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PHARMACOGNOSTICAL STUDY AND EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAVES OF *ZIZIPHUS OENOPLIA* (L.) MILL.

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ABSTRACT

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physic chemical analyses are few of the basic protocol for standardization of herbals. Free radicals have been implicated in the etiology of several human diseases as well as ageing[20-21]. But it has to be emphasized that ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signalling molecules. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant materials. Attempts are also made to identify and evaluate antioxidants.

***Keywords*: :** Herbal Medicine Ziziphus oenoplia (L.) Pharmacognostical Antioxidant Activity.

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Introduction

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant. To ensure reproducible quality of herbal medicines, proper control of starting material is authentication followed by creating numerical values of standards for comparison. [8] Pharmacognostical parameters for easy identification like leaf constants, microscopy & physic chemical analyses are few of the basic protocol for standardization of herbals. Hence forth the current dissertation work has been tiled and designed so as to create standards for both the plants by performing Pharmacognostic evaluation, phytochemical screening, followed by validating some of its traditional pharmacological claims by conducting few *In-vitro* pharmacological assays.

Materials and Methods

The leaves of plant (*Ziziphus oenoplia* (L.) Mill., Rhamnaceae), are collected from surroundings of Nalgonda, Andhra Pradesh, India, in the month of January. The plant material was identified and authenticated by Dr.P.S.UDAYAN M.Sc., M.Phil., Ph.D, Senior Scientist (Taxonomy), Centre for Medicinal Plants Research (CMPR), Aryan Vaidya Sala, Kottakkal. The plant specimen was prepared and submitted in the Department of Pharmacognosy under the voucher no: 06709.

Pharmacognostic study

Morphological study

The drug was evaluated by its colour, odour, taste, size, shape and special features, like texture, touch, etc. evaluation was carried based on the morphological and sensory profiles of whole drug.

Microscopic Study

Fresh leaf of *Ziziphus oenoplia* (L.) Mill., were used for this purpose, section of leaf was taken with the aid of potato. Phloroglucinol, toluedine blue, hydrochloric acid and glycerin were used as a stain and mounted on a glass slide and focused under a microscope.

Powder microscopy

Shade dried leaves of *Ziziphus oenoplia* (L.) Mill., were powdered with the help of an electric grinder till a fine powder was obtained .This fine powder of leaves were subjected to powder microscopy, as per standard. Slide was prepared with help of a brush and then focused under a microscope.

Determination of leaf constants Stomatal index, vein-islet number, vein termination number were determined and performed.

Quantitative microscopy

Quantitative microscopy of leaf of *Ziziphus oenoplia* (L.) Mill., were carried and all the measurements were determined for individual parts to differentiate the plant from its simulating species

Determination of ash values

This test was used to determine quality and purity of a crude drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalate, silica, carbonate content of crude drug affects 'Total ash value'. Such variables were the removed by treating with acid (as they are soluble in hydrochloric acid) and then acid insoluble ash value was determined.

Determination of extractive values

Extractive values of leaf of *Ziziphus oenoplia* (L.) Mill., were determined separately determination of alcohol-soluble extractives The percentage w/w of extractive with reference to the air-dried drug was calculated and water-soluble extractives also determined Chloroform water was used instead of alcohol (Chloroform acts as a preservative) as per standard procedure and values were reported.

Determination of moisture content (loss on drying)

About 10 g of the powdered drug was weighed and transferred into a flat and thin porcelain dish, allowed to dry in hot air oven at 100^0c for 3 hours. Then cooled in a desiccator and weighed. The loss in weight was calculated by using the following formula:

Loss on drying = $\frac{W2-W3}{W2-W1} \times 100$

Where,*W1* – Weight of empty sample ;*W2* – Weight of sample ;*W3*– Weight of bottle with sample after 3 hours of drying.

Successive solvent extraction

Powdered leaves were successively extracted separately with given solvents based on increasing polarity using Soxhlet's apparatus.

Initially 25gm of crude powder was taken and packed in a packing paper. This packing was placed in a soxhlet extractor for 18 hrs (approximately) with different solvents *i.e.* Benzene, ethyl acetate, alcohol and water. Temperature was adjusted as per the solvent been used in the extraction. After successive extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery.

Preliminary phytochemical analysis

The successive extracts of leaves of *Ziziphus oenoplia* (L.) Mill., were subjected to preliminary photochemical analysis and results were reported.

Thin layer chromatography (TLC) [66-67]

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures of chemical compounds. It is the most basic method of confirming the presence of a phytochemical compound. All the successive extracts (leaf of *Ziziphus oenoplia* (L) Mill.), were subjected to TLC and their profiles were noted.

TLC for Simple Phenols and Phenolic acids

Adsorbent	**:**	Pre-coated Silica Gel GF 254
Solvent system	**:**	Acetic acid: Chloroform (1:9)
Spraying reagent	**:**	Folin reagent and Vanillin-HCl.
Visualization	**:**	UV Chamber

TLC for Flavonoids

Adsorbent	**:**	Pre-coated Silica Gel GF 254

Solvent system : Forestal (Acetic acid: Conc.HCl: Water (30:3:10)).

Visualization : UV Chamber

Estimation of total Phenolic content

The total Phenolic content of successive extracts leaf of *Ziziphus oenoplia* (L.) Mill., was determined according to the Folin–Ciocalteau method[68-69], with slight modifications. Series of Gallic acid standard solutions at a concentration range of 20-200 µg/ml and test sample 1000 µg/ml in methanol were prepared. 0.5ml of each Gallic acid dilution was mixed with 5ml of 10% reagent and 4ml of 1M aqueous Sodium Carbonate. The mixture was allowed to stand for 15 mins and absorbance of reaction mixture (blue colour) was measured at 765nm using UV visible spectrophotometer. Standard graph was plotted with the series of concentrations (20-200 µg/ml) on X-axis and absorbance on Y-axis. The total Phenolic concentrations were determined from standard graph and results were expressed in terms of Gallic acid equivalents mg GAE/gm dry extract.

Estimation of total Flavonoids content

Total Flavonoids content of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was determined by aluminium chloride colorimetric method[58]. Series of Rutin standard solutions at a concentration range of 10-100 µg/ml in methanol and 1000 µg/ml of sample were prepared. 1ml of sample is mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water, and then allowed to stand at room temperature for 30min. The absorbance of the reaction mixture (pink color) is measured at 415nm. Standard graph was plotted with the series of concentrations (10-100 µg/ml). Flavonoids are expressed in terms of mg of Rutin equivalents/gm dry extract.

***In vitro* antioxidant activity**

In vitro antioxidant potentials of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., were determined by various systems.

DPPH (2, 3-diphenyl-1-picrylhydrazyl) radical scavenging assay

The DPPH free radical scavenging capability of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was performed as the method described by Braca et al., (2001)[70] with slight modifications. 1ml of various concentrations (20-100 µg/ml.) of plant extract dissolved in methanol was added to 3 ml of 0.1 mM methanolic solution of DPPH. Extract volume was replaced by methanol and used as control. Reaction mixture was incubated in dark room at room temperature for 30 minutes. The Absorbance of the reaction mixture was measured at 517 nm using UV/Visible spectrophotometer. The percentage inhibition of DPPH radical was calculated according to the following equation:

Percentage inhibition (%) = $\frac{Acontrol-Asample}{Acontrol} \times 100$

Where *Acontrol* is the absorbance of control and *Asample* is the absorbance of the sample.

IC_{50} (minimum concentration at which 50% of radicals were scavenged) values were estimated graphically using a non linear regression algorithm.

Evaluation of ROS inhibition

Evaluation of ROS inhibition was carried by using different systems involved with different types of reactive species.

Superoxide anion scavenging assay

Superoxide radical scavenging assay of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was determined by the nitroblue tetrazolium reduction method[71]. 1ml of Nitro Blue Tetrazolium (NBT) solution (156 µM NBT in 100mM phosphate buffer, ph 7.4) and 1 ml of NADH solution (468µM NADH in 100mM phosphate buffer, ph 7.4) and 0.1ml of the samples (20-200 µg/ml) were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS) solution (60µM PMS in 100 ml phosphate buffer, Ph 7.4) to the mixture. The reaction mixture was incubated at 25^0C for 5min, and the absorbance at 560nm was measured against blank samples, containing all the reagents except the PMS. The positive control (the sample was replaced with trolox) and the negative control (only the solvent was added) were subjected to the same procedure described above as the sample. The percentage inhibition of superoxide radical radical was calculated according to the following equation:

Percentage inhibition (%) = $\frac{Acontrol-Asample}{Acontrol} \times 100$

Where *Acontrol* is the absorbance of control and *Asample* is the absorbance of the sample.

IC_{50} values were estimated graphically using a non linear regression algorithm.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was determined according to the method of Ebrahimzadeh et al (2010a)[72]. Various concentrations (20-200 µg/ml.) 1ml. of plant extracts were added to 2 ml of hydrogen peroxide solution (10 mM) in phosphate buffer (50 mM, pH 7.4). The extract was replaced by methanol for control. Reaction mixture was incubated at room temperature for 30 min. The unreacted hydrogen peroxide was determined by measuring the absorbance of the reaction mixture at 230 nm with respect to the blank (methanol) using UV/visible spectrophotometer. The percentage inhibition was calculated according to the following equation:

Percentage inhibition (%) = $\frac{Acontrol-Asample}{Acontrol} \times 100$

Where *Acontrol* is the absorbance of control and *Asample* is the absorbance of the sample.

IC_{50} values were estimated graphically using a non linear regression algorithm.

Hydroxyl radical scavenging assay

The scavenging capacity for hydroxyl radical of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was determined according to the method of Olabinri et al. (2010)[73], with slight modifications. 60 µl of $FeSO_4.7\ H_20$ (1 mM) was added to 90 µl of aqueous 1, 10 phenantroline (1 mM). 2.4 ml of 0.2 M phosphate buffer pH 7.4 was added to the mixture Followed by addition of 150 µl of hydrogen peroxide (0.17 mM) and 1.5 ml (20-200 µg/ml) of sample in sequence. Extract was replaced by methanol for control. The mixture was incubated for 5 min at room temperature. The absorbance of the mixture was read at 560 nm, Using UV-Visible spectrophotometer against blank. The percentage inhibition of hydroxyl radical was calculated according to the following equation:

Percentage inhibition (%) = $\frac{Acontrol-Asample}{Acontrol} \times 100$

Where *Acontrol* is the absorbance of control and *Asample* is the absorbance of the sample.

IC_{50} values were estimated graphically using a non linear regression algorithm.

Nitric oxide radical inhibition assay

The nitric oxide radical scavenging of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was carried out according to the method of Olabinri et al. (2010), with slight modifications. 2ml of sodium nitroprusside (10 mM) in 0.5ml of phosphate buffered saline was mixed with 0.5ml of sample (20-200 µg/ml) dissolved in methanol. The same reaction mixture without the sample but with equivalent amount of methanol served as the control. The mixture was incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Greiss reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-(1-napthyl) ethylene diamine dihydrochloride was added. The absorbance of the mixture was read at 546 nm using UV/Visible spectrophotometer.

The percentage inhibition of nitric oxide radical was calculated according to the following equation:

$$\text{Percentage inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} \times 100$$

Where *Acontrol* is the absorbance of control and *Asample* is the absorbance of the sample.

IC_{50} values were estimated graphically using a non linear regression algorithm.

Metal chelating assay

The metal chelating ability of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was determined according to the method of Ebrahimzadeh et al. (2010b)[74], with little modifications. 1 ml of various concentrations of extract (20-100 µg/ml) was added to a solution of 2mM $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5 mM Ferrozine (0.2 ml). The mixture was shaken vigorously and left standing at room temperature for 10min. The sample was replaced by methanol in the reaction mixture. The mixture was incubated for 10 minutes at room temperature. The absorbance of the mixture was measured at 562 nm using UV-Visible spectrophotometer. The percentage inhibition of ferrozine-Fe^{2+} complex was calculated according to the following equation:

$$\text{Percentage inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} \times 100$$

Where *Acontrol* is the absorbance of control and *Asample* is the absorbance of the sample.

IC_{50} ((minimum concentration at which 50% of ferrozine-Fe^{2+} complexes were inhibited) values were estimated graphically using a non linear regression algorithm

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Reducing power assay

The Fe^{3+} reducing power of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill., was determined by the method of Zhao et al. (2006)[75], with slight modifications. The extract 0.75 ml at various concentrations (20-100 µg/ml) were mixed with 0.75 ml of phosphate buffer (0.2 M, pH.7.0) and 0.75 ml of potassium hexacyanoferrate $[K_3Fe(CN)_6]$ (w/v 1 %), followed by incubating at 50^0 C in a water bath for 20min. The reaction was stopped by adding 0.75ml of trichloroacetic acid solution (TCA) (10 %) and then centrifuged at 3000rpm for 10 min. Then 1.5 ml of supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride solution (0.1 %w/v). After 10 min. the absorbance at 700 nm was measured as the reducing power. Higher the absorbance of the reaction mixture indicated greater the reducing power.

Results and Discussions

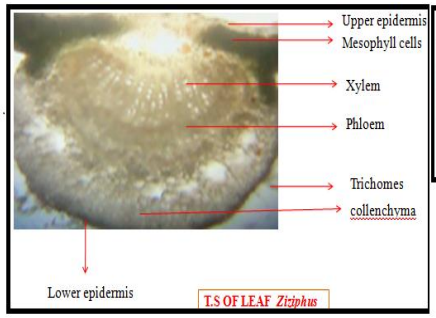
Morphological studies

Leaf powder of *Ziziphus oenoplia* (L.) Mill., exhibiting characteristic odour, with very smooth touch for leaf powder.

Table 01

Colour	Green
Odour	Odourless
Taste	Tasteless
Size	2-9 cm long : 1.5-5 cm width
Shape	Ovate
Texture	Hairs on surface
Touch	Smooth

Microscopic studies[78-80]



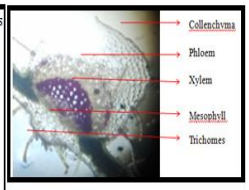
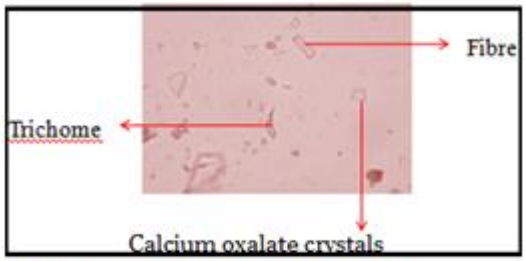


Fig 01: MICROSCOPY OF *Ziziphus oenoplia* (L.) Mill. LEAF



Powder microscopy of leaf of *Ziziphus oenoplia* (L.) Mill.

Table: 02 Leaf constants of *Ziziphus oenoplia* (L.) Mill.

Parameter	value
Stomatal number	
Upper surface	**3-4-5**
Lower surface	**4-5-6**
Stomatal index	**13.04-16.66-20**
upper surface	**16.66-20-23.07**
lower surface	
Vein islet number	**6-8-9**
Vein let termination	**8.6-11-12**

Table 03 Quantitative microscopy of leaf of *Ziziphus oenoplia* (L.) Mill.

Parameter	Leaf	
	Length (µ)	**Width (µ)**
Phloem fibres	117.6-161.7-205.8	17.64-26.46-32.34
Xylem vessels	132.3-176.4-220.5	19.11-27.93-35.28

Parameter	Leaf
Ash value	
Total ash	10.834±0.7638
Acid insoluble ash	2±0.5
Water soluble ash	2.5±0.6245
Sulphated ash	16.16±0.3512
Extractive values	
Water soluble	21.15±1.185
Alcohol soluble	5.49±0.4692
Benzene soluble	7.32±0.3911
Moisture content	22±1.0
Crude fibre content	7.16±3.01

Proximate analysis

PARAMETER	ALCOHOL EXTRACT	WATER EXTRACT
(%w/w) yield	23.09	21.84
Consistency	Solid	Semi-solid
Colour (Day light	Green	Green
Colour (Short UV)	Pale green	Light green
Colour (Long UV)	Thick green	Dark green
Nature of extract	Amorphous	Amorphous

Extractive values

Fig:01 Proximate Analysis and Extractive values

Preliminary phytochemical analysis of successive extracts of leaf:

Test	Benzene	Ethyl acetate	Alcohol	Water
Carbohydrates	-	-	+	+
Amino acids	-	-	-	-
Proteins	-	-	-	-
Alkaloids	-	-	+	+
Tannins	-	-	-	-
Steroids	-	-	-	-
Flavonoids	-	+	+	+
Saponins	-	-	-	-
Glycosides	-	-	+	+
Phenols	+	+	+	+

Fig:02 Preliminary Phyto chemical screening

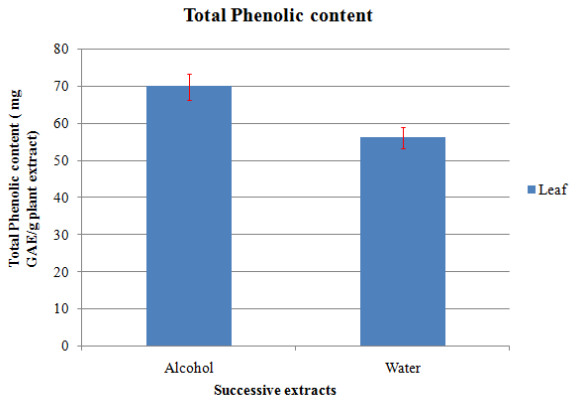
REAGENTS	DAY LIGHT	SHORT WAVE LENGTH	LONG WAVE LENGTH
Conc. Hcl	Pale yellow	Greenish yellow	black
Dil.Hcl	Light brown	Pale brown	Brownish black
Conc.H2SO4	Brownish black	Brown	Black
Dil.H2SO4	Light brown	Greenish brown	Black
Conc.HNO3	Brown	Pale brown	Brownish black
Dil. HNO3	Greenish brown	Brown	Brownish black
Ninhydrin reagent	Light green	Pale brown	Black
Wagner's reagent	Brown	Brown	Brown
Chloroform	Light green	Green	Brown
Glycerine	Pale green	Light green	Brown
Dragendorff's	Light brown	Light green	Black
Benedict's reagent	Light brown	Light green	Black
Hager's reagent	Light brown	Brown	Black
Methanol	Brown	Brownish black	brown

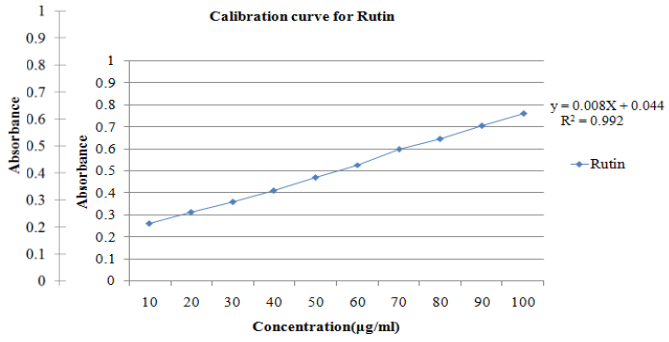
Fluorescence analysis of leaf powder of *Ziziphus oenoplia* (L.)

SOLVENT SYSTEM	LONG WAVELENGTH		SHORT WAVE LENGTH		DAY LIGHT	
	SPOT COLOR	Rf	SPOT COLOR	Rf	SPOT COLOR	Rf
acetic acid: chloroform (1:9)	Bright fluorescence	0.91	Pale brown	0.91	Brownish yellow	0.91
	Yellow fluorescence	0.88	Pale brown	0.88	Brownish yellow	0.88
Acetic acid: Conc.HCl: Water (30:3:10)	White fluorescence	0.875	Pale brown	0.875	Brownish yellow	0.875
	Brownish yellow fluorescence	0.857	Light brown	0.857	Brownish yellow	0.857
	Brown fluorescence	0.821	Light brown	0.821	Brownish yellow	0.821
	Bright fluorescence	0.75	Pale brown	0.75	Brownish yellow	0.75
	Brown fluorescence	0.714	Pale brown	0.714	Brownish yellow	0.714
	Light fluorescence	0.71	Light brown	0.71	Brownish yellow	0.71
	Light fluorescence	0.642	Light brown	0.645	Brownish yellow	0.645
	Light fluorescence	0.63	Light yellow	0.63	Brownish yellow	0.63
	Light fluorescence	0.625	Pale brown	0.625	Brownish yellow	0.625
	Brown fluorescence	0.6	Pale brown	0.6	Brownish yellow	0.6

Total Phenolic Content of successive extracts of leaf of *Ziziphus oenoplia* (L.) Mill.

	mg Gallic acid equivalents/g of extract	
	Alcohol extract	Water extract
Leaf	69.89±0.0953	56.14±0.90

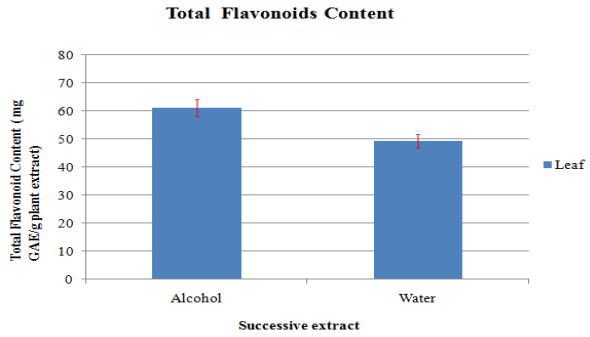




Total Flavonoids Content of successive extracts of leaf of *Ziziphus oenoplia*(L.) Mill.

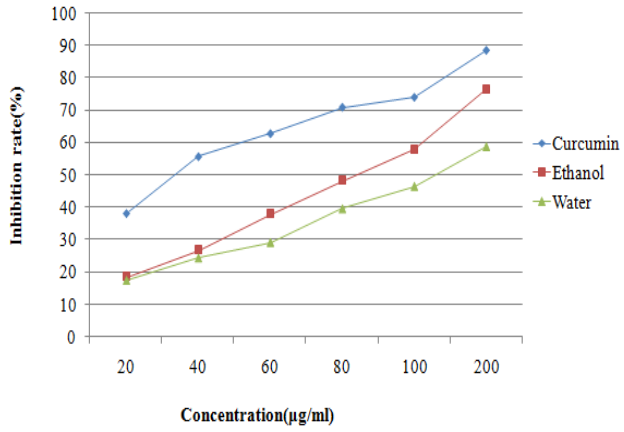
	mg Rutin equivalents/g of extract	
	Alcohol extract	Water extract
Leaf	61.22±1.132	49.34±0.8502

Each value is expressed as mean±SD (n=3).



ANTIOXIDANT ACTIVITY

DPPH radical-scavenging activity of successive leaf extracts of *Ziziphus oenoplia*(L.) Mill.[70]

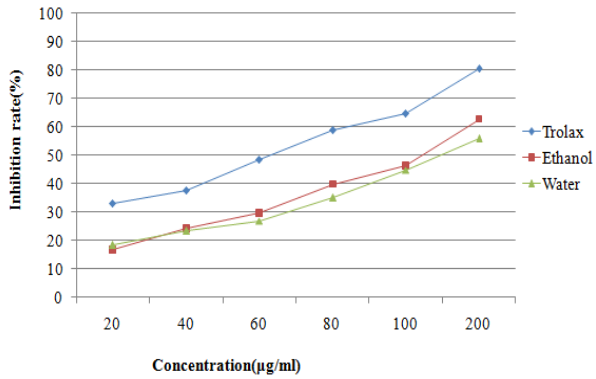


DPPH radical-scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition(%)		
	Curcumin	Alcohol	Water
20	38.10±5.045	18.53±2.574	17.55±2.019
40	55.75±2.482	26.89±2.99	24.43±2.26
60	62.91±2.886	37.85±4.616	29.25±0.2784
80	71.09±4.086	48.30±0.799	39.62±4.167
100	74.16±1.985	57.96±4.563	46.54±2.455
200	88.74±3.586	76.50±3.245	58.77±5.5
IC_{50}	35.12	83.08	137.36

Each value is expressed as mean±SD (n=3).

Fig:04 DPPH radical-scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

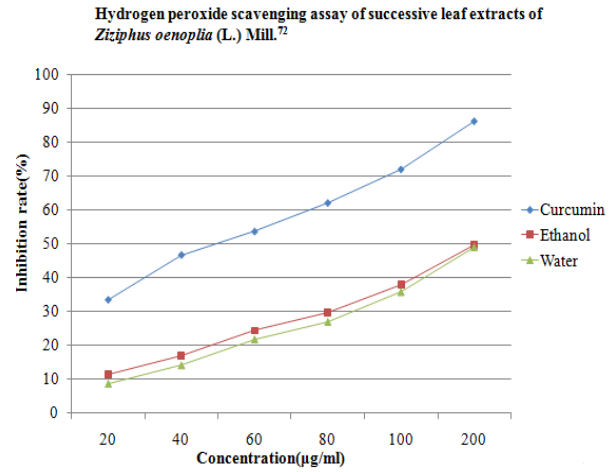


Super oxide radical scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition(%)		
	Trolax	Alcohol	Water
20	32.97±1.01	16.92±2.603	18.47±1.845
40	37.78±3.516	24.32±2.934	23.69±0.7690
60	48.33±4.352	29.58±4.419	27.04±1.95
80	59.01±3.795	39.97±1.749	35.34±1.941
100	64.88±1.987	46.23±2.671	44.97±3.625
200	80.64±2.389	62.87±2.688	56.0±2.0
IC_{50}	64.02	120.9	150.8

Each value is expressed as mean±SD (n=3).

Fig: 05 Super oxide radical scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

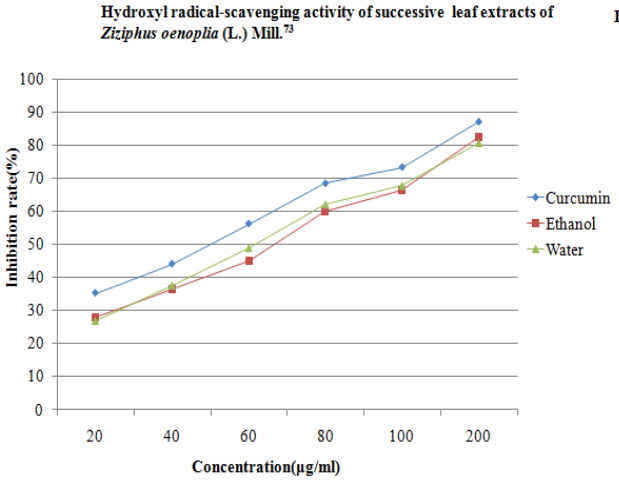


Hydrogen peroxide scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition(%)		
	Curcumin	Alcohol	Water
20	33.33±3.592	11.41±3.62	8.57±3.449
40	46.60±4.820	17.0±2.0	14.0±2.0
60	53.56±2.011	24.32±3.609	21.69±2.398
80	62.02±3.596	29.60±3.377	26.83±2.083
100	71.97±2.536	37.78±1.164	35.66±3.326
200	86.235±3.998	49.62±3.423	48.89±1.767
IC_{50}	50.23	198.02	199.16

Each value is expressed as mean±SD (n=3).

Fig:06 Hydrogen peroxide scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

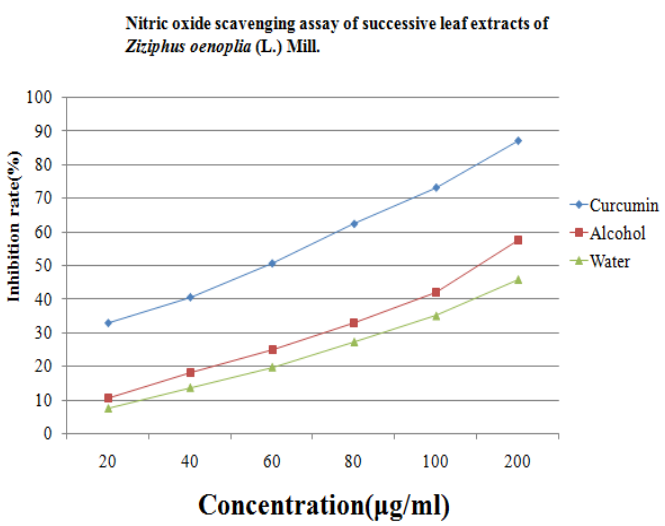


Hydroxyl radical scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition(%)		
	Curcumin	Alcohol	Water
20	35.21±2.714	27.95±3.597	26.72±2.432
40	44.06±2.627	36.41±2.697	37.44±3.72
60	56.30±5.07	45.0±2.0	48.78±3.745
80	68.54±2.611	60.0±3.606	61.94±1.01
100	73.44±2.721	66.50±4.652	67.61±4.348
200	87.20±4.978	82.67±2.423	80.56±2.85
IC_{50}	50.92	62.86	67.58

Each value is expressed as mean±SD (n=3).

Fig: 07 Hydroxyl radical-scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.[73]

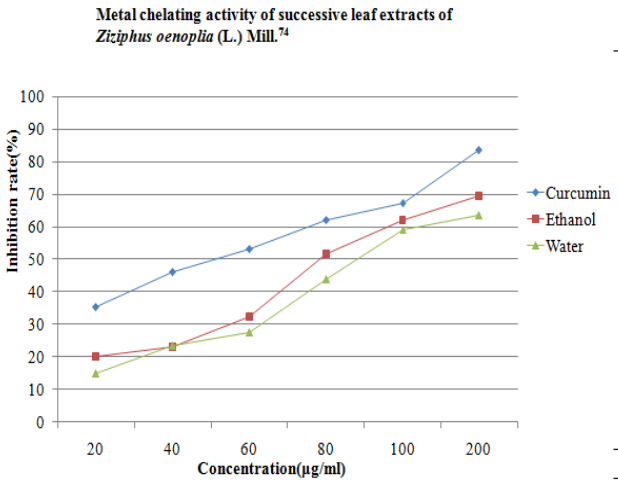


Nitric oxide scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition(%)		
	Curcumin	Alcohol	Water
20	32.81±4.77	10.65±2.379	7.30±1.386
40	40.50±2.835	18.37±2.266	13.46±2.75
60	50.6±2.865	25.10±3.572	19.56±3.529
80	62.37±3.159	33.0±2.0	27.06±1.985
100	73.19±2.727	42.21±2.62	34.81±2.188
200	87.139±1.998	57.64±3.556	45.61±3.501
IC_{50}	**59.80**	150.62	>200

Each value is expressed as mean±SD (n=3).

Fig:08 Nitric oxide scavenging activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

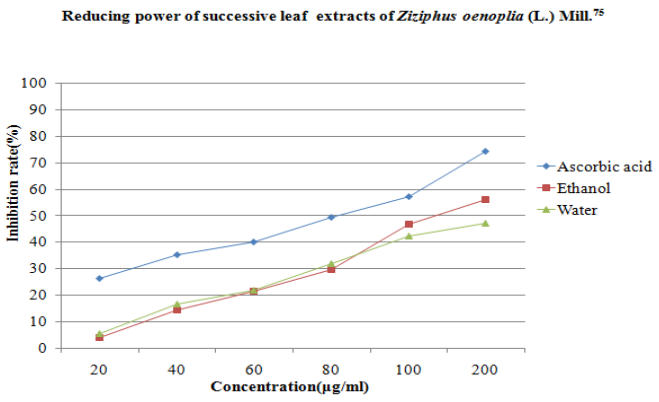


Metal chelating activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition rate (%)		
	Curcumin	Alcohol	Water
20	35.23±0.8516	20.10±1.664	15.0±2.646
40	46.26±2.87	23.0±2.828	23.5±2.498
60	53.20±0.8318	32.5±1.7	27.5±4.652
80	62.099±3.979	51.7±2.846	43.92±3.94
100	67.25±4.396	62.10±2.565	59.20±7.555
200	83.62±3.859	69.41±4.37	63.57±1.845
IC_{50}	51.76	95.24	123.0

Each value is expressed as mean±SD (n=3).

Fig:09 Metal chelating activity of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.



Reducing power of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill.

Concentration (µg/ml)	Inhibition(%)		
	Ascorbic acid	Alcohol	Water
20	26.24±1.664	3.9±0.9625	5.4±1.916
40	35.28±3.912	14.38±2.423	16.6±2.8
60	39.87±2.98	21.36±2.825	21.739±1.675
80	49.29±4.663	29.77±4.097	32.0±2.646
100	57.20±2.578	46.72±2.581	42.29±3.65
200	74.39±3.715	55.98±3.596	47.167±1.998
IC_{50}	82.1	134.24	>200

Each value is expressed as mean±SD (n=3).

Fig: 10 Reducing power of successive leaf extracts of *Ziziphus oenoplia* (L.) Mill

Conclusion

The study of Pharmacognostic features of Ziziphus oenoplia had shown the standards which will be useful the detection of its identity and authenticity. The other study viz. Physical evaluation, preliminary phytochemical test and Fluorescence analysis add to its quality control and quality assurance for proper identification. Preliminary phytochemical screening of leaves of plant extracts revealed the presence of Alkaloids, Flavonoids, Phenols, Glycosides and Carbohydrates. Thin layer chromatography for Flavonoids and Phenols were performed to confirm the presence of these active constituents. The antioxidant activities of successive extracts were evaluated in

seven different test systems DPPH, superoxide, hydrogen peroxide, hydroxyl, nitric oxide, metal chelating and reducing power. The successive alcohol extract of leaf exhibited the highest antioxidant effect followed water extract of leaf.

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