Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 12, Issue 10, October 2021 :3423 – 3427

Research Article

# Evaluation of In-Vitro free Radical Scavenging Potential of Whole Plant of Ziziphusxylopyrus Retz.

**M.Prabhu*[1], G.Alagumanivasagam[2] and Sureshkumar R.B[3]**

*prabhupharma80@gmail.com*
*[1, 2] Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamilnadu, India.*
*[3] Scientist, Novartis Healthcare Private Limited, Bangalore.*

**Abstract**

The antioxidant activity of the entire plant of *Ziziphusxylopyrus* Retz. was studied in this study using several in-vitro techniques. Total antioxidant activity (Phosphomolybdic acid technique), FRAP test with reference standard ascorbate, and total flavonoids concentration were utilised to assess antioxidant activity. The total antioxidant activity of the ethanolic extract of *Ziziphusxylopyrus* Retz was determined to be modest. The $IC_{50}$ values of the ethanolic extract of *Ziziphusxylopyrus* Retz and ascorbate were found to be 470µg/ml and 410µg/ml respectively. The ethanolic extract of *Ziziphusxylopyrus* Retz was found moderate effective in FRAP assay. However, as compared to the ethanolic extract containing ascorbate (standard), the *Ziziphusxylopyrus* Retz ethanolic extract performed better. There are a lot of flavonoids in the ethanolic extract of *Ziziphusxylopyrus* Retz. Furthermore, the findings were shown to be concentration dependant. All of the in-vitro tests show that the ethanolic extract of *Ziziphusxylopyrus* Retz has higher antioxidant activity. These in-vitro tests show that this plant extract is a greater source of natural antioxidants, which may aid in the prevention of different oxidative stresses.

**Key words:** *Ziziphusxylopyrus* Retz, *In-vitro* antioxidant, Total antioxidant activity, FRAP assay, Total flavonoids.

## 1.0 Introduction

Antioxidants act as a major defenseagainst radical-mediated toxicity by protecting against the damages caused by free radicals[1]. Oxidative damage to the cells occur due to cellular oxidative stress. The cellular antioxidant status is usually altered during oxidative stress and it determines the susceptibility to cellular oxidative damage.[2]

In the prevention of various human diseases, anti-oxidants are known to play an important role. Antioxidants obtained from natural sources such as leafy vegetables and seeds (like ascorbic acid, vitamin E and phenolic compounds)posses the ability to reduce the cellar oxidative damage associated with wide variety of diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing[3,4,5,].

Jujab is the English name for *Ziziphusxylopyrus* (Retz) Wild (F. Rhamnaceae)[6]. It's a tiny tree or huge trailing shrub with spines that grows up to 4 metres tall. Fruits are globose, three-celled (sometimes two or four-celled), and have a seed in each cell. They are hard and

woody. India, Pakistan, and China[7] are all home to this species. Different sections of this plant, according to ethno medicinal literature, have a variety of therapeutic properties[8]. It also possesses anthelmintic[9], antimicrobial[10] antidepressant[11], analgesic and anti-inflammatory activities[12]. Quercetin is found in the leaves. Tannins may be found in the fruits, including oleanolic acid, l-leucocyanidin, l-epicatechin, and 3, 3, 4-tri-O-methyl-ellagic acid. Tannins, d-7, 3', 4'-trihydroxy flavan-3, 4-diol, and oleanolic acid are all found in the bark. Alkaloids (xylopyrine-A and B), triterpenoids, and flavonoids have been found in the plant's stem wood[13]. Leaves are reported to contain flavonoids like quercetin, which can ameliorate the oxidative stress caused by liver damage[14]. A great number of medicinal plants containing antioxidant properties in the form of phenolic compounds and help to protect the cells against the oxidative damage by the free radicals[15].

However, there is no information in the literature about the antioxidant properties of the *Ziziphusxylopyrus* entire plant. As a result, we conducted this study to test the antioxidant activity of an ethanolic extract of the entire plant of *Ziziphusxylopyrus* using several in vitro models.

## 2.0 Materials and Methods:

### 2.1Collection and authentication of plant material:

The whole plant *Ziziphusxylopyrus* Retz.were collected and authenticated by P.Sara. Lecturer in Botany, Deparment of Botany &Micorbiolgy, P.R.Govt.College, Kakinada, Andhra Pradesh, India. The authentication number is DBGCAP125. The whole plant of *Ziziphusxylopyrus* was dried under shade and powdered by pulverising in the mechanical grinder and the powder is passed through the 40mesh sieve.

### 2.2 Preparation of the extract

The powdered plant material was successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus for about 24 hours. The concentration of the extracts was done by rotary evaporator and was subjected to freeze drying in the lyophilizer until dry powder was obtained. The extracts were suspended in 2% tween 80[16].

## 3. Evaluation of Antioxidant activity by *in-vitro* Techniques:

### 3.1 Total antioxidant activity (Phosphomolybdic acid method)[17]

Converting Mo (VI) to Mo (V) to create phosphomolybdenum complex was used to determine the antioxidant activity of ethanolic extract (Prieto et al., 1999). In a vial with 4 ml of reagent solution, an aliquot of 0.4 ml of ethanolic extract was added (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were sealed and incubated for 90 minutes in a water bath at 95°C. Cool the samples to room temperature. After that measure the absorbance at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

### 3.2 FRAP Assay [18]

Benzie and Strain A modified method of (1996)16 was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mMHCl and 20 mM FeCl3. 6H2O. the fresh solution was prepared, in that 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml FeCl3.6H2O was mixed. Prior to use, The solution was heated to $37^0$ Celsius. For 30 minutes in the dark, ethanolic extract (0.15 ml) was allowed to react with 2.85 ml of FRAP solution. The readings of the coloured product

(Ferrous tripyridyltriazine complex) were taken at 593 nm. Between 200 and 1000 M FeSo4, the standard curve was linear. The results were compared to those of ascorbic acid and represented in μM (Fe (II) /g dry mass.

### 3.3 Total flavonoids[19]

The 0.2g of ethanolic extract was ground in two different ratios of ethanol-water, 9:1 and 1:1, respectively. These two ratios were blended after the homogenate was filtered. This was evaporated and dried until all traces of ethanol were gone. The resulting aqueous extract was extracted with chloroform or hexane in a separating funnel. The extracted aqueous layer of solvent was concentrated, and a 0.5 ml aliquot was pipette-out into a test tube. 4 mL vanillin reagent (1 percent vanillin in 70% conc. H2SO4) was added and maintained for 15 mints in a hot water bath. At 360 nm, the absorbance was measured. Catechol(110 μg/ml) was used as a standard.

## 4.0 Results and Discussion

Antioxidant chemicals can operate as scavengers of free radicals, initiators of pro-oxidant metal complexes, reducing agents, and quenchers of singlet oxygen formation[20]. Due to the presence of phenolic compounds and flavonoids as main ingredients, most plants have been documented to have antioxidant and free radical scavenging activities [21]. As a result, the relevance of finding natural antioxidants has grown in recent years, and many academics have focused on this topic. [22].

### 4.1Total antioxidant activity (Phosphomolybdic acid method)

Table 1 shows the proportion of total antioxidant activity of an ethanolic extract of *Ziziphusxylopyrus* Retz. At 1000 μg/ml, the ethanolic extract of *Ziziphusxylopyrus* Retz had a maximum total antioxidant activity of 68.73 percent, whereas ascorbate (standard) had a maximum total antioxidant activity of 55.23 percent. The IC50 of *Ziziphusxylopyrus* Retz and ascorbate ethanolic extract was determined to be 470μg/ml and 410μg/ml, respectively.

**Table 1: Total antioxidant activity of Ethanolic extract of** *Ziziphusxylopyrus*Retz**.**

| S.No | Concentrations (μg/ml) | % of activity (±SEM*) | |
|---|---|---|---|
| | | Sample (Ethanolic extract) | Standard (EDTA) |
| 1 | 125 | 24.36 ± 0.042 | 26.87 ± 0.076 |
| 2 | 250 | 35.30 ± 0.024 | 30.30 ± 0.054 |
| 3 | 500 | 48.16 ± 0.042 | 60.64 ± 0.022 |
| 4 | 1000 | 68.73 ± 0.032 | 55.23 ± 0.014 |
| | | **$IC_{50}$ = 470μg /ml** | **$IC_{50}$ = 410μg /ml** |

### 4.2 FRAP assay

The capacity of Ziziphusxylopyrus Retz to decrease TPTZ-Fe (III) complex to TPTZ-Fe (II) complex was determined using the FRAP assay (II). Table 2 shows the results of testing the reducing ability of the ethanolic extract of Ziziphusxylopyrus Retz and ascorbate at various concentrations (125, 250, 500, 1000 μg/ml). At 1000 μg/ml, ethanolic extract and ascorbate had maximal reduction abilities of 76.43 percent and 98.04 percent, respectively. The IC50 values for ethanolic extract and ascorbate were 236 and 50 g/ml, respectively.

**Table 2: FRAP assay of Ethanolic extract of** *Ziziphusxylopyrus* Retz

| S.No | Concentrations (µg/ml) | % of activity (±SEM*) | |
|---|---|---|---|
| | | Sample (Ethanolic extract) | Standard (EDTA) |
| 1 | 125 | 32.73 ± 0.032 | 72.02 ± 0.012 |
| 2 | 250 | 47.81 ± 0.025 | 81.03 ± 0.025 |
| 3 | 500 | 64.24 ± 0.034 | 88.04 ± 0.033 |
| 4 | 1000 | 76.43 ± 0.026 | 98.04 ± 0.023 |
| | | **$IC_{50}$ = 236µg /ml** | **$IC_{50}$ = 50 µg /ml** |

### 4.3 Total flavonoids

Flavonoids, which are found in plant-based foods, are also potential antioxidants[23]. Flavonoids' antioxidant and chelating properties are credited with the majority of their positive effects,[24,25]. Table 3 shows the total quantity of flavonoids in an ethanolic extract of the complete plant of *Ziziphusxylopyrus* Retz.

**Table 3: The total flavonoids content of ethanolic extract of whole plant of** *Ziziphusxylopyrus*Retz

| S.No. | Extract | Total flavonoids content (mg/g) (±SEM)* |
|---|---|---|
| 1 | Ethanolic extract of *Ziziphusxylopyrus*Retz | 3.165 ± 0.034 |

* Values are expressed as mean ± SEM for all three determinations

Based on the result the ethanolic extract of *Ziziphusxylopyrus* Retz was found higher content of flavonoids.

### 5.0 Conclusion

The present study shows when compared to standard ascorbate, the ethanolic extract of *Ziziphusxylopyrus* Retz demonstrated modest antioxidant activity as measured by total antioxidant activity and FRAP test. Furthermore, the ethanolic extract of *Ziziphusxylopyrus* was discovered to have a significant quantity of flavonoids, which play an important role in antioxidant regulation.

### References

1. Mallika J, Shyamala Devi CG. In-vitro and in-vivo evaluation of free radical scavenging potential of Cissusquadranguloris. Afr J Biomed Res 2005; 8: 95-9.
2. Bhor VM, Raghuram N, Sivakami S. Oxidative damage and altered antioxidant enzyme activities in the small intestine of Streptozotocin induced diabetic rats. Int J Biochem Cell Biol 2004; 36: 86-97.
3. Halliwell B (1994) lancet 344, 721-724.
4. Duh P d (1998) J Am Oil Chem. Soc 75, 455-461.
5. Espin JC,Soler Rivas C & Wichers HJ (2000)J Agric food chem. 48, 648-656.
6. Pietta P, Simonetti P and Mauri P, Antioxidant activity of selected medicinal plants, J Agric Food Chem, 1998, 46, 4487-90.
7. Lee KG, Mitchell AE and Shibamoto T, Determination of antioxidant properties of aroma extracts from various beans, J Agric Food Chem, 2000, 48, 4817-20.

8. Middleton E, Kandaswamy C and Theoharides TC, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer, Pharmacol Rev, 2000, 52, 673-751.
9. Part-1. Vol.5. Delhi: Govt of India, Department of ISM and H, Published by the Controller of Publications; Department of AYUSH. Ayurvedic Pharmacopoeia of India. 2006; p: 47.
10. X-Z. Vol. 11. New Delhi: NISCAIR, CSIR; Anonymous. The Wealth of India: Raw Materials Series; 1976; pp: 123- 4.
11. Sudhakar Reddy C, Reddy KN, Murthy EN and Raju VS: Traditional medicinal plants in Seshachalam hills, Andhra Pradesh, India. Journal of Medicinal Plants Research 2009; 3(5): 408-12.
12. Mishra US, Mishra AM, Murthy PN, Bal T and Jena B: Screening of chloroform extract of Bark of Ziziphusxylopyrus Wild for Anthelmintic activity. International Journal of Pharma and Bio Sciences 2008; 2(2): 103-6.
13. Pullaiah T: Ziziphurxylopyrus (Retz.) Willd.Encyclopedia of world medicinal plants, 2006; 5: 2104.
14. Vimal KS, Nagendra SC, Santram L and Singhai AK: Anti-Depressant activity of Ziziphusxylopyrus. International Journal of Phytomedicine 2009; 1:12-7.
15. Mishra US, Murty PN and Parida SK: Analgesic and antiinflammatory activities of Indian medicinal plant Ziziphusxylopyrus stem barks in experimental animal models. Elixir Pharmacy 2012; 44:7265-70.
16. G.Alagumanivasagam, A.Kottai Muthu, R. Manavalan, *In vivo* Antioxidant and Lipid Peroxidation Effect of Methanolic Extract of whole plant of *Teramnus labialis* (Linn.) in Rat fed with high Fat Diet, International Journal of PharmTech Research, Vol.4, No.3, pp 1233-1237, July-Sept 2012.
17. Jangir S, Mathur K, Goyal M and Yadav SK: A Review on *Cocculuspendulus* (J. R. Forst. & G. Forst.) diels: traditional uses, phytochemistry and pharmacological properties. World Journal of Pharmacy and pharmaceutical sciences 2016; 5(4): 672-681.
18. Prieto, P., Pineda, M., Aguilar, M. Spectrophotometricquantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. Anal. Biochem, 1999, 269, 337-41.
19. Benzie IEF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem, 1996, 239, 70-76.
20. Cameron GR, Milton RF and Allen JW.Measurement of flavonoids in plant samples. Lancet, 1943, 179.
21. Christensen Lars P. Tuliposides from *Tulipasylvestris*and *T. turkestanica*, Phytochemistry, 1999, 51 (8), 969-74.
22. Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. Antibacterial and antioxidant activities of grape (*Vitisvinifera)* seed extract, Food Res. Int, 2003, 36, 117-22.
23. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP and Rice Evans C, Polyphenolicflavonoids as scavengers of aqueous phase radicals and as chain-breaking antioxidants, Arch BiochemBiophys, 1995, 322(2), 339-46.
24. Van Acker SABE, Van den Vijgh WJF and Bast F, Stuctural aspects of antioxidant activity of flavonoids, Free Rad Bio Med, 1996, 20(3), 331-42.
25. Hassig A, Liang WX, Shwabl K and Stampfl K, Flavonoids and tannins: plant based antioxidants with vitamin character, Med Hypotheses, 1999, 52, 471-81.