Journal of Plant Biochemistry & Physiology

ISSN: 2329-9029

Daniel and Kumari, J Plant Biochem Physiol 2019, 7:1
DOI: 10.4172/2329-9029.1000232

Research article Open Access

Free Radical Scavenging Activity of Aqueous (Hot) Extract of *Eugenia uniflora* (L.) Leaves

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Rec date: January 10, 2019; **Acc date:** March 18, 2019; **Pub date:** March 25, 2019

Abstract

In recent years, there is an increasing interest in the study of free radicals since free radicals are the reason for various human diseases. Different forms of radicals are generated by human body as a result of certain metabolic pathways. Normally there is a balance in the levels of free radicals and antioxidants for proper physiological conditions. Overproduction of the free radicals causes oxidative damage to the biomolecules like lipids, DNA and proteins. This leads to chronic diseases like cardiovascular diseases, neuronal disease, cataract, cancer etc. The present study was designed to evaluate the potential of aqueous leaf extract of *Eugenia uniflora* as an antioxidant lead by using various *in vitro* models like lipid peroxidation (FTC and TBA method, total antioxidant capacity and reducing power scavenging assays using standard procedures. IC_{50} values were calculated respectively. In all these studies, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. These results clearly indicated that leaf extract of *Eugenia unflora* could be a potential source of natural antioxidant and effective against free radical mediated diseases.

Keywords: Antioxidant; Free radicals; Scavenging; Aqueous extract; *Eugenia uniflora*; IC_{50} (Inhibitory concentration)

Introduction

Medicinal plants are the major source of molecules with medicinal properties due to the presence of many natural compounds. During ancient and also in modern culture, medicinal plants play an important role in the protection of human health. Two- third of the world's plant species contain medicinal properties. Medicinal plants contain several components with therapeutic value that can be used as drug formulations to treat various human diseases. According to the previously given report the availability, cost effectivity and the non-toxic nature, drugs from the medicinal plants acts as good source of therapeutic agents. WHO reported [1] that 80% of the earth populations rely on traditional medicines for their primary health care needs which involve the use of plant extracts and their active components? The presence of active phytochemicals like steroids, terpenoids, alkaloids, phenolic compounds, flavonoids, tannins, amino acids makes them effective for the treatment of various diseases.

Several literatures reveal that, due to the presence of all the above phytochemicals, these medicinal plants possess strong antioxidant activity which helps to protect the oxidative damage caused by free radicals. Free radicals are generated in human body through the aerobic respiration or from various exogenous sources which causes damage at their higher concentration. Free radicals react with various biological molecules like lipids, protein, DNA resulting in the imbalance between oxidants and antioxidants. Due to the presence of several antioxidants, medicinal plant scavenges these free radicals and protects human health against several diseases. Compared to synthetic antioxidants, medicinal plant species have high favanoids and flavanols that have strong scavenging capacity against free radicals.

Eugenia uniflora, is an evergreen shrub belonging to Myrtaceae family. Various study report suggests that *Eugenia uniflora* is a functional food which contains different compounds with antioxidant, anti-diabetic, anti-inflammatory effects. Previous studies have reported the therapeutic effects of various parts of *Eugenia uniflora*. Leaves of *Eugenia uniflora* have been used for treating gout, hypertension, inflammation, digestive disorders, rheumatism, cough, hepatic diseases, sore throat, haemorrhoids and amygdalitis. Pharmacological studies in *Eugenia uniflora* as hypothermic, antinociceptive, antitumor, intestinal effects, prolongation of sleeping time have proved the medicinal application of this herb. *Eugenia uniflora* also have antibacterial, antifungal, antiviral and antprotozoal activities. Various metabolites like flavanoids, terpenes, tannins, steroids, phenols, essential oil etc., were identified in in *Eugenia uniflora*, these metabolites contribute for the biological properities [2-9].

Considering all the pharmacological aspects of the plant under study, present study focuses in the radical scavenging assays in aqueous extract of *Eugenia uniflora* leaves based on standard procedures.

Materials and Methods

Plant collection and authentication

Fresh leaves of *Eugenia uniflora* (Linn), Family- Myrtaceae, were collected from Wayanad district, Kerala during the month of April 2014. Taxonomic authentication was done by Dr. V.S Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India.

Sample processing

The leaves were washed, shade dried at room temperature and powered in a mixer grinder.

Hot water decoction: 10 g of the powdered sample was dissolved in 100 ml of distilled water which was boiled for one and half hours and filtered. The decoction was stored at 4°C for further usage.

Chemicals

All chemicals used for the evaluation were in analytical grade and obtained from either Sigma–Aldrich or Merck.

Radical scavenging assays in aqueous hot extract of *Eugenia uniflora* (L.) leaves

In present study radical scavenging assays were carried out in aqueous extract of *Eugenia uniflora* leaves based on standard procedures. The standard procedures used for free radical scavenging assay of aqueous extract of *Eugenia uniflora* leaves is presented in Table 1.

Parameters	References
Lipid peroxidation (FTC method)	Chang et al. [10]
Lipid peroxidation (TBA method)	
Total antioxidant capacity	Prieto et al. [11]
Reducing power assay	Oyaizu [12]

Table 1: Radical scavenging assays in aqueous hot extract of *Eugenia uniflora* (L.) leaves.

Percentage inhibition and IC_{50} value were calculated using standard formula. IC_{50} is the half maximal inhibitory concentration is the measure of effectiveness of a substance in inhibiting a specific biological function. Graphical representation was done for better understanding.

Statistical analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation.

The scavenging activity of superoxide radical effect was calculated as follows:

% of Scavenging=A control – A test/A control × 100

Where: A control=Absorbance of the control in the absence of sample A sample=Absorbance of sample.

Results and Discussion

Lipid peroxidation scavenging assay (FTC method and TBA method)

Lipid peroxidation is a metabolic process in which ROS results in oxidative damage of lipids. The combination of excess production of lipid peroxides results in the depletion of antioxidants. Peroxidation of the lipids can disturb the assembly of membranes which causes changes in permeability, fluidity, ion transport and inhibition of metabolic process and results in cellular dysfunction [10-13].

In present study aqueous extract of *Eugenia uniflora* leaves was investigated to scavenge lipid peroxides by FTC (Ferric thiocyanate) method and TBA (Thio barbituric acid) method. FTC test measures the amount of peroxides in the initial stages of lipid peroxidation. TBA test is used to measure the secondary products of oxidation like aldehydes and ketones. The result obtained for the assay is presented in Tables 2 and 3 and Figures 1-3. The result is compared with standard α-tocopherol. From the result obtained it is concluded the aqueous extract have significant lipid peroxidation inhibition activity. This activity was related to the presence of phytochemicals present in the extract. The IC_{50} value of the extract was calculated as 57.83 ± 0.56 µg/ml for FTC method and The IC_{50} value of the extract was calculated as 49.66 ± 0.45 µg/ml for TBA method.

Concentration (µg/ml)	% Inhibition of standard	% Inhibition of extract	IC_{50} value of the extract (µg/ml)
20	21.78 ± 0.23	17.87 ± 0.45	57.83 ± 0.56
40	39.98 ± 0.98	34.89 ± 0.87	
60	58.01 ± 0.45	51.98 ± 0.12	
80	76.87 ± 0.29	68.67 ± 0.67	
100	90.05 ± 0.56	85.78 ± 0.34	
Values are expressed as mean ± SD of three samples, Standard- α-tocopherol, Extract- Aqueous extract of *Eugenia uniflora* leaves			

Table 2: Lipid peroxidation scavenging assay (FTC method).

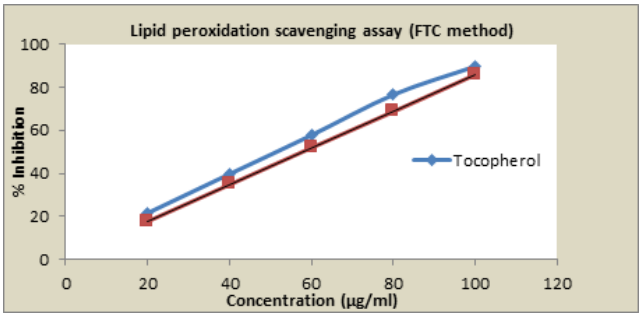

Figure 1: Lipid peroxidation scavenging assay (FTC method).

Concentration(µg/ml)	% Inhibition of standard	% Inhibition of extract	IC_{50} value of the extract (µg/ml)
20	38.23 ± 0.78	20.76 ± 0.12	49.66 ± 0.45
40	48.09 ± 0.27	41.56 ± 0.57	
60	66.89 ± 0.09	61.76 ± 0.18	
80	87.98 ± 0.17	82.98 ± 0.45	
100	92.21 ± 0.34	89.34 ± 0.67	
Values are expressed as mean ± SD of three samples, Standard- α-tocopherol, Extract- Aqueous extract of *Eugenia uniflora* leaves			

Table 3: Lipid peroxidation scavenging assay (TBA method).

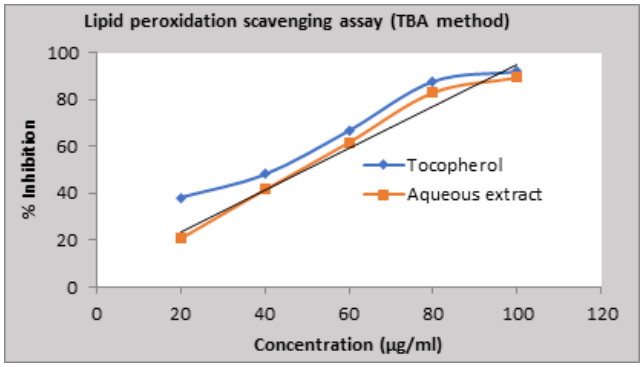

Figure 2: Lipid peroxidation scavenging assay (TBA method).

Total antioxidant capacity assay (Phosphomolybdnum method)

Concentration (µg/ml)	% Inhibition of standard	% Inhibition of extract	IC_{50} value of the extract (µg/ml)
20	24.07 ± 0.98	19.85 ± 0.37	105.48 ± 0.89
40	25.98±0.56	20.05 ± 0.43	
60	32.56 ± 0.19	29.86 ± 0.67	
80	48.12 ± 0.08	39.70 ± 0.16	
100	53.79 ± 0.12	49.98 ± 0.98	

Values are expressed as mean ± SD of three samples, Standard- Ascorbic acid, Extract- Aqueous extract of *Eugenia uniflora* leaves

Table 4: TAC assay -Phosphomolybdnum method.

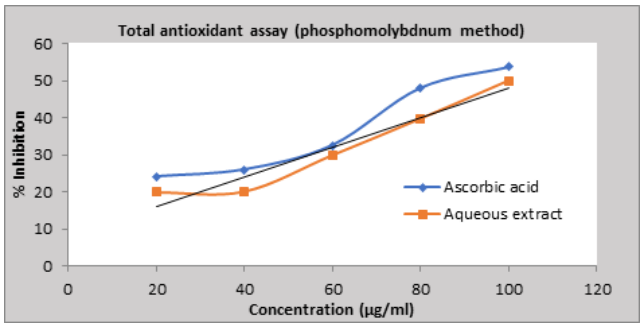

Figure 3: Total antioxidant capacity assay by phosphomolybdnum method.

TAC assay summarizes the overall activity of the antioxidants. In early stages of oxidative stress, total antioxidants stress is eliminated by the stock organ antioxidants from liver and adipose tissues. TAC falls in the later stages of oxidative stress due to the depletion of antioxidants. Assessment of antioxidant status is one of the markers of oxidative stress. Phosphomolybdnum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound resulting in the formation of green phosphate Mo (V) complex [14-16]. The result obtained is presented in Table 4 and Figure 4. The result obtained was compared with ascorbic acid. The IC_{50} value of the extract was calculated as 105.48 ± 0.89 µg/ml.

Reducing power assay

Reducing power shows a significant reflection of antioxidant capacity of an extract. Compounds exhibiting reducing power show that they are electron donors and they can reduce the oxidized intermediates of lipid peroxidation process. In this assay antioxidant compounds converts oxidative form of Fe3+ ion to ferrous ion. Yellow color of the solution changes to green and blue depending on the reducing power of the test specimen [17,18]. The result obtained for the reducing power assay of aqueous extract of Eugenia uniflora leaves is presented in Table 5 and Figure 4. Reducing power of the extract increased with increase in concentration. The result is compared with standard ascorbic acid. The IC50 value of the extract was calculated as 50.07 ± µg/ml.

Concentration (µg/ml)	% Inhibition of standard	% Inhibition of extract	IC_{50} value of the extract (µg/ml)
20	0.18 ± 0.12	0.17 ± 0.15	50.07 ± 0.07
40	0.43 ± 0.07	0.37 ± 0.34	
60	0.64 ± 0.32	0.55 ± 0.17	
80	0.82 ± 0.08	0.74 ± 0.26	
100	0.98 ± 0.18	0.94 ± 0.09	

Values are expressed as mean ± SD of three samples, Standard- Ascorbic acid, Extract- Aqueous extract of *Eugenia uniflora* leaves

Table 5: Reducing power assay.

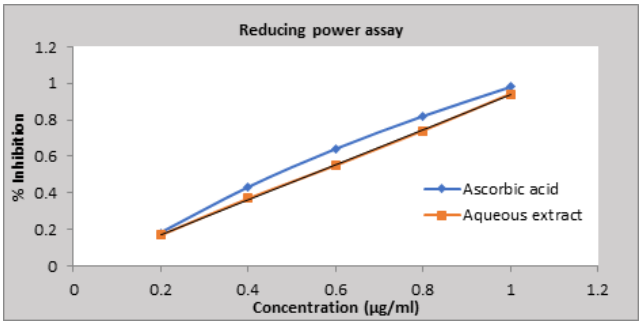

Figure 4: Reducing power assay.

Conclusion

Medicinal plants have been used to treat diseases since thousands of years. The research on plants of medicinal importance is growing at international level. Addition to this, people are increasingly interested in medicinal plants because of their low toxicity, strong antioxidant capacity and good therapeutic performance. Reactive oxygen species are concerned for many diseases and antioxidant therapy has gained much importance. Various medicinal plants have high phenolic and

large amounts of flavanoids and flavanols. They are used in therapeutic agents worldwide.

The present informative note suggests that aqueous leaf extract of *Eugenia uniflora* possess antioxidant potential and the best supplement for the disease associated with oxidative stress. However, *in vivo* studies are required before they are applied in the market as a preventive medicine. Systematic investigation should be done to identify the antioxidant potential of the extract.

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