

Original Research Article

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Embelin Isolated from *Embelia ribes* Burm. f. As A Plant-Derived Photocatalyst for The Degradation of Coomassie Brilliant Blue R-250

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ABSTRACT

Embelin, a naturally occurring benzoquinone isolated from the berries of *Embelia ribes* Burm. f., was investigated as a plant-derived photocatalyst for the degradation of Coomassie Brilliant Blue R-250 (CBB R-250). *E. ribes* is a medicinal plant traditionally used in various systems of medicine, and embelin is recognized as one of its major bioactive constituents (Lal and Mishra, 2013; Poojari, 2014). In the present study, embelin was isolated from dried berries by chloroform extraction and crystallization, and its identity was confirmed by thin-layer chromatography, FT-IR, and ¹H and ¹³C NMR analyses. The isolated embelin was immobilized in a chitosan–agar matrix to enhance its photocatalytic efficiency. A 10-ppm solution of CBB R-250 was treated with free and immobilized embelin at 1 µg/mL under sunlight, and dye degradation was monitored spectrophotometrically at 590 nm. Free embelin showed 45% degradation after 70 min, whereas immobilized embelin exhibited markedly enhanced activity, achieving 96% degradation within the same period. The control treatments showed no appreciable degradation. The enhanced activity of immobilized embelin may be attributed to its improved distribution and increased surface availability within the chitosan–agar matrix (Roshitha et al., 2019). The novelty of the present study lies in demonstrating the photocatalytic application of isolated embelin from *E. ribes* for CBB R-250 degradation and in establishing chitosan–agar immobilization as an effective approach for substantially enhancing its dye-degradation efficiency. The findings highlight the potential of embelin as a natural, plant-derived photocatalyst for the eco-friendly treatment of dye-contaminated water.

Keywords

Embelia ribes Burm. f.; Embelin; Plant-derived photocatalyst; Coomassie Brilliant Blue R-250; Photocatalytic degradation; Chitosan–agar; Dye remediation.

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Introduction

Embelia ribes Burm. f and Its Medicinal Importance

Embelia ribes Burm. f., commonly known as false black pepper or Vidanga, is a medicinal woody climber belonging to the family Myrsinaceae. It is widely distributed in India and other parts of Asia (Lal and Mishra, 2013). The fruits have a long history of traditional medicinal use, particularly as an anthelmintic in Ayurveda and Unani medicine (Sudhakaran, 2015). Its medicinal properties are associated with diverse phytochemicals, including alkaloids, flavonoids, glycosides, phenols and steroids (Zeeshan *et al.*, 2018). The fruits are a rich source of the bioactive benzoquinone derivative embelin (Lal and Mishra, 2013; Sudhakaran, 2015).

Embelin as a Bioactive Constituent

Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) is a major bioactive constituent of *E. ribes* Burm. f. It is a para-benzoquinone derivative containing hydroxyl and carbonyl groups and contributes to the characteristic orange-red colour of the fruits. Embelin has demonstrated antimicrobial, antioxidant, anti-inflammatory and anticancer activities (Poojari, 2014; Zeeshan *et al.*, 2018; Sheng *et al.*, 2020). Its quinone and phenolic groups are also of interest because of their potential redox activity.

Coomassie Brilliant Blue R-250 as a Model Dye

Synthetic dyes released from industrial activities can cause significant aquatic pollution. Coomassie Brilliant Blue R-250 (CBB R-250) is a triphenylmethane dye widely used as a protein-staining reagent in biochemical analysis (Georgiou *et al.*, 2008). Its persistence makes the removal or degradation of dye-containing wastewater an important environmental concern.

Photocatalytic Degradation of Organic Dyes

Various physical, chemical and biological methods have been investigated for dye removal. Among these, advanced oxidation processes and heterogeneous photocatalysis have attracted considerable attention because of their ability to degrade complex organic pollutants (Khataee and Kasiri, 2010). Light-induced

electron-transfer reactions generate reactive species that promote the degradation of organic dyes. Increasing interest in naturally derived materials has encouraged the exploration of plant compounds as potential photocatalytic materials. The redox-active nature of embelin suggests its possible application in such processes (Bishop, 1961; Sheng *et al.*, 2020).

Immobilization of Embelin for Enhanced Photocatalytic Activity

Immobilization of an active compound in a suitable polymeric matrix can improve its handling, stability and interaction with pollutants. Chitosan possesses useful adsorption and film-forming properties, while agar provides structural support. Polymer-supported photocatalytic systems have been reported to improve dye-degradation performance (Roshitha *et al.*, 2019). Therefore, a chitosan–agar matrix may provide a suitable platform for immobilizing embelin.

Rationale and Novelty of the Present Study

Although embelin has been extensively investigated for its medicinal properties, its application as a plant-derived photocatalyst for synthetic dye degradation remains comparatively unexplored.

The present study investigates embelin isolated from the berries of *E. ribes* Burm. f. and characterized by FT-IR and NMR spectroscopy for the photocatalytic degradation of CBB R-250 under sunlight. Its activity was evaluated in free form and after immobilization in a chitosan–agar matrix.

The novelty of the present study lies in demonstrating the photocatalytic application of isolated embelin from *E. ribes* Burm. f. for CBB R-250 degradation and evaluating chitosan–agar immobilization as an approach to enhance its dye-degradation efficiency.

Materials and Methods

Plant Material and Authentication

Dried berries of *Embelia ribes* Burm. f. (250 g) were procured from a local market in Chennai, India, and authenticated by Dr. Pandikumar, Taxonomist, Entomology Research Institute, Loyola College, Chennai.

Extraction and Isolation of Embelin

Dried berries of *Embelia ribes* Burm. f. were powdered and extracted with chloroform by cold percolation for 48 h. The filtered extract was concentrated using a rotary evaporator and refrigerated overnight to precipitate embelin. The precipitate was filtered and recrystallized from petroleum ether to obtain red-orange needle-shaped crystals. The isolated embelin (approximately 4 g) showed a melting point of 142 °C. Embelin is reported as a major bioactive constituent of *E. ribes* Burm. f. fruits (Poojari, 2014; Sheng *et al.*, 2020).

Characterization of Isolated Embelin

The isolated compound was initially examined by TLC on silica gel. The isolated embelin was further characterized by FT-IR and NMR spectroscopy. The structural characteristics were evaluated based on the functional groups and spectral features corresponding to embelin, a hydroxybenzoquinone derivative containing phenolic hydroxyl and quinone groups (Sudhakaran, 2015; Sheng *et al.*, 2020).

Immobilization of Embelin

Embelin was immobilized using a chitosan-agar matrix. Briefly, 20 mg of isolated embelin was added to 9 mL of 1% acetic acid containing 1 g of chitosan and 0.25 g of agar-agar. The mixture was heated at 80 °C with mild stirring for 20 min to obtain a homogeneous preparation.

The mixture was subsequently transferred into a Petri plate containing 1.5% sodium chloride solution to facilitate cross-linking. After hardening, the resulting chitosan-agar matrix containing embelin was cut into small cubes and used for the photocatalytic degradation experiments. Chitosan-based immobilized systems have previously been investigated for photocatalytic dye degradation and have shown enhanced dye-removal efficiency (Roshitha *et al.*, 2019).

Photocatalytic Degradation of CBB R-250

The photocatalytic activity of free and immobilized embelin was evaluated using Coomassie Brilliant Blue R-250 as the model dye. A 10 ppm aqueous solution of CBB R-250 was prepared, and 1 µg/mL of isolated free embelin was added to the dye solution. A separate treatment containing 1 µg/mL of immobilized embelin

was also maintained. Two controls were included: (i) 10 ppm CBB R-250 without catalyst and (ii) 10 ppm CBB R-250 containing chitosan- agar cubes without embelin.

All treatment and control tubes were exposed to natural sunlight at approximately 12: 00 noon. Duplicate tubes were maintained for each experimental condition. The photocatalytic degradation was monitored at 10-min intervals. At each interval, 2.0 mL of the reaction mixture was withdrawn and centrifuged at 3000 rpm for 5 min. The supernatant was collected and its absorbance was measured at 590 nm using a UV-visible spectrophotometer. The decrease in absorbance was used to determine the extent of CBB R-250 degradation. Photocatalytic degradation of CBB dyes using different catalytic systems has been previously reported (Khataee and Kasiri, 2010; Baradaran and Sadeghi, 2019; Singh *et al.*, 2019).

Calculation of Dye Degradation

The percentage of dye degradation was calculated using the following equation:

$$\text{Dye degradation (\%)} = [(C_0 - C) / C_0] \times 100$$

Where C_0 represents the initial concentration of CBB R-250 and C represents the dye concentration after photocatalytic treatment. The degradation efficiency was determined at each sampling interval based on the reduction in absorbance at 590 nm.

Results and Discussion

Isolation and Preliminary Characterization of Embelin

The chloroform extract obtained from the dried berries of *Embelia ribes* Burm.f. yielded red-orange crystals after concentration, refrigeration and crystallization with petroleum ether. The isolated compound showed a melting point of 142 °C and a yield of approximately 4 g. Preliminary chromatographic and chemical tests indicated the presence of a quinone-containing phenolic compound. TLC analysis showed a single spot with an R_f value of 0.61 using benzene (1: 1) as the solvent system. The isolated compound produced a purple colour with alcoholic ferric chloride and a pink colour with alcoholic sodium hydroxide, confirming the presence of phenolic and quinone functionalities, respectively.

The isolated compound was identified as embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) based on its physical properties, colour reactions and spectroscopic characteristics.

The spectral data were also compared with previously reported data as described in the manuscript.

FT-IR and NMR Characterization of Embelin

The FT-IR spectrum of the isolated compound exhibited characteristic absorption bands at 3310, 2922, 2850, 1616, 1329 and 1195 cm^{-1} , together with additional bands at 982, 944, 902, 860, 768, 708 and 696 cm^{-1} .

The spectral characteristics were consistent with the functional groups present in embelin. The molecular formula of the isolated compound was determined as $\text{C}_{17}\text{H}_{26}\text{O}_4$, with a molecular weight of 294.4.

The ^1H NMR and ^{13}C NMR spectral data further supported the identity of the isolated compound as embelin.

The combined TLC, chemical, FT-IR and NMR results confirmed the successful isolation and identification of embelin from *E. ribes* Burm. f.

Photocatalytic Degradation of CBB R-250 by Free Embelin

The photocatalytic activity of free embelin was evaluated against 10 ppm Coomassie Brilliant Blue R-250 (CBB R-250) under sunlight. Free embelin was used at a concentration of 1 $\mu\text{g/mL}$, and the reaction mixture was exposed to sunlight at approximately 12: 00 noon. Samples were collected at 10-min intervals up to 70 min, centrifuged and the absorbance of the supernatant was measured at 590 nm.

A gradual decrease in absorbance was observed during sunlight exposure, indicating degradation of CBB R-250. At the end of 70 min, free embelin showed approximately 45% degradation of the dye.

The original time-dependent absorbance profile presented in the manuscript should be retained as the free-embelin degradation graph, because the intermediate absorbance values are available graphically rather than as a numerical table.

Photocatalytic Degradation of CBB R-250 by Immobilized Embelin

The photocatalytic efficiency of embelin was further evaluated after immobilization in a chitosan-agar matrix. The immobilized embelin preparation was exposed to 10 ppm CBB R-250 under sunlight using the same experimental conditions applied for free embelin.

A pronounced decrease in dye absorbance was observed during the treatment period. Immobilized embelin exhibited substantially higher photocatalytic activity than free embelin and produced approximately 96% degradation of CBB R-250 within 70 min.

The original time-dependent graph for immobilized embelin should be retained as the immobilized-embelin degradation graph.

Comparison of Free and Immobilized Embelin

The photocatalytic performance of free and immobilized embelin was compared based on the percentage degradation of CBB R-250 after 70 min of sunlight exposure.

Free embelin produced 45% degradation, whereas immobilized embelin produced 96% degradation under the same experimental conditions.

Thus, the immobilized preparation showed a markedly greater dye-removal efficiency than free embelin.

The immobilized embelin therefore demonstrated approximately 2.1-fold higher percentage degradation than free embelin based on the reported endpoint values.

Control Experiments

Control experiments were performed using CBB R-250 alone and CBB R-250 with chitosan-agar cubes without embelin. No appreciable degradation of the dye was observed in either control.

These observations indicate that the chitosan-agar matrix alone did not account for the photocatalytic degradation observed in the embelin-containing treatment.

These results support the contribution of embelin to the observed photocatalytic activity.

Table.1 Preliminary characterization of the isolated embelin

Parameter	Observation
Plant material	<i>Embelia ribes</i> Burm. f. berries
Extracting solvent	Chloroform
Appearance	Red-orange crystals
TLC solvent system	Benzene (1: 1)
Rf value	0.61
Ferric chloride test	Purple colour
Alcoholic NaOH test	Pink colour
Melting point	142 °C
Isolated yieldh	4 g
Molecular formula	C ₁₇ H ₂₆ O ₄
Molecular weight	294.4

Table.2 Spectral characteristics of the isolated embelin

Spectroscopic analysis	Characteristic observation
FT-IR	3310, 2922, 2850, 1616, 1329, 1195 cm ⁻¹
Additional FT-IR bands	982, 944, 902, 860, 768, 708, 696 cm ⁻¹
¹ H NMR	Characteristic proton signals corresponding to embelin
¹³ C NMR	Characteristic carbon signals corresponding to embelin
Molecular formula	C ₁₇ H ₂₆ O ₄
Molecular weight	294.4

Table.3 Photocatalytic degradation of CBB R-250 by free embelin

Parameter	Experimental condition/result
Dye	CBB R-250
Initial dye concentration	10 ppm
Free embelin concentration	1 µg/mL
Light source	Sunlight
Exposure time	70 min
Detection wavelength	590 nm
Degradation at 70 min	45%

Table.4 Photocatalytic degradation of CBB R-250 by immobilized embelin

Parameter	Experimental condition/result
Dye	CBB R-250
Initial dye concentration	10 ppm
Immobilized embelin concentration	1 µg/mL
Immobilization matrix	Chitosan-agar
Light source	Sunlight
Exposure time	70 min
Detection wavelength	590 nm
Degradation at 70 min	96%

Table.5 Comparative photocatalytic efficiency of free and immobilized embelin

Treatment	Degradation after 70 min	Remaining dye*
Free embelin	45%	55%
Immobilized embelin	96%	4%

*Remaining dye percentage calculated as 100 – percentage degradation.

Table.6 Control experiments for CBB R-250 degradation

Experimental group	Observation
CBB R-250 alone	No appreciable degradation
CBB R-250 + chitosan-agar cubes without embelin	No appreciable degradation
CBB R-250 + free embelin	45% degradation after 70 min
CBB R-250 + immobilized embelin	96% degradation after 70 min

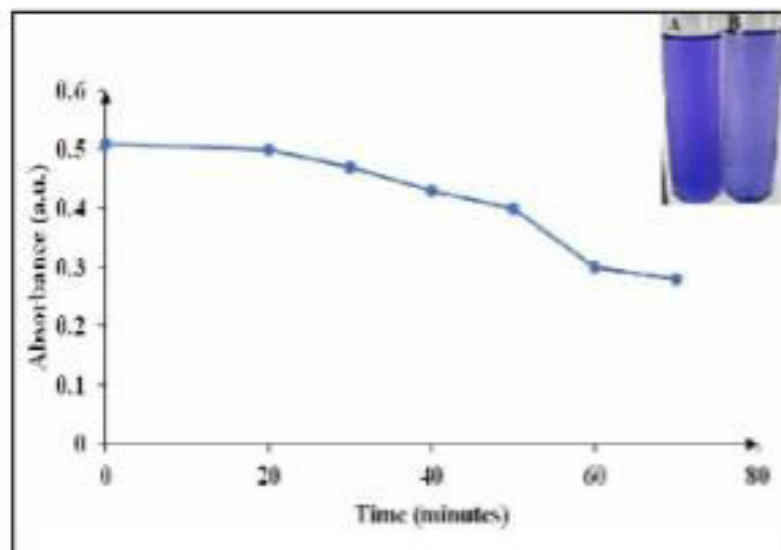
Table.7 Visual observations after 24 h

Sample	Observation after 24 h
A – CBB R-250 control	Dye remained in the solution
B – CBB R-250 + chitosan-agar	No appreciable degradation
C – CBB R-250 + immobilized embelin	Greater dye separation/deposition
D – CBB R-250 + free embelin	Small amount of dye deposition

Graph.1 Exact comparison from the study Photocatalytic degradation of CBB R-250 by embelin.

Reported degradation of 10 ppm CBB R-250 after 70 min of sunlight exposure.

Figure 1. Photocatalytic degradation of CBB R-250 by free embelin

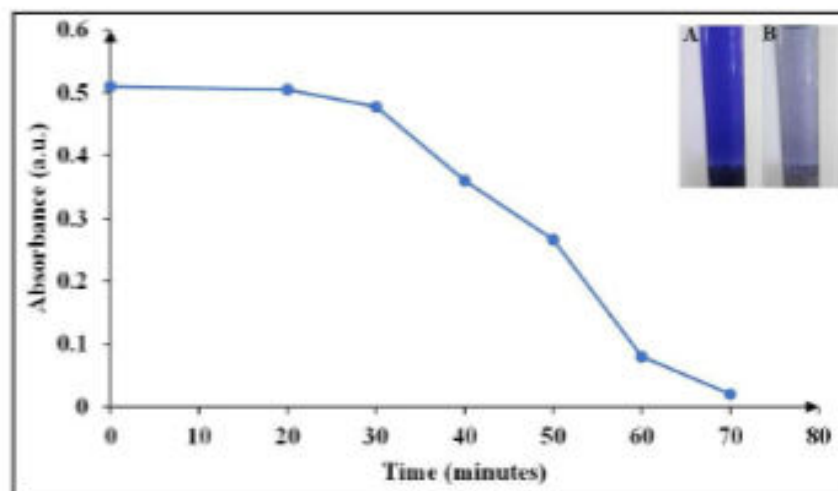


Degradation of CBB R-250 by free Embelin

(A) Control (10 ppm dye)

(B) Sample (10 ppm dye+free Embelin)

Figure 2. Photocatalytic degradation of CBB R-250 by immobilized embelin

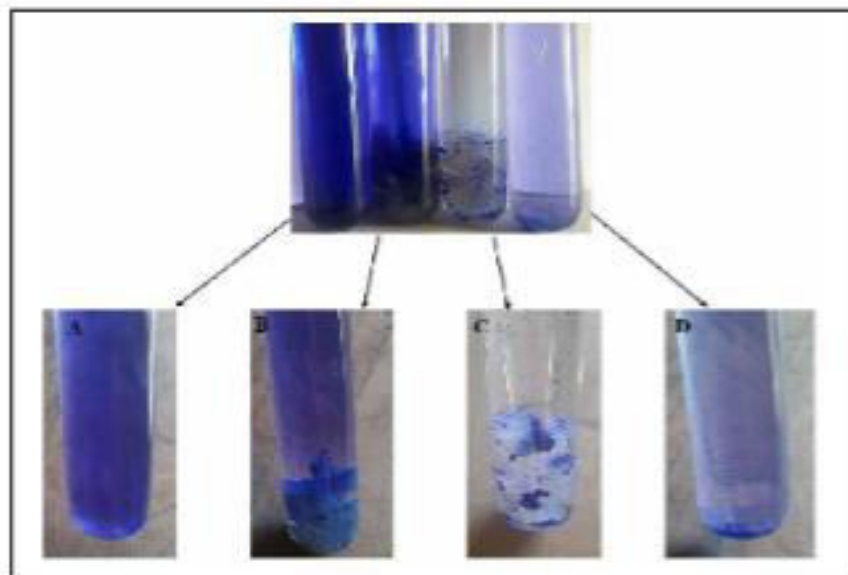


Degradation of CBB R-250 by free Embelin

(A) Control (10 ppm dye)

(B) Sample (10 ppm dye+free Embelin)

Figure 3. Visual observation of CBB R-250 after 24 h



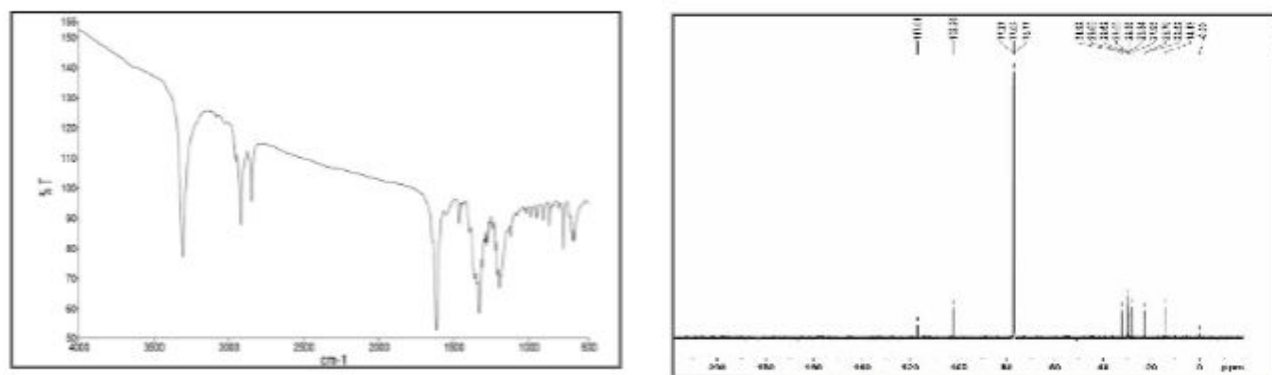
Observation after 24 h

- (A) Control: 10 ppm dye,
- (B) Control: 10 ppm dye+ Chitosan-agar cubes,
- (C) Sample: 10 ppm dye + Embelin immobilized in Chitosan-agar,
- (D) Sample: 10 ppm dye + free Embelin

Figure 4 – Preliminary characterization of embelin

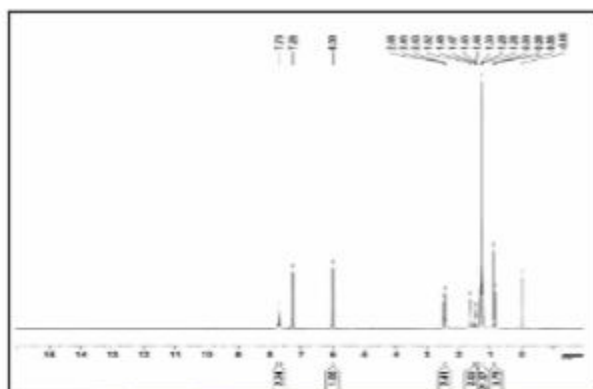


Figure 5 – FT-IR and NMR characterization



IR spectrum of embelin

^{13}C NMR of Embelin



^1H NMR of Embelin

Table 3 – Free embelin degradation

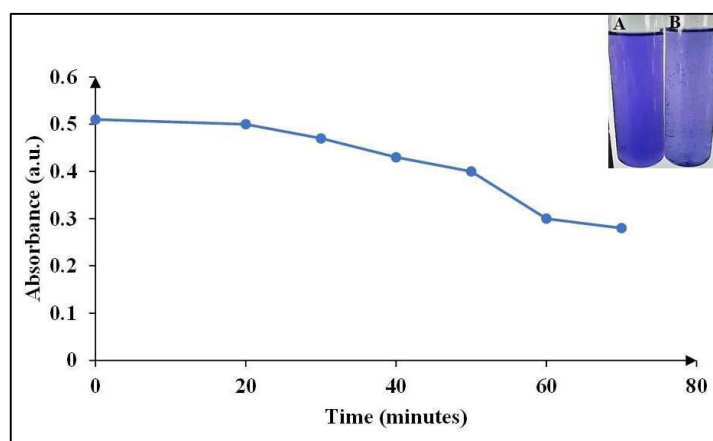
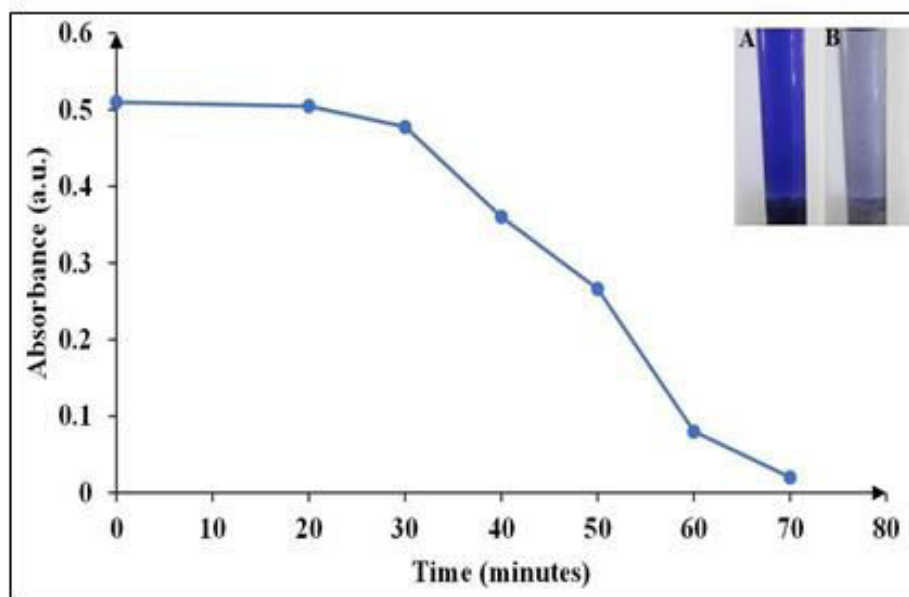


Table 4 – Immobilized embelin degradation



Post-treatment Observation

Following 70 min of sunlight exposure, the reaction mixtures were allowed to stand at room temperature for 24 h. The dye was observed to separate from the water and become associated with the immobilized embelin preparation. A smaller amount of dye deposition was observed in the free-embelin treatment. In contrast, no comparable degradation or deposition was observed in the control tubes or in the absence of sunlight.

The corresponding photographs in the original manuscript provide visual evidence of the differences among the control, immobilized-embelin and free-embelin treatments.

Overall Photocatalytic Performance

The results demonstrated that embelin possesses photocatalytic activity against CBB R-250 under sunlight. Free embelin showed 45% degradation after 70 min, whereas immobilization of embelin in the chitosan-agar matrix increased the degradation efficiency to 96% within the same period. The absence of significant degradation in the controls further supports the role of embelin in the observed dye degradation.

The improved performance of immobilized embelin may be associated with its distribution within the supporting matrix and the increased effective surface available for

interaction with the dye. The manuscript also relates this observation to previously reported photocatalytic systems based on chitosan-containing materials.

Embelin (2,5-dihydroxy-3-undecyl-1, 4-benzoquinone) was successfully isolated from the dried berries of *Embelia ribes* Burm. f. as red-orange crystals with a melting point of 142 °C. The positive phenol and quinone tests, together with the FT-IR and NMR spectral data, supported its identity in agreement with previously reported data (Kaur *et al.*, 2015).

The present study demonstrates the photocatalytic potential of embelin for the sunlight-assisted degradation of Coomassie Brilliant Blue R-250 (CBB R-250). Free embelin achieved 45% dye degradation after 70 min, whereas embelin immobilized in a chitosan-agar matrix showed markedly enhanced degradation of 96% within the same period. Neither the dye control nor the chitosan-agar matrix without embelin showed appreciable degradation, indicating that embelin was responsible for the observed photocatalytic activity.

The enhanced performance of immobilized embelin may be associated with its uniform distribution within the polymer matrix and the greater available surface area for interaction with the dye. This observation is comparable with previous findings on immobilized chitosan-based photocatalytic systems, which demonstrated efficient dye degradation (Roshitha *et al.*, 2019).

The novelty of the present study lies in demonstrating embelin, a naturally occurring quinone from *E. ribes* Burm. F. as a plant-derived photocatalyst for sunlight-assisted degradation of CBB R-250 and in showing that its immobilization in chitosan–agar substantially improves the degradation efficiency. The observed post-treatment adsorption of dye onto immobilized embelin further indicates its potential for application in dye-contaminated water treatment.

In conclusion, the present study investigated the isolation of embelin from the dried berries of *Embelia ribes* Burm.f. and evaluated its photocatalytic activity against Coomassie Brilliant Blue R-250 (CBB R-250). Embelin was isolated as red-orange crystals and characterized by FT-IR and NMR spectroscopy. Free and chitosan–agar-immobilized embelin were evaluated for sunlight-assisted dye degradation using 10 ppm CBB R-250. Free embelin showed 45% dye degradation after 70 min, whereas immobilized embelin achieved 96% degradation within the same period. The controls showed no appreciable degradation, confirming the contribution of embelin to the observed photocatalytic activity.

The novelty of the present study lies in demonstrating the photocatalytic potential of isolated embelin as a plant-derived photocatalyst and, importantly, in showing that its immobilization in a chitosan–agar matrix markedly enhances sunlight-assisted degradation of CBB R-250. This approach provides a simple and potentially eco-friendly strategy for improving the applicability of a plant-derived photocatalyst in dye-contaminated water treatment.

Collectively, the findings identify embelin as a promising plant-derived photocatalyst for sunlight-assisted degradation of CBB R-250, while chitosan–agar immobilization offers an effective means of enhancing its degradation efficiency.

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Author Contributions

M. Priyanka: Conceptualized the study, conducted the research, performed data analysis, and prepared the

manuscript. V. Hemamalini: Supervised the study, provided methodological guidance, contributed to interpretation of the results, and critically reviewed the manuscript. R. Kaviya: Assisted with data collection and analysis and contributed to manuscript review and revision. J. Anandhalakshmi: Experimental assistance, data analysis, and review of the manuscript. S. Gayathri: Experimental assistance, literature review, and review of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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