In vitro antioxidant activity of *Erycibe paniculata* Roxb. – An ethnomedicinal plant

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Abstract

Background: *Erycibe paniculata* Roxb. (Family-Convolvulaceae) has been reported for its potential ethno medicinal value. Leaf, stem, bark, and root of this plant are being used either single or in the mixture of the whole part in different disease conditions by different tribes. **Aims and Objectives:** The aim and objective of this study is to assess the antioxidant activity of methanolic extracts of different parts (leaf, stem, bark, and root) of *Erycibe paniculata* Roxb *(E. paniculata)*. **Materials and Methods:** Different *in-vitro* assay such as free radical-scavenging assay by 2,2-diphenyl-1-picryl-hydrazyl-hydrate method, reducing power, super oxide radical scavenging, nitric oxide, and hydrogen peroxide scavenging assays were used to determine the antioxidant activity of different parts of *E. paniculata*. Ascorbic acid, sodium nitrite, and gallic acid were used as the standards for antioxidant activity. **Results:** The percentage inhibition for all methods were plotted against different concentration and suggested that the obtained activities were concentration and dose depended. Inhibitory concentration (IC_{50}) value of methanolic extract of leaf, stem, bark, and root of *E. paniculata* in different *in vitro* activities exhibited significant antioxidant activity. Methanolic extract of bark showed higher IC_{50} value in all antioxidant assays than other parts of *E. paniculata*. **Conclusion:** Methanolic extract of leaf, stem, bark, and root of *E. paniculata* has potential antioxidant activity.

Keywords: Antioxidant, *Erycibe paniculata*, ethno-medicinal, *in vitro*, *Kari, Khoil Khamar*

Introduction

Antioxidants are an inhibitor of the process of oxidation even at relatively small concentration, and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers and help in converting the radicals to less reactive species.[1] A variety of free radical-scavenging antioxidants are found in dietary sources such as fruits, vegetables and tea. Medicinal plants are an important source of antioxidants. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke. The secondary metabolites such as phenolic and flavonoids from plants have been reported to be potent free radical scavengers.[2] They are found in all parts of plants such as leaves, fruits, seeds, roots, and bark. Due to toxicological concerns of synthetic antioxidants,[2,3] there have been increasing interests in identifying phenolic compounds in plants to minimize or retard lipid oxidation in lipid-based food products. Therefore, the role of antioxidants in human health are surmount popularities. In Ayurveda, many drugs have been listed for their *Rasayana* properties. Plants such as *Haridra* (*Curcuma longa* L.), *Rasona* (*Allium sativum* L.), *Amalaki* (*Emblica officinalis* Gaertn.), *Guduchi (Tinospora cordifolia* Thunb. Miers), *Karvellaka* (*Momordica charantia* L.) and *Tulasi* (*Ocimum sanctum* Linn) have been proved for their antioxidant activity.[4] Screening of plants for antioxidant activity is being carried out through various standard *in-vitro* models such as 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, nitric oxide method; ferric-reducing ability of plasma (FRAP) method, super oxide dismutase method, and hydrogen peroxide method.[5]

Erycibe paniculata Roxb *(E. paniculata)*, family – Convolvulaceae is a climbing shrub; young branch

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Quick Response Code:

Website: www.ayujournal.org

DOI: 10.4103/ayu.AYU_282_19

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How to cite this article: Patel MR, Patel AG, Gamit RV, Nariya MK, Acharya R. *In vitro* antioxidant activity of *Erycibe paniculata* Roxb. – An ethnomedicinal plant. AYU 2019;40:256-61.

Submitted: 03-Oct-2019 **Revised:** 17-Jan-2020

Accepted: 14-Jul-2020 **Published:** 14-Jan-2021

lets rusty-pubescent or-tomentose, leaves obovate, fairly common in the forest of Odisha (India), Sri Lanka and the surrounding region and known as Chain Katho, Kari and Khoil Khamar in odiya.[6] Various parts of *E. paniculata* have been reported for their ethno-pharmacological activities. Bark is used to manage cholera;[7] roots to treat post-delivery complications; extract of young leaves to treat night blindness;[8] whole plant is reported as a diuretic and hypotensive; plant infusion as a gargle to treat inflamed gum; bark decoction administrated once a day for a seven days in case of chronic malaria; and bark powder is used in diarrhoea and fever.[9] Many of these potential biological activities may be due to its antioxidant properties which have not been reported yet. Therefore, the present study is attempted to evaluate *in vitro* antioxidant activities of different parts of *E. paniculata* through established methods.

Materials and Methods

Drug

E. paniculata was first identified by the botanist and then identified on the basis of its morphological characters with the help of local flora[6] and the collected from its natural habitat Paikmal (Altitude, latitude, and longitude – 869 ft, 20.5°N and 82.4°E, respectively), Odisha, during the month of December–January 2018. Plant herbarium was authenticated from Botanical survey of India (BSI), Kolkata (CNH/Tech. II/2019/41) as *E. paniculata* Roxb. of family-convolvulaceae. A specimen of the plants herbarium has also been deposited in the Pharmacognosy laboratory, Institute for Postgraduate Teaching & Research in Ayurveda (Specimen No. IPGT and RA. Phm. 6294/18-19) for future reference.

Preparation of extract

The collected plant samples (leaves, stem, bark and root of *E. paniculata*) were washed under running fresh water to remove adherent soil and dirt. Leaves, stem, bark and root were detached from the plants., and individually shade dried. After proper drying, all the parts were powdered individually through mechanical grinder, 5 g of each part was macerated with 100 ml methanol, in a conical flask, for 24 h, shaking frequently during six hours and allowed to stand for eighteen hours. After 24 h, filtered, dried on evaporating and methanol extracts of different parts were collected.[10] Coding of samples as: Leaf (EL), stem (ES), bark (EB) and root (ER).

Preparation of stock solution

The standard such as ascorbic acid, sodium nitrite, gallic acid and extract of each part was accurately weighed into clean and dry volumetric flasks, dissolved in methanol and the volume was made up to 10 ml using the same solvent to make the concentration of the solution as 1 mg/ml.

Chemicals and glassware

All the chemicals were used of analytical grade (Sigma-Aldrich, SRL, Merck and Renkem), and glassware used in the present study was calibrated and of scientific grade (Borosil).

Antioxidant Assays

2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical-scavenging activity

0.1 mM solution of DPPH in ethanol was prepared. 1 ml of this solution was added to 3 ml of methanol extract of *E. paniculata* at different concentrations (100, 200, 300, 400, 500 and 600 µg/ml). The mixture was shaken vigorously and allowed to stand at the room temperature for 30 min and then absorbance was measured at 517 nm by using the UV-VIS spectrophotometer 1800 (Shimadzu). Reference standard compound being used was ascorbic acid. The inhibitory concentration (IC_{50}) value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical was calculated using log dose-inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.[11]

Ferric reducing antioxidant power assay

Different concentrations (10, 20, 40, 60, 80 and 100 µg/ml) of all the extracts were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of potassium hexacynoferrate solution (1% w/v). The mixture was incubated at 50ºC in water bath for 20 min. After incubation, 2.5 ml of trichloroacetic acid (10% w/v) was added to terminate the reaction and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was mixed with equal volume of distilled water and 0.5 ml of ferric chloride solution (0.1% w/v) and the absorbance was measured at 700 nm against an appropriate blank solution. Ascorbic acid at various concentrations (10–100 µg/ml) was used as a standard. Increased absorbance of the reaction mixture indicated increased reducing power.[12]

Superoxide radical scavenging activity

Different concentrations (25, 50, 75, 100, 200 and 400 µg/ml) of all the four extracts were mixed with 0.5 ml of each reagent. After noticing the initial reading, all the mixtures were kept under the incandescent lamp for 15 min. Then, absorbance was measured at 530 nm by using UV-VIS spectrophotometer 1800 (Shimadzu). Ascorbic acid at various concentrations (25–400 µg/ml) was used as a standard.[13]

Nitric oxide radical scavenging activity

Sodium nitroprusside (10 mM) in phosphate-buffered saline was mixed with different concentrations of all three extracts of plant dissolved in their respective solvents at different con centration (25, 50, 75, 100, 150 and 200 µg/ml) and incubated at the room temperature for 150 min. After the incubation period, 0.5 ml of griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the chromophores formed was read at 546 nm. Sodium nitrite was used as standard.[14]

Hydrogen peroxide scavenging assay

The ability of extracts to scavenge hydrogen peroxide was determined by little modification. The solution of hydrogen peroxide (100 mM) was prepared of 40 mM in phosphate buffer saline of PH 7.4 , at various concentration of methanolic, extracts (20-120 µg/ml) were added to hydrogen

peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. Absorbance was taken at 230 nm.[15]

Results and Discussion

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPHH) scavenging assay method

DPPH scavenging assay method is one of the most widely used method to evaluate the free radical-scavenging activity. DPPH free radical process is based on electron transfer that produces a violet solution in alcohol. Reactive oxygen compounds (ROS) are essential cellular components, enzymatically generated in aerobic living organisms, which play a key role in different pathological and physiological processes. Particularly at low levels, ROS take part in signal transduction, gene transcription, and regulation of soluble guanylate cyclase activity.[16, 17] This assay is mainly based on the theory that a hydrogen donor is an antioxidant. It measures the compounds that are radical scavengers.[11]

In present study, it was observed that the percentage inhibition verses concentration showed the IC_{50} value as Erycibe leaf (EL) 439.78 (R^2 = 0.9953), Erycibe stem (ES) 458.96 (R^2 = 0.9631), Erycibe Bark (EB) 430.69 (R^2 = 0.9542), Erycibe root (ER) 452.13 (R^2 = 0.9939) and STD-420.44 (R^2 = 0.9983) µg/ml for same methenolic extract and ascorbic acid, respectively [Table 1 and Figure 1a and b]. Above data exhibited that the bark extract has captured more free radicals formed by DPPH than the leaf, root and stem respectively when compared with ascorbic acid as a standard.

Ferric reducing antioxidant power assay

Reducing power is the ability of a chemical to reduce other substances. It is another way of saying redox potential. Reduction of a ferric complex to ferrous form, which has an intense bluish green color, this color changes absorbance is directly related to the total reducing power of the electron-donating antioxidants.[18] In present study, it was observed that the percentage of inhibition verses concentration showed the IC_{50} values as EL-34.1 (R^2 = 0.9869), ES-39.5 (R^2 = 0.9634), EB-33.06 (R^2 = 0.9877), ER-36.98 (R^2 = 0.9900) and STD-22.02 (R^2 = 0.9898) µg/ml for same methanolic extract and ascorbic acid respectively [Table 2 and Figure 2a and b]. Above data exhibited that the EB has marked antioxidant value, whereas EL, ER and ES have moderate values compared with ascorbic acid as a standard. Thus, methanolic extract of leaf, stem, bark, and root showed potential antioxidant properties when compared to ascorbic acid as a standard.

Superoxide radical

The super oxide anion is one of the most important radical in the formation of reactive species. It is an enzyme that helps to break down potentially harmful oxygen molecules in cells, which might prevent damage to tissues.[19] It is being researched to see if it can help conditions where oxygen molecules are believed to play a role in disease. In present study, it was observed that the percentage of inhibition verses concentration showed the IC_{50} values as EL-331.4 (R^2 = 0.9911), ES-335 (R^2 = 0.99776), EB-324.37 (R^2 = 0.9887), ER-332.8 (R^2 = 0.9895) and STD-301.4 (R^2 = 0.9955) µg/ml for same methenolic extract and ascorbic acid respectively [Table 3 and Figure 3a and b]. Above data exhibited that the EL has marked antioxidant value, whereas EL, ER and ES have moderate values compared to ascorbic acid as a standard. Thus, methenolic extract of leaf, stem, bark and root showed potential antioxidant properties when compared to ascorbic acid as a standard.

Table 1: 2, 2-diphenyl-1-picryl-hydrazyl-hydrate free radical-scavenging activity

Concentration (µg/ml)	Percentage inhibition				
	STD	EL	ES	EB	ER
100	88.30	81.87	77.19	74.85	77.78
200	78.36	70.76	71.35	66.08	71.35
300	64.33	61.40	56.73	59.06	60.23
400	51.46	53.22	53.22	58.48	53.80
500	40.94	46.20	47.37	42.11	47.37
600	28.07	35.09	41.52	36.26	38.01
IC50 (µg/ml)	420.44	439.78	458.96	430.69	452.13

STD: Standard, EL: Erycibe leaf, ES: Erycibe stem, EB: Erycibe bark, ER: Erycibe root, IC50: Inhibitory concentration

Table 2: Ferric reducing antioxidant power assay

Concentration (µg/ml)	Percentage inhibition				
	STD	EL	ES	EB	ER
10	44.30	36.91	39.37	37.14	35.79
20	49.44	42.28	43.18	43.18	41.83
40	60.18	51.01	45.41	54.14	51.01
60	69.35	63.09	56.38	61.74	60.63
80	85.01	78.97	69.13	78.52	71.81
100	99.78	95.08	78.97	92.62	87.70
IC50 (µg/ml)	22.02	34.1	39.5	33.06	36.98

STD: Standard, EL: Erycibe leaf, ES: Erycibe stem, EB: Erycibe bark, ER: Erycibe root, IC50: Inhibitory concentration

Table 3: Superoxide radical-scavenging activity

Concentration (µg/ml)	Percentage inhibition				
	STD	EL	ES	EB	ER
25	5.63	1.40	1.87	3.75	4.22
50	8.45	4.69	2.81	6.57	5.63
75	14.08	11.73	7.98	7.51	9.38
100	18.77	11.26	10.79	14.55	16.90
200	36.61	32.39	27.69	34.27	33.33
400	64.78	59.62	58.68	60.56	58.68
IC50 (µg/ml)	299.69	331.41	345.03	324.37	332.29

STD: Standard, EL: Erycibe leaf, ES: Erycibe stem, EB: Erycibe bark, ER: Erycibe root, IC50: Inhibitory concentration

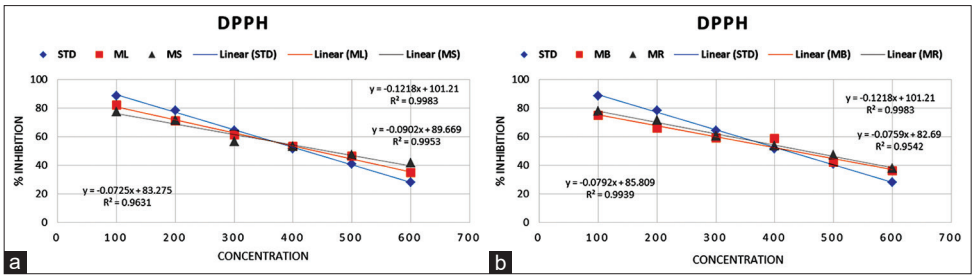

Figure 1: (a) 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical-scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe leaf (EL) and Erycibe stem (ES), (b) 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical-scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe bark (EB) and Erycibe root (ER)

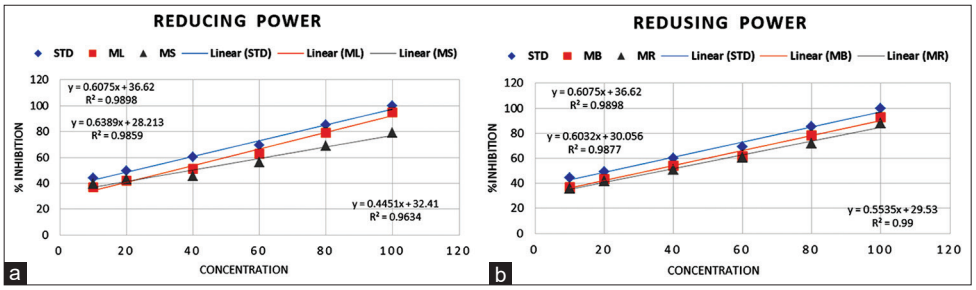

Figure 2: (a) Reducing power assay (ferric reducing ability of plasma)- % inhibition versus concentration graph of standard and test drug Erycibe leaf (EL) and Erycibe stem (ES), (b) Reducing power assay (ferric reducing ability of plasma)- % inhibition versus concentration graph of standard and test drug Erycibe bark (EB) and Erycibe root (ER)

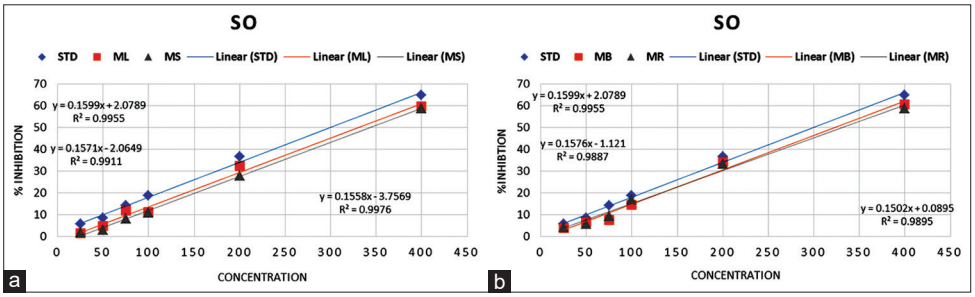

Figure 3: (a) Superoxide radical-scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe Leaf (EL) and Erycibe Stem (ES), (b) Superoxide radical scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe Bark (EB) and Erycibe Root (ER)

Nitric oxide

Nitric oxide is a free radical and important signalling molecule that consists of single unpaired electron. It is converted to nitrous acid and nitric acid when it reacts with water and oxygen.[20,21] It is estimated by using the griess reagent. In the presence of test (extracts) compound, which is a scavenger, the amount of nitrous acid will decrease. In present study, it was observed that the percentage of inhibition verses concentration showed the IC_{50} values were found as EL-94.88 (R^2 = 0.9710), ES-98.75 (R^2 = 0.9946), EB-92.81 (R^2 = 0.9931), ER-93.3 (R^2 = 0.9671), and STD-65.94 (R^2 = 0.9892) µg/ml for same methenolic extract and sodium nitrite, respectively [Table 4 and Figure 4a and b]. Above data exhibited that the bark is having more amount of decrease is in nitrous acid and reflect in scavenging than the, leaf, root and stem, respectively, compared with sodium nitrite as a standard.

Table 4: Nitric oxide

Concentration (µg/ml)	Percentage inhibition				
	STD	EL	ES	EB	ER
25	32.88	26.03	23.29	23.29	24.66
50	41.10	28.77	30.14	31.51	30.14
75	56.16	45.21	41.10	43.84	49.32
100	65.75	52.05	53.42	56.16	56.16
150	83.56	75.34	67.12	72.60	69.86
200	98.63	83.56	87.67	89.04	83.56
IC50 (µg/ml)	65.91	94.88	98.75	92.81	93.30

STD: Standard, EL: Erycibe leaf, ES: Erycibe stem, EB: Erycibe bark, ER: Erycibe root, IC50: Inhibitory concentration

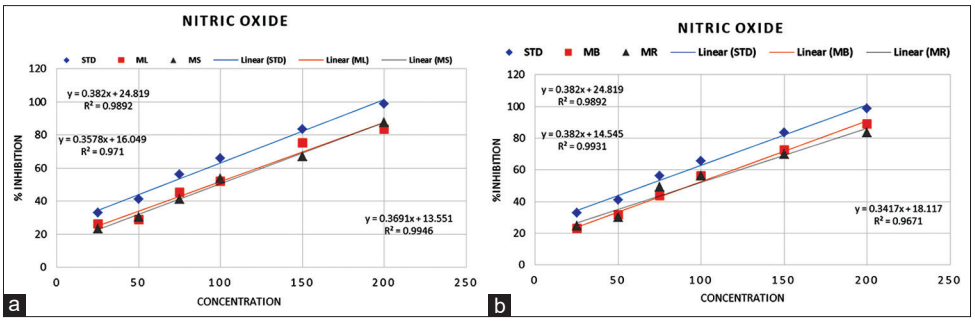

Figure 4: (a) Nitric oxide radical-scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe leaf (EL) and Erycibe stem (ES), (b) Nitric oxide radical scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe bark (EB) and Erycibe root (ER)

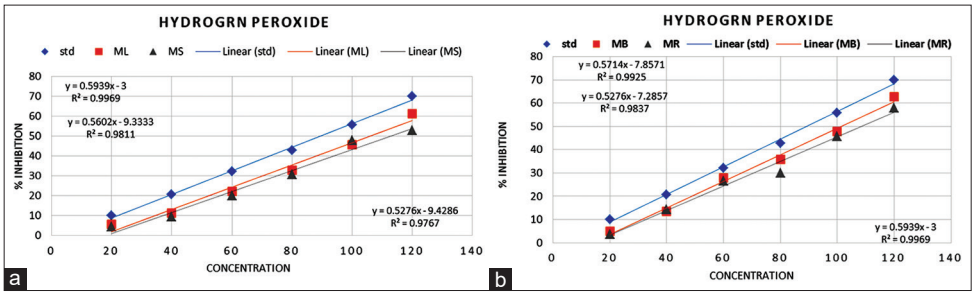

Figure 5: (a) Hydrogen peroxide radical-scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe Leaf (EL) and Erycibe Stem (ES), (b) Hydrogen peroxide radical scavenging activity- % inhibition versus concentration graph of standard and test drug Erycibe Bark (EB) and Erycibe Root (ER)

Hydrogen peroxide scavenging assay

Hydrogen peroxide (H_2O_2) itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, the removal of H_2O_2 is very important for the protection of food systems. Hydrogen peroxide-scavenging activity, especially of phenolic compounds is assigned to their electron-donating ability. In present study, it was observed that the percentage of inhibition verses concentration showed the IC_{50} value were found as EL105.91 (R^2 = 0.9811), ES112.63 (R^2 = 0.9767), EB101.25 (R^2 = 0.9925), ER108.57 (R^2 = 0.9837) and STD 89.24 (R^2 = 0.9969) µg/ml for same methenolic extract and gallic acid respectively [Table 5 and Figure 5a and b]. Data revealed that the bark has the highest ability to scavenge H_2O_2 molecules followed by leaf, root and stem when compared with ascorbic acid as a standard.

The data revealed that, all the free radical-scavenging activities of different parts of *E. paniculata* may be due to potential phytoconstituents such as phenol, flavonoids and tannin. Several researchers showed that most of these compounds (phenols and flavonoids) have antioxidant properties.[1,22]

Table 5: Hydrogen peroxide

Concentration (µg/ml)	Percentage inhibition				
	STD	EL	ES	EB	ER
20	10.00	5.71	4.29	5.00	3.57
40	20.71	11.43	9.29	13.57	14.29
60	32.14	22.14	20.00	27.86	26.43
80	42.86	32.86	30.71	35.71	30.00
100	55.71	45.71	47.86	47.86	45.71
120	70.00	61.43	52.86	62.86	57.86
IC50 (µg/ml)	89.24	105.91	112.63	101.25	108.57

STD: Standard, EL: Erycibe leaf, ES: Erycibe stem, EB: Erycibe bark, ER: Erycibe root, IC50: Inhibitory concentration

Conclusion

Present study concluded that, methanolic extracts of leaf, stem, bark and root of *E. paniculata* plant showed better antioxidant potential by using DPPH, ferric reducing antioxidant power, superoxide, nitric oxide and hydrogen peroxide scavenging *in-vitro* methods. Among all the parts, bark is having more potential antioxidant than the leaf, stem and root. *E. paniculata* is reported to have promising antioxidant activity and may be useful in various diseases involving stress-induced free radical generation.

Acknowledgement

IPGT&RA, Jamnagar for providing faith to carry-out research work.

Financial support and sponsorship

Nil.

Conflicts of interest

No

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