

CHAPTER 6

Phytochemical screening and Thin Layer Chromatography (TLC) of bark of *Alstonia scholaris* (Apocynaceae)

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Abstract: Qualitative phytochemical screening of *Alstonia scholaris* (L.) R.Br. bark was carried out using different extracts (aqueous, ethanol, and acetone). The results showed that tannins and saponins were commonly present in all three extracts, followed by both flavonoids and reducing sugars, which were present in ethanol and acetone extracts but absent in aqueous extract. Steroids are only present in acetone extract and absent in aqueous and ethanol extracts. The presence of various phytoconstituents indicates the pharmacological potential of *A. scholaris* bark. Therefore, thin layer chromatography analysis was carried out and only one spot was observed in the acetone extract. Additional experimental work is needed for the evaluation of different phytoconstituents and their biological activities of *A. scholaris* bark for the development of novel pharmaceuticals.

Keywords: Biological activities, novel pharmaceuticals, phytoconstituents, pharmacological potential

Introduction

Apocynaceae is a family of the order Gentianales (Sennblad and Bremer, 2002). It is a flowering plant that includes trees, shrubs, herb, succulents, and climbers. It is also known as the Dogbane family because some plants were used as dog poisons (Patil *et al.*, 2023). It has five subfamilies and 4600 species (Perez-Lopez *et al.*, 2024). Most of the plants in this family have ornamental and medicinal values. They are also known for their phytochemicals and bioactivities. In India, the most common genera of this family are *Aganosma*, *Alstonia*, *Carissa*, *Catharanthus*, *Holarrhena*, *Ectocarpus*, *Rauvolfia*, *Vallaris*, *Wrightia*, etc. (Khyade *et al.*, 2014). Among the genus of Apocynaceae, *Alstonia* is very common, and they

usually produce milky latex. They are used for medicinal and ornamental purposes (De *et al.*, 2024). They also do phytoremediations (Singh *et al.*, 2023). Keeping in mind the importance of the genus *Alstonia*, an attempt has been made to evaluate the phytochemicals available in the bark of *A. scholaris*, followed by thin-layer chromatography studies. *A. scholaris* is commonly known as Chhatian, Chatni, Palgaruda, etc. It is a large tree with milky latex and dark gray bark that is nearly smooth and lenticellate (Figures 4-5). Leaves are usually six in a whorl, glabrous, and have many secondary nerves (Figure 2). Flowers are greenish white or cream. Fruits are terrate and narrow (Saxena and Brahmam, 1995). In natural habitats, it is commonly available in valleys, but in urban and rural areas, it is often planted (Figure 1). It has ethnomedicinal significance and plant parts are used to cure diarrhoea, wounds, rheumatic swellings and skin infections (Nayak and Kumar, 2023). The present study highlights the medicinal importance of the bark of *A. scholaris*.

Methodology

Collection of plant parts and preparation of extracts

The bark of *A. scholaris* were collected from CDA 13, Cuttack district of Odisha state in June 2024 (Figures 3-5). The plant was identified by the senior author. The Soxhlet method of extraction was adopted using different solvents for phytochemical analysis. Detection of nine secondary metabolites was carried out using standard methods (Devi *et al.*, 2023; Jena *et al.*, 2024).

Qualitative phytochemical analysis

Test for tannin

About 1 ml of the bark extract was taken. 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 bark extract was taken and added with 1 ml of distilled water. After addition shaken it well and observed the persistent froth formation confirmed the presence of saponin.

Test for flavonoids

About 1 ml of the bark extract was taken. Added 2 ml of 2% NaOH solution and 3 to 4 drops of dilute HCL. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This change in colours confirmed the presence of flavonoids.

Test for terpenoids

About 1 ml of the filtrate was added with 6 drops of chloroform and place it in the water bath for few minutes. Then 6 drops of concentrated H₂SO₄ were added. The reddish-brown interface confirmed the positive result for the presence of terpenoids.



Figure 1: Whole plant of *A. scholaris* is in semi-urban areas



Figure 2: Leaves of *A. scholaris* in wild

Test for phenolic compounds

About 1 ml of the filtrate was taken. Few drops of 5% Ferric chloride solution were added. The dark bluish black provides the positive result of the test for phenolic groups.

Test for reducing sugars

About 1 ml of the bark extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept it in water bath for some time. The presence of red orange precipitate confirmed the presence of reducing sugar.

Test for steroids

About 1 ml of the bark extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added into it. The appearance of upper red and lower yellow with green fluorescence provide the presence of steroids.

Test for alkaloids

About 1 ml of the bark extract was taken and added 3 to 4 drops of Dragendroff's reagent. The formation of reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds

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

TLC 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). Then the slides were activated at 50° C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (3:2) was used as mobile phase. Then spots were observed and recorded the R_f (Retention factor) values (Kumar and Jena, 2014).



Figure 3: Collection of *A. scholaris* bark



Figure 4: Bark of *A. scholaris*



Figure 5: Collected barks of *A. scholaris* for experimental work

Results and discussion

The phytochemical screening revealed that tannin and saponins were commonly present in all three extracts (aqueous, ethanol and acetone) of *A. scholaris* bark followed by flavonoids and reducing sugars were present in both ethanol and acetone extracts while absent in the aqueous extract (Table 1; Figures 6-8). Steroids only present in acetone extract which could have potential for the antioxidant and anti-inflammatory activities (Pant *et al.*, 2017). Thin layer chromatography analysis was observed with only one spot in the acetone extract and there were no spots in aqueous and ethanol extracts were visualized (Table 2). Many researchers have reported about the pharmacological activities of different plant parts of *A. scholaris* like the methanol extract of leaf, stem bark and root bark show antituberculosis properties (Arulmozhi *et al.*, 2007). Ethanolic leaf extract has anticancer activities. The crude methanolic extract of leaf, stem and root have antibacterial properties (Kaushik *et al.*, 2011). Singh and Sangwan, (2011) also reported the antimicrobial activities of *A. scholaris*. The literature survey indicates that there is a need of more works in ethnopharmacological aspects.

Table 1: Phytochemical screening of secondary metabolites using different extracts of *A. scholaris* bark

Bioactive compounds	Aqueous	Ethanol	Acetone
Tannin	+ve	+ve	+ve
Saponin	+ve	+ve	+ve
Flavonoids	-ve	+ve	+ve
Terpenoids	-ve	-ve	-ve
Phenolic compounds	-ve	-ve	-ve
Reducing sugar	-ve	+ve	+ve
Steroids	-ve	-ve	+ve
Alkaloids	-ve	-ve	-ve
Carbonyl compounds	-ve	-ve	-ve

(+ve: positive; -ve: negative)

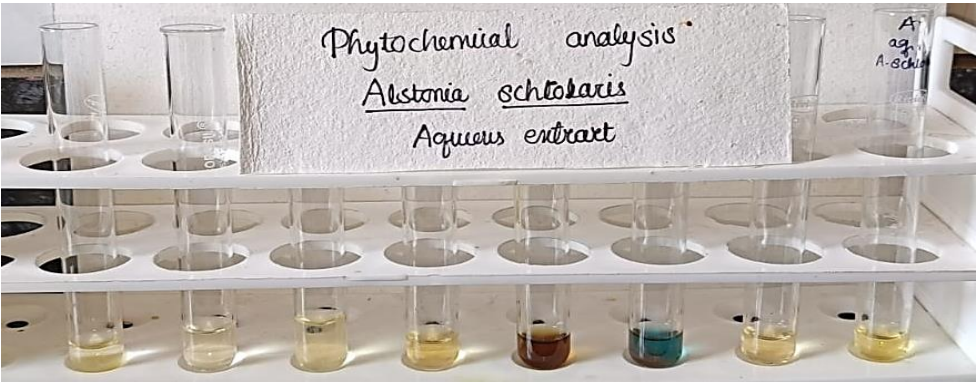


Figure 6: Detection of secondary metabolites using aqueous extract of *A. scholaris* bark

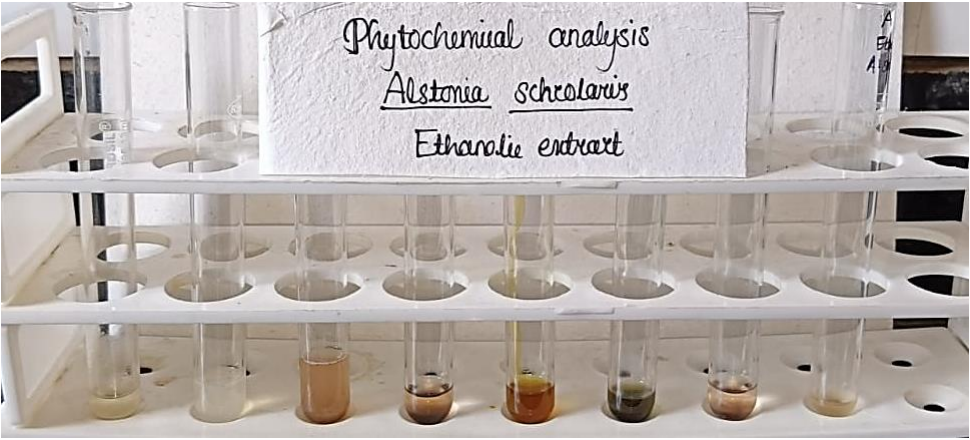


Figure 7: Detection of secondary metabolites using ethanolic extract of *A. scholaris* bark



Figure 8: Detection of secondary metabolites using acetone extract of *A. scholaris* bark

Table 2: TLC analysis of acetone extract of *A. scholaris* bark

Mobile phase	Extracts	Rf value of spots
Chloroform: Methanol (2:3)	Acetone	0.9
	Ethanol	Nil
	Aqueous	Nil

Conclusion

The study concludes that *Alstonia scholaris* is an important medicinal tree of India. It has medicinal uses and bark is rich with diverse secondary metabolites. TLC studies show that acetone extract of bark might has some active constitutes which could be an important source for the isolation and characterization of novel or derived bioactive compounds. Further, more experimental works are required using bark of *A. scholaris*.

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