J. Biodivers. Conservation 10(2): 130-136
2026

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

Research Article

Assessment of radical scavenging capacity in *Aegle marmelos* (L.) Corrêa fruits using DPPH assays

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DOI: https://doi.org/10.5281/zenodo.20454601

Article Details: Received: 2026-04-12| Accepted: 2026-05-30| Available online: 2026-05-30

Abstract: *Aegle marmelos*, commonly known as bael, is a medicinal plant widely used in traditional systems of medicine across South and Southeast Asia. The present study aimed to explore the antioxidant potential of fruit pulp extracts from *A. marmelos*, using solvents with different polarities (n-hexane, ethanol and water) through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The experiment was carried out using the extracts at different concentrations ranging from 0.125 to 1.0 mg/ml. The results showed that all three extracts had a concentration-dependent ability to scavenge radicals, with the aqueous extract showing the highest inhibition, achieving an impressive 98.31% inhibition at 1.0 mg/ml and 91.32% at 0.125 mg/ml. The ethanolic extract also performed well, with inhibition values between 85.26% and 89.37% across the same concentration range. Meanwhile, the n-hexane extract had a lower but steady scavenging ability, with inhibition values from 64.09% to 66.91%. This trend in antioxidant effectiveness based on polarity suggests that the key bioactive compounds responsible for scavenging free radicals in *A. marmelos* fruit pulp are mainly hydrophilic, including phenolics, flavonoids and other polar secondary metabolites. The present findings highlight the traditional uses of bael fruit

and highlight its potential as a natural source of antioxidants for nutraceutical and future pharmaceutical formulation.

Keywords: Antioxidant potential, free radical scavenging, hydrophilic, nutraceutical and pharmaceutical

Introduction

Natural antioxidants from plants have caught a lot of attention from scientists in recent years, mainly because of their potential to help fight oxidative stress-related issues like cancer, heart disease and neurodegeneration (Szymanska et al., 2016; Muscolo et al., 2024). Free radicals, especially reactive oxygen species (ROS), are known to cause cellular damage and contribute to disease progression, which is the reason of finding effective radical scavengers, a key focus in both pharmacological and nutraceutical research (Chaudhary et al., 2023; Gulcin, 2025). *Aegle marmelos*, commonly known as bael or wood apple, is a tropical deciduous tree that hails from the Indian subcontinent and is found throughout South and Southeast Asia (Khanal et al., 2023; Shetty et al., 2026). This plant is highly regarded in Ayurvedic, Unani and Siddha traditional medicine systems, where its leaves, bark, roots and fruits have been used for centuries to treat various ailments like diarrhea, dysentery, diabetes, respiratory issues and inflammation (Monika et al., 2023; Mujeeb et al., 2025).

Figure 1: Leaves and fruit of *A. marmelos*

The fruit, in particular, is seen as one of the most valuable parts of the plant, packed with a variety of secondary metabolites such as alkaloids, flavonoids, tannins, coumarins and terpenoids (Indriyani et

al., 2023; Patil et al., 2024; Shetty et al., 2026). Even though *A. marmelos* has a long history of use in traditional medicine, there hasn't been enough systematic scientific research to validate the antioxidant properties of its fruit pulp across different solvent systems (Sharma et al., 2022; Khanal et al., 2023). The polarity of solvents is crucial for effectively extracting phytochemicals; polar solvents like water and ethanol are great for pulling out hydrophilic compounds, while non-polar solvents like n-hexane are better for dissolving lipophilic substances (Ponphaiboon et al., 2023; Tsaturyan et al., 2025). Therefore, comparing the antioxidant profiles of the plant material across solvents with varying polarities can give us a more comprehensive understanding of the antioxidant profile (Sharifi et al., 2025). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a popular method for assessing how well substances can scavenge free radicals in vitro (Baliyan et al., 2022; Kato et al., 2025). In current analysis, the stable DPPH radical gets reduced when it encounters a hydrogen-donating antioxidant, leading to a noticeable drop in absorbance that correlates directly with the extract's ability to inhibit free radicals (Gulcin, 2025). The present study aims to evaluate and compare the radical scavenging abilities of *A. marmelos* fruit pulp extracts made with n-hexane, ethanol and water using the DPPH assay. The goal was to create an antioxidant profile based on polarity and to provide scientific backing for the traditional medicinal uses of this important species for future therapeutics and pharmaceuticals.

Methodology

The present study is based on field survey, experimental analysis and a thorough review of existing literature on *A. marmelos*. Scientific databases, including Google Scholar, Scopus, PubMed and Web of Science, were referenced to retrieve peer-reviewed research articles, review papers, ethnobotanical surveys and pharmacological studies. To find relevant publications, authors used keywords such as "*A*egle *marmelos*," "medicinal uses," "bioactive compounds" and "potent scavenging bioactive compounds". The field survey was carried out in March - April 2026, right during the peak fruit-ripening season for *A. marmelos*. The plant identification was complied with reference to flora guide (Saxena and Brahmam, 1994). The experimental analysis was also conducted to assess the antioxidant potential of *A. marmelos* fruits using the DPPH assay (Rajurkar and Hande, 2011; Chaves et al., 2020; Baliyan et al., 2022).

Antioxidant DPPH assay

Collection of *A. marmelos* fruit was 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 (Kaur et al., 2026; Kumar et al., 2026). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate the filtered extract following Baliyan 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× 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 present study evaluated the DPPH radical scavenging activity of *A. marmelos* fruit pulp extracts at four different concentrations (0.125–1.0 mg/ml). All three extracts showed a concentration-dependent inhibition, meaning their activity increased with the rise in concentration (Table 1). The aqueous extract exhibited with the highest scavenging activity, achieving an impressive 91.32% inhibition at 0.125 mg/ml and reaching a peak of 98.31% at 1.0 mg/ml. The ethanolic extract displayed moderate yet steady activity, ranging from 85.26% to 89.37%. In contrast, the n-hexane extract had the lowest inhibition (64.09–66.91%), with a notably narrow range across concentrations, suggesting early saturation of its scavenging capacity. The DPPH radical scavenging activity showed polarity-dependent decreasing trend in various extracts in the order, aqueous > ethanolic > n-hexane, indicating that the dominant antioxidant components in *A. marmelos* fruit pulp are likely hydrophilic, such as phenolics, flavonoids and tannins, which tend to dissolve better in polar solvents (Figures 1 and 2). These findings align with previous reported phytochemical profiles of *A. marmelos* fruit and support its significant ethnomedicinal relevance as a powerful natural antioxidant.

Table 1: Antioxidant potential of *Aegle marmelos* fruit pulp extracts

Concentration (in mg/ml)	Inhibition (%)		
	n-Hexane	Ethanolic	Aqueous
1.0	66.91	89.37	98.31
0.5	65.94	89.13	97.82
0.25	65.21	88.16	94.22
0.125	64.09	85.26	91.32

Research gaps

While the antioxidant properties of *A. marmelos* have been highlighted in various ethnopharmacological studies, there are still notable gaps in the current research. Many earlier studies have focused on just one solvent system, which limits the overall understanding of the antioxidant profile. The recent study aims to fill that gap by using a comparative, polarity-based extraction method. However, the specific phytochemical compounds that contribute to the radical scavenging activity in each extract haven't been isolated or characterized yet. Moreover, the research relies solely on the DPPH assay, which only assesses hydrogen-donating antioxidant capacity and doesn't capture the full range of antioxidant mechanisms. The lack of IC_{50} values, standard reference comparisons and in vivo validation further restricts how applicable these findings are in real-world scenarios. Additionally, the variations in

phytochemical composition of *A. marmelos* fruit pulp based on geography and season have not been thoroughly explored in the existing literature.

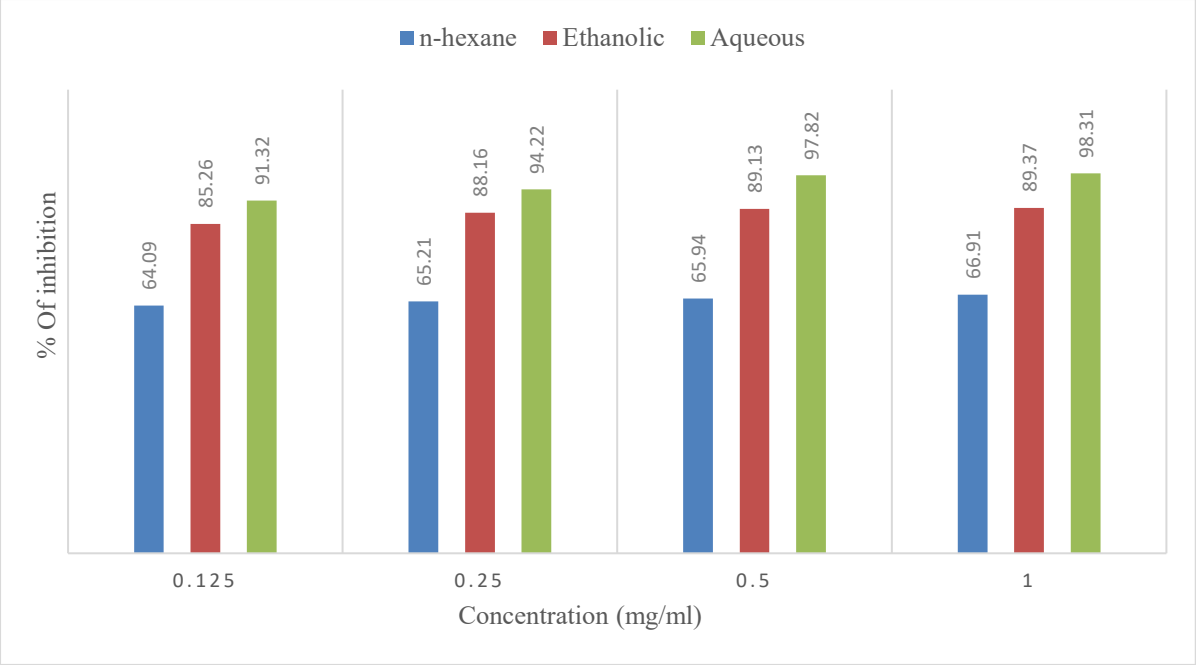

Figure 2: Antioxidant activities of *A. marmelos* fruit extracts

Future aspects

Future research should really focus on isolating and identifying the active antioxidant compounds found in both aqueous and ethanolic extracts that showed the strongest inhibitory effects. It would be beneficial to use complementary assays like ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), FRAP (Ferric Reducing Antioxidant Power) and superoxide radical scavenging alongside DPPH to get a more comprehensive view of antioxidant capacity. Determining IC_{50} values with suitable positive controls, such as ascorbic acid or BHT (Butylated Hydroxytoluene), would enhance the quantitative analysis of the results. Additionally, in vivo studies using appropriate animal models are necessary to confirm the in vitro findings and evaluate bioavailability. Exploring how factors like fruit maturity, geographic origin and seasonal changes affect antioxidant yield would significantly aid in standardization efforts. Lastly, research into encapsulation and formulation that looks at incorporating *A. marmelos* fruit extracts into functional foods, nutraceuticals or topical antioxidant products could open up exciting new avenues for applied research.

Conclusion

The present study revealed that extracts from the fruit pulp of *A. marmelos* show impressive DPPH radical scavenging activity and this effect increases with concentration across all three solvent systems were tested. Among them, the aqueous extract showed as the most effective antioxidant, followed by the ethanolic and n-hexane extracts. This pattern highlights a clear relationship with polarity, suggesting that hydrophilic compounds are the main players in the radical scavenging action. These results lend

scientific support to the traditional medicinal use of bael fruit and highlight its potential as a valuable natural source of antioxidants for future pharmaceutical and nutraceutical developments.

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