Phytochemical Screening and Evaluation of Antioxidant Activity of Hydroalcoholic extract of *Butea Monosperma* Roots

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Abstract:

The present study investigates the phytochemical profile and antioxidant potential of *Butea monosperma* root extract. Proximate analysis was conducted to determine ash values and extractive yields, which serve as key indicators of the quality and purity of the crude drug. The total ash (3.90 ± 1.012%), acid-insoluble ash (0.96 ± 0.02%), and water-soluble ash (2.97 ± 0.01%) were within standard pharmacopoeial limits, indicating minimal adulteration. Water-soluble (12.21 ± 0.04%) and alcohol-soluble (3.47 ± 0.09%) extractive values suggested a predominance of polar constituents. The hydroalcoholic extract yielded 17.01% dried extract. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, carbohydrates, steroids, and proteins. Total phenolic content was quantified using the Folin–Ciocalteu method and expressed as gallic acid equivalents. The antioxidant activity was evaluated using DPPH and hydrogen peroxide radical scavenging assays. The extract demonstrated dose-dependent scavenging activity, with 65.00% inhibition of hydrogen peroxide at 100 µg/mL, compared to 82.50% by ascorbic acid. These findings suggest that *Butea monosperma* roots contain bioactive constituents with moderate antioxidant potential, justifying further studies for isolation and characterization of active compounds for possible therapeutic applications.

Keywords: Antioxidant; Characterization; Phytochemical; Therapeutic

1. Introduction

The biodiversity of plants and animals on the Indian subcontinent is incredibly rich due to its varied topography and agroclimatic conditions. Both ancient and modern medical systems are currently integrated into the Indian healthcare system. Ayurveda, Siddha, Unani, and other traditional techniques, as well as many types of folk medicine, are still very popular and important in managing health. Interestingly, Ayurveda and Siddha, which have their roots in India, are still widely used today; around 75% of people living in rural areas get their primary medical treatment from these traditional systems [1-5]. The phytochemical profile of plants, in particular the presence of flavonoids, phenolic compounds, and other reducing agents, greatly influences their antioxidant capability [6-7]. There is an urgent need to conduct a systematic assessment of Butea monosperma roots' bioactive potential due to the paucity of scientific literature on their antioxidant and phytochemical properties. One of the best methods for extracting a variety of phytoconstituents, including polar and semi-polar molecules, is hydroalcoholic extraction, which usually involves a solution of ethanol and water. For the initial screening and bioactivity assessment of medicinal plant components, this makes it the perfect solvent system [8-10].

2. Methodology

Proximate Analysis of *Butea monosperma roots*

The powdered roots of Butea monosperma were analyzed to determine various ash values, including total ash, acid-insoluble ash, water-soluble ash, and extractive values in water and alcohol [11].

Total Ash Content

One gram of the powdered root sample was placed in a previously weighed silica crucible, which had been pre-dried. The sample was then incinerated in a muffle furnace until it became carbon-free. The remaining ash was allowed to cool and subsequently weighed to determine the total ash content.

Acid-Insoluble Ash

To the crucible containing the total ash, 25 ml of dilute hydrochloric acid was added. The mixture was covered with a watch glass and gently boiled for 5 minutes. The insoluble matter obtained was filtered through an ashless filter paper, thoroughly washed with hot water until the filtrate turned neutral, then dried and ignited to a constant weight. The final residue was cooled in a desiccator for 30 minutes before weighing [12-15].

Water-Soluble Ash

Twenty-five milliliters of distilled water were added to the crucible containing the total ash, and the mixture was gently boiled for 5 minutes. The resulting insoluble material was collected on an ashless filter paper, washed thoroughly with hot water, and ignited in a crucible for 5 minutes. The weight of the residue was then subtracted from the total ash to determine the water-soluble ash content [16-18].

Extractive Values (Water and Alcohol)

Approximately 2 g of accurately weighed, homogenized powdered drug was placed in a glass-stoppered

conical flask and macerated with 100 ml of solvent (water or alcohol) for 6 hours with frequent shaking. It was then allowed to stand for 18 hours. The mixture was filtered carefully, ensuring no loss of solvent. An aliquot of 25 ml of the filtrate was transferred to a pre-weighed, flat-bottom dish and evaporated to dryness on a water bath. The residue was dried in an oven at 105°C to constant weight, cooled in a desiccator for 30 minutes, and then weighed [19-20].

Extraction of Plant Material

Fresh, mature roots were washed thoroughly under running tap water to remove dust, dirt, and other impurities. The cleaned roots were shade-dried under ambient conditions, then coarsely powdered. The powdered material was macerated at room temperature using a hydroalcoholic solvent (80:20 v/v). The extract was filtered, concentrated using a rotary vacuum evaporator, and finally freeze-dried. The dried extract was stored in an airtight glass container for further analysis [21-22].

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the root extract was conducted using standard procedures to identify the presence of bioactive compounds such as flavonoids, alkaloids, steroids, saponins, tannins, carbohydrates, and proteins [23].

Estimation of Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method. In this assay, 1 ml of the root extract (10 mg in 5 ml) was mixed with 5 ml of Folin–Ciocalteu reagent (10% v/v) and vortexed for 5 minutes. Afterward, 5 ml of 7.5% sodium carbonate solution was added to the mixture**.** The reaction mixture was incubated at room temperature for 2 hours. From this, 2 ml of the solution was transferred to a test tube and diluted to 5 ml with distilled water. After standing for 15 minutes, the absorbance was measured at 765 nm. A calibration curve was prepared using standard gallic acid solutions. The stock solution of gallic acid (5 mg/100 ml) was used to prepare six different concentrations: 50 µg, 100 µg, 150 µg, 250 µg, 350 µg, and 500 µg in 50 ml. The same procedure as above was followed for each standard concentration [24-25].

Antioxidant Assay

The antioxidant activity of the hydroalcoholic extract was assessed using two in vitro chemical assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide scavenging methods. Stock solutions of both the crude extract and the standard (ascorbic acid) were prepared in methanol at a concentration of 10 mg/10 ml (based on dry weight). These were then serially diluted with methanol to yield five concentrations: 20, 40, 60, 80, and 100 µg/ml [26].

Hydrogen Peroxide Radical Scavenging Assay

Phosphate buffer (pH 7.4) was prepared by combining 50 ml of 0.2 M potassium dihydrogen phosphate solution with 39.1 ml of 0.2 M sodium hydroxide, and the final volume was adjusted to 200 ml using distilled water, following the guidelines of the Indian Pharmacopoeia (1996). An equal volume of hydrogen peroxide solution was then added to the phosphate buffer and allowed to stand at room temperature for 5 minutes to generate reactive radicals. To assess scavenging activity, 1 ml of the root extract (prepared in

distilled water) was added to 0.6 ml of the hydrogen peroxide solution. The absorbance was recorded at 230 nm using a UV–Visible spectrophotometer against a blank containing only phosphate buffer (without hydrogen peroxide)[27-30]. The percentage of hydrogen peroxide scavenged was calculated using the following formula:

$$\text{Percent scavenge } (H_2O_2) = \frac{A0-A1}{A0} \times 100$$

Where:

A_0 = Absorbance of control (without extract)

A_1 = Absorbance with plant extract or standard

DPPH Free Radical Scavenging Assay

A 0.1 m DPPH solution was prepared in methanol and kept in the dark for 30 minutes to stabilize. To measure antioxidant activity, 1 ml of plant extract or standard (ascorbic acid) was added to 9 ml of the DPPH solution. The mixture was left at room temperature for 30 minutes. A blank solution was prepared by mixing 1 ml of methanol with 9 ml of DPPH solution. All tests were conducted in triplicate. The decrease in absorbance was measured at 517 nm using a UV–Visible spectrophotometer [31-37]. The DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{Ac-At}{Ac} \times 100$$

Where:

A_a = Absorbance of control (DPPH + methanol)

A_t = Absorbance with test sample

The antioxidant activity was expressed as IC_{50}, the concentration of extract required to inhibit 50% of free radicals. A lower IC_{50} value indicates stronger free radical scavenging activity.

3. Result and discussion

Ash Value analysis and extractive value analysis

Total ash, water soluble ash, acid insoluble ash, water soluble and alcohol soluble extractive values were determined. The total ash value (3.90 ± 1.012%) indicates the total amount of inorganic residue remaining after incineration, which reflects both physiological and non-physiological ash content. The acid-insoluble ash value (0.96 ± 0.02%) is low, suggesting minimal contamination with silica, sand, or earthy matter. The water-soluble ash value (2.97 ± 0.01%) indicates the presence of water-soluble inorganic components, which may include essential mineral elements. These values are within acceptable pharmacopoeial limits, indicating the purity and quality of the crude drug and absence of excessive inorganic adulterants. The water-soluble extractive value was found to be relatively high (12.21 ± 0.04%), indicating a good amount of

polar compounds such as glycosides, sugars, tannins, or mucilage present in the extract. In contrast, the alcohol-soluble extractive value (3.47 ± 0.09%) was lower, suggesting a smaller concentration of alcohol-soluble constituents like alkaloids, flavonoids, or resins. The significant difference between water- and alcohol-soluble extractives implies that polar compounds dominate the phytochemical profile of the root sample. This helps guide future solvent selection for extraction during phytochemical studies or formulation development.

Table no. 01: Results of ash and extractive value.

Parameter	**Total ash**	**Value**
Ash values (%)	Acid Insoluble ash	0.96±0.02%
	Water soluble ash	2.97±0.01%
Extractive values (%)	Water soluble	12.21±0.04%
	Alcohol soluble	3.47±0.09%

Extraction - Percentage yield of hydroalcoholic extract of root was 17.01%

Phytoconstituents Analysis

The hydroalcoholic extract underwent preliminary analysis to identify the presence of primary and secondary phytoconstituents. The results revealed that the extract contains steroids, flavonoids, carbohydrates, alkaloids, proteins, and tannins.

Table no. 02: Phytoconstituents analysis of root extract.

Plant constituents	**Test/Reagent**	**Hydroalcoholic extract**
Steroids	Salkowski reaction	+
Steroids	Liebermann-Burchard test	+
Alkaloids	Dragendorff’s reagent	-
Alkaloids	Mayer’s reagent	+
Alkaloids	Hager’s reagent	+
Alkaloids	Wagner’s reagent	+
Tannins	Ferric chloride test	+
Tannins	Lead acetate test	+
Tannins	Potassium dichromate	+
Flavonoids	Shinoda test	+
Carbohydrates	Molish’s test	+
Carbohydrates	Barfoed’s test	-

Proteins	Biuret test	-
Proteins	Xanthoproteic test	+
Saponins	Foam test	-

Standard gallic Acid Calibration Curve for Total Phenolic Content

A calibration curve was constructed using gallic acid as the standard reference compound to quantify the total phenolic content in the plant extract. A stock solution of gallic acid (5 mg/100 ml) was prepared in distilled water, and from this, serial dilutions were made to obtain concentrations of 50, 100, 150, 250, 350, and 500 µg/50 ml. Each standard solution was treated with 5 ml of 10% Folin–Ciocalteu reagent, followed by the addition of 5 ml of 7.5% sodium carbonate solution. The mixtures were incubated at room temperature for 2 hours to complete the color development. After incubation, 2 ml of each solution was transferred into separate test tubes and diluted to 5 ml with distilled water. The absorbance was then measured at 765 nm using a UV–Visible spectrophotometer. A standard curve was plotted with absorbance (Y-axis) against concentration of gallic acid (µg/ml) (X-axis). The resulting linear regression equation was used to calculate the total phenolic content of the plant extract, expressed in terms of mg of gallic acid equivalent (GAE) per gram of dry extract.

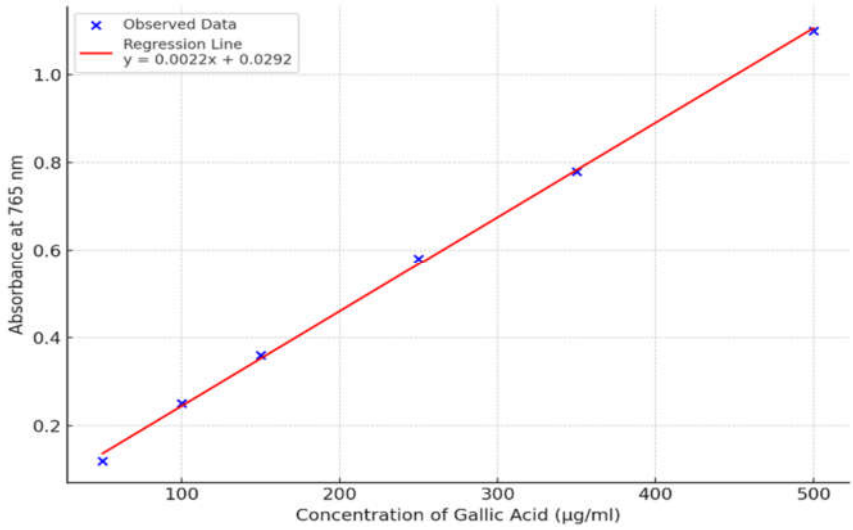

Fig. 01: Standard gallic acid calibration curve for total phenolic content.

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical scavenging activity of the plant extract and standard ascorbic acid was evaluated at different concentrations ranging from 20 to 100 µg/mL. The percentage of scavenging activity was calculated using the decrease in absorbance relative to the control (A_0 = 0.800). Both the extract and ascorbic acid demonstrated a dose-dependent increase in hydrogen peroxide scavenging activity. The extract showed 22.50% inhibition at 20 µg/mL, increasing to 65.00% at 100 µg/mL. In comparison, ascorbic acid

exhibited higher activity, with 37.50% scavenging at 20 µg/mL and up to 82.50% at 100 µg/mL. Hydrogen peroxide is a non-radical reactive oxygen species that can generate highly reactive hydroxyl radicals in the presence of metal ions, leading to oxidative damage in cells. The ability to scavenge hydrogen peroxide is a valuable indicator of antioxidant potential. In this study, the plant extract showed significant hydrogen peroxide scavenging activity, increasing with concentration. This suggests that the extract contains antioxidant phytochemicals capable of reducing hydrogen peroxide to water, possibly through electron or hydrogen atom donation. When compared to the standard antioxidant ascorbic acid, the root extract demonstrated moderate but consistent activity, though lower at each concentration. Ascorbic acid showed greater scavenging effects, which is expected due to its well-known and potent antioxidant properties. These findings indicate that the plant extract possesses noticeable antioxidant activity, supporting its traditional or ethnomedicinal use. However, its lower activity compared to ascorbic acid suggests the need for further fractionation, isolation of active compounds, or formulation strategies to enhance its efficacy.

Table no. 03: Scavenging ability of the root extract.

S. No.	Concentration (µg/mL)	Absorbance of Control (A_0 = 0.800)	Absorbance (Extract)	% Scavenging (Extract)	Absorbance (Ascorbic Acid)	% Scavenging (Ascorbic Acid)
1	20	0.8	0.62	22.5	0.5	37.5
2	40	0.8	0.55	31.25	0.38	52.5
3	60	0.8	0.46	42.5	0.3	62.5
4	80	0.8	0.35	56.25	0.21	73.75
5	100	0.8	0.28	65	0.14	82.5

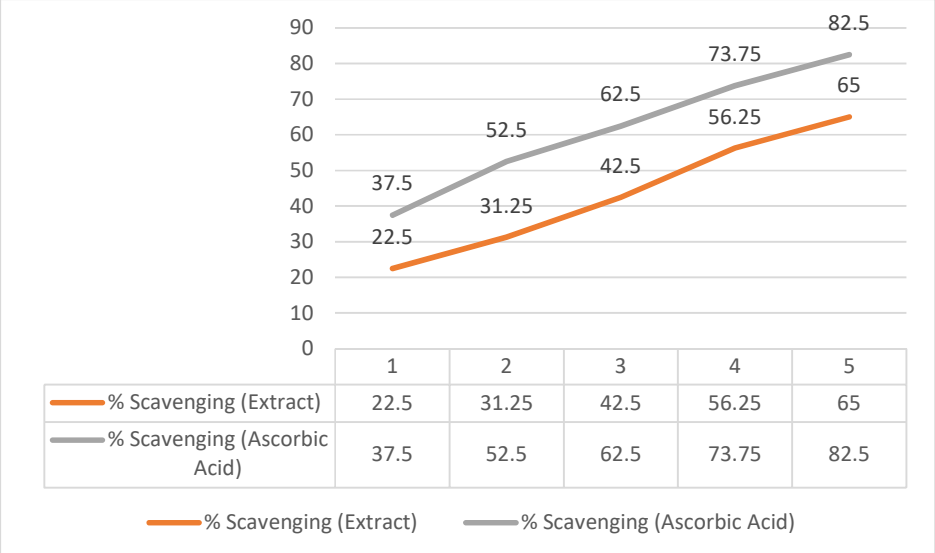

Fig.02: The scavenging ability of the extract.

DPPH free radical scavenging assay

The antioxidant potential of the root extract and the standard ascorbic acid was evaluated using the DPPH free radical scavenging assay across concentrations ranging from 20 to 100 µg/mL. The percentage scavenging activity of both samples increased in a dose-dependent manner.At 20 µg/mL, the root extract exhibited 18.75% scavenging activity, which progressively increased to 70.00% at 100 µg/mL. In comparison, ascorbic acid displayed stronger activity, with 40.00% scavenging at 20 µg/mL and reaching 87.50% at 100 µg/mL. These results indicate that while both the root extract and ascorbic acid possess antioxidant properties, the standard exhibits significantly higher free radical scavenging activity at all tested concentrations.

Table no. 04: The scavenging ability of the extract and standard ascorbic acid on DPPH.

S. No.	Concentration (µg/mL)	Absorbance of Control (A_0 = 0.800)	Absorbance (Root Extract)	% Scavenging (Extract)	Absorbance (Ascorbic Acid)	% Scavenging (Ascorbic Acid)
1	20	0.8	0.65	18.75	0.48	40
2	40	0.8	0.53	33.75	0.35	56.25
3	60	0.8	0.43	46.25	0.25	68.75
4	80	0.8	0.32	60	0.18	77.5
5	100	0.8	0.24	70	0.1	87.5

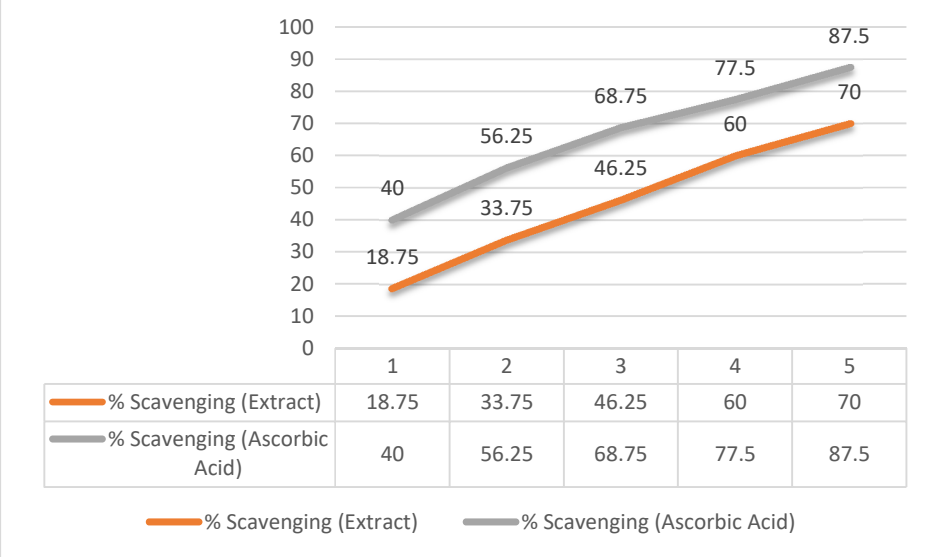

Fig.03: The scavenging ability of the extract and standard on DPPH.

4. Conclusion

The present study on the phytochemical screening and antioxidant activity of the hydroalcoholic extract of *Butea monosperma* roots reveals important pharmacognostical and biological insights into this traditional medicinal plant. Proximate analysis confirmed the quality and purity of the raw material, with ash values within permissible limits and a high water-soluble extractive value indicating the abundance of polar bioactive constituents. The percentage yield of the hydroalcoholic extract was 17.01%, confirming the efficiency of the extraction method. Preliminary phytochemical screening showed the presence of key secondary metabolites, including flavonoids, tannins, alkaloids, steroids, and carbohydrates, many of which are known for their antioxidant and therapeutic properties. The absence of saponins and limited protein detection further help characterize the extract's profile. The total phenolic content, estimated using the Folin–Ciocalteu method, demonstrated a measurable presence of phenolic compounds, which are potent natural antioxidants and contribute significantly to the plant's free radical scavenging ability. Antioxidant evaluation using DPPH and hydrogen peroxide scavenging assays showed dose-dependent scavenging activity of the root extract. Although ascorbic acid exhibited stronger activity in both assays, the extract still demonstrated considerable antioxidant potential, with 65% inhibition in hydrogen peroxide and 70% in DPPH at 100 µg/mL. These results suggest the presence of compounds capable of neutralizing free radicals, potentially reducing oxidative stress. In conclusion, *Butea monosperma* root extract exhibits promising antioxidant activity, which supports its traditional use and encourages further pharmacological exploration. The moderate activity compared to the standard suggests that future studies should focus on fractionation, isolation, and characterization of active constituents, along with in vivo validation, to develop effective antioxidant formulations or nutraceuticals based on this medicinal plant.

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