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Phytochemical elucidation and antimicrobial screening of *Didelotia afzelia*

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Abstract

Background: Antimicrobial resistance motivates exploration of plant-based agents. *Didelotia afzelia* is used in Sierra Leonean ethnomedicine and as a fish poison, suggesting potent bioactives (Ken Fern, 2021; Friday, 2018; Odugbemi, 2008; World Health Organization, 2020) [4, 5, 8, 12].

Objective: To profile phytochemicals from leaves, stem, bark, and roots of *Didelotia afzelia* using three solvents and to screen antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Shigella dysenteriae*, and *Pseudomonas aeruginosa*.

Materials and Methods: Air-dried powders were macerated in methanol, water, or petroleum ether. Qualitative phytochemical tests followed Sofowora/Trease & Evans/Harborne (Sambo *et al*., 2015) [9]. Agar-well diffusion on Mueller-Hinton agar used 0.5 McFarland suspensions; zones were read after 24 h at 37 °C.

Results: Methanol and aqueous extracts contained abundant alkaloids, glycosides, terpenoids, tannins, and saponins; steroids were undetected. Petroleum ether extracts showed few constituents. Bark extracts produced the largest inhibition zones especially against *Staphylococcus aureus* with methanol generally exceeding aqueous activity. TLC indicated more non-polar constituents in leaves; bark produced the fastest piscicidal effect.

Conclusion: *Didelotia afzelia* harbors diverse phytochemicals and exhibits in-vitro antibacterial effects (notably bark/methanol), supporting traditional use and motivating isolation, quantitation, MIC/MBC testing, and safety evaluation (Truong *et al*., 2019; Dirar *et al*., 2019; Kneifel *et al*., 2002) [10, 3, 7].

Keywords: *Didelotia afzelia*, phytochemical screening, agar well diffusion, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Shigella dysenteriae*, TLC, maceration, antibacterial

Introduction

Medicinal plants remain vital to healthcare and drug discovery (Odugbemi, 2008; Twilley *et al*., 2020) [8, 11]. WHO underscores standards for safety, quality, and efficacy (World Health Organization, 2020) [12]. *Didelotia afzelia* (Fabaceae) is used for hemorrhoids and infected sores and as a fish poison in West Africa (Ken Fern, 2021; Friday, 2018) [4, 5]. This study characterizes its phytochemicals across solvents and screens antibacterial activity against priority bacteria.

Materials and Methods

Study Design and Setting

Qualitative laboratory study at COMAHS-USL and the Pharmacy Board of Sierra Leone Microbiology Laboratory (April-September 2022).

Plant Collection and Authentication

Leaves, stem, bark, and roots of *Didelotia afzelia* were collected in Bumban, Bombali District, authenticated at Fourah Bay College; WHO collection guidance was followed (World Health Organization, 2020) [12].

Extraction

Air-dried powders (10 g) were macerated in 250 mL methanol, petroleum ether for 72 h with intermittent shaking and aqueous extraction was performed for 24 h. Filtrates were reserved for phytochemistry, TLC, acute toxicity (fish), and antibacterial testing (Abubakar & Haque, 2020) [1]. Solvent choice considered polarity-yield relationships (Dirar *et al*., 2019; Truong, 2019) [10, 3].

Phytochemical Tests
Carbohydrates (Molisch, Benedict, Fehling), glycosides, alkaloids (Mayer's), saponins (emulsification), tannins ($FeCl_3$), flavonoids (alkaline reagent), anthraquinones (Bornträger), steroids, and terpenoids as per Sofowora, Trease & Evans, and Harborne (Sambo *et al*., 2015) [9].

TLC
Silica gel plates; mobile phases: petroleum ether:ethyl acetate (9:1), petroleum ether:ethyl acetate (17:3), and petroleum ether:ethyl acetate:methanol (18:1:1); Rf values recorded.

Bacterial Isolates and Antimicrobial Assay
Standard isolates *Staphylococcus aureus*, *Streptococcus pyogenes*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* were tested by agar-well diffusion on Mueller-Hinton agar (0.5 McFarland; 37 °C; 24 h); zones (mm) were measured.

Acute Toxicity (Fish)
Tilapia were exposed to dispersions of plant parts in water; time to 100% mortality recorded to compare relative piscicidal effects.

Results

Table 1: Mass of powdered plant parts

Plant part	Mass (g)
Leaves	225.29
Bark	333.12
Roots	147.29
Stem	65.90

Table 2: Acute toxicity results

Plant part / Control	Time to 100% mortality
Bark	25 minutes
Leaves	1 hour
Roots	58 minutes
Stem	1 hour
Control (water)	5 hours

Time to 100% mortality (tilapia) following exposure to water dispersions of powdered plant parts. The bark produced the shortest time, indicating greater acute toxicity; the stem produced the longest time, indicating the lowest acute toxicity among plant parts.

Table 3: Phytochemical screening observations

Phytochemical tests	Observations
Carbohydrate — Molisch's test	Bluish-violet ring at the interface
Carbohydrate — Fehling's test	Brick-red precipitate observed
Carbohydrate — Benedict test	Brick-red precipitate observed
Alkaloids	Creamy-white precipitate observed
Glycosides	Oily layer formed on the surface
Steroids	No visible reaction
Terpenoids	Reddish-brown coloration
Tannins	Dirty-green precipitate
Saponins	Stable foam observed
Flavonoids	Pale-brown coloration
Anthraquinones	Pinkish solution observed

Methanol, petroleum ether, and aqueous extracts of the stem, leaves, bark, and roots of *Didelotia afzelia* were screened qualitatively; observations are summarized below
Phytochemical screening results (test observations)

Table 4: Phytochemical composition of plant parts in aqueous extracts

Components	Stem	Leaves	Bark	Root
Carbohydrate	+	+++	+++	++
Alkaloids	+++	+++	+++	+++
Glycosides	++	+++	+++	++
Terpenoids	+++	+++	+++	++
Tannins	++	+++	+++	++
Saponins	++	++	+++	++
Flavonoids	+	+	++	++
Anthraquinones	-	+	+	-
Steroids	-	-	-	-

Key: +++ abundance (excess); ++ moderate; + trace; - absent.

Table 5: Phytochemical composition of plant parts in methanol extracts

Components	Stem	Leaves	Bark	Root
Carbohydrate	++	++	+++	+++
Alkaloids	++	+++	+++	+++
Glycosides	++	+++	+++	+++
Terpenoids	++	+++	+++	+++
Tannins	++	+++	+++	++
Saponins	+	+++	+++	++
Flavonoids	+	++	-	++
Anthraquinones	-	-	-	-
Steroids	-	-	-	-

Key: +++ abundance (excess); ++ moderate; + trace; - absent.

Table 6: Phytochemical composition of plant parts in petroleum ether extracts

Components	Stem	Leaves	Bark	Root
Carbohydrate	+	+	+	+
Alkaloids	-	-	-	-
Glycosides	-	-	-	-
Terpenoids	-	-	-	-
Tannins	-	+	+	-
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Anthraquinones	-	-	-	-
Steroids	-	-	-	-

Key: +++ abundance (excess); ++ moderate; + trace; - absent.

Table 7: Antimicrobial susceptibility Zones of inhibition (mm) produced by aqueous extracts of *Didelotia afzelia*

Test isolates	Stem	Leaves	Bark	Roots	Control (N. sativa)
Pseudomonas aeruginosa	6.00	7.00	13.00	8.00	16.00
Staphylococcus aureus	8.00	12.00	17.00	10.00	19.00
Streptococcus pyogenes	6.00	6.00	13.00	7.00	14.00
Shigella dysenteriae	4.00	7.00	13.00	8.00	15.00

Fig 1: Zones of inhibition for aqueous extracts of *D. afzelia*.

Table 8: Zones of inhibition (mm) produced by methanol extracts of *Didelotia afzelia*

Test isolates	Stem	Leaves	Bark	Roots	Control (N. sativa)
Pseudomonas aeruginosa	8.00	9.00	15.00	10.00	16.00
Staphylococcus aureus	9.00	13.00	19.00	10.00	18.00
Streptococcus pyogenes	5.00	7.00	13.00	10.00	15.00
Shigella dysenteriae	5.00	8.00	13.00	7.00	14.00

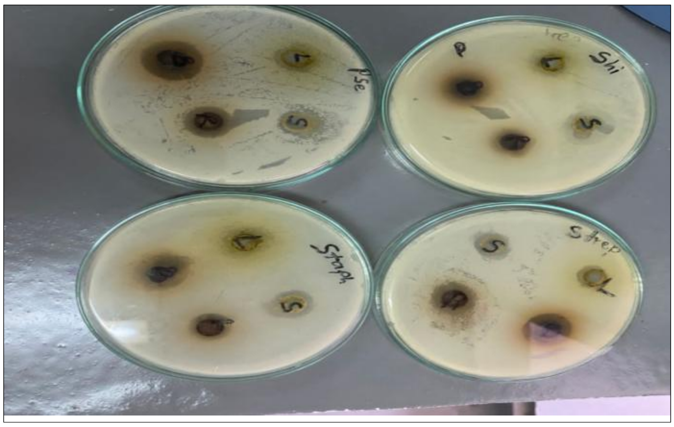

Fig 2: Zones of inhibition for methanol extracts of *D. afzelia*

Thin-layer chromatography (TLC)

Methanol extracts of *Didelotia afzelia* exhibited multiple bands across solvent systems, indicating compounds spanning a range of polarities. Leaves showed the highest number of non-polar bands.

Table 9: TLC analysis of methanol extracts (spot length / Rf) Leaves

Spot	PEL-EA 9:1	PE-EA 17:3	PE-EA-MeOH 18:1:1
A	0.5 / 0.07	0.4 / 0.06	0.8 / 0.11
B	1.7 / 0.24	0.8 / 0.11	1.3 / 0.19
C	2.4 / 0.34	1.2 / 0.17	2.0 / 0.29
D	4.9 / 0.70	1.8 / 0.26	3.0 / 0.43
E	—	2.4 / 0.34	4.0 / 0.57
F	—	3.0 / 0.43	—
G	—	4.3 / 0.61	—
H	—	5.3 / 0.76	—
Roots			
Spot	PEL-EA 9:1	PE-EA 17:3	PE-EA-MeOH 18:1:1
A	0.5 / 0.07	1.3 / 0.19	0.5 / 0.07
B	1.0 / 0.14	2.0 / 0.29	1.1 / 0.18
C	—	2.7 / 0.39	1.6 / 0.22
Stem			
Spot	PEL-EA 9:1	PE-EA 17:3	PE-EA-MeOH 18:1:1
A	0.4 / 0.06	1.5 / 0.21	0.7 / 0.10
B	1.4 / 0.20	2.2 / 0.31	1.3 / 0.19
C	—	3.5 / 0.50	3.3 / 0.47
D	—	5.3 / 0.76	—
E	—	5.9 / 0.84	—
Bark			
Spot	PEL-EA 9:1	PE-EA 17:3	PE-EA-MeOH 18:1:1
A	0.9 / 0.13	2.2 / 0.31	1.4 / 0.20
B	5.4 / 0.77	2.5 / 0.36	1.8 / 0.26

Discussion

Solvent polarity and plant part clearly shaped both chemistry and bioactivity in *Didelotia afzelia*. Polar solvents (methanol, water) recovered broader classes than petroleum ether consistent with polarity-driven extraction reported elsewhere (Dirar, 2019; Truong, 2019) [10, 3] while steroids remained undetected. These richer profiles translated into stronger antibacterial effects: bark extracts produced the largest zones, and methanol consistently outperformed water, with the strongest activity against *Staphylococcus aureus*. The relatively greater susceptibility of the Gram-positive *S. aureus* versus the Gram-negatives is in line with known permeability barriers.

The activity pattern is mechanistically plausible. Bark showed abundant tannins and saponins, which can disrupt proteins/membranes (Barbehenn & Constabel, 2011) and are associated with piscicidal effects matching the rapid fish mortality observed for bark (Grib, 2006; Friday, 2018) [5]. TLC of methanolic extracts revealed numerous bands, particularly in leaves, including non-polar constituents; however, bark remained the most bioactive, suggesting either higher concentrations or more potent components despite fewer spots.

This work is preliminary. Limitations include qualitative phytochemistry, non-identifying TLC, and absence of MIC/MBC and cytotoxicity data; rigorous quality and safety evaluation are needed before standardization and use (Kneifel, 2002) [7].

Conclusion

This study demonstrates that solvent polarity and plant part markedly influence the chemistry and antibacterial activity of *Didelotia afzelia*. Polar extracts (methanol, water) contained broader phytochemical classes than petroleum ether, and bark particularly the methanolic extract consistently produced the largest inhibition zones, with the strongest effect against *Staphylococcus aureus*. TLC profiles confirmed substantial chemical diversity across parts, while the rapid fish mortality observed for bark highlights the presence of membrane-active constituents and the need for safety evaluation. Collectively, these findings support the ethnomedicinal use of *D. afzelia* and identify bark methanol extract as a promising source of antibacterial leads. Future work should quantify key phytochemical classes, establish MIC/MBC and time-kill kinetics against priority pathogens, apply bioassay-guided fractionation to isolate active principles, and conduct cytotoxicity and in-vivo tolerability studies to define a therapeutic window and enable standardization.

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