



DPPH Antioxidant potential of *Leea macrophylla* Roxb. ex Hornem

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Abstract:

Medicinal plants are the most valuable bioresource, providing a wealth of drugs for several traditional medicine systems worldwide. *Leea macrophylla* very commonly known as Hastikarna Palasa, a wild medicinal herbaceous shrub which owns its place into Vitaceae family. It has been a valued component of herbal medicine for its therapeutic properties, which are used to alleviate a range of disorders in traditional Indian remedies. For generations, tribal communities have relied on the medicinal properties of *Leea macrophylla* plant parts to treat a range of health issues and to support overall nutrition.

Alcoholic extracts of tuberous root, stems, and leaves were used for antioxidant activity with DPPH assays. The antioxidant study found that ethanolic and methanolic extracts of the tuberous root possessed remarkable antioxidant properties, but with slightly reduce efficacy compared to standard i.e., ascorbic acid. Furthermore, the results indicated that leaves extracts had notable potential, whereas stems extracts exhibited relatively weaker antioxidant potential. Given its rich antioxidant properties, *Leea macrophylla* can be considered a valuable plant source, offering immense potential for the development of natural antioxidant agents, enriched with a multitude of bioactive compounds that can enhance the efficacy of various remedies.

Keywords: Prehistoric civilization, Hastikarna Palasa, DPPH.

Introduction:

Plants are the essential part of our life. It is looked on from an antiquity of co-evolution between human kind and plants. Forest are home to a diverse inhabitant of medicinal plants and a huge number of indigenous people rely on the forest resources for their traditional medicines. Absolutely, plants have been providing beautiful nature, life, shelter, food and above all these, medicines to all kind of life. As well, the plants have been ameliorating effects on the environment and it is also evident that from past history, plants are consequence in traditional along with modern medicines.

Plants are grown for obtaining food, fodder, fruits, flowers, seeds and also, many plants are being cultivated for medicines¹. All plants are given a thought to be the mainstay of life on earth and vital resource for humans along with animals². These are the essential source of therapeutic drugs which play a key role into the preparation of traditional medicines. Medicinal plants, in addition with their products have been utilized into the Indian traditional system of medicine and shown experimental clinical anti-diabetic, anti-cancer, activity^{3,4}. Further, World Health Organization has also advocated the evaluation of traditional plants treatments for diabetes or cancer⁵. The medicinal plants are being identified and used right throughout

the human history⁶. The botanical family Vitaceae, used as ayurvedic and traditional purpose. The plants come to this family are herbs or shrubs which is used as a source of vitamins, minerals, for anti-bacterial, anti-oxidant, anti-cancer, anti-diabetic, anti-inflammatory, arthritis, and much more. It is generally famous for its berries commonly called grapes and traditionally, the plants are used in cancer, diabetes, nephrolithiasis, body ache, sexual disability, rheumatism, snake bites, bone fractures blood effusion⁷. Vitaceae family is rich in alkaloids, tannins, steroids, sugars, etc⁸.

Leea macrophylla Roxb. ex Hornem. which own its place to Vitaceae family, very commonly called as Hathikana or Hastikarna Palasa⁹. This plant is wild edible, herbaceous shrub, with lots of nutritive value in terms of vitamins and mineral contents^{10,11}. *Leea macrophylla* Roxb. ex Hornem., is identified to established its medicinal properties through few scientific techniques and tools.

Methods and Materials:

Plant collection and its authentication

Whole plant (Fig.1) was brought from ICAR-IIAB Garhkhatanga, Ranchi during the month of September to November 2024 and further plant authentication identification were confirmed by flora “The Botany of Bihar and Orissa” by H. H. Haines.



Fig. 1: Photograph of habitat of *Leea macrophylla* Roxb. ex Hornem.

Plant extract preparation

Firstly, tuberous roots, stems, and leaves were separated from the collect whole plant, washed with running tap water twice- thrice to remove earthy contaminants. After being washed, all parts were chopped into small pieces and kept at a clean area for drying for a week. Then all dried materials were grinded into powder and were soaked with methanol in the ratio of 1:10 into a conical flask for next 72 hours. Later the obtained methanolic extract of tuberous root, stems, leaves were filtered with the help of Whatman filter paper no 1, and kept in dark for 5-6 days to evaporate the methanol solvent to get completely dried extract. Further all of the three extracts were scratched and diluted with ethanol and methanol to make the final concentration of the extracts.

In vitro Antioxidant activity

Antioxidant activity of ethanolic and methanolic extract of tuberous root, stems, and leaves on the stable radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) was estimated by the method described by Feresin et. al¹³.

2.0 mL of ethanolic and methanolic solution of sample at different concentration were mixed with 3.0 ml of freshly DPPH ethanolic and methanolic solution. After 30 minutes of reaction period at normal room temperature in a very dark place. The absorbance was measured at 517 nm against ethanol and methanol as blank by UV Spectrophotometer 30 minutes after the reaction was started.

Inhibition of free radical scavenging activity DPPH (%RSA) or percentage inhibition (I%) was calculated with the help of following formula:

$$\% \text{ RSA (radical scavenging activity)} = \frac{\text{Control Absorbance} - \text{Sample Extract Absorbance}}{\text{Control Absorbance}} \times 100$$

The ethanolic and methanolic extract concentration that provides IC₅₀ value µg/mL was calculated from graph plotted laid out inhibition percentage against the ethanolic and methanolic extract concentration. The value of IC₅₀ is the concentration of plant sample that can scavenge 50% of DPPH free radical.

Result and Conclusion:

DPPH assay tuberous root showed highest activity was observed in ethanol extract of 98.02 ± 0.03, while lowest observed 33.9 ± 0.01 in methanol extract. The IC₅₀ values demonstrated the concentration required to scavenge 50% of DPPH radicals, ranged from 16.78 µg/ml to 19.42 µg/ml. In stems the highest activity was observed in methanol extract of 97.86 ± 0.01, while lowest observed 26.29 ± 0.03 in methanol extract. The IC₅₀ values demonstrated the concentration required to scavenge 50% of DPPH radicals, ranged from 37.78 µg/ml to 38.69 µg/ml. Leaves showed the highest activity was observed in methanol extract of 96.53 ± 0.01, while lowest observed 34.4 ± 0.02 in ethanol extract. The IC₅₀ values demonstrated the concentration required to scavenge 50% of DPPH radicals, ranged from 26.03 µg/ml to 26.69 µg/ml as given in tables 1-6.

IC ₅₀ of Tuberous root		
Working concentration	Ethanol	Methanol
15 µg/mL	37.76	33.90
30 µg/mL	43.96	51.46
60 µg/mL	76.18	72.92
120 µg/mL	93	83.80
240 µg/mL	98.02	94.83
IC ₅₀ µg/mL	16.78	19.42

Table 1: Antioxidant activity 50% inhibition of ethanolic and methanolic extract of Tuberous root

IC ₅₀ of Stems		
Working concentration	Ethanol	Methanol
15 µg/mL	29.67	26.29
30 µg/mL	52.68	42.13
60 µg/mL	61.42	73.36
120 µg/mL	80.49	88.65
240 µg/mL	91.96	97.85
IC ₅₀ µg/mL	37.78	38.69

Table 2: Antioxidant activity 50% inhibition of ethanolic and methanolic extract of Stems

IC ₅₀ of Leaves		
Working concentration	Ethanol	Methanol
15 µg/mL	34.40	35.91
30 µg/mL	55.65	52.16
60 µg/mL	62.52	61.78
120 µg/mL	73.11	86.91
240 µg/mL	81.83	96.53
IC ₅₀ µg/mL	26.03	26.69

Table 3: Antioxidant activity 50% inhibition of ethanolic and methanolic extract of Leaves

Ascorbic acid equivalent antioxidant activity (mg/AAE/g)		
Working concentration	Ethanol	Methanol
15 µg/mL	37.76 ± 0.02	33.90 ± 0.01
30 µg/mL	43.97 ± 0.05	51.46 ± 0.03
60 µg/mL	76.18 ± 0.03	72.93 ± 0.02
120 µg/mL	93.01 ± 0.02	83.80 ± 0.03
240 µg/mL	98.02 ± 0.03	94.83 ± 0.02

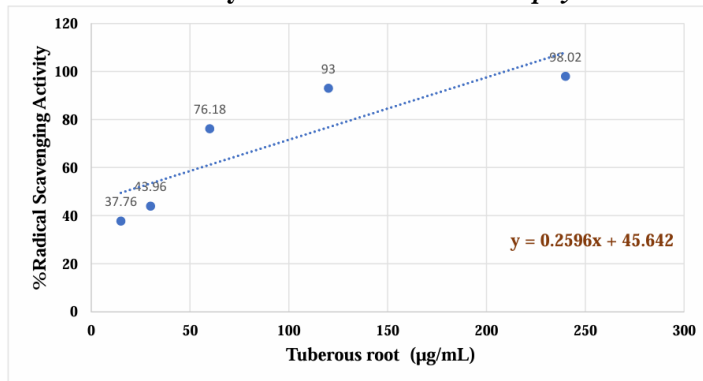
Table 4: Antioxidant activity of Tuberous root of *Leea macrophylla* Roxb. ex Hornem

Ascorbic acid equivalent antioxidant activity (mg/AAE/g)		
Working concentration	Ethanol	Methanol
15 µg/mL	29.68 ± 0.04	26.29 ± 0.03
30 µg/mL	53.69 ± 0.61	42.13 ± 0.01
60 µg/mL	61.43 ± 0.03	73.37 ± 0.01
120 µg/mL	80.49 ± 0.05	88.66 ± 0.01
240 µg/mL	91.96 ± 0.02	97.86 ± 0.01

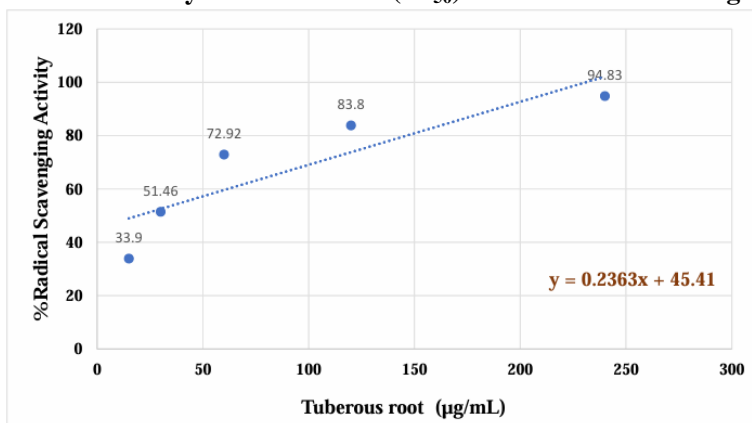
Table 5: Antioxidant activity of Stems of *Leea macrophylla* Roxb. ex Hornem

Ascorbic acid equivalent antioxidant activity (mg/AAE/g)		
Working concentration	Ethanol	Methanol
15 µg/mL	34.40 ± 0.02	35.91 ± 0.01
30 µg/mL	55.66 ± 0.03	52.17 ± 0.03
60 µg/mL	62.52 ± 0.03	61.79 ± 0.01
120 µg/mL	73.11 ± 0.03	86.91 ± 0.03
240 µg/mL	81.83 ± 0.01	96.53 ± 0.01

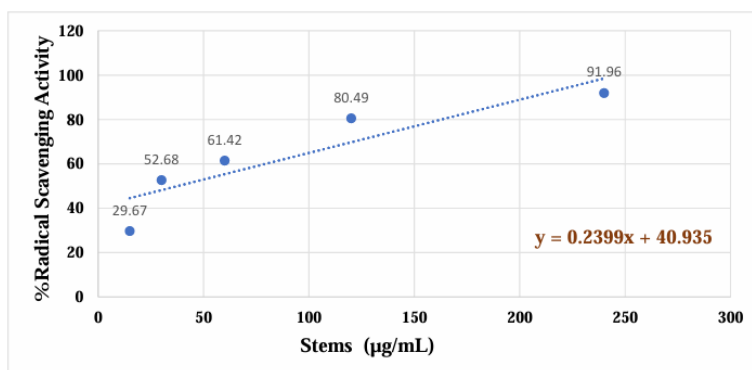
Table 6: Antioxidant activity of Leaves of *Leea macrophylla* Roxb. ex Hornem



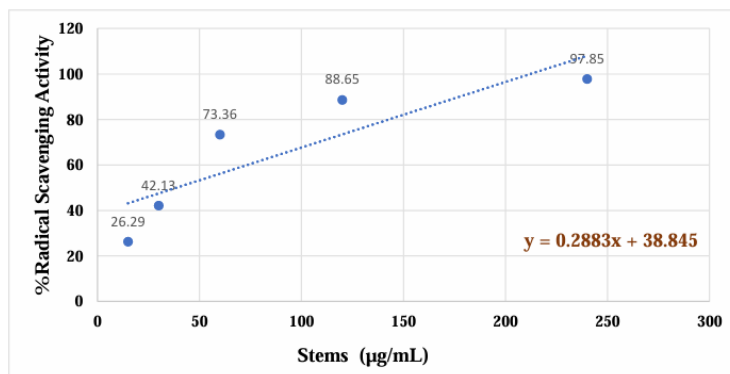
Graph 1: Antioxidant activity 50% inhibition (IC₅₀) of Tuberous root using ethanol extract



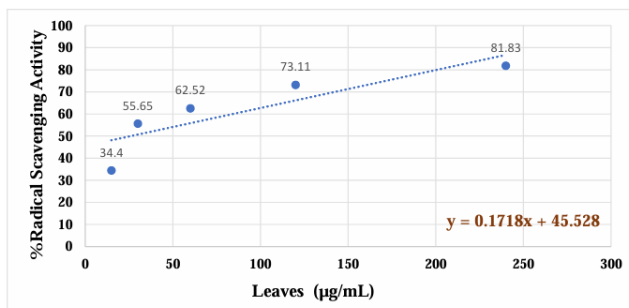
Graph 2: Antioxidant activity 50% inhibition (IC₅₀) of Tuberous root using methanol extract



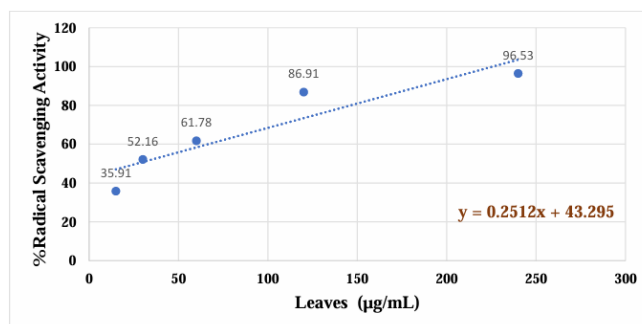
Graph 3: Antioxidant activity 50% inhibition (IC₅₀) of Stems using ethanol extract



Graph 4: Antioxidant activity 50% inhibition (IC₅₀) of Stems using methanol extract



Graph 5: Antioxidant activity 50% inhibition (IC₅₀) of Leaves using ethanol extract



Graph 6: Antioxidant activity 50% inhibition (IC₅₀) of Leaves using methanol extract

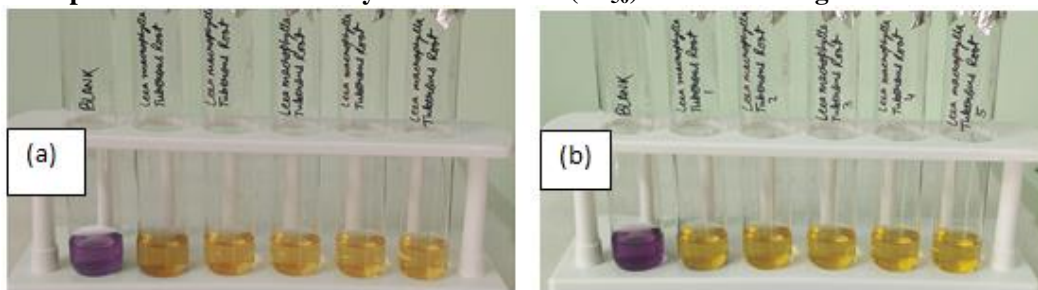


Fig. 2: Photograph showing antioxidant activity of Tuberos root using (a) ethanol and (b) methanol extract.

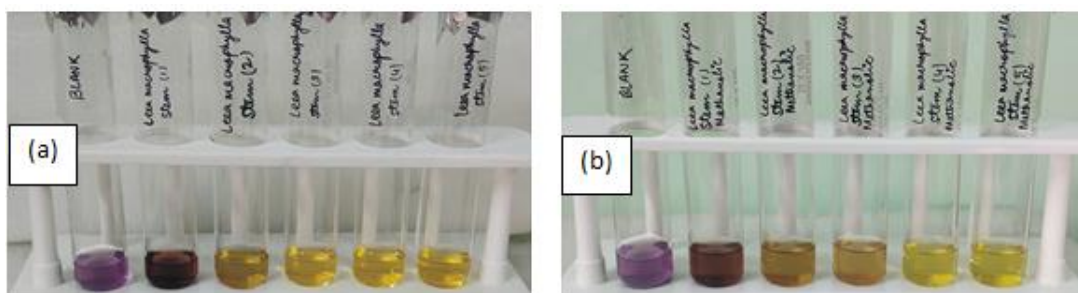


Fig. 3: Photograph showing antioxidant activity of Stems using (a) ethanol and (b) methanol extract

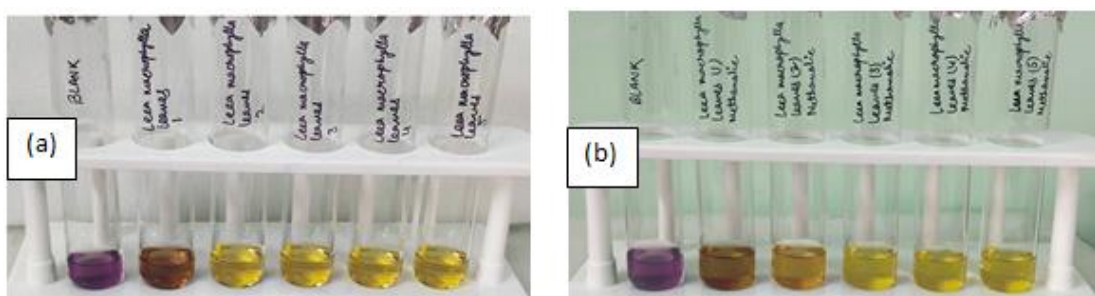


Fig. 4: Photograph showing antioxidant activity of Leaves using (a) ethanol and (b) methanol extract.

The authors declared that they have no completing conflicts of interest to publish under the current issue of the journal.

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