

**IN VITRO ANTIOXIDANT ACTIVITY AND FOURIER TRANSFORM INFRARED ANALYSIS OF
ELAEAGNUS CONFERTA ROXB. LEAF EXTRACT**LALITHA G^{1*}, NAZEEMA TH²¹Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamil Nadu, India. ²Department of Biochemistry, Michael Job College of Arts and Science for Women, Coimbatore, Tamil Nadu, India. Email: lalithajune3@gmail.com

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ABSTRACT

Objective: The objective of this study was to carry out the antioxidant potential of *Elaeagnus conferta* (EC) Roxb. leaves that were worked on various assay 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization, total reducing power was studied by ferric reducing antioxidant power (FRAP) and total radical trapping antioxidant potential (TRAP) method. The spectroscopic technique Fourier transform infrared (FTIR) spectrophotometer was performed to detect the characteristic peak values and their functional groups of EC Roxb.

Methods: Ethanol solvent is used ABTS, DPPH, FRAP, TRAP, and FTIR analysis.

Results: ABTS assay, IC₅₀ value was found to be 60 µg/ml compared with standard ascorbic acid 64 µg/ml. DPPH IC₅₀ value of EC Roxb. compared with standard ascorbic acid was found to be 0.43 µg/ml and 0.62 µg/ml. FRAP IC₅₀ value along with standard ascorbic acid was found to be 0.582 µg/ml and 0.596 µg/ml. The IC₅₀ value of TRAP along with standard ascorbic acid was found to be 0.39 µg/ml and 0.62 µg/ml. In FTIR spectroscopic study, it revealed different characteristic peak values with various functional compounds such as phenols, alcohols, amide, alkanes, carboxylic acids, alkenes, primary amines, aromatics, ester, alkyl halides, and aliphatic amines compounds.

Conclusion: EC Roxb. leaves have a potent rich source of antioxidant. In FTIR analysis, it is confirmed with the presence of phenolic compounds that can be emphasized for efficacy in pharmaceuticals.

Keywords: *Elaeagnus conferta* Roxb., 2,2-Diphenyl-1-picrylhydrazyl hydrate, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay, Ferric reducing antioxidant power, Total radical trapping antioxidant potential method, Fourier transform infrared analysis.

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INTRODUCTION

Over 175,000 cancers are expected each year globally. Trends in cancer survival differ depending on the regions. Lung cancer is one of the most common world widely seen cancers. It is a condition that causes cells to divide in the lungs uncontrollably [1]. Hence, nowadays, a large proportion of the world population depends on their traditional medicine due to scarcity and high costs of orthodox medicines [2]. *Elaeagnus conferta* (EC) Roxb. is a vast majority of the species that are abundantly found in subtropical regions of Asia. It nearly consists of about 50–70 species that are found in the family of Elaeagnaceae. It is a flowering one and its fruit is an edible one; it is used as a natural remedy. Some bioconstituents such as alkaloids, flavonoids, carbohydrates, phenol, and tannin mostly seen in plants [3]. Polyphenolic compounds are usually present in the natural products that are beneficial in reducing the risk of cancer [4]; the presence of the antioxidant defense is universal [5]. Reactive nitrogen species and reactive oxygen species cause damage to the normal function of the cell due to exposure and unhealthy diet as it damages in sugars, lipids, protein, and nucleic acid in the cells [6]. Antioxidant compounds broadly consist of major groups as carotenoids, vitamins, and phenolics, these compounds are found to be rich in free radical scavenging, it leads to a potential benefit to human health prevent from diseases [7]. Although the antioxidant activity of different plants, including their leaves, bark, roots, fruits, and seed, has been studied extensively, synthetic antioxidants cause toxicity and carcinogenicity [8]. Similarly, to identify the chemical structure for the phytoconstituents, Fourier transform infrared (FTIR) analysis is used to get an idea on various functional groups, the chemical bonds in a molecule can be determined, that is, responsible for their medicinal

purposes [9]. Hence, this subject has focused on free radical scavenging assay and analysis of FTIR of the plant EC Roxb. leaves to identify those constituents responsible for the beneficial effects.

MATERIALS AND METHODS**Collection of the plant material**

Fresh, green mature EC Roxb. leaves were collected from Coimbatore, Anaikatti Area, Ghats (Tamil Nadu, India) and have been authenticated for this project from the Botanical Survey of India. The authenticated number is BSI/SRC/5/23/3018/2785/Tech.

Preparation of plant extract

The leaves were thoroughly washed, air-dried, and powdered. One hundred grams sample was mixed with 500 ml of ethanol with occasional shaker. The extract was taken using a rotary evaporator; it was used for *in vitro* antioxidant activity and FTIR analysis.

In vitro* free radical scavenging activity of EC Roxb. leaf extract*2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay**

EC Roxb. leaves of ethanol extract were using ABTS⁺ solution, it was followed by reference of Re *et al.* [10]. Sample and standard were taken on different concentrations (20–100 µg/ml); 1 ml of the ABTS⁺ solution was added and read at 734 nm. The standard used to be ascorbic acid.

$$\% \text{ Inhibition of ABTS} = (\text{Control OD} - \text{Test OD} / \text{Control OD}) \times 100$$

From the graph, the IC₅₀ (50% inhibition) value was calculated.

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) assay

EC Roxb. leaves of ethanol extract were using DPPH solution, it was followed with reference of Elizabeth and Rao [11,12]. Sample and standard were taken on different concentrations ranging from 20 to 100 µg. One milliliter of DPPH solution was added and read at 517 nm. The standard used to be ascorbic acid.

$$\% \text{ Inhibition of DPPH} = (\text{Control OD} - \text{Test OD} / \text{Control OD}) \times 100.$$

From the graph, the IC₅₀ (50% inhibition) value was calculated.

Ferric reducing antioxidant power (FRAP) assay

EC Roxb. leaves of ethanol extract were using FRAP solution, it was followed by reference of Deuschla *et al.* [13]. Sample and standard were taken on different concentrations ranging from 20 to 100 µg. One milliliter of FRAP solution was added and read at 593 nm. The standard used to be ascorbic acid.

$$\% \text{ Inhibition of FRAP} = (\text{Control OD} - \text{Test OD} / \text{Control OD}) \times 100.$$

From the graph, the IC₅₀ (50% inhibition) value was calculated.

Total radical trapping antioxidant potential (TRAP) assay

EC Roxb. leaves of ethanol extract were using TRAP solution, it was followed with reference of Giradi *et al.* [14]. Sample and standard were taken on different concentrations ranging from 20 to 100 µg. One milliliter of TRAP solution was added and read at 490 nm. The standard used as ascorbic acid.

$$\% \text{ Inhibition of TRAP} = (\text{Control OD} - \text{Test OD} / \text{Control OD}) \times 100.$$

From the graph, the IC₅₀ (50% inhibition) value was calculated.

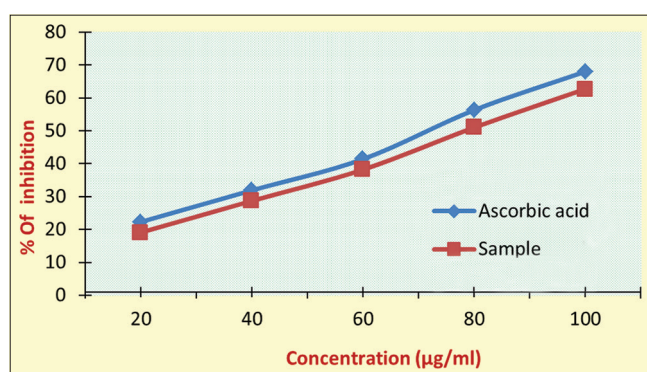


Fig. 1: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity of ethanol extract of the leaves of *Elaeagnus conferta* Roxb., values expressed are mean±standard deviation (n=3)

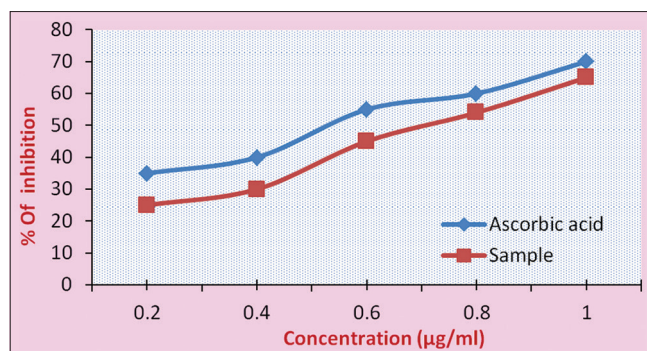


Fig. 2: 2,2-diphenyl-1-picrylhydrazyl hydrate scavenging activity of the ethanol extract of leaves of *Elaeagnus conferta* Roxb., values expressed are mean±standard deviation (n=3)

FTIR spectroscopic analysis

Ten milligrams of dried powder of the ethanol extract of EC Roxb. leaves were encapsulated in 100 mg of KBr pellet, to prepare translucent sample discs. The powdered sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) with a scan range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹. Using the infrared absorption spectrum, the different wavelength shows the presence of chemical bonds in a molecule [15,16].

RESULTS AND DISCUSSION

From this study, EC Roxb. leaves of ethanol extract on ABTS⁺ are shown in Fig. 3, ascorbic acid used as a reference. ABTS has a role in hydrogen donor, the presence of phenol or flavonoid content has more ability to quench radicals as they could be as good antioxidant [17]. Since the percentage activity of the ethanol extract of EC Roxb. leaves shows that the maximum inhibition is about 70% at 100 µg/ml, as the standard shows that the maximum value is about 65% at 100 µg/ml. IC₅₀ value is 0.60 µg/ml, which was similar to the standard value of 0.64 µg/ml, as it shows the potent of antioxidants [18,13].

From the DPPH results, an IC₅₀ value shows that the percentage of inhibition was decreasing with decreased concentration of test sample and standard. Ascorbic acid used as a standard, Fig. 2. IC₅₀ values of the test sample and standard ascorbic acid were found to be 0.43 µg/ml and 0.62 µg/ml, respectively. DPPH scavenging is due to hydrogen donor, the depletion of absorbance appears the presence of DPPH. Recent studies on various medicinal plants have been reported that a positive correlation between the flavonoid and phenolic content has a potent antioxidant [19]. Similar results also revealed from Deuschla *et al.* [13] reported that IC₅₀ value is nearer to the standard; hence, it shows

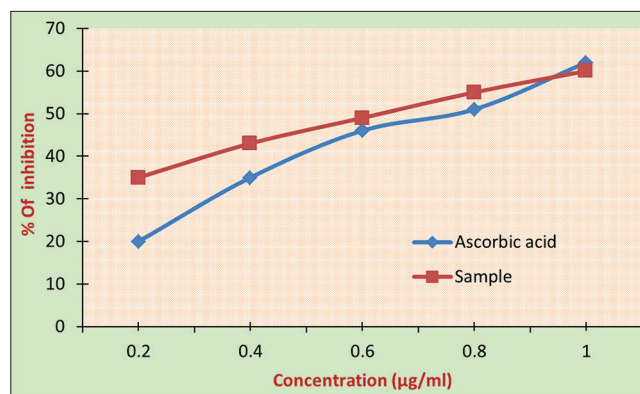


Fig. 3: Ferric reducing antioxidant power scavenging activity of the ethanol extract of leaves of *Elaeagnus conferta* Roxb., values expressed are mean±standard deviation (n=3)

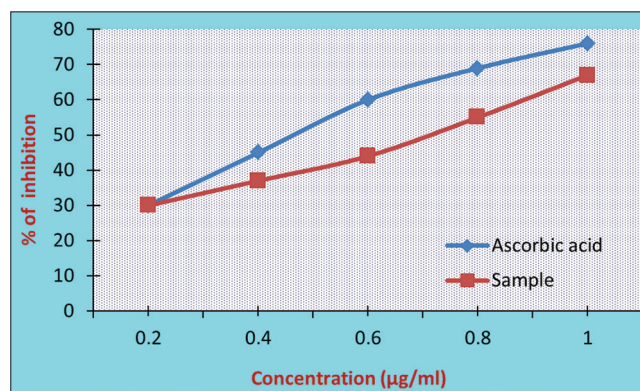


Fig. 4: Total radical trapping antioxidant potential scavenging activity of the ethanol extract of the leaves of *Elaeagnus conferta* Roxb., values expressed are mean±standard deviation (n=3)

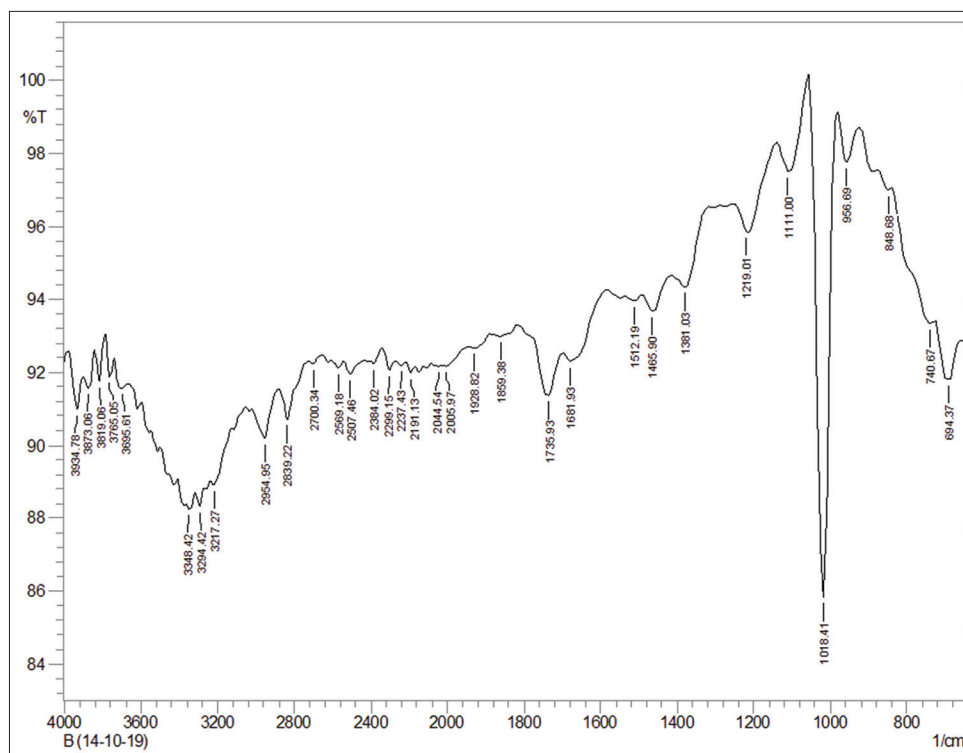


Fig. 5: Fourier transform infrared analysis spectrum of the ethanol extract of the leaves of *Elaeagnus conferta* Roxb.

the scavenging effect that leads to be a potential to be developed as a medicinal properties [20,21].

FRAP assay results are shown in Fig. 3. Most of them suggested that most of the secondary metabolites are redox-active compounds, they are assessed by FRAP assay from the food products [22-24]. Therefore, the antioxidant potential of an aqueous extract of EC Roxb. leaves was estimated for their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) along with standard ascorbic acid, FRAP shows a potent antioxidant activity [25]. From the results, it was found that the percentage of inhibition was increased with increasing concentration of plant extract (60% at 100 µg/ml) and for standard (62% at 100 µg/ml). The IC_{50} value for a sample and standard ascorbic acid is 0.582 µg/ml and 0.596 µg/ml.

TRAP value is used to monitor the reactions involved in the radical scavenging for the presence of antioxidants [26-28], as shown in Fig. 4. From the results, it was found that the percentage of inhibition was increased with increasing concentration of test sample (68% at 100 µg/ml) and for standard (70% at 100 µg/ml). This was one of the methods in oxygenated/nitrogenated species applicable to lipophilic and hydrophilic interactions; hence, this involves in radical trapping that used for the potential of antioxidants [29]. Similarly, this IC_{50} value for the sample and standard ascorbic acid is found to be 0.39 µg/ml and 0.62 µg/ml, respectively.

Functional groups of EC Roxb. leaves were identified using an FTIR spectrum of the active components based on the peak value in the region of the infrared radiation. The FTIR spectrum profile is illustrated in Table 1. It displays a number of absorption peaks, reflecting its complex nature, as shown in Fig. 5. FTIR study indicated the carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) groups, which are mainly involved in antioxidant activity. This study correlates with Collins *et al.* [30]. The peaks around 1018.41/cm may correspond to C-O stretching vibrations, and also typically, the peaks at 3217.27/cm may correspond to O-H bond. The peaks around 3873.08/cm may correspond to phenolic compounds, it will be useful in higher potential in antioxidant.

Table 1: Fourier transform infrared spectrum of functional groups of the ethanol extract of the leaves of *Elaeagnus conferta* Roxb.

S. No.	Frequency	Functional groups
1.	3873.08	Phenols
2.	3819.08	Phenols
3.	3785.05	Alcohols
4.	3695.61	Phenols
5.	3348.42	Alcohol and phenol
6.	3294.42	Alcohol and phenol
7.	2954.95	Alkanes
8.	2839.22	Alkanes
9.	2700.34	Carboxylic acid
10.	2569.18	Carboxylic acid
11.	2237.43	Alkynes
12.	2191.13	Alkynes
13.	1735.93	Ester and aromatic
14.	1681.93	Ester and aromatic
15.	1111.00	C-N group
16.	1018.41	C-O group
17.	956.69	Aromatic
18.	740.67	Aromatic
19.	694.74	Aromatic

CONCLUSION

The above results clearly confirmed that EC Roxb. leaves are a rich source of antioxidants, which can scavenge free radicals such as DPPH, ABTS, FRAP, and TRAP. It is also evident that from FTIR analysis, there is a presence of phenolic compounds; hence, further work can be emphasized for the presence of active new compounds responsible for efficacy and bioactivity.

AUTHORS' CONTRIBUTIONS

Both the authors had contributed equally in performing the assays and writing the manuscript.

COMPETING INTEREST

There are no conflicts of interest.

AUTHORS' FUNDING

Both the authors contribute equal.

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