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PHYTOCHEMICAL SCREENING AND IN VITRO ANTIBACTERIAL ACTIVITY OF BARK AND POD EXTRACTS OF *PROSOPIS CINERARIA* (L.) DRUCE AGAINST *ESCHERICHIA COLI*

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Prosopis cineraria (L.) Druce, a leguminous desert tree of the family Fabaceae widely known as khejri, jand, or sangri, is used across the arid tracts of north-western India in traditional medicine. This study qualitatively screened the phytochemical constituents of bark and pod extracts of *P. cineraria* in petroleum ether, ethyl acetate, and methanol, and evaluated the in vitro antibacterial activity of the same bark and pod extracts against *Escherichia coli*. Plant material was collected from the campus of Maharshi Dayanand Saraswati University, Ajmer, in March-April, shade-dried, powdered, and extracted by Soxhlet apparatus, with dry-weight extractive yield calculated for each solvent-part combination. Extracts were screened for alkaloids, carbohydrates, proteins, saponins, tannins, flavonoids, and starch using standard colour and precipitation reactions. The antibacterial activity was assessed by the agar well diffusion method across a concentration gradient (0.05-2 mg/mL), with ampicillin and DMSO as positive and negative controls. Petroleum ether extracts of bark and pods showed comparatively few positive phytochemical reactions, whereas ethyl acetate and methanolic extracts of bark and pods showed a broader positive profile, including alkaloids, carbohydrates, tannins, and flavonoids, consistent with methanol and ethyl acetate's greater capacity to solubilize both polar and non-polar metabolites than non-polar petroleum ether. Methanol also gave the highest dry-weight extractive yield in both tissues (7.5% for bark, 3.2% for pods), with bark yielding more extract by mass than pods in all three solvents. Bark extracts inhibited *E. coli* at markedly lower concentrations than pod extracts in both petroleum ether and ethyl acetate; the ethyl acetate bark extract was the most active preparation, producing a zone of inhibition of 32 mm at 1 mg/mL, approaching the ampicillin positive control (approximately 36 mm), while pod extracts in all three solvents required concentrations above 1.2-1.6 mg/mL before comparable inhibition was observed. These findings support the traditional use of *P. cineraria* bark in infection-related remedies and identify bark ethyl acetate extract as the most promising material for further bioassay-guided fractionation.

Keywords: Antibacterial activity; *Escherichia coli*; Minimum inhibitory concentration; Phytochemical screening; *Prosopis cineraria*; Soxhlet extraction.

Introduction

Medicinal plants have supplied remedies for infectious disease throughout human history and remain central to primary healthcare in much of the developing world, where they continue to effect both traditional practice and modern drug discovery[1]. Interest in plant-derived antibacterials has intensified alongside growing concern over antimicrobial

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resistance (AMR): a comprehensive global analysis estimated that bacterial AMR was associated with 4.95 million deaths and directly attributable to 1.27 million deaths in 2019 alone[2]. This crisis has redirected scietific attention toward plant-derived phytochemicals like alkaloids, flavonoids, tannins, phenolics, and terpenoids among them, as alternative antibacterial agents capable of supplementing or extending the useful life of conventional antibiotics[3]. A review of plant compounds, extracts, and essential oils described between 2016 and 2021 found that polyphenols and terpenes were the most frequently active phytochemical classes and that disruption of the bacterial plasma membrane was the most commonly reported mechanism of action[4], and with specific reference to *Escherichia coli*.

Prosopis cineraria (L.) Druce belongs to the family Fabaceae, subfamily Mimosoideae, within the genus *Prosopis,* a group of roughly 40-45 species of trees and shrubs distributed across the arid and semi-arid regions of Asia, Africa, and the Americas. Locally it is known by many vernacular names — khejri, jand, or janti in Rajasthan, kandi in Sindh, banni in Karnataka, vanni or jambu in Tamil Nadu, and sami or sumri in Gujarat[5] and in Ayurvedic and Siddha literature it is termed kalpataru, popularly rendered as the Golden Tree or Wonder Tree of the desert, in recognition of the fact that nearly every part of the tree is put to some traditional use.

P. cineraria is a small to medium, irregularly branched, spiny tree with thick, rough, ash-grey bark showing deep longitudinal fissures. Its taproot is exceptionally deep, reportedly penetrating vertically 20 metres or more in search of groundwater, an adaptation that allows it to persist through prolonged drought. Flowers appear around March and are a valued source of honey, and the pods that follow are cylindrical, elongated (8-19 cm), glabrous, with a thin, brittle pericarp; naturally dried pods are locally known as sangri and consumed as a vegetable[6].

The species is native to the arid tracts of Arabia and extends across India through Rajasthan, Haryana, Punjab, Gujarat, western Uttar Pradesh, and the drier parts of the Deccan as far south as South India, and is similarly found across Iran, Afghanistan, and Pakistan. It is especially dominant in the Thar Desert of Rajasthan, where it is recognised as the state tree, and where the present study's plant material was collected.

Ecologically and economically, *P. cineraria* is valued for soil improvement and sand-dune stabilization in addition to its role as fodder and food; as a nitrogen-fixing legume it enriches soil fertility beneath its canopy, and its deep taproot allows it to survive where shallower-rooted vegetation cannot, making it a keystone species of desert agroforestry systems[6]. Wood ash from the tree is used as a source of potash, and Prosopis sawdust has separately been used as an adsorbent for dye removal from aqueous solution and as a precursor for activated carbon[7], underscoring the plant's broader utility beyond direct medicinal or nutritional use.

The bark has documented abortifacient and laxative properties, and crude bark extracts have shown supportive benefit against protein and mineral deficiency[6]. Aqueous bark extracts are applied externally to treat skin disease, disinfect wounds, and promote healing, and *P. cineraria* stem bark has documented traditional use in rheumatism, cough, common cold, anthelmintic disorders, dysentery, bronchitis, asthma, leucoderma, piles, and muscle tremor[8]. Dried pods are traditionally consumed to help prevent protein and mineral deficiency and have documented traditional applications spanning pain, hypercholesterolaemia, diabetes, anaemia, and kidney and liver disorders[8]. A wider survey of the plant's chemical constituents catalogued antibacterial, antihyperglycaemic, antihyperlipidaemic, antidepressant, skeletal-muscle-relaxant, bronchodilator, vasodilatory, detoxifying, anticancer,

analgesic, antidiarrhoeal, anticonvulsant, and antioxidant activity attributed to the plant, including activity against multidrug-resistant bacterial and fungal strains[8], while a review of the plant's traditional usage in the United Arab Emirates, where it is locally called ghaf, documented antitumour bioactivity of bark extracts, including inhibition of MCF-7 breast cancer cell proliferation and activity against liver tumour cells, alongside analgesic and antipyretic effects[9].

Phytochemically, *P. cineraria* as a whole contains carbohydrates, proteins, tannins (including gallic acid), steroids (stigmasterol, campesterol, sitosterol), flavone derivatives (prosogerin A-E), alkaloids (spicigerine, prosophylline), and terpenes distributed across most of its plant parts[7], but the relative composition differs by tissue. Leaves have been reported to contain phenolic acid derivatives and unsaturated fatty acids, including linoleic and oleic acid, alongside alkaloids, flavonoids, tannins, and saponins on qualitative screening[10]. Bark and stem bark are comparatively rich in tannins and phenolics and have yielded alkaloids, proteins, carbohydrates, flavonoids, and saponins on qualitative screening in alcohol and aqueous extracts[7]. Pods contain tannins, alkaloids, flavonoids, and glycosides[10], with free amino acid and protein content increasing during pod development from the young to the ripened stage[6]. Flowers contribute measurable glycoside content and are a source of honey[10], and seeds have shown flavones on qualitative testing[10]. A solvent-polarity screening study across pods, flowers, stem, and seeds found ethanol and water to be the best solvents for recovering these metabolites, with pods and seeds proposed as a plausible alternative protein source in addition to yielding compounds of antibacterial relevance[11].

Because different plant parts of *P. cineraria* are prepared and used differently in traditional practice, and because the phytochemical survey above shows that tannin, flavonoid, alkaloid, and glycoside content varies meaningfully between leaves, bark, pods, flowers, and seeds, a single plant part cannot be assumed to represent the antibacterial potential of the species as a whole. Prior work has separately shown antibacterial activity in bark[12] and, in related assays, in other tissues, but few studies have directly compared plant parts collected from the same site and season across a shared solvent gradient and test organism, making it difficult to judge from the existing literature alone which tissue offers the most concentration-efficient antibacterial lead.

This study focuses on bark and pods. Both are widely used traditionally, bark for wounds and infections, and pods (sangri) as a common food but they have rarely been compared directly for antibacterial activity under the same conditions. Bark and pods were therefore collected from a single site and extracted using three solvents of increasing polarity: petroleum ether, ethyl acetate, and methanol. For each of the resulting extracts, dry-weight yield was recorded, major phytochemical classes were screened qualitatively, and antibacterial activity against *Escherichia coli* was tested across a range of concentrations. The aim was to identify which plant part and solvent combination gives the strongest, most concentration-efficient antibacterial result, to guide future bioassay-guided fractionation work.

Material and Methods

Plant Material and Sample Collection:

Fresh bark and pods of *Prosopis cineraria* were collected from the campus of Maharshi Dayanand Saraswati University, Ajmer, Rajasthan, India, during March-April, when the maximum-to-minimum ambient temperature ranged from 38 to 25 ˚C. Samples were transported to the laboratory, washed under running tap water followed by distilled water to remove

adhering dust and debris, and shade-dried for 5-7 days. The dried material was ground to a coarse powder using a mechanical grinder and stored in airtight containers at room temperature until further use.

Soxhlet Extraction and Extractive Yield:

Extraction followed the general Soxhlet extraction protocol described for plant antimicrobial screening[13], adapted for three solvents of increasing polarity like wise petroleum ether, ethyl acetate, and methanol[11]. For each plant part, 100 g of coarsely powdered material was loaded into the thimble of a Soxhlet extractor, and solvent was added to the round-bottom flask beneath the extractor and condenser assembly on a heating mantle. The solvent was heated to boiling and continuously evaporated, condensed, and siphoned back through the powdered material for approximately 16 hours per extraction cycle. Before extraction, each empty round-bottom flask/beaker was weighed; after extraction, the flask containing the dried extract was reweighed, and the net weight of extract was obtained by subtraction. Because the process involves continuous heating of flammable solvents alongside circulating condenser water, the apparatus was operated carefully and monitored regularly rather than left unattended overnight, with gloves used throughout as standard personal protective equipment[13]. Percentage dry-weight extractive yield was calculated for each solvent-plant part combination as (net weight of dried extract, g / weight of powdered plant material extracted, g) x 100.

Qualitative Phytochemical Screening:

Each extract was screened qualitatively for carbohydrates, alkaloids, starch, proteins, tannins, flavonoids, and saponins using standard colour and precipitation reactions[7].

Test for Alkaloids - Alkaloids were tested by Dragendorff's method: a few milligrams of extract were dissolved in 5 mL distilled water, acidified with 2 M HCl, and treated with 1 mL of Dragendorff's reagent, an orange-to-reddish-brown precipitate indicating a positive result; this colorimetric approach is consistent with the standard spectrophotometric method for estimating Dragendorff-precipitable alkaloids in plant material[14].

Test for Carbohydrates - Carbohydrates were tested by Fehling's test (1 mL of a 1:1 mixture of Fehling's solutions A and B added to 2 mL aqueous extract and boiled), a brick-red or yellow-green colour indicating a positive result.

Test for Proteins - Proteins were tested by the Ninhydrin test (addition of 0.1% w/v Ninhydrin reagent in n-butanol), a purple colour indicating a positive result.

Test for Saponins - Saponins were tested by the foam test: 5 mL of extract was treated with a few drops of sodium bicarbonate, shaken vigorously, and left to stand; persistent honeycomb-like froth indicated a positive result.

Test for Tannins - Tannins were tested with the ferric chloride test (a few drops of 5% FeCl3 added to 1-2 mL of extract), with dark green, bluish-black, or greenish-black colouration indicating a positive result.

Test for Flavonoids - Flavonoids were tested using the alkaline reagent test (addition of sodium hydroxide solution to the test extract), a yellow colour, or its disappearance on acidification with dilute HCl, indicating a positive result.

Test for Starch - Starch was assessed using the iodine test, a blue-black colour indicating a positive result.

Each test was interpreted as present or absent based on the described colour or precipitation change, and each plant part-solvent combination was screened independently.

Antibacterial Activity Against *E. coli*:

Preparation of Antibiotic Stock and MIC Reference Concentration - Ampicillin was used as the reference antimicrobial agent. Following standard practice for minimum inhibitory concentration (MIC) determination by agar or broth dilution[15], the weight of antibiotic required to prepare a 10 mg/mL stock solution was calculated using W = (C x V) / P, where W is the weight of antimicrobial agent to be dissolved (mg), C is the desired final concentration of the stock solution (micrograms/mL), V is the desired volume (mL), and P is the potency stated by the manufacturer (micrograms/mL). With a manufacturer-stated potency of 500 mg and a target stock concentration of 10 mg/mL, 200 mg of ampicillin was weighed using sterile containers and a sterile spatula and dissolved in sterile distilled water to prepare the stock solution used as the positive control in the antibacterial assays below.

Test Organism and Antibacterial Assay:

Escherichia coli was used as the test organism. Bacterial stock cultures were maintained at 4 °C and sub-cultured at 37 °C for 24 hours prior to testing. Test samples of bark and pod extracts in petroleum ether, ethyl acetate, and methanol were prepared by dissolving the extracts in dimethyl sulfoxide (DMSO) at a concentration gradient from 0.05 to 2 mg/mL; DMSO alone served as the negative control, and the ampicillin stock solution served as the positive control[16]. Antibacterial activity was assessed by the agar well diffusion method: Luria-Bertani (LB) agar was autoclaved at 121 ˚C and 15 psi, cooled to 45-50 ˚C, and poured into sterile Petri dishes to form solid plates. Wells 6 mm in diameter and 3 mm deep were bored into the solidified agar. *E. coli* was spread evenly across the plate surface using a sterile cotton swab, and the bark or pod extract at each test concentration was loaded into the wells, with ampicillin and DMSO loaded into separate wells as positive and negative controls. Plates were left for 2 hours to allow extract diffusion, then sealed with parafilm and incubated at 37 ˚C for 24 hours. Antibacterial activity was quantified by measuring the diameter of the zone of inhibition (mm) around each well, with each concentration tested in triplicate and the mean of the three replicates reported[16].

Results and Discussion

Extractive Yield (Dry Weight):

The weight of each extract was determined by weighing the extraction vessel before and after Soxhlet extraction of 100 g of powdered bark or pod material per solvent. Percentage dry-weight yield, calculated as (net extract weight / 100 g) x 100, is given in Table 1.

Table 1. Dry-weight extractive yield of *P. cineraria* bark and pod extracts by solvent

Solvent	***Plant part***	***Weight of beaker before extraction (g)***	***Weight of beaker after extraction (g)***	***Net weight of extract (g)***	***Extractive yield (% w/w)***
Petroleum ether	Bark	46.272	47.488	1.216	1.22
Petroleum ether	Pods	47.952	48.661	0.709	0.71
Ethyl acetate	Bark	101.844	102.053	0.209	0.21
Ethyl acetate	Pods	109.894	110.087	0.193	0.19
Methanol	Bark	95.224	102.773	7.549	7.55
Methanol	Pods	88.537	91.690	3.153	3.15

Methanol gave by far the highest extractive yield for both tissues (7.55% for bark, 3.15% for pods), followed by petroleum ether (1.22% bark, 0.71% pods) and ethyl acetate

(0.21% bark, 0.19% pods), consistent with methanol's intermediate polarity and its capacity to solubilize a wide range of both polar and non-polar constituents (Fig.1.). Notably, bark gave a higher extractive yield than pods in all three solvents tested, indicating that, on a simple dry-weight basis, bark is the more extractable tissue of the two, a finding that complements the antibacterial results below, where bark extracts were also more potent than pod extracts at equivalent concentrations.

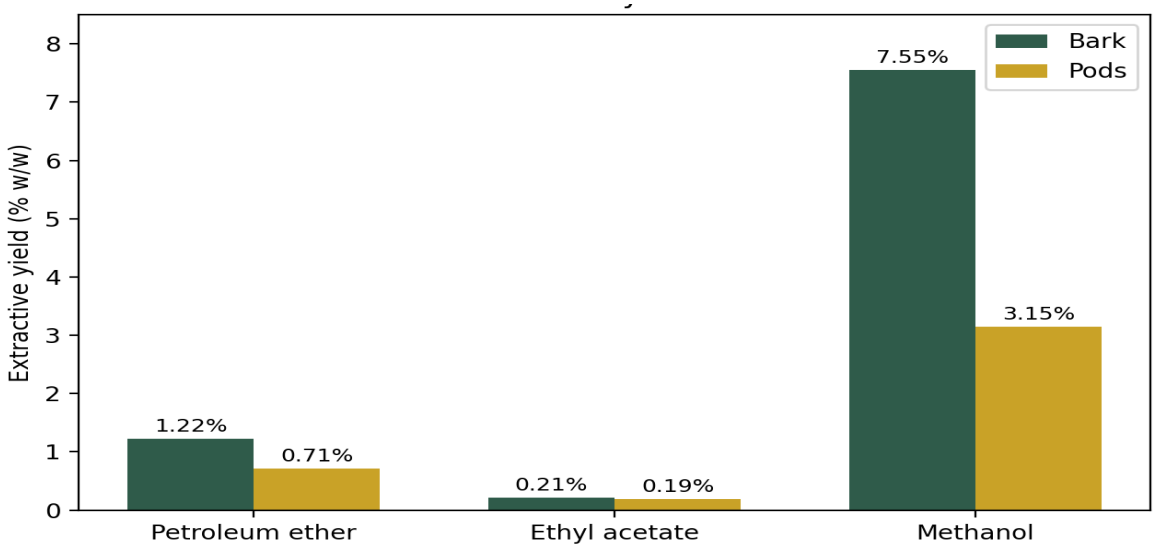

Fig. 1. Dry-weight extractive yield (% w/w) of *P. cineraria* bark and pod extracts by solvent, calculated from the data in Table 1.

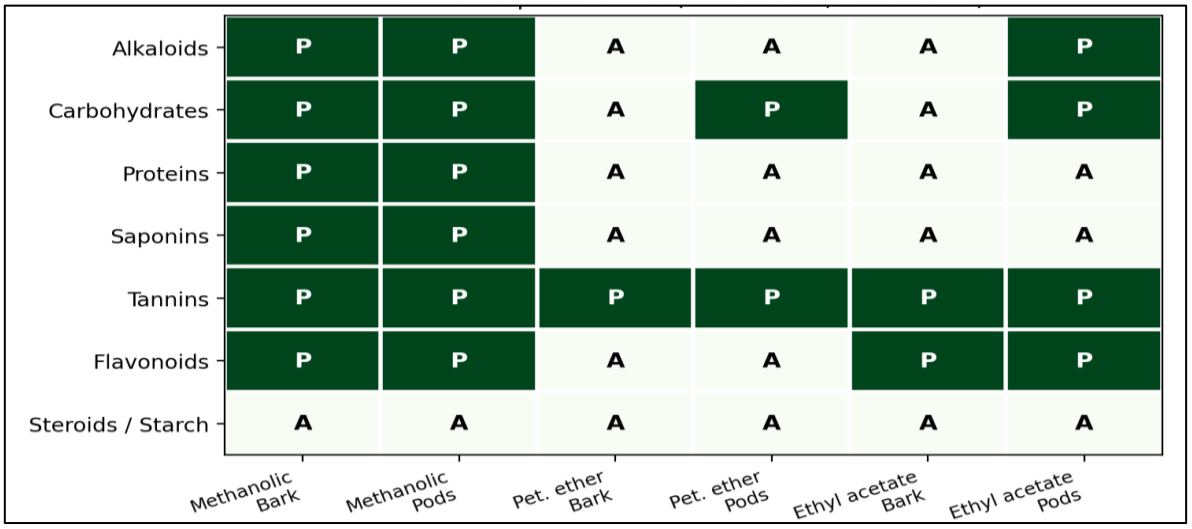

Fig. 2. Heat map summary of qualitative phytochemical screening results across the six *P. cineraria* bark and pod extract combinations examined (Tables 2a-2f). Dark green = present; white = absent.

Qualitative Phytochemical Screening:

Six plant part-solvent combinations were screened: methanolic, petroleum ether, and ethyl acetate extracts of bark and pods.

Table 2 and the heat map in Fig. 2 together showed a clear solvent-dependent pattern. Petroleum ether extracts of bark and pods were predominantly negative across the seven classes tested, with only tannins (by ferric chloride test) positive in both tissues, and carbohydrates (by Fehling's test) additionally positive in pods; alkaloids, proteins, saponins, flavonoids, and starch were absent from both petroleum ether

extracts. This is consistent with petroleum ether's non-polar character, which favours extraction of waxes, fatty material, and some terpenoids over the more polar alkaloid, flavonoid, saponin, and protein-associated metabolites. Ethyl acetate extracts of bark and pods, by contrast, tested positive for alkaloids, tannins, and flavonoids, and the pod extract additionally tested positive for carbohydrates, indicating that ethyl acetate's intermediate polarity recovers a broader range of metabolites than petroleum ether. Tannins were the only class present in every one of the six extract combinations, visible in Fig. 1 as the only fully dark-green row, while steroids/starch were absent everywhere, the only row that is uniformly unshaded. Methanolic extracts of both bark and pods gave the broadest positive profile, testing positive for alkaloids, carbohydrates, proteins, saponins, tannins, and flavonoids alike, differing only in that pod carbohydrate content tested positive by both Anthrone's and Fehling's tests, whereas bark carbohydrate tested positive by Anthrone's but not Fehling's test.

Table 2. Phytochemical Screening of *P. cineraria* Bark and Pods

Phytochemical class	***Test***	***Methanolic Bark***	***Methanolic Pods***	***Pet. Ether Bark***	***Pet. Ether Pods***	***Ethyl Acetate Bark***	***Ethyl Acetate Pods***
Alkaloids	Dragendorff's test	Present	Present	Absent	Absent	Present	Present
Carbohydrates	Fehling's test	Absent	Present	Absent	Present	Absent	Present
Proteins	Ninhydrin test	Present	Present	Absent	Absent	Absent	Absent
Saponins	Foam test	Present	Present	Absent	Absent	Absent	Absent
Tannins	Ferric chloride test	Present	Present	Present	Present	Present	Present
Flavonoids	Alkaline reagent test	Present	Present	Absent	Absent	Present	Present
Starch	Iodine test	Absent	Absent	Absent	Absent	Absent	Absent

Table 3. Zone of inhibition (mm, mean of triplicate readings) of *P. cineraria* bark and pod extracts against *E. coli*, by solvent and concentration

Solvent	***Plant part***	***Concentration range tested***	***Zone of inhibition range (mean, mm)***
Petroleum ether	Bark	0.2 - 1.0 mg/mL	8.7 (at 0.2) -> 25.0 (at 1.0)
Petroleum ether	Pods	1.6 - 2.0 mg/mL	9.0 (at 1.6) -> 16.0 (at 2.0)
Ethyl acetate	Bark	0.3 - 1.0 mg/mL	15.0 (at 0.3) -> 32.0 (at 1.0)
Ethyl acetate	Pods	1.3 - 2.0 mg/mL	8.7 (at 1.3) -> 20.3 (at 2.0)
Methanol	Bark	0.3 - 1.0 mg/mL	8.3 (at 0.3) -> 23.7 (at 1.0)
Methanol	Pods	1.2 - 2.0 mg/mL	10.3 (at 1.2) -> 27.3 (at 2.0)
Ampicillin (positive control)	--	Fixed reference concentration	~36.0 (all plates)
DMSO (negative control)	--	Fixed reference concentration	0.0 (all plates)

Overall, methanol produced the broadest positive phytochemical profile among the solvents tested for both bark and pods, ethyl acetate produced an intermediate profile (positive for alkaloids, tannins, and flavonoids but not proteins or saponins), and petroleum ether produced the narrowest profile of the three — a pattern consistent with the solvent-polarity principles described in prior phytochemical surveys of the species[11], in which ethanol and water were similarly identified as the most effective solvents for metabolite recovery from P. cineraria. This qualitative ranking is further supported by the quantitative extractive-yield data in Table 1, where methanol likewise gave the highest dry-weight yield for both tissues.

Antibacterial Activity Against *E. coli*:

Bark and pod extracts in petroleum ether, ethyl acetate, and methanol were evaluated for antibacterial activity against *E. coli* across a concentration gradient by the agar well diffusion method, with ampicillin (positive control) consistently producing a zone of inhibition of approximately 36 mm and DMSO (negative control) producing no zone of inhibition (0 mm) in every plate, confirming assay validity.

All three solvents can be compared directly for pods (Table 3, Fig. 4), methanol reached the highest zone of inhibition of the three at the top of its tested range (27.3 mm at 2 mg/mL), exceeding ethyl acetate (20.3 mm at 2 mg/mL) and petroleum ether (16.0 mm at 2 mg/mL) at the same concentration, and methanol pod extract was also active from a slightly lower starting concentration (1.2 mg/mL) than ethyl acetate (1.3 mg/mL) or petroleum ether (1.6 mg/mL) pod extract. This mirrors the extractive-yield ranking in Table 1, where methanol likewise gave the highest yield for pods, and suggests that, for pod tissue specifically, methanol is the more effective solvent for both metabolite recovery and antibacterial performance, even though it does not overtake ethyl acetate bark extract as the single most active preparation overall.

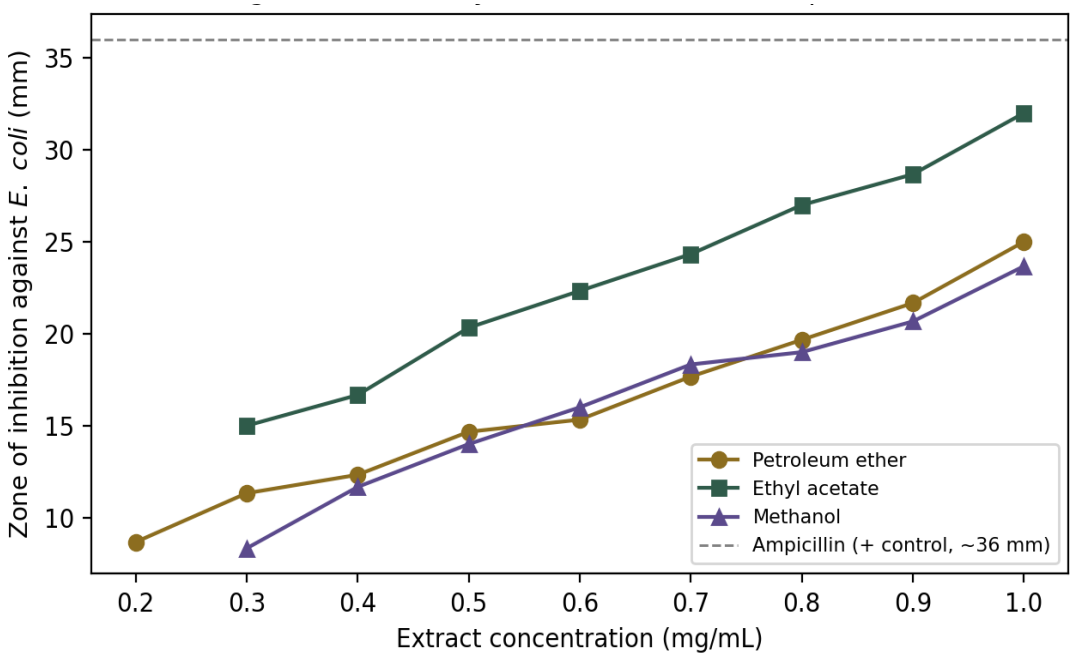

Fig. 3. Dose-response relationship between extract concentration and zone of inhibition against *E. coli* for *P. cineraria* bark extracts in three solvents, with the ampicillin positive control shown as a reference line (mean of three replicates).

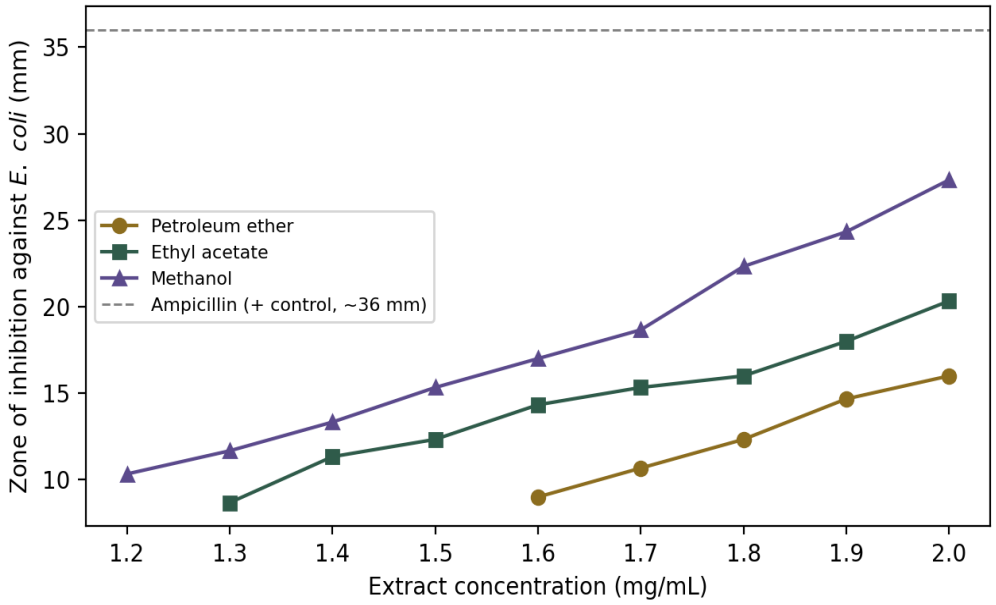

Fig. 4. Dose-response relationship between extract concentration and zone of inhibition against *E. coli* for *P. cineraria* pod extracts in three solvents, with the ampicillin positive control shown as a reference line (mean of three replicates).

The most striking pattern in Table 3 and Figs. 3-4 remains that bark extracts produced measurable inhibition zones at concentrations roughly four to eight times lower than pod extracts in the same solvent: bark extracts in petroleum ether and ethyl acetate showed activity from as low as 0.2-0.3 mg/mL, whereas pod extracts in all three solvents required concentrations of 1.2-1.6 mg/mL before any measurable zone of inhibition appeared. This indicates that, on a concentration-for-concentration basis, bark extract is considerably more potent against *E. coli* than pod extract, a pattern now corroborated by the extractive-yield data in Table 1, which shows bark also yielding more extract by mass than pods in every solvent tested so bark is therefore both the more extractable and the more antibacterially potent of the two tissues examined here.

Among the three solvents tested on bark, ethyl acetate produced the strongest activity at every matched concentration for which data were available, reaching a zone of inhibition of 32 mm at 1 mg/mL which was approaching the ampicillin positive control (~36 mm) and could be compared with 25 mm for petroleum ether and 23.7 mm for methanol bark extract at the same 1 mg/mL concentration. This ranking is consistent with the phytochemical screening pattern in Table 2, where ethyl acetate bark extract tested positive for alkaloids, tannins, and flavonoids; these phytochemical classes are repeatedly implicated in antibacterial activity against Gram-negative organisms such as *E. coli*[4] and with prior bioassay-guided fractionation of *P. cineraria* aerial parts, in which the ethyl acetate fraction likewise showed the strongest antibacterial activity among fractions tested[17].

Conclusion

This study determined the dry-weight extractive yield of, and screened for major phytochemical classes in, bark and pod extracts of *Prosopis cineraria* across petroleum ether, ethyl acetate, and methanol, and evaluated the antibacterial activity of the same extracts against *Escherichia coli*. Methanol gave the highest extractive yield for both tissues (7.55% bark, 3.15% pods) and the broadest positive phytochemical profile; petroleum ether gave the lowest yield and narrowest phytochemical profile, and bark

outperformed pods on both extractive yield and antibacterial potency in every solvent tested. Antibacterial testing showed that bark extracts inhibited *E. coli* at markedly lower concentrations than pod extracts in the same solvent, and that ethyl acetate was consistently the most effective solvent for bark, producing a zone of inhibition (32 mm at 1 mg/mL) that approached the ampicillin positive control, while methanol was the most effective solvent for pods. *Prosopis cineraria* can be considered an alternative protein source for populations facing energy-protein malnutrition, and the plant produces a range of compounds — alkaloids, tannins, flavonoids, saponins, carbohydrates, and proteins — some of which, as shown here, exhibit antibacterial activity. Bark ethyl acetate extract in particular is identified as the most promising material, among those tested, for future bioassay-guided fractionation, compound isolation, and testing against a wider panel of Gram-positive and Gram-negative organisms.

Acknowledgments

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