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Research Article

Comparative Phytochemical Screening and Antioxidant Activity of *Azadirachta Indica* and *Phyllanthus Emblica*

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ABSTRACT

Background: Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and endogenous antioxidant defenses, is a critical factor in the pathogenesis of chronic diseases such as carcinoma, diabetes mellitus, and cardiovascular disorders. Medicinal plants, rich in secondary metabolites, serve as indispensable sources of natural antioxidants. Traditionally, *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla) have been utilized in Ayurvedic medicine for their diverse therapeutic potentials. **Objective:** The study aimed to perform a comparative phytochemical screening and evaluate the in-vitro antioxidant efficacy of ethanolic leaf extracts of *Azadirachta indica* and *Phyllanthus emblica*. **Methods:** Dried leaf powders were subjected to 72-hour ethanolic maceration. Preliminary phytochemical screening followed standardized qualitative protocols. Antioxidant capacity was evaluated via the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay, using ascorbic acid as a reference standard. **Results:** Phytochemical analysis revealed a high density of phenolics and flavonoids in both species. In the DPPH assay, *Phyllanthus emblica* demonstrated superior potency with an IC_{50} of 41.2 $\mu\text{g/mL}$ compared to 63.5 $\mu\text{g/mL}$ for *Azadirachta indica* (Standard IC_{50} : 25.6 $\mu\text{g/mL}$). FRAP results confirmed concentration-dependent reducing power, with *P. emblica* reaching higher absorbance (~ 0.9) than *A. indica* (~ 0.6) at 100 $\mu\text{g/mL}$. **Conclusion:** Both extracts possess significant antioxidant activity; however, *Phyllanthus emblica* exhibits superior radical scavenging and reducing potential, likely due to its high concentration of hydrolyzable tannins and gallic acid.

INTRODUCTION

Traditional medicine systems, notably Ayurveda, represent the foundational heritage of modern

CONTEXTUAL BACKGROUND

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pharmacognosy. While historical practice focused on holistic physiological balance, contemporary pharmaceutical research seeks to validate these traditional claims through the isolation and characterization of bioactive secondary metabolites. This transition from empirical tradition to evidence-based pharmacology allows for the integration of botanical agents into modern clinical frameworks.

ANTIOXIDANT THEORY

Free radicals are highly unstable molecular species possessing unpaired electrons that initiate deleterious chain reactions, leading to oxidative damage of lipids, proteins, and DNA. Antioxidants act as protective agents by neutralizing these radicals through two primary mechanisms: chain-breaking (radical quenching) and prevention of radical formation (metal chelation). Endogenous systems include enzymatic (e.g., SOD, Catalase) and non-enzymatic (e.g., Glutathione) components, which often require supplementation from dietary plant-based sources to maintain cellular homeostasis.

PLANT PROFILES

***Azadirachta indica* A. Juss. (Neem):**

A member of the Meliaceae family, neem is a versatile medicinal tree native to the Indian subcontinent. It is renowned for its antimicrobial and anti-inflammatory properties, driven by a complex profile of limonoids, flavonoids, and polyphenolics.

***Phyllanthus emblica* L. (Amla):**

Belonging to the Phyllanthaceae family, amla is categorized as a premier *Rasayana* (rejuvenative) in Ayurveda. It is exceptionally rich in hydrolyzable tannins—specifically Emblicanin A and B—as well as gallic acid and Vitamin C,

which collectively impart profound antioxidant and immunomodulatory effects.

RATIONALE OF THE STUDY

The long-term safety concerns associated with synthetic antioxidants (e.g., BHA, BHT) have catalyzed the search for safe, plant-derived alternatives. While both Neem and Amla are staples of traditional medicine, a systematic comparison of their leaf extracts is necessary to quantify their relative radical scavenging efficiencies. This study provides necessary scientific validation for their application as natural antioxidant sources in pharmaceutical and nutraceutical sectors.

MATERIALS AND METHODS

Plant Collection and Authentication

Mature, healthy leaves of *Azadirachta indica* A. Juss. and *Phyllanthus emblica* L. were collected from the Ravulapalem region. Authentication was performed by a resident taxonomist at the Department of Pharmaceutical Analysis, Lydia College of Pharmacy. Voucher specimens were deposited in the institutional herbarium.

Preparation of Extract

Leaves were shade-dried for 7–10 days and pulverized. Maceration was conducted by soaking 50 g of powdered material in 250 mL of ethanol for 72 hours with periodic agitation. The extracts were filtered and concentrated under reduced pressure at temperatures below 45°C. To ensure scientific accuracy, the percentage yield was calculated on a weight-to-weight (w/w) basis: $\text{Percentage Yield} = (\text{Weight of dry extract} / \text{Weight of starting material}) \times 100$.

Phytochemical Screening



The extracts were subjected to the following qualitative assessments:

- **Carbohydrates:** Molisch's test (violet ring), Fehling's test (brick-red precipitate), and Benedict's test (red/orange precipitate).
- **Proteins:** Biuret test (violet/pink color) and Ninhydrin test (blue-purple color).
- **Alkaloids:** Detection using Mayer's (cream precipitate), Dragendorff's (orange-brown precipitate), Hager's (yellow precipitate), and Wagner's (reddish-brown precipitate) reagents.
- **Glycosides:** Bornträger's and Modified Bornträger's tests (pink color in ammoniacal layer) for anthraquinone derivatives.
- **Saponins:** Foam Test (persistent froth > 15 mins).
- **Tannins:** Ferric Chloride (blue-black/greenish color) and Lead Acetate (white precipitate) tests.
- **Flavonoids and Polyphenolics:** Aluminium Chloride and Folin-Ciocalteu reagent methods (used as markers for phenolic hydroxyl groups).

Antioxidant Assays

- **DPPH Assay:** 0.5 mL of varying concentrations (20–100 µg/mL) were added to 2.5 mL of 0.1 mM methanolic DPPH. After 30 minutes of dark incubation, absorbance was measured at 517 nm.
- $\% \text{ Inhibition} = [(Absorbance \text{ of Control} - Absorbance \text{ of Sample}) / Absorbance \text{ of Control}] \times 100$.

- **FRAP Assay:** The FRAP reagent (acetate buffer pH 3.6, TPTZ, and FeCl₃ in 10:1:1 ratio) was mixed with 0.1 mL of extract. Following incubation at 37°C for 30 minutes, absorbance was recorded at 593 nm.

RESULTS

Phytochemical Profile

Phytochemical screening confirmed a rich diversity of metabolites. Notably, *A. indica* tested positive for alkaloids via Dragendorff's and Wagner's tests, contrary to previous reports of absence.

Table 1: Phytoconstituents of *A. indica* and *P. emblica* Extracts

Phytoconstituents	<i>Azadirachta indica</i>	<i>Phyllanthus emblica</i>
Carbohydrates	(+)	(++)
Proteins	(+)	(++)
Alkaloids	(+)	(++)
Glycosides	(+)	(+)
Saponins	(++)	(+)
Tannins	(+)	(++)
Flavonoids	(++)	(++)
Polyphenolics	(++)	(++)

DPPH Activity

Both extracts displayed dose-dependent scavenging. *P. emblica* consistently outperformed *A. indica* at every concentration.

Table 2: DPPH Radical Scavenging % Inhibition

Concentration (µg/mL)	<i>A. indica</i> (%)	<i>P. emblica</i> (%)	Ascorbic Acid (%)
20	28.4	35.6	48.2
40	39.7	51.8	65.3
60	52.5	68.9	81.6
80	64.3	82.4	92.5
100	73.8	91.2	97.4

Note: Ascorbic acid served as the reference standard.



Calculated IC₅₀ Values:

- *Azadirachta indica*: 63.5 µg/mL
- *Phyllanthus emblica*: 41.2 µg/mL
- *Ascorbic Acid*: 25.6 µg/mL

FRAP Activity

The reducing power (Fe³⁺ to Fe²⁺) increased linearly with concentration. *P. emblica* demonstrated significantly higher absorbance values (reaching ~0.9 at 100 µg/mL) compared to *A. indica* (~0.6 at 100 µg/mL), indicating a superior electron-donating capacity.

DISCUSSION

Phytochemical-Antioxidant Correlation

The data demonstrates a robust correlation between secondary metabolite density and radical scavenging efficiency. *Phyllanthus emblica* showed higher intensity (++) for tannins and polyphenolics. While Vitamin C is a known contributor, the superior activity of Amla is largely attributed to its unique hydrolyzable tannins, specifically Emblicanin A and B, and high levels of gallic acid. These polyphenols possess multiple hydroxyl groups that facilitate efficient hydrogen atom donation, which directly explains the significantly lower IC₅₀ value (41.2 µg/mL) of Amla compared to Neem.

Comparative Analysis

In the comparative analysis, *P. emblica* was found to be the more potent antioxidant across both assay systems. The DPPH assay, which measures radical quenching via hydrogen/electron donation, and the FRAP assay, which measures ion reduction, both favored Amla. This suggests that Amla's metabolites are superior electron donors in both

pH-dependent (FRAP) and pH-independent (DPPH) environments. *Azadirachta indica* still represents a valuable source of antioxidants, particularly through its flavonoid and saponin content, but its radical scavenging efficiency is approximately 35% lower than that of Amla.

Validation of Traditional Use

These in-vitro results provide definitive scientific evidence supporting the long-standing use of both plants in Ayurveda. The high radical scavenging activity validates their roles in preventing cellular degradation associated with aging and metabolic stress.

CONCLUSION

Final Verdict

The investigation confirms that ethanolic extracts of *Azadirachta indica* and *Phyllanthus emblica* are potent reservoirs of natural bioactive antioxidants.

Key Finding

Phyllanthus emblica demonstrated superior antioxidant potential compared to *Azadirachta indica*, evidenced by lower IC₅₀ values and a higher capacity for ferric ion reduction.

Future Directions

Subsequent research should prioritize bioassay-guided isolation of Emblicanins and specific neem limonoids, followed by in-vivo pharmacokinetic evaluations to determine their efficacy in biological systems.

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