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Comparative Evaluation of Free Radical Scavenging Activity of *Pterocarpus marsupium Roxb.*

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Abstract: *Pterocarpus marsupium Roxb.* is an important medicinal tree of the Fabaceae family that has been traditionally used in Indian systems of medicine and is recognized for its diverse pharmacological properties. The plant contains a wide range of biologically active phytoconstituents, particularly phenolic compounds, flavonoids, stilbenes and other secondary metabolites that may contribute to its antioxidant potential. The present study was undertaken to determine the free radical scavenging activity of different plant parts and in vitro-derived tissues of *P. marsupium* as part of a broader investigation on mass multiplication and pharmacological profiling of the species. Plant materials were collected from three sites of Barnawapara, Chhattisgarh, India, during June 2022 to May 2024. Leaves, stems, roots, flowers, bark, seeds, pods, in vitro plants and in vitro seedlings were evaluated for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH assay was based on the ability of antioxidant constituents to donate hydrogen or electrons to the stable DPPH radical, resulting in a decrease in absorbance at 517 nm. The antioxidant activity showed considerable variation among plant tissues. The highest DPPH inhibition was recorded in in vitro seedlings (83.8%), followed by in vitro plants (81.9%) and bark (80.3%). Roots and leaves exhibited 72.1% and 68.4% inhibition, respectively, whereas pods, stems, flowers and seeds showed comparatively lower activities of 52.7%, 45.2%, 39.6% and 25.4%, respectively. The strong radical scavenging capacity of bark and in vitro-derived tissues corresponded with their relatively higher phenolic and flavonoid contents. In vitro seedlings also showed the highest total phenolic content (23.4 mg/g) and flavonoid content (26.34 mg/g), supporting the association between secondary metabolite accumulation and antioxidant activity. The findings demonstrate substantial antioxidant potential in P. marsupium, particularly in bark and in vitro-derived tissues, and indicate that tissue culture may provide an alternative source of antioxidant-rich plant material.

Keywords: *Pterocarpus marsupium*, antioxidant activity, DPPH, free radical scavenging, phenolic compounds, flavonoids, in vitro culture, medicinal plant.

Introduction - *Pterocarpus marsupium* commonly known as Indian kino, Vijaysar or Bijaka, is a medicinal tree belonging to the Fabaceae family. Different parts of the plant, including bark, heartwood, leaves, flowers and gum, have traditionally been used for various therapeutic purposes. The bark and wood are especially associated with antidiabetic applications, while other plant parts have been reported to possess antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, analgesic and related biological properties. The pharmacological importance of *P. marsupium* is closely related to its chemical diversity. Previous investigations have reported flavonoids, phenolic compounds, stilbenes, terpenoids, saponins, tannins, glycosides and other constituents in different tissues. Important compounds reported from the species include pterostilbene, epicatechin, pterosupin, marsupin, liquiritigenin and related polyphenolic constituents. These compounds have been associated with several biological activities, particularly antioxidant and antidiabetic effects.

Oxidative stress results when the generation of reactive oxygen species and other free radicals exceeds the capacity of biological antioxidant defense systems. Free radicals possess unpaired electrons and are highly reactive. Excessive free radical generation can damage biomolecules such as proteins, lipids and nucleic acids and has been associated with cellular injury and several chronic pathological conditions. Antioxidants can neutralize reactive species by donating electrons or hydrogen atoms, thereby reducing oxidative damage.The antioxidant properties of *P. marsupium* are particularly relevant because several phytochemicals reported from this species are capable of contributing to radical-scavenging processes. Phenolic compounds and flavonoids can act as electron or hydrogen donors, while other secondary metabolites may contribute

synergistically to overall antioxidant activity.

The DPPH assay is one of the commonly used in vitro methods for preliminary evaluation of antioxidant capacity. DPPH is a stable nitrogen-centered free radical characterized by a deep violet color in solution. When an antioxidant donates a hydrogen atom or electron to DPPH, the radical is reduced and the intensity of the violet color decreases. The reduction can be quantified spectrophotometrically, commonly at 517 nm.

The specific objective addressed in this article was the determination of free radical scavenging activity of *P. marsupium*. The study evaluated nine tissue types—leaves, stems, roots, flowers, bark, seeds, pods, in vitro plants and in vitro seedlings—using the DPPH assay. The antioxidant results were considered together with the quantitative phenolic and flavonoid profiles reported elsewhere in the thesis to provide a more integrated interpretation of antioxidant potential.

Materials and Methods

Plant material and collection: Different plant parts of *P. marsupium* were collected from three different sites of Barnawapara, Chhattisgarh, India, during June 2022 to May 2024. The collection was carried out in the morning. Healthy plant material was selected for analysis. The collected materials were cleaned with distilled water to remove foreign particles and were processed for biochemical analysis.

Preparation of extracts: The thesis describes Soxhlet extraction as the principal hot extraction procedure. Powdered plant material was placed in a porous thimble and continuously extracted with the selected solvent through repeated cycles of vaporization, condensation and siphoning. Extraction was considered complete when the solvent in the siphon tube became clear. The extracts obtained from different plant tissues were subsequently used for the antioxidant investigation.

DPPH radical scavenging assay: Free radical scavenging activity was determined using the DPPH assay following the method cited in the thesis as Kumari (2013), with the calculation of activity percentage based on Shekhar and Anju (2014).

A 0.3 mM solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared in methanol and protected from light. For the assay, 2 mL of extract at the respective concentration was mixed with 1 mL of DPPH solution. Ethanol was used instead of DPPH for the blank, while ethanol was used instead of plant extract for the control. The reaction mixtures were maintained in the dark for 30 min, after which absorbance was recorded at 517 nm. Measurements were performed in triplicate.

DPPH inhibition (%) = [(Absorbance of control " Absorbance of sample) / Absorbance of control] × 100

Comparative phytochemical analysis: To interpret the antioxidant results, the DPPH values were considered alongside the total phenolic and total flavonoid contents reported in the thesis. Total phenolic content was determined for different plant parts, with values ranging from 5.1 to 23.4 mg/g. Total flavonoid content ranged from 6.5 to 26.34 mg/g. This comparison was descriptive and was used to examine whether tissues with higher levels of these secondary metabolites also exhibited stronger DPPH radical-scavenging activity.

Results

Variation in DPPH radical scavenging activity: The DPPH assay revealed marked variation in radical-scavenging activity among the different tissues of P. marsupium.

Plant material	DPPH inhibition (%)
Leaves	68.4
Stems	45.2
Roots	72.1
Flowers	39.6
Bark	80.3
Seeds	25.4
Pods	52.7
In vitro plant	81.9
In vitro seedling	83.8

The highest DPPH inhibition was recorded in in vitro seedlings (83.8%), followed by in vitro plants (81.9%) and bark (80.3%). Roots showed 72.1% inhibition, while leaves exhibited 68.4%. Intermediate activity was observed in pods and stems, with 52.7% and 45.2% inhibition, respectively. Flowers showed 39.6% inhibition and seeds exhibited the lowest value of 25.4%.

Phenolic content and relationship with antioxidant activity: Total phenolic content varied substantially among the tissues examined. Seeds showed the lowest reported value of 5.1 mg/g, whereas in vitro seedlings showed the highest value of 23.4 mg/g. Bark contained 18.4 mg/g, in vitro plants 19.8 mg/g, roots 15.6 mg/g and leaves 12.5 mg/g.

The general pattern of phenolic accumulation corresponded with the DPPH findings. In vitro seedlings, which had the highest phenolic content, also showed the highest DPPH inhibition. Bark and in vitro plants also contained relatively high phenolic concentrations and exhibited strong radical-scavenging activity.

Flavonoid content and antioxidant activity: A similar trend was observed for flavonoids. In vitro seedlings contained 26.34 mg/g total flavonoids, followed by in vitro plants at 23.91 mg/g and bark at 20.1 mg/g. Leaves contained 15.4 mg/g, while stems, roots, flowers, seeds and pods showed lower values.

The tissues with the highest flavonoid concentrations were therefore largely the same tissues that demonstrated the highest DPPH inhibition. This parallel pattern supports the possibility that phenolic and flavonoid compounds contributed substantially to the antioxidant activity observed in the extracts.

Discussion: The present investigation demonstrated considerable differences in free radical scavenging capacity

among tissues of *P. marsupium*. The most important finding was the strong antioxidant performance of in vitro-derived tissues. In vitro seedlings recorded 83.8% DPPH inhibition, while in vitro plants showed 81.9%. Bark also exhibited high activity at 80.3%. These results demonstrate that the antioxidant potential of P. marsupium is not restricted to traditionally used mature tissues and that tissue-cultured material may also possess substantial radical-scavenging capacity.

The DPPH method measures the ability of antioxidant constituents to reduce a stable free radical. Therefore, the observed differences among tissues can reflect differences in the type and concentration of extractable compounds capable of donating electrons or hydrogen atoms.

High antioxidant activity of bark: Bark showed 80.3% DPPH inhibition and represented one of the strongest naturally occurring tissues tested. The high antioxidant activity of bark is biologically plausible because bark serves as an external protective tissue and is exposed to physical injury, pathogens, herbivores and environmental stress. Plants often accumulate defense-related secondary metabolites in tissues that are exposed to such challenges. The total phenolic content of bark was 18.4 mg/g and its total flavonoid content was 20.1 mg/g. Both values were relatively high compared with several other natural tissues. This biochemical profile provides a reasonable explanation for its high DPPH activity.

Strong antioxidant activity of roots: Roots demonstrated 72.1% DPPH inhibition, making them the second strongest naturally occurring tissue after bark. Root tissues interact continuously with soil microorganisms and environmental stresses and therefore possess a range of chemical defense mechanisms.

The relatively high total phenolic content of roots (15.6 mg/g) and flavonoid content (12.3 mg/g) may contribute to their antioxidant activity. Root-associated phenolic compounds can participate in defense against microorganisms and oxidative stress.

Antioxidant activity of leaves: Leaves displayed 68.4% DPPH inhibition. Although lower than bark, roots and in vitro tissues, this represents substantial antioxidant capacity. Leaves are metabolically active organs that continuously encounter light, oxygen and environmental fluctuations. Photosynthetic processes generate reactive oxygen species, requiring efficient antioxidant systems to maintain cellular homeostasis.

The leaf phenolic content was 12.5 mg/g and flavonoid content was 15.4 mg/g. These metabolites may contribute to protection against oxidative stress caused by environmental exposure.

Lower antioxidant activity in stems, flowers, pods and seeds: Stems, flowers, pods and seeds demonstrated lower DPPH inhibition compared with bark, roots and in vitro-derived tissues. Stem activity was 45.2%, flower activity 39.6%, pod activity 52.7%, and seed activity only 25.4%.

The comparatively low activity in seeds is particularly notable. Seeds can contain high levels of storage compounds, but the antioxidant profile depends strongly on species, developmental stage and the specific compounds accumulated. In the present study, seeds showed relatively low phenolic and flavonoid levels compared with in vitro seedlings, which may partly explain the lower DPPH activity.

Enhanced antioxidant activity in *in vitro* tissues: One of the most significant findings of the study was the high DPPH activity of in vitro-derived tissues. In vitro seedlings showed the highest value of 83.8%, while in vitro plants showed 81.9%. These values exceeded all naturally collected tissues evaluated in the study.

The enhanced antioxidant capacity of tissue-cultured material may be related to the controlled nutritional environment, availability of carbon sources, growth regulators and physiological conditions associated with in vitro culture. Tissue culture can alter plant metabolism and may stimulate secondary metabolite biosynthesis under particular conditions.

In vitro seedlings had the highest total phenolic content (23.4 mg/g) and highest total flavonoid content (26.34 mg/g), together with the highest DPPH activity. In vitro plants also showed high phenolic content (19.8 mg/g), flavonoid content (23.91 mg/g) and DPPH activity (81.9%). This simultaneous elevation of antioxidant-associated phytochemicals and radical-scavenging activity is an important observation.

Association between phenolics, flavonoids and DPPH activity: A clear descriptive relationship was observed between phenolic/flavonoid accumulation and DPPH activity. The highest three DPPH values were found in in vitro seedlings, in vitro plants and bark. These tissues also showed high phenolic and flavonoid concentrations.

In vitro seedlings had 23.4 mg/g phenolics and 26.34 mg/g flavonoids, with 83.8% DPPH inhibition. In vitro plants contained 19.8 mg/g phenolics and 23.91 mg/g flavonoids, with 81.9% inhibition. Bark contained 18.4 mg/g phenolics and 20.1 mg/g flavonoids, with 80.3% inhibition.

Conversely, seeds exhibited 5.1 mg/g phenolics, 6.5 mg/g flavonoids and only 25.4% DPPH inhibition. This parallel trend suggests that phenolic and flavonoid compounds may be important contributors to the radical-scavenging capacity of the species.

However, it is scientifically important not to conclude that these compounds alone are responsible for the observed antioxidant activity. Plant extracts contain complex mixtures of compounds, and interactions among phenolics, flavonoids, saponins, anthocyanins and other constituents may influence the overall response.

Pharmacological significance: The antioxidant activity demonstrated by *P. marsupium* supports its continued investigation as a source of bioactive compounds. The results are particularly relevant to the known

pharmacological importance of *P. marsupium*, including its traditional association with antidiabetic applications.

Nevertheless, the DPPH findings should be interpreted as evidence of in vitro chemical radical-scavenging capacity, rather than direct evidence of therapeutic efficacy in humans. Further studies involving additional antioxidant assays, compound isolation, mechanistic investigations, cellular models and in vivo validation would be required before pharmacological claims can be made.

Importance of *in vitro*-derived antioxidant material: The most notable implication of the study is the high antioxidant activity observed in in vitro-derived tissues. Conventional harvesting of bark and heartwood can place additional pressure on medicinal tree populations because these tissues are obtained by destructive collection. In contrast, tissue culture provides a controlled system for generating plant biomass without repeatedly harvesting mature trees. If future studies confirm that in vitro-derived tissues consistently maintain high concentrations of target antioxidant compounds, optimized tissue culture systems could potentially be developed for production of bioactive biomass.

Conclusion: The present study demonstrated substantial variation in free radical scavenging activity among different tissues of *Pterocarpus marsupium*. DPPH inhibition ranged from 25.4% in seeds to 83.8% in in vitro seedlings. The highest activities were observed in in vitro seedlings (83.8%), in vitro plants (81.9%) and bark (80.3%), followed by roots (72.1%) and leaves (68.4%). Stems, pods and flowers showed intermediate-to-low activity, whereas seeds showed the lowest antioxidant response.

The strong antioxidant activity observed in in vitro seedlings and in vitro plants was accompanied by comparatively high phenolic and flavonoid contents. In vitro seedlings, in particular, contained the highest reported phenolic and flavonoid concentrations and simultaneously demonstrated the highest DPPH inhibition. This pattern indicates a strong association between secondary metabolite accumulation and radical-scavenging capacity.

Bark also showed substantial antioxidant activity and contained high levels of phenolics and flavonoids, supporting its recognized pharmacological importance. The results further demonstrate that antioxidant potential differs markedly among tissues and that selective utilization of high-activity tissues may be more appropriate than assuming uniform pharmacological properties throughout the plant.

The findings provide experimental support for the antioxidant potential of *P. marsupium* and highlight the importance of in vitro-derived tissues as promising alternative sources of antioxidant-rich biomass. In combination with the successful in vitro propagation work reported in the thesis, these results suggest that plant tissue culture may have dual significance for conservation and sustainable production of pharmacologically valuable material.

However, DPPH activity alone cannot establish therapeutic efficacy. Future investigations should quantify individual antioxidant compounds using advanced analytical techniques such as HPLC, HPTLC, GC-MS or LC-MS, determine concentration-response relationships and IC50 values, compare multiple complementary antioxidant assays, and investigate the biological mechanisms underlying the observed activity.

Pterocarpus marsupium possesses considerable free radical scavenging potential, with particularly strong activity in *in vitro*-derived seedlings and plants. These findings contribute to the pharmacological profiling of the species and provide a basis for further research into sustainable production and utilization of its antioxidant phytochemicals.

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