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Evaluation of Antibacterial Activity of Methanolic and Ethanolic Extracts of *Celastrus paniculatus* Willd. against *Escherichia coli*

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Abstract

Celastrus paniculatus Willd. (Celastraceae), commonly known as Malkangani or Jyotishmati or Kujri, is an important medicinal plant traditionally used in Indian systems of medicine. Different parts of the plant, particularly the seeds, roots, leaves, and stems, have been associated with traditional applications in neurological disorders, rheumatism, skin disorders, wounds, gastrointestinal complaints, and other ailments . The present study evaluated the antibacterial activity of methanolic and ethanolic extracts prepared from different parts of *C. paniculatus* against *Escherichia coli* strain 3099 using the Kirby–Bauer disc diffusion method. Mature leaves, stems, roots, and seeds were collected from Khunti district, Jharkhand, India, during October 2024. The plant was taxonomically authenticated at the Central National Herbarium, Botanical Survey of India, Howrah, CNS/31/2025/Tech. II. Dried and powdered plant materials were separately extracted using methanol and ethanol. Antibacterial activity was assessed at extract volumes of 20, 40, 60, and 80 µL, while tetracycline (TE 30) was used as the positive control. Among the methanolic extracts, the seed extract exhibited the greatest antibacterial activity, producing inhibition zones of 11, 14, 18, and 19 mm at 20, 40, 60, and 80 µL, respectively, with a mean inhibition zone of 15.50 ± 3.70 mm. The methanolic root extract showed the next highest activity, with a mean inhibition zone of 10.75 ± 0.50 mm. Among the ethanolic extracts for which data were available, the seed extract demonstrated the highest activity, with inhibition zones of 9, 13, 15, and 16 mm and a mean of 13.25 ± 3.10 mm. Tetracycline produced considerably larger inhibition zones, ranging from 30 to 34 mm. Overall, the results indicate that the seed extract of *C. paniculatus*, particularly the methanolic extract, exhibited comparatively greater antibacterial activity against *E. coli* than the other plant parts investigated. These findings provide preliminary evidence supporting further investigation of *C. paniculatus* seeds for antibacterial constituents. Further studies incorporating independent biological replicates, standardized extract concentrations, statistical hypothesis testing, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and phytochemical characterization are required to establish the antibacterial potential and identify the bioactive constituents responsible for the observed activity.

Keywords: *Celastrus paniculatus* willd.; Malkangani; Jyotishmati; Celastraceae; antibacterial activity; *Escherichia coli*; methanolic extract; ethanolic extract;

1. Introduction

Medicinal plants have played an important role in traditional healthcare systems and remain important sources of chemically diverse natural products. Increasing antimicrobial resistance and the continuing need for new antibacterial compounds have stimulated renewed interest in medicinal plants and their secondary metabolites as potential sources of bioactive molecules. [1, 5]

Celastrus paniculatus Willd. belongs to the family Celastraceae and order Celastrales. Under the Angiosperm Phylogeny Group IV classification, Celastraceae is placed within Celastrales [6]. The currently accepted name is *Celastrus paniculatus* Willd. and current taxonomic databases recognize the species as native from the Indian subcontinent to southern China and Indochina [7]. The plant is commonly known as Malkangani, Jyotishmati, Staff Tree, Kujri, Intellect Plant, and by several regional names in India. [2, 7]

The species is a woody climbing or scrambling shrub. It occurs principally in forested and hilly habitats and is widely distributed in India. Indian floristic resources describe it as an unarmed climbing shrub with simple elliptic, ovate or obovate leaves, unisexual flowers arranged in panicles, globose capsules, and seeds enclosed by a conspicuous scarlet aril [8, 9].

Different parts of *C. paniculatus*, especially seeds and seed oil, have a long history of use in Ayurvedic and other traditional medicinal systems. Traditional applications have included neurological and cognitive complaints, paralysis, epilepsy, insomnia, rheumatism, arthritis, sciatica, skin disorders, wounds, and gastrointestinal ailments [1–4]. The seeds and their oil have received particular attention because of their traditional association with memory and cognitive functions [2–4].

Ethnobotanical records from Jharkhand also document medicinal use of *C. paniculatus*. A biodiversity resource of the Government of Jharkhand reports the use of the roots together with seeds, prepared as a paste, for body ache and joint pain [10]. A handbook of medicinal plants of Jharkhand further records the use of seeds and seed oil in traditional preparations for several ailments, including rheumatic pain, wounds, paralysis and skin disorders [11]. Such ethnobotanical information provides a rationale for further pharmacological investigation but should not be interpreted as evidence of clinical efficacy.

The phytochemical profile of *C. paniculatus* is chemically diverse. Reported constituents include sesquiterpenes, β-dihydroagarofuranoids, alkaloids, flavonoids, terpenoids, sterols, fatty acids, and other secondary metabolites [2–4]. Recent reviews have summarized a broad range of reported pharmacological properties, including antioxidant, anti-inflammatory, wound-healing, neuroprotective, antimicrobial and other activities [1–3].

Experimental studies have also reported antibacterial activity associated with *C. paniculatus*. A large-scale screening study of ethnomedicinal plants reported antibacterial activity of methanolic *C. paniculatus* extracts against Gram-positive and Gram-negative bacteria, including *E. coli* [12]. Earlier investigations summarized in subsequent reviews have also reported antibacterial activity of seed-derived preparations against *E. coli* and other bacterial species [3, 13]. These findings support further investigation of different plant organs and extraction solvents.

The extraction solvent can influence the qualitative and quantitative recovery of phytochemicals and consequently affect the biological activity of plant extracts. Methanol and ethanol differ in polarity and extraction characteristics, and comparative evaluation may therefore provide useful information regarding the relative antibacterial activity of different plant parts.

Therefore, the present study was undertaken to comparatively evaluate the antibacterial activity of methanolic and ethanolic extracts of leaves, stems, roots, and seeds of *C. paniculatus* against *Escherichia coli* strain 3099 using a disc diffusion assay. The study particularly examined whether antibacterial activity varied according to plant part, extraction solvent, and volume of extract applied.

2. Botanical and Ethnobotanical Profile

2.1 Taxonomic classification

The taxonomic placement of *Celastrus paniculatus* follows the Angiosperm Phylogeny Group IV classification [6], while current nomenclature and distribution were checked against current taxonomic resources [7, 8].

Kingdom: Plantae
Order: Celastrales
Family: Celastraceae
Genus: *Celastrus* L.
Species: *Celastrus paniculatus* Willd.

Accepted botanical name: *Celastrus paniculatus* Willd.

Common names: Malkangani, Malkangni, Jyotishmati, Staff Tree, Intellect Plant, Kujri.

The current Plants of the World Online record recognize *Celastrus paniculatus* Willd. as an accepted species in Celastraceae [7]. India Flora Online also records the species under Celastraceae and provides regional vernacular names and distribution information [8].

2.2 Botanical description

Celastrus paniculatus Willd. is an unarmed woody climbing or scrambling shrub. The leaves are simple, generally elliptic, ovate or obovate, with crenate to serrate margins. The flowers are small, unisexual and yellowish-green or whitish, and occur in terminal or axillary panicles. The fruit is a globose or subglobose capsule that becomes yellowish when mature and is loculicidally three-valved. The seeds are generally ovoid or ellipsoid and are enclosed by a conspicuous fleshy scarlet aril [8, 9].

The species is characteristically associated with forest margins, moist deciduous forests and semi-evergreen habitats in parts of its Indian range [8, 9].

2.3 Distribution

Celastrus paniculatus Willd. has a native distribution extending from the Indian subcontinent to southern China and Indochina [7]. In India, it has been reported from several regions including parts of the Himalayan and peninsular areas and a number of forested and hilly localities [8, 9].

India Flora Online records the species from numerous Indian districts and describes its habitat as moist deciduous and semi-evergreen forest, with occurrences extending to approximately 1400 m in some regions [8].

The present plant material was collected from Khunti district, Jharkhand, India, where the species is also represented in traditional medicinal knowledge [10, 11].

3. Traditional Medicinal and Ethnobotanical Importance

Celastrus paniculatus willd. has been used traditionally in Ayurveda and other Indian systems of medicine. The seeds and seed oil are particularly important medicinal parts and have traditionally been associated with cognitive and neurological applications [1–4].

Traditional reports describe the use of seeds and seed oil for neurological complaints, memory-related applications, rheumatism, arthritis, paralysis, insomnia, wounds, and certain skin disorders [2–4, 11]. Root and other plant parts have also been used in traditional preparations for various ailments.

Ethnobotanical information from Jharkhand provides additional evidence of local medicinal use. Government biodiversity documentation reports that root together with seeds may be pounded into a paste and applied for body ache and joint pain [10]. A medicinal-plant handbook for Jharkhand records traditional use of the seeds and seed oil for conditions including rheumatic pain, wounds, paralysis and certain skin ailments [11].

The ethnomedicinal importance of the species has also been reviewed extensively in recent literature. Kumar and Jaitak [1] summarized traditional uses, phytochemistry, pharmacological activities and safety considerations, while Nagpal et al. [2] reviewed the phytochemical and pharmacological literature on the species.

Traditional use, however, does not establish therapeutic efficacy. Controlled laboratory studies are required to determine biological activity, potency, mechanism of action, safety, and the identity of responsible constituents.

4. Materials and Methods

4.1 Collection and authentication of plant material

Mature leaves, stems, roots, and seeds of *Celastrus paniculatus* Willd. (Family Celastraceae) were collected during October 2024 from Khunti district, Jharkhand, India.

Roots were carefully excavated from the soil, and adhering soil particles were removed by shaking followed by washing with water. Healthy leaves, stems, roots and mature seeds were collected from mature plants and transported to the laboratory in clean containers.

The plant material was taxonomically authenticated at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. CNS/31/2025/Tech. II

Sl. No.	Scientific Name	Family
1	***Celastrus paniculatus*** Willd.	Celastraceae

4.2 Processing of plant material

The collected leaves, stems, roots and seeds were separately washed with water and shade-dried at room temperature with occasional turning to facilitate uniform drying.

The dried plant materials were separately pulverized using a mechanical grinder. The powdered materials were passed through sieve No. 40 to obtain relatively uniform powder.

The powders were separately stored in clean, dry, airtight containers until extraction.

4.3 Preparation of methanolic extracts

For preparation of methanolic extracts, 100 g of powdered leaf, stem, root and seed material was separately soaked in 200 mL methanol in clean, wide-mouthed, airtight containers.

The mixtures were allowed to undergo extraction with occasional shaking. Following extraction, each mixture was filtered through Whatman No. 40 filter paper to remove plant residues.

The filtrates were concentrated using a rotary evaporator at approximately 50°C until the solvent was removed and concentrated crude extracts were obtained.

The extracts were transferred to sterile containers and stored under refrigerated conditions until antibacterial testing.

4.4 Preparation of ethanolic extracts

Ethanolic extracts were prepared using the same general procedure.

Briefly, 100 g of each powdered plant material was separately extracted with 200 mL ethanol in clean, airtight containers. The mixtures were allowed to extract with occasional shaking and subsequently filtered through Whatman No. 40 filter paper.

The filtrates were concentrated using a rotary evaporator at approximately 50°C. The resulting crude extracts were transferred into sterile airtight containers and stored under refrigerated conditions until antibacterial evaluation.

4.5 Test organism

The antibacterial activity of the prepared plant extracts was evaluated against *Escherichia coli* strain 3099.

A fresh bacterial suspension was prepared before the assay and standardized according to the laboratory procedure used for the experiment.

The exact culture collection/source and strain identification should be stated in the final manuscript if available.

4.6 Antibacterial assay

Antibacterial activity was evaluated using the Kirby–Bauer disc diffusion method. The general principles of standardized antimicrobial disc susceptibility testing are described in the Clinical and Laboratory Standards Institute M02 standard [14].

The prepared plant extracts were tested at volumes of 20, 40, 60 and 80 µL. Following incubation under the conditions used in the laboratory, the diameter of the inhibition zone surrounding each test disc was measured in millimetres.

Because the exact agar medium, inoculum standardization, disc diameter, incubation temperature and incubation period were not provided in the available experimental record, these parameters should be added before submission.

4.7 Positive control

Tetracycline (TE 30) was used as the positive control. The inhibition zones produced by the plant extracts were compared with those produced by tetracycline under the experimental conditions.

4.8 Data analysis

Antibacterial activity was expressed as the diameter of the inhibition zone in millimetres.

Mean values and standard deviations were calculated from the available measurements.

Because the supplied dataset does not specify the number of independent biological or technical replicates, inferential statistical testing was not performed. The reported mean ± SD values are therefore presented descriptively.

5. Results

5.1 Antibacterial activity of methanolic extracts

The methanolic leaf extract produced inhibition zones of 7, 7, 9 and 10 mm at 20, 40, 60 and 80 µL, respectively, with a mean inhibition zone of 8.25 ± 1.50 mm.

The methanolic stem extract produced inhibition zones of 7, 8, 11 and 9 mm, respectively, giving a mean value of 8.75 ± 1.71 mm.

The methanolic root extract produced inhibition zones of 10, 11, 11 and 11 mm, respectively, with a mean inhibition zone of 10.75 ± 0.50 mm.

The methanolic seed extract demonstrated the greatest antibacterial activity among the tested plant parts. It produced inhibition zones of 11, 14, 18 and 19 mm at 20, 40, 60 and 80 µL, respectively, resulting in a mean inhibition zone of 15.50 ± 3.70 mm.

Tetracycline produced inhibition zones of 31, 34, 32 and 30 mm in the corresponding assays.

Table 1. Antibacterial activity of methanolic extracts of *Celastrus paniculatus* Willd.against *E. coli*

Plant part	20 µL (mm)	40 µL (mm)	60 µL (mm)	80 µL (mm)	Mean ± SD (mm)	Tetracycline (mm)
Leaf	7	7	9	10	8.25 ± 1.50	31
Stem	7	8	11	9	8.75 ± 1.71	34
Root	10	11	11	11	10.75 ± 0.50	32
Seed	11	14	18	19	15.50 ± 3.70	30

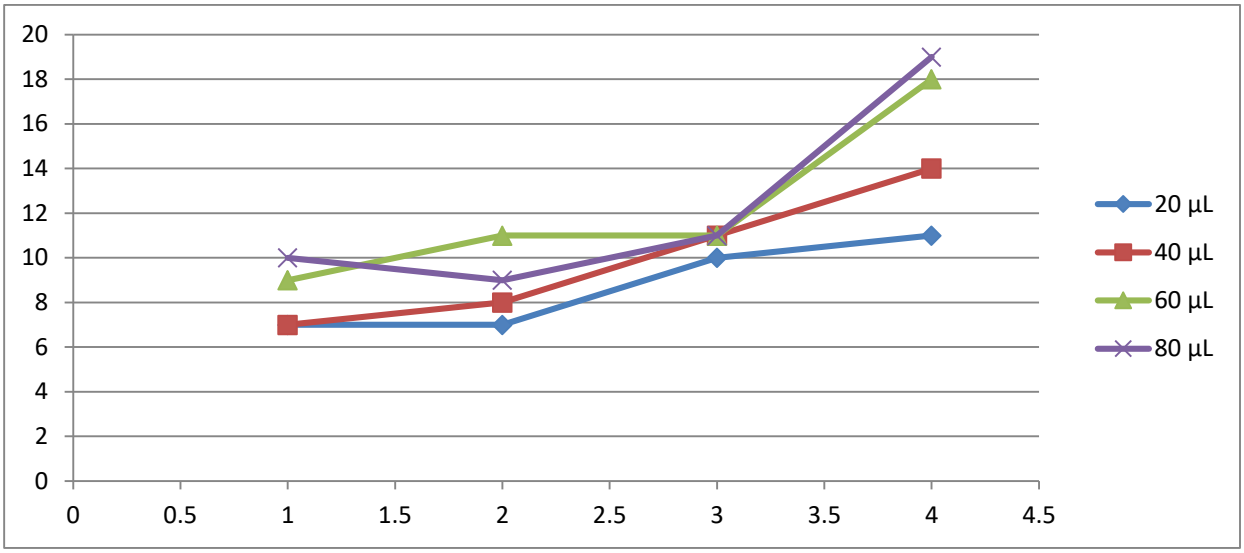

Figure:1. Antibacterial activity of methanolic extracts of *Celastrus paniculatus* Willd. leaf, stem, root and seed against E. coli at different extract concentrations.

5.2 Antibacterial activity of ethanolic extracts

The ethanolic stem extract produced inhibition zones of 9, 9, 10 and 11 mm at 20, 40, 60 and 80 µL, respectively, with a mean inhibition zone of 9.75 ± 0.96 mm.

The ethanolic root extract produced inhibition zones of 8, 10, 11 and 11 mm, respectively, resulting in a mean inhibition zone of 10.00 ± 1.41 mm.

The ethanolic seed extract showed the greatest activity among the ethanolic extracts for which data were available. It produced inhibition zones of 9, 13, 15 and 16 mm at 20, 40, 60 and 80 µL, respectively, with a mean inhibition zone of 13.25 ± 3.10 mm.

Tetracycline produced inhibition zones of 34, 31 and 30 mm in the corresponding stem, root and seed assays.

5.3 Comparative activity of methanolic and ethanolic seed extracts

The seed extract demonstrated the highest antibacterial activity among the plant parts examined.

At 80 µL, the methanolic seed extract produced an inhibition zone of 19 mm, whereas the corresponding ethanolic seed extract produced a zone of 16 mm.

The mean inhibition zone of the methanolic seed extract was 15.50 ± 3.70 mm, compared with 13.25 ± 3.10 mm for the ethanolic seed extract.

Thus, under the conditions of the present experiment, the methanolic seed extract exhibited comparatively greater inhibition of *E. coli* than the ethanolic seed extract.

Table 2. Antibacterial activity of ethanolic extracts of *Celastrus paniculatus* willd.against *E. coli*

Plant part	20 µL (mm)	40 µL (mm)	60 µL (mm)	80 µL (mm)	Mean ± SD (mm)	Tetracycline (mm)
Stem	9	9	10	11	9.75 ± 0.96	34
Root	8	10	11	11	10.00 ± 1.41	31
Seed	9	13	15	16	13.25 ± 3.10	30

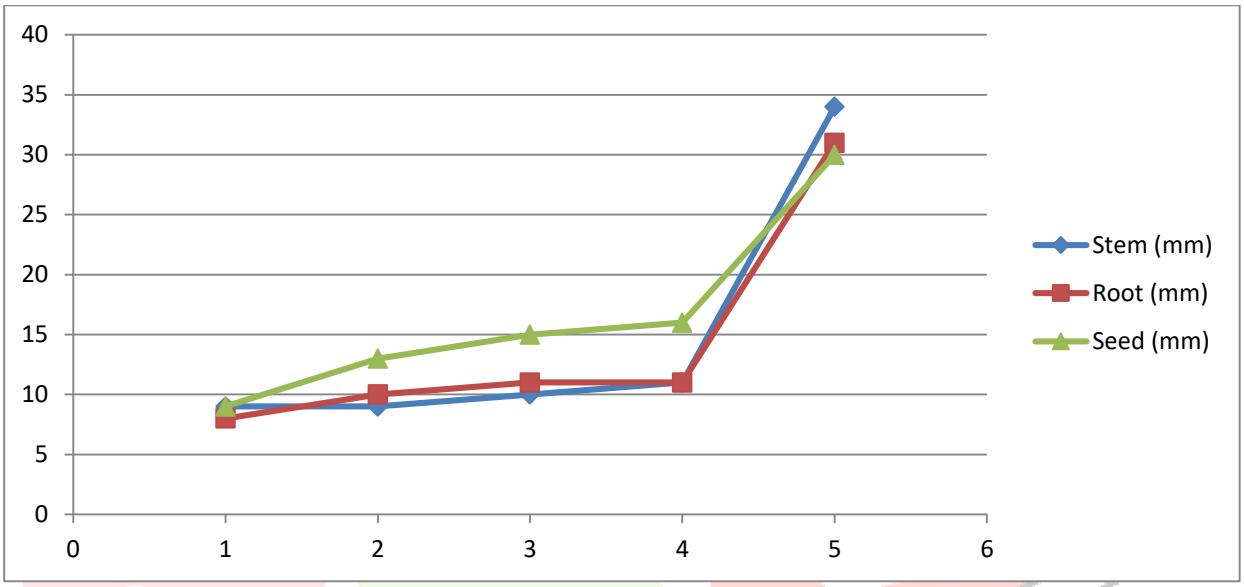

Figure: 2. Antibacterial activity of ethanolic extracts of *Celastrus paniculatus* willd. stem, root and seed against E. coli at different extract concentrations.

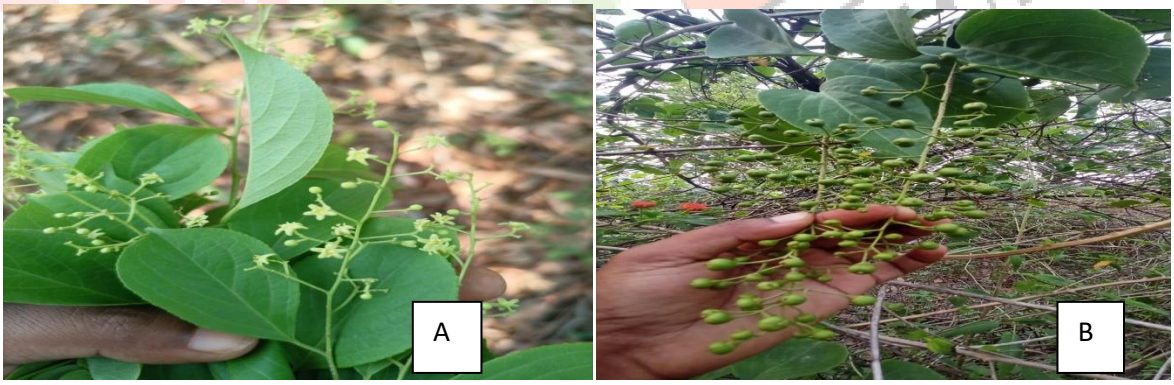

Figure: 3. (A-B) - Flowers and Fruits of *Celastrus paniculatus* Willd.

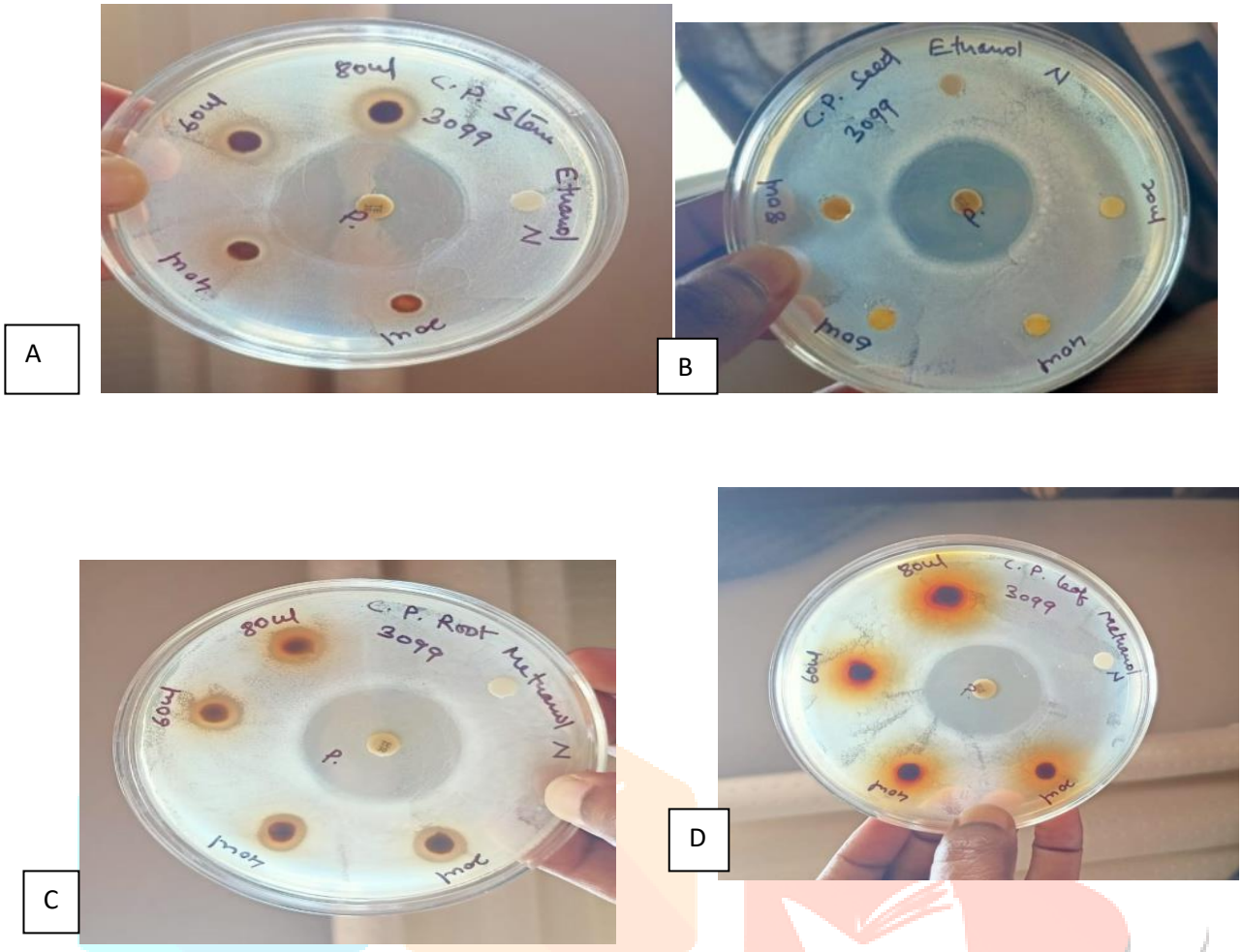

Figure: 4. Screening of plant extracts of *Celastrus paniculatus* Willd. (A) – stem extracts (Ethanol). (B) Seed extracts (Ethanol) (C)- Root extracts (Methanol). (D) - Leaves extract (Methanol).

6. Discussion

The present study demonstrated measurable antibacterial activity of *Celastrus paniculatus* willd. extracts against *Escherichia coli*. The magnitude of activity varied according to plant part and extraction solvent.

The methanolic seed extract demonstrated the greatest activity, producing a maximum inhibition zone of 19 mm at 80 µL and a mean inhibition zone of 15.50 ± 3.70 mm. The ethanolic seed extract also showed comparatively strong activity, reaching 16 mm at 80 µL and a mean of 13.25 ± 3.10 mm. Thus, the seed consistently demonstrated the strongest antibacterial response among the plant parts for which data were available.

The observed seed activity is consistent with previous literature describing biological activity associated with *C. paniculatus* seeds and seed-derived preparations [2–4, 12, 13]. A large-scale screening study of ethnomedicinal plants reported antibacterial activity for methanolic *C. paniculatus* extracts against Gram-positive and Gram-negative bacteria, including *E. coli* [12]. Earlier reports summarized in reviews have also documented antibacterial activity of seed-derived preparations against *E. coli* and other bacterial species [3, 13].

The relatively greater activity of the seed extract may be associated with the chemically diverse constituents reported from *C. paniculatus* seeds. The species contains sesquiterpenoids, β-dihydroagarofuranoids, alkaloids, terpenoids, flavonoids, sterols and fatty acids, among other constituents

[1–4]. However, the present study did not perform phytochemical characterization, and therefore no specific compound can be identified as responsible for the observed antibacterial effect.

The methanolic root extract showed the second-highest activity among the methanolic preparations, with a mean inhibition zone of 10.75 ± 0.50 mm. The ethanolic root extract showed a similar mean value of 10.00 ± 1.41 mm. The relatively comparable activity between methanolic and ethanolic root extracts suggests that both solvents may recover constituents capable of inhibiting *E. coli* under the experimental conditions.

The leaf and stem extracts generally showed lower activity than the seed extracts. The methanolic leaf and stem extracts produced mean inhibition zones of 8.25 ± 1.50 and 8.75 ± 1.71 mm, respectively, while the ethanolic stem extract produced a mean inhibition zone of 9.75 ± 0.96 mm.

Differences between plant organs may reflect variation in phytochemical composition and concentration. The distribution of secondary metabolites is frequently organ-dependent, and the efficiency with which these compounds are recovered may also vary according to solvent polarity and extraction conditions [1–3].

A concentration-related increase in antibacterial activity was particularly evident for the seed extracts. The methanolic seed extract increased from 11 mm at 20 µL to 19 mm at 80 µL, while the ethanolic seed extract increased from 9 mm to 16 mm over the same range.

This pattern suggests that increasing the amount of extract applied to the assay increased the quantity of potentially active constituents available for interaction with bacterial cells. Nevertheless, inhibition-zone diameter is affected not only by antibacterial potency but also by compound diffusion through agar, extract composition and concentration. Therefore, the observed increase should not be interpreted as a definitive dose-response relationship.

The methanolic seed extract showed greater inhibition than the corresponding ethanolic extract at all tested volumes. At 80 µL, the methanolic seed extract produced a 19-mm zone compared with 16 mm for the ethanolic extract.

This difference may reflect differences in extraction efficiency or in the chemical profiles of methanol and ethanol extracts. Recent phytochemical work on *C. paniculatus* has demonstrated that methanolic and ethanolic seed extracts can contain different sets of detectable compounds, supporting the possibility that extraction solvent influences the recovered chemical profile [15].

However, solvent efficiency cannot be concluded solely from inhibition-zone measurements. Extract yield, extract concentration, compound solubility, stability and diffusion characteristics can all influence the size of an inhibition zone.

The present findings are also consistent with the broader literature describing antibacterial properties of *C. paniculatus*. Reviews have summarized antibacterial activity among the diverse pharmacological effects reported for the species [1–4]. In addition, studies involving *C. paniculatus*-derived preparations or fractions have demonstrated activity against several bacterial organisms [3, 13].

The tetracycline control produced substantially larger inhibition zones than the plant extracts, ranging from 30 to 34 mm. This finding indicates that the standard antibiotic showed considerably stronger antibacterial activity against the tested *E. coli* strain under the experimental conditions.

The plant extracts should therefore be interpreted as showing preliminary antibacterial potential, rather than antibacterial efficacy comparable to tetracycline.

An important consideration is that disc diffusion assays alone cannot establish antibacterial potency. Standardized concentration-dependent testing such as MIC and MBC determination is required to quantify antibacterial activity. Furthermore, appropriate independent biological replicates and statistical analysis are necessary before differences between extracts can be considered statistically significant.

The results nevertheless provide a useful preliminary indication that the seeds of *C. paniculatus*, particularly the methanolic extract, warrant further investigation. The traditional medicinal importance of the species, together with its chemically diverse phytochemical profile and previously reported biological activities, provides a reasonable basis for subsequent isolation and characterization studies [1–4].

Future work should therefore include standardized extract preparation, determination of extraction yield, reporting of extract concentration in mg/mL, MIC and MBC determination, testing against a broader panel of bacterial pathogens, appropriate biological and technical replication, statistical hypothesis testing, phytochemical profiling using chromatographic and spectrometric techniques, and isolation of active compounds.

7. Limitations of the Study

Several limitations should be considered when interpreting the present findings.

First, the supplied dataset does not specify the number of independent biological or technical replicates for each concentration. Therefore, the reported mean ± SD values should be interpreted descriptively and not as evidence of statistically significant differences.

Second, the extracts were applied as volumes of 20–80 µL rather than as standardized mass concentrations such as mg/mL. Because inhibition-zone size depends partly on the amount of extract applied, future experiments should report extract concentration and the actual mass of extract applied per disc.

Third, the exact extraction duration was not specified in the available methodology. Extraction time and other extraction parameters should be standardized and reported to improve reproducibility.

Fourth, data for the ethanolic leaf extract were not supplied. Consequently, no value has been estimated or extrapolated.

Fifth, the present study evaluated only one bacterial species and one strain. The antibacterial spectrum of *C. paniculatus* therefore cannot be generalized to other Gram-negative or Gram-positive organisms.

Sixth, disc diffusion results alone do not provide MIC or MBC values. Additional quantitative antimicrobial assays are required to establish antibacterial potency.

Finally, phytochemical characterization was not performed. Consequently, the active constituents responsible for the observed antibacterial effect remain unidentified.

8. Conclusion

The present study provides preliminary evidence that methanolic and ethanolic extracts of *Celastrus paniculatus* Willd. possess antibacterial activity against *Escherichia coli* strain 3099.

The antibacterial response varied according to plant part and extraction solvent. Among the plant materials investigated, the seed extract demonstrated the greatest antibacterial activity. The methanolic seed extract was particularly active, producing a maximum inhibition zone of 19 mm at 80 µL and a mean inhibition

zone of 15.50 ± 3.70 mm. The ethanolic seed extract produced a maximum inhibition zone of 16 mm at 80 µL and a mean of 13.25 ± 3.10 mm.

Root extracts demonstrated moderate activity, whereas leaf and stem extracts generally showed lower activity. Tetracycline produced considerably larger inhibition zones than the plant extracts, indicating substantially stronger antibacterial activity under the experimental conditions.

The findings suggest that *C. paniculatus*, particularly its seeds, warrants further investigation as a potential source of antibacterial compounds. However, the present results should be considered preliminary. Standardized extract concentrations, independent biological replicates, statistical analysis, MIC and MBC determination, broader antimicrobial screening, and phytochemical characterization are required before conclusions regarding antibacterial potency or active constituents can be established.

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