Chapter 5

Bioactive richness of the forest ebony: *Diospyros sylvatica* Roxb. (Ebenaceae)

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ABSTRACT

The forest ebony *Diospyros sylvatica* Roxb., a hardy tree belonging to the family Ebenaceae, thrives in dry deciduous forests and is valued for both its strong ebony wood and traditional medicinal uses associated with its genus. Present study was carried out to explore the bioactive richness of this species by analyzing the phytochemical composition and antibacterial activity of its extracts. Qualitative screening revealed the presence of important secondary metabolites such as tannins, saponins, phenolics, and reducing sugars, which are known for their medicinal properties. The hydroethanolic extract showed promising antibacterial effects, suggesting that the active compounds may help combat bacterial growth. This study highlights *D. sylvatica* as a valuable source of natural bioactive compounds with potential therapeutic applications, encouraging further scientific exploration.

Keywords: Antibacterial, ebony, secondary metabolites

INTRODUCTION

Members of the family Ebenaceae are renowned for their remarkable resistance to harsh climatic conditions and are predominantly native to dry deciduous and semi arid habitats (POWO, 2025). Their ability to thrive in such constrained environments (Wallnöfer, 2001) contributes to the

development of dense and durable wood highly valued in carpentry and internationally traded under the name ‘ebony.’ Beyond their economic and ecological significance, many species within this family possess rich ethnomedicinal histories (Ribeiro *et al.,*2023), being used traditionally to treat a variety of ailments ranging from skin infections to gastrointestinal and respiratory disorders (Fankam *et al.,*2025).



Figure 1: Fruits of *Diospyros sylvatica*

Among them, *Diospyros sylvatica* Roxb., commonly known as forest ebony, stands out as a lesser explored species with promising pharmacological potential. Traditionally, various parts of genus *Diospyros* have been employed in indigenous medicine for their therapeutic effects, including pharmacological properties (Rao *et al.,*2024). Despite the traditional importance, scientific documentation on this particular species *sylvatica*'s phytochemical composition and bioactivity remains limited. Investigating the bioactive compounds present in *D. sylvatica* is therefore crucial not only for validating its ethnomedicinal relevance but also for identifying potential natural compounds that may serve as leads in modern drug discovery. The present study aims to explore the bioactive richness of *D. sylvatica* through qualitative phytochemical screening and antibacterial evaluation of its fruits (Figure 2), shedding light on its pharmacological potential and conservation value.

METHODOLOGY

The fruits of *Diospyros sylvatica* were collected from forests of Chandaka-Dampara Wildlife Sanctuary, Odisha, India. The plant species was identified by the authors (Figure 1). The maceration extraction method was adopted using hydroethanolic extract for the following experiments (Devi *et al.,*2023; Jena *et al.,*2025).

Detection of secondary metabolites: Detection of secondary metabolites was conducted following Devi et al. (2023).

Test for tannin: 1 ml of plant extract is mixed with 4-5 drops of 10% lead acetate solution. The formation of a light-yellow precipitate confirms the presence of tannins.

Test for saponin: 1 ml of plant extract is mixed with 1 ml of distilled water,and after vigorous shaking, the formation of a stable, persistent frothconfirms the presence of saponins.

Test for flavonoids: To 2 ml of plant extract, a few drops of dilute sodiumhydroxide (NaOH) are added. The appearance of an intense yellow colourindicates the presence of flavonoids. Subsequent addition of 4-5 drops of dilute sulphuric acid causes the colour to fade, further confirming the presence of flavonoids.



Figure 2: Collected fruit sample of *D. sylvatica*

Test for terpenoids: A mixture of 1 ml of plant extract and 2 ml of chloroform is prepared, and an equal volume of concentrated sulphuric acid is added. The appearance of a reddish-brown coloration at the interface confirms the presence of terpenoids.

Test for phenolic compounds: 1 ml of plant extract is treated with 5 drops of 1% ferric chloride solution, resulting in the formation of a bluish-black coloration, which indicates the presence of phenolic compounds.

Test for reducing sugars: 1 ml of plant extract is added with 3 drops of Fehling's solution A and B, and kept in water bath for a few minutes. The formation of an orange-red precipitate confirms the presence of reducing sugars.

Test for steroids: 1 ml of plant extract is mixed with 2 ml of chloroform, followed by the addition of 2 ml of concentrated sulphuric acid. The resulting formation of two distinct phases, characterized by an upper red layer and a lower yellow layer with green fluorescence, confirms the presence of steroids.

Test for alkaloids: 1 ml of plant extract is mixed with 3-4 drops of Dragendorff's reagent and kept in water bath for a few minutes. The formation of an orange-red precipitate confirms the presence of alkaloids.

Antibacterial activity assessment: It was studied by determining the minimum inhibitory concentration (MIC) using *Escherichia coli* using standard methods (Jena *et al.,*2025).

RESULTS AND DISCUSSION

Phytochemical screening of *Diospyros sylvatica* fruit extracts revealed variation in metabolite composition between non polar and polar solvents (Table 1; Figure 2-3). The hydroethanolic (polar) extract detected many of the tested secondary metabolites, including tannins, saponins, phenolic compounds, reducing sugars, and alkaloids. In contrast, the n-hexane (non polar) extract displayed the presence of only reducing sugars, indicating limited solubility of polar phytoconstituents. The presence of these bioactive compounds, particularly tannins and phenolics, suggests potential antioxidant and antimicrobial properties associated with the hydroethanolic extract.

Table 1: Qualitative phytochemical analysis of *Diospyros sylvatica* using different extracts

Bioactive compounds	n-Hexane	Hydroethanolic
Tannin	Absent	Present
Saponin	Absent	Present
Flavonoids	Absent	Absent
Terpenoids	Absent	Absent
Phenolic compounds	Absent	Present
Reducing sugars	Present	Present
Steroids	Absent	Absent
Alkaloids	Absent	Present

Therefore, broth microdilution method was used to study the antibacterial potential of the hydroethanolic extract. It exhibited moderate antibacterial activity with a minimum inhibitory concentration (MIC) of 100 mg/mL (Table 2; Figure 5). No bacterial growth was observed in the antibiotic control, confirming assay validity, while the nutrient broth showed no inhibition, serving as a negative control. These observations suggested that the antibacterial property of *D. sylvatica* may be attributed to the presence of tannins, saponins, phenolic compounds, and alkaloids, which help stop bacterial growth by weakening the cell membrane and blocking vital enzymes (Vaou *et al.,*2021).

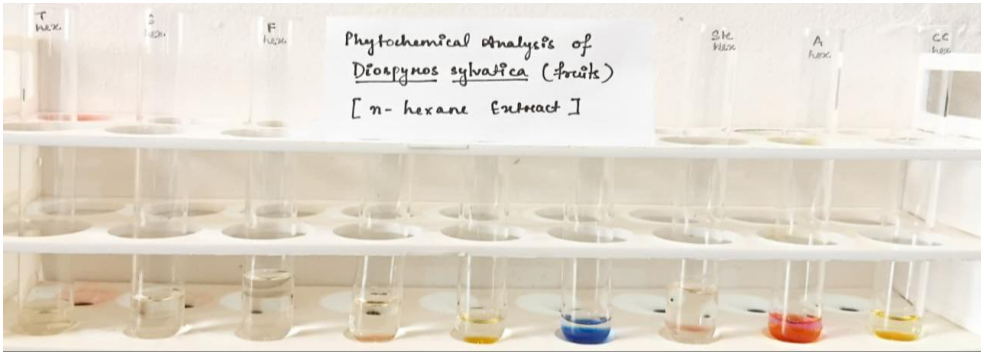


Figure 3: Detection of secondary metabolites in n-hexane extract

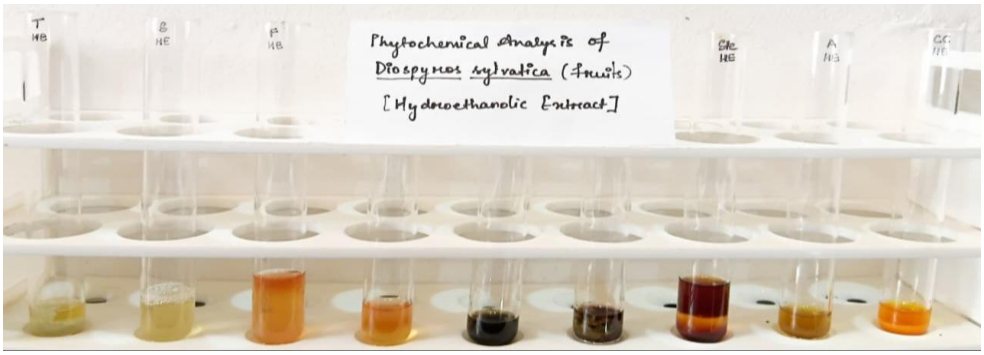


Figure 4: Detection of secondary metabolites in hydroethanolic extract

Table 2: Estimation of Minimum Inhibitory Concentration (MIC)

Extract Type	Tested Organism	Range of Concentrations (mg/mL)	MIC (mg/mL)	Antibacterial Strength
Hydroethanolic extract (*Diospyros sylvatica* fruits)	*E. coli*	12.5 – 100	100	Moderate
Antibiotic control	*E. coli*	Standard	-	Strong
Nutrient broth	-	-	-	Negative

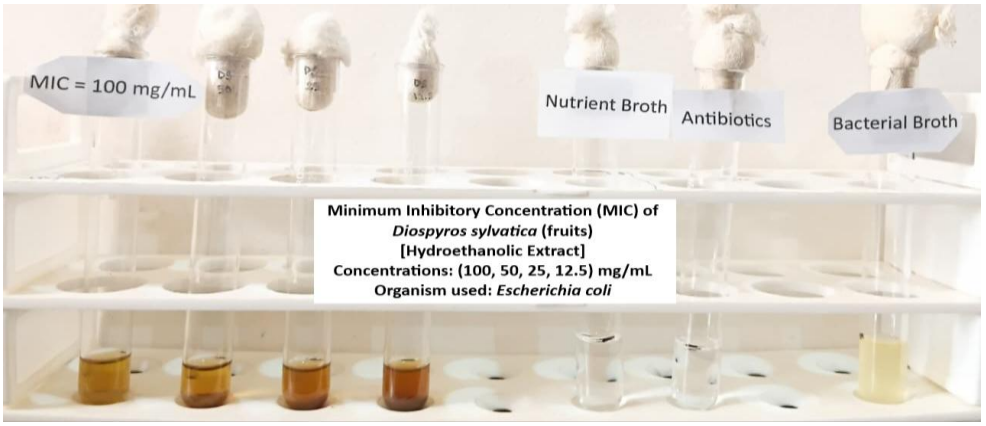


Figure 5: Determination of minimum inhibitory concentration (MIC)

CONCLUSION

The results of this study revealed that *Diospyros sylvatica* is not only a symbol of ecological endurance but also a reservoir of valuable phytochemicals. The presence of diverse bioactive compounds and noticeable antibacterial activity indicates that this species holds great promise for developing natural remedies and plant based antibacterial agents. By bridging traditional knowledge with modern science, the study reaffirms the medicinal potential of forest ebony and underlines the need for its conservation and further pharmacological research.

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