RESEARCH PAPER

Phytochemical, Antioxidant, and Antibacterial Investigation of Stem Bark Extracts of Selected Medicinal Plants

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Abstract
Stem barks of *Pithecellobium dulce*, *Limonia acidissima*, and *Moringa oleifera* were evaluated for their pharmacognostic characteristics, phytochemical composition, antioxidant capacity, and antibacterial potential. Methanolic extracts were prepared by cold maceration and screened using standard qualitative tests, thin-layer chromatography (TLC), and high-performance thin-layer chromatography (HPTLC). Total phenolic content ranged from 98.27 ± 2.14 to 102.83 ± 3.07 mg GAE/g, and total flavonoid content from 22.56 ± 1.48 to 58.40 ± 2.31 mg RE/g. DPPH radical scavenging activity was dose-dependent across all three extracts, with IC_{50} values of 28.7 ± 1.2 µg/mL (*P. dulce*), 31.4 ± 0.9 µg/mL (*M. oleifera*), and 42.8 ± 1.6 µg/mL (*L. acidissima*). Antibacterial screening against *Staphylococcus aureus* ATCC 25923 revealed zones of inhibition between 2.6 ± 0.3 and 4.9 ± 0.4 mm for individual extracts. A polyherbal combination produced a larger zone (6.3 ± 0.5 mm) and a minimum inhibitory concentration of 150 mg/mL. Although the antibacterial potency was modest, these preliminary findings point to the stem bark as a reservoir of bioactive molecules warranting further fractionation and *in vivo* investigation.
Keywords: *Pithecellobium dulce*; *Limonia acidissima*; *Moringa oleifera*; phytochemical screening; antioxidant; antibacterial
How to cite this article: Khandare S, Sharma R, Vengurlekar S, Jain SK. Phytochemical, antioxidant, and antibacterial investigation of stem bark extracts of selected medicinal plants. Int J Drug Deliv Technol. 2026;16(75s): 347-356. DOI: 10.25258/ijddt.16.75s.39

Introduction

Medicinal plants have served as a backbone of traditional healthcare across Asia, Africa, and the Americas for thousands of years, and the World Health Organization estimates that roughly 80% of the population in developing countries still relies on herbal preparations for primary care [1,2]. Despite this deep-rooted dependence, the scientific understanding of many plant parts remains incomplete. While leaves, fruits, and seeds of popular species have attracted considerable research attention, the stem bark—which often accumulates secondary metabolites in unusually high concentrations—has been somewhat neglected. Three species that illustrate this imbalance are *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae), *Limonia acidissima* Groff (Rutaceae), and *Moringa oleifera* Lam. (Moringaceae).*Pithecellobium dulce*, commonly known as Manila tamarind, has a long ethnobotanical record in wound care and glycemic management. Earlier investigations on its leaf and fruit tissues have documented a varied repertoire of alkaloids, flavonoids, and terpenoids [3,24]. *Limonia acidissima*, whose tangy fruit pulp is eaten fresh or made into chutneys across South and Southeast Asia, has attracted attention for its reputed efficacy against diabetes, infections, and certain inflammatory conditions [4]. *Moringa oleifera*—the widely celebrated "miracle tree"—has been studied intensively for the antioxidant power of its leaves, yet its stem bark chemistry remains comparatively poorly mapped [3]. To date, no single study has placed the pharmacognostic profiles and biological activities of stem bark extracts from all three species side by side under a common experimental framework.

The rationale for investigating antioxidant and antibacterial properties together is straightforward. Free radicals generated during normal aerobic metabolism can damage lipids, proteins, and nucleic acids when endogenous defenses are overwhelmed, and a growing body of evidence links oxidative stress to cardiovascular disease, cancer, and neurodegenerative disorders [4,5]. Plant-derived phenolics and flavonoids are known to intercept radical chain reactions by donating hydrogen atoms or electrons, and many of these same molecules also disrupt bacterial cell membranes or interfere with microbial enzyme systems [20,21]. With antibiotic resistance spreading at an alarming pace worldwide, the search for new antimicrobial leads from natural sources has taken on fresh urgency. Our objective,

therefore, was to carry out a systematic pharmacognostic characterization, qualitative and quantitative phytochemical profiling, and preliminary biological screening of the methanolic stem bark extracts of these three species, so as to create a baseline dataset that could inform future isolation and formulation work.

Materials and Methods

Plant Material

Stem bark of *P. dulce*, *L. acidissima*, and *M. oleifera* was collected during November 2024 from healthy, mature trees growing in the vicinity of Aherawadi, Purna, District Parbhani, Maharashtra, India (19.27° N, 76.27° E). The material was authenticated at the Botanical Survey of India, Western Regional Centre, Pune, and a voucher specimen was deposited under accession number BSI/WRC/Tech/2024/JWD-106. Bark pieces were washed under running tap water to remove surface debris, shade-dried at ambient temperature (28–32 °C) for 10–12 days, and then ground in an electric grinder to pass through a 40-mesh sieve. The resulting powder was stored in airtight glass containers at room temperature until extraction.

Extraction

Each powdered bark sample (50 g) was extracted by cold maceration with 250 mL of analytical-grade methanol in a stoppered Erlenmeyer flask for 72 hours, with intermittent manual shaking every 6 hours. The macerate was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure on a rotary evaporator at 40 °C. The dry residue was weighed, the percentage yield was calculated relative to the starting dry weight, and the extract was stored at 4 °C until further use [9].

Organoleptic Evaluation

Dried bark samples were inspected for color, odor, taste, and surface texture as a quick identity check, following the approach outlined by Chanda [6].

Physicochemical Parameters

Total ash, acid-insoluble ash, and water-soluble ash were determined by the methods described in the WHO guidelines for quality control of medicinal plant materials [7]. Approximately 2 g of powder was ignited at 450 °C in a tared silica crucible until the residue was carbon-free. For acid-insoluble ash, the total ash was boiled with 25 mL of 2 M HCl for 5 minutes and the insoluble fraction was filtered, ignited, and weighed. For water-soluble ash, the procedure was repeated with distilled water, and the soluble portion was obtained by difference [8]. All determinations were carried out in triplicate and results are reported as mean ± SD.

Preliminary Phytochemical Screening

Fresh methanolic extracts were subjected to a battery of standard color-reaction tests for carbohydrates (Molisch, Fehling, Benedict), proteins (Biuret, Millon), amino acids (Ninhydrin), steroids and triterpenoids (Salkowski, Liebermann–Burchard), glycosides (Borntrager), flavonoids (Shinoda, lead acetate, sulfuric acid), tannins and phenolics (ferric chloride, phenazone, gelatin), and alkaloids (Hager, Wagner, Dragendorff, Mayer). Reagents and protocols followed Harborne [9], Banu and Cathrine [10], and Agidew [11].

TLC Fingerprinting

Aliquots of each extract were spotted on silica gel 60 F_{254} TLC plates (Merck) and developed in solvent systems optimized for each: benzene–methanol–ethyl acetate (8:1:1, v/v/v) for PD-ME, benzene–ethyl acetate–acetic acid (4:1:0.1) for LA-ME, and chloroform–acetic acid–methanol (4:2.5:3.5) for MO-ME. Developed plates were visualized under short-wave UV (254 nm) and long-wave UV (366 nm) light, then sprayed with anisaldehyde–sulfuric acid or 10% sulfuric acid reagent. R_f values were recorded for every resolved spot. Because R_f values are sensitive to adsorbent batch, layer thickness, chamber saturation, and ambient humidity, each run was performed in triplicate under tightly controlled conditions [12].

HPTLC Analysis

Each extract (100 mg) was dissolved in 10 mL of methanol by vortexing and sonicating for 10 minutes (final concentration 10,000 ppm). Solutions were filtered through 0.22-µm syringe filters and applied as 8-mm bands onto HPTLC silica gel 60 F_{254} S plates (Merck) using a Camag Linomat 5 applicator. Development was carried out in a Camag twin-trough chamber pre-saturated for 20 minutes with Whatman No. 1 filter paper, using toluene–ethyl acetate–methanol–formic acid (6.0:2.0:1.5:0.1, v/v/v/v) as the mobile phase (development distance 80 mm). Scanning was performed at 254 nm and 366 nm on a Camag TLC Scanner 3.

Total Phenolic Content (TPC)

TPC was estimated by the Folin–Ciocalteu method [13]. Gallic acid served as the reference standard (10–50 µg/mL; calibration equation $y = 0.0127x + 0.0432$, $R^2 = 0.997$). To 1 mL of extract or standard, 0.5 mL of Folin–Ciocalteu reagent was added. After 5 minutes, 2 mL of 20% sodium bicarbonate was introduced and the volume was adjusted to 10 mL. After 60 minutes at room temperature, absorbance was read at 765 nm on a UV-visible spectrophotometer. Results are expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). All readings were taken in triplicate.

Total Flavonoid Content (TFC)

TFC was measured by the aluminum-chloride colorimetric method using rutin as the standard (10–50 µg/mL; calibration equation $y = 0.0098x + 0.0215$, $R^2 = 0.995$) [14]. To 1 mL of sample or standard in a 10-mL flask, 0.30 mL of 5% $NaNO_2$ was added. Five

minutes later, 0.3 mL of 10% $AlCl_3$ was introduced, and after a further 5 minutes 2 mL of 1 M NaOH was added. The volume was made up with distilled water and absorbance was read at 510 nm. Results are expressed as milligrams of rutin equivalents per gram of dry extract (mg RE/g). Determinations were performed in triplicate.

DPPH Free-Radical Scavenging Assay

Antioxidant capacity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of Brand-Williams et al. [25]. Briefly, 2 mL of a freshly prepared 0.1 mM DPPH solution in methanol was mixed with 2 mL of extract (25–125 µg/mL) and 1 mL of methanol. Rutin, gallic acid, and ascorbic acid were used as positive controls at the same concentrations. Tubes were incubated in the dark for 30 minutes, and absorbance was measured at 517 nm against a methanol blank. Percentage scavenging was calculated as: $[(A_{control} - A_{sample}) / A_{control}] \times 100$. IC_{50} values (the concentration producing 50% scavenging) were determined by non-linear regression of the dose–response curves. Each concentration was tested in triplicate [15,16].

Antibacterial Activity

Antibacterial screening was carried out by the agar well-diffusion method [15–17]. A reference culture of *Staphylococcus aureus* ATCC 25923 was obtained from the departmental culture collection. An overnight broth culture was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and spread onto Mueller–Hinton agar plates with a sterile swab. Wells (6 mm diameter) were cut with a cork borer, and 50 µL of each extract (15 mg/mL) was added. Ciprofloxacin (5 µg/mL) served as the positive control and 0.5% DMSO as the negative control. After incubation at 37 °C for 24 hours, zones of inhibition (including the well diameter) were measured with a Vernier caliper. Each test was done in triplicate.

We acknowledge that testing against a single Gram-positive organism is a limitation; work on Gram-negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) is currently under way in our laboratory and will be reported separately.

Minimum Inhibitory Concentration (MIC)

A polyherbal extract, prepared by combining equal weights of the three bark extracts, was dissolved in 0.5% DMSO at concentrations of 15, 30, 60, 90, 120, 150, 300, 450, and 600 mg/mL. MIC determination followed the broth macrodilution method [17,18]. Each concentration was tested in triplicate in Mueller–Hinton broth inoculated with *S. aureus* ATCC 25923. After 24 hours at 37 °C, the MIC was read as the lowest concentration showing no visible turbidity.

Statistical Analysis

All experiments were performed in triplicate unless stated otherwise. Data are expressed as mean ± standard deviation (SD). Group comparisons were made by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant. Calculations were done using GraphPad Prism 9.0.

Results

Organoleptic Characteristics

Table 1 summarizes the sensory features of the three dried bark powders. Each sample had a rough texture and an unpleasant smell. *M. oleifera* was the only one with a markedly bitter note alongside its pungent taste.

Table 1. Organoleptic characteristics of stem bark samples.

Plant	Color	Odor	Taste	Texture
P. dulce	Light whitish-grey	Unpleasant	Pungent	Rough
L. acidissima	Dark grey	Unpleasant	Pungent	Rough
M. oleifera	Grey	Unpleasant	Bitter, pungent	Rough

Physicochemical Evaluation

Ash values and extractive yields are set out in Tables 2 and 3. *L. acidissima* had the highest total ash (5.5 ± 0.3%), suggesting a comparatively high mineral load, while *P. dulce* gave the largest methanolic extractive yield (19.5 ± 0.8%), nearly double that of *L. acidissima* (10.0 ± 0.6%).

Table 2. Ash values (%, mean ± SD, n = 3).

Plant	Total Ash	Acid-Insol. Ash	Water-Sol. Ash
P. dulce	4.0 ± 0.2	0.75 ± 0.05	2.5 ± 0.2
L. acidissima	5.5 ± 0.3	2.0 ± 0.1	6.0 ± 0.4
M. oleifera	3.5 ± 0.2	1.0 ± 0.1	5.0 ± 0.3

Table 3. Methanolic extractive values (%, mean ± SD, n = 3).

Plant	Solvent	Yield (%)
P. dulce	Methanol	19.5 ± 0.8
L. acidissima	Methanol	10.0 ± 0.6
M. oleifera	Methanol	15.5 ± 0.7

Qualitative Phytochemical Screening

Table 4 lays out the results of the color-reaction tests. Every extract gave positive signals for at least some alkaloids, flavonoids, glycosides, and tannins/phenolics. One detail that caught our eye was that *M. oleifera* was the only bark in which all four alkaloid tests—Hager, Wagner, Dragendorff, and Mayer—came back positive, pointing to a particularly rich nitrogen-containing metabolite pool in this species.

Table 4. Qualitative phytochemical screening. (+) detected; (−) not detected.

Category	Test	PD	LA	MO
Carbohydrates	Molisch's	+	+	+
Carbohydrates	Fehling's	−	+	−
Carbohydrates	Benedict's	−	+	−
Proteins	Biuret	+	+	−
Proteins	Millon's	+	+	+
Amino acids	Ninhydrin	+	+	+
Steroids	Salkowski	+	−	+
Steroids	Liebermann–Burchard	−	+	+
Glycosides	Borntrager's	+	+	+
Glycosides	Foam test	−	+	−
Flavonoids	Shinoda	+	+	+
Flavonoids	Lead acetate	+	+	+
Flavonoids	H_2SO_4 test	+	+	−
Tannins/Phenolics	Lead acetate	+	+	+
Tannins/Phenolics	$FeCl_3$	+	−	−
Tannins/Phenolics	$K_2Cr_2O_7$	−	+	+
Tannins/Phenolics	Dil. HNO_3	−	+	−
Alkaloids	Hager's	+	+	+
Alkaloids	Wagner's	−	−	+
Alkaloids	Dragendorff's	−	−	+
Alkaloids	Mayer's	−	−	+

TLC Fingerprinting

Table 5 presents the TLC data. The moringa extract resolved into four distinct spots—the most complex pattern of the three—hinting at a wider spectrum of secondary metabolites. An interesting observation was that PD-ME and LA-ME shared a spot at around R_f 0.64, which may indicate one or more compounds common to all three species [12].

Table 5. TLC fingerprinting of stem bark extracts.

Sr.	Extract	Solvent System	Ratio	Spray Reagent	Rf	Color
1	PD-ME	Benzene:MeOH:EtOAc	8:1:1	Anisaldehyde-H_2SO_4	0.20	Pink
2			8:1:1	Anisaldehyde-H_2SO_4	0.32	Purple
3			8:1:1	Anisaldehyde-H_2SO_4	0.64	Yellow
4	LA-ME	Benzene:EtOAc:AcOH	4:1:0.1	10% H_2SO_4	0.29	Brown
5			4:1:0.1	10% H_2SO_4	0.43	Lt. yellow
6			4:1:0.1	10% H_2SO_4	0.66	Purple
7	MO-ME	$CHCl_3$:AcOH:MeOH	4:2.5:3.5	10% H_2SO_4	0.64	Lt. orange
8			4:2.5:3.5	10% H_2SO_4	0.54	Pink
9			4:2.5:3.5	10% H_2SO_4	0.42	Yel. green
10			4:2.5:3.5	10% H_2SO_4	0.21	Green

HPTLC Fingerprinting

Figure 1 shows the composite HPTLC plate image, and the densitometric chromatograms at 254 nm and 366 nm for each species appear in Figures 2–7.

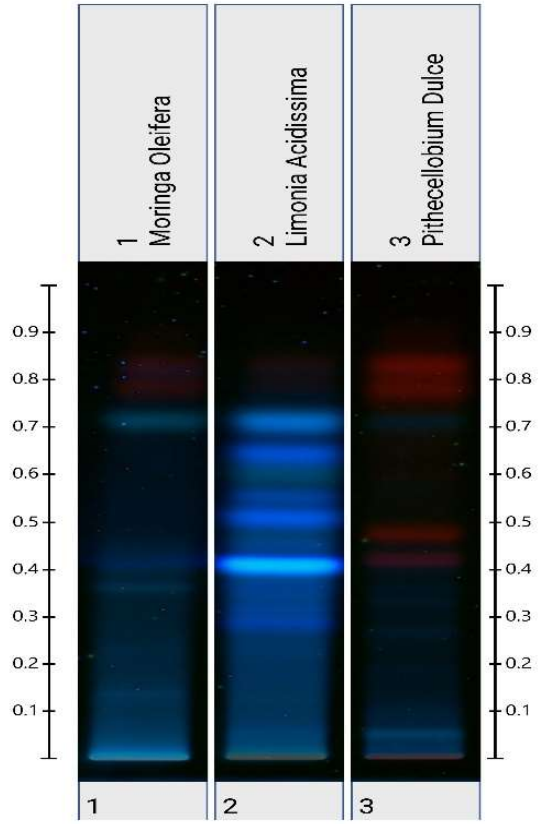
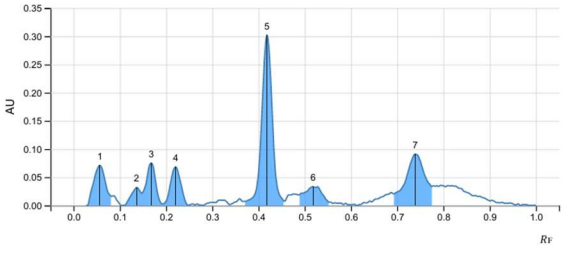


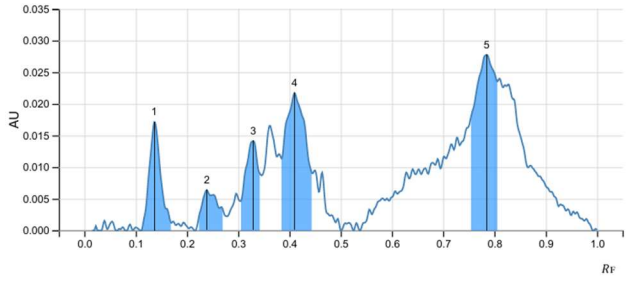
Figure 1. HPTLC plate showing band profiles of all three stem bark extracts.

Seven peaks were detected in the *M. oleifera* track at 254 nm (Figure 2), with the two tallest—at R_f 0.418 (39.54% of total peak area) and 0.739 (21.78%)—likely representing major phenolic or flavonoid constituents. At 366 nm (Figure 3), only five peaks remained, but the bands at R_f 0.785 (38.33%) and 0.410 (29.40%) became markedly more prominent, suggesting that some compounds fluoresce more efficiently at longer wavelengths—a behavior typical of conjugated flavonoids or chlorophyll-related pigments.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.031	0.0000	0.057	0.0707	10.54	0.082	0.0162	0.00211	10.37
2	0.107	0.0000	0.136	0.0318	4.74	0.147	0.0216	0.00072	3.53
3	0.147	0.0216	0.168	0.0749	11.16	0.192	0.0030	0.00179	8.77
4	0.193	0.0027	0.221	0.0681	10.14	0.247	0.0002	0.00174	8.52
5	0.367	0.0071	0.418	0.3016	44.93	0.456	0.0075	0.00806	39.54
6	0.489	0.0191	0.518	0.0333	4.96	0.553	0.0058	0.00152	7.48
7	0.693	0.0205	0.739	0.0909	13.53	0.775	0.0335	0.00444	21.78

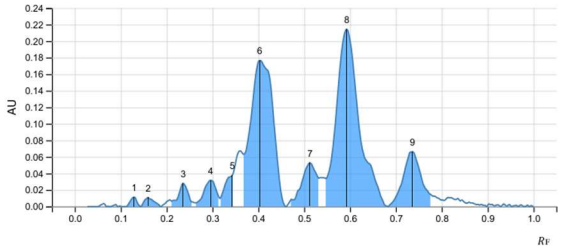
Figure 2. HPTLC chromatogram of M. oleifera at 254 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.111	0.0000	0.136	0.0171	19.67	0.169	0.0012	0.00045	13.14
2	0.221	0.0011	0.239	0.0064	7.33	0.272	0.0027	0.00023	6.89
3	0.305	0.0047	0.329	0.0141	16.21	0.344	0.0088	0.00042	12.24
4	0.385	0.0114	0.410	0.0217	24.93	0.444	0.0090	0.00100	29.40
5	0.752	0.0169	0.785	0.0277	31.86	0.806	0.0235	0.00130	38.33

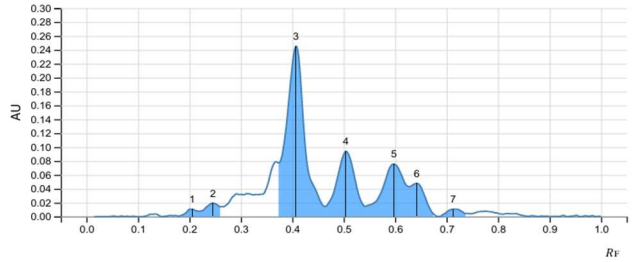
Figure 3. HPTLC chromatogram of M. oleifera at 366 nm.

L. acidissima yielded the richest profile at 254 nm: nine peaks (Figure 4), dominated by bands at R_f 0.593 (40.76%) and 0.403 (32.85%). At 366 nm (Figure 5), the principal peak shifted to R_f 0.407 (48.84%), indicating that the major UV-absorbing compound also possesses strong fluorescent character.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.111	0.0000	0.129	0.0113	1.80	0.142	0.0004	0.00018	0.59
2	0.143	0.0000	0.160	0.0109	1.72	0.188	0.0000	0.00026	0.86
3	0.211	0.0065	0.236	0.0281	4.46	0.256	0.0054	0.00068	2.25
4	0.264	0.0045	0.296	0.0317	5.03	0.315	0.0102	0.00099	3.28
5	0.317	0.0099	0.343	0.0376	5.97	0.345	0.0392	0.00077	2.53
6	0.368	0.0631	0.403	0.1766	28.05	0.460	0.0000	0.00993	32.85
7	0.469	0.0068	0.512	0.0528	8.39	0.532	0.0343	0.00200	6.60
8	0.547	0.0339	0.593	0.2143	34.05	0.682	0.0003	0.01232	40.76
9	0.683	0.0000	0.736	0.0663	10.53	0.781	0.0124	0.00311	10.28

Figure 4. HPTLC chromatogram of L. acidissima at 254 nm.

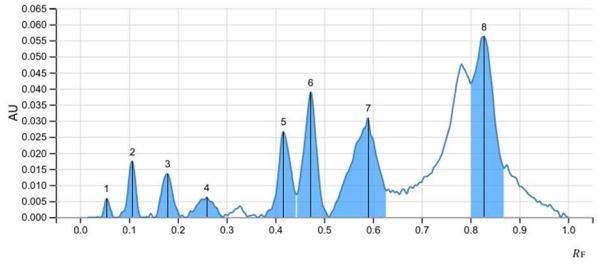


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.184	0.0013	0.206	0.0105	2.10	0.226	0.0089	0.00032	1.56
2	0.226	0.0089	0.246	0.0193	3.85	0.262	0.0143	0.00057	2.76
3	0.374	0.0773	0.407	0.2450	48.73	0.463	0.0146	0.01010	48.84
4	0.464	0.0145	0.504	0.0939	18.67	0.542	0.0147	0.00394	19.06
5	0.542	0.0147	0.597	0.0755	15.02	0.626	0.0430	0.00384	18.56
6	0.628	0.0429	0.642	0.0478	9.51	0.678	0.0016	0.00154	7.44
7	0.683	0.0000	0.713	0.0107	2.12	0.738	0.0041	0.00037	1.78

Figure 5. HPTLC chromatogram of L. acidissima at 366 nm.

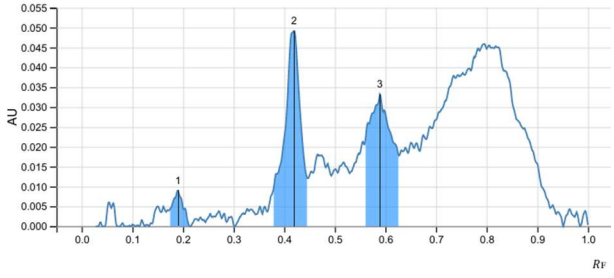
P. dulce behaved quite differently. Eight peaks appeared at 366 nm (Figure 6), the two strongest being

at R_f 0.828 (36.17%) and 0.590 (24.95%)—relatively high R_f values that suggest non-polar constituents, possibly terpenoids or lignans with inherent fluorescence. At 254 nm (Figure 7), only three peaks were resolved, but two of them (R_f 0.419 at 48.41% and 0.589 at 45.74%) accounted for almost the entire absorbance, pointing to a simpler UV-absorbing profile.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.044	0.0003	0.054	0.0058	2.96	0.069	0.0000	0.00007	0.97
2	0.076	0.0000	0.107	0.0173	8.89	0.125	0.0000	0.00031	4.13
3	0.151	0.0000	0.179	0.0135	6.93	0.206	0.0002	0.00035	4.63
4	0.223	0.0007	0.260	0.0061	3.14	0.292	0.0000	0.00025	3.30
5	0.378	0.0002	0.417	0.0265	13.60	0.443	0.0071	0.00079	10.46
6	0.444	0.0071	0.472	0.0388	19.89	0.508	0.0001	0.00116	15.40
7	0.510	0.0000	0.590	0.0308	15.78	0.628	0.0083	0.00189	24.95
8	0.801	0.0416	0.828	0.0562	28.82	0.869	0.0153	0.00273	36.17

Figure 6. HPTLC chromatogram of P. dulce at 366 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.172	0.0040	0.190	0.0090	9.83	0.211	0.0005	0.00022	5.85
2	0.379	0.0096	0.419	0.0491	53.80	0.446	0.0121	0.00182	48.41
3	0.561	0.0226	0.589	0.0332	36.36	0.626	0.0178	0.00172	45.74

Figure 7. HPTLC chromatogram of P. dulce at 254 nm.

Total Phenolic and Flavonoid Content

Phenolic and flavonoid levels are shown in Tables 6 and 7. All three extracts had phenolic contents in a fairly narrow band (98–103 mg GAE/g), with *L. acidissima* marginally on top. Flavonoid content, by contrast, varied more widely: *M. oleifera* led at 58.40 ± 2.31 mg RE/g, nearly 2.5 times the value for *P. dulce* (22.56 ± 1.48 mg RE/g).

Table 6. Total phenolic content (mean ± SD, n = 3).

Extract	Conc. (µg/mL)	Abs. (765 nm)	TPC (mg GAE/g)
MO	50	0.772 ± 0.018	100.32 ± 2.65
LA	50	1.199 ± 0.025	102.83 ± 3.07
PD	50	0.705 ± 0.014	98.27 ± 2.14

Table 7. Total flavonoid content (mean ± SD, n = 3).

Extract	Conc. (µg/mL)	Abs. (510 nm)	TFC (mg RE/g)
MO	50	0.093 ± 0.004	58.40 ± 2.31
LA	50	0.313 ± 0.011	49.80 ± 1.95
PD	50	0.104 ± 0.006	22.56 ± 1.48

DPPH Radical Scavenging Activity

All three extracts scavenged DPPH radicals in a clear dose-dependent manner (Table 8). At 125 µg/mL, *P. dulce* reached 92.85 ± 1.4% inhibition—a figure that came remarkably close to ascorbic acid (93.84 ± 0.9%). *M. oleifera* peaked at 91.53 ± 1.1% at 100 µg/mL, while *L. acidissima* plateaued around 86.0 ± 1.7% at the top dose. Calculated IC_{50} values were 28.7 ± 1.2, 31.4 ± 0.9, and 42.8 ± 1.6 µg/mL for PD, MO, and LA, respectively, confirming that *P. dulce* bark possessed the strongest radical-quenching capacity of the three ($p < 0.05$ vs. LA).

Table 8. DPPH scavenging activity (% inhibition, mean ± SD, n = 3).

Conc.	Rutin	Gallic A.	Ascorbic A.	MO	LA	PD
25	89.84 ±1.2	80.61 ±1.5	85.07 ±1.0	86.92 ±1.3	73.5 ±1.8	85.23 ±1.1
50	92.76 ±0.9	82.92 ±1.3	86.30 ±0.8	88.61 ±1.0	75.8 ±1.6	87.54 ±1.2
75	94.46 ±0.7	85.69 ±1.1	88.76 ±0.9	89.38 ±0.8	81.3 ±1.4	86.12 ±1.3
100	96.30 ±0.5	90.60 ±0.8	90.00 ±0.7	91.53 ±1.1	85.2 ±1.2	90.54 ±0.9
125	97.69 ±0.4	94.76 ±0.6	93.84 ±0.9	86.61 ±1.5	86.0 ±1.7	92.85 ±1.4

Antibacterial Activity

Table 9 presents the inhibition-zone data. Individually, *M. oleifera* was the most active (4.9 ± 0.4 mm), followed by *P. dulce* (3.8 ± 0.3 mm) and *L. acidissima* (2.6 ± 0.3 mm). When the three were combined at equal weight (5 mg each, 15 mg/mL total), the polyherbal mix produced a zone of 6.3 ± 0.5 mm (Figures 8 and 9). It should be noted that these zones are small by conventional standards—ciprofloxacin, for instance, produced a zone of 28 ± 1.2 mm under the same conditions—so the observed activity, while

measurable and statistically greater than the DMSO control ($p < 0.05$), should be described as modest rather than potent. The wider zone produced by the mixture does, however, suggest an additive or mildly synergistic interaction among the constituent phytochemicals.

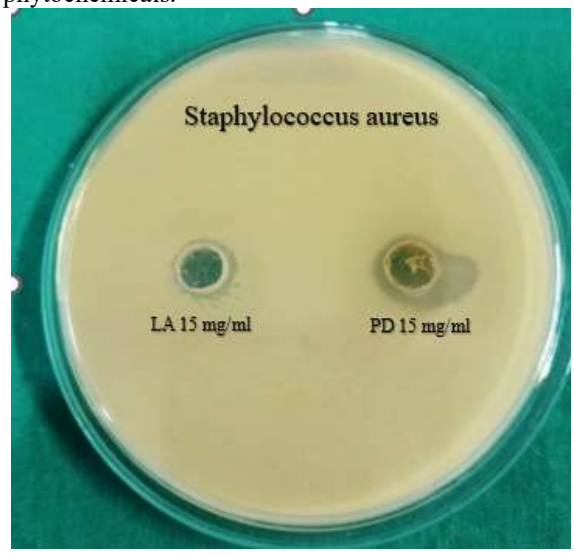


Figure 8. Zones of inhibition produced by individual bark extracts against S. aureus ATCC 25923.

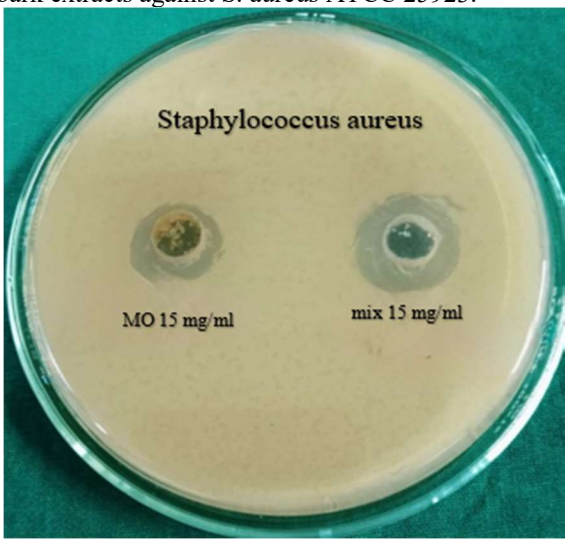


Figure 9. Zone of inhibition produced by the polyherbal extract.

Table 9. Zones of inhibition against *S. aureus* ATCC 25923 (mean ± SD, n = 3).

Sr.	Sample	Conc.	ZOI (mm)	p vs. DMSO
1	*L. acidissima*	15 mg/mL	2.6 ± 0.3	< 0.05
2	*M. oleifera*	15 mg/mL	4.9 ± 0.4	< 0.01
3	*P. dulce*	15 mg/mL	3.8 ± 0.3	< 0.05
4	Polyherbal mix	15 mg/mL	6.3 ± 0.5	< 0.01
5	Ciprofloxacin (+)	5 µg/mL	28 ± 1.2	—
6	0.5% DMSO (−)	—	0	—

Minimum Inhibitory Concentration

Visible turbidity persisted through 120 mg/mL but disappeared at 150 mg/mL, making this the MIC of the polyherbal blend against *S. aureus* ATCC 25923 (Table 10; Figures 10–12). A value of 150 mg/mL is high relative to purified antimicrobial compounds and underscores that these are crude extracts; nonetheless, the result provides a useful starting point for bioassay-guided fractionation aimed at isolating the active constituents.

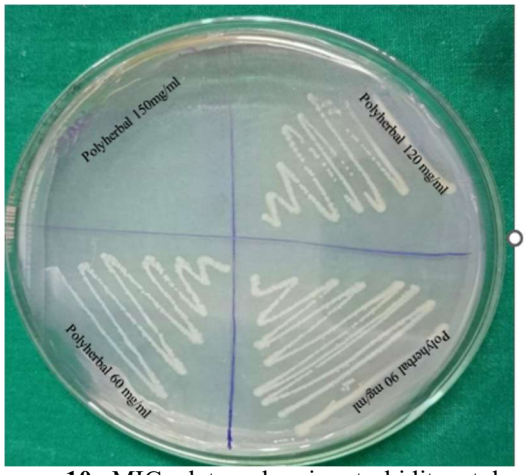


Figure 10. MIC plate showing turbidity at lower concentrations.

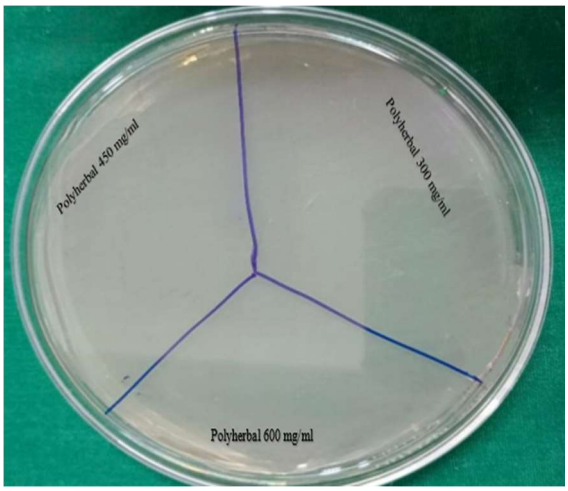


Figure 11. MIC plate showing the transition from turbid to clear.

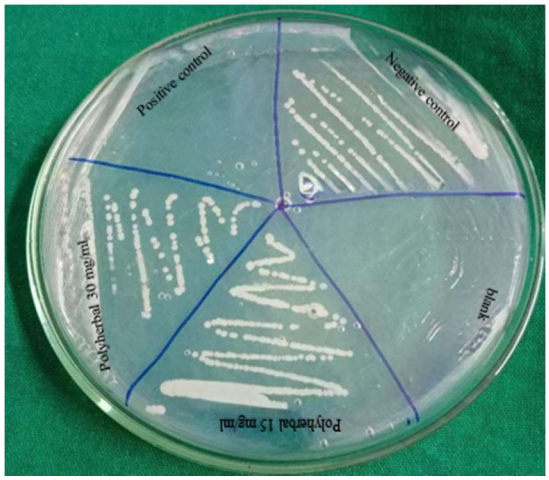


Figure 12. MIC determination of polyherbal extract against S. aureus.

Table 10. MIC of polyherbal extract against *S. aureus* ATCC 25923.

Sr.	Sample	Organism/Detail	Conc.	Observation
1	Negative control	Bacterial suspension	—	Turbid
2	Positive control	Ciprofloxacin	5 µg/mL	Clear
3	Vehicle control	0.5% DMSO	—	Clear
4	Polyherbal		15 mg/mL	Turbid
5	Polyherbal		30 mg/mL	Turbid
6	Polyherbal		60 mg/mL	Turbid
7	Polyherbal		90 mg/mL	Turbid
8	Polyherbal		120 mg/mL	Turbid
9	Polyherbal		150 mg/mL	Clear
10	Polyherbal		300 mg/mL	Clear
11	Polyherbal		450 mg/mL	Clear
12	Polyherbal		600 mg/mL	Clear

Discussion

Three broad observations emerge from this study, and each deserves honest appraisal. The first is that all three stem barks harbor a surprisingly rich repertoire of secondary metabolites. Across the board, the qualitative tests flagged flavonoids, alkaloids, glycosides, tannins, and phenolics—the same broad classes that other groups have reported in leaf and fruit tissues of these species [19,20]. The consistency of detection across very different plant families reinforces a growing view in the literature that bark tissue, because of its interface between vascular cambium and the external environment, functions as a natural defensive barrier accumulating bioactive chemistry [21]. The HPTLC data added a layer of nuance: *M. oleifera* bark routinely produced the most complex chromatographic profile, with the greatest number of peaks at both 254 nm and 366 nm, whereas *P. dulce* bark gave a simpler UV-absorbing profile but a richer fluorescence pattern. These differences underscore the fact that broad-brush descriptors like "rich in phenolics" can mask meaningful species-level variation.

The second observation concerns antioxidant potency. All three extracts scavenged DPPH radicals in a clean dose-dependent fashion, and the calculated IC_{50} values—ranging from about 29 to 43 µg/mL—fall well within the range that the phytochemical literature considers pharmacologically interesting [21,25]. *P. dulce* bark was the most active, with an IC_{50} of 28.7 µg/mL, not far from the ascorbic-acid reference (IC_{50} ≈ 20 µg/mL under our conditions). The high phenolic content across all three extracts (98–103 mg GAE/g) provides a plausible chemical explanation for this activity, since polyphenolic hydroxyl groups are well-established hydrogen donors to free radicals [21]. *M. oleifera* bark, despite having the highest flavonoid content, ranked second in the DPPH assay, a reminder that total flavonoid quantity alone does not dictate antioxidant performance; structural features such as the number and position of hydroxyl groups on the B ring, degree of conjugation, and synergistic or antagonistic interactions with co-extracted compounds all play a role [21].

Third, the antibacterial data, while promising in direction, must be interpreted cautiously. The zones of inhibition produced by the individual extracts (2.6–4.9 mm) and even by the polyherbal mixture (6.3 mm) are

small compared with the ciprofloxacin positive control (28 mm). In practical terms, an MIC of 150 mg/mL for a crude extract is not exceptional; many published studies on crude plant extracts report MICs in the 1–16 mg/mL range for genuinely active preparations [22]. We therefore characterize the observed antibacterial effect as modest. That said, the wider zone produced by the three-way combination is noteworthy because it exceeded the sum of what any single extract achieved alone at the same total concentration, hinting at an additive or mildly synergistic interaction that could become more pronounced once the active fractions are concentrated. This pattern is consistent with the well-documented phenomenon of multi-target synergy in herbal formulations [20].

Several limitations should be borne in mind when interpreting these data. First, only methanol was used as the extraction solvent; water-soluble compounds and highly lipophilic constituents may have been missed. Second, antibacterial testing was confined to a single Gram-positive species (*S. aureus* ATCC 25923). Work on representative Gram-negative strains is under way and will be reported separately. Third, all activity measurements were performed *in vitro*; *in vitro* potency does not translate automatically into clinical efficacy, particularly for crude extracts whose bioavailability and metabolism are unknown. Finally, although all experiments were done in triplicate, the small sample size inherent in a laboratory screening study means that the statistical comparisons should be treated as preliminary rather than definitive.

Going forward, the most productive next steps would be bioassay-guided fractionation to pin down which specific molecules are responsible for the antioxidant and antibacterial effects, followed by *in vivo* efficacy and toxicity testing. Stability studies under accelerated storage conditions would also be needed before any of these extracts—alone or in polyherbal combination—could realistically be considered for development into a standardized herbal product.

Conclusion

This study provides a side-by-side pharmacognostic and biological comparison of methanolic stem bark extracts from *P. dulce*, *L. acidissima*, and *M. oleifera*. All three proved rich in phenolics, flavonoids, alkaloids, glycosides, and tannins. *M. oleifera* bark showed the greatest chromatographic complexity, while *P. dulce* bark exhibited the strongest antioxidant activity (IC_{50} 28.7 µg/mL), approaching synthetic standards. A polyherbal blend of all three extracts produced a wider antibacterial zone against *S. aureus* ATCC 25923 than any extract alone, although the overall potency remained modest (MIC 150 mg/mL). These findings lend preliminary scientific support to the ethnomedicinal use of these plants and justify further fractionation, compound isolation, and *in vivo* validation.

Acknowledgments

We are grateful to Oriental University, Indore, for providing laboratory facilities and to the Botanical Survey of India, Western Regional Centre, Pune, for authenticating the plant specimens.

Declaration of Interest Statement

The authors report there are no competing interests to declare.

Data Availability Statement

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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