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## *In Vitro* Phytochemical Screening and Antioxidant Activity of Jamun (*Eugenia jambolana* Linn) Plants Parts Collected from Lahore, Pakistan

Nureen Zahra<sup>1\*</sup>, Momna Nadir<sup>1</sup>, Arif Malik<sup>1</sup>, Ayesha Shaukat<sup>2</sup>, Asia Parveen<sup>1</sup>, Muhammad Tariq<sup>3</sup>

### Affiliation:

<sup>1</sup>Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan

<sup>2</sup>University Institute of Radiology Sciences and Medical Imaging technology, Pakistan

<sup>3</sup>Faculty of pharmacy and alternative medicine, The Islamia University, Bahawalpur, Pakistan

\*Corresponding author: Nureen Zahra, Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan, Tel: +92 (0)42 111-865-865, E-mail: [nureenzahra@ymail.com](mailto:nureenzahra@ymail.com)

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### Abstract

Our research work based on the qualitative and quantitative phytochemical studies including the antioxidant activity of extracts (ethanolic and aqueous) obtained from leaf, stem and seed of *Eugenia jambolana* plant. Qualitatively phytochemical analysis was done by biochemical testing, quantitative estimation was taken spectrophotometrically and antioxidant activity of extracts was tested by DPPH radical scavenging. Qualitative and quantitative outcomes of our study explain that ethanol is applicable solvent system that comprises high quantity of bioactive compounds than water solvent system. Antioxidant activity revealed that ethanolic leaf, seed and stem extracts removed free radicals 68%, 98% and 33% respectively in contrast with water extracts of leaf, seed and stem 5.09%, 1.25% and 7.26% respectively. Ascorbic acid was taken as standard that kills 97% free radical species. Extracts in ethanol solvent system displayed protruding antioxidant activity and also contains high amount of phyto-chemicals.

**Keywords:** Antioxidant, *Eugenia jambolana*, Phytochemicals, Statistical analysis.

### Introduction

*Eugenia jambolana* (family Myrtaceae) has venerable curative properties for treatment of various disease. It is one of the broadly utilized therapeutic plants all through the world for the treatment of the numerous infections mainly diabetes mellitus. The characteristic parts of *E. jambolana* plant included leaves, seed, stem and fruit have excessive remedial worth [1].

*E. jambolana* habitually grows in the rain forest [2]. It is most frequently cultivated in Indian subcontinent, and also in many others adjoins regions of South Asia such as India, Bangladesh, Burma, Nepal, Pakistan, Sri Lanka, Indonesia, Philippines and Africa [1]. In 1911 it was introduced in Florida, United State. And also found in, Australia, and New Caledonia, Malagasy and southwestern region of the Pacific Islands, Hawaii and New Zealand [3].

It grips countless beneficial compounds that divulge numerous health benefits. A number of primary and secondary phytochemical bioactive compounds like phenol, flavonoids, alkaloids, saponins, tannins, terpenoids and steroids, which are valuable for spawning drugs for action numeral of illness covering with diabetes [1].

They give away various pharma-logical actions such as diabetes mellitus, stomach disorders, antioxidant, anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, anti-HIV, antifungal, antidiarrheal and antifertility [4]. *Eugenia jambolana* is highly rich in compounds covering alkaloid and glycoside is an effective for diabetes controlling and healing stomach disorders or inflammation bronchitis dysentery and controlling blood pressure.

The biochemical formation and antioxidant action of *E. jambolana* fruits have intended that tannins take out from *E. jambolana* fruit appear as better DPPH radical scavenging activity [5,6]. *E. jambolana* comprehends substances that have capability to keeps against oxidation damage and lessen swelling. *E. jambolana* plays vital role in liver protection, anti-hyperglycemic, anti-inflammatory, cardioprotective, and antioxidant [7].

### Research Aim

The aim of this research was to carry out detailed qualitative and quantitative phytochemical studies and antioxidant activities of the leaf, stem and seed of *E. jambolana* using ethanolic and aqueous extracts.

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## Materials and Methods

### Collection of Plant Material

The natural and aseptic plant parts Leaf, Stems and Seeds of *Eugenia jambolana* were collected carefully in November 18, 2016 from Lahore, Pakistan. The plant materials leaf, stems and seeds were properly cleaned and kept in room temperature.

### Preparation of extracts

Table 1 and Table 2

EJ.L.E	<i>Eugenia jambolana</i> leaf in ethanol
EJ.L.W	<i>Eugenia jambolana</i> leaf in water
EJ.S.E	<i>Eugenia jambolana</i> stem in ethanol
EJ.S.W	<i>Eugenia jambolana</i> stem in water
EJ.SE.E	<i>Eugenia jambolana</i> seeds in ethanol
EJ.SE.W	<i>Eugenia jambolana</i> seeds in water

Table 1: Six collected crude extracts.

Ethanolic Fractions (95% ethanol)		Dry Extracts	Water Fractions	Dry Extracts
EJ.L	40g	1.2g	60g	1.8g
EJ.S	40g	2.3g	40g	1.2g
EJ.SE	20g	1.06g	30g	1.4g

Table 2: Ethanolic Fractions extracts.

### Qualitative phytochemical analysis

Qualitative phytochemical analysis was investigated by biochemical testing with the extracts of leaf, stem and seeds of *Eugenia jambolana* to detect the presence or absence of numerous primary and secondary metabolites with the help of standard protocols [8-11].

### Quantitative Phytochemical Analysis

Quantitative phytochemical screening done by spectrophotometer method of ethanolic and aqueous extracts of *E. jambolana* to measure the total flavonoids, total alkaloids, total carbohydrate, total steroid, total phenol, total tannins and total carotenoids with the help of standard protocols [9].

### DPPH scavenging antioxidant assay

1ml of ethanolic and water extract of each sample was taken and 1ml of DPPH was pipetted in all the samples very carefully. The reaction mixture was kept in dark for 24h. After incubation period, absorbance was measured at 517nm. Standard curve was drawn by taking ascorbic acid as standard.

## Result

### Qualitative phytochemical analysis

To check the qualitative phytochemical analysis of *E. jambolana* leaves, stem and seeds having the solvent water and ethanol. Current study showed the presence of primary and secondary phytoconstituents in ethanol and water extracts of *E. jambolana*. Some of the active ingredients present in each plant part under different extraction method were carbohydrate, saponins (Table 3).

### Quantitative phytochemical analysis

Current study exposed that ethanolic leave and seed extracts contain highest quantity of bioactive compounds as compared to water solvent system as shown in Table 4. These bioactive compounds have great medicinal importance as it attributed high anti-diabetic, anti-oxidant, anti-inflammatory, anti-microbial, anti-bacterial, anti-HIV, and anti-fungal (Table 4) (Figures 1-6).

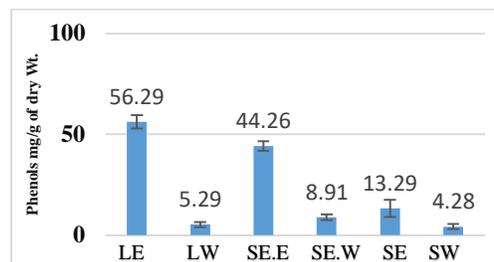


Figure 1: Quantification of Phenol.

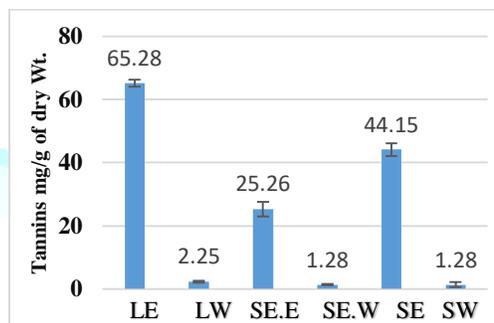


Figure 2: Quantification of Tannins.

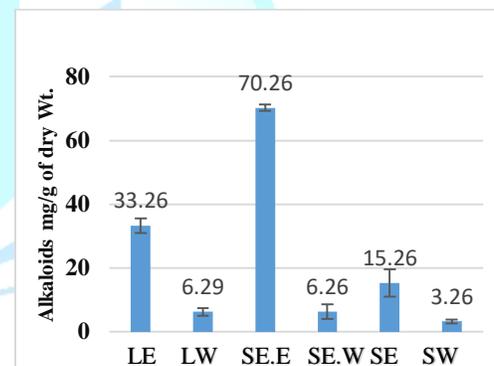


Figure 3: Quantification of Alkaloids.

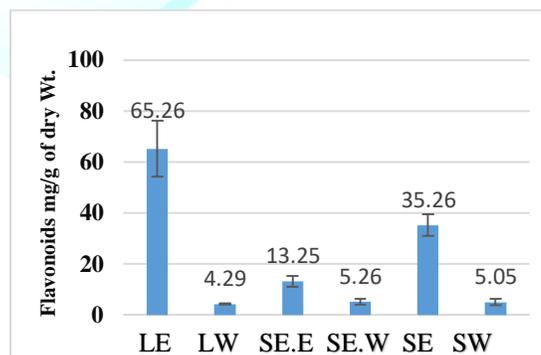


Figure 4: Quantification of Flavonoids.

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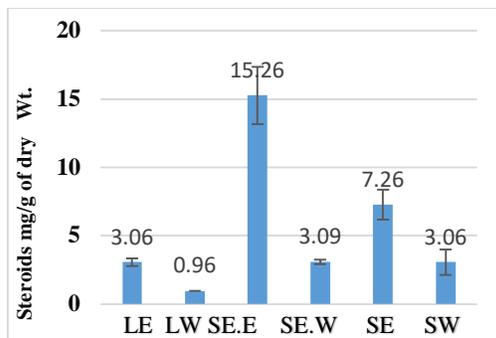


Figure 5: Quantification of Steroids.

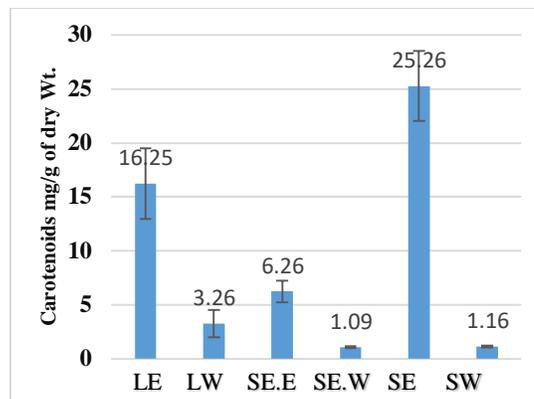


Figure 6: Quantification of Carotenoids.

Phytochemicals	EJ.L.W	EJ.L.E	EJ.S.W	EJ.S.E	EJ.SE.W	EJ.SE.E
Carbohydrate	-	-	+	+	+	+
Saponins	+	+	+	+	-	+
Proteins	-	-	-	+	+	-
Phenol	+	+	-	+	+	+
Tannins	+	+	-	+	+	+
Glycosides	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Steroids	+	-	+	+	+	+
Terpenoids	+	+	+	+	+	-

Table 3: Qualitative phytochemical constituents of *Eugenia jambolana* leaves, stems and seeds extracts in the water and ethanol solvent system.

Solvent extractions mg/g of dry extract	Phenols	Tannins	Alkaloids	Flavonoids	Carotenoids	Steroids
EJ.L.E	56.29±3.26	65.28±1.15	33.26±2.35	65.26±11.06	16.25±3.28	3.06±0.26
EJ.L.W	5.29±1.25	2.26±0.30	6.29±1.22	4.29±0.28	3.26±1.26	0.96±0.004
EJ.S.E	13.29±4.18	44.15±2.09	15.26±4.26	35.26±4.28	25.26±3.25	7.26±1.09
EJ.S.W	4.28±1.16	1.28±0.8	3.26±0.60	5.05±1.28	1.16±0.09	3.06±0.91
EJ.SE.E	44.26±2.29	25.26±2.26	70.26±1.06	13.25±2.09	6.26±1.00	15.26±2.08
EJ.SE.W	8.91±1.29	1.28±0.16	6.26±2.26	5.26±1.08	1.09±0.08	3.09±0.16

Each value is the average of three analyses (Mean) ± standard deviation (SD).

Table 4: Phytochemical characterization of *Eugenia jambolana*.

## Result of Antioxidant activity

DPPH radical scavenging activity assay make known the significant antioxidant activity of *Eugenia jambolana* ethanolic seed and leaves extract than other extracts as shown in Table 5 and Figure 7.

Plant Extract	%
Leaf Ethanol (LE)	68.29±4.29
Leaf Water (LW)	5.09±1.39
Seed Ethanol (SE.E)	98.29±4.26
Seed Water (SE.W)	1.25±0.055
Stem Ethanol (SE)	33.29±4.26
Stem Water (SW)	7.26±1.56
Ascorbic Acid	97.86±6.77

Table 5: DPPH free radical scavenging activity of *Eugenia jambolana* (%).

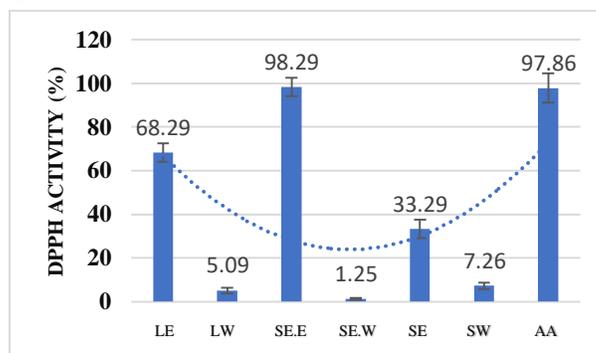


Figure 7: DPPH scavenging activity of *Eugenia jambolana* in ethanol and water solvent.

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## Discussion

Qualitative analysis of phytochemical compounds of *Eugenia jambolana* in methanolic extract and reveals the presence of amino acid, flavonoids, alkaloids, glycosides, saponins, tannins, steroids and triterpenoids. Recent results indicates that some phytochemicals components are present in both solvents, some presents only in ethanolic solvent and others are present in water solvent system [11]. A higher content of both total phenolics and flavonoids were found in the methanolic extract compared with other extracts [9]. Our current study of spectrophotometric analysis to quantified phytochemicals revealed that phenols, tannins, alkaloids, flavonoids, carotenoids and steroids present in high amount in ethanol extracts as comparison with water extracts.

Antioxidant activity of all extracts of *Eugenia jambolana* were examined by using two methods, namely DPPH and FRAP. In both methods, the methanol extract exhibited a higher antioxidant activity than methylene chloride and essential oil extracts [9]. Our present results demonstrate that ethanolic extracts contained maximum antioxidant activity than water extracts. The series of distribution of antioxidant activity was ethanolic seed extract>ethanolic leave extract>ethanolic stem extract>water stem extract>water leave extract>water seed extract.

## Conclusion

The purpose of present research work was to determine the phyto-constituents present in different parts of *Eugenia jambolana* qualitatively and quantitatively phytochemical screening, and to investigate the antioxidant activity *E. jambolana* leaves, seeds and stem in ethanolic extracts exhibits great amount of primary and secondary metabolites than the extracts of water solvent system. Quantification results of ethanolic and water extracts presented that it contains secondary metabolites while ethanolic leaves and seed contains maximum secondary phyto-constituents. Quantified result moreover demonstrated that some bioactive components are also extant in extracts of *Eugenia jambolana* in average amount but they were not identified by qualitative phytochemical screening. *Eugenia jambolana* shows noticeable antioxidant activity in ethanolic solvent system as compared to water solvent system while ethanolic seeds and leaves keeps high antioxidant activity because it contains rich amount of phytochemicals.

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