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IN-VITRO FREE RADICAL SCAVENGING ACTIVITY STUDIES OF EXTRACTS AND ISOLATED COMPOUNDS OF *EUGENIA JAMBOLANA* LAM. SEEDS

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ABSTRACT

Background: *Eugenia jambolana* seeds of *Myrtaceae* family are substantial ayurvedic medicine in treating diabetes. It is also narrated to have anti-inflammatory, hypoglycemic, antiulcerative, hypolipidemic, antiviral, ameliorative and antioxidant activities due to flavonoids, tannins, triterpenoids, saponins and glycosides. **Objective:** Various successive Soxhlet extractives [petroleum ether (PEE), $CHCl_3$ (CE), ethyl acetate (EAE) and methanol (ME)] and isolated compounds [IC1 and IC2] of *E. jambolana* seeds were inflicted to free radical scavenging activities employing DPPH and H_2O_2 radicals procedure which could be the main cause of degenerative diseases. **Materials and Methods:** All the extracts and isolated compounds were established to predicate good antioxidant activity against DPPH and H_2O_2 radicals as compared to standard Ascorbic acid. PEE and CE showed very less antioxidant activity when compared to standard, EAE, ME, IC1 and IC2. Standard, PEE, CE, EAE, ME, IC1 and IC2 offered utmost DPPH radical scavenging activity of 88.49, 17.40, 22.59, 61.08, 72.12, 54.55 and 66.62 µg/ml at concentration of 500 µg/ml and for H_2O_2 scavenging, ultimate activity of 82.09, 24.25, 27.23, 67.61, 78.85, 60.53 and 71.36 µg/ml at concentration of 50 µg/ml. The antioxidant activity was extreme in ME and IC2. **Results:** The IC_{50} of Standard, EAE, ME, IC1 and IC2 for DPPH radical and H_2O_2 radical was

pioneered 265, 360, 295, 380 and 220 µg/ml and 26, 33, 27,37 and 27 µg/ml respectively. **Conclusion:** The separation and identification of flavonoids, tannins, glycosides and triterpenoid saponins present in seeds of *E. jambolana* can help the researchers to detect new molecules as natural antioxidants.

KEYWORDS: Antioxidant, DPPH, *Eugenia jambolana*, H_2O_2, IC_{50}, Isolated compound.

INTRODUCTION

Antioxidants are substances, which help to defend the body against cell damage by various free radicals. Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Free radicals are molecules containing unpaired electrons. Most of the free radicals are produced by mitochondria and most free radicals in biological system are derivatives of oxygen ("Reactive Oxygen Species" ROS), but there are also derivatives of nitrogen ("Reactive Nitrogen Species" RNS). Synthetic anti-oxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) have restricted use in food industry as they are suspected to be carcinogenic. Moreover, the synthetic anti-oxidants also show low solubility and moderate anti-oxidant activity. Therefore, many researchers are in search for anti-oxidants of natural origin.[1]

Eugenia jambolana Lam. (Synonym: *Syzygium cumini* Linn.) of family *Myrtaceae*, is widely spread in the plains from sub-Himalayas to South India[2] and is a popular traditional medicinal plant in India. *E. jambolana* is prescribed for management of diabetes and several-marketed formulations contain the seeds of *Eugenia jambolana* as an ingredient. Seed powder is widely used in diabetes and urine related troubles. It is also used in indigestion, loss of appetite, vomiting and fever. It has been reported to have hypoglycemic, antidiabetic, ameliorative, antioxidant, anti-hyperlipidemic, antiviral, anti-inflammatory which may be due to the presence of flavonoids, saponins, glycosides and triterpenoids in the extract.[3-24] Therefore, our aim was to evaluate the free radical scavenging activity of various successive extracts and isolated compounds of *E. jambolana* seeds to determine its anti-oxidant properties and its uses in different diseases.

MATERIALS AND METHODS

Raw material characterization and preparation

The seeds of the plant *E. jambolana* were acquired from the forest region of Coimbatore District of Tamil Nadu, India. The seed material of the plant was ascertained by Agronomy

Dept., The Tamil Nadu Agricultural University, Coimbatore, India. The accrued material was dried under shade, powdered well with a mechanical grinder and stored in an air-tight container.

Chemicals

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) was received from Sigma Chemicals, USA. The other chemicals and solvents of analytical reagent grade accomplished from S. D. Fine Chemicals Ltd., India, Qualigens Fine Chemicals Ltd., Mumbai, India and Ranbaxy Chemicals Ltd., New Delhi, India.

Extraction

The seed powder was condemned to successive extraction with petroleum ether, chloroform, ethyl acetate and methanol utilizing Soxhlet Extractor at 30-45 ºC (5 days for each extraction).

Preliminary phytochemical evaluation

The preliminary phytochemical screening of the four consecutive extracts was executed to know the aberrant constituents existing in the seeds of *E. jambolana* as per the standard procedures. The extracts were tested for tannins, flavonoids, chlorophyll, alkaloids, glycosides and triterpenoids.[25-27] The presence of flavonoids and tannins was confirmed by Thin layer chromatographic analysis using mobile phase as $CHCl_3$:CH_3COCH_3: HCOOH (75:16.5:8.5 %v/v/v) and detected under UV light.[28]

Isolation

Two unknown isolated compounds were recovered from ethyl acetate extract by Preparative TLC method using mobile phase as $CHCl_3$: CH_3COCH_3: HCOOH (75:16.5:8.5 %v/v/v) at the R_F values of 0.4 (IC1) and 0.9 (IC2). The isolated compounds were checked for purity (single spot/compound) by TLC, HPTLC and HPLC analysis. The spots were distinguished under UV light because the extract contains fluorescence compounds which avoid using spray reagents. The extracts and isolated compounds obtained were weighed and stored at -4 ºC until further analysis.

DPPH free radical scavenging activity[29]

Free radical scavenging potentials of PEE, CE, EAE and ME of *Ej* seeds and IC1 & IC2 were proven against a methanolic solution of DPPH.[29] 0.1 mM solution of DPPH in methanol was

prepared and 1.0 ml of this solution was added to 3.0 ml of different concentrations (100-500 µg/ml) of PEE, CE, EAE and ME of *Ej* seeds prepared in water. It was incubated at room temperature for 30 minutes and the absorbance was measured at 517 nm against the corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contained DPPH and distilled water without any extract using the following equation.

% Scavenging Activity = [(Ac- As) / Ac] × 100

Where, Ac is the absorbance of the control reaction and As is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Scavenging of Hydrogen Peroxide[30]

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by recording the absorbance at 230 nm. Different concentrations of PEE, CE, EAE and ME of *Ej* seeds and IC1 & IC2 (10-50 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by PEE, CE, EAE and ME of *Ej* seeds and IC1 & IC2 and ascorbic acid (standard) was calculated using following equation.

% Scavenged [H_2O_2] = [(Ac - As)/Ac] × 100

The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of hydrogen peroxide radicals by 50%.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the in-vitro antioxidant activity of crude plant extracts.[31-32] DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[33] The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by

antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation.[34]

Fig. 1 and fig. 2 epitomize DPPH scavenging activity of PEE, CE, EAE and ME and of IC1 and IC2 are compared with standard Ascorbic Acid. Table No.1 emphasizes % maximum antioxidant activity and IC_{50} values of standard, PEE, CE, EAE, ME, IC1 and IC2.

Scavenging of Hydrogen Peroxide

Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems. Scavenging of H_2O_2 by antioxidants may be due to donation of electrons to H_2O_2, thus neutralizing it to water.[35]

Fig. 3 and fig. 4 delineate H_2O_2 scavenging activity of PEE, CE, EAE and ME and of IC1 and IC2 are compared with standard Ascorbic Acid. Table No. 2 annotates % maximum antioxidant activity and IC_{50} values of standard, PEE, CE, EAE, ME, IC1 and IC2.

Figures

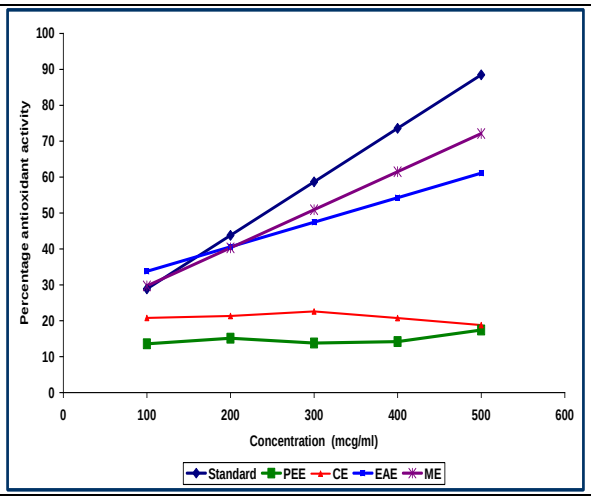


Fig. 1 - DPPH Scavenging Activity of PEE, CE, EAE and ME are compared with Standard Ascorbic Acid

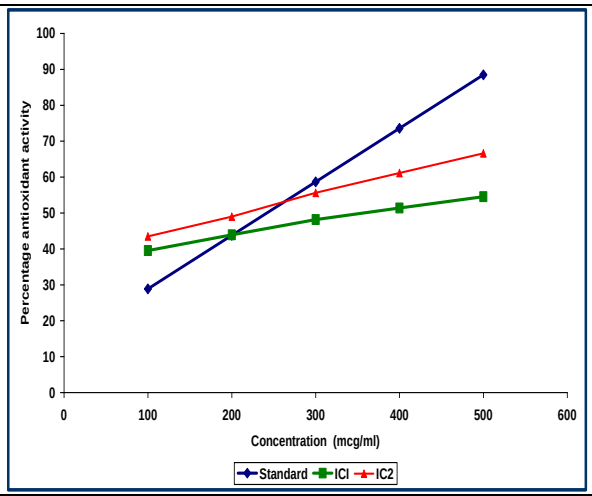


Fig. 2 - DPPH Scavenging Activity of IC1 and IC2 are compared with Standard Ascorbic acid

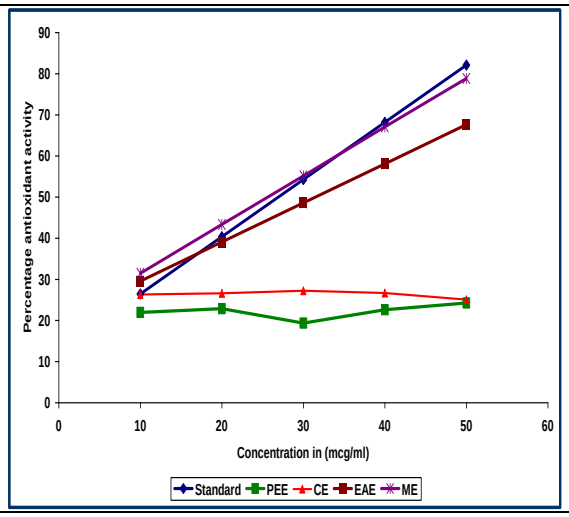


Fig. 3 - Hydrogen Peroxide Scavenging Activity of PEE, CE, EAE and ME are compared with Standard Ascorbic Acid

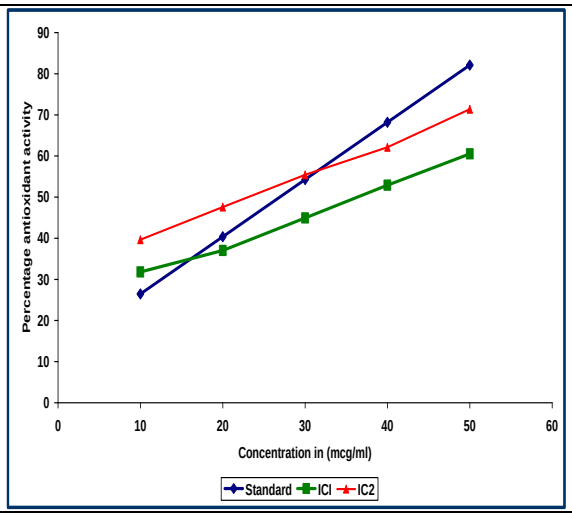


Fig. 4 - Hydrogen Peroxide Scavenging Activity of IC1 and IC2 are compared with Standard Ascorbic Acid

Table No. 1 - % Maximum DPPH free radical scavenging activity and IC_{50} of Standard, PEE, CE, EAE, ME, IC1 and IC2

S. No.	Sample	% maximum Anti-oxidant activity [%]	IC_{50}
1	Standard	88.4878	265
2	PEE	17.3969	–
3	CE	22.5945	–
4	EAE	61.0825	360
5	ME	72.1219	295
6	IC1	54.5533	380
7	IC2	66.6237	220

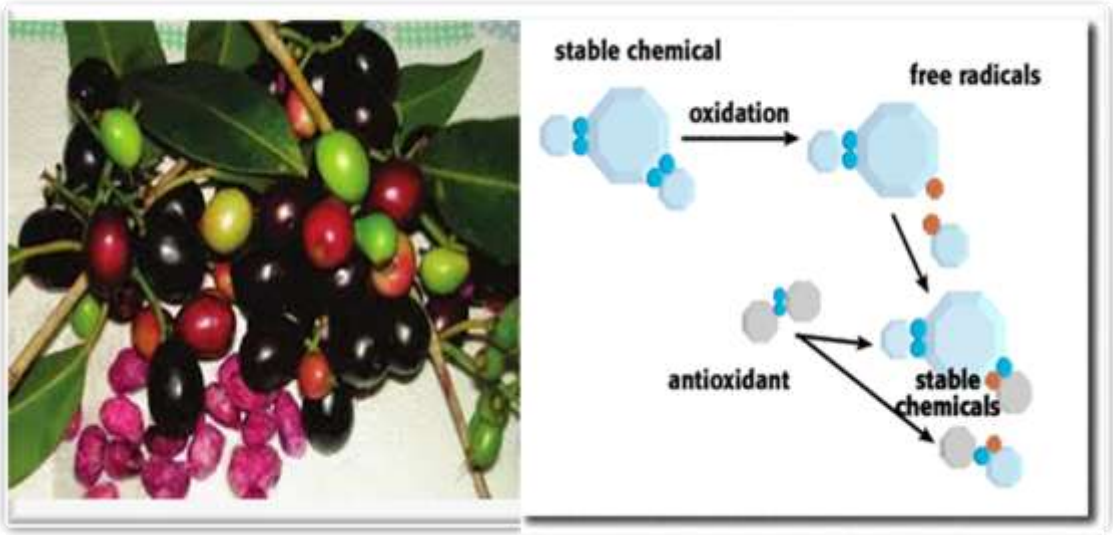
Note: PEE – Petroleum ether extract; CE – Chloroform extract; EAE – Ethyl acetate extract; ME – Methanol extract; IC1 – Isolated compound 1; IC2 – Isolated compound 2; IC_{50} – 50% Inhibitory concentration.

Table No. 2 - % Maximum H_2O_2 free radical scavenging activity and IC_{50} of Standard, PEE, CE, EAE, ME, IC1 and IC2

S. No.	Sample	% maximum Anti-oxidant activity [%]	IC_{50}
1	Standard	82.0938	26
2	PEE	24.2550	–
3	CE	27.2350	–
4	EAE	67.6082	33
5	ME	78.8546	27
6	IC1	60.5338	37
7	IC2	71.3656	27

Note: PEE – Petroleum ether extract; CE – Chloroform extract; EAE – Ethyl acetate extract; ME – Methanol extract; IC1 – Isolated compound 1; IC2 – Isolated compound 2; IC_{50} – 50% Inhibitory concentration

PICTORIAL ABSTRACT



CONCLUSION

The preliminary phytochemical investigation and TLC studies indicate the presence of flavonoids, tannins, triterpenoid saponins and glycosides in seeds of *E. jambolana* Lam. Polyphenols like flavonoids and tannins are the well known natural antioxidants. So, the antioxidant potential of extracts and isolated compounds may be due to the presence of flavonoids and tannins. The results of the present study show that the extracts and isolated compounds of seeds of *E. jambolana* Lam. possess good antioxidant activity when compared with standard Ascorbic acid. The antioxidant activity was highest in methanolic extract and IC2. PEE and CE showed very less antioxidant activity when compared with other extracts (EAE and ME) standard and isolated compounds. Hence, *E. jambolana* Lam. seeds can be used as the potential source for natural antioxidants. Characterization and formulation development of IC1 and IC2 can be used in modern medicine for treating the diseases caused by free radicals.

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