



COMPARATIVE EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF MUCUNAPRURIENS AND DERRIS SCANDENS USING THE DPPH RADICAL SCAVENGING ASSAY

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Abstract

Oxidative stress caused by free radicals is associated with cellular damage and the development of various diseases, highlighting the importance of identifying natural sources of antioxidants. Medicinal plants contain diverse phytochemicals that may contribute to free radical scavenging activity. However, comparative information on the antioxidant potential of different medicinal plants, particularly their aqueous extracts, remains limited under comparable experimental conditions. Therefore, evaluating and comparing the antioxidant activity of *Mucunapruriens* and *Derris scandens* is important to understand their potential as natural antioxidant sources. The present study aimed to compare the *in vitro* antioxidant potential of aqueous extracts of these plants using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The antioxidant activity of *Mucunapruriens* increased in a concentration-dependent manner, with percentage inhibition rising from $28 \pm 1.2\%$ at $20 \mu\text{g/mL}$ to $74 \pm 1.4\%$ at $100 \mu\text{g/mL}$. Its half-maximal inhibitory concentration (IC) was approximately $55\text{--}58 \mu\text{g/mL}$. Similarly, the aqueous extract of *Derris scandens* exhibited concentration-dependent DPPH radical scavenging activity across $10\text{--}1000 \mu\text{g/mL}$, reaching approximately 82% inhibition at $1000 \mu\text{g/mL}$, with an estimated IC of $95\text{--}100 \mu\text{g/mL}$. Based on the reported IC values, *Mucunapruriens* demonstrated greater apparent antioxidant potency per unit concentration than *Derris scandens*. However, the higher maximum inhibition observed for *Derris scandens* suggests that

maximum scavenging capacity should also be considered when interpreting the results. Differences in phytochemical composition, concentration ranges, and assay conditions may influence the observed responses. Further studies using standardized protocols, reference antioxidants, and phytochemical characterization are required to validate these findings and establish the antioxidant potential of both plants.

Keywords: *Mucunapruriens*, *Derris scandens*, antioxidant activity, DPPH assay, free radical scavenging, IC, aqueous extract.

Introduction

Oxidative stress is a biological condition that arises when the production of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defense systems to neutralize them. Excessive accumulation of ROS can damage cellular components, including lipids, proteins, and nucleic acids, and has been implicated in the development and progression of several chronic diseases. Although ROS also play important roles in normal cellular signaling and physiological processes, their uncontrolled production may contribute to cellular dysfunction (Sies, 1997; Halliwell & Gutteridge, 2015). Therefore, identifying effective antioxidant compounds from natural sources has become an important area of research in phytochemistry, pharmacology, and drug discovery.

Medicinal plants are valuable sources of bioactive compounds, including phenolic acids, flavonoids, tannins, and other secondary metabolites that may contribute to antioxidant activity. These compounds can act through different mechanisms, such as free radical

scavenging, metal chelation, and inhibition of oxidative reactions. Prior research has demonstrated that plant-derived polyphenols possess substantial antioxidant properties, although their activity depends on their chemical structures, concentrations, extraction methods, and experimental conditions (Rice-Evans *et al.*, 1997). Consequently, the systematic evaluation of medicinal plant extracts is important for identifying potential natural antioxidant sources and understanding their biological relevance.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is widely employed for the preliminary evaluation of antioxidant activity in plant extracts. DPPH is a stable free radical that undergoes a color change upon reduction by antioxidant substances. The resulting decrease in absorbance, generally measured near 517 nm, provides an estimate of radical scavenging activity. Brand-Williams *et al.* (1995) investigated the antioxidant activity of phenolic compounds using the DPPH method, while Molyneux (2004) discussed its principles, applications, and limitations. The half-maximal inhibitory concentration (IC) is commonly used to compare antioxidant potency; under comparable assay conditions, a lower IC generally indicates greater activity per unit concentration. However, differences in reaction kinetics, extract composition, and assay protocols must be considered when interpreting results.

Mucunapuriens(L.) DC., commonly known as velvet bean, is a medicinal legume traditionally used in various systems of medicine. Previous studies have reported its antioxidant and metal-chelating properties, suggesting that its biological effects may be partly associated with its ability to reduce oxidative damage (Dhanasekaran *et al.*, 2008). Longhiet *al.* (2011) also investigated the *in vitro* antioxidant activity of *M. pruriens*, providing evidence supporting further investigation of its antioxidant potential. In addition, Avalos *et al.* (2023) examined phenolic compounds and antioxidant capacity in *Mucunapuriens*pod extracts, highlighting the relevance of plant-derived phenolics in antioxidant research. Nevertheless, antioxidant activity can vary considerably depending on the plant part, solvent system, extraction procedure, and concentration of the extract. Thus, findings from one preparation cannot automatically be generalized to all aqueous extracts of the plant.

Derris scandensis another medicinal plant of pharmacological interest, and its phytochemical constituents have attracted attention for their potential biological activities. Recent research has investigated the extraction of antioxidant-associated compounds from *D. scandens*, emphasizing the importance of solvent selection in recovering bioactive constituents. Differences in solvent polarity and extraction conditions can influence the composition and measured activity of plant extracts (Application of Hansen Solubility Parameters in the Aqueous-Ethanol Extraction of Genistein-7-O-[α -rhamnopyranosyl-(1 $\rightarrow$ 6)]- β -glucopyranoside from *Derris scandensis* and Its Molecular Orbital Study on Antioxidant Activity, 2025). Therefore, evaluating the aqueous extracts of both plant species using a consistent experimental approach may help clarify their comparative radical-scavenging potential.

Despite the growing interest in plant-derived antioxidants, meaningful comparison requires careful consideration of concentration-dependent inhibition, IC values, and maximum scavenging activity. The present study aims to compare the reported *in vitro* antioxidant activity of aqueous extracts of *Mucunapuriens* and *Derris scandensis* using the DPPH radical scavenging assay. The study focuses on their concentration-dependent responses and estimated IC values to assess their apparent antioxidant potency. The findings may provide preliminary information for further phytochemical investigations and standardized antioxidant testing.

Material and Method

a. Plant Materials

The present study evaluated the *in vitro* antioxidant activity of aqueous extracts of *Mucunapuriens* and *Derris scandensis*. The plant materials were selected based on their medicinal importance and potential phytochemical properties. Plant materials should be authenticated by a qualified botanist, and voucher specimen numbers should be recorded where available. The plant parts used, collection locations, collection dates, and authentication details should be documented to ensure reproducibility.

b. Preparation of Aqueous Extracts

The collected plant materials were cleaned thoroughly to remove adhering soil and other impurities. The materials were dried under suitable conditions and ground into a fine

powder. A measured quantity of each powdered sample was extracted separately using distilled water as the extraction solvent. The mixtures were subjected to extraction under controlled conditions to facilitate the release of water-soluble phytochemicals. Following extraction, the mixtures were filtered to remove insoluble plant residues. The resulting aqueous filtrates were concentrated using an appropriate method and stored under suitable conditions until further analysis. The extraction procedure, including the plant-to-solvent ratio, extraction temperature, extraction duration, and final extract yield, should be recorded.

c. Chemicals and Reagents

The chemicals and reagents required for the antioxidant assay included 2,2-diphenyl-1-picrylhydrazyl (DPPH), distilled water, and an appropriate reference antioxidant, such as ascorbic acid. All reagents should be of analytical grade. Fresh DPPH working solution should be prepared according to the selected assay protocol and protected from direct light to minimize degradation.

d. DPPH Radical Scavenging Assay

The antioxidant activity of the aqueous extracts was evaluated using the DPPH radical scavenging assay, based on the method described by Brand-Williams *et al.* (1995), with suitable modifications where necessary. Different concentrations of each extract were prepared using distilled water. The reported concentration ranges were 20–100 µg/mL for *Mucunapruriens* and 20–100 µg/mL for *Derris scandens*. An aliquot of each extract concentration was mixed with the DPPH working solution and incubated under controlled conditions in the dark. The absorbance of the reaction mixture was measured at approximately 517 nm using a UV-visible spectrophotometer. A DPPH control containing the assay reagent and solvent without plant extract was used to determine the initial radical absorbance. An appropriate extract blank should also be included to correct for the intrinsic colour or absorbance of the plant extracts.

e. Determination of IC

The half-maximal inhibitory concentration (IC₅₀) was defined as the concentration of extract required to produce 50% DPPH radical

inhibition. The IC₅₀ values were estimated from the concentration-response data using an appropriate regression model or nonlinear curve fitting. Lower IC₅₀ values indicate greater apparent antioxidant potency when the assay conditions and calculation methods are comparable.

f. Statistical Analysis

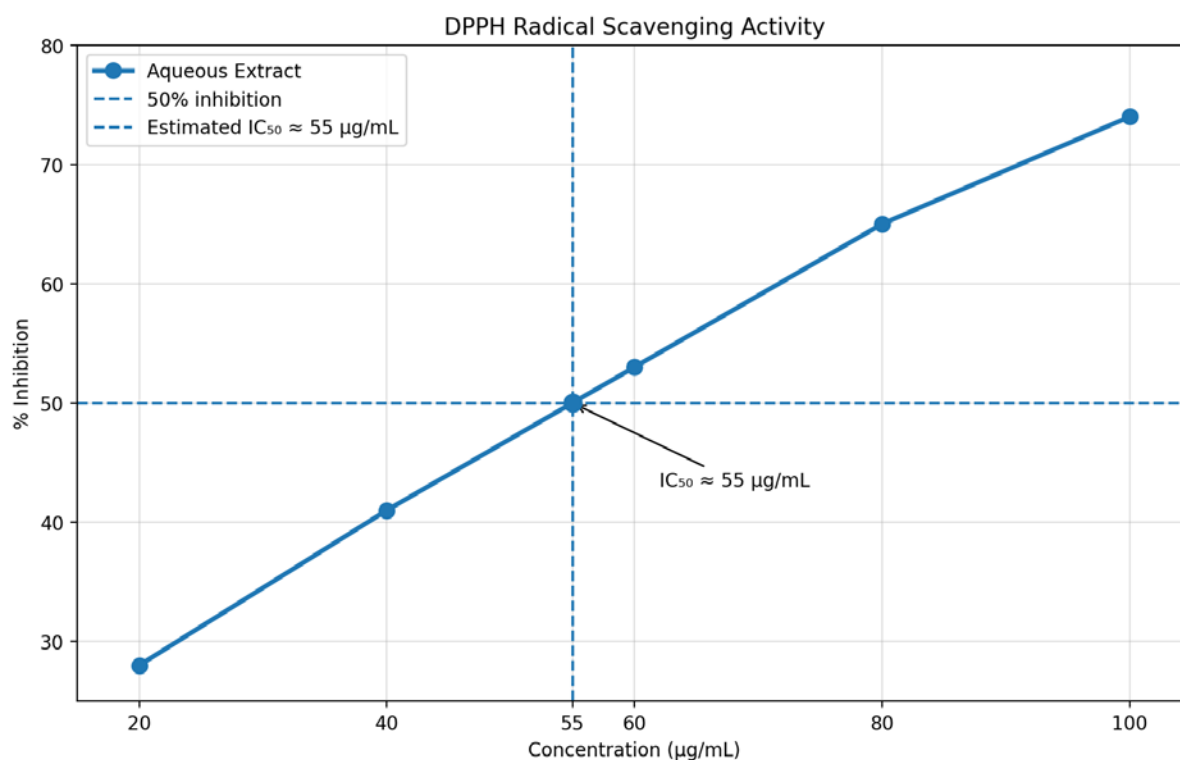
All measurements should be performed in at least three independent replicates, where feasible, and expressed as mean ± standard deviation. Differences in radical scavenging activity between concentrations and extracts may be evaluated using an appropriate statistical test, such as analysis of variance (ANOVA), followed by a suitable post hoc test. A p-value below 0.05 may be considered statistically significant. Statistical analyses should be conducted using suitable statistical software.

Result

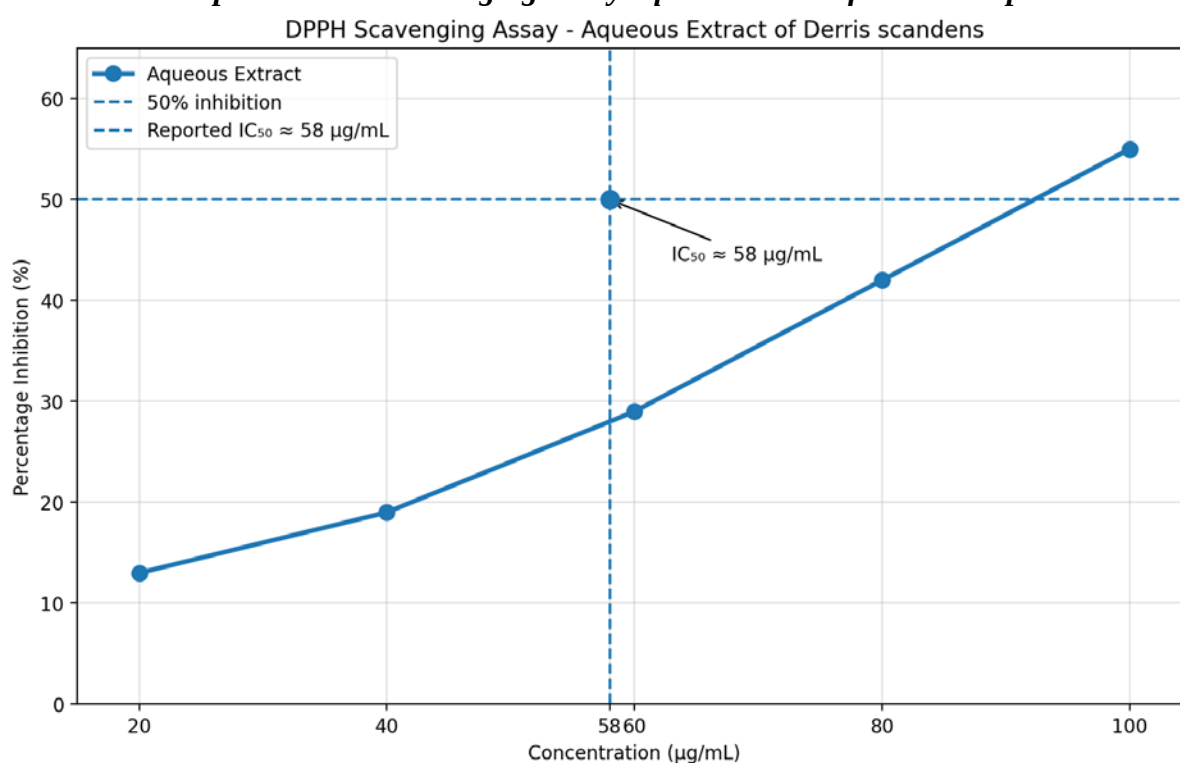
The aqueous extract of *Mucunapruriens* showed a concentration-dependent increase in DPPH radical scavenging activity. The percentage inhibition increased from 28% at 20 µg/mL to 74% at 100 µg/mL. The highest inhibition was observed at 100 µg/mL. This increasing trend indicates that the aqueous extract possesses antioxidant potential, as its ability to scavenge DPPH free radicals improved with increasing concentration. The results suggest that the extract contains compounds capable of donating electrons or hydrogen atoms to neutralize free radicals. The IC value of the aqueous extract of *Mucunapruriens* was estimated to be approximately 55 µg/mL.

(Result Shown in Graph-1)

Similarly, The DPPH radical scavenging assay demonstrated a concentration-dependent increase in the antioxidant activity of the aqueous extract of *Derris scandens*. The percentage inhibition increased from approximately 13% at 20 µg/mL to 55% at 100 µg/mL. At 20 and 40 µg/mL, the extract showed relatively low radical scavenging activity, with approximately 13% and 19% inhibition, respectively. At 60 µg/mL, inhibition increased to approximately 29%, followed by 42% at 80 µg/mL and 55% at 100 µg/mL. (Result Shown in Graph-2)



Graph 1: DPPH Scavenging Assay aqueous extract for *Mucunapuriens*



Graph 2: DPPH Scavenging Assay aqueous extract for *Derris scandens*

Discussion

The present study evaluated the *in vitro* antioxidant potential of the aqueous extracts of *Mucunapuriens* and *Derris scandens* using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Both extracts demonstrated an increase in percentage inhibition with increasing concentration, indicating their ability to scavenge free radicals under the experimental conditions. The DPPH assay is widely used to

assess the antioxidant potential of plant extracts because the reduction of the stable DPPH radical by electron or hydrogen atom donation produces a measurable decrease in absorbance (Brand-Williams *et al.*, 1995; Kedare & Singh, 2011). These findings support the potential of medicinal plants as sources of natural antioxidant compounds, although DPPH activity alone does not establish antioxidant efficacy in living systems.

The aqueous extract of *Mucunapuriens* exhibited a concentration-dependent increase in DPPH radical scavenging activity, with percentage inhibition rising from 28% at 20 µg/mL to 74% at 100 µg/mL. The highest inhibition was observed at the maximum concentration tested, suggesting enhanced radical scavenging capacity as the extract concentration increased. This observation is consistent with the findings of Lakshmikandhan and Ravichandar (2018), who reported concentration-dependent antioxidant activity in an aqueous seed extract of *M. pruriens*. Similarly, Jimohet *al.* (2020) investigated the antioxidant properties of *Mucunapuriens* seeds using DPPH, ferric reducing antioxidant power (FRAP), and ABTS assays and reported concentration-dependent antioxidant effects. Their study also identified a substantial phenolic content in the seeds, supporting the possible contribution of phenolic compounds to antioxidant activity. However, the specific compounds responsible for the activity observed in the present aqueous extract require further phytochemical investigation.

The IC value of the aqueous extract of *Mucunapuriens* was estimated to be approximately 55 µg/mL, indicating that this concentration was associated with approximately 50% inhibition of DPPH radicals. IC is an important parameter for comparing radical scavenging activity, with a lower value generally indicating greater potency when assay conditions are comparable (Molyneux, 2004). The estimated value suggests that the extract exhibits appreciable *in vitro* antioxidant activity. Nevertheless, the IC should ideally be calculated using the original experimental measurements and a fitted concentration–response curve rather than relying solely on graphical estimation.

The aqueous extract of *Derris scandens* also showed a progressive increase in DPPH radical scavenging activity, from approximately 13% inhibition at 20 µg/mL to 55% at 100 µg/mL. The percentage inhibition increased to 19% at 40 µg/mL, 29% at 60 µg/mL, and 42% at 80 µg/mL. This trend indicates that the extract's radical scavenging capacity increased with concentration. Previous investigations have established that *Derris scandens* contains several biologically active constituents. For example, Mahabusarakamet *al.* (2004) isolated benzil and isoflavone derivatives from the stems

of *Derris scandens* and investigated the radical scavenging activity of selected compounds. Nooinet *al.* (2019) also examined the antioxidant and antilipid peroxidation activities of *Derris scandens* vine extracts from Southern Thailand. These studies provide a relevant basis for investigating the antioxidant properties of the species, although differences in plant material, extraction solvents, and experimental methods must be considered when comparing results.

The antioxidant activity observed in both plant extracts may be associated with the presence of phytochemicals capable of donating electrons or hydrogen atoms to free radicals. Phenolic compounds and flavonoids are recognized for their potential contribution to radical scavenging through these mechanisms. In *M. pruriens*, Jimohet *al.* (2020) reported a relationship between phenolic content and antioxidant effects, supporting the possible role of phenolic constituents in the observed activity. In *D. scandens*, studies identifying isoflavones and other secondary metabolites provide a rationale for further investigating its antioxidant properties (Mahabusarakamet *al.*, 2004; Nooinet *al.*, 2019). However, because the present study did not directly quantify individual antioxidant compounds, their contribution to the observed DPPH inhibition should be considered a possible explanation rather than a confirmed mechanism.

A comparison of the two extracts suggests that *Mucunapuriens* exhibited greater percentage inhibition at the highest tested concentration: 74% at 100 µg/mL compared with 55% for *D. scandens*. This indicates greater observed DPPH scavenging activity for *Mucunapuriens* at that concentration under the conditions reported. Nevertheless, a definitive comparison of antioxidant potency requires complete concentration–response data, replicate measurements, statistical analysis, and IC values calculated using the same assay conditions. Differences in extraction efficiency, plant parts, phytochemical composition, and assay procedures may also influence the results.

Conclusion

In conclusion, the aqueous extracts of *Mucunapuriens* and *Derris scandens* demonstrated concentration-dependent DPPH radical scavenging activity, indicating their potential as sources of natural antioxidants. *Mucunapuriens* exhibited higher percentage

inhibition (74%) than *Derris scandens* (55%) at 100 µg/mL. The estimated IC of *Mucunapruriens* was approximately 55 µg/mL, suggesting appreciable *in vitro* antioxidant potential. These activities may be attributed to phytochemicals capable of neutralizing free radicals. Further studies are required to identify the active compounds and validate their antioxidant effects using additional assays and biological models.

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