Determination of Phenolic, Flavonoid and Antioxidant Activity of leaves of *Broussonetia papyrifera* (L.) L'Hérit. ex-Vent.

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ABSTACT

Antioxidants are biomolecules with the potential to mitigate various diseases in the human body by scavenging free radicals and neutralizing reactive oxygen species. Phenols and flavonoids, as key antioxidants, have become integral to current pharmacognosy research. This study investigates the antioxidant activity of *Broussonetia papyrifera* (L.) L'Herit. ex Vent through the estimation of phenols and flavonoids using spectrophotometric methods. Comparative analyses were performed using ethanolic, methanolic, and aqueous extracts of mature and young leaves. Notably, the ethanolic extracts of fresh leaves demonstrated higher phenolic content (62.30–64.36 mg/g) and flavonoid content (48.00–60.35 mg/g), indicating significant antioxidant potential. These findings highlight the plant's substantial antioxidant properties, reinforcing its value as a promising candidate for pharmacognosy research. This study concludes that ethanolic extracts exhibit superior antioxidant activity compared to methanolic and aqueous extracts.

Keywords: Antioxidant activity, Phenols, Flavonoids, *Broussonetia papyrifera*

INTRODUCTION

Broussonetia papyrifera (L.) L'Herit. ex Vent., belonging to the family Moraceae, is a shrub or tree widely distributed across Asia and Pacific regions, including China, India, Burma, Japan, Laos, Thailand, and parts of North America. It has also been introduced as an exotic species in countries like Hungary, Indonesia, Italy, Spain, Tonga, Uganda, and Zimbabwe. This plant holds significant value in traditional Chinese medicine, where it is used to treat conditions such as hernias, dysentery, tinea, and oedema.

In traditional Chinese practices, the leaves of *Broussonetia papyrifera* are used to prepare medicinal tea due to their reported antihepatotoxic, antioxidant, and antifungal properties. Leaf juice is also utilized for treating excessive menstrual bleeding, uterine bleeding, haemoptysis, and hematemesis, and in Hawai'i, it is traditionally used for bleeding stomach ailments.

Apart from its nutritional value, *Broussonetia papyrifera* is rich in phenolic compounds, which contribute to its medicinal properties. Recently, there has been a growing interest in plants like

Broussonetia papyrifera due to their dual role as dietary supplements and sources of bioactive compounds with therapeutic potential. Plants rich in active compounds, particularly dietary antioxidants, play a crucial role in neutralizing free radicals such as reactive oxygen species, thereby promoting health and preventing disease.

Over the past few years, several studies have explored the phytochemical composition and pharmacological properties of *Broussonetia papyrifera*. Archana Naik and Vaishali Bachchhav (2012) reported the phytochemical and physicochemical properties of this plant. Yang et al. (2014) isolated and characterized novel phenolic compounds with oestrogen biosynthesis-inhibiting and antioxidant activity from *Broussonetia papyrifera* leaves. Zhao et al. (2011) investigated the antioxidant and anti-inflammatory properties of Chushizi oil derived from the fruit, while Sun et al. (2012) analysed the chemical composition and antioxidant activities of the plant's fruits.

MATERIALS AND METHODS

Plant Collection

The plant materials were collected from the campus of Savitribai Phule Pune University, Pune, Maharashtra, between January and April 2016. The collected plant material was authenticated and identified by the Botanical Survey of India, Western Circle Head Office, Pune, Maharashtra. Fresh leaf samples were thoroughly washed under tap water, dried using blotting paper, and further air-dried under shade. The dried leaves were ground into fine powder using an electric grinder. The powder was sieved to ensure uniform particle size and stored in airtight screw-cap bottles for further analysis.

Macroscopic and organoleptic evaluations were performed following the protocol described by Wallis (1967). These included observations of external morphological features such as the shape and size of stems and leaves, colour, taste, and texture. Fresh plant material was used for organoleptic evaluation.

Determination of Percent Extractive

The percentage extractive values were determined using the method outlined in the Indian Pharmacopoeia (Anonymous, 1955).

1. Exactly 10 grams of shade-dried powdered leaves were macerated with 100 mL of a selected solvent (petroleum ether, chloroform, toluene, methanol, acetone, ethanol, or diethyl ether) in a closed screw-cap conical flask.
2. The mixture was shaken at regular intervals for 24 hours.
3. After 24 hours, the solution was filtered rapidly to avoid any loss of solvent.
4. Approximately 20 mL of the filtrate was evaporated to dryness in a porcelain dish, dried at 110°C, and weighed.
5. The percentage extractive was calculated based on the weight of the air-dried drug.

Total Phenolics Content

Phenolic compounds, which contain aromatic hydroxyl groups, are ubiquitous in plants and

play a key role in defense mechanisms against diseases and pests. They include a wide range of compounds such as tannins and flavonols. The total phenolic content was estimated using the Folin - Ciocalteau reagent.

Procedure:

1. Weigh 1 gram of fresh plant material and grind it in 10 mL of 80% ethanol using a mortar and pestle.
2. Centrifuge the homogenate at 10,000 rpm for 20 minutes.
3. Collect the supernatant and re-extract the residue with five volumes of 80% ethanol. Centrifuge again and pool the supernatants.
4. Evaporate the combined supernatant to dryness.
5. Dissolve the dried residue in 5 mL of distilled water.
6. Prepare different aliquots (0.3, 0.6, 0.9, 1.2, and 1.5 mL) of the extract in test tubes and make up the volume to 3 mL with distilled water.
7. Add 0.5 mL of Folin-Ciocalteau reagent to each test tube.
8. After 3 minutes, add 2 mL of 20% Na_2CO_3 solution to each test tube.
9. Mix thoroughly and place the test tubes in boiling water for exactly 1 minute, then allow them to cool.
10. Measure the absorbance at 650 nm against a reagent blank.
11. Prepare a standard curve using different concentrations of catechol.

TOTAL FLAVONOID CONTENT

Flavonoids are plant secondary metabolites with significant antioxidant properties. The total flavonoid content was estimated as follows:

Procedure:

1. Weigh exactly 1 gram of fresh plant material and grind it in 10 mL of methanol using a mortar and pestle.
2. Allow the mixture to settle, then collect the supernatant.
3. Take 0.5 mL of the supernatant and add 1.5 mL of methanol.
4. Add 0.5 mL of 10% aluminum chloride solution.
5. Add 0.1 mL of 10% potassium acetate solution.
6. Add 2.8 mL of distilled water and mix thoroughly.
7. Incubate the mixture at room temperature for 30 minutes.
8. Measure the absorbance at 415 nm using a UV-Vis spectrophotometer.

ANTIOXIDANT ASSAY

The free radical scavenging activity of the plant extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is based on the reduction of the DPPH radical by antioxidants.

Principle:

DPPH is a stable free radical with a characteristic absorption at 515 nm. Upon reaction with an antioxidant, the DPPH radical is reduced, resulting in a color change from deep violet to light yellow. This change in absorbance is used to determine the antioxidant activity.

Preparation of Plant Extract

1. Leaves of *Broussonetia papyrifera* were dried, ground to a fine powder, and used for extraction.
2. Ten grams of powdered plant material were extracted with 70 mL of methanol by a cold extraction method at room temperature overnight.
3. The mixture was sonicated the following day, filtered, and concentrated using a rotary vacuum evaporator.
4. The extracts were dissolved in DMSO and stored at -20°C until further use.

Procedure for DPPH Assay:

1. Prepare a 0.5 mM solution of DPPH in methanol.
2. Mix 1 mL of the DPPH solution with 10 µL of plant extract samples at varying concentrations (2, 4, 6, 8, and 10 µg/mL for each reaction).
3. Shake the mixtures and incubate them in the dark at room temperature for 30 minutes.
4. Measure the absorbance at 517 nm using a double-beam UV-Vis spectrophotometer.
5. Use methanol as the blank and ascorbic acid as the positive control.
6. Calculate the percentage of inhibition using the following formula: \text{% Inhibition} = \left[\frac{\text{Ac} - \text{As}}{\text{Ac}} \right] \times 100 Where:
    - **Ac** = Absorbance of the blank (control sample)
    - **As** = Absorbance in the presence of the leaf extract

Radical Scavenging Activity:

The antioxidant activity is expressed as the percentage of reduction in the initial DPPH absorbance, calculated using the equation above.

RESULTS AND DISCUSSION

The total phenolic content (TPC) in *Broussonetia papyrifera* was measured in both young and mature leaves. The polyphenol concentration in young leaves was found to be 3.052 mg/g of fresh weight, which is nearly double that of mature leaves, which contained 1.536 mg/g of fresh

weight. This indicates that phenolic content decreases as the leaves mature (Graph No. 1 and Table No. 1)

In contrast, the flavonoid content showed an opposite trend. Young leaves contained 48 mg/g of fresh weight, while mature leaves had a higher concentration of 68 mg/g of fresh weight, indicating that flavonoids increase with leaf maturity (Graph No. 2 and Table No. 2)

To evaluate the antioxidant properties of *Broussonetia papyrifera*, extracts were prepared using methanol, ethanol, and water as solvents. Among these, the ethanol extract exhibited the highest antioxidant content, followed by methanol and water extracts (Graph No. 3).

Graph No. 1-Standard graph of Catechol (mg/gm) for total phenolic content

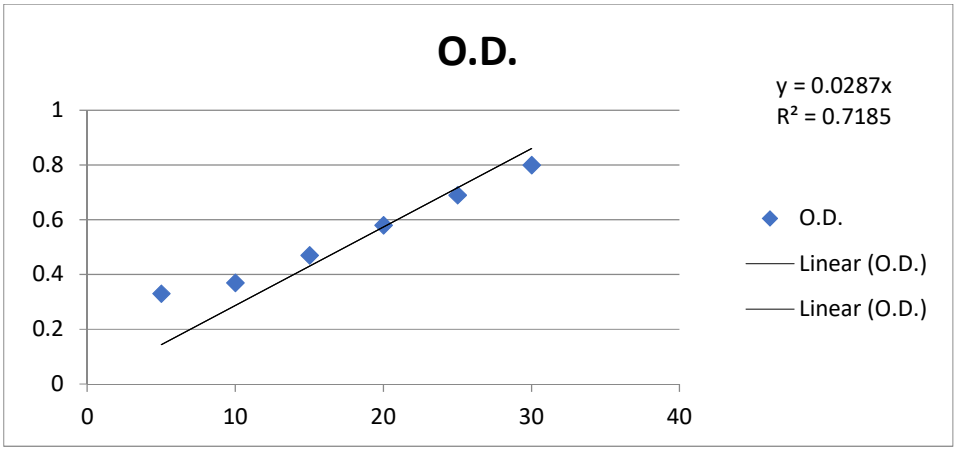


Table No. 1-. Total phenolic content

Sr. No.	**Polyphenols**	**mg/gm.** of fresh wt.
1	Young leaves	3.052
2	Mature leaves	1.536

Graph No.2-Standard graph of Quercetin (1mg/100ml) flavonoid

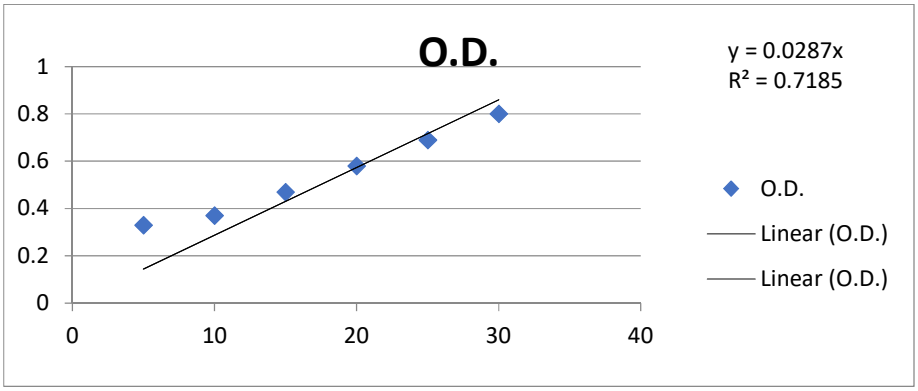
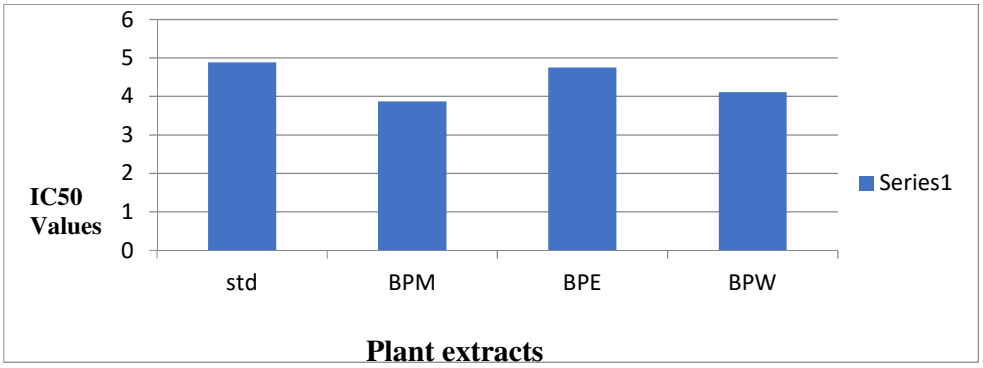


Table No. 2-Total Flavonoid content in *Broussonetia papyrifera.*

Sr. No.	**Total Flavonoid**	**mg/gm.** of fresh wt.
1	Young leaves	48
2	Mature leaves	68

Graph No. 3-Antioxidant assay:



Where, STD= Standard,

BPM= *Broussonetia papyrifera* methanol extract.

BPE= *Broussonetia papyrifera* ethanol extract.

BPW= *Broussonetia papyrifera* water exract.

CONCLUSION

1. Based on the IC50 values, it can be concluded that the ethanolic extract exhibits higher antioxidant activity compared to methanolic and aqueous extracts.
2. The flavonoid content is significantly higher in mature leaves than in young leaves of *Broussonetia papyrifera*.
3. In contrast, the total phenolic content is greater in young leaves compared to mature leaves of *Broussonetia papyrifera*.

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