



Research Article



Foliage of *Dalbergia sissoo* Roxb.: A potential antioxidant agent

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This study explores the antioxidative potential of *Dalbergia sissoo*, commonly known as Indian Rosewood, focusing on the methanol extracts from its foliage leaves. Findings revealed high levels of phenolic compounds, correlating with strong antioxidant properties evidenced by Total phenolic content as 34.95 ± 1.26 mgGAE/g and low IC₅₀ value in the DPPH assay ($104.04 \pm 1.5 \mu\text{g/ml}$), suggesting higher antioxidant capacity. The FRAP assay ($2577.5 \pm 3.2 \mu\text{M}$) further supported these results by demonstrating the effective free radical scavenging activity of extract. These outcomes affirm the substantial antioxidative potential of *D. sissoo* leaves, indicating their promise for therapeutic applications, particularly in combating oxidative stress-related disorders.

Keywords: *Dalbergia sissoo*, Phenolic content, DPPH, FRAP, Antioxidant

1. Introduction

Medicinal plants have been used for time immemorial as remedies for various health conditions¹. These plants have bioactive compounds that offer potential therapeutic benefits and form part of pharmaceuticals, herbal supplements, and traditional medicine systems². Further research on medicinal plants has included modern-day research exploring their potential use in medicine for efficacy or opportunities for replacing synthetic drugs. Last decade has seen as a considerable global inclination towards phytomedicine³. Free radicals are molecules whose one of the orbitals has an unpaired electron, which can exist independently. They are generally formed during oxidative processes, but disease, stress etc. can increase the formation of these radicals⁴. These radicals can lead to cells being damaged in chain reactions. Antioxidants are able to control the level of ROS in the cell mainly by scavenging the produced free radicals, limiting lipid peroxidation, and at the same time chelating the metal ions that catalyze the reactions⁵. Search for natural plant-based antioxidants is the need of the hour⁶. *Dalbergia sissoo* Roxb. (family Fabaceae) is commonly known as Shisham, Sison, or Indian Rosewood. It is a deciduous tree that has its origin from the subcontinent of India and southern Iran⁷.

It falls under the family of Fabaceae, known for its hardness and economic value. It reaches up to 30 meters in height and has a diameter of about 1.5-2 meters. It features a long, straight bole with smooth, thin bark ranging from gray to dark brown⁸. The tree has pinnate, leathery leaves with alternating leaflets that are ovate and acuminate, ending in fine points with petiolate bases⁹. The upper surface of the leaves remains dark green and glossy, while the lower surface is lighter. Small, fragrant flowers about 5-8 mm long form in racemes ranging from pale to pink and are nearly sessile, blooming densely along short peduncles during spring¹⁰. After flowering, flat, brownish, pod-like fruits develop, which are 1 to 4-seeded and dispersed by water and animals. It thrives in diverse climatic conditions but prefers riverbanks and moist places, often forming riparian forests. This species is tolerant of poor and saline soils and can withstand drought conditions¹¹⁻¹². This plant has been introduced globally as a timber crop and ornamental tree. *D. sissoo* wood is highly valued for its hardness, durability, and resistance to decay, extensively used in furniture, building materials, and woodcrafts. It also serves as fuel wood and for making charcoal. The tree's environmental benefits include soil stabilization and erosion control, as well as nitrogen fixation that enhance soil fertility, making it useful in agroforestry systems⁹.

Biologically active compounds found in its leaves, flowers, stem bark, and pods include isoflavone-O-glycoside, sissotrin, biochanin A, 7-O-Methyl tectorigenin, meso-inositol, 4-Rhamnoglucoside, dalbergione, dalbergin, methyl dalbergin, and isotectorigenin¹². Traditionally, Indian Rosewood has been used in Ayurvedic medicine to treat various health issues. Its bark, rich in phenolic compounds, tannins, and flavonoids, is employed for stomach disorders, leucoderma, dysentery, ulcers, vomiting, and various skin conditions¹³.

The leaves are used to treat gonorrhea, cardiovascular problems, and syphilis¹⁴, while the seeds¹⁵ help alleviate itching, skin burning, and scabies. Its plantation not only aids in environmental conservation but also supports local biodiversity by providing habitat and food for various wildlife species¹⁶.

1. Material and methods

2.1 Collection and Extraction

Samples of *D. sissoo* leaves were collected from Tonk district, Rajasthan, India. A reference sample was preserved in the herbarium of the Department of Bioscience and Biotechnology at Banasthali Vidyapith. The collected leaves were first cleaned with tap water and then left to dry in open air at room temperature for 10 days. After drying, the leaves were ground into a fine powder. Five grams of this powdered leaf sample were then soaked in 80% methanol and placed on an orbital shaker (Metrex, MRS-100C—37 °C; 120 rpm) to agitate for 24 hours¹⁷. After the agitation period, the mixture was centrifuged at 3000 g for 15 minutes. The supernatant from each sample was collected and stored at 4 °C for subsequent analysis.

2.2 Collection and Extraction

0.125 ml of an ethanolic extract was mixed in the same volume of Folin–Ciocalteu reagent. 3% sodium bicarbonate was added to this mixture, and thereafter, it was made up to a final volume of 3 ml. All the test tubes that contained the mixture were further incubated for a total period of 90 minutes, and after this, the determination of the absorbance was made at a characteristic wavelength of 760 nm. The result was recorded in milligrams of Gallic acid equivalent (GAE) per gram of the sample's dry weight¹⁸.

2.3 DPPH

The assay was carried out according to the method by Vats and Kamal¹⁹. Plant extract at a volume of 1 mL was mixed with 0.3 mM DPPH at a volume of 1 mL and allowed to stand for 30 min in the dark at room temperature. The absorbance of the mixture was measured at 517 nm, and the IC₅₀ (in µg/ml) was determined. The capability to scavenge the DPPH radical was calculated using equation.

$$\text{DPPH Scavenged (\%)} = (\text{AB} - \text{As}) / \text{AB} \times 100$$

Where AB is the absorbance of the blank solution and AS is DPPH radical + plant extract. The results were expressed as minimum Inhibitory concentration (IC₅₀).

2.4 FRAP

A 300 mM acetate buffer at pH 3.6 was prepared along with a 10 mM 2,4,6-tripyridylstriaizine (TPTZ) solution in 40 mM hydrochloric acid (HCl) and a 20 mM ferric chloride hexahydrate in the preparation of the working solution, 25 mL of acetate buffer was mixed with 2.5 mL of TPTZ and the same quantity of the FeCl₃.6H₂O solutions. Therefore, in the assay test, 50 µL of the extract was dispensed into 1.5 mL of the FRAP reagent. The absorbance of the mixture was read at 593 nm after a reaction period of 5 min, as described by Vats and Tiwari²⁰.

2.5 Statistical analysis

The experiments were repeated five times and the data were presented as mean ± standard deviation (SD).

3. Result and Discussion

3.1 Total Phenolic Content

The standard curve of gallic acid had a correlation coefficient (R²) of 0.9959. The total phenolic content in extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram weight. The

extracts had phenolic content was present in leaves 34.95±1.26mgGAE/g.

Phenolic are the main and thoroughly studied classes of phytochemical constituents¹⁷. The hydroxyl groups are critical in phenols and flavonoids for conferring redox properties necessary for these compounds to exert their ability as antioxidants²¹. It has been shown that *D. sissoo* has the highest levels of phenolic compounds. *D. sissoo* leaves extracted through methanol had the highest TPC this observation is in line with similar findings by Yasmeen and Gupta²², who noted that a significantly high TPC of methanol extract was observed in leaves of *D. sissoo*. Such concord among these studies presents prospective use of *D. Sissoo* harbors a very good number of phenolic compounds, which are well known to demonstrate antioxidant activity. Such consistency not only proves the reproducibility of the determination of the phenolic content but also points out that *D. sissoo* could be further explored for its therapeutic potentials, particularly in antioxidative applications.

3.2 DPPH

IC₅₀ values represent the concentration of the plant extract required to neutralize 50% of the free radicals, and hence are one of the representative activities of plant extract radical scavenging against DPPH. Lower IC₅₀ values signify more potent antioxidant properties, whereas higher values suggest weaker activities. The extract of the plant contains many secondary metabolites, like glycosides, phenolic, and tannins, which change the colour of the DPPH solution due to its ability to donate hydrogen, showing they have strong bioactivity²³. The extract exhibited antioxidant activity, but to different degrees and was mostly of polar radical-inhibitory compounds, as

expected in the extraction with methanol solvent known to effectively extract polar. The antioxidant power was determined according to the criteria established by use of spectrometric method²⁴. The methanolic extract of the leaves of *D. sissoo* showed the highest IC₅₀ value of 104.04±1.5µg/ml from the above results obtained in the present study, it may be concluded that methanolic extract showed maximum antioxidant activity. *D. sissoo* leaves showed higher percentage in radical scavenging activity compared to other plant extract. *D. sissoo* leaves is a natural reservoir of antioxidants and might be useful in treating a variety of ailments²⁵.

3.3 FRAP

FRAP assay is employed to assess the antioxidant capacity of plant extracts by observing their ability to donate electrons. Compounds within the extract convert Fe³⁺ (ferric ions) in a potassium ferricyanide complex to Fe²⁺ (ferrous ions), thereby reducing potassium ferricyanide into potassium ferrocyanide. The resultant ferrocyanide then reacts with ferric chloride to form a blue ferric-ferrous complex, indicating the presence of antioxidant activity²⁶. This colour change is measured by the intensity of blue coloration, with a higher absorbance at 530 nm reflecting stronger reducing power and more potent antioxidant activity. Additionally, reductants in the sample can inhibit peroxide formation by interacting with peroxide precursors, further demonstrating the extract's antioxidative properties. The highest ferric reduction was shown by *D. sissoo* leaves is 2577.5 ±3.2µM.

4. Conclusion

The present study on *D. sissoo* leaves highlights the significant antioxidative potential of its methanolic extract, as demonstrated through various assays including TPC, DPPH radical scavenging activity, and FRAP. The findings confirm the presence of high levels of

phenolic compounds, which are well-known for their effective antioxidant properties. The IC₅₀ values from the DPPH assay suggest that the antioxidant capacity of *D. sissoo* leaves underscoring the plant's potential as a source of natural antioxidants. *D. sissoo* can be a promising candidate for further research into its therapeutic potentials, especially in the prevention and management of oxidative stress-related disorders.

Compliance with Ethical Standards

Conflict of Interest: The authors declared that there are no conflicts of interest.

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