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IN-VITRO ANTIOXIDANT ACTIVITY OF THE SUCCESSIVE EXTRACTS OF *ZIZIPHUS MAURITIANA* LEAVES

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ABSTRACT

The importance of medicinal plant has been emphasized from time to time. It is believed that the drug of natural origin shall play an important role in health care particularly in rural areas of India. *Ziziphus mauritiana* belongs to family Rhamnaceae and commonly known as Indian jujube or ber .The leaves are alternate and elliptic. Flowers are small and bisexual .The leaves are about 2.5 – 3.2 cm long. Commercially it is cultivated in China & India. *Ziziphus mauritiana* is small to medium sized spiny tree. *Ziziphus mauritiana* contains fructose, galactose, malonic acid, malic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid and vanillic acid .The antioxidant activities of the plant extract and pure compounds were assessed by Reduction of NBT (Nitro Blue Terazolium) and Nitric Oxide Radical Inhibition activity method. The five successive extract (benzene, petroleum ether, chloroform, methanol and aqueous extract) of *Ziziphus mauritiana* Leaves and one standard (Ascorbic acid) were tested for in-vitro antioxidant activity. The result was expressed as IC_{50} values and percentage inhibition at different concentrations (25, 50, 100, 200, 250, and 500 in µg/ml). The methanol extract showed maximum antioxidant activity with IC_{50} value of 36.34±0.16µg/ml and percentage inhibition 33.60±0.06, 41.08±0.12, 64.40±1.32, 76.36±0.56, 81.42±0.64 and 87.23±0.04 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively.

INTRODUCTION: *Ziziphus mauritiana* belongs to the family Rhamnaceae and is generally propagated by seeds. Several cultivars have been selected among the seedling populations for their superior fruit quality. This fruit is commonly consumed in households as fresh and is dehydrated for later use. The powder from the fruit is used for baking and to prepare jam and a traditional loaf. Mature green fruits (unripe) are used in India to prepare chutney, pickle and jelly [1]. The leaves are simple, shining green above and whitish tomentose beneath, commonly sub-orbicular to ovate-oblong, rounded at both ends, highly variable in shape and size [2]. The flowers are small and yellow green in colour.The fruit is an edible drupe with yellowish brown colour and 1-5 cm long, sweet and sugary in taste [3].

Ziziphus mauritiana contains fructose, galactose, malonic acid, malic acid, p-hydroxybenzoic acid ,caffeic acid,ferulic acid and vanillic acid [4]. *Ziziphus mauritiana* mostly contains Alkaloids, Sapogenin & Flavonoids .The leaves also contains carotenoids, protein and tannin while fruit contains Mg, Ca, Zn, Mn, Fe & P in mesocarp.

The bark and root bark contain pentacyclic triterpenoid, zizyberanalic acid,lupeol and betulic acid [1, 5, 6]. The hepatoprotective activity of ethanol extract of *Ziziphus mauritiana* leaf against carbon tetrachloride –induced liver damage in rat were reported [7]. *Ziziphus mauritiana* also shows the presence of soluble sugars like fructose, glucose, galactose and non volatile organic acids like citric acid, malic acid and malonic acid by using TLC, HPLC, PC & determine the R_f value in the fruits of *Ziziphus mauritiana*.

The phenolic compounds were also detected by HPLC method. The major phenolic compounds quantified were p-hydroxybenzoic acid and caffeic acid which is responsible for antioxidant properties. The dietary intake of *Ziziphus mauritiana* fruit may reduced incidence of human disease like cancer and cardiovascular disorder, in which free radicals are involved [4].

The antioxidant compounds can be used to counteract oxidative damage by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers [8]. Numbers of epidemiological studies have demonstrated an association of increase intake of natural antioxidants such as vitamins A, C, E, flavonoids and reduced mortality and morbidity of cardiovascular disease [9].

On the basis of above trends, the present work deals with the study of antioxidant activity of *Ziziphus mauritiana* leaves.

MATERIALS AND METHODS: The fresh leaves of *Ziziphus mauritiana* was collected from Allahabad district (Uttar Pradesh, India) and identified by Dr. D.D. Patra, Scienticist, Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The fresh leaves were dried under shade, powdered and pass through 40 mesh sieve and stored in closed containers for further use. The powder was extracted with different solvents ranging from non-polar to polar solvents.

***In-vitro* Antioxidant activity:**

1. **Reversal of NBT reduction by superoxide anions Method:** Reduction of NBT (Nitro Blue Terazolium) is the most popular method for determination of antioxidant activities. This method is based on generation of super oxide radical by auto oxidation of riboflavin in the presence of light. The super oxide radical reduces NBT to a blue coloured formazon that can be measured at 560 nm [10]. The reaction mixture was prepared with 50 mM KH_2PO_4-KOH, PH= 7.4 containing 1 mM EDTA, 0.5 ml of 100 µM hypoxanthine, 0.5 ml of 100 µM NBT. The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 µl of phosphate buffer and 0.5 ml of the test extract in saline.

   The reactions mixture incubated at 25^{o}C for 5 minute and absorbance was determined at 560 nm. Ascorbic acid was used as standard compound. Decrease in the absorbance of reaction mixture indicates an increase in superoxide anion scavenging activity. The result was expressed in IC_{50} value and the percentage inhibition of NBT reduction rate [11]. Successive extract of *Ziziphus mauritiana* Leaves at different concentrations were studied by above method and result shown in **Table 1**.

2. **Nitric Oxide Radical Inhibition Activity methods:** Nitric oxide, because of its unpaired, it is classified as a free radical. This method is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in phosphate buffer saline. Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide which interacts with oxygen to produce nitrite and measured by Griess reagents. Ascorbic acid was used as standard blank solution.

   The reaction mixture (6.0 ml) containing sodium nitroprusside (10 mM, 4.0 ml), phosphate buffer saline (1.0 ml) and extracts (1.0 ml) at different concentrations were incubated at 25^{0}C for 150 minutes. After incubation, 0.5 ml of reaction mixture containing nitrite was removed; 1.0 ml of sulphanilic acid reagents (0.33 % in 20 % Glacial Acetic Acid) was mixed well and allowed to stand for 5 minutes for completing diazotization and then 1.0 ml (0.1 %) 1-Naphthylamine was added, mixed and allowed to stand for 30 minutes. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding standard blank solution.

The activity is expressed as percentage reduction of nitric oxide. IC_{50} value is the concentration of sample required to inhibit 50 % of nitric oxide radical [10, 12]. Result was shown in **Table 2**.

Percentage inhibition (I %) = 100 X (A_0-A_t / A_0)

A_0 – Absorbance of control, A_t – Absorbance of test compounds

RESULTS AND DISCUSSION: The five successive extract (benzene, petroleum ether, chloroform, methanol and aqueous extract) of *Ziziphus mauritiana* Leaves and one standard (Ascorbic acid) were tested for *in-vitro* antioxidant activity. Among all five extract of *Ziziphus mauritiana* Leaves the methanol extract showed maximum antioxidant activity with IC_{50} value of 36.34±0.16µg/ml and percentage inhibition 33.60± 0.06, 41.08±0.12, 64.40±1.32, 76.36±0.56, 81.42±0.64 and 87.23±0.04 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively followed by petroleum ether extract with IC_{50} value 64.25±1.23 µg/ml and percentage inhibition 22.34±1.62, 36.04± 1.46, 54.86±0.48, 60.06±2.56, 72.23±0.04, and 74.64± 2.40 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively while standard ascorbic acid having IC_{50} value 18.64±0.36 and percentage inhibition 34.66±4.24, 44.52±2.98, 70.18±2.36, 83.64± 2.38, 89.42±1.36, 92.56±0.34 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively and minimum with aqueous extract by NBT (Nitro Blue Terazolium) method (**Table 1**). All the five successive extract of *Ziziphus mauritiana* Leaves and one standard (Ascorbic acid) were tested for in-vitro antioxidant activity by using Nitric Oxide Radical Inhibition activity method which is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in phosphate buffer saline. The result was expressed as IC_{50} values and percentage inhibition at different concentrations (25, 50, 100, 200, 250, and 500 in µg/ml).

Among all five extract of *Ziziphus mauritiana* Leaves the petroleum ether extract showed maximum antioxidant activity with IC_{50} value of 32.16±2.08µg/ml and percentage inhibition 27.44±2.62, 38.12±0.74, 46.08±2.67, 63.14±1.22, 71.86±2.48 and 83.14±0.34 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively followed by benzene extract with IC_{50} value 38.98±2.74µg/ml and percentage inhibition 29.02±1.23, 36.55±0.04, 43.28±0.32, 58.90±0.33, 68.05±2.18, and 76.58±3.52 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively while standard ascorbic acid having IC_{50} value 18.38±1.23 and percentage inhibition 32.48±0.65, 43.92±1.70, 58.05±0.03, 67.82±1.24, 78.40±0.25 and 87.58±1.47 at 25, 50, 100, 200, 250, and 500 in µg/ml concentrations respectively and minimum with aqueous extract (**Table 2**).

TABLE 1: *IN-VITRO* ANTIOXIDANT ACTIVITY OF *ZIZIPHUS MAURITIANA* LEAVES BY REVERSAL OF NBT REDUCTION BY SUPEROXIDE ANIONS METHOD

Extract	% Inhibition (Concentration in µg/ml)						IC_{50} ± S.E (µg/ml)
	25	50	100	200	250	500	
Benzene Extract	16.48±2.16	42.32±0.90	58.24±2.36	63.12±0.06	68.90±1.24	70.06±1.02	174.56±0.48
Pet. Ether Extract	22.34±1.62	36.04±1.46	54.86±0.48	60.06±2.56	72.23±0.04	74.64±2.40	64.25±1.23
Chloroform Extract	18.20±1.84	26.64±0.08	48.53±1.82	54.26±0.12	60.82±2.46	63.48±1.66	196.37±0.32
Methanol Extract	33.60±0.06	41.08±0.12	64.40±1.32	76.36±0.56	81.42±0.64	87.23±0.04	36.34±0.16
Aqueous Extract	14.20±2.44	25.62±1.58	34.06±0.28	44.45±2.78	51.16±1.13	55.82±2.26	248.12±1.24
Ascorbic Acid	34.66±4.24	44.52±2.98	70.18±2.36	83.64±2.38	89.42±1.36	92.56±0.34	18.64±0.36

TABLE 2: *IN-VITRO* ANTIOXIDANT ACTIVITY OF *ZIZIPHUS MAURITIANA* LEAVES BY NITRIC OXIDE RADICAL INHIBITION ACTIVITY METHOD

Extract	% Inhibition (Concentration in µg/ml)						IC_{50} ± S.E (µg/ml)
	25	50	100	200	250	500	
Benzene Extract	29.02±1.23	36.55±0.04	43.28±0.32	58.90±0.33	68.05±2.18	76.58±3.52	38.98±2.74
Pet. Ether Extract	27.44±2.62	38.12±0.74	46.08±2.67	63.14±1.22	71.86±2.48	83.14±0.34	32.16±2.08
Chloroform Extract	21.48±0.03	27.62±0.74	32.88±3.02	43.12±1.62	50.38±1.72	60.92±0.03	73.08±0.06
Methanol Extract	28.62±0.17	31.92±1.68	40.32±2.74	52.08±0.22	60.12±3.18	72.13±2.08	52.04±1.55
Aqueous Extract	17.68±1.44	23.82±0.63	30.78±1.04	37.50±0.28	43.65±0.38	53.82±1.64	84.64±0.20
Ascorbic Acid	32.48±0.65	43.92±1.70	58.05±0.03	67.82±1.24	78.40±0.25	87.58±1.47	18.38±1.23

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