenolics are present in ethanol fraction. The chloroform fraction shows presence of alkaloids, while in aqueous fraction flavonoids are present. (Table 1). The anthelmintic activity reveals that total alcoholic extract & its ethanol & aqueous fractions showed significant activity at all the concentrations. The total alcoholic extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of alcoholic & aqueous fraction when compared with standard. (Table 2). As flavonoids, tannins & phenolics have been identified in the total alcoholic extract, ethanol fraction & aqueous fraction, we believe that the anthelmintic activity of this extract is probably due to presence of flavonoids, tannins, phenolics in the extract.

Conclusion

It could be concluded & confirmed that the total alcoholic extract, ethanol fraction & aqueous fraction of root of *Zizyphus oenoplia* showed significant anthelmintic activity comparable with standard drug, which is effective against parasitic infection of humans. Further in future it is necessary to identify & isolate the possible active phytoconstituents responsible for the anthelmintic activity & study its pharmacological actions.

References

1. William DA & Lemke TL, Parasitic infection-Helminthes In: Foyes principal of medicinal chemistry. 5th edition, New york; Lippincot William & Wilkins 2002.
2. http://www.expresspharmaonline.com/20060331/research02.shtml27/11/2008.
3. Prajapati ND, Purohit SS. Agro's colour atlas of medicinal plants published by Agrobios (India) Jodhpur. 2004.
4. Paul cos, Arnold J, Vlietinck. Antiinfective potential of natural products.Journal of ethnopharmacology 2006; April 18, (4):290-302.

5. Prusti A, Mishra SR. Antibacterial activity of some Indian medicinal plants.Ethnobotanical leaflets 2008; 12:227-230.
6. Wallia TE, Text book of Pharmacognosy. 5th ed new Delhi: CBS Publishers and Distributors 1985.
7. Khandelwal KR, Kokate CK, Pawar AP, Gokhle SB, Practical Pharmacognosy. 1st ed., Nirali prakashan, 1995.
8. Vigar Z. Atlas of medical parasitology, 2nd edition, Singapore; P.G. Publication house 1984; P.216-217.
9. Ajaiyeoba EO, Onocha PA & Olarenwaju OT. In virtro anthelmintic properties of *Buchholzia coriaceae* & *Gynandropsis gynandra* extract. Pharm Bio 2001; 39; 217-220.
10. Sollmann T. Anthelmintics: their efficiency as tested on earthworms. J Pharmacol Exp. Therap 1984; 12:129-170.
11. Thorn GW, Adams RD, Braunwald E, Isselbacher KJ & Peterdrof RG. New York: Harrisons principles of internal medicine. McGraw Hill Co. 1977.
12. Jain ML & Jain SR. Therapeutic utility of Ocimum basilicum var. album. Planta Med.1972; 22; 66-70.
13. Suresh PGK, Kar DM, Ganpaty S& Panda SB. Evaluation of Evolvulus alsinoids Linn. For anthelmintic & antimicrobial activities. J Nat Remedies.2002; 2:182-185.

Table 1: Preliminary phytochemical constituents present in total alcoholic extract and fractions of *Zizyphus oenoplia*.

Sl. No.	Phyto constituents	Chemical tests	Alcoholic Extract	Pet. ether fraction	Eth. fraction	Chloroform fraction	Aqueous fraction
1	Alkaloids	Dragendorff's test	+	-	-	+	-
		Mayer's test	+	-	-	+	-
		Hager's test	+	-	-	+	-
		Wagner's test	+	-	-	+	-
2	Carbohydrate	Molish test	-	-	-	-	-
		Fehling's test	+	-	-	-	-
		Benedict's test	+	-	-	-	-
		Barfoed's test	-	-	-	-	-
3	Proteins	Biuret test	+	-	-	-	-
		Millons test	-	-	-	-	-
		Precipitation test	+	-	-	-	-
4	Amino tests	Ninhydrin test	+	-	-	-	-
		Xanthoproteic test	-	-	-	-	-
5	Steroids	Salkowaski test	+	+	-	-	-
6	Flavonoid	Shinoda test	+	-	+	-	+
7	Glycoside	Borntragers test	-	-	-	-	-
		Legals	-	-	-	-	-
8	Tannins and Phenolics	Zinc HCL test	+	-	+	-	+
		With 5% Ferric chloride	+	-	+	-	+
		With $KMnO_4$	+	-	+	-	-
		With lead acetate	+	-	+	-	+

+ indicates positive, - indicates negative result

Table 2: Showing time required for paralysis & death of earthworm at various concentrations.

Sr. No.	Treatment	Conc. (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Control (normal saline)	-	-	-
2	Piperazine citrate (Standard)	25	27±0.4	33±0.8
		50	20±0.9	28±0.4
		100	13±0.5	20±0.4
3	Total alcoholic extract	25	50±0.2	64±0.5
		50	36±0.6	49±0.3
		100	30±0.6	40±0.4
4	Ethanol fraction	25	65±0.8	96±0.1
		50	45±0.1	59±0.6
		100	32±0.2	45±0.4
5	Aqueous fraction	25	70±0.2	82±0.2
		50	50±0.1	66±0.7
		100	35±0.7	47±0.4

All values represent mean±SD; n=6 in each group, comparisons made between standard versus treated group.