Determination of in vitro antioxidant activity of the leaves extracts of *Ehretia pubescens*

Krishnamoorthy Meenakumari¹, Giridharan Bupesh*¹,2, Mayur Mausoom Phukan²

¹Research and Development Wing, Sree Balaji Medical College and Hospital (SBMCH), Central Research Laboratory, BIHER, Chrompet, Chennai - 600044, Tamil Nadu India, India
²Department of Forest Science, School of Science, Nagaland University(Central), Lumami, Zenheboto, Nagaland-798627, India

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**ABSTRACT**

The foods from plants were known to ensure against degenerative diseases and maturing because of their antioxidant activity credited to their high polyphenolic content. Information on antioxidant activity of Indian medicinal plant is abundant. To the best of our knowledge, biological properties have not been accounted in the literature for this species of *Ehretia*. As a point, this is the first results to assess the anti-oxidant activity of the plant *Ehretia pubescens* Benth which belongs to the family *Ehretiaceae*. The antioxidant activity of Methanol, Hexane, Ethyl acetate and Aqueous extracts of *E. Pubescens* was determined using the DPPH free radical scavenging activity, ABTS radical scavenging activity and reducing power assay. The DPPH scavenging activity showed higher activity observed in methanolic extract (63%) than ethylacetate (54%), hexane (44%) and aqueous (30%). Similarly the ABTS assay showed highest inhibition in methanolic extract (58%) than ethylacetate (43%), hexane (38%) and aqueous (32%) extracts. The reducing power assay of different extracts was increased in methanolic extract (54%) than ethylacetate (40%), hexane (34%) and aqueous (28%) extracts. Overall, the methanolic and ethyl acetate extract had higher antioxidant properties than other extract. However, in this study, *Ehretia Pubescens* Benth extracts exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

*Corresponding Author
Name: Giridharan Bupesh
Phone: +91 8012405965
Email: bupeshgiri55@gmail.com

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**INTRODUCTION**

Free radicals are electrically charged atoms that can attack different cells, tearing through impermeable cell layers to respond with the nucleic acids, proteins and compounds present in the body. Free radicals are normally happening by-products of our own digestion (*Gardeli et al., 2008; Fang et al., 2002*). Free radicals are the causative agent in lipid peroxidation in nourishments which prompts their deterioration. Oxidation is known as to be the significant reason for nourishments and materials degradation. Oxidation is a synthetic procedure that permit move of electron from a substance to an oxidizing operator (*Atoui, 2005; Kumbhare et al., 2012*). Oxidation response can be produced by different free radicals (*Pourmorad et al., 2006; Chanda et al., 2009*).

Antioxidants can also be classified based on their sizes, small molecular antioxidants and large molecular antioxidants. The small molecule antioxidants
neutralize the ROS in a method called radical scavenging and kill it. Large-molecular antioxidants are enzymes (SOD, GSHPx and CAT) that absorb ROS and prevent it from attacking others and other proteins which are very essential (Berker et al., 2010; Kahrizi et al., 2012).

Medicinal plants used in conventional medicines are well-known effective sources of natural antioxidants. Natural antioxidants which are derived from the medicinal plants are in the form of chemical constituents or raw extracts which are very effective in blocking the process of oxidation by neutralizing free radicals. This is also widely accepted that medicinal products extracted from plant products are healthier than their synthetic counterparts; however, most medicinal plants have toxicity in them and this toxicity has not been comprehensively evaluated. While considering the significant health effects, the extraction methods of natural antioxidants, the correct assessment of antioxidant activity, as well as their key resources from food and medicinal plants, attract a great deal of interest in food science and nutrition. (Maisuthisakul et al., 2007; Jang, 1997). It is additionally acknowledged that drugs taken from plant items are more secure than their engineered partners; in any case, the poisonous quality profile of most therapeutic plants have not been extensively surveyed (Dragsted et al., 1993; Raja and Pugalendi, 2010). Be that as it may, due to having an enormous assortment of restorative plants species, the entirety of the major therapeutic plants have not yet been assessed for their therapeutically properties, including cell reinforcement movement (Halliwell, 2007; Belboukhari et al., 2013).

Plants are a basic constituent of the world and individuals and people have utilized plants for medication and to cure numerous diseases (Chaabi et al., 2008; David et al., 2015). Plants contain a significant source of medications and a great deal of drugs has been literally derived from them (Kenganora and Hukkeri, 2008; Chanvitayapongs et al., 1997). The free radicals produce numerous infections in human, for example, maturing, cardiovascular illnesses, neural scatters, arteriosclerosis, and malignancies etc (Prieto et al., 1999; René et al., 2010; Santhi et al., 2019). The best route is to do evacuation of an assortment of free radicals which can cause the oxidative pressure is called as anti-oxidative resistance systems (Yan and Asmah, 2010; Zineb et al., 2013). Cell reinforcements or Antioxidant are the substances which have properties of breaking free radical (Prieto et al., 1999; Meir et al., 1995).

The plant *Ehretia Pubescens* Benth belongs to the genus *Ehretia* which contains about species 70 species, appropriated for the most part in the tropical. Various types of *Ehretia* have been investigated to separate alkaloids, phenolic acids, flavonoids, benzoquinones. Literature review has revealed no research work to evaluate the antioxidant activity of fruits and leaves of *Ehretia Pubescens*. In perspective on that, the present comprehensive in vitro antioxidant study has been done in the leaves of *Ehretia Pubescens* in various solvents based on the polarity.

**MATERIALS AND METHODS**

**Plant Collection**

The fresh leaves of plant *Ehretia Pubescens* Benth were collected from Auroville botanical garden, Villupuram, Tamilnadu, India. And the selected plant was authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy and Research centre, Chennai 600045. Authentication Number is PARC/2016/3326.

**Preparation of Plant extract**

The leaves of plant were washed thoroughly with running tap water, and it was tend to dried at room temperature and grind it to a coarse powder. The powdered leaves were extracted with four solvents i.e methanol, Hexane, Ethyl acetate and Aqueous separately by Soxhlet extraction method. 20 grams of sample was packed in muslin cloth and it was processed with 250ml of solvent in rb flask for soxhlet extraction. 2 cycles of soxhlet extraction were done with all the solvents. The extract was then lyophilised using alpha christ lyophiliser. Further it is stored in -20 degree deep freezer.

**ABTS⁺ decolourization assay**

The ABTS⁺ decolourisation assay was carried out following the method developed by (Re et al., 1999). The stock solution was prepared by making a solution of ABTS (0.038 g) in deionised water (10 mL) and then potassium persulfate (0.27032 g) was added. The stock solution was mixed thoroughly and placed in dark for 19 h. The working solution was prepared by diluting the stock solution with phosphate buffer saline (PBS) till the absorbance of 0.700 (+0.02) was reached at 734 nm and equilibrated at 30 °C. Then 10 μL of the sample (1 mg/mL) was dissolved in diluted ABTS solution (2.99 mL) and absorbance was measured at 734 nm after every 0.5 min for 8 min.

**DPPH radical scavenging assay**

The antioxidant activity of the methanol, ethyl acetate, hexane and aqueous extracts of dried leaves of the plant *Ehretia pubescens* was settled the extent that hydrogen giving or radical looking through
Figure 1: Abts Radical Decolourization Assay of the Plant *Ehretia pubescens*

Figure 2: DPPH radical scavenging activity of the plant *Ehretia pubescens*

Figure 3: Reducing power assay of the plant *Ehretia pubescens*
limit using the consistent radical DPPH finished by using the methodology of (Molyneux, 2004). The response blend containing 1 mL of DPPH arrangement (0.1 mmol/L, in 95% ethanol v/v) with various groupings of the concentrate was shaken and incubated for 30 min at room temperature and the absorbance was perused at 517 nm. The radical scavenging movement was estimated as a lessening in the absorbance of DPPH and determined utilizing the accompanying condition,

\[
\text{Effect of scavenging (\%) =} \left[ \frac{1 - A \text{ sample} (517 \text{nm})}{A \text{ control} (517 \text{nm})} \right] \times 100
\]

Reducing power assay

This assay was determined according to the method of (Oyaizu, 1986). The extracts of dried leaves of the plant *Ehretia pubescens* (20-200 µg/ml, 3 ml) was mixed with 3 ml of 200 mM sodium phosphate buffer and 3 ml of 1% potassium ferricyanide and the mixture was incubated at 50°C for 25 min. After the addition of 3 ml of 15% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 15 min. About 5 ml of the upper layer was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power.

RESULTS AND DISCUSSION

**ABTS radical cation decolourization assay**

The basic principle involved in the ABTS⁺ decolourization assay is that ABTS, on reaction with Potassium persulphate, forms a greenish blue radical cation with 734 nm as one of its wavelength maxima. Sample solutions that are able to transfer an electron to ABTS radical cation reduce the colour of the solution proportionate to its amount. The extent of scavenging of the radical depends upon both the concentration and nature of the compound under analysis. ABTS assay showed highest inhibition in methanolic extract (58%) than ethylacetate (43%), hexane (38%) and aqueous (32%) extracts. The reducing capacity in terms of EC₅₀ value of the synthesized molecules was determined (Figure 1).

**DPPH radical scavenging activity**

The DPPH examine is simply founded on the assumption that an antioxidant fills in as a hydrogen giver and therefore decreases the DPPH free radicals. This examine is known as a fundamental and speedy apparatus to do assessment of cancer prevention agent action of plant extricates. Right now, indicated that all plant extracts had critical degrees of radical scavenging activity in a portion subordinate way (Figure 2). The DPPH-determined IC₅₀ estimations of plant extracts are additionally shown in Table 1. The methanolic, Hexane, Ethyl acetate and Aqueous extract of plant inhibited DPPH upto 65.31%, 56.15%, 62.72% and 30.72% at concentration 120µg/ml. Amongst the plant extracts of *Ehretia Pubescens*, methanolic and ethyl acetate extracts were found to be the most potent DPPH scavengers, as they could inhibit DPPH free radicals up to 65.31%, at 120µg/ml concentration compared to the rest Hexane, Ethyl acetate and Aqueous extract.

**The reducing power assay**

The extracts of plant *Ehretia Pubescens* were tested in the present study which is illustrated in Figure 3. The findings revealed that the values of reducing power of the plant extracts of *Ehretia Pubescens* were based on their concentrations. In this study, results showed that all plant extracts had significant levels of Reducing Power activity in a dose dependent manner. At concentration 120µg/ml, methanolic, ethyl acetate, hexane and aqueous extract had reducing power values 52.51%, 43.12%, 51.72% and 28.12% as compare to standard. At this concentration, methanolic extract showed a remarkable reducing power that was significantly greater than those of the hexane, Ethyl acetate and Aqueous extract.

Oxidative stress is assumed to be the major cause of different diseases, for this cause that antioxidant activities is one of the frequently determined biological activities in extracts of plants. (Zara et al., 2012) assessed antioxidant exercises of different dissolvable concentrates of fruits and leaves of *E. serrata*. The ethyl acetate fraction of leaves contain the most noteworthy flavonoid just as phenolic content. It is additionally seen as generally dynamic against free radicals and diminishing specialists when explored by different measures. This investigation demonstrates that the ethanol extract acquired from the leaves and stem of the therapeutically significant plant *E. laevis* contain high measure of phenolic and flavonoid mixes. (Sarkodie et al., 2015) examined the antihyperglycaemic, cancer prevention agent and antimicrobial exercises of ethanolic concentrate of *E. cymosa*. Pharmacological exercises, for example, cytotoxic, pytotoxic, antibacterial, cell reinforcement pain relieving, revealed that these plants are good for human and different creatures and ought to be prescribed for extraction of different dynamic constituents liable for explicit impact. These discoveries ought to be utilized to get ready minimal
Table 1: Antioxidant capacity and IC_{50} Value (µg/ml) of Ehretia pubescens in different solvents

| Solvents     | IC_{50} Value (µg/ml ± SD) | DPPH Assay | ABTS Assay | Reducing Power Assay |
|--------------|----------------------------|------------|------------|----------------------|
| Aqueous      | 3.025±0.48                 | 2.48±0.19  | 34.89±0.1  |
| Hexane       | 2.48±0.08                  | 3.21±0.84  | 29.87±0.38 |
| Ethyl Acetate| 1.017±0.7                  | 1.078±0.48 | 1.012±0.87 |
| Methanol     | 1.003±0.03                 | 1.025±0.07 | 1.005±0.04 |

CONCLUSIONS

The antioxidant agent was estimated by the free radical scavenging methods such as DPPH, ABTS and Reducing power measure was demonstrated to be high. The methanolic and ethyl acetate concentrates of the plant E. pubescens demonstrated high antioxidant activity as demonstrated by their low IC_{50} value. This shows the capability of the concentrates as a source of common cancer prevention agents or nutraceuticals with potential application to diminish oxidative stress with resulting medical advantages. Addition to it, the leaves of E. pubescens recognized as having high antioxidant action might be utilized to design for further investigations for novel treatment procedures for disorders related with free radicals induced tissue harm.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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