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Phytochemical Profiling and Antioxidant Potential of *Acacia farnesiana* (L.) Willd. Bark Collected from Three Distinct Indian Localities

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ABSTRACT

***Acacia farnesiana* (L.) Willd. is a medicinal plant widely used in traditional medicine; however, scientific evidence regarding the influence of geographical variation on its phytochemical composition and antioxidant potential remains limited. The present study aimed to comparatively evaluate the phytochemical profile and in-vitro antioxidant activity of *A. farnesiana* bark collected from three distinct Indian localitiesRajasthan, Haryana, and Punjab. Ethanolic bark extracts were subjected to qualitative phytochemical screening, quantitative estimation of total phenolic and flavonoid contents, and antioxidant evaluation using DPPH and ABTS radical scavenging assays. The results revealed marked regional variation in phytochemical composition, with Rajasthan bark exhibiting higher levels of phenolics and flavonoids compared to samples from Haryana and Punjab. Correspondingly, the Rajasthan extract demonstrated the strongest antioxidant activity, reflected by lower IC_{50} values in both assays. A positive correlation was observed between phenolic/flavonoid content and antioxidant efficacy. These findings highlight the significant impact of geographical factors on the bioactive profile of *A. farnesiana* bark and support its potential as a natural antioxidant source.**

INTRODUCTION

Nearly four-fifths of the world's population relies on traditional herbal medicine as a primary component of healthcare, particularly in regions where access to modern medical facilities remains limited [1]. A significant proportion of these therapeutic practices originate from long-established medical traditions in China, India, and several African countries. This continued dependence on plant-based treatments reflects both the deep historical roots of indigenous healthcare systems and present-day challenges related to affordability, availability, and infrastructure of conventional healthcare services in resource-constrained settings [2].Medical systems such as Traditional Chinese Medicine, Ayurveda from the Indian subcontinent, and diverse African ethnomedicinal practices encompass centuries of empirical knowledge regarding the medicinal value of plant-derived compounds and their therapeutic applications. These systems remain deeply embedded in local cultures and are widely employed for the management of a broad spectrum of health conditions, including infectious diseases, inflammatory disorders, and chronic illnesses [3].

The growing global reliance on herbal remedies also underscores persistent inequalities in access to modern pharmaceuticals, especially in developing nations where traditional medicine often represents the most accessible and cost-effective healthcare alternative for large populations. In recent years, the use of herbal medicines has expanded beyond traditional settings, driven by increasing scientific interest in plant-derived bioactive constituents for the treatment of conditions ranging from microbial infections to cancer. This resurgence highlights the therapeutic potential of natural products and supports ongoing efforts to scientifically validate traditional medicinal plants [4].

Acacia farnesiana (L.) Willd., belonging to the family Fabaceae, is a well-known medicinal plant widely distributed across tropical and subtropical regions of India. Traditionally, different parts of the plantincluding bark, leaves, flowers, and podshave been used in folk medicine for the management of diarrhea, dysentery, inflammation, skin disorders, and wound healing. The bark of *A. farnesiana* is particularly rich in secondary metabolites and has been reported to possess astringent, antimicrobial, and anti-inflammatory properties [5].

However, systematic scientific validation of its phytochemical composition and antioxidant potential remains limited. Phytochemical composition of medicinal plants is influenced by several factors such as geographical location, climatic conditions, soil characteristics, altitude, and environmental stress. These variations can significantly affect the concentration and profile of bioactive constituents, thereby influencing the therapeutic efficacy of herbal raw materials. Therefore, comparative studies assessing the impact of geographical origin on phytochemical content and biological

activity are essential for crude drug standardization, quality control, and selection of superior plant sources for herbal formulations [6].

Despite the traditional importance of *A. farnesiana*, there is a lack of comprehensive comparative data on the phytochemical profile and antioxidant activity of its bark collected from different Indian localities. Understanding such regional variations is critical for establishing quality benchmarks and ensuring reproducibility in pharmacological investigations. Therefore, the present study aims to perform a comparative phytochemical screening and evaluate the in-vitro antioxidant potential of *Acacia farnesiana* bark samples collected from three distinct Indian geographical regions. The findings of this study are expected to provide valuable insights into the influence of geographical variation on phytochemical composition and antioxidant activity, thereby supporting the rational selection and standardization of *A. farnesiana* bark as a potential natural antioxidant source.

MATERIALS AND METHODS

2.1. Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2″-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS), Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, potassium acetate, sodium acetic acid, glacial acetic acid, ascorbic acid, quercetin, and gallic acid were purchased from Sigma-Aldrich (St. Louis, Mo, USA). All chemicals were of analytical grade.

2.2. Plant Material

The bark of *Acacia farnesiana* (L.) Willd. was collected from three geographically distinct locations (Rajasthan, Haryana and Punjab) in India, representing variations in climatic and edaphic conditions. Botanical identification and authentication of the plant material were carried out by a senior scientist of Himachal Pradesh State Biodiversity Board, Shimla, Himachal Pradesh.

2.3. Preparation of Extracts

Firstly, the plant bark were washed with water to remove dirt and other foreign matters were separated and shade dried. Dried leaves were then milled to a coarse powder and then passed over sieve No. 14. The obtained dried powdered bark of *A. farnesiana* (20 g) were placed in the tube of Soxhlet apparatus in the form of a thimble and extracted with ethanol (300 mL) at 60–65 °C for 3–4 h. The obtained ethanolic extracts of all three-region sample, were filtered while hot and dried by evaporation using a rotary vacuum evaporator and the final dried extract samples were kept at low temperature in the fridge for further study. The residue obtained from each extract was dissolved in the same solvent for further analysis.

2.4. Total Polyphenols and Flavonoid Contents

The total phenolic content (TPC) and flavonoid (TFC) content of each *A. farnesiana*bark extracts were determined using the earlier reported method. TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g of extract, while the TFC was expressed as mg of quercetin equivalents (QE) per 100 g [7-9].

2.5. Antioxidant Activity

2.5.1. DPPH Radical-Scavenging Activity

The free radical scavenging capability of extracts solution on the DPPH radical was determined as previously described. Ethanolic extracts of three resion sample were prepared at different concentrations from 20 to 100 µg/mL. The DPPH radical solution (50 µM) was added to the solution of various plant extracts concentrations and standard ascorbic acid individually. The reaction mixtures were shaken thoroughly and kept in the dark for 30 min. The control solution was prepared by adding 2 mL of methanol with 2 mL of DPPH solution. The absorbance of all the reaction mixtures and control solution was measured at 517 nm [10]. The percentage inhibition was calculated by the following formula:

$$\%\ inhibition = \frac{(Ac517 - As517)}{Ac517} X\ 100$$

where, AC is the absorbance of Control and AS is the absorbance of the Sample.

The graph was plotted between % inhibition and different concentrations of plant extracts and ascorbic acid and IC_{50} value was determined.

2.5.2. ABTS Assay

The reducing power of the crude extracts was determined using the ABTS assay as described earlier. ME, EAE, and AE solutions were prepared at varying concentrations from 20 to 100 µg/mL.

One milliliter of distilled dimethyl sulfoxide was mixed to 0.2 mL of varying concentrations of the samples and 0.16 mL ABTS solution was added to obtain a volume of 1.36 mL. The absorbance was analyzed spectrophotometrically, after 20 min at 734 nm with a UV spectrophotometer [11]. Control remained without a sample. ABTS scavenging capacity of the ABTS was expressed as IC_{50} (µg/mL) and the percentage of inhibition was calculated using the following formula:

ABTS scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

where, A_0: absorbance of the control, A_1: absorbance of the sample.

2.6 Statistical Analysis

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical comparison among samples from three different locations was carried out using one-way ANOVA, followed by appropriate post hoc tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

1.1. Comparative Qualitative Phytochemical Screening of Bark Extracts

The qualitative phytochemical screening of *Acacia farnesiana* bark ethanolic extract revealed a distinct regional variation in the distribution and intensity of major secondary metabolites, as shown in Table 1

Table 1 Comparative qualitative phytochemical profile of bark ethanolic extract

Phytoconstituent	Rajasthan	Haryana	Punjab
Phenols	+++	++	++
Flavonoids	+++	++	+
Alkaloids	++	++	+
Tannins	+++	++	++
Saponins	++	+	+

The presence of phenolic compounds was most prominent in the Rajasthan bark extract (+++), whereas Haryana and Punjab samples showed moderate presence (++). Phenols are well known for their antioxidant, anti-inflammatory and protective roles in plants, and their higher abundance in Rajasthan bark may be attributed to environmental stress conditions such as high temperature and low water availability, which stimulate phenolic biosynthesis.

Similarly, flavonoids were detected in high intensity (+++) in Rajasthan samples, moderate levels (++) in Haryana, and low levels (+) in Punjab. Flavonoids function as UV protectants and stress-responsive metabolites, and their elevated presence in Rajasthan bark supports the adaptive role of secondary metabolites under harsh climatic conditions
.

The alkaloid content was found to be moderately present (++) in both Rajasthan and Haryana bark samples, while only weak presence (+) was observed in Punjab. Alkaloids are nitrogen-containing compounds associated with plant defense mechanisms, and their comparatively reduced presence in Punjab samples may reflect favourable growth conditions with reduced biotic stress.

Tannins showed strong presence (+++) in Rajasthan bark and moderate presence (++) in Haryana and Punjab samples. Tannins contribute to astringent properties and possess antimicrobial and antioxidant activities, further indicating the superior pharmacological potential of Rajasthan bark.

The presence of saponins followed a decreasing trend from Rajasthan (++) to Haryana and Punjab (+). This observation correlates well with the higher foaming index observed in Rajasthan samples, thereby validating the qualitative phytochemical findings.

Overall, the comparative qualitative phytochemical profile clearly demonstrates that *A. farnesiana* bark ethanolic extract from Rajasthan contains a richer and more diverse spectrum of bioactive secondary metabolites than samples from Haryana and Punjab, emphasizing the strong influence of geographical and environmental factors on phytochemical composition.

1.2. Comparative Quantitative Phytochemical Content (Bark)

The quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) in the ethanolic bark extracts of *Acacia farnesiana* collected from Rajasthan, Haryana and Punjab revealed significant regional variation, as shown in Table 2.

Table 2. Comparative TPC and TFC of bark Ethanolic Extract

Parameter	Rajasthan	Haryana	Punjab
TPC (mg GAE/100 g)	98.4 ± 2.6	85.2 ± 2.2	78.6 ± 2.0
TFC (mg QE/g)	52.8 ± 1.9	44.6 ± 1.7	38.2 ± 1.5

The TPC values ranged from 78.6 ± 2.0 to 98.4 ± 2.6 mg GAE/100 g, with the highest phenolic content observed in the Rajasthan bark extract. Phenolic compounds are primary contributors to antioxidant potential due to their hydrogen- and electron-donating ability. The elevated TPC in Rajasthan samples may be attributed to environmental stress factors such as high temperature, intense sunlight and limited water availability, which are known to enhance the biosynthesis

and accumulation of phenolic compounds as part of the plant's adaptive defense mechanism.
Similarly, the TFC values showed a decreasing trend from Rajasthan (52.8 ± 1.9 mg QE/g) to Haryana (44.6 ± 1.7 mg QE/g) and Punjab (38.2 ± 1.5 mg QE/g). Flavonoids play a crucial role in protecting plants against oxidative stress, UV radiation and microbial attack. The higher flavonoid concentration in Rajasthan bark further supports the stress-induced enhancement of secondary metabolite production under arid climatic conditions.

1.3. Comparative Antioxidant Activity of Bark

The in-vitro antioxidant activity of *Acacia farnesiana* bark ethanolic extracts from Rajasthan, Haryana and Punjab was evaluated using DPPH and ABTS radical scavenging assays, and the results are summarized in Table 3. Both assays demonstrated a clear concentration-dependent and region-dependent antioxidant response.

Table 3. Comparative IC_{50} values (µg/mL)

Sample	DPPH	ABTS
Rajasthan bark	34.2 ± 1.1	31.8 ± 1.0
Haryana bark	40.6 ± 1.3	37.9 ± 1.2
Punjab bark	46.9 ± 1.5	44.1 ± 1.4
Ascorbic acid	18.4 ± 0.6	15.2 ± 0.4

The Rajasthan bark ethanolic extract exhibited the lowest IC_{50} values in both DPPH (34.2 ± 1.1 µg/mL) and ABTS (31.8 ± 1.0 µg/mL) assays, indicating the strongest free radical scavenging activity among the three regional samples. This enhanced antioxidant potential correlates well with the higher total phenolic and flavonoid contents observed in the Rajasthan bark, as phenolic hydroxyl groups play a crucial role in neutralizing free radicals through hydrogen or electron donation.

The Haryana bark extract showed moderate antioxidant activity, with IC_{50} values of 40.6 ± 1.3 µg/mL (DPPH) and 37.9 ± 1.2 µg/mL (ABTS), whereas the Punjab bark extract demonstrated comparatively weaker activity, reflected by higher IC_{50} values. This gradual decline in antioxidant efficiency from Rajasthan to Punjab mirrors the decreasing trend in quantitative phytochemical content, particularly phenolics and flavonoids.

The standard antioxidant, ascorbic acid, exhibited significantly lower IC_{50} values than all bark extracts, confirming the validity and sensitivity of the experimental assays. However, the comparatively strong activity of the Rajasthan bark extract indicates its substantial natural antioxidant potential.

Furthermore, the slightly lower IC_{50} values observed in the ABTS assay compared to the DPPH assay for all samples suggest that the bark extracts are more effective in scavenging hydrophilic radicals, which is consistent with the predominance of polar phenolic compounds in the ethanolic extracts.

Overall, the comparative antioxidant evaluation confirms that *Acacia farnesiana* bark collected from Rajasthan possesses superior free radical scavenging ability, which can be directly attributed to its higher phenolic and flavonoid content. These findings support the potential therapeutic relevance of the bark and justify its further exploration for antioxidant-based pharmacological applications. These variations clearly demonstrate the impact of geographical and climatic conditions on the bioactive profile of *A. farnesiana* bark.

CONCLUSION

The present study provides a comprehensive comparative evaluation of the phytochemical composition and antioxidant potential of *Acacia farnesiana* bark collected from three distinct Indian localities. Both qualitative and quantitative analyses revealed marked geographical variation in the distribution and concentration of bioactive secondary metabolites, particularly phenolics, flavonoids, tannins, and saponins. Among the three regions examined, bark samples collected from Rajasthan consistently exhibited a richer phytochemical profile, with significantly higher total phenolic and flavonoid contents compared to samples from Haryana and Punjab.The enhanced phytochemical richness of the Rajasthan bark was reflected in its superior in-vitro antioxidant activity, as demonstrated by lower IC_{50} values in both DPPH and ABTS radical scavenging assays. The strong correlation observed between phenolic/flavonoid content and antioxidant efficacy underscores the central role of these compounds in mediating the free radical scavenging potential of *A. farnesiana* bark. The comparatively weaker antioxidant activity observed in Haryana and Punjab samples further emphasizes the influence of geographical, climatic, and environmental factors on the biosynthesis of secondary metabolites.These findings highlight the importance of geographical origin in determining the pharmacological quality of herbal raw materials and reinforce the need for region-specific selection and standardization of medicinal plant resources. The superior antioxidant potential of *A. farnesiana* bark from Rajasthan suggests its suitability as a promising natural source of antioxidant compounds for use in herbal formulations and nutraceutical applications.Overall, this study contributes valuable scientific evidence supporting the traditional use of *Acacia*

farnesiana and provides a foundation for future investigations focusing on the isolation, characterization, and mechanistic evaluation of its bioactive constituents. Further in-vivo and molecular studies are warranted to substantiate its therapeutic potential and facilitate its rational utilization in evidence-based herbal medicine.

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