INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

*IJPAR |Vol.7 | Issue 3 |*
*Journal Home page: www.ijpar.com*

*Research article* *Open Access*

# In-Vitro antioxidant activities of ethanolic extract of whole plant of Ziziphus xylopyrus (Retz.)

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## ABSTRACT

*In vitro*antioxidantactivities of ethanol extract of whole plant of *Ziziphus xylopyrus*(Retz.) was investigated. The free radical scavenging activity to evaluate by DPPH (2, 2-diphenyl -1- picrylhydrazyl) method, superoxide anion scavenging and iron chelating activity. DPPH radical scavenging activity of ethanolic extract and reference standard Rutin $IC_{50}$ values was found to be 820 µg/ml and 460 µg/ml, nitric oxide scavenging activity of ethanolic extract and reference standard ascorbic acid $IC_{50}$ values was found to be 510µg/ml and 420µg/ml and iron chelating activity of ethanolic extract and reference standard EDTA $IC_{50}$ values was found to be 320 µg/ml and 63µg/ml respectively. The above result of possess good an antioxidant activity when compare to the above all standard.

**Keywords:** Antioxidant, *Ziziphus xylopyrus* (Retz.), DPPH method, Nitric oxide scavenging activity, Iron chelating activity.

## INTRODUCTION

The role of free radicals in many ailments and diseases including inflammation,rheumatoid arthritis, cancer and cardiovascular diseases has been widely established [1].Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids,lignins, stilbenes, tannins, flavonoids, quinones,coumarins, alkaloids, amines, betalains, and other metabolites, which are affluent in antioxidant activity [2,3]Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity [4]

*Ziziphus xylopyrus*(Retz.) Willd, a large, straggling shrub, is commonly distributed throughout the North-Western India, Pakistan, and China. Various reports claimed the traditional uses of the different parts of the plant for the treatment of different ailments such as obesity, urinary troubles, diabetes, skin infections, fever, diarrohea,

insomnia, digestive, and liver disorders. [5] Different types of the plant extracts have been advocated pharmacologically to exhibit the anti steroidogenic, [6] anticonvulsant, anti nociceptive, anti-inflammatory, [7] antidepressant [8] as well as wound healing activity.[9-11]

*Z. xylopyrus* leaves were reported to contain flavonoids, *viz.*, Quercetin and querceitrin, [12] which can ameliorate oxidative stress-mediated liver damage. It is also used in AragvadhadiKvathChurna and AbharakBhashma formulations.[13,14] The link between antioxidant and hepatoprotective mechanisms has been previously established.[15] Therefore, various parts of *Z. xylopyrus* exhibit potential to be included as active component of functional food. [16].

However, no data are available in the literature on the antioxidant activity of whole plant of *Ziziphus xylopyrus*(Retz.). Therefore we undertook the present investigation to examine the antioxidant activities of ethanolic extract of whole plant of *Ziziphus xylopyrus*(Retz.) through various *in vitro* models.

## MATERIALS AND METHODS

### Collection and identification of plant materials

The aerial parts of *Ziziphus xylopyrus*(Retz.), were collected form Senkottai, Tirunelveli District of Tamil Nadu,India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government ofIndia. Palayamkottai. The aerial parts of *Ziziphus xylopyrus*(Retz.) were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

### Preparation of extracts

The above powered materials were successively extracted with Petroleum ether (40-600C) by hot continuous percolation method in Soxhlet apparatus [**17**] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-780C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

### Evaluation of antioxidant activity by *in vitro* techniques:

#### *DPPH photometric assay*

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001) **[18].** A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

### Nitric oxide radical scavenging activity

Nitric oxide generated from aqueous solution of sodium nitroprusside at physiological pH interacts with oxygen to produce nitrite ions,which was measured by the following method **[19].**The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M), 0.5 ml of ethanolic extract were incubated at 250C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Further, 1 ml of naphthyl ethylene diamine dihydrochloride (1% NEDA) was added to the above mixer and allowed to stand for 30 mins. Aqueous solution of Sodium nitroprusside at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of GriessIllosvery reaction at 540 nm.

### Iron chelating activity

The method **[20]** was adopted for the assay. The principle is based on the formation of O-henanthroline-Fe2+ complex and its disruption by the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% OPhenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations of extract ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was

measured at 510 nm.EDTA was used as a classical metal chelator. The experiment was performed in triplicates

## RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases**[21].**They are also involved in autoimmune disorders like rheumatoid arthritis etc**[22]** Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same. **[23].**

### DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 518 nm, which is induced by antioxidants. The percentage of DPPH radical scavenging activity of ethanolic extract of *Ziziphus xylopyrus*(Retz.) presented in Table I. The ethanolic extract of *Ziziphu sxylopyrus*(Retz.) exhibited a maximum DPPH scavenging activity of 55.46% at 1000 µg/ml whereas for Rutin(standard) was found to be 65.72% at 1000 µg/ml. The IC50 of the ethanolic extract of *Ziziphus xylopyrus*(Retz.) and Rutin were found to be 820µg/ml and 460µg/ml respectively.

**Table I: Effect of Ethanolic extract of whole plant of** *Ziziphus xylopyrus*(Retz.) **on DPPH assay**

| S.No | Concentration (µg/ml) | % of activity (±SEM*) | |
|---|---|---|---|
| | | Sample (Ethanolic extract) | Standard (Rutin) |
| 1 | 125 | 13.64±0.046 | 16.82 ± 0.072 |
| 2 | 250 | 21.36±0.057 | 24.06 ± 0.044 |
| 3 | 500 | 35.54±0.055 | 48.20 ± 0.023 |
| 4 | 1000 | 55.46±0.063 | 65.72 ± 0.012 |
| | | **$IC_{50}$ = 820µg/ml** | **$IC_{50}$ = 460µg/ml** |

*All values are expressed as mean ± SEM for three determinations

### Nitric oxide radical scavenging activity

Free radical scavenging activity of the ethanolic extract of *Ziziphus xylopyrus*(Retz.) **(Linn)** was determined by Nitric oxide radical scavenging activity.

The percentage of Nitric oxide radical scavenging activity of ethanolic extract of *Ziziphus xylopyrus*(Retz.)presented in Table II. The free radical scavenging potential shown maximum activity is 75.75% at 1000µg/ml for as Standard (ascorbate) was found to be 64.21% at 1000 µg/ml. The IC50 of the ethanol extract of *Ziziphus xylopyrus*(Retz.) and standard (ascorbate) was found to be 420µg/ml and 410µg/ml better antioxidant is respectively.

**Table II Effect of Ethanolic extract of whole plant of *Ziziphus xylopyrus*(Retz.) on Nitric oxide Free radical scavenging method**

| S.No | Concentration (µg/ml) | % of activity (±SEM*) | |
|---|---|---|---|
| | | Sample (Ethanolic extract) | Standard (Ascorbate) |
| 1 | 125 | 24.31±0.09 | 26.13 ± 0.066 |

| | | | |
|---|---|---|---|
| 2 | 250 | 37.12±0.06 | 48.43 ± 0.054 |
| 3 | 500 | 49.76±0.12 | 53.22 ± 0.032 |
| 4 | 1000 | 75.75±0.09 | 64.21 ± 0.024 |
| | | **$IC_{50}$ = 510µg/ml** | **$IC_{50}$ = 420µg/ml** |

*All values are expressed as mean ± SEM for three determinations

## Iron chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components**[24,25].** .It causes lipid peroxidation through the Fenton and Haber-weiss reaction and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can perpetuate the chain reactions(26) and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can perpetuate the chain reactions(27)

Iron binding capacity of the ethanolic extract of *Ziziphus xylopyrus*(Retz.) and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table III. Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 81.34% and 96.87% respectively. The IC50 value of ethanolic extract of *Ziziphus xylopyrus*(Retz.) and EDTA was recorded as 320µg/ml and 63µg/ml respectively.

**Table III: Effect of Ethanolic extract of whole plant of** *Ziziphus xylopyrus*(Retz.) **on** Iron chelating method

| **S.No** | **Concentration (µg/ml)** | **% of activity (±SEM*)** | |
|---|---|---|---|
| | | **Sample (Ethanolic extract)** | **Standard (EDTA)** |
| 1 | 125 | 27.52 ± 0.14 | 54.64 ± 0.013 |
| 2 | 250 | 41.33 ± 0.15 | 63.17 ± 0.016 |
| 3 | 500 | 71.53 ± 0.21 | 85.82 ± 0.032 |
| 4 | 1000 | 81.34 ± 0.05 | 96.87 ± 0.012 |
| | | **$IC_{50}$ = 320µg/ml** | **$IC_{50}$ = 63µg/ml** |

*All values are expressed as mean ± SEM for three determinations

## CONCLUSION

From the results obtained in the present study, it is concluded that a whole plant of ethanolic extract of *Ziziphus xylopyrus*(Retz.) which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract and *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.future studies for this plant for antihyperlipidemic activities work.

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