

Chapter 7

Evaluation of phytochemical constituents, cytotoxic potential, and chromatographic profiling of fruits of *Alphonsea lutea* (Roxb.) Hook.f. & Thomson

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Abstract: Present study investigated the phytochemical composition, cytotoxic potential, and thin-layer chromatography (TLC) profile of the fruits of *Alphonsea lutea* (Roxb.) Hook.f. & Thomson, a lesser-known member of the Annonaceae family. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, reducing sugar, phenolics, and saponins. TLC analysis was employed to develop a chemical fingerprint of the fruit extracts, highlighting key phytoconstituents. The cytotoxic activity was assessed using the brine shrimp lethality assay, demonstrating moderate to significant toxicity, suggesting the presence of bioactive compounds with potential pharmacological applications. These findings provide a foundation for further investigation into the therapeutic potential of *A. lutea* fruits.

Keywords: Chemical fingerprint, phytoconstituents, therapeutic potential

Introduction

Alphonsea lutea (Roxb.) Hook.f. & Thomson, belonging to the family Annonaceae, is a lesser known yet potentially valuable medicinal plant native to parts of South and Southeast Asia, including India (Srivastava and Mehrotra, 2025). It is a deciduous or semi-evergreen tree, typically found in tropical and subtropical forest regions (Katole et al., 2018). Though not extensively studied, *A. lutea* is recognized in some traditional systems of medicine for its therapeutic properties (Bakri et al., 2017). Local communities have used different parts of the plant including fruits, bark, and leaves for treating ailments such as fever, inflammation, digestive disorders, and microbial infections (Kumar and Dash, 2012). Despite its traditional relevance, scientific validation of its phytochemical profile and biological activities remains limited, creating an opportunity for further Pharmacognostic exploration.



Figure 1: Fruits of *A. lutea*

Recent global health challenges, particularly those related to antimicrobial resistance, chronic diseases, and the rising burden of cancer, call for the discovery of novel, plant-derived bioactive compounds (Chaachouay and Zidane, 2024). *A. lutea*, with its reservoir of secondary metabolites such as alkaloids, flavonoids, tannin, saponins and reducing sugar, holds promise as a source of natural therapeutic agents (Sahu et al., 2021). This study aims to bridge the gap between ethnomedicinal knowledge and scientific validation, supporting the global pursuit of sustainable and accessible healthcare alternatives.

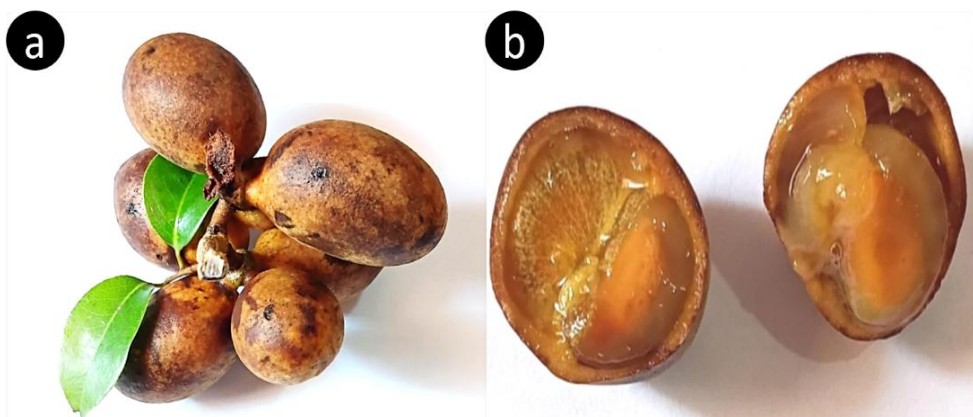


Figure 2: Fruits of *A. lutea* and their parts; a) Fruits and b) Fruit pulp and seeds

Methodology

The fruits of *A. lutea* were collected from Khordha district of Odisha, India (Figure 2). The plant species was identified by the authors by the help of relevant taxonomic literature (Saxena and Brahmam, 1994; Figure 1). The maceration extraction method was adopted using different solvents (n-hexane, ethanol and aqueous) for experimental analysis (Figure 3). Detection of nine secondary metabolites was conducted using standard methods (Jena et al., 2025; Majhi et al., 2025; Nithish et al., 2025). Details of phytochemical analysis is given below.



Figure 3: Filtrate extracts of *A. lutea* fruits for experimental analysis

Test for tannin: 1 ml of fruit extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was added to it. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of distilled water was added to 1 ml of the fruit extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

Test for flavonoids: About 1 ml of the fruit extract was taken, and 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The color initially turned to an intense yellow color with NaOH solution and later became colorless, which confirmed the presence of flavonoids.

Test for terpenoids: To 1 ml of the fruit extract, 5-6 drops of chloroform were added. Then, 4-6 drops of concentrated sulphuric acid were added to it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the fruit extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the fruit extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: To 1 ml of the fruit extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the fruit extract was taken, and 3-4 drops of Dragendroff's reagent were added to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of fruit extract was taken, and 3-4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent were added. The formation of yellow crystals confirmed the presence of carbonyl compounds.

Cytotoxicity analysis: For cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35°C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml⁻¹, 31.25 mg ml⁻¹, 62.5 mg ml⁻¹, 125 mg ml⁻¹ and 250 mg ml⁻¹) of the *A. lutea* fruit extracts were taken and 3.5% saline water were used to make the volume 2 ml in each test tube. 1 % DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative

controls were prepared using 3.5% saline water and vincristine sulphate (5 mg ml⁻¹) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 cm glass slides using silica gel powder. Slides were washed with clinical laboratory detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring and finally poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 °C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *A. lutea* fruits revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, alkaloids and reducing sugars (Table 1: Figures 4-6). These compounds were found to be present in different extracts having various ethnomedicinal uses and pharmacological potential. Traditionally, *Alphonsea* species are medicinally used to treat rheumatism and edema (Bakri et al., 2017). The presence of wide range of phytoconstituents suggests that *A. lutea* fruits may possess medicinal properties.

Table 1: Qualitative phytochemical analysis of *Alphonsea lutea* fruits using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Absent	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Present	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Absent
Reducing sugars	Absent	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Present
Carbonyl compounds	Absent	Absent	Absent

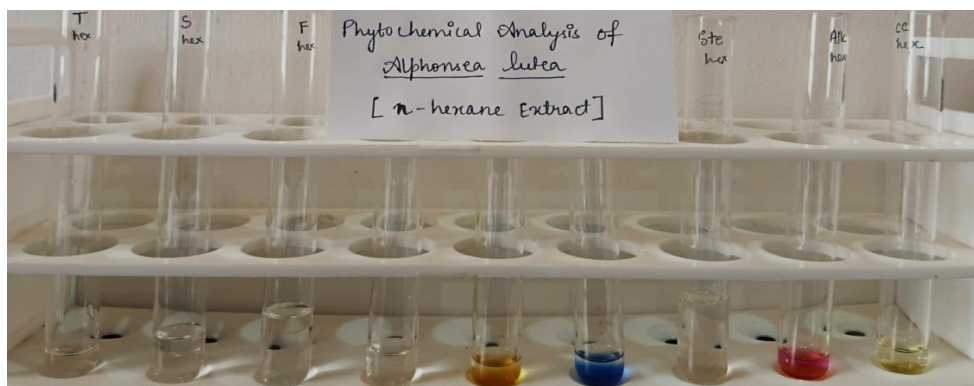


Figure 4: Detection of secondary metabolites of *A. lutea* fruits using n-hexane extract

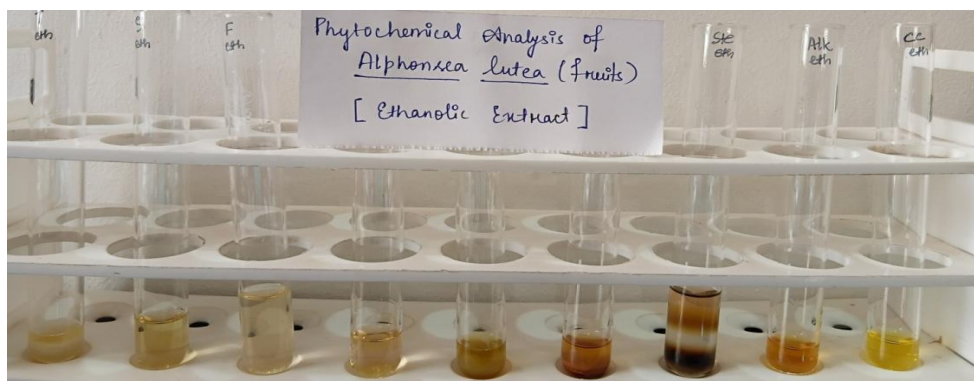


Figure 5: Detection of secondary metabolites of *A. lutea* fruits using ethanolic extract



Figure 6: Detection of secondary metabolites of *A. lutea* fruits using aqueous extract

The cytotoxicity analysis of *A. lutea* fruit extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 7-9). In contrast, the aqueous extract exhibited moderate lethality response. Present findings suggest that *A. lutea* fruit may possess specific bioactive compounds that contribute to toxicity.

Table 2: Cytotoxicity analysis of *Alphonsea lutea* fruits using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n- hexane	15.62	10	2	8	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	1	10	10	10	100
	125	10	10	10	10	10	100
	250	10	1	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100

	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	0	2	2	2	20
	31.25	10	4	10	10	10	100
	62.5	10	5	10	10	10	100
	125	10	5	10	10	10	100
	250	10	4	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

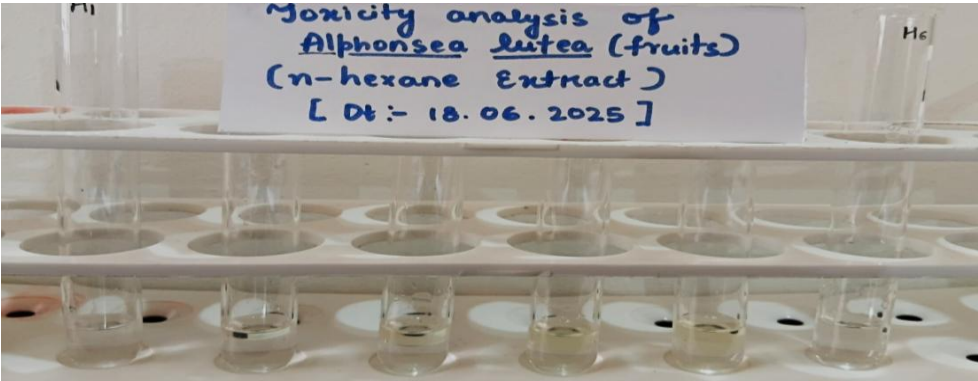


Figure7: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *A. lutea* fruits



Figure 8: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *A. lutea* fruits



Figure 9: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *A. lutea* fruits

The chromatographic analysis of hydroethanolic extract of *A. lutea* fruit revealed distinct bioactive components, with different mobile phases separating various phytochemicals based on their R_f values. The presence of different spots in the TLC plates confirmed the presence of bioactive compounds in *A. lutea* fruits, which may be responsible for its medicinal properties (Table 3).

Table 3: TLC analysis of *Alphonsea lutea* fruits using different mobile phases

	Mobile Phases	Rf value of Spots	
		S1	S2
Hydroethanolic	Methanol: Chloroform (1:1)	-	-
	Ethyl acetate: Chloroform (1:9)	-	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.6	0.67

Conclusion

Present study highlighted the phytochemical richness of *A. lutea* fruits, which supports its traditional medicinal uses and suggests potential applications in pharmaceutical and therapeutic research. The observed cytotoxic activity further indicated the plant's potential role in anticancer or antimicrobial drug development. Biological potential of *A. lutea* stands as a strong candidate for

further pharmacological investigations that can contribute to the discovery of novel natural products against the global health challenges.

References

- Bakri YM, Talip MA and Azziz SSSA. (2017). A mini review on *Alphonsea* sp. (Annonaceae): traditional uses, biological activities and phytochemistry. Journal of Applied Pharmaceutical Science. 7(10): 200-203. DOI: 10. 7324/JAPS.2017.71030
- Chaachouay N and Zidane L. (2024). Plant-derived natural products: a source for drug discovery and development. Drugs Drug Candidates. 3(1): 184-207.
- Jena N, Vimala, Singh B, Patra A, Sharma BP, Hossain E and Kumar S. (2025). Methods for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for pharmacological evaluation of medicinal plants. Journal of Biodiversity and Conservation. 9(2): 87-107.
- Katole RM, Gautam JM and Mokat DN. (2018). Phytochemical study of- *Annona squamosa* L. and *Annona reticulata* L. International Journal of Research. 5(12). 156-175.
- Kumar S and Dash D. (2012). Flora of Nandan Kanan Sanctuary. Medicinal plants with their role in health care. International Journal of Pharmacy & Life Sciences. 3(4): 1631-1642.
- Kumar S and Jena PK. (2014). Chromatographic, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. Plant Science Research. 36(1&2): 83-90.
- Kumar S, Tripathy, PK and Jena PK. (2012). Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and its toxicity to *A. salina* L. Plant Science and Research. 34(1&2): 93-96.
- Majhi S, Samantray PK, Jena N and Kumar S. (2025). Qualitative phytochemical investigation of leaves & fruits of *Ventilago denticulata* Willd. (Rhamnaceae). Plants and Secondary Metabolites, Volume 8. Ambika Prasad Research Foundation, Odisha, India.
- Mishra S and Bhatnagar S. (2024). Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. World Journal of Pharmaceutical and Life Sciences. 10(2): 116-118.

- Nayak SR, Choudhury R, Kumar S, Solanki HA and Biswal SK. (2021). Analysis of pharmacological values of *Vanda tessellata* and its toxicity to *A. salina* L. International Journal of Green and Herbal Chemistry. 10(3): 238-248.
- Nithish SA, Das K and Jena N. (2025). Exploring the phytoconstituents of leaves of *Centratherum punctatum* Cass. (Asteraceae). Plants and Secondary Metabolites, Volume 8. Ambika Prasad Research Foundation, Odisha, India.
- Sahu A, Ghosh G and Rath G. (2020). Identification and molecular docking studies of bioactive principles from *Alphonsea madraspatana* Bedd. against uropathogens. Current Pharmaceutical Biotechnology. 21(7): 613-625. DOI: 10.2174/1389201021666200107114846
- Sahu A, Swain SS, Pradhan D, Sahu DK, Biswasroy P, Kar B, Rath G and Ghosh G. (2021). Identification of bioactive compounds from *Alphonsea madraspatana* leaves against multi drug resistance bacteria. Asian Journal of Chemistry. 33(6): 1273-1279.
- Saxena HO and Brahmam M. (1994). The Flora of Orissa, Volume 1. Regional Research Laboratory, Bhubaneswar & Orissa Forest Development Corporation Limited, Bhubaneswar, Odisha, India.
- Srivastava G and Mehrotra RC. (2013). First fossil record of *Alphonsea* Hk. f. & T. (Annonaceae) from the Late Oligocene sediments of Assam, India and comments on its phytogeography. PLoSOne. 8(1): e53177. DOI: 10.1371/journal.pone.0053177