**Research Article** **CODEX: IJRPJK** **ISSN: 2319 – 9563**

**International Journal of Research in Pharmaceutical and Nano Sciences**

Journal homepage: **www.ijrpns.com**

# *IN VITRO* ANTIOXIDANT ACTIVITIES OF VARIOUS EXTRACTS OF *EUGENIA JAMBOLANA* LEAVES

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## ABSTRACT

The world today is not restricted to the use of synthetic medicine but also focusing more upon the herbal medication for curing various ailments. The present research was subjected to screen *in vitro* antioxidant activity of hydroalcoholic extract *Eugenia jambolana* leaves. Preliminary Phytochemical investigation was carried out on the successive extract Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Hydroalcoholic extract of *Eugenia jambolana* leaves. It indicates the presence of Carbohydrates, Alkaloids, glycosides, Saponins, Tannins, Terpeoids, Phenols, Flavonoids, Protein, Sterols and steroids. The antioxidant activity was determined by *in vitro* methods such as Scavenging of ABTs Radical Method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, Antilipid peroxidation Assay, Scavenging of Hydrogen Peroxide, Scavenging of Hydroxyl Radical by p-NDA Method, Nitric Oxide Radical Inhibition, Evaluation of total antioxidant capacity and Estimation of Total Phenol and Flavonol Contents. The $IC_{50}$ value of hydroalcoholic extract of *Eugenia jambolana* leaves for DPPH and hydrogen peroxide scavenging activity showed significant antioxidant activity in all antioxidant assays The results of this research work are promising thus indicating the utilisation of the leaves of *Eugenia jambolana* as a significant source of natural antioxidants.

## KEY WORDS

Hydroalcoholic, Scavenging of hydroxyl radical, Total phenol and Antioxidant.

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## INTRODUCTION

An antioxidant is a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate. Antioxidants get their name because they combat oxidation[1]. They are substances that protect other chemicals of the body from damaging oxidation

reactions by reacting with free radicals and other hindering the process of oxidation. During this reaction the antioxidant sacrifices itself by becoming oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical[2]. Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation. A free radical is any atom with at least one unpaired electron in the outermost shell, and is capable of independent existence[3]. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom[4]. The *Eugenia jambolana* plant is well known plant for its medicinal properties in indigenous medicine, earlier studies carried out in *jambolana* species have proved its anti-inflammatory, diuretic, and antibacterial activities, anti-diabetic, anti-oxidant etc. Based on the traditional uses as well as earlier work on other species of *Eugenia jambolana*. *In vitro* antioxidant activity studies were selected in Eugenia jambolana leaves.

## MATERIAL AND METHOD

### Collection and Authentication of *E. jambolana* Leaves

The leaves of *E. jambolana* was collected in Vangapally (Village), Nalgonda (District) and authenticated by a botanist Dr. S. Srinivas rao, Swami Degree College, Bhongir (Mandal), Nalgonda (District), where voucher specimen was retained for further reference. The plant materials were dried under shade, coarsely powdered and separately subjected to extraction

### Preparation of successive extracts of *E. jambolana* leaves

The leaves of *E. jambolana* was shade dried and powdered and extracted (300 g) successively with 1.5 l of each of petroleum ether (60–80° C), chloroform, ethyl acetate and ethanol in a Soxhlet extractor for 18-20 h. The extract was concentrated under reduced pressure and controlled temperature (40-50° C). The petroleum ether extract yielded a dark brown sticky residue, weighing 10.20 g (3.40 % w/w), the reactive oxygen species within the body, hence chloroform extract yielded a dark green semi-solid residue, weighing 9.40 g (3.13 % w/w), the ethyl acetate extract yielded a dark green residue, weighing 8.50 g (2.83 % w/w) and ethanol extract yielded a greenish brown semisolid residue, weighing 10.80 g (3.60 % w/w), respectively.

### Preparation of Hydro-alcoholic Extract of *E. jambolana* Leaves

The dried leaves of *E. jambolana* was coarsely powdered and 200 g was extracted with 1 l of hydro-alcohol (Water 50 %: Ethanol 50 %) in a Soxhlet apparatus for 48 hrs. The extract was concentrated under reduced pressure and controlled temperature (40-50° C). The hydro-alcoholic extract yielded a dark green semisolid residue, weighing 21.8 g (10.90 % w/w). The extract was stored in air-tight container in a refrigerator at 4° C until further use.

### Phytochemical Investigation of Hydro-alcoholic Extracts of *E. jambolana* Leaves

The Preliminary phytochemical studies of hydroalcoholic extract of *E. Jambolana* leaves was carried out according to standard procedures.

## EXPERIMENTAL DESIGN

### *In Vitro* Antioxidant Studies of Extracts

To study the comparative antioxidant activity of the successive and hydroalcoholic extracts of *E. jambolana in vitro* antioxidant studies were carried out. The extracts having better antioxidant property were chosen for *in vivo* studies. The scavenging activities of the different extracts against different radicals were carried out according to the procedures described below. In all the experiments, the absorbance was measured against a blank solution that contained the extract or standard, without the reagent. A control was performed without adding extract or standard. The final concentration of the extract or standard was 1000 to 0.45 µg/ml. The results of all the *in vitro* antioxidant scavenging activities were expressed in terms of $IC_{50}$, which is the concentration of the sample required to scavenge 50 % of free radicals.

**Scavenging of ABTS Radical Method**

ABTS (54.8 mg) was dissolved in 50 ml of distilled water to 2 mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. It was left to stand at room temperature overnight in dark before usage. To 0.2 ml of various concentrations of the extract or standard added 1.0 ml of freshly distilled DMSO and 0.16 ml of ABTS solution to make a final volume of 1.36 ml. Absorbance was measured after 20 min at 734 nm[5].

**DPPH Assay**

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of various concentrations of the extract or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37º C for 30 min. Absorbance was measured at 490 nm using ELISA reader[6].

**Antilipid Peroxidation Assay**

Lipid peroxidation was initiated by adding ferric chloride (100 µM, 0.25 ml) to a mixture containing the rat brain homogenate (0.25 ml) and different concentrations of the extract or standard (0.25 ml) in a total volume of 0.75 ml. The reaction mixture was incubated for 20 min at 37° C. After incubation, the reaction was stopped by adding 1 ml of ice–cold 0.25 N HCl containing 15 % trichloroacetic acid, 0.38 % thiobarbituric acid and 0.05 % BHT. Followed by heating at 80° C for 15 min, samples were cooled, centrifuged at 1000 rpm for 10 min and absorbance of the supernatant was measured at 532 nm[7].

**Scavenging of Hydrogen Peroxide**

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide. The absorbance was measured after 10 min at 230 nm[8].

**Scavenging of Hydroxyl Radical by p-NDA Method**

To a reaction mixture containing ferric chloride (0.1 mM, 0.5 ml), EDTA (0.1 mM, 0.5 ml), ascorbic acid (0.1 mM, 0.5 ml), hydrogen peroxide (2 mM, 0.5 ml) and p–NDA (0.01 mM, 0.5 ml) in phosphate buffer (pH 7.4, 20 mM), were added various concentrations of the extract or standard in freshly distilled DMSO (0.5 ml) to give a final volume of 3 ml. Absorbance was measured at 440 nm[9, 10].

**Nitric Oxide Radical Inhibition**

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), PBS (pH 7.4, 1 ml) and the extract or standard solution (1 ml) were incubated at room temperature for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 540 nm[11].

**Evaluation of total antioxidant capacity**

An aliquot of 0.1 ml of sample solution containing a reducing species in freshly distilled DMSO was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95° C for 90 min. The samples were cooled to room temperature, and the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.

**Estimation of Total Phenol and total flavonol Content**

In a test tube, 200 µl of the extract (1 mg/ml to 0.1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 h for reaction. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml. Using the standard curve, the total phenol content of the extract was determined and expressed as gallic acid equivalent in mg/g of the extract. A known volume (0.5 ml) of the extract was mixed with 1.5 ml methanol, and 0.1 ml of 1 M potassium acetate. After 5 min, 0.1 ml of 10 % aluminium chloride was added and finally the mixture is diluted with 2.8 ml of distilled water. After

incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm in spectrophotometer. The total flavonol content was expressed as rutin equivalent in mg/g of the extract.

**Acute toxicity study as per OECD guideline 425**

Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labeling. LD (medium lethal 50 doses), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 % of animals when administered by the oral route. Healthy Wistar rats weighing between 180-220 g were used to carry out acute oral toxicity studies by the 'staircase' method. All successive extracts of *Eugenia jambolana* leaves in 0.5 % tween 80 was administered orally by gavages in graduated dose to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0,1,2,4 and 24 h for any mortality[12].

**RESULTS AND DISCUSSION**

Phytochemical screening of all the extract of *E. jambolana* leaves showed the presence of various chemical constituents, mainly tannins, saponins and flavonoids which may be responsible for it anti-oxidant properties was shown in Table No.1. The antioxidant activity of successive and hydro-alcoholic extracts of *E. Jambolana* leaves were carried out using ABTS, DPPH method, lipid peroxidation assay, hydrogen peroxide method, hydroxyl radical by p-NDA method and nitric oxide method with total antioxidant capacity and total phenol and flavonol contents. The scavenging activities extracts against different radicals were carried out according to the standard procedures. The results of all the *in vitro* antioxidant scavenging activities were expressed in terms of $IC_{50}$, which is the concentration of the sample required to scavenge 50 % of free radicals. Total antioxidant capacity was also carried out using the standard phosphomolybdenum method was show in Table No.2 to Table No.9. Acute toxicity test at 3000mg/kg of leaf extracts of *E. jambolana* leaves produced no mortality after 24 hours of observation. The median lethal dosage (LD50) of the hydro-alcoholic leaves extract was greater than 3000 mg/kg body weight. The extract did not produce any grossly negative behavioural changes such as excitement, restlessness, respiratory distress, convulsions or coma. However, a reduction in body weights of the rats was observed. The reduction in weight may be due to reduced fluid and water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract. Despite the above side effects, the very high value of the LD50 indicated that the extract of *E. jambolana leaves* is practically non-toxic.

**Table No.1: Qualitative Phytochemical Analysis of Successive and Hydro-alcoholic Extracts of *E. jambolana* Leaves**

| S.No | Tests | Successive extracts | | | | Hydroalcoholic extract |
|---|---|---|---|---|---|---|
| | | Petroleum ether | Chloroform | Ethyl acetate | Ethanol | |
| 1 | Carbohydrates | - | - | - | + | + |
| 2 | Alkaloids | - | + | + | + | + |
| 3 | Glycosides | - | - | + | + | + |
| 4 | Saponins | - | + | + | + | + |
| 5 | Tannins | - | - | + | + | + |
| 6 | Terpenoids | + | + | - | - | + |
| 7 | Flavonoids | - | - | + | + | + |
| 8 | Proteins and amino acids | - | - | + | + | + |
| 9 | Steroids and sterols | + | + | - | - | + |

***(+ Present, - Absent)**

**Table No.2: Antioxidant Activity of Various Extracts of *E. jambolana* Leaves by ABTs Radical Method**

| S.No | Extracts/ standards | $IC_{50}$ values (µg/ml)* |
|---|---|---|
| | | ***E. jambolana*** |
| 1 | Petroleum ether | >1000 |
| 2 | Chloroform | 132.84 ± 1.16 |
| 3 | Ethyl acetate | 51.92 ± 0.86 |
| 4 | Ethanol | 32.78 ± 1.06 |
| 5 | Hydro-alcoholic extract | 21.54 ± 1.24 |
| 5 | Ascorbic acid | 10.69 ± 0.35 |
| 6 | Rutin | 3.18 ± 0.14 |

***Average of three determinations.**

**Table No.3: Antioxidant Activity of Various Extracts of *E. jambolana* Leaves by DPPH Method.**

| S.No | Extracts/ standards | $IC_{50}$ values (µg/ml)* |
|---|---|---|
| | | ***E. jambolana*** |
| 1 | Petroleum ether | >1000 |
| 2 | Chloroform | 432.76 ± 0.86 |
| 3 | Ethyl acetate | 351.43 ± 0.46 |
| 4 | Ethanol | 123.54 ± 0.36 |
| 5 | Hydro-alcoholic extract | 82.38 ± 1.02 |
| 5 | Ascorbic acid | 2.69 ± 0.05 |
| 6 | Rutin | 3.19 ± 0.1 |

***Average of three determinations.**

**Table No.4: Antioxidant Activity of Various Extracts of *E. jambolana* Leaves by Anti lipid Peroxidation Assay**

| S.No | Extracts/ standards | $IC_{50}$ values (µg/ml)* |
|---|---|---|
| | | ***E. jambolana*** |
| 1 | Petroleum ether | >1000 |
| 2 | Chloroform | 268.85 ± 2.62 |
| 3 | Ethyl acetate | 196.92 ± 1.54 |
| 4 | Ethanol | 124.78 ± 1.28 |
| 5 | Hydro-alcoholic extract | 107.54 ± 1.56 |
| 6 | BHA | 95.26 ± 1.02 |

***Average of three determinations**

**Table No.5: Antioxidant Activity of Various Extracts of *E. jambolana* Leaves by Hydrogen Peroxide**

| S.No | Extracts/ standards | $IC_{50}$ values (µg/ml)* |
|---|---|---|
| | | ***E. jambolana*** |
| 1 | Petroleum ether | >1000 |
| 2 | Chloroform | 166.76 ± 1.12 |
| 3 | Ethyl acetate | 187.24 ± 1.09 |
| 4 | Ethanol | 135.36 ± 1.76 |
| 5 | Hydro-alcoholic extract | 104.68 ± 0.56 |
| 6 | Rutin | 32.46 ± 0.32 |

***Average of three determinations.**

**Table No.6: Antioxidant Activity of Various Extracts of *E. jambolana* Leaves by Scavenging of Hydroxyl Radical by p-NDA Method**

| S.No | Extracts/ standards | $IC_{50}$ values (µg/ml)* |
|---|---|---|
| | | ***E. jambolana*** |
| 1 | Petroleum ether | >1000 |
| 2 | Chloroform | 382.76 ± 1.34 |
| 3 | Ethyl acetate | 189.92 ± 1.42 |
| 4 | Ethanol | 214.45 ± 0.88 |
| 5 | Hydro-alcoholic extract | 207.54 ± 1.56 |
| 6 | Rutin | 194.26 ± 2.16 |

***Average of three determinations.**

**Table No.7: Antioxidant activity of Various Extracts of *E. jambolana* Leaves by Nitric Oxide Radical Inhibition**

| S.No | Extracts/ standards | $IC_{50}$ values (µg/ml)* |
|---|---|---|
| | | ***E. jambolana*** |
| 1 | Petroleum ether | >1000 |
| 2 | Chloroform | 282.76 ± 0.78 |
| 3 | Ethyl acetate | 213.34 ± 0.48 |
| 4 | Ethanol | 148.64 ± 1.32 |
| 5 | Hydro-alcoholic extract | 98.86 ± 0.88 |
| 6 | Rutin | 64.62 ± 1.42 |

***Average of three determinations**

**Table No.8: Total Antioxidant Capacity of Various Extracts of *E. Jambolana* Leaves**

| S.No | Extracts | Total antioxidant capacity (µM/g) |
|---|---|---|
| 1 | Petroleum ether | 0.56 ± 0.08 |
| 2 | Chloroform | 0.96 ± 0.04 |
| 3 | Ethyl acetate | 1.34 ± 0.06 |
| 4 | Ethanol | 1.86 ± 0.12 |
| 5 | Hydro-alcoholic extract | 2.46 ± 0.06 |
| 6 | α- tocopherol | 3.14 ± 0.04 |

***Average of three determinations**

**Table No.9: Total Phenol and Flavonol Contents of Various Extracts of *E. Jambolana* Leaves**

| S.No | Extracts | Total phenol content (mg/g)* | Total flavonol content (%mg/g)* |
|---|---|---|---|
| 1 | Petroleum ether | - | - |
| 2 | Chloroform | 66.96 ± 0.14 | 18.44 ± 0.24 |
| 3 | Ethyl acetate | 72.84 ± 0.16 | 22.94 ± 0.16 |
| 4 | Ethanol | 87.84 ± 0.12 | 34.56 ± 0.22 |
| 5 | Hydro-alcoholic extract | 94.16 ± 0.14 | 41.36 ± 0.12 |

***Average of three determinations.**

## CONCLUSION

The dried leaves were powdered and successive extracts were prepared by Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and ethanol as solvents. Hydro-alcoholic extract also prepared. The percentage yield of hydro-alcoholic extract was more when compared to all extracts. Qualitative phytochemical analysis for all the extracts was carried out by using standard procedures. The Presence of alkaloids, tannins, saponins. Flavonoids, glycosides were observed in all the extracts. In vitro antioxidant activity of all the extracts was carried out by different methods. In all methods the hydro alcoholic extract showed significant activity. Total phenol and total flavonol contents estimation was carried out for all extracts depending on the *in vitro* estimations. The

further scope for research in isolating and characterize the active phytoconstituents from the extract and to carry out the other biological properties of the extracts as well as isolated constituents. There is also a need to establish the mechanism of the observed activities.

**ACKNOWLEDGEMENT**

The authors are sincerely thankful to the CMJ University, Shillong, and Meghalaya, India for providing the facilities to complete this research work.

**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

**REFERENCES**

1. Halliwell B and Gutteridge J M C. The chemistry of oxygen radicals and other oxygen-derived species, *In Free Radicals in Biology and Medicine, New York, Oxford University Press,* 2nd edition, 1985, 20-64.
2. Acworth I N and B Bailey. Reactive Oxygen Species, in the handbook of oxidative metabolism. Massachusetts, *ESA Inc,* 1(1), 1997, 1- 4.
3. Alessio H M and Blasi E R. Physical activity as a natural antioxidant booster and its effect on a healthy lifestyle, *Res. Q. Exerc. Sport,* 68(4), 1997, 292-302.
4. Halliwell B and Chirico S. Lipid peroxidation its mechanism, measurement, and significance, *Am. J. Clin. Nutr,* 57(5), 1993, 715-725.
5. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization, *Free Radic Biol Med,* 26(9-10), 1999, 1231-1237.
6. Sreejayan N. and Rao M N A. Free radical scavenging activity of curcuminoids, *Drug Res,* 46(2), 1996, 169-171.
7. Sudheer Kumar M, Jagadish P C, Sridhar Reddy B, Kiran Babu S, Unnikrishnan M K. In vitro evaluation of antioxidant properties of *Cocos nucifera* Linn, *Water Food Nahrung,* 47(2), 2003, 126-131.
8. Guddadaranga vanahally K J, Lingamallu J R, Kunnumpurath K S. Antioxidant activities of flavidin in different *in vitro* model systems, *Bio org Med Chem,* 12(19), 2004, 5141-5146.
9. Kunchandy E and Rao M N A. Oxygen radical scavenging activity of curcumin, *Int J Pharma,* 58(3), 1990, 237-240.
10. Halliwell B, Gutteridge J M C, Aruoma O I. The deoxyribose method a simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals, *Anal Bioche,* 165(1), 1987, 215-9.
11. Marcocci L, Packer L, Droy-Lefaix M T, Sekaki A, Gardes-Albert M. Antioxidant action of *Ginkgo biloba* extract Methods, *Enzy mol,* 234, 1994, 462–75.
12. OCED 425 guidelines. OCED Guidelines for testing animals, 26, 2001, 1-26.

**Please cite this article in press as:** Shankar M and Suthakaran R. *In vitro* antioxidant activities of various extracts of *Eugenia jambolana leaves, International Journal of Research in Pharmaceutical and Nano Sciences,* 1(2), 2012, 317-326.