

Chapter 1

Qualitative investigation of secondary metabolites of *Eugenia roxburghii* DC., flowers

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ABSTRACT

The global health threat of antimicrobial resistance and drug failures necessitates rapid screening of medicinal plants for bioactive compounds. Present study addresses this need by investigating the secondary metabolites of *Eugenia roxburghii* flowers, revealing the presence of tannins, saponins, flavonoids, phenols, reducing sugars, and alkaloids, while terpenoids, steroids, and carbonyl compounds were not detected, offering valuable insights into the plant's medicinal potential.

Keywords: Antimicrobial resistance, medicinal plants, Myrtaceae

INTRODUCTION

Coastal ecosystems, including sand dunes, play a vital role in maintaining ecological balance and supporting biodiversity (Sahu et al., 2019; Anbarashan et al., 2022; Massarelli et al., 2023). Sand dunes serve as natural barriers against storms, erosion, and sea-level rise, protecting inland areas and infrastructure (Rodil et al., 2015). Moreover, these ecosystems harbour unique plant species adapted to harsh conditions, such as high salinity, strong winds, and shifting sands (Miller, 2015). Coastal plants, including those found on sand dunes, have evolved remarkable survival strategies, making them valuable resources for understanding ecological resilience (Duran and Moore, 2013). These plants also provide essential ecosystem services, including medicinal plants, habitat creation, nutrient cycling, and carbon sequestration (Yang and Tang, 2019; Kumar, 2021). Furthermore, coastal ecosystems support a wide range of wildlife, from migratory birds to marine species (Sievers et al., 2022). Due to several stress in coastal plants, they may develop different types of secondary metabolites which can be used to develop future therapeutic agents (Ramakrishna and Ravishankar, 2011; Marndi and Kumar, 2018). Therefore, present study on bioactive compounds of coastal plants, such as flowers of *Eugenia roxburghii*, (Figure 1) can provide valuable insights into their medicinal properties and potential applications. It belongs to the family Myrtaceae (Giri et al., 2022a). It comes under minor wild edible fruits (Giri et al., 2022b). Given the growing interest in natural products (Vaou et al., 2021; Devi et al., 2023) and conservation, exploring the phytochemicals of coastal plants can contribute to the discovery of new bioactive compounds and inform strategies for sustainable management and conservation of these critical ecosystems.



Figure 1: Flower of *E. roxburghii*

METHODOLOGY

The flowers of *E. roxburghii* were collected from Puri district of Odisha, India (Figure 2). The plant species was identified by Dr. Sanjeet Kumar. The Soxhlet extraction method was adopted using ethanol, acetone and distilled water for phytochemical analysis. Detection of selected secondary metabolites were conducted using standard methods (Devi *et al.*, 2023; Marndi *et al.*, 2024).

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

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



Figure 2: Collected flowers of *E. roxburghii* for experimental work

Test for flavonoids: About 1 ml of the flowers extract was taken. Added 2ml of NaOH solution and 3-5 drops of dilute HCl to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless which confirmed the presence of flavonoids.

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

Test for phenols: About 1 ml of the flowers 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 flowers 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 few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroid: To 1 ml of the flowers extract, added 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid into it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

Test for alkaloid: About 1 ml of the flowers extract was taken. Added 3-4 drops of Dragendroff's reagent to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

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

TLC Thin layer chromatography) analysis: TLC plates were prepared on 6 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 was poured over the slides and slides were left undisturbed till the drying of silica layer (Kumar *et al.*, 2013; Kumar and Jena 2014). Then the slides were activated at 50° C for 15-20 minutes before running the TLC. Methanol:

chloroform (50%), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then the spots were marked, recorded the R_f (Retention factor) values and visualized under UV chamber (Mishra and Bhatnagar 2024).

RESULTS AND DISCUSSION

The phytochemical screening of *E. roxburghii* flowers revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, phenols, reducing sugars, and alkaloids, in different extracts (Table 1; Figures 3-5). The widespread presence of these compounds highlights the potential medicinal value of the plant.

Table 1: Qualitative phytochemical screening of *Eugenia roxburghii* flowers using different extracts

Bioactive compounds	Extracts		
	Ethanol	Acetone	Aqueous
Tannin	Detected	Detected	Detected
Saponin	Detected	Detected	Detected
Flavonoids	Detected	Detected	Not detected
Terpenoids	Not detected	Not detected	Not detected
Phenols	Detected	Detected	Detected
Reducing sugar	Detected	Detected	Detected
Steroid	Not detected	Not detected	Not detected
Alkaloid	Detected	Detected	Detected
Carbonyl compound	Not detected	Not detected	Not detected



Figure 3: Detection of secondary metabolites of *E. roxburghii* flowers using ethanol extract

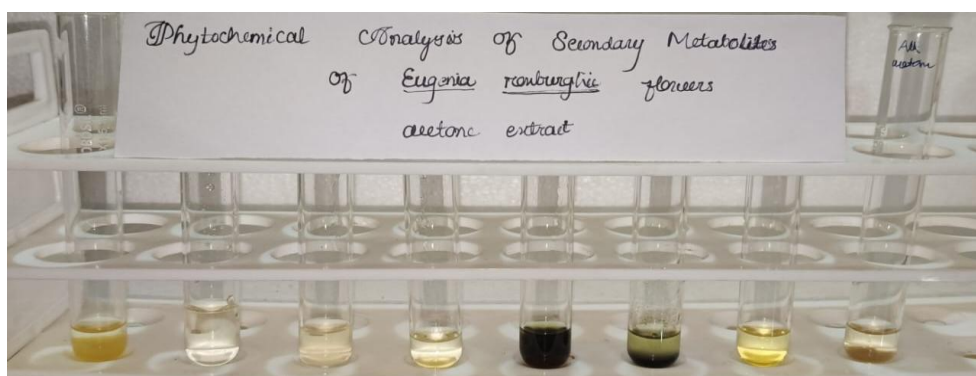


Figure 4: Detection of secondary metabolites of *E. roxburghii* flowers using acetone extract



Figure 5: Detection of secondary metabolites of *E. roxburghii* flowers using aqueous extract

Table 2: TLC analysis of *Eugenia roxburghii* flowers using different mobile phases

Mobile phases	Extracts	Rf value of spots	
		S ₁	S ₂
Chloroform: methanol	Hydroethanolic aqueous	0.31	0.51
Ethyl acetate: chloroform	Hydroethanolic aqueous	0.91	0.95
Ethyl acetate: Water: Acetic acid	Hydroethanolic aqueous	0.76	0.83

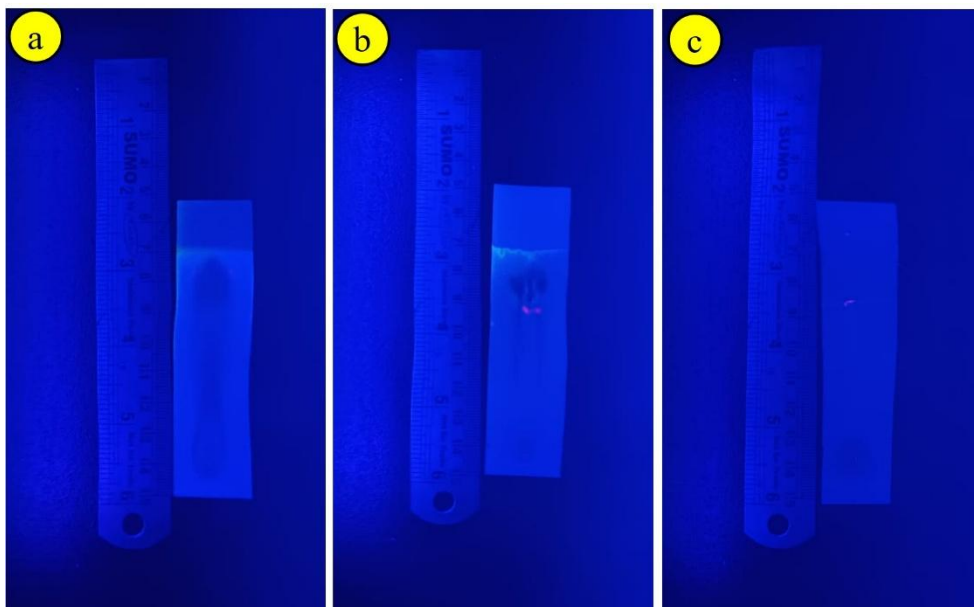


Figure 6: Spots on TLC under UV light; a) (Chloroform: methanol) hydroethanolic extract; b) (Ethyl acetate: chloroform) hydroethanolic extract of *E. roxburghii* flowers

The detection of tannins, phenols, and flavonoids suggests antioxidant and anti-inflammatory properties, while saponins and alkaloids indicate potential antimicrobial and pharmacological activities. The absence of terpenoids, steroids, and carbonyl compounds in all extracts may indicate their limited role in the plant's medicinal properties or specific environmental factors influencing their production. The TLC analysis further confirmed the presence of distinct bioactive compounds with varying R_f values, depending on the mobile phase used (Table 2; Figure 6). These findings provide a foundation for further research into the specific bioactive compounds and their potential therapeutic applications.

CONCLUSION

Present study demonstrates the presence of diverse bioactive compounds in *Eugenia roxburghii* flowers, highlighting their potential medicinal value. The findings provide a foundation for further research into the plant's therapeutic properties and potential applications, contributing to the discovery of natural medicines and informing conservation strategies for this valuable plant species.

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