Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 4, 2012

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *ZIZYPHUS OENOPLIA (L) MILL.* ROOT EXTRACT

SURYAKANT A. JADHAV*[1], DEEPALI S. CHAVAN[2]

*[1]Indira Institute of pharmacy, Devrukh, Ratnagiri, Maharashtra, [2]Rajaram Bapu college of pharmacy, Kasegaon, Sangli, Maharashtra, India.

Received: 09 Jun 2012, Revised and Accepted: 13 July 2012

ABSTRACT

The aim of present study is to investigate in vitro antioxidant effect of ethyl alcohol & aqueous extracts of root of *Zizyphus oenoplia.* The ethyl alcohol and aqueous extract of *Zizyphus oenoplia* exhibited promising result at higher concentration. BHA was used as standard antioxidant and positive control. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The reducing power of extract was carried out with ascorbic acid as a standard reducing agent. The results suggest that both the ethanol & aqueous extract posses significant antioxidant activity.

Keywords: Antioxidant activity, Reducing power, DPPH scavenging, *Zizyphus oenoplia.*

INTRODUCTION

It is recognized that naturally occurring substances in higher plants have antioxidant activity. Recently, there is an increased interest in oxygen containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders. That is why; attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them[1]. Flavanoids and flavones are widely distributed secondary metabolite with antioxidant and antiradical properties. Plant based natural constituents can be derived from any part of plant's bark, leaves, flowers, roots, fruits, seeds etc. that is any part of the plant may contain active components.[2]

Free radicals play an important role in a number of biological processes including intracellular killing of bacteria and certain cell signaling processes. Free radicals are derived from molecular oxygen under reducing conditions. Excess amount of these free radicals can lead to cell injury, which results in many diseases like cancer and diabetes[3]. Recently there has been a surge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. The main characteristics of an antioxidant are its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids and DNA and can initiate degenerative diseases. Antioxidant compounds like phenolics acids, Polyphenols and flavanoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.[4]

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.[5,6]

MATERIAL & METHODS

Plant material

Fresh roots of *Zizyphus oenoplia* was identified and authenticated by 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.

Preparation of extract

Ethyl alcohol extract

The powdered material was exhaustively extracted with 95% ethyl alcohol using a soxhlet apparatus. The extract was concentrated in vaccum to a syrupy consistency. The percentage yield of extract was found to be 16%.

Aqueous extract

The dried powder (200g) was kept for maceration with 1000ml of distilled water for 24hrs.The extract was double filtered by using muslin cloth and Whatmann no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 18%.

Antioxidant assay

The antioxidant activity of plant extracts were determined by different in-vitro methods such as the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in ethanol at the concentration of 2mg/ml. All the assays were carried out in triplicate and average value was considered.

b) DPPH radical scavenging activity

DPPH scavenging activity of the plant extract was carried out according to the method of Koleva I.I *et al* 2002[7]; Mathiesen *et al* 1995[8]. 0.2 ml of ethyl alcohol solution of plant extract samples at different concentration (20- 100µg ml-l) was mixed with 0.8 ml of Tris Hcl buffer (100Mm, pH 7.4). One ml DPPH (500 M in ethanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting solution was measured at 517nm UVVisible Spectrophotometer (Shimadzu 1700, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 ml of ethyl alcohol (without plant extract) was added. Percentage of DPPH scavenging activity determined as follows:

% DPPH radical scavenging = [Absorbance of control –Absorbance of test sample / Absorbance of control] × 100

Control was the DPPH solution without plant extract. Purified sample 2mg/ml in ethyl alcohol of *Zizyphus oenoplia* extracts were taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant. Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity. In this study ethyl alcohol and aqueous extracts of *Zizyphus oenoplia* were used.

(b) Reducing power

This was carried out as per the method of Yildrim *et al* 2001[9]; Lu and Foo[10]. 1ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K_3Fe (CN_6)] (10g/l), then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which

was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $Fecl_3$ (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Shimadzu 1700, INDIA). As a control, ascorbic acid was used (final concentration 10 mg/ml).

RESULTS & 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, steroids, flavanoids and tannins are present in ethanol extract, where as carbohydrates, flavonoids, tannins and phenolics are present in aqueous extract. (Table no. 1)

Table 1: shows phytochemical constituents of *Zizyphus oenoplia.*

Phytochemical constituent	Ethanol extract	Aqueous extract
Carbohydrate	+	+
Steroids	+	-
Alkaloids	+	-
Saponins	-	-
Triterpenes	+	-
Tannins	+	+
Flavanoids	+	+
Polyphenols	+	+

It has been recognized that alkaloids and flavanoids shows antioxidant property and their effects on human nutrition and health care considerable11.Mechanism of action of alkaloids are through inhibition of peroxidation.12, 13 Compounds such as Flavanoids are responsible for the inhibition of lipid peroxidation.14Scavenging activity of free radicals of 1, 1 diphenyl-1, 2-picryl hydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of plants. Free radical scavenging activity of ethyl alcohol and aqueous extracts was quantitatively determined using DPPH and reducing power assay.

The DPPH radical scavenging activity increases with increasing concentration. Therefore in this study, the antioxidant properties of ethyl alcohol and aqueous extract of root of *Zizyphus oenoplia* were examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Table 2a and 2b as comparable with known antioxidant BHA. In term of antioxidant activity, all the extracts investigated exhibited a rather high degree of ability.

Table 2a: Shows Antioxidant activity of ethyl alcohol extract of *Zizyphus oenoplia*

Concentration	OD 517 nm			% of activity	
	sample	standard		sample	standard
50µl	1.012	2µl	0.845	7.65	22.6
100µl	1.023	4µl	0.652	5.5	39.0
150µl	0.915	6µl	0.471	18.5	56.3
200µl	0.900	8µl	0.325	19.2	68.3
250µl	0.812	10µl	0.260	26.4	75.2

Control OD at 517 nm-1.124

Table 2b: Shows Antioxidant activity of aqueous extract of *Zizyphus oenoplia*

concentration	OD 517 nm			% of activity	
	sample	standard		sample	standard
50µl	1.050	2µl	0.894	5.1	19.5
100µl	0.983	4µl	0.681	11.4	38.0
150µl	0.936	6µl	0.482	16.2	56.0
200µl	0.869	8µl	0.370	20.9	66.4
250µl	0.784	10µl	0.272	29.0	75.1

Control OD at 517 nm- 1.113

Ethyl alcohol and aqueous extract of *Zizyphus oenoplia* exhibited good reducing power. The reducing power of both extracts of *Zizyphus oenoplia* along with that of ascorbic acid at concentration between 100-500mg/l. High absorbance indicates high reducing power. The reducing power of ethyl alcohol and aqueous extracts of *Zizyphus oenoplia* increases with increase in the concentration. (Table 3a and 3b)

Table 3a: Shows reducing power of ethyl alcohol extract of *Zizyphus oenoplia*

Sample	Concentration (mg/l)	Absorbance (700nm)
control	0	0.06±0.04
Ethyl alcohol extract of *Zizyphus oenoplia*	100	0.15±0.012
	200	0.29±0.042
	500	0.46±0.08
Ascorbic acid	5	0.39±0.005
	10	0.75±0.006
	15	1.10±0.006

Control was test sample without plant extract. High absorbance indicates high reducing power

Table 3b: Shows reducing power of aqueous extract of *Zizyphus oenoplia*

Sample	Concentration (mg/l)	Absorbance (700nm)
control	0	0.08±0.05
Aqueous extract of *Zizyphus oenoplia*	100	0.21±0.011
	200	0.41±0.041
	500	0.57±0.08
Ascorbic acid	5	0.39±0.005
	10	0.72±0.006
	15	1.13±0.007

Control was test sample without plant extract. High absorbance indicates high reducing power

CONCLUSION

The findings of the present study suggested that *Zizyphus oenoplia (L) Mill* could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. Further in future it is necessary to identify & isolate the possible active phytoconstituents responsible for the antioxidant activity & study its pharmacological actions.

REFERENCES

1. Larson RA. The antioxidants of higher plants, *Phytochemistry*, 1988; 969-978.
2. Halliwel B. Free radicals, antioxidants and human disease: Curiosity, cause or Consequence. , 1994; 344: 505.
3. Ashwini M, Nisha lather, Shivaji bole, Vedamutrhy AB, Sam balu. In vitro antioxidant and anti inflammatory activity of *coccinia grandis.* Int J Pharm Pharm Sci, Vol 4, Issue 3, 239-242.
4. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, Phenols, Flavanoid contents of selected Iranian Medicinal Plants. S.Afr. J. Biotechnol 2006; 1142-1145.
5. http://www.expresspharmaonline.com/20060331/research02.shtml27/11/2008.
6. Prajapati ND, Purohit SS. Agro's colour atlas of medicinal plants, Agrobios (India) Jodhpur. 2004.
7. Koleva II., Van Beek TA, Linssen JPH., De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis. 2002; 13, 8-17.
8. Mathiesen, L., Malterud KE and Sund RB. Antioxidant activity of fruit exudate and methylated dihydrochalcones from Myrica gale. Planta Med., 1995; 61, 515-518.

9. Yildirim, A., Mavi A. and Kara AJ. Agric. Food Chem. 2001; 49, 4083-4089.
10. Lu, Y. and Foo, Y. Antioxidant activities of polyphenols from sage (Salvia officinalis.)Food Chem, 2000; 75, 197-202.
11. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, the Royal Society of Chemistry, UK, 1999; 178-187.
12. Kessler M,Ubeaud G,Jung L. Anti and prooxidant activity of rutin and quercetin derivatives.J. Pharm and Pharmacol.2003; 55,131-142.
13. Cook NC, Samman S. Flavanoids-chemistry, metabolism, cardio-protective effects and dietary source.Nutr.Biochem.1996; 7, 66-76.
14. Das NP, Pereira TA. Effects of Flavanoids on Thermal auto-oxidation of palm oil: Structure activity relationship. J.Am.Oil Chem.Soc. 1990; 67,255- 258.