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# ANTHELMINTIC PROPERTY OF *ZIZYPHUS OENOPLIA*

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## ABSTRACT

Only fewer medicinal plant shows anthelmintic property in which *Zizyphus oenoplia* is one of them. The worm disease cause by pheretima pootums is common in eastern U.P. (India). The aim of present study was to investigate the preliminary phytochemical constituent and anthelmintic activity of *Zizyphus oenoplia* against earthworm (Pheretima postuma). The concentrations (20, 50 & 100 mg/mL) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worms. The total alcoholic extract and its ethanolic and 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 and normal saline water as control. So it could be confirmed that the total alcoholic extract, ethanol fraction and aqueous fraction of root of *Zizyphus oenoplia* showed significant anthelmintic properties comparable with standard durg, which is effective against parasitic infection of humans.

**Key words**: *Zizyphus oenoplia*, Anthelmintic property.

## INTRODUCTION

*Zizyphus oenoplia* is widely distributed throughout the north eastern U.P. of India. The root, stem, bark of this plant is an important source of chemicals namely cyclopeptide alkaloids zizyphine (A-G) and abyssinine A and B. Root, bark contains zizyphine A, zizyphine B and betulnic acid. The roots of the plant *Zizyphus oenoplia* posses astringent, bitter, anthelmintic, digestive and antiseptic. They are useful in hyperacidity, ascaris infection, stomachalgia and healing of wounds[1,2]. This plant is being for the treatment of pain, gastrointestinal disorders and infectious diseases. It is widely used for healing of wounds and is also effective against bacteria, fungus and chronic white discharge[3,4]. There is no previous record and research work available on the traditional medicinal values of *Zizyphus oenoplia*. Most of the ancient knowledge systems continued to survive by oral communication from generation to generation in rural as well as in tribal communities. The preliminary phytochemical studies reveal the presence of flavonoid glycosides flavones-

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flavonoids ligands and fatty acids. Therefore the present study was undertaken to demonstrate scientifically the antinociceptive and anti-inflammatory activities[5-7] of the alcoholic and petroleum ether extracts of *Zizyphus oenoplia*, whole plant material in experimental animals.

## EXPERIMENTAL

### Material and methods

The whole plant *Zizyphus oenoplia* was collected from field and authenticated by Dr. S. B. Singh, Department of Taxonomy (Botany) S. G. R. P. G. College Dobhi, Jaunpur (U.P.).

The fresh roots of *Zizyphus oenoplia* was identified and collected. 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.

### Extraction and fractionation

Air-dried and powdered whole plant of *Zizyphus oenoplia* was extracted with different solvents. The extracts were concentrated and fractionated to solid residues.

After seven hour extraction with dried powdered of whole plant yield 2.1% yield, however when it again extracted with petroleum ether (Ketone free) gave 5.6% alcoholic extract. This extract was trialed with T. L. C. for compound identification resolute flavonoids terpenoid and alkaloid. 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-siolation 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.

### Phytochemical screening

Petroleum ether and alcoholic extract was charge in column chromatography shows presence of active compound. During this study, we have taken Mayer’s, Wagner’s and Dragendroff’s reagents for alkaloid identification, Shinoda test for flavoniod, $KMnO_4$, lead acetate and 5% $FeCl_3$ for seven phenolic active compounds.

### Animals

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

### Anthelmintic Activity

For antihelmintic study, total alcoholic extract and its fractions from the root of *Zizyphus oenoplia* were investigated for their anthelmintic activity against earthworm as pheretima postuma various concentration (20, 50, 100 mg/mL) of total alcoholic extract and its ethanol and aqueous fraction were tested in bioassay, which involved determination of time of paralysis and time of death of worms[8,9]. Piperazine citrate was included as standard reference and normal saline water as control. The anthelmintic assay was carried as per the method of 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. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compound *in vitro*. In the first set of experiment, six groups of six earthworms were released into 50 mL of solution of piperazine citrate, total alcoholic extract ethanol fraction and aqueous fraction of root of *Zizyphus oenoplia* (25, 50, 100 mg/mL each) in distilled water.

All drugs and 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 and 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. Earthworm pheretima postama, which exist in eastern U.P. in India are more sensitive towards alkali metals. The alkali salts of glucosides active principal are enhanced antihelminthetic activity were as heavy metals glucoside prolong sensitivity in earthworm.

## RESULTS AND DISCUSSION

It is well aware that active principles are more reactive than any aqueous or organic solvent. According to polarity maximum active principle compounds itself or with glucoside extract with alcohol. According to phytochemical investigation in plant *Zizyphus* preliminary phytochemical screening reveals that the presence of flavonoids, carbohydrates, tannins,

steroids, triterpenes and phenol also. The present paper indicates that 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 as indicated in Table 1.

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

| Phyto constituents | Chemical tests | Alcoholic extract | Pet. ether fraction | Eth. fraction | Chloroform fraction | Aqueous fraction |
|---|---|---|---|---|---|---|
| Alkaloids | Dragendorff's test | + | – | – | + | – |
| | Mayer's test | + | – | – | + | – |
| | Hager's test | + | – | – | + | – |
| | Wagner's test | + | + | + | + | – |
| Carbohydrate | Molish test | – | – | – | – | – |
| | Fehling's test | + | + | + | – | – |
| | Benedict's test | + | – | – | – | – |
| | Barfoed's test | – | – | – | – | – |
| Proteins | Biuret test | + | + | + | – | + |
| | Millons test | – | – | – | – | – |
| | Precipitation test | + | – | + | – | – |
| Amino tests | Ninhydrin test | + | – | – | – | – |
| | Xanthorproteic test | – | – | – | – | – |
| Steroids | Salkowaski test | + | + | + | + | – |
| Flavonoid | Shinoda test | + | – | – | – | + |
| Glycoside | Borntragers test | + | – | – | – | – |
| | Legals | + | + | – | – | – |
| Tannins and Phenolics | Zinc HCl test | + | – | – | – | + |
| | With 5% ferric chloride | + | – | – | – | + |
| | With $KMnO_4$ | + | + | – | – | – |
| | With lead acetate | + | – | – | – | + |

+ indicates positive, - indicates negative result

The anthelmintic activity reveals that total alcoholic extract and its ethanol and aqueous fractions showed significant activity at all the concentrations. The total alcoholic extract showed more significant effect on paralyzing the earthworms, in terms of paralysis time, at every concentration compared to that of alcoholic and aqueous fraction when compared with standard in (Table 2). As flavonoids, tannins and phenolics have been identified in the total alcoholic extract, ethanol fraction and aqueous fraction, so we believe that the anthelmintic activity of this extract is probably due to presence of flavonoids, tannins, phenolics in the extract.

**Table 2: Time required for paralysis and death of earthworm (*Pheretim postiman*) at various concentrations**

| S. No. | Treatment | Conc. (mg/mL) | Time taken for paralysis (min) | Time taken for death (min) |
|---|---|---|---|---|
| 1 | Control (normal saline) | - | - | - |
| 2 | Piperazine citrate (Standard) | 25 | 28 ± 0.5 | 34 ± 0.9 |
| | | 50 | 21 ± 0.9 | 29 ± 0.5 |
| | | 100 | 14 ± 0.6 | 21 ± 0.5 |
| 3 | Total alcoholic extract | 25 | 51 ± 0.3 | 65 ± 0.6 |
| | | 50 | 37 ± 0.7 | 50 ± 0.4 |
| | | 100 | 31 ± 0.7 | 40 ± 0.5 |
| 4 | Ethanol fraction | 25 | 66 ± 0.9 | 97 ± 0.2 |
| | | 50 | 46 ± 0.8 | 60 ± 0.7 |
| | | 100 | 33 ± 0.3 | 46 ± 0.5 |
| 5 | Aqueous fraction | 25 | 71 ± 0.3 | 83 ± 0.3 |
| | | 50 | 51 ± 0.2 | 67 ± 0.8 |
| | | 100 | 36 ± 0.8 | 48 ± 0.5 |

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

Therefore the present plant active compounds alongwith there glucosides shows quick anthelmintic properties. It also prove with the present of alkaloid, proteins, carbohydrates, steroid, flavoid, glucoside, tannin with their chemical test as mentioned in Table 1.

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