J. Biodivers. Conservation 10(2): 122-129
2026

ISSN: 2457-0761 (online)
http://www.aprfjournal.in

Research Article

Evaluation of the protective role of neem (*Azadirachta indica* A. Juss.) bud extracts against oxidative stress-induced damage

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DOI: https://doi.org/10.5281/zenodo.20423000

Article Details: Received: 2026-03-16| Accepted: 2026-05-28| Available online: 2026-05-28

Abstract: Oxidative stress, resulting from an imbalance between reactive oxygen species overproduction and inadequate antioxidant defenses, contributing to the pathogenesis of chronic diseases. Medicinal plants rich in bioactive compounds exhibit potent free radical scavenging activities, positioning them as promising natural therapeutic agents against oxidative stress-associated pathologies. *Azadirachta indica* A. Juss, commonly known as neem, is a remarkable medicinal plant celebrated for its wide range of therapeutic benefits. Present study explored the antioxidant potential of neem bud extracts that were prepared using three different solvents: n-hexane, ethanol and water. The current experimental analysis assessed their effectiveness against oxidative stress through the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, testing concentrations from 0.125 to 1.0 mg/ml. All the extracts showed a concentration-dependent ability to inhibit oxidative stress. Notably, the ethanolic extract stood out with the highest scavenging activity, ranging from 89.35% to 96.32%. The aqueous extract followed, showing an activity between 71.49% and 80.91%, while the n-hexane extract, although lower, maintained a stable inhibition rate of 68.05% to 70.52%. The impressive performance of the ethanolic extract is likely due to its effective extraction of polar phenolic and flavonoid compounds. These findings indicate that neem bud extracts, particularly those prepared with ethanol, have great potential as natural antioxidants to combat oxidative stress-

related damage, thereby offering a valuable base for future novel drug discoveries and the development of plant-based pharmaceutical formulations.

Keywords: Flavonoid compounds, natural antioxidants and therapeutic benefits

Introduction

Oxidative stress is a significant factor in the development of many chronic and degenerative diseases, such as cancer, diabetes, heart problems and neurodegenerative disorders (Pizzino et al., 2017; Leyane et al., 2022). It occurs when there's an imbalance between the production of reactive oxygen species (ROS) and the body's ability to defend itself with antioxidants (Qin et al., 2020; Liu et al., 2025). In recent years, there has been a growing concern about the side effects and costs of synthetic antioxidants, which has sparked a lot of interest in natural alternatives derived from plants (Gulcin. 2025). Medicinal plants, known for their rich content of phenolic compounds, flavonoids and other beneficial phytochemicals, have been studied as sustainable sources of powerful antioxidants (Tungmunnithum et al., 2018). One such plant is *Azadirachta indica* A. Juss, commonly known as neem, which holds a significant place in traditional medicine throughout South and Southeast Asia (Tufail et al., 2025). Almost every part of the neem tree - its leaves, bark, seeds, flowers and buds has been recognized for its various biological activities, including antimicrobial, anti-inflammatory, anticancer and antioxidant effects (Debnath et al., 2025; Dhakad et al., 2025).

Figure 1: Buds and flowers of *A. indica*

While there has been extensive research on neem leaves and seeds, the bud fraction is still relatively unexplored, especially regarding its antioxidant activity depending on the solvent used for extraction (Veerendrakumar et al., 2023; Toson et al., 2026). The choice of extraction solvent is crucial because it affects the yield and characteristics of bioactive compounds, as the polarity of the solvent influences the solubility of phenolics, flavonoids, and terpenoids (Sutrisno et al., 2024). Thus, present study aims to assess and compare the free radical scavenging activity of neem bud extracts using solvents of different polarities - n-hexane, ethanol and water by employing the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a reliable and widely accepted method for evaluating antioxidant activity in vitro. The DPPH radical scavenging assay is a widely recognized and trusted method for assessing the in vitro antioxidant activity of plant extracts (Shahidi and Samarasinghe, 2025). This technique relies on the ability of hydrogen-donating antioxidant compounds to convert the stable, purple-hued DPPH radical into its yellow, non-radical form. The extent of this color change is measured spectrophotometrically, indicating the percentage of inhibition (Pillai and Simelane, 2024; Polile et al., 2024). Thanks to its simplicity, reproducibility and sensitivity, this assay is particularly effective for comparing the antioxidant capacities of various solvent extracts. When it comes to neem bud extracts, the DPPH assay allows for a detailed examination of how the polarity of solvents affects the extraction efficiency of radical-scavenging compounds (Chekuri et al., 2018). Typically, ethanolic extraction, which tends to favor polar phenolics and flavonoids, is expected to produce higher antioxidant activity than non-polar solvents like n-hexane, which mainly dissolve lipophilic components such as terpenoids and fatty acids. Aqueous extracts, in contrast, provide insight into the biological availability of antioxidant compounds under physiological conditions (Azzahra et al., 2025; Ojong et al., 2026). By measuring inhibition percentages across a concentration range of 0.125 to 1.0 mg/ml for each extract, the present study offers a thorough and comparative look at the antioxidant potential found in neem buds, contributing to the larger goal of identifying and validating plant-based agents that protect against oxidative stress-related cellular damage.

Methodology

The present study is based on field survey, experimental assay and an extensive survey of published literature related to *A. indica* scientific databases, including Google Scholar, Scopus, PubMed and Web of Science, are consulted to retrieve peer-reviewed research articles, review papers, ethnobotanical surveys and pharmacological studies. Keywords including "*A. indica*," "medicinal uses," "bioactive compounds," and "potent scavenging bioactive compounds" were used to identify relevant publications. The field survey was carried out during March, April and May of 2026, when the budding season of *A. indica* at its peak. Identification was complied with reference to flora guide (Saxena and Brahmam, 1994). The experimental analysis was carried out to validate the antioxidant potential of *A. indica* buds using the DPPH assay (Dehar et al., 2026).

Antioxidant DPPH assay

Collection of *A. indica* buds were done from nearby Mahanadi areas of Cuttack District, Odisha, India (Figure 1). The fruit was thoroughly washed, broken and the pulp was macerated with different solvents

like n-hexane, ethanol and distilled water separately (Dehar et al., 2026; Figure 1). The DPPH radical scavenging assay was used to evaluate the filtered extract following Dehar et al., (2022) with minor modifications. 1 ml of 0.1 mM DPPH solution prepared in methanol was added to prepared concentrations of aqueous, ethanolic and n-hexane extracts (1.0, 0.5, 0.25 and 0.125 mg/mL) using the respective solvents adjusting the final volume to 2 ml. 1 mL 0.1 mM DPPH in 1 mL methanol was used as control. Sample blanks (without DPPH) were used for background correction of absorbance. Reaction mixtures were exposed to dark incubation at room temperature for 20 minutes and the absorbance was spectrophotometrically taken at 517 nm. Percentage of radical scavenging activity was calculated using the following formula (Table 1).

% Inhibition= $A_0 - A_s / A_0 \times 100$

Where, A_0 is the absorbance of the control and A_s is the absorbance of the sample after blank correction.

Results and discussion

The antioxidant activity of neem bud extracts was assessed at four different concentrations (0.125–1.0 mg/ml) for n-hexane, ethanolic and aqueous fractions. All three extracts showed a concentration-dependent increase in antioxidant activity, with higher concentrations leading to greater inhibition. Among them, the ethanolic extract stood out, showcasing the highest scavenging activity across all tested concentrations, with inhibition values of 89.35%, 92.68%, 94.71% and 96.32% at 0.125, 0.25, 0.5 and 1.0 mg/ml, respectively. This impressive performance is likely due to ethanol's ability to effectively extract polar bioactive compounds like phenolics and flavonoids, which are known for their role as hydrogen donors that can neutralize free radicals. The aqueous extract displayed moderate inhibition, ranging from 71.49% to 80.91%, indicating the presence of water-soluble antioxidant components. In contrast, the n-hexane extract produced the lowest inhibition values (68.05–70.52%), showing a nearly flat response across concentrations, which suggests that its non-polar lipophilic constituents may reach saturation at lower doses. Overall, the ranking of antioxidant activity was ethanolic > aqueous > n-hexane, aligning with previous findings on plant extracts of varying polarity (Table 1; Figure 2). This reinforces the idea that polar phenolic compounds are key players in the antioxidant capacity of neem buds. These results highlight the potential of neem bud extracts, especially those prepared with ethanol, as effective natural agents against oxidative stress-related damage.

Table 1: Antioxidant potential of *A. indica* bud extracts

Concentration (in mg/ml)	Inhibition (%)		
	n-Hexane	Ethanolic	Aqueous
1.0	70.52	96.32	80.91
0.5	69.44	94.71	78.74
0.25	68.31	92.68	71.98

0.125	68.05	89.35	71.49

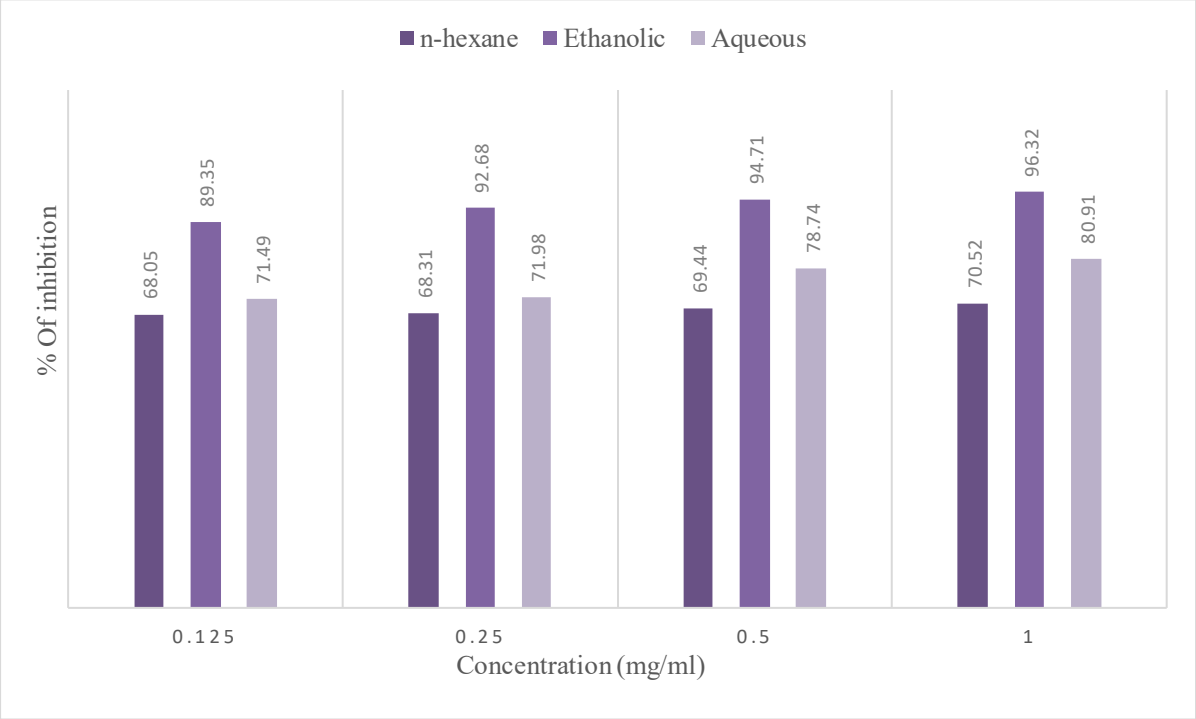

Figure 2: The hierarchy of antioxidant potential of *A. indica* bud extracts

Research gaps

While the present study sheds light on the antioxidant potential of neem bud extracts, there are still quite a few gaps in the current research. Most earlier investigations into *A. indica* have mainly concentrated on the leaves, bark and seeds, leaving the buds largely overlooked. Relying solely on the DPPH assay gives us a narrow view of antioxidant activity, as it doesn't fully capture the intricate mechanisms of oxidative stress in biological systems. We still haven't pinpointed or measured the specific phenolic and flavonoid compounds that contribute to the observed scavenging activity, which leaves a hole in our understanding of how neem buds work. Additionally, since the findings are based on in vitro studies, we can't directly apply them to in vivo situations, as we don't know how well the active compounds are absorbed or how they behave in the body. The toxicological profile, the effects of where the neem is grown, and how seasonal changes impact the antioxidant potential of neem buds are also areas that need more thorough investigation.

Future aspects

For future research, it would be beneficial to use advanced phytochemical profiling techniques like HPLC (High-Performance Liquid Chromatography), GC-MS (Gas Chromatography-Mass Spectrometry) and LC-MS (Liquid Chromatography-Mass Spectrometry). These methods can help identify and quantify the bioactive compounds that contribute to the antioxidant activity found in neem bud extracts. To get a more thorough evaluation of antioxidant properties, complementary assays such as ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), FRAP (Fluorescence Recovery After

Photobleaching) and nitric oxide scavenging should also be included. Conducting in vivo studies with animal models experiencing oxidative stress is crucial to confirm the biological significance of these findings in real-life conditions. Isolating individual bioactive fractions and examining their structure-activity relationships would enhance our understanding of the mechanisms involved. Before considering any clinical applications, it's vital to conduct cytotoxicity and safe dosage studies. Moreover, exploring nanoformulation and encapsulation strategies could significantly improve the bioavailability and stability of these active compounds, paving the way for potential pharmaceutical and nutraceutical advancements.

Conclusion

The present study revealed that extracts from neem buds show impressive DPPH free radical scavenging activity and this effect increases with concentration across all three solvent fractions currently analysed. The ethanolic extract stood out with the highest antioxidant potential, ranging from 89.35% to 96.32%, followed by the aqueous extract at 71.49% to 80.91%, and the n-hexane extract at 68.05% to 70.52%. This highlights the key role that polar phenolic and flavonoid compounds play in providing antioxidant protection. The experimental results suggest that neem buds could be a valuable and relatively untapped source of natural antioxidants, offering promising protection against damage caused by oxidative stress. To further validate and expand on these findings for potential therapeutic uses, more in vivo studies and phytochemical analyses are recommended.

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